Effects of oil pollution in the ecology, behaviour and physiology of the sand lizard (Acanthodactylus scutellatus) in Kuwait.

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Effects of Oil Pollution on the Ecology, Behaviour and Physiology of the Sand Lizard (*Acanthodactylus scutellatus*) in Kuwait

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DEDICATION

To my late parents, may their souls rest in perfect peace

SUMMARY

The effects of oil pollution on a sand lizard (*Acanthodactylus scutellatus*) were studied throughout 2002 and 2003 at Greater Al-Burgan oil fields in Kuwait. This study examined the effects on this species because large land-based oil spills (such as in Kuwait in 1991) present a new environmental concern.The most unexpected outcome of the study was that assumptions made about the degrees of contamination based on physical evidence (such as soot and tar) were not supported. All the classes of contaminated sites appeared to be equally contaminated. Population studies of the lizards and their ant prey seem to have many interpretational problems. The population sizes of *A. scutellatus* did not vary markedly among the study sites using the pitfall trap and transect methods which could be due to the fact that all the sites were equally polluted with oil. Initially, the daily behaviour of *A. scutellatus* was observed. Field observations included the timing of morning emergence as well as basking and foraging behaviours. These behaviours seemed influenced by oil pollution with lizards on the highly polluted sites emerging earlier than the other sites. The presumably highly polluted sites exhibited the highest substrate temperatures, influencing basking of *A. scutellatus*. Basking duration decreased as the degree of pollution increased. Foraging behaviour did not differ between the study sites because the lizards continued as a 'Sit and wait' predators at all the locations and did not have to increase their foraging. Lizards were examined in the laboratory for their substrate preference. They were monitored using a digital video camera and the times spent on polluted and/or nonpolluted substrates were recorded. Lizards collected from the tar mat sites preferred to remain on the dark substrate whereas those collected from the control sites chose the light substrate. The strength of this response suggests that the behaviour is highly adaptive because possessing this cryptic colouration is essential to avoid predators. Lizard body size and weight were measured and adult lizards were larger on the tar mat and soot sites than on the clear and control sites. Food appeared to be available in greater quantities on the tar mat and soot sites and consuming prey with high levels of fat resulted in lizards accumulating adipose tissue in their bodies. Crude oil contains heavy metals with nickel and vanadium generally being the predominant elements. An attempt was also made to determine if heavy metals in the environment influenced sand

lizards. Concentrations of these elements were determined in soil and whole body tissues of lizard using ICP/AES analysis. There was a significant variation in nickel concentration in soil between the control and the soot and tar mat sites. Nickel concentration differed in lizard samples from the control and the tar mat sites. Vanadium concentration in soil differed between the control and the tar mat sites but did not show any difference in lizard tissues between the different study sites. Sixteen PAHs (EPA priority pollutants) were studied using GC/MS in lizard and ant whole body tissues to investigate their presence and concentration. Of the 16, phenanthrene, fluoranthene and benzo[a]anthracene were present in the polluted sites but undetectable in the control sites for both lizards and ants. Although 12 years have passed since the Kuwait oil spill catastrophe, all sites are still contaminated with PAHs (there was no distinction between tar mat, soot and clear sites). The effects of PAHs and heavy metals on the histopathology of *A. scutellatus* vital organs such as liver were also investigated. Hepatocytes showed remarkable responses to PAHs and heavy metals. Swollen hepatocytes, ballooning degeneration of cytoplasm and dead cells were the most common cytopathological signs observed. This research confirms that *A. scutellatus* is a suitable bioindicator species for ecotoxicological studies on the effect of PAH compounds. The importance of lizards was emphasized in hope that they be included in ecological risk assessments as well as studies on environmental contamination in desert locations such as Kuwait. This is because lizards are an important component of biodiversity, and many such species are listed as threatened or endangered.

DECLARATIONS AND STATEMENTS

DECLARATION

This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree.

Signed ... . **(Candidate)**

Date ..12.-.01.-.2006.....

STATEMENT 1

This thesis is the result of my own investigations, except where otherwise stated.
Other sources are acknowledged by footnotes giving explicit references.
A bibliography is appended.

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STATEMENT 2

I hereby give my consent of my thesis, if accepted, to be available for photocopying and for interlibrary loan, and for the title and summary to me made available to outside organizations.

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Date ...12.-.01.-.2006.....

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ABBREVIATIONS

AHs	Aliphatic hydrocarbons
Aminoil	American Independent Oil Company
ANOVA	Analysis of Variance
BP	British Petroleum
bw	Body Weight
Ci	Confidence Intervals
cm	Centimetres
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
df	Degrees of freedom
DMBA	9,10 di-methyl benz(1-2)anthracene
ERA	Ecological Risk Assessment
GC/MS	Gas Chromatography/Mass Spectrometry
GESAMP	The Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection
HCs	Hydrocarbons
H&E	Haematoxylin and eosin
IARC	International Agency for Research on Cancer
ICP/AES	Inductively Coupled Plasma/Atomic Emission Spectroscopy
IUCN	International Union for the Conservation of Nature
KISR	Kuwait Institute for Scientific Research
KNPC	Kuwait National Petroleum Company
KOC	Kuwait Oil Company
KPC	Kuwait Petroleum Company
KW.EPA	Kuwait Environmental Protection Authority
LD_{50}	Median Lethal Dose

LSD	Least Significant Difference
m	metres
mm	millimetres
MOOPAM	Manual of Oceanographic Observations and Pollutant Analysis Methods
MPM	Moves per minute
ms	Mean of squares
n	Estimate of population size
NIOSH	National Institute for Occupational Safety and Health
OAPEC	Organization of Arab Petroleum Exporting Countries
OPEC	Organization of Petroleum Exporting Countries
PAHs	Polycyclic aromatic hydrocarbons
ppm	Parts per million
Rfc	Reference Concentration (risk)
Rfd	Reference Dose (risk)
ROPME	Regional Organization for the Protection of the Marine Environment
rpm	Revolutions per minute
s.d.	Standard deviation
SVL	Snout-vent length
SW	Sit-and-Wait
UXO	Unexploded Ordnance
VTL	Vent-tail length
WF	Widely Foraging

CHAPTER 1

General Introduction

1.1 Location and Area

The state of Kuwait covers an area of approximately 17,818 km^2. It is situated at the head of the Arabian Gulf between latitudes 28° and 30° north and longitudes 46° and 48° east. A 240 km border with Iraq runs along the north and west of Kuwait and in the south a 250 km border is the boundary with Saudi Arabia, while the Arabian Gulf marks the eastern limit (Fig. 1.1, p 2).

The state of Kuwait also includes nine offshore islands. The only inhabited island is Failaka, situated in the mouth of Kuwait Bay. The other islands are Bubian and Warbah to the north, Umm An Namel, Auhah, Kubbar, Qaruh, Miskan and Umm Al Maradim to the south (Clayton and Pilcher, 1983).

1.2 Surface and Topography

The mainland of Kuwait is a generally flat, desert landscape. A few oases, undulating sand hills and the occasional escarpment or depression break the uniformity of the interior plateau (Fig. 1.2, p 3). The Wadi Al Batin forms one of the Kuwait's prominent topographic features. The Wadi (a generally dry river bed) runs south-west to north-east, making a distinctive western border with Iraq and, in places, cutting some 45 metres (m) into the plateau. To the north-east, beyond Jabal Sanam, the bed of the Wadi becomes a track indistinguishable from the surrounding countryside. The Zor escarpment, stretching approximately 60 km from Atraf north-eastward to Al Bahrat, forms another of Kuwait's major physical features. The plateau of Kuwait slopes gently towards the north-east and exhibits many shallow basins and playas across its entire extent. In the far north-eastern area of Kuwait, the countryside has a number of conspicuous sand dunes. With the exception of the Wadis along the coast, which drain into the Gulf, the Wadi Systems of Kuwait all drain into the interior. The most mature Wadi systems are situated in the northern part of Kuwait in the Rawdatain and Umm Al Aish regions (Clayton and Pilcher, 1983; Clayton and Wells, 1987).

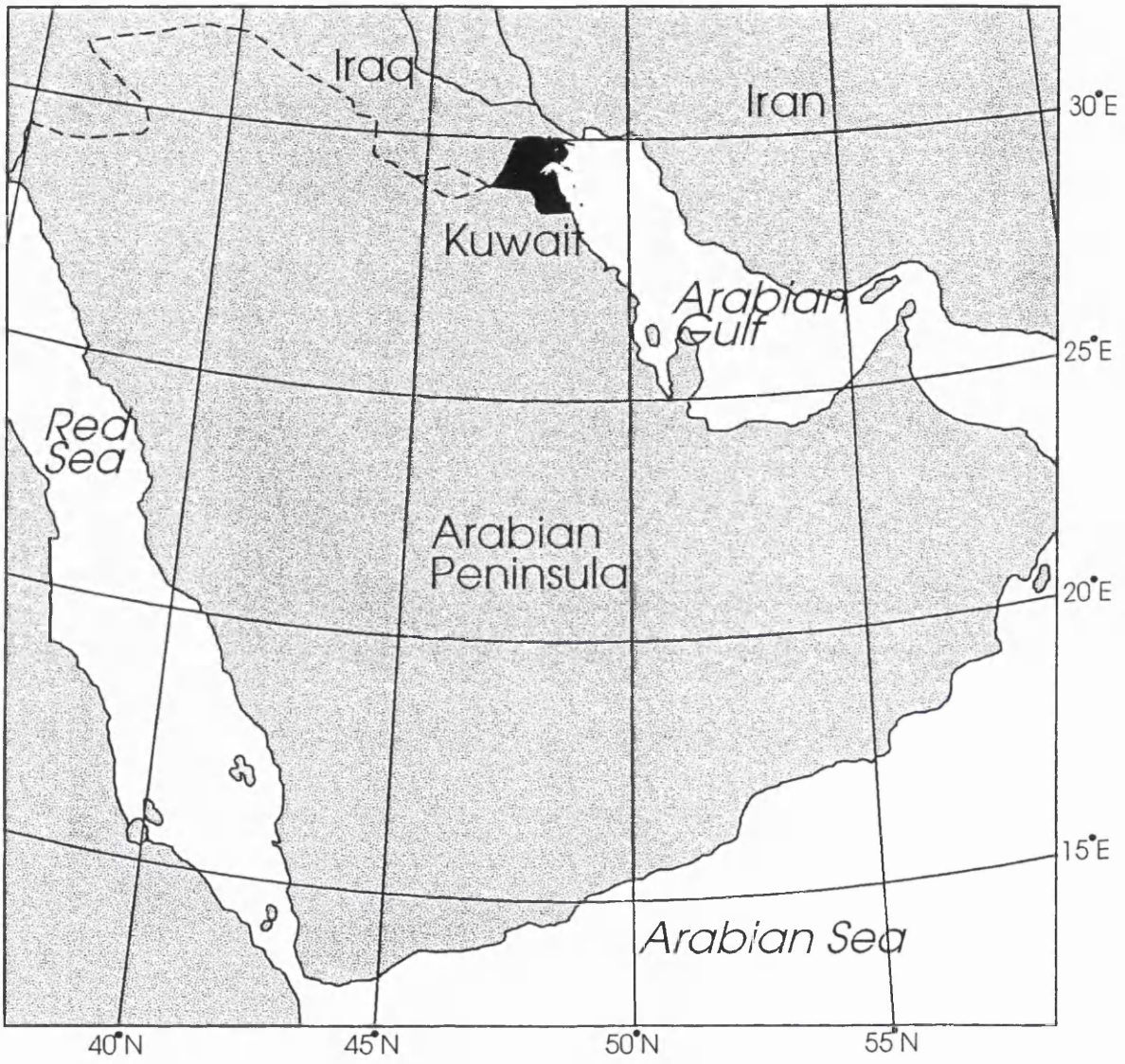

Figure 1.1 Geographical location of Kuwait (From Omar, S.; Misak, R.; Bhat, N.; Shahid, S. and Delima, E. (2003) Assessing Damage Magnitude and Recovery of the Terrestrial Ecosystem/ Follow Up of Natural and Induced Desert Recovery (FA015C). *Final Report Submitted to: Public Authority for Assessing Compensation for Damages Resulting from Iraqi Aggression, September)*

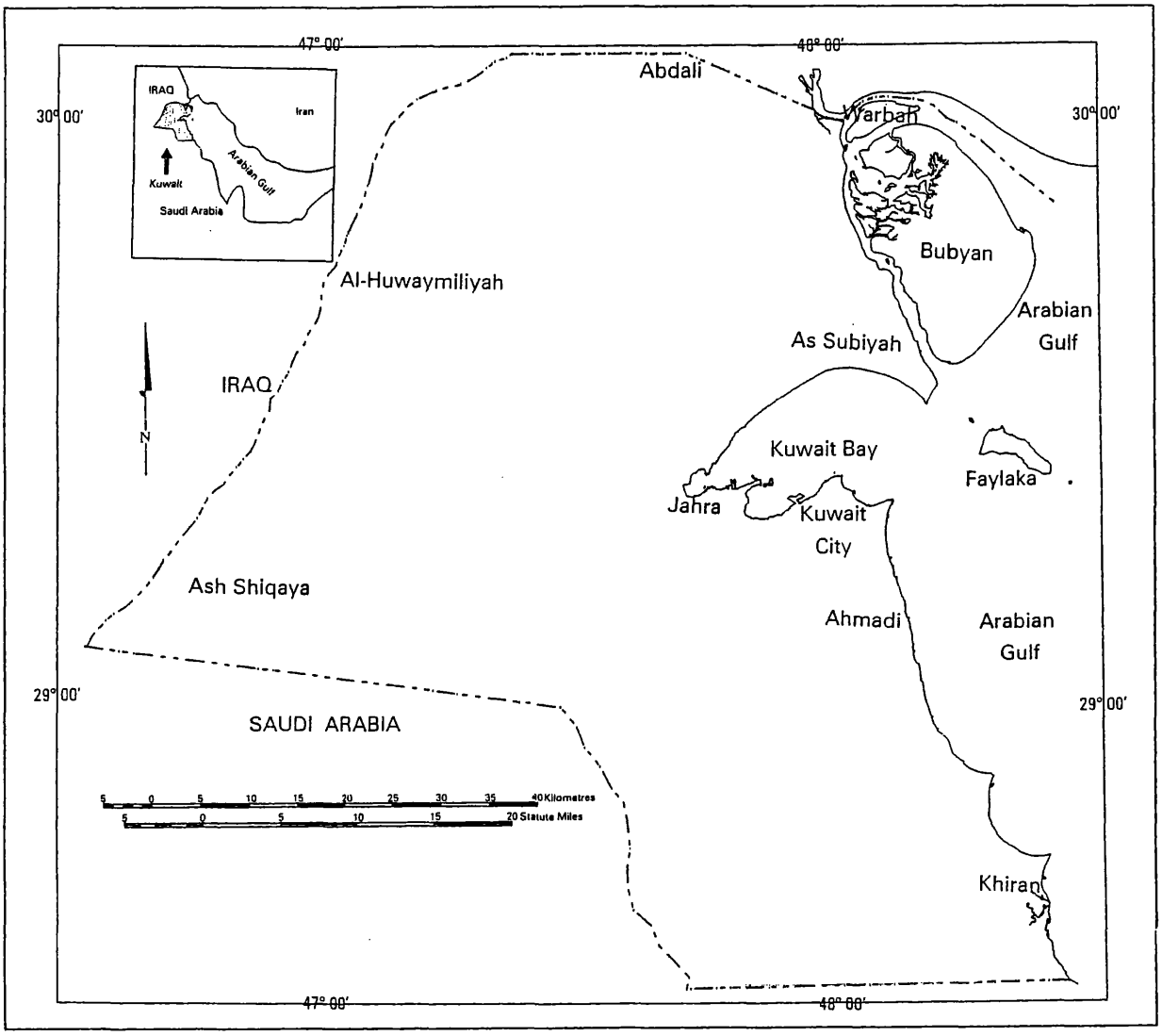

Figure 1.2 Map of Kuwait showing Kuwait Bay and some Kuwaiti Islands (From Omar, S.; Misak, R.; Bhat, N.; Shahid, S. and Delima, E. (2003) Assessing Damage Magnitude and Recovery of the Terrestrial Ecosystem/ Follow Up of Natural and Induced Desert Recovery (FA015C). *Final Report Submitted to: Public Authority for Assessing Compensation for Damages Resulting from Iraqi Aggression, September)*

The topography of north-eastern Kuwait is thought to be due to the gradual sinking of the Arabian Gulf geosyncline (a large trough, presently filled by the plains of southern Iraq and the Arabian Gulf). As the geosyncline has subsided, sediments have accumulated in the form of gravel beds. This has given rise to a series of gravel-topped ridges extending in a north-easterly direction. These ridges are only about 1 m in height and completely lack vegetation. The long, narrow, sandy depressions and basins running between the gravel ridges are usually covered in scrub throughout the year.

The topography of southern Kuwait is relatively devoid of physical features and vegetation. There are a few mature Wadi systems and occasional patches of coarse grass in the spring.

1.3 Climate

Summer in Kuwait falls between May and September and the winter between November and March. The seasonal temperatures vary considerably. Summer temperatures are extremely high, often exceeding 45°C during July and August. Winter temperatures can vary considerably, rising to over 20°C during the day and then falling rapidly to 3° or 4°C during the night. It is not unusual for night frosts to occur inland, particularly when a cold north-westerly wind is blowing. Kuwait has very low average annual rainfall (around 111 mm), with most rain falling as light winter showers brought by westerly depressions, especially in January (Husain, 1995). The average humidity in the early summer is generally lower than during winter. Between July and October, however, intermittent south-easterly winds laden with moisture from the Gulf, combined with the severe heat, elevate the humidity to over 80%, making life uncomfortable (Al-Kulaib, 1975).

Dust and sandstorms are common throughout the year. They are more frequent in the winter months and in mid-summer, when hot, dry air is blown in from the interior deserts by the As Somoum wind.

1.4 Oil Development in Kuwait

Since the granting of oil concessions in 1934 to the Anglo-Persian Oil Company (now British Petroleum or BP) and the Gulf Oil Corporation of America, Kuwait's development has seen dramatic changes. The importance of oil to Kuwait can be clearly seen by the fact that oil revenues account for over 94% of the country's total income.

BP and Gulf Oil formed an operating unit, the Kuwait Oil Company (KOC), with each holding 50% of the share capital. Various exploratory wells were drilled in the concession area during the late 1930s and, in 1938, a large oil field was discovered in Burgan area. The Second World War temporarily halted drilling operations and development. Operations resumed in 1945 and Kuwait exported approximately 797,350 tonnes (797,350,000 kg) of crude oil in June 1946. Over the next fifteen years, concessions were granted to other oil companies and the neutral zone between Kuwait and Saudi Arabia was granted to the American Independent Oil Company (Aminoil) in 1948. Aminoil built an oil refinery at Mina Abdulla in 1958. In 1961, the Khafji oil field was discovered as a result of concessions being granted in 1958 to the Japanese Oil Company in the maritime area adjacent to the neutral zone. Also in 1961, the Kuwait Shell Petroleum Company was granted a lease to explore the Kuwait sea-bed but explorations were halted a few years later due to disputes over territorial rights (KOC, 1982; Clayton and Pilcher, 1983).

As is evident above, the early years of Kuwait's oil development involved exploiting the reserves of oil by a system of concessions to foreign companies rather than by the State. In order to integrate both the exploration and production of the industry under the auspices of the State, the Kuwait National Petroleum Company (KNPC) was set up in 1960. The intention was that KNPC would oversee the production, refining and marketing of oil with 60% of the capital controlled by the government and 40% by the Kuwaiti public. Shortly afterwards, the government took control of the whole of KNPC's capital and, in order to supervise the country's oil reserves to their full potential, took over the control of KOC in 1975. The Aminoil Company (owned by an American Company who had a joint operating agreement with the Getty Oil Company) was taken over and nationalized as the Kuwaiti Wafrah Oil

Company in 1977. In 1978, this company was dissolved and merged with the KOC. The Japanese-owned, Arabian Oil Company is the only Non-Kuwaiti company holding off-shore concessions in the Neutral Zone, granted to them by the government in 1957 and 1958. In 1979, the Kuwait Petroleum Corporation (KPC) was formed by amalgamating KNPC, KOC, the Petroleum Industries Company and the Kuwait Oil Tanker Company. The KPC has centralized oil sales and strengthened Kuwait's marketing position.

Kuwait is a member of both Organization of Petroleum Exporting Countries (OPEC) and the Organization of Arab Petroleum Exporting Countries (OAPEC). The policy of the Kuwaiti government with respect to its oil industry is based on four activities, namely:

a. The efficient management of oil and natural gas reserves as a plentiful and inexpensive source of energy and raw materials that can be used to maximize the country's economic and industrial development.
b. The careful monitoring and regulation of the revenues received from the oil industry to continue with planned regional and international scheme.
c. Noting local and world economic trends, to adjust the price of oil and the amount produced to ensure the maximum use is made of the revenues from the oil industry.
d. Investing in facilities in consumer countries, so that Kuwait will benefit in the long term by having some control of these markets and will gain income from any joint endeavours.

1.5 Oil Well Fires and Terrestrial Oil Pollution

The Iraqi invasion of Kuwait on 2 August 1990 and the subsequent war activities have left many scars on the desert ecosystem. Mechanical actions involving military equipment, vehicles, and machinery, as well as heavy bombing and trench digging, removed vegetation and increased soil erosion. Indeed, the increased sand movement eventually led to the covering of huge amounts of ammunition, including mines and unexploded cluster bombs (Al-Hassan, 1992; Omar *et al.*, 2000). The most severe element in Kuwait's environmental crises was, however, the burning of oil wells. Over

730 oil wells were set on fire by the Iraqi forces. More than 80% of the wells blazed while the rest gushed oil to the soil's surface, covering wide areas of the desert. Fire fighters used millions of gallons of seawater to extinguish these fires, causing further environmental problems (Omar *et al.*, 2000). There was also massive destruction of refineries, storage facilities, desalination and power plants, infrastructure and factories. The Iraqi troops left behind a country totally devastated as well as a deeply traumatized population.

The fires, resulting from the destruction of oil wells throughout Kuwait, produced a substantial cloud of darkness and pollution. The country (and then progressively the whole region) was covered by an oily film from the growing black cloud. Dark clouds blocked the sunlight for months, resulting in air temperatures decreasing by an average of 10^{o} C and even the sea temperature declined by several degrees. The temperatures around the blazing oil wells in Kuwait were very high. Their effects on soil reached some 300 m around the well centre. This elevated temperature killed the flora and fauna of the area. Substantial numbers of small mammals, birds (Fig. 1.3, p 8) and insects were incinerated (Al-Sayegh, 2000).

The oil fires caused the release of particles, organic and inorganic gases, hydrocarbons (HCs) and oil droplets (Al-Hassan, 1992). Oil spills, aerosol deposits, and seawater use have all had adverse effects on the desert ecosystem. The explosion of oil wells in Burgan and Ahmadi produced enormous volumes of soot and unburned oil in the form of oil-mist that was carried to distant areas. In areas covered by oil-soot, a thin black crust of about 2-5 mm of slightly compacted superficial soil was formed on the surface. Unburned oil escaping from exploded oil wells accumulated in low-lying areas to form oil-logged soil where oil penetrated 15 cm or more in the soil profile (Omar *et al.*, 2000).

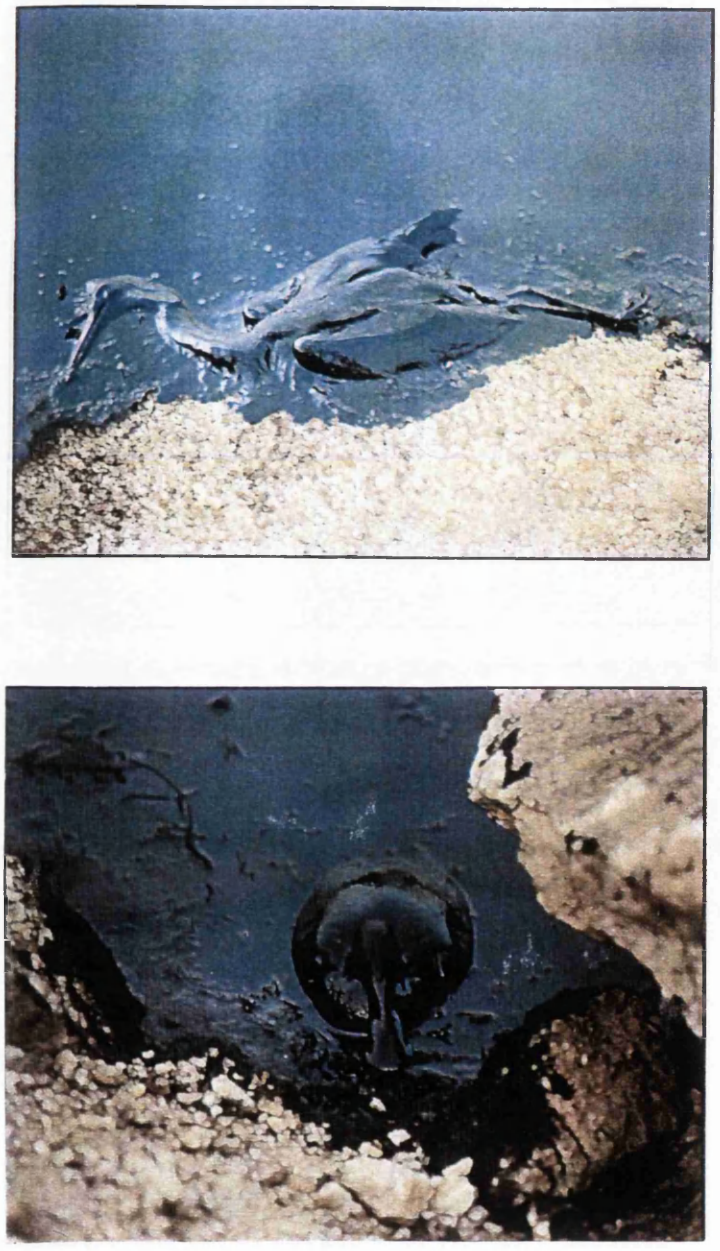

Figure 1.3 Sea birds falling victim in the oil lakes (From Al-Hassan, J.M. 1992 *Iraqi Invasion of Kuwait: An Environmental Catastrophe*. Kuwait, Fahad Al-Marzouk Publishers)

Just as the oil fires were a source of pollution to land, sea and air, the oil lakes became a source of pollution *per se*. The formation of the lakes resulted from discharge of oil from damaged wells that acted as gushers and burning wells, whose discharge rate was greater than could be consumed by the flame (such that the spray of oil finally landed back on the ground). The oil subsequently collected on the ground and ran into streams, following slopes and contours of desert topography. Soon there were running streams followed by the formation of lakes. Scientists from the Kuwait Institute of Scientific Research (KISR) established the depth of penetration of crude oil as being 10-70 cm at the edge of the lakes, and earth removal by the KOC contractors indicated that the depth of penetration in lakes at some damaged well heads exceeded 4m. The extent of seepage was dependent on the geological properties of the soil under the lake, as well as the chemical nature and specific gravity of the crude oil *per se* (Al-Hassan, 1992).

1.6 What is Oil?

1.6.1 Chemical Composition of Crude Oil

The name 'petroleum' is derived from the Latin words *petra* (rock) and *oleum* (oil). Crude oils are reservoired petroleum produced from a well at atmospheric pressure. Natural crude oil classification is strongly linked to the organic and sedimentary facies of the parent source rocks, from which the crude oils are generated. These facies influence the input and preservation of the organic matter as well as depositional setting of the sediments. The knowledge of these factors makes it possible to predict types and amounts of HCs generated and expelled (primary migration) from a given source rock type at the right levels of thermal maturity. Thus the formation of crude oils is a result of oil-prone organic source matter (kerogen) subjected to high temperatures (approximately $100^{\circ} - 150^{\circ}C$) over a time span of several million years.

After expulsion from the source rock, the oils migrate by the forces of buoyancy and capillary pressure through the porous network of sandstones to traps (reservoirs) from which they are extracted. The chemical composition of this crude oil is of relevance to exploration, oil production, pipeline transport, refinery process and the environment. Since crude oils are extremely complicated mixtures, with thousands of

components, different schemes for oil classification are in use, each depending upon the branch of the petroleum industry involved (Townshend, 1995).

Crude oil is a complex mixture of chemicals: HCs and HC-like chemicals comprised of atoms of nitrogen, oxygen or sulphur (N-S-O compounds) (Hatch and Matar, 1981). There are also metals in oil, such as vanadium (V) and mercury (Hg). HCs occur in two major chemical subdivisions: those made up of benzene rings called 'aromatic hydrocarbons' (AHs) and other chains known as 'aliphatic hydrocarbons'. Every petroleum oil consists of hundreds of different HCs and N-S-O compounds, and no two oils have the same chemical composition.

1.6.2 The Process of Weathering

As much more has been written about marine pollution by oils, the basic information from such studies will be included followed by reference to terrestrial habitats (where those exist).

After spillage, the chemical composition of oil immediately begins to change at a rate that varies with environmental conditions. The process by which the composition of oil changes after it is spilled is referred to collectively as "weathering". Weathering is a continuous process that is generally completed in about one year. The main processes of weathering are evaporation and the formation of emulsions of oil-in-water or of water-in-oil. This later process produces a sticky mess called a "mousse". The dispersion of those emulsions involves the eventual dissolving into water, photo-oxidised sedimentation or biological conversion into carbon dioxide and water. At any point in the process, the oil may become buried in soil or sediments. In such cases, the weathering process may be suspended for years until the oil is re-exposed, e.g. when the beach or sediment is disturbed (perhaps by a storm).

The concentration of potentially poisonous chemicals in oil increases, especially during the first few weeks of weathering. Volatile components evaporate quickly, leaving behind the less volatile molecules. The less volatile AHs and related N-S-O compounds are the components of petroleum oils most likely to act as serious poisons.

1.6.3 Impact of Spilled Oil in the Environment

Spilt petroleum oil can affect organisms by direct physical coating or by altering essential elements of the habitat. Oil is an unusual pollutant as, when it is spilled into water, it remains in a concentrated mass on the surface, which is only slowly changed and degraded by the process of weathering. Oil has its most pronounced effects on organisms that make use of the water surface or that inhabit shorelines. This effect extends to benthic organisms when large quantities of oil are incorporated into sediments.

1.6.3.1 Impacts on soils

The oil that poured from the burning wells and the destroyed pipes, transporting oil from the wells to the collection depots and on to the ports, formed 246 oil lakes in the north and south of Kuwait. They covered a combined area of about 49.15 km^2. The oil, which accumulated in these lakes, was estimated to be 3927 litres. Some of these lakes were five km long, 500 m wide and 60-120 cm deep. The pollution will persist in the soil for a long time, especially when compared to the oil pollution in a marine environment. In the case of the marine environment, the petroleum oils are spread over a wide area on the surface of water. This increases the surface exposed to sunlight which, in turn, enhances the processes of evaporation and photochemical oxidation. In contrast, the surface spread of oil in soil is constrained, reducing the effectiveness of these processes (Al-Ghunaim, 1997).

Petroleum affects soil properties and microbial populations. Bacterial numbers may increase, including aerobic nitrogen-fixing bacteria. Eventually, the nitrogen content of the soil increases as a consequence (IUCN, 1983).

1.6.3.2 Impacts on plants

Marsh grass, mangrove and intertidal plant communities showed high mortalities due to spilled oil, and may require one to two decades to recover. Floating algae may be killed or may grow more abundantly in response to oil, depending on conditions and the oil's concentration. Variable responses to oil by plants can result in

major shifts in the relative abundance of plant species in polluted environments. This can also apply to terrestrial plants.

Oil pollution can kill plants and inhibit growth (IUCN, 1983) by various mechanisms including:

(a) Acting as a physical barrier and preventing gaseous exchange;

(b) Being directly toxic;

(c) Changing soil properties;

(d) Affecting the abilities of other plants to grow and compete.

Photosynthesis may be reduced by oil pollution, because some oil films limit carbon dioxide entry. Light intensity reduction may also decrease photosynthetic rates although, in many cases, plants have been found growing through very dark-coloured oils. The inhibition of photosynthesis may reduce the sugar and dissolved solid contents of plants (*op. cit.*). Seed germination and seedling growth are very susceptible to oil pollution, affecting future populations. Seedling density was reduced in oiled mangrove swamps compared with unoiled counterparts (*op. cit.*). Oil pollution of the soil has direct and indirect impacts on plant communities. It has a harmful effect on the parts of the plant, such as the roots and the surface of leaves. The oil drizzle on the plant leaves makes a dark layer on the surface, impeding the passage of sunlight to the plant's chloroplasts and hampering photosynthesis (Al-Sayegh, 2000).

The impact on the vegetation cover and microorganisms of the soil varies according to the type and quantity of the oil pollutants in the soil. In the areas heavily polluted by oil, herbs are quickly affected, whereas trees (especially those in cold areas) are less influenced. The oil pollution in Kuwait's soil was particularly dangerous as it was enhanced by the type of soil present which absorbs pollutants and the fact that the plant cover largely consists of herbs. Oil pollution upsets soil aeration as it prevents the oxygen reaching the areas of the oil-dissolving bacteria. The lack of nutrients in the soil means that the substrate takes longer to recover its former nature such that plants can grow in it again (Al-Ghunaim, 1997).

1.6.3.3 Impacts on invertebrates

The main groups affected by marine oil pollution are molluscs, crustaceans, polychaetes, echinoderms, corals, zooplankton, and a variety of fresh water invertebrates (IUCN, 1983). Invertebrates in intertidal zones affected by spilled oil are generally virtually totally eliminated. Again, the affects of oil include physical smothering, loss of food and direct chemical toxicity. Invertebrates may be eliminated from sediments contaminated by oil for many years, since oil can chronically persist in such locations.

Fresh oil can cause heavy mortalities of invertebrates. In many cases, invertebrate mortalities may have profound repercussions on other species. For example, intertidal mudflats rich in worms and crustaceans are often important feeding grounds for birds and fish. Subtidal invertebrates also form a major portion of the diet of many commercial fish species (*op. cit.*). Al-Bakri and Kittaneh (1998) indicated that the prevailing harsh environmental conditions (especially high temperature and salinity) after the huge oil spill of the Gulf war in 1991 restricted benthic fauna diversity and led to the development of a fragile intertidal ecosystem. The fauna inhabiting this zone were dominated by a few species probably living at their limit of tolerance.

Al-Hassan *et al.* (2000) confirmed that petroleum HCs were increased at this time in the Arabian Gulf. There are reports showing that the concentrations of total petroleum HCs in the coastal and offshore sediments as well as fish in the Gulf are increasing with time (Al-Lihaibi and Ghazi, 1997; Talat *et al.*, 1995). The time-dependent increases of Polycyclic Aromatic Hydrocarbon (PAH) and Aliphatic HC concentrations in barnacles may be due to the persistent presence of HCs in the Gulf waters leading to contamination of the food chain. Accumulation of PAHs in barnacles is alarming, since these chemicals are potent human carcinogens. Their presence at high concentrations is a marker for the Gulf marine environmental pollution, potentially contaminating edible fish in this region (Al-Hassan *et al.*, 2000). Transportation of crude oil and oil production related activities have neither increased steeply nor has there been a major oil spill in the Gulf marine environment since the 1991 Gulf war. This suggests

that the increased concentration is due to the crude oil deposited in the sediment from the oil spill during the 1991 Gulf war slowly becoming bioavailable (*op. cit.*).

Jones *et al.* (1998) conducted quantitative surveys of the intertidal macrobiota between 1991 and 1995 in the Saudi Arabian Gulf along permanent transect lines. These were established within the area impacted between Ras az-Zaur and Abu Ali that was impacted by the 1991 Gulf war, and now forms the Jubail Marine Wildlife Sanctuary. By December 1991, between 50 and 100% mortality of biota had occurred on the upper shore as a result of pollution but, by 1995, species diversity on the lower shore was similar to that found on unpolluted shores. This work was the first long-term quantitative monitoring of recovery on Gulf shores and hence provides an invaluable baseline for future oil spill management and mitigation. It is essential that monitoring is continued until all habitats reach their fully recovered state so that a definitive recovery time can be provided for future environmental planners.

The oil spill in Kuwait was catastrophic because oil continued to flow into the water for a long period affecting a wide range of organisms, including migrant and resident birds, marine mammals, fish and invertebrates. Where the oil washed ashore, it seeped into sandy beaches, and sank progressively deeper into the substrate. As lighter fractions evaporated, the residual oil formed asphalt several inches thick. Even where the beaches appeared clean, oil was found in a grayish layer just below the surface, where it continued to influence marine invertebrate populations.

1.6.3.4 Impacts on fish

Fayad *et al.* (1996) noticed that concentrations of HCs from oil in fish populations from the Gulf varied according to fish species and sampling location. N-alkane concentrations in the Hamour (*Epinephelus multinotatus*) populations collected from the nearshore fishing grounds appeared to be about three times the concentrations found in the same species collected from offshore populations. Light oils such as gasoline (which are highly toxic), have been known to cause substantial fish kills when spilled in restricted inshore localities (GESAMP, 1997). Malins and Hodgins (1981) concluded "There is ample evidence that fish exposed to petroleum in sediments, water

or through their diet accumulate hydrocarbons in tissues and body fluids. The tissues are purged of these hydrocarbons when the fish are no longer exposed."

Al-Hassan *et al.* (2000) indicated that sharks accumulated noticeable levels of PAHs and AHs, irrespective of the depth at which they swim in Gulf waters, their feeding modes and the areas in which they were caught. Gulf waters are known to contain oil-derived pollutants through oil transportation activity, oil spills and intentional discharge of oil from shipping. These results suggest that shark meat consumers in the Gulf should be alarmed about the contamination of the fish by potent carcinogens, which may afflict many consumers, especially pregnant mothers. Periodic and long-term checks on the levels of PAHs and AHs in sharks at different locations and at different times of the year are required to monitor these toxicants (Al-Hassan *et al.*, 2000).

1.6.3.5 Impacts on amphibians and reptiles

Little information on the deleterious effects of oils on reptiles is available. The death of two small turtles, one of which was found with tar in its mouth and the other found covered with oil was reported by Witham (1978). Crocodiles, alligators, frogs and toads may all be at risk, mainly in fresh water habitats but occasionally in estuaries of near shore waters (IUCN, 1983).

The oil pollution in Kuwait caused the soil temperature to drop. The soil temperature influences sexual determination in reptile eggs. When the temperature drops, the proportion of males increases. The reverse is true if the soil temperature rises. Thus oil pollution may not only cause the direct death of such animals (Fig. 1.4, p 16) because of poisoning, suffocation in their holes or under the soil, but also change population structure (Al-Ghunaim, 1997).

1.6.3.6 Impacts on birds

Birds are often the most obvious victims of oil spills and much effort is often expended in cleaning and releasing them (Fig. 1.3, p 8). A wide variety of bird species may be affected by oil pollution but animals that spend much of their time on the sea surface

are, of course, particularly at risk. Newman *et al.* (2000) stated that variables which are likely to influence survivorship of oiled and rehabilitated birds include petroleum type, environmental factors associated with the geographical locations of spills, the season,

Figure 1.4 A sand lizard caught in the tar mat at the Al-Burgan oil field, May 24, 1996 (From Kwarting, A.Y. and Al-Ajmi, D. 1997 Satellite Remote Sensing Applications in the State of Kuwait. Kuwait, Kuwait Institute for Scientific Research)

the pre-existing condition of birds, facilities and trained personnel available to care for oiled wildlife and available biomedical techniques.

Crude oil exerts a multitude of effects on birds. The feathers of seabirds repel water and thereby contribute to buoyancy and thermoregulation. The oiling of feathers causes a loss of water repellency, a collapse of plumage, and the displacement of their insulating layers of air, resulting in hypothermia. Following external contact with oil, the animals naturally try to clean the oil off the surfaces of their bodies by preening their

feathers. This action results in oil consumption and to pathological states with consequences for a variety of physiological processes including development, reproduction, immunity, osmoregulation and nutritional status (Briggs *et al.*, 1996).

1.6.3.7 Impacts on Mammals

The impact of oil on most marine mammals is generally much less than on birds. The exceptions are mammals such as Sea otters that rely on fur rather than blubber for thermal insulation. Here, the impact of oil can be severe and similar to that on birds.

In various places throughout the world, domestic animals (sheep, cattle and horses) graze on salt marshes. The possibility of contamination of important grazing areas should be considered. Additionally, sheep sometimes graze on seaweed-dominated shores and following the 'Esso Bernicia' accident in Sullom Voe, Shetland, UK, 50 sheep were killed and the fleeces of over 2000 damaged by oil (Richardson, 1979).

1.7 Statement of the Problem

There is a substantial scientific literature on oil pollution but relatively few studies have been made on the effects of oil pollution on terrestrial habitats (partly because there are fewer cases of such pollution). Those cases that have been reported often say little about the effects on the vegetation. The tundra regions of Alaska and the Delta region of the Niger are examples of areas where oil production is developing and where terrestrial habitats have been affected (IUCN, 1983).

Although the Kuwait environmental catastrophe was unexpected, it should be remembered that some oil pollution of terrestrial habitats also occurs through blowouts, pipeline and storage tank breakages, roads or rail accidents and by general industrial discharges. It should be noted that, although almost ten years have passed at time of present study since the Kuwait oil spill, damage to organisms and their habitats continues. Oil lakes of different sizes, dry oil lakes, tar mat and soot are features still found in the Greater Al-Burgan oil field area. Very little is known about the wildlife living in this area and the effects of oil pollution on the behaviour, physiology and daily

activity on terrestrial wildlife needs to be investigated. This provides the focus of the present study.

1.8 Objectives of the Study

Omar and Zaman (1995) recommended that action was needed to reclaim polluted areas where destroyed vegetation and reduced productivity potentials were evident. The biological diversity of the terrestrial environment has to be regularly monitored in order to develop practical measures for its protection and management. Re-introduction of wildlife species needs to be investigated for future preservation of lost fauna.

In line with the above recommendations, the aim of this present study was to assess the impact of oil pollution on the behaviour and physiology of reptiles, specifically, the sand lizard (*Acanthodactylus scutellatus*) in the Greater Al-Burgan oil field area.

The study had the following objectives: namely to:-

a. Assess whether oil pollution changes the behaviour of *A. scutellatus*
b. Assess whether oil pollution alters the population size of *A. scutellatus*
c. Determine whether food resources in the Al-Burgan oil field affect the sand lizard's populations
d. Determine the effects of heavy metals on *A. scutellatus*
e. Quantify the effects of PAHs on the physiology of sand lizards
f. Quantify the effects of PAHs on ants (the lizard's main prey)
g. Establish whether oil pollution has an impact on liver histology (indicative of pathology) of *A. scutellatus*

CHAPTER 2

The Effects of Oil Pollution on the Basking Durations and Substrate Preferences Shown by *Acanthodactylus scutellatus*

2.1 Introduction

Lizards are generally terrestrial tetrapods that can move quickly, have good vision, low energy and water demands, can accelerate physiological processes cheaply by basking and can invade relatively dry habitats. Many lizards are small insectivores consuming large food items using their heavy jaws and kinetic skulls. The genus *Acanthodactylus* includes lacertids with a very widespread geographical distribution. The majority of the species are psammophilous, being associated with sandy habitats such as those typical of deserts like the Sahara, the Arabian sandy desert, the Iranian desert and the Thar desert of west India (Arnold, 1983; Arnold and Burton, 1978; Salvador, 1982).

The *Acanthodactylus* genus includes diurnal, ground-dwelling reptiles that feed mainly on insects and other small invertebrates. All species of this genus are oviparous and the clutch size ranges from two to seven eggs. The breeding season usually commences in spring, but can start earlier. In some species, the reproductive season extends throughout the year, a feature that can facilitate multiple clutches. The young of most species of the genus become sexually mature in the following spring. There are, however, some species that reach sexual maturity within four months of hatching whereas others take two years to reach sexual maturity (Arnold, 1983). Lizards, as all reptiles, are ectotherms obtaining the energy they need to raise their body temperatures to levels permiting normal activity from the sun. They do this either directly (by basking in the sunlight) or indirectly (by resting on a warm surface such as a rock that has been previously heated by the sun). In contrast, endotherms (such as birds and mammals) produce heat by metabolizing carbohydrates, lipids, and proteins from the food they eat.

Many ectotherms control their body temperatures at high levels and within narrow limits during their periods of activity. Lizards, especially species that live in

open, sunny habitats, provide the best examples of the effectiveness of ectothermal thermoregulation. Many species of lizards can maintain their body temperatures between 35°C and 42°C while they are thermoregulating (i.e. they have body temperatures as high as most birds and mammals). Diurnal lizards spend their nights in their burrows. During the night, the lizard's body temperature falls to the temperature of its burrow (about 20°C in summer). In the morning and after sunrise, lizards emerge from their burrows so their body temperature rises to 35°C. Outside the burrow, lizards move through a mosaic of sunny and shaded areas. Their body temperature rises to 37°C and stabilizes at that level, rising and falling slightly from minute to minute as lizards move from sun to shade. By late morning, the sun is nearly overhead and the desert is too hot. The lizards can no longer maintain a safe body temperature, and they cease activity and retreat to their burrows. In the burrow, the lizards cool again to a body temperature near 20°C and remain at this until they re-emerge. This is a typical pattern of daily temperature change for an ectotherm – warm when it is active, and cool when it is in its retreat site (Pough *et al.*, 2001).

2.1.1 The Species Studied

A. scutellatus was chosen for this study because it has a wide distribution. This species is a typical medium–sized lacertid with a cylindrical body with a reticulate pattern and with well-developed limbs (Fig. 2.1, p 21) (Leviton, 1992; Salvador, 1982). The tail is long and the head is wide with an elongated pointed snout. A fringe of scales on the trailing edge of each (especially the 4^{th}) toe, facilitates locomotion on loose sand and gives these animals their common name of 'fringe-toed lizards'. The lizard is capable of generating substantial propulsive force and can achieve considerable speeds when fully active. This species is insectivorous, feeding mainly on ants, flies, small beetles and insect larvae. Its' food is most abundant in Kuwait in March, April, September and October. Stomach contents of 66 *A. scutellatus* examined by Perry and Dmi'el (1994) showed that ants comprised a large proportion of the diet (some 36% of food items). The abundance of plant fragments may, however, be surprising because herbivory is unusual in small lizards (Pough, 1973) and the quantities found do not

indicate accidental ingestion. Herbivory in small lizards may be seasonal. Perry and Dmi'el (1994) found that *A. scutellatus* was usually observed in sandy areas with little or no plant cover: 80% of 108 animals observed were found in areas with 0-5% plant cover, 11% in areas with 5-20% plant cover, and only 9% in more densely vegetated areas. *A. scutellatus* often appears in isolated populations, colonizing the mobile sand dunes (Mellado and Olmedo, 1991). *A. scutellatus* may live for 3-5 years (Perry and Dmi'el, 1994).

A. scutellatus is an oviparous lizard, meaning that, like many other reptiles, it produces eggs so that the embryo is able to develop outside the body of the mother (Bou-Resli, 1976). The adult, non-breeding female usually has two large fat bodies or sacs in the abdominal cavity in June and July. The gravid female has generally two to three (rarely four) mature eggs in the oviducts, and many small eggs in the ovaries (*op. cit.*). The breeding season may extend over many months, but it is locally generally from March to the beginning of May. The timing probably depends mainly on climatic conditions but the young lizards are usually found in May and June.

Figure 2.1 *A. scutellatus* in its natural habitat (author's own image)

2.1.2 Adaptation of Lizards to Desert Environments

The extreme physical and climatic conditions of the desert biome have generated a number of interrelated morphological, behavioural, and physiological adaptations. The most critical limiting factors in desert habitats are temperature (Cloudsley–Thompson, 1972; Mayhew, 1968) and rainfall (Seely and Louw, 1980). Many desert lizards overcome the harsh desert temperature problem by simply avoiding high temperature, particularly at midday. Small size and also the ability to burrow, allow desert lizards to seek out suitable microhabitats thus avoiding high levels of solar radiation (Louw and Seely, 1982).

2.1.3 Effects of Oil Pollution on Behaviour of *A. scutellatus*

Lizards are important components of terrestrial ecosystems, forming an important link in food chains between invertebrate prey and predatory vertebrates such as birds and snakes (Lambert, 1997a; b). Lizards have rarely been used as bioindicators of pollution for a variety of reasons, including difficulty in sampling sufficient numbers, and their relative lack of economic importance (Loumbourdis, 1997). Since, however, invertebrates are the prey of most lizards, the uptake of any chemical contaminants ingested by invertebrates is an important pathway by which pollutants enter the body of lizards. These chemicals can also result from the incidental ingestion of soil (small stones are often found in the digestive tracts of the lizards and are used to break up gut contents as in bird gizzards). Lizards have consequently recently been seen as potential bioindicators of pesticides entering the environment (Lambert, 1993).

It has been agreed that behavioural changes are the most sensitive indicators of environmental contamination (Brain *et al.*, 1994). Because no data is available on the effect of oil pollution on the behaviour of reptiles, a main purpose of this thesis was to ascertain whether *A. scutellatus* can be used as a bioindicator of oil pollution in desert locations. Behaviour was one of the aspects selected for detailed investigation.

2.2 Materials and Methods

2.2.1 Study Area Description

2.2.1.1 Reference (Control) Area Description

The Agriculture Research Station at Kabd was established in 1975. The research station covers a total area of 20 km^2, being 4 km (east to west) x 5 km (north to south) (Fig. 2.2, p 24). The ground elevation varies between 130m (to the west) and 75m (to the north-east) above sea level. The area is covered by a series of surface sediments including granules and pebbles, loose sands, and silt. The soils of the Agricultural Research Station are dominantly sandy. The Muslan series is the major soil type covering around 75% of the station area. This soil type is very common throughout the country being very porous and easily carried away by winds. The perennial shrub *R. epapposum* dominates the location. The ground is covered by a mixture of perennial shrubs and grasses in addition to the annual grasses. Among the important research activities carried out at the Agricultural Research Station are assessments of the flora and fauna. Wildlife including mammal, bird and reptile species are monitored around the site. Small ponds were established at Sulaibiya field station to achieve two objectives, namely to provide a source of water for wildlife species in the area and provide a food source for wildlife species that are part of the food chain (such as algae, insects, birds and mammals). These ponds are regularly observed and monitored for changes in the microhabitats and the impact of such changes on the flora and fauna in the vicinity of the pond. Sulaibiya station is fenced and permission from by KISR is required to enter the site.

2.2.1.2 Study Sites in the Oil-Polluted Area

The Greater Al-Burgan oil field has an area of 349.65 km^2 and lies to the south of Kuwait City (Fig. 2.2, p 24). The landscape is flat to gently rolling. The underlying geology consists of sandstones and limestones, while the featureless surface is dominated by torripsamment soils and loose, massive sands with depths ranging from 50 to 150 cm. Organic matter makes up between 0 to 0.5% by weight and the pH varies

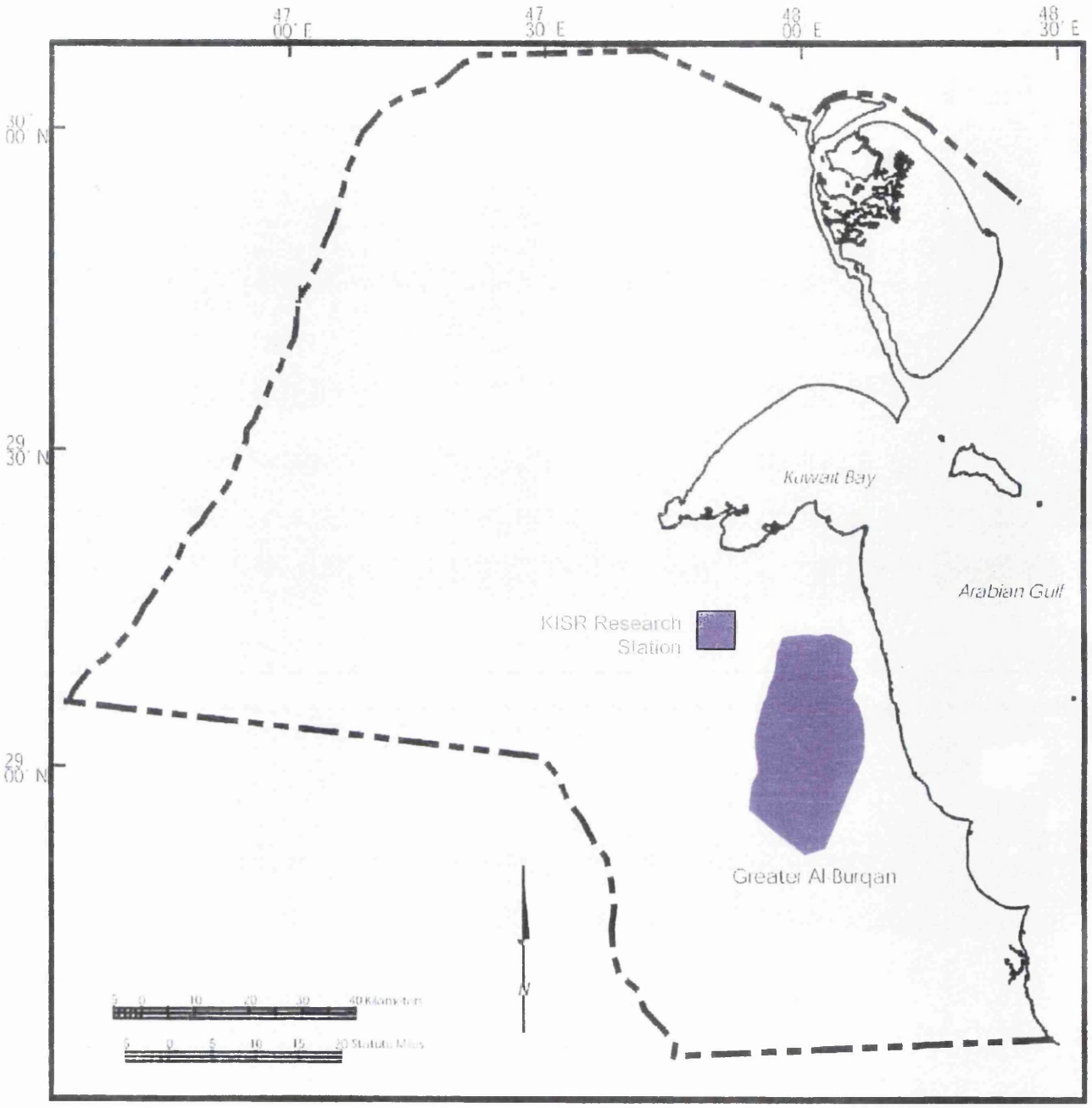

Figure 2.2 A map showing the location of the control (KISR Research Station) and contaminated study sites (Greater Al-Burgan Oil-Field) (From Omar, S.; Misak, R.; Bhat, N.; Shahid, S. and Delima, E. (2003) Assessing Damage Magnitude and Recovery of the Terrestrial Ecosystem/ Follow Up of Natural and Induced Desert Recovery (FA015C). *Final Report : Submitted to: Public Authority for Assessing Compensation for Damages Resulting from Iraqi Aggression, September)*

between 7.5 and 8.5. Currently, the land is solely used for oil and gas production and is fenced to prevent unauthorized entry.

Types of contaminated soils were categorized according to the ground observations. The types used were designated as tar mat, soot and clear. The tar mat areas had a soil surface that had been solidified by oil, forming a layer about 1 cm thick that could be peeled off the underlying clean soil. The soot areas were found within the upper layer of soil and could be a 1-8 mm in depth. Contamination is, however, sometimes continuous and at other times discontinuous. The clear sites had no visual evidence of soil pollution. Two sites for each category of contaminated soils were located (Fig. 2.3, p 26). All sites had been used previously by KISR for botanical and soil surveys in areas declared by the military to be safe from unexploded ordnance (UXO).

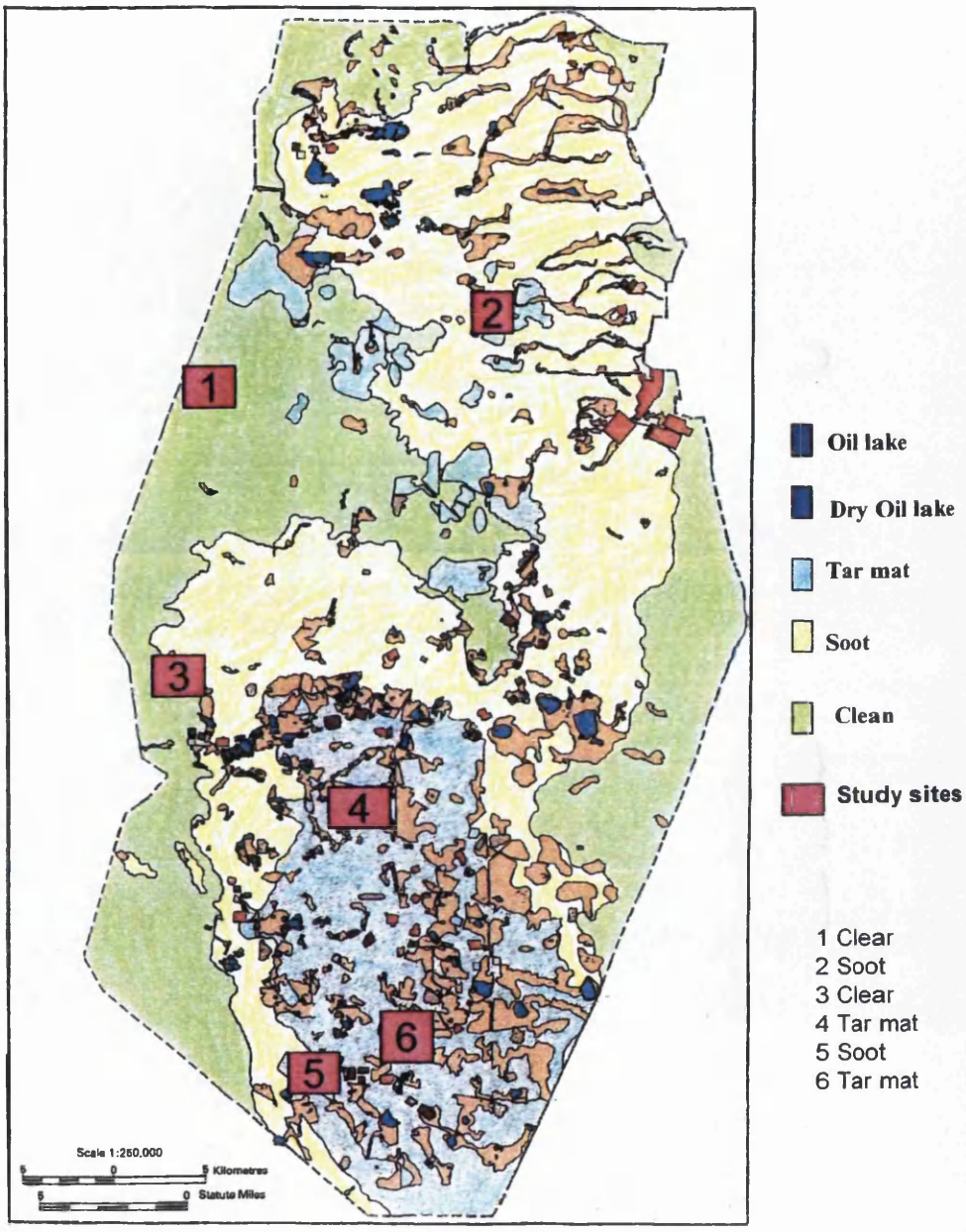

Figure 2.3 A map showing the different contaminated sites at Al-Burgan oil field (From Omar, S.; Misak, R.; Bhat, N.; Shahid, S. and Delima, E. (2003) Assessing Damage Magnitude and Recovery of the Terrestrial Ecosystem/ Follow Up of Natural and Induced Desert Recovery (FA015C). *Final Report : Submitted to: Public Authority for Assessing Compensation for Damages Resulting from Iraqi Aggression, September)* with the numbered locations of the 6 study sites used in this thesis

2.2.2 Flora of the Study Areas

The current classification system divides the flora of Kuwait into eight vegetation units (Omar, 2000). Three of these occur in the study area, namely the Cyperietum (Fig. 2.4, p 28), Stipagrostietum (Fig. 2.5, p 29) and Rhanterietum (Fig. 2.6, p 29) units. The Cyperietum unit covers 26% of the country but accounts for over 90% of the study area. The characteristic species is *Cyperus conglomeratus*, a tough perennial that reaches about 60 cm in height and is a good sand stabilizer. Associated species include annuals such as *Astragulas annularis* (Asab-Al-Arous), *Brassica tournefortii* (Harraizah) and *Plantago boissieri* (Rublah). Although the Stipagrastietum unit occupies 39% of Kuwait, it occurs only in very restricted localities in the extreme south of the Al-Burgan area. This area is dominated by *Stipagrostis plumosa*, a perennial grass that forms dense tufts reaching about 40 cm in height. Two other perennials, *Centropodia forsskalii* and *Moltkiopsis ciliata* also occur. The Rhanterietum unit only occupies 2% of the country and is found only in the extreme northwest corner of the study area. The dominant species is *Rhanterium epapposum*, a tough perennial member of the Compositae that grows up to 60 cm in height. The Rhanterietum unit is much favoured by grazing animals. Associated species include several other perennials: a shrub, *Convolulus oxyphyllus* as well as two grasses, *Centropodia forsskalii* and *Stipagrostis plumosa*. The desert vegetation is thorny or spiny and armed with stout twigs, and is thus generally not conducive to predators of lizards but provides suitable cover for these reptiles.

2.2.3 Fauna of the Study Areas

Common arthropods include beetles, ants, termites, scorpions, spiders and flies. In terms of reptiles, in addition to *A. scutellatus*, there is a congeneric species *A. schmidti*. There are also two species of Mesalina, *Mesalina brevirostris* and *M. adramitana*, and the large herbivorous lizard *Uromastix microlepis*, a desert monitor or waral (*Varanus griseus)* plus agamids, gekkoes and snakes. A number of bird species are seen in the study area during winter and spring, in particular, species of Lark (*Calandrella brachydctyla* and *Alaemon alaudipes*) and Wheatears (*Oenanthe*

pleschanka, O. desert and O. oenanthe) but with seasonal variations including birds of passage and winter residents. The mammal fauna includes the Long-eared hedgehog (*Hemiechinus auritus*), the Red fox (*Vulpes vulpes*), Jerboas (*Allactaga* spp.), Jirds (*Meriones* spp.) and Gerbils (*Gerbillus* spp.).

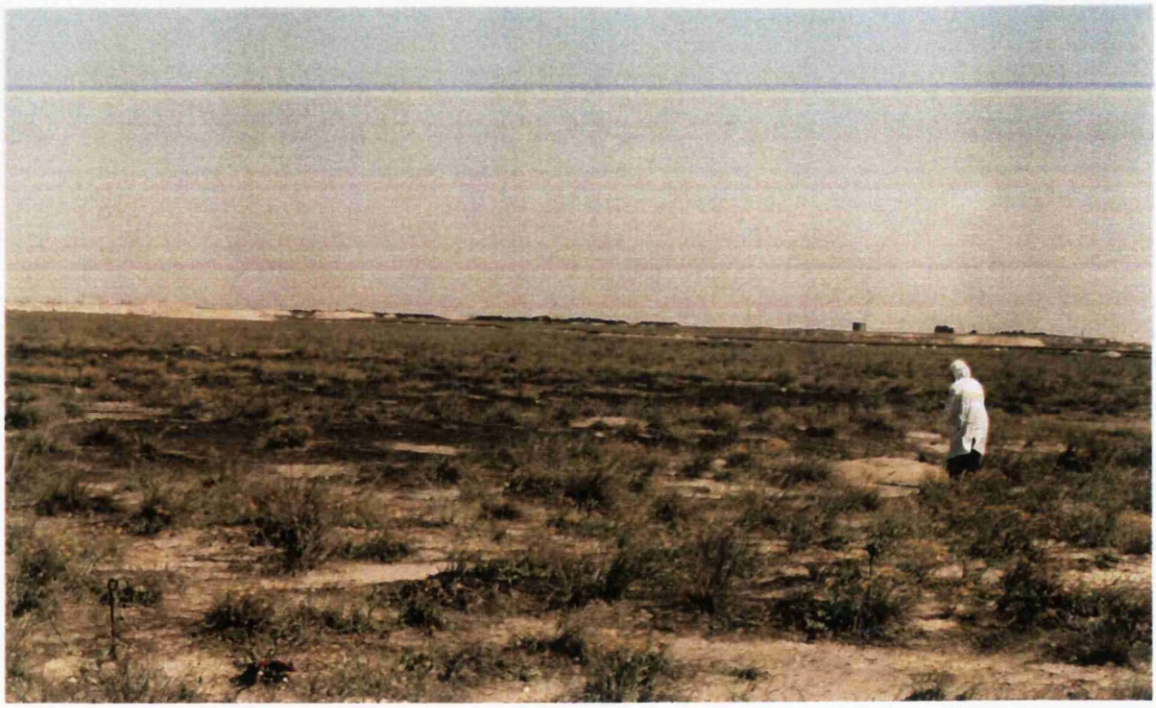

Figure 2.4 Cyperietum Community (author's own image)

Figure 2.5 Stipagrostietum Community (author's own image)

Figure 2.6 Rhanterietum Community (author's own image)

2.2.4 Behavioural Studies of Sand Lizards

Ten lizards (5 of each sex) from each polluted site (tar mat, soot and clear) and the control sites were observed in the field at the times where they were highly active in 2002 and 2003. This study was performed during the population monitoring phase of the investigation, so only mark-bearing lizards were used to overcome the problem of data repetition with the same lizard. Marked lizards were monitored for morning emergence time, kind of shelter used and durations of foraging and basking behaviours.

Individual lizards were watched, with 7 x 50 binoculars (Pentax, Asahi Optical Co., Ltd., Japan) where necessary, for extended periods of the year but mainly between February and April when they were highly active. Three readings were taken for each observed lizard at the 2 replicates of all the study sites and the average reading was recorded. The air, substrate and burrow temperatures were recorded using mercury and thermocouple thermometers (Model No. 8528-10, Diqi-Sense, Cole-Palmer Instrument Company-Chicago, Illinois USA). These temperatures were measured in the 2 replicates of each study site 3 times for each observed lizard and the average reading was recorded. The individual temperatures were taken 30 times at each site category over 30 days. These temperatures were taken during the times when the lizards were observed at each study site. Burrow temperature was taken by inserting the probe of the thermocouple thermometer 8cm inside the lizard's burrow.

Ten lizards from the 2 replicates of each study site were observed in the behavioural studies (e.g. five lizards from the first replicate and 5 lizards from the second replicate of each study site giving total of 10 lizards [N = 10] for each study site); i.e. ten lizards from the control site, 10 lizards from the clear site, 10 lizards from the soot site and 10 lizards from the tar mat site.

Each lizard was observed 3 times for morning emergence, 3 times for basking behaviour and 3 times for foraging behaviour. The average of these 3 measurements was recorded for each lizard, then the mean of the 10 averages (of 10 lizards) was taken for each study site. This procedure was also performed with air, substrate and burrow temperatures. During each observation, these individual temperatures were measured, again 3 readings for each lizard and the average of these 3 readings was recorded. The

mean of 10 averages (of 10 lizards) was taken for each study site for each individual temperature.

Activities of *A. scutellatus* were monitored by visually searching the study areas each day at 08:00, 10:00, 13:00 hours local time during the study period. When the weather became warm during Spring, monitoring was started one hour earlier. The study sites were slowly walked following an established route and each animal seen was watched for an extended period, so its behaviour could be quantified.

2.3 Statistical Analysis

Data were analyzed with Minitab, version 13.32 (2003) and SPSS, version 11.0 (2001). One-way analysis of variance (ANOVA) was used for morning emergence, basking behaviour and foraging behaviour data. For substrate preference experiments, Chi-square test was used. In all tests, significance was accepted at the $P < 0.05$ level.

2.4 Results

2.4.1 Lizard Habitats

A. scutellatus was found in the study sites associated with areas of pure sand but vegetation was very important because it provided good feeding sites, attracting insects and other invertebrates. As noted previously, the vegetation was also used by lizards as cover from their predators. Vegetation also provided protection from high levels of radiation, particularly at midday. The roots of the plants support and stabilize sand, thus enabling lizards to dig their burrows in these locations. Burrows of other animals (including small mammals) were also utilized by small lizards, especially when avoiding predators. The structure of these burrows with many exits and entrances afford the lizards a very good chance of escape from predators. Lizards in the study sites showed little hesitation in using any burrow when disturbed or chased. This type of habitat may not, however, be without disadvantages because the high number of small mammals attract many predators such as snakes, foxes and birds of prey which consume both mammals and lizards.

The control area (Sulaibiya) is a fenced reserve protected from livestock and human interference. People are not allowed to enter without permission. Consequently,

the area is highly vegetated (Fig. 2.7 below) especially in spring. At the highly contaminated (tar mat) sites used in this study, the soil was covered with a thick layer of deposits (Fig. 2.8, p 33). Under the tar mat, the sand was very loose and it was very easy for lizards to burrow under it when chased (Fig. 2.9, p 33).

Figure 2.7 Sulaibiya Station (the control location) in Spring (author's own image)

Figure 2.8 Tar mat at Burgan oil field; the soil is covered with a thick layer of deposits (author's own image)

Figure 2.9 Entrance of a lizard's burrow at the tar mat site (shown by the position of the pen) (author's own image)

2.4.2 Morning Emergence

During times of elevated activity, emergence of *A. scutellatus* was observed when air temperatures were around 20-25°C and substrate temperatures 25-35°C. Emergence could take place earlier or later depending on the study site and on the weather (cloud cover and wind conditions), but most emergence occurred 2 to 3 hours after sunrise in the tar mat and clear sites but some 3 to 4 hours after sunrise in the other study sites. In most cases, the lizard would push out its head first, then after few minutes it would expose the entire body. The data are presented in figure 2.10 (below).

Figure 2.10 Mean (± s.d.) air temperatures, substrate temperatures, burrow temperatures and morning emergence times at the different study sites

Location	Mean air temp. (°C)	Mean substrate temp. (°C)	Mean burrow temp. (°C)	Mean morning emergence times (minutes since sunrise)
Control	21.18 ± 2.24 (N=10)	28.87 ± 1.86 (N=10)	18.32 ± 2.19 (N=10)	243.80 ± 15.76 (N=10)
Clear	21.63 ± 1.73 (N=10)	30.55 ± 3.08 (N=10)	19.06 ± 1.74 (N=10)	185.30 ± 11.78 (N=10)
Soot	20.82 ± 2.27 (N=10)	28.63 ± 2.73 (N=10)	18.16 ± 2.18 (N=10)	228.00 ± 23.12 (N=10)
Tar mat	22.32 ± 1.86 (N=10)	31.49 ± 3.0 (N=10)	19.40 ± 1.63 (N=10)	163.50 ± 20.82 (N=10)

One-way analysis of variance (ANOVA) revealed significant differences in the emergences between the sites ($F_{3,36} = 40.78$, $P < 0.0001$). There was no significant overall difference in air temperature between the study sites, which means that air temperature could not account for the emergence time variation. There was no significant difference of substrate temperatures that was taken during morning emergence between the study sites. One-way ANOVA also showed no significant difference in burrow temperatures between the study sites.

The *post hoc* tests (Scheffe' test) showed that there was no difference in morning emergence times of sand lizards between the control and the soot sites ($P = 0.31$). The lizards from the control and the soot sites emerged, however, significantly later than the clear and tar mat sites ($P < 0.0001$). This suggests that animals at the clear and tar mat sites can emerge earlier and start feeding. It suggests that the view that clear sites are less polluted than the soot sites and that the tar mat sites are most polluted is an over simplification.

The correlation coefficients (Pearson's Product Moment) between the emergence time, air, substrate and burrow temperatures are shown is figure 2.11 (p 36).

2.4.3 Basking

Following the morning emergence, *A. scutellatus* would bask in the sunlight to raise its body temperature to the level required for the normal activity. Basking usually took place in close proximity to the lizard's burrow. Lizards generally lie motionless close to their burrows but were alert and would retreat if disturbed. In order to warm up, the lizard widely spreads its ribs and orients itself so the long axis of its body is perpendicular to the rays of the sun with the legs stretched out. In this situation, the sun strikes the lizard's entire dorsal surface and the reptile intercepts the maximum amount of solar radiation and casts a relatively large shadow on the ground. In contrast, when the lizard is hot and trying to minimize the amount of solar radiation received, it compresses its ribs against its body and faces directly into the sun. In this position the sun strikes perpendicularly only on the lizard's head and shoulders, and the reptile casts a much smaller shadow.

Figure 2.11 Correlation coefficients (Pearson's Product Moment) between the emergence times (in minutes), air, substrate and burrow temperatures (°C)

Site		Emergence time	Air temperature	Substrate temperature	Burrow temp.
Control	Emergence time				
	Air temperature	0.964 (*P*=0.0001, N= 10)			
	Substrate temperature	0.923 (*P*=0.0001, N= 10)	0.861 (*P*=0.001, N=10)		
	Burrow temperature	0.894 (*P*=0.0001, N= 10)	0.955 (*P*=0.0001, N= 10)	0.825 (*P*=0.003, N=10)	
Clear	Emergence time				
	Air temperature	0.768 (*P*=0.009, N=10)			
	Substrate temperature	0.700 (*P*=0.024, N=10)	0.710 (*P*=0.021, N=10)		
	Burrow temperature	0.905 (*P*=0.0001, N= 10)	0.921 (*P*=0.0001, N= 10)	0.656 (*P*=0.034, N=10)	
Soot	Emergence time				
	Air temperature	0.983 (*P*=0.0001, N= 10)			
	Substrate temperature	0.954 (*P*=0.0001, N= 10)	0.970 (*P*=0.0001, N= 10)		
	Burrow temperature	0.961 (*P*=0.0001, N= 10)	0.978 (*P*=0.0001, N= 10)	0.953 (*P*=0.0001, N= 10)	
Tar mat	Emergence time				
	Air temperature	0.833 (*P*=0.003, N=10)			
	Substrate temperature	0.866 (*P*=0.001, N=10)	0.955 (*P*=0.0001, N= 10)		
	Burrow temperature	0.892 (*P*=0.001, N=10)	0.907 (*P*=0.0001, N= 10)	0.905 (*P*=0.0001, N= 10)	

The amount of time allocated to basking was measured for thirty minutes for each lizard after morning emergence at the types of oil polluted and the control sites (Fig. 2.12, below). More details on how data were collected on basking behaviour, the number of lizards observed and the total animals studied are discussed in section 2.2.4 (p 30).

Figure 2.12 Mean (± s.d.) air temperatures, substrate temperatures and basking durations at the different study sites

Location	Mean air temp. (°C)	Mean substrate temp. (°C)	Mean basking duration (minutes)
Control	20.32 ± 1.07 (N = 10)	29.28 ± 1.71 (N = 10)	27.10 ± 2.13 (N = 10)
Clear	21.56 ± 2.05 (N = 10)	29.93 ± 2.39 (N = 10)	24.20 ± 2.39 (N = 10)
Soot	21.90 ± 1.95 (N = 10)	31.37 ± 2.52 (N = 10)	21.20 ± 3.82 (N = 10)
Tar mat	22.41 ± 1.98 (N = 10)	32.13 ± 2.56 (N = 10)	18.20 ± 3.15 (N = 10)

One-way ANOVA was used to investigate the basking seen on the varied sites. Basking duration showed significant variation between the study sites ($F_{3,36} = 16.87$, $P < 0.0001$).

The *post hoc* tests (Scheffe' test) showed that there was no difference in basking period of sand lizards at the control and the clear sites. There was a significant difference between the control and the soot sites ($P < 0.001$), the control and the tar mat sites ($P < 0.0001$) and the clear and the tar mat sites ($P < 0.001$) in basking period of sand lizards. In essence, the mean basking duration was shortest at the presumably highly polluted tar mat sites and it was shorter in the soot sites than in the clear sites. Mean basking duration was longest at the control sites.

There was no significant variation of air temperatures between the study sites, indicating that variations in air temperatures did not account for the varied basking durations. The substrate temperatures were measured by mercury and thermocouple thermometers when air temperatures were recorded after morning emergence at the different study sites. The means showed a significant variation ($F_{3,36} = 3.15$, $P < 0.03$). Consequently, variations in substrate temperatures could be an important factor in producing this difference among the study sites (control sites and the tar mat sites are both shaded by vegetation but the tar mat sites appeared to absorb solar radiation more effectively).

The correlation coefficient (Pearson's Product Moment) between basking periods and the different study sites is shown in figure 2.13 (p 39).

2.4.4 Foraging

As used by Pianka *et al.* (1979), the mean number of moves per minute (MPM) made were used to compare the foraging strategies of *A. scutellatus* at the different sites presumably varying in their degree of oil pollution. MPM in this study was determined after the animal basks in the sun and becomes active, any movement included to capture flying insect or creeping insect larvae or walking insects was recorded. During observations, care was taken to avoid disturbing the focal animal or of observing the same animal twice. Consequently, only 10 marked lizards from each study site were included in these measurements. Although it was impossible to completely exclude all other activities during such a study, data collected while animals appeared involved in other activities such as fighting, mating, thermoregulation and predation avoidance were eliminated from the analysis. The mean MPM at the 4 different sites are shown in Figure 2.14 (p 40).

Figure 2.13 Correlation coefficients (Pearson's Product Moment) between the basking duration (in minutes), air and substrate temperatures (°C)

Site		Basking duration	Air temperature	Substrate temperature
Control	Basking duration			
	Air temperature	-0.849 (*P*=0.002, N=10)		
	Substrate temperature	-0.641 (*P*=0.04, N=10)	0.845 (*P*=0.002, N=10)	
Clear	Basking duration			
	Air temperature	0.650 (*P*=0.04, N=10)		
	Substrate temperature	0.641 (*P*=0.04, N=10)	0.956 (*P*=0.0001, N=10)	
Soot	Basking duration			
	Air temperature	0.923 (*P*=0.0001, N=10)		
	Substrate temperature	0.804 (*P*=0.005, N=10)	0.952 (*P*=0.0001, N=10)	
Tar mat	Basking duration			
	Air temperature	0.508 (*P*=0.13, N=10)		
	Substrate temperature	0.563 (*P*=0.09, N=10)	0.978 (*P*=0.0001, N=10)	

Figure 2.14 Mean (± s.d.) foraging behaviour (MPM) at the different study sites

Location	MPM
Control (N = 10)	0.17 ± 0.07
Clear (N = 10)	0.16 ± 0.07
Soot (N = 10)	0.15 ± 0.05
Tar mat (N = 10)	0.19 ± 0.08

The one-way ANOVA test revealed no significant variance in foraging activity at the different study sites ($F_{3,36} = 0.77$, $P = 0.52$). It worth noting, however, that the lizards at the tar mat site showed the most MPM followed by the lizards from the control site. The lizards from the soot site showed the lowest MPM value.

2.4.5 Substrate Preferences

Experiments on the substrate preference were performed in the laboratory to investigate the effect of oil pollution on the behaviour of *A. scutellatus* collected from tar mat contaminated sites and from the control (Sulaibiya station) sites. In this study, substrate preference is determined when the animal chooses one type of substrate and prefers to stay at this substrate until the end of the experiment. It would have been preferable to have studied lizards from all 4 types of location but constraints of time and availability of subjects precluded this.

A box made of glass was designed (Fig. 2.15, p 41). It was divided into three chambers, separated by two movable partitions. The box was covered on all sides except one that was used for monitoring by using a video camera (Digital Handycam, DCR-TRV 310E, Sony Corporation, Japan). The sides were covered to allow the observer to freely remove the partitions without disturbing the animals. One chamber was filled with tar mat that was ground to form a black substrate. The other chamber was filled with

sand collected from Sulaibiya and ground into soft light sand. The middle chamber was left empty. The room and substrate temperatures were fixed at 25°C through all the experiments and were regularly measured during and after each experiment using a mercury thermometer

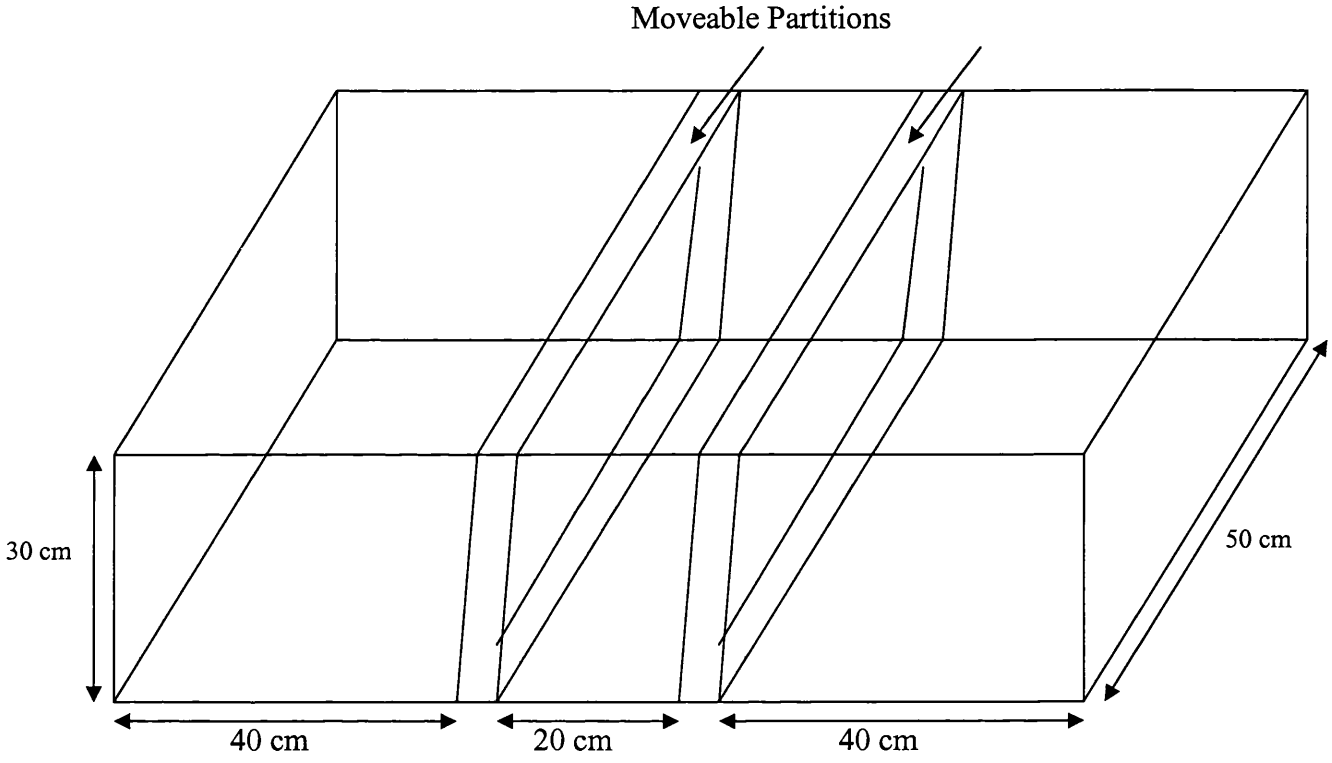

Figure 2.15 Glass box used for substrate preference experiments

The lizard was first kept in the middle chamber with the partitions in place and the video camera was operated by a remote control. After few seconds, the partitions were slowly removed by the observer positioned such that a lizard was unable to see her. Monitoring was undertaken for 15 minutes for each lizard. The box was kept on a table where the number of the experiment could be visualized by the video camera (e.g. S11, S12, B11, B12... etc.). The initial time taken to move (latency) and the times spent in the areas of light/or dark substrate were the measures recorded.

Figure 2.16 *A. scutellatus* from a control site showing normal colour appearance (author's own image)

Figure 2.17 This *A. scutellatus* from a tar mat site showing darker colouration (author's own image)

An attempt was made to determine whether the characteristics of the substrate (soil polluted with tar mat or light sand) influenced substrate choice in sand lizards. Twenty lizards were collected from the presumably most polluted field sites (tar mat) and twenty from the control (Sulaibiya) sites and were kept in the laboratory.

Lizards were fed with beetle larvae and were maintained basking within their cages in the garden. The cages used to house the lizards before the experiment were the same size and shape as the box used for the experiment but they lacked the partitions. Animals collected from the tar mat locations were housed in one cage provided with tar mat substrate and the lizards brought from the control locations were kept in another cage with a 5cm layer of sand brought from the control area. Active lizards were then moved back to laboratory ready to perform the substrate preference experiments.

The data were analyzed using SPSS 11.0 (2001). Chi-square test was used to compare between the two samples. The test was applied on the duration of time spent on light and dark substrates to investigate if there were any significant differences between the lizard subjects from the polluted and the control sites.

Figure 2.18 (below) compares the numbers favouring the light and dark substrates and the median times they spent on these surfaces.

Figure 2.18 Preferences of lizards from control and tar mat sites for the light and dark substrates and median time spent on these substrates by in 15 minute tests

Location	Number favouring the light substrate	Number favouring the dark substrate	Median time spent on the light substrate (minutes)	Median time spent on the dark substrate (minutes)
Control	14	6	13.32 (10.16-30.49)	2.54 (1.02-3.20)
Tar mat	3	17	3.05 (2.25-4.25)	13.10 (9.20 – 15.25)

The data indicate that most lizards used from the control site chose the light substrate and most lizards from the tar mat site chose the dark substrate. Chi-square test was run to see the association between site and substrate. The association was found to be significant ($\chi^2 = 12.38$, $P < 0.01$). Lizards from the tar mat sites preferred the dark substrate to the light one and *vice versa*.

The median time spent by animals from the control sites on the light substrate differed significantly from those animals from the tar mat sites ($t = -5.71$, $P < 0.0001$). The median time spent by lizards from the tar mat sites on the dark substrate also differed significantly from those of the control sites ($t = 10.47$, $P < 0.0001$). The lizards from the control site spent more time on the light substrate and the lizards from the tar mat site spent more time on the dark substrate. The duration of time on the preferred substrate was similar for both populations of lizards.

2.5 Discussion

This study shows that *A. scutellatus* populations at the clearly oil polluted and the non polluted (control) sites show differences in their daily behaviour. This was clearly demonstrated in the results of morning emergence of *A. scutellatus* between the different study sites. At the highly polluted sites (tar mat), the lizards emerged earlier than the other sites. In spite of the ambient temperature conditions being similar, field observations also provided strong evidence that substrate temperature varied among the different sites of study and had an impact on the basking of *A. scutellatus*. The presumably highly polluted site (tar mat) exhibited the highest substrate temperature. It appears that the morning emergence and the duration of basking are good evidence of the effect of oil pollution on the behaviour of *A. scutellatus*. In general, pollution resulted in the lizards being active earlier and potentially for longer.

The studies suggest that animals at the tar mat sites required less time basking to raise their temperatures to functional levels (the dark surface appearred to result in more rapid solar gain). It was difficult to interpret the emergence times but the later emergence of lizards in the control and soot areas suggests they will have less time for foraging behaviour. The final laboratory experiment confirms that operating on the tar

mat changes the animal's preference for at least a number of days. Basically, the findings suggest that the energetics are such that lizards on the tar mat areas will obtain a working temperature more rapidly, be able to obtain their food requirements more quickly and will consequently reduce their potential exposure to predators.

Many authors (e.g. Pianka, 1966) have recognized two basic modes of foraging used by carnivorous lizards, commonly called "sit-and-wait" (SW) and "widely foraging" (WF) styles. SW foragers tend to capture mobile prey whereas WF foragers generally feed on sedentary prey (Huey and Pianka, 1981). The frequency of prey that wander by a SW predator is positively correlated with prey density, so the success of a SW forager declines with prey scarcity (Schoener, 1969). SW foraging is more energetically conservative than active foraging (Huey *et al.*, 1983) and is generally favoured in reptiles when mobile prey are sufficiently abundant. When mobile prey are limited or prey abundance is reduced, active searching appears to be favoured. The foraging mode varies between species of reptiles and is correlated with patterns of habitat and prey use (Huey and Pianka, 1981; Petranka, 1998). The foraging movements of WF reptiles are naturally more extensive than those of SW counterparts (Pietruszka, 1986). There have been few studies on the foraging behaviour of lizards in the wild, so there is an incomplete understanding of the detailed behaviours of WF and SW predators (Day *et al.*, 1999). Arnold (1984) explained that vision is a main sensory system needed for SW predators while hearing and olfaction are often involved in WF predators. Vulnerability to visual predators is low in SW foragers because lizards are often immobile and can forage without detection in exposed positions if they possess good crypsis. Vulnerability to visual predators is, however, higher in WF strategists because their movement draws attention to these lizards. Consequently, unless predators are absent, active searching is only likely to take place in protected situations such as in vegetative cover. Prey mobility must be high if these are to reach SW foragers and their visibility should be high as they are often detected at considerable distances (Arnold, 1984).

Although the lizards at the tar mat site showed the greatest number of MPM in foraging at these sites, the result was not significantly different from that for lizards at

the other sites. Perry *et al.* (1990) measured the MPM for *A. scutellatus,* finding a mean of 1.01. In this present study, the mean of MPM was 0.199 for *A. scutellatus* from the tar mat sites and 0.172 for the controls. The difference between these results in the two studies might be related to food resource availability or the biodiversity of prey species. Various diets will be assorted with different efficiencies and energy requirements. The predators may increase their MPM to fulfill their dietary requirements or may reduce this index because the food is abundant and needs little energy to be consumed. A proximate reason for a predator to limit its movements could be reducing its own chance of being predated. Risk of predation usually increases with the amount of movement so the SW strategy of lizards avoids moving in order to not draw the attention of their own predators. Huey and Pianka (1981) suggested that SW strategists generally rely on crypsis to avoid being detected. The MPM study confirms that the lizard persists as a SW at all the locations and does not apparantly have to increase its active foraging.

Integumentary colour changes are known in lizards and snakes and they are categorized as being physiological or morphological (Cooper and Greenberg, 1992). Physiological colour change usually occurs in response to proximal environmental stimuli, such as change in general illumination, background and social behaviour. In reptiles, physiological colour changes are controlled by hormonal or neural action on chromatophores. Animals having a particular body colouration may behaviourally select habitats in which they are undectable against the background. In some species, such as chameleons, the animal can change body colour rapidly to match the current background (Cooper *et al.*, 1990). Physiological colour change is also believed to occur in *Anolis carolinensis* (Medvin, 1990) and *Chamaeleo chamaeleon* (Raxworthy and Nussbaum, 1995) where lizards use chromatophores in their integument to match their colouration to that of the substrate. Morphological colour changes may also gradually shift body colouration to match the predominant background by manufacturing extra melanin. The lacertid lizard, *Podarcis taurica*, changes colour seasonally, being bright green in spring and early summer and becoming dark on olive green to brown in late summer (Chondropoulos and Lykakus, 1983). Animals from polluted locations preferred to stay on polluted substrate rather than non-polluted substrate. The strength of this response

suggests that the behaviour is highly adaptive and if they do not change colour they would easily be seen and captured by predators. Oil pollution influences the lizard's behaviour and makes them prone to contamination by direct contact with the substrate or via the consumption of contaminated food. Lizards from the tar mat sites (dark substrate) were darker than those from the control sites (light substrate). This may be because, living on a dark substrate for many years after oil pollution in 1991 and the formation of tar mat, has selected sand lizards possessing the new cryptic colouration which is essential to avoid predators in these areas with darker substrate (Figures 2.16 and 2.17, p 41). In the chapparal of southern California, periodic fires blacken the stalks of shrubs for several years. After the charred surface has worn off the stalks, the stalks are lighter in colour. The darkly coloured iguanid lizard *Sceloporus occidentalis* perches selectively on the dark stalks. After the stalks lighten and the lizards are no longer cryptic, they switch perch performance to rock outcrops. In the laboratory, the lizards strongly preferred to perch on dark branches rather than light ones (Lillywhite *et al.*, 1977). The explanation for colour matching is that crypsis lessens the likelihood of predation. Luke (1989) in his laboratory tests showed that lizards (*Uta stansburiana*) that have colouration contrasting with that of the substrate are more likely killed by avian predators. Similar results were shown by lizards (*Sceloporus undulatus*) differing from background colouration have higher frequencies of tail breaks in the field suggesting that they are subject to greater predation pressure (Gillis, 1989). Colouration has important effects on behaviour in that individuals having colouration that allows more rapid warming to preferred body temperature may have more time available for numerous activities. Because many behaviours of ectotherms are thermally dependent (Waldschmidt *et al.*, 1985), the performance efficiences of the behaviours may vary with colouration (Luke, 1989).

"Of course, it would have been interesting to take lizards from the different substrates and maintain them on the alternative background for an extended period of time (e.g. animals from the clear sites on the tar mat background) and to see whether the subjects adapted their colouration. There was, however, no time to complete such a study".

A number of predators of the sand lizard *A. scutellatus* were seen in the study sites. Horned and common sand vipers (*Cerastes* spp.) are known to take large numbers of lizards especially during summer when those predators become very active and abundant. One of those snakes was found preying on sand lizards at the end of April 2002. The Waral was also observed in the study areas during late spring and early summer. Such animals become very active at noon when most sand lizards retreat to their burrows to avoid high temperatures. These large monitor lizards were seen actively searching burrows for prey and were often seen near several burrows used by sand lizards. Large adult *A. scutellatus* were also predators of hatching lizards of their own species. Birds of prey such as the Common kestrel (*Falco tinnunculus*) and the Lesser kestrel (*F. naumanni*) were commonly seen in the study areas during winter and spring.

Living in an open desert habitat with little vegetative cover, results in a lizard's anti-predator strategies being limited to concealment and speed. These two strategies were both employed by *A. scutellatus*.

CHAPTER 3

Effects of Oil Pollution on the Population Sizes of *Acanthodactylus scutellatus* and Ants at the Different Study Sites

3.1 Introduction

All organisms need to obtain finite resources (e.g. food, nesting sites and shelter) from their environment. The maximum number of a species that a habitat can support throughout their complete life cycle is called the 'carrying capacity'. This sets an upper limit to population size, but does not explain why population numbers change in the way they do. The factors predicting population trends may act at any stage in the life cycle, and there may be competition between or within species for available resources, direct interaction between species (e.g. parasitism or predation) as well as effects of the abiotic environment (such as drought, flood, heat or cold).

Populations of animals are rarely constant for any extended period of time. The apparent effects of pollutants on population size can be greatly influenced by many other factors. Exposure to a pollutant may coincide with decrease in population size of one or more species, but this does not show that the pollutant has necessarily directly altered populations of that species. Evidence that a pollutant has killed some individuals does not necessarily indicate that the population size will be affected. Conversely, a pollutant that kills no individuals but has sublethal effects on a significant proportion of individuals could have a severe impact on its viability (Moriarty and Walker, 1987).

It is unlikely that any potential pollutant will only affect one species but differences in species' susceptibility can be important (Blanck *et al.*, 1984; Sloof *et al.*, 1986). A striking field illustration of unexpected differential toxicity was generated when the insecticide Dieldrin was banned as a seed dressing to protect winter wheat from the Wheat bulb fly in the UK because of its presumed effects on seed-eating birds. The organophosphorus insecticide Carbophenothion was employed as a potential

substitute for Dieldrin, but several incidents of deaths of Greylag geese (*Anser anser*) and of Pink-footed geese (*Anser fabalis brachyrhynchus)* were reported from 1971 to 1975 in birds that had fed in fields sown with the newly dressed grain. One notable feature of these incidents was that only geese of the genus *Anser* were found dead, although other species of grain-eating birds were present on these fields. In laboratory tests, Westlake *et al.* (1978) showed that an oral dose of 25 mg Carbophenothion/kg body weight was sufficient to kill Greylag and Pink-footed geese. In contrast, Canada geese (*Branta canadensis*) [with an estimated LD_{50} of 29 – 35 mg/kg] as well as pigeons (*Columba livia*) and chickens *(Gallus domesticus*) were unaffected. This clearly illustrates that one cannot predict with complete confidence from toxicity data gathered for one species what the effects of the same chemical will be on another species (even if it is closely related). Species can differ appreciably in the way they ingest, accumulate, distribute, and eliminate pollutants. It should also be anticipated (for genetic and other reasons) that there will be distinct differences in susceptibility between individuals within populations.

Generally the most noticeable and publicized effect of oil spills is from the oil slick itself. Sea birds become covered with oil and die. It is difficult to estimate numbers but it is reasonably certain that, in the seas around North-western Europe, spilt oil kills some tens of thousands of seabirds annually (Dunnet, 1982). There is, however, little evidence that the populations of these seabirds are reduced by this mortality, even though many of these species have relatively low breeding rates and do not breed until they are several years of age. If environmental conditions become less favourable, however, oil pollution might well have an adverse effect on these species. Where species have declined, factors such as climatic change appear to be largely responsible (Clark, 1984). Habitat vulnerability and species susceptibility both influence the effects of an oil spill. One of the basic difficulties in scientific studies of oil pollution is that one cannot readily quantify the duration of exposure or the dose.

Sand lizard populations were monitored to compare abundance in areas with apparently differing degrees or levels of oil-polluted soils. Before commencing the study in 2000-2001, a preliminary survey was carried out on the study areas to choose

the locations for the pitfall traps and the transects. The intention was to establish whether monitoring this animal was useful in relation to oil pollution in a desert environment.

3.2 Materials and Methods

3.2.1 Monitoring Methods for Sand Lizards

A map produced in KISR's GIS Section was used to select the study sites. This map had been used by KISR personnel for investigation of soil studies and the same sites were used for safety reasons (only areas known to be clear of UXO could be used as study sites).

The study sites in Al-Burgan oil fields and the control areas were selected according to the apparent degree of pollution. Two sites were chosen in each pollution category, a trapping unit was installed at each. A transect (25m x 25m) was also plotted at each site.

3.2.1.1 Drift Fence and Pitfall Traps

Drift fences and pitfall traps were used to catch sand lizards and to identify different species of arthropods found in the same area. At each of the studied sites, a 40 cm high 18 m drift fence was set, keeping within the safety zone. Fences were made of canvas, leading to sunken buckets from which lizards could not escape. These were permanent installations which were checked daily when they were in use. When they were not in use, the entrances were sealed, and bricks were kept over the seals in case strong winds occurred. Pitfall traps consisted of 5 gallon (22.7 litre) green buckets (28 cm diameter, 30 cm depth). Pitfall traps were set at 3, 6, 9, 12 and 15 m from either end of the drift fences, and were numbered in sequence from 1 to 5. The design of the drift fence and pitfall traps are shown in figures 3.1 and 3.2 (p 53). It was necessary to install mesh filters in the bottom of traps to enable various taxa to avoid each other.

Live specimens of sand lizards were permanently marked by toe clipping and temporarily (for easy visual recognition) by painting bands on the animal's back using

nail polish (Fig. 3.3, p 54). Toe clipping followed specific manner by numbering the toes in the order 1-5, starting with the left forelimb (lizard on its abdomen), the right forelimb, the left hindlimb and finally (if necessary) the right hindlimb using sharp fingernail scissors. Each of the five toe positions corresponds to a colour for all limbs (1 = red, 2 = green, 3 = black, 4 = pink and 5 = white). These colours are used in painting bands of the lizard being marked using nail polish. For example, if the first toes of the forelimbs and the left hindlimb are used, then 3 red bands will be painted on the lizard's back. Two marking schemes were applied in order to facilitate animal observations in the field. All lizards captured in the pitfall traps were recorded in the associated data sheets.

Ant numbers were estimated by counting them in all 5 traps in the 2 replicates of each study site. The ants were almost all dead in the traps, so they were recorded in the data sheet and were collected in jars cleaned with ethanol and were frozen at $-20^{\circ}C$ for later chemical analysis. Total ant numbers were estimated per 324 m^2 because the area of drift fence and pitfall traps was 18m x 18m as advised by a consultant and was then calculated per $1km^2$ for the entire study area. Each pollution category has two replicates.

Air and substrate temperatures were recorded also in the pitfall data sheet during this study according to a timed schedule starting with sites having higher air and substrate temperatures. The live specimens were then released about 10m from the traps. Dead specimens were collected in jars cleaned with ethanol and were frozen at $-20^{\circ}C$ for later chemical analysis.

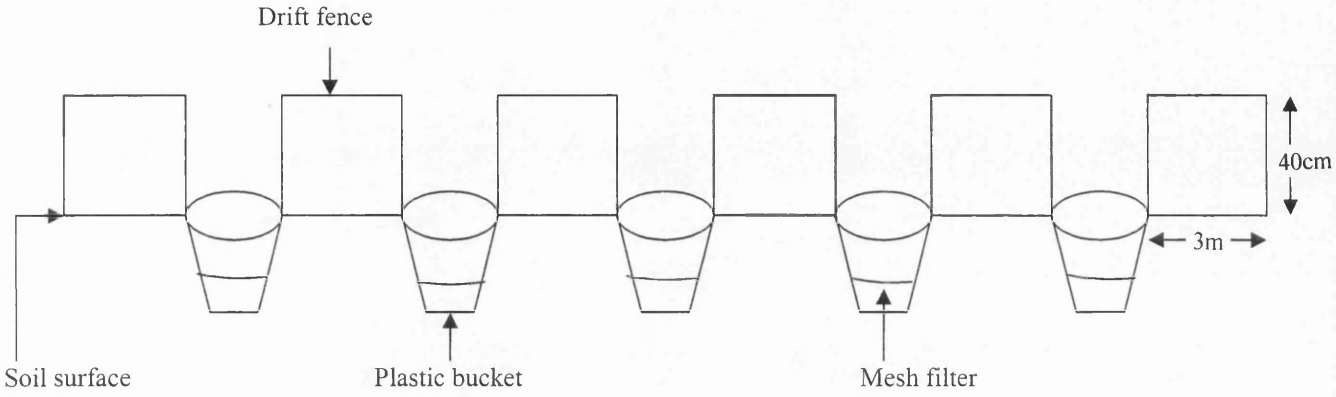

Figure 3.1 Schematic design of the drift fence with pitfall traps used in the study

Figure 3.2 Drift fence and pitfall traps in the field (author's own image)

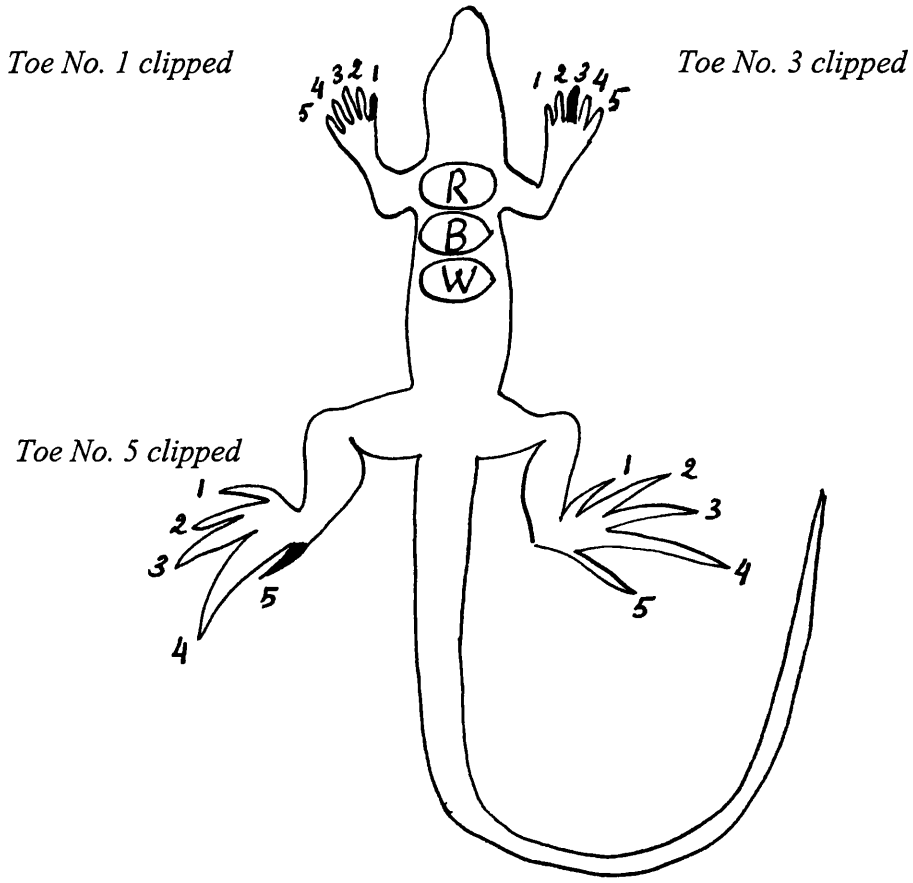

Figure 3.3 Manner of marking lizards by clipping toes and painting bands with nail polish. Numbers (1-5) the order of marking toes. The lizard illustrated has been marked 135 or RBW by clipping the first toe on leg one, the third toe on leg two, and the fifth toe on leg three. Each of the five toe positions corresponds to a colour, and these colours are used in painting bands on the back of the lizard being marked (after Degenhardt, 1966).

The Schnabel method of population estimation was used in this study. A series of samples were used in which there is a 2^{nd}, 3^{rd}, 4^{th}, ...n^{th} sample. Individuals caught at each sample were first examined for marks, then marked and released. Marking occurred at each sampling session. Only a single type of mark was used (Krebs, 1999), since there was a need to distinguish only two types of individuals namely, marked (signifying caught in one or more prior samples) and unmarked (signifying never previously caught)

An estimation of population size (N) was given by:

$$\hat{N} = \frac{\Sigma_t (C_t \ M_t)}{\Sigma_t \ R_t}$$

C_t = Total number of individuals caught in the sample $_t$

R_t = Number of individuals already marked when caught in sample $_t$

M_t = Number of marked individuals just before sample $_t$ was taken

The data of pitfall traps were grouped in tables including information on sample number, Schnabel method symbols, date, area, site number, air temperature, soil temperature and number of ants. This was carried on separately for each site and year of study. $\acute{N}$, lower and upper 95% confidence interval, degrees of freedom and standard error of $1/\acute{N}$ is also included with each table.

The Schnabel estimator is calculated on the reciprocal of N:

$$\text{Variance } (1/\hat{N}) = \frac{\Sigma \ R_t}{(\Sigma \ C_t \ M_t)^2}$$

$$\text{Standard error of } 1/\hat{N} = \sqrt{\text{Variance } (1/\hat{N})}$$

Schumacher and Eschmeyer (Krebs, 1999) pointed out that if graph paper is used to plot x-axis : M_t, number of individuals previously marked (before time t)

y-axis : R_t / C_t, proportion of marked individuals in the t-th sample,

the plotted points should lie on a straight line of slope (1/N) passing through the origin. Consequently one can use linear regression techniques to obtain an estimate of the slope (1/N) and hence the population size. The appropriate formula for this estimation is

$$\hat{N} = \frac{\sum_{t=1}^{s} (C_t M_t^2)}{\sum_{t=1}^{s} (R_t M_t)}$$

Where s = Total number of samples

The variance of Schumacher estimator is obtained from linear regression theory as the variance of the slope of the regression. In terms of mark recapture data,

$$\text{Variance of } (1/\hat{N}) = \frac{\Sigma (R^2_t/C_t) - [(\Sigma R_t M_t)^2 / \Sigma C_t M^2_t]}{s-2}$$

Where s = Number of samples included in the summations.

The standard error of the slope of the regression is obtained as follows:

$$\text{Standard error of } \left(\frac{1}{\hat{N}}\right) = \sqrt{\frac{\text{Variance of } (1/\hat{N})}{\Sigma (C_t M_t^2)}}$$

The Schnabel method makes assumptions that the population size is constant without recruitment or losses, that sampling is random, and that all individuals have an equal chance of capture in any given sample (obviously this is likely to only apply to relatively short durations of study).

A different mark-recapture method, the Jolly-Seber, was applied to obtain estimates from an open population. In this method, mark-recapture samples are taken on three or more occasions. The important point here is to be able to answer, for each marked animal in the sample: when was this marked individual last captured?. The samples are usually point samples of short duration, and separated by a long duration from the next sample. The time interval between samples need not be constant, and any number of samples can be accommodated, so that series of data extending over many years can be used in this method (Krebs, 1999). This method not only allows for gains

and losses but provides estimates of the number of animals entering the population and the survival rate, though the former includes both immigrants and births and 'survival' is the complement of both emigration and mortality (Sutherland, 1996).

3.2.1.2 Transect Method

This method was intended to locate sand lizards and record them. Five permanent transect lines, each 25m long and 5m wide were installed randomly in the

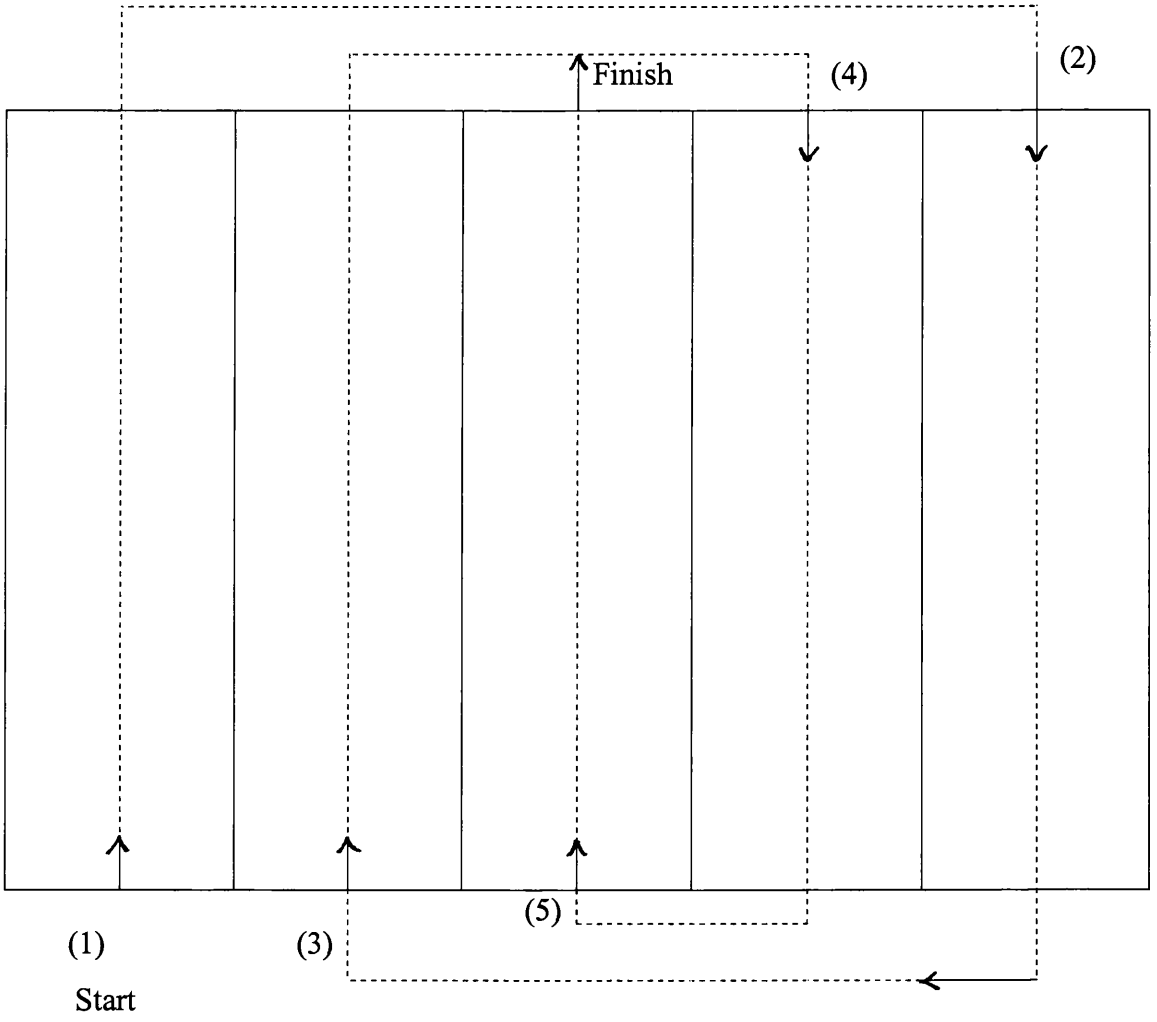

Figure 3.4 Route followed in lizard and ant quadrat census (author's own design)

study sites. They were plotted in a quadrat form as shown in figure 3.4 (p 57). The lizards were recorded as numbers per 625m² and the totals from most quadrats gave that number directly. Number of ants was recorded by walking the transects and counting ants present in the manner indicated in figure 3.4 (p 57). The total number of ants was recorded as numbers per 625 m² because the quadrat was 25m x 25m as advised by a consultant and then was calculated per 1 km² for the entire study area. Estimates of lizard and ant numbers were then converted into populations per km². The lizards and ants on each quadrat were recorded by walking the rows in the manner indicated according to a schedule with respect to time and temperature, starting with sites where lizards emerge earlier in the morning and ending with sites where lizards take longer to emerge.

Transects were only carried out on warm and clear days during the periods of highest lizard activity (February-April). The transects were carried out 10 times in 2002 and 14 times in 2003 for each replicate of the study sites. The rate of travel on the transect was adjusted by the observer. Generally, the rate was 5-10 minutes per 625m^2 of transect. Air temperature with substrate temperatures at start of walking the transect and when finished were recorded. During transect walks, lizard activity levels initially increased as the substrate temperature warmed, but decreased as the heat became excessive. All lizards seen while the investigator was inside the quadrat were plotted on the data sheets.

3.2.2 Lizard Body Size Measurements

Information on the body size of *A. scutellatus* was gathered in the field between 2002 and 2003 using capture and recapture methods. Lizards used in laboratory studies for behavioural and chemical analysis studies were also used for body size measurements.

Snout-vent length (SVL) and Vent-tail length (VTL) of all lizards captured during this study were measured by placing the animals on their backs alongside a metal ruler. Body weights (BW) were determined by weighing the lizards in a plastic bag (of known weight) using Pesola spring balance (Parco-No. 142259, Pesola AG,

Baar, Switzerland). Ten adult males and 10 adult females from each site were used for measuring lizard's body size and weight. Because of the small numbers of juveniles captured, the data for these were excluded. The data for the lizard's body size and weight were carefully recorded at the various study sites.

3.3 Results

3.3.1 Monitoring methods

The number of lizards and ants at all study sites obtained by pitfall traps are shown in figures 3.5 (below) and 3.6 (p 61) for 2002 and 2003 respectively and by using the transects in figures 3.7 (p 64) and 3.8 (p 65) for 2002 and 2003 respectively.

Figure 3.5 Mean (± s.d.) population sizes of lizards and ants/ km² and air and substrate temperatures (°C) recorded whilst monitoring the pitfall traps in 2002

Location	Mean population size of lizards/ km²	Mean number of ants/km²	Mean air temperature (°C)	Mean substrate temperature (°C)
Control	5658 ± 318.9 (N = 2)	30666 ± 1338.8 (N=12)	23.4 ± 0.62 (N=12)	32.4 ± 1.4 (N=12)
Clear	3170 ± 308.4 (N = 2)	169933 ±18545.5 (N=12)	24.1 ± 0.11 (N=12)	33.5 ± 0.42 (N=12)
Soot	6239 ± 130.4 (N = 2)	35479 ± 1678.2 (N=12)	24.8 ± 0.88 (N=12)	35.5 ± 0.54 (N=12)
Tar mat	2432 ± 206.3 (N = 2)	398533 ± 51100.2 (N=12)	25.2 ± 0.37 (N=12)	35.6 ± 0.55 (N=12)

One-way ANOVA showed no difference in lizard population sizes ($F_{3,4} = 1.15$, $P = 0.43$) between the different study sites using pitfall method in 2002 (Schnabel method). A slight difference was observed in ant population sizes between the sites ($F_{3,4} = 6.39$, $P = 0.05$). Air ($F_{3,4} = 3.93$, $P = 0.10$) and substrate ($F_{3,4} = 3.99$, $P = 0.10$) temperatures did not vary significantly between the sites.

The data of lizard and ant population sizes by using the pitfall trap method in 2002 showed very high numbers of lizards at the soot and control sites whereas the tar mat sites showed the lowest numbers of lizards, followed by the clear sites. The mean number of ants/km^2 also varied between the sites. The highest numbers of ants were at the tar mat sites and the lowest numbers were at the control sites. The mean air temperatures recorded during monitoring of pitfall traps showed very close values between the different sites. The same results were obtained for the means of substrate temperatures recorded during monitoring the pitfall traps.

The Jolly-Seber method was further applied to the existing data set of pitfall trap in 2002. One-way ANOVA showed no significant difference in lizard population size between the study sites ($F_{3,4} = 1.32$, $P = 0.38$) by using the pitfall method in 2002.

Figure 3.6 Mean (± s.d.) population sizes of lizards and ants/ km² and the means of air and substrate temperatures recorded whilst monitoring the pitfall traps in 2003

Location	Mean population size of lizards/ km²	Mean number of ants/ km²	Mean air temperature (°C)	Mean substrate temperature (°C)
Control	6396 ± 235.5 (N = 2)	34215 ± 1932.3 (N=13)	21.4 ± 0.82 (N=13)	28.37 ± 1.5 (N=13)
Clear	5080 ± 402.7 (N = 2)	144184 ±15813.8 (N=13)	22.8 ± 0.79 (N=13)	29.2 ± 1.18 (N=13)
Soot	2811 ± 253.6 (N = 2)	33413 ± 1482.2 (N=13)	22.8 ± 0.96 (N=13)	29.2 ± 0.81 (N=13)
Tar mat	3599 ± 318.5 (N = 2)	230153 ± 3829.5 (N=13)	20.7 ± 1.18 (N=13)	26.7 ± 1.62 (N=13)

One-way ANOVA showed no significant difference in lizards population size between the study sites ($F_{3,4}$ = 0.63, P = 0.63) by using the pitfall method in 2003 (Schnabel method). No significant variance was found in ant population size between the study sites ($F_{3,4}$ = 2.70, P = 0.18). Air ($F_{3,4}$ = 2.54, P = 0.19) and substrate ($F_{3,4}$ = 1.56, P = 0.33) temperatures did not show a significant difference between the sites.

The data in figure 3.6 (above) for the pitfall trap method in 2003 showed that the control sites had very high numbers of lizards, followed by the clear sites. The soot sites had the fewest lizards compared to the other study sites with the tar mat sites having slightly more lizards. The ant population size was highest at the tar mat sites and lowest at the soot sites. The mean air and substrate temperatures recorded during monitoring of pitfall traps showed very similar values at the different study sites.

One-way ANOVA showed no significant difference in lizard population size between the different study sites ($F_{3,4}$ = 0.54, P = 0.67) by using the pitfall method in 2003 (Jolly-Seber method).

Two-way ANOVA test was used to contrast the measures obtained with the pitfall trap method in 2002 and 2003 (Schnabel method). No significant difference was observed between years ($F_{1,15}$ = 0.005, P = 0.94), or sites ($F_{3,15}$ = 0.88, P =0.48), or the interaction between year and site ($F_{3,15}$ = 0.82, P = 0.51) in lizard population sizes. Ant population sizes revealed no significant difference between years ($F_{1,15}$ = 1.15, P = 0.31), but a significant difference was observed between sites ($F_{3,15}$ = 8.87, P ‹ 0.006) and the interaction between year and site showed no significant difference ($F_{3,15}$ = 0.82, P = 0.51). The air temperature showed a significant difference between years ($F_{1,15}$ = 37.61, P ‹ 0.0001), but no significant difference was observed between sites ($F_{3,15}$ = 3.39, P = 0.07) and the interaction between year and site was not significant ($F_{3,15}$ = 2.43, P = 0.14). The substrate temperature revealed a significant difference between years ($F_{1,15}$ = 92.05, P ‹ 0.0001), but no significant difference was observed between sites ($F_{3,15}$ = 1.9, P = 0.21) and the interaction between year and site showed no significant difference ($F_{3,15}$ = 3.25, P = 0.08).

Two-way ANOVA test was used to contrast the measures obtained with the pitfall trap method in 2002 and 2003 (Jolly-Seber method). No significant difference was observed between years ($F_{1,15}$ = 0.034, P = 0.85), or sites ($F_{3,15}$ = 1.03, P =0.43), or the interaction between year and site ($F_{3,15}$ = 0.383, P = 0.77) in lizard population sizes.

The data of lizard and ant numbers using the transect method in 2002 are shown in figure 3.7 (p 64). It was obvious that the control sites had the highest lizard numbers followed by the tar mat sites whereas the soot sites had the fewest lizards. The ant numbers were also very high at the tar mat and the clear sites and were lowest at the control and the soot sites. The air and substrate temperatures at start and when finished walking the transects were very similar at the different sites of study. Similar search times were used on the different study sites.

The one-way ANOVA test of the data recorded for lizards and ants numbers using transect method for the year 2002 showed no significant variation in lizard number between the different study sites ($F_{3,4} = 1.06$, $P = 0.45$). Ant numbers also did not vary between the study sites ($F_{3,4} = 0.86$, $P = 0.53$). Air temperature showed a slight variance between the sites ($F_{3,4} = 15.53$, $P < 0.01$). The substrate temperature at the start ($F_{3,4} = 8.96$, $P < 0.03$) and finish ($F_{3,4} = 7.52$, $P < 0.04$) of the transect walk differed slightly between the sites. No significant difference was shown in search time between the different study sites ($F_{3,4} = 0.75$, $P = 0.57$).

Figure 3.7 Mean (± s.d.) population sizes of lizards and ants, as well as air and substrate temperatures (°C) at the start and finish, and mean search times recorded when walking the transects in 2002

Location	Mean lizard numbers / km²	Mean ant numbers / km²	Mean air temp.	Mean substrate temp. at start	Mean substrate temp. at finish	Mean search time (minutes)
Control	4012 ± 872.9 (N = 2)	34259 ± 436.5 (N = 10)	24.4 ± 0.11 (N = 10)	30.1 ± 0.41 (N = 10)	31.2 ± 0.46 (N = 10)	9.7 ± 0.14 (N = 10)
Clear	2623 ± 109.2 (N = 2)	57407 ± 3099.5 (N = 10)	24.8 ± 0.18 (N = 10)	31.1 ± 0.12 (N = 10)	32.1 ± 0.01 (N = 10)	9.7 ± 0.42 (N = 10)
Soot	1741 ± 155.6 (N = 2)	32561 ± 1113.4 (N = 10)	24.6 ± 0.13 (N = 10)	32.0 ± 0.43 (N = 10)	32.9 ± 0.45 (N = 10)	9.4 ± 0.14 (N = 10)
Tar mat	3086 ± 218.4 (N = 2)	62500 ± 3382.7 (N = 10)	23.1 ± 0.48 (N = 10)	30.9 ± 0.39 (N = 10)	31.9 ± 0.31 (N = 10)	9.7 ± 0.14 (N = 10)

The data using the transect method in 2003 is presented in figure 3.8 (p 65) and shows very high numbers of lizards in the clear sites followed by the control sites. The tar mat sites had lower lizard numbers than the clear and control sites but did not differ markedly from lizard numbers at the control sites. The numbers of ants were much higher at the tar mat sites than the other locations followed by the clear and soot sites. The control sites had the lowest number of ants of all the study sites. The air temperature and the substrate temperatures at the start and the finish of walking the

transects showed similar values at the different sites of study. Search times used on the different sites were also similar.

The one-way ANOVA test did not show any significant variance in lizard numbers between the different study sites ($F_{3,4} = 0.08$, $P = 0.96$) as assessed using the transect method in 2003. This was also true for the ant numbers ($F_{3,4} = 0.87$, $P = 0.52$). Air temperatures did not differ between the sites ($F_{3,4} = 0.59$, $P = 0.65$) and the substrate temperatures ($F_{3,4} = 0.18$, $P = 0.90$) on starting and when finishing ($F_{3,4} = 0.11$, $P = 0.94$) the transect walk showed no significant variation between the sites. This was also true for the search times which showed no significant variance between the sites ($F_{3,4} = 1.24$, $P = 0.40$).

Figure 3.8 Mean population sizes of lizards and ants, mean air and substrate temperatures at start and finish and the means of search time ± s.ds recorded during walking the transects in 2003

Location	Mean lizard numbers / km²	Mean ant numbers / km²	Mean air temp.	Mean substrate temp. at start	Mean substrate temp. at finish	Mean search time (minutes)
Control	5291 ± 935.4 (N = 2)	54122 ± 1917.2 (N = 14)	21.5 ± 0.94 (N = 14)	28.3 ± 1.69 (N = 14)	29.2 ± 1.63 (N = 14)	9.4 ± 0.40 (N = 14)
Clear	5731.9 ± 249.2 (N = 2)	81679 ± 6874.5 (N = 14)	22.8 ± 0.48 (N = 14)	28.9 ± 0.88 (N = 14)	29.6 ± 0.91 (N = 14)	9.4 ± 0.30 (N = 14)
Soot	4614.8 ± 332.8 (N = 2)	60846 ± 2961.7 (N = 14)	22.4 ± 2.0 (N = 14)	29.2 ± 2.60 (N = 14)	29.9 ± 2.8 (N = 14)	9.2 ± 0.10 (N = 14)
Tar mat	5180.8 ± 358.4 (N = 2)	113866 ± 2447.4 (N = 14)	21.4 ± 1.2 (N = 14)	29.5 ± 1.21 (N = 14)	30.2 ± 1.41 (N = 14)	9.7 ± 0.01 (N = 14)

Two-way ANOVA test was used to contrast the measures obtained with the transect method in 2002 and 2003. A significant difference was observed in lizard numbers between years ($F_{1,15} = 6.53$, $P < 0.03$), but no significant difference was found between sites ($F_{3,15} = 0.45$, $P = 0.72$), or the interaction between year and site ($F_{3,15} = 0.21$, $P = 0.89$). No significant difference was shown in ant numbers between years ($F_{1,15} = 3.48$, $P = 0.09$), or between sites ($F_{3,15} = 1.56$, $P = 0.27$), or the interaction between year and site ($F_{3,15} = 0.17$, $P = 0.91$). The air temperature showed a significant difference between years ($F_{1,15} = 23.26$, $P < 0.001$), but no significant difference was observed between sites ($F_{3,15} = 2.24$, $P = 0.16$), or the interaction between year and site ($F_{3,15} = 0.27$, $P = 0.84$). A significant difference was found in the substrate temperature at start between years ($F_{1,15} = 11.65$, $P < 0.009$), but no significant difference was shown between sites ($F_{3,15} = 0.92$, $P = 0.47$), or the interaction between year and site ($F_{3,15} = 0.19$, $P = 0.89$). The substrate temperature at finish showed a significant difference between years ($F_{1,15} = 12.31$, $P < 0.008$), but no significant difference was shown between sites ($F_{3,15} = 0.622$, $P = 0.62$), or the interaction between year and site ($F_{3,15} = 0.17$, $P = 0.91$). No significant difference was observed in the search times between years ($F_{1,15} = 2.01$, $P = 0.19$), or sites ($F_{3,15} = 1.75$, $P = 0.23$), or the interaction between year and site ($F_{3,15} = 0.28$, $P = 0.83$).

3.3.2 Lizard Body Size Measurements

Lizard body size (SVL) and weights (BW) for animals captured at the different study sites are presented in Figures 3.9 and 3.10 below, respectively.

Figure 3.9 Mean (± s.d.) SVL (cm) of male and female *A. scutellatus* at the different study sites

Location	Sex	Mean
Control (N = 20)	Male (N = 10)	55.70 ± 0.67
	Female (N = 10)	52.70 ± 2.62
Clear (N = 20)	Male (N = 10)	58.30 ± 1.16
	Female (N = 10)	53.00 ± 2.49
Soot (N = 20)	Male (N = 10)	59.50 ± 2.55
	Female (N = 10)	53.80 ± 1.54
Tar mat (N = 20)	Male (N = 10)	60.40 ± 3.83
	Female (N = 10)	54.10 ± 2.64

Figure 3.10 Mean (± s.d.) BW (g) of male and female *A. scutellatus* at the different study sites

Location	Sex	Mean
Control (N = 20)	Male (N = 10)	4.74 ± 0.25
	Female (N = 10)	4.23 ± 0.48
Clear (N = 20)	Male (N = 10)	5.32 ± 0.42
	Female (N = 10)	4.36 ± 0.62
Soot (N = 20)	Male (N = 10)	5.78 ± 0.84
	Female (N = 10)	4.34 ± 0.41
Tar mat (N = 20)	Male (N = 10)	6.09 ± 1.19
	Female (N = 10)	4.47 ± 0.34

The one-way ANOVA test showed significant variance between the study sites in the adult male lizard's SVL ($F_{3,36}$ = 7.23, P< 0.001). This variation with respect to location was further analyzed using *post hoc* Scheffe' tests. The SVLs of male lizards at the control sites were shorter than subjects taken at the soot sites (P < 0.01) and the tar mat sites (P < 0.001) but did not differ from counterparts collected at the clear sites (P = 0.13). No significant differences were observed in SVLs between the clear and soot sites, the clear and tar mat sites, or the soot and tar mat sites. The means of female SVL did not differ between the study sites ($F_{3,36}$ = 0.77, P = 0.51).

One-way ANOVA test showed a significant difference in the male lizard body weight between the different sites ($F_{3,36}$ = 5.81, P < 0.002). The *post hoc* Scheffe' tests showed no significant difference between the male body BW at the control and the clear sites but the BW was lower in subjects from the control than these from the soot (P < 0.04) or the tar mat (P < 0.005) sites. Mean BWs were greatest in male lizards caught at tar mat sites and least in counterparts caught at the control sites. No significant difference was observed in the BW of adult female lizards between the study sites ($F_{3,36}$ = 0.42, P = 0.73).

Further comparisons of SVLs and BWs were carried out between male and female lizards at each study site and at all study sites to see if any evidence of sexual dimorphism exists. One-way ANOVA test between the male and female SVLs at all sites showed a very significant difference ($F_{1,78}$ = 73.38, P < 0.0001). A similar result was obtained between the male and female BWs at all study sites ($F_{1,78}$ = 49.48, P < 0.0001). One-way ANOVA test showed a significant difference between the male and female SVLs (M > F) at the control sites ($F_{1,18}$ = 12.23, P < 0.003), at the clear sites ($F_{1,18}$ = 37.12, P < 0.0001), at the soot sites ($F_{1,18}$ = 36.50, P < 0.0001) and at the tar mat sites ($F_{1,18}$ = 18.29, P < 0.0001). The One-way ANOVA test also showed a significant difference between the male and female BWs (M > F) at the control sites ($F_{1,18}$ = 8.95, P < 0.008), at the clear sites ($F_{1,18}$ = 15.75, P < 0.001), at the soot sites ($F_{1,18}$ = 23.54, P < 0.0001) and at the tar mat sites ($F_{1,18}$ = 17.01, P < 0.001).

3.4 Discussion

Population monitoring by drift-fence and pitfall traps are the most common methods of assessing lizard numbers. Trapping by pitfalls is less investigator-dependent than the line transect method in that the investigator must only ensure that the traps are carefully installed so that surface irregularities do not vary from year to year or investigator to investigator.

Predation within the traps was a potential problem. The Sand viper (*C. cerastes*) was found eating small lizards three times during the field work. One of these snakes was found dead 1 m away from the traps (it had obviously been attacked by a bird of prey). On other occasions a Sand viper that had shed its skin, was found very close to the pitfall traps. This indicates that this type of predator can easily enter the traps, eat the contained prey and then often exit without leaving a trace of their victims. Since it is likely that traps are attacked deliberately, one cannot readily remove the bias they create. For this reason, a pitfall trapping protocol involving checking traps less than once per day is likely to produce unacceptable errors in this type of monitoring of live animals. Even with daily trap-checking (as in this present study), there will be some undetected mortality in the traps. On a number of occasions during this study, other lizard species (Warals) were seen within or immediately outside the transect bounds.

The pitfall traps of the present study were 30cm deep, which was reasonably safe for quantitative monitoring of sand lizards and arthropods of a variety of sizes. Mark-recapture results revealed that the number of lizards at the tar mat sites was almost half of that at the control sites. The presumably most polluted sites (the tar mat and soot locations) were actually highly vegetated. Some 12 years since the oil spill, the desert habitats appeared dominated by a variety of small shrubs and grasses which encouraged arthropod abundance in some of the study sites. Having a mat of tar at particular sites essentially provided cover that preserved moisture and organic matter in the underlying soil. These conditions will encourage desert plant seeds to germinate, producing local concentrations of desert plants even if the weather is generally dry. This, in turn, generates a very high local density of invertebrates including ants in these presumably highly polluted sites.

Both the Schnabel (designed for closed populations) and Jolly-Seber (designed for open populations) methods were applied to the existing data. The two methods generated similar results at 2002 and 2003 by using the pitfall trap method suggesting that the population estimates were correct for both years. Although the applied methods were designed for different kinds of populations, the statistical analyses were similar indicating that the Schnabel method showed good results. These results were supported with Jolly-Seber method which gives more biologically realistic situation of open populations. Most populations are constantly changing in size because of births, deaths, immigration and emigration.

The pitfall trap and transect methods used in this study generated similar results for lizard and ant numbers. The means of both measures differed, however, slightly over the 2 years and with the type of method used.

Because environmental characteristics, resource availability and predation pressure vary between geographic regions or even localities within a region, they have influences on population numbers. Although the mean estimated lizard numbers were lowest at the tar mat sites, the ant number in this location was greatest, meaning that food availability was highest at these sites. This suggests any reduction in the numbers of lizards is unrelated to low resource availability. The lizard numbers at the tar mat sites could be depressed by some property of the pollutants. The presence of large lizards at the tar mat sites could preclude other smaller lizards of the same species from these locations, reducing the total numbers.

The soot sites had higher numbers of lizards in 2002 which might be attributed to the warmer weather as compared to 2003, increased food availability or reduced predation. More predators were observed at the soot sites in 2003. Perhaps also the large lizards at the tar mat sites could preclude other lizards from these locations which will reduce the population size at these locations.

The adult lizards were bigger at the tar mat and soot sites than the clear and control sites. This was unexpected as it was thought that presumably severe oil pollution would decrease body size. One obvious explanation for this phenomenon is the great availability of food in both the tar mat and soot sites. Another explanation

might be that the food resource is affected by oil pollution (see chapter 5) and that lizards consuming prey with high levels of fat will accumulate toxins in their adipose tissues in their bodies, especially the liver (see chapter 6).

The variation in microhabitat characteristics may affect territorial behaviours such as aggressiveness which was clearly observed in animals from the tar mat sites, which were often conflict with members of their own species over control of a particular territory. In many occasions, especially during the breeding season, males were fighting and were using tail movements and perform different displays to defend territories. Chases in which one individual rapidly runs or jumps at the other individual, and the latter rapidly runs and jumps away were also observed in male lizards from the tar mat sites.

The results of this study show that there is sexual dimorphism in body size with males being generally larger than females at all the study sites. Such sexual dimorphism is quite common in the animal kingdom (Krebs and Davis, 1993) and often seems related to the breading systems adapted by the species. In many animals the males have to fight for females or for access to sites where they are located. A larger body size gives them a competitive advantage in encounters with male conspecifics.

CHAPTER 4

Heavy Metal Concentrations in Soil and *Acanthodactylus scutellatus* Tissues at the Different Study Sites

4.1 Introduction

The term 'heavy metals' refers to metallic elements occurring in natural environments in small amounts and that, when present in sufficient bioavailable concentrations, are toxic to living organisms. They include antimony, arsenic, barium, beryllium, boron, cadmium, chromium, cobalt, copper, lead, manganese, mercury, molybdenum, nickel, selenium, silver, thallium, tin, vanadium and zinc. The United States Environmental Protection Agency (U.S. EPA) has included 13 metals in its priority pollutants list, namely (in order of their importance): silver, arsenic, beryllium, cadmium, chromium, copper, mercury, nickel, lead, antimony, selenium, thallium, and zinc.

Metals external to the organism will only cause adverse effects to the body once they have been absorbed and assimilated. In essence, the metals have to come in contact with the organism to be of any biological consequence and be in a form in which they will be able to enter the organism (Adriano, 2001). This last mentioned property is termed 'Bioavailability' and refers to the property of being available to be taken up by the organism and reacting with its metabolic machinery (Campbell, 1995).

Background information is only provided on the two metals (vanadium and nickel) which were found in samples in the present study. Nickel and vanadium were specified in this study because these 2 metals are the most predominant metals in the chemical composition of Kuwait crude oil.

4.1.1 Vanadium

Vanadium is a shiny, silvery metal with few naturally-occurring isotopes. It appears to be required in comparatively small quantities for normal growth and differentiation in all organisms but whether the element is essential and what its precise

function is remains obscure (Golden and Golden, 1981; Nielsen, 1984). Vanadium is found in many foods, there being significant amounts in milk, seafoods, cereals, and vegetables. The element has a natural affinity for fats and oils (cooking oils often have high concentrations of this metal).

Occupational exposure to vanadium has been observed in several industrial processes. For example, vanadium-pentoxide is found in iron and steel production and may be inhaled by workers as a dust or in fumes. Vanadium oxides are also used in the manufacture of pigments, printing inks and paints, in photography, in insecticides, in the glass industry and in the cleaning and repairing of oil-fired boilers (particularly in electricity generating stations). The adverse effects of exposing humans to the combustion products of vanadium-bearing residual oils and to fumes and dusts in metallurgical refining have stimulated interest in the toxicology of this material. The toxic action of vanadium is largely confined to the respiratory tract but industrial exposure may also irritate the skin and eyes. Studies in animals and humans have shown compounds of this metal cause damage in many systems. Signs of toxicity and clinical symptoms associated with vanadium poisoning include conjunctivitis, rhinitis, pharyngitis, bronchitis, ataxia, tremors, paralysis, depression and cardiovascular disorders (Waters, 1977).

The mechanism by which vanadium exerts its toxic effects are poorly understood, but interference with the normal kinetics and macromolecular binding of the body's other essential metals (such as zinc, cadmium, magnesium, iron and copper) appear to play significant roles. It is well known that non-essential metals, such as lead and mercury exert toxic effects by interacting with essential elements, thereby adversely affecting various metabolic processes (Goyer, 1978; Chertok *et al.*, 1981). Manganese increases the hepatic content of copper in rats (Murthy *et al.*, 1981) but vanadium (in contrast) diminishes the uptake of copper by the liver of animals (Witkowska *et al.*, 1988).

Although man-made emissions of vanadium into the environment have been increasing by burning of fossil fuels, the environmental behaviour of this element has rarely been studied. This paucity of studies is related to the absence of any obvious

environmental problem caused by this element and, unlike mercury the lack of sensitive analytical methods for its analysis. There has been even less information available regarding vanadium's effects on behaviour and distribution in wild animals (Sperling *et al.*, 2000). Saeki *et al.* (1999) observed high concentrations of vanadium in the liver, hair and bone of Northern fur seals (*Callorhinus ursinus*). This increase was caused by an elevated retention in the nuclear and mitochondrial fraction of the cells in these tissues.

Heavy metals are known to influence cytochrome P-450 monooxygenases and conjugating enzymes in the liver (Dierickx, 1982). Cytochrome P-450 serves as the terminal electron acceptor and substrate-binding site of the mixed-function oxidase system and is probably the most versatile and unique biological catalyst known. Although this enzyme system was formerly believed to function primarily in detoxification, it is now known to metabolically activate many protoxins and procarcinogens to reactive metabolites that initiate toxic and carcinogenic events (Conney, 1982). Chakraborty *et al.* (1995) showed that, unlike many other heavy metals, vanadium caused a steady increase in the cytochrome P-450 level with time in the Indian catfish (*Clarias batrachus*). This increase in cytochrome P-450 level may be of considerable significance because several inducers of liver microsomal enzymes are promotors of liver tumours in a variety of fish species as well as in humans (Payne *et al.*, 1987).

Although the study of Loumbourdis (1997) on the lizard *Agama stellio stellio* included many heavy metals, vanadium was not one of them, so no information is available on the potential toxic effect of this metal on lizard species.

4.1.2 Nickel

Nickel is a silvery-white, lustrous, malleable and ductile metal that resists corrosion and is soluble in all acids except concentrated nitric acid (Pais *et al.*, 1997; Adriano, 2001). Although nickel is toxic to organisms at elevated concentrations, trace amounts of this element are required for several biological processes.

The major uses of refined nickel in industry include electroplating, alloy production and fabrication, the manufacture of nickel-cadmium batteries and electronic components. Most nickel is used in alloys that are strong and corrosion resistant such as stainless steel. Thus, nickel is found in a wide variety of commodities such as automobiles, batteries, coins, jewelry, surgical implants and kitchen appliances (Adriano, 2001). Igneous rocks, upon weathering, are the primary source of nickel in soils. The soil content of nickel is very variable but the world's average value is reported to be around 20 ppm.

Nickel deficiency produces a variety of physiological effects in plants. In soybeans, urea accumulates to toxic levels in leaflet tips of nickel-deficient plants as a result of depressed levels of urease activity (Adriano, 2001). Similarly, when grown under condition of low nickel supply, tomato plants developed chlorosis in the youngest leaves leading to necrosis of their meristematic tissues.

Since nickel is ubiquitous in the environment, it is a normal constituent of plant tissues. Mean values of about 0.20 to 4.5 ppm have been reported for nearly 2000 specimens of field crops and natural vegetation from the United States. In normal plants, the following ranges of values have been reported (Kabata–Pendias and Pendias, 1992): grasses, 0.10 to 1.7 ppm dry weight; clovers, 1.2 to 2.7 ppm; vegetables, 0.20 to 3.7 ppm; cereals, average of 0.5 ppm. Generally, the level of nickel in most plant species that may produce phototoxicity ranges from 10 to 100 ppm.

Tochimoto *et al.* (2003) found that nickel concentration in larvae of aquatic insect (*Stenopsyche marmorata*) is mainly taken from their food source. Herkovits *et al.* (2000) concluded that the exposure threshold for nickel to exert acute lethal effects on *Bufo arenarum* embryos is 96 h of treatment

4.2 Heavy Metals and Bioindicators

Contamination from heavy metals is a serious problem in most countries of the world. The environment of the Arabian Gulf region has been a subject of study in this respect in recent years due to the 1991 oil spill. Direct and indirect methods have been developed to detect heavy metals quantitatively and qualitatively and to evaluate the

level of pollution in this area. Direct methods are applied to soil and water; indirect methods use plants and animals as bioindicators. Reptiles have rarely been used as bioindicators of pollution. It can be argued, however, that lizards would be good indicators of the quality of terrestrial habitats because of their relatively restricted mobility (Lambert, 1993). As invertebrates form a substantial part of the diet of most lizards, they are likely to concentrate metals that have been incorporated in the bodies of insects by eating plants. Heavy metals thus accumulate through the ingestion of contaminated food, and also by the incidental ingestion of soil (Loumbourdis, 1997). In the few studies using reptiles (especially turtles, e.g. Albers *et al.*, 1986) accumulation of high concentrations of these metals, confirms that reptiles are likely to be good pollution bioindicators.

Very few studies analysed heavy metal concentrations in lizards. Beck (1956) included copper concentrations found in lizard livers. Lance *et al.* (1995) analyzed blood plasma in 16 different species of lizards to determine normal levels of zinc that were present. There are also heavy metal studies involving lizards from Brazil (Schmidt, 1984), India (Kaur, 1988) and Puerto Rico (Burger *et al.*, 1992). Loumbourdis (1997) analyzed aluminum, barium, cadmium, cesium, chromium, cobalt, copper, lead, manganese, molybdenum, nickel, rubidium, strontium and zinc concentrations in the whole body (minus liver and digestive tract) and the liver of the lizard *A. stellio stellio* in a high altitude urban site and a low-altitude agricultural area in Greece. This study was ground-breaking in that so many heavy metals were analyzed and lizards were used as the focus animals.

4.3 Materials and Methods

4.3.1 Heavy Metal Analysis

4.3.1.1 Soil Analysis

Eight soil samples (initial weight for each is 500g) from each of the two control, clear, soot and tar mat sites were taken from the field, air dried and sieved using 2mm mesh and kept in sealed bottles. One gram of each sample was weighed and transferred into 100 ml conical flask before adding 30 ml of 37% concentrated (12N) hydrochloric

acid (Fluka Wacker Ghemie GmbH, Munich, Germany). The sample was digested at a temperature near 200°C where it did not boil vigorously and froth was not formed. Digestion continued for 2 – 3 hours until the volume was reduced to 3 ml. The resultant solution was cooled and then filtered through Whatman No. 1 filter paper (Fluka Wacker Ghemie GmbH, Munich, Germany) into 25 ml volumetric flask (while transferring from conical to volumetric flask, the sides of the conical flask were washed down using distilled water which diluted the acid). The elute was analyzed using the Inductively Coupled Plasma/Atomic Emission Spectrophotometry (ICP/AES) methodology.

4.3.1.2 Lizard Samples

Ten lizards (5 of each sex) from each study site were collected from the field, weighed, humanely killed by keeping them at very low temperature until they were ecologically dead and then they were kept in jars in a freezer at - 20°C. They were then freeze-dried and weighed. The samples were ground with pestle and mortar and kept in sealed bottles for further analysis.

One gram of the ground sample was taken in 100 ml conical flask. Twenty ml of 70% analytical grade concentrated (16N) nitric acid (Fluka Wacker Ghemie GmbH, Munich, Germany) and 10 ml analytical grade perchloric acid (S.D. Fine Chemical Ltd. Mumbai, India) were added. The sample was digested without frothing and vigorous boiling using a temperature just below 200°C until the sample was reduced to 3 ml. The digested sample was then allowed to cool and made up to a volume of 25 ml using distilled water. The sample was then analyzed using the ICP/AES methodology.

4.4 Results

The data obtained for heavy metal concentrations in soils and lizards tissues are shown in figures 4.1 (p 78) and 4.2 (p 79).

Figure 4.1 Mean (± s.d.) heavy metal concentrations (ppm) in soil samples

Location	Nickel	Vanadium
Control	17.9 ± 0.64 (N =16)	20.3 ± 0.49 (N =16)
Clear	21.2 ± 2.55 (N =16)	22.9 ± 2.69 (N =16)
Soot	22.9 ± 1.34 (N =16)	22.8 ± 0.92 (N =16)
Tar mat	24.9 ± 2.05 (N =16)	27.9 ± 2.26 (N =16)

One-way ANOVA test showed that there is no significant difference in nickel concentrations in soil between the different study sites ($F_{3,4} = 5.62$, $P = 0.06$). The t-test was used to compare between each two categories. There was no difference in nickel concentration in soil between the control and clear sites (t = - 3.35, P = 0.42). A significant difference was observed between the control and soot sites (t = - 9.5, P ‹ 0.04) and the control and tar mat sites (t = - 9.7, P ‹ 0.04). One-way ANOVA test showed that there is no difference in vanadium concentrations in soil between the different study sites ($F_{3,4} = 6.12$, $P = 0.05$). The t-test showed no significant difference vanadium concentration between the control and clear sites (t = - 2.65, P = 0.60) and the control and soot sites (t = - 2.5, P = 0.63). A significant difference was observed between the control and tar mat sites (t = - 9.65, P ‹ 0.04).

The mean nickel concentrations were highest in the tar mat soils and lowest in the control soils. Mean concentrations of vanadium were also highest in the tar mat soils but there were similar mean concentrations in the soot and clear soils. The lowest mean concentration of vanadium was in the control soils.

Figure 4.2 Mean (± s.d.) heavy metal concentrations (ppm) in total lizard tissues

Location	Nickel	Vanadium
Control	3.1 ± 0.14 (N =10)	0.55 ± 0.14 (N =10)
Clear	4.8 ± 1.34 (N =10)	0.83 ± 0.04 (N =10)
Soot	3.6 ± 1.13 (N =10)	0.53 ± 0.11 (N =10)
Tar mat	4.5 ± 0.18 (N =10)	0.84 ± 0.25 (N =10)

One-way ANOVA test showed that there is no significant difference in nickel concentrations in lizard tissues between the different study sites ($F_{3,4} = 1.60$, $P = 0.32$). The t-test showed no significant difference in nickel concentrations in lizard tissues between the control and clear sites ($t = - 4.62$, $P = 0.31$) and the control and soot sites ($t = - 3.42$, $P = 0.33$). A significant difference was evident between the control and tar mat sites ($t = - 10.2$, $P < 0.03$). One-way ANOVA test showed that there is no difference in vanadium concentrations in lizard tissues between the different study sites ($F_{3,4} = 2.36$, $P = 0.21$). The t-test showed no significant difference in vanadium concentration of lizard tissues between the control and clear sites ($t = -3.5$, $P = 0.41$), the control and soot sites ($t = - 4.1$, $P = 0.26$) and the control and tar mat sites ($t = - 6.3$, $P = 0.08$).

The mean nickel concentrations in lizard whole body tissue were highest at both the clear and tar mat sites and values were lowest at the control sites. Mean concentrations of vanadium in lizard tissues were similar at the tar mat and clear sites but were low at both soot and control sites.

4.5 Discussion

The statistical analysis of the results of the present study showed some variation in nickel content between the sites in both soil and lizard tissues. Variation in vanadium content was only evident in soil but did not differ in lizard tissues between the different study sites.

Nickel concentrations in the liver and carcass of the lizard *A. stellio stellio* were found to be 3.60 $\pm$ 3.11 and 33.83 $\pm$ 22.12 (ppm) respectively (Loumbourdis, 1997). In the present study, the highest mean nickel concentration of the sand lizard *A. scutellatus* was found to be 4.80 $\pm$ 1.34 (ppm) for the whole body tissues. Thus the total concentration in the carcass of nickel in *A. stellio stellio* was much higher than that of the present study in *A. scutellatus*. Feeding habits may have a great effect on the exposure of organisms to metals as well as local concentrations of the metal. The overall few reptile species studied in the literature regarding nickel and vanadium do not facilitate easy conclusions concerning dynamics of incorporation of these elements.

Analytical chemistry is almost always required at some point to assess or predict toxic hazards of heavy metals to wildlife. Chemical analysis often serves to identify those contaminants present, and their respective concentrations and may often provide for some diagnosis of an observed wildlife problem. In many instances, however, chemical residue data obtained from field samples will prompt further laboratory and field investigations to determine if any undetected biological effects are occurring.

This chapter provided some information about the presence of nickel and vanadium in the tissues of *A. scutellatus* and although the values of these metals are relatively modest, they might well have severe effects on lizard's survival and health.

CHAPTER 5

Polycyclic Aromatic Hydrocarbon Concentrations in *Acanthodactylus scutellatus* Tissues and Ants at the Different Study Sites

5.1 Introduction

PAHs are a group of chemicals formed during the incomplete burning of coal, oil and gas. They are also found in garbage, creosote and in road, as well as roofing tar. PAHs can be man-made (anthropogenic) or may occur naturally. Indeed, they are ubiquitous environmental pollutants found in air, water and soil. A few PAHs are used in medicines and to make dyes, plastics and pesticides. Obvious human sources of PAHs include automobile exhaust systems, industrial stacks, household cooking and fireplaces. Since aromatic products are more stable than their precursors, high temperatures are needed for their formation (Dias, 1987).

The 16 U.S.E PA priority PAHs are classified into two major groups. PAHs with 2-to 4- benzene rings may be non-carcinogenic or carcinogenic and include: naphthalene, acenaphthene, anthracene, phenanthrene, acenaphthylene, fluorene, fluoranthene and pyrene. Carcinogenic PAHs with 4-to 6- benzene rings include: benzo[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno(1,2,3,c-d)pyrene, dibenzo[a-h]anthracene, chrysene, and benzo[g,h,i]perylene.

Although PAHs generally behave similarly in the environment, each PAH compound has a unique set of physical and chemical properties. At ambient temperatures, pure PAH compounds are solids. They generally have high melting and boiling points, low vapour pressures, and very low water solubilities. Because of their hydrophobic nature, PAHs tend to be absorbed by the organic matter in soil. Smith *et al.* (1999) illustrated the correlation between the environmental fates of PAHs and their molecular weight or number of benzene rings. For example, PAH compounds with more benzene rings tend to be strongly absorbed to the organic matter of soils or sediments. In contrast, lower molecular weight compounds tend to be more volatile,

soluble, bioavailable and generally have a higher rate of biodegradation. The solubilities of 16 PAHs are inversely proportional to the number of fused benzene rings. For example, the solubility of naphthalene in distilled water at 25°C is approximately 31.69 mg/L whereas that of benzo[g,h,i]perylene is approximately 0.00026 mg/L.

Some of these PAHs are probable human carcinogens. Their distribution in the environment and the possible exposure of human beings has been the focus of much research. The total potential dose of carcinogenic PAHs for humans from water, air, sediment, soil, and food has been estimated by Menzie *et al.* (1992). Many PAHs cause cancers, affecting a variety of tissues. In the PAH class, 16 compounds in potable water and waste waters and 22 compounds in soil and solid wastes are listed under priority pollutants by the EPA. However, benzo[a]pyrene is a potent human carcinogen, while benzo[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, chrysene, dibenzo[a,h]anthracene and indeno(1,2,3c-d)pyrene have shown sufficient evidence of carcinogenicity in animals to be causes of concern (Patnaik, 1999).

5.2 Materials and Methods

5.2.1 Insect and lizard samples analysis

Sixteen PAHs (EPA priority pollutants) were studied in lizard (5 lizards from each study site, there being a total of 40 lizards from polluted and control sites) and ant tissues at the different study sites to investigate their presence and concentration in wildlife tissues. Lizards were humanely killed, kept in jars that had been washed with ethanol (Fluka Wacker Chemie GmbH, Munich, Germany) and stored in a freezer at - 20 °C until analysis. The method used for the analysis of petroleum HCs in the biota followed techniques used in the Manual of Oceanographic Observations and Pollutant Analysis Methods (MOOPAM, 1999). For analysis, the samples were defrosted and prepared for solvent extraction. To achieve satisfactory recovery of the petroleum HCs, samples were chopped, freeze-dried and weighed.

Five grams of the freeze-dried sample were extracted with a Soxhlet extractor with 250 ml of methanol in a flask. Twenty ml of 0.7M reagent grade potassium

hydroxide (KOH) (Readel'den han AG. D00016, Sleeze, Germany) and 30 ml of distilled water were added to the flask to saponify the lipids overnight. The content of the extraction flask was transferred into a separation funnel and extracted with 90 ml of analytical grade hexane (Laboratory Supplies, Poole, BH15 ITD, UK) and re-extracted twice with 50 ml of hexane. Subsequently, all hexane extracts were combined, filtered through glass wool and dried with reagent grade anhydrous sodium sulphate (ZT Baker Chemical Company, Philipsburg, Germany) until a volume of 5 ml was reached, after which they were transferred to a clean-up column.

Silica gel-aluminium oxide and glass wool were prepared. First, they were cleaned for 8 hr with methanol and then for 8 hr with hexane. They were dried in an oven at 60°C for 45 min to remove the solvent and subsequently at 150°C overnight. The items were kept separate in amber light glass bottles. Before use, they were activated at 200°C for 4 hr and partially deactivated with 5 % water. A chromatography column was prepared using a 50 ml burette in which a piece of glass wool was added near the stopcock to maintain the packing material. Ten ml of silica were transferred into the column, then 10 ml of alumina and on top 1g of sodium sulphate was added in order to avoid the disturbance of the first layer when solvents were poured into the column.

The sample was applied on top of the column, then a first fraction (Fraction 1) was obtained by eluting the sample with 20 ml of hexane. As this fraction contained saturated aliphatics, it was discarded. The second fraction containing the aromatic hydrocarbons, was obtained by eluting the sample with 30ml of a mixture (90 : 10) of hexane and analytical grade dichloromethane (Sharlau Chemie S.A., Barcelona, Spain). It contained the aromatic HCs. This was then reduced in volume using a rotary evaporator (Brinkmann Instruments Inc., New York, USA) until a volume of 5ml was reached and the sample transferred into a concentrator (eBay Inc., Los Angeles, USA) where nitrogen gas was used to reduce it to a final volume of 1 ml. This sample was then ready for the gas chromatography/ mass spectrometry (GC/MS) analysis of PAHs.

5.3 Results

The results of PAH estimates in ant and lizard tissues are shown in figure 5.1 below.

Figure 5.1 Mean ± s.d of total PAH concentrations (ng/g) in ant and lizard tissues from the different study sites

Location	Ants	Lizards
Control	Not detectable	Not detectable (N =10)
Clear	27.43 ± 2.5	91.6 ± 22.5 (N =10)
Soot	23.92 ± 2.0	80.7 ± 21.3 (N =10)
Tar mat	39.3 ± 3.2	136.5 ± 37.5 (N =10)

The one-way ANOVA test showed a highly significant difference in the mean total PAH concentrations in ants ($F_{3,4} = 572$, $P < 0.0001$) over the four study sites. The *post hoc* Scheffe' tests showed that the total PAH concentrations in the control sites differed significantly from the clear, soot and the tar mat sites (all $P < 0.0001$). The total PAH concentrations in ants at the clear sites did not differ from values at the soot sites ($t = 3.87$, $P = 0.07$), but they significantly differed from the tar mat sites ($t = -12.8$, $P < 0.001$). The PAH concentrations of ants in the soot sites differed significantly from samples from the tar mat sites ($t = -16.6$, $P < 0.0001$).

The one-way ANOVA test showed highly significant variance in the mean of total PAH concentrations in lizard whole body tissues ($F_{3,4} = 1416.88$, $P < 0.0001$) from the different study sites. *Post hoc* Scheffe' tests showed a significant difference between the PAH concentrations of lizard tissues at the control and the clear ($t = -42.5$, $P < 0.0001$), the soot ($t = -37.7$, $P < 0.0001$) and the tar mat ($t = -63.9$, $P < 0.0001$) sites. The PAH concentrations in lizards at the clear sites were also significantly different

from the soot (t = 4.8, $P < 0.03$) and tar mat (t = -21.4, $P < 0.0001$) sites. These concentrations in lizard tissues significantly differed at the tar mat and soot sites (t = -26.2, $P < 0.0001$).

Three PAHs, namely, phenanthrene, fluoranthene and benzo[a]anthracene out of 16 PAHs were present in lizard and ant whole body tissues. They were proved to be present in samples from the tar mat, soot and clear sites but they were not detected in the reference (control) sites. The tar mat sites exhibited the highest PAH concentrations in both lizards and ants. No previous study appears to have been carried out on reptiles on the concentrations of PAHs as a result of oil pollution, and the results suggest that the levels of PAHs found in lizards and ants living in the Kuwait desert could be high enough to affect vital organs such as liver. Very low levels of PAHs (ng/g) might be toxic to lizards as was discussed in chapter 6 (6.4). The results also suggest that these species have the ability to not only tolerate, but to concentrate potentially harmful materials, such as PAHs.

5.4 Discussion

Lizards are vulnerable to habitat changes through their limited powers of migration and dispersal but this vulnerability makes them potentially excellent indicators of local contamination of terrestrial habitats (Lambert, 1993). Culley and Applegate (1967a) sampled wildlife from cotton fields, desert and desert periphery within a 30-mile radius of Presidio, Texas. Dichlorodiphenyldichloroethylene (DDE) residues as high as 7.0 μg/g were found in the tail muscle of Whiptail lizards (*Cnemidophorus* spp.) collected from cotton fields and desert periphery sites (*op. cit.*). Pesticide concentrations decreased in samples gathered at greater distances from the cotton fields. Gravid females had an average of 16.4 μg/g DDE in their eggs but only 3.4 μg/g DDE in their muscle tissue (*op. cit.*). In a subsequent study, DDE residues of up to 49.9 μg/g were measured in the liver of Whiptail lizards from cotton-field sites (Culley and Applegate, 1967b). High levels of DDE were still found in the Rio Grande and Pecos River drainages of New Mexico and Texas when White and Krynitsky (1986) extensively sampled vertebrates in these areas in 1982 and 1983. A Whiptail

lizard from Pecos, Texas had the highest DDE concentration (104 μg/g, whole body) of all animals (birds, bats and lizards) examined in this study. The high concentrations found in such lizards provided strong support for the view that the source of contamination was local.

Lambert (1993) monitored the impact of ground spraying with DDE on lizards in Mopane woodlands and gritstone outcrops of northwestern Zimbabwe in 1989 and 1990. In the woodland-dwelling lizard *Mabuya striata wahlbergi*, whole-body total dichlorodiphenyltrichloroethane (DDT) levels increased significantly with the number of annual treatments. Highest DDT and DDE concentrations found in these lizards were 15.38 μg/g (dry weight) and 6.00 μg/g (dry weight), respectively over the treatment period. Lizards from the outcrops (*Mabuya quinquetaeniata margaritifer* and *Agama kirkii*) showed some accumulation of residues, but these were significantly lower than in the woodland species.

Heavy spillage from a pesticide store that was bombed near Hargesia, Somaliland caused the contamination of 3700m^2 of soil (Lambert, 1997b). Lizards and frogs were used as bioindicators of contamination in the area. In lizards, the highest levels of Dieldrin and total DDT were found in *Chalcides ragazzi* and *Mabuya striata striata* from three sites in Hargesia that were 4.1 to 9.0 km downstream of the spillage site (*op. cit*). Other lizards (*Hemidactylus parkeri*) from the spillage vicinity and an area 350m downstream, had the highest concentrations of beta-hexachlorocyclohexane residues.

In this present study, PAHs that are strong indicators of petrogenic HC contamination, were detected in lizard and ant samples. The analyses of *A. scutellatus* and ant samples collected from sites of different levels of oil pollution from Burgan oil fields produced evidence of contaminations with oil HCs. This study confirmed the presence of highly significant concentrations of phenanthrene, fluoranthene and benzo[a]anthracene in lizards and ants from the oil polluted sites (tar mat, soot, clear), while these PAHs were undetectable in the control sites. These three PAH compounds were dominant in both lizard and ant samples, confirming that one of the sources of

PAHs contamination in lizards is by ingesting contaminated food. Ants are a major food source for *A. scutellatus*.

The clear sites had higher PAH concentrations than the soot sites in both ant and lizard samples. This was an interesting result because the clear sites have generally been thought to be clear of oil pollution. The present study proves that the clear sites are polluted perhaps to a greater extent than the supposedly more polluted soot sites.

Few other studies were located concerning PAH levels in lizards especially those dealing with dietary contamination. An exception is Wikelske *et al.* (2002) who explained the high mortality of Marine iguanas (*Amblyrhynchus cristatus*) in the aftermath of the oil spill in terms of the oil having had a direct toxic effect either on the iguanas themselves or on the algae they consume. The iguanas may become endangered because they decline to eat because their food had been fouled or because their hindgut becomes poisoned such that they can no longer digest the food they eat.

The natural background levels of PAHs in lizards are still largely unknown but the results suggest that higher total PAHs in the tar mat sites for both lizards and their prey may be responsible for reducing the lizard's survival (see chapter 3), and may be responsible for histopathological features (see chapter 6) that link tissue damage to prey contamination. In some areas of Kuwait desert, natural weathering has converted the spilled crude oil to a hard residue containing high levels of toxic PAHs. Because of their low metabolic rates and relatively simple enzyme systems, lizards may not be able to detoxify complex chemical compounds that they inhale or ingest with contaminated invertebrate prey as quickly as do endotherms (Walker and Ronis, 1989).

The major conclusion that can be drawn from this present study is that, although 12 years have passed on Kuwait oil spill at the Burgan oil fields, the terrestrial environment is still contaminated with PAHs (including some carcinogenic compounds). The sand lizard *A. scutellatus* is confirmed to be a suitable bioindicator species for studies on the effect of PAH compounds. Further, this study suggests that the clear sites are, in fact, contaminated with PAHs rather than being free of these materials as is generally assumed from the lack of physical signs of contamination. Perhaps the major difference between the contaminated sites is in their physical

properties, with the tar mat being obviously very different from the clear site in forming a protective crust over the underlying soil.

CHAPTER 6

Evidence of Hepatotoxicity in *Acanthodactylus scutellatus* from the Different Study Sites

6.1 Introduction

Fry and Lowenstine (1985) showed during histological examination of oil contaminated Common murres (*Uria aalge*), that emphasis should be placed on sections of liver, kidney and duodenum as these appeared to be the organs most susceptible to damage by crude oil. Examination of stained tissue sections from these birds consistently revealed histopathological changes in these structures. Liver necrosis was common but congestion of the liver, fatty degeneration and dissociation of hepatocytes were also observed. There was also evidence of pigment (haemosiderin) accumulation in hepatocytes of all examined birds.

The liver of lizards (as in other vertebrates) is the largest extrinsic digestive gland and is the site of initial processing of materials. One consequence is that toxic materials in absorbed food can enter the liver and affect its structure and functions (Schaffner, 1998). No studies have been located that have assessed the effects of oils on the histopathology of tissues in lizards. This chapter consequently attempts to look at the long term effects of crude oil on liver cells (hepatocytes) of the sand lizard *A. scutellatus*.

6.2 Materials and Methods

Ten adult male and female sand lizards were collected from both the presumably highly polluted (tar mat) and from the control sites. The animals were dissected and the liver was gently removed. It was cut into 2 pieces and placed on a clean piece of card. One piece was fixed by dropping Bouin's solution on it and the other fixed by dropping formal-saline on it. Both pieces were then further cut into small sizes with a sharp blade. To complete fixation, the liver sections were immersed in the

same fixatives that had been used for each main piece and were kept in vials and placed on a rotator (2 rpm) (Taab Laboratory Equipment Ltd. Aldermaston, Berkshire, UK) for 24-48 hours at room temperature. After fixation, the tissues were washed overnight in 70 % ethanol, dehydrated in 90% ethanol overnight followed by two changes for 3 hours each of absolute alcohol (Fluka Wacker Chemie GmbH, Munich, Germany). The sections were then cleared in toluene (Fluka, Wacker Chemie GmbH, Munich, Germany) for 24 hours at room temperature. The tissues were incubated in fresh liquid paraffin wax (Fluka Wacker Chemie GmbH, Munich, Germany) and kept in an oven at 60°C overnight to allow the wax to penetrate the tissues. After that, they were embedded in plastic moulds ready for sectioning.

Once the blocks had been prepared for sectioning, they were fixed on a rotary microtome (Leica, Laboratory Talk, Knowlhill, Milton Keynes, UK). Paraffin sections were cut at 5 μm thickness in the form of a ribbon. Good sections were selected using a brush to place them in a water bath at 45°C for flattening. The sections were then placed on albuminized slides and dried on a hot plate.

The slides were dewaxed using two 5 minute changes of xylene (Fluka Wacker Chemie GmbH, Munich, Germany). The specimens were rehydrated by two changes of absolute alcohol, followed by 90% ethanol and then 70% ethanol. All changes were of 2 minutes duration. The slides were washed with tap water for 2 minutes, followed by immersion in haematoxylin (Shandon Scientific Ltd., Cheshire, UK) for 5 minutes to stain the nuclei of the tissue cells. Excess stain was removed by washing the slides for 2 minutes in water before bluing them with ammoniated alcohol and re-rinsing in water. Specimens were then kept in 70% and 90% ethanol respectively for 2 minutes. To stain the cytoplasm, the slides were transferred to eosin (Shandon Scientific Ltd., Cheshire, UK) for 3 minutes, then rinsed in absolute alcohol and were dehydrated by two successive absolute alcohol immersions of 2 minutes each. They were then cleared with 2 changes of xylene each for 5 minutes. To preserve the specimens, the slides were mounted using 1-2 drops of a mixture of distyrene, a plasticizer, and xylene (DPX) (Fluka Wacker Chemie GmbH, Munich, Germany) while it was wet and sealed by a cover slip. The specimens were dried by keeping the slides on a hot plate for 5-10

minutes. Finally, the sections were examined under the light microscope with different objective lenses, and photomicrographs of areas of interest were taken.

Twenty sections (ten from males and 10 from females) from each tar mat and control sites were then examined for cell diameter and nuclear measurements using CAS200 (Cell Analysis Systems, Inc. Watlow, St Louis, USA) which consists of a microscope attached to a computer system. This was performed to find out if there is any difference in cell diameters and nuclei of hepatocytes taken from lizards from the presumably highly contaminated sites and the control sites. One hundred cells from the tar mat and 100 cells from the control sample were chosen equally from male and female subjects for cell and nuclei measurements. Random cells were chosen by moving the slide randomly by the examiner while it is on the microscope, then the examiner will stop and move the mouse of the computer randomly and stops without looking at specific area of the liver section. The examiner will look at the randomly chosen cell by the mouse of the computer and perform the required measurements. The length and the width of each examined cell were recorded and then the average cell diameter was estimated. The nuclei diameter for each cell was also recorded.

Twenty sections (10 from male and 10 from female subjects) from the tar mat and control sites were randomly chosen and examined under the light microscope. An area of 100 cells was randomly selected using an eye piece graticule, the numbers of swollen cells (inflammation in the hepatocytes probably as a result of water accumulation), cells with cytoplasm degeneration (cytoplasm has lost definition and the cell margins are indistinct) and dead cells (nuclear changes accompanied by cytoplasmic changes, nuclei become fragmented into several particles which represent pieces of degenerate nuclear material) were counted. Random selection of cells was performed by moving the slide randomly while it is on the microscope, then the examiner will stop and the area shown was chosen and examined.

6.3 Results

The mean cellular diameters and nuclear measurements in sections of livers from male and female subjects from control and tar mat sites are shown in figure 6.1 (p 92).

The corresponding mean numbers of swollen cells, cells with cytoplasm degeneration and dead cells are shown in figure 6.2 (p 93).

In samples from the control sites, the liver consisted of intersinusoidal cords of hepatocytes and the cytology of hepatocytes was similar to that described in other reptiles (Schaffner, 1998). The occasional degenerating cells that were found appeared to be part of a normal cycle of apoptosis of hepatocytes. Dead cells were abundant in the sections of lizard livers from the tar mat sites and occurred in notably greater numbers than the sections of livers of animals from the control sites.

Figure 6.1 Mean ± s.d cell and nuclear diameters (μm) in liver sections of male and female *A. scutellatus* from the control and tar mat sites

Location	Sex	Cell diameter (N = 100)	Nuclear diameter (N = 100)
Control (N = 20)	Male (N = 10)	10.26 ± 1.30 (N = 50)	4.09 ± 0.77 (N = 50)
	Female (N = 10)	09.01 ± 0.85 (N = 50)	3.14 ± 0.54 (N = 50)
Tar mat (N = 20)	Male (N = 10)	17.37 ± 3.48 (N = 50)	4.00 ± 0.62 (N = 50)
	Female (N = 10)	12.39 ± 1.83 (N = 50)	3.59 ± 0.70 (N = 50)

A two-way ANOVA comparing the data in terms of site and sex as well as their interaction was carried out. Both sex ($F_{1,98}$ = 108.15, P ‹ 0.0001) and site ($F_{1,98}$ = 307.05, P ‹ 0.0001) were highly significant factors in terms of their impact on cell diameter. The interaction between sex and study sites was also significant ($F_{1,98}$ = 38.90, P ‹ 0.0001). Sex ($F_{1,98}$ = 51.32, P ‹ 0.0001) and site ($F_{1,98}$ = 3.69, P = 0.05) also had significant impacts on nuclear diameter. The interaction between sex and the study

site was also significant ($F_{1,98} = 8.29$, $P < 0.004$). Examination of the data confirms that the cell and nuclear diameters in liver samples of males were generally greater than those of corresponding females. The data also show that the sections obtained from animals in the tar mat sites had greater cellular diameters than counterparts from the control sites. In terms of nuclear diameter change, male sections from the tar mat sites showed reduced measures whereas females showed an increase in this index.

Figure 6.2 Mean numbers of ± s.d swollen cells, cells with cytoplasm degeneration and dead cells in liver sections of male and female *A. scutellatus* from the control and tar mat sites

Location	Sex	Swollen cells (N = 100)	Cells with cytoplasmic degeneration (N = 100)	Dead cells (N = 100)
Control (N = 20)	Male (N = 10)	0.20 ± 0.42 (N = 50)	0 (N = 50)	0.20 ± 0.42 (N = 50)
	Female (N = 10)	0.23 ± 0.43 (N = 50)	0.10 ± 0.32 (N = 50)	0.10 ± 0.31 (N = 50)
Tar mat (N = 20)	Male (N = 10)	82.80 ± 7.33 (N = 50)	27.80 ± 6.46 (N = 50)	5.90 ± 1.66 (N = 50)
	Female (N = 10)	65.40 ± 6.52 (N = 50)	18.0 ± 1.49 (N = 50)	5.60 ± 1.43 (N = 50)

A two-way ANOVA test looking at sex and site as factors as well as their interaction was carried out. In terms of numbers of swollen cells, both sex ($F_{1,98} = 31.34$, $P < 0.0001$) and study site ($F_{1,98} = 2261.8$, $P < 0.0001$) were significant factors as was the interaction between these two variables ($F_{1,98} = 31.35$, $P < 0.0001$). Number of

cells showing cytoplasmic degeneration were also influenced by sex ($F_{1,98} = 21.35$, $P < 0.0001$) and study site ($F_{1,98} = 474.05$, $P < 0.0001$) and the interaction between these variables was highly significant ($F_{1,98} = 22.24$, $P < 0.0001$). The numbers of dead cells observed in sections was influenced by the study site ($F_{1,98} = 246.49$, $P < 0.0001$) but not by the sex of the lizard ($F_{1,98} = 0.31$, $P = 0.57$) and there was no interaction between sex and study site ($F_{1,98} = 0.07$, $P = 0.78$).

Essentially the numbers of swollen cells, cells with cytoplasmic degeneration and dead cells (all signs of liver damage) were much higher in liver sections taken from lizards from the tar mat sites. Sex also influenced the numbers of swollen and cytoplasmically damaged cells with males showing greater impacts at the tar mat sites. The number of dead cells did not, however, significantly vary with sex.

Representative photomicrographs of the liver sections are presented as figures 6.3–6.10 (pp 95–98). The hepatocytes of *A. scutellatus* from the control sites appeared normal in all slides examined. The hepatic lobules were typical and the hepatocytes radiated from the central vein toward the periphery of the lobule (Fig. 6.3, p 95). The cells from this male lizard appeared normal with typical polygonal shape and prominent nuclei, and surrounding cytoplasm was normal with distinct plasma membrane. Hepatocytes of male lizards from the tar mat sites were very clearly swollen and showed ballooning degeneration of the hepatic cytoplasm owing to hydropic degeneration (Fig. 6.4, p 95). Hepatocytes of female lizards from the control sites were comparable to those of males from the same sites, being normal with distinct plasma membranes and normal surrounding cytoplasm (Fig. 6.5, p 96). Swollen hepatocytes and ballooning of cytoplasm was also clearly shown in the female lizards from the tar mat sites (Fig. 6.6, p 96). No dead cells were shown in the control male lizard hepatocytes whereas the numbers of such cells were clearly enhanced in the male lizards from the tar mat sites (Fig. 6.7 and 6.8, p 97). Similarly, no dead hepatocytes were shown in the control female lizards but many dead hepatocytes were clearly observed in the female lizards of the polluted sites (Fig. 6.9 and 6.10, p 98).

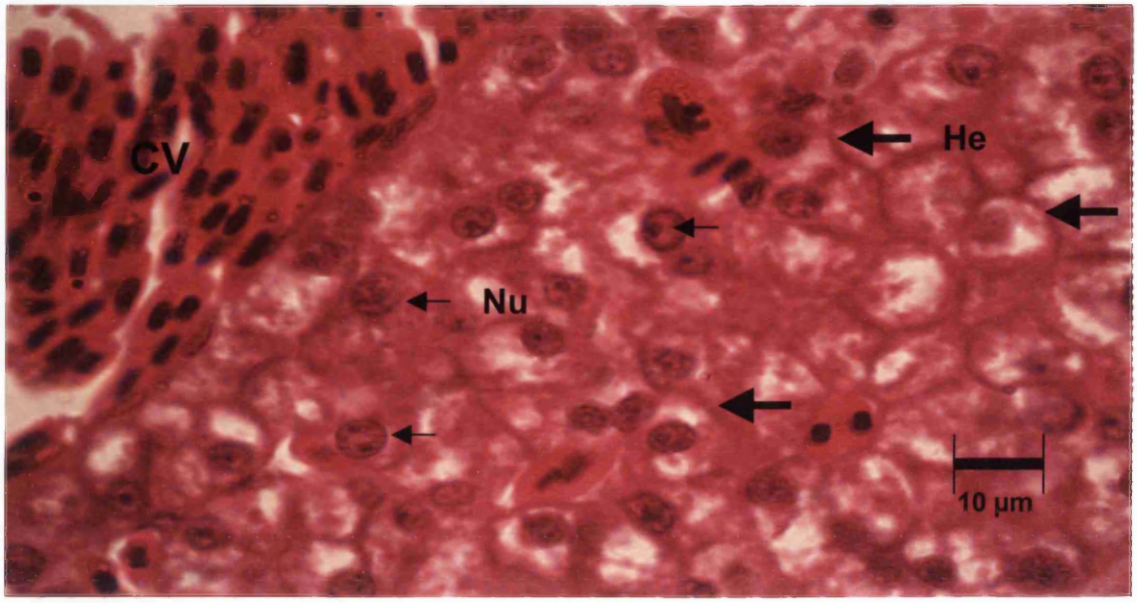

Figure 6.3 High power photomicrograph of liver tissue in sections from a male lizard from a control site stained with H & E where hepatocytes (He) (thick arrows) are surrounded by a distinct plasma membrane; hepatocyte nuclei (Nu) (thin arrows) are very distinct. Central venule (CV) is shown. (author's own image)

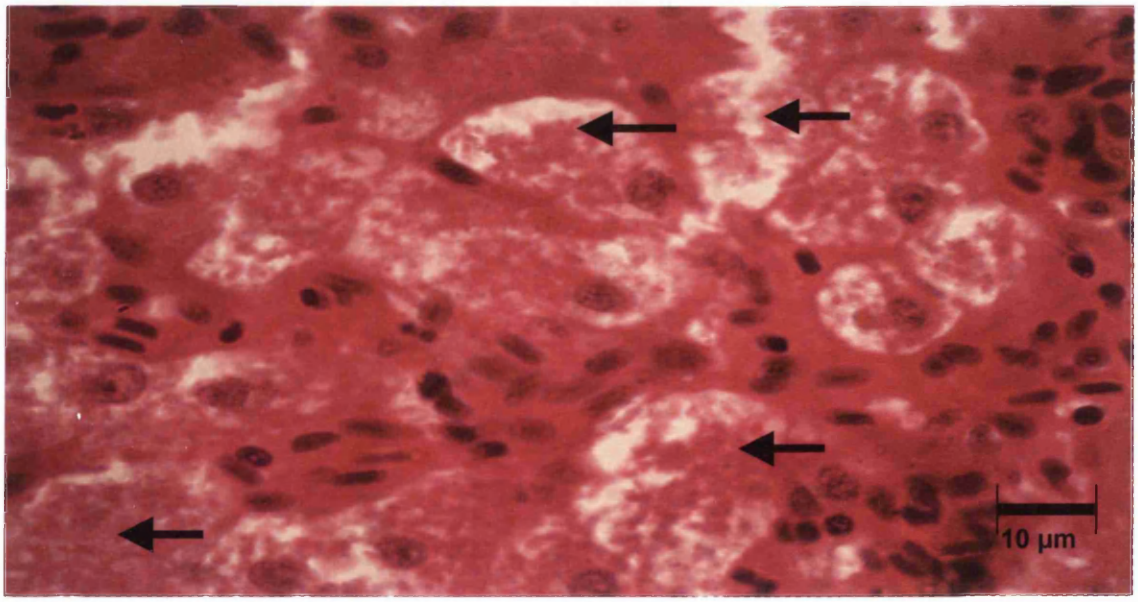

Figure 6.4 High power photomicrograph of liver tissue in sections from a male lizard from a tar mat site stained with H & E showing swelling of hepatocytes and ballooning degeneration of hepatic cytoplasm (arrows). (author's own image)

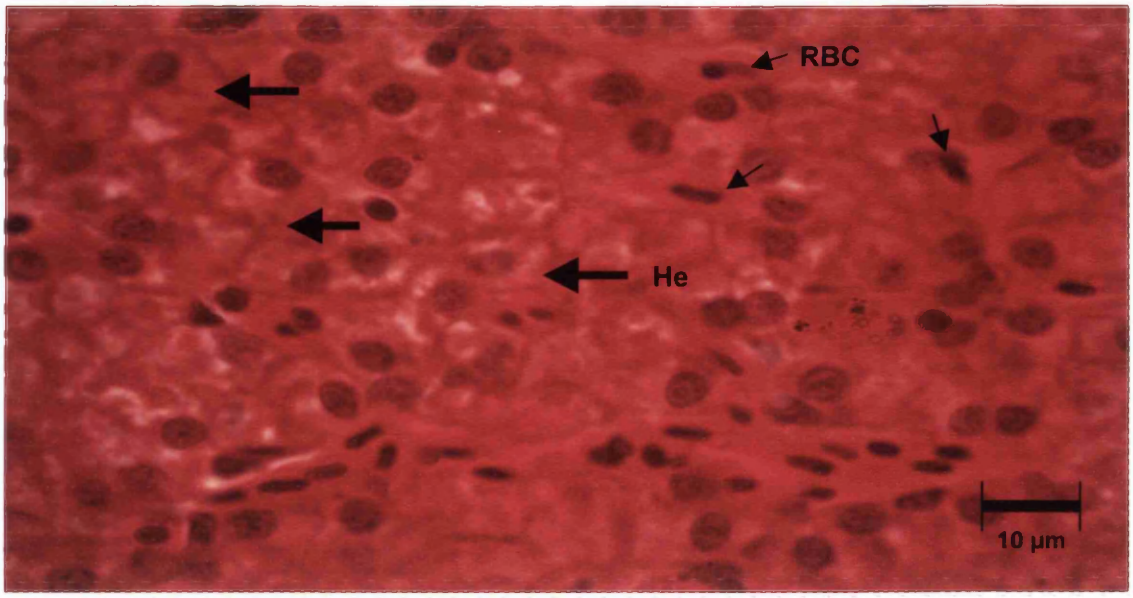

Figure 6.5 High power photomicrograph of liver tissue in sections from a female lizard from a control site stained with H & E showing normal hepatocytes with distinct plasma membranes (long thick arrows) and normal cytoplasm surrounding the nuclei. Red blood cells (RBCs) (short thin arrows) are shown. (author's own image)

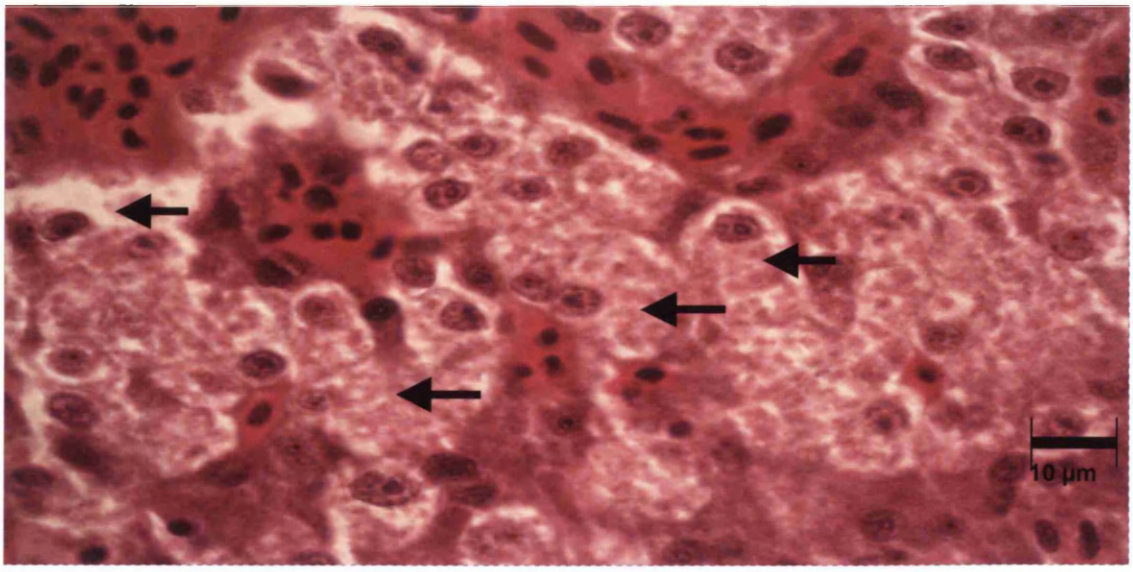

Figure 6.6 High power photomicrograph of liver tissue in sections from a female lizard from a tar mat site stained with H & E showing swollen hepatocytes and ballooning degeneration of the cytoplasm (arrows). (author's own image)

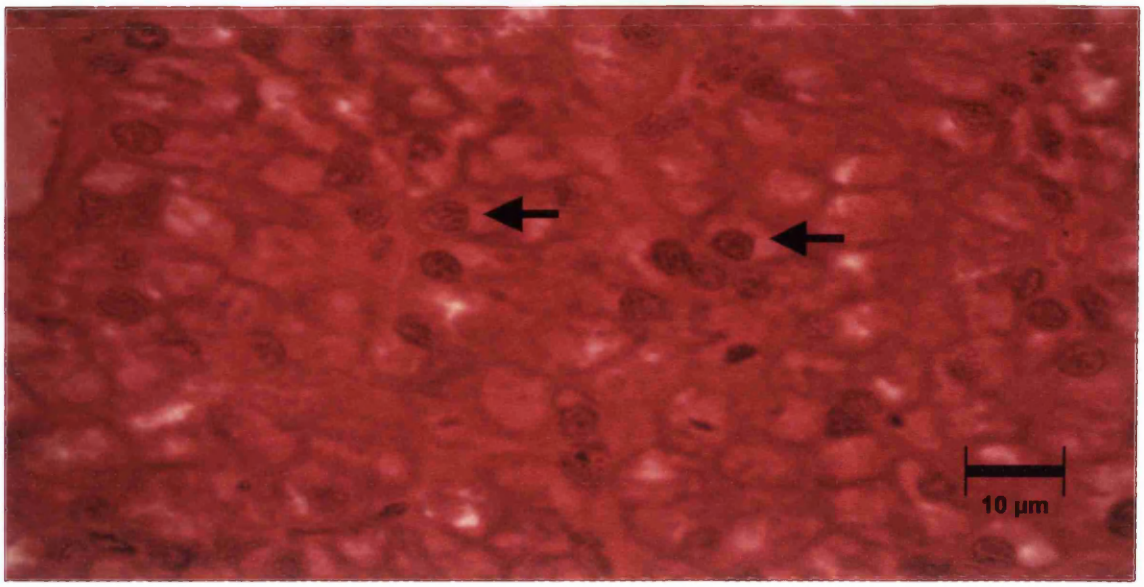

Figure 6.7 High power photomicrograph of liver tissue in sections from a male lizard from a control site stained with H & E showing normal hepatocytes (arrows). No dead cells are evident. (author's own image)

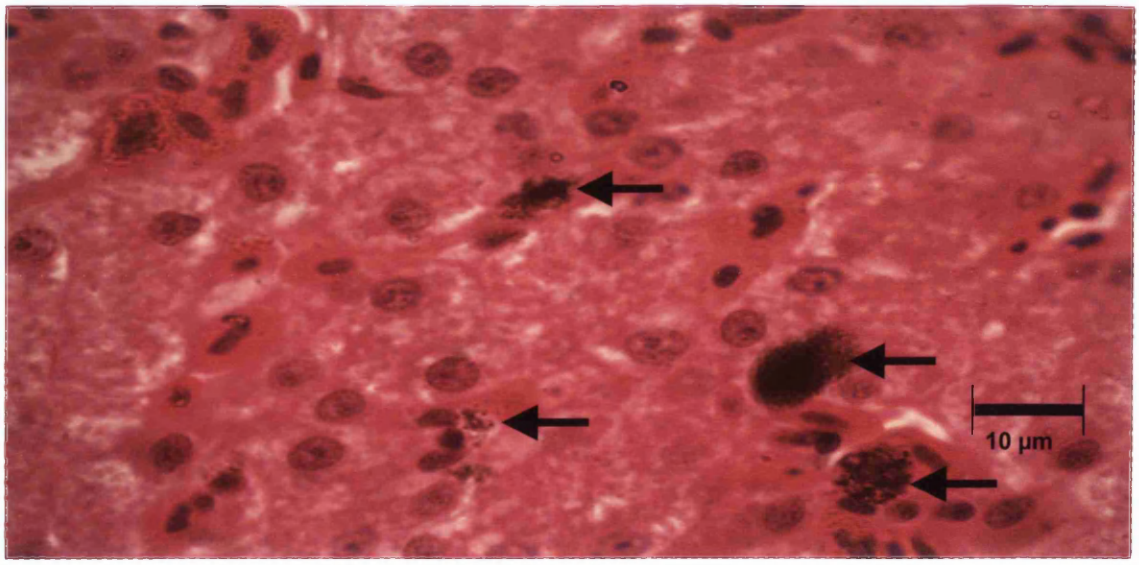

Figure 6.8 High power photomicrograph of liver tissue in sections from a male lizard from a tar mat site stained with H & E showing many dead hepatocytes (arrows). (author's own image)

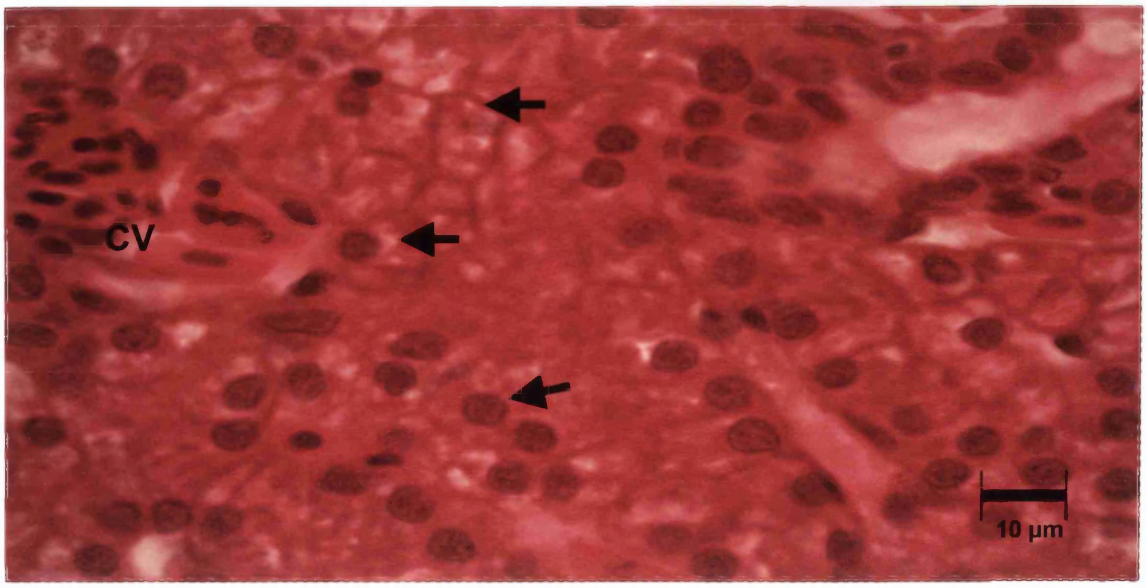

Figure 6.9 High power photomicrograph of liver tissue in sections from a female lizard from a control site stained with H & E showing normal hepatocytes (arrows). There are no obvious dead cells. (author's own image)

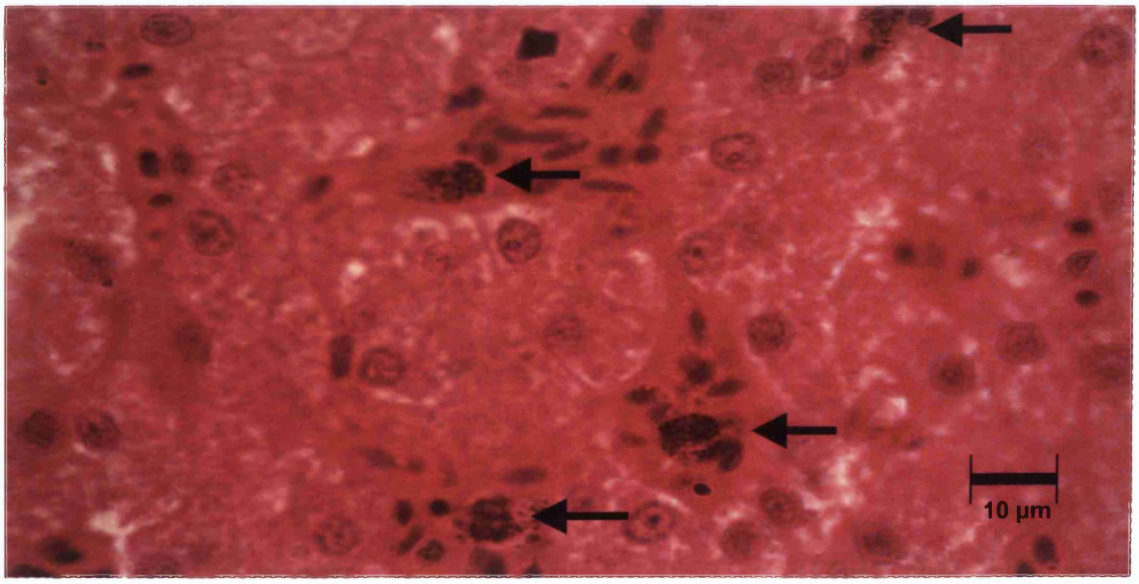

Figure 6.10 High power photomicrograph of liver tissue in sections from a female lizard from a tar mat site stained with H & E showing dead hepatocytes (arrows). (author's own image)

6.4 Discussion

Changing environments demand a considerable degree of cellular adaptability in liver tissue. Many adaptations are accompanied by structural changes which are visible microscopically (Stevens *et al.*, 2002). If a cell makes a successful adaptation to an environmental change, it will either return to normal or it may make an adaptive change. If a cell is unable to respond successfully to an environmental change, then the cell may die. Once exposed to a harmful stimulus, the cell may be affected as a whole or the damage may affect selected sub-cellular systems. If there is mild damage to cellular components then a cell may develop morphological changes termed cellular degeneration. The most common structural changes are swelling, hydropic degeneration and fatty change. These morphological changes are reversible if the causative environmental change is removed. Under such circumstances, a cell may return to normal after removal of damaged organelles or it may still die because it can not respond successfully to the environmental change (*op. cit.*).

It was clear from the results that the hepatocytes of lizards collected from the tar mat sites were damaged compared to the normal hepatocytes collected from lizards of the control sites. The most remarkable feature observed was swelling of hepatocytes, ballooning degeneration of the hepatic cytoplasm and cell death. The swelling of hepatocytes occurred in both male and female lizard but it was more obvious in the males. Male *A. scutellatus* from the tar mat sites had much greater in the hepatocyte diameters than all other examined groups, but the nuclear diameters of these males were not significantly changed by oil pollution. Females from the polluted sites were also affected by oil pollution by having larger hepatocyte diameters than females of *A. scutellatus* of the control sites but their nuclei were also affected by oil pollution, being larger than female nuclei in sections from the control sites.

Swollen hepatocytes, cells with cytoplasm degeneration and dead cells were clearly observed in males and females from the tar mat sites but were not evident in either sex from the control sites. Swollen hepatocytes are identified by becoming buffy or enlarged compared to normal hepatocytes. Cells with cellular degeneration are characterized by having cytoplasm with no distinct borders with the cytoplasm being

diluted and dispersed. Dead cells have condensed nuclei due to chromatin clumping which is accompanied by cytoplasmic changes. The cytoplasm has lost definition and the cell margins (plasma membranes) are indistinct, also nuclei of dead cells become fragmented compared to living cells. Males appeared more affected than females by oil pollution in having larger numbers of swollen hepatocytes and greater cytoplasmic degeneration. This morphological change in lizard hepatocytes may continue in the polluted sites or in tissues from animals from the tar mat sites and cells may eventually die. Dead hepatocytes were clearly evident in males and females from the tar mat sites but were not observed in the livers of either sex from the control sites.

Contaminants can impact organisms in two different ways, via organization or activation. First, embryonic exposure can cause organizational abnormalities by altering the chemical signals required for normal development and thus permanently changing the endocrine function and or response of an organ or organism. Second, they can alter cellular signaling in mature systems, that is, disrupt activational signaling that would lead to altered organ system or organism performance (Guillette *et al.*, 2003). Contamination may be one of many factors influencing sex steroid concentrations which can also result from alterations in hepatic biotransformation and metabolism of hormones. The morphological cellular change between male and female lizards under pathological conditions remains one of the future needs studies.

Swelling of hepatocytes is caused by accumulation of water and is referred to as hydropic change. This hydropic change may result from exposure to toxins or as a result of oxygen reduction. Hydropic change is sometimes caused by toxins that might affect the respiration mechanism or the oxygen content of the hepatocytes*. Fish treated with Dieldrin and Malathion (Mathur, 1965; Dutta *et al.*, 1993) and mice treated with Phosphamidon (Bhatnagar and Nirmala, 1986) show similar changes in their liver tissues.

* (Anim and Matheo, personal communication)

The presence of injured or dead cells in the livers of lizards from the tar mat sites is another sign that the organ is not normal (such cells were not evident or were only found as part of regular cycle of apoptosis of the hepatocytes of normal lizards). Akbar

(1996) indicated that 9, 10 di-methyl benz (1-2) anthracene (DMBA) (a known carcinogen) causes cytoplasmic degeneration and swelling of hepatocytes. This PAH also showed similar effects to those of oil polluted samples in mouse livers.

Ganser *et al.* (2003) demonstrated the accumulation of arsenic, cadmium, copper, selenium, strontium and vanadium in livers of Southern Watersnakes (*Nerodia fasciata fasciata*) fed fish from a coal-ash contaminated site. Liver fibrosis and proliferation of collagen fibers with increasing mass of intersinusoidal parenchyma were the most prevalent histopathological features observed.

In conclusion, there is ample evidence in the literature that prolonged exposure to oil pollution (with a mixture of PAHs and heavy metals) may result in increased accumulation of contaminants and may cause severe liver pathology in a range of wild organisms. Such effects are likely to alter an organism's growth, survival and reproduction. These studies on *A. scutellatus* confirm that the same applies to these lizards at the presumably 'most polluted' tar mat sites. It is unfortunate that there was no time to carry out similar investigations on subjects from the 'clear' and 'soot' sites. Data in chapters 4 and 5 strongly suggest that these sites are also contaminated with PAHs and heavy metals. It would have been interesting to establish whether they produced effects or whether all the contaminated sites (irrespective of the amount of visible evidence) had equally deleterious effects on the liver histology of these lizards.

CHAPTER 7

Discussion and Conclusions

7.1 Ecological Risk Assessment and Reptiles

Ecological Risk Assessment (ERA) is a set of formal, scientific methods for estimating the probability and magnitude of an adverse impact on the ecology of a specified area posed by a particular stressor occurring at a particular frequency or concentration. The stressors are often (but not always) chemical in nature. ERAs should focus on a full complement of the relevant members (plants and animals) of the systems being studied. Having said this, it would obviously be difficult, time-consuming and expensive to assess the impact of, for example, particular chemical contaminants on all animals and plants within an ecological system. Interpretation of such complex data would also be very difficult. There has consequently been a tendency to simplify and speed ERAs by focusing attention on 'indicator species' that (hopefully) reliably reflect whether problems associated with contamination are increasing or in decline.

In general, the characteristics of a useful indicator species for ERA purposes (Sparling *et al.*, 2000) include their:

- High likelihood of being exposed to the stressor of interest,
- Having a special-status (e.g. endangered or threatened),
- Being a key component within a specific trophic level or guild,
- Toxicity data being available, and
- Being recreationally or commercially valued.

Each of those attributes can be associated with reptiles. Indeed, Five reptilian species are presented in USEPA; Wildlife Exposure Factors Handbook (USEPA, 1993) as having such characteristics. These are the Painted turtle (*Chrysemys picta*), Eastern

box turtle (*Terrapene carolina*), Common snapping turtle (*Chelyda serpentina*), Racer (*Coluber constrictor*), and Northern water snake (genus *Nerodia*). These species were selected on the basis of their wide geographic distribution within North America, the wealth of natural-history data available on them compared to other reptiles, and their representation of different ecological niches.

The above is, however, only true of North America. Although basic ecological information is available for a large number of reptiles, the lack of sufficient toxicity data in the literature often precludes their being used as indicator species in ERAs. The need for further research on toxicity of chemicals and other environmental stressors on reptiles has been effectively presented by Hall and Henry (1992).

However, reptiles are rarely included in ERAs either because contaminant data are not available or these animals are not considered important in ecosystem functions (Campbell and Campbell, 2000). It can be argued, however, that they are important constituents of ecosystems and comprise a large percentage of the faunal biomass in many terrestrial and aquatic locations. The life histories of reptiles make their roles in food webs diverse and important. They are predators of some vertebrates and many invertebrates, as well as being prey of larger reptiles, birds and mammals. Reptiles may be especially pertinent in risk assessments performed for terrestrial arid ecosystems (Vonder Valk, 1997). In contrast to birds and mammals, lizards appear to be good indicators of the quality of terrestrial habitats because of their relatively restricted mobility (Lambert, 1993). In addition, lizards are sensitive to many pesticides as the result of being directly exposed or following intake via their prey (these are often insects that come into intimate contact with these chemicals or feed upon plants that are contaminated by these agents).

Many reptilian species throughout the World are in danger of extinction and chemical contaminants may affect their survival. Toxic equivalency factors developed for use in risk assessment for fish, birds and humans are not, however, necessarily predictive of toxicity in reptiles (Bishop and Gendron, 1998). In fact, the impacts of pollutants on reptiles have rarely been quantified and monitored in the wild and the toxicity of most chemicals to reptiles in arid lands is virtually unknown (Everts, 1997). Contaminants that find their way into the environment could even reach humans

through bioconcentration pathways that include lizards. However, lizards have been either ignored or represented as incidental components of contaminant studies.

The present series of studies provides detailed information on the effects of oil pollution in desert locations in Kuwait on the behaviour, population size and liver histology of the Sand lizard *A. scutellatus*. PAHs and heavy metals, as major constituents of oil pollution were also detected in both Sand lizards and their prey (ants). No other study has extensively investigated the effects of oil pollution on lizard populations (or even wildlife in general) for any part of Greater Al-Burgan oil fields. This study is the most comprehensive currently available of the effects and accumulation of environmental contaminants on lizards.

It can be argued that there is a *prima facie* case for representative species of lizards being included in ERAs, especially when studies are carried out in areas where these reptiles are abundant and diverse and thus play major roles in ecosystem processes. Many of the results presented in this thesis support the view that lizards are ideal bioindicators of oil pollution in arid areas. They are also important in their own right as a component of biodiversity.

One unexpected outcome of the entire study was that assumptions made about the degrees of contamination based on physical evidence (such as soot and tar) were not supported. All the classes of contaminated sites appeared to be equally contaminated. This finding throws into doubt relying on physical appearance as an indicator of oil pollution and suggests that many of the earlier investigations using this feature will have to be re-evaluated.

Population studies of the lizards and their ant prey were important in terms of providing a 'snap shot' of biodiversity and the current status of the animals but seem to have too many interpretational problems to be used in ERAs. Sites and replicates had to be chosen with consideration to the researcher's safety. In addition, this type of investigation that involves different sites and many replicates of (apparent) oil pollution categories (which were very distant from each other) would require more than one researcher because it is time and labour intensive. Even if this was not the case, the population sizes of *A. scutellatus* did not vary markedly among the study sites. This may be a consequence of the number of replicates used for each type of pollution

categories or could be due to the fact (as suggested earlier that all the sites were equally polluted with oil). Comparisons between the lizard population sizes using the pitfall trap and transect methods in 2002 and 2003 revealed essentially similar results, suggesting that values are broadly accurate.

Noting the lizard's changing behaviour seemed, however, to reflect oil pollution in arid areas. This was not, however, simply a consequence of being exposed to chemicals. The present study confirmed that morning emergence and basking times varied in Sand lizards among the different 'pollution' categories of site. Most notably, animals on the tar mat appeared to bask earlier. It appeared that this is because oil pollution produces physical changes in tar mat causing the substrate temperatures to rise more quickly in the morning as a result of solar gain. This gives the lizards the opportunity to start eating earlier, giving them an advantage in terms of energetics (perhaps, in turn, influencing their rates of growth and fecundity). These findings suggest that interpretation of the effects of oil pollution on populations of *A. scutellatus* is complex. Lizards on the tar mat sites could benefit from the physical characteristics of the location (e.g. increased solar gain and perhaps greater prey abundance) but be damaged by the ingestion of heavy metals and PAHs (see later).

A. scutellatus living in the tar mat and soot sites were also darker in colour than those living in the clear and control sites. In some species of reptile, the animal can change body colour rapidly to match the current background (Cooper *et al.*, 1990). Such 'physiological colour change' occurs, for example, in *Anolis carolinensis* (Medvin, 1990) and *Chamaeleo chamaeleon* (Raxworthy and Nussbaum, 1995) where the lizards use chromatophores in their integument to match their colouration to that of the substrate. 'Morphological colour changes', where the animal gradually shifts its body colouration to match the predominant background by manufacturing extra melanin, is seen in other lizard species. The lacertid lizard *Podarcis taurica*, changes colour seasonally in such a manner, being bright green in spring and early summer and becoming dark olive green to brown in late summer (Chondropoulos and Lykakus, 1983). It is uncertain what mechanism accounts for the colour change in *A. scutellatus* but rapid colour change was not observed in this species. Of course, darkly-coloured lizards would warm up more rapidly while basking than lightly-coloured counterparts,

also increasing their energetic advantage. The lizards clearly selected their habitat by matching their body colour to the substrate. Lizards (*Uta stansburiana*) having a body colouration contrasting with their substrate are more likely to be killed by a predator than are substrate-matched lizards (Luke, 1989). In a similar vein, lizards (*Sceloporus undulates*) differing from their background colouration show higher frequencies of tail breaks, suggesting that they are subject to greater predation pressure (Gillis, 1989).

Contamination by heavy metals resulting from sources such as oil spills is a serious problem recognized in most countries. Chemical analysis is required in this type of study to assess the toxic hazards of heavy metals to wildlife. The present study focused on nickel and vanadium in lizard whole body tissues and the soils of the study sites. The 'clear' sites were actually confirmed to have elevated heavy metal values c.f. the control sites, meaning that the physical presence of soot and tar mat is not a reliable guide to the degree of contamination. It also suggests that contamination is more widespread than might have been suspected on the basis of earlier surveys. No information is currently available on the bioaccumulation of heavy metals in lizards, making it difficult to evaluate this study.

The presence of PAHs was determined in Sand lizards and ant (a major food resource for these lizards) tissues. Again subjects from all the 'different' oil polluted sites showed increased concentrations of these chemicals but levels were undetectable at the control sites. The concentrations of PAHs did not vary between the categories of polluted sites confirming that, even when the visual signs of pollution are lacking, the sites are as contaminated (or even more contaminated) than the sites with soot or tar mat. As the same PAH compounds present in lizards were also found in ants, one may suggest that the lizards essentially ingest contaminated food. The dearth of information concerning PAHs in lizards makes it difficult to assess the impact of oil pollution on wildlife.

Further study was performed on the histology of the Sand lizard's liver, a vital organ believed to be affected by heavy metals and PAHs. Liver damage was clearly observed in lizards from the polluted sites. This damage was seen as dead and swollen hepatocytes, cytoplasm disintegration and loss of cellular membranes of liver cells which might be a response to carcinogenicity. Because nickel and benzo[a]anthracene

are carcinogenic compounds in humans, the strong possibility exists that they may account for damage to hepatic cells in *A. scutellatus*.

7.2 Recommendations and Future Studies

The oil contamination of the terrestrial ecosystems in Kuwait is said to be one of the worst in history. The impact on the environment (always one of the victims of war throughout mankind's history according to Charrier 1998) will take decades even to partially disappear.

The general characteristics of reptiles (such as their relatively small home ranges, high trophic-level position, oviparity and longevity) appear to make them excellent bioindicators for land-based environmental contamination studies. Although, these initial studies on Sand lizards support the view that they could be helpful in assessing the impact of oil pollution in arid locations, very little is known about reptilian ecotoxicology, compared to what is known for frogs, mammals and birds. There is a great need for further research in this area if lizards in locations outside North America are to be accepted as bioindicators. Given the fact that even the clear sites have chemical contamination but lack a protective layer (as in the case of the tar mat), it would have been instructive in these studies to have compared lizards from control and clear sites. This might well have produced more direct and tangible evidence of any detrimental effects of the exposure of these reptiles to the chemicals.

Studies on the effects of transportation of organic and heavy metal contaminants from females to eggs and from hatchlings to adults might well help to determine if some stages of the life cycle are more sensitive to these compounds. Death or morbidity in younger or older individuals produces different repercussions on the viabilities of populations. Molecular studies might well facilitate our understanding of the mechanisms involved in interactions between foreign chemical molecules and organisms at the tissue level. It would be very nice to be able to 'track' the movement of some contaminants or their metabolites through the rather simple ecosystems in the arid areas.

Information on a wide range of species (including lizards) collected at Al-Burgan oil fields should be continued in order to record the long-term impact of oil

pollution on wildlife. Monitoring efforts should be extended throughout the Al-Burgan oil fields to ensure that a complete picture of the changes is obtained. The area is essentially a natural laboratory for assessing the impact of oil pollution on an arid area over the long term.

APPENDIX A

Pitfall trap data for the varied sites 2002 - 2003

Table 1 Pitfall trap data for estimation of population size of lizards and ants in control site in 2002.

Sample	C_t	R_t	M_t	$C_tM_t^2$	R_tM_t	R_t^2/C_t	R_t/C_t	Date	Air temp. °C	Soil temp. °C	ants
1	6	0	0	0	0	0	0	04/02/02	18.3	19.1	7
2	6	1	6	216	6	0.16	0.16	05/02/02	16.8	21.1	11
3	3	1	11	363	11	0.33	0.33	06/02/02	21.5	27	5
4	1	0	13	169	0	0	0	07/02/02	22.6	35.8	8
5	4	2	14	784	28	1	0.5	16/02/02	23.6	34	10
6	1	0	15	225	0	0	0	17/02/02	22.3	33	9
7	4	2	16	1024	32	1	0.5	20/02/02	22.9	35	15
8	2	0	18	648	0	0	0	21/02/02	21	32.5	13
9	2	1	20	800	20	0.5	0.5	02/03/02	24	32	16
10	4	3	21	1764	63	2.25	0.75	03/03/02	25	34	22
11	2	1	22	968	22	0.5	0.5	09/03/02	26.2	34	18
12	6	1	23	3174	23	0.16	0.16	01/04/02	31	39	25
Sum				10135	205	5.91		Mean	22.93	31.38	13.25

N=	49.44			DF=	10
Var(1/N)=	0.177			SE(1/N)=	0.00418
CI(1/N)=	0.011	,	0.03		
CI(N)=	33.85	,	91.62 ←	95% CI	

C_t = Total number of individuals caught in sample t

R_t = Number of individuals already marked when caught in sample t

M_t = Number of marked individuals in the population just before sample t is taken

Table 2 Pitfall trap data for estimation of population size of lizards and ants in control site in 2002.

Sample	C_t	R_t	M_t	$C_tM_t^2$	R_tM_t	R_t^2/C_t	R_t/C_t	Date	Air temp. °C	Soil temp. °C	ants
1	5	0	0	0	0	0	0	04/02/02	20.3	24	10
2	3	2	5	75	10	1.33	0.66	05/02/02	18.4	24.8	8
3	2	2	6	72	12	2	1	06/02/02	22	28.5	14
4	3	1	6	108	6	0.33	0.33	07/02/02	23.2	36	20
5	2	1	8	128	8	0.5	0.5	16/02/02	24	36	18
6	2	1	9	162	9	0.5	0.5	17/02/02	22.7	34	25
7	1	0	10	100	0	0	0	20/02/02	24.5	36	20
8	1	0	11	121	0	0	0	21/02/02	22	34	26
9	3	2	12	432	24	1.33	0.66	02/03/02	24.7	34	32
10	2	2	13	338	26	2	1	03/03/02	25.5	35	36
11	1	0	1	1	0	0	0	09/03/02	27	38	40
12	4	1	14	784	14	0.25	0.25	01/04/02	31.5	41	52
Sum				2321	109	8.25		Mean	23.82	33.44	25.08

N=	21.29			DF=	10
Var(1/N)=	0.313			SE(1/N)=	0.01161
CI(1/N)=	0.021	,	0.073		
CI(N)=	13.73	,	47.43 ←	95% CI	

C_t = Total number of individuals caught in sample t

R_t = Number of individuals already marked when caught in sample t

M_t = Number of marked individuals in the population just before sample t is taken

Table 3 Pitfall trap data for estimation of population size of lizards and ants in clear site in 2002.

Sample	Ct	Rt	Mt	$CtMt^2$	$RtMt$	Rt^2/Ct	Rt/Ct	Date	Air temp. °C	Soil temp. °C	ants
1	5	0	0	0	0	0	0	09/02/02	22.3	30.4	80
2	3	1	5	75	5	0.33	0.33	10/02/02	22.4	29	65
3	5	2	7	245	14	0.8	0.4	11/02/02	23	30	92
4	3	0	10	300	0	0	0	12/02/02	22.9	30.1	86
5	3	3	13	507	39	3	1	13/02/02	23.1	31	78
6	3	1	13	507	13	0.33	0.33	18/02/02	24	31	107
7	4	3	15	900	45	2.25	0.75	19/02/02	22.8	32	150
8	4	1	16	1024	16	0.25	0.25	04/03/02	25.3	35.2	280
9	3	1	19	1083	19	0.33	0.33	05/03/02	25	35.8	320
10	3	2	21	1323	42	1.33	0.66	06/03/02	24.9	36	340
11	3	1	22	1452	22	0.33	0.33	03/04/02	26.3	38	300
12	1	1	24	576	24	1	1	04/04/02	26.4	40	360
Sum				7992	239	9.96		Mean	24.03	33.21	188.17

N=	33.44			DF=	10
Var(1/N)=	0.282			SE(1/N)=	0.00594
CI(1/N)=	0.017	,	0.043		
CI(N)=	23.18	,	59.98 ←	95% CI	

Ct = Total number of individuals caught in sample t

Rt = Number of individuals already marked when caught in sample t

Mt = Number of marked individuals in the population just before sample t is taken

Table 4 Pitfall trap data for estimation of population size of lizards and ants in clear site in 2002.

Sample	Ct	Rt	Mt	$CtMt^2$	$RtMt$	Rt^2/Ct	Rt/Ct	Date	Air temp. °C	Soil temp. °C	ants
1	1	0	0	0	0	0	0	09/02/02	22.8	31	12
2	1	0	1	1	0	0	0	10/02/02	23	30	18
3	1	1	2	4	2	1	1	11/02/02	22.5	29.8	22
4	1	0	2	4	0	0	0	12/02/02	23.5	31.2	20
5	1	1	3	9	3	1	1	13/02/02	24	32	17
6	1	1	3	9	3	1	1	18/02/02	23.5	32.3	25
7	1	0	3	9	0	0	0	19/02/02	23.2	34	28
8	1	1	4	16	4	1	1	04/03/02	25.8	36	32
9	1	1	4	16	4	1	1	05/03/02	25.7	36.2	26
10	2	1	4	32	4	0.5	0.5	06/03/02	24.2	35.8	30
11	1	0	5	25	0	0	0	03/04/02	25.9	37.6	27
12	1	1	6	36	6	1	1	04/04/02	26.1	39.8	34
Sum				161	26	6.5		Mean	24.18	33.81	24.25

N=	6.192			DF=	10
Var(1/N)=	0.23			SE(1/N)=	0.03781
CI(1/N)=	0.077	,	0.246		
CI(N)=	4.07	,	12.94 ←	95% CI	

Ct = Total number of individuals caught in sample t

Rt = Number of individuals already marked when caught in sample t

Mt = Number of marked individuals in the population just before sample t is taken

Table 5 Pitfall trap data for estimation of population size of lizards and ants in soot site in 2002.

Sample	*Ct*	*Rt*	*Mt*	$CtMt^2$	*RtMt*	Rt^2/Ct	*Rt*/*Ct*	Date	Air temp. °C	Soil temp. °C	ants
1	4	0	0	0	0	0	0	09/02/02	23.5	31	7
2	2	1	4	32	4	0.5	0.5	10/02/02	22.8	32	9
3	2	1	5	50	5	0.5	0.5	11/02/02	24.6	32.1	8
4	2	0	6	72	0	0	0	12/02/02	23.4	32.9	12
5	4	2	8	256	16	1	0.5	13/02/02	23.5	34	16
6	4	1	10	400	10	0.25	0.25	18/02/02	23.8	36.2	20
7	5	2	13	845	26	0.8	0.4	19/02/02	23.6	35.5	17
8	3	1	16	768	16	0.33	0.33	04/03/02	26	37.3	28
9	4	1	18	1296	18	0.25	0.25	05/03/02	26.2	37.6	25
10	5	3	21	2205	63	1.8	0.6	06/03/02	26.9	39.2	32
11	2	0	23	1058	0	0	0	03/04/02	27.3	42	45
12	2	2	25	1250	50	2	1	04/04/02	27.5	42.5	56
Sum				8232	208	7.43		Mean	24.93	36.03	22.92

N=	39.58			DF=	10
Var(1/N)=	0.218			SE(1/N)=	0.00514
CI(1/N)=	0.014	,	0.037		
CI(N)=	27.23	,	72.43 ←	95% CI	

Ct = Total number of individuals caught in sample *t*

Rt = Number of individuals already marked when caught in sample *t*

Mt = Number of marked individuals in the population just before sample t is taken

Table 6 Pitfall trap data for estimation of population size of lizards and ants in soot site in 2002.

Sample	Ct	Rt	Mt	$CtMt^2$	$RtMt$	Rt^2/Ct	Rt/Ct	Date	Air temp. °C	Soil temp. °C	ants
1	5	0	0	0	0	0	0	09/02/02	23.6	30	8
2	3	2	5	75	10	1.33	0.66	10/02/02	22.4	31.2	7
3	2	2	6	72	12	2	1	11/02/02	23.8	31.1	12
4	2	1	8	128	8	0.5	0.5	12/02/02	23.2	33.4	9
5	4	2	10	256	20	1	0.5	13/02/02	23.4	34.2	17
6	4	1	14	784	14	0.25	0.25	18/02/02	23.5	35.2	19
7	1	0	18	100	0	0	0	19/02/02	23.8	35.5	16
8	3	1	19	108	19	0.33	0.33	04/03/02	26.2	36.3	32
9	1	0	22	100	0	0	0	05/03/02	26.3	37.6	28
10	2	2	23	338	26	2	1	06/03/02	26.7	39.2	25
11	1	0	25	1058	0	0	0	03/04/02	27.2	41	48
12	2	0	26	1352	0	0	0	04/04/02	27.3	41.5	58
Sum				4371	109	7.41		Mean	24.54	35.93	23.82

N=	19.85			DF=	10
Var(1/N)=	0.281			SE(1/N)=	0.00314
CI(1/N)=	0.015	,	0.034		
CI(N)=	24.33	,	62.43 ←	95% CI	

Ct = Total number of individuals caught in sample t

Rt = Number of individuals already marked when caught in sample t

Mt = Number of marked individuals in the population just before sample t is taken

Table 7 Pitfall trap data for estimation of population size of lizards and ants in tar mat site in 2002.

Sample	Ct	Rt	Mt	$CtMt^2$	$RtMt$	Rt^2/Ct	Rt/Ct	Date	Air temp. °C	Soil temp. °C	ants
1	2	0	0	0	0	0	0	09/02/02	23.1	29.5	58
2	1	0	2	4	0	0	0	10/02/02	22.1	30	72
3	2	1	3	18	3	0.5	0.5	11/02/02	24.3	31.2	65
4	2	1	4	32	4	0.5	0.5	12/02/02	22.8	31	95
5	1	0	5	25	0	0	0	13/02/02	23	33.2	188
6	3	2	6	108	12	1.33	0.66	18/02/02	22.5	34.2	260
7	2	1	7	98	7	0.5	0.5	19/02/02	22	34.5	240
8	3	2	8	192	16	1.33	0.66	04/03/02	25.4	35.2	200
9	1	0	9	81	0	0	0	05/03/02	25.8	36	270
10	1	1	10	100	10	1	1	06/03/02	26	38	300
11	6	2	10	600	20	0.66	0.33	03/04/02	26.6	39.5	450
12	6	2	14	1176	28	0.66	0.33	04/04/02	27	41	520
Sum				2434	100	6.5		Mean	24.22	34.44	226.50

Ń=	24.34	DF=	10
Var(1/N)=	0.239	SE(1/N)=	0.00991
CI(1/N)=	0.019 , 0.063		
CI(N)=	15.83 , 52.64 ← 95% CI		

Ct = Total number of individuals caught in sample t

Rt = Number of individuals already marked when caught in sample t

Mt = Number of marked individuals in the population just before sample t is taken

Table 8 Pitfall trap data for estimation of population size of lizards and ants in tar mat site in 2002.

Sample	Ct	Rt	Mt	$CtMt^2$	$RtMt$	Rt^2/Ct	Rt/Ct	Date	Air temp. °C	Soil temp. °C	ants
1	1	0	0	0	0	0	0	09/02/02	24.5	31.2	45
2	1	0	1	1	0	0	0	10/02/02	23.8	33.5	30
3	1	1	2	4	2	1	1	11/02/02	26	35	55
4	1	0	2	4	0	0	0	12/02/02	23.6	33.2	80
5	1	1	3	9	3	1	1	13/02/02	25.3	35.8	150
6	1	1	3	9	3	1	1	18/02/02	24.1	36.3	220
7	2	1	3	18	3	0.5	0.5	19/02/02	23.7	36	300
8	1	1	4	16	4	1	1	04/03/02	26.8	37.2	450
9	1	0	4	16	0	0	0	05/03/02	26.5	37.5	400
10	1	1	5	25	5	1	1	06/03/02	26.8	40	550
11	2	1	5	50	5	0.5	0.5	03/04/02	27	41	410
12	1	1	6	36	6	1	1	04/04/02	27.5	42.8	570
Sum				188	31	7		Mean	25.47	36.63	271.67

N=	6.065			DF=	10
Var(1/N)=	0.189			SE(1/N)=	0.03169
CI(1/N)=	0.094	,	0.236		
CI(N)=	4.246	,	10.61 ←	95% CI	

Ct = Total number of individuals caught in sample t

Rt = Number of individuals already marked when caught in sample t

Mt = Number of marked individuals in the population just before sample t is taken

Table 9 Pitfall trap data for estimation of population size of lizards and ants in control site in 2003.

Sample	C_t	R_t	M_t	$C_tM_t^2$	R_tM_t	R_t^2/C_t	R_t/C_t	Date	Air temp. °C	Soil temp. °C	ants
1	4	0	0	0	0	0	0	05/02/03	18.1	21.3	5
2	2	0	4	32	0	0	0	06/02/03	18.7	22	8
3	5	1	6	180	6	0.2	0.2	15/02/03	20.3	25.5	6
4	4	0	10	400	0	0	0	16/02/03	20.9	25	10
5	4	2	14	784	28	1	0.5	20/02/03	19.3	22.5	9
6	4	2	16	1024	32	1	0.5	22/02/03	22.5	28	14
7	4	1	18	1296	18	0.25	0.25	26/02/03	23.7	32	12
8	3	2	21	1323	42	1.33	0.66	27/02/03	19.8	30	10
9	3	1	22	1452	22	0.33	0.33	02/03/03	17.5	25.2	16
10	2	0	24	1152	0	0	0	03/03/03	19.8	26.3	20
11	3	2	26	2028	52	1.33	0.66	09/03/03	21.3	29.4	18
12	3	2	27	2187	54	1.33	0.66	10/03/03	25	33	17
13	3	1	28	2352	28	0.33	0.33	12/03/03	24.1	35	22
Sum				14210	282	7.11		Mean	20.85	27.32	12.85

N=	50.39			DF=	11
Var(1/N)=	0.138			SE(1/N)=	0.00312
CI(1/N)=	0.013	,	0.027		
CI(N)=	37.44	,	77.04 ←	95% CI	

C_t = Total number of individuals caught in sample t

R_t = Number of individuals already marked when caught in sample t

M_t = Number of marked individuals in the population just before sample t is taken

Table 10 Pitfall trap data for estimation of population size of lizards and ants in control site in 2003.

Sample	C_t	R_t	M_t	$C_tM_t^2$	R_tM_t	R_t^2/C_t	R_t/C_t	Date	Air temp. °C	Soil temp. °C	ants
1	1	0	0	0	0	0	0	05/02/03	18.6	23	6
2	1	0	1	1	0	0	0	06/02/03	19.1	24	10
3	2	1	2	8	2	0.5	0.5	15/02/03	21	26.3	20
4	2	0	3	18	0	0	0	16/02/03	21.4	24	35
5	2	0	5	50	0	0	0	20/02/03	20.5	24.6	22
6	3	1	7	147	7	0.33	0.33	22/02/03	23	29.6	37
7	4	2	9	324	18	1	0.5	26/02/03	23.1	34.6	28
8	2	1	11	242	11	0.5	0.5	27/02/03	23.5	34.3	25
9	2	1	12	288	12	0.5	0.5	02/03/03	18.9	28.2	30
10	3	0	13	507	0	0	0	03/03/03	21	29.5	35
11	1	1	16	256	16	1	1	09/03/03	24.5	33	44
12	3	2	16	768	32	1.33	0.66	10/03/03	25.6	35	41
13	1	0	17	289	0	0	0	12/03/03	25.9	36.5	56
Sum				2898	98	5.16		Mean	22.01	29.43	29.92

N=	29.57				DF=	11
Var(1/N)=	0.168				SE(1/N)=	0.00762
CI(1/N)=	0.017	,	0.051			
CI(N)=	19.76	,	58.69	← 95% CI		

C_t = Total number of individuals caught in sample t

R_t = Number of individuals already marked when caught in sample t

M_t = Number of marked individuals in the population just before sample t is taken

Table 11 Pitfall trap data for estimation of population size of lizards and ants in clear site in 2003.

Sample	C_t	R_t	M_t	$C_tM_t^2$	R_tM_t	R_t^2/C_t	R_t/C_t	Date	Air temp. °C	Soil temp. °C	ants
1	3	0	0	0	0	0	0	08/2/03	22.5	26	60
2	3	1	3	27	3	0.33	0.33	12/2/03	22.5	25	90
3	1	0	5	25	0	0	0	13/2/03	21.3	24.9	100
4	3	1	6	108	6	0.33	0.33	17/2/03	25.3	29	80
5	2	1	8	128	8	0.5	0.5	18/2/03	23	26	70
6	3	0	9	243	0	0	0	19/2/03	22.5	28	100
7	2	1	12	288	12	0.5	0.5	23/2/03	21	27.6	120
8	3	2	13	507	26	1.33	0.66	24/2/03	20.3	29.1	200
9	5	1	14	980	14	0.2	0.2	04/3/03	20	30	150
10	3	0	18	972	0	0	0	05/3/03	22	30	200
11	3	2	21	1323	42	1.33	0.66	11/3/03	25	32.2	300
12	3	1	22	1452	22	0.33	0.33	15/3/03	21	29.1	260
13	3	1	24	1728	24	0.33	0.33	16/3/03	23.4	32.3	350
Sum				7781	157	5.2		Mean	22.29	28.40	160.00

N=	49.56			DF=	11	
Var(1/N)=	0.185			SE(1/N)=	0.00487	
CI(1/N)=	0.009	,	0.031			
CI(N)=	32.36	,	105.8 ←	95% CI		

C_t = Total number of individuals caught in sample t

R_t = Number of individuals already marked when caught in sample t

M_t = Number of marked individuals in the population just before sample t is taken

Table 12 Pitfall trap data for estimation of population size of lizards and ants in clear site in 2003.

Sample	C_t	R_t	M_t	$C_tM_t^2$	R_tM_t	R_t^2/C_t	R_t/C_t	Date	Air temp. °C	Soil temp. °C	ants
1	1	0	0	0	0	0	0	08/02/03	24.2	27.4	25
2	1	0	1	1	0	0	0	12/02/03	23.2	25	10
3	1	1	2	4	2	1	1	13/02/03	21.6	24.9	20
4	1	0	2	4	0	0	0	17/02/03	25.6	31	18
5	2	1	3	18	3	0.5	0.5	18/02/03	23	26	25
6	2	1	4	32	4	0.5	0.5	19/02/03	22.8	30.1	15
7	1	1	5	25	5	1	1	23/02/03	23.5	30.5	12
8	1	0	5	25	0	0	0	24/02/03	21.3	30.5	28
9	2	1	6	72	6	0.5	0.5	04/03/03	22.4	31.8	22
10	1	0	7	49	0	0	0	05/03/03	23.2	32	20
11	2	0	8	128	0	0	0	11/03/03	24	32.5	16
12	1	1	10	100	10	1	1	15/03/03	23.9	34	24
13	1	1	10	100	10	1	1	16/03/03	25.8	35.3	28
Sum				558	40	5.5		Mean	23.42	30.08	20.23

N=	13.95			DF=	11
Var(1/N)=	0.239			SE(1/N)=	0.02071
CI(1/N)=	0.026	,	0.117		
CI(N)=	8.528	,	38.31 ←	95% CI	

C_t = Total number of individuals caught in sample t

R_t = Number of individuals already marked when caught in sample t

M_t = Number of marked individuals in the population just before sample t is taken

Table 13 Pitfall trap data for estimation of population size of lizards and ants in soot site in 2003.

Sample	Ct	Rt	Mt	$CtMt^2$	$RtMt$	Rt^2/Ct	Rt/Ct	Date	Air temp. °C	Soil temp. °C	ants
1	2	0	0	0	0	0	0	08/02/03	22.5	30	10
2	3	0	2	12	0	0	0	12/02/03	22	28	8
3	3	2	5	75	10	1.33	0.66	13/02/03	21.2	27.1	12
4	3	1	6	108	6	0.33	0.33	17/02/03	24	29.5	6
5	3	2	8	192	16	1.33	0.66	18/02/03	21.5	26.8	15
6	2	1	9	162	9	0.5	0.5	19/02/03	23	30.2	20
7	2	1	10	200	10	0.5	0.5	23/02/03	21	29	14
8	3	2	11	363	22	1.33	0.66	24/02/03	20.3	29.3	25
9	2	1	12	288	12	0.5	0.5	04/03/03	20.8	29.6	30
10	1	0	13	169	0	0	0	05/03/03	22.1	31	20
11	1	1	14	196	14	1	1	11/03/03	25.3	34.1	30
12	1	0	14	196	0	0	0	15/03/03	21.7	31	50
13	2	2	15	450	30	2	1	16/03/03	23	32	40
Sum				2411	129	8.83		Mean	22.18	29.82	21.54

N=	18.69			DF=	11
Var(1/N)=	0.176			SE(1/N)=	0.00853
CI(1/N)=	0.035	,	0.072		
CI(N)=	13.83	,	28.8 ←	95% CI	

Ct = Total number of individuals caught in sample t

Rt = Number of individuals already marked when caught in sample t

Mt = Number of marked individuals in the population just before sample t is taken

Table 14 Pitfall trap data for estimation of population size of lizards and ants in soot site in 2003.

Sample	Ct	Rt	Mt	$CtMt^2$	$RtMt$	Rt^2/Ct	Rt/Ct	Date	Air temp. °C	Soil temp. °C	ants
1	1	0	0	0	0	0	0	08/02/03	22.5	27	12
2	1	0	1	1	0	0	0	12/02/03	22.5	26	6
3	1	1	2	4	2	1	1	13/02/03	21.2	27.2	10
4	1	0	2	4	0	0	0	17/02/03	24.5	28.5	12
5	1	1	3	9	3	1	1	18/02/03	23.5	27.6	15
6	1	1	3	9	3	1	1	19/02/03	23	29.2	15
7	1	0	3	9	0	0	0	23/02/03	22	29.5	22
8	1	1	4	16	4	1	1	24/02/03	21.3	29.8	25
9	1	1	4	16	4	1	1	04/03/03	20.7	30.6	28
10	2	1	4	32	4	0.5	0.5	05/03/03	22.5	32	16
11	1	0	5	25	0	0	0	11/03/03	25.5	32.1	20
12	1	1	6	36	6	1	1	15/03/03	22.6	34	40
13	2	1	6	72	6	0.5	0.5	16/03/03	23.8	34.5	30
Sum				233	32	7.00		Mean	23.25	30.02	22.45

N=	8.951				DF=	11
Var(1/N)=	0.196				SE(1/N)=	0.01853
CI(1/N)=	0.075	,	0.172			
CI(N)=	6.183	,	13.8	← 95% CI		

Ct = Total number of individuals caught in sample t

Rt = Number of individuals already marked when caught in sample t

Mt = Number of marked individuals in the population just before sample t is taken

Table 15 Pitfall trap data for estimation of population size of lizards and ants in tar mat site in 2003.

Sample	Ct	Rt	Mt	$CtMt^2$	$RtMt$	Rt^2/Ct	Rt/Ct	Date	Air temp. °C	Soil temp. °C	ants
1	2	0	0	0	0	0	0	08/02/03	19.8	25.1	50
2	2	0	2	8	0	0	0	12/02/03	20	21.8	40
3	2	0	4	32	0	0	0	13/02/03	18.4	22	60
4	2	1	6	72	6	0.5	0.5	17/02/03	21.6	25.3	30
5	4	2	7	196	14	1	0.5	18/02/03	17.9	22.1	80
6	4	1	9	324	9	0.25	0.25	19/02/03	21.5	27	60
7	2	1	12	288	12	0.5	0.5	23/02/03	20	28	130
8	2	1	13	338	13	0.5	0.5	24/02/03	18	26	190
9	3	1	14	588	14	0.33	0.33	04/03/03	17	25	170
10	2	1	16	512	16	0.5	0.5	05/03/03	19	26	250
11	4	1	17	1156	17	0.25	0.25	11/03/03	23.5	30	280
12	2	1	20	800	20	0.5	0.5	15/03/03	20	26.9	400
13	2	1	21	882	21	0.5	0.5	16/03/03	21.6	27.8	350
Sum				5196	142	4.83		Mean	19.87	25.62	160.77

N=	36.59			DF=	11
Var(1/N)=	0.087			SE(1/N)=	0.004
CI(1/N)=	0.018	,	0.036		
CI(N)=	27.54	,	54.52 ←	95% CI	

Ct = Total number of individuals caught in sample t

Rt = Number of individuals already marked when caught in sample t

Mt = Number of marked individuals in the population just before sample t is taken

Table 16 Pitfall trap data for estimation of population size of lizards and ants in tar mat site in 2003.

Sample	Ct	Rt	Mt	$CtMt^2$	$RtMt$	Rt^2/Ct	Rt/Ct	Date	Air temp. °C	Soil temp. °C	ants
1	1	0	0	0	0	0	0	08/02/03	21.5	27	20
2	1	0	1	1	0	0	0	12/02/03	21.5	24.9	10
3	1	1	2	4	2	1	1	13/02/03	20	24	30
4	1	0	2	4	0	0	0	17/02/03	24.1	29	20
5	1	1	3	9	3	1	1	18/02/03	22	25	60
6	3	1	3	27	3	0.33	0.33	19/02/03	21.2	28.5	50
7	1	1	5	25	5	1	1	23/02/03	20.5	29	80
8	1	0	5	25	0	0	0	24/02/03	20	28.6	150
9	2	2	6	72	12	2	1	04/03/03	20	29	200
10	3	2	6	108	12	1.33	0.66	05/03/03	21.5	29	180
11	1	0	7	49	0	0	0	11/03/03	24	32	250
12	1	1	8	64	8	1	1	15/03/03	21	28	300
13	3	3	8	192	24	3	1	16/03/03	22.8	29	300
Sum				580	69	10.66		Mean	21.55	27.92	126.92

N=	8.406				DF=	11
Var(1/N)=	0.223				SE(1/N)=	0.01963
CI(1/N)=	0.076	,	0.162			
CI(N)=	6.166	,	13.2	←	95% CI	

Ct = Total number of individuals caught in sample t

Rt = Number of individuals already marked when caught in sample t

Mt = Number of marked individuals in the population just before sample t is taken

APPENDIX B

Transect data for the varied sites 2002 - 2003

Table 1 Transect data for estimating population size of lizards and ants in control site in 2002

Sample	Lizards	ants	Date	Air temp. °C	Soil temp. at start °C	Soil temp. at finish °C	Time at start (h)	Time at finish (h)	Time spent searching (min)
1	2	13	12/02/02	22	26.2	27	09:10	09:20	10
2	1	24	13/2/02	23.6	28	28.9	09:15	09:24	9
3	2	33	14/2/02	24.1	29	29.6	09:30	09:40	10
4	2	0	02/03/02	24.5	29.5	30.6	09:45	09:55	10
5	1	0	03/03/02	24.9	30	31.1	10:05	10:15	10
6	2	16	09/03/02	25.2	30.5	31.8	09:20	09:30	10
7	1	16	10/03/02	23.6	29.6	30.5	09:30	09:40	10
8	2	0	11/03/02	24.3	30.5	31.6	09:40	09:48	8
9	1	0	06/04/02	25.8	31.6	32.8	09:25	09:35	10
10	1	8	07/04/02	25.6	33.5	34.7	09:43	09:52	9
Mean	1.50	11.00		24.36	29.84	30.86	0.40	0.40	9.60
S.D.	0.53	11.55		1.13	1.97	2.12	0.01	0.01	0.70

Table 2 Transect data for estimating population size of lizards and ants in control site in 2002

Sample	lizards	ants	Date	Air temp. °C	Soil temp. at start °C	Soil temp. at finish °C	Time at start (h)	Time at finish (h)	Time spent searching (min)
1	0	10	12/02/02	22.3	26.8	27.6	09:40	09:50	10
2	1	10	13/02/02	23.5	28	28.7	09:45	09:55	10
3	1	12	14/02/02	24.5	29.8	30.5	09:55	10:05	10
4	1	11	02/03/02	24.8	30	31.6	10:15	10:25	10
5	1	10	03/03/02	25	31	31.7	10:30	10:40	10
6	1	12	09/03/02	25.6	31.2	32.6	10:45	10:55	10
7	1	10	10/03/02	23.8	30.2	31.4	10:00	10:10	10
8	2	7	11/03/02	24.5	31.1	32.3	10:10	10:18	8
9	1	10	06/04/02	26	33	34.2	10:15	10:25	10
10	2	20	07/04/02	25.2	33.2	34.6	10:20	10:30	10
Mean	1.10	11.20		24.52	30.43	31.52	0.42	0.43	9.80
S.D.	0.57	3.39		1.09	1.98	2.18	0.01	0.01	0.63

Table 3 Transect data for estimating population size of lizards and ants in clear site in 2002

Sample	lizards	ants	Date	Air temp.°C	Soil temp. at start °C	Soil temp. at finish °C	Time at start (h)	Time at finish (h)	Time spent searching (min)
1	1	14	09/02/02	23.5	27.5	28.2	11:20	11:28	8
2	1	25	10/02/02	24.2	28.6	29.8	11:50	11:59	9
3	2	30	11/02/02	25.3	29	29.8	11:20	11:30	10
4	1	25	18/02/02	24.6	29.3	30.2	11:30	11:39	9
5	1	10	19/02/02	22.9	30.6	31.5	11:38	11:48	10
6	0	25	04/03/02	25	32	33.2	11:20	11:30	10
7	2	33	05/03/02	25	32.8	33.6	12:05	12:15	10
8	1	20	06/03/02	25.3	33	34.2	10:52	11:00	8
9	1	40	03/04/02	25.3	33.5	34.3	11:20	11:30	10
10	1	35	04/04/02	26.2	34.2	35.8	11:35	11:45	10
Mean	1.10	25.7		24.73	31.05	32.06	0.48	0.49	9.40
S. D.	0.57	9.31		0.97	2.35	2.50	0.01	0.01	0.84

Table 4 Transect data for estimating population size of lizards and ants in clear site 2002

Sample	lizards	ants	Date	Air temp.°C	Soil temp. at start °C	Soil temp. at finish °C	Time at start (h)	Time at finish (h)	Time spent searching (min)
1	0	9	09/02/02	23.6	28.1	28.9	11:55	12:05	10
2	1	20	10/02/02	24.5	28.9	29.5	12:25	12:35	10
3	1	12	11/02/02	25.6	28.8	29.5	12:00	12:10	10
4	1	7	18/02/02	24.8	29	29.8	12:05	12:15	10
5	0	11	19/02/02	23.2	30	30.8	12:10	10:20	10
6	1	6	04/03/02	25.2	32.3	33.7	12:00	12:10	10
7	1	12	05/03/02	25.5	33	33.6	12:35	12:45	10
8	0	18	06/03/02	25.6	33.5	34.6	11:30	11:40	10
9	1	15	03/04/02	25.5	33.8	34.5	12:00	12:10	10
10	0	5	04/04/02	26.4	34.8	35.6	12:15	12:25	10
Mean	0.60	11.50		24.99	31.22	32.05	0.50	0.50	10.00
S.D.	0.52	5.02		0.98	2.50	2.58	0.01	0.03	0.00

Table 5 Transect data for estimating population size of lizards and ants in soot site in 2002

Sample	lizards	ants	Date	Air temp.°C	Soil temp. at start °C	Soil temp. at finish °C	Time at start (h)	Time at finish(h)	Time spent searching (min)
1	1	7	09/02/02	22.8	28.5	29.2	10:15	10:25	10
2	0	12	10/02/02	23.6	28.2	28.7	10:40	10:50	10
3	0	8	11/02/02	24.8	28.5	28.9	10:15	10:25	10
4	0	10	18/02/02	24.2	29.4	30	10:30	10:38	8
5	1	0	19/02/02	22.4	30.3	31	10:30	10:39	9
6	1	12	04/03/02	24.7	32.2	33.5	10:10	10:18	8
7	1	20	05/03/02	25.2	34.6	35.9	09:55	10:05	10
8	0	18	06/03/02	25.7	34.5	35.5	09:50	10:00	10
9	1	23	03/04/02	25.6	35.4	36.5	10:08	10:18	10
10	1	21	04/04/02	25.9	35.6	36.7	10:30	10:40	10
Mean	0.60	13.10		24.49	31.72	32.59	0.43	0.43	9.50
S.D.	0.52	7.29		1.22	3.08	3.36	0.01	0.01	0.85

Table 6 Transect data for estimating population size of lizards and ants in soot site in 2002

Sample	lizards	ants	Date	Air temp.°C	Soil temp. at start °C	Soil temp. at finish °C	Time at start (h)	Time at finish (h)	Time spent searching (min)
1	0	6	09/02/02	23.2	29.5	30.6	10:50	11:00	10
2	1	4	10/02/02	24	29.4	30	11:20	11:28	8
3	0	4	11/02/02	25	29.8	30.5	10:50	11:00	10
4	0	7	18/02/02	24.7	30.6	31.2	11:00	11:09	9
5	0	8	19/02/02	22.8	31.2	31.8	11:05	11:14	9
6	1	11	04/03/02	24.8	33	34.2	10:50	11:00	10
7	0	4	05/03/02	25.2	34.1	35.3	10:30	11:38	8
8	1	12	06/03/02	25.6	34.5	35.2	10:20	10:29	9
9	0	10	03/04/02	25.5	35.6	36.8	10:38	10:48	10
10	0	14	04/04/02	26	35.7	36.8	11:00	11:10	10
Mean	0.30	8.00		24.68	32.34	33.24	0.45	0.46	9.30
S.D.	0.23	3.62		1.05	2.53	2.70	0.01	0.01	0.82

Table 7 Transect data for estimating population size of lizards and ants in tar mat site in 2002

Sample	lizards	ants	Date	Air Temp.°C	Soil temp. at start °C	Soil temp. at finish °C	Time at start (h)	Time when at finish (h)	Time spent searching (min)
1	1	9	09/02/02	22	29	30.2	9:50	10:00	10
2	1	7	10/02/02	21.5	27.5	28.5	9:30	9:40	10
3	1	9	11/02/02	23	28.3	28.8	9:10	9:19	9
4	2	18	18/02/02	22	28	28.4	9:25	9:35	10
5	2	21	19/02/02	20.9	29	29.5	9:15	9:25	10
6	1	28	04/03/02	24	32.1	33	9:40	9:50	10
7	1	55	05/03/02	25.1	34.1	35.8	9:22	9:31	9
8	2	45	06/03/02	25.5	34	35.2	9:12	9:20	8
9	1	40	03/04/02	25	35.2	36.1	9:35	9:45	10
10	3	48	04/04/02	25.8	35.5	36.2	10:00	10:10	10
Mean	1.50	28.00		23.48	31.27	32.17	0.40	0.40	9.60
S.D.	0.71	17.87		1.82	3.22	3.41	0.01	0.01	0.70

Table 8 Transect data for estimating population size of lizards and ants in tar mat site in 2002

Sample	lizards	ants	Date	Air temp.°C	Soil temp. at start °C	Soil temp. at finish °C	Time at start (h)	Time at finish (h)	Time spent searching (min)
1	0	7	09/02/02	21	28.9	29.5	9:10	9:20	10
2	1	5	10/02/02	23	30.8	31.5	10:00	10:10	10
3	1	8	11/02/02	24	29	29.8	9:40	9:50	10
4	0	10	18/02/02	23.5	30.2	31.1	9:55	10:03	8
5	1	6	19/02/02	21.5	30.3	31.8	9:50	10:00	10
6	1	5	04/03/02	22	31.3	32.6	9:15	9:25	10
7	0	10	05/03/02	24	33.2	34.5	8:55	9:05	10
8	1	25	06/03/02	23.8	32.3	33.4	8:40	8:50	10
9	0	35	03/04/02	23.5	33.1	34	9:00	9:10	10
10	0	14	04/04/02	21.6	28	29	8:55	9:05	10
Mean	0.50	12.50		22.79	30.71	31.72	0.39	0.40	9.80
S.D.	0.53	9.90		1.15	1.79	1.91	0.02	0.02	0.63

Table 9 Transect data for estimating population size of lizards and ants in control site in 2003

Sample	Lizards	ants	Date	Air temp. °C	Soil temp. at start °C	Soil temp. at finish °C	Time at start (h)	Time at finish (h)	Time spent searching (min)
1	2	35	03/02/03	18	22.3	23	09:10	09:18	8
2	1	4	04/02/03	18.8	23.2	23.9	09:25	09:34	9
3	1	18	15/02/03	17.3	21.1	22	09:20	09:30	10
4	1	6	16/02/03	19.2	24	24.6	09:35	09:45	10
5	2	3	20/02/03	20.9	21.5	22.1	08:55	09:05	10
6	3	7	22/02/03	18.8	21.9	22.6	09:00	09:10	10
7	2	10	26/02/03	21.5	27	27.9	09:15	09:25	10
8	3	15	27/02/03	22.3	34.8	35.4	09:20	09:29	9
9	1	20	02/03/03	17.5	25.6	26.2	09:45	09:55	10
10	2	35	03/03/03	19.5	26.1	28.1	09:50	10:00	10
11	3	34	09/03/03	24.1	30.5	31.6	09:20	09:30	10
12	2	45	10/03/03	23.2	30.2	31.9	09:45	09:55	10
13	3	50	12/03/03	25.1	35	36.8	09:15	09:25	10
14	1	25	19/03/03	26	35.6	36.1	09:10	09:20	10
Mean	1.93	21.9		20.87	27.06	28.01	0.39	0.40	9.71
S.D.	0.83	15.6		2.88	5.27	5.42	0.01	0.01	0.61

Table 10 Transect data for estimating population size of lizards and ants in control site in 2003

Sample	lizards	ants	Date	Air Temp.°C	Soil temp. at start °C	Soil temp. at finish °C	Time at start (h)	Time at finished (h)	Time spent searching (min)
1	0	6	03/02/03	18.9	23.2	23.7	09:30	09:38	8
2	1	4	04/02/03	18.5	22.6	23.2	09:45	09:55	10
3	2	5	15/02/03	19.6	24.4	24.9	09:50	09:57	7
4	2	4	16/02/03	20	25.1	25.8	10:00	10:07	7
5	1	24	20/02/03	22	25.3	26.4	09:35	09:45	10
6	2	13	22/02/03	23.5	30.7	32.3	09:40	09:48	8
7	2	20	26/02/03	23	34.5	35	09:40	09:50	10
8	1	15	27/02/03	22.9	34.4	35.2	09:45	09:55	10
9	2	11	02/03/03	19.8	28.5	29.3	10:30	10:40	10
10	1	13	03/03/03	21	29.3	30.9	10:30	10:39	9
11	2	22	09/03/03	24.5	32.1	32.8	09:50	10:00	10
12	2	20	10/03/03	25	32.2	33.7	10:20	10:29	9
13	2	15	12/03/03	25.8	34.5	35.1	09:45	09:55	10
14	1	12	19/03/03	26.4	35.6	36.2	09:45	09:55	10
Mean	1.50	13.1		22.21	29.46	30.32	0.41	0.42	9.14
S.D.	0.65	6.72		2.63	4.62	4.69	0.01	0.01	1.17

Table 11 Transect data for estimating population size of lizards and ants in clear site in 2003

Sample	lizards	ants	Date	Air Temp.°C	Soil temp. at start °C	Soil temp. at finish °C	Time at start (h)	Time at finish (h)	Time spent searching (min)
1	3	22	08/02/03	23	26.2	26.4	10:45	10:54	9
2	4	30	12/02/03	21.3	22.6	22.8	10:25	10:34	9
3	2	27	13/02/03	20.2	23.1	23.3	10:30	10:40	10
4	3	46	17/02/03	26	29.1	29.7	12:05	10:34	9
5	2	30	18/02/03	22.6	24.6	25.1	11:25	11:35	10
6	3	41	19/02/03	23	28.9	29.8	11:10	11:19	9
7	2	24	23/02/03	22	28	28.7	11:05	11:15	10
8	2	30	24/02/03	19.6	27.8	28.5	11:20	11:30	10
9	2	25	04/03/03	19.2	29	29.8	10:50	11:00	10
10	2	54	05/03/03	22.7	30.5	31.6	10:35	10:45	10
11	3	40	11/03/03	24.2	31.3	32.1	11:05	11:15	10
12	1	62	15/03/03	22.5	29.2	30.8	11:10	11:20	10
13	3	90	16/03/03	24.1	32.9	33.5	11:10	11:20	10
14	2	70	17/03/03	24	32	33.6	10:50	10:59	9
Mean	2.43	42.2		22.46	28.23	28.98	0.46	0.46	9.64
S.D.	0.76	20.2		1.90	3.15	3.46	0.02	0.01	0.50

Table 12 Transect data for estimating population size of lizards and ants in clear site in 2003

Sample	Lizards	ants	Date	Air Temp.°C	Soil temp. at start °C	Soil temp. at finish °C	Time at start (h)	Time at finish (h)	Time spent searching (min)
1	0	7	08/02/03	23.2	26.4	26.6	11:15	11:23	8
2	2	4	12/02/03	22	23.6	23.8	11:00	11:10	10
3	1	6	13/2/03	20.6	23.8	24	11:15	11:24	9
4	1	6	17/2/03	26.6	30.5	31.1	11:10	11:19	9
5	1	16	18/2/03	23.5	26.2	26.9	12:05	12:14	9
6	3	6	19/2/03	23.6	30	30.5	11:50	11:59	9
7	1	3	23/2/03	22.9	29.6	30.8	11:50	11:58	8
8	1	8	24/2/03	20.5	28.6	29.7	12:05	12:14	9
9	1	5	04/03/03	21	29.3	31	11:30	11:39	9
10	1	15	05/03/03	22.6	31.7	32.1	11:25	11:35	10
11	1	8	11/03/03	24.8	32.3	33.8	11:55	12:05	10
12	1	32	15/3/03	24.5	34.2	35.4	11:40	11:50	10
13	2	12	16/3/03	23	32.2	33	12:02	12:11	9
14	2	22	17/3/03	25.3	34.3	35.1	12:00	12:10	10
Mean	1.29	10.7		23.15	29.48	30.27	0.49	0.49	9.21
S.D.	0.73	8.14		1.78	3.45	3.73	0.02	0.02	0.70

Table 13 Transect data for estimating population size of lizards and ants in soot site in 2003

Sample	lizards	ants	Date	Air Temp.°C	Soil temp. at start °C	Soil temp. at finish °C	Time at start (h)	Time at finish (h)	Time spent searching (min)
1	2	11	08/02/03	21.9	28	28.3	09:50	09:58	8
2	3	8	12/02/03	20.5	24.8	25	09:30	09:38	8
3	1	18	13/02/03	19.5	21.6	21.9	09:20	09:28	8
4	2	23	17/02/03	23.6	27.2	27.8	09:05	09:14	9
5	1	54	18/02/03	20	24.4	24.9	09:50	09:59	9
6	1	11	19/02/03	22.5	28.5	29	09:35	09:45	10
7	2	46	23/02/03	19.6	27.8	28.5	09:35	09:45	10
8	1	40	24/02/03	19.4	27.2	27.9	09:55	10:04	9
9	1	22	04/03/03	19.2	27.4	28.1	09:30	09:40	10
10	2	7	05/03/03	20	28.3	28.9	09:55	10:04	9
11	1	32	11/03/03	24.2	32.4	33.2	09:35	09:45	10
12	1	25	15/03/03	20.3	27	27.9	09:40	09:49	9
13	2	51	16/03/03	21.5	28.6	29.1	09:50	09:59	9
14	2	23	17/03/03	21.9	30.1	30.5	09:30	09:40	10
Mean	1.57	26.5		21.01	27.38	27.93	0.40	0.41	9.14
S.D.	0.65	15.9		1.61	2.57	2.67	0.01	0.01	0.77

Table 14 Transect data for estimating population size of lizards and ants in soot site in 2003

Sample	lizards	ants	Date	Air temp. °C	Soil temp. at start °C	Soil temp. at finish °C	Time at start (h)	Time at finish (h)	Time spent searching (min)
1	0	3	08/02/03	23.5	30.8	31	11:45	11:54	9
2	0	2	12/02/03	22.6	26	26.3	11:35	11:44	9
3	1	17	13/02/03	21.9	24.3	24.7	11:50	11:58	8
4	0	9	17/02/03	27.2	32.3	33.1	11:45	11:55	10
5	2	21	18/02/03	24.3	30.3	30.9	12:36	12:44	9
6	0	17	19/02/03	24.2	31.2	32.9	12:25	12:33	8
7	0	4	23/02/03	23.5	30.6	31.8	11:50	11:58	8
8	0	2	24/02/03	21.5	30.5	31.9	12:45	12:55	10
9	1	20	04/03/03	22.3	31.9	32.6	12:10	12:20	10
10	1	21	05/03/03	23	32.6	33.1	12:10	12:20	10
11	0	19	11/03/03	25.3	33.9	34.5	12:35	12:45	10
12	2	8	15/03/03	23.3	30.2	31.5	12:40	12:50	10
13	0	18	16/03/03	25.8	34.6	35.8	12:40	12:49	9
14	0	20	17/03/03	25.3	35.6	36.1	12:20	12:30	10
Mean	0.49	12.9		23.84	31.06	31.87	0.51	0.52	9.29
S.D.	0.32	7.76		1.62	3.03	3.15	0.02	0.02	0.83

Table 15 Transect data for estimating population size of lizards and ants in tar mat site in 2003

Sample	lizards	ants	Date	Air temp. °C	Soil temp. at start °C	Soil temp. at finish °C	Time at start (h)	Time at finish (h)	Time spent searching (min)
1	2	10	08/02/03	21.5	29	29.9	09:25	09:35	10
2	2	20	12/02/03	20.9	26	26.3	08:55	09:05	10
3	3	13	13/02/03	18.9	25.3	25.5	08:35	08:44	9
4	3	23	17/02/03	22.3	28.6	28.9	08:30	08:40	10
5	4	42	18/02/03	18.2	25	25.6	09:10	09:20	10
6	2	35	19/02/03	22	30	30.8	09:00	09:10	10
7	3	30	23/02/03	20	28.1	28.5	09:01	09:10	9
8	2	27	24/02/03	18.6	28.2	28.8	09:05	09:15	10
9	2	34	04/03/03	17.5	26.2	26.5	09:01	09:10	9
10	2	55	05/03/03	19.1	29.1	29.9	09:06	09:15	9
11	3	70	11/03/03	24	33.2	33.8	09:00	09:10	10
12	3	65	15/03/03	20.7	29.1	29.6	09:00	09:10	10
13	2	96	16/03/03	22.2	32.2	32.9	09:05	09:15	10
14	2	75	17/03/03	21.5	30.2	31.6	08:50	09:00	10
Mean	2.50	42.5		20.53	28.59	29.19	0.37	0.38	9.71
S.D.	0.65	25.8		1.87	2.42	2.59	0.01	0.01	0.47

Table 16 Transect data for estimating population size of lizards and ants in tar mat site in 2003

Sample	lizards	ants	Date	Air temp.°C	Soil temp. At start °C	Soil temp. at finish °C	Time at start (h)	Time at finish (h)	Time spent searching (min)
1	1	8	08/02/03	22.6	31.5	31.9	10:15	10:24	9
2	3	29	12/02/03	22	28.6	29.4	09:55	10:05	10
3	2	27	13/02/03	21	26.2	26.9	09:50	09:59	9
4	2	44	17/02/03	25	29.2	29.9	09:45	09:55	10
5	0	15	18/02/03	22.3	28.6	28.3	10:30	10:40	10
6	1	17	19/02/03	22	31.2	32	10:15	10:25	10
7	0	30	23/02/03	21.3	29.1	30.5	10:25	10:34	9
8	0	25	24/02/03	20	30.9	31.7	10:35	10:45	10
9	0	35	04/03/03	20.1	29.1	30.2	10:05	10:15	10
10	0	40	05/03/03	22	31.2	32.9	09:47	09:56	9
11	0	50	11/03/03	24.5	33.5	34.1	10:20	10:30	10
12	1	43	15/03/03	21.8	28.5	29.2	10:25	10:35	10
13	1	50	16/03/03	23.3	34.4	35.6	10:30	10:40	10
14	1	25	17/03/03	22.7	32.3	34.1	10:05	10:15	10
Mean	0.86	31.3		22.19	30.31	31.19	0.42	0.43	9.71
S.D.	0.95	13		1.43	2.23	2.45	0.01	0.01	0.47

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