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Ecological and genetic studies on the Arabian spiny-tailed lizard Uromastyx aegyptia microlepis (BLANFORD, 1875) in the State of Kuwait

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Ecological and genetic studies on the Arabian spiny-tailed lizard *Uromastyx aegyptia microlepis* (Blanford, 1875) in the State of Kuwait

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DECLARATION

I hereby declare that this thesis is the results of my own investigations, except where otherwise stated. All other sources are acknowledged by bibliographic references. This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree unless, as agreed by the University, for approved dual awards.

ABSTRACT

In order to assess the global driving factors affecting the status and population threads of desert lizards IUCN Red List database were reviewed covering the years 2006 to 2021. The findings show that residential/commercial development, agriculture, invasive species, human disturbance, energy/mining, pollution, transportation and biological use had a profound negative influence on both critical status and population trends. The Arabian spiny-tailed lizard *Uromastyx aegyptia microlepis* is a common lizard species in the arid habitats of Kuwait. The ecology and genetics of this species were investigated through a series of field studies and laboratory experiments carried out between 2017 and 2019 at four sites in Kuwait desert (two protected and two unprotected areas). The study targeted the prioritization of the most important areas for the conservation of this species using the Maximum Entropy Distribution Model (MaxEnt). Based on the results of MaxEnt, distribution and prioritisation maps for the conservation of this lizard in Kuwait were developed. The results showed that Al-Huwaimliya and Nuwaiseeb areas were the most important areas for conservation of *Uromastyx aegyptia microlepis*. To assess the condition of the populations of this lizard, a morphometric study was conducted at the four study sites adopting the Scaled Mass Index (SMI). The results showed that the protected areas did not affect the SMI of this lizard. The study also revealed that the lizard is capable of adapting to the scarcity of resources in unprotected areas. Microsatellite genotyping was used to investigate genetic diversity within the different *Uromastyx* populations at the four study sites. The results show that protected areas did not promote genetic heterozygosity within the Arabian spiny-tailed lizard, while unprotected habitats had no impact on genetic diversity within populations of this species. Based on the results of the studies conducted, it is recommended that the conservation responses of the government sectors be restructured and modified to better protect the hotspot habitats of this species to produce more positive impacts on it. Furthermore, the results of the study call for a greater understanding of the ecology of this species for more effective conservation actions at the local, regional and international levels.

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Table of Contents

CHAPTER 1

CHAPTER 2

CHAPTER 3

CHAPTER 4
THE SIGNIFICANCE OF PROTECTED AREAS FOR THE CONSERVATION OF *UROMASTYX AEGYPTIA MICROLEPIS* IN THE STATE OF KUWAIT 74

CHAPTER 5
POPULATION GENETICS OF *UROMASTYX AEGYPTIA MICROLEPIS* IN THE STATE OF KUWAIT 109

CHAPTER 6

List of Figures

List of Tables

List of Abbreviations

AI	Artificial Intelligence
AUC	Area under the ROC Curve
ANOVA	Analysis of Variance
BCI	Body Condition Index
BMI	Body Mass Index
CBD	Convention on Biological Diversity
CIS	Condition Indices
CITES	Convention on International Trade in Endangered Species of Wild Fauna and Flora
CSV	Comma Separated Values
DEM	Digital Elevation Model
DZ	Demilitarized Zone
EPA	Environment Public Authority, State of Kuwait
GCC	Gulf Cooperation Council
GIS	Geographic Information System
GLM	Generalized Linear Model
GPS	Global Positioning System
IOC	Inverse Optimal Control
IUCN	International Union for Conservation of Nature
KEPS	Kuwait Environment Protection Society
KISR	Kuwait Institute for Scientific Research
KOC	Kuwait Oil Company
MaxEnt	Maximum Entropy Distribution Model
NDVI	Normalized Difference Vegetation Index
NGO	Non-Governmental Organization
NIR	Near-Infrared
OLS	Ordinary Least Squares
PA	Protected Area
PAAF	Public Authority for Agriculture Affairs and Fish Resources, State of Kuwait
PCR	Polymerase Chain Reaction
PIT	Passive Induced Transponder
RMI	Relative Mass Index
SMI	Scale Mass Index
SNP	Single Nucleotide Polymorphism
SPSS	Statistical Package for the Social Sciences
STR	Short Tandem Repeats
SVL	Snout to Vent Length
UN	United Nations
UNCED	United Nations Conference on Environment and Development
UNDP	United Nations Development Programme
UNESCO	United Nations Educational, Scientific and Cultural Organization
WGS	Whole Genome Sequencing

Chapter 1

General Introduction

1.1 Conservation of biodiversity

Conservation is an attempt to sustain, restore and/or reclaim the integrity of the processes as the biogeochemical cycles, interactions within the biotic elements and the abiotic constituents and between the biotic and abiotic components of the ecosystems (Brooks et al., 2006; Hoffmann et al., 2008; Hoffmann et al., 2010b; Rawat & Agarwal, 2015). On earth, conservationists endeavour to mitigate, control or adapt to the sources of disturbances imposed on the ecosystems around the biomes caused by mankind (Goldstein, 1999; Swinton et al., 2007). An important aspect of conservation is the sustainability of biodiversity to benefit from species ecosystem services to maintain equilibrium state in ecosystems by maximizing the ecosystems' resistance and the resilience of ecosystems (Goldstein, 1999; Hobfoll, 2011).

Due to the increasing demands of humans on the earth resources and the continuous pressures imposed on such balanced systems, the biodiversity trends of ecosystems, habitats, species and genetic components are decreasing (Pereira & Cooper, 2006). Many species as a result are threatened or declared extinct in the wild or are completely extinct (Chu & Karr, 2017; Gülsoy et al., 2022). Other drivers of the environmental issues have emerged because of population and economic growth such as urbanization, climate change, invasive alien species which magnified the need for conservation efforts targeting ecosystems, habitats and species (McKinney, 2002; Brunel et al., 2013). Biodiversity conservation has become a global priority in the last century due to its crucial role in ensuring the survival of humanity.

An important cornerstone of conservation is the establishment of protected areas (PAs) (Naughton-Treves et al., 2005). Many success stories of protected areas shed light on the importance of this conservation procedure. Protected areas sustain multiple components of ecosystems such as physiography, geological features, rangelands, vegetation, and species (Janishevski et al., 2015). Further, social aspects could be conserved such as human heritage, and traditional knowledge and technologies which is noted with the establishment of world heritage sites governed by the United Nations Educational, Scientific and Cultural Organization (UNESCO). In the xeric ecosystems of the Arabian Peninsula, military activities, population, and economic growth are drivers of change toward increasing the impacts of anthropogenic activities as urbanization, which is causing tremendous pressures and adverse results on the environment.

The establishment of protected areas was initially aimed at mitigating the impact of resource degradation on biodiversity loss. The Convention on Biodiversity (CBD) calls for the protection of 17% of terrestrial area and 10% of the world's oceans, through effectively and equitably managed, ecologically representative, and well-connected systems (PA) and other effective area-based conservation measures, by 2020 (CBD, 2010). In arid lands, such as the deserts of the Arabian Peninsula, protected areas play major roles in the survival of ecosystems and species. Due to the immense pressures of intensified military settlements, camping, mining, and the excessive use of 4x4 vehicles, protected areas that free ecosystems from such pressures show significant differences in species richness of several living organisms.

In the deserts of the Arabian Peninsula, the contrast is very obvious between protected and unprotected areas especially in terms of vegetation cover (Janishevski et al., 2015). Therefore, many governmental bodies in the Gulf Cooperation Council (GCC) countries have adopted protected areas as the first line of conservation of deteriorated ecosystems (Chape et al., 2005). Success stories in protected areas are achieved for numerous native species such as the Houbara bustard *Chlamydotis undulata*, the Arabian oryx *Oryx leucoryx*, and the Arabian sand gazelles *Gazella marica* in Mahazat As-Sayed and the Empty Quarter (Uruq Bani Maarid) in Saudi Arabia (Abuzinada, 2003; Islam et al., 2011; Wronski et al., 2011). In Oman, the Arabian leopard *Panthera pardus nimr* was successfully rehabilitated in the Dhofar area in the Jabal Samhan Protected Area (Spalton et al., 2006; Said et al., 2013). Additionally, in 2015 the Arabian Gazelle *Gazella arabica* was also managed to maintain its numbers in the Jabal Samhan Protected Area (Al Hikmani et al., 2015).

1.2 Conservation in the State of Kuwait

Kuwait has recognised the value of biological resources as an integral part of its natural heritage with the potential to provide long-term benefits to human's well-being and establish a foundation for sustained economic development. Therefore, in June 1992, it signed along with 155 other states, the Convention on Biological Diversity (CBD) at the United Nations Conference on Environment and Development (UNCED) held in Rio de Janeiro. The ratification of the CBD Convention took place on 2 August 2002, while the Nagoya Protocol and Cartagena Protocol were ratified on 5 June 2017. Therefore, the State of Kuwait is internationally committed to protect its biodiversity (EPA, 2019).

Kuwait has joined many global action conventions related to the protection of natural habitats and biodiversity. In addition to joining the Convention on Biodiversity, Kuwait has joined the IUCN in 1993 as a state member. Previously, in 1992, the Kuwait Environment Protection Society (KEPS) joined IUCN, while the Kuwait Institute for Scientific Research (KISR) later joined in 2000. In 2015, Kuwait ratified the Ramsar Convention on Wetlands of International Importance Especially as Waterfowl Habitat (also known as the Convention on Wetlands). Currently, there is only one designated Ramsar site in Kuwait (Mubarak Al-Kabeer) with an area of 638 km^2 (RAMSAR, 2023).

The main governmental authority for implementing the CBD and protocol requirements in Kuwait is the Environment Public Authority (EPA). In 1998, Kuwait, with the help of the United Nations Development Programme (UNDP) and the International Union for Conservation of Nature (IUCN), formulated its National Biodiversity Strategy. The immediate purpose of this Strategy was to place biodiversity considerations in national planning and mainstream development processes (EPA, 2019). The underlying theme of the National Strategy is improved coordination and effective harmonisation of sector policies, programmes and legislation to fill institutional gaps and reduce any overlaps that have direct effects on biodiversity. The most common conservation plan within protected areas is to conserve the function of ecosystems in order to restore their integrity.

The Strategy is designed to establish a robust and cohesive policy framework dedicated to the preservation of biodiversity and the sustainable use of Kuwait's biological resources (EPA, 2019). The primary objectives of the Strategy are multifaceted. First, it aims to identify and address the challenges and elements of biodiversity in Kuwait and proposing viable solutions. Second, it involves conducting a detailed examination of local developments and issues related to biodiversity conservation. Third, the Strategy emphasises the continuous update of Kuwait's National Biodiversity Strategy, providing scientific advice to decision makers.

This includes formulating recommendations for adoption at regional and international conventions. Finally, the Strategy involves rigorous follow-up on the implementation of decisions and recommendations from the States' Parties meetings and the review and endorsement of proposals and recommendations related to the relevant protocols of the CBD.

Furthermore, one of the main actions taken by the EPA for the protection of biodiversity in Kuwait is the establishment of protected areas. The CBD defines a protected area as a "geographical area that is regulated and managed to achieve specific conservation objectives". The IUCN has defined a global classification system for different types of protected areas with nature conservation as its main goal (IUCN, 2022). The IUCN has recommended that a minimum of 10% of each country's surface area should be under effective conservation management.

In addition to institutional protected area designations, there are several other *de facto* protected areas in Kuwait, including experimental range enclosures, exclusive rights areas controlled by the Kuwait Oil Company (KOC) and designated military areas and underground water extraction, in addition to Kuwait Airport. There is also a strictly enforced Demilitarised Zone along the Kuwait-Iraq border that has the potential to become a permanent border peace park. Currently, the notion of protected areas is established in the State of Kuwait.

The total protected areas in Kuwait are 2,074 km^2, which represents about 12% of the total area of the State of Kuwait (Kuwait Alyoum, 2016). The selection of these protected areas was made by the National Biodiversity Committee based on their location, type of habitat and biodiversity. Each area is classified differently based on its location, topography, biological diversity and intended uses using the widely accepted IUCN categorization of protected areas.

Kuwait has twelve protected areas, 10 of them are terrestrial and two are marine. There are additional proposed areas, which are still under consideration. As of now, terrestrial protected areas make up 10.7% of the total area of the country compared to 1.4% for marine protected areas. Protected areas are located in different parts of the country and cover deserts, islands, coastlines, sand dunes, gravel, mud flats and gypsum escarpment ecosystems (Fig. 1.1).

The pressures imposed on Kuwait's ecosystems have magnified in the last two decades. Population and economic growth in Kuwait have led to increased political demands to change the use/cover of natural habitats (Al-Awadhi et al., 2005; Al-Awadhi et al., 2014; Uddin, 2014). In particular, the demand for housing projects has jeopardised the well-being of the desert environment and has led to the disappearance of large areas of natural habitats.

Figure 1.1 Protected areas of the State of Kuwait (eMISK, 2022).

The negative impacts imposed on the implementation of habitat protection are mainly contributed by uncontrolled anthropogenic activities such as camping, poaching, overgrazing, urbanisation, mining and agriculture (Fig. 1.2). The most severe incident that occurred in 1991 was the Gulf War. Military activities that occurred during the war were responsible for exacerbating the deterioration of desert habitats in Kuwait. Military waste and severe deformation of surface topography in the desert resulting from this war have greatly pressurised the Kuwait desert toward the loss of habitats (Toukan, 1991). Furthermore, climate change and alien species have added more uncertainty about the success of protected areas.

The State of Kuwait has serious environmental problems that lead to the degradation of the remaining biodiversity and biological resources of the nation. The widespread desertification of rangelands, the weak enforcement of environmental legislation, the unregulated hunting of wild animals and excessive patterns of resource consumption are the main problems of concern (EPA, 2019). As a result of the serious challenges facing the habitats of the Kuwait desert, several species are declining and some have become extinct (EPA, 2019).

The declining large carnivores are the Arabian wolf *Canis lupus arabs* and the caracal *Caracal caracal*, which have become extremely rare. Among exterminated mammals are the dorcas gazelle *Gazella dorcas*, the mountain gazelle *Gazella gazella*, the Arabian sand gazelle *Gazella marica*, and the Asiatic cheetah *Acinonyx jubatus venaticus*. Due to these growing concerns, Kuwait has taken strong measures to ensure the protection and conservation of its ecosystems and biodiversity.

In fact, since 2010 the Kuwaiti EPA has established a long-term plan within the National Biodiversity Strategy to install an adequate, representative and ecologically viable system of protected areas in the terrestrial and marine environments of Kuwait to conserve indigenous wildlife and plant life. As a response, the most important legislation for the protection of biodiversity in the State of Kuwait is Legislation Number 42/2014 (Environmental Protection Law). Despite this legislation, the issue of the conservation of species and habitats in the State of Kuwait is not highly prioritised.

Figure 1.2 Total habitat destruction of the Kuwait desert due to: (a) Camel raising, (b) Extensive camping activities, (c) Garbage dumping (Photos by W. Behbehani).

1.3 Study region

The State of Kuwait is a small, flat, gently undulating desert country located in the most north-western corner of the Arabian Gulf. It is bordered by the Kingdom of Saudi Arabia to the south and the southwest, and the Iraq Republic to the north and northwest. The total area of Kuwait is 17,818 km^2 of land and about 1,000 km^2 of nine offshore islands (Amr et al., 2021). The only inhabited island is Failaka. the other islands are Boubyan and Warba to the north. Awhah, Kubbar, Qaruh, Miskan and Umm Al Maradim to the south in addition to Umm Al Namel in Kuwait Bay (Fig. 1.3).

The climate is characterised by extremely hot, dry summers with long and intense hours of sunshine and moderately cool, short winters with occasional rain. Dust storms often occur during the summer months. Relative humidity increases in summer but is generally low and temperatures sometimes reach 50°C in the shade. During winter, the temperature occasionally reaches 18°C but also drops below 0°C at night. Winter rainfall is minimal and irregular, varying from year to year and averaging about 115 mm a year (fluctuating between 25 and 250 mm). However, the rate of evaporation is very high, ranging from 3.1 to 21.6 mm per day. Rainfall occurs between mid-October and April and is sufficient to induce the germination of desert annuals in November. Strong, dry and hot north westerly winds prevail during summer, particularly in June and July (Omar et al., 2009).

Kuwait's soil is predominately sandy, poor in organic matter and low in water retention capacity. The dominant soil types are Aridisols (70.8%) and Entisols (29.2%) (Omar et al., 2001). Land use is dominated by range land (75.2%), which is mainly used for camel and sheep grazing, as well as recreational activities such as camping, human settlements, industrial sectors, power plants, oil fields, sand and gravel quarries and agricultural farms. The desert topsoil of Kuwait has been severely damaged mainly due to military activities during and after the Gulf War of 1991 and vehicular activities since then. These changes have produced alterations in surface sediment and morphological characteristics that result in environmental degradation (Omar et al., 2001).

Figure 1.3 General map of the State of Kuwait (eMISK, 2022).

1.4 Kuwait desert biodiversity

Despite harsh arid conditions, the Kuwait desert has a relatively rich fauna and flora. According to the IUCN publication "The State of Biodiversity in Kuwait" (Amr et al., 2021) there are one amphibian species, 39 reptile species, 30 mammal species, 407 bird species (mostly migrants), four scorpion species, and three spider species. Additionally, there are 492 insect species (Al-Houty, 2009). Furthermore, there is a high diversity of flora (451 species) consisting mainly of annual Angiospermae (Abdullah & Al-Dosari, 2022). Of the 39 reptile's species 26 are lizards indicating high diversity. A total of six families are represented: Gekkonidae, Agamidae, Trogonophidae, Scincidae, Lacertidae, and Varanidae (Table 1.1). The most common and largest lizards in the Kuwait desert is the Arabian-spiny-tailed lizard *Uromastyx aegyptia microlepis* (Amr et al., 2021).

Table 1.1 Total number of lizard species in the Kuwait desert belonging to different families (Amr et al., 2021).

Family	**Number of species**
Gekkonidae	9
Agamidae	5
Trogonophidae	1
Scincidae	4
Lacertidae	6
Varanidae	1
Total	**26**

1.5 Global status of desert lizards

Biodiversity around the world is impacted by constantly increasing pressures such as species extinction, habitat loss and distribution shifts (Pereira et al., 2010; Mora & Sale, 2011). Habitat loss is one of the main pressures that result in changes in structure, biotic composition and resource availability (Cuarón, 2000; Hobbs et al., 2009; Abdullah et al., 2019). Due to this, the disturbance negatively affects the biodiversity of the desert (Gonthier et al., 2014; Alvarez-Berríos et al., 2016).

Globally, deserts are deteriorating due to growing uncontrolled human activities that make all terrestrial vertebrates face many threatening processes. Habitat degradation, overexploitation, invasive species, and climate change are considered the most important processes (Thomas et al., 2004; Hoffmann et al., 2010a; Sinervo et al., 2010; Foden et al., 2013; Böhm et al., 2016). Multiple threatening processes can be exacerbated for most species (Cardillo et al., 2005; Hayward, 2011; Böhm et al., 2013).

Reptiles are a paraphyletic assemblage of approximately 11,136 species among which are important major contributors toward natural ecosystems (Read, 1998; Carroll, 2001; Raxworthy et al., 2008; Uetz & Hošek, 2020). The diversity of reptiles reflects the health of the environment and is a good indicator of viable ecosystems (Amr et al., 2021). Despite their biological and ecological importance, the conservation status of only half of the reptiles has been evaluated on the IUCN Red List (Böhm et al., 2013; Meiri & Chapple, 2016). Therefore, the statuses of most reptiles remain undetermined, which presents a real challenge for herpetologists, ecologists and conservationists seeking to ensure the existence of reptiles in the future.

In general, reptiles are essential biotic providers of ecosystem services toward a balanced ecosystem upon their interactions with abiotic and other biotic components. They control insects and arthropods, as well as facilitate the spread of seeds of native plants at different sites, by creating microhabitats for other living species (Valido & Olesen, 2007; Valencia-Aguilar et al., 2013).

Additionally, reptiles are an important food resource in resource-poor environments for a variety of predators (foxes, wolves, large birds of prey and monitors). Lizards are the largest group of reptiles with 6,827 species, which make up approximately 61% of reptiles with 163 recently described species up to 2020 (Uetz & Hošek, 2020). Many lizard species are threatened worldwide (Gibbons et al., 2000; Huey et al., 2010). Many reasons are given for the decline in their populations, including habitat loss, the introduction of invasive species, environmental pollution, and diseases, as well as unsustainable harvest/use and global climate change (Gibbons et al., 2000).

1.6 The targeted species

The subfamily Uromastycinae within the Agamidae family comprises 18 species, three of which are in the genus *Saara* and 15 within the genus *Uromastyx* (ITIS, 2024). The genus *Uromastyx* is a diverse group of lizards among which *Uromastyx aegyptia* (Forsskål, 1775) occurs in the Arabian Peninsula (Wilms et al., 2009). There are three subspecies in the Arabian Peninsula *U. a. aegyptia* (Forsskål, 1775), *U. a. microlepis* (Blanford, 1875), and *U. a. leptieni* (Wilms & Böhme, 2000). *Uromastyx* is prevalent in the deserts of North Africa and along the Arabian Peninsula toward Iran. This genus has undergone multiple vicariance and dispersal events during Saharo-Arabian colonisation due to tectonic movements and habitat fragmentation that resulted in the separation of Arabia from Africa and the expansion and contraction of arid areas in the region (Tamar et al., 2018).

Uromastyx has been listed in Appendix II of CITES in its entirety since 1977. Based on CITES data, more than 215,000 *Uromastyx* were legally traded between (1977-2001), almost all were wild-caught primarily from Egypt and Mali (Knapp, 2004). Of the known *Uromastyx* species, eight are listed on the IUCN Red List of Threatened Spices, with two assessed as Vulnerable, three as Near Threatened, and three as Least Concerned (IUCN, 2015).

The Arabian spiny-tailed commonly called "dhub" or "dabb" in Arabic (Fig. 1.4), has a wide distribution spanning deserts and semideserts of the Arabian Peninsula, including Kuwait, Yemen, Oman, Saudi Arabia, Jordan, Syria, Iraq, and immediate neighbouring countries such as Iran (Arnold, 1986; Leviton et al., 1992; Naldo et al., 2009; The Reptile Database, 2021; IUCN, 2022).

The Arabian spiny-tailed lizard is an ectothermic agamid that uses macro- and microhabitat shuttling to regulate its body temperature (Kotler & Brown, 1988; Bartholomew & Ebeid, 2011). It is a strong and tenacious vertebrate that can tolerate the extreme weather conditions of the desert (IUCN, 2022).

Figure 1.4 *Uromastyx aegyptia microlepis* in the desert of Kuwait (Photo by W. Behbehani).

The spiny-tailed lizard prefers to bask right after sunrise, at sunset and in the afternoon (Cunningham, 2000). It measures a body length of more than 700 mm and a mass of up to 2,500 g. It spends most of the year alone, while during May and June males and females join for mating and laying eggs in the burrow (Cunningham, 2000; Wilms et al., 2009). The hatchlings are seen close to the burrows in August and September (Wilms et al., 2010). The ecology of this lizard is affected by the marked seasonality of the desert environment (temperature, humidity, precipitation) that affects food availability and plant diversity.

This lizard prefers to live in a burrow to escape the extreme temperatures of the summer season and hibernates in the winter. By excavating its burrows, the lizard indirectly creates shelter for birds, reptiles and arthropods (Wilms et al., 2009; Al-Sayegh et al., 2020; Amr et al., 2021). The hoopoes and desert larks use lizard burrows to escape excessive heat during the middle of the day, where temperatures could reach 50°C. Weather conditions, soil type, vegetation and elevation are considered the factors determining the lizard's habitat use (Wilms et al., 2009; Aghanajafizadeh & Mobaraki, 2018). Aghanajafizadeh and Mobaraki (2018) found that slope, geographical direction of burrow opening, soil texture, plant cover, distance from water, roads and vegetation are influential in defining the distribution of the Arabian spiny-tailed lizard in Iran. In Saudi Arabia, Wilms et al., (2009) discovered that the ambient temperature inside and outside the burrow is an important characteristic of lizard habitats.

One supporting factor that enforces the success in lizards is their flexibility to change their trophic levels from herbivorous to total carnivorous, consuming a wide range of native plants, insects and arthropods (Bouskila, 1985; Robinson, 1995; Cunningham, 2000; Al-Johany, 2003; Sarhan & Al-Qahtani, 2007; Cunningham, 2009; Castilla et al., 2011a; Castilla et al., 2011b).

The habitat of this lizard is being lost due to overgrazing of livestock, human settlement and large-scale agricultural expansion, land reclamation, solid waste disposal and off-road vehicle travel (IUCN, 2022). These disturbances have fragmented the lizard's populations throughout its range (Omar, 1991; Brown 2003; Abd El-Wahab, 2016). Despite these problems, the Arabian spiny-tailed lizard is still locally common in some places of the Arabian Peninsula, especially in protected areas (Wilms et al., 2012). However, this species has a spatial conservation value since it is captured and traded in Kuwait and Saudi Arabia where its population is declining (Aloufi et at., 2019). The decline in the Arabian spiny-tailed lizard in most habitats is expected to continue and may already be close to meeting a 30% decline rate over the past 15 years or three generations (Wilms et al., 2012). This trend needs to be further evaluated by conducting a more in-depth population surveys, because the Arabian spiny-tailed is believed to be a good proxy for indicating ecosystem health (Al-Sayegh, 2017).

1.7 Niche of research

This thesis reviews and analyses the status, population trends, threats and conservation actions of desert lizards to pinpoint the conservation means applied to them around the globe. Furthermore, the thesis describes research related to the ecology and conservation of a specific endangered flagship lizard species *Uromastyx aegyptia microlepis* from the State of Kuwait. This approach is quite applied in nature and uses some established methodologies to investigate the distribution of species within the country, the factors that affect it, body condition, genetic diversity, and to identify and allocate the best suitable sites to promote its conservation.

1.8 Aims and Objectives

This study was aimed at assessing various attributes of *Uromastyx aegyptia microlepis* such as distribution, morphometrics and genetic attributes to measure its performance in the desert ecosystem. The general hypothesis states that protected areas positively affect the well-being of this lizard.

The objectives of this study were the following:

1- Assess the factors influencing the population of desert lizards around arid lands in deserts around the world using the IUCN Red List database.
2- Identify the most suitable habitats for *Uromastyx aegyptia microlepis* in the desert of Kuwait using the Maximum Entropy Distribution Model (MaxEnt).
3- Quantify the Scaled Mass Index (SMI) of the species using phenotypic measurements to illustrate how the season and protection status of the study site affect it within the sampled populations.
4- Evaluate the genetic diversity of *Uromastyx aegyptia microlepis* at protected and unprotected sites using microsatellite primers.

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Chapter 2

Global Driving Factors Affecting the Desert Lizards Status and Population Threats in Contrast with Conservation Action

Reviewing the IUCN Red List Database

2.1 Introduction

The IUCN Red List of Threatened Species is the most widely used and accepted system worldwide in the conservation status of biodiversity (Rodrigues et al., 2006). The Red List is the most comprehensive resource available that details the global conservation status and trends of the world's biodiversity. It is also a rich collection of information on threats, ecological requirements, and species habitats, as well as conservation actions that can be or have been taken to reduce or prevent species extinction.

The IUCN Red List is based on an objective system for assessing the risk of species extinction based on past, present, and projected threats. Species assessments by IUCN experts involve a rigorous process based on the highest standards of scientific documentation (Rodrigues et al., 2006). In addition, the experts are asked to go beyond status assessments and list threats associated with species. This process provides an opportunity to identify targeted conservation management actions to alleviate the threat status (Hayward, 2009). Yet, because this process also requires conducting explicit studies, which require high budget and excessive time, which are, in many cases, limited; therefore, data generated from estimations or qualitative methods may be feasible as an initial step. Such a study framework is an important approach for future studies that face similar circumstances.

This study reviews the effects of conservation actions targeted at desert lizards around the globe on the population status and trend of the population and threats, as an exploratory step to acquire knowledge about the protection of the desert lizard population around the world. The specific objectives of this research are to assess the fallowing: the status and population trends of desert lizards, the threats faced by desert lizards and the conservation action being carried out to protect desert lizards using the IUCN Rest List Database. This is an exploratory deductive study, meaning that no assumptions were made for a certain relationship; the complete process was reviewed.

In this study, descriptive data from the IUCN Red List were entered as numerical nominal values. Using descriptive data forms a gap when expressing the situation in comparison with using numbers. Using subjective words weakens the representation of the actual situation compared to continuous quantitative data.

Indicators represented in continuous data make better indicators than descriptive expressions; yet this does not rule out their significance in circumstances where quantification of a certain variable is unattainable. In other words, measurements are more accurate than describing a status in words.

2.2 Materials and Methods

The IUCN Red List Database (IUCN Red List of Threatened Species, 2022) were reviewed for all desert lizard species. Only entries created during the period 2006-2021 were used. The taxonomy as inclusion criteria was restricted to Animalia-Chordata Reptilia-Squamata, with all lizard families selected. Furthermore, desert was selected as the main habitat constraint while hot, temperate, and cold deserts were selected as sub-habitats. The data extracted cover the variables: status, population trends and threats that affect desert lizards. Out of 504 data entries for the species, a total of 391 species were used for the analysis. One hundred eleven species which were considered by the IUCN Red List of Threatened Species (2024c) as data deficient due to the inadequate information they convey, were removed. In other words, data that did not consist of sufficient information to make either direct or indirect estimations were omitted. The presence of various disturbance factors was evaluated in a binary fashion (present or not present): invasive species (invaders), agriculture, transport, biological use, climate change, energy mining, natural system modification, pollution, and residential/commercial development. All these factors are best described as descriptive data criteria for threats and stresses depending on the percentage of the total populations affected by the threat or threats (IUCN Red List of Threatened Species, 2024a, 2024b).

IBM SPSS version 24 was used to perform the statistical analysis. Descriptive analysis of both dependent variables (critical status and population trends) was in the form of percentage distribution graphed in a horizontal bar chart. Generalised Linear Models (GLM) (McCullagh & Nelder, 1989) was used to assess whether or not there was a statistically significant linear relationship of the dependent variables on the two dependent variables studied. Thus, there were two GLM models.

One GLM model was used when critical status was the multi-categorical dependent variable (with five categories: least concern, nearly threatened, vulnerable, endangered and critically endangered). A multinomial logistic model was used and a cumulative logit link function. The second GLM model was for population trends which was coded as a binary dependent variable (with two categories: decreasing or nondecreasing) and analysed using a binomial model and a logit link function. This allows modelling of a dependent scale variable based on the hypothesized linear relationship to either categorical or scale predictors (independent variables). The categorical predictor is considered a factor while the scale predictor is considered as covariates.

GLM is a natural generalization of classical linear models that include as special cases, linear regression and analysis-of-variance models, logit and probit models for quantal responses, log-linear models and multinomial response models for count and some commonly used models for survival data (McCullagh & Nelder, 1989). The main hypothesis tested within GLM is that whether or not there is a statistically significant linear relationship between the dependent variable (critical status or population trends) and the threat factors.

A total of 11 threat factors were investigated including: residential / commercial development, agriculture, energy mining, transport, biological use, human disturbance, natural system modification, invasive species, pollution, climate change and other threats. In addition, four conservation actions were tested including: researching / monitoring, protected areas, species management, and education. The statistical significance considered is 5% or 1%. Any *p*-value less than 0.05 was denoted with a (*) being statistically significant at 5% and any *p*-value less than 0.01 was denoted with a (**) statistically significant at 1%.

2.3 Results

The results of the status and population trends of the desert lizards over a period of 15 years (2006 to 2021) are presented in Figures 2.1 and 2.2. Assessment of the status of the desert lizards (Fig. 2.1), suggests that 342 species (87.5%) are identified as least concern, 11 species (2.8%) as nearly threatened, 14 species (3.6%) as vulnerable, 16 species (4.1%) as endangered, and only 8 species (2.0%) as critically endangered. The results reveal that the population trend (Fig. 2.2) of 334 species (85.4%) of desert lizard species increased or was stable, collectively called the non-decreasing population, while 57 species (14.6%) have a decreasing trend.

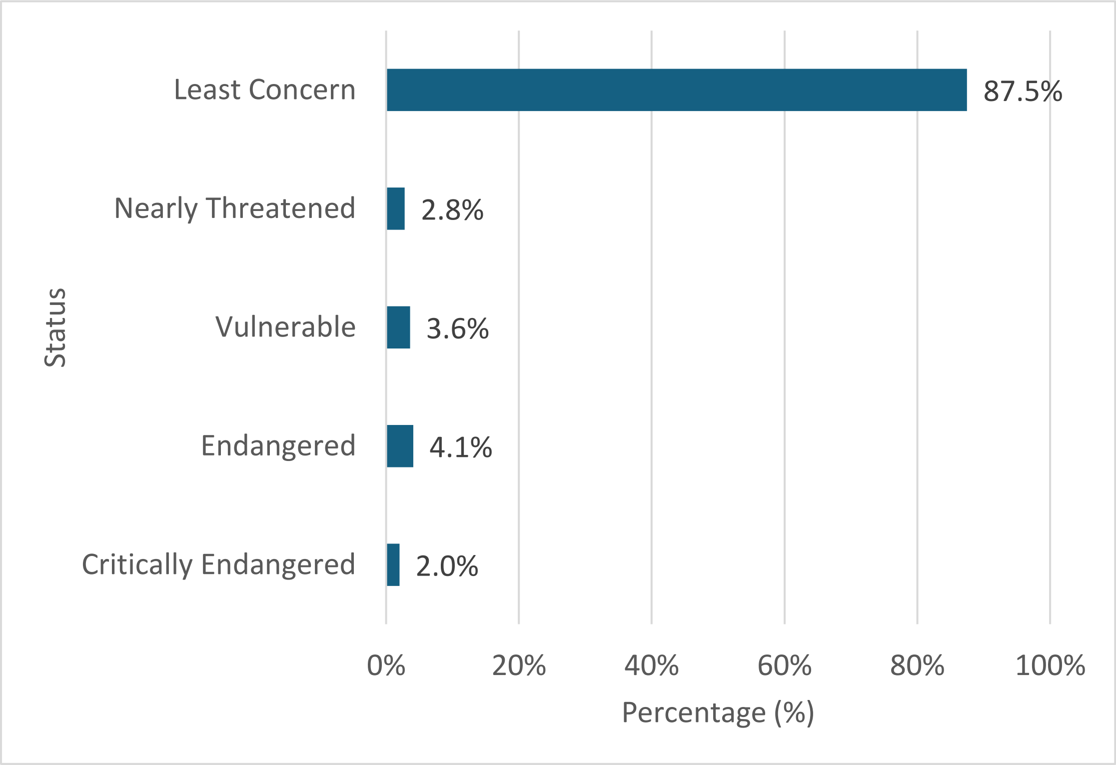

Figure 2.1 Critical status of 391 desert lizard species covering the period 2006 – 2021 (IUCN Red List 2021).

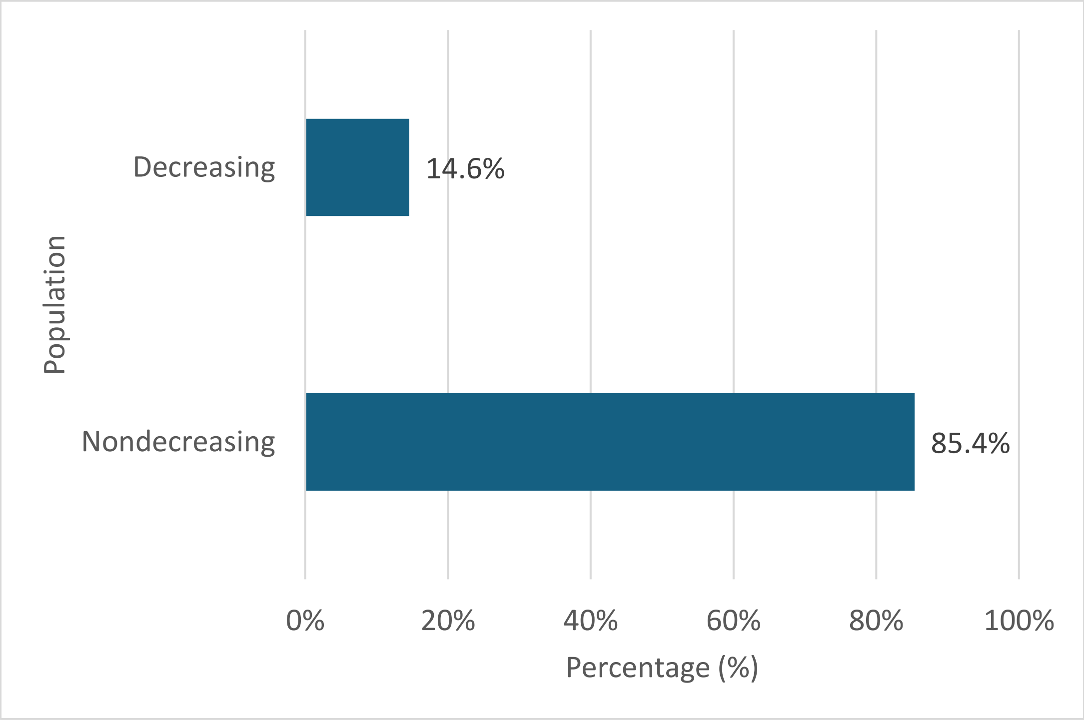

Figure 2.2 Population trend of 391 desert lizard species covering the period 2006 – 2021 (IUCN Red List 2021).

Generalised linear models incorporated the 11 key variables predicted to influence the desert lizard's critical status (Table 2.1) and population trends (Table 2.2). Four conservation predicted factors were identified to influence desert lizards critical status (Table 2.3) and population trends (Table 2.4). In the GLM model 1, only four of the identified threats statistically influenced the critical status at 1% significance levels. The associated Wald Chi-square scores were respectively 12.3 (*p* = <0.001) for residential/commercial development, 17.7 (*p* = <0.001) for agriculture, 9.5 (*p* = 0.002) for energy/mining and 8.3 (*p* = 0.004) for invasive species (Table 2.1). Collectively, these four factors negatively influenced the critical status of 93 of 342 least concern species (27.2%), 9 of 11 nearly threatened species (81.8%), 11 of 14 vulnerable species (78.6%), 16 of 16 endangered species (100%) and 8 of 8 critically endangered species (100%). The other threat factors tested were not statistically significant and none of the conservation actions were statistically significant in affecting the critical status, since *p*-value was greater than 0.05 (Table 2.2).

In the GLM model 2, the Omnibus test showed a likelihood Chi-square ratio of 144.6 (*P* = <0.001; df = 15), indicating that this model including the hypothesized threat factors outperforms the null model and accordingly this model is concluded to be adequate. The Wald Chi-square scores were statistically significant at 5% or 1% in five threat factors, 30.5 (*p* = <0.001) for residential/commercial development, 18.5 (*p* = <0.001) for agriculture, 4.0 (*p* = 0.044) for energy/mining, 4.1 (*p* = 0.043) for human disturbances and 6.5 (*p* = 0.01) for invasive species (Table 2.3). Residential/commercial development negatively affected 35 species (9.0%), agriculture affected 39 species (10.0%), energy/mining affected 19 species (4.9%), human disturbances affected 18 species (4.6%), and invasive species affected 12 species (3.1%). The other threat factors tested were not statistically significant since the *p*-values exceeded 0.05. Furthermore, none of the conservation actions was statistically significant in affecting population trends (Table 2.4).

2.4 Discussion

The findings of this study show that residential/commercial development, agriculture, invasive species and energy/mining had a profound negative influence on both the critical status and population trends. Although agriculture was ranked second as an influencing factor for population

Table 2.1 The effect of threats on the critical status of the desert lizards.

Predictor (Independent Variable)	β	Standard error	Hypothesis Test	
			Wald Chi-Square	*p*-value
Residential/commercial development (code = 0)	-1.527**	.4348	12.341	<.001
Agriculture (code = 0)	-1.795**	.4267	17.699	<.001
Energy mining (code = 0)	-1.441**	.4683	9.465	.002
Transport (code = 0)	.676	.7427	.829	.363
Biological use (code = 0)	1.041	.5846	3.169	.075
Human disturbance (code = 0)	-.011	.5103	.000	.982
Natural system modifications (code = 0)	-.417	.7754	.290	.590
Invasive species (code = 0)	-1.685**	.5864	8.257	.004
Pollution (code = 0)	-.200	.8118	.060	.806
Climate change (code = 0)	-.518	.7223	.514	.473
Other (code = 0)	-1.114	1.3152	.717	.397
Geological events (code = 0)	--	--	--	--

* Statistically significant at 5% (*p*-value < 0.05); ** statistically significant at 1% (*p*-value < 0.01).

Table 2.2 The effect of threats on the population trend of the desert lizards.

Predictor (Independent Variable)	**β**	**Standard error**	**Hypothesis Test**	
			Wald Chi- Square	*p*-value
Residential/commercial development (code = 0)	-2.406**	.4354	30.530	<.001
Agriculture (code = 0)	-1.803**	.4194	18.481	<.001
Energy mining (code = 0)	-1.013*	.5039	4.040	.044
Transport (code = 0)	.999	.8512	1.377	.241
Biological use (code = 0)	-.422	.5655	.558	.455
Human disturbance (code = 0)	-1.106*	.5463	4.102	.043
Natural system modifications (code = 0)	-.770	.7642	1.017	.313
Invasive species (code = 0)	-1.725*	.6753	6.526	.01
Pollution (code = 0)	-1.687	1.2018	1.971	.160
Climate change (code = 0)	-.211	.8151	.067	.796
Other (code = 0)	-1.184	1.8486	.410	.522
Geological events (code = 0)	--	--	--	--

* Statistically significant at 5% (*p*-value < 0.05); ** statistically significant at 1% (*p*-value < 0.01).

Table 2.3 The effect of conservation means on the critical status of the desert lizards.

Predictor (Independent Variable)	**β**	**Standard error**	**Hypothesis Test**	
			Wald Chi-Square	*p*-value
Researching/monitoring (code = 0)	-.176	.8652	.041	.839
Protected areas (code = 0)	.583	.6181	.889	.346
Species management (code = 0)	-1.332	.8879	2.250	.134
Education (code = 0)	-.062	.7737	.006	.936

* Statistically significant at 5% (*p*-value < 0.05); ** statistically significant at 1% (*p*-value < 0.01).

Table 2.4 The effect of conservation means on the population trend of the desert lizards.

Predictor (Independent Variable)	**β**	**Standard error**	**Hypothesis Test**	
			Wald Chi-Square	*P*-value
Researching/monitoring (code = 0)	.012	.9497	.000	.990
Protected areas (code = 0)	.586	.6694	.768	.381
Species management (code = 0)	-1.445	.9542	2.293	.130
Education (code = 0)	-.646	1.0203	.401	.526

* Statistically significant at 5% (*p*-value < 0.05); ** statistically significant at 1% (*p*-value < 0.01).

trends of desert lizards, it was the biggest offender, affecting population trends in 39 of the 391 species of desert lizards (10.0%). Interestingly, these eight threats do not affect the critical status of the least concerned species (27.2%). However, these threats are the most significant for nearly threatened species, vulnerable species, endangered species, and critically endangered species. The invasion of species did not change the native species occurrence lists but it affected their niches (Núñez-Tobajas et al., 2024).

The effect of human presence combined with habitat degradation and pollution has been identified as a major threat to lizard assemblages (Gibbons et al., 2000). The possible mechanisms by which lizard populations decline may be associated with humans removing lizards from their habitats (poaching) for recreation, food, trade or by accidental roadkill (Kaur et al., 2020). Other indirect effects can manifest as a result of natural to agriculture habitat conversion and can lead to altering other species' occurrence yielding additional disturbance of the reptiles (AlRashidi et al., 2021). Habitat loss due to the conversion of human land use for agriculture, housing, and commercial forest use can also negatively affect lizards by limiting their ability to meet their ecological needs for survival and reproduction (Todd et al., 2010). Contrary to these observations, others have claimed that there is no evidence that any lizard species is negatively affected by habitat disturbances (Smart et al., 2005). According to these authors, some species are more common in communal lands with their species richness and diversity being higher due to the reduced numbers of their predators and competitors (Smart et al., 2005). The present study has predicted environmental pollution as an important limiting factor in the status and trends.

The findings of this study suggest that the use of the most effective conservation actions to address these threatening factors in desert lizard populations is currently lacking, and the species conservation assessors of the IUCN Red List do not acknowledge this problem when considering protected areas as the sole mitigation for all threats. The results indicate that desert lizards are threatened by multiple factors. Therefore, a more integrated conservation approach is likely to improve outcomes. Some factors that cause habitat loss such as agriculture, residential and mining can be easier to mitigate than others (Hayward, 2011). Although creating a protected area was the only conservation action included in the IUCN conservation measures for desert lizards, other actions, such as laws prohibiting habitat destruction of undesignated protected and hunting areas are additionally needed to improve the status of desert lizards (Hoffmann et al., 2010). Assessors on the Red List should consider reviewing these measures in future conservation strategies.

One in five lizard species is threatened at some level (IUCN Red List of Threatened Species, 2022). This is likely due, to a large part, to life's historical attributes; the prolonged exposure of desert lizards to the threats in association with urban settlements. These factors have provided the lizards with opportunities to adapt to the manmade habitat modifications and made them less susceptible to decline from anthropogenic factors. Notably, many lizards that occur at high population densities, have short generation times and high fecundity. Consequently, lizards can adjust rapidly to environmental changes or rebound quickly from short-term population reductions (Todd et al., 2010). The findings of the present study also show that desert lizards are affected by some anthropogenic factors. Lizard species at greater risk are those that typically have specific attributes, such as endemism, restricted geographic ranges, large body size, long lives, late maturity, or low fertility (Pianka & Vitt, 2003). These attributes tend to make lizard species more susceptible to population declines from anthropogenic factors.

Additionally, pollution from agricultural activities, such as the excessive use of pesticides and irrigation with untreated, primary or secondary treated effluent water, was also identified as a threat to desert lizards. Numerous environmental contaminants, such as heavy metals, pesticides, herbicides, and radioactive waste associated with human activities, have direct and indirect effects on amphibians and reptiles. The release of these contaminants into the environment has been listed as one of six major contributors to the global decline of reptiles (Hinton et al., 1990; Hall & Henry, 1992; Gibbons et al., 2000). Furthermore, some reptiles are long-lived and have small home ranges compared to similar-sized endotherms, making them susceptible to long-term exposure and subsequent bioaccumulation (Hopkins, 2000; Shelby & Mendonca, 2001; Bergeron et al., 2007).

Pollution by heavy metals can affect lizard metabolism by accumulating in their vital organs, such as liver and kidneys (Oyekunle et al., 2012). Although not necessarily directly driving mortality, the sub-lethal effects of these contaminants may be more detrimental to the long-term persistence of reptile populations. High tissue loads of various contaminants have been documented from reptiles in the field e.g., lizards and snakes; reviewed in Campbell & Campbell, 2000, 2001; Bergeron et al., 2007. The sub-lethal effects of contaminants on reptile locomotor performance have also been documented in Holem et al. (2006); Hopkins & Winne (2006) as well as their metabolic energy consumption in Hopkins et al. (2002); DuRant et al. (2007). Although these studies provide information on the mechanisms that link sub-lethal exposure to population dynamics, few studies have attributed declining reptile populations to sub-lethal contaminate exposure. Furthermore, the effects of mining pollution have been documented to decrease the reproduction rate of some lizards associated with low energy supply due to low food availability (Sasaki et al., 2016).

Mining is also a threat to reptiles, as it is a highly growing activity leading to habitat loss. Mining processes transforms the desert habitats and therefore, numerous reptile habitats could be lost accordingly. Mining activities also affect the herpetofauna leading to compositional changes in reptile communities (Jackson & Sax, 2010; Mayani-Parás et al., 2019). In many living organisms, such as mammals, amphibians and reptiles, habitat loss due to several anthropogenic activities as mining deteriorates the status of biodiversity in those habitats leading to either delayed immigration or to the disappearance of reptile species in the disturbed areas (Linkie et al., 2003; Sasaki et al., 2016; Plante et al., 2018).

The effect of human activities associated with residential or commercial development and pollution may have varied consequences on the critical state of the desert population and population trends. The “intermediate disturbance hypothesis” states that diversity is highest under an intermediate disturbance regime and lowest at very high or very low levels of disturbance (Grime, 1973; Huston & Huston, 1994).

Previous studies of lizard populations experiencing intermediate disturbance levels have provided some support for this idea. For example, lizard abundance and species richness peaked at intermediate urbanization levels in Tucson, Arizona (Germaine & Wakeling, 2001). The Tucson study also showed that the low to moderate levels of residential development had a positive effect on lizard assemblages, but lizard populations declined once development levels reached beyond that point. Although the definition of ‘intermediate disturbance’ is subjective and therefore problematic, it has been suggested that some lizard taxa may benefit from an intermediate disturbance (Smart et al., 2005). For example, terrestrial lizards (lacertids, in particular) became more abundant as the ground cover became sparser. This type of disturbance generally affects discrete areas, resulting in a patchy landscape and increased habitat heterogeneity.

Meiri and Chapple (2016) have highlighted the ecology and biology data gaps that exist within IUCN database entries. They have suggested adopting an integrated approach to bridge the “assessment gap in lizards.” This approach will improve the regional and taxon-specific working groups associated with the IUCN's Global Reptile Assessment. Furthermore, the use of predictive modelling will improve our understanding of lizard distribution, biology, and taxonomy. The present study specifically highlights the lack of recommended conservation actions for desert lizards and the key threats.

Consequently, none of the conservation actions had a significant influence on either population trends or critical status. However, this data set is highly biased toward conservation action in protected areas (357 out of 399 actions, 89.5%) since most lizards were identified as natural living or purposely placed in national park reserves. More research and monitoring is recommended to further improve species critical status and population numbers of desert lizards.

2.5 Conclusions and recommendations

Desert lizards are affected by multiple factors that impact their population trend and critical status. In most cases, the impact is due to combined factors. The present study shows that residential and commercial development, energy mining and agriculture activities to be the top three factors that affect desert lizard population trends and their critical status.

Furthermore, the diversity and abundance of desert lizards are affected by some threatening processes. Understanding what threats affect biodiversity is crucial to developing conservation plans for desert lizards and other species. It is crucial that declines in desert lizard populations are closely monitored and assessed so that appropriate mitigation measures can be applied. However, some mitigation actions could be undertaken pre-emptively to proactively reduce any negative effects on desert lizard populations. As such, it is recommended that large areas be used for conservation or restoration programs. This mitigation measure will not only reduce the threat to desert lizards but will also reduce threats to biodiversity.

Agriculture activities that may cause pollution must be treated on site and no pollutants should be allowed to leave a site. Energy mining has similar direct habitat impacts to residential and commercial development - potential problems of pollution. Therefore, mitigation measures that address both of these factors are necessary. Conservation managers are advised to pay special attention to the combined effects of residential and commercial development, energy mining, and agriculture activities that threaten desert lizard populations. Because important desert lizards such as *Uromastyx aegyptia microlepis*, the landscaper of the desert, are threatened or vulnerable. Immediate action is needed to confront these threats. All available conservation recourse should be considered as mitigation measures for the protection of desert lizards.

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Chapter 3

The Conservation of *Uromastyx aegyptia microlepis* in the State of Kuwait: Modelling the Most Suitable Habitat

3.1 Introduction

The species distribution model is a mathematical tool used in mapping processes of spatial data to describe or predict species status (Elith & Leathwick, 2009; Miller, 2010). It is a powerful tool to represent and demonstrate the status and important characteristics of a targeted species (Kalboussi & Achour, 2018; Asadalla et al., 2021; Abdullah et al., 2022). This model is becoming a priority tool in many ecological applications such as restoration of ecosystems and conservation (Castelar et al., 2015; Kalboussi & Achour, 2018; Moradi et al., 2019). The outcomes of distribution models can be further processed in software that integrate distribution model maps with conservation areas and their costs in decision making as Marxan (Watts et al., 2009; Watts et al., 2017) to conduct feasibility studies prior to designating protected areas. Moreover, the habitats of targeted species can be evaluated in terms of suitability by the use of species distribution models applying machine learning statistics software, such as Maximum Entropy Distribution Model (MaxEnt) (Phillips et al., 2004).

This mapping tool has made fieldwork more efficient, in comparison with conventional mapping methods. In the conventional mapping methods, larger surveillance areas and more manpower are needed in comparison with using machine-learning statistics where maps are forecasted from smaller surveillance areas. This tool is considered an important proactive step towards the conservation of species. The probability of species occurrence in certain habitats is used to indicate the ecosystem health (Zhao et al., 2019; Fu et al., 2021).The application of species distribution and ecological niche models in ecology has recently increased (Sillero et al., 2021). Using presence-only records in the form of geographic coordinates, the distribution and suitable habitats can be estimated (Soberón & Peterson, 2005). Species distribution models shed light on the potential geographic distribution of a species, while ecological niche models focus on the parameters of the actual niche of the species (Feng et al., 2019).

Species distribution models such as Maximum Entropy Distribution Model (MaxEnt) (Phillips et al., 2004) use training presence-only points from systematically selected sites to detect the distribution of the species in the whole map extent partaking into account biotic and abiotic factors. In other words, MaxEnt model determines the possibility of species occurrence within a given map extent. Another implication that can be made from Maxent models is to determine the most suitable habitats for a target species (Abdullah et al., 2022).

MaxEnt modelling is an Artificial Intelligence (AI) application. In general, AI software consists of training datasets and variables (Oke, 2008). When the model is run, the entire continuum will be predicted for the target domain based on the training data sets. Deep learning and neural network analysis are other examples of machine learning statistics (Lek & Guégan, 1999; Aitkenhead et al., 2004; Han et al., 2019; Emmert-Streib et al., 2020). The results are obtained by comparing the test data with the training part. If the model is random, that is, if the variables had no effect on the training datasets, the Inverse Optimal Control (IOC) would be equal to 0.5. The MaxEnt model assumes a random distribution of the species when none of the environmental variables has control or reinforcement effects on the training points. However, the alternative assumption in the model is that the variables do influence the distribution of the species, as was expected. In this case, the IOC of the Area under the ROC Curve (AUC) value would be greater than 0.75. The closer the value of AUC moves to the value of one, the more obvious the effect is and the more precise is the model (Elith et al., 2011).

The MaxEnt ecological niche model is used to determine factors affecting the distribution of the target species to map its most suitable habitat in any given region (Elith et al., 2011). MaxEnt is a model that enables the researcher to pinpoint the factors that affect the distribution of a target species. This is important because when the factors that influence the distribution of the species are known, initial restoration programs of the target species, habitat, or ecosystem can be developed (Alatawi et al., 2020; Abdullah et al., 2022). One application of MaxEnt modelling is in the domain of invasive alien species. The model helps predict and identify factors that facilitate the distribution of alien species (West et al., 2016). This machine learning, or presence-only, model assumes that every point in the map is likely to contain the sampled species.

Sample coordinate training points and covariate layers can alter the randomness of the model (Phillips et al., 2004; Phillips et al., 2006; Phillips & Dudík, 2008). In other words, the model compares the training and test (pseudo-presence) points to determine the factors that are likely to drive the distribution of the speices. The model ranks the factors that most influence the distribution of a species through jackknife analysis (Elith & Leathwick, 2009; Elith et al., 2011).

Despite the capability of ArcGIS in multivariate analysis and other statistical tools, it does not provide jackknife analysis the same way as MaxEnt does. Therefore, numerous researchers have used MaxEnt models to predict the species richness, geographic distribution, habitat suitability, niche and the effect of climate change on living organisms (Wilms et al., 2009; Khanum et al., 2013; Farashi & Shariati, 2017; Escobar et al., 2018; Zhang et al., 2018; Alatawi et al., 2020).

Determining hotspot habitats using conventional methods is difficult because it requires a great deal of time and tremendous human resources. Therefore, with the help of remote sensing and Geographic Information System (GIS) techniques and machine learning statistics, hotspot habitats can be predicted as an initial stage to prepare for field research. Excluding the less important habitats and delineating the important ones will help focus future tasks on limited areas for more efficient performance.

The many advantages of using MaxEnt modelling include: (1) high performance with presence-only data (Elith et al., 2011); (2) in cases of limited accessibility to targeted areas for survey, high-quality performance with low sample sizes (Phillips et al., 2004); (3) not requiring the removal of correlated variables unless the variable is ecologically irrelevant (Elith et al., 2011); (4) a technical specification of having a built-in regularization that is known to perform well (Hastie et al., 2009); and (5) being among the most advanced techniques that provide an extra step in predicting the distribution of invasive species and in exploring the impacts of climate change on species distribution (Radosavljevic & Anderson, 2014; Elith et al., 2010; Lissovsky & Dudov, 2021).

The living species is a single component of a community or a population operating under the population system (Royama, 1981). The distribution and dispersion of the species is one of its survival strategies that enables it to perform many physiological functions such as foraging and reproducing (Wharton, 2004). Through that, the species situates itself at a certain trophic level and plays the role of a producer or a consumer. In other words, each species strives to find its niche in the ecosystem. Geographical distribution and ecological niche modelling are key approaches to describing and studying any species.

The present study predicts the distribution of the Arabian spiny-tailed lizard and assesses the factors that affect its distribution in natural and urban scenarios. Using MaxEnt modelling, the factors that contribute towards either reinforcing or controlling the distribution model of the lizards are classified. Climatic, bioclimatic, anthropogenic, and natural models were selected to conduct the MaxEnt modelling to represent the story for this lizard in natural desert ecosystems and in peripheral urban ecosystems.

3.2 Materials and Methods

3.2.1 Training points for MaxEnt model

Training points were obtained by marking active burrows through visual encounter method (Eekhout, 2010) using Global Positioning System (GPS) (Mulualem, 2016; Estes-Zumpf et al., 2017). Burrows were considered as active (inhabited or visited) either if an *Uromastyx* was observed directly or if fresh tracks were found near an open and clean burrow entrance (Wilms et al., 2010). A total of 972 burrows were recorded in different parts of Kuwait. The points were obtained once per sampling period. The different areas were visited to ensure that the points were obtained from scattered parts of Kuwait at the most accessible sites. Sampling from evenly distributed sites in the targeted vicinity improves the performance of the model by avoiding omission and commission errors, false negatives and positives respectively (Kramer-Schadt et al., 2013). The points were pre-processed in an Excel sheet and converted from the Excel file to a comma-delimited Comma Separated Values (CSV) file which is compatible with MaxEnt modelling software. Acquired points are shown in (Fig. 3.1). The instructions followed while sampling were as follows:

- The driving speed was less than 25 km/h.
- During sampling, active burrows were marked.
- Visit the site once; the GPS markings were kept on the device to prevent double sampling.
- The sightings were marked using the degree decimal coordinate.

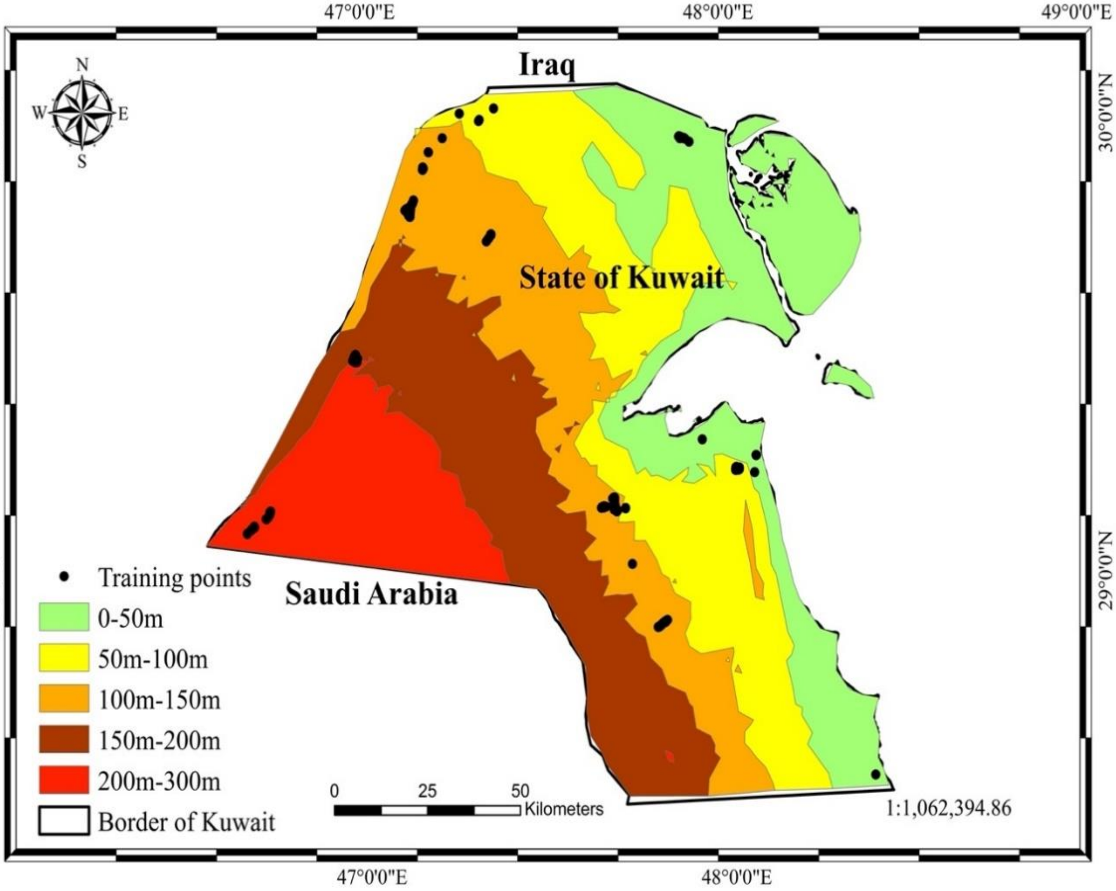

Figure 3.1 Obtained training points for *Uromastyx aegyptia microlepis* in the State of Kuwait. The black dots represent the surveyed areas.

3.2.2 Required data description

3.2.2.1 Climatic and bioclimatic data

Climatic data were used as variables in the model (Hijmans, et al., 2005a). Raster layer files represent the variables consisting of monthly maximum, minimum and average temperatures, and precipitation. From the above, nineteen bioclimatic layer variables (Hijmans, 2004; Hijmans, et al., 2005a) were used to detect the distribution of the Arabian spiny-tailed lizard MaxEnt models. These variables are described as interpolated ground station data that included gridded time series variables for the years 1960–1990. Layers of resolution (30”, 1 km^2) files were downloaded, creating a grid for the State of Kuwait. The files were then processed using Diva GIS Version 7.5 (Hijmans, et al., 2005b), which were then converted to a stack layer and trimmed to Kuwait map. The trimmed grid layer was converted to ArcGIS and then to ASCII format raster files.

3.2.2.2 Vegetation, soil and elevations maps

Covariate vector and raster layers of elevation, soil type, and vegetation were included, representing natural environmental data (Higmans et al., 2015a) and a land use layer (including all man-made facilities and establishments such as housing, industrial, military and other facilities) representing an anthropogenic model were constructed, based on available data (Aghanajafizadeh & Mobaraki, 2018; Eskandarzadeh et al., 2018). A Digital Elevation Model (DEM) raster layer was obtained from the United States Geological Survey (USGS, 2020).

Vector and raster map layers, such as soil type, vegetation, DEM and land use were converted to shape file layers using ArcGIS version 10.4. The sampled coordinates bioclimatic and anthropogenic variables were entered into the MaxEnt model. Through the Model platform, the model was set to test 80% of the sampling points and assign 20% for the validation process. In other words, the model randomly selected 80 percent of the training points for the testing step and left out the rest for validation. Additionally, the settings were selected to obtain response curves and jackknife analysis for all variables.

The Normalized Difference Vegetation Index (NDVI) (Rouse, 1973) was applied to satellite imagery depending on the combination of two spectral bands, red and Near- Infrared (NIR), which was calculated using the following equation:

$$\text{NDVI} = \frac{NIR - R}{NIR + R}$$

3.2.2.3 Land use variable

Land use vector layers were obtained from Meuser (2019), including government buildings, markets, malls and residential areas, as shapefiles. Land use characteristics included all anthropogenic activities that alter the natural ecosystem for different uses. These areas were maintained by digitising satellite images using ArcGIS software. Soil types and vegetation covariate vector layers were obtained from Omar et al. (2001) as shapefiles. Finally, the raster and vector layers were converted to ASC raster layers and coupled with the climatic and bioclimatic raster files via QGIS (QGIS, 2019).

3.2.3 MaxEnt modelling

In MaxEnt, parts of the targeted domain Version 3.4.1 (Phillips et al., 2004) was used to predict and classify the suitable habitats for the Arabian spiny-tailed lizard in the Kuwait desert and the factors affecting the suitability of the habitat. In other words, the model predicts the potential species distribution mode.

3.2.3.1 Selection of significant climatic and bioclimatic factors (Pre-trial step)

Before beginning the main MaxEnt model run, a pre-trial model was run to exclude the insignificant climatic bioclimatic variables and select the top six most influencing variables from the jackknife analysis results. The climatic and bioclimatic (ASC) raster layers were run with the sampled points. The variables that contributed >5% to the model were included (Table 3.1), while all other variables that contributed <5% were excluded to simplify the model because they were insignificant and did not affect the model. This step helped make the main MaxEnt model run faster and avoid system halts.

Table 3.1 Selected climatic and bioclimatic variables used in the pre-trial MaxEnt to be applied in Natural and anthropogenic models.

Variable	**Meaning**
TMax	Maximum Temperature
Prec. 11	Annual Precipitation
Bio2	Mean Diurnal Range (mean of monthly temp (max temp – min temp))
Bio3	Isothermality (Bio2/Bio7) * 100
Bio10	Mean Temperature of Warmest Quarter
Bio15	Precipitation Seasonality (Coefficient of Variation)

*These factors were used as a single layer in the models and were not subdivided into classes, subcategories, or components; this layer was used as a single layer. The areas also included the peripheral zones surrounding the land use areas.

3.2.3.2 Natural and anthropogenic models

Two MaxEnt models were conducted; one illustrating the distribution of the Arabian spiny-tailed lizard in natural ecosystems while the other conveying the distribution of the lizard in urban ecosystems. Combining the urban and natural ecosystems in one MaxEnt would only show the model representing the cities and other scattered facilities. Therefore, in the natural model, the land use variable was not used as it was in the anthropogenic model.

The natural (environmental) model was run using only climatic, bioclimatic, and natural covariables. In the anthropogenic model, the climatic and bioclimatic variables, natural and land use covariables were used in the model on the lizard distribution. The two models were used separately because the land use variables of the anthropogenic model will deviate the results of suitable habitats in the natural ecosystems. Knowing that one of the advantages of MaxEnt modelling is solving the matter of collinearity, the natural and the anthropogenic models were separated to prevent the collinearity issues, if any were present. The variables used in both natural and anthropogenic MaxEnt models are shown in Table 3.2.

Both the natural and the anthropogenic models were iterated 15 times to ensure more precise results. After the models, the area under the receiver operating characteristic curve (AUC) was obtained. The program was assigned to test a random 20% of the records after every 15 iterations to validate the model. The model was assigned to perform a jackknife analysis to rank the most key factors in percentages. Additionally, the average response curve for each variable was recorded. For both models, the expected results are the contribution factors for each variable: average AUC value, standard deviation and response curves distinguishing influencing and controlling factors.

Table 3.2 Natural and anthropogenic factors used in the MaxEnt model.

Covariable	**Data Description**
Anthropogenic	
Elevation*[1]	Altitude variation in the State of Kuwait.
Soil types	Different classes of soil types in Kuwait.
Vegetation (Veg 2002) *	NDVI map showing green cover in the State of Kuwait including most common annual communities.
Land use*	Housing units, government- and private-sector buildings, and other urban facilities. Land use area also includes the peripheral zone surrounding the areas.
Natural	
Elevation*	Altitude variation in the State of Kuwait.
Soil types*	Different classes of soil types in Kuwait. For more details refer to Omar et al. (2001).
Vegetation*[1]	NDVI map showing green cover in the State of Kuwait including most common annual communities (Asadalla et al., 2021).

*****These factors were used as a single layer in the models and were not subdivided into classes, subcategories, or components; this layer was used as a single layer. The areas also included the peripheral zones surrounding the land use areas.

3.2.3.3 Processing MaxEnt distribution maps produced from this study

The outcomes of the MaxEnt modelling are: (1) Distribution map ASCII file format; (2) AUC analysis report, jackknife analysis; and (3) response curves of the variables used in both models. Using ArcMap GIS, the ASCII files of the predicted maps of the lizard were reclassified into five classes from highly prioritized areas for conservation of lizards to least prioritized and converted to shapefile. The final produced maps were converted to JPG files of 600 dpi.

3.2.4 Prioritized *Uromastyx aegyptia microlepis* conservation hotspots

The outcome of the Asadalla et al. (2021) map for native plants was added to the habitat suitability map via maths tools in ArcGIS. The map of restoration prioritization map in Asadalla et al. (2021) (which has three restoration classes) is added to the lizards' natural MaxEnt model map of this study using ArcGIS. This step was done to merge the outcomes of the Asadalla et al. (2021) as a base map with the map obtained from this study.

In ArcGIS, visual interpretation of merging the two layers would not be scientific. Therefore, the addition tool of ArcGIS was used as a spatial conservation prioritization tool (Wilson et al., 2009; Lehtomaki & Moilanen, 2013). This step is to delineate the overlap of the most prioritized areas in both maps and designate them as most prioritized. Because the first map (Asadalla et al., 2021) has priority attribute values of one to three and the second map (of this study) has the priority attribute values from one to five, the two maps were added via ArcGIS addition tool. Thus, the outcome of that will result in showing class values from two to eight. In other words, the polygons codded from one to three of Asadalla et al. (2021) are added to the polygons (codded from one to five) of the MaxEnt model map of this study. The ArcGIS produced a map with polygons numbered from two to eight. Then the newly produced final map was reclassified into five classes, representing conservation priority, ranging from the least to the highest prioritization areas. The higher the class value, the more prioritized it is for conservation (Fig. 3.2).

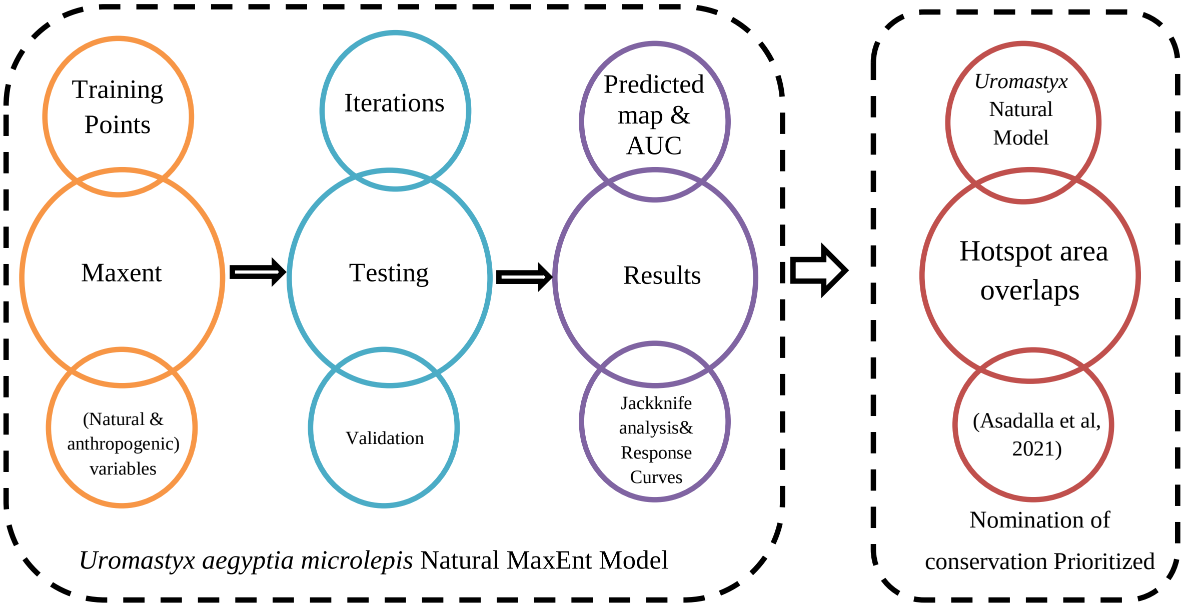

Figure 3.2 Prioritization of conservation sites for *Uromastyx aegyptia microlepis*.

3.3 Results

3.3.1 Natural (Environmental) MaxEnt Model

Natural modelling of the distribution of the Arabian spiny-tailed lizards revealed that the suitable habitats for this species are mainly in north-western, south-western, and south-eastern Kuwait between 94 and 150 meters above sea level (Fig. 3.3). The AUC was 0.92, and the standard deviation was 0.059, while the test gains were equal to 1.5, indicating a robust model. The results of the jackknife analysis showed that the most important variables determining the suitability of the habitat of the Arabian spiny-tailed lizard are elevation, vegetation cover/greenness (NDVI), Bio2, and Bio15, contributing 14.9%, 13.9%, 13.6%, and 12.8%, respectively (Table 3.3).

The curves for the elevation and vegetation cover variables showed their sustaining effect on the distribution, which means that the elevation gradient helps increase the probability of the occurrence of the lizard. The response of the Arabian spiny-tailed lizard to altitude and vegetation is showing reinforcing effects in Figures. 3.4 a and b, while other variables show controlling effects (Figs. 3.4 c, d, e, and f). jackknife analysis indicates that vegetation and elevation are the most contributing factors affecting the distribution of the lizard in the natural model.

3.3.2 Anthropogenic MaxEnt modelling

The species distribution map generated by the MaxEnt model, which included anthropogenic covariates, revealed that suitable habitats are in the midwestern parts and midcoastal lines of Kuwait (Fig. 3.5). The AUC was 0.852 with a standard deviation of 0.302, while the test gain was 1.35, indicating a robust model. Land use and elevation are the most important variables (68.9% and 14.1%, respectively). The response curves revealed that land use and elevation are the reinforcing factors that increase the probability that the lizard occurs at any site.

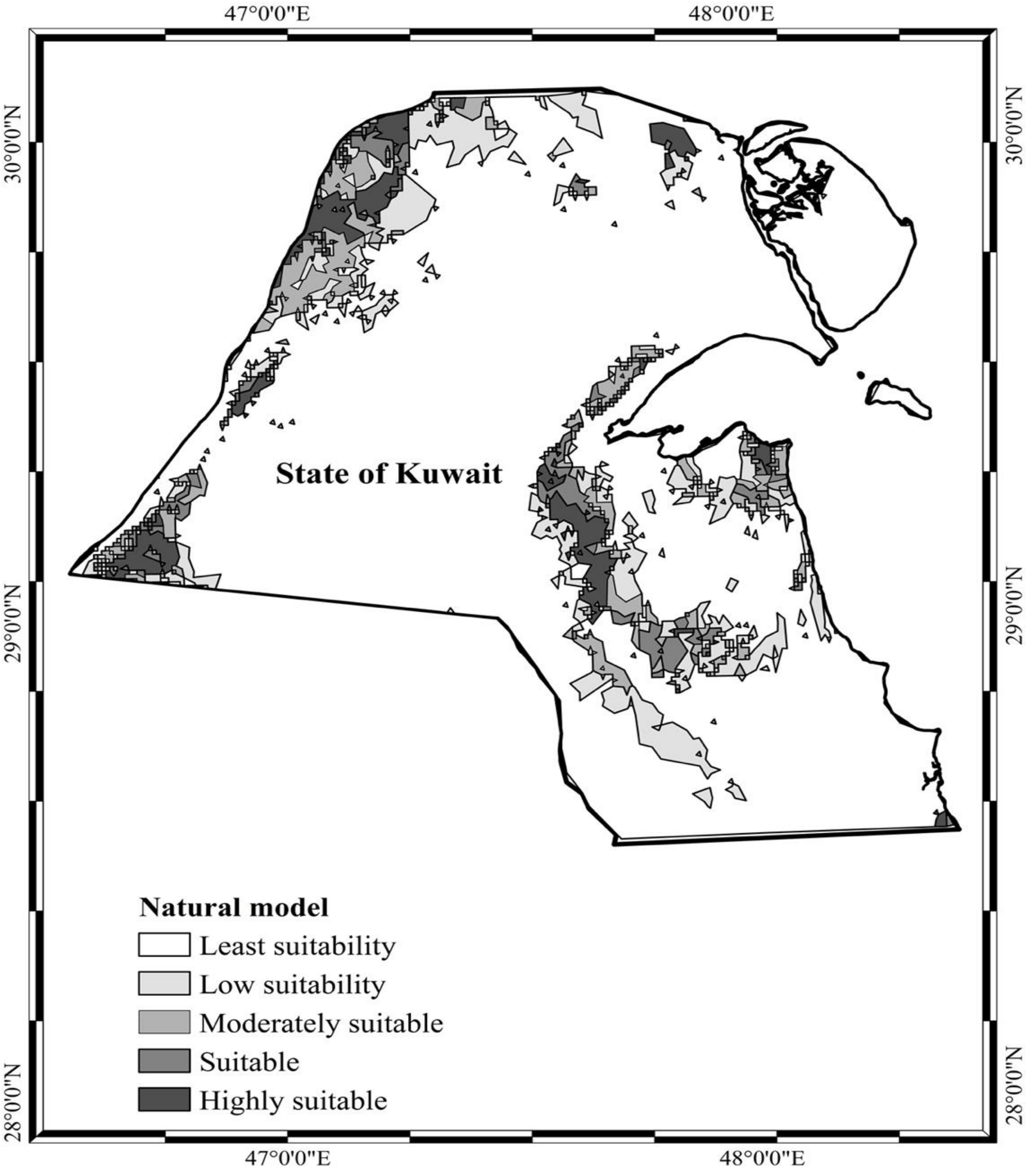

Figure 3.3 Species distribution map for the natural model for *Uromastyx aegyptia microlepis.* Suitability scales range from 1 (lowest suitability) to 5 (highest suitability).

Table 3.3 jackknife analysis for ranking the importance of natural variables and the elevation, vegetation and soil type covariables.

Variable	**Percent contribution**
Elevation	14.9
Vegetation 2002	13.9
Bio 2	13.6
Bio 15	12.8
Bio 10	9.8
Prec. 11	9.5

***** To view all variables, refer to (Phillips et al., 2006; Phillips & Dudík, 2008).

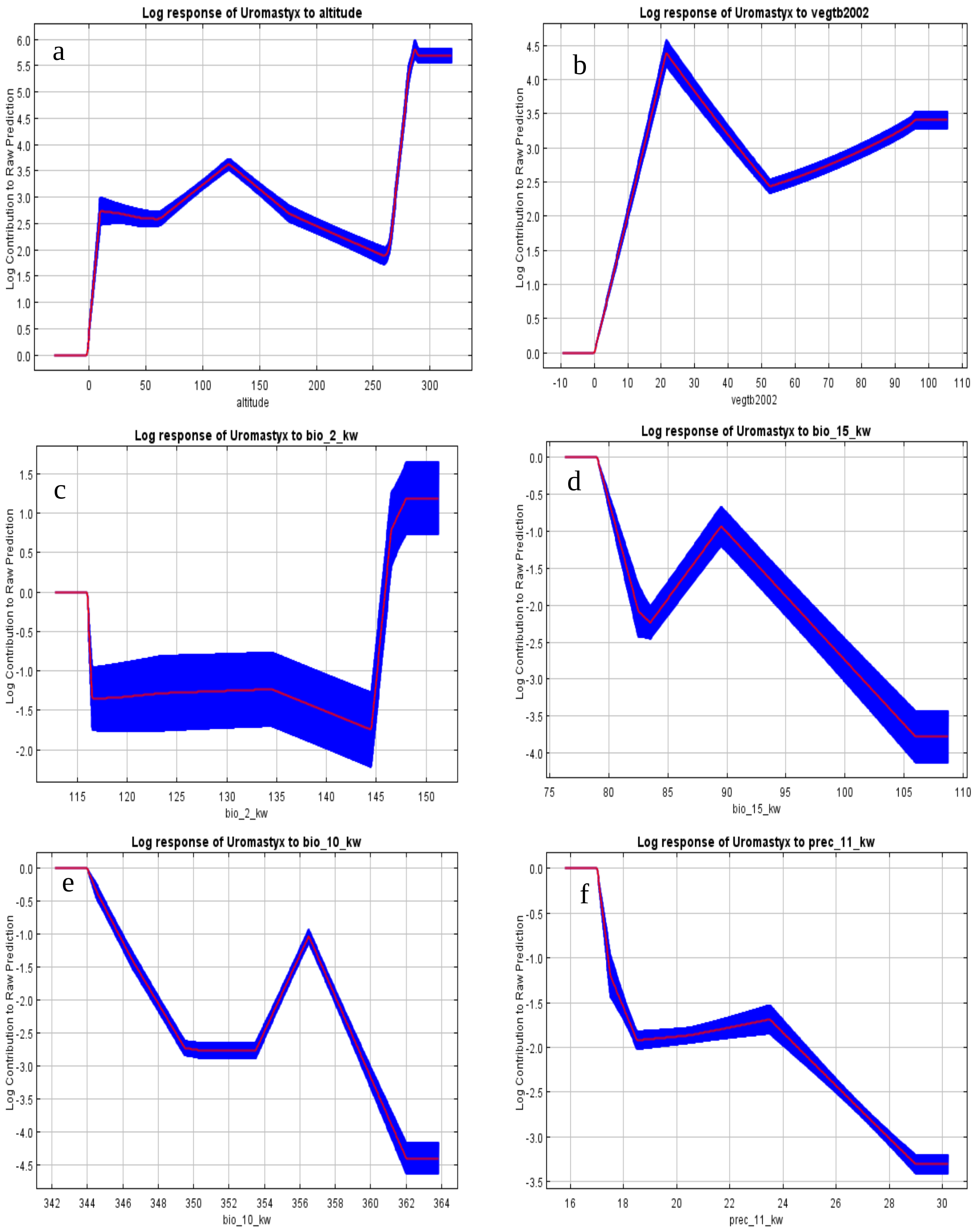

Figure 3.4 Average response curves in (red) and ±1 SD in (blue) for: (a) elevation; (b) vegetation; (c) Bio 2; (d) Bio 15; (e) Bio 10 and (f) Prec. 11 variables.

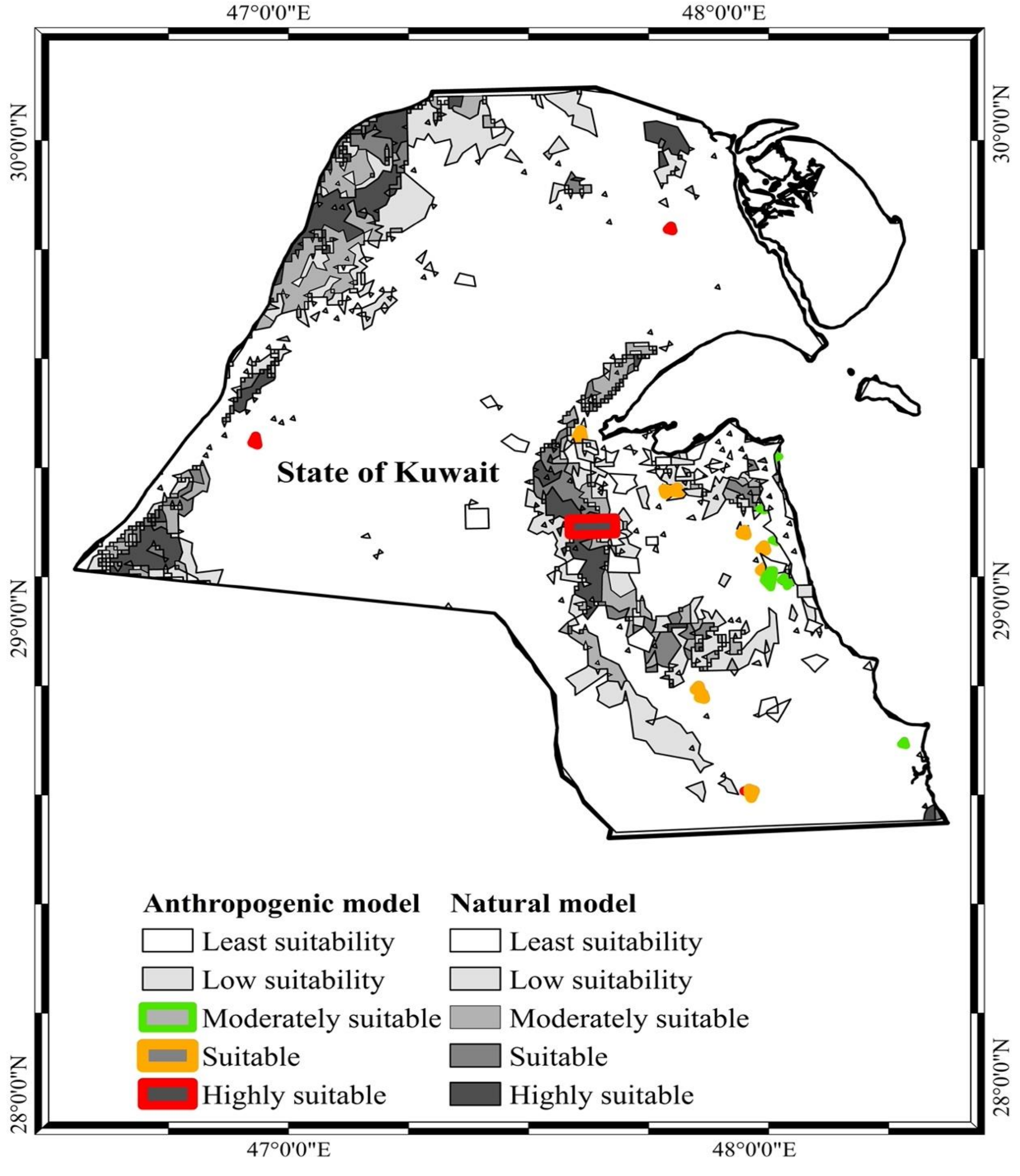

Figure 3.5 Species distribution map for the anthropogenic model for *Uromastyx aegyptia microlepis.* Suitability scales range from 1(lowest suitability) to 5 (highest suitability).

The process of urbanization seems to reinforce the distribution of this lizard especially at peripheral areas around the cities. (Figs. 3.6 a and b), while vegetation cover exhibits a decreasing trend (controlling factor) (Fig. 3.6 c) in contrast to the natural model. Therefore, land use and elevation covariables are reinforcement variables. In general, the elevation variable influenced both natural and anthropogenic models. Vegetation played an important role in the distribution of the lizard in the natural model only, while it was least important in the anthropogenic model. Interestingly, the land use variable influenced the distribution of this lizard in the model (Table 3.4).

3.3.3 Prioritization of *Uromastyx aegyptia microlepis* conservation areas

Based on the addition of the restoration prioritization map of Asadalla et al. (2021) with the natural MaxEnt model of this study, the results classified the conservation prioritization areas for *Uromastyx aegyptia microlepis* (Fig. 3.7). Hotspot sites for the conservation of this lizard are in the middle and southern parts of Kuwait. The northern parts included moderate to least prioritised areas for conservation. Most of the high priority conservation areas in the middle and southern areas of the country are under the supervision of the Kuwait Oil Company (KOC). Therefore, these *de facto* protected areas are excluded from conservation projects, while few protected areas are located in the highly prioritized conservation sites.

3.4 Discussion

The Arabian spiny-tailed lizard is a robust species partially due to its trophic flexibility. It can survive without vegetation, feeding on insects, arthropods and small lizards (Castilla et al., 2011a; Castilla et al., 2011b; The IUCN Reptile Database, 2021). Similarly to the natural model, elevation influences the formation of water catchments by guiding water surface runoff to depressions, which may increase vegetation cover (Omar et al., 2001). Although partially due to the deterioration of vegetation cover, which facilitates the success of the lizards, around urban areas, the lizards are still successful in survival. This supports the resistance of the lizard to limited vegetation cover because the reptile can switch its diet to consuming smaller lizards and arthropods. Also, annual shoots may be available for consumption in the spring rainy season. By modifying land cover/ land use in cities, the species occurrence can be altered toward the peripheral

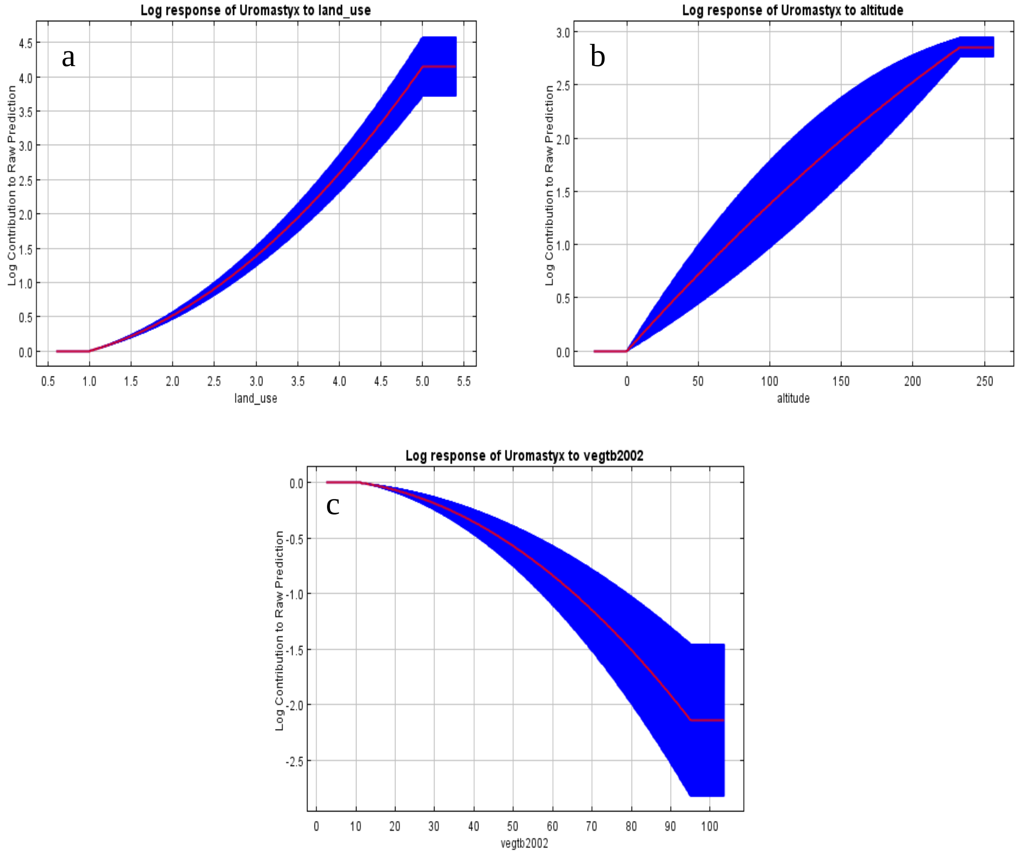

Figure 3.6 Average response curves in (red) and ±1 SD in (blue) for: (a) land use; (b) elevation and (c) vegetation.

Table 3.4 Jackknife analysis for ranking the most important variables in the anthropogenic model.

Variable	**Percent contribution**
Land use	68.9
Elevation	14.1
Veg 2002	5.4

AUC = 0.852.

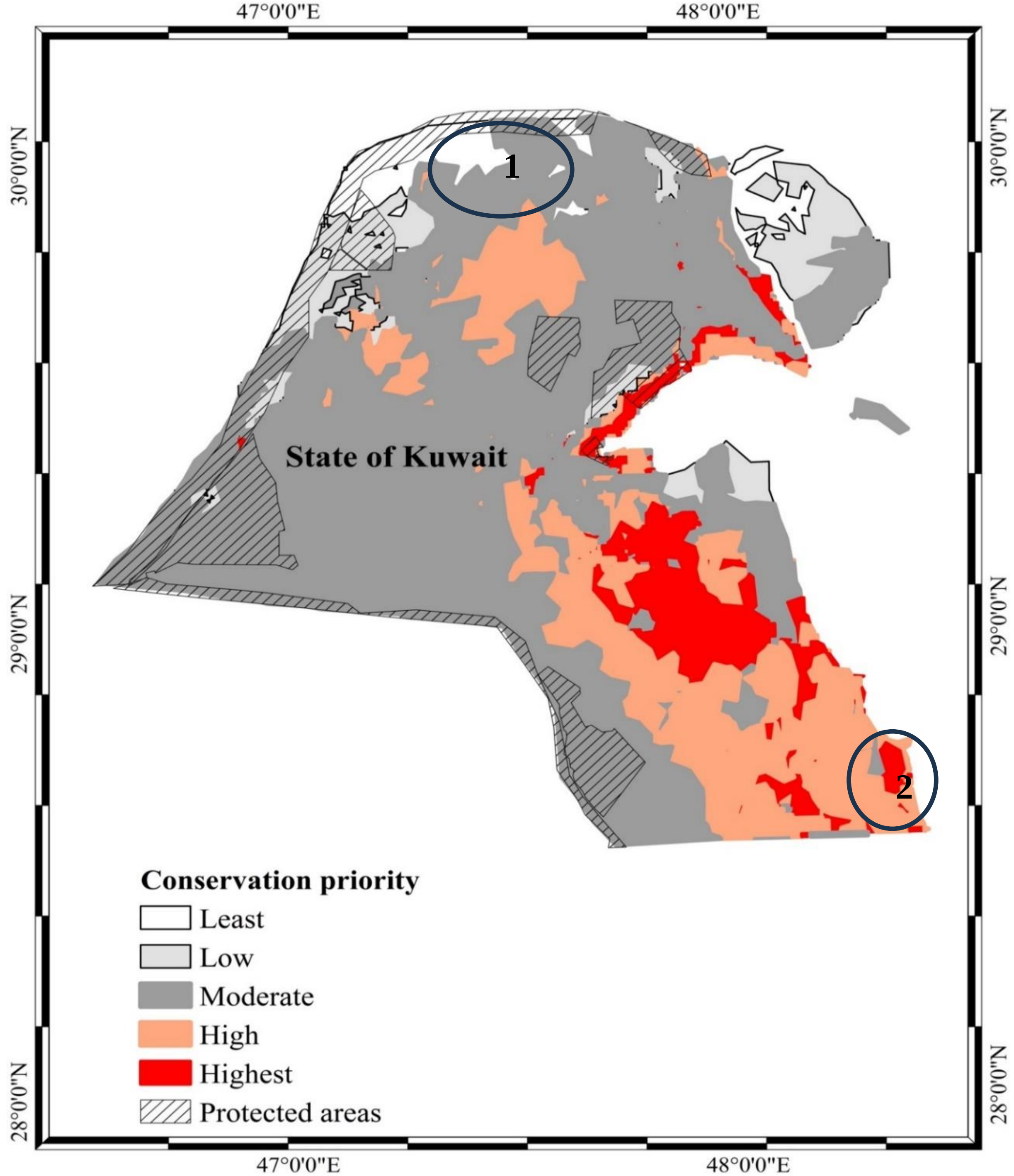

Figure 3.7 Prioritized areas for the conservation of *Uromastyx aegyptia microlepis* (1) Al- Huwaimliya, (2) Nuwaiseeb, based on the addition of the restoration prioritization map of Asadalla et al. (2021) with the natural MaxEnt model map of this study.

areas of cities (Chace & Walsh, 2006; French et al., 2018; McKinney, 2008). Native living organisms are forced out to the peripheral areas (marked in Figure. 3.5 on the land use suitability scale) around the urban settlements, where they are exposed to mid-level pressures that lizards may be able to survive.

Based on jackknife analysis, elevation was also an important variable in both models, as Kalboussi and Achour (2018) have reported in their study of six snake species (which are also ectotherms). Similarly, Eskandarzadeh et al. (2018) stated that elevation is a factor that determines the distribution of the Arabian sand boa *Eryx jayakari*. The sand boas and the Arabian toad-head agama *Phrynocephalus arabicus* (Amr et al., 2021) partially overlap with the spiny-tailed lizards in the preferred habitats and partially share the same trophic level as they both consume arthropods (Al-Sadoon & Al-Otaibi, 2014; Eskandarzadeh et al., 2018). The Arabian spiny-tailed lizard uses mounds and small hills for detecting prey, basking, shelter and for burrow excavation (Al-Sirhan & Brown, 2010). The western and northern parts of Kuwait have more mounds that are favourable sites for the lizard compared to the eastern parts of Kuwait where the area is mostly flat.

In the natural model, the results of this study support those of Aghanajafizadeh and Mobaraki (2018) in that the vegetation factor, as a source of food, plays a role in the distribution of lizards. Additionally, vegetation can provide shelter, as reported by Melville and Schulte (2001) and Wilms et al. (2009), who has reported that desert lizards (Agamids) are semi-arboreal (depending partially on trees or shrubs) as are other saxicolous or ground-dwelling species. Moreover, Wilms et al. (2009) agreed that vegetation is one of the most important variables for lizards as a food resource. These findings were anticipated because vegetation is one of the main dietary choices for *Uromastyx* lizards (Bouskila, 1985).

Additionally, in the natural model, the seasonal precipitation in October (Prec. 10) and annual precipitation (Bio 15) variables play less of a role in the distribution of lizards, since lizards can efficiently extract water from plants on which they forage (Krakauer et al., 1968). Yet, precipitation is a key factor because in the rainy season the lizards' potential for survival increases. As a result, the Arabian spiny-tailed lizard possesses high resistance to xeric (dry or low moisture) habitats of Kuwait because precipitation increases the sources of green water, especially the stored water in native plants (Bouskila, 1985; Al-Sayegh, 2017; AlRashidi et al., 2021). Similarly, the mean temperature in the warmest quarter (Bio10) did not influence the distribution of the lizard, meaning that this lizard is resistant to the extreme high temperatures of the desert environment, a claim supported by Wilms et al. (2009; 2010; 2011).

In the anthropogenic model, land use covariables significantly affected the distribution of the Arabian spiny-tailed lizard. This case was also observed in the Sabhan area. Peripheral areas around urban areas were found to be suitable habitats, and the impact of urban areas on the surrounding peripheral zones could cause the clearance of most native species except for the highly resistant ones, allowing the Arabian spiny-tailed lizard to succeed. This phenomenon is known as the edge effect (Atauri & de Lucio, 2001; Bennett & Saunders, 2010); in this case, they are the processes of development which free the habitat from the natural predators, such as the honey badger *Millivora capensis* and the desert monitor *Varanus griseus*, which usually coexist with this lizard in its natural habitat. This ecological scenario is known as the enemy release hypothesis (Richardson et al., 2011).

Moreover, since this lizard is a generalist in terms of dietary requirements as flexible omnivores, its ability to shift its diet to consuming insects and arthropods maximises its resistance to urban sprawl. Fragmented habitats within urban land use areas do not limit the distribution of lizards in general (Tolley et al., 2010).

In particular, the energy requirement per day for lizards, such as ectotherms, is low compared to that of endotherms such as mammals (Pough, 1983). Therefore, lizards possess an intrinsic ability to survive with limited food resources in fragmented areas that would be intolerable to predators, especially carnivorous mammals.

In addition, the broad diet of the lizard has enabled them to overcome the challenges posed by the scarcity of vegetation, as illustrated by the remains of reptiles found in the faeces of the species (Castilla et al., 2011a; Kevork & Al-Uthman, 1972) which explains why vegetation in the anthropogenic model did not play a role in the distribution of lizards. It is logical to find that vegetation is sparse in fragmented habitats of urban areas, such as the coastal line south of Kuwait Bay (Bennett & Saunders, 2010). After the hibernation season ends in March, the Arabian spiny-tailed lizard is able to survive on vegetation shoots and other sources of food, such as insects and arthropods (Wilms et al., 2010).

Also, in the anthropogenic model, elevation covariate affected the distribution of lizards. As was the case in the natural model, the most reasonable explanation is that the lizard uses elevation for the detection of predators, prey and basking and to avoid inundation of its burrow during the rainy season The lizard burrows, as reported by Aghanajafizadeh and Mobaraki (2018) were mainly seen in regions with a mean slope of 10%.

Overall, the elevation covariate in both MaxEnt models influenced the distribution of the Arabian spiny-tailed lizard, as reported previously (Aghanajafizadeh & Mobaraki, 2018; Eskandarzadeh et al., 2018; Wilms et al., 2010). The elevation variable (the mounds are formed by some native plants such as *Haloxylon sp.* trapping sand drift movements at their roots) created suitable habitats for the lizards as was the case on the eastern coast of Saudi Arabia (Alatawi et al., 2020). The Arabian spiny-tailed lizard also excavates its burrow at slightly elevated surfaces in an open and less dense vegetated landscape as a survival strategy (AlRashidi et al., 2021).

Desert microhabitats play an important role in the distribution of lizards. MaxEnt models only consider macrohabitats. Because the climatic data used in this study are surface measurements (Elith & Leathwick, 2009), which only represent macrohabitats (Hijmans, et al., 2005a), the areas that were assigned as being least suitable for the lizard may be inhabited by the lizard because the lizard is capable of creating suitable microhabitats as burrows. By doing this, it could adapt to the climatic and bioclimatic data layers that would otherwise limit their occurrence. The results of the present study indicate that future studies should consider that microhabitats are needed to construct a method for determining the distribution of the Arabian spiny-tailed lizard.

Optimal habitats for the lizard are best described as flat to slightly sloping surfaces with coarse sandy soil. The vegetation of the habitat would be less dense than in the surrounding areas. In these suitable habitats, arthropods such as scorpions, smaller agamids such as the Arabian toad-head agama, and lizards such as *Acanthodactylus boskianus* and *Stenodactylus doriae* would be abundant and suitable for lizards to forage on after their hibernation period. Sloped surfaces would be the first features that would indicate an optimum habitat for the lizard in which new populations would be found.

To re-establish populations, elevation and vegetation cover are essential natural features that would assist in the process of translocation. Protection should be allocated to areas that are less exposed to disturbances and possess the natural survival elements such as vegetation and elevation heterogeneity. The results of the present study seem to match previous findings by Aghanajafizadeh & Mobaraki, (2018); Wilms et al. (2009); Wilms et al. (2010); Wilms et al. (2011) that lizards are very flexible omnivorous reptiles, favouring habitats with heterogenic elevations and foraging on different plants such as *Haloxylon* and 18 other plant species (Castilla et al., 2011b). Since knowing that disturbance of an ecosystem through anthropogenic activities is one of the main factors that limit the survival of lizards, protection of an area controls human access to protected areas and enhances the survival potential of the species. This was one of the reasons that the Kuwaiti government authorities protected some areas that could be used for the conservation of the biotic and abiotic ecosystems in general. To magnify the output of conservation, it is recommended to assess whether protected sites are achieving the conservation of key fauna species such as *Uromastyx aegyptia microlepis* and, if not, to designate new unprotected areas. Software such as Marxan (Watts et al., 2009; Watts et al., 2017) is an effective tool for the designation of the protected areas that allows a more systematic approach for decision making.

The combination of the MaxEnt model in this study and the data in Asadalla et al. (2021) identified the following areas: Al-Huwaimliya in northern Kuwait (Fig. 3.7, number 1) and Nuwaiseeb in Southern Kuwait (Fig. 3.7, number 2), which are located outside of the protected sites and away from residential and agricultural areas for protection. That is why these two sites were prioritised for the conservation of the lizard. Al-Huwaimliya is a sand dune ecosystem that is mostly composed of a community of two plant genera, *Haloxylon* and *Panicum* (Halwagy & Halwagy, 1974; Halwagy et al., 1982).

There is a small dense region in the southwest corner of the State of Kuwait that contains communities of two or more plant species. The area is the beginning of a sand dune belt extending south-east toward the Wafra area. According to Wilms et al. (2009), the Arabian spiny- tailed lizard favours sandy soil for establishing its burrows. Nuwaiseeb is the southern land of Kuwait that is characterised as a foraging area occupied by *Rhanterium* sp., *Lecium shawi* trees and other perennial grasses. The area can also be designated by the Kuwaiti EPA as a protected area.

3.5 Conclusion and recommendations

In conclusion, the Arabian spiny-tailed lizard shows resistance to anthropogenic pressures, such as changes in land use and land cover in the peripheral areas of cities. Mounds, small hills, and slopes are preferred by the lizards; therefore, the availability of these features affect the distribution of this species.

The lizard distribution maps produced using the MaxEnt model show that the northern region of Kuwait and other protected areas, such as the Sabah Al-Ahmad Natural Reserve north of Kuwait Bay, are major hotspots for the occurrence of this lizard due to the abundance of vegetation cover and the heterogeneous elevations of the sites. Since 1992, the United Nations has assigned the northern areas of Kuwait as a demilitarised zone, banning the public from free access. As a result, the vegetation cover has nearly recovered. A comparison between future and historical climatic and bioclimatic data is recommended for future studies to assess the effect of climate change on the distribution of the Arabian spiny-tailed lizard.

Several environmental factors influence the Arabian spiny-tailed lizard populations, and the interaction of these variables is important to fully understand the potential for conversation of a particular species. The limitation of this study lies in the inability to account for microhabitats, which may be of great importance for the survival of the lizard. The temperatures in summer were extreme, reaching 60°C in the sun. There were many inaccessible areas belonging to the different governmental sectors. This study, however, provides a summary based on compiled information from previous work conducted on the Arabian spiny-tailed lizard for the purpose of its conservation. In general, this study recommends that the Kuwaiti EPA considers immediately establishing conservation posts in Al-Huwaimliya and Nuwaiseeb to conserve the Arabian spiny-tailed lizard populations in Kuwait.

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Chapter 4

The Significance of Protected Areas for the Conservation of *Uromastyx aegyptia microlepis* in the State of Kuwait

4.1 Introduction

The body condition of an animal (condition or corpulence index) refers to its health and fitness state (Jakob et al., 1996; Labocha et al., 2014). An animal in good condition is assumed to have more energy reserves than an animal in poor condition. For instance, individuals with larger energy reserves may have better fasting endurance and higher survival than individuals with smaller reserves (Millar and Hickling 1990). Other fitness parameters related to reproduction and survival have been found to correlate with body condition in many taxa (Dobson, 1992; Dobson & Michener, 1995; Wauters & Dhondt, 1995; Bachman & Widemo, 1999; Shine et al., 2001).

Body condition is a major concept in ecology addressed in countless studies. A variety of non-destructive methods are used to estimate the condition of individuals based on the relationship between body mass and measures of length (Peig & Green, 2009). Body conditions are an estimate of an individual animal's fitness, or coefficient of the relevant size of energy stores compared with structural body components (Green, 2001).

Biologists have developed a number of simple metrics to assess the health and energetic status of individual organisms and populations. Body Conditions Index (BCI) is assumed to indicate the health, quality and well-being of the lizards (Schulte-Hostedde et al., 2001; Peig & Green, 2009; Labocha et al., 2014). The BCI does not measure the amount of energy or energy reserves of lizards; yet it is a linear relation between body length and mass (Hayes & Shonkwiler, 2001; Schulte-Hostedde et al., 2005). The BCI can be directly related to the survival potentials of lizards (Millar & Hickling, 1990; Linden et al., 1992; Le Galliard et al., 2004).

The BCI index is a set of measurements used to quantify the survival fitness of the species in contrast to ecosystem health. In other words, BCI is the best result of how species benefit from the ecosystem provisions of suitable habitats that provide food, climate, and other necessary features for survival (Dudek et al., 2015; Al-Sayegh et al., 2020). Understanding the relationship between climate, habitat characteristics, and the ecology and physiology of the animal provides a better vision for conservation programs (Stark et al., 2022).

The BCI index uses mass and body length as old Body Mass Index (BMI) in humans ; yet the most known method is the Ordinary Least Squares (OLS) regression of body mass against length (Peig & Green, 2009). Condition indices (CIs) such as BMI (Madsen & Shine, 1999, 2002), Ratio and Residual index (Brown, 1996), Tail-base and Relative Mass Index (RMI) (Willemsen & Hailey, 2002; Willemsen et al., 2002) express the fitness and health of species in terms of phenotypic properties (Vervust et al., 2008). The indices are used for habitat integrity, assessing human disturbance (Orians & Soulé, 2001), breeding success (Atkinson & Ramsay, 1995) and predation pressure (Vervust et al., 2008). The BCI and Scaled Mass Index (SMI) are among the important CIs that represent a set of measurements used to quantify the health and fitness of the species in contrast with the ecosystem integrity (Warner et al., 2016; McCaffrey et al., 2023).

The most suitable CI based on Peig and Green (2009) is the SMI which gives consideration to fat content because the test targets Snout Vent Length (SVL). The SMI is a better representation than BCI (McCaffrey et al., 2023) because it relies on the fat component in the body regardless of the lizard size. Whereas BCI represents the size more than indicating the actual energy reserve which matters for the health of the lizards (Peig & Green, 2009; Siliceo-Cantero & García, 2014).

The Arabian spiny-tailed lizard is one of the crucial biotic components of arid ecosystems in Kuwait (Al-Sayegh et al., 2020). The assessment of their health is vital for their sustainability in the desert ecosystem to conserve the ecosystem's integrity. Currently, the description of BCI for the reptiles in protected areas lacks species-specific data; the status of the conservation and performance quality of the species are still partially unknown (Milenkaya et al., 2015). Therefore, the present study aimed to investigate how protected areas impact the health and fitness of the spiny-tailed lizard. This was achieved by comparing lizards in protected and unprotected areas measuring their phenotypic characteristics, especially the SMI. The findings of this study provide an evaluation of the effectiveness of current government protected areas for this species. Future conservation strategies to ensure the sustainability of species are recommended.

4.2 Materials and Methods

4.2.1 Study sites

The study was carried out at four selected sites for a period of 18 months, from May 2017 to April 2019. The sampling sites consisted of two protected sites (Kabd and Khabari Al-Awazem) and two unprotected sites (Al-Abraq and Sabhan) (Fig. 4.1).

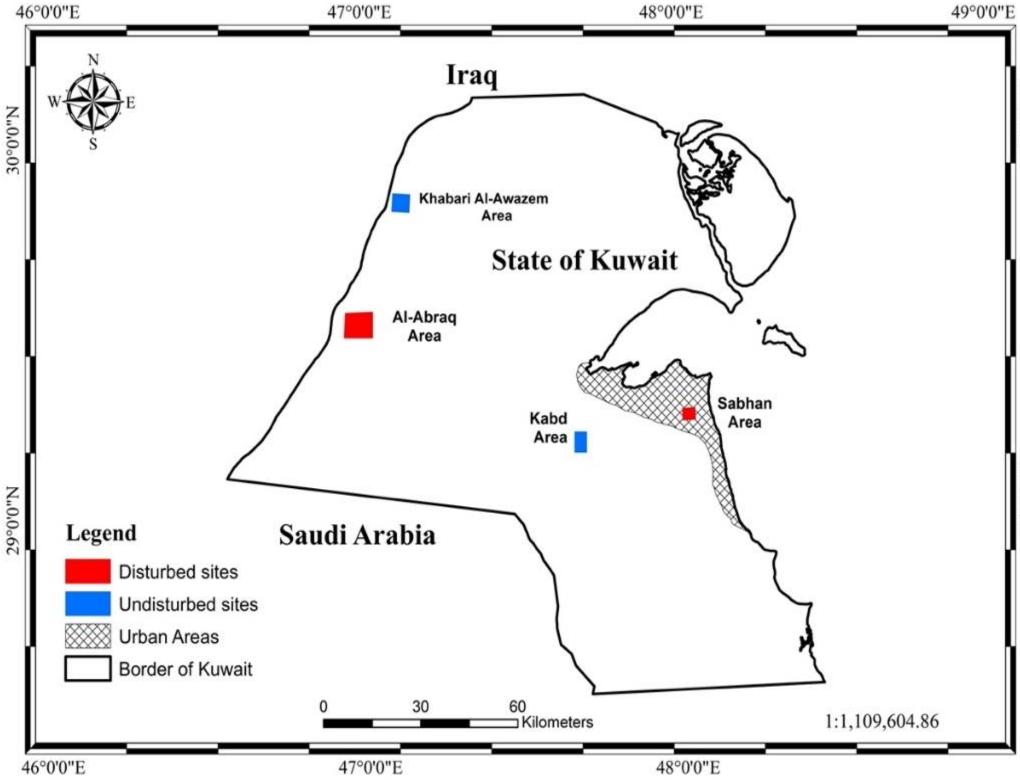

Figure 4.1 Sampling-site locations for *Uromastyx aegyptia microlepis* in the State of Kuwait.

All selected sites were affected by the Gulf War (1990), military activities, camping, mining, and other practices. All of the above activities severely affected the natural properties of ecosystem components, such as soil and vegetation, resulting in the loss of habitats and living organisms. The study sites were allocated according to the information from locals regarding the presence of the species in sufficient numbers and the accessibility of the sites; since many sites in which the lizard lives are restricted and require permits due to being military areas, protected areas or under the control of the Kuwait Oil Company (KOC). The number of sites was limited to four, due to the limitation of manpower and to synchronize the season factor over all sampling sites. Simultaneous field visits of numerous teams are needed to conduct invasive site surveys to obtain the required morphometric measurements in the targeted seasons.

4.2.1.1 Site 1: Agriculture Research Station (Kabd) *de facto* protected area

The Agriculture Research Station was established by the Kuwait Institute for Scientific Research (KISR) in 1975, covering a total area of 40 km^2. The main objective of establishing the station is to protect and conserve desert plants and animals in Kuwait. It is also intended to make possible the conducting of research activities and studies related to animal protection, plant production, rangeland and ecology. This protected area is fundamental for ecological studies on the flora and fauna of Kuwait and the optimization of animal grazing activities. During the last 26 years, several research projects and activities have been completed at the Station. These projects have been in the areas of range management, irrigated pasture production, sheep production and poultry production. In addition, a Desert Animal Centre was established at the Station in 1999.

Originally the total area of the Station was 20 km^2. In 2001, it was decided to double the area by adding another 20 km^2 to increase the protected area in Kuwait for the conservation and protection of more desert animals and plants. The elevation of the ground in this area varies between 130 m (to the west) and 75 m (to the north – east) above sea level. Through the Station, KISR has succeeded in conserving and protecting several plants such as; *Rhanterium epapposum*, C*yperus conglomeratus*, *Gynadriris sisyrinchium*, *Panicum turgidum* and *Farsetia aegyptia* and some desert animals such as the red fox *Vulpes vulpes*, the monitor *Varanus griseus* and the lizard *Uromastyx aegyptia microlepis* (Fig. 4.2).

4.2.1.2 Site 2: Khabari Al-Awazem - protected area

This area is 150 km northwest of Kuwait City on the Kuwaiti side of the Demilitarised Zone (DZ), which was allocated by the United Nations (UN) in 1991 between Iraq and Kuwait under the Security Council Resolution NO. 689-1991. The area extends in the form of a border strip between the two countries, covering approximately a distance of 200 km and an average width of 5 km. The DZ was established in 2003 as a passage point check for the allied troops between the United States of America and Iraq under the governance of the Kuwaiti government and was inaugurated in 2007.

The General Department of Border Security of the Ministry of Interior of Kuwait controls this zone. This corridor has heterogeneous physiological characteristics. It is located in a wadi (valley) that accumulates rainwater seasonally and forms swamps. As a result, the area consists of various annual shrubs and plants such as *Haloxylon salicornicum* (Moq.), *Rhanterium epapposum* (Oliv.), and *Calligonum comosum* (L'Hér.) along with annuals that flourish in rainy seasons compared to nearly bare unprotected areas. This area is highly diversified compared to adjacent open areas (Fig. 4.3).

4.2.1.3 Site 3: Al-Abraq – unprotected area

The Al-Abraq area is located northwest of Kuwait City. The site is a deteriorated rangeland that is heavily grazed by cattle and camels (Fig. 4.4). Additionally, the area is considered a hunting ground for game birds such as the common quail and the Macqueen houbara bustard by use of 4x4 vehicles. The surface topsoil is heavily eroded and compacted; therefore, vegetation cover is scarce. The site has a very flat topography with little or no shade cover. In the past, Al-Abraq was a route of passage for Bedouin nomads between Kuwait, Saudi Arabia, and Iraq. The

Figure 4.2 (a) *Rhanterium epapposum*, the main native plant in Kabd Station. (b) *Uromastyx aegyptia microlepis* at the Station (Photos by W. Behbehani).

Figure 4.3 (a) *Rhanterium epapposum* (b) The monitor *Varanus griseus* at Khabari Al-Awazem area (Photos by W. Behbehani).

Figure 4.4 Various types of human activities pressurizing the natural habitats of *Uromastyx aegyptia microlepis*. (a & b) Overgrazing (c) Anthropogenic waste as a source of pressure on the desert ecosystem (Photos by W. Behbehani).

area got its name because of the existence of alveoli rocks (located on the protected side of the area) that appear shiny white compared to adjacent sandy soils.

4.2.1.4 Site 4: Sabhan – unprotected aera

Sabhan is an area under the administrative responsibility of the Ministry of Defence. The Sabhan Industrial Area, south of Kuwait City, is surrounded by equine stables and light industries. The land has vehicles and horse trails. There is a solid waste dump and a pipeline that passes through the site. The site is also disturbed, most likely due to its proximity to the Kuwait International Airport and military bases.

The site is sporadically covered by natural vegetation, which grows in response to recent precipitation. Annual vegetation, such as *Malva parviflora* and other grasses, occur there. Many burrows of *Uromastyx aegyptia microlepis* are buried due to surface runoff or human intervention. Sabhan area was a natural desert that was used as a rangeland for livestock and camels. However, at present, the pressure of hunting game birds by 4 x 4 vehicles and camping pressurised the soil, leading to further desertification (Fig. 4.5). In March 2018, the Ministry of the Interior was permitted to reclaim a large area of Sabhan site. The allocated area has undergone complete transformation by placing recreational and camping facilities on it (Figs. 4.6 & 4.7).

4.2.2 Data collection

The fieldwork was conducted at the four selected sites twice a week during the study period going from closer sites to the farthest ones. Sample replicates per season were not obtained due to limited manpower. For each captured lizard the coordinates (latitude and longitude) were marked using a Global Positioning System (GPS) device (GARMIN, OREGON 650). Lizards were captured by hand or using cages (metal mesh gravity action trigger traps) designed by Al-Sayegh, 2017 (Fig. 4.8). The cages were placed at the entrance of a burrow into which a lizard was seen retreating. Each captured lizard was tagged with a Passive Integrated Transponder (PIT) (Soorae et al., 2008) to ensure that the same lizard was not recaptured and to avoid double records in the data analysis. A dab of tissue glue (Vetbond

Figure 4.5 (a) *Uromastyx aegyptia microlepis* near its burrow. (b) Vegetation covers the area after the rainy season (Photos by W. Behbehani).

Figure 4.6 (a, b and c) Transformation of a large section of Sabhan site by placing recreational and camping facilities (Photos by W. Behbehani).

Figure 4.7 (a, b and c) Transformation of Sabhan site into a camping site (Photos by W. Behbehani).

Figure 4.8 Captured *Uromastyx aegyptia microlepis* in a metal mesh gravity action trigger placed at the entrance of the lizard’s burrow (Photos by W. Behbehani).

Tissue Glue – 3M Vetbond TM, St. Pau Minnesota, USA) was used to close the skin after needle puncture to prevent accidental loss of PIT and infection. The SVL was measured using a digital calibre (0-150 mm) and the body mass was weighed in grams using dinamometro digital balance (VALEX). Body temperatures were recorded using a digital thermometer through the cloaca. While the ambient temperature was measured using the data of the Metrological Department of the Kuwait General Directorate of the Civil Aviation. After the measurements were taken, the lizards were released at the same place they had been captured. A total of 112 adult lizards were captured from the four study sites, 52 (46.4%) females and 60 (53.6%) males (Table 4.1).

Table 4.1 Number of males and females of captured *Uromastyx aegyptia microlepis* at the selected sites.

	Khabari Al-Awazem (PA)	**Sabhan (PA)**	**Al-Abraq (UPA)**	**Kabd (UPA)**	**Total**
Total sample size	28	34	23	27	112
Female (♀)	11	19	8	14	52
Male (♂)	17	15	15	13	60

4.2.3 Body condition measurements

Body conditions was calculated by "Scaled Mass Index", a novel CI method (Peig & Green, 2009), which standardize body mass at a fixed value of a linear body measurement based on the scaling relationship between mass and length according to the following equation:

$$M_i = M_i \ \left(SVL_0/SVL_i\right)^{b_{SMA}}$$

Where for each individual i, M_i is the body mass (in grams), and SVL_i is the body measurement (mm), SVL_0 is the mean SVL across the measured lizard, and b_{SMA} is the scaling exponent which is calculated by dividing the slope of the linear model between log-scaled mass and log-scaled SVL by Pearson’s correlation coefficient between the variables.

Finally, the calculated Scaled Mass Index (M_i) represent the scaled body mass when the SVL is standardized to SVL_0. Data were categorized by site protection status bivalent nominal value (protected, unprotected) and sex type (male or female). Juveniles were not included in this study because in some species individuals of different ages differ significantly in their body proportions (Dudek et al., 2015). Additionally, data were sorted per summer, spring, and fall. No lizards were captured during winter due to hibernation.

4.2.4 Statistical analysis

In this study qualitative data with nominal values such as sex, study site and site protection status were used with the morphometric quantitative measurement with continuous values such as body length, SVL and body temperature. Therefore, the mixed method (Östlund et al., 2011; Adatho, 2011) was adopted because a combination of qualitative and quantitative approaches were used to assess the effect of protected sites, sex, seasonality, body, and ambient temperatures on SMI.

Therefore, nominal (for qualitative variables) and ratio (for quantitative variables) entry types were used for the variables. Thus, categorial and numerical variables were obtained. Finally, dummy variables were used in relation to multiple regression to express the variable attributes and avoid bias. This study assumes that SMI in protected sites indicate better performance in terms of fitness and health than the lizards at unprotected sites.

A *post hoc* test was used to apply on each sex separately to check that each sex has no effect on the other. Locations and seasons were set as predictors (independent variable) while the respondent factor used was SMI. Multiple regression analysis was used for the whole population and for each sex separately; the reason for that is to evaluate the effect of each sex and the effect of both sexes on SMI. In other words, after viewing the effect of each sex separately; both sexes were combined to see whether one sex has an effect on SMI in the presence of the other sex.

The following continuous data were used: 1) SVL and body mass (from which SMI was extrapolated); 2) Body temperature; and 3) Ambient temperature. A two-way analysis of variance ANOVA was used to assess the effect of the sex and site protection status on SMI. The reason for selecting body temperatures at captures indicates metabolic rate, energy consumption and in general the fitness and performance in nature. While the ambient temperature variable indicates the suitability of the habitat in supporting the lizard’s activity. Snout-vent length expresses how does the lizard invest in energy in relation with its organs development (Olsson & Shine, 1997). The categorical data used were location; season and sex (Only used in the analysis of the whole population). Multiple regression was used to determine the best predictor of SMI. Ambient and body temperature of the lizards along with sex, locations and seasons categorical variables were used as predictors, whereas, SMI was used as the dependent variable in multiple regression analysis.

Dummy variables were used to assign a numerical value for the attributes of each categorical variable. For instance, three attribute groups were assigned for Al- Abraq, Kabd, Khabari Al- Awazem, and Sabhan study sites. The number of numerical groups assigned equalled their degree of freedom. In this case, three numerical groups (attributes) were assigned for the study sites. Moreover, the levels of measurement used in the subgroups were nominal; meaning that the value 2 is not twice as much as 1; the numbers were used instead of the text format of the sites’ names. One numerical code was used combining the three attributes of the variable.

For instance, if the sample belonged to Al-Abraq site, the code would be (100) while the code for a sample belonging to Kabd would be (010) and if the sample belonged to Sabhan site, the code would be (000). Another example, if we named four colour variables green, white, red, and blue, the following codes (100) for green and (010) for white, (001) for red and for blue the code (000) is used. Note that the number of dummy variables = number of responses -1. JMP software performs this step by default. All statistical analyses were performed in JMP software Version 7 with a level of significance $p = 0.05$.

4.3 Results

4.3.1 The relationship between seasons and location on lizards' SMI

Males and females showed that seasonality weakly affect the SMI ($p = 0.0026$ and $p = 0.047$ respectively). Yet, the R^2 values of 0.18 for female lizards and for males 0.11 are very weak. Therefore, it can be generalised that male and female lizards are very similar (Figs. 4.9 and 4.10) and (Tables 4.2 and 4.3).

4.3.2 The effect of variables on SMI for male and female lizards

The results for male and female lizards show that ambient temperature, Khabari Al-Awazem Site (Protected), sex, spring season (Seasonality) and body temperature affected the SMI at significance Values of 0.008, 0.043, 0.0275, 0,046 and 0.001 respectively. The overall significance for the SMI valued 0.0001, $R^2 = 0.99$ (Fig. 4.11 and Table 4.4).

4.3.3 The effect of variables on SMI for each sex separately

Multiple regression in male lizards only shows that protection actions did not affect their SMI; the variables spring season, ambient temperature, and body weight affected SMI at p -values equalled 0.026, 0.034 and <0.001 respectively. The overall significance of the comparison between the actual and the predicted SMI for male lizards equalled $p < 0.001$ and $R^2 = 0.99$ (Fig. 4.12 and Table 4.5). Multiple regression for female lizards separately shows that the protected Khabari Al-Awazem site and total body weight affected SMI at p -values 0.017 and <0.001 respectively and $R^2 = 0.99$. (Fig. 4.13 and Table 4.6).

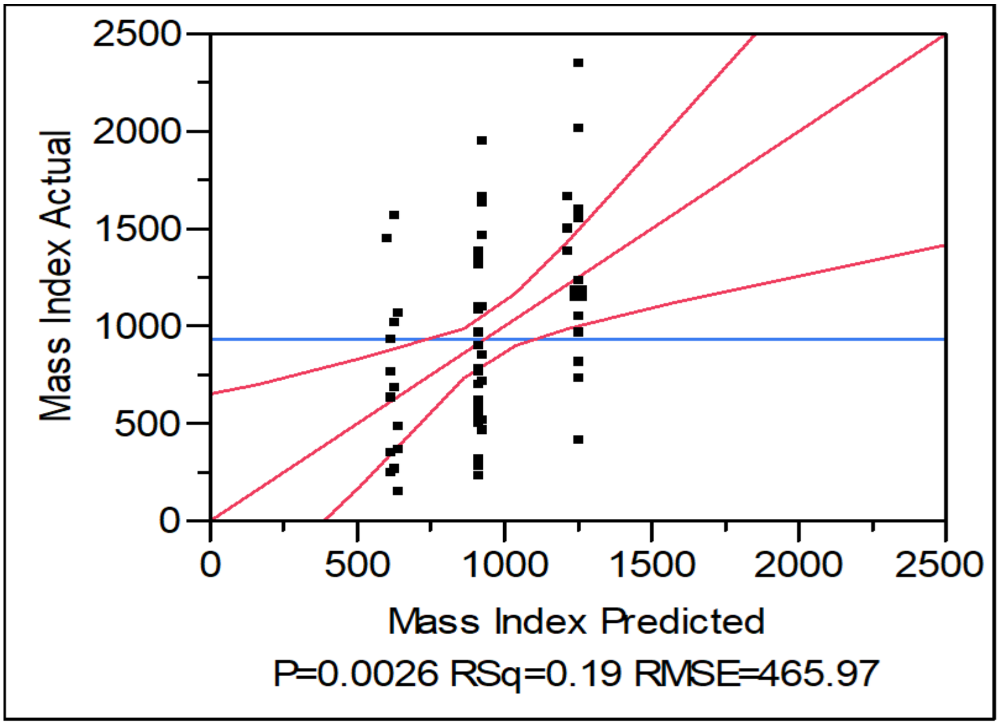

Figure 4.9 Actual Vs. Predicted mass index plots for *Uromastyx aegyptia microlepis* females sample group.

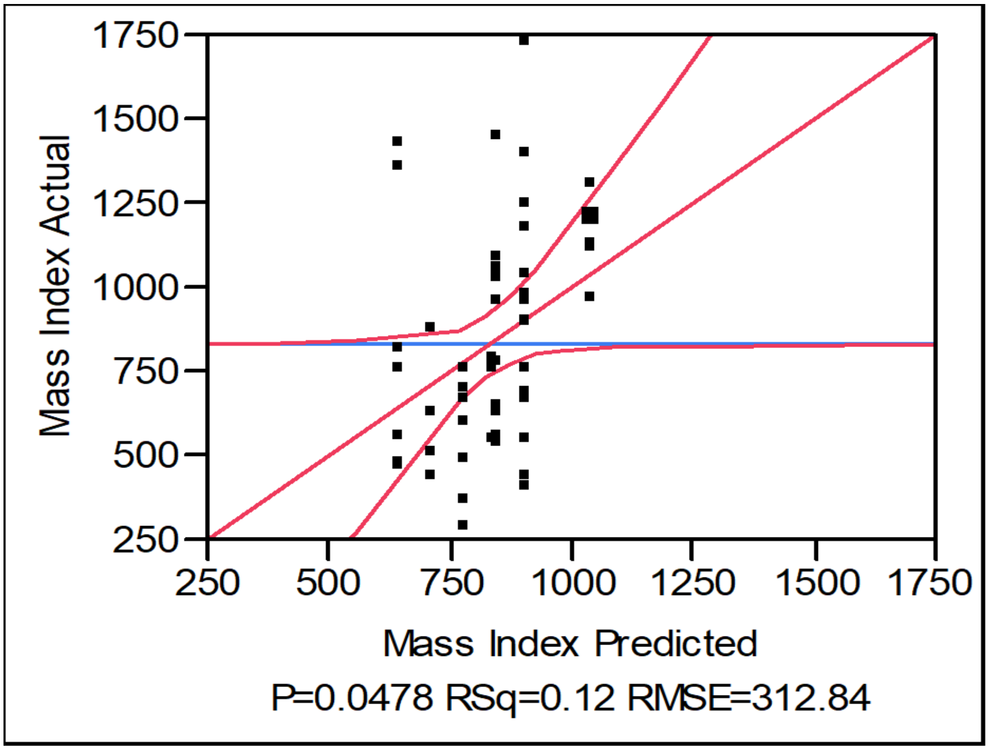

Figure 4.10 Actual Vs. Predicted mass index plots for *Uromastyx aegyptia microlepis* males sample group.

Table 4.2 Two-way ANOVA variables estimate for *Uromastyx aegyptia microlepis* females.

Term	**Estimate**	**Std Error**	**t Ratio**	**Prob>|t|**
Season	-133.6056	61.90341	-2.16	0.0358*
Location	-65.55504	37.71528	-1.74	0.0885**

*Significance level 0.05, **Significance level <0.001

Table 4.3 Two-way ANOVA variables estimate for *Uromastyx aegyptia microlepis* males.

Term	**Estimate**	**Std Error**	**t Ratio**	**Prob>|t|**
Season	-305.4096	87.40946	-3.49	0.0009**
Location	10.930509	53.82769	0.20	0.8398*

*Significance level 0.05, **Significance level <0.001

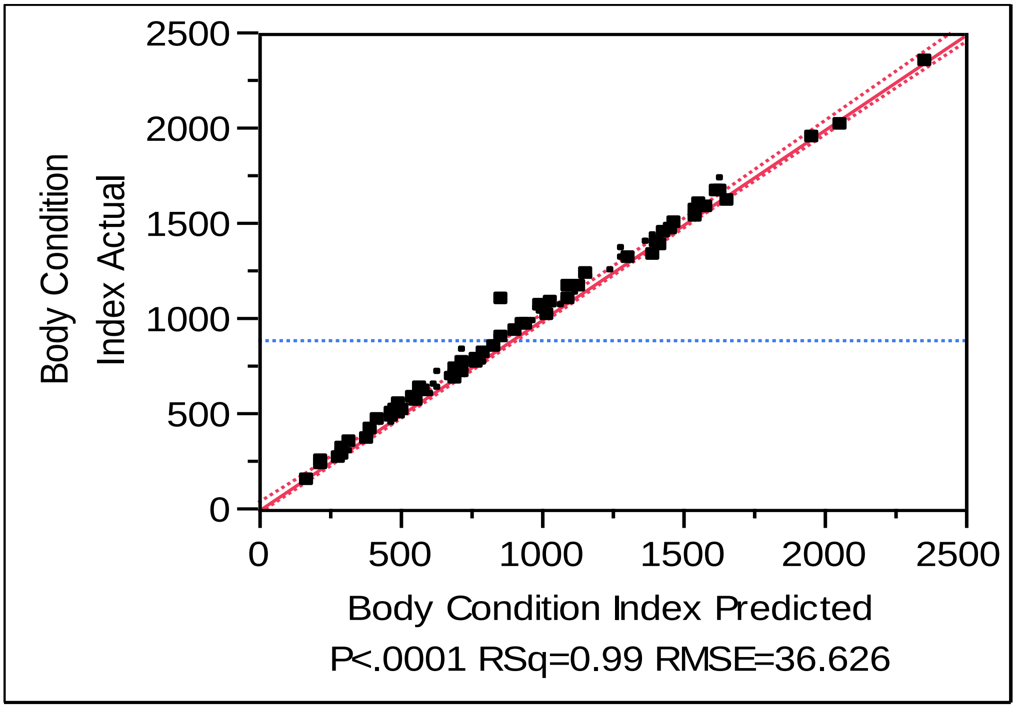

Figure 4.11 Actual Vs Predicted SMI of the whole population of the sampled *Uromastyx aegyptia microlepis* lizard.

Table 4.4 Parameters estimate for the multiple regression on the variables affecting SMI and the effect test for the variables on SMI for the *Uromastyx aegyptia microlepis* lizard.

Term or source	**Estimate**	**Std Error**	**t Ratio**	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Body Temperature	-0.153819	0.613274	-0.25	1	1	84	0.0629	0.8025
Ambient air Temperature	3.0710629	1.134175	2.71	1	1	9836	7.3319	0.0080*
Al-Abraq DV	-7.611481	5.660332	-1.34	1	1	2426	1.8082	0.1817
Kabd DV	-13.7846	13.39746	-1.03	2	2	5904	2.2004	0.1160
Khabari Al-Awazem DV	-14.70156	7.177688	-2.05	1	1	5628	4.1953	0.0431*
Male DV	8.367791	3.74067	2.24	1	1	6713	5.0041	0.0275*
Spring DV	-17.95563	8.917973	-2.01	1	1	5438	4.0539	0.0467*
Summer DV	-9.720092	9.212152	-1.06	1	1	1493	1.1133	0.2939
Body temperatures	0.8723498	0.00837	104.22	1	1	14572108	10862.79	<.0001*

*Significance level 0.05, **Significance level <0.001

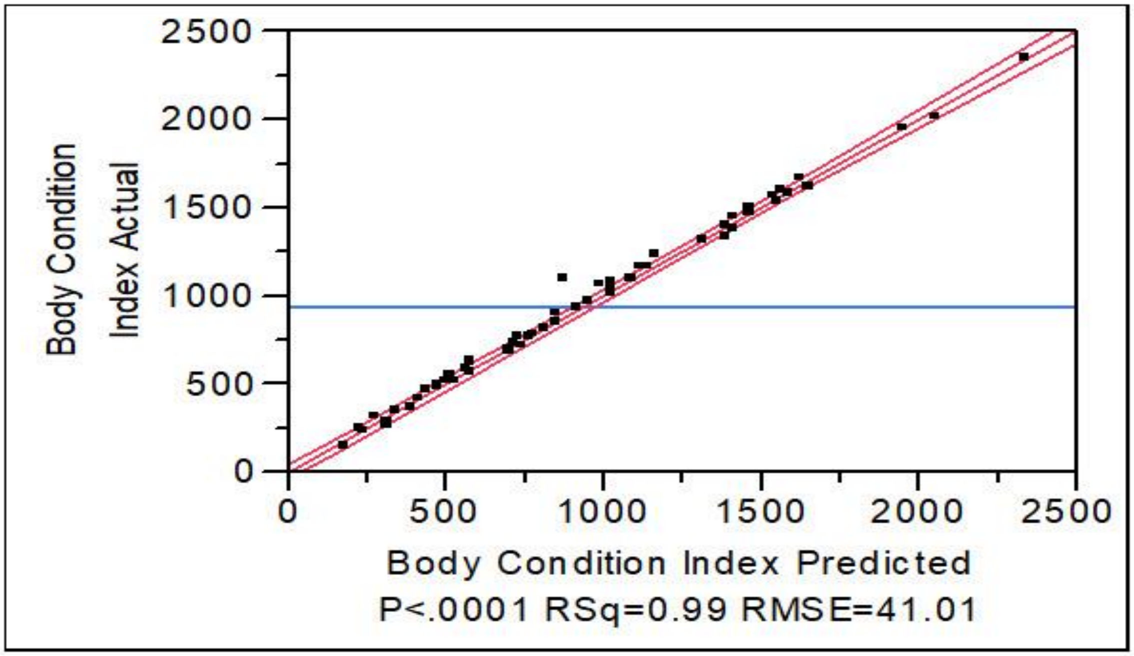

Figure 4.12 Actual Vs Predicted SMI for the *Uromastyx aegyptia microlepis* males.

Table 4.5 Parameters estimate for the variables affecting the SMI and the Test effects for the variables affecting the SMI of *Uromastyx aegyptia microlepis* males.

Term or source	Estimate	Std Error	t Ratio	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Total Body Weight g	0.8547437	0.011772	72.61	1	1	8867019.7	5272.380	<.0001**
Body Temperature	-0.665716	0.735306	-0.91	1	1	1378.5	0.8197	0.3696
Ambient air Temperature	3.3729497	1.548502	2.18	1	1	7979.3	4.7446	0.0341*
Al-Abraq DV	-10.11533	9.323841	-1.08	1	1	1979.4	1.1770	0.2832
Kabd DV	-21.4225	16.49822	-1.30	2	2	7338.3	2.1817	0.1235
Khabari Al-Awazem DV	-18.26661	13.35979	-1.37	1	1	3144.0	1.8695	0.1777
Spring DV	-29.92327	13.06036	-2.29	1	1	8828.3	5.2494	0.0262*
Summer DV	-16.7987	17.98848	-0.93	1	1	1466.7	0.8721	0.3549

*Significance level 0.05, **Significance level <0.001

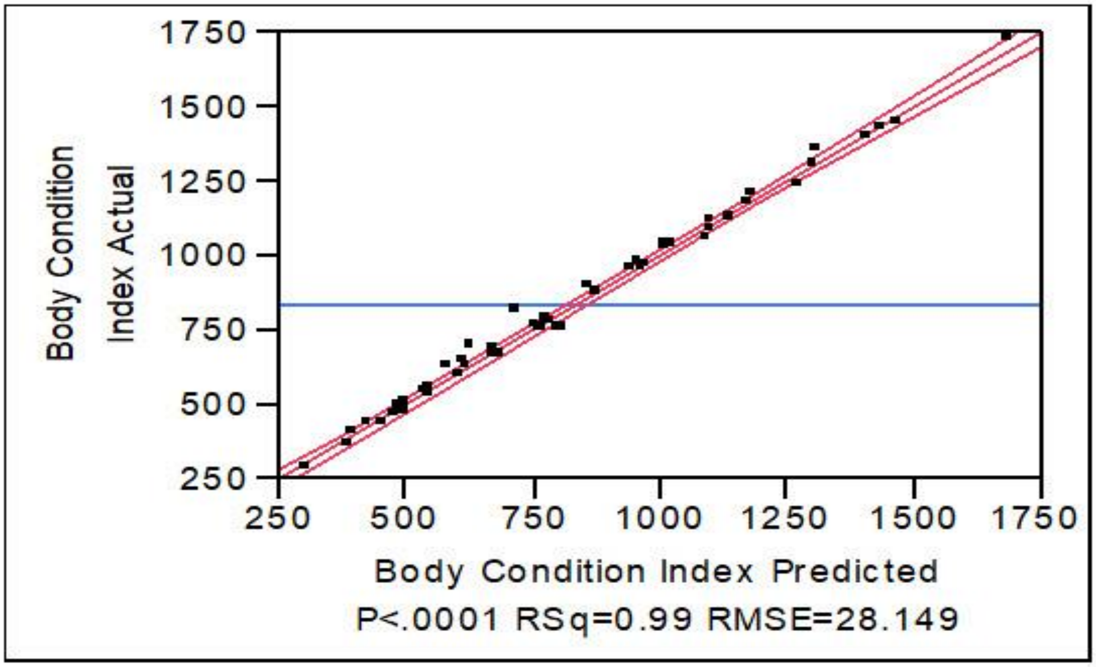

Figure 4.13 Actual Vs Predicted SMI for the *Uromastyx aegyptia microlepis* females.

Table 4.6 Parameters estimate for the variables affecting the SMI and the Test effects for the variables affecting the SMI of *Uromastyx aegyptia microlepis* females.

Term or source	**Estimate**	**Std Error**	**t Ratio**	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Body Temperature	0.8176956	1.532218	0.53	1	1	225.7	0.2848	0.5963
Ambient air Temperature	2.3376903	1.836156	1.27	1	1	1284.3	1.6209	0.2098
Al-Abraq DV	-2.091176	7.249932	-0.29	1	1	65.9	0.0832	0.7744
Kabd DV	-11.10135	6.078181	-1.83	1	1	2643.1	3.3358	0.0747
Khabari Al-Awazem DV	-19.38878	7.857187	-2.47	1	1	4824.8	6.0893	0.0177*
Spring DV	0.6148547	14.10164	0.04	1	1	1.5	0.0019	0.9654
Summer DV	0.4111918	11.97654	0.03	1	1	0.9339807	0.0012	0.9728
Total Body Weight g	0.9108483	0.013097	69.55	1	1	3832473.1	4836.902	<.0001**

*Significance level 0.05, **Significance level <0.001

4.4 Discussion

An important finding of this study is that protecting an area does not affect the SMI of the lizards. More, seasonality overruled other predictors for testing SMI. This finding is somewhat unexpected given the fact that other research works (Gaston et al., 2008; Treves, 2009) showed that protecting an area is a cornerstone step for enhancing the fitness of targeted species in general including reptiles. Also, protection of a habitat could allow the native plants to flourish and diversify providing more suitable foraging habitats than that of the unprotected sites (Norton-Griffiths & Said, 2010).

The results further reveal that Khabari Al-Awazem protected site affects the SMI of the lizards. This makes sense when knowing that the area is classified by Asadalla et al. (2021) as high prioritised conservation area because it hosts multiple native plants making the area an important foraging site for many desert species. Another explanation would be that the sandy soil type of Khabari Al-Awazem, the potential occurrence of native communities of perennial plant species are preferred by lizards (Williams et al., 1999; Wilms et al., 2009; Alrashidi et al., 2021).

Protection at Kabd area did not affect the fitness of the lizards because the green cover density in the area is limiting the occurrence of the lizards (Alrashidi et al., 2021); lizards have more preference to areas of less dense vegetation to be more efficient in monitoring their predators (Arnold, 1984). Protected areas did not promote the fitness of the lizards because the fences did not limit the access of the lizards to the outer unprotected areas. mainly, the features of the unprotected sites were sufficient for sustaining the lizard population and protection of the areas did not add an advantage on the population health over the ones in the unprotected sites.

It is possible that the unmeasured extent of human intervention in the Kabd protected area could have an adverse result on the fitness of the lizards despite the proper protection measures applied in the site. This indicates that conservationists should not assume that protection of the sites would promote the well-being of the lizards; yet they must make sure that variables as the vegetation density, species assemblage and other variables promote the suitability of the habitat to host the targeted Arabian spiny-tailed lizard.

The results obtained were expected in the unprotected sites. Part of Sabhan site, at the time of sampling was transformed into a governmental facility, to be used for camping, military parade and for display purposes. Therefore, the area was heavily disturbed. In addition, Al-Abraq site, which mostly consists of bare lands, where lizards coexisted with predators such as Honey Badgers *Mellivora capensis*, red foxes *Vulpes vulpes*, and stray dogs (Cowan, 2013; Amr et al., 2021) could limit the habitat suitability and affect the fitness of the lizards.

Most of the protected areas in Kuwait are established to conserving a whole ecosystem, landmark site, engineered ecosystems; the protection measures did not aim for conserving a targeted species (Kingswood et al., 2001; UNEP-WCMC, 2023). Thus, the only species that benefit from the protection measures are the ones whose hotspot habitats are protected. This fact may hold true for lizards in Kuwait.

The dimorphic characteristics of lizards are noticed within their hormonal, behavioural and the reflection in response to the attributes of ambient habitats. Spring season is the recovery season for lizards after winter season as a preparation step to summer season where the Arabian spiny-tailed lizards reproduce. Therefore, the results of this study show that spring has a significant effect on male lizards only particularly on their androgens activities and SMI seems logical (Abu-Zinadah, 2008; Wilms et al., 2009).

The approach depending on SMI of the spiny -tailed lizard may be misleading at times. The idea of conservation depending on establishing protected areas may not turn out with positive results. Establishing the protected areas without exclusive evaluation of the biotic and abiotic indicators ‘suitability for the protected area to host the lizard will not produce significant results; yet the protection could benefit a predator not the targeted species creating a stressful habitat. For instance, protection as a conservatory step was not the main factor affecting SMI of the lizard. The results do not reflect the assumption of this study that protection would positively affect the lizards’ SMI. The main reason for that may be relevant to the method of the selection of the study areas.

In statistics, it is known that larger sampling size and sites yield more accurate and representative results. Because the sampling sites were allocated for this study based on traditional locals’ knowledge and the instruction and regulations of the Ministry of Interior, limited study sites were surveyed. This incident limited the sampling sites and size. Therefore, the results may not reflect the actual situation. More precise results for the effects on SMI are expected when hormonal responses partially due to anthropogenic threats, seasonality, physiological parameters, physiographic attributes are considered (Tracy et al., 2005; Wilms et al., 2009).

Because of the limitations in manpower, the simultaneous visits to multiple study sites were not possible. Synchronizing the timings of sampling at the allocated sites provides more chances of obtaining precise results and granting the chances for conducting comparative studies between study sites and the seasons. Further, the extreme temperatures in summer season are intolerable. Some areas that would grant more chances to obtain better findings could not be accessed as being private and governmental sites. The Ministry of Interior instructed the team to the accessible areas. The access banned areas would have offered more chances to survey more lizard groups.

The methodology of this study is the most important point of improvement. Reconsidering more sampling sites may alter and strengthen the outcomes of the study. It is suggested to assess whether the SMI is a proper predictor for the fitness of the lizards. Moreover, the opportunity to include burrow density, production success and home range among the variables used in the study were missed.

For future studies, biotic factors such as vegetation cover and abiotic factors as soil type, elevations and burrow attributes should be used in the multiple regression to test the effects on SMI of this lizard. Moreover, the resistance and resilience of the lizards could be tested to understand how lizards adopt survival strategies in a habitat. Telemetric studies on the daily coverage area of the Arabian spiny-tailed lizard in protected and unprotected areas is highly recommended.

Delineating the daily coverage area for the lizard would add more value to the study targeting the dispersion of the species. Moreover, investigating the feeding ecology of the species (food availability) types of food eaten and relative frequency of each food type should be an excellent topic to study as an additional approach to assess the well-being of the lizards in protected and unprotected areas.

4.5 Conclusions

Protecting a species hotspot is more effective than protecting an ecosystem. Conservation of native reptiles in the desert habitat of Kuwait requires further redirection towards prioritizing the conservation of the species' hotspot habitats to increase the efficiency of sustaining the species in the country. Further, at non-hotspot habitats, habitat modifications could be applied towards promoting the suitability of the habitat. For example, native species that are preferred by the lizards could be propagated to enrich the rangeland. In Addition, the predators of the lizards could be culled in population to provide a better potential for the lizards to increase in populations. The last modification needs more investigations because modifying other native species for the conservation of a targeted species may not yield the expected results (Phillips & Union, 2002; Hoffmann et al., 2008; McGowan et al., 2017). Apparently, the verified notion that protected areas are a suitable magic bullet for conservation for all species should be reconsidered.

The results of this type of study benefits the protected areas management bodies and helps them to understand the most acceptable means of allocating areas for protection and how to meet the required attributes of a suitable habitat for the lizards. This study has joined the ecological studies of native wildlife with conservation management approach to develop a better approach for species base management.

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Chapter 5

Population Genetics of *Uromastyx aegyptia microlepis* in the State of Kuwait

5.1 Introduction

Genetic diversity is at the heart of population health and evolutionary potential (Ellegren & Galtier, 2016) and is directly connected with complex interactions at higher levels of biological order such as mutation, random genetic drift, gene flow and natural selection from species to ecosystems (Reusch et al., 2005; Hughes et al., 2008; Pelletier et al., 2009; Post & Palkovacs, 2009; Schoener, 2011; Kokko et al., 2017). Across species, the distribution and patterns of genetic diversity are influenced by the interaction between different eco-evolutionary processes, such as genetic drift, gene flow, natural selection, as well as simple survival and reproduction (Vellend & Geber, 2005; Sork, 2016; Burak et al., 2018). These processes are also influenced by the spatial distribution and size of the population, which fluctuate over time in response to historical and contemporary biotic and abiotic factors. Deciphering the relative impact of these processes on current diversity patterns is a challenging task that relies primarily on the evaluation of historical influences. However, this is a critical step in building on relevant conservation plans.

Protected areas (PA) are an efficient tool to preserve genetic diversity and minimise the impact of global changes (Geldmann et al., 2019). In many cases, PAs are designed following opportunistic rather than scientific criteria, leading to important mismatches between protected area and biological diversity components (Dudley & Stolton, 2008). In fact, genetic diversity and related processes are barely considered even though they are crucial for the effective functioning of PAs (Kahilainen et al., 2014; Coates et al., 2018). Among the eco-evolutionary processes impacting successful conservation strategies, particular attention should be paid to connectivity within PAs, as well as between PAs and neighbouring unprotected areas. Correct identification of any homogenising or disruptive effects such as inbreeding, climate change, pollution, destruction of habitats, invasive species, overexploitation of the natural environment, etc. that may induce population depletion will allow the maintenance and replenishment of genetic diversity as was observed by Madsen et al. (2020) were deleterious effects of inbreeding in an isolated population of adders *Vipera berus* was reduced by genetic rescue. Therefore, an important step to improve PA management is the identification of populations and their genetic diversity.

One main obstacle to genetic diversity is inbreeding. Inbreeding can be defined as the mating of closely related individuals within the same population (Van Dyke, 2008). The level of inbreeding can be assessed by measuring shared ancestry in the maternal and paternal lineages of an individual, genetic drift in a finite population in terms of the decrease in heterozygosity relative to a random mating population, and the mating system specific to a reproducing population. Each of these three dimensions of inbreeding grows stronger in its effects as the population size decreases. Such effects can and must be measured to make informed and appropriate management decisions regarding breeding and conservation strategies for small populations (Templeton & Read, 1994). Inbreeding can be used as a measure of a mating system in a population, quantified as a value called the panmictic index f. The panmictic index measures inbreeding as a deviation from a reference population, which has a system of mating in which alleles at a locus are paired in proportion to their frequencies in the overall population (by definition, random mating). The panmictic index evaluates deviations from the heterozygosity frequencies expected under random mating:

$$f = 1 - H_o/H_e$$

H_e is the expected heterozygosity under random mating, and H_o is the observed heterozygosity. (H_o) can be calculated from genetic measurements of sampled individuals. If the observed heterozygosity is $f < 0$, then the population has a reproductive system that avoids inbreeding. On the contrary, if the observed heterozygosity is $f > 0$, inbreeding is not avoided. The value of the panmictic index can be used to quantify the degree of avoidance of inbreeding in a population. When populations are inbred, genotypic frequencies are skewed towards higher proportions of homozygous individuals, and heterozygosity decreases. As the proportion of homozygous individuals increases, so will the manifestation of recessive traits, which can only be expressed in a homozygous condition, but which are maintained in the population by heterozygous carriers. In environments that select against recessive conditions, inbreeding can predictably lead to inbreeding depression. Inbreeding depression is a pattern of reduced reproduction and survival that occurs on account of inbreeding (Frankham et al., 2002) and can happen when historically large, outcrossing populations suddenly decline to only a few individuals, in addition to reduced survival and fecundity.

The inbreeding measure can also be used with the panmictic index in combination with the Hardy-Weinberg equilibrium law, a foundational principle of modern genetics and population genetics (Crow, 1988; Namipashaki et al., 2015):

$$p^2 + 2pq + q^2 = 1$$

For an autosomal-diploid variant, the principle establishes that genotype frequencies achieve a stable composition in one generation of time and will remain in the absence of disturbing forces. For biallelic variants, this implies that genotype frequencies will have relative frequencies (AA = p^2, AB = 2pq, BB = q^2), where p and q are the allele frequencies of A and B, respectively, with p + q = 1. The Hardy-Weinberg principle becomes more complicated if one considers the X chromosome (Crow & Kimura, 1972), multiple alleles (Hernandez & Weir, 1989; Guo & Thompson, 1992; Aoki, 2003; Huber et al., 2006), null alleles (Carlson et al., 2006; McCarroll et al., 2006), copy number variation (Lee et al., 2008; Recke et al., 2015), or polyploidal species (Meirmans et al., 2018; Sun et al., 2021). The Hardy-Weinberg law plays a crucial role in probability calculations and in the analysis of microsatellite data (Morin et al., 2009). In genetic subpopulation studies, statistical tests for the Hardy-Weinberg principle are routinely applied to autosomal short tandem repeats (STR), single nucleotide polymorphisms (SNP) and micro-haplotypes as part of quality control procedures and to calculate observed homozygosity and heterozygosity ratios (Kidd et al., 2014; Waples, 2015; Chen et al., 2017).

Microsatellite loci are polymorphic DNA markers in eukaryotic species (Morin et al., 2009). A high mutation rate in several short repeat regions constitutes the basis for their extremely high level of polymorphism in most species. In addition to having the potential to show a high level of variability, microsatellite techniques also require very small amounts of tissue, allowing the release of the animal immediately after sampling. This makes them ideal tools for studying genetic variability in small and inbred populations when conventional markers, such as allozymes and mitochondrial DNA, fail for this purpose. During the past three decades, DNA fingerprinting and amplification of microsatellite loci have greatly increased the potential to detect high levels of genetic variability in reptilian populations, due to the high mutation rate that quickly drives new genetic variation at these loci (Cooper et al., 1997; Gardner et al., 2000; Broquet et al., 2007; Ariani et al., 2013; Shaney et al., 2016).

The objective of this study was to evaluate the genetic diversity of the *Uromastyx aegyptia microlepis* lizard by comparing the genetic makeup of the protected and unprotected subpopulations in the Kuwait desert using microsatellite primers. Protected habitats are those where access is limited as they are fenced and permission is required to enter. Unprotected habitats are habitats that are not protected from environmental factors such as urbanization, poaching and industrialization.

The hypotheses for this study were the following: 1. Genetic heterozygosity within the Arabian spiny-tailed lizards' population is not affected by the protection status of the habitats in the State of Kuwait; 2. The unprotected habitats in Kuwait have a negative impact on genetic diversity within the populations of the Arabian spiny-tailed lizards.

5.2 Materials and Methods

In this study, genetic data was obtained from seven microsatellite primers that were used to monitor the genetic diversity of *Uromastyx aegyptia microlepis* in the State of Kuwait. The study was carried out on this lizard at two protected sites: Khabari Al- Awazem and Kabd and two unprotected sites: Al-Abraq and Sabhan (Fig. 5.1).

5.2.1 Collection of DNA samples

At each study site, lizards were captured using cages, snares, or hands. Buccal cells were collected from 129 lizards using CytoSoft Cytology collection brushes (Medical Packaging Corporation, Camarillo, CA) as follows; Khabari Al-Awazem (31 samples), Kabd (33 samples), Al-Abraq (29 samples), Sabhan (36 samples). Buccal cells were collected by swabbing firmly against the inside of both cheeks, swabbing up and down at least three times for each cheek to ensure a higher yield of cells. The swabs were cut into 1.5 ml dry micro-centrifuge tubes. The tubes were tightly closed, labelled, and kept on ice for the duration of the field trip. Once in the laboratory, the swabs were frozen at -80 ° C until DNA extraction.

5.2.2 DNA extraction

DNA extraction was performed using the Gentra Puregene Buccal Cell Kit protocol (Qiagen, Maryland, United States). The extracted DNA was quantified using a NanoDrop nd1000 spectrophotometer (Thermo Fisher Scientific). Genomic DNA quality was determined using

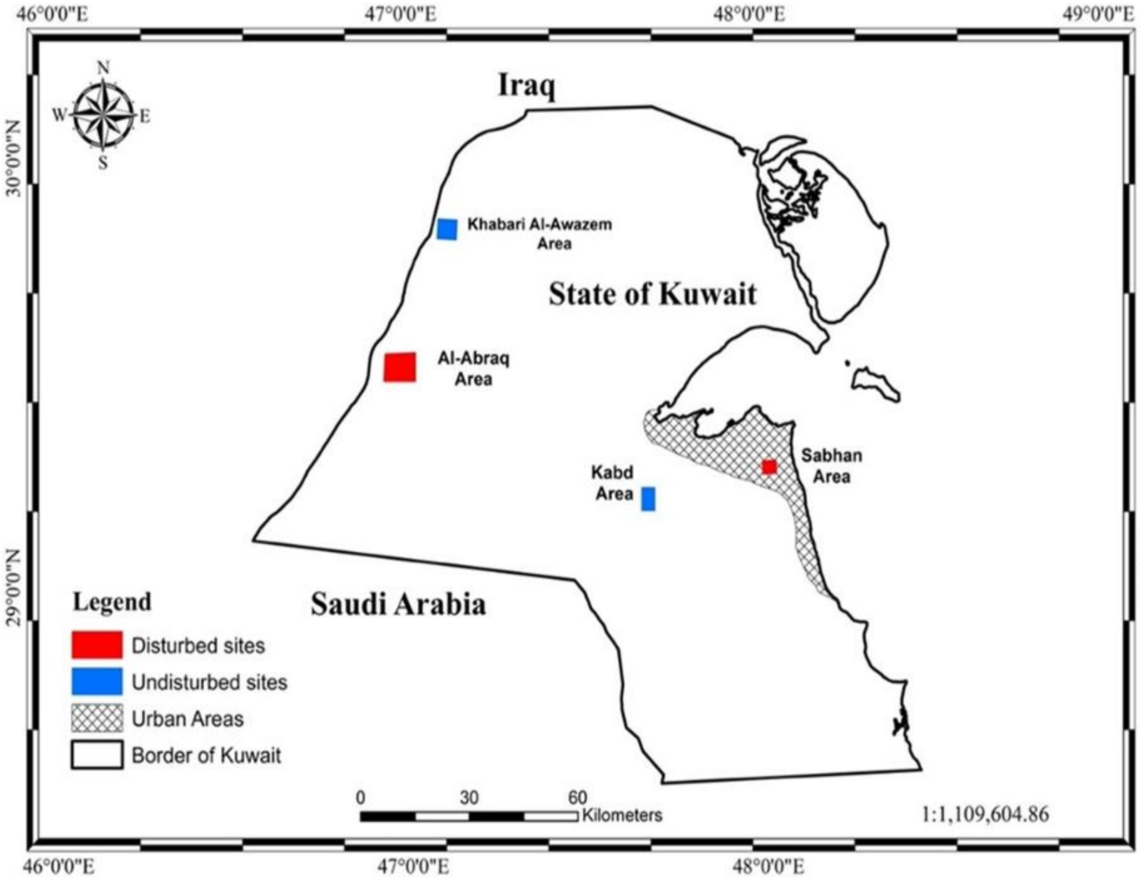

Figure 5.1 Sites for collection of genetic samples from *Uromastyx aegyptia microlepis* in the State of Kuwait.

the 260/280 spectra ratio. Samples outside the ratio of 1.6-1.8 were repeated or rejected. For quality assurance purposes, only 112 samples that met the high purity criteria were selected from the 129 buccal swap samples. The DNA concentration range varied from 30 ng/µl to 738 ng/µl.

5.2.3 PCR Conditions and analysis

PCR was carried out on 112 samples using 12 microsatellite primer pairs produced by Invitrogen primer development services (Shaney et al., 2016). These 12 microsatellite primer pairs amplify penta- and hexa-nucleotide repeat regions. The microsatellite primers were obtained from *Uromastyx benti* (Spiny tailed lizards), from North African and the Middle East which is a squamate reptile species that is used for reptile trade or is of conservation concern. However, five of the 12 microsatellite primers were omitted from the study, as they did not give any amplification after PCR. DNA was amplified by polymerase chain reaction (PCR) in one reaction for each sample. The PCR reaction mix consisted of ddH_2O- 2.5 µl; 1X DreamTaq Green PCR Master Mix; 0.31 µM Forward Primer: 0.31 µM Reverse Primer and diluted DNA- 5ng. The total volume of the PCR reaction mix was 20 µl. A summary of the primer sequences and the annealing temperature used for PCR of the seven successfully amplified loci is provided in Table 5.1. The annealing temperature was empirically determined by gradient PCR. Subsequently, the optimised reaction was performed individually for each primer set.

The finalised and optimised conditions for the primer pairs used to sequence the relevant region employed the following cycling conditions: Initial Denaturation at 95 °C for 3 min, followed by 40 cycles of denaturation at 95 °C for 30 sec, annealing for 30 sec and extension at 72 °C for 1 min, followed by final extension at 72 °C for 7 min. The PCR products were loaded onto 1.2% agarose electrophoresis gels (Sigma, Burlington, MA, United States). Images were taken using a SynGene Digital Imaging System (A Division of Synoptics Ltd) and gel band analysis was performed using AlphaView software (Version 3.4).

Table 5.1 The list of primer sequences and the annealing temperatures selected for each primer pair for the amplification of the 112 samples.

Sl. No.	Primer Name	Primer Sequence	Annealing Temperature
1	F-P2	CAGAAGCTGCACAAGAAGTATGC	58° C
	R-P2	GTTCTGTTTCAAGCCACCCC	
2	F-P4	TGGGCAGCAAATTACACAGC	53° C
	R-P4	CCGAGAGTAGCACACTCACACC	
3	F-P7	TACCCAGCCTTTCAGTGTGC	53° C
	R-P7	TGTGCACGCTTAAGGTTTCC	
4	F-P8	CAGTGAAGGGATCCTCAAGC	58° C
	R-P8	GCTACAGAAGATGGCAGAAACC	
5	F-P10	ACCCTGAGACCAGGCAGC	53° C
	R-P10	CAGACATTCCCTTGTAACAGGC	
6	F-P11	TCAAACTGCACTTGAACCCC	49° C
	R-P11	ACACCCTCTTCCCATTCAGG	
7	F-P12	AAGATGTCCAAGGGTGCTGC	52° C
	R-P12	CCTGGCCTTTTCCCTAATCC	

5.2.4 Analysis of microsatellite data

Hardy-Weinberg genotypic expectations (the probability test option) were carried out with GENEPOP ver.4.7.5 (Raymond & Rousset, 1995). From the GENEPOP analysis, I was able to calculate the Hardy-Weinberg probability, linkage disequilibrium, population differentiation, Nm estimates, observed/expected homozygotes, and heterozygotes, F_{ST}, and other correlations. The data set consisted of seven microsatellite loci and four population groups with a total of 112 samples.

5.2.4.1 Population differentiation and gene flow (Nm)

The Hardy-Weinberg exact probability test was used to calculate the probability that each population was in the Hardy-Weinberg equilibrium. The genetic differentiation for all populations was calculated using the exact G test. The *p*- value obtained gives a measure of genetic variation random process disequilibrium, and population differentiation test were estimated using the Markov chain method. Markov chain method is a random process with Markov property where the probability of a random process transitioning to the next state is only dependent on the current state and it is independent of states that preceded the current state. The parameters of the Markov chain were dememorization-1000, batches-100, and iterations per batch-1000. The ploidy was estimated for the diploid data using the estimate of Nm (Private allele method) option in the GENEPOP software ver. 4.7.5. Gene flow (Nm) estimates the flow of individuals and genetic materials between populations.

5.2.4.2 Gene diversity (Allele frequencies)

Basic information and Gene diversities and F_{IS} were calculated using the sub-option in GENEPOP software ver. 4.7.5. Basic information included genotypic matrices, number of observed/expected homozygotes and heterozygotes, and allele frequencies. Ploidy was calculated for the multi-locus estimates for the diploid data set. This test option gives us the observed and expected homozygotes/heterozygotes for all loci across all populations, the alleles present per locus for protected and unprotected areas, the allele richness (AR) and the coefficient of inbreeding (F_{IS}).

5.2.4.3 F_{ST} and other correlations - Allele identity (F_{ST} stats) for all populations

F_{ST} statistics (F_{IS}, F_{ST} and F_{IT}) were calculated for all loci using the F_{ST} and other correlation options in the GENEPOP software ver.4.7.5. Allele identity (F_{ST}) stats for all population pairs were also obtained. The fixation index (F_{ST}) indicates genetic differentiation between populations. The inbreeding coefficient (F_{IS}) was calculated to measure the degree of inbreeding within the population.

5.3 Results

With the Hardy-Weinberg exact probability test, it was possible to calculate the probability of equilibrium for each population. The probability of Khabari Al-Awazem, Al-Abraq and Sabhan population was <0.001 and of Kabd was <0.05 (Table 5.2). This suggests that the *Uromastyx aegyptia microlepis* population in both the protected areas and the unprotected areas are not in the Hardy-Weinberg equilibrium. The overall population probability was < 0.001, suggesting population deviation from the Hardy-Weinberg equilibrium. Population differentiation occurs when there is genetic variation within and between populations. The *p*-value at all loci was <0.001 which indicates the presence of population variation. The results also show that there is restricted gene flow (Nm) between populations. Gene flow (Nm) estimates the flow of individuals and genetic materials between populations. For these population sets, Nm (number of migrants) was observed after size correction to be 0.36 migrants (Table 5.3), suggesting restricted gene flow between populations.

Of the seven primers used, the result of only six are presented, as the seventh primer (Primer 12) did not return any value when used for analysis using Genepop software as Primer 12 had only single allele at the loci. Hence, the primer 12 results were not included in the Tables. In all populations, the observed homozygotes at all loci were 0 and differed from the expected values (Table 5.4). On the contrary, the observed values of heterozygotes were not equal to 0 and differed from the expected values (Table 5.5), suggesting that there is restricted flow of genetic material between populations. From the observed homozygotes and heterozygotes, it can be inferred that the populations in protected areas and unprotected areas are heterozygous. Alleles present in protected and unprotected populations at all loci are listed in Table (5.6).

Table 5.2 Summary of the Hardy-Weinberg probability test for *Uromastyx aegyptia microlepis* in the Kuwait desert.

Population	**Chi-squared Or X^2**	**df**	***p* -Value**
Khabari Al-Awazem	>40.0961	12	$p < 0.001$
Kabd	>24.8627	12	$p < 0.05$
Al-Abraq	>62.2060	10	$p < 0.001$
Sabhan	>46.5729	8	$p < 0.001$
All	**>173.7376**	**42**	$\boldsymbol{p < 0.001}$

Table 5.3 Estimates of Nm (gene flow) for the diploid data set.

Mean sample size	**15.25**
Mean frequency of private alleles p (1)	0.150726
Number of migrants for mean N=10	0.544266
Number of migrants for mean N=25	0.31599
Number of migrants for mean N=50	0.232116
Number of migrants after correction for size	0.356896

Table 5.4 Expected and observed homozygotes for six loci of *Uromastyx aegyptia microlepis* in different sites in the Kuwait desert.

	Khabari Al-Awazem (PA)		**Kabd (PA)**		**Al-Abraq (UPA)**		**Sabhan (UPA)**	
Loci	Expected	Observed	Expected	Observed	Expected	Observed	Expected	Observed
P 2	1.31	0	0.71	0	2.13	0	0.71	0
P 4	0.67	0	0.67	0	0.80	0	-	-
P 7	0.82	0	0	0	0.57	0	-	-
P 8	1.84	0	0.93	0	-	-	0.80	0
P10	0.55	0	0.33	0	3.24	0	2.68	0
P11	4.12	0	4.58	0	5.41	0	4.16	0

Table 5.5 Expected and observed heterozygotes for six loci of *Uromastyx aegyptia microlepis* in different sites in the Kuwait desert.

	Khabari Al-Awazem (PA)		**Kabd (PA)**		**Al-Abraq (UPA)**		**Sabhan (UPA)**	
Loci	Expected	Observed	Expected	Observed	Expected	Observed	Expected	Observed
P2	5.69	7	3.28	4	5.87	8	3.28	4
P 4	4.33	5	1.33	2	7.20	8	-	-
P 7	5.18	6	3	3	3.43	4	-	-
P 8	4.16	6	7.06	8	-	-	2.20	3
P10	4.44	5	4.67	5	21.75	25	13.32	16
P11	19.87	24	16.41	21	21.58	27	17.83	22

Table 5.6 Number of Alleles per locus observed for *Uromastyx aegyptia microlepis* in protected and unprotected sites in the Kuwait desert.

Loci	**Khabari Al-Awazem (PA)**	**Kabd (PA)**	**Al-Abraq (UPA)**	**Sabhan (UPA)**
Locus 2	7	4	8	4
Locus 4	5	2	8	0
Locus 7	6	3	4	0
Locus 8	6	8	1	3
Locus 10	5	5	25	16
Locus 11	24	21	27	22

The standard indices of genetic diversity for protected and unprotected areas, allele richness (AR), observed heterozygosity (Ho), expected heterozygosity (He) and coefficient of inbreeding (F_{IS}) are shown in Table 5.7. As seen in Table 5.7 allele richness (AR) was more in the unprotected area of Al-Abraq which indicates higher genetic diversity in this area while the remaining areas had similar AR.

The observed negative F_{IS} value in all the regions implies that there is an avoidance of inbreeding. Additionally, the inbreeding coefficients (F_{IS}) for the Khabari Al-Awazem and Kabd protected areas were -0.213 and -0.202 while the (F_{IS}) for the unprotected Al-Abraq and Sabhan areas were -0.203 and -0.228 (Table 5.7). The average inbreeding coefficient relative of an individual to the subpopulation (F_{IS}), total population (F_{IT}) and fixation index (F_{ST}) for all populations across all loci was -0.21, -0.04 and 0.14 (Table 5.8). The F_{IS} values varied from -0.14 to – 0.31 across all loci. The negative F_{IS} in both the protected and unprotected subpopulations and the negative average F_{IS} values (-0.21) show the absence values as they ranged from 0.10 to 0.15 across all loci. An average F_{ST} value of 0.14 across all loci in all four populations shows that there is genetic variability of 14% between the populations at the different sites (Table 5.8). The highest F_{ST} was observed for locus 11 (0.15) while the lowest F_{ST} was observed of inbreeding between individuals in the subpopulations and the population. The same is true for F_{ST} for locus 7 (0.1).

The highest F_{ST} was observed between the populations of the open and geographically Distant site pairs of Al- Abraq and Sabhan (0.19) and the lowest F_{ST} was between the geographically close fenced Khabari Al- Awazem and the disturbed Al- Abraq (0.10) areas (Table 5.9). An average F_{IT} value represents the breeding potential of an individual relative to the total population which was also negative (-0.04). The F_{IT} value ranged from -0.02 to 0.0083. The positive F_{IT} value at locus primer 8 shows the presence of interbreeding, hence exchange of genetic material is happening when comparing an individual to the total population.

Table 5.7 Standard indices of genetic diversity for each sampling site in the Kuwait desert, N = number of samples; AR = allele richness; Ho = observed heterozygosity; He = Expected Heterozygosity; F_{IS} = coefficient of inbreeding.

Sample Site	**N**	**AR**	**(Ho)**	**(He)**	**F_{IS}**
Khabari Al-Awazem	28	8.83	53	43.69	-0.213
Kabd	28	7.17	43	35.77	-0.202
Al-Abraq	28	12	72	59.83	-0.203
Sabhan	28	7.5	45	36.65	-0.228

Table 5.8 F_{ST} and other correlations for each locus for *Uromastyx aegyptia microlepis.*

Locus	**F_{IS}**	**F_{ST}**	**FIT**
Primer 2	-0.31	0.14	-0.13
Primer 4	-0.18	0.13	-0.02
Primer 7	-0.14	0.10	-0.03
Primer 8	-0.16	0.14	0.0083
Primer 10	-0.16	0.12	-0.02
Primer 11	-0.25	0.15	-0.06
All	**-0.21**	**0.14**	**-0.04**

Table 5.9 F_{ST} values for all population pairs of *Uromastyx aegyptia microlepis* at different sites in the Kuwait desert.

Population	**Khabari Al-Awazem**	**Kabd**	**Al-Abraq**
Kabd	0.12	-	-
Al-Abraq	0.10	0.11	-
Sabhan	0.16	0.18	0.19

5.4 Discussion

The Hardy-Weinberg law plays an important role in the field of population genetics and often serves as a basis for genetic inference (Guo and Thompson, 1992). A population is said to be in Hardy-Weinberg equilibrium if five assumptions are met. The five assumptions are: no mutation, random mating, no gene flow, infinite population size, and no selection (Alghamdi & Padmanabhan, 2014). When one or more of the five assumptions is not met, as shown in other studies, (Karlsson & Mork, 2005), the populations are considered to be in disequilibrium. From the Hardy-Weinberg exact probability test, the Hardy-Weinberg equilibrium probability value of each Arabian spiny-tailed lizard population was calculated. The overall Hardy-Weinberg equilibrium *p*- value was less than 0.05, suggestive that the Arabian spiny-tailed lizard population in Kuwait significantly deviated from the Hardy-Weinberg equilibrium. Since a significant deviation from Hardy-Weinberg equilibrium is detected it suggests that one or more of the five assumptions is not met (Karlsson & Mork, 2005). This has also been observed in several studies of genetic diversity in lizards (Harris et al., 2007) and other species (Jablonski et al., 2021).

The populations of the Arabian spiny-tailed lizard in protected and unprotected areas are probably connected in more ways than one. As part of species management, government and NGOs deliberately translocate lizards in different parts of the country. The relative geographic proximity of some regions makes it possible for both natural and anthropogenic action to contribute to this connectivity. This could play a role in increasing the heterozygosity of populations in protected areas due to damaged fences or the ability of the lizard to dig burrows under the fences. Furthermore, fences around protected areas may limit anthropogenic activities and access to these areas.

However, the fence does not block the movement of lizards in and out of the protected areas. This point is validated by the high degree of variation ($p<0.001$) between subpopulations. Also, gene flow value of 0.357 (Table 5.3) means that low flow of genetic material is present between the Arabian spiny tailed lizard populations in the protected and adjacent unprotected areas.

In the present study on the Arabian spiny-tailed lizard, the observed number of heterozygotes was higher than the expected. Also, since the observed homozygotes in the lizard population in protected and unprotected areas were zero, it implies that the lizard population in protected and unprotected areas comprises solely of heterozygotes. High number of heterozygotes denotes greater genetic variability. Furthermore, high heterozygosity indicates that subpopulations, either naturally or through human management, maintain diversity in their gene pool (López-Cortegano et al., 2019). However, this also may be the expected scenario if the populations were recently isolated and started inbreeding, since inbreeding may take several generations before the effects become evident within a population (Manoel et al., 2012).

The genetic diversity of the Arabian spiny-tailed lizard in the State of Kuwait was found to be highest in Al-Abraq site (Al-Abraq, AR =12, $H_o = 72$, $H_e = 59.83$). Higher heterozygosity has been reported in the endangered Spanish sheep breeds, *Churra tensina* and *Churra lebrijana*, despite their small population size (Calvo et al., 2011). Similarly, greater heterozygosity has also been reported in Balearic sheep breeds (Sharma et al., 2016), and those from Croatia, Bosnia and Herzegovina (Salamon et al., 2014), and Turkey (Yilmaz et al., 2015). According to these studies, a higher heterozygosity suggests avoidance of inbreeding within the subpopulations. Since the F_{IS} values were approximately -0.2 for all populations, it indicates absence of inbreeding within the Arabian spiny- tailed lizard population in both the protected and the unprotected areas.

The fixation index (F_{ST}) indicates genetic differentiation among the populations. It reflects the variance in allele frequency in a population. More diverse populations are likely to have individuals that are genetically different and therefore are less likely to resemble each other. An F_{ST} value of 0-0.05 indicates low genetic differentiation, 0.05-0.5 indicates moderate differentiation, and an F_{ST} value greater than 0.25 shows significant genetic differentiation. The F_{ST} values are widely used for population diversity studies to estimate genetic diversity. Salvi et al. (2009) used F_{ST} to report on genetic diversity in the rock lizard *Archaeolacerta bedriagae*. They reported that a high F_{ST} value ($F_{ST} = 0.172$), indicated that genetic diversity between subpopulations accounts for less than 20% of the genetic variation observed in *A. bedriagae*.

Similarly, in the present study on the Arabian spiny-tailed lizard, the F_{ST} values ranged from 0.10 to 0.15 at all loci. F_{ST} values >0.05 indicate genetic diversity between populations. Calvo et al. (2011) observed a high F_{ST} of (0.143) while studying the Spanish mouflon insect *Graellsia isabellae* and inferred that Hardy–Weinberg disequilibrium in a population may be the main cause. Similarly, the high F_{ST} value in the present study is an indication of high genetic diversity.

The total F_{ST} value for the tested populations across all loci was 0.14 suggesting moderate differentiation between subpopulations. All pairs of populations showed moderate F_{ST} values. The population pair with slightly higher F_{ST} value than the others was Sabhan and Al-Abraq (0.19), indicating slightly more genetic differentiation than the other population pairs. This might be because both Sabhan and Al-Abraq areas are geographically distinct and far from each other. The population pair with the lowest F_{ST} value and hence the least genetic differentiation was Khabari Al-Awazem and Al-Abraq (0.10). Khabari Al-Awazem and Kabd are fenced areas while the others are not fenced. These selected study areas are not connected to each other. Moreover, Sabhan is located in a peripheral urban zone whereas Khabari Al-Awazem and Kabd are located within the active sand encroachment zone which creates isolating challenging conditions. The fence does not block the lizards' routes to the peripheral areas around the protected areas. The populations in and outside of the protected areas are still sustaining their connectivity. And therefore, the fence does not appear to reduce variability within the population genetics. This explains the F_{ST} of 10% observed between Khabari Al-Awazem and Al-Abraq and F_{ST} of 11% observed for Al-Abraq and Kabd.

Fixation indices (F statistics) are applied to mating systems in populations, in which consanguine mating is either avoided as much as possible or pursued as much as is possible without any disruption of the group (Wright, 1965). Low inbreeding was observed in all population areas, with an average F_{IS} of -0.213 in Khabari Al- Awazem and -0.202 in Kabd both of which are protected areas, and -0.203 in Al- Abraq and -0.228 in Sabhan, the unprotected areas.

Positive F_{IS} value denotes presence of inbreeding and negative F_{IS} value means absence of inbreeding, resulting in more heterozygosity and a definite drift from the Hardy-Weinberg equilibrium. The values of F_{IS} range from -1 to 1, but as it approaches -1, there is a heterozygous excess and a homozygous deficit (Wright, 1965). Since the F_{IS} values are slightly negative for lizard populations in protected and unprotected areas, it indicates the absence of inbreeding, and this may be the reason why the heterozygotes were observed in these areas.

The sum of evidence from the study of selected microsatellites in the four populations indicates that despite the various stressors, the four populations appear to have a healthy genetic pool with little inbreeding and good gene diversity. Microsatellites in genetic studies have a long established history in helping understand population structures, migration etc. (Gonzalez et al., 2014; Gariboldi et al., 2016) but more novel approaches such as double digest Restriction-site associated DNA (ddRAD- seq) that use Single Nucleotide Polymorphism (SNP) to detect single changes in nucleotides may be more informative in genotyping (Thrasher et al., 2018).

Furthermore, low-coverage whole genome sequencing which determines the order of bases in the organism in one process (Homburger et al., 2019) is now possible and achievable. Sequencing approaches such as Restriction-site associated DNA sequencing (RAD-seq) and Whole Genome Sequencing (WGS) of individuals in protected and unprotected areas will give more detailed insight about the extent to which the species are diverse in these areas. Genomic information obtained from RAD-seq and WGS can help in the conservation, planning, and management of commercially exploited species. Due to computational and sequencing development, we can envision a future where genome analysis will become a routine task and would help in getting genomic information from endangered species rapidly. WGS can provide a better understanding of the genetic source of inbreeding depression (Saremi et al., 2019).

The obtained genetic data can be used to uncover the cause of inbreeding depression and assist in the planning of breeding programs to avoid the inclusion of individuals carrying deleterious mutations that can help recover the wild or captive population. However, the lack of genomic resources for endangered species under conservation and the high cost of high throughput sequencing and the high demand for computing resources still constrain the application of RAD-seq and WGS in population genetic studies.

The fact remains that microsatellites protocols, as used in this study, remain of lower cost in comparison to the novel ones noted above. More importantly, they allow us to compare our findings with a wealth of published information.

5.5 Conclusions

In conclusion, the Arabian spiny-tailed lizard population within the protected areas (Khabari Al- Awazem and Kabd) and unprotected areas (Al-Abraq and Sabhan) avoid inbreeding and do not show signs of inbreeding depression. Despite restriction of individual animal movement and what appears to be low genetic flow between populations, genetic diversity is prevalent with marked heterozygosity across all the populations in all sites. It appears that erecting barriers in the form of fences around protected areas has had a minor effect on the genetic diversity of this species. The observed heterozygosity in one of the unprotected areas (Al-Abraq) was only slightly higher than the other sites. Also, the fixation index of the Arabian spiny- tailed lizard populations in the open areas of Al-Abraq and Sabhan showed them to be slightly more divergent than the rest of the population pairs. These findings confirm the hypothesis that genetic heterozygosity within the Arabian spiny-tailed lizards' population is not affected by the protection status of the habitats in Kuwait. Furthermore, the lack of protection of some sites in the State of Kuwait has had no impact on genetic diversity within the Arabian spiny-tailed lizard populations. These findings could aid conservationists to adjust their views and actions towards how restoration and rehabilitation of habitat programs are implemented for the Arabian spiny-tailed lizard in Kuwait to ensure the success of conservation efforts especially in disturbed areas. More elaborate genetic studies, covering more sites, using whole genome sequencing is necessary to obtain a more accurate and representative view on the genetic diversity of the various populations of the Arabian spiny-tailed lizards in the State of Kuwait.

5.6 References

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Chapter 6

General Discussion

The present thesis combined field and laboratory components to improve our understanding of the ecology and genetics of a common reptile species in the arid habitats of Kuwait, the Arabian spiny-tailed lizard *Uromastyx aegyptia microlepis*. Reptiles play an integral role in ecosystems where they live (Read, 1998; Carroll, 2001; Raxworthy et al., 2008; Uetz & Hošek, 2020; Amr et al., 2022). The diversity of reptiles reflects the health of the environment and is a good indicator of viable ecosystems (Al-Sayegh et al., 2020). In arid regions, such as Kuwait desert, reptiles flourish and show high diversity in terms of species composition and niche selection, which are characteristics of a diversified ecosystem (Amr et al., 2022).

According to the spatial conservation prioritization of the Arabian spiny-tailed lizard using MaxEnt modelling, Al-Huwaimliya and Nuwaiseeb sites are recommended as prioritized for protection of the species. The other results reveal that the current measures followed in the establishment of protected areas, despite their importance for the conservation of many species and habitats, did not show a significant contribution to the communities of lizards. The well-being of the condition and the genetic heterogeneity of the species in both protected and unprotected areas did not show significant differences. This may be partly, due but not restricted, to its robustness and tremendous ability to convert its trophic level from herbivorous to carnivorous (Bouskila, 1985; Robinson, 1995; Cunningham, 2000; Al-Johany, 2003; Sarhan & Al-Qahtani, 2007; Cunningham, 2009; Castilla et al., 2011a; Castilla et al., 2011b) and to the connectivity between protected and unprotected communities. For other native species such as the Asian houbara bustard *Chlamydotis macqueenii,* for instance, protected areas highly contribute towards its conservation by providing more suitable habitats that support multiple stages of its life cycle (Combreau & Smith, 1997; Aghanajafi-Zadeh et al., 2010; Zadeh et al., 2010; Hardouin et al., 2015). The houbara bustard is a robust bird that converts from herbivorous to carnivorous (Mian, 1999; Tigar & Osborne, 2000) just like the Arabian spiny-tailed lizard, but needs a separate habitat for mating, foraging, sheltering, and nesting (Gelinaud et al., 1997). Preferably, more sensitive species to ecosystem modification are used as indicators to assess the changes in habitats. For example, sand cats are more sensitive to habitat changes than the Arabian spiny-tailed lizards (Ghadirian et al., 2016; Ghafaripour et al., 2017; Mugerwa et al., 2020; Amin et al., 2021).

Therefore, to assess the effect of protected areas in the deserts on condition indices, adaptability and ecology, the robust species that are resistance to habitat change should not be considered. It is better to select the Arabian spiny-tailed lizards to study the mechanisms or processes of its robustness, resistance and resilience factors.

Finally, by reviewing studies related to the conservation of endangered species, together with the results of the current study, the following recommendations and proposals for future studies are presented:

1- Conduct more in-depth studies on the important ecological characteristics of *Uromastyx aegyptia microlepis* such as home range activity, biotic and abiotic characteristics of their habitats, population system attributes, diversity of vegetation species, frequency, importance, cover for the habitats and burrows characteristics.

2- More field investigations with long-term monitoring studies to be conducted to determine the effectiveness of protected areas in the conservation of *Uromastyx* lizards within their boundaries and throughout the State of Kuwait.

3- More genetic studies on the spiny-tailed lizard of Kuwait to be conducted using sequencing approaches such as Restriction-site associated DNA sequencing (RAD-seq) and Whole Genome Sequencing (WGS) in protected and unprotected areas to gain details about the species diversity in these areas. Genomic information obtained from RAD- seq and WGS can help in the establishment of genetic information repositories for conservation, planning and management of commercially exploited species.

4- Propose the prioritization map produced in this research to the relevant governmental sectors to amend the current legislation on protected areas to include Al-Huwaimliya and Nuwaiseeb sites among the protected areas in the country.

5- Adopt modifications to the criteria for protecting areas to include habitat hotspots of flagship species such as the Arabian spiny-tailed lizard.

6- Launch awareness campaigns in collaboration with government and nongovernmental organizations to educate the public on the importance of the ecosystem services of the spiny-tailed lizards and the conservation of the endangered animal in maintaining the balance of the ecosystem.

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