A description of reproduction, diet, nematode infection and sexual dimorphism in *Agama anchietae* and *Pedioplanis undata undata* (Reptilia: Lacertilia) in Namibia

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Abstract

The objectives of this study were to describe reproduction, diet, nematode infection and sexual dimorphism in *Agama anchietae* and *Pedioplanis undata undata*, two Namibian lizard species with wide geographic distributions for which such baseline ecological information is still lacking. The specimens used in the study came from the preserved Herpetological Collection of the National Museum of Namibia.

Agama anchietae occupies rocky areas and the findings were as follows, *reproduction*: (i) it reproduces in spring and summer, the warm and rainy season of Namibia when insect food abounds, presumably to optimize the survival of its offspring, (ii) the gonads were inactive during autumn and winter, (iii) the mass of its abdominal fat bodies and liver decreased during the reproductive period, presumably because they serve as sources of energy during that time; *diet*: (i) prey items consumed by the sexes belonged to the orders Hymenoptera, Coleoptera, Lepidoptera and Mantodea, (ii) in addition to these an item belonging to the Diptera was also found in males, (iii) no significant numerical differences were found between the sexes with respect to the items consumed, (iv) the fact that hymenopterans occurred in substantially higher numbers suggest that the species relies mainly on sit-and-wait foraging; *nematode infection*: (i) in both sexes a larger number of nematodes occurred in the stomach than in the intestine, (ii) no significant numerical differences in infection were found between the sexes; *sexual dimorphism*: (i) males were larger than females in absolute body size, (ii) relative hind-limb but not forelimb length of males was significantly longer than that of females; (iii) relative head breath, length and width of males were all significantly greater than that of females.

Pedioplanis undata undata occurs in flat open areas and the findings were as follows, *reproduction*: (i) a similar pattern with respect to the activity of the gonads as reported for *A. anchietae* above was also found in this species, (ii) the same also applied to the mass fluctuations of the abdominal fat bodies and the liver; *diet*: (i) males consumed items belonging to the orders Hymenoptera, Isoptera, Coleoptera, Lepidoptera, Blatoidea, Solifugida as well as larvae, (ii) in females the prey items belonged to the Hymenoptera, Orthoptera, Coleoptera, Hemiptera and Aranea, (iii) no significant numerical differences were found between the sexes for similar prey items, (iv) the low numbers of hymenopterans but fairly high diversity of prey types suggest that this species is a wide forager; *nematode infection*: (i) in both the stomach and intestine nematode infection was low with that of the stomach marginally higher than that of the intestine, (ii) no significant numerical differences were found between males and females; *sexual dimorphism*: (i) males and females were of similar body size, (ii) males had significantly longer fore-and hind-limbs than females, and may therefore be able to run faster, (iii) relative head breath, length and width of males were all significantly greater than that of females.

List of Publication(s)/Conference(s) proceedings

A comparative analysis of the reproductive biology of *Agama anchietae* and *Pedioplanis undata undata* (Reptilia: Lacertilia) in Namibia, and related aspects of their ecology presented at University of Namibia's 5th Annual Science Research Conference on 15 November 2017.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ABBREVIATIONS

FLL : Front-limb length

HDH : Head height

HDL : Head length

HDW : Head width

HLL : Hind-limb length

SSD : Sexual size dimorphism

SVL : Snout-vent length

TL : Tail length

SPSS : Statistical Package for Social Scientists

SMR : State Museum Reptile Accession number

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Dedication

This thesis is dedicated to my fellow young scientists all over the world who long to deepen their walk in herpetological research, move into everything it has for them, and become everything they are meant to be.

Declarations

I, Bertha N. Buiswalelo, hereby declare that this study is my own work and is a true reflection of my research, and that this work, or any part thereof has not been submitted for a degree at any other institution.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the study

Namibia is the most arid country in sub-Saharan Africa, and is well-known for its high diversity and endemicity of reptiles comprising crocodiles, lizards, snakes, terrapins, tortoises, turtles, and worm lizards (Conradie *et al*., 2012; Herrmann and Branch, 2013). Although they are a common feature of just about all ecosystems in Namibia, baseline empirical studies of the ecology of many lizard species still remain to be done; *Agama anchietae* and *Pedioplanis undata undata* being two examples.

In order to gain an understanding of the role of an organism in helping to maintain the integrity of an ecosystem, it is obviously important to understand its basic ecology, i.e., what it requires to survive and reproduce in the habitat where it occurs. The aspects studied and described in this study are some of the most basic ecological parameters in that regard and is obviously not exhaustive given time constraints.

Although some baseline ecological studies of Namibia's lizard species have been carried out, much still remains to be done. Heideman (1994) compared and contrasted the reproductive strategies, including the roles of the abdominal fat-bodies and liver as sources of energy, in *Agama aculeata aculeata and Agama planiceps planiceps* females in Windhoek. This was followed-up by a similar study of males of the two species (Heideman, 1995). Reproduction was also described in *Agama etoshae*, the only endemic agama of Namibia by Heideman (1998). Comparative studies of the diet of these agamas were also undertaken (Heideman, 1998, *A. etoshae*; Heideman, 2002, *A. a. aculeata* and *A. p. planiceps*). Findings on gut cestode and nematode infection in these agamas have also been reported.

Oochoristica truncata (Cestoda) infection of *A. a. aculeata* and *A. p. planiceps* in the Windhoek area was reported by Heideman (1991), as well as nematode infection in them (Heideman, 1995). A comparative analysis of the effect of these worm infections on body condition and fecundity in these agamas was also undertaken (Heideman, 1997). A new species of the arthropod endoparasite, *Raeillietiella namibiensis*, (Pentastomida: Cephalobaenida), was found in the lungs of *A. a. aculeata* and *A. p. planiceps* (Heideman, 2002).

Finally a few studies have described sexual dimorphism in the Namibian agamas, including *A. etoshae* (Heideman, 1998), and *A. a. aculeata* and *A. p. planiceps* (Heideman, 1995). Although some empirical studies on lacertid species of Namibia have been undertaken (see subsequent chapter), nothing on the ecology of the genus *Pedioplanis* of the country has been reported.

1.2 Statement of the problem

Agama anchietae and *P. u. undata* are Namibian lizard species that vary in terms of their morphology and the type of habitat in which they occur. The former species is the larger of the two and inhabits rocky outcrops while the latter species lives in open flat sandy areas. *Pedioplanis u. undata* is a fast moving lizard, often seen darting between bushes, while *A. anchietae* is less active, possibly to enhance the effectiveness of its cryptic body colouration as a means of evading detection by predators.

No empirical studies of reproduction, diet, nematode infection and sexual dimorphism have been conducted on the two species yet. This constitutes a gap in knowledge which needs to be addressed in order to begin to understand their basic ecology and life history strategy for long-term persistence. This information will also form part of the body of knowledge needed to understand their role in ecosystems where they occur.

1.3 Objectives of the study

The objectives of the study were the same for the two species and compared and contrasted only the aspects listed below intersexually within each species:

a) To describe their annual reproductive process in terms of its timing, duration and possible environmental factors regulating it.

b) To describe changes in the mass of their abdominal fat bodies and liver as potential energy stores during reproduction.

c) To describe their diet composition in terms of prey item found in their stomach and intestine.

d) To describe the levels of nematode infection in each species and whether infection levels differed between the stomach and intestine of individuals.

e) To describe the degree of head, body and limb dimorphism in each specie.

1.4 Hypotheses of the Study

Reproduction: In order for a species to persist it has to be able to survive and reproduce successfully in the environment where it occurs. Successful reproduction in lizards requires males and females to be in optimal physiological condition to do so and for their offspring to arrive at a time when their chances of survival are also optimal. The following hypotheses were therefore tested:

H_0: The physiological condition of males and females of the two study species, and the timing of the arrival of their offspring does not play a role in their reproductive success

H_1: The physiological condition of males and females of the two study species, and the timing of the arrival of their offspring does play a role in their reproductive success

Diet: Studies of the diet of lizard species in Namibia have generally found that they are insectivorous. Insectivorous lizards can either be sit-and-wait foragers or wide foragers. This can be deduced from the diversity and numbers of particular food items found in their gut or by actual observation. The following hypotheses were therefore tested:

H_0: The two study species have no particular food preference and that their mode of foraging cannot be deduced from their diet.

H_1: The two study species are insectivorous and that their mode of foraging can be deduced from their diet.

Nematode infection: Nematode infection of the gut is common to probably all vertebrates. The type of nematode found in the stomach and intestine can differ as some thrive in the acidic environment of the stomach while others prefer the alkaline environment of the intestine. The following hypotheses were therefore tested:

H_0: Nematodes do not infect the gut of the study species.

H_1: Nematodes do infect the gut of the study species and that different species and levels of infection are found between the stomach and intestine.

Sexual dimorphism: Body size and head shape dimorphism is a common phenomenon in agamas but is poorly studied and therefore not well known among lacertids. Various selective and ecological factors drive the evolution of these phenomena. The following phenomena were therefore tested:

H_0: The species are not sexually dimorphic with respect to body and limb size as well as head shape

H_1: The species are sexually dimorphic with respect to body and limb size as well as head shape, and that the phenomenon manifests itself more strongly in the agama species.

1.5 Significance of the study

By describing the reproductive process, diet, levels of nematode infection and sexual dimorphism in *A. anchietae and P. u. undata,* we can begin to understand their general biology and the life history strategy which enable them to persist. These aspects are also fundamental to ultimately clarifying their role in the ecosystems they occupy.

1.6 Limitations of the study

Limitations encountered by the study included small samples per month and their uneven seasonal spread per sex which negatively impacted the drawing of firm conclusions. This was due to the museum specimens often being fragile due to long storage in ethanol, rendering them unusable and therefore reducing sample size for the particular month or season.

1.7 Delimitation of the study

This study focused on two Namibian lizard species (*Agama anchietae* and *Pedioplanis undata undata*) in the preserved lizard collection of the National Museum of Namibia, collected from only certain parts of the country.

CHAPTER TWO

2.0 LITERATURE REVIEW

Namibia, a hot and dry country on the subcontinent, has remarkable reptile diversity, namely, about 258 species, of which more than 50% are endemic to the country (Marais and Alexander, 2007). Given the general harshness of their habitats, these reptiles have evolved interesting adaptations, making their study an exciting undertaking (Marais and Alexander, 2007). Eight species of agama are recognized in Namibia, of which one is endemic, *A. anchietae, A. atra, A. aculeata, A.armata, A. etoshae* (endemic)*, A. hispida, A. planiceps, and Acanthocercus atricollis,* commonly known as the southern tree agama (Griffin, 2003). While the range of the genus extends across the whole country, *A. anchietae* occurs in the southern and western parts of the country. Ten species of *Pedioplanis* occur in the country, of which 6 are endemic (Griffin, 2003; Uetz and Jiri, 2018). These species are *Pedioplanis bunguellensis, P. breviceps* (endemic), *P. gaerdesi* (endemic)*, P. inornata* (endemic), *P. laticeps, P. lineocellata, P. rubens* (endemic)*, P. undata* (endemic)*, P. husabensis* (endemic) *and P. namaquensis*. *Pedioplanis undata* occurs in the Naukluft mountain range, north of Rehoboth and in the north-western part of the country.

As part of the southern African sub-region, Namibian species have been included in several comprehensive studies (Griffin, 2003). *A. a. aculeata* and *A. p. planiceps* were found to reproduce in summer, the rainy season, when vegetation blooms as well as insect food, which presumably optimizes the survival of their offspring according to Heideman (1994, 1995). In the latter studies, abdominal fat bodies and liver mass decreased during the breeding probably because they serve as the main sources of energy at this time. A similar breeding pattern was also observed in

Agama etoshae, the only agama species endemic to Namibia, which occurs in its northern border area (Heideman, 1998). The latter study reported that males caught in autumn and winter had inactive testes while those caught in spring and summer showed early to full spermatogenic activity. These agama species thus seem to optimize their reproductive success by timing the arrival of their offspring to coincide with the bio-meteorological conditions most favourable for their survival.

Formal studies of lacertids are limited in Namibia and restricted mainly to species from the Namib Desert such as the genus *Meroles* (seen in Nkosi et al, 2004). Some of these are anecdotal in taxonomic works (e.g. Fitzsimons, 1945). Geographic centres of herpetological research have been the Namib Desert and areas around Windhoek, leaving wide parts of the country hardly touched (FitzSimons, 1945; Hermann and Branch, 2013). In addition to these, comments are also found in the checklist summarizing fifty years of herpetological research in the Namib Desert by Herrmann and Branch (2013). Empirical research has also been conducted on *Meroles anchietae* (Larcertidae) and *Gerrhosaurus skoogi* (Gerrhousauridae) by Robinson and Barrows (2013). In the analysis of the reproductive cycle of *M. anchietae*, these workers found that changes related to prey abundance sharply influenced reproduction, directed by the amount of energy stored. With adequate prey abundance males and females could produce throughout the year (Robinson and Barrows, 2013). In the latter study, reproductive success trailed the uneven rainfall patterns which in turn influenced prey species richness and abundance, and males and females could produce gametes all year round. Abdominal fat bodies, however, showed a dissimilar seasonal cycle that related to food abundance. Rain related changes in prey abundance and the amount of energy warehoused by males and females strongly predisposed them to the likelihood of reproduction (Robinson and

Barrows, 2013).

Several studies of the diet of Namibian lizards have been undertaken, including that of *A. etoshae* (Heideman, 1998) and *A. a. aculeata* and *A. p. planiceps* by Heideman (2002). In all three species ants were present in the highest number of all the prey items found, suggesting that they are primarily sit-and–wait foragers. In *M. anchietae* Robins and Barrows (2013) found that diet varied with prey species richness and abundance which tracked the irregular rainfall pattern of the Namib.

Studies of the levels of stomach and intestinal parasitic worm infection in Namibian lizard species have been undertaken, although few in number. These include studies of cestodes and nematodes in *A. a. aculeata* and *A. p. planiceps* by Heideman (1991) and Heideman (1995, 1997). The study of Heideman (1997) looked for a possible correlation between levels of infection and body condition and fecundity in the two species studied. Helminths were also reported in other lizard species including *A. aculeata, A. atra, A. planiceps, Chamaelo dilepis, Chamaeleo namaquensis, Cordylus pustulatus, Gerrhosaurus nigrolineatus, Meroles cuneirostris, Meroles suborbitalis, and Pedioplanis namaquensis*. A new species of blunt-hooked lung pentastomid, an arthropod, was also reported in *A. a. aculeata* and *A. p. planiceps* by Heideman (1998, 2002).

Sexual dimorphism is a common feature among Namibia’s lizards, although still not comprehensively documented. Sexual size dimorphism was reported in *A. etoshae* (Heideman, 2001) and in *A. a. aculeata* and *A. p. planiceps* (Heideman, 1995), with the conclusion that sexual selection, differential growth, or intraspecific food niche segregation were likely to have been the underlying drivers. The latter study also addressed the question as to whether the latter species was better adapted for high speed running than the former, by comparing their relative limb lengths.

CHAPTER THREE

3.0 RESEARCH METHODS

3.1. Research design

The study specimens all came from the preserved Herpetological Collection of the National Museum of Namibia. As the study required the use of adult specimens of *A. anchietae* and *P. undata*, only specimens with a snout-vent length (SVL) of at least 80% of the published maximum SVL for each species were utilized.

3.2 Analysis of the reproductive process

3.2.1 Males cycle

The testes of specimens were exposed by dissecting their body along its ventral midline. In those instances where the testes were difficult to detect, a stereo-microscope was used to locate them in the body cavity. Only the testis on the left side of the body cavity was removed in both species, so that the other one remained intact. The testis was blotted with a paper towel to remove as much of the alcohol preservative as possible, and weighed on an appropriately sensitive balance to the nearest 0.01 grams. The testes were then individually placed in vials with 70% EtOH and kept until histological sectioning. The sectioning was carried out by the histopathology lab of the Medical School of the University of the Free State, following their own protocol. The protocol involved dehydrating the tissue through a series of increasing EtOH concentrations (50%, 75%, 95% and four changes of 100%), using a VIP 300E automatic tissue processor. The tissue was then cleared in xylene (two changes of 100%) and then manually embedded in paraffin wax. The histological sections were cut at 10 μm on a manual microtome and stained with Meyer’s hematoxylin and eosin. The spermatogenic activity was analysed per season by assessing the histological status of the testes and categorizing them according to the stages described by Licht (1971).

3.2.2 Females cycle

The ovaries of specimens were exposed by dissecting their body along its ventral midline. In those instances where the ovaries were difficult to detect, a stereo microscope was used to locate them in the body cavity. The oogenic status of the ovaries was assessed *in situ* per season using the stages described by Van Wyk (1991). The oviducts were also examined for the presence of eggs and their number in each oviduct recorded.

3.2.3 Abdominal fat body and liver mass cycles

Only the abdominal fat body on the left side was removed along with the liver. Both were blotted with a paper towel to remove as much of the alcohol preservative as possible, and both weighed to the nearest 0.01 grams. Their mass fluctuations were then analysed per season.

3.3 Diet composition and nematode infection

The stomach and intestine was removed from the dissected specimens and their contents placed in separate petri dishes filled with water. A stereo microscope was then used to identify the prey items based mainly on the identification and counting of prey head capsules. Identification of the prey items was done to the lowest possible taxonomic level (usually order) with the assistance of entomologists. Nematodes in the stomach and intestine were counted and recorded for each section. They were not identified due to a lack of expertise.

3.4 Sexual dimorphism

Two aspects were studied, body size dimorphism and head shape dimorphism. In the case of *A. anchietae* sample size was 32 specimens for females and 46 for males. For *P. u. undata* it was 27 females and 63 males. Morphometric measurements were taken with a Vernier calliper to the nearest 0.1mm.

3.4.1 Body size dimorphism

The snout-vent length (SVL) and tail length (TL) were measured in each specimen to the nearest 0.1mm. Snout-vent length was measured from the tip of the snout to the anterior edge of the cloaca. Tail length was measured from the posterior edge of the cloaca to the tip of the tail. In addition to this the lengths of the fore- (FLL) and hind limbs (HLL) were also measured in each species.

3.4.2 Head shape dimorphism

The measurements of the head taken were head height (HDH), head length (HDL) and head width (HDW). Head length was measured from the tip of the snout to the posterior edge of the parietal scale. Head width was measured at the widest part while viewing the head dorsally. Head height was measured in-line with the middle of the parietal scale.

CHAPTER FOUR

4.0 DATA ANALYSES

Prior to analyses, the distribution of data sets were tested for normality using the Kolmogorov-Smirnoff test in order to select the appropriate test. Seasonal variation in testis and abdominal fat body mass was analysed using either parametric or non-parametric analysis of variance, followed by the appropriate post-hoc test where significant variation among the data sets were found. Counts of male and female dietary items, and gut nematodes were compared using ANOVA followed by the LSD post-hoc test where necessary. Intersexual differences in body, limb and head linear dimensions were investigated using analysis of covariance (ANCOVA) with SVL as covariate. All the statistical analyses were carried out using the software programme STATISTICA for Windows, 2014. Differences between datasets were considered significant at $P \leq 0.05$.

CHAPTER FIVE

5.0 RESEARCH ETHICS

Ethical clearance was obtained from the University of Namibia’s Research Ethics Committee (UREC) and permission from the National Museum of Namibia for the use of its specimens. On completion of the study, organs together with specimens were returned to the museum to be re-archived for future use.

CHAPTER SIX

RESULTS

6.1 Reproductive process

6.1.1 Male cycle

Using histological slides, the seasonal stages of spermatogenesis were described on the basis of the seven stages outlined in Table 1.

Table 1. Stages of spermatogenesis in seasonal breeding lizards as presented by Licht (1971)

Stage	Spermatogenetic condition
1	Seminiferous tubules involuted with only spermatogonia; epididymis atrophic and empty
2	Seminiferous tubules involuted with primary spermatocytes
3	Secondary spermatocytes and early spermatids present
4	Transforming spermatids with a few spermatozoa
5	Spermatozoa abundant with maximum spermatogenesis
6	Spermatozoa abundant but spermatocytes and spermatids greatly reduced
7	Only spermatogonia, epididymis hypertrophied and empty

Agama anchietae specimens collected in winter (May through August) all had inactive testes (stage 1), except for three specimens collected in July which had testicular activity at stage 2. One individual collected in autumn (March) had active testes (stage 5). Of ten specimens collected in spring (October) three had testes at stage 3, four were at stage 4 and three were at stage 6. Summer collected specimens (November through February) all had testes at stage 5, except one each from November and February which had testicular activity at stages 3 and 4, respectively.

In *A. anchietae* significant variation in testis mass was found among seasons (F = 6.171, $P = 0.002$) and among biomes (F = 9.877, $P = 0.000$), respectively. The LSD post-hoc test showed that testis mass of specimens collected in summer was significant greater than those collected in the other three seasons ($P < 0.05$, in all cases). That of spring was significantly greater than that of winter ($P = 0.001$). Testis mass of autumn and winter collected specimens did not differ significant ($P= 0.506$). The testis mass of specimens collected from the Karoo and desert differed significantly as well as Karoo and savanna collected specimens ($P = 0.000$, in both cases), but no significant difference was observed between the savanna and desert specimens ($P > 0.05$).

In *P. u. undata* no specimens for autumn were available in the museum collection. Specimens collected in winter (May through August) all had testes at stage 1, except for one specimen collected in August which had testicular activity at stage 6. In spring (September and October) two specimens had transforming spermatids with a few spermatozoa (stage 4) while all other specimens showed maximum spermatogenic activity (stage 5). One specimen from October had inactive testes (stage 1). In summer (November through February) a January and February specimen each had testes at stages 1 and 4, respectively. The rest of the specimens collected in February were all at full spermatogenesis (stage 5).

In *P. u. undata*, significant variation in testis mass was also found among seasons (F = 4.29; $P = 0.009$) and biomes (F= 9.768; $P= 0.000$). The LSD post-hoc test showed that testis mass of specimens collected in summer and spring was significant higher than that of the specimens collected in winter ($P = 0.0501$ and $P = 0.000$, respectively). The testis mass of autumn collected specimens did not differ significant from that of any of the other three seasons ($P > 0.05$, in all cases. No significant differences were found between the desert and savanna collected specimens (*P* > 0.05). However, testis mass of specimens collected from the Karoo were significantly heavier than those from the desert and savanna (*P* = 0.000, in both cases).

6.1.2 Female cycle

In *A. anchietae* total and average egg mass did not vary significantly between biomes (F = 0.825 and 3.248, respectively; *P* > 0.05 in both cases). Ovarian mass, however, varied significantly between seasons (F = 51.622; *P* = 0.000) and between biomes (F= 56.734; *P* = 0.000). The LSD post-hoc test showed that its mass in summer collected specimens was significantly higher than that of winter (*P* = 0.000), spring (*P* = 0.008) and autumn (*P* = 0.000) collected specimens. That of spring collected specimens was significantly higher than that of winter (*P* = 0.036) and autumn (*P* = 0.017) collected specimens. Ovarian mass of autumn and winter collected specimens did not differ significantly (*P* > 0.05).

In *P. u. undata* total and average egg mass also did not vary significantly between biomes (F = 0.373 and 0.832, respectively; *P* > 0.05 in both cases). Ovarian mass, however, varied significantly between seasons (F = 5.90; *P* = 0.013) but not between biomes (F= 2.073; *P* > 0.05). The LSD post-hoc test showed that its mass in summer collected specimens was significantly higher than that of winter (*P* = 0.001) and spring (*P* = 0.047) collected specimens. No autumn collected specimens were available and that of spring collected specimens did not differ from that of winter (*P* > 0.05) collected specimens.

6.1.3 Abdominal fat body and liver mass cycles

In *A. anchietae* males, abdominal fat body mass varied significantly among the seasons (F = 4.7402; *P* = 0.0062) and biomes (F = 6.477; *P* = 0.0035). The LSD post-hoc test showed that specimens collected in winter had heavier abdominal fat bodies than those collected in summer (*P* = 0.029). No significant differences were found between the rest of the seasons (*P* > 0.05, in all cases). The abdominal fat bodies of specimens collected in the desert biome were significantly heavier that those collected in the savanna and Karoo biomes (*P* = 0.036). Liver mass did not vary significantly among the seasons or biomes (*P* > 0.05, in all cases).

In *P. u. undata* males, abdominal fat body mass did not vary significantly among season (F = 0.153; *P* > 0.05) or biomes (F = 0.032; *P* > 0.05). Liver mass showed significant seasonal variation (F = 3.594; *P* = 0.021). The LSD post-hoc test showed that liver mass of specimens collected in summer was significantly lower than that of ones collected in winter and spring (*P* = 0.006 and *P* = 0.013, respectively).

In *A. anchietae* females, abdominal fat body mass varied significantly among seasons (F = 2.634; *P* = 0.033) and biomes (F = 2.634; *P* = 0.033). The LSD post-hoc test showed that abdominal fat body mass of specimens collected in summer and spring was significantly greater than that of ones collected in winter (*P* = 0.000 and *P* = 0.033, respectively). For the biomes, significant variations occurred between Karoo and savanna collected specimens (*P* = 0.019), and savanna and desert collected specimens (*P* = 0.020). Liver mass did not vary significantly between seasons (F = 0.133; *P* > 0.05).

No seasonal variation in abdominal fat body mass was found in *P. u. undata* females (F = 0.153; *P* > 0.05). Liver mass showed significant seasonal variation (F = 3.594; *P* = 0.021). The LSD post-hoc test showed that liver mass of specimens collected in summer was significantly lower than that of ones collected in winter and spring (*P* = 0.006 and *P* = 0.013, respectively).

6.2 Diet composition and nematode infection

The findings with respect to the diet of the two species are given in the respective table below.

Table 2 Numerical contribution of different prey orders to the diet of *A. anchietae*. N refers to sample size.

Order	**N**	**Mean**	**Min**	**Max**	**St. Dev**
	Males				
HYMENOPTERA (ants)	29	92.9	2	318	92.9
COLEOPTERA (beetles)	5	1.2	1	2	0.4
LEPIDOPTERA (moths)	4	2.5	1	4	1.3
MANTODEA (praying mantis)	1	1.0	1	1	
	Females				
HYMENOPTERA (ants)	41	56.6	1	488	98.1
COLEOPTERA (beetles)	5	2.0	1	6	2.2
LEPIDOPTERA (moths)	1	1.0	1	1	
MATOIDEA (praying mantis)	0				
DIPTERA (flies)	1	1.0	1	1	

Ants were clearly the major prey item in both males and females of *A. anchietae* with the other types making minor contributions. Where appropriate, ANOVA was carried out and in all cases showed that the diet of males and females did not differ significantly ($P > 0.05$).

Table 3. Numerical contribution of different prey orders to the diet of *P.u. undata.* N refers to sample size

Order	N	Mean	Min	Max	St. Dev
	Males				
HYMENOPTERA (ants)	2	1.5	1	2	0.7
ISOPTERA (termites)	8	16.5	1	78	25.9
ORTHOPTERA (locusts)	0				
COLEOPTERA (beetles)	1	1	1	1	
HEMIPTERA (bugs)	0				
LEPIDOPTERA (moths)	2	3.5	1	6	3.5
BLATOIDEA (cockroaches)	1	2	2	2	
ARANEA (spiders)	0				
SOLIFUGIDA (solifuges)	1	1	1	1	
LARVAE	1	1	1	1	
	Females				
HYMENOPTERA (ants)	1	1	1	1	
ISOPTERA (termites)	0				
ORTHOPTERA (locusts)	2	1.5	1	2	0.7
COLEOPTERA (beetles)	1	1	1	1	
HEMIPTERA (bugs)	1	1	1	1	
LEPIDOPTERA (moths)	0				
BLATOIDEA (cockroaches)	0				
ARANEA (spiders)	1	1	1	1	
SOLIFUGIDA (solifuges)	0				
LARVAE	0				

No clear trend emerged from the analysis in terms of major prey items consumed by *P. u. undata* males and females, although high numbers of termites were found in the males. Where appropriate ANOVA was carried out and in all cases showed that the diet of males and females did not differ significantly ($P > 0.05$).

6.2.1 Levels of nematode infection

Seventy-eight specimens of *A. anchietae* were examined of which 39 had nematodes in their stomach and 24 in their intestine. Fifteen had nematodes in both their stomach and intestine.

Table 4. Nematode count in the stomach in the stomach and intsestine of *A. anchietae* males and females. N refers to sample size.

REGION	Females					Males				
	N	Mean	Min	Max	St. Dev	N	Mean	Min	Max	St. Dev
STOMACH NEMATODES	16	5.5	1	25	7.2	23	21.2	1	121	35.4
INTESTINAL NEMATODES	8	2.6	1	6	1.7	16	4.9	1	38	9.1

Where appropriate to apply, ANOVA found no significant difference in the levels of stomach nematode infection between males and females (F = 3.05, P = 0,089), and also in the levels of intestinal infection between the sexes (F = 0.47, P = 0.50) in *A. anchietae*.

Table 5. Nematode count in the stomach and instestine of *P.u. undata* males and females. N refers to sample size.

REGION	Females					Males				
	N	Mean	Min	Max	St. Dev	N	Mean	Min	Max	St. Dev
STOMACH NEMATODES	2	1	1	1	0	5	1.4	1	3	0.9
INTESTINAL NEMATODES	1	7	7	7		3	3	1	5	2

Where appropriate to apply, ANOVA found no significant difference in the levels of stomach nematode infection between males and females (F = 0.36, *P* = 0,58), and also in the levels of intestinal infection between the sexes (F = 3.0, *P* = 0.22) in *P. u. undata*.

6.3 Sexual dimorphism

***Table 6.* Body, head and limb measurements (in mm) for *A. anchietae*, given as the mean ± 1SD; the abbreviations are explained in the text**

SVL	TL	HDH	HDL	HDW	FLL	HLL
Females, n = 32						
79.05 ± 7.84	95.30 ± 15.90	13.84 ± 1.60	22.63 ± 2.33	19.25 ± 2.09	41.88 ± 4.15	51.83 ± 5.48
Males, n = 47						
84.60 ± 12.14	113.25 ± 18.50	15.24 ± 4.42	25.26 ± 4.42	22.37 ± 3.87	44.00 ± 5.61	57.00 ± 7.05

In *A. anchietae* mean absolute body length of females was 174.35 mm and males 197.84 mm. Relative SVL and tail length of males was significantly larger than that of females (*P* = 0.025 and *P* = 0.00039, respectively). Head width (P = 0.000), HDH (P = 0.011) and HDL (P = 0.017) of males were all significantly greater than that of females. Relative hind limb length of males was significantly greater than that of females (*P* > 0.01). No difference was found with respect to their relative fore-limb length (*P* > 0.05).

***Table 7.* Body, head and limb measurement in mm for *P. u. undata*, given as the mean ± 1SD; the abbreviations are explained in the text.**

SVL	TL	HDH	HDL	HDW	FLL	HLL
Females, n = 27						
51.6 ± 4.13	97.45 ± 20.34	5.29 ± 0.53	12.29 ± 1.44	7.22 ± 0.51	18.18 ± 1.70	31.63 ± 7.67
Males, n = 63						
50.33 ± 5.58	105.15± 26.65	5.47 ± 0.62	12.8 ± 0.98	7.43 ± 0.64	19.38 ± 2.40	31.78 ± 3.51

In *P. u. undata* no significant intersexual differences in relative SVL and tail length were found (*P* > 0.05, in both cases). Relative HDW (*P* = 0.0097), HDH (P = 0.028) and HDL (P = 0.000) was significantly greater in males than females. Relative fore- and hind limb length of males was significantly greater than that of females (*P* = 0.00019 and *P* = 0.026, respectively).

CHAPTER SEVEN

7.0 DISCUSSION

7.1 Reproductive cycles

The results showed that reproduction in *A. anchieatae* took place seasonally. The species reached peak reproductive condition in summer, thus showing a pattern corresponding with that of the other Namibian agama species *A. a. aculeata* and *A. p. planiceps* (Heideman, 1995) and *A. etoshae* (Heideman 1997). The first signs of spermatogenesis appeared in July and it peaked in November through February. In females, reproduction was also seasonal and reached a peak in summer. The biome in which specimens occurred did not seem to affect the timing of the reproductive period. Oviducal eggs were found in summer (December through February) and autumn (March through April). The reproductive period corresponded with the rainfall season (summer) that brings about a vegetation bloom and therefore an abundance of insect food. These conditions are well suited for successful incubation of the eggs and the survival of the hatchlings. The findings thus support the alternate hypothesis proposed on the timing of reproduction in the species. The similar output of eggs for the two periods is interesting and does not seem to fully support the energy proposition made. The reason for this difference requires further investigation.

Pedioplanis u. undata males and females showed the same summer pattern of reproduction as *A. anchietae*. The first signs of spermatogenesis appeared in September and it peaked through February. Oviducal eggs were found in summer (November and February; average 6 eggs) and autumn (March; average 5 eggs). Again, the similar output of eggs for the two periods is interesting and does not support the energy proposition made above. The reason for this difference will have to be investigated. The biome in which specimens occurred also did not seem to affect that timing of the reproductive period. The alternate hypothesis regarding the timing of reproduction was thus also supported in this species. This reproductive pattern found is similar to the one reported for *Pedioplanis burchelli* of South Africa by Nkosi *et al*. (2004).

7.2 Abdominal fat body and liver cycles

In *A. anchietae* males abdominal fat body mass peaked in winter prior to the onset of breeding in summer, which can be interpreted as consistent with good physiological condition thus supporting the alternate hypothesis being tested. Abdominal fat body mass reached its lowest level in summer, presumably because it serves as energy source for the reproductive process. This pattern is consistent with the findings of Heideman (1995**)** in *A. a. aculeata* and *A. p. planiceps*. The absence of significant seasonal variation in liver mass was unexpected and differed from the seasonal variation found in *A. a. aculeata* and *A. p. planiceps* (Heideman, 1995). In females the alternate hypothesis was also supported regarding the timing of reproduction and physiological condition as reflected by peak abdominal fat body mass at the start of the reproductive period. The significant negative correlation between abdominal fat body mass and reproduction again suggests its utilization as energy source during this period. The absence of significant seasonal variation in liver mass was again unexpected and differed from the findings in *A. a. aculeata* and *A. p. planiceps* (Heideman, 1995). The findings concurs with the conclusion of Aguilar-Kirigin and Naya (2013) and Ramirez-Bautista *et al*., (2006) that season is a better predictor of fat body mass variation, brought about by reproduction which is energy dependent.

In P. *u. undata* males and females a pattern similar to that found in *A. anchietae* was not found, thus not supporting the alternate hypothesis on physiological condition and the timing of reproduction. Liver mass, however, showed a clear pattern being the highest during winter and spring and lowest in summer. This pattern is associated with summer reproductive budgets (Ashby, 1998).

7.3 Diet composition

Agama anchietae is insectivorous and consumes a variety of insect prey items. The fact that ants (Hymenoptera) occurred in substantially larger numbers than any of the other prey items found, suggests that the species is primarily a sit-and-wait forager. In *P. u. undata*, on the other hand, fewer ants were found but the diet included representatives from a larger number of insect orders, and in addition to these were also larvae. The prey items are more dispersed, suggesting that this species is a wide forager. This is also supported by the large number of termites found in males. The alternate hypothesis tested regarding the diet of the two species was therefore supported.

7.4 Levels of nematode infection

As far as nematode infection was concerned, the alternate hypothesis was only partially supported, in that nematode were found in both species but no significant differences in infection levels were found between the stomach and intestine.

7.5 Sexual dimorphism

In *A. anchietae* TL and SVL was significantly greater in males than females. Head size (HDW, HDH and HDL) were also greater in males than females. Although males had larger HLL than females, they had similar FLL. It was expected that males would be larger than females due to intra-sexual selection driven by male-male combat, expected especially during the reproductive period. The significantly longer tails of males than females presumably has a selective advantage in male-male combat for females which was also suggested by Verrastro and Ely (2013) for similar findings in *Liolaemus occipitalis*. Relatively larger head dimensions in males compared to females could nevertheless also be the outcome of natural selection to minimize competition for food (Nkosi *et al*, 2004). The alternate hypothesis was thus supported to a large extent in this species

In *P. u. undata* despite the significant variation in limb length and head size (males > females), TL and SVL was similar between the sexes. The relatively longer limbs of males may enable them to have faster running speed, running speed being the product of stride length and stride rate. Males having relatively larger heads in *P. u. undata* which may have been caused in the same way as suggested in *A. anchietae* above. The lack of size dimorphism with respect to TL and SVL does not support the alternate hypothesis being tested, and suggests that the optimal state of these body measurements is similar for the sexes

CHAPTER EIGHT

CONCLUSIONS

Agama anchietae and *P. u. undata* reproduce in summer, which is also the rainfall season, presumably to optimize their reproductive output. At this time males and females are in optimal physiological condition as deduced from the state of their abdominal fat bodies. The warm and moist conditions are conducive to the successful incubation of their eggs and with the abundance of insect food at this time, the survival of the hatchlings is also optimized. Both species are insectivores, *A. anchietae* being a sit-and-wait predator and *P. u. undata* wide foraging predator. No intersexual dietary differences were found in the two species. While nematodes were found in both, no differences were found between the levels if infection of their stomach and intestine. Sexual dimorphism was more common in *A. anchietae* than in *P. u. undata*. Relatively longer fore- and hind-limbs in *P. u. undata* males may enable them to run faster. The majority of alternate hypotheses tested by this study was supported.

CHAPTER NINE

RECOMMENDATIONS

Some of the unexplained and conflicting findings of the study warrant further investigation. Ideally, this should be done using freshly caught specimens rather than preserved museum specimens which do not always provide accurate data. Sufficiently large sample sizes in order to confidently draw conclusions, are important, as was one of the shortcomings of the present study. For example, when assessing the influence of different biomes on the aspects investigated good sample sizes evenly spread over them are needed. With the great diversity of lizards in Namibia, more research is needed to fully appreciate and understand their adaptations and role in its ecosystems.

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