Demography and genetic diversity of the Mexican black iguana *Ctenosaura pectinata*.

*By*

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A thesis submitted to the University of Plymouth in partial fulfillment for the degree of

**DOCTOR OF PHILOSOPHY**

School of Biological Sciences

Faculty of Science

Director of Studies: Dr Miguel Franco
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Demography and genetic diversity of the Mexican black iguana *Ctenosaura pectinata*. Victor Aguirre-Hidalgo

**ABSTRACT**

The hunting of the black iguana (*Ctenosaura pectinata*) in some regions of Mexico constitutes an acute problem because mature, gravid females are killed and eaten just before they lay their eggs. This practice thus impacts both the survival of adults and the otherwise imminent recruitment of new individuals to the population. The objective of this project was to compare the demographic behaviour and the genetic variability of two population of the black iguana, one protected and one subject to hunting. This information allowed us to project their population dynamics and investigate the likely population-level consequences of hunting. A clear difference in age structure was observed between the two populations, with younger reproductive individuals and shorter lifespan in the hunted population. Body size was also different and reproductive individuals tended to be bigger and produce bigger clutch sizes in the hunted population than in the protected one. Survival rate of hatchlings and yearlings was estimated in both populations using soap moulds. A clear difference in survival was observed. This difference is related to the absence of natural predators in the more disturbed (hunted) area. Genetic diversity was estimated using mitochondrial DNA and differences in haplotype and nucleotide diversity between the two populations were found. It was therefore concluded that the hunted population (Nizanda) is at higher risk of suffering genetic bottlenecks. Age-based matrix projections and population viability analysis revealed that under the present conditions both populations can persist. They, however, show that current hunting levels in a closed population would cause local extinction in the short term. The study makes it possible to infer that, although black iguanas are resilient to a substantial amount of disturbance and modification of their population parameters, the gradual loss of numbers and genetic variability will increase their risk of extinction in the near future. Management actions to diminish this risk are suggested.
TABLE OF CONTENTS

COPYRIGHT STATEMENT................................................................. 1

ABSTRACT .................................................................................. iii

ACKNOWLEDGEMENTS ............................................................... xi

AUTHOR'S DECLARATION ............................................................ xiii

Chapter 1 The exploitation of the black iguana (*Ctenosaura pectinata*) ......... 1

1.1. INTRODUCTION ........................................................................ 2

1.2. *Ctenosaura pectinata* ............................................................... 5

1.1. STUDY AREA ........................................................................... 6

1.3.1 Chamela .................................................................................. 8

1.3.2 Nizanda .................................................................................. 8

1.2. OBJECTIVES .......................................................................... 11

1.3. HYPOTHESIS .......................................................................... 11

1.4. STRUCTURE OF THE THESIS .............................................. 11

Chapter 2 Life History .................................................................. 13

2.1. INTRODUCTION .................................................................... 14

2.1.1. Age Estimation ................................................................. 15

2.1.2. Survival ............................................................................. 17

2.1.3. Modification of Population Parameters ............................. 17

2.2. MATERIALS AND METHODS ........................................... 20

2.2.1 Reproduction ....................................................................... 20

2.2.2 Skeletochronology ............................................................. 21

2.3. DATA ANALYSES ................................................................ 22
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.4</td>
<td>RESULTS</td>
<td>24</td>
</tr>
<tr>
<td>2.5</td>
<td>DISCUSSION</td>
<td>33</td>
</tr>
<tr>
<td>2.5.1</td>
<td>Reproduction</td>
<td>34</td>
</tr>
<tr>
<td>2.5.2</td>
<td>Population Structure and Life History</td>
<td>35</td>
</tr>
<tr>
<td>Chapter 3</td>
<td>Experimental estimation of predation of hatchlings and juveniles</td>
<td>37</td>
</tr>
<tr>
<td>3.1</td>
<td>INTRODUCTION</td>
<td>39</td>
</tr>
<tr>
<td>3.1.1</td>
<td>Principal Predators of <em>Ctenosaura pectinata</em></td>
<td>40</td>
</tr>
<tr>
<td>3.1.2</td>
<td>Population Stability and Predation Rate in Early Stages of Life</td>
<td>40</td>
</tr>
<tr>
<td>3.2</td>
<td>MATERIALS AND METHODS</td>
<td>43</td>
</tr>
<tr>
<td>3.2.1</td>
<td>Study Sites</td>
<td>43</td>
</tr>
<tr>
<td>3.2.2</td>
<td>Construction of Replicas</td>
<td>43</td>
</tr>
<tr>
<td>3.2.3</td>
<td>Pre-dispersal Mortality</td>
<td>44</td>
</tr>
<tr>
<td>3.2.4</td>
<td>Post-dispersal Mortality</td>
<td>45</td>
</tr>
<tr>
<td>3.3</td>
<td>DATA ANALYSIS</td>
<td>48</td>
</tr>
<tr>
<td>3.3.1</td>
<td>First-year Survival</td>
<td>48</td>
</tr>
<tr>
<td>3.4</td>
<td>RESULTS</td>
<td>49</td>
</tr>
<tr>
<td>3.4.1</td>
<td>Pre-dispersal death rate</td>
<td>49</td>
</tr>
<tr>
<td>3.4.2</td>
<td>Post-dispersal death rate</td>
<td>49</td>
</tr>
<tr>
<td>3.4.3</td>
<td>Survival</td>
<td>52</td>
</tr>
<tr>
<td>3.5</td>
<td>DISCUSSION</td>
<td>54</td>
</tr>
<tr>
<td>3.5.1</td>
<td>Use of Replicas to Estimate Predation</td>
<td>54</td>
</tr>
<tr>
<td>3.5.2</td>
<td>Pre-dispersal predation</td>
<td>55</td>
</tr>
<tr>
<td>3.5.3</td>
<td>Post-dispersal predation</td>
<td>56</td>
</tr>
</tbody>
</table>
### Chapter 4 Genetic Diversity ......................................................... 58

4.1 INTRODUCTION .......................................................................... 59

4.1.1 Conservation Genetics ............................................................... 59

4.2 MATERIALS AND METHODS ......................................................... 60

4.2.1 Study Areas ............................................................................... 60

4.2.2 DNA Extraction ......................................................................... 61

4.3 RESULTS .......................................................................................... 65

4.4 DISCUSSION .................................................................................. 68

4.4.1 Genetic Variability ...................................................................... 69

4.4.2 Habitat Disturbance and Human Pressure ................................. 70

### Chapter 5 Asymptotic population dynamic and population viability analysis ........................................................................ 72

5.1 INTRODUCTION .............................................................................. 73

5.1.1 Population Demography ............................................................. 74

5.1.2 Population Viability Analysis (PVA) .............................................. 77

5.2 MATERIALS AND METHODS ......................................................... 79

5.2.1 Asymptotic Projections ............................................................... 79

5.2.2 Population Viability Analysis ....................................................... 84

5.3 RESULTS .......................................................................................... 89

5.3.1 Asymptotic population parameters .............................................. 89

5.3.2 Projected stable vital rates ......................................................... 93

5.3.3 Population Viability Analysis ....................................................... 96

5.4 DISCUSSION .................................................................................. 99

5.4.1 Asymptotic Population Dynamics ............................................... 99
Chapter 6 General Discussion

6.1. Reproductive Traits ................................................................. 107
6.2. Population Structure ............................................................... 108
6.3. Hatchling Survival ................................................................. 108
6.4. Genetic Diversity ................................................................. 109
6.5. Asymptotic Analyses .............................................................. 109
6.6. PVA Models ........................................................................... 110
6.7. Further Work ....................................................................... 111

REFERENCES .................................................................................. 114

APPENDIX 1 ................................................................................. 140
  Histological Protocol ................................................................. 140

APPENDIX 2 ................................................................................. 142
  Haplotype list ........................................................................ 142
LIST OF FIGURES AND TABLES

FIGURES

Figure 1-1. Area of distribution of Ctenosaura pectinata ............................................ 7
Figure 1-2. Habitat and geographic position of Chamela ............................................ 10
Figure 1-3. Habitat and geographic position of Nizanda ............................................. 10
Figure 2-1. Relationship between clutch size and age ................................................ 26
Figure 2-2. Relationship between snout vent length (SVL) and age ........................... 27
Figure 2-3. Relationship between clutch size and body size .................................... 28
Figure 2-4. Fitted, standardised age frequency distributions (proportion of individuals in each age class) for populations of the black iguana at Chamela (continuous line) and Nizanda (dashed line) ........................................................................................... 30
Figure 2-5. Logarithmic survival model fit ................................................................. 31
Figure 2-6 Standardised logarithmic survival curves ................................................ 32
Figure 2-7. Relationship between the force of mortality and age ................................ 32
Figure 3-1. Hatchling and juvenile soap moulds ......................................................... 47
Figure 3-2. Probability of predation outside and inside the Chamela reserve ............ 50
Figure 3-3. Survivorship curves estimated inside and outside the Chamela reserve employing two different estimates of mortality ......................................................... 53
Figure 4-1. The relative frequency of mtDNA haplotypes in Chamela and Nizanda .66
Figure 4-2. Haplotype network of Nizanda and Chamela’s black iguana ............... 67
Figure 5-1. Relative contribution of hatchlings, juveniles and adults to changes in population growth at Chamela under four different scenarios ................................................. 92

Figure 5-2. Survivorship curves at stable stage distribution assuming maximum, and two times the maximum, hatchling survival in two populations of the black iguana employing two different models of juvenile-adult survival ........................................... 94

Figure 5-3. Reproductive Value at stable stage distribution (Vₙ) assuming maximum and two times the maximum hatchling survival in two populations of the black iguana employing two different models of juvenile-adult survival ........................................ 95

Figure 5-4. Predicted population size at Nizanda and Chamela .................................. 97

TABLES

Table 1-1. Habitat characteristics at Chamela and Nizanda.............................................. 7

Table 2-1. Snout-vent length (SVL), clutch size and mean age of gravid females .... 25

Table 2-2. Coefficients of variation (CV) of body size, clutch size and age................. 25

Table 2-3. Number of iguanas in each of twelve age categories obtained from skeletochronological analysis at Chamela and Nizanda......................................................... 29

Table 3-1. Factorial analysis of variance among sites and periods............................... 51

Table 3-2. Analysis of variance of predation rate during the pre-dispersion phase.... 51

Table 3-3. Analysis of variance of predation during the post-dispersion phase.......... 52

Table 4-1. Genetic diversity of iguanas in Chamela and Nizanda............................... 66

Table 5-1. Age specific survival (pₙ) and fecundity (fₙ) values used for Chamela and Nizanda matrices based on the age at death model (AD) and the distribution fit model (DF) ................................................................. 82
Table 5-2. Life history parameters used for population viability analysis .......... 87

Table 5-3. Vortex model input parameters specified for each scenario .............. 88

Table 5-4. Population parameters projected by STAGECOACH ....................... 90

Table 5-5. The elasticity of age-specific survival ($p_x$) and fecundity ($f_x$) for the DF and AD models with maximum, two times maximum hatchling survival ............... 91

Table 5-6. Vortex sensitivity analysis .............................................................. 98
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AUTHOR'S DECLARATION

At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University award without prior agreement of the graduate committee.

I declare that the work submitted in this thesis is the result of my own investigation except where reference is made to published literature and where assistance is acknowledged.

Presentations and Conferences Attended:

VII Reunión Nacional sobre iguanas (May 2004)
Demografía y estructura genética de la iguana negra *Ctenosaura pectinata*.

VIII Reunión Nacional sobre Iguanas en México (May 2005)
Uso de réplicas de crías de iguana negra *Ctenosaura pectinata* como un método alternativo para estimar su tasa de mortalidad a consecuencia de la depredación.

IX Reunión Nacional sobre Iguanas (May 2006)
Aprovechamiento de la iguana negra *Ctenosaura pectinata* en Chamela, Jalisco y Nizada, Oaxaca.
Moisés Minor Sánchez, **Víctor Aguirre-Hidalgo**, María del Carmen Corona Vargas.

Synergy Meeting of the ecology groups from the University of Exeter and the University of Plymouth (December 2006).
Poster presentation: Erosion of genetic diversity in hunted black iguanas (*Ctenosaura pectinata*).
**Víctor Aguirre-Hidalgo**, Miguel Franco and Lise Dupont

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Poster presentation: Erosion of genetic diversity in hunted black iguanas (*Ctenosaura pectinata*).
**Víctor Aguirre-Hidalgo**, Miguel Franco and Lise Dupont

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Chapter 1

The exploitation of the black iguana (Ctenosaura pectinata)
1.1. INTRODUCTION

The current, mostly unregulated use of wildlife as a source of income and food endangers its future. In particular, consumption of meat from wild species ("bushmeat") is a practice that impacts many vertebrate species all over the world (Carpaneto & Fusari 2000; Fa et al. 2002). This practice often has a long tradition in rural communities because it represents a means of generating income, employment opportunities, and access to alternative sources of protein (Loibooki et al. 2002; Milner-Gulland & Bennett 2003). In rural areas, family income often increases at a lower rate than the price increase of basic products, making wild meat an economically attractive resource. In addition to this, increased human population in and around natural habitats puts an ever increasing pressure on the species consumed. An increasing rural population inevitably leads to over-hunting and may be responsible for the local extinction of numerous species (Milner-Gulland & Bennett 2003; Whitfield 2003). In some countries the magnitude of wild meat hunting is dramatic. For example, Carpaneto & Fusari (2000) found that in Tanzania a total of 236 animals belonging to 37 species were killed from December 1995 to February 1996. Similarly, Bennett & Rao (2002) reported that the demand for wild meat in many parts of south-east Asia is increasing dramatically and wildlife populations are consequently declining.

Bakarr et al. (2002) highlighted the necessity of complementing efforts between government agencies, NGOs, scientists, producers and rural people to safeguard wildlife and to generate management options designed to help the
remaining populations of wild species. Among the urgent tasks scientists have in this effort is to compile reliable information on the population-level effects of hunting, particularly for those species regarded as most susceptible (Ortiz 2002).

An interesting case of wild meat hunting in the Mexican countryside is that of iguanine lizards, in particular the black iguana Ctenosaura pectinata. This species is endemic to Mexico and is classified as threatened with extinction by the Mexican Official Norm of Ecology No. 059 (SEMARNAT 2002). The main causes for this classification are habitat loss and over-exploitation (Fitch et al. 1982; Alberts 2000). Black iguanas are hunted for human consumption in many rural areas (Reynoso-Rosales 2000). The existing records suggest that this reptile has been exploited since pre-Columbian times. In consequence, its consumption probably dates back several hundred years, and possibly more (Garibay 1989).

Lack of information on the demographic status of this species has limited our ability to quantify the likely consequences of hunting. What is known is that mature gravid females are hunted and eaten just before they lay their eggs (Fitch et al. 1982). This practice thus impacts both the survival of female adults and the otherwise imminent recruitment of new individuals to the population. If this practice produces low population size and, as would be expected, reduces genetic variability, it is likely to increase the risk of local extinction (Lande 1998; Vucetich & Waite 1999; Brook et al. 2002b; Reed et al. 2003). It is therefore necessary to quantify the vulnerability of this species and to recommend corrective measures if evidence on impending peril
exists. Gathering this evidence requires detailed population monitoring on which to base sustainable harvesting programmes (Stephens et al. 2002).

The usefulness of demographic models in the proposition of specific conservation measures has been evident in other species. For example, in marine turtles efficient conservation programs have been characterized in terms of the relative effect that harvest-induced mortality on different stages of the life cycle has on population growth (Sensitivity and elasticity analyses; Crouse et al. 1987; Congdon et al. 1993; Crowder et al. 1994; Heppell et al. 1996a; Heppell et al. 1996b). One possible advantage of iguanas over turtles, as subject of study, is that it is still possible to study control populations where harvesting is minimal or non-existent. This happens in areas where humans are few and iguanas are protected, for example, in relatively isolated nature reserves. Information collected in such areas would allow identification of the factors that regulate the populations in the absence of hunting. It would also allow comparison with currently exploited populations as a means to quantify both the demographic consequences and the selective effect that hunting has had on population parameters. For example, in over exploited species of fish it is known that an increase in adult mortality has favoured the evolution of shorter lifespan and smaller body size at sexual maturity (Rochet 2000; Gardmark et al. 2003). Demographic information is therefore essential to predict not only the short-term dynamics of the populations, but to make inferences on the likely long-term life history consequences.
Previous studies on the life history of Mexican iguanas have been conducted
(Aguirre-Hidalgo et al. 1998; Aguirre-Hidalgo & Reynoso-Rosales 1998; Salas-Tapia et al. 1999; Salas-Tapia & Reynoso-Rosales 2000; Reynoso-Rosales 2000; Salvatore-Olivares 2001; González-Montfil 2002; Aguirre-Hidalgo 2002). These were carried out in areas impacted by hunting (Nizanda, Oaxaca State, Mexico) and provide important information on life history, reproductive biology and reproductive behaviour, as well as on current levels of harvesting. These studies show that iguanas are hunted at two times of the year. The first is during the courtship season, when iguanas are exposed while searching for potential mates. The second period occurs during hatching, when gravid females are hunted before they lay their eggs. The impact that these practices have on the populations must be carefully evaluated.

Within this context, the present study’s main objective was to compare the demography, reproductive biology and genetic diversity of two populations of the black iguana. The first population is found in the locality of Nizanda, in the State of Oaxaca, Mexico. The second population is located in the Chamela-Cuixmala reserve, in the State of Jalisco, Mexico. While the population at Nizanda has undergone hunting for possibly hundreds of years, that in Chamela has been isolated in a sparsely populated area during historical, and possibly also pre-historical times.

1.2. Ctenosaura pectinata

The black iguana is a slow growing, long-lived species of reptile inhabiting the Pacific Coast of Mexico, from southern Sinaloa State to northern Chiapas State (Fig.
1. It is also found in the islands Isabel and Tres Marias. It exhibits a marked dimorphism. Adult males possess a dorsal spiny crest and are generally bigger than adult females (Köhler & Streit 1996). Newborn iguanas are green and become darker with age (Köhler 2002). The adult has dark cross bands along the tail, but patterns of black and white or even colours such as orange, pink, yellow or blue may also be present in some individuals (Flores-Villela 1991; Suazo & Alvarado 1994). The natural habitat of the black iguana is tropical and subtropical vegetation between 0 and 1400m above sea level, where the mean temperature ranges from 20°C to 26°C (Flores-Villela 1991). This includes deciduous and subdeciduous tropical forests, thorny forest, grasslands, oak forest, mangroves, coastal dunes and perturbed areas in and around human communities (Suazo & Alvarado 1994). Adult black iguanas nest wherever a hiding place or burrow can be found. \textit{C. pectinata} feeds on plants, insects, newborn rodents and small birds; it can also feed on carrion (Flores-Villela & Gerez 1988). As with all iguanine species, \textit{C. pectinata} is oviparous. It reproduces once a year with eggs being laid from March to May (Aguirre-Hidalgo & Reynoso-Rosales 1998).

**1.1. STUDY AREA**

The study areas (Nizanda and Chamela) have similar climatic conditions and are dominated by deciduous and semi-deciduous forest with summer precipitation (Garcia 1988). Nizanda, however, is slightly more productive (it has higher precipitation and taller forest structure) than Chamela (Table 1-1).
Table 1-1. Habitat characteristics at Chamela and Nizanda.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Chamela</th>
<th>Nizanda</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geographic location</td>
<td>19°29’N 105°01’W</td>
<td>16°39’N 95°00’W</td>
</tr>
<tr>
<td>Vegetation</td>
<td>Deciduous and semi-deciduous dry forest</td>
<td>Deciduous and semi-deciduous dry forest</td>
</tr>
<tr>
<td>Climate</td>
<td>Tropical sub-humid, with marked seasonality.</td>
<td>Tropical sub-humid, with marked seasonality.</td>
</tr>
<tr>
<td>Mean Annual Temp.</td>
<td>24.6</td>
<td>25</td>
</tr>
<tr>
<td>(°C)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Annual Rainfall (mm)</td>
<td>788</td>
<td>1000</td>
</tr>
<tr>
<td>(Jun.-Oct.)</td>
<td></td>
<td>(May-Oct.)</td>
</tr>
</tbody>
</table>

Climatic information from Garcia-Oliva et al. (2002), for Chamela, and from Pérez-García et al. (2001), for Nizanda.
1.3.1 Chamela

The Chamela-Cuixmala Reserve (19° 30' N, 105° 03' W; Fig. 1-2) is located approximately 120 km north of Manzanillo, Colima, in the municipality of La Huerta. It has an extension of 13,000 ha and most of this area is below 150 m of altitude. This reserve is the only one in Mexico specifically designed to protect tropical dry forests. In addition to the dominant tropical dry forest, tropical semi-deciduous forest and wetlands exists along the river courses. The relief is dominated by hills and an alluvial plain on the riverbanks of the Cuitzmala River. Superficial currents are scarce and small streams carry water only during the rainy season or during cyclones. Chamela is characterized by a marked seasonality. Most of the precipitation (annual total ~800mm) is concentrated from July to October, with a prolonged dry season. The mean annual temperature is 24.9 °C. The area contains more than 1,100 vascular plant species (Lott 1993). The terrestrial vertebrates consist of 72 mammal species, 271 species of birds and 87 species of herpetofauna (Bullock 1986).

1.3.2 Nizanda

Nizanda is in the Isthmus of Tehuantepec, Juchitan District, Oaxaca (16° 39' N; 95° 00' W Fig. 1-3). The altitude in the area varies between 100 and 700 meters above sea level, with the most frequent altitude being 200m. The records for the area show 746 species of vascular plants in seven terrestrial vegetation types: gallery forest, thorn scrub, xeric scrub, savannah, dry tropical forest, semievergreen forest and semideciduous tropical forest (Pérez-García et al. 2001). Three streams converge in
Nizanda, but only one carries water throughout the year. As in Chamela, there is a marked seasonality and the rainy season occurs from May to October. The annual precipitation is ~1000 mm and the mean annual temperature is 25°C (Pérez-García, Gallardo, & Meave 2001).
Figure 1-2. Habitat and Geographic position of Chamela.

Figure 1-3. Habitat and Geographic position of Nizanda.
1.2. OBJECTIVES

The objective of this study was to contrast the reproductive biology, demography and genetic diversity of two populations of the black iguana, one subject to hunting (Nizanda) and the other protected (Chamela).

1.3. HYPOTHESIS

The continuous extraction of gravid iguanas and the consequent elimination of potential offspring must exert a selective pressure on the reproductive biology and life history characteristics of the black iguana. This is likely to result in reduced genetic variability and an increased risk of extinction.

1.4. STRUCTURE OF THE THESIS

This study is organised in five chapters and a general discussion. Chapter 1 has provided a general background to the demographic and genetic problems that hunted species face. It presents the justification for population studies and their relevance in the prediction of potential population decline. This chapter also describes the biology of the species and the climatic conditions of the two areas where the study was conducted. Finally, the objective and basic hypothesis of the study are stated. The remaining four chapters cover specific aspects of the population biology of the black iguana.

Chapter 2 investigates the age structure and survival schedules of hunted and protected populations of the black iguana. It explains the individual age estimations.
obtained with the technique of skeletochronology. Demographic and life history differences between the two populations are presented, with the hunted population having earlier age at sexual maturity, shorter lifespan and larger reproductive individuals with bigger clutch sizes than the protected population.

Chapter 3 describes an experiment designed to quantify survival of hatchlings throughout their first year of life. Estimations were made employing soap models, recording the numbers lost and damaged by predators. Predation was higher in protected areas, presumably as a consequence of the absence of natural predators in hunted areas with strong human influence.

Chapter 4 compares the genetic diversity within and between hunted and non hunted populations. Genetic diversity was lower in the hunted population.

Chapter 5 incorporates the results from previous chapters into a model of the dynamics of the two populations. Two types of models are explored: (i) the asymptotic dynamics of the populations as a means to investigating their dynamic and life history differences, and (ii) population viability analyses that allow simulation of the likely consequences that different hunting scenarios would have. The final goal was to assess the sustainability of current hunting practices. Despite a loss of genetic diversity, the black iguana is able to sustain high levels of hunting. It is doubtful, however, that these will be sustainable in the long run, particularly if the practice continues to expand.
Chapter 2

Life history
2.1. INTRODUCTION

Life history is the pattern of survival, growth and reproduction that the average individual in a population has. The diversity of life histories is a consequence of the history of selective pressures acting on genetically-determined morphological, physiological or behavioural traits. A study of life history must therefore document the changes in survival, growth and reproduction through the life cycle of the individuals making up a particular population. In this chapter we investigate the life history of the black iguana in two contrasting populations, one protected and the other subject to regular, intense hunting. Because we are hypothesising that differences in mortality (hunting pressure) have resulted in modifications to life history of the two studied populations, we need to quantify differences in patterns of survival, body size and reproductive contribution (fecundity) with age. Unless the individual were to live forever (something which, by the simple laws of probability, is impossible, Medawar 1952) reproductive outcome is the ultimate currency of evolutionary success. Thus, in addition to obvious differences in growth (e. g., size of the adult) and survival (e. g., longevity) between species, the variety of life histories is apparent in the diversity of reproductive output. In a single reproductive bout, mature individuals can generate one (e. g., anoles; geckos; sea birds and big-sized mammals) to thousands of offspring (e. g., some fishes and plants; Pianka 2000).

Life history theory attempts to explain the manner in which the energy available to an individual is allocated to different functions and how this results in the observed variation in reproductive output (Partridge & Harvey 1988; Stearns 1992;
Several parental investment models for organisms with variant clutch size suggest the existence of optimal clutch/egg size under a given set of conditions (Brockelman 1975). An important element in these models is the assumption of trade-offs between different life history traits. The concept of trade-off is based on the premise that each individual has a finite amount of available energy which must be optimally distributed between reproduction, maintenance, growth and storage (Bonnet et al. 2001). Allocation to one of those traits will necessarily lead to fewer resources for the others (Stearns 1976; Stearns 1992).

Studies of reproductive patterns have attempted to assess the effects of i) demography (Pianka 1972; Heino & Godo 2002), ii) resource availability (Ballinger 1977; Dunham 1978; Bronikowski & Arnold 1999; Du 2006) and iii) phylogeny (Miles & Dunham 1992). More recently, the intensive exploitation of economically important wild species (e.g., fish) has drawn attention to its strong selective effects. It has been hypothesised, and confirmed in some studies, that this additional selective pressure acting on the larger adults promotes rapid, irreversible changes in life history and genetic structure (Conover & Munch 2002; Olsen et al. 2004; Reznick & Ghalambor 2005; de Roos et al. 2006).

2.1.1. Age Estimation

Because life histories unfold over time, age of the organisms is an important element in the investigation of how survival, growth and fecundity change with it. The determination of the age of organisms in the wild is difficult, costly and time-
consuming (De Buffrenil & Castanet 2000). It is, however, possible to determine the age of individual animals through methods that employ hard body parts that grow seasonally, such as scales, feathers and bones, or soft body parts such as reproductive organs or even blood samples (Dudzinski et al. 1977; Scalet et al. 1996; Haussmann & Vleck 2002).

Analysis of cross sections of hard bones for assessing the age of animals (skeletochronology) has been employed to estimate age in species with indeterminate growth. This technique has the advantage of not altering the original population structure (Castanet & Smirina 1990). Skeletochronology is based on the periodic accumulation of growth layers – broader growth zones delimited by lines of arrested growth (LAGs). In the majority of cases these growth marks correspond to one year (growth and dormant/arrested periods, but see Bjorndal et al. 1998, for some inconsistencies). These marks provide evidence of the number of growth cycles that the animal has lived through (Castanet & Smirina 1990). Care must be taken when estimating age in bones because of the possibility of missing rings due to endosteal resorption or over-estimating the true age by counting false rings (Castanet & Smirina 1990). Notwithstanding these potential sources of error, skeletochronology has been used successfully to estimate age in several groups of ectothermic animals, such as anurans (Wake & Castanet 1995; Driscoll 1999; Khonsue et al. 2000; Pancharatna et al. 2000; Reaser 2000; Khonsue et al. 2001; Kumbar & Pancharatna 2001; Jakob et al. 2002; Khonsue et al. 2002), and reptiles (Parham & Zug 1997; Bjorndal, et al. 1998; El Mouden et al. 1999; De Buffrenil & Castanet 2000; Androne et al. 2005).
2.1.2. Survival

Survival is an important demographic parameter and a major determinant of the transient dynamics of a population as it tends to converge towards a demographic harvesting equilibrium. It is also an essential component of life history (Stearns 1992; Stearns 2000). For example, using long term monitoring studies, Gaillard et al. (2000) showed that variation of adult survival has more influence on population growth than variation in recruitment. They also observed that adult survival was less variable than juvenile survival.

2.1.3. Modification of Population Parameters

The population dynamics of wild organisms is modified by factors such as environmental fluctuations, predation, natural disturbances, animal release and relocation, and selective harvesting (Stearns & Koella 1986; Koons et al. 2005). Such factors can alter life history (e.g., maturation, reproduction and mortality; Coltman et al. 2003), modify population structure (Clodert et al. 1998; Whitman et al. 2004), decrease population stability (Stearns 1992; Lande 1998; Whitman, et al. 2004; Benton et al. 2006) and negatively affect population growth, thus increasing the probability of extinction (Lande 1993; Gotelli 1998; Morris & Doak 2002). In general, modification of vital rates (e.g., fecundity, survival, maturation, recruitment) can produce changes in age and size structure (Keyfitz 1971; Koons 2006).
Hunting creates an additional source of mortality, affecting population growth and both the number and type of individuals remaining in the population (Calvet & Gauthier 2005). The effect of this additional mortality will depend on the individual’s contribution to population growth (Hunter & Caswell 2005). Hunting, fishing and harvesting continuously modify population structure and drive populations away from equilibrium (Hall 1999a; Peres 2000; Ferguson 2002; Coltman, et al. 2003; Coulson et al. 2004). Although their effect on transient dynamics may be difficult to predict, their end result may be an increase in the risk of extinction (Gordon et al. 2004).

**Human influence on life history evolution**

Evidence of human effect on life histories of wild animals has been obtained primarily on fish species (Pitcher & Hart 1992). Using information from fisheries, several harvest models have been developed. De Roos et al (2006) propose that fished populations can lead to three different scenarios. In the first one, fishing can generate a decrease in age and size at maturation. The second probable scenario is an increase in size at maturation, but no increase in age at maturation. Finally, harvesting can result in decreasing age at first reproduction, but a limited change in maturation size. Changes in age and size at maturation have been documented in several exploited fish populations, such as silverside (*Menidia menidia*), Atlantic cod (*Gadus morhua*) and black spot sea bream (*Pagellus bogaraveo*) (Conover & Munch 2002; Olsen, et al. 2004; Munch et al. 2005; Erzini et al. 2006). Similar phenotypic changes have also been reported in terrestrial vertebrates such as rams (*Ovis canadensis*) and red deer.
(Cervus elaphus), where body weight and horn size changes have occurred in hunted populations (Langvatn & Loison 1999; Coltman, et al. 2003).

Traits such as age and size at maturation are important life history qualities because they have direct influence on survival, reproductive effort, growth rate, offspring survival, length of the reproductive lifespan, and expected lifetime fecundity (Stearns 1976; Stearns & Koella 1986; Stearns 2000). Estimating survival in harvested populations can help to determine whether a population is in its stable age distribution and allow detailed analysis of its population dynamics (Koons 2006). Given these findings, research on the interaction between both age and size at sexual maturity and fecundity in exploited and unexploited populations is required to infer the magnitude to which human selective pressure is influencing life history. Thus, in this chapter the essential reproductive characteristics, age structure and patterns of survival of two populations of C. pectinata with different degrees of exploitation are compared.
2.2. MATERIALS AND METHODS

2.2.1 Reproduction

During the periods of March to April 1999 and March to April 2005 in Nizanda, and April to June 2004 and April-June 2005 in Chamela, adult individuals were collected and measured. Depending on the size of the iguanas, several capture methods were used. One year old iguanas were caught by hand or with a running noose at the end of a pliable aluminium pole. Adult iguanas were also captured with this pole. In Chamela, iguanas spotted in the field usually ran to the nearest hole or crack in the rocks or tree. They were extracted by hand or trapped when they left the hole by using a running noose situated in front of the hole's opening and tied to a piece of buried wire. The information recorded for each individual was its gender and snout-vent length (SVL).

Gender and reproductive status was established by examining secondary sexual characters in adult males and palpating the presence of eggs in gravid females. In juveniles, sex was determined by cloacal inspection (González-Monfil 2002). Once the data were recorded, the iguanas were released near their capture site or, in the case of some gravid females, maintained in individual cages of 1 x 1.05 x 1 m. Each cage had a ~50cm soil mound in the middle, with a 30 cm PVC tube partially buried under it. Sample sizes for gravid females were 20 and 28 iguanas in Nizanda and 12 and 28 individuals in Chamela. After oviposition, each female was weighed and liberated in the area where it was captured, and had her eggs counted.
2.2.2 Skeletochronology

During the same period of collection of reproductive data, small portions of digits were clipped from captured specimens and kept in 98% ethanol. Samples were collected from 194 iguanas in Chamela and 182 iguanas in Nizanda. The mutilation does not seem to have serious consequences for the future survival of the iguana (Zug & Rand 1987) and the necessary permits were obtained from the Mexican authorities. Castanet and Smirina (1990) procedures were used to estimate age from the finger portions collected. The skin and flesh were removed and the bones soaked in tap water for 24 hours. They were then demineralised in 5% nitric acid for 10-24 hrs. After demineralisation, the tissue was washed overnight to remove excess acid. Diaphyses were cross-sectioned and both portions dehydrated and embedded in paraffin wax. The cut-end sections were finally cross-sectioned three or more times using a rotary microtome. Slides were prepared with at least six sections 6µm in thickness. Each prepared slide was stained with haematoxylin-eosin, and then mounted in Canada balsam (see Appendix 1 for complete protocol). Every slide prepared was examined under a light microscope to count hematoxylinophilic rings and photographs were taken.

The objective of the technique was to determine the number of growing marks, in the form of lines of arrested growth (LAGs) that separate annual increments of bone deposition. LAGs were counted from the phalangeal cross-sections starting from the marrow cavity and counting towards the marginal section of the bone. This technique had already been used in iguanas with good results (Zug & Rand 1987). Given the
marked seasonality at both localities, annual periodicity in the formation of bone rings was assumed for both populations.

2.3. DATA ANALYSES

Differences in age at reproduction between both populations were tested by a Student’s t-test. Age class distributions were compared with a Kolmogorov-Smirnov test. The relationship between clutch size and body size was estimated using regression analyses. Despite King’s (2000) recommendation concerning the advantages of log-transformed data (based on an allometric argument), regression analyses were performed on untransformed data to emphasise the differences in the size and reproductive contribution of gravid females between the two populations. All statistics analyses were carried out with STATISTICA 7. 1 (StatSoft 2005).

Estimates of survival were calculated based on the distribution of age obtained from the skeletochronological analysis. A flexible, cumulative distribution function (Franco et al. 2007) was used to describe the age distribution of the populations. This function has the form:

\[ n_s = N \left( 1 - \left( 1 - \frac{g}{1 + e^{-m_r x_a}} \right)^r \right) \]

In the context of the age distributions described here the variables and parameters are:
\( N \): total population size \( N = \sum_{x} n_x \).

\( n_x \): number of individuals of age \( x \).

\( g \): a measure of the rate of accumulation of individuals as consecutive age classes are added to the count.

\( b \): a measure of monotonic change in the rate of accumulation of individuals; a shape parameter.

\( z \): a lag in the accumulation of individuals in the first age classes; a measure inversely proportional to recruitment.

The parameters of the model were estimated employing the nonlinear fit module of MatLab (The Mathworks 2007). Notice that dividing both sides of the equation by total population size, \( N \), yields the proportion of individuals in each age class, allowing direct comparison of the age distribution of the two populations.

For comparison, an alternative estimation of relative survival was obtained with the logarithmic model \( n_x = b_0 + b_1 \ln(x) \). Because individuals are difficult to observe during their first two years of life, for the analysis of the population at Chamela (stages 1 and 2 years) and Nizanda (first four years), the immature age stages were omitted from this model fit. The total numbers of individual’s data not employed in these regressions were 26 and 31 for Chamela and Nizanda, respectively, which correspond to 13 and 17 percent of the total number of individuals in the
corresponding sample (see Table 2-4). These two models (distribution fit and logarithmic) allowed construction of their corresponding static life tables, from which the proportion of surviving individuals \( l_x = n_x / n_0 \), the proportion dying \( d_x = l_{x-1} - l_x \), and the force of mortality \( \mu_x = -\ln(l_x / l_{x+1}) \) during each age interval were calculated.

2.4. RESULTS

There were no differences in the relationship between SVL and clutch size in the two years of study in either Nizanda (ANCOVA \( F_{1,45} = 0.77158, p = 0.38440 \)) or Chamela (ANCOVA: \( F_{1,37} = 0.07543, p = 0.78512 \), with year as the categorical predictor, clutch size as the dependent variable and SVL as the continuous predictor). Consequently, information collected on individuals measured in two different years were grouped and analysed as one sample for each of the two populations.

The variation of SVL, clutch size and age for reproductive females from Nizanda and Chamela are shown in Table 2-1. Adult iguanas tended to reach larger body size and to have larger clutch size in Nizanda than iguanas of the same age from Chamela. Similarly, the SVL of adults in Nizanda was significantly larger than the SVL of adults from Chamela \( (t = 10.764, d. f. = 86, P < 0.05) \). Clutch size in Nizanda was also significantly larger than in Chamela \( (t = 6.88, d. f. = 86, P < 0.05) \).

Reproductive age, however, was not significantly different between the two populations \( (t = -0.119, d. f. = 58, P > 0.05) \). The variability of SVL, clutch size and age
estimated through the coefficient of variation (CV = SD/Mean), was consistently higher in Chamela than in Nizanda (Table 2-2).

In Nizanda, the relationship between clutch size and SVL was significant. In contrast, those between clutch size and age (Fig. 2-1), and between SVL and age (Fig. 2-2) were not significant. In Chamela, on the other hand, all three relationships were significant (Fig. 2-1 and 2-2).

<table>
<thead>
<tr>
<th>Period</th>
<th>N</th>
<th>SVL (cm)</th>
<th>Clutch size</th>
<th>Age (yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
</tr>
<tr>
<td>Chamela</td>
<td></td>
<td>Range</td>
<td>Range</td>
<td>Range</td>
</tr>
<tr>
<td>2004</td>
<td>12</td>
<td>25.75±3.02</td>
<td>34.08±15.25</td>
<td>5.66±1.66</td>
</tr>
<tr>
<td>2005</td>
<td>28</td>
<td>24.30±2.46</td>
<td>29.96±9.65</td>
<td>5.50±1.55</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>24.74±2.69</td>
<td>31.20±11.57</td>
<td>5.55±1.57</td>
</tr>
<tr>
<td>Nizanda</td>
<td></td>
<td>30.56±20.0</td>
<td>44.66±9.56</td>
<td>5.5±1.43</td>
</tr>
<tr>
<td>1999</td>
<td>20</td>
<td>27.5-35</td>
<td>28-65</td>
<td>4-9</td>
</tr>
<tr>
<td>2005</td>
<td>28</td>
<td>30.52±2.57</td>
<td>46.35±6.83</td>
<td>32-60</td>
</tr>
<tr>
<td>Total</td>
<td>48</td>
<td>30.53±2.36</td>
<td>45.64±8.03</td>
<td>28-65</td>
</tr>
</tbody>
</table>

Table 2-1. Snout-vent length (SVL), clutch size and mean age of gravid females collected in two localities (Chamela and Nizanda) in two reproductive seasons.

<table>
<thead>
<tr>
<th></th>
<th>SVL (cm)</th>
<th>Clutch size</th>
<th>Age (yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nizanda</td>
<td>0.0077</td>
<td>0.1759</td>
<td>0.26</td>
</tr>
<tr>
<td>Chamela</td>
<td>0.1087</td>
<td>0.37</td>
<td>0.2828</td>
</tr>
</tbody>
</table>

Table 2-2. Coefficients of variation (CV) of body size, clutch size and age in two populations of the black iguana.
Figure 2-1. The relationship between clutch size and age in female black iguanas from Chamela (circles and continuous line) and Nizanda (open triangle and dashed line). The regression equations and their $r^2$ were: Chamela: Clutch size = $11.8936 + 3.4786\text{age}$, $r^2 = 0.12$; Nizanda: Clutch size = $49.9385 - 9615\text{age}$, $r^2 = 0.02$. 
Figure 2-2. The relationship between snout vent length (SVL) and age in female black iguanas from Chamela (filled circles and continuous line) and Nizanda (open triangles and dashed line). The regression equations and their \( r^2 \) were: Chamela: \( SVL = 19.1384 + 1.0273 \text{age} \), \( r^2 = 0.35 \); Nizanda: \( SVL = 29.6836 + 0.1603 \text{age} \), \( r^2 = 0.01 \).

A quadratic model provided a good fit to the relationship between clutch size and SVL in both populations, suggesting that the increase in clutch size with age has a limit or may even decrease at old age (Fig. 2-3). This pattern was more evident in Nizanda, where bigger individuals were registered, than in Chamela (Fig. 2-3). Linear relationships confirmed that the increase in clutch size is steeper in Chamela than in Nizanda.
The population at Chamela contained 10 reproductive age classes plus ages 1 and 2 which contained juveniles and sub adults, respectively. The oldest recorded individual in Chamela was 12 years old. The population at Nizanda contained 7 reproductive age groups, plus the same two initial categories of juveniles and subadults. The oldest individuals in Nizanda were 9 years old (Table 2-3).
Table 2-3. Number of iguanas in each of twelve age categories obtained from skeletochronological analysis at Chamela and Nizanda.

<table>
<thead>
<tr>
<th>Age</th>
<th>Chamela</th>
<th>Nizanda</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>33</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>38</td>
<td>24</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>51</td>
</tr>
<tr>
<td>6</td>
<td>29</td>
<td>50</td>
</tr>
<tr>
<td>7</td>
<td>22</td>
<td>32</td>
</tr>
<tr>
<td>8</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>9</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>10</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

Adult (i. e., reproductive) iguanas at Chamela had a mean age of 5.53±2.102 years (range: 3-12yr, n=168). Adult iguanas at Nizanda had a mean age of 5.76±1.30 years (range: 4-9yr, n=182). Thus, although with an apparently different maximum longevity, mean age was not significantly different between the two populations (t=−1.106, d. f. =348, P>0.05). However, their age distribution was significantly different (Kolmogorov-Smirnov $D_{max}=0.085$, P<0.05).
Figure 2-4. Fitted, standardised age frequency distributions (proportion of individuals in each age class) for populations of the black iguana at Chamela (continuous line) and Nizanda (dashed line).

The fitted age distribution revealed a lower variance in the age distribution of Nizanda’s iguanas, with a larger proportion of individuals in the 5th to 6th age classes. On the other hand, the age distribution in Chamela had a higher variance with a longer tail of increasingly older individuals (Fig. 2-4). Both of these models had an adjusted $r^2 > 0.98$.

On the other hand, the $r^2$ values of the logarithmic model fit for 83% of the sample in Chamela and 87% of the sample in Nizanda (i.e., younger categories were excluded; see methods section) was 0.80 and 0.92, respectively (Fig. 2-5). This model revealed a Deevey (1947) type II survivorship curve for both populations throughout their adult life followed by a sharper period of mortality (Deevey’s type I mortality).
near the end of life. This drop in survival at advanced ages occurred earlier and was more pronounced in Nizanda than in Chamela (Fig. 2-6). From the life table constructed with this logarithmic fit, the force of mortality slightly decreased during the first two years of life, stayed low and relatively constant for a few years and then increased at later ages (Fig 2-7). This pattern was similar in both populations but senescence, as evidenced by an increase in mortality at older age, occurred earlier in Nizanda.

Figure 2-5. Logarithmic survival model fit for Chamela (filled circles and continuous line: \( N = -26.134 \ln(Age) + 67.222 \)) and Nizanda (open triangles and dashed line, \( N = -87.14 \ln(Age) + 197.92 \)).
Figure 2-6 Standardised logarithmic survival curves for populations of the black iguana at Chamela (continuous line) and Nizanda (dashed line).

Figure 2-7 The relationship between the force of mortality and age in Chamela (filled circles and continuous line) and Nizanda (open triangles and dashed line). Mortality is expressed as a relative instantaneous rate at each age category.
2.5. DISCUSSION

Populations of the black iguana *Ctenosaura pectinata* have been hunted, perhaps for centuries, in several regions of Mexico. Little is known, however, on the effects that this practice has had on its population dynamics and life history.

Differences in body and clutch size were observed between the populations of *C. pectinata* at Nizanda and Chamela. Nizandas' iguanas tended to be bigger, and to lay more eggs per reproductive event. Comparisons of life-history traits in several groups of lizards have revealed variation in phenotypic and reproductive traits across their distribution range. These differences have been ascribed to environmental and genetic factors, and their interaction (Ballinger 1977; Sorci et al. 1996). Climatic conditions are considered an important factor associated with the evolution of life histories (Shine 1983; Caley & Schwarzkopf 2004) and is a factor that cannot be discounted here. Although differences in climatic conditions between Chamela and Nizanda may be relatively small, whatever differences exist, they are accompanied by differences in associated flora and fauna. We can therefore only assume that differences in hunting pressure have further contributed to the differences in life history observed (Winemiller 2005), as with the chuckwalla iguana (*Sauromalus obesus*) and the green iguana (*Iguana iguana*) whose life history is modified by the combination of climatic conditions, period of activity, abundance of food resources, social interactions among individuals, population density, and mortality (Lichtenbelt & Albers 1993; Tracy 1999).
2.5.1 Reproduction

As most reptiles, *C. pectinata* grows continuously throughout its life. The fact that *C. pectinata* is oviparous and that fecundity is correlated with body size implies that body size restricts the number of eggs produced in one reproductive season. Although some differences were expected, the difference in individual growth between iguanas at Chamela and iguanas at Nizanda is noticeable. The expected positive relationship between clutch size and age was only observed in Chamela. In this population, bigger size corresponded to older individuals. In Nizanda age was not related to body size. Because, on average, iguanas from Nizanda were bigger than iguanas from Chamela, they must grow faster and slow down their growth considerably past a certain size. This means that the overall relationship between clutch size and size (SVL) shown in figure 3 would be the same for both populations (and perhaps other populations of the same species), but the iguanas at Chamela never reach the hump of this relationship because they grow slowly and die before reaching such sizes. Similar differences have been reported for the Bahamas’ iguana *Cyclura cychlura* (Knapp 2001). However, unlike *C. cychlura* where bigger iguanas mature at an earlier age, individuals of *C. pectinata* at Nizanda did not reach sexual maturity at a younger age than those at Chamela. In fact, the opposite was the case: from the sampled iguanas, those at Chamela seem to reach sexual maturity at ~3 years of age, while those at Nizanda do so at ~4 years of age.

In animals with indeterminate growth, life history is usually correlated with body size (Miles & Dunham 1992). Clutch size shows a positive relationship with
body size in several reptile groups (Congdon & Sels 1993; Tinkle et al. 1993; In den Bosch & Bout 1998; Du et al. 2005). The same pattern has been described in several species of the iguanine group, for example, *Iguana iguana*, *Ctenosaura pectinata*, *Sauromalus obesus* and *Cyclura cyanea* (Fitch & Henderson 1977; Lichtenbelt & Albers 1993; Aguirre-Hidalgo 2002; Knapp et al. 2006). Minimum size at sexual maturity was smaller in Chamela (19.1 cm) than in Nizanda (26 cm). Although it has been suggested that sexual maturity in the chuckwalla iguana (*S. obesus*) depends on size, not age (Tracy 1999), it has also been suggested that chuckwalla populations are connected and thus gene flow between them may prevent the fixation of local characteristics. Similar results have been obtained in *Varanus salvator* (Shine et al. 1998). Unlike these two species, however, the Chamela and Nizanda populations are separated by a distance of ~1,100 km and, although the distribution of the black iguana is assumed to be more or less continuous (see Fig. 1.1); there are many different habitats and barriers to movement along this range. Therefore, gene flow must be severely limited (see also Chapter 4). It is therefore likely that the differences observed are at least partially genetically determined.

2.5.2 Population Structure and Life History

The Nizanda population was dominated by organisms in their fifth year and the maximum longevity was nine years. On the other hand, although the population in Chamela was also dominated by individuals in the fifth category the proportion of the population in this age was lower than in Nizanda. In Chamela, the oldest recorded individual was twelve years old. This means that, other things being equal, iguanas
from Chamela potentially have three or four more reproductive episodes than iguanas from Nizanda. Although our evidence of correlated changes between hunting and life history is only circumstantial, it is generally accepted that exploitation can induce adaptive changes in life history traits (Ernande et al. 2004). Thus, it would appear that hunting in Nizanda may be responsible for an increased mortality in the adult stages and a shorter lifespan. Conclusive evidence would probably require experimental manipulation and/or detailed demographic monitoring over many years.

The time between first and last reproduction has a direct influence on survival and reproductive output and, consequently, on fitness. Age at first reproduction was similar in both populations, but evidently reproductive lifespan was shorter in Nizanda than in Chamela. Although for both populations the fifth age class had a higher frequency, this may be due to the difficulty of observing the youngest stages of the life cycle.

Evidence from other studies suggest that hunting mortality is additive to natural mortality (Gauthier et al. 2001), and this should be reflected in overall lower survival in hunted populations (Calvet & Gauthier 2005). A lower survival/higher mortality was observed at all ages in the Nizanda population compared to the Chamela population (Figs. 2-5 and 2-6). This essentially provides two breeding seasons to the majority of adult individuals in Nizanda before mortality suddenly increases. In Chamela, on the other hand, adult mortality is lower and does not increase substantially until the 10th age class. This gives individuals at Chamela more opportunities to reproduce.
Given that iguanas at Nizanda are subject to higher mortality (by hunting or otherwise), than those at Chamela, we would expect them to reach sexual maturity at a younger age and size (Nobili & Accordi 1997; Guichon et al. 2003). However, age at maturity in Nizanda was slightly larger than in Chamela, while body size went in the opposite direction, iguanas of the same age being larger in Nizanda than in Chamela. There are two reasons why these predictions did not hold in the study populations. First, these relationships are expected in organisms with determinate growth (Charnov 1991), such as mammals (e.g., Harvey & Zammuto 1985; Guichon et al. 2003), but not necessarily in organisms with indeterminate growth (e.g., Franco & Silvertown 1996; Bauwens & Diaz-Uriarte 1997). Secondly, there are other factors that could be influencing optimal development. Organisms not only respond to prevailing environmental conditions, but to their variability or predictability (Stearns 1992; Stearns 2000). Climatic variability at Chamela is known to be high both within and between years (Bullock 1986). If Chamela is more unpredictable than Nizanda, this could have the opposite effect on life history that reduced adult mortality would. A more detailed demographic analysis presented in chapter 5 will further explore these patterns.
Chapter 3

**Experimental estimation of predation of hatchlings and juveniles**
3.1. INTRODUCTION

*Ctenosaura pectinata* is an iguana endemic to Mexico and is threatened with extinction (SEMARNAT 2002). The three main threats are habitat loss, over-exploitation and introduction of domestic and feral fauna (Fitch et al. 1982; Reynoso-Rosales 2000; Alberts 2000). Predation, either from natural enemies or from domesticated, introduced fauna is likely to affect young iguanas more than experienced, older ones. To protect iguanas from this threat, it is necessary to monitor the causes of mortality in hatchlings and juveniles (Fitch et al. 1982).

Census techniques are the usual method to estimate demographic parameters of populations. However, an important assumption of these methods is that all the individuals have the same possibility of being counted (Francis & Cooke 1993). We have observed that studying the population dynamics of the early stages of the life of *C. pectinata* is complicated by the difficulty to observe, handle and monitor them. An alternative method is experimentation through the use of surrogates, similar in size and general appearance to the real organism. Thus, in this chapter, a simple technique to quantify predation of young stages of *C. pectinata* is presented. As will be discussed later, although the results cannot be taken to represent an actual measure of the intensity and impact of predation, the technique is useful to quantify the relative risk of predation in different areas.
3.1.1 Principal Predators of *Ctenosaura pectinata*

The black spiny tailed iguana *C. pectinata* inhabits both undisturbed and perturbed areas in tropical and subtropical areas including areas in close proximity to human settlements (Suazo & Alvarado 1994). Its principal natural predators include various species of reptiles, birds and mammals. Egg predators include the Virginia opossum, *Didelphis virginiana*; coatis, *Nasua* sp; skunks, *Conepatus* sp and *Mephitis* sp, the Mexican python, *Lachesis bicolour*; and the scorpion, *Heloderma horridum*. Hatchling predators are the Lyre snake, *Trimorphodon biscutatus*; the vine snake, *Oxibelis aeneus*; the brown basilisk, *Basiliscus viaticus*; the whiptail, *Cnemidophorus* sp; the great-tailed grackle, *Quiscalus mexicanus*; the roadside hawk, *Buteo magnirostris*; and the roadrunner, *Geococcyx* sp. Predators of juveniles and adults are fewer and include the ocelot, *Felis pardalis*; coyote, *Canis latrans*; domestic dog, *Canis familiaris*; gray fox, *Urocyon cineroargenteus*; and puma, *Puma concolor* (Vandevender 1982; Mora 1987; Suazo & Alvarado 1994; Salvatore-Olivares 2001; Hidalgo-Mihart et al. 2001; De Villa Meza et al. 2002).

3.1.2 Population Stability and Predation Rate in Early Stages of Life.

Studies with mammals, birds and reptiles agree that predation in the early stages of life can be extremely high (Caughley 1966; Frazer 1986; Bijlsma 1990; Iverson 1991; Heppell et al. 1996a; Sarno et al. 1999). Predation in the early stages of life has also been suggested as a factor that may cause high annual variability in population.
dynamics (Gaillard 1998). It is therefore important to obtain detailed information on mortality in these early stages.

In order to estimate predation intensity in hatchlings, previous authors have used predation exclusion (Ferguson & Fox 1984; Janzen et al. 2000a), recorded frequency of injuries (Schoener & Schoener 1980), employed mark-recapture techniques (Civantos et al. 1999; Janzen et al. 2000b), radioisotope markers (O'Brien et al. 1965; Ward 1976), radio-transmitters (Sarno et al. 1999) and the spool-and-line technique (Salvatore-Olivares 2001). An alternative is to use model replicas of prey. This method has been effective in studies of predation in bird’s nests (Greenberg et al. 2002; Mezquida et al. 2004; Thompson & Burhans 2004), reptiles (Castilla & Labra 1998; Stuart-Fox et al. 2003; Webb & Whiting 2005) and fish (Caley & Schluter 2003). One disadvantage of this method is that surrogates do not show antipredator behaviour. Replicas tend to overestimate both the risk of predation and the diversity of real predators (Thompson & Burhans 2004). On the other hand, lack of prey odour may make it difficult for the predator to detect potential prey. A major advantage of replicas is that they are amenable to manipulation, can be used in designed experiments, and it is also possible to obtain a good quantity of data (Major & Kendal 1996) without the problem of separating the effects of predation from those of emigration (See Francis & Cooke 1993).

Because of the difficulty to observe hatchling of *C. pectinata* in the wild, the use of replicas to estimate predation in this species could be useful. Hatchling are bright-green in colour and spend a good part of the day camouflaged in the vegetation.
Also, perhaps as an anti-predator behaviour, hatchlings spend a lot of their time immobile. When disturbed, however, they quickly jump and run. Thus, they are difficult to observe and record in the wild and, when spotted, they are difficult to catch.

Some attempts have been made to estimate predation rate in iguana hatchlings, but it is not clear if those estimations are measures of predation, emigration or both (Vandevender 1982). Also, depending on the structure of the vegetation, young iguanas may stay within a few metres of the hatching site for several days or move away within one day of hatching (Salvatore-Olivares 2001).

The survival of black iguana's hatchlings has been assumed to be similar to that of the green iguana *Iguana iguana* (Suazo & Alvarado 1994). Although these two species are closely related, anatomical and behavioural differences (e.g., green iguana’s hatchling are bigger than those of black iguana; green iguanas have more fidelity to a site than black iguanas) make this assumption questionable (Vandevender 1982).

This chapter describes experimental work employing soap replicas to estimate relative mortality in black iguanas during their first year of life.
3.2. MATERIALS AND METHODS

3.2.1 Study Sites
Due to the arduous, time consuming nature of the study, it could only be conducted in one of the two study sites. We chose Chamela because despite not having the direct human impact that the Nizanda population has; it was possible to compare the incidence of predators within and outside the reserve. Two areas differing in habitat structure, diversity, and, judged from previous observations, abundance of predators were chosen for the experiment. Area 1 was adjacent to, but outside the Chamela Reserve, between the Chamela and Cuitzmala rivers. This area was cleared of natural vegetation some time ago and is currently dominated by mango and papaya plantations, or used for cattle and goat grazing. Area 2 was inside the Chamela Reserve and the vegetation consists of seasonal deciduous tropical forest (Garcia-Oliva et al. 2002). The study was conducted in five periods: July, August and November 2004, and February and April 2005.

3.2.2 Construction of Replicas
Seven preserved newborn individuals and 5 preserved juveniles of the black iguana were embedded in rubber-silicon to construct casting moulds. During initial trials, replicas were created using coloured non-toxic wax, but this became brittle and disintegrated when attacked by a predator. They also tended to melt at the high temperatures encountered in the field, often >30°C. It was therefore decided to experiment with soap. This was easier to shape and withstood higher temperatures than wax (Aguirre-Hidalgo et al. 2005). Hatchling replicas were the size of an average
hatchling, ~59 mm SVL (Aguirre-Hidalgo 2002), whereas juvenile replicas varied between 69 and 90 mm, the size range of juvenile black iguanas (Arcos-García et al. 2001).

3.2.3 Pre-dispersal Mortality

In July 2004, 2370 replicas were displayed in the field. Area 1 contained 1200 replicas and Area 2 included 1170 replicas. Each area was visited every second day. A transect line was laid down in areas where gravid females had been spotted previously. In area 1 (outside of the reserve) 150 replicas, divided in 5 groups or clutches of 30 replicas each, were positioned on the floor at 50m intervals along the transect. This was repeated eight times during a three week period. In area 2 (inside of the reserve) the same procedure was employed with the exception that the last, eighth set only had 120 replicas divided in 4 groups of 30 individuals each. All replicas were displayed for a total of 17-19 hours. Replicas within clutches were situated within an approximate area of one square metre, resembling the pre-dispersal clumping of newborn iguanas. After the exposure period, all replicas were collected and the number lost or with signs of having been attacked was recorded. Because real hatchlings attempt to flee the area during an attack, if more than one replica had wound marks only one replica per group was counted as “killed”. This procedure was meant to avoid over-estimation of predation. It would, however, still tend to overestimate predation because it assumed that one hatchling would be killed whenever an attack occurred. To prevent learning and recognition of artificial iguanas
by predators, different locations (i.e., transects) were used at each of the eight sampling dates.

3.2.4 Post-dispersal Mortality

Potential post-dispersal predation of newborn iguanas was investigated during the months of August and November 2004, and February and April 2005. During these periods, replicas were placed in the same general areas where the pre-dispersal experiment was conducted. In Area 1 the corresponding number of replicas was 347, 470, 487, and 245 respectively. In Area 2 the number of replicas laid down in August, November, February and April were 400, 470, 485 and 265 respectively.

In August, 70 replicas were displayed every second day in each area. These replicas were also exposed to predation for 17-19 hours. For November, February and April, however, 50, 50 and 25 replicas were positioned in each area during a single day and recorded every other day. The main difference with the method used in the pre-dispersal experiment was that in the post-dispersal experiment a single replica was positioned every 25 m along two transects each recording day. In total, 3169 replicas were employed during the post-dispersal experiment.

All replicas were located in plain, unobstructed view at ground level, simulating the position of a basking iguana. Small plastic flags were placed near each replica to help relocating it. Because all flags were identical in colour and size, possible differences between the two areas cannot be attributed to the presence of the flag. After data collection, all flagging and replicas were removed. As in the pre-
dispersal experiment, wound marks were recorded as well as their size and whether the model had been completely broken or went missing. Also with the pre-dispersal experiment, different transects were employed every day to prevent learning and recognition of artificial iguanas by predators.

It was important to use the correct colour of iguanas in all the experiments. They were green during the pre-dispersal experiment and the first three work periods of the post-dispersal experiment. However, the April 2005 replicas were bigger and coloured with dark and light blotches resembling the patterned colour and size of juvenile iguanas (Fig. 3.1).
Figure 3-1 Hatchling and juvenile replicas used in predation experiment. (A) Hatchlings casts, (B) a few of the green hatchlings moulds used in the pre- and post-dispersal experiments, (C) pre-dispersion arrangement of "clutches" in the field, (D) post-dispersal arrangement, (E and F) wound marks in model iguanas, (G) Juvenile mould, (H) Predation marks in juvenile model.
3.3. DATA ANALYSIS

Predation rates were estimated as the proportion of damaged plus missing replicas (one per clutch) per day. All statistical analysis were carried out with STATISTICA (StatSoft 2005). In order to compare the probability that a clutch would be attacked in each of the two areas during the pre-dispersal phase, the binomial event attack vs no-attack was analysed employing Cochran's Q test. In order to have the same number of clutches in the two areas, one of the 40 clutches in area 1 was discarded at random from the analysis. This gave us 39 clutches for each site. A 2x5 (area by time of the year) factorial analysis of variance previous angular transformation of the data (arcsin√x) and a nested analysis of variance (transect within area) were used to compare predation between periods and areas, and between areas and more specific sites within them. Although analyses were performed using angular transformation, the figures are plotted using the untransformed data.

3.3.1 First-year Survival

Pre and post-dispersal mortality were used to develop estimates of survival for the first year of life of newborn iguanas. Mean, maximum and minimum survival was estimated for each of the five periods and these were combined to obtain overall estimations of survival (survival curves) for the first year of life.
3.4. RESULTS

3.4.1 Pre-dispersal death rate.
Predation was prevalent in both areas and most of the groups (clutches) lost at least one individual during the time exposed to predation. Proportion of daily predation before dispersal was higher outside of the reserve (area 1) than inside the reserve (Fig. 3-2). A Cochran's Q test on the binomial data (attack vs no attack of each of 39 clutches in the two areas) indicated significant differences in the probability of attack between the two areas $Q=8.89$, d.f.=1, $p<0.01$, $n=39$. This probability of attack was bigger outside of the reserve than inside it (56.4% and 23.1%, respectively).

3.4.2 Post-dispersal death rate.
Predation was recorded in both areas in four seasons. Unlike pre-dispersal predation, post-dispersal predation was higher inside than outside the reserve. The range of daily predation outside the reserve in summer, autumn, winter and spring was 0.0-0.08; 0.02-0.18; 0.02-0.18 and 0.0-0.16, respectively. Inside the reserve, this range was 0.01-0.10; 0.06-0.46; 0.0-0.26; 0.0-0.24. The season with the highest incidence of predation in both areas was autumn (Fig. 3-2). Autumn was also the season when a significant difference in predation was found between the two areas.
Figure 3-2. Probability of predation (proportion of predated iguanas day$^{-1}$) outside (open triangles and dashed line) and inside the Chamela reserve (circles and continuous line). Vertical bars indicate 95% confidence intervals.

A 2×5 factorial ANOVA used to test differences between sites and seasons revealed a significant main effect of both areas and period. It also revealed a significant interaction between area and season (Table 3-1).
Table 3-1. Factorial analysis of variance among sites and periods (pre-dispersion, Summer, Autumn, Winter and Spring). Data were arcsine transformed before the analysis.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>DF</th>
<th>SS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area</td>
<td>1</td>
<td>0.042</td>
<td>14.804</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Period</td>
<td>4</td>
<td>0.415</td>
<td>35.826</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Area X Period</td>
<td>4</td>
<td>0.076</td>
<td>6.630</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

A nested ANOVA showed significant differences in the probability of predation before dispersion between the two areas and a significant interaction between area and transects (Table 3-2). On the other hand, ANOVA did not show evidence of significant interaction in the probability of post-dispersal predation between areas and transects, but it showed significant differences between the two areas (Table 3-3).

Table 3-2. Analysis of variance of predation rate during the pre-dispersion phase. Data were arcsine transformed before the analysis.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>DF</th>
<th>SS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area(Transcct)</td>
<td>14</td>
<td>0.299</td>
<td>1.937</td>
<td>0.038</td>
</tr>
<tr>
<td>Area</td>
<td>1</td>
<td>0.084</td>
<td>7.621</td>
<td>0.007</td>
</tr>
</tbody>
</table>

51
Table 3-3. Analysis of variance of predation during the post-dispersion phase. Data were arcsine transformed before the analysis.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>DF</th>
<th>SS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area</td>
<td>1</td>
<td>0.108</td>
<td>4.156</td>
<td>0.047</td>
</tr>
<tr>
<td>Area(Transect)</td>
<td>21</td>
<td>0.354</td>
<td>0.648</td>
<td>0.858</td>
</tr>
</tbody>
</table>

3.4.3 Survival

The maximum and average probability of predation gave annual survival values small enough to be considered essentially zero (Fig. 3-3). Only two conditions produced values of survival after one year that would be likely to lead to viable populations, those with minimum mortality either inside or outside of the reserve. Still, the estimated maximum survival of newborns after one year was one order of magnitude bigger outside the reserve than inside it (Fig. 3-3B).
Figure 3-3. Survivorship curves estimated inside and outside the Chamela reserve employing three different estimates of mortality: maximum, mean and minimum daily mortality. Open squares = maximum mortality inside the reserve; crosses = maximum mortality outside reserve, open triangles = mean inside; dots = mean outside; open circles = minimum inside; continuous line = minimum outside. Only the last two curves are likely to produce viable populations. Survivorship curves are shown in arithmetic and logarithmic scales for visual clarity.
3.5 DISCUSSION

An experimental approach to estimate risk of mortality was tested with the spiny-tailed black iguana (*Ctenosaura pectinata*). The experiments conducted suggest that predation of hatchlings is high during their first year of life and that this risk increases during the year. Interestingly, predation was higher in reserve areas than in disturbed areas. Three previous studies have investigated mortality in iguanas: Snell & Tracy's (1985) study of the land iguana (*Conolophus subcristatus*), Abts' (1987) study of the Chuckwalla iguana (*Saurodalis obesus*) and Iverson et al's (2004) investigation of the Allen Cay rock iguana (*Cyclura cychlura*). These studies, however, did not document variation in the risk of mortality due to season or area. Also, although some of their work involves long term records, information on survival in the early stages of the life cycle is scant. The results presented here are therefore the most comprehensive attempt to estimate mortality risk in the first year of life of an iguanine lizard.

3.5.1 Use of Replicas to Estimate Predation.

The evidence obtained from the experiments conducted with replicas of iguanas' hatchlings suggests they were good enough to fool predators. They also had the advantage that in at least some of them it was possible to distinguish the type of predator that was involved. Damage in the form of mammal licks and tooth bites, bird pecks, and crab pincers was observed. These resulted in lost limbs, tails and other portions of the body. When the whole body was mangled, this suggested a large mammal, possibly a carnivore. The use of replicas to estimate predation has an
important disadvantage; replicas do not exhibit anti-predator behaviour/avoidance. Nonetheless, they estimate potential risk of predation (Castilla & Labra 1998). The technique also avoids the problem of confounding predation and immigration, a difficulty that Harris (1982) and Vandevender (1982) could not avoid in their studies.

The method also allowed us to investigate how the risk of predation varies throughout the year. A trend towards increasing predation with a peak in autumn was discerned. It is possible that the increase in predation is caused by changes in vegetation structure. Autumn is the season where vegetation starts to lose the leaves and probably the new born iguanas were less cryptic, leaving them exposed to predators (Alberts 2000). By this time, their body size has only reached between two and ten grams (Arcos-Garcia et al. 2001), which is probably still not sufficient to allow them quick escape from their natural predators. The higher incidence of predation after dispersion in undisturbed areas may be a consequence of a higher abundance and diversity of natural predators, such as birds, foxes, ocelots, coyotes, and a variety of snakes, in the protected area. These predators are less abundant and frequent in modified, mostly agricultural areas and small rural settlements around the reserve.

3.5.2 Pre-dispersal predation.

During the hatchling season, it is frequent to see groups of newborn iguanas perched and basking (Pers. obs.). In green iguanas, it is also common to observe them walking slowly in groups during the course of dispersion (Vandevender 1982; Drummond & Burghardt 1982). The behaviour of the hatchlings of black iguanas is somewhat
similar (Salvatore-Olivares 2001). Salvatore-Olivares (2001) determined that hatchlings of the black iguana disperse slowly during the next few days after emerging from the nest-holes. As a consequence, there is a short period when a high density of newborn iguanas concentrate in a relatively small area, with the possible consequence of attracting the attention of potential predators.

Although hatchlings have been seen basking and walking in disturbed environments, and it is possible to find nest holes of this species on these areas, it is not clear whether these habitats are appropriate for newborn iguanas. Previous works with others iguanas, such as those with the Cyclura group, concur that deforested areas have a big impact on their populations (Alberts 2000).

3.5.3 Post-dispersal predation.

After dispersion, an increase in the probability of predation was observed. However, in contrast to the pre-dispersal phase, predation was higher in the protected area than in the disturbed one. As discussed in section 3.5.1, animals such as ocelot, coyote, grey fox, lyre snake, and vine snake, all of them potential predators of newborn and juvenile iguanas are less frequent in human-dominated areas. This suggests that, unlike the West Indian iguana Cyclura spp (Case et al. 1992) and the Fijian iguana Brachylopus (Iverson 1978; Wiwanda 1982), which are impacted by predation from introduced/domesticated animals, the black iguana benefits from human presence until it reaches bigger sizes. Certainly, in Nizanda black iguanas thrive in disturbed habitats using inaccessible cavities in rural buildings. They can therefore subsist in close proximity with humans. Nonetheless, iguanas can suffer attacks from introduced
species such as rats, cats and dogs. During this study it was not uncommon to see limbless iguanas or with wounds or scars, possibly caused by domestic animals, in areas near human settlements. Despite these observations, and the fact that introduced and domestic animal have been a cause of population decline in others species of iguanas (Alberts & Philips 1994), the results here presented indicate that, overall, predation in the first year of life is higher in undisturbed, protected habitat than in more disturbed, human-influenced areas.
Chapter 4

Genetic Diversity
4.1 INTRODUCTION

Over-hunting of wild populations produces a number of demographic, behavioural and genetic changes with potentially important evolutionary consequences. Apart from its effects on life history, some of which have been discussed in chapter 2, a decrease in population size may lead to a decrease in genetic variability. This is because, by reducing the opportunities for mating among fewer adults, the effective population size is reduced and this, in turn, decreases genetic flow and, in turn, genetic variability (Frankham et al. 2002). It is therefore of interest to investigate whether a decreased genetic diversity is found in hunted populations of the black iguana. In the end, an understanding of the genetic consequences of environmental modification (e.g., disturbance, hunting, etc) should be an integral component of the management and conservation of every species of conservation concern (DeYoung & Honeycutt 2005).

4.1.1. Conservation Genetics

Conservation genetics is an emerging field in conservation biology. Its principal aims are to provide information concerning loss of genetic diversity and to develop strategies that promote species persistence (Moyle et al. 2003). Conservation genetics has proved to be important in conservation and management of wild and/or socioeconomically important species. For instance, using estimations of genetic diversity, such as the variation of alleles and genotypes within populations, it has been possible to reveal genetic problems such as inbreeding depression, loss of genetic diversity and genetic drift. These can potentially reduce the ability of the species to
respond to environmental change, and consequently increase its probability of extinction (Frankham et al. 2002; Freeland 2005). Specifically, modern molecular techniques provide a family of efficient methods to address questions ranging from population genetic variability and dispersal to social patterns within and among populations (Hoelzel 1999; Clark et al. 1999; Diaz et al. 2000; Goldsworthy et al. 2000; Larson et al. 2002b; Hartl et al. 2003; Branch et al. 2003; Martins et al. 2003; Wu & Fang 2005; Huang et al. 2005; Jiang et al. 2005). In this chapter, molecular markers are employed to test whether the hunted population at Nizanda has lower genetic variability that the protected population at Chamela. To this end, the mtDNA marker Cytochrome b (Cyt. b) was used to infer the inter- and intra-population genetic variability in the two localities.

4.2 MATERIALS AND METHODS

4.2.1 Study Areas

The study was undertaken in the two areas, Chamela and Nizanda, already described. At Chamela, iguanas from three areas, differing in habitat structure and diversity were chosen for this part of the study. Two of the areas were located in the communal agricultural areas of Francisco Villa and Emiliano Zapata, at the south, and Chamela, at the north of the reserve; the third one was situated inside the reserve. The areas near the reserve were cleared of natural vegetation some time ago, are dominated by mango and papaya plantations and are also used for cattle and goat grazing. The area
inside the reserve zone consists of seasonal deciduous tropical forest (Lott 1993). Iguanas are not hunted by humans either inside or outside the reserve.

At Nizanda, samples came from two adjacent areas situated at the foot of two hills: “Cerro Verde” and “Cerro de Tilo” and on the boundaries of two intermittent rivers “Arroyo Mazahua” and “Arroyo Chilona”. The natural dominant vegetation consist of seasonal deciduous and sub-deciduous dry forest (Pérez-García et al. 2001). Many areas have been cleared of natural vegetation and are used for the seasonal cultivation of maize, courgettes and beans. There is also secondary vegetation, where fields have been abandoned, dominated by Mesquite (Prosopis sp). Hunters avidly search and hunt gravid iguanas in late spring.

4.2.2 DNA Extraction.
Mitochondrial DNA (mtDNA) marker is an extranuclear DNA enclosed in the mitochondria of cells and maternally inherited (DeYoung & Honeycutt 2005). mtDNA primers have been useful tools in applied and basic ecology studies because they provide unique information related to genetic population characteristics, such as average allele size, effective number of alleles per loci, mean heterozygosis, observed heterozygosis, and proportion of polymorphic loci. These characteristics allow us to make inferences on the adaptability and persistence of populations (Charlesworth & Giesel 1972; Dobson et al. 1992; Vazquez-Dominguez et al. 1999). The technique has been frequently used to assess information on genetic variation, genetic structure, phylogeography patterns, and phylogeny (Passoni et al. 2000; Frantzen et al. 2001; Su
et al. 2001; Feral 2002; Storz et al. 2002; Larson et al. 2002a; Martins et al. 2003; Vigilant & Bradley 2004; Song et al. 2005). These characteristics make mtDNA an ideal molecule for within- and between-species comparisons (DeYoung & Honeycutt 2005). Tail tissues were collected from specimens of both populations and used as a source of mtDNA coding for Cytochrome b (Cyt. b). Samples were fixed separately in 98% ethanol until processed in the laboratory. DNA was extracted by finely slicing approximately a 5 mm section of tissue and incubated overnight at 55°C in 170 μl suspension containing 150 μl Buffer-TGB (0.05 EDTA, 0.1M Tris pH 7.4, 0.5 % SDS), and 20 μl of Proteinase K (20 mg/ml). After incubation, DNA was extracted following the phenol-chloroform protocol of Sambrook et al. (1989), resuspended in 100 μl 0.1x TE buffer and stored at −20°C. In order to test DNA quality and concentration, 5 μl of this DNA was run on agarose gel.

DNA amplification using Cyt. b.

Cyt. b sequences were established for 44 iguanas from Chamela and 45 iguanas from Nizanda. In Chamela 31, 6 and 7 samples were obtained, respectively, from the southern area outside the reserve, from inside the reserve and from the northern area outside the reserve. In Nizanda, as there are no hunting restrictions, all the samples came from the route that hunters commonly used to catch the animals.

DNA was amplified using the Polymerase Chain Reaction (PCR). A pair of universal primers [L14724 5'-CGAAGCTTGATATGAAAAACCATCGTTG-3'] (Irwin et al. 1991) and H15149 5'-
AACTGCAGCCCTCAGAATGATATTTGTCCCTCA-3' (Kocher et al. 1989)]

located in the Cyt. b control region were employed for the PCR reaction. The names of the universal primers are conformed by a capital letter followed by a numerical code. The capital letters L and H make reference to the sequence of light and heavy strands, respectively. Code numbers refer to their position in the human mtDNA (Anderson et al. 1981).

The reaction mix consisted of one microlitre of DNA together with the following reagents: 1x Buffer, 0.5 mg/ml BSA, 0.25 μM DNTPs, 1.5 μM MgCl₂, 0.5 μM of each primer, 1 unit of Taq polymerase. The mix was contained in a total volume of 20 μl and was run in 35 amplification cycles (PCRs). Negative water controls were run in each set of PCR to identify any possible contamination. The parameters used for the PCR were, with a little variation, those followed by Ozawa et al. (1997): one initial denaturing cycle of 94°C for 4 minutes, 35 cycles of denaturing at 94°C for 45 sec, annealing at 51°C for 1:30 min, and extension at 72°C for 2 min. One final extension was carried out at 72°C for 10 min. After the last cycle, samples were incubated at 10°C. PCR products were examined using 1% agarose gel (1x TAE), stained with ethidium bromide and purified using microCLEAN® (microzone).

**DNA sequencing**

Following purification, products were sequenced with 3.2 pM of each primer, 3.5 units of buffer, 1 units of BigDye reaction mix, and 4.5 μl of PCR product. The new thermal cycling parameters consisted of one initial denaturing at 96°C for 60 sec, 25
cycles of denaturing at 96°C for 10 sec, annealing at 52°C for 5 sec, and extension at 60°C for 240 sec. After the last cycle, samples were incubated at 4°C. Sequences were analyzed by an ABI 3100 automated sequencer (Applied Biosystems).

**Sequence analysis**

All sequences were aligned using the program BIOEDIT, Ver. 7.0.5.2 (Hall 1999b) and visually checked. MEGA version 3.1 (Kumar et al. 2004) was used to identify haplotypes. The number of haplotypes, haplotype diversity (h); and nucleotide diversity (π) between populations were obtained using DnaSP version 4.10 (Rozas et al. 2003). Haplotype diversity (h) describes the numbers and frequencies of mitochondrial haplotypes, and is calculated as $h = 1 - \sum_{i=1}^{m} x_i^2$, where $x_i$ is the frequency of haplotype $i$, and $m$ is the number of haplotypes found. A relevant piece of information is the nucleotide dissimilarity between any two sequences. This is estimated by the nucleotide diversity ($\pi$), which quantifies the mean divergence between sequences. Nucleotide diversity is calculated as $\pi = \sum i, j f_i f_j p_{ij}$, where $f_i$ and $f_j$ represent the frequencies of the $i^{th}$ and $j^{th}$ haplotypes in the population, and $p_{ij}$ represents the proportion of the sequence in the sample (Freeland 2005).

Genetic differentiation between populations was estimated using Arlequin version 3.01 (Excoffier et al. 2005). The construction of the reticulated relationship within and among haplotypes was obtained using the median-joining method (Bandelt

4.3 RESULTS

A Cytochrome b fragment of 398 bp was determined in all 89 samples. The analysis identified 17 polymorphic sites, 15 in Chamela’s and 2 in Nizanda, resulting in 8 haplotypes (6 in Chamela and 2 in Nizanda, Table 4-1). The base sequences of all the haplotypes are displayed in Appendix 2.

In Chamela, eleven of the observed mutations were transitions and four were transversions. In Nizanda, the two observed mutations were transitions. The base composition in Chamela was 32.98% C; 25.15% T; 28.17% A and 13.70% G. This composition was very similar in Nizanda (32.74%; 25.76%; 27.82% and 13.89% for C, T, A and G, respectively). The total mean haplotype diversity (h) was estimated to be 0.797, and differences between populations were perceptible (h=0.76 for Chamela and h=0.42 for Nizanda). The index of dissimilarity (Fₛ) indicated differences in genetic (i.e., nucleotide) diversity (Fₛ=0.744, P< 0.05). In terms of distribution and relative frequency, haplotypes were not shared between the two populations. In Chamela, the predominant haplotypes (H₁, H₂ and H₄) were present in 13, 10 and 14 individuals; the less frequent haplotypes, H₃, H₅ and H₆, were present in 4, 2 and 1 individuals, respectively. On the other hand, from the two haplotypes sequenced in Nizanda one of them (H₆) was found in 32 individuals, more than double the
frequency of the other haplotype, which was found in 13 individuals. The relative frequency of the eight haplotypes is displayed in Figure 4-1.

Table 4-1. Genetic diversity of iguanas in Chamela and Nizanda. N: number of individuals sampled, N_hap: number of haplotypes, h: haplotype diversity, π: nucleotide diversity.

<table>
<thead>
<tr>
<th>Study area</th>
<th>N</th>
<th>N_hap</th>
<th>h</th>
<th>π</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chamela</td>
<td>44</td>
<td>6</td>
<td>0.766±0.029</td>
<td>0.014±0.007</td>
</tr>
<tr>
<td>Nizanda</td>
<td>45</td>
<td>2</td>
<td>0.420±0.05</td>
<td>0.002±0.001</td>
</tr>
<tr>
<td>Total</td>
<td>89</td>
<td>8</td>
<td>0.797±0.025</td>
<td>0.020</td>
</tr>
</tbody>
</table>

Figure 4-1. The relative frequency of mtDNA haplotypes in Chamela (solid columns) and Nizanda (dashed columns).
The reticulated network analysis revealed a clear division between the two populations (Fig. 4-2). In Nizanda the dominance of haplotype H7 is apparent. In Chamela, the network analysis showed the formation of two well differentiated clusters. Cluster A1 was shared by 14 individuals and cluster A2 was shared by 30 individuals. Haplotype H2 was found mainly in juvenile individuals inhabiting the closest village (Chamela), situated to the north-west of the reserve. On the other hand, haplotype H3 was found in adult individuals inhabiting protected areas in the Chamela-Cuixmala Reserve, approximately 6 km from the village. The other four haplotypes (H1, H4, H5 and H6) were found in disturbed areas around the Francisco Villa and Emiliano Zapata villages.

Figure 4-2. Network depicting the phylogenetic relationship of Nizanda and Chamela's black iguana haplotypes based on Cytochrome b sequences. (A1) and (A2) Chamela's bases. (B) Nizanda's bases. mv1 and mv2=median vectors. Circle size is proportional to the corresponding haplotype frequency.
The median vectors, the hypothesised sequences that connect the haplotypes with maximum parsimony, suggest a close relationship between the two haplotypes in Nizanda and a more distant relationship between the two clusters in Chamela. The two clusters from Chamela are as separated from each other as they are from Nizanda.

4.4 DISCUSSION

Hunting of wild species frequently involves the alteration of population traits (such as population size, population density, age distribution and dispersal pattern), life history characteristics (such as age and size at reproduction, generation time, number of newborns per reproductive cycle, and longevity), as well as the alteration of social structure and behaviour (Hedrick 1995; Frankham 1996; O'Grady et al. 2004; Comer et al. 2005; DeWoody 2005). These modifications have negative consequences on reproduction, survival and genetic diversity (Hedrick 2000; Spielman et al. 2004; Novaro et al. 2005).

There is evidence that unrestricted hunting/poaching of wild species has biased the frequency of certain phenotypic traits via shifts in the genetic structure of the population (Frankel & Soule 1981). This limits the capacity of the population to respond to environmental changes (Frankel & Soule 1981) and increases the risk of extinction.

In the present study, the analysis of nucleotide sequence of Cytochrome b provided an initial quantification of genetic diversity in the black iguana. Using this
molecular marker, it was possible to quantify the degree of genetic variation within and among populations. Iguanas from Nizanda have likely been hunted since pre-Columbian times and habitats in this area have undergone substantial modification. On the contrary, there is no evidence that iguanas from Chamela have ever suffered sustained hunting by humans; the habitat in this area is remarkably well preserved. This difference in the amount of human impact is likely to have influenced the interactions among individuals and the structuring of the population into demes. This would be expected to result in differential alterations to the genetic composition and variability of the two populations (Sugg et al. 1996; Gaggiotti 2003).

4.4.1 Genetic Variability

A clear difference in genetic haplotype and nucleotide diversity was observed between the populations at Chamela and Nizanda. While six haplotypes were identified in Chamela, only 2 haplotypes were found in Nizanda. Although it would be premature to conclude that differences in hunting pressure are responsible for this difference, evidence from other studies suggests it may have played an important role. Studies of the sea otter, Enhydra lutris, the northern elephant seal, Mirounga angustirostris, and the Guadalupe fur seal, Arctocephalus townsendi, suggest that genetic diversity diminished as a consequence of the reduction in population size following their exploitation (Weber et al. 2000; Larson et al. 2002b; Weber et al. 2004). Although there is no evidence that population size per se has been dramatically affected by hunting at Nizanda (see next chapter), the likely disruption to the social
structure, dispersion ability and, consequently, deme structure would lead to
modification of the genetic structure and composition of the population.

4.4.2 Habitat Disturbance and Human Pressure

Habitat disturbance can modify the social interactions and dispersal behaviour of
animals (Travis et al. 1995; Lacy & Martins 2003; Kuehn et al. 2003; Berry et al.
2005). Because iguanas tend to form social breeding groups with specific mating and
clutching areas (Dugan & Wiewandt 1982), the formation of spatial genetic structure
is also promoted (Bock & Mccracken 1988). Although information regarding changes
in social interactions in the study populations is required, alterations to the pattern of
social interactions attributable to habitat disturbance have been observed in the Cuban
rock iguana, Cyclura nubila nubila (Lacy & Martins 2003). These authors concluded
that groups in disturbed areas were more tightly knit, increasing antagonistic
behaviours among adults compared to undisturbed populations. It is possible that this
could be occurring with the black iguana in Mexico. Apart from the protection from
hunting provided by the reserve, people around the protected area in Chamela do not
show interest in eating or hunting iguanas. It is therefore common to observe adult
iguanas perching and basking on human constructions, or using openings in walls and
buildings as refuges. Human disturbance in Chamela has provided iguanas with
protection from natural predators. This situation discourages mobility and may
promote a more spatially structured distribution of haplotypes. This would account for
both the higher variability observed and its spatial patchiness.
In contrast, we hypothesise that the regular extraction of adults during the breeding season, together with the ability of the black iguana to coexist with humans in disturbed areas in Nizanda, have impoverished the gene pool while promoting higher dispersal ability. This would account for both a reduced number of haplotypes and the dominance of one of the two haplotypes. Corroboration of this conjecture would require a more detailed investigation on the movement of marked individuals. It is also known that dispersal is influenced by vegetation structure (Salvatore-Olivares 2001). In open areas, such as those that dominate in Nizanda, hatchlings quickly disperse moving ≤60 m day⁻¹. In Chamela, however, hatchlings tend to climb trees and are probably able to disperse from tree to tree. On the other hand, adults in both Chamela and Nizanda tend to remain in the same area. It is unlikely that the observed variability misrepresents reality in Nizanda because the total linear distance surveyed was more than five kilometres.

To mitigate the loss of genetic variability observed in Nizanda, a relocation programme could help to revert the negative consequences of inbreeding depression. This, however, would have to be monitored to investigate the success of relocated individuals. In conclusion, this study provides the first indication of a probable loss of genetic variability in a population of the black iguana subject to regular and intense hunting pressure. A wider study covering many populations throughout its distribution range would help to differentiate the effects of habitat from that of hunting and contribute to develop specific conservation strategies guided by genetic evidence.
Chapter 5

Asymptotic population dynamics

and population viability analysis
5.1. INTRODUCTION

The life cycle of an organism follows the sequence of birth, growth, reproduction and death. In this loop, parents are replaced by newborns and, on average, parents contribute with at least one reproductive individual before they die (Aubone 2004). The vicissitudes encountered throughout the life cycle by the average organisms in a population is known as life history. The quantitative analysis of the life history is done employing the tools of demography. Of particular relevance in this study are the differences in the life history of the two study populations, as determined by differences in their population dynamics. Studies of population dynamics result in quantitative estimators of the current status of a population. They help to pinpoint critical stages of the life cycle and allow evaluation of possible fates given different ecological scenarios (Caswell 2001). Both the natural variability in survival and reproduction, and measures of the intensity and timing of harvest (including the proportions and types of individuals harvested) comprise the essential information required to develop management systems that maximize both the persistence and economic return of exploited populations (Olmsted & Alvarez-Buylla 1995; Hunter & Caswell 2005).

Evaluating the sustainability of hunting is of paramount importance for species of economical and cultural value. This is essential to ensure their conservation, guarantee the long term structural stability of their ecosystem and guide the responsible exploitation of natural resources (Redford 1992; Novaro et al. 2000). There are relatively few studies of the population dynamics of harvested species with
which to draw general conclusions on sustainable harvest strategies (Bulte & Horan 2002; Ling & Milner-Gulland 2006). What is clear from these studies, however, is that each species (and the conditions under which it is found) needs to be carefully and independently evaluated. By providing quantitative assessments of the state and likely fate of individual populations, these studies allow us to address and give an approximate answer to the question of the likelihood that the population will go extinct within a foreseeable future. This requires detailed, reliable demographic information. The existence of this information is particularly crucial for long lived species where the effects of hunting may be cumulative and only detectable after many years (Fujiwara & Caswell 2001).

5.1.1 Population Demography

Population demography is based on the estimation of survival and reproduction (Morris & Doak 2002). For species whose survival and reproduction vary with the age or stage of the life cycle of the organism, the tool of choice is matrix models (Caswell 2001). Population projections matrices are relatively simple to produce and yield a number of informative statistics. The basic matrix population model has the form

$$N_{t+1} = AN_t$$

where \( N \) denotes an age/stage column vector, whose elements are the number of individual in each age or stage category at two successive time intervals, \( t \) and \( t+1 \), and \( A \) represents a square population-projection matrix which summarises the average contribution that an individual in each and every age/stage \( j \) makes to each and every other age/stage \( i \) during the projection interval \( t \) to \( t+1 \) (i. e., these contributions are \( A = \{a_{ij}\} \). Following this labelling of the subscripts of each element
of matrix A, for an age-classified life cycle the first subdiagonal of the matrix contains the survivorship probabilities from one year to the next \((a_{i-1,i})\) and the first row contains fecundities. This is called a Leslie matrix after Leslie (1945). Thus, the elements of matrix A contain the fecundity and survivorship records of the average individual as it ages (Caswell 1997; Caswell 2001).

Under the assumption of constancy of vital rates (survival and fecundity) in each age class, the model converges onto \(N_{t+1} = \lambda N_t\), where \(\lambda\) denotes the finite rate of population growth and each and every one of the categories in the population grows at this rate. This equal growth rate for every category means that the population has reached a stable age distribution (SAD; Caswell 1982) where the proportion of individuals in each stage class remains the same over time. Leslie (1945) demonstrated that, mathematically, the finite rate of population increase is equivalent to the dominant eigenvalue of the matrix, and that the stable age distribution is equivalent to its associated right eigenvector (Caswell 2001).

A couple of additional, valuable tools in demography are sensitivity and elasticity analyses (Caswell 1978; De Kroon et al. 1986; De Kroon et al. 2000). These measure, respectively, the absolute and relative effects that a change in each of the elements of the matrix would have on population growth rate. Being a relative measure of the effect that each element of the matrix has on population growth, elasticity allows us to make comparisons on a proportional scale of the effect that changes in different elements of the matrix would make to population growth. Analytically, sensitivity, \(s_{ij}\), and elasticity, \(e_{ij}\), are defined as:
\[ s_{ij} = \frac{\partial \lambda}{\partial a_{ij}} \quad \text{and} \quad e_{ij} = \frac{\partial \lambda}{\partial a_{ij}} \frac{a_{ij}}{\lambda} \]

As Caswell (1978) has shown, the sensitivity of each matrix element can be calculated from the elements of the left \((v)\) and right \((w)\) eigenvectors associated to \(\lambda\) divided by their scalar product:

\[ s_{ij} = \frac{\partial \lambda}{\partial a_{ij}} = \frac{v_i w_j}{\lambda} \]

In consequence, elasticity can be calculated as (de Kroon et al. 1986):

\[ e_{ij} = \frac{\partial \lambda}{\partial a_{ij}} \frac{a_{ij}}{\lambda} = \frac{v_i w_j}{\lambda} \]

The analysis of projection matrices also provides a range of measures of population structure and dynamic behaviour that allow comparisons to be made among populations (Franco & Silvertown 2004).

Although asymptotic analyses are a useful tool to understand the natural tendency of the population, a formal viability assessment can provide a more accurate answer to the question of how deterministic (habitat lost, fragmentation of habitat, over-exploitation, introduction of exotic species and pollution, Caughley 1994) and stochastic (demographic, environmental and genetic, Lande 2002) factors may affect the probability of a population persisting over time (Hubbell & Werner 1979; Akcakaya & Sjögren-Gulve 2000; Rails et al. 2002).
5.1.2 Population Viability Analysis (PVA)

PVA is a collection of analytical or stochastic simulation models that evaluates the probability of population persistence over a specific time interval into the future (Beissinger 2002). It uses estimates of demographic vital rates and other biological information such as age structure, density-dependence, cost of inbreeding depression and environmental stochasticity (catastrophes and bonanzas) to estimate the likelihood that the population would go extinct within a given time interval. PVA has been shown to have good predictive accuracy given reliable data (Brook et al. 1999; Brook et al. 2000; Brook et al. 2002a). This tool has been applied to an ample range of taxa, e.g., reptiles, mammals, birds, arthropods and plants (e.g., Armbruster & Lande 1993; Brook & Kikkawa 1998; South et al. 2000; Armstrong & Ewen 2001; Rivera & Fernandez 2004; Heinsohn et al. 2004; Schtickzelle & Baguette 2004; Haines et al. 2005; Adams et al. 2005; Maschinski et al. 2006). PVA addresses three main issues: 1) evaluation of risk of extinction, success of species reintroduction, or likelihood of species recovery; 2) quantification of the influence of specific life-history traits, life cycle stages, habitat connection among habitat patches, translocation of individuals, climatic changes and environmental stochasticity on population growth rate, and 3) identification of conservation problems, management plans and potential habitats of a species. PVA can also be used to elucidate which information is necessary to clarify these issues, to organise the related data and assumptions about a species or population (Akcakaya & Sjögren-Gulve 2000), to quantify human impact on exploited populations, and to explore the consequences of different assumptions on its predictions (Boyce 1992; Ralls et al. 2002). PVA is currently taking momentum as a
tool to formulate or even implement conservation policy and legislation (Lindenmayer & Possingham 1993; Possingham et al. 1993; Mills 2007).

In this chapter, two types of analysis of the demographic information collected in the field over two annual seasons are described. These are standard asymptotic demographic analyses and Population Viability Analysis. These tools are employed to address the issue of the sustainability of exploitation of the black iguana in Mexico. In particular, the analyses address four questions:

1. What is the likelihood that the exploited population will go extinct?
2. Which are the most sensitive population parameters driving the dynamics of the populations?
3. What magnitude of changes in these parameters would cause population instability?
4. What future risks are the populations likely to confront?
5.2. MATERIALS AND METHODS

5.2.1 Asymptotic Projections

The acquisition of the information employed in this chapter was described in previous chapters. Data on hatchling survival comes from the experiments conducted in Chamela described in chapter 3. It was assumed that the natural mortality that hatchlings would undergo in Nizanda would be similar to that recorded in the modified habitats outside of the Chamela reserve. The information on fecundity and age-dependent survival of juveniles and adults was presented in chapter 2. Overall, information on age structure and survival was derived from 194 adult individuals from Chamela and 182 individuals from Nizanda.

Two types of life tables were obtained. The first one was derived assuming the recorded age structure reflected the age at death (AD) (Deevey 1947). This method uses the total number of dead animals to reconstruct the survival schedule for the population. The total number of dead individuals is considered as the starting size of a cohort, and the number of survivors in each category is calculated by successively subtracting the number of dead individuals from each consecutive age class until the last class is reached (See Neal 2004, pages 225-227). Two important assumptions are implicit in this method: 1) The age of the individuals is measured accurately and 2) There is no bias in the loss of the material used for aging because of different process of natural degradation. This is adequate for the population at Nizanda because here adult iguanas from which the life table was reconstructed were hunted. In comparison, however, iguanas caught in Chamela were freed after collecting toe and tail samples.
(chapter 2). Thus, we are assuming that our sampling reflected both the static structure of the population and the risk of death of the individuals captured in each age category. The second type of life table was constructed using data from the cumulative distribution function (DF) obtained from chapter 2. As specified in that chapter, relative age survival was obtained employing the logarithmic model

\[ n_x = b_0 + b_1 \ln(x) \]; ages 1 and 2 from Nizanda and 1-4 from Chamela were omitted because of small sample size, i.e., these categories were difficult to observe in the field. The estimation of survival in these young age classes was extrapolated from the model fits.

**Construction of transition matrices**

Individuals were grouped in 10 age classes, with hatchlings corresponding to age class zero (newborns) and the last category being nine years old or older. Reproduction occurs in pulses at a specific breeding season (spring) and census of both adults and offspring took place at this time of the year. Thus, calculation of survival and fecundity schedules took place after breeding (Caswell 2001, pp 25-29). The non reproductive age classes were 0-3 for Nizanda and 0-2 for Chamela. In consequence, Nizanda had 6 reproductive age categories (4-9y old). On the other hand, due to small sample in the last 4 age categories in Chamela, the last age category, 9+ contained individuals 9-12 years old. The Chamela transition matrix therefore had 7 reproductive categories (3-9+ y old).
Because four out of six estimations of hatchling survival predicted extinction of the population (see Fig. 3-3), only the two scenarios with minimum mortality (inside and outside the Chamela reserve; upper two curves in Fig. 3-3) were employed in the projections. The matrix projections were repeated with twice the maximum values of seasonal hatchling survival observed extrapolated over an annual interval, as in Fig. 3-3. For Chamela, the maximum and two times the maximum seasonal hatchling survival produced annual survival values of 0.001 and 0.035, respectively. For Nizanda, these values were 0.049 and 0.224. These for values were employed for each of the two survival model types (AD and DF), producing a total of eight scenarios. Mean fecundity values, the entries for the first row of the matrix, were obtained from gravid females maintained in captivity until oviposition (see chapter 2 for details). Because González-Monfil (2002) estimated a 1:1 sexual proportion in hatchlings, the total number of hatchlings was divided by two to estimate the number of female newborns to be used in the matrix analyses.

Transition probabilities between age classes were estimated by calculating the probability of surviving into the next age class from the life table data ($p_{ij} = l_{ij} / l_i$). Because the AD model for both Nizanda and Chamela was constructed from the information on adult reproductive individuals, it was necessary to use the transition probabilities obtained from the DF model (Chapter 2) in some of its entries. For Chamela, these values corresponded to age category 1, while for Nizanda, the values corresponded to the first and second age categories (Table 5-1).
Table 5-1. Age specific survival ($p_x$) and fecundity ($f_x$) values used for Chamela and Nizanda matrices based on the age at death model (AD) and the distribution fit model (DF). In columns for survival ($p_x$) age categories 0-8 correspond to elements in the first subdiagonal ($p_{x+1}$) except for Chamela’s age category 9+ where $p_x$ corresponds to the stasis element in the main diagonal, i. e. $p_{90}$. Fecundity values represent the average number of female hatchlings per adult female in each age category. Projections were conducted employing two scenarios of hatchling survival (transition $a_{10}$): maximum, and two times maximum hatchling survival. For Chamela, these values were: 0.001 and 0.035. For Nizanda, they were: 0.049 and 0.224.

<table>
<thead>
<tr>
<th>Age</th>
<th>Chamela DF</th>
<th>Chamela AD</th>
<th>Nizanda DF</th>
<th>Nizanda AD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$p_x$</td>
<td>$f_x$</td>
<td>$p_x$</td>
<td>$f_x$</td>
</tr>
<tr>
<td>0</td>
<td>$a_{10}$</td>
<td>0</td>
<td>$a_{10}$</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>0.73</td>
<td>0</td>
<td>0.73</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0.78</td>
<td>0</td>
<td>0.88</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0.8</td>
<td>11</td>
<td>0.8</td>
<td>11</td>
</tr>
<tr>
<td>4</td>
<td>0.81</td>
<td>12</td>
<td>0.72</td>
<td>12</td>
</tr>
<tr>
<td>5</td>
<td>0.81</td>
<td>16</td>
<td>0.85</td>
<td>16</td>
</tr>
<tr>
<td>6</td>
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</tr>
<tr>
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</tr>
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<td>8</td>
<td>0.76</td>
<td>19</td>
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<td>19</td>
</tr>
<tr>
<td>9+</td>
<td>0.55</td>
<td>23</td>
<td>0.38</td>
<td>23</td>
</tr>
</tbody>
</table>

Matrix projections

Matrices were projected with the program STAGECOACH (Cochran and Ellner, 1992). This program yields a series of scalars, vectors and matrices useful in population studies, such as eigenvalue and eigenvector spectra, sensitivity and elasticity matrices, and several age-related life history parameters. Because the matrices for Nizanda are strictly age-based (i. e., Leslie matrices), these age-related parameters can be obtained directly from the life table. However, the matrices for Chamela involve stasis in the last category (i. e., a proportion of individuals live beyond the 9th year of age) and STAGECOACH can estimate some of these age-related parameters. The parameters that we use here are:
1. $\lambda_1$, the dominant eigenvalue of each matrix, equivalent to the asymptotic
growth rate of the population described by the matrix.

2. $G$, generation time (the mean age of parents of offspring produced at stable
age distribution); equation 26 in Cochran and Ellner’s (1992) paper.

3. $L$, the total lifespan (the expected age at death for individuals that have already
reached the last age of the life cycle); equation 6 in Cochran and Ellner (1992).

4. $R_n$, the net reproductive rate (the average production of female offspring per
female during her lifetime); this was calculated directly from the life table
rather than by STAGECOACH.

Elasticity analysis was used to evaluate how much a change in survival or
reproduction in a group of age classes would affect the population growth rate ($\dot{N}$).
This was done by adding elasticities within and across particular age-classes. Thus,
the total sum of elasticities in columns for hatchlings (column 0) juveniles (columns
1-2 for Chamela and 1-3 for Nizanda) and adults (the remaining columns) gave an
estimation of the contribution to population growth rate by each of these three age
groups.

STAGECOACH was also used to calculate age-specific survival ($l_i$), fecundity ($m_i$),
and reproductive value ($V_i$) at stable age distribution. The reproductive value is the
expected future contribution to reproduction of individuals that have reached a
particular age. Finally, employing the range of eigenvalues for each matrix (survival
model × hatchling survival scenario), the damping ratio ($\rho$) and the period of
oscillation $P_f$ were calculated. The damping ratio is an estimation of the rate at which
the population would converge to stability. The period of oscillation ($P_i$, where $i$ correspond to the highest possible complex eigenvalue) represents the average duration of an oscillation as the population converges to equilibrium. These two parameters correspond to equations 4.90 and 4.99 of Caswell (2001).

5.2.2 Population Viability Analysis

Population viability analyses were performed using the free computer program VORTEX version 9.50 (Lacy 1993a). VORTEX is a stochastic individual-based population model. It is based on Monte Carlo methods that sample the population distribution and project a population into the future by varying vital rates for each time step. It models the effects of demographic, environmental and genetic variability, as well as periodic catastrophes, (Lacy 1993a). VORTEX can also model sub-populations with complex dispersal patterns and to model harvesting scenarios (Clark et al. 1991; Lacy 1993b; Brook et al. 1999; Lindenmayer et al. 2000; Heinoth et al. 2004).

As AD and DF produced similar survivorship patterns, the PVA was conducted using mortality rate ($q_x = \frac{I_x - I_{x+1}}{I_x}$) from the DF model as core data. All simulations were run for a period of 100 years using as guide the time interval, 50 years, that the World Conservation Union (IUCN) and the Convention of International Trade in Endangered Species of Wild Fauna and Flora (CITES) use before considering a species extinct.
Because Vortex requires an estimate of absolute total density, density was that estimated by Zurita-Carmona et al. (2004) in the municipality of Santos Reyes Nopala, Oaxaca, 230 km west of Nizanda. These authors estimated that population density was 18-101 iguanas km$^{-2}$. Thus, in Nizanda, the maximum number of iguanas inhabiting the approximately 5 km$^2$ that hunters use as poaching area should be about 500. Taking into account that poaching may keep population density below its local carrying capacity, the number of iguanas in the hunted area may be lower than the maximum registered in Nopala. Following this premise, we decided to subtract 180 individuals (the number caught by poachers during the season when hunting was recorded at Nizanda) to the estimated 500 iguanas in 5 km$^2$, and round-off this figure down to 300. This was therefore the initial population size with which the model was run. All the VORTEX models use a carrying capacity of 400 individuals. The same population size and carrying capacity were employed for the simulations corresponding to Chamela. Because hunting scenarios caused extinction in all simulations (see figure 5-4), it was not necessary to run simulations with different parameter values at low densities.

Similarly, because we lack information on critical population size for black iguanas, we used the “only one sex remaining” criterion of VORTEX to define extinction. Tables 5-2 and 5-3 specify the life-history parameters and scenarios used for the different simulations. Black iguanas are polygamous and exhibit male monopolization within a breeding area (pers. obs.). Adult mortality for Chamela (ages 3 to 12) and Nizanda (ages 4 to 9) was set using a quadratic equation fitted to the relationship between age-specific mortality ($q_x$) and age ($x$).
For Chamela the best fit was: $q_x = 0.022x^2 - 0.145x + 0.378 \ (r^2 = 0.89; \ P < 0.05)$.

For Nizanda this was: $q_x = 0.043x^2 - 0.429x + 1.296 \ (r^2 = 0.98; \ P < 0.05)$.

In the supplementation scenario, the minimum number of individuals with which the population remained stable (i.e., $\lambda = 1$) was obtained by adding (doubling) or subtracting (halving) the number of individuals in successive runs. Sensitivity analyses were conducted to evaluate how changes in parameter values affected population persistence. As with equilibrium density, input parameters were modified manually until conditions for population stability were reached.
Table 5-2. Life history parameters used for population viability analysis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Values used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at first reproduction for females</td>
<td>Chamela 3, Nizanda 4</td>
</tr>
<tr>
<td>Age at first reproduction for males</td>
<td>Chamela 3, Nizanda 4</td>
</tr>
<tr>
<td>Maximum (reproductive) age</td>
<td>Chamela 12, Nizanda 9</td>
</tr>
<tr>
<td>Sex ratio at birth(%)</td>
<td>Chamela 50, Nizanda 50</td>
</tr>
<tr>
<td>% adult females breeding</td>
<td>Chamela 100, Nizanda 100</td>
</tr>
<tr>
<td>No. of Hatchlings mean ± st. dev.</td>
<td>Chamela 31.2±11.57, Nizanda 45.64±8.03</td>
</tr>
<tr>
<td>Annual mortality (age 0-1)</td>
<td>Chamela 26.9, Nizanda 30.5</td>
</tr>
<tr>
<td>Annual mortality (age 1-2)</td>
<td>Chamela 21.5, Nizanda 25.6</td>
</tr>
<tr>
<td>Annual mortality (age 2-3)</td>
<td>Chamela 19.5, Nizanda 24.5</td>
</tr>
<tr>
<td>Annual mortality (age 3-4)</td>
<td>Chamela -, Nizanda 25.2</td>
</tr>
<tr>
<td>Annual mortality (adults)</td>
<td>See model inputs in text</td>
</tr>
</tbody>
</table>
Table 5-3. Vortex model input parameters specified for each scenario.

<table>
<thead>
<tr>
<th>Model scenarios</th>
<th>Best</th>
<th>Worst</th>
<th>With supplementation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inbreeding depression</td>
<td>NO</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>Lethal equivalents</td>
<td>NO</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>% Due to recessive alleles</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>Reproduction correlated with survival</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>Polygamous mating system</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>Density-dependence reproduction</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>% Males in breeding pool</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>% Males successfully siring offspring</td>
<td>28.8</td>
<td>28.8</td>
<td>28.8</td>
</tr>
<tr>
<td>Mean # of offspring/Successful sire</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Stable age distribution</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>Initial population size</td>
<td>300</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td>Carrying capacity (K)</td>
<td>400</td>
<td>400</td>
<td>400</td>
</tr>
<tr>
<td>Future changes in K</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>Harvest</td>
<td>NO</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>First year of harvest</td>
<td>-</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Last year of harvest</td>
<td>-</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>Interval between harvests</td>
<td>-</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Optional criterion for harvest</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Population supplementation</td>
<td>NO</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>First year of supplementation</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Last year of supplementation</td>
<td>-</td>
<td>-</td>
<td>100</td>
</tr>
<tr>
<td>Interval between supplementation # of adult females supplemented</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td># of adult males supplemented</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Genetic management</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
</tr>
</tbody>
</table>
5.3. RESULTS

5.3.1 Asymptotic population parameters

The asymptotic projection of the two populations employing models DF and AD predicted viable populations (i.e., $\lambda \geq 1$) in all but one case, that where hatchling survival was maximum employing either the DF or the AD model in Chamela (Table 5-4). Since the population at Chamela does not seem to be in any danger, minimum hatchling mortality must have been overestimated by the predation experiment. Regardless of the two values of hatchling survival assumed, model DF produced higher values of $\lambda$ in Chamela, while model AD produced higher values of $\lambda$ in Nizanda.

Generation time, the mean age of the parents of a cohort (Caswell 2001), was ~6 years for Nizanda and ~7 years for Chamela when population growth rate was positive. For Chamela, however, it increased to more than 8 years when $\lambda < 1$ (Table 5-4). The net reproductive rate ($R_0$), the expected number of offspring produced by an individual during its lifetime (Caswell 2001), increased with $\lambda$ in both populations and models. For a given value of hatchling survival, the DF survival model produced slightly higher $R_0$ values than the AD model in Chamela. The opposite, i.e., higher values of $R_0$ employing the AD survival model, was observed in Nizanda.

The damping ratio ($\rho$), the antilogarithm of the exponential rate of convergence towards the stable stage distribution (Caswell 2001), was consistently higher for the Chamela population than for the one at Nizanda. Thus, the time that it would take for the effect of $\lambda_1$ to be twice that of $\lambda_2$ would vary between 6.9
(minimum $\rho$ for Nizanda) and 1.9 years (maximum $\rho$ for Chamela) (see equation 4.93 in Caswell 2001). When population growth was positive, the period of oscillation was ~7 years in Chamela and ~6 years in Nizanda, irrespective of the model employed. As expected (Caswell 2001), these periods of oscillation were similar to the generation time. On the other hand, when population growth was negative (Chamela with minimum hatchling survival), the period of oscillation was higher (7.5 and 9.1) and was also higher than the corresponding generation time. That is, the population tends to extinction with oscillations exceeding the generation time.

Table 5-4. Population parameters projected by STAGECOACH employing the AD and DF models at two levels of hatchling survival (maximum, and twice this maximum) suggested by the experiments described in chapter 3. Ch. =Chamela; Niz. =Nizanda. DF=Distribution fit model; AD=Age at death model. $R_0$ and $\rho$ are dimensionless.

<table>
<thead>
<tr>
<th>Model and hatchling survival</th>
<th>Finite rate of population increase, $\lambda$ (¥⁻¹)</th>
<th>Generation time (¥)</th>
<th>Net reproductive rate, $R_0$</th>
<th>Damping ratio, $\rho$</th>
<th>Period of oscillation, $P_1$ (¥)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DF max</td>
<td>Ch. 0.69 Niz. 1.04 Ch. 11.45 Niz. 6.56</td>
<td>Ch. 0.04 Niz. 1.27 Ch. 1.21 Niz. 1.17</td>
<td>Ch. 10.88 Niz. 6.56</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AD max</td>
<td>Ch. 0.63 Niz. 1.07 Ch. 8.73 Niz. 6.28</td>
<td>Ch. 0.03 Niz. 1.54 Ch. 1.19 Niz. 1.12</td>
<td>Ch. 9.25 Niz. 6.21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DF2xmax</td>
<td>Ch. 1.04 Niz. 1.32 Ch. 6.91 Niz. 6.17</td>
<td>Ch. 1.31 Niz. 5.80 Ch. 1.37 Niz. 1.15</td>
<td>Ch. 5.08 Niz. 5.98</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AD2xmax</td>
<td>Ch. 1.02 Niz. 1.37 Ch. 6.21 Niz. 5.99</td>
<td>Ch. 1.15 Niz. 7.06 Ch. 1.43 Niz. 1.11</td>
<td>Ch. 7.10 Niz. 5.89</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The elasticity analyses of the DF and AD models (Tables 5-5) show that the stages of the life cycle that would influence population growth the most are the non reproductive categories, i.e., age categories 1-3 for Nizanda and 1-4 for Chamela (Fig. 5-1).
Table 5-5. The elasticity of age-specific survival ($p_s$) and fecundity ($f_s$) for the DF and AD models with maximum and two times maximum hatchling survival. Notation is the same as that employed in Table 5-1. Thus, in the survival column ($p_s$), age categories 0-8 correspond to elements in the first subdiagonal of the matrix ($p_{s+1,s}$) except for category 9+ where $p_s$ corresponds to the stasis element in the main diagonal, i.e. $p_{99}$.

<table>
<thead>
<tr>
<th>Age</th>
<th>Chamela DF</th>
<th>Chamela DF</th>
<th>Chamela AD</th>
<th>Chamela AD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.087</td>
<td>0.145</td>
<td>0.114</td>
<td>0.161</td>
</tr>
<tr>
<td>1</td>
<td>0.087</td>
<td>0.145</td>
<td>0.114</td>
<td>0.161</td>
</tr>
<tr>
<td>2</td>
<td>0.087</td>
<td>0.145</td>
<td>0.114</td>
<td>0.161</td>
</tr>
<tr>
<td>3</td>
<td>0.085</td>
<td>0.002</td>
<td>0.113</td>
<td>0.027</td>
</tr>
<tr>
<td>4</td>
<td>0.082</td>
<td>0.003</td>
<td>0.095</td>
<td>0.023</td>
</tr>
<tr>
<td>5</td>
<td>0.077</td>
<td>0.005</td>
<td>0.072</td>
<td>0.023</td>
</tr>
<tr>
<td>6</td>
<td>0.071</td>
<td>0.006</td>
<td>0.051</td>
<td>0.021</td>
</tr>
<tr>
<td>7</td>
<td>0.064</td>
<td>0.007</td>
<td>0.036</td>
<td>0.015</td>
</tr>
<tr>
<td>8</td>
<td>0.056</td>
<td>0.009</td>
<td>0.024</td>
<td>0.013</td>
</tr>
<tr>
<td>9+</td>
<td>0.054*</td>
<td>0.056</td>
<td>0.007*</td>
<td>0.024</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age</th>
<th>Nizanda DF</th>
<th>Nizanda DF</th>
<th>Nizanda AD</th>
<th>Nizanda AD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.152</td>
<td>0.162</td>
<td>0.159</td>
<td>0.167</td>
</tr>
<tr>
<td>1</td>
<td>0.152</td>
<td>0.162</td>
<td>0.159</td>
<td>0.167</td>
</tr>
<tr>
<td>2</td>
<td>0.152</td>
<td>0.162</td>
<td>0.159</td>
<td>0.167</td>
</tr>
<tr>
<td>3</td>
<td>0.152</td>
<td>0.162</td>
<td>0.159</td>
<td>0.167</td>
</tr>
<tr>
<td>4</td>
<td>0.112</td>
<td>0.0841</td>
<td>0.102</td>
<td>0.060</td>
</tr>
<tr>
<td>5</td>
<td>0.069</td>
<td>0.043</td>
<td>0.052</td>
<td>0.050</td>
</tr>
<tr>
<td>6</td>
<td>0.037</td>
<td>0.031</td>
<td>0.024</td>
<td>0.028</td>
</tr>
<tr>
<td>7</td>
<td>0.015</td>
<td>0.022</td>
<td>0.008</td>
<td>0.016</td>
</tr>
<tr>
<td>8</td>
<td>0.004</td>
<td>0.011</td>
<td>0.002</td>
<td>0.006</td>
</tr>
<tr>
<td>9</td>
<td>0.004</td>
<td>0.002</td>
<td>0.001</td>
<td>0.000</td>
</tr>
</tbody>
</table>

*The values of $p_s$ reported for the last category in Chamela (9+) correspond to those projected divided by four, which is the number of ages in this category (9-12y). This allows direct comparison with the other categories whose duration is one year.
Figure 5-1. Relative contribution of each age category to changes in population growth at Chamela (A) and Nizada (B) under four different scenarios. For explanation of scenarios, see text. Dashed line=maximum survival estimation. Solid line=two times maximum survival estimation. Open square marks=DF model. Asterisk marks=AD model. Although elasticities values do not represent a continuous function, they are shown as linear plots for visual clarity.
5.3.2 Projected stable vital rates

Projected survivorship was similar for both populations and models throughout most of the life cycle when assuming maximum and two times maximum hatchling survival (Figure 5-2). The two populations, however, differed in survival in the early and late stages of the life cycle, with Nizanda maintaining higher hatchling survival and lower adult survival than Chamela. Despite these differences, all curves resembled a Deevey type 3 curve with a sharp drop during this first stage of the life cycle, followed by a Deevey type 2 curve with relatively constant mortality at intermediate ages, and a Deevey type 1 curve towards the end of life. The AD model consistently showed lower survival at older ages than the DF model.

The reproductive value when hatchling survival was maximal had a maximum at two (Chamela) or five years of age (Nizanda) (Fig. 5-3A), regardless of the adult survival model employed (AD or DF). When hatchling survival was assumed to be twice the maximum, reproductive value peaked at 5-6 years of age (Fig. 5-3B).
Figure 5-2. Survivorship curves at stable stage distribution assuming maximum (A), and two times the maximum (B), hatchling survival in two populations of the black iguana employing two different models of juvenile-adult survival. AD and DF (see text). Solid line = AD model, dashed line = DF model. Open circles = Nizanda, open triangles = Chamela.
Figure 5-3. Reproductive value at stable stage distribution (\( J^t \)) assuming maximum (A), and two times the maximum hatchling survival (B) in two populations of the black iguana employing two different models of juvenile-adult survival, AD and DF (see text). Solid line = AD model, dashed line = DF model. Open triangles = Chamela, open circles = Nizanda.
5.3.3 Population Viability Analysis

The best scenario (without hunting) shows a growing population in both areas. On the other hand, when half of the population was removed every year (worst scenario), both populations became extinct within 3 and 6 years. The supplementation scenario revealed that extinction risk can be avoided, although at low densities, with as little as two new reproductive individuals (one male and one female) being added after hunting (Fig. 5-4).

Modifying input parameters from the best scenario input through the sensitivity analyses option of VORTEX (Table 5-6), it was observed that: 1) The minimum population size in Nizanda needs to be bigger than that in Chamela. 2) In both populations hatchling mortality needed to be as high as 98% for the populations not to become unstable. 3) In both populations the non reproductive juvenile categories (but not hatchlings) can withstand mortality rates almost as high as those that hatchlings would seem to be able to support before extinction of at least one of the 100 simulated populations occurs. 4) In order to avoid extinction, the maximum number of hunted individuals in Nizanda must be <24 adult females and <26 adult males (i.e., 50 individuals in total). 5) Current hunting levels can only be maintained if a minimum of 4 females and 1 male migrate into the hunted area. However, if hunting were occurring in Chamela with the same intensity as in Nizanda, a minimum immigration of 7 adult females and 1 adult male would be required to prevent the population from going extinct.
Figure 5-4. Predicted population size at Nizanda (solid line) and Chamela (dashed line) (A) Without harvesting, (B) With harvesting but no supplementation, and (C) With harvesting and immigration of one adult male and one adult female per year.
Table 5-6. Vortex sensitivity analysis – the minimum conditions required to keep the populations stable.

<table>
<thead>
<tr>
<th>Scenarios</th>
<th>Chamela</th>
<th>Nizanda</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum population size</td>
<td>8</td>
<td>14</td>
</tr>
<tr>
<td>Maximum hatchling mortality %</td>
<td>98</td>
<td>98</td>
</tr>
<tr>
<td>Maximum mortality %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-2 age category</td>
<td>97</td>
<td>97</td>
</tr>
<tr>
<td>2-3 age category</td>
<td>97</td>
<td>96</td>
</tr>
<tr>
<td>3-4 age category</td>
<td>95</td>
<td>-</td>
</tr>
<tr>
<td>Maximum hunted adult females</td>
<td>44</td>
<td>24</td>
</tr>
<tr>
<td>Maximum hunted adult males</td>
<td>54</td>
<td>26</td>
</tr>
<tr>
<td>Minimum supplemented individuals with current hunting</td>
<td>7♀</td>
<td>4♀</td>
</tr>
<tr>
<td></td>
<td>1♂</td>
<td>1♂</td>
</tr>
</tbody>
</table>
5.4. DISCUSSION

Growing evidence indicates that many reptile species are in decline as a consequence of habitat loss and degradation, introduced species, pollution, overhunting and climate change (Gibbons et al. 2000). Black iguanas have been hunted for hundreds, perhaps even thousands of years and there is probably some level of resilience that their populations have to this source of mortality. With the increase of the rural population, however, there is concern that this practice may become unsustainable. Unlike other species, such as ungulates, whose hunting began to be regulated in the 1970s (Gross 1969; Walters & Gross 1972), iguanas are not protected by law. The regular harvesting experienced by the black iguana in Nizanda, where a number of hunters maintain a constant pressure during the oviposition period, simulates the kind of exploitation known as constant-effort harvesting (Stephens et al. 2002). According to these authors, this type of harvest is more commonly associated with high probabilities of extinction, particularly if no corrective measures are implemented.

Interestingly, it would seem that disturbance has had beneficial effects in Nizanda by eliminating the natural predators of iguanas, thereby increasing hatchling and juvenile survival.

5.4.1 Asymptotic Population Dynamics

The results of this study indicate that both study populations are growing or stable, and that only exceedingly high levels of hatchling mortality would threaten their immediate future. This is true, whatever model of survival is employed, AD or DF.
The most sensitive stages of the life cycle were the four or five initial ages, regardless of site, survival model and level of hatchling mortality assumed. Iguanas in these early stages are difficult to observe and record in the field. It is therefore likely that their elusiveness may be responsible for maintaining the populations despite increased mortality in later stages from natural predation (in Chamela) and hunting (in Nizanda). Similar results were obtained in sea turtles by Heppell et al. (2000) and they recommended protection of these stages from fishing.

Survival was similar in both populations with mortality being higher at either end of the life cycle (Fig. 5-2) and suggesting the onset of senescence (Rose 1991). On the other hand, although varying with mortality level, survival model and site, the reproductive value peaked at intermediate ages (Fig. 5-3). The differences observed in the reproductive value curves are essentially determined by differences in the patterns of survival.

5.4.2 PVA.

Although elasticity analyses provide information on how \( z \) would change if individual coefficients of the matrix were modified, they cannot be extrapolated to situations where population density is substantially modified, as is the case with hunting (Enright et al. 1995; De Kroon, Van Groenendaal, & Ehrln 2000). In this case, numerical simulations using PVA can help better understand the population consequences of additional mortality.

Despite the regular, sustained exploitation that black iguanas have suffered, the results from the PVA indicate low probabilities of population extinction. This
suggests that the relatively high fecundity and apparent high resilience to habitat
disturbance, which tends to increase hatchling and juvenile survival, play important
roles in the recovery of the population. Another important, difficult to measure event
is immigration. PVA suggested that a few immigrants can help to dilute the effect of
the loss of reproductive individuals due to hunting. This would require the existence
of protected populations outside the hunted area to maintain a constant influx of
individuals. The evidence of erosion of genetic diversity (chapter 4) suggests
immigration may not be occurring at a sufficiently high rate in Nizanda. This limits
our ability to categorically assert whether the current levels of hunting of black
iguanas in Nizanda are sustainable. Continuous monitoring of the populations and a
wider study of genetic diversity across the distribution range of the species is
necessary. Although with higher genetic diversity than the population at Nizanda, the
evidence of spatially structured demes in Chamela suggests that the social structure of
black iguanas, with polygamous, territorial males may further contribute to reduce
genetic diversity in heavily hunted areas. The PVA also indicate that current hunting
levels would be likely to cause local extinction in a closed population. The fact that
iguanas have not been extinguished from the hunted area suggest that either local
recruitment was underestimated or immigration is occurring at a rate which is
currently numerically sufficient to keep population numbers reasonably stable.
Whatever its current value is, immigration would be facilitated if exclusion areas were
established around the hunting areas (e.g., Novaro et al. 2000).

An important aspect that requires detailed investigation is the dispersal ability
of different stages of the life cycle. Apart from Salvatore-Olivares' (2001) work with
hatchlings, there is no information on dispersal rates of juveniles and adults. The PVA indicated that a dispersion of 4 adult females and 1 adult male for the hunted population and 7 females and 1 male for the unhunted one would be sufficient to maintain the populations at sizes similar to those they currently have. This is in agreement with Pulliam (1988) suggestion that low levels of dispersal may be sufficient to maintain populations numerically stable. Studies of hunted populations would therefore benefit from taking into account the spatial context in which both hunting and dispersal occur.

Despite some gaps in our understanding of the population dynamics of the black iguana, this work has made it possible to infer that they are able, or at least have been able so far, to sustain substantial modifications to both their habitat and their population parameters. However, given their reduced genetic diversity and the relentless exploitation that they will continue to suffer, the creation of exclusion zones, the management of the habitat to favour migration between populations, and perhaps also the translocation of iguanas between different areas are some of the management practices that could help guarantee viable populations.
Chapter 6

General discussion
Wildlife resources have traditionally been and still are an important component of the diet of, and a source of income for, many people in the world, and it would be ideal if these resources were to be managed sustainably (Roth & Merz 1996; Chardonnet et al. 2002). However, increased levels of exploitation are currently the cause of population decline and even extinction of many species (Mills 2007). A now infamous example is that of the passenger pigeon. This species, which was once described as "the most abundant gregarious species ever known in any land" (Forbush 1913), with estimates of 1-5 billion individuals, was hunted to extinction throughout the nineteenth century. By the time it was realized the species was in trouble, there was nothing that could be done to prevent the collapse of the small remaining populations. Another once common species driven near the point of extinction were the plain and the wood bisons (Bison bison bison and B. b. athabascae; Hornaday 1889). Although not necessarily representative of the majority of species, these examples demonstrate that large population size does not constitute a guarantee against extinction in the wild from over-exploitation.

The exact mechanisms determining the minimum viable population size (defined, in reality, not as a fixed threshold number, but as population density with an associated probability of extinction within a certain timeframe) vary from species to species. Despite this uncertainty, or perhaps precisely because of this uncertainty, it seems clear that in order to quantify the probability that a population would go extinct under a set of conditions (e.g., hunting intensity), a quantification of these conditions and their effect on population growth and stability is necessary. Essential information
includes, but is not limited to, population density over consecutive time intervals, geographic distribution and local distribution patterns, life history traits, feeding ecology, behaviour and social structure, patterns of population connectivity among populations, and predator-prey interactions. In addition to recording some of this basic information, when attempting to conserve and manage exploited populations it is also necessary to quantify the magnitude and frequency of harvest, the extent to which wildlife responds to these pressures, both in ecological and evolutionary terms, how wildlife exploitation is integrated into the local culture of the people, and the technological development on the area were hunting is occurring (Njiforti 1996; Roth & Merz 1996; Sarno et al. 1999; Linklater 2003; Mangel et al. 2006; Mills 2007).

This is obviously a monumental task. In practice, given the limitation of time and resources, plans for the conservation and management of hunted or threatened species more often have to be done with incomplete information, or guided by the experiences of previous studies.

Many reptiles, including turtles, tortoises, crocodiles, snakes and lizards, undergo what seems, on the face of their declining numbers, excessive exploitation. The common life history characteristics of these species are large size, relatively long life spans, delayed sexual maturity and large clutch size (Fitzgerald 1994a). The products usually obtained from reptiles are meat and skin (Fitch et al. 1982; Fitzgerald et al. 1994; Fitzgerald 1994b; Klemens & Thorbjarnarson 1995; Shine et al. 1998; Shine et al. 1999; Ottenwalder et al. 2000; Fitzgerald & Painter 2000; Minor-Sánchez 2006), and unless substitute products are found or developed, these reptiles are
unlikely to see their fate improved. On the other hand, it may be possible to implement sustainable management programme based on a detailed understanding of their populations’ ability to withstand harvesting. For example, because of its long lifespan, the tegu lizard seems to be able to withstand years of poor recruitment in the face of high adult mortality (Fitzgerald 1994a).

In Latin America, iguanid species are exploited for human consumption. The most common exploited species include the green iguana (*Iguana iguana*), the brown iguana (*Ctenosaura similis*), and the black iguana (*Ctenosaura pectinata*) (Fitch et al. 1982; Harris 1982; Muñoz et al. 2003). In some countries, eggs are the main product and can fetch twice the price of chicken eggs by weight. On the contrary, iguana meat does not sell well because it is considered of low quality (Harris 1982). This perception, however, varies from place to place. Thus, in the southern region of Mexico, meat and eggs of *C. pectinata* are equally consumed and in constant demand (Reynoso-Rosales 2000). Because in these regions iguanas are abundant, local people do not see the need to develop conservation and management plans (e.g. Mooney & Sala 1993). Trading of this reptile now occurs not only at a local level, but has extended to trading with other communities. This means that iguanas are nowadays also transported and sold in big regional markets. The hunting of adult iguanas has increased considerably in the past few years (pers. obs.) and the sustainability of the practice needs to be investigated. Farming of iguanas has not been developed and, when attempted, it has not been commercially successful. If wild populations are to continue to be demographically viable, it may be necessary to increase the likelihood
of population stabilisation through the optimisation of their life history parameters (Singh & Kaumanns 2005).

For all these reasons, and given the current situation of the black iguana in the Mexican countryside, the present work attempted to quantify the basic elements of the population dynamics and genetic variability of one hunted and one protected population. This information was then used to investigate, by means of computer simulations the ability of the populations to sustain different levels of harvest. Specifically, five different aspects were investigated: reproductive traits, population dynamics, genetic diversity, asymptotic population dynamics and population viability analysis.

6.1. Reproductive Traits

There was a clear difference in body size between the iguanas of the hunted and non-hunted populations. The hunted population tended to produce larger reproductive individuals with a bigger clutch size per reproductive event than individuals from the non-hunted population. This suggests that growth rate is connected with habitat factors such as food availability, climatic conditions, and perhaps even social interactions (Stearns & Koella 1986; Werner 1987; Niewiarowski & Roosenburg 1993; Reznick & Bryant 2007). Limited food availability is an important cause of reduction of growth rate, clutch size and reproductive effort in the tree lizard *Urosaurus ornatus* and in the black iguana *C. pectinata* (Ballinger 1977; Arcos-Garcia et al. 2001). However, age at
maturity was similar for both populations, suggesting limited plasticity in the age at
sexual maturity in this species.

6.2. Population Structure

Population structure is important because it is related to the life cycle and because
each age/stage may be affected differently by environment perturbations (Cameron &
Benton 2004; Benton et al. 2006). In this study, hunted and non-hunted populations
showed differences in population structure. The hunted population had shorter
longevity and, therefore, fewer reproductive age classes than the non-hunted
population. The shorter longevity at the hunted site may be due to the hunters’
preference for large adult iguanas (Aguirre-Hidalgo 2002). This higher mortality in
the adult stages of the hunted population imposes a selective pressure on life history.
It is therefore possible that the observed differences in longevity between the two
populations may be at least partially genetically determined.

6.3. Hatchling Survival

After dispersal, hatchlings and juveniles are difficult to observe in the field, yet their
survival is an essential piece of information in demographic studies. This study
constitutes the first attempt to quantify the risk of death from predation during the
iguanas’ first year of life. Risk of predation is the result of three main factors: 1) the
time the individual is vulnerable to attack, 2) the probability of encounter with
potential predators and 3) the probability of escaping an attack (Lima & Dill 1990).
The relative importance of these three factors is a consequence of landscape conditions and predator abundance and behaviours (Werner & Gilliam 1984). Using hatchling models as surrogates tends to over-estimate predation because the models do not show anti-predator behaviour. Despite this limitation, the results can help to define the time of the year and areas where hatchlings are more vulnerable to predators. The experiments confirm that predation of hatchlings varies with season, possibly as a consequence of the changing habitat structure. Predation was higher in disturbed areas before hatchlings disperse. On the contrary, predation was higher in undisturbed habitat after hatchling dispersal. These results highlight the changing incidence of predation in time and space.

6.4. Genetic Diversity

The result of this part of the investigation provided evidence of differences in haplotype and nucleotide diversity between the two study sites, with minor genetic variability in hunted population and the possible structuring in sub-populations (demes), particularly in the protected area. Dominance by one of the two haplotypes found in the hunted area was also evident. The hunted population is at higher risk of suffering genetic bottlenecks.

6.5. Asymptotic Analyses

Despite the apparently more precarious status of the hunted population, the majority of the asymptotic projections indicated growing or stable populations. The only
exception was that of the protected populations if hatchling survival was low. Thus, these analyses provide no indication of impending peril for either population. Since hatchlings are not targeted by hunters, and they also have reduced mortality from natural predators at the unprotected site, their survival at this site guarantees a continuous replenishment of the population.

6.6. PVA Models

Population viability analyses confirmed the findings of the asymptotic analyses of a low probability of extinction of the two populations. PVA suggested that the unprotected, hunted area may be acting as a population sink, in which migration from adjacent areas may be preventing extinction. Detailed studies of the movement of individual iguanas throughout their life, both in the hunted zone and in adjacent areas, would help to elucidate their ability to replenish the more heavily impacted area. More detailed studies employing molecular markers would also help to answer this question.

An important limitation of the PVAs conducted was that the period for which demographic records were made did not allow quantification of temporal environmental and demographic variability. They therefore implicitly assume that this variability is relatively unimportant. This variability, however, needs to be quantified in future studies.
6.7. Further Work

Programs such as RAMAS GIS are opening the possibility of linking PVA with landscape data, giving us the opportunity to investigate the population dynamics of individual species in a heterogeneous spatial context. Investigating and modelling the metapopulation dynamics of the black iguanas would allow more precise estimates of the contribution of migration to population growth. Obtaining the necessary field data to calibrate these models represents a big task, but this task must be accomplished if conservation plans are to contribute to the development of effective conservation actions. In addition to this need for detailed field data, the lack of a clear understanding of the taxonomic relationships in the iguanine group limits our ability to extrapolate results from one population or species to another. More precise quantification of the levels of human-induced mortality over a larger area/higher number of populations would help gauge the true impact of hunting.

As with the Bahamian rock iguana, Cyclura cychura (Hayes et al. 2004), the population differences observed in the black iguana seem to be determined by a combination of environmental adaptations and demographic forces. Studies of animal development may help to disentangle their relative effects. Some of these investigations could be conducted in collaboration with the emerging iguana producers.

Population differences in phenotypic traits may also be accounted for by differences in food availability and feeding habits. Although C. pecintata is
categorized as insectivore in the juvenile stage, but herbivore in the adult stage (Durtsche 2000), information on the seasonal variation of their diet is lacking. Stable isotopes techniques can help to elucidate seasonal variation in diet because often isotopes reflect average diet more accurately than traditional ("one-off meal") dietary studies (Deniro & Epstein 1981). Ecological studies of black iguanas should consider the temporal and spatial utilization of food, the foraging strategies employed, foraging time, and food quality in disturbed and undisturbed areas (Fuller & Sievert 2001). This type of information can be valuable for successful habitat and population management (Eberhardt 2002), particularly in disturbed habitats.

Because *C. pectinata* is a territorial animal with a well established hierarchical (social and mating) structure (Evans 1971), it is necessary to carry out behavioural studies to identify the effects of local habitat conditions on behaviour, particularly in relation to the iguanas' ability to occupy and exploit both the modified and the surrounding habitat.

Lack of microsatellite markers for this species limited our ability to genetically identify individuals. Further work on microsatellites markers is needed to help to determine aspects of reproductive behaviour, and to quantify inbreeding depression and dispersal rates (Freeland 2005). Studies of population genetics would help to identify if the species requires active management in the form of relocation, repatriation or translocation (Dodd & Seigel 1991).
Market-based incentives for sustainable harvesting must balance a number of often competing forces. They must protect the interests of current and future generations of humans, as well as the biological potential of the resource. The conservation and management of *C. pectinata* must therefore take into account the interest of local communities because strict protection and regulation are frequently not efficient options (Oldfield 2003). The key point is to move toward systems of use that do not cause irrecoverable population damage (i.e., over a given period of time). Social studies can be very useful in this respect because they can give local people the opportunity to participate in planning the levels and areas of hunting and define the feasibility of environmental projects. Understandably, social (traditions, culture, perception, attitudes, intentions, choice, social values, needs, etc), economic (production, consumption) and ecological factors need to be combined to reach consensus (Caddy & Seijo 2005; Bulte & Damania 2005; Campbell 2005).
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Demography and genetic diversity of the Mexican black iguana *Ctenosaura pectinata*.
Victor Aguirre-Hidalgo

*Appendices*
APPENDIX 1

Histological Protocol

After decalcification tissues were placed in labelled plastic cassettes and dehydrated following the next process.

- Dehydrated protocol.
  1. 10% Formalin, 1 hr
  2. 90% alcohol, 1 hr
  3. 96% alcohol, 1½ hr (two times)
  4. 100% alcohol, 1½ hr (three times)
  5. 1:1 Alcohol-Xylene 2 hr
  6. Xylene 2 hr (two times)
  7. Molten wax 2 hr, 60-65°C (two times)

- Embedding.
  The embedding process was carried out with a Tissue Tek embedding centre using stainless steel cassette moulds. The plastic labelled cassettes were incorporated into the finished block. Finally, blocks were cooled and removed from the mould once the wax was set hard.

- Cross-section
  6 µm sections were cut from the blocks using a rotary microtome, placed into a warm water bath, lifted out into a glass microscope slide, and allowed to dry on a hot plate set just below the melting point of the wax. Once dried the sections were stained with the haematoxylin-eosin method.

- Haematoxylin-Eosin Staining Method
  - Deparaffination
    1. Xylene I, 20 min
    2. Xylene II, 20 min
    3. 1:1 Alcohol: Xylene, 20 min
4. 100% Alcohol, 15 min
5. 96% Alcohol, 15 min
6. Water rinse (three times)

- **Staining**
  1. Harris’ haematoxylin 10 min
  2. Wash in running tap water
  3. Rinse 1% HCl-Alcohol 10 sec.
  4. Water rinse (three times)
  5. water-Li₂CO₃ (saturated solution) 30 sec
  6. Washing with running tap water 10 min
  7. Eosin solution 20 sec

- **Dehydration and immersion**
  1. 96% Alcohol 20 sec
  2. 100% Alcohol 20 sec
  3. Alcohol-Xylene 20 sec
  4. Xylene 20 sec (two times)

Mount of section in Canada balsam with a micro cover glass.
APPENDIX 2

Haplotype list. Haplotypes 1 to 6 are from Chamela. Haplotypes 7 and 8 are from Nizanda

Haplotype 1

\[\text{ACCCAAACCCTAAAATAATCAATAACCTTATGCAGGCTTACCAACACCCCCTATACTTCGAGATGAAACCTCGGCTCACTACTAGGACTCTGGCTAAT} \]
\[\text{CATCGAGATGGAACCTCGGCTCACTACTAGGACTCTGGCTAAT} \]
\[\text{GAAATGAAACCTCGGAGTATTTCTCTACTACTACGTAGGCTACGTACTACCTGAGGACAAATATCATTTC} \]

Haplotype 2

\[\text{ACCCAAACCCTAAAATAATCAATAACCTTATGCAGGCTTACCAACACCCCCTATACTTCGAGATGAAACCTCGGCTCACTACTAGGACTCTGGCTAAT} \]
\[\text{CATCGAGATGGAACCTCGGCTCACTACTAGGACTCTGGCTAAT} \]
\[\text{GAAATGAAACCTCGGAGTATTTCTCTACTACTACGTAGGCTACGTACTACCTGAGGACAAATATCATTTC} \]

Haplotype 3

\[\text{ACCCAAACCCTAAAATAATCAATAACCTTATGCAGGCTTACCAACACCCCCTATACTTCGAGATGAAACCTCGGCTCACTACTAGGACTCTGGCTAAT} \]
\[\text{CATCGAGATGGAACCTCGGCTCACTACTAGGACTCTGGCTAAT} \]
\[\text{GAAATGAAACCTCGGAGTATTTCTCTACTACTACGTAGGCTACGTACTACCTGAGGACAAATATCATTTC} \]

Haplotype 4

\[\text{ACCCAAACCCTAAAATAATCAATAACCTTATGCAGGCTTACCAACACCCCCTATACTTCGAGATGAAACCTCGGCTCACTACTAGGACTCTGGCTAAT} \]
\[\text{CATCGAGATGGAACCTCGGCTCACTACTAGGACTCTGGCTAAT} \]
\[\text{GAAATGAAACCTCGGAGTATTTCTCTACTACTACGTAGGCTACGTACTACCTGAGGACAAATATCATTTC} \]
Haplotype 5
ACCCAAATCCAAAAATTATCAAAATACCTACATTTCATCGAACCCTACCCACCCCTCTTAAATCTCTGCAATGAAACTTCGCTCAGTTCTAGGACTCTGCTATTATCTCTCCGCATGATGAAACTTCGGCTCACTACTAGGACTCTGCTATTAAT

Haplotype 6
ACCCAAATCCTAAAATTATCAAAATACCTACATTTCATCGAACCCTACCCACCCCTCTAATATCTCTGCAATGAAACTTCGCTCAGTTCTAGGACTCTGCTATTATCTCTCCGCATGATGAAACTTCGGCTCACTACTAGGACTCTGCTATTAT

Haplotype 7
ACCCCAATCCTGAAAAATTATCAAAATACCTACATTTCATCGAACCCTACCCACCCCTCTAATATCTCTGCAATGAAACTTCGCTCAGTTCTAGGACTCTGCTATTATCTCTCCGCATGATGAAACTTCGGCTCACTACTAGGACTCTGCTATTAT

Haplotype 8
ACCCGAATCCTGAAAAATTATCAAAATACCTACATTTCATCGAACCCTACCCACCCCTCTAATATCTCTGCAATGAAACTTCGCTCAGTTCTAGGACTCTGCTATTATCTCTCCGCATGATGAAACTTCGGCTCACTACTAGGACTCTGCTATTAT