CHAPTER 3

MATERIALS AND METHODS

BIO-INVENTORY STUDIES AND REPTILIAN COMMUNITY STRUCTURE

Field Studies: The present study was conducted during 2006-2008 covering all the seasons. Various methods and techniques were employed for recording the reptilian diversity and sampling their population through time and space. Visual Encounter Survey (VES) was primarily used to prepare a checklist for the intensive study areas (ISA) and further expand the same on a broad scale (state level). Transects were used for a systematic sampling of the communities at various study sites. These surveys were done during times of maximum reptilian activity.

Visual Encounter Survey (VES)

Visual encounter survey, a standard technique given by Crump (1971) was largely used to record the diversity of reptiles in the selected ISAs. Followed by VES active combing operations were done in all the seasons to document the reptiles, as many of the species are highly cryptic and reveal their presence only when disturbed. Moreover, the combing operations also helped analyzing the micro-habitat requirements of different species. Reptiles in different sites were sampled and checklists of species with their relative abundance in terms of broad occurrence patterns were prepared. The surveys were conducted during day and night times so as to cover diurnal as well as nocturnal species. Thorough searches were made in all the seasons and all the possible habitats (small bushes, leaf litter, tree barks, hutments and houses etc.) that reptiles could occupy were explored for their presence. Small rocks, boulders and fallen logs were upturned and thoroughly examined for the presence of species. Hand-capturing method (Blomberg and Shine, 1996) was used for those species that could not be identified in the field. They were brought to the laboratory and carefully studied for their taxonomic characters. Identification of the species was done using standard monographs (Gunther, 1864; Boulenger, 1890; Smith, 1935; Daniel, 2002 and Das, 2008).

Transect Sampling

Transect sampling such as line transect, belt transect and quadrat sampling (Southwood and Henderson, 2000) was used to sample the reptilian communities in the selected ISAs. Suitable technique was selected and used based upon the topography and terrain of the site. Line Transect of 1 km each at various locations was laid in the dry deciduous hill forest of Pavagadh. This technique is best and recommended for a terrain with a slope, undulations and varied vegetation. However, belt Transect was largely employed for the scrublands and grasslands. Narrow strips of 4m X 6m were randomly placed in the pre-marked areas. These strips were then surveyed in a zigzag manner and the reptiles therein were recorded. Reptiles that were encountered right on the border of the belt were not counted as slightest disturbance caused them to move out of the belt thereby leading to an error. Similarly, adjoining belts were avoided to minimize the duplication of individuals that can increase the error factor. Quadrate Sampling was used in all the ISAs as laying a small quadrate of 5m X 5m at random offered not much difficulty in any of the study sites. However, the number of quadrates varied from site to site. After the quadrates were laid, they were surveyed starting from centre walking towards the periphery along a circular path forming concentric circles. Care was taken while recording the individuals in quadrat sampling as well.

Analytical Methods

Diversity, dominance, evenness, ecological distribution, niche breadth and niche overlap of reptile assemblages at all the study sites were studied using the formulae from Krebs (1999). The results obtained were computed and analyzed using standard statistical softwares BD Pro Version 2.0 and EcoSim.

Standard Formulae (Krebs, 1999)

1. Jackknife Index of species richness:

$$\hat{S} = s + \frac{(n-1)}{n} k$$

Where $\hat{S}$ = Jacknife estimate of species richness
s = Observed total number of species present in n quadrats
n = Total number of quadrats sampled
k = Number of unique species

2. Simpson's index of diversity:

$$1 - D = 1 - (p_i)$$

Where $(1 - D)$ = Simpson's index of diversity

p_i = Proportion of individuals of species 'i' in the community

3. Shannon-Wiener Diversity Index:

$$H' = \sum_{i=1}^{s} (p_i)(\log_2 p_i)$$

Where H'= Index of Species Diversity

S = Number of Species

p_i = Proportion of total sample belonging to i^{th} species

4. Margalef's Equation for Evenness:

$$J = \frac{H'}{H_{max}}$$

Where J = Evenness Index

H' = Shannon-Wiener diversity index

$H_{max} = \log_2 S$

S = Number of species

5. Levins's Measure of Niche Breadth:

$$B = \frac{1}{\sum p_j^2}$$

Where B = Levins's measure of niche breadth

p_j = Proportion of individuals found in or using resource (microhabitat) state j

6. Levins's Measure of Standardized Niche Breadth:

$$B_A = \frac{B - 1}{n - 1}$$

Where B_A = Levins's standard niche breadth

B = Levins's measure of niche breadth

n = Number of possible resource (microhabitat) states

7. Pianka's Index of Niche Overlap:

$$O_{jk} = \frac{\sum_{i=0}^{n} p_{ij}\, p_{ik}}{\sqrt{\sum_{i=1}^{n} (p_{ij}^2)(p_{ik}^2)}}$$

Where O_{jk} = Pianka's measure of niche overlap between species *j* and species *k*
p_{ij} = Proportion of resource *i* is of the total resources utilized by species *j*
p_{ik} = Proportion of resource i is of the total resources utilized by species *k*
n = Total number of resource states

8. Jaccard's Index of Similarity Coefficient:

$$SC_j = \frac{c}{A + B - c}$$

Where SC_j = Similarity Coefficient
c = number of common species
A = total number of species in site A
B = total number of species in site B

9. Coefficient of Community:

$$C = \frac{2W}{a - b}(100)$$

Where C = Coefficient of community
a = sum of scores for one site
b = sum of scores for second site
W = Sum of lower scores for each species

STUDIES ON *SITANA PONTICERIANA*

Population Sampling

Correct identification of a species is an important pre-requisite to an ecological study. During the inventory studies (Chapter 3) it was observed that *Sitana ponticeriana,* a medium sized lizard belonging to family Agamidae was found common in all the study sites. It was found to be an abundant species in the ISAs and hence one of the ISA – Scrubland at Sindhrot village was selected to estimate the population of this common agamid lizard. Sampling of *S. ponticeriana* was done with the help of 'Capture-Mark-Recapture Technique' (Seber, 1982). The first ecological use of this technique was done by Peterson a Danish fisheries biologist in the year 1896 (Ricker, 1975) for studying the movement and migration in fishes. He developed a systematic method and a formula for the population estimation studies known as the 'Peterson Method'. Later many workers (Lincoln, 1930; Jackson, 1933; Green and Evans, 1940) used the capture-mark-recapture technique for sampling populations of different animal groups. Seber (1982) has described this method as one of the best method for animals that are less motile and hold more chance of encounter during each of the field survey, thus the method is recommended for many of the herpetofaunal species like lizards. Capture-mark-recapture techniques could be used for both closed and open populations (Otis *et al.*, 1978; Pollock *et al.*, 1990). In the current study Peterson Method was used to estimate the population of *S. ponticeriana,* and results with 95% confidence limits were obtained. Individuals of *S. ponticeriana* were hand captured (Blomberg and Shine, 1996) and were marked by the toe clipping method. Woodbury (1956) suggested using marks that can be applied using some sort of coding system, so a large number of combinations could be obtained. Toe clipping is one such method wherein numbering of toes and clipping each at a specific point can lead to a multitude of combinations. Initially it was thought to mark the individuals using some paint (nail polish works well) as it was a non invasive method but two disadvantages of paint marking *viz.* paint mark might make the animal more visible to predators and secondly the mark will wear off in the long run as animal will moult the skin; discouraged one from using this method. Other methods like scale clipping, cauterizing or branding are also suggested for marking of reptiles (Woodbury, 1956). However, these methods become cruel and harmful to the animal if not carried out by experienced, trained herpetologists or under the supervision of a veterinarian and also these methods are less applicable to the species that are small in size. In the current study, toe clipping was found to be an ideal method for marking the individuals of *S. ponticeriana.*

Peterson Method for Population Estimation:

$$\check{N} = \frac{(M+1)(C+1)}{(R+1)} - 1$$

Where $\check{N}$ = Estimate of Population size at the time of marking
M = Number of individuals marked in the first sample
C = Total number of individuals captured in the second sample
R = Number of individuals in second sample that are marked

Morphometric Studies

Morphometry of *S. ponticeriana* was done based upon age and sex of the individuals.

Individuals were divided into three different age groups namely, hatchlings, juveniles and adults. Further adults being sexually dimorphic, Morphometric measurements were separately recorded for males and females. Hand-capturing technique (Blomberg and Shine, 1996) was used to capture the lizards from their habitat and the measurements were recorded in the field itself using calibrated dialer Vernier Caliper (Mitutoyo, Japan). Since majority of the morphometry work was done in the field and the captured animals were released back into their habitat but in few cases collection of a holotype was essential. Live animals were collected from the field and brought to the laboratory and preserved using 5% - 30 % formalin depending on the body size of the animal, the preserved specimen was then deposited in the museum of the Department of Zoology, The M. S. University of Baroda. Photo documentation of the animals was done using both analog SLR camera – Canon T70 and a Digital Camera – Olympus C770.

Gut Content Analysis

Individuals of *Sitana ponticeriana* that were captured and brought to the laboratory were subjected to the examination of their gut content to know the diet composition of the species. Animals collected were sacrificed with chloroform fumes and their alimentary canal was dissected out. Region from stomach to rectum was excised out and the content therein was flushed out into a Petri dish containing reptilian saline solution (composition in millimolar concentration NaCl, 145; KCl, 2.5; $CaCl_2$, 3.6; $MgSO_4$, 1.8; KH_2PO_4, 1.0 HEPES, 5.0; NaOH as needed for pH 7.4). The content was then examined thoroughly under a Binocular Research Microscope (Leica MZ 16A) and recorded.

Measurements of Dietary Preferences

An animal when faced with a variety of possible food types, it prefers some and avoids others. Thereby it is obvious to measure preference very simply by comparing usage and availability. Manly *et al.* (1993) has recently reviewed the problem of resource selection by animals and they provide a detailed statistical discussion of the problems of measuring preferences. Resource selection may involve habitat preferences, food preferences or nest site preferences, however in the present work we concentrated on the dietary preference of *S. poniticeriana.* Based upon the gut content analysis and in field observation on the foraging mode of *S. ponticeriana* foraging ratio for the species was derived through the simplest measure of preference *Forage Ratio* first suggested by Savage (1931) and later by Williams and Marshall (1938).

$$w_i = \frac{o_i}{p_i}$$

Where w_i = Forage ratio for species *i*
o_i = Proportion or percentage of species *i* in the diet
p_i = Proportion or percentage of species *i* available in the environment

Further, based upon the forage ratio the utilized food types (prey species) were broadly grouped into two-prey case and choice made by the animal between these two prey species was analyzed Murdoch's Index (1969).

$$C = \left(\frac{ra}{rb}\right)\left(\frac{nb}{na}\right)$$

Where C = Murdoch's Index of preference
r_a, r_b = Proportion of prey species *a, b* in diet
n_a, n_b = Proportion of prey species *a, b* in the environment

ENVIRONMENTAL PARAMETERS

Temperature

Being ectothermic (poilkilotherms), temperature plays a key role in governing the activity budget of reptiles. A sharp rise or fall in the temperature is directly depicted through a change in the lifestyles of these cold blooded creatures. Moreover, optimum temperature is required to begin the daily activities again. Ambient temperature and humidity were recorded on a regular basis during the field visits using digital Humitherm. Meteorological data were procured from state meteorological department.

Soil Parameters

Since the current study focused mainly on the terrestrial reptiles, soil becomes an important abiotic factor directly linked with the biota present. Soil was collected only from the ISA and was analyzed for parameters such as Soil Surface Hardness or Penetrability, Soil Texture, pH of the soil, Soil Temperature and Soil Moisture (Jones and Reynolds, 1996).

1. Soil Surface Hardness or Penetrability

Soil penetrability is an important property for seed germination, and for animals which dig or scrape the ground for food or shelter. Though *S. ponticeriana* is a ground dwelling agamid lizard, it is not fossorial in its habit. It does take shelter into the burrows at times when threatened but these burrows are not the self burrows and perhaps dug by some rodent. The only time when *S. ponticeriana* needs to dig the soil is at the time of laying its clutch. Penetrability of the surface soil was examined with the simplest method the 'BJPS' or "Bob James' Pointy Stick' method. A bamboo stick sharpened at one end was consistently dropped from the shoulder height and the extent to which the stick penetrated in the soil was measured using a standard graduated scale.

2. Soil Texture

Soil texture is determined based upon the percentage content of its various constituent particles. The standard texture scales that are most widely used are developed by United States Department of Agriculture (USDA). Soil collected from the ISA was first of all hand strained and all the large sized particles like gravels and pebbles were removed manually. Later this soil was sieved through a standard sieve so as to eliminate the particles greater than 2 mm in diameter as they are to be excluded in the texture analysis. Thereafter the pre-weighed soil was sieved through a column of sieves staked one above the other with variable and precise pore size by a constant rotating action. Soil particles then separated out through these sieves were then individually weighed and their relative percentage was calculated.

3. Soil pH

pH of the soil is an essential criteria for evaluating the fertility of the soil and thus has a direct impact on the cultivation or is important for the microflora and microfauna. As this property is not of much relevance when it comes to any terrestrial fauna still one examined the same so as to establish any kind of correlation if existed. In a clean, dry beaker 1:5 suspension solution of soil was prepared using distilled water, the pH of this suspension was then determined using hand held pH meter (Eutech Cybernetics – Model pHScan2) . The pH of soil collected from the ISAs ranged from 6.5 to 9, which were found suitable for the existence of both flora and fauna. Soils with exceeding acidic or alkaline conditions are unsuitable for cultivation as well as for the microflora and microfauna.

4. Soil Temperature

Temperature of the soil is an important parameter to be considered for the fossorial forms. The sub-surface temperature is usually lower than the ambient temperature and also the humidity is relatively higher thus making soil an ideal place of shelter for the burrowing animals. Though, *S. ponticeriana* is not a burrowing lizard but as a typical agamid, it lays its clutch by digging a nest in the soil. As soil acts as an incubation medium, therefore recoding the soil temperature becomes essential. Soil temperature was thus measured using standard calibrated soil thermometer.

5. Soil Moisture

Soil moisture is an essential ecological property which differs in the availability of the water to the plants. It could be further considered into three categories, (1) Free-draining or Gravitational water that drains away soon after rain, (2) Plant-available or Capillary water that occupies the spaces between the soil particles and is most important and (3) Plant-unavailable or hygroscopic water that forms a thin film on the surface of the particles. In the current study, capillary water was taken into consideration for determining the soil moisture and the approximation was done by the loss in weight of the soil at field capacity. Wet soil immediately after the rain was collected from the ISA and 100 gm was weighed and spread onto a newspaper for a week. After the moisture had evaporated the soil was weighed again and the loss in weight was taken as the percentage of moisture present in the soil.