

Cobra

Vol.-XI Issue -II

July - December 2017



Half-yearly Journal
Hof the Chennai Snake Park Trust

Annual subscription : Individual – Rs. 75/-. Institution – Rs.150/-.

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Cover

Keeled Rock gecko (*Cyrtopodion scabrum*)

A night-active, rock-dwelling lizard found in dry arid parts of central and northwestern India.
Feeds on insects. Lays eggs. Natural history poorly-known.

Photo : Asish K. Jangid

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Volume : XI Issue 2

July - December 2017

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EFFECTS OF MONOCHROTOPHOS ON THE HISTOLOGY OF LIVER AND KIDNEY OF THE INDIAN SKIPPER FROG *EUPHLYCTIS CYANOPHLYCTIS*

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Abstract: We studied the toxic effects of the organophosphorus pesticide monochrotophos 36% SL, on the skipper frog *Euphlyctis cyanophlyctis*. Frogs were injected with the selected doses of monochrotophos 36% SL exhibited changes in their behavioural pattern such as loss of balance, immobilization and lack of co-ordination. The sublethal dose of monochrotophos 36% SL at 24 hours was found to be 0.036 ppt and that of median lethal and lethal dose at 24 hours was 0.17 ppt & 0.36 ppt. Histology of liver of control frogs showed polyhedral hepatocytes. Histopathological changes in liver were noticed after the pesticide treatment. Liver of treated frogs showed derangements in cords with cellular necrosis and appearance of cavuoles. The enlargement of hepatocyte cells in all dosages were not statistically significant. Histology of the kidney of control frogs showed glomerulus and internal cells. Histopathological changes in kidney were disturbed glomerulus, appearance of large number of vacuoles, cell enlargement and disappearance of nucleus in the internal cells. The enlargement of internal cells except sublethal dose was statistically significant.

Introduction

Amphibians are ecologically important bio-indicator organisms that are susceptible to environmental changes, particularly pollution (Mohandy-Hejmadi et al., 1979). Hence they act as an index of ecosystem health. Pesticides toxicants may induce changes in the behavior and physiology of organisms which inevitably leads to ecological crisis (Indra et al., 1998). Toxicological works carried out on amphibians are meager when compared to fishes. So there is a great need for ascertaining the toxicity effects of more commonly used pesticides on amphibians. Monochrotophos 36% SL is used extensively for pest control in agriculture fields of the study area; it has a wide spectrum activity and low mammalian toxicity;

more residue of monochrotophos 36% SL is detected in all the components of the environment to which it was sprayed; it is a water soluble compound. We here undertake a study to get a basic understanding of the toxic effects of the widely used commercial pesticide Monochrotophos 36% SL – an organophosphate on a common Indian frog species.

In the present investigation the pesticide monochrotophos 36% SL has been selected because it has a lethal effect on the non-target organisms [amphibians and fishes] when it is predominantly used to control sucking, mining and chewing insect pests and acarine pests of agricultural crops of the study area. The skipper frog *Euphlyctis cyanophlyctis* has been selected in the present study because this frog is more susceptible to pesticide in the agricultural fields and in water bodies; are very good bio-controllers of agricultural insect pests (Mohandy-Hejmadi et al., 1979; Battish and Sandhu 1988) economically important as their reduction in number due to aquatic pollution may adversely affect our agricultural production. The present study aims to study the Sublethal, medianlethal and lethal doeses of monochrotophos 36% SL at 24 hours; to observe the effect of Monochrotophos 36% SL on the behavior due to exposure; to study the histological and histopathological effects of monochrotophos 36% SL. on liver and kidney of *E. cyanophlyctis*.

Materials & Methods

Animal model: *Euphlyctis cyanophlyctis*, one of the most commonly available frog and collected from ponds and agricultural paddy fields of Nallathukkudi area. They were collected from water bodies using a sweep net or picked at night from the bunds of the paddy fields. The collected frogs were housed in cement cisterns in appropriately spaced and furnished animal enclosures. The booms of the cisterns were filled with water and a few bricks were also provided to serve as resting places for the frogs. They were maintained under normal day/night and at a temperature of 28-30°C. They were fed with live earthworms and small alive insects like grasshoppers. The water in the cistern was changed every day. The frogs weighing approximately 15 to 22 gm and 50 to 60 mm snout-vent length and in good condition, were selected for experimental studies. Physical verification of frogs was also done for any signs of diseases, damage and mortality. Frogs of abnormal size, overweight and damaged by injuries were discarded. The frogs were divided into two different batches. One set of the frogs were kept in a cistern for experimental study. The other set was maintained separately as a control. Both the

control and the experimental frogs were forcibly starved for one day prior to the commencement of experiment.

Toxicity studies: Monochrotophos 36% was selected for the acute toxicity studies. In the present investigation a stock solution of this commercial grade pesticide was prepared by dissolving one ml of the pesticide in 359 ml of water. From this stock solution the following different concentrations of Monochrotophos 36% SL were prepared.

- i) 0.1 ppt solution was prepared by adding 0.1 ml of the stock solution to one liter of water.
- ii) 0.2 ppt solution was prepared by adding 0.2 ml of the stock solution to one liter of water.
- iii) Other concentrations were prepared in the same manner.

Range finding tests: To simplify the procedure preliminary small scale tests were conducted to obtain the approximate range of concentration to determine the LC 100, LC50 and LC0 of the pesticide taken (Monochrotophos 36% SL.) This range was then covered in full-scale tests. Solutions were first prepared over a wide range of concentration from 0.02 ppt to 5 ppt. Two acclimated frogs were kept in jars and the rate of mortality was observed. The highest concentration at which all the frogs survived and the lowest concentration at which most of the frogs died were obtained from this experiment.

Full scale test: Based on the observations of the range finding tests, for the fullscale test, solutions of 0.03 ppt to 0.36 ppt were taken. The collected frogs were acclimatized to the laboratory condition for 10 days. After acclimation the healthy frogs were sorted out and used for experimental studies. 1 ml of the pesticide solution containing the required dose of the pesticide solution. (Solution were prepared over a wide range of concentrations from 0.5 mg/kg to 5.5 mg/kg) and administered intraperitoneally using a hypodermic syringe. The rate of mortality was observed at 3,6,9,12,15,18,21 and 24 hrs. Individuals showing no buccal movement, lack of co-ordination by extending their hind limbs backwards were considered as dead and were removed immediately. The least concentration at which 100 percent mortality was observed with in 24 hrs was considered as sub lethal concentration (LC_0).

LC₅₀ value: Median lethal concentration (LC₅₀) is the minimum quantity required to kill at least 50% of the population of the test animals. The LC₅₀ value was obtained by the probity analysis method based on the observed percentages of the test animals surviving at concentrations lethal to more than half and less than half of the test subjects (Finney 1971).

Histological studies: The liver and kidney were dissected out from the control and treated frogs were cut into several bits. The control and treated tissues were separately fixed in aqueous Bouin's fluid, which gave better results. After dehydration in alcohol (alcohol 70%) the tissues were transferred into absolute alcohol or acetone for complete dehydration. Then the tissues were transferred into Xylol for clearing, till the material become transparent, and the tissues were embedded in paraffin wax (BDH 58- 60%). Sections were cut at 5 microns thickness and the deparaffinised sections were hydrated and stained in Heidenhain iron alumhaematoxylin and counterstained in aqueous eosin for microscopic observations.

Results

In the present work the effect of monochrotophos 36% SL on the behavioural and histological parameters of *Euphlyctis cyanophlyctis* were studied and the observations were as follows:

Behavioural changes: Intraperitoneal injection of the monochrotophos 36% SL to *Euphlyctis cyanophlyctis* exhibited restlessness and showed some breathing difficulties. The frog administered with monochrotophos 36% SL exhibited irregular movements and tried to rest up on each other using their forelimbs, where as their hind limbs began to lose their co-ordination gradually. Some of them tried to jump out of the container but failed and also the body of the frog was covered with excess mucous and there was reddening of the toes, finally the frog died, with a shrill characteristic cry, others which lost their coordination of limbs and died without any cry.

Acute toxicity studies: The toxic impact of the pesticide monochrotophos 36% SL on *Euphlyctis cyanophlyctis* was obtained from the percentage mortality of the test animal were given in Table – 1. When the animals were exposed to different concentrations of pesticide, death occurred at different times. The percentage of mortality increased at the end of each interval of time up to 15 hrs. Hundred percent survivals of the frogs after 24 hrs was observed only at the concentration of 0.036 ppt and that was taken as sub lethal concentration of 24 hrs LC₀ hundred percent

mortality was observed at 0.36 ppt and it was considered as the lethal concentration of 24 hrs of this pesticide to this frog. At the concentration of 0.1659 ppt 50% of the test population survived after 24 hrs and hence it was taken as LC50 for 24 hrs for this pesticide for this frog.

Table-1. Percentage mortality of *Euphlyctis cyanophlyctis* exposed to different concentration of monochrotophos 36% SL

Lethal Concentration	3 hrs	6 hrs	9 hrs	12 hrs	15 hrs	18 hrs	21 hrs	24 hrs
0.036*	0	0	0	0	0	0	0	9
0.085	0	0	0	9	9	12	12	12
0.094	0	9	9	12	12	15	12	15
0.11	9	9	12	12	15	15	25	24
0.14	12	12	15	15	25	25	35	35
0.17**	15	15	25	25	35	48	48	48
0.22	25	25	35	35	48	48	63	63
0.26	25	25	35	35	48	48	63	72
0.33	25	35	48	63	72	72	86	86
0.36***	35	48	63	72	86	98	98	98

* LC 00 24 hrs

** LC 50 24 hrs

*** LC100 24 hrs

Histology: The size of control and experimental frog's liver, kidney cells were measured with the aid of a filer or drum micrometer. Hepatocytes and interrenal cells of the frogs were selected randomly and measured from various place of the prepared slides. Average size of hepatocytes and interrenal cells were calculated and tabulated. In the same way the size of the interrenal cells of kidney and hepatocytes of liver of sublethal dose, median lethal dose and lethal dose treated frogs were measured. The size of the hepatocytes and interrenals cell of the control and treated frogs were compared.

Histology of liver (Experimental frogs): Liver of frogs treated with monochrotophos 36% SL showed derangements in the cords, cellular necrosis, and disappearance of nucleus and appearance of vacuoles. The hepatocyte cells were measured with the aid of a filer micrometer, the size ranged from 550 μ to 860 μ

and the average size of hepatocytes was calculated as $702 \pm 142 \mu$. The size of the hepatocyte cells of the sublethal dose, median lethal dose, and Lethal dose treated frogs were measured and presented In Table -2. From an analysis of the table-4 it may be observed that there is slightly increase in the size of hepatocyte cells in all experimental animals. The enlargement of hepatocyte cells in sub lethal dose, median lethal dose and lethal dose treated frogs were not statistically significant.

Table-2. Size of Hepatocyte cell in *Euphlyctis cyanophlyctis* exposed to different concentration of monochrotophos 36% SL.

Dosage	Control frog	Experimental frog	T value	P
Sub lethal dose	702 ± 142	744 ± 70.1	0.8496	$P > 0.05$
Median lethal dose	702 ± 142	782 ± 50.1996	1.5968	$P > 0.05$
Lethal dose	702 ± 142	784 ± 92.89	0.9905	$P > 0.05$

Histology of kidney (Control frogs): Kidney of control frogs showed glomerulus formed by the little cluster of small blood vessels and is held together by connective tissue. Internal cells were distributed at maximum in the center of the kidney. The internal cells were contain compact and clear.

Histology of kidney (Experimental frogs): Kidney of treated frogs showed disturbed network of glomerulus and lost their compactness. The appearance of large number of vacuoles, cell enlargement and disappearance of nucleus were also observed. The internal cells were measured using the filar micrometer the size ranged from 710 microns to 1170 microns and the average size of internal cells calculated was $912 \pm 185 \mu$ microns. The size of the internal cells of the sub lethal dose, median lethal dose, lethal dose treated frogs internal cells were measured and presented in Table 3.

Table – 3. Size of internal cell in *Rana cyanophlyctis* exposed to different concentration of monochrotophos 36% SL

Dosage	Control frog	Experimental frog	T value	P value
Sub lethal dose	912 ± 185	980 ± 173	0.4988	$p > 0.05$
Median lethal dose	912 ± 185	1106 ± 265	2.9293	$p < 0.05$
Lethal dose	912 ± 185	5732 ± 1497	7.4691	$p < 0.05$

From an analysis of the table-3 increase in the size of internal cells in all the experimental animals was observed. The enlargement of internal cells in sub lethal dose treated frogs was not statistically significant while the median lethal dose and the lethal dose induced significant increase in the size of the internal cells.

Discussion

A behavioural response may be the initial reaction to the chemical exposure in the natural environment (Mayer et al, 1978). In the present investigation injection of monocrotophos 36% SL on *Euphlyctis cyanophlyctis* produced drastic changes in the behavior. They exhibited restlessness, breathing difficulties and some of them tried to jump out of the container. The body of the frogs were covered with excess mucous, loss of co-ordination of limbs and there was reddening of the toes and finally the frog expired. Treated frogs exhibited restlessness, however this results was not in accordance with Anderson and Prahlad (1976) who worked on *Xenopus laevis* embryos, and found them to be lethargic with slightly retarded trunks and tails. Desi et al., (1976) had concluded that pesticides affected the cholinesterase activity, which eventually lead to embalance of the animal. Monocrotophos 36% SL is a fairly potent inhibitor of cholinesterase which is an essential constituent of the nervous system in these frogs.

Similar behavioural changes were noticed in *Anabas testudineus* exposed to sublethal (0.56 ml/l for 120 hrs) and lethal (1.53 mg/l for 24 hrs) concentration of the carbonate pesticide Carbofuron (Bakthavathsalam, 1980). Several authors have reported the loss of co-ordination of limbs which was coinciding with the present study. Pawar and Katdare (1983) treated the tadpoles of *Microhyla ornate* with Sumithion and found loss of co-ordination and balance. This was also observed by Lorette and John (1983) when adult *Rana pipiens* were administered with different pyrethroids. The present result was consistent with the result obtained by Naiyara Yasmeen and Nayeemunnia (1985) who studied the effects of methylparathion on the rate of oxygen consumption of tadpoles of *E. cyanophlyctis*.

Vijayakumaran (1985) studied the effect of endosulfan on the frog *E. cyanophlyctis* and observed that the frogs secreted excess amount of mucous, floated on water surface, showed signs of lack of co-ordination and became motionless (Ramaswamy 1999). Bharathi (1999) who studied the effect of phosphomidon 85% SL on *E. cyanophlyctis* observed reddening of toes as in the case of present study also. Mudgal and Patil (1987) conducted acute toxicity tests on *E. cyanophlyctis* and found that mercury was more toxic than Cadmium. For mercury 96 hrs LC₅₀ values

for both male and female were 2.5 and 3.16 mg/l respectively. For Cadmium the 96 hrs LC_{50} value were 56.6 mg/l for female and 75.0 mg/l for male. Alam and NoorAlam (1989) investigated the effect of two agricultural pesticides metacid 50 (Parathion methyl) and Ekalux (Quinophos) on tadpoles of *E. cyanophlyctis*. The safe level (LC_0), median lethal (LC_{50}) and lethal dose (LC_{100}) for 96 hrs exposures in water of Parathionmethyl were 4.0, 8.75 and 9.5 ppm and for Quinophos was 5.0, 8.0 and 9.0 ppm respectively.

Sheela et al. (1991) observed the toxicity of Dipterex to *E. cyanophlyctis* tadpoles and reported that 0.001% to 0.005% of the pesticide didn't produce any mortality but 0.1% produced 100 % mortality within 24 hrs. The LC_{50} for *E. cyanophlyctis* at 96 hours of Cythion was 1 ml / litre was found by Patil and Kulkarni (1993). Vijayakumaran (1995) obtained 24 hours sublethal, median lethal and lethal values as 0.075 ppm, 0.0958 ppm and 0.150 ppm of endosulfan on *E. cyanophlyctis*. Ramaswamy (1997) observed the toxicity values of endosulfan 3.75 mg/kg, 2.08 mg/kg and 0.42 mg/kg as the lethal dose, median lethal dose and sublethal dose of the pesticide respectively. Bharathi (1999) obtained 120 hours sublethal, median lethal dose values as 0.96 mg/kg, 2.26 mg/kg and 3.36/kg of phosphomidon for *E. cyanophlyctis*. In the present analysis the effect of monochrotophos on the skipper frog *E. cyanophlyctis* was studied the sublethal, median lethal and lethal doses for 24 hrs were 0.036 ppt, 0.17 ppt and 0.36 ppt respectively. When compared with Ramaswamy (1997) who worked on *E. cyanophlyctis* the lethal dose of this result was less. When compared with the studies of Alam and NoorAlam (1989) in *Rana cyanophlyctis* the obtained lethal dose of was less. LC_{50} concentration reported by Abbasi and Soni (1984) was less when compared to the present study. They conducted the experiment with tadpoles of *E. cyanophlyctis* but the present study was carried out in adult *E. cyanophlyctis*. Thus the LC_{50} concentration values were varying widely from species to species, even in the same species depending upon the stage in their life history.

As regards the Histology, in a vertebrate body, the liver is the most important target organ as it is the chief it is the chief metabolic and detoxification center. Within the hepatic cells virtually all the reactions or intermediary metabolism takes place (Pesch and Topper, 1963). Many reports indicate that it is the organ with the highest concentration of the pesticide (Holden, 1972; Cough, 1975) and with the greatest damage or impairment (Eller, 1971) due to pesticide treatment. In the present study the liver of control frogs showed polyhe dral hepatocytes. Bile

capillaries are present in between the cells. The broad irregular thin sinusoids are present. Histology of the liver of frogs were described by Gurr (1956). He described that the liver of frogs were the largest reddish brown gland consisting of two lobes right and the left. These two lobes remain connected with each other by a narrow bridge. The left lobe is again subdivided into two lobes. The liver is covered with an intact outer protective membrane. The liver cells or hepatic cells are polygonal in shape while some faces of the polygonal hepatic cells are in contact with adjoining cells, others are exposed to the blood capillaries. The blood is carried through the liver in sinusoids. These are loosely arranged blood channels lined by endothelial cells. Between adjacent liver cells run the bile canaliculi. The nucleus of hepatocytes are spherical with regular surface and show uniform size, shape and orientation. The histology of liver of frog was also described by Moore (1964), Rajam (1987) and Mia et al. (1988).

The study of the damages of the liver cells helps in understanding the effects of the toxicants on cells. The liver of the frog was highly damaged after pesticide treatment. Rajam (1987) have reported the effects of nuvucron on *E. cyanophlyctis*, the frog liver showed necrosis of the hepatocytes, rupture of the outer membrane, vacuolization, cytoplasmolysis, nuclear enlargement and karyolysis. The present findings of the work are in good agreement with observations of Amminikutty and Rege (1977) on *Gymno corymbus tarzetzii*, Duogall and Patil (1986) and Bharathi (1999) on *E. cyanophlyctis*. In the present investigation on *E. cyanophlyctis*, treated with monochrotophs histological changes in the liver after treatment includes derangements in the cords, cellular necrosis and appearance of Vacuoles in hepatic cells.

In our work the kidney of control frogs showed glomerulus formed by the little cluster of small blood vessels. Internal cells were distributed at maximum in the centre of the kidney. Kidney of control frogs was described by Ditrich and Lamatschwandtner (1992). The kidney of frogs is composed of renal corpuscle. Renal corpuscle consists of two parts, a glomerulus and a capsule. The glomerulus is circulating system, which is formed of compact little cluster of small blood vessels. The capsule is a double-layered hemisphere. The capsule and the glomerulus together form the roughly spherical renal corpuscle. Internal cells were found distributed at maximum in the center of the kidney.

In our study the kidney of treated *E. cyanophlyctis* showed glomerular damage and lost their compactness. The appearance of large number of vacuoles,

cell enlargement and disappearance of nucleus were observed in the internal cells. Celentano et al., (1979) observed that several pesticides, toxicity is thought to be the binding of lead to sulphhydryl groups and disruption of sulphur bridges. This would change the structure of membrane proteins leading to damage in kidney of aquatic organisms (Verma et al., 1979, Rao and Ramana, 1979). Histopathological changes in kidney to the Fish *Heteropneustes* fossils due to impact of pollutant showed degeneration of uriniferous tubules impairment of normal function of kidney (Kumar and Kumari, 1995). The present study reveals a cause for conservation concern by demonstrating the potentially deleterious effects of organophosphorus pesticide monochrotophos 36% SL, on the skipper frog *Euphlyctis cyanophlyctis*.

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TEMPERATURE AND RAINFALL DEPENDENCY OF GECKOS FROM CENTRAL RAJASTHAN, INDIA

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Introduction

Reptiles are ectotherms and they maintain their body temperature on the basis of surrounding environments (Heath, 1970; Werner, 1983; Sievert & Hutchinson, 1988). Many of the reptiles as well as anuran species cannot survive at critically low temperature thus, specialized behavioral and ecological adaptation event “Hibernation” will be performed that is regularly seen in the North India due to fluctuating and extreme climatic conditions (Sharma & Vazirani, 1977). Hibernation is a physiological and biochemical process, in which animal reserves the food materials at first, then it slows down the metabolic rates, so the consumption of energy will be very less, only in breathing and some essential processes (Sharma & Vazirani, 1977; Gregory, 1982). The geckos are well adapted for various geographical and environmental conditions (Prasad, 1916; Prakash, 1973). They can be observed as arboreal, scansorial, fossorial, cursorial, glider on the basis of their behavior (Sharma, 2005a). In India, geckos are found in various environmental conditions of temperature, humidity and rainfall although density and frequency of the species can vary in different climatic zones. Temperature fluctuations regulate the different behavioral activities of geckos (Bennett, 1980; Adolph & Porter, 1993; Angilletta *et al.*, 1999).

There are around 100 species of geckos found in India, among which six species are reported from central Rajasthan province (Sharma, 2005a). The study has been carried out at central Aravalli region (Figure 1), where there are five species of geckoes (Sharma *et al.*, 2013). To observe the association of Gekkonidae

members with two critical environmental factors temperature and rainfall, we selected these five species Yellow bellied house gecko *Hemidactylus flaviviridis*, Kushmore house gecko *H. kushmorensis*, bark gecko *H. leschenaulti*, Termite hill gecko *H. triedrus* and Keeled rock gecko *Cyrtopodion scabrum*. These five species are having good population at central Aravalli mountain ranges. *Hemidactylus flaviviridis* is widely distributed at North, West and Central India. *Cyrtopodion scabrum* is a rare and range-restricted *H. kushmorensis* and *H. flaviviridis* are widely distributed in Northwestern and Northern India, while *H. leschenaulti* and *H. triedrus* are widespread throughout India. Specially, *Cyrtopodion scabrum* has a very small range of distribution, in North Western India (Tikader & Sharma, 1992; Sharma, 2005a; Vyas, 2011).

Methodology

An year-long study was carried out to understand the relationship of geckos with the temperature and rainfall as the dependency in their respective habitats. We aimed to observe the consequences of temperature and rainfall on geckos at central Aravalli mountain region geographically covered by Ajmer city (26.58°N 74.52°E- 26.32°N 74.73°E), surrounded by hillocks of Aravalli ranges. The study area was selected due to its location between arid desert and semi arid dry deciduous landscapes, recognized as ecotone among the Thar Desert and Aravalli Mountain Ranges (Champion & Seth, 1968). The study has been performed in urban as well as forest areas. The forest is mainly covered by dry deciduous kind of vegetation, which mainly includes *Ziziphus jujube*, *Azadirachta indica*, *Acacia nilotica*, *Prosopis juliflora*, *Prosopis cineraria*, *Tecomella undulata*, *Boswellia serrata*, *Ficus spp.*, *Anogeissus pendula*, *Pongamia pinnata*, *Euphorbia nulia*, *Dalbergia sissoo*, *Acacia senegal*, *Butea monosperma*, *Aegle marmelos*, *Cassia fistula*, *Buchanania lanzan* etc (Champion & Seth, 1968). The rocky terrain and such habitat are favorable to some of target species group.

The study has been conducted in four different microhabitats to solve the statistical complexity, categorized as study site 'A' (Urban Land), 'B' (Semi Urban Land), 'C' (Vegetation cover zones on the plains, including shrubs and trees) and 'D' (Rocky Terrain) respectively. These microhabitats were classified on the basis of their terrain and vegetation occurrences. All the five species of geckos occur in these four habitats at the study area. Study area was classified into equal sized grids of 4 kilometers square area. 60-70% of whole study area was sampled with equal microhabitats size.

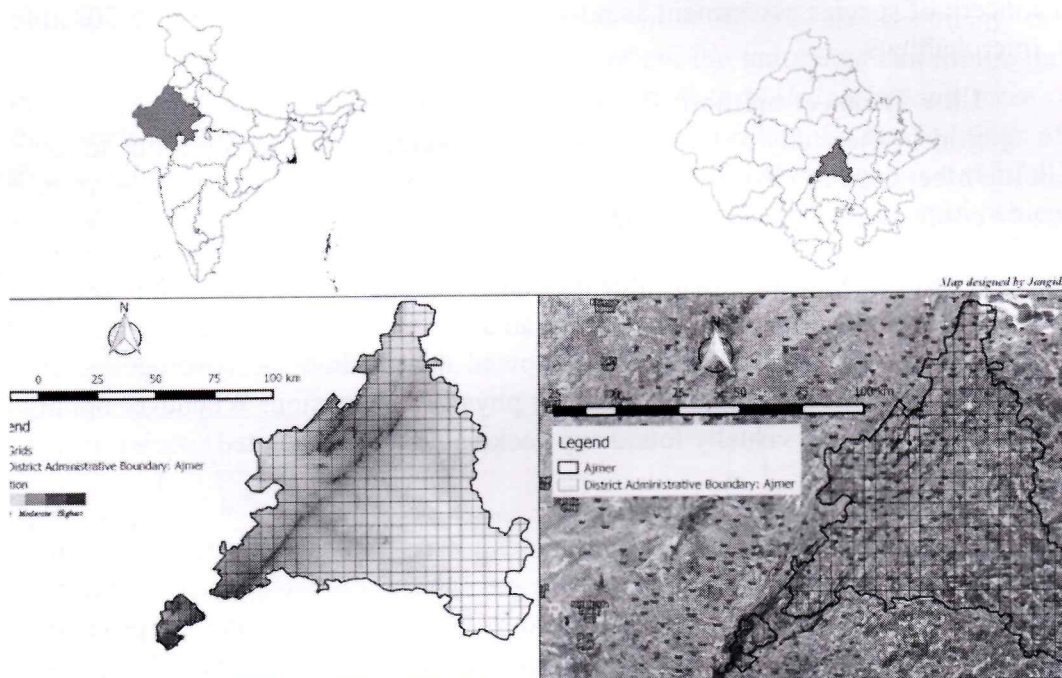


Figure 1: Study Area focusing Ajmer, Rajasthan, India

Species identification of sighted lizards were based on high resolution photographs, identified using various standard diagnostic keys (Biswas & Sanyal, 1977; Sharma, 1996; Tikader & Sharma, 1996; Sharma, 2005a). We categorized Zone “A” as the wall of houses, crevices between of walls, walls of wells, toilets and other domesticated areas as urbanized area. Railway tracks, human dominated wetlands, main roads were counted as semi urban area Zone “B”. The vegetation including shrubs and trees were counted in Vegetation cover forests Zone “C”. The elevation and top of high undulations from normal ground surface was counted in Zone “D”.

Mainly these survey methods were employed to achieve the goals of study.

Point Count method

This method consists of laying out a series of grids and thoroughly searched at 10 selected oblique points with interval of 500 meters in each sampling grid. At each selected point, we performed point count within 50 metres radius. The selected grids were of 4 kilometers square, in which accessible grids were covered

in concern of species assessment as possible. We tried to cover at least 60-70% of all microhabitats.

- **Line Transect method:** We walked through a oblique line in pre-decided grid in the study area with a defined distance of 5 kilometers maximum. In between of destination place, whatever species has been seen, were recorded with their sighting numbers.
- **Visual or Audible Encounter method:** Audible method deals with the calling pattern of species like Amphibian and Avian fauna, to identify the species too (Sharma, 2005b). We followed this method, because geckos are also able to make calls during their physical depression. Whenever opportunistically we visually found any gecko among five selected species, it was recorded.

Point count and Line transect methods were performed in same grid, which was designed for the sampling. Mainly the data collection was based on stratified random sampling (Greenwood & Robinson, 2006). For the study purpose, we selected all twelve months of year 2016 to see the changes of sightings of the species with respect to temperature and rainfall. We surveyed thrice in each month using grid and line transect method. Visual encounter method was performed eventually, whenever found any gecko. Temperature and rainfall were manually recorded and also compared with data of Rajasthan state meteorological department.

Results

The observed gekkonids were mainly nocturnal but sometimes they were seen during days too. *Hemidactylus flaviviridis* was found in close proximity to human dominated landscapes, preferring wall crevices, old blocks of stones, dark places etc. Sometimes, *H. flaviviridis* uses the vertical places or resting, where rain water is less likely to approach. So its number of individual were increasing with temperature and showed directly proportional relation. Rainfall did not change the sighted numbers of individuals similarly. *Hemidactylus kushmorensis* also prefers vertical places as well as ground, so the dependency of these free roaming lizards varied on the basis of temperature as well as rainfall. As the rainfall increases, the rain water fills the ground crevices, utilized for esting and retreat by these species and they get flushed out and move in search of suitable retreat places. Similar observation was made with *H. triedrus* and *Cyrtopodion scabrum*. As both these species mainly live on land, when the rain water floods the crevices the habitats of

both species gets damaged. In case of *H. leschenaulti*, the species uses mainly the dark coloured substrates like tree trunks etc., where the individual can mimic the background in camouflage. So rain water doesn't disturb *H. leschenaulti* sites to the extent like *H. triedrus* and *Cyrtopodion scabrum* habitats. So the sightings of this species mainly depends on the temperature, and partially depends on rainfall. During this study, we found a critical value range of both the parameters, which changed the behaviour and sighting patterns of these species.

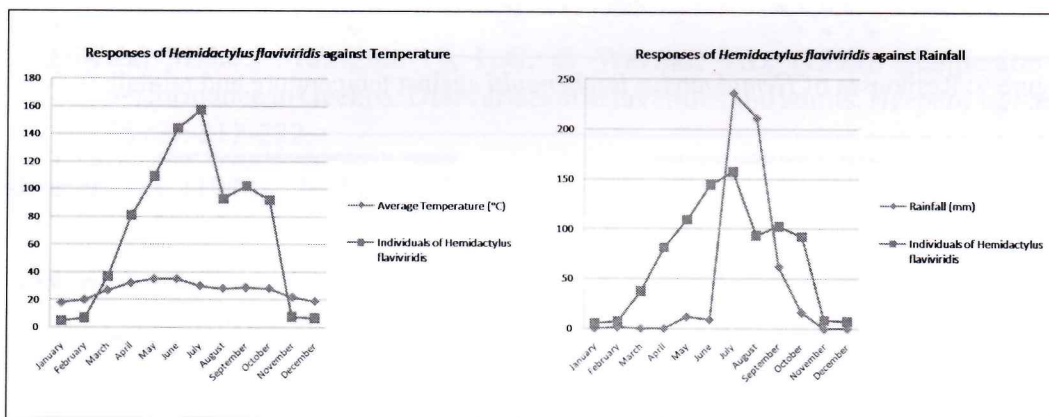


Figure 7: Responses of *Hemidactylus flaviviridis* against temperature and rainfall

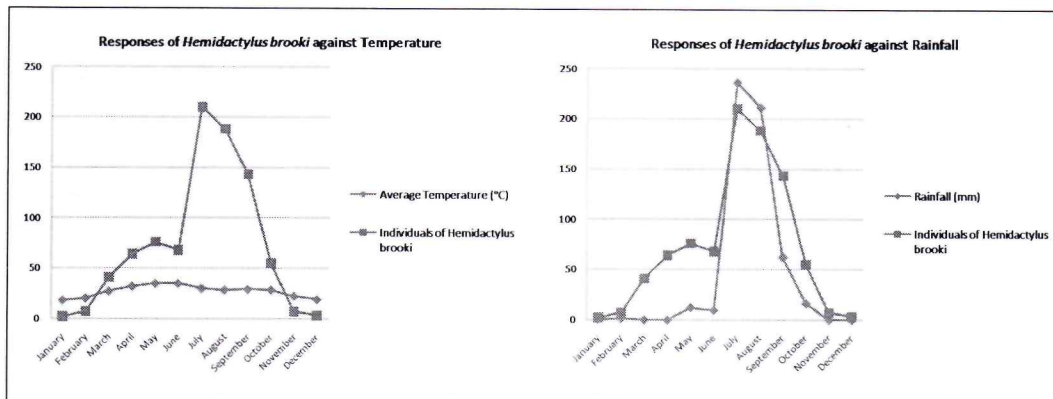


Figure 8: Responses of *Hemidactylus kushmorensis* against temperature and rainfall

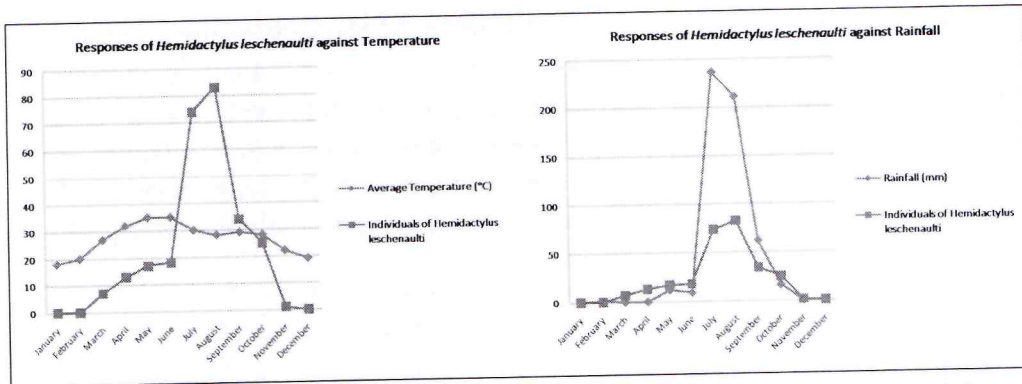


Figure 9: Responses of *Hemidactylus leschenaulti* against temperature and rainfall

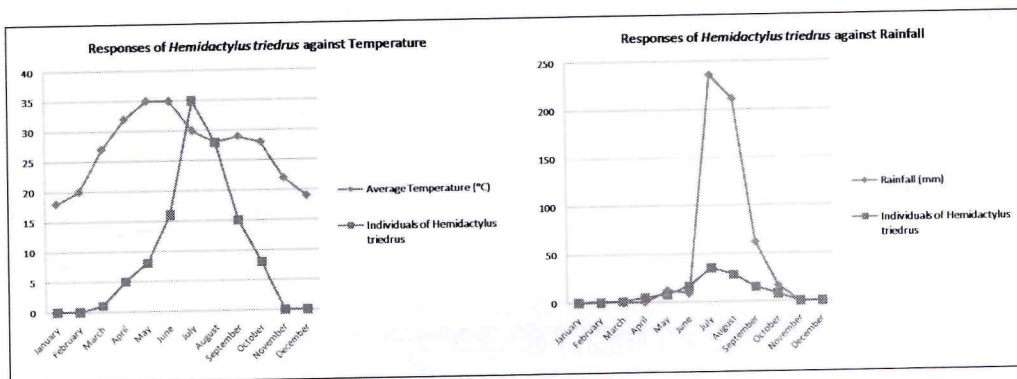


Figure 10: Responses of *Hemidactylus triedrus* against temperature and rainfall

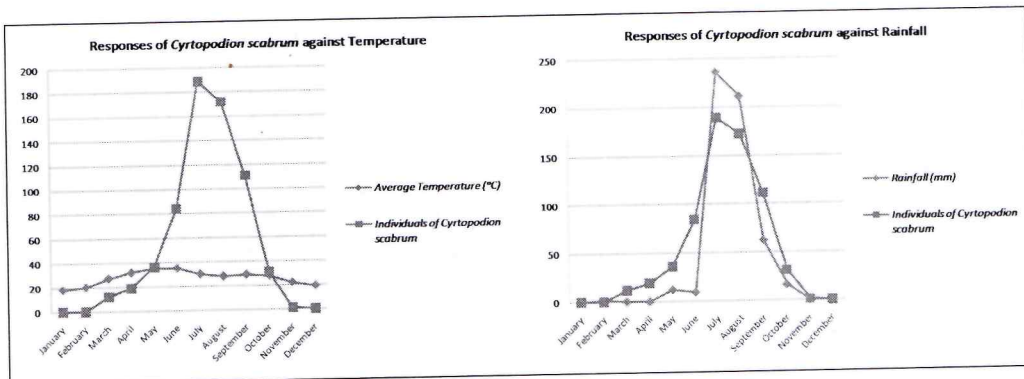


Figure 11: Responses of *Cyrtopodion scabrum* against temperature and rainfall

This study calculates the critical values of temperature and rainfall for the five species of geckos. According to study, 30-35°C temperature and approx. 200

mm rainfall can be defined as critical values of temperature and rainfall in central Aravalli region for geckoes. These small creatures are not well understood to be part of wildlife. While, the conservation of megafauna like big mammals go on, lizards that are also part of ecosystem with their unique roles get spaced out. It is important to conserve geckoes, otherwise they can be extinct due to anthropogenic pressures.

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BATH BY THE STARRED TORTOISE *GEOCHELONE ELEGANS* (SCHOEPFF) IN WATER TROUGH DURING SEVERE SUMMER IN SOUTHERN RAJASTHAN

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The Starred tortoise *Geochelone elegans* (Schoepff, 1795) is a common terrestrial herbivorous reptile which inhabits rocky, grassy areas, scrublands, semi-arid and desert tracts having plenty of vegetation (Daniel, 2002; Tikader & Sharma 1985). This species is present in Thar desert, Aravallian hilly tracts and eastern plains of Rajasthan. It is quite at home in desert conditions. It is a diurnal terrestrial species which feeds during early morning and evening to avoid heat of the day. This pattern of feeding is much adopted during summer season to avoid the scorching sun. However, it may be active throughout the day during the rainy season (Das 1985, 1995 & 2002). During day time of summer season, starred tortoise spend hot hours below some bushes or other shady places. In this note, I present my observation on bathing behaviour of starred tortoises in cement tanks.

On May 27, 2016 at about 1400 hrs, while inspecting enclosure of starred tortoise in Sajjangarh Biological Park, Udaipur, close to Sajjangarh Wildlife Sanctuary, I observed 12 tortoises (9F, 3M) inside the water trough (Figure 1). The trough was a shallow structure hardly having any depth of water about 3-4 cm. The tortoises were stationary at their location and lower part of their bodies were in water. Their carapaces and head were out of water. They were crowded in the water with their bodies touching each other. Their faecal matter was also present here and there in the water trough. The outside temperature of the park was about 40°C while it was 44°C at few localities inside Udaipur city.

I enquired about this behavior from the care-taker of the enclosure. He told me that tortoises are showing this behavior from many days owing to the heat wave.

They enter the water around 1130 hrs and leave the water by about 1600 hrs, he added. Obviously, they were doing so to avoid severe heat of the day. Though shade was available inside the enclosure but nearly 40% population of tortoises were opting water for shelter. The size of through was not big enough to accommodate all the tortoises. This behaviour is not common for starred tortoises, though it is usual for aquatic turtles and terrapians. Such behaviour in the wild will make starred tortoise a competitor for the aquatic turtles and terrapians. Sharma (1990) has recorded a Common Garden Lizard (*Calotes versicolor*) taking bath in water to cool itself during a hot day. Terrestrial reptiles generally avoid getting into water, but if temperature is high and opportunity is available or options are limited, they may enter the water to get rid of excessive heat and temperature.

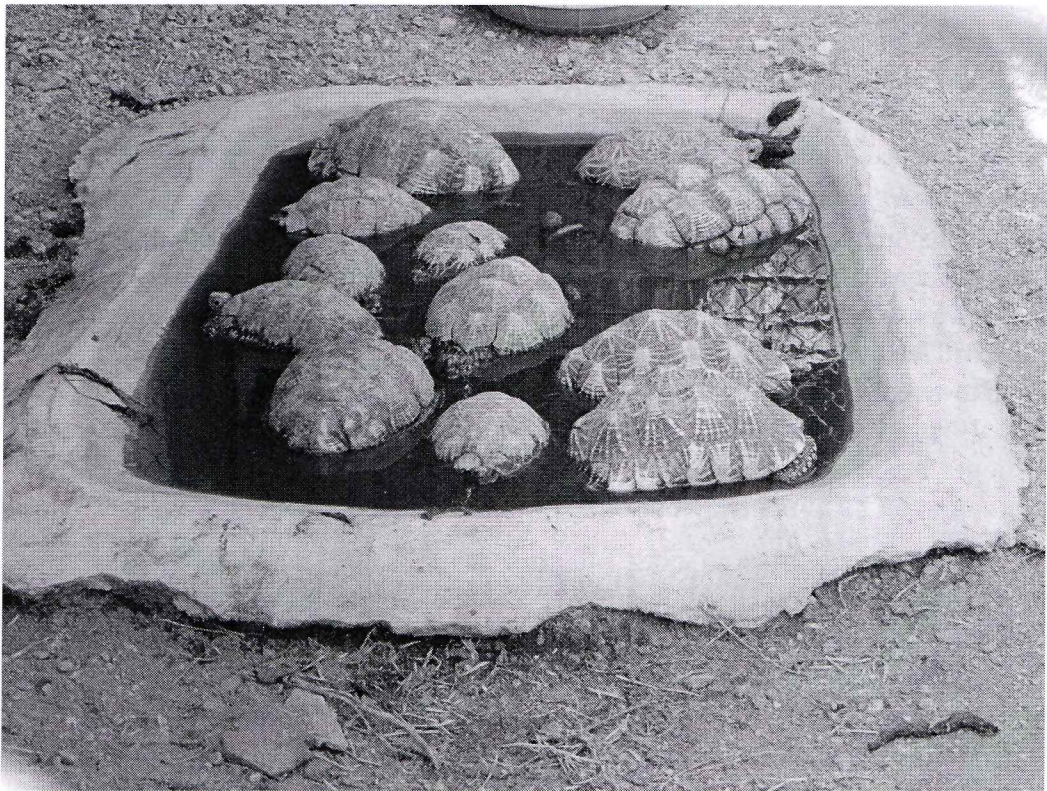


Figure 1. Starred Tortoise Bathing in cemented pond water



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HIBERNATION OF *CALOTES VERSICOLOR* IN RODENT HOLES IN RAJASTHAN

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The Indian garden lizard *Calotes versicolor* (Daudin) is a common, arboreal lizard which occupies all biotopes from dry desert to thick forests (Daniel, 2002, Tikader & Sharma, 1992). In Rajasthan, this lizard is cold blooded animal which goes for hibernation in winters. Winters are very severe in many parts of Rajasthan. Especially nights are chilling in the Thar desert in western Rajasthan. Temperature starts decreasing in the month of October in the state. November to February is the normal winter season. From February, temperature starts rising. Sometimes low temperature conditions are seen in first half of March in many pockets of the state. During winter season, most of terrestrial, arboreal and house dwelling reptiles hide themselves in safer sites to pass the winter snugly. In this article, I present my observations on the *Calotes versicolor* using rodent-holes as hibernation sites during cold seasons in the Thar desert.

While wandering in many pockets of the state where barrenness is prevailing, I observed that reptiles like Common Garden Lizard (*Calotes versicolor*) hibernates in abandoned rodent holes. It is evident from observations taken in Bhilwara and Pali districts (Table 1). Where rodent holes are easily available, reptiles opt to hibernate there. The Kushmore Gecko *Hemidactylus kushmorensis* (formerly under the catch-all name *H. brookii*) has been seen taking shelter in holes of Indian Gerbil (*Tatera indica*) in Udaipur zone of southern Rajasthan (Sharma 2015). *Hemidactylus kushmorensis* not only hibernates in the holes of rodents but take shelter during day time in non-winter season also. When young trees have no cracks on their bark to provide retreat space to the geckos, rodent holes become more safe to spend day time. After sunset, the geckos venture out at night for their biological activities.

While studying the hibernation behavior of *Calotes versicolor* in Bhilwara and Pali districts, it came to notice that no old trees were present there. If old trees were present having hollowness in their stem and branches, they could have use

them for hibernation. In Phulwari-ki-Nal Wildlife Sanctuary of southern Rajasthan old aged trees with hollowness are available in plenty. Tree hollows and crevices of rocks are the best hibernation sites for reptiles in forested hilly areas of Phulwari-ki-Nal Sanctuary. Due to decaying process, old aged trees develop hollowness in their stem and thick branches. Primary hole nester birds also make their holes for nesting in trees. The hollows and holes available on trees are also used for hibernation by the cold blooded reptiles (Sharma, 2007). Few hibernation records in Phulwari-ki-Nal Sanctuary in tree hollow are given below.

Table 1. Hibernation of *Calotes versicolor* in rodent holes in Bhilwara and Pali districts.

Date	District	Site	Number of holes occupied by the Garden Lizard	Rodent that dug the hole	Location of hole	Sex of user animal and number
29.01.2016	Bhilwara	Pastureland of Mukundgarh village (Teh. Mandalgarh) Area surveyed 4 ha.	1	Indian Gerbil (<i>Tatera indica</i>)	Below of small piece of rock	Male <i>Calotes versicolor</i> No.1
26.02.2017	Pali	Pastureland of Vayad village (Teh. Rohat) area surveyed 5 ha.	3	Indian Gerbil (<i>Tatera indica</i>)	Near small bushes	Adult male <i>c. versicolor</i> in each hole
2.12.2016	Pali	Pastureland of Khariya Neev village (Teh. Sojat) Area surveyed 3 ha.	1	Indian Desert Jird (<i>Meriones hurrianae</i>)	Open sandy area having spar grasses	Adult female No. 1
2.12.2016	Pali	Pastureland of Chawandiyak village (Teh. Sojat) Area surveyed 5 ha.	1	Indian Gerbil (<i>Tatera indica</i>)	Bund of pastureland	Adult female No. 1

Table 2. Reptiles seen hibernating during winters of 2002-04 in Rajasthan.

S. No.	Date of record	Location	Host tree	Hibernating Species
1	18.2.2002	Champa Khet	<i>Madhuca indica</i>	<i>Coelognathus helena</i>
2	27.9.2003	Birothi	<i>Ficus religiosa</i>	<i>Amphiesma stolatum</i>
3	18.3.2004	Dhedmariya	<i>M.indica</i>	<i>Varanus bengalensis</i>
4	18.3.2004	Dhedmariya	<i>M.indica</i>	<i>Calotes versicolor</i>

It is evident from above text that different types of hibernating sites are used by *Calotes versicolor* and other reptiles in different parts of the state. In Udaipur district, *C. versicolor* has been seen hibernating in deserted nest of purple sunbird (Sharma, 2004). It seems that those shelter sites readily available in natural habitat are used in an easy way for hibernation by the reptiles.

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LIZARDS OF CHENNAI AND ITS ENVIRONS, TAMIL NADU

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Lizards are the commonest of all living reptiles, and many different kinds live in different habitats in India (Daniel, 2002; Das, 2002; Tikader and Sharma, 1992; Murthy, 1990). Lizards are ectotherms that embrace a wide geographic distribution. Most species are small. They are arboreal or ground dwelling with body temperatures changing according to the surroundings and they hardly look after their young ones. There are about 3,000 species of lizards in the world of which about 200 are found in India. In India there are 20 different families of lizards that includes the agamas, chameleons, skinks, monitors, legless lizards, lacertids and worm lizards, Lizards are important for several ecological reasons. They feed on insects and control pests. But they are also being exploited in the country. The flesh of some larger species such as the monitors is eaten by tribals throughout India and their skin much sought after for the manufacture of high quality leather articles. This trade in skin is a threat to monitors and several species are close to extinction in some parts of the world (Das and Whitaker, 1997).

Chennai region (12° 55' N and 79° 54' E) is a bustling urban area located in northeastern Tamil Nadu. This is the capital of the state and is one of the four megacities of India. It is home of thousands of people, but at the same time, has some pockets of greenery still left – Guindy NP, IIT campus, Theosophical Society, Adyar Poonga, Vadnalur, Nanmangalam RF, Chengelpet, Tirukkalukundram hills, Neelankarai, Kovalam and Vadanemmeli beaches to name a few. It mainly experiences northeast monsoon and to some extent the southwest monsoon too. This city and its surroundings includes a variety of habitats such as hillocks surrounded by reserve forests to the southwest, coastal sand-dune vegetation along the east, scrub jungles surrounding lakes and ponds, tree plantations of both native and exotic species and extensions of villages and towns with concrete and semi concrete structures such as huts and *pucca* houses. These are in fact important habitats for some species of lizards that range in size from the insectivores nocturnal geckoes to the large endangered predatory monitor lizard. In this work, I summarize my

past field observations on lizards sighted in and around Chennai for over a decade. Sighted lizards were identified using field guides (Das, 2002; Daniel, 2002; Murthy, 1990; Tikader & Sharma, 1992).

A total of 16 species of lizards belonging to 8 genera and 5 families have been known from in and around Chennai. Three species were represented from the family Agamidae, five from the family Gekkonidae, four from the family Scincidae and one each from the families Chamaeleonidae and Varanidae. Many of these lizards are human-commensals and are very common. Such species include the garden lizard *Calotes versicolor*, the geckoes *H. parvimaclatus*, *H. frenatus*, *H. leschenaultii* and the skinks *Eutropis carinata*, *E. macularia*, *Lygosoma punctatum*. Other lizards are rare or range-restricted, uncommon species. Such species include the agamids fan-throated lizard (*Sitana ponticeriana*) and rock agama (*Psammophilus blandfordanus*), the rock gecko (*Hemidactylus graniticolus*) and reticulated gecko (*Hemidactylus reticulatus*), sand skink (*Eutropis bibronii*) and white-dotted garden skink (*Lygosoma albopunctatum*), chameleon (*Chamaeleo zeylanicus*) and monitor lizard (*Varanus bengalensis*). The monitor is endangered and listed in Schedule I of the Indian Wildlife Protection Act (1972).

Table 1: Lizards known from Chennai, Tamil Nadu and places of my sightings.

Sl.No.	Species	Microhabitat	Places
1	Fan-throated lizard <i>Sitana ponticeriana</i>	Terrestrial, on sand dunes and near open / rocky areas	Neelankarai, Guindy NP, Nanmangalam RF
2	Garden lizard <i>Calotes versicolor</i>	Tree and bush-dwelling, more near human habitations	Throughout
3	Rock agama <i>Psammophilus blandfordanus</i>	Rock-dwelling, only near rock boulders / hillocks	Chengelpet, Vandalur, Tirukalukundram
4	House gecko <i>Hemidactylus frenatus</i>	Arboreal, on building walls, avenue trees	Throughout
5	Bark gecko <i>Hemidactylus leschenaultii</i>	Arboreal, on tree trunks, also near human settlements and in gardens	Guindy NP, IIT campus, Adyar Poonga
6	Spotted house gecko <i>Hemidactylus parvimaclatus</i>	Arboreal, on building walls, avenue trees	Throughout

7	Termite-hill gecko <i>Hemidactylus triedrus</i>	Terrestrial, under logs, stones, within mounds and in burrows	Guindy NP, IIT campus, Theosophical Society
8	Reticulated gecko <i>Hemidactylus reticulatus</i>	In rocky outcrops, under stones	Chengelpet, Vandalur, Tirukkalukundram
9	Rock gecko <i>Hemidactylus garniticolus</i>	In rocky hillocks, within caves and rock crevices	Tirukkalukundram, Chengelpet hillocks
10	Sand skink <i>Eutropis bibronii</i>	Terrestrial, on sandy shores only near coastline, in the bushy creepers	Neelankarai, Kovalam,
11	Grass skink <i>Eutropis carinata</i>	Terrestrial, on leaf litter, ground vegetation and also human settlements	Throughout
12	Bronze skink <i>Eutropis macularia</i>	Terrestrial, on leaf litter, ground vegetation and also human settlements	Throughout
13	Snake skink <i>Lygosoma punctatum</i>	Terrestrial and often found under stones, leaf litter and ground vegetation	Throughout
14	White-dotted skink <i>Lygosoma albopunctatum</i>	Terrestrial, under stones, fallen logs, inside humus mounds	Velachery (see Daniels, 2002)
15	Indian chameleon <i>Chameleo zeylanicus</i>	Arboreal, found on plants and trees, rarely comes to ground	Guindy NP, Adyar Poonga, Vandalur, Nanmangalam RF, Tirukkalukundram, Chengelpet,
16	Monitor lizard <i>Varanus bengalensis</i>	Found in caves, rock crevices, burrows near farmlands, tree holes inside reserve forests	Nanmangalam RF, Guindy NP,

These apart, a very old report of the gecko *Hemidactylus scabriceps* from Adayar also exist (Tikader & Sharma, 1992). The monitor lizard being a top predator is an indicator of diversity of small vertebrates living in reserve forests here, that are protected by law. Southwest and northeast monsoon provided ideal conditions of temperature and food (mostly ground dwelling arthropods including insects) to the lizards and played a major role in the reproductive ecology and biology of different species of lizards. This work on Chennai regions' lizards could be considered as a platform for more intensive monitoring.

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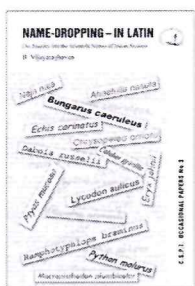
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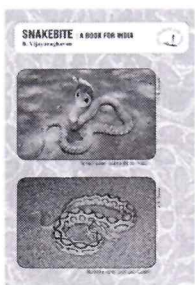
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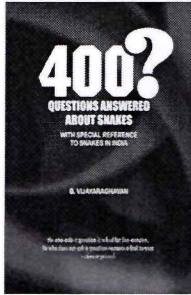
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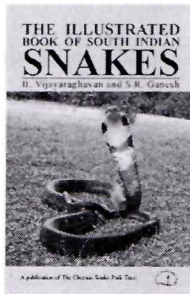
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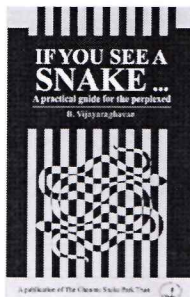


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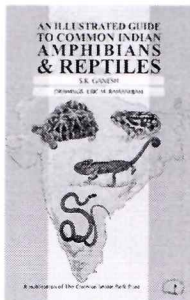


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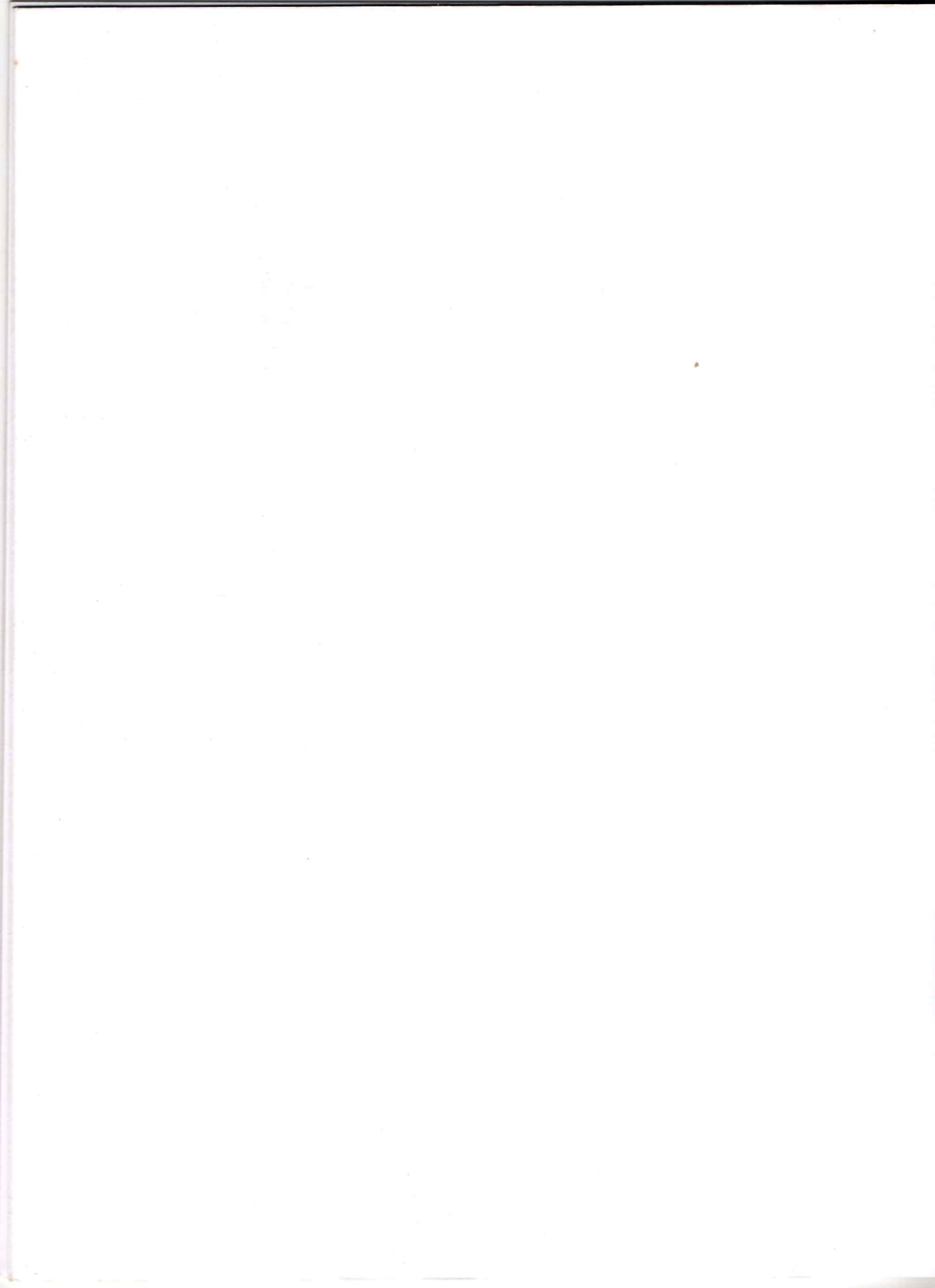
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Registered with the Registrar Office of Newspapers for India
(Ministry of Information and Broadcasting)
Registration No. TNENG/2007/19859
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Payable at Chennai.

Chennai Snake Park Trust
Rajbhavan Post, Chennai – 600 022. India.
Phone: 91-044-22353623
E-mail: cspt1972@gmail.com
Website: cspt.in

Printer : N. Arunachalam, Students Offset Services
Shop No. 3, Apex Chambers, 20, Thiagaraya Road, Pondy Bazar, Chennai - 600 017.
Publisher : R. Rajarathinam on behalf of Chennai Snake Park Trust. Published by Chennai Snake Park
Trust, Rajbhavan Post, Chennai - 600 022
Editor : Dr. S. Paulraj, IFS (Retd.)

