“TOADS ON ROADS”

EFFECT OF LINEAR BARRIER ON THE MOVEMENT ECOLOGY OF COMMON ASIAN TOAD

(*Duttaphrynus melanostictus*)

DISSERTATION SUBMITTED TO SAURASHTRA UNIVERSITY, RAJKOT, IN PARTIAL FULFILMENT OF THE MASTERS DEGREE IN WILDLIFE SCIENCE

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by

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What is the use of running when we are not on the right road?

-German proverb

For the love of toads

भारतीय वन्यजीव संस्थान
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CERTIFICATE

This is to certify that **Ms. Preeti Sharma** has carried out an original piece of research in partial fulfilment of Master's Degree in Wildlife Science of the Saurashtra University, Rajkot. The topic of her dissertation is **"Toads on Roads: Effect of Linear barrier on movement ecology of Common Asian Toad *Duttaphrynus melanostictus*".** The study was carried out under my supervision from December 2014 to June 2015. I hereby certify that this work has not been submitted for any degree to any university.

Date: 13 June, 2015
Place: Dehradun

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Love to my only companions in field.....my Moped and Bacchu!

Preeti Sharma

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EXECUTIVE SUMMARY

Amphibians in India are threatened by population decline and extinction. Conservation efforts are hindered by scanty knowledge on their movement pattern, habitat use and dispersal. Thus, I conducted first ever radio telemetry study on an Indian Amphibian species in Krishnan kovil, a village in Virudunagar district in Tamil Nadu state of India. The study was conducted over a stretch of 1.5 km on both sides of Kollam-Thirumangalam highway (NH 208). The objective was to know if there is any effect of road on the movement and population abundance of *Duttaphrynus melanostictus*, commonly known as common Asian toad. Reason for selecting a widespread perianthropic species is that such species can better indicate how gene flow is structured in an urban landscape. Total 9 adult individuals (5 ♀ and 4 ♂) were tracked ranging between 12 to 41 days from February 1 – April 9, 2015.

Findings of the study revealed that NH 208 has a significant effect (estimate 0.007589; standard error 0.003108) on linear displacement of toads that increases away from the road. However in case of village roads the linear displacements were higher near to the roads (estimate -0.01569; standard error 0.002977). This indicates that NH may be affecting the movement of amphibians as a potential physical barrier. However, village roads still seem to be permeable by the toads as they are using it for movement. Orientation data shows no significant orientation (Chisquare value 1.19, p value 0.55) of movement of the toads. However, field observations show that movement of toads with NH was always along or away but never across it. Home range estimates show that animal # 6 (NHR6M) used largest home range of 4262.92 sq. meters and showed maximum linear displacement in one night up to 333m.

Another aspect of the study was to determine effect of NH on population abundance of common Asian toad. I conducted mark recapture studies in one hectare plot at three sites. The abundance estimates are 228.97 (S.E. 14.09) individuals per hector for site 1, 125.59 (S.E. 9.68) individuals per hector for site 2, 365.94 (S.E. 46.85) individuals per hector for site 3. Abundance estimates at the 3 sites show an increasing trend with the increasing perpendicular distance from the NH. This indicates that the road may have an effect on the population of *Duttaphrunusmelanostictus*.

The study is limited to only one non breeding season (3 months), thus, is biased towards movement in less activity period. The study provides hitherto unknown information on toad movement with respect to linear barrier. Such studies are useful in developing realistic, predictive models useful for mitigation and restoration of dispersal corridors in a rapidly changing urban landscape. Results of this first ever amphibian radio telemetry study from India add to a new dimension in Indian amphibian research- beyond taxonomy.

KEY WORDS: Amphibian • Movement • Ecology • Radio telemetry • Mark capture recapture

1. INTRODUCTION

Amphibians (caecilians, frogs, and salamanders) are anamniotic tetrapod vertebrates currently represented by 7416 species worldwide (amphibian web, 2015). Amphibians are particularly sensitive to local environmental changes because they possess following traits: ectothermy, moist permeable skin, eggs without any protective cover, gill respiration in larval form, require both aquatic and terrestrial habitat to complete life history, a high degree of philopatry, relatively small home ranges, limited dispersal ability and extremely seasonal mode in reproduction. Habitat changes thus can modify amphibian community composition or seriously impact fitness in some amphibian species (deMaynadier and Hunter, 1995; Patrick et al 2006, Werner et al, 2007). However, mechanisms that cause such changes in amphibian population are poorly understood.

Amphibians are found in a great variety of ecosystems from tropical rainforests to barren deserts (Stebbins and Cohen 1995). Amphibian habitats are generalized to be confined to wetlands and other aquatic environments, but surprisingly, a large number of species are entirely terrestrial e.g., plethodontid salamanders and eleutherodactylid frogs (Stebbins and Cohen 1995). This diversity in habitat requirements between species and even between life-stages of the same species emphasizes that a simplistic approach to amphibian conservation can not address the serious threats being faced by amphibians.

The most serious threat being posed on amphibians is the changing landscape and loss of habitat. Habitat destruction, alteration and fragmentation are probably the most serious causes of current as well as future amphibian population declines and species extinctions (Dodd and Smith 2003).

Tools to trace the physiology, behavior and energetic status of free ranging animals are termed as Biotelemetry. The technique that involves receiver, sensors and radio-transmitter has enabled researchers to know, how a free ranging creature adapt in an environment. Since 1990's this technology was extensively used in case of mammals and birds and some large reptiles such as marine turtles that yielded information that has been challenging to collect historically from animals in their naturally associated environment. Biotelemetry, thus given us tools to know about aspects of life history strategies that could not be gathered in other techniques such as in drift fence, visual encounter surveys (Crump and Scott, 1994).

Large birds and mammals have received much attention from researchers studying home range size and space use. However, with the discovery of really small transmitters (1.5 to <2 gm) and advent of enhanced technology, radio telemetry was found to be suitable tool even for small and secretive species such as reptiles and amphibians. Extensive use of such technology to study movement pattern, home range, hibernation, energetic status in herpetofauna have been conducted in Europe, North America and Australia. Scanty studies so far made in Asia and none so far in South Asia on Amphibians.

So far in India, amphibian research is focused on aspects of systematics justified by the fact that documentation of the basic diversity is yet to be achieved. In this scenario, the use of advance technology such as radiotelemetry to understand the spatial ecology of small vertebrates is a new development. A deeper understanding of the pattern of habitat use and identifying the factors influencing these patterns are likely to uncover aspects of spatial ecology hitherto unknown for Indian amphibians.

As a result of rampant development many amphibian species with a formerly continuous spatial distribution are now turned into meta population, in which individuals likely to move among multiple, spatially separated patches (e.g. breeding sites, and foraging habitat); in either case movement between patches often necessitate crossing man-made barriers. Unless mitigation technologies and best-practices protocol for reducing herpetofaunal mortality and maintaining connectivity are implemented some species are likely to decline in fast developing areas. Population declines may be a consequence of persistence mortality, or due to reduced habitat connectivity that disable animals to cross them. Radio telemetry and mark recapture studies thus expected to provide information on mechanism of movement related to age, sex and environment that will enable us to generate information on population dynamics. This gap in understanding is increasingly problematic as more species and population face threats of decline and extinction due to global climate change, habitat fragmentation and change in land use. Especially amphibians where at least 43% of species worldwide are experiencing population decline which is highest among all vertebrates (Stuart et al 2004, Wake and Vredenburg, 2008).

Most amphibian species shows a patchy distribution restricted by availability of life history traits such as moisture and waterbodies. Thus, amphibians are considered as classical metapopulation models. However, some species possess ability to breed in various habitat types and uses human dominated landscape for population built-ups. These species might be expected to be distributed almost continuously and show panmixia. However, such extent of dispersal is dependent on landscape features that may hamper their dispersal activity.

My study species *Duttaphrynus melanostictus* is one such widely distributed perianthropic habitats. However, recent molecular studies have shown that population of this well adapted species showed great degree of genetic homogenization, low level of population genetic structure and differentiation (Janani, 2014). However, ecological factors leading to such genetic bottleneck is largely unknown and is the basis of this study.

2. STUDY SPECIES

The study species is one of the most common species which we see daily in our backyard, the common Asian toad, *Duttaphrynus melanostictus (*Schneider 1799).

Class: Amphibia (7416 species)
Order: Anura (6300 species)
Family: Bufonidae (576 species across 50 genera)
Genus: *Duttaphrynus* (29 species)
Species: *melanostictus*
Common names: Asian common toad, Asian toad, Black-spectacled toad, Common Sunda toad, Javanese toad.

2.1 Taxonomic note: Amphibians of the family Bufonidae Gray, 1825 is naturally distributed in all continents and major Islands (except Australia and Antarctica) (Dubois and Ohler, 1999). *Duttaphyrynus* provisionally contains the *Bufo melanostictus*, *Bufo scaber*, and *Bufo stomaticus* groups. The genus currently contains 29 species.

Duttaphrynus melanostictus (Schneider, 1799) is the type species of the genus and the type locality is not precise and is given as "India orientali". Schneider's (1799) description of the species is based on two specimen (syntypes) currently housed at Berlin Museum collection.

2.2 Distribution: Southwestern and southern China (including Taiwan and Hainan) throughout southern Asia from northern Pakistan, Nepal, and Bangladesh through India to Sri Lanka, Andaman Is., Maldives, and Malaya, Sumatra, Java, Borneo, and Bali, up to 2000 m in some places; introduced onto Sulawesi, East Timor, and Papua New Guinea (Frost et al 2015). It was known to be distributed below 1800m.

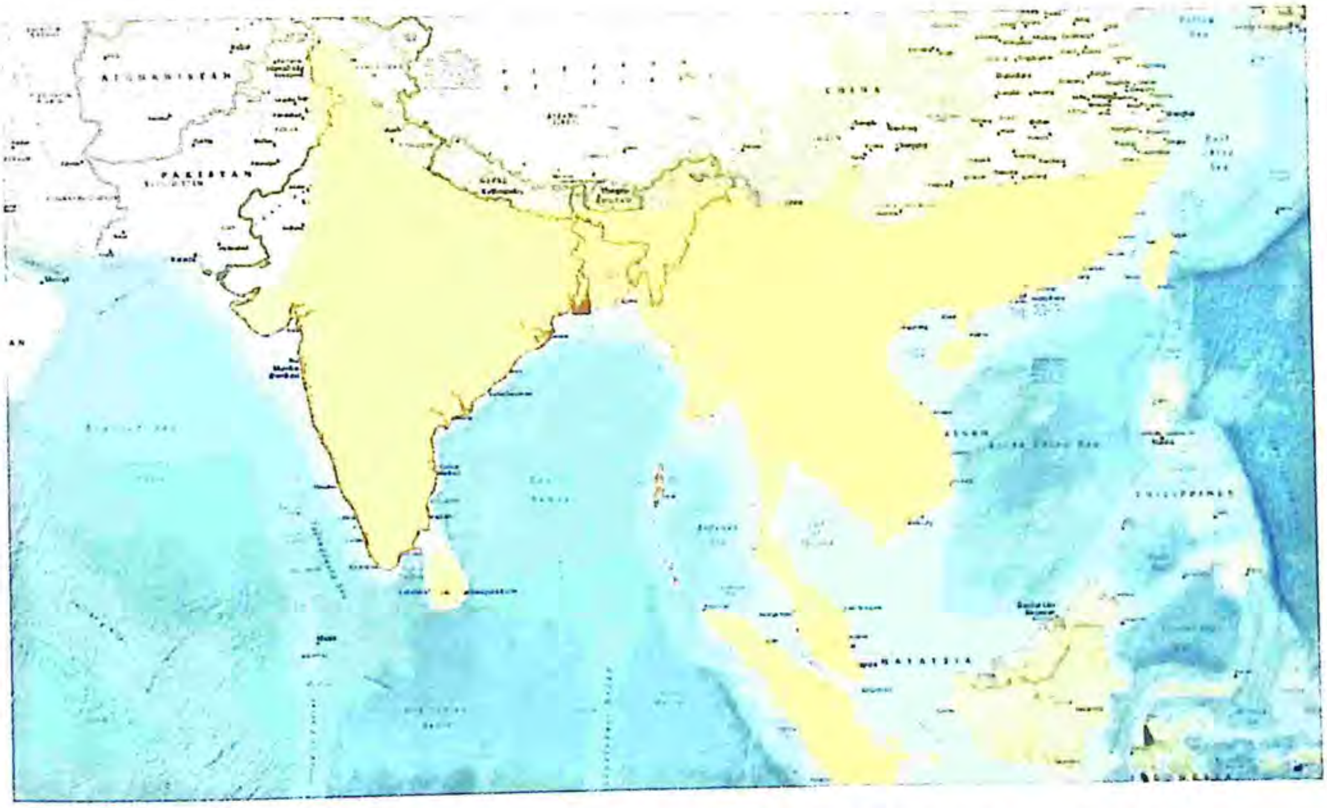

Figure 1 Distribution map of *Duttaphrynus melanostictus* (Schneider 1799).

2.3 Morphology and Biology: This is a large growing species of toad with a head distinctly wider than long. Snout is slightly pointed and densely arranged warts on the head and dorsum have black tips. A characteristic cranial ridge consisting of canthal ridge, preorbital and postorbital and supraorbital ridge. Supratympanic ridge are only indicated and the parietal ridges absent. Pupil horizontal, tympanum distinct and the round or vertical oval, contacting the posterior end of postorbital ridge. Finger tips are rounded and black. Relative finger length 2<1<4<3. Toes webbed with webbing formula I0-0II0-0III0-2 IV2-0V. Dorsal warts are small to medium sized. Dorsal warts are also used as individual identity (Daniel, 1994). Longevity of female toads is reported as twelve years in nature (Nayak et al 2006). Female lives longer than males. Both male and female attains sexual maturity in second year after metamorphosis. Maximum SVL 140 mm for a specimen from 140 mm (Mitchell and Zug 1986). Average weight of adult males vary between 50-55 gm (Gosh et al 1990) and female weight varies from 23-146 g. During this study maximum weight for the female was found to be 200 g and for male 120 g.

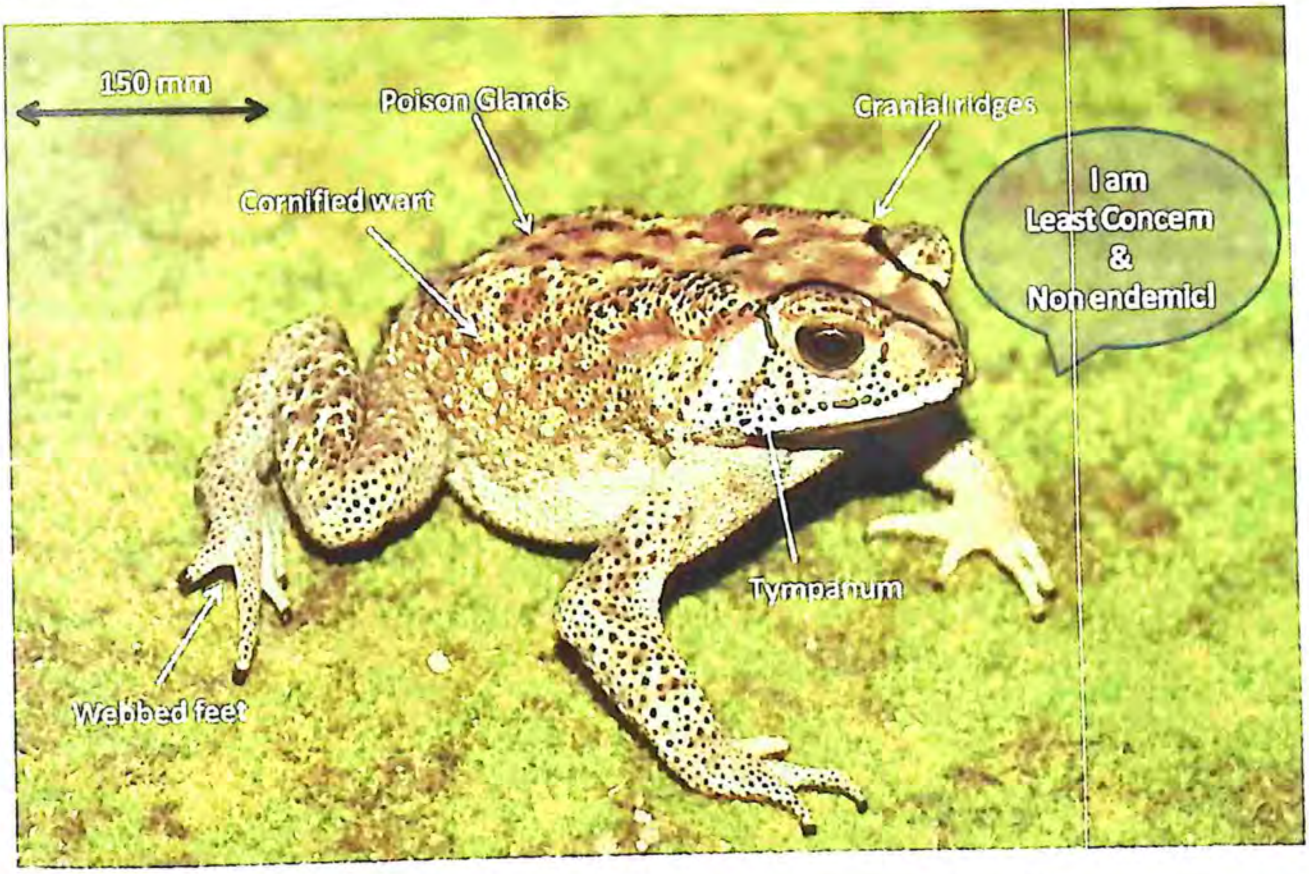

Figure 2 Study species: *Duttaphrynus melanostictus* (Schneider 1799)

Dorsal basic coloration ranges from yellowish olive through reddish to grey or brown with a pattern of irregular dark olive to blackish brown spots. Males during breeding season attain yellowish dorsal colouration and the vocal sc becomes orange colour. Sexual dimorphism is pronounced in terms of body size. Males also develop nuptial pads on the first two toes.

The species is perianthropic (Scheich and Kastle, 2002) found in wide variety of habitats such as clearings, near streams, paddy fields. It prefer open area and around human habitation. It plays an outstanding role in the control of mosquitoes and agricultural pests (Atwal, 1986). Activity is largely crepuscular and nocturnal with the exception of diurnal activities only during peak reproductive period. On a cultivated land in China Lazell and Wei-Ping (1987) observed a population density of 80 specimen/ha, 82% of them are females. Mating generally initiate in peak monsoon and varies in different places in India. In North India it is usually in the month of February and March. In the study are the peak breeding season is in September to November. Amplexus is axillary. Eggs are deposited in double strings and are fastened to aquatic plants. The clutch size varies from 1000-8000 eggs. Larvae hatch after 2-4 days. Metamorphosis happens within two months.

3. LITERATURE REVIEW

Amphibians are the most threatened group of vertebrates, with approximately one third of global amphibian species are now threatened with extinction (Stuart et al. 2004). Reasons for decline are identified as habitat loss, exploitation for food and medicine, UV-B radiation, introduced species, disease and climate change (Beebee and Griffiths 2005). Understanding the cause and extent of the declines requires an understanding of ecological and biological factors that might affect the movement and dynamics of a species.

3.1 Movement ecology: Movement is a fundamental aspect of an organisms' ecology (Nathan et al, 2008) that defines spatial and temporal scale of its interaction with other organisms, resource and environment (Clobert et al 2001, 2009). An animal move either to disperse to new sites or settle near natal site, which has strong consequences for individual fitness, gene flow, natural selection, adaptation, population persistence, metapopulation dynamics and species distribution (Knowlton & Graham, 2010). Despite the importance of movement to the persistence of species, there are considerable gaps in our understanding of movement processes (e.g., Bonte et al., 2012; Clobert et al., 2009; Ronce, 2007). Funk et al, (2005) studied the dispersal in frogs and related their vulnerability to habitat fragmentation. Huste et al, (2006) studied movement and site fidelity of threatened Natterjack toad and Miaud et al, (2000) studied terrestrial movement of the same species. Series of studies being made to understand the dynamics of fragmented Amphibian population that often represent classical metapopulation in a landscape where they were once widely distributed. Understanding the interaction between such populations is key to understand the dynamics (Waples & Gaggiotti, 2006; Baguett & Van Dyck, 2007, Ribeiro et al 2011) and thus prevent extinction of local population of the species. Landscape connectivity refers to functional (how dispersal is affected by landscape structure and elements) and structural connectivity (spatial configuration of habitat patches in the landscape, e.g. vicinity or presence of barriers, Taylor et al, 1993) and thus crucial in understanding functioning of metapopulation. Thus, dispersal is a key behavioural trait, constituting the focus of functional connectivity (Clobert *et al.*, 2001; Taylor et al, 2006). A review of mark-recapture studies on 53 anuran species revealed that dispersal distance of 56% of species was less than 1 km, but that of four species exceeded 10 km (Smith & Green, 2005). The longest documented movement of a marked amphibian amounted to 34 km in a subadult *B. fowleri* (Smith & Green, 2006), almost equalling the annual range expansion of 55 km of *B. marinus* in Australia (Phillips *et al.*, 2007). These observations have modified our view on amphibians as sedentary ectothermic vertebrates. Studies also shown that dispersal distances do not seem to be sex biased, but those of juveniles are larger (Funk et al., 2005) or equal (Smith & Green, 2006) compared with adults. A Kernel density estimate for 13 temperate zone amphibians demonstrated that 50% of individuals stayed within a radius of 93 m around the breeding site (Rittenhouse & Semlitsch, 2007).

3.2 Radiotelemetry: Radio telemetry is recently used to study the movement and habitat use of terrestrial and aquatic frogs. Johnson et al, (2007) studied spatial distribution in *Hyla versicolor*. McGarrity and Johnson (2010) recorded microhabitat characteristic and habitat use by invasive *Osteopilus septentrionalis* in Florida. Grafe (2011) has delineated movement pattern and homerange size in river frog (*Limnonectes leporinus*) from Sundaland. Sinsch (2012) radio tracked natterjack toad to determine the connectivity of local amphibian population. Anuran amphibians are constrained by Physiology, shape and size of their body. External attachment although is challenging but more extensively used in amphibian studies (Richter et al, 2001, McGarrity, 2010 and Johnson, 2010). In India radio telemetry studies are available in many mammalian taxa and birds (*see* Habib et al 2014). However, So far no radio telemetry studies being made on amphibians in India.

3.3 Metapopulation ecology: Population is an evolutionarily significant unit of biological conservation. However, interaction between of local population of a species is crucial for understanding of metapopulation dynamics (Waples & Gaggiotti, 2006; Baguett & Van Dyck, 2007, Ribeiro et al 2011). Metapopulation ecology of amphibians and their conservation require information on functional and structural landscape connectivity (Safner et al 2011). Migratory capacity (Sum of all movement between two successive breeding periods) of an amphibian species may determine its ability to disperse, colonize or re-colonize breeding sites and to maintain genetic integrity among local population.

3.4 Habitat ecology: Microhabitat selection likely to vary according to season and along a gradient. Microhabitat selection may vary as daily, breeding or during hibernation. Homing ability was reported earlier in Anuran species and particularly in stream frog species (Grafe, 2011). In such studies it was shown that each individual returned to its core activity zone after long migration.

4. STUDY AREA

The study was conducted in the Krishnan Kovil village in Virudhunagar district, Tamil Nadu. Srivilliputhur is located at the foot hills of the Western Ghats. The average elevation of the area is 137.2 meters above mean sea level. It receives scanty rainfall with an average of 811 mm (31.9 in) annually, which is lesser than the state average of 1,008 mm (39.7 in). The area receives scanty rainfall on the onset of South west monsoon in June and lasts up to August. The area also receives North East monsoon in the months of October, November and December. Thus, this particular site was selected keeping in view the breeding and post breeding time of common Asian toad in this area. Also there is prior information available on the genetic bottleneck of Duttaphrynus melanostictus in the study area (Janani 2015).

There are intact forests in this area located on the eastern slopes of the Western Ghats. Nearest protected is grizzled squirrel Wildlife sanctuary. These forests are rich in biodiversity consisting of varieties of flora and fauna found along the mountain slopes.

The Srivilliputhur municipality maintains a total of 54.576 km of roads. The town has 15.63 km (9.71 mi) concrete roads, 38.526 km (23.939 mi) bituminous roads and 0.4 km (0.25 mi) earthen roads. The national highway (NH 208) connects Madurai and Kollam passes through the town and connects surrounding urban centers like Rajapalayam and Tenkasi. According to 2011 census, Srivilliputhur had a human population of 75,396. As of 1994, a total 255.11 ha (45%) of the land in Srivilliputhur was used for residential, 17.89 ha (44.2 acres) (3%) for commercial, 15.04 ha (3%) for industrial, 30.808 ha (5%) for public & semi public, 12.677 ha (2%) for educational and 238.61 ha (24%) for non urban purposes like agriculture and irrigation.

4.1 Intensive study site

My intensive study sites were along NH-208; the Kollam-thirumangalam highway on both the sides of the road (Figure 2). A stretch of 1.5 km was chosen to conduct the study. Three plots were selected at 0 meters, 150-200 m and 600 m respectively from the NH-208. A list of geo-coordinates of the study site provided in appendix (1).

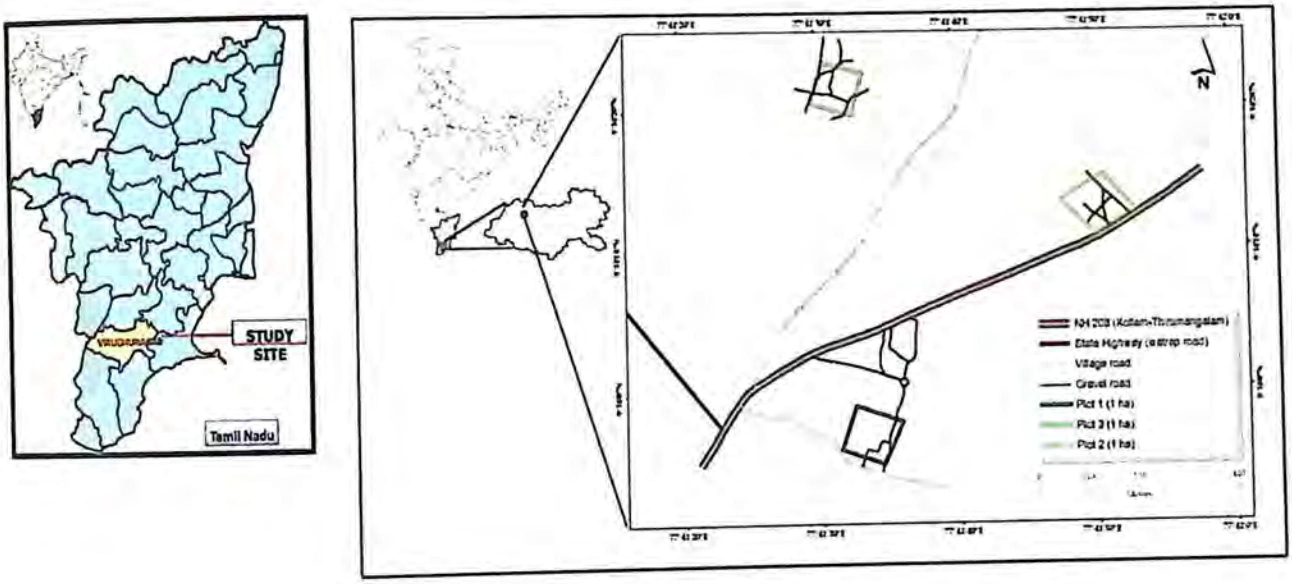

Figure 3 Map of Intensive Study Area in Krishnan Kovil, Tamil Nadu showing three study locations

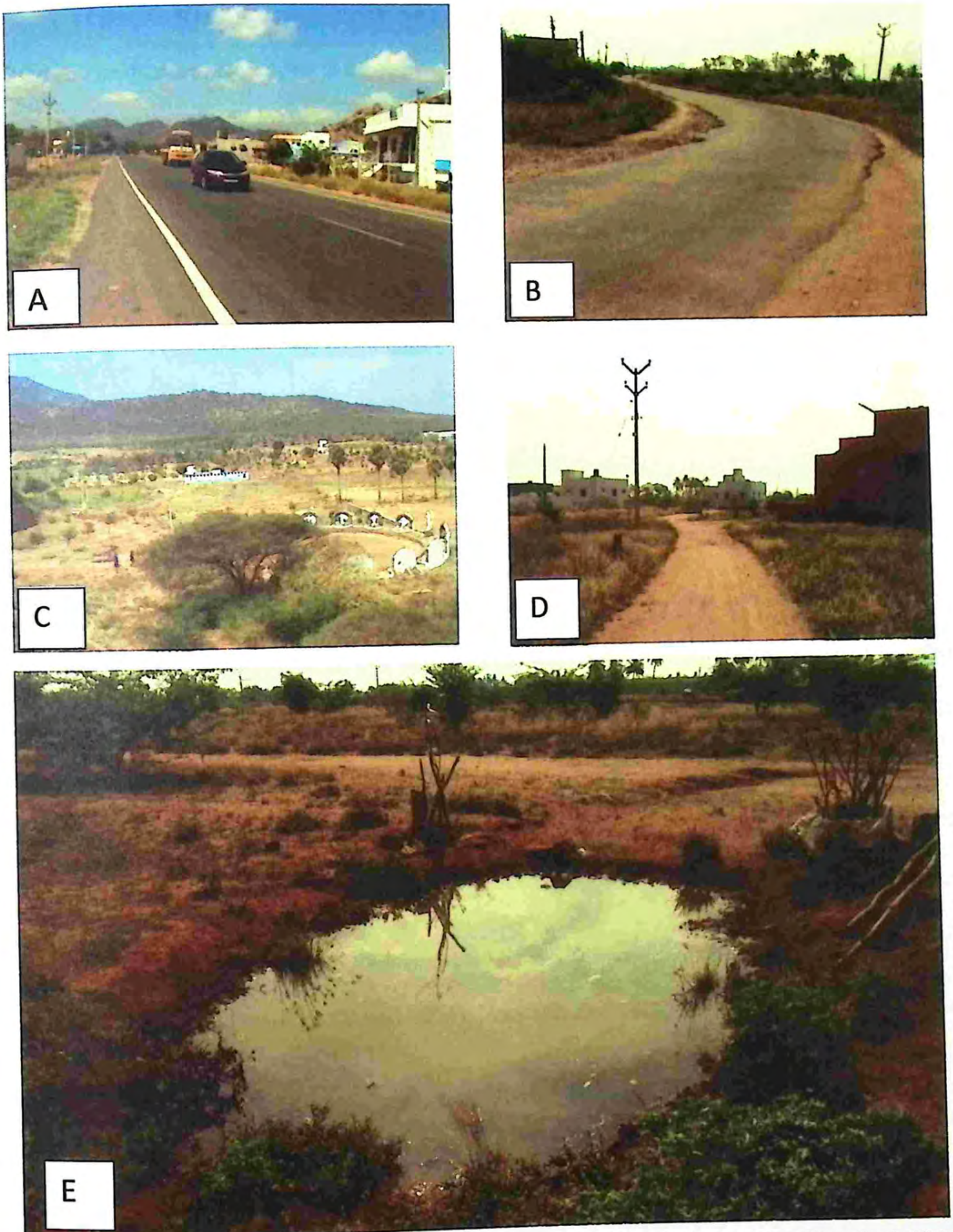

Fig 4: A. NH 208, B. Village road, C. view of typical landscape, D. Mud road, E. a typical toad breeding habitat

5. JUSTIFICATION OF THE STUDY

Urban landscapes are characterized by species that we often see and refer as "human commensal". These species are at zenith of their evolution and adapted themselves quite well in a modified landscape, yet few species or groups showed remarkable decline (e,g, vultures, house sparrow). We know little about such enigmatic decline. We need to know basic ecology, spatial behavior and movement patterns of those species to save them. Having such information in a landscape which is undergoing continuous change is crucial to understand urban ecology.

To gain information on the microhabitat use, home range and movement of a species, it is often necessary to remotely track individuals in the field. Radio telemetry is commonly used to track amphibians, but can only be used on relatively large individuals (Rowley and Alford 2007)

Habitat fragmentation due to changes in the landscape is a factor that affects meta-population dynamics and patterns which is of considerable importance to biodiversity conservation. The study thus, is justified on the ground that hitherto no information exists on movement any Indian amphibian species. This biological indicator group (Welsh, and Ollivier, 1998) is grossly overlooked in any environment impact assessment study. The dynamics of such fragmented population needs to be understood to development mitigation measures.

Population genetic study on *Duttaphrynus melanostictus* showed genetic bottleneck in population separated by roads (Janani, 2014). However, my study showed that the average linear displacements of the toads along and away from the road are almost the same. The results however, show a response of the toads of whether or not to move if they are along or away from the road. This decision may also be due the fact that either the NH is an absolute barrier for the species or the toads are behaviorally avoiding the roads.

Why a Common species??

Experimentation with rare animal is difficult and they generally face local extinction with urbanization. The common Asian toad appears to be a good model in understanding the impact of linear barrier on an amphibian species as it is widely distributed in human dominated landscapes that are now fragmented for their safe movement.

a. It may Provide measurable response (having biphasic life and thus sensitive to the disturbance or stress but do not go extinct readily).

b. Response may reflect the whole population/community/ecosystem response.

c. Adequate local population density, common, relatively stable despite moderate climatic and environmental variability.

d. Life history well known, taxonomy stable and easy and cheap to survey.

Since the species is widely distributed and least concerned, radio-telemetry and mark-recapture studies were easy to do and a considerably fair amount of data set was obtained to understand how linear barriers operate in populations. This otherwise would not have been possible for any such study on a rare species.

Being a close associate to humans Common Asian Toad serves the purpose best since it can be used as best role model to understand how gene flow is restricted in an urban landscape. Such studies can then further be reciprocated in many important amphibian habitats of India facing similar crisis.

6. RESEARCH OBJECTIVES

I. To determine how linear barrier such as roads influence movement pattern of common Asian toad.

The home range and the maximum distance moved should give some idea how much a toad move. The orientation of animal with respect road should provide us clues about avoidance of the linear barrier. We also expect changes in the maximum linear distance moved and the number of switches in direction made in these sites. Since the animals are tagged at varying distances from the road, the impact of linear barrier on the movement pattern could be quantified. The data collected will also help identify hotspots of toad activity and thereby areas where crossings can be facilitated.

II. Understand the influence of linear barrier on population density of common Asian toad.

The linear barrier would have both direct and indirect effects on the population, i.e., direct effect is the physical barrier on movement and dispersal, the indirect effect is noise, pollution and modification of the habitat for the toads and impacting their population. In the case of the toad, the population estimation at different distances away from the road will result in population size or density, age class distribution and sex ratios. The direct effect of the linear barrier would result in accumulation of small-body size sub-adult individuals increasing density near the road and change the population structure; while away from the road, the same age group will be freely dispersing, resulting in fewer recaptures of the age group. Indirect effects such as noise, light and pollution would also alter the population towards reducing reproductive success close to the roads.

6.1 Hypothesis for objective I

Road may be acting as a barrier for an otherwise equilibrium meta-population of common Asian toad.

Collecting information about how different organisms move in relation to landscape structure and resource availability will help identify drivers of population decline. Thus, I am interested to know;

A) How far an individual toad moves i.e., daily, weekly and seasonally (taking into account only one season during the study period) with respect to road?
B) Looking at the movement pattern, if it is along or across the road?
C) To determine the home range of male and female toads? Are there any site fidelity and variation in movement of male and female?

6.2 Hypothesis for objective II

Population density of toads is directly proportional to the increasing perpendicular distance from the road.

A. What is the population density of toads at different distances from the road?
B. If there is any trend in population densities observed at various distances from the National highway.

7. METHODOLOGY

7.1 Objective I – Study design

7.1.1 The Harness

Tag attachment can alter the behaviour of amphibians (Rowley and Alford, 2007). Thus, I designed a simple and light weight harness using a Teflon tube of 1mm internal diameter and 2 mm outer diameter.

Figure 5 Harness; Radiotransmitter attached to leather piece and teflon tube

The harness weighed less than 5% of the body weight of each animal tagged. I used a leather piece of 4 cm as base of transmitter fitting and put it around the Teflon tube using araldite glue. The transmitters were attached to the leather piece using epoxy glue. An elastic thread piece was passed through the Teflon tube and was used to tie it to the waist of the toad.

7.1.2 Radio Transmitters

I used 6 VHF Implant-glue on transmitters (Model R1640) of Advanced Telemetry Systems to conduct the radio telemetry study on the study species.

Figure 6 *Duttaphrynus melanostictus*; adult femlae with radiotransmitter attcahed to the waist

Every VHF transmitter had a fixed frequency to which it responded. I used radio transmitters of the following frequencies:

151.440; 151.460; 151.480; 151.500; 151.520; 151.540

A small magnet attached to the transmitter was a switch to put the transmitter in ON and OFF mode. The length of the transmitter was 22 cm that weighed 2 g with a battery life of 140 days.

Table 1 Specifications of VHF Radio transmitters used during the study.

Model	Description	
R1640	Implant-Glue on Transmitter (Toad)	
	Pulser R	30 ppm
	Pulser W	18 ms
	Weight	2 gr
	Battery Life	140 days
	Circuit	None
	Antenna Material	30# Black Steel
	Antenna Angle	8 in
	Frequency Xtal Type	Epson Standard

7.1.3 <u>Tracking equipments</u>

For the radio telemetry study I used a 3-elemnt collapsible Yagi antenna and an ATS receiver.

The receiver was connected to the antenna using a coaxial cord.

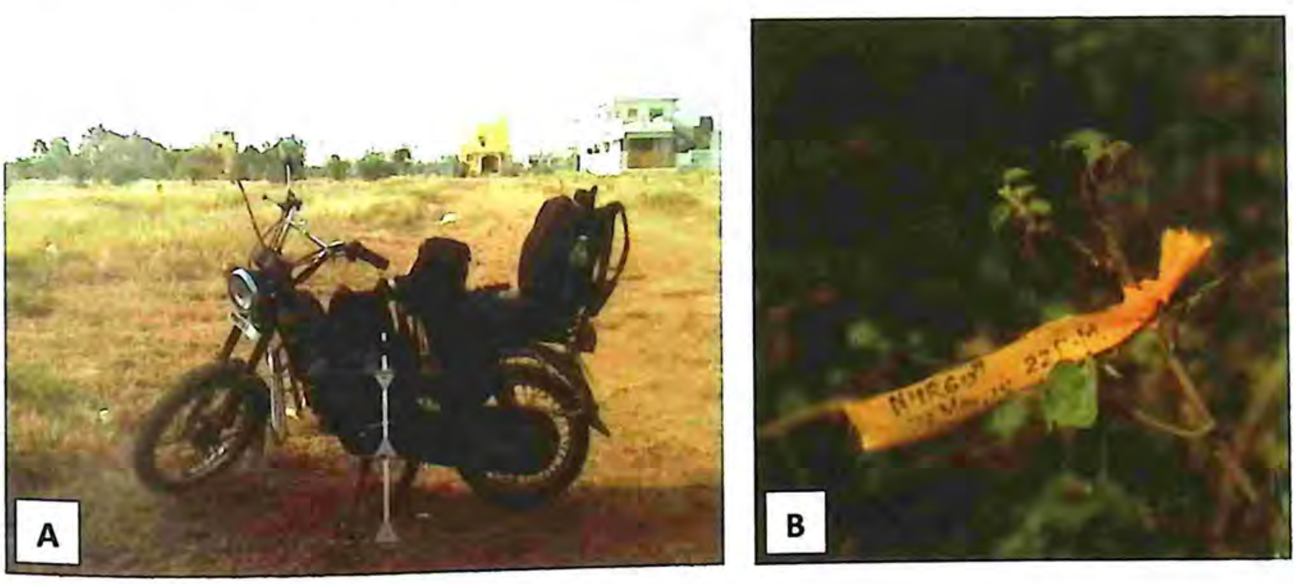

Figure 7 A. Field and tracking equipments; B. Marking of toad locations

7.1.A. Tracking

Initial Experimentation

On December 22, 2014 the first female toad was attached with the waist belt harness, kept under observation for a day and once it seemed comfortable with the harness, it was released the next day on 23 December, 2014. This adult female was tagged near a church area which was at the base of the hill. The female was tracked for five days and I lost the signal and the animal. This was the first transmitter (Frequency 1.523) to go missing. After that the transmitters were put on several individuals but there were problems regarding the design of the harness. The toads gave locations for a couple of days but it was found that the toads were putting the transmitters off their body by simply stretching their legs and removing the transmitter. After the transmitter were fallen off the toad's body it was a challenge to get the transmitters back since the location of the tagged individuals had ground vegetation of 20-60 cm, had rocky formations of huge rocks, human settlements. Searching a transmitter of 12.9 mm in width strained the eyes completely. Finally the Teflon tube was attached to a cotton thread along with the elastic thread and then tied to the waist to give more adherence of the harness on toad's body. This method worked till the end of the study period. Radio belt attachment is known to affect movement of some frogs (Blomquist and Hunter 2007). Therefore, I conducted a locomotory performance test where I movement of toad with harness and toad without harness in an experimental area within a fixed time.

The Initiation

A total of 9 individuals were tracked continuously, starting from February 1, 2015 to April 9, 2015, for different number of days at distances ranging from 44 m to 550 m from the National Highway. The telemetry was done in morning and evening hours so as to get minimum 2 locations in a day for every individual. To get more continuous movement data, I radio tracked several individuals for different number of days depending on the feasibility to conduct the telemetry. I used the ATS receiver, a 3-element folding Yagi antenna and glue on ATS transmitters attached to the waist belt harness (Fig 7A). Each toad was located using homing in method twice, once in morning and evening every day. At every location ribbons of different colors were put with the date, location ID and animal ID (Fig 7 B).

The weight of the transmitter was taken care so that it accounted for 3% of the body weight for females and 5.88% for males thus ensuring that the weight of the transmitter had no impact on toad movement.

If no signal was detected for a particular toad at a particular point, I walked 300-500 m in each direction away from that site, trying to reach elevated positions for maximum detection range. If no signal could be picked up (15-45 minutes depending on terrain and vegetation type), I began searching for the next toad. The time for tracking varied between 7 am to 11 am in the morning and 7 pm to 2 am in the evening. On locating each animal environmental parameters (air temperature, relative humidity, substrate temperature, soil pH and soil salinity), and habitat parameters like distance of the located toad from NH, village metal road, mud road, human habitation and water source. Only in February end and first week of March there were few showers and linear displacement was recorded up to 180 to 333 m for few individuals. Although it rained only for a few days but tracking was done during rainy days also. To get a natural movement behavior the toads were not disturbed. If the individuals were hidden or in very steep terrain, the waypoint was taken by getting as close as possible. The number of tracking days also included the Capture and Recapture days for every animal.

All the animals were released during evening hours exactly at their capture locations. There were no observations made on the day of release to avoid stress on the animals. For all the tagged toads the linear displacements was calculated from each location where the animal was located to the next location of the toad.

Figure 8 A. Transmitter fitted toad (NHR 2 ♀); B. A diurnal retreat among thorny vegetation of NHR 2 ♀

The total distance moved by each toad was calculated by summing up all the linear displacements moved. All the distances (using range finder and meter tape) and GPS locations have been collected from field and represent the true ground control points for every location.

7. 2 Objective II – Study design

A mark-recapture study was conducted at 3 sites at Krishnan kovil and Saptagiri Nagar from 11 February, 2015 to 7 April, 2015. The study was conducted along Kollam-Thirumanagalam highway (NH 208) in Tamil Nadu. Keeping in view the behavior of the Common Asian Toad (*Duttaphrynus melanostictus)* of being a habitat generalist, the selection of the 3 sites focused primarily on human dominated areas in the landscape e.g human settlements. The primary aim of the study was to look into the densities of the toads at varied distances from the NH 208. Based on the hypothesis designed it was speculated that the numbers of the toads should increase at increasing perpendicular distances from the NH 208. Thus, to understand the indirect effects of NH, mark recapture study was designed targeting the human settlements at different perpendicular distances from NH 208. The three sites were selected such that the first site (Krishnan kovil) was 250 m, second site (Saptagiri Nagar) 0 m and the third site (Krishnan kovil) was 600 m away from the NH. Site three acted as a control site ie. negligible direct and indirect effects from NH 208.

The location of the three sites ensured that each site was an independent unit and it was assumed that there was no movement of animals between the three sites. All the three sites had almost equal number of human settlements; 10 to 12 houses and at least one man made source of water.

Initial experimentation

To estimate the population densities there are various techniques designed like VIE tagging, Pit tags, toe clipping and others. (Heyer et al. 1993). To start with Visual Implant Elastomers (VIE) were deployed to mark the toads at different sites. VIE tags give the luxury to use the coloured material in different colours to make multiple combinations. The coloured material and the curing agent are mixed in a ratio of 1:10 and a silicone mixture is prepared. Various combinations can be made in order to give individual IDs to the toads. The IDs were made based on the geographical location, year of capture and the age class of the individuals. There have been a lot of studies where VIE tags have been used successfully for fishes, frogs and other species. But for this particular species (*Duttaphrynus melanostictus*) the main challenge being faced was marking on its rough warty skin which made the penetration of injecting syringe containing the VIE tag a real tuff task.

The smudging of colors was observed on the dorsal side which made the individual identification ambiguous. After the dorsal side of the toad did not serve the purpose, the animals were marked on the ventral side where the skin was smooth and it was comparatively easy to do the tagging. The toads were marked near the femur region and were kept under observation for a period of 12-24 hours. It was observed that although the animals were doing fine but the silicone mixture did not solidify and was found to be moving around in the belly of the toads. Thus, the option to put VIE tags on the toads was dropped and other methods were given a thought.

Due to the availability of PIT tags, it was the next possible option to mark the individuals. But the feasibility to use huge number of PIT tags and the cost involved with it strained the methodology and the duration of the study. Thus, the idea to do PIT tagging was also dropped.

7.2.A. Toe tipping

Mark recapture technique was conducted using the most reliable, least expensive and oldest method for the amphibian population surveys and ecological research (Donnelly et al. 1994). Toe tipping has historically been regarded as the most appropriate marking technique in which unique combinations of multiple digits are removed from the hands and/or feet when the animal is first captured, to allow recognition of each individual (Donnelly et al. 1994). A variety of coding schemes for use with toe tipping have been developed. For this study Donnelly (1989) scheme was deployed.

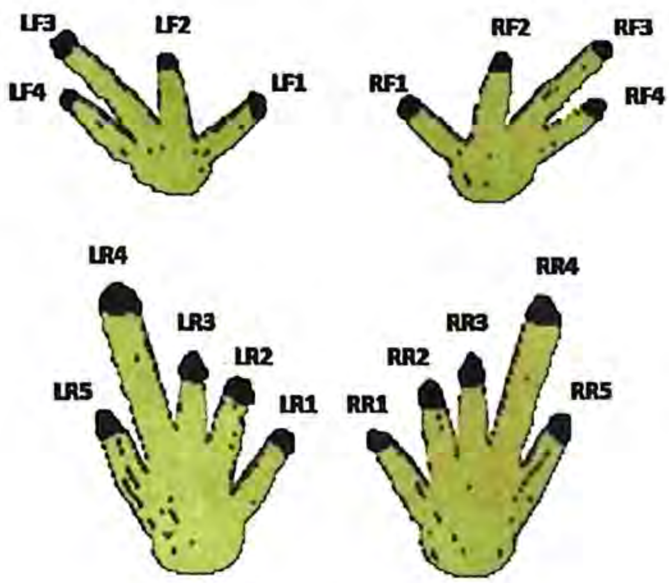

Figure 9 Clip code scheme for marking amphibians: After Donnelly (1989) scheme.

In Donnelly (1989) scheme, toes are clipped in sequence, but they do not correspond to numbers. The code associated with each toe refers to foot location (left, right, front, rear) and toe position (innermost=1, outermost= 4 or 5) (Donnelly et al. 1994).

7.2.1 <u>Sampling design</u>

A 100X100 m i.e. 1 Hectare plot was laid on all the 3 sites which was treated as an 'Intensive sampling area.' Each one hectare plot was then identified with various 'sampling units' based on the availability of congregations of individuals seen in the one hectare plot. This made the sampling easy and convenient. Continuous 8 days of capture sessions were carried out in each plot starting from February to April for the respective sites. After the end of 8^{th} Capture session gap of 5 days were given for the population to mix. This also helped reduce the biases during recaptures. The first recapture session was conducted on the 6^{th} day after the end of 8^{th} Capture session followed by three more recapture sessions at an interval of 1-2 days.

Table 2. Mark recapture sampling design

<table>
<tr><th colspan="8">Mark recapture Sampling design</th></tr>
<tr><td>DAY 1</td><td>DAY 2</td><td>DAY 3</td><td>DAY 4</td><td>DAY 5</td><td>DAY 6</td><td>DAY 7</td><td>DAY 8</td></tr>
<tr><td>Capture I</td><td>Capture II</td><td>Capture III</td><td>Capture IV</td><td>Capture V</td><td>Capture VI</td><td>Capture VII</td><td>Capture VIII</td></tr>
<tr><td colspan="8">Interval: 5 Days (DAY 9 – 13)</td></tr>
<tr><td colspan="2">DAY 14</td><td colspan="2">DAY 17</td><td colspan="2">DAY 20</td><td colspan="2">DAY 23</td></tr>
<tr><td></td><td colspan="2">Interval: 2 Days (DAY 15-16)</td><td colspan="2">Interval: 2 Days (DAY 18-19)</td><td colspan="2">Interval: 2 Days (DAY 21-22)</td><td></td></tr>
</table>

Mark recapture plot I

For the first plot, Krishnan kovil which was at a perpendicular distance of 250 m on right side of the NH 208, total of 118 individuals were marked at the end of 8^{th} capture session. At the end of the 4^{th} recapture session the number of individuals marked went up to 182.

Mark recapture plot 2

The second plot was at Saptagiri Nagar exactly along the NH 208 on the left side of the road. Number of marked individuals at the end of 8^{th} capture was 78 which went up to 101 individuals by the end of 4^{th} recapture session.

Mark recapture plot 3

The third plot was the control site which faced negligible effects from the NH 208. A total of 177 individuals were marked during the 8 capture sessions which then rose to 236 individuals that were marked in the III Plot.

Table 3: A detailed description of Capture and Recapture sessions for Plot I, II and III.

Sites	MRC PLOT 1			MRC PLOT 2			MRC PLOT 3		
Capture history	C	R	NC	C	R	NC	C	R	NC
C 1	11	_	_	21	_	_	29	_	_
C 2	15	_	_	16	_	_	23	_	_
C 3	36	_	_	11	_	_	23	_	_
C 4	12	_	_	2	_	_	25	_	_
C 5	18	_	_	4	_	_	30	_	_
C 6	13	_	_	10	_	_	9	_	_
C 7	4	_	_	10	_	_	27	_	_
C 8	9	_	_	4	_	_	11	_	_
R 1	61	33	28	27	23	4	43	22	21
R 2	33	21	12	20	16	4	37	27	10
R 3	51	33	18	37	28	9	42	34	8
R 4	39	33	6	21	15	6	63	43	20
Total Marked		182			101			236	

C= capture; R= recapture; NC= new capture

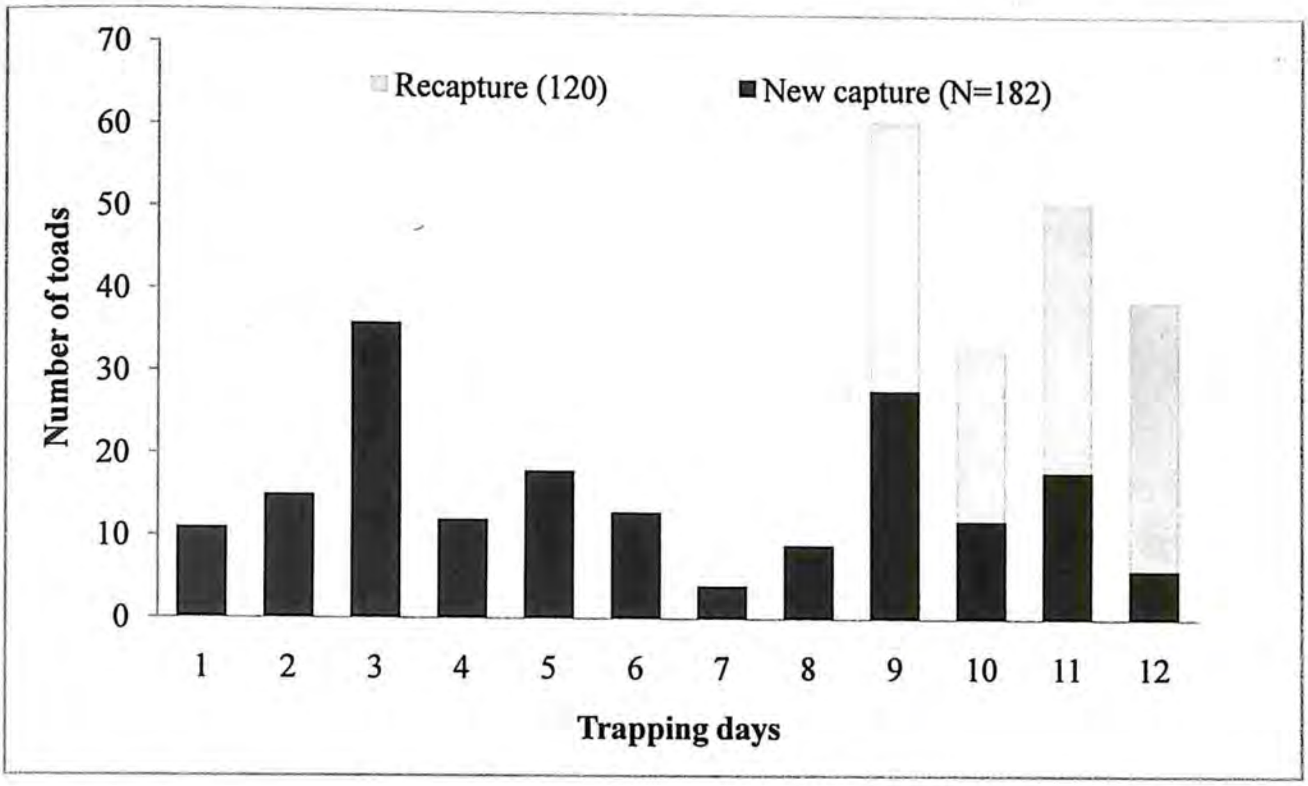

Figure 10 Capture history for plot 1; Krishnan kovil (200 m)

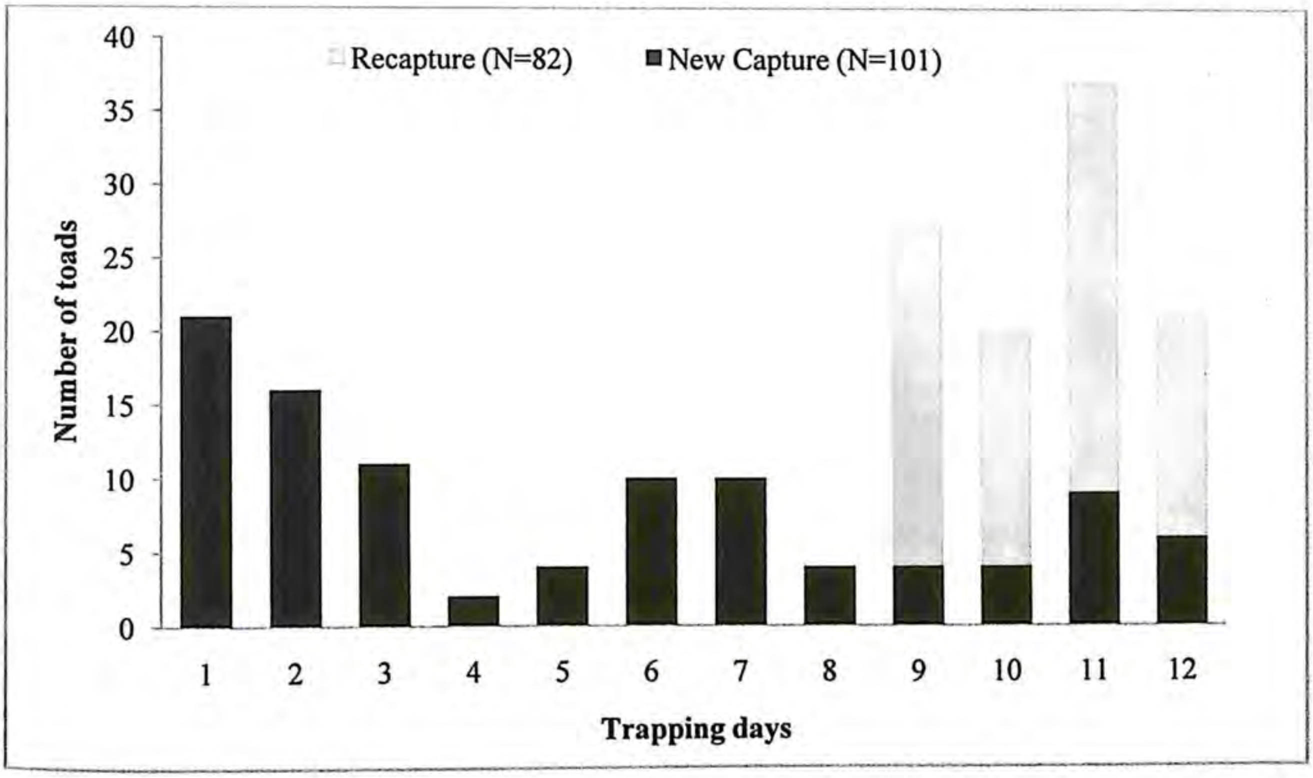

Figure 11 Capture history for Plot 2; Saptagiri Nagar (Zero meters)

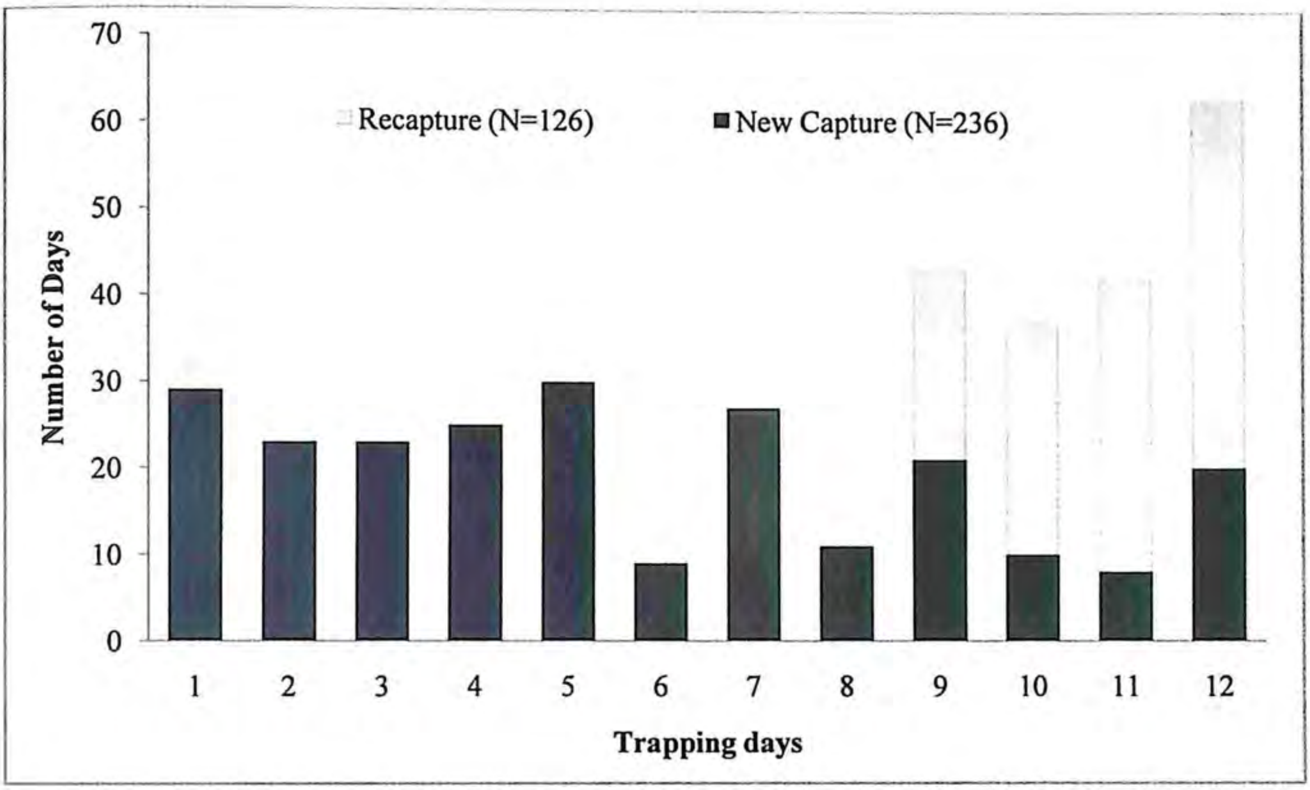

Figure 12 Capture history of Plot 3; Krishnan Kovil (600 m)

8.RESULTS

8.1 Objective I

8.1.1 Movement pattern

Total 9 individuals of *Duttaphrynus melanostictus* were tracked for a minimum of 12 to 41 days starting from February 1 –9 April, 2015. Although the number of individuals tracked and the tracking days may not represent the exact pattern but are important from the point of view of a base line information about general movement of a male and a female toad.

Table 4 Tracking summary of 9 Adult *Duttaphrynus melanostictus* tagged with radio transmitters

Toad ID	SVL (mm)	Weight (g)	Sex	Tracking days	Locations	Distance (from capture location)		
						NH 208	Village metal road	Mud road
NHR 1	93.68	141	F	15	25	134 m	206 m	12 m
NHR 2	100.1	200	F	15	25	219 m	90 m	4 m
NHR 3	80.89	58	M	17	28	134 m	208 m	20 m
NHC 4	95.86	100	F	37	60	550 m	285 m	6 m
NHR 5	96.88	100	F	14	23	245 m	42 m	1 m
NHR 6	93.33	60	M	41	80	257 m	29 m	9 m
NHR 7	83.45	55	M	12	9	44 m	206 m	21
NHL 8	81.51	51	M	29	56	63 m	355 m	3 m
NHL 9	99.46	106	F	29	56	112 m	337 m	2 m

8.1.2 Home Range

For the nine individuals that I tracked, the maximum home range observed (Minimum convex polygon 47557.10 sq m) was for adult male NHR 6 ♂ who was tracked for a period of 41 days. However home range between males and females differed since males showed high vagility than females. The maximum home range estimates among the females were of NHL9♀ (Minimum convex polygon 5076.01)

Table 5 Home range estimates (MCP; 100%) for nine tagged toads

HOME RANGE ESTIMATES

Individual_ID	MCP (sq m)
NHR1 ♀	591.57
NHR2 ♀	1394.7
NHR3 ♂	3030.6
NHC4 ♀	3288.3
NHR5 ♀	219.71
NHR6 ♂	47557.1
NHR7 ♂	1637.17
NHL8 ♂	679.21
NHL9 ♀	5076.01

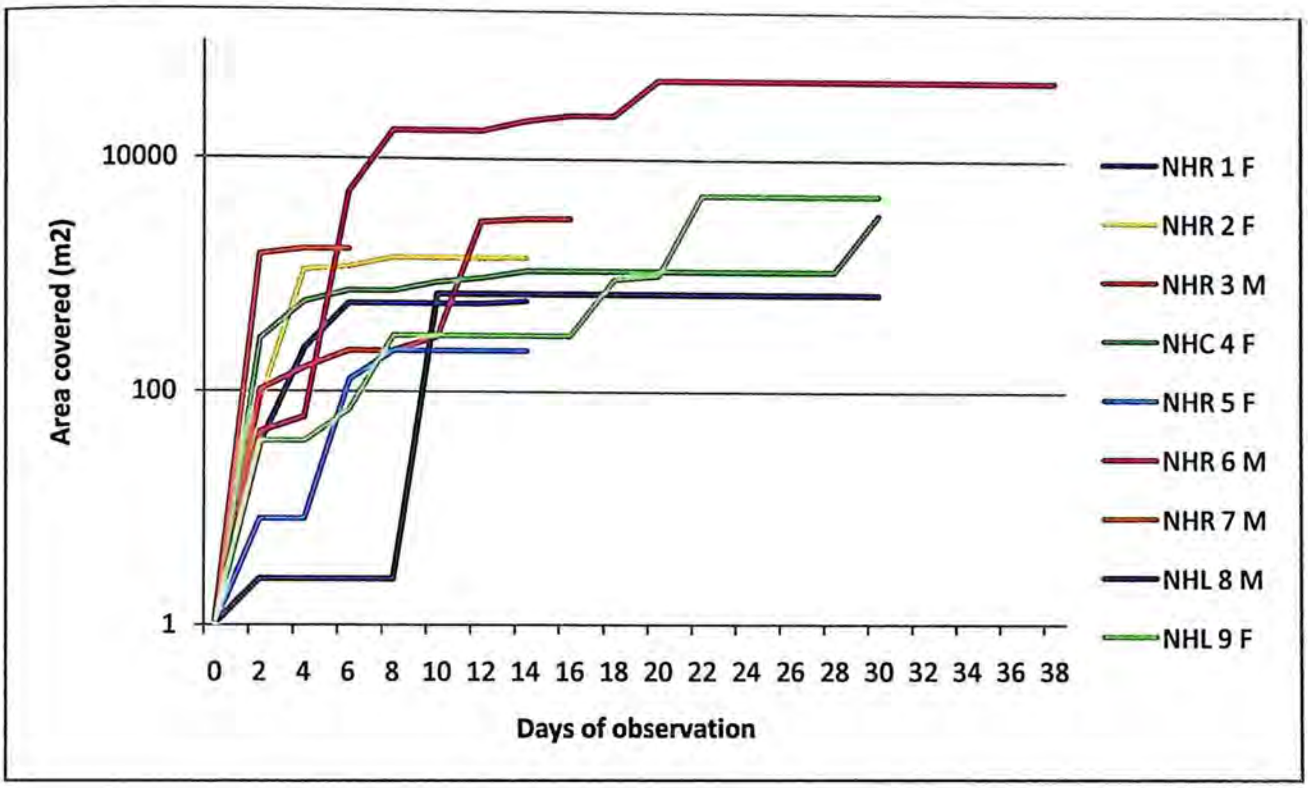

Figure 13 Cumulative area covered by *Duttaphrynus melanostictus* with increasing number of observations

Home range for all the 9 tagged individuals was calculated using Hawths tool in ArcGIS using Minimum Convex Polygon (MCP; 100%). The movement pattern for NHR 1♀, NHR 2♀, NHR 3♂, NHC 4♀, NHR 5♀, NHR 6♂, NHR 7♂, NHR 8♂ and NHL 9♀ are represented in the graphs below.

The star symbolizes the start point for the individual (initiation of telemetry), the diamond depicts the end point which marks the end of telemetry. The waypoints or the locations at which the toad was located is depicted by green dots. The black lines are showing the movement pattern of the toad. The bold black lines depict the maximum area covered by the toad using Hawths tool in ArcGIS using MCP at 100%. The location of the toad with respect to the NH 208 can referred from the inset.

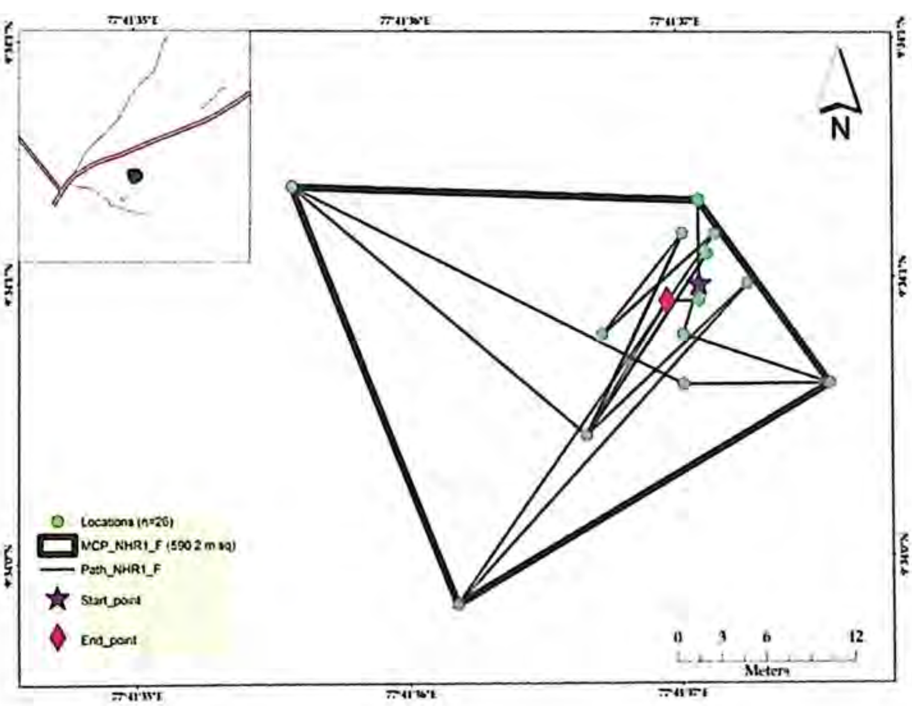

Figure 14 Movement pattern of NHR 1♀

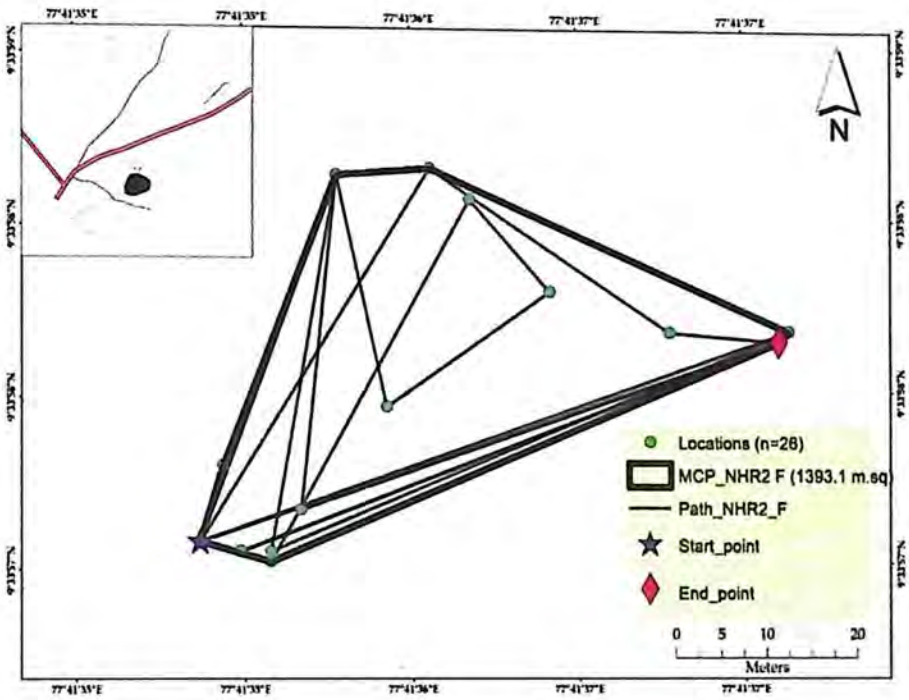

Figure 15 Movement pattern of NHR 2♀

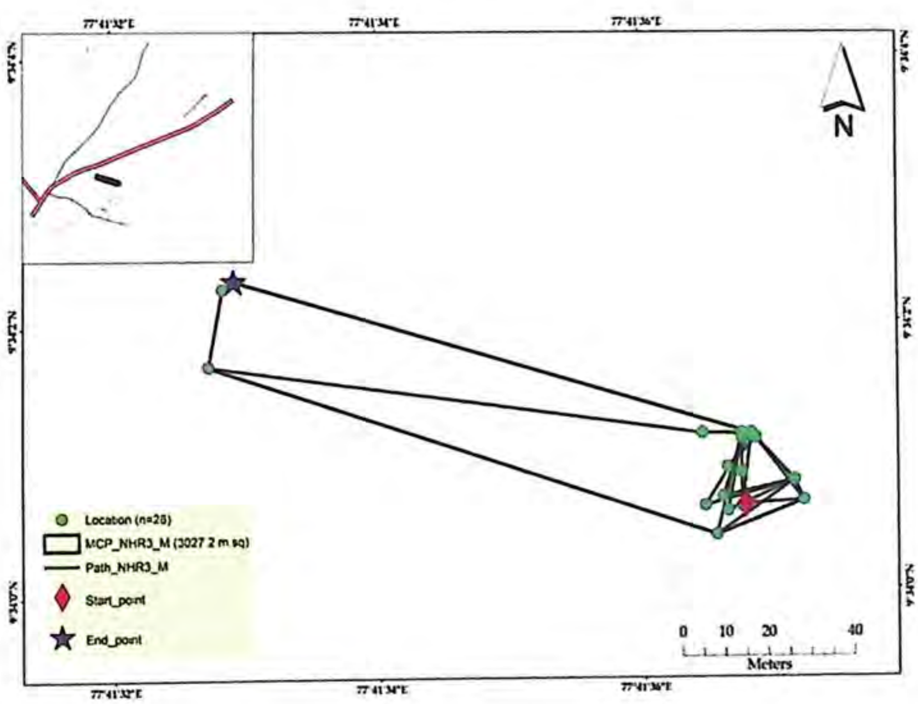

Figure 3 Movement pattern of NHR 3♂

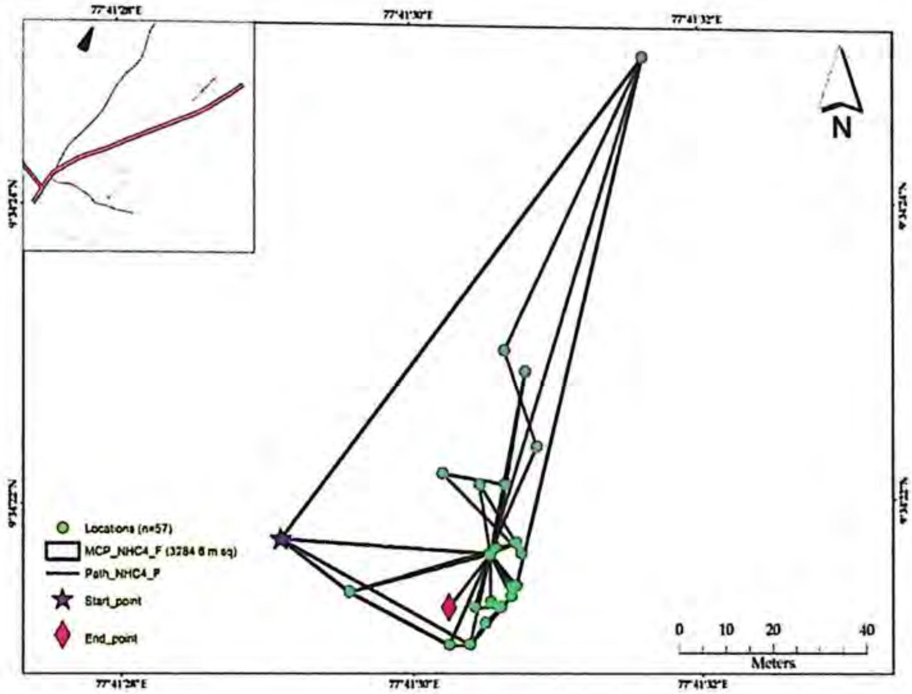

Figure 47 Movement pattern of NHC 4♀

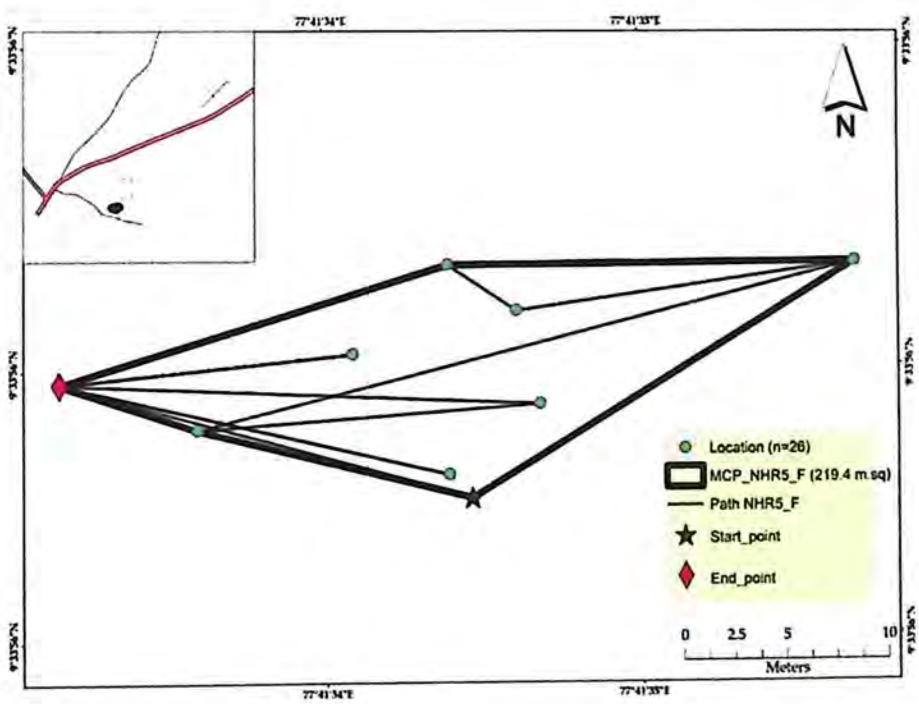

Figure 58 Movement pattern of NHR 5♀

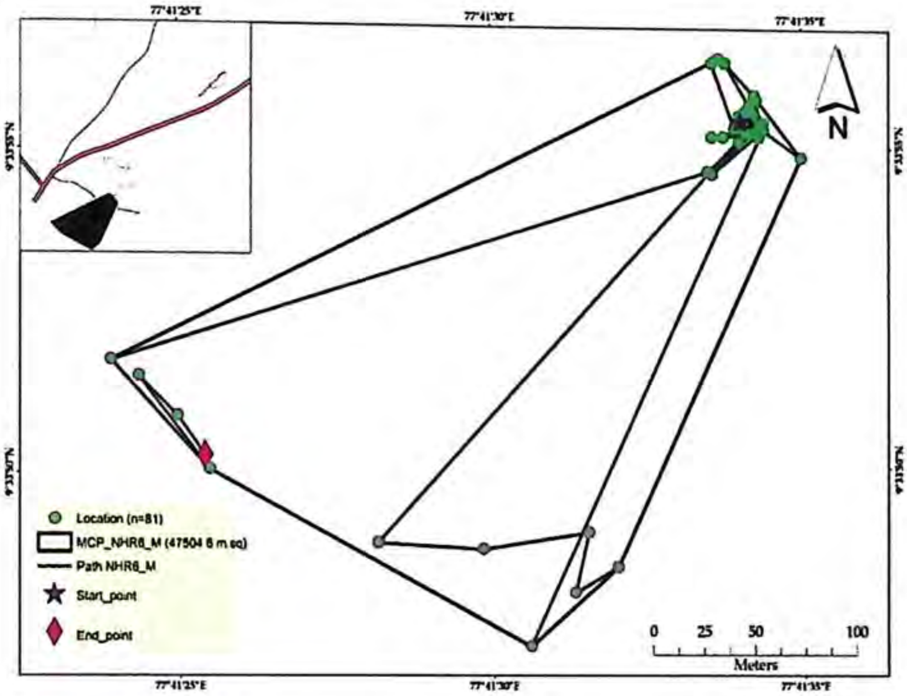

Figure 69 Movement pattern of NHR 6♂

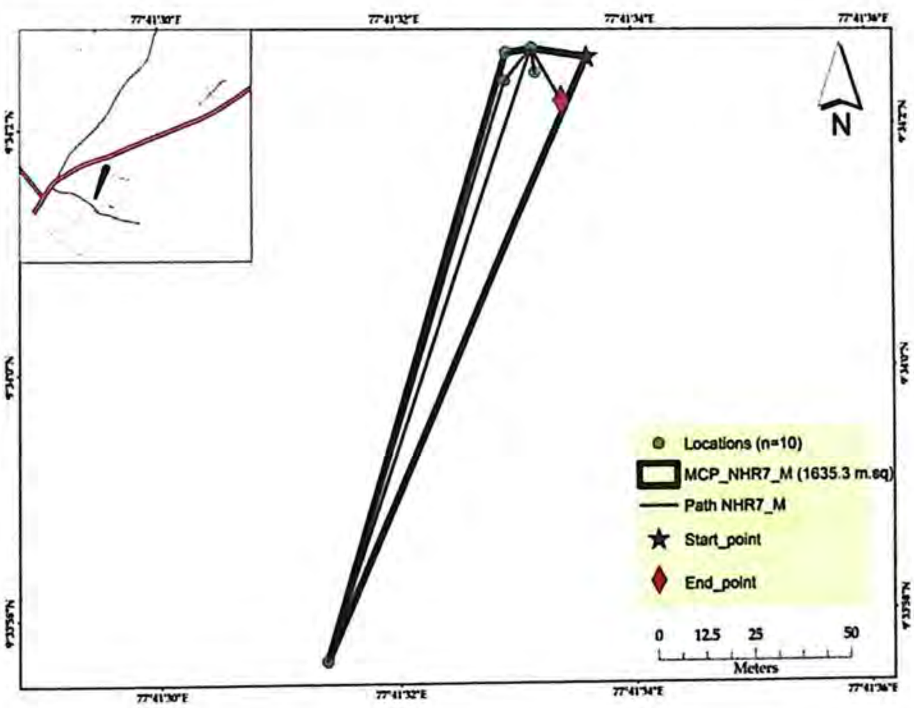

Figure 20 Movement pattern of NHR 7♂

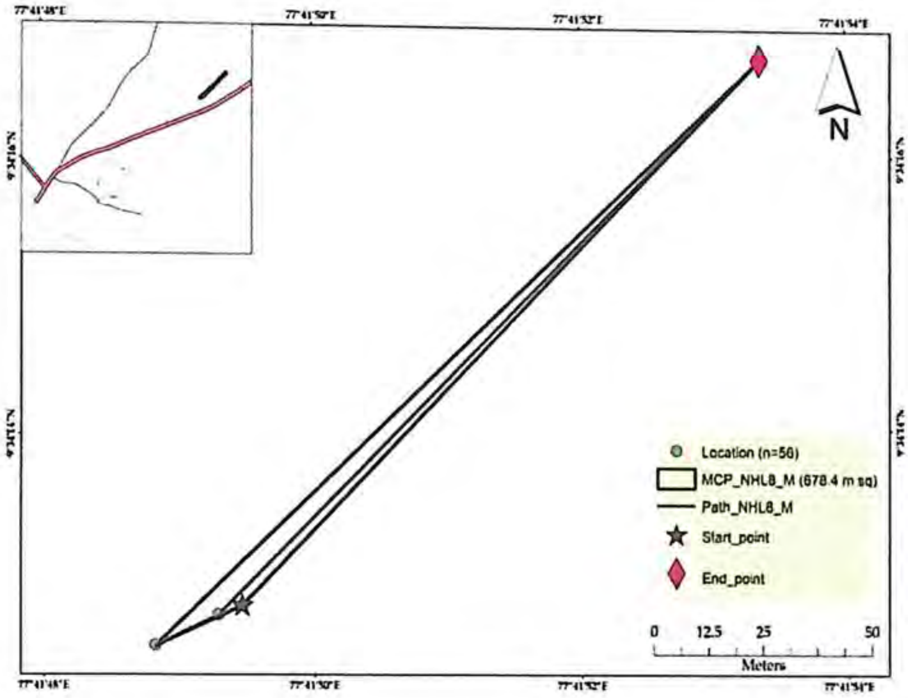

Figure 21 Movement pattern of NHR 8♂

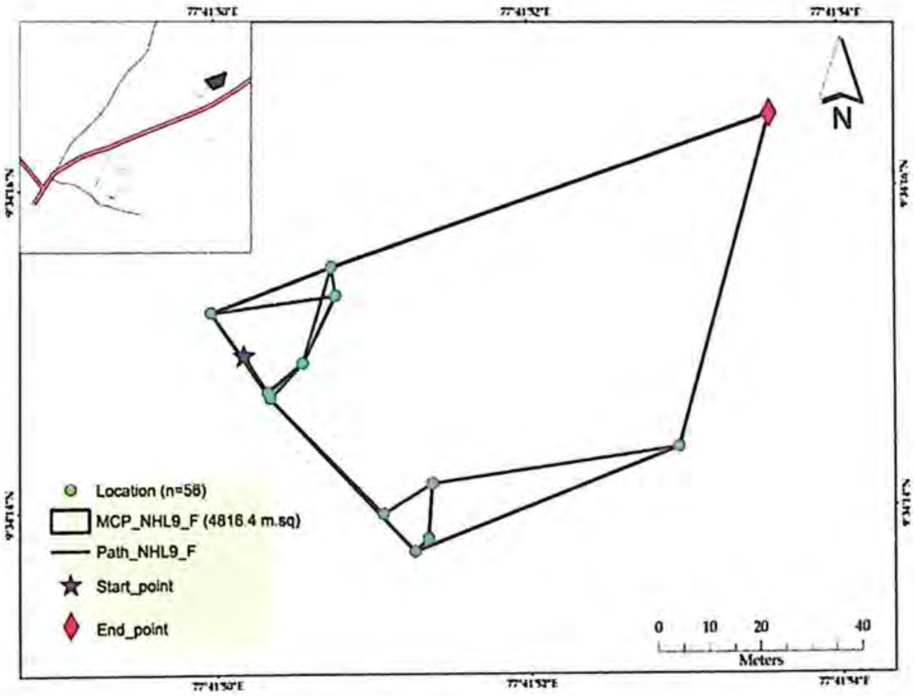

Figure 22 Movement pattern of NHR 9♀

8.1.3 Linear displacement of toads with respect to various road forms

The movement data collected from telemetry study gave information on the distance moved by each toad from first location to second and to third and so on. The data was categorized such that the movement Vs no movement responses were given binary values and were treated as binary variables. These binary variables were modeled into distance classes from the NH. The distance classes were taken as 0-150 (along the road), 150-300 (average distance from road), 300-600 (away from road). Other forms of roads (village metal road and gravel road) were also considered in order to see if there was actually an affect of road on the movement of toads.

The results showed significant trend in the linear displacements made by the toads with respect to distances from various road forms. With respect to NH the radio tagged individuals showed an increasing displacement away from road (Estimate 0.007589; Standard error 0.003108). However, for village metal road the toads did not show any avoidance (Estimate -0.01569; Standard error 0.002977).

Table 6 Model selection table for linear displacement of toads with respect to various road forms.

Model selection table

Model	(Int)	dst_mud	dst_vll	dst_nh	Df	logLik	AICc	delta	weight
1	2.533	-0.02475	-0.01569	0.007589	5	-189.15	388.5	0	0.844
2	3.764		-0.01617		3	-192.885	391.8	3.37	0.156
3	-3.759			0.02107	3	-205.98	418	29.56	0
4	0.2498				2	-220.478	445	56.52	0
5	0.3347	-0.00639			3	-220.42	446.9	58.44	0

Table 7 Parameter estimates for linear displacement of toads with roads.

Parameter estimates

	Estimate	Standard Error	z value	Pr (>|z|)
(Intercept)	2.533181	1.244381	2.036	0.0418
dist_mud	-0.02475	0.020344	-1.216	0.2238
dist_vill	-0.01569	0.002977	-5.27	1.37E-07
dist_nh	0.007589	0.003108	2.442	0.0146

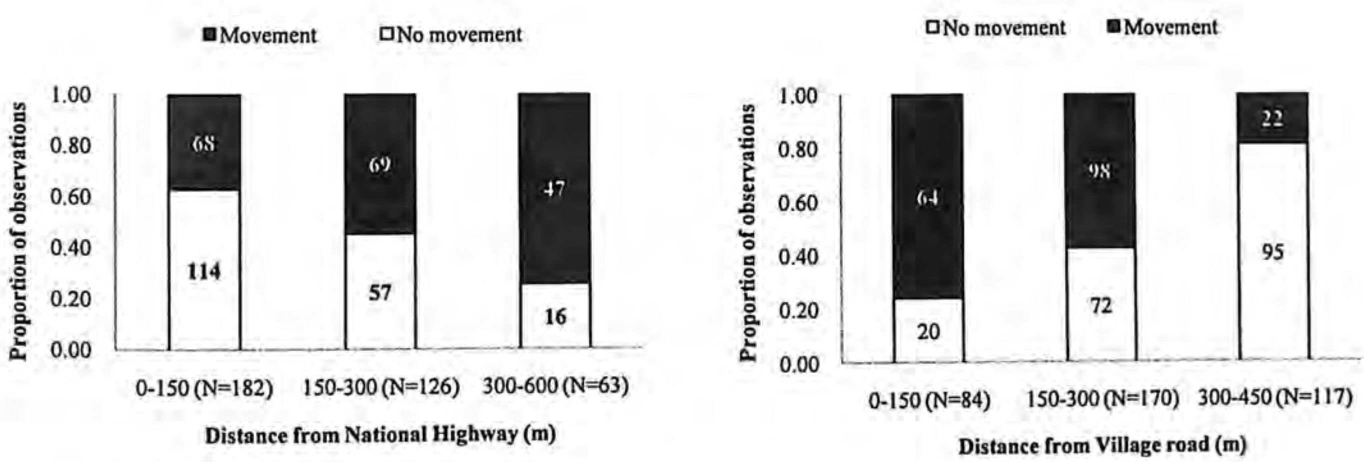

Figure 23 Linear displacement of toads at various distance classes from NH 208 and Village metal road

8.1.4 Distance moved

Out of the 9 individuals radio tracked maximum movement was shown by the adult male NHR 6 which was tagged 257 m inside the NH on 28 February, 2015. The total distance moved by this male was 1764 m for 80 locations. Based on number of locations NHR 2 Female showed comparatively higher movement than all the toads. A summary of the distance moved with respect to the number of locations is given in the table below:

Table 8 Distance travelled by 9 tagged *Duttaphrynus melanostictus*

No. of Individuals	1	2	3	4	5	6	7	8	9
No. of locations	25	25	28	60	23	80	9	56	56
No. of tracking days	15	15	17	37	14	41	12	29	29
Total Distance travelled (m)	280.4	901.2	417.1	903.4	155	1764	325	249	324.4
One night movement (m)	27.6	71	112	110	30	333	131	183	67

Distance moved by each animal with respect to the number of tracking observations made is depicted in following the graph below.

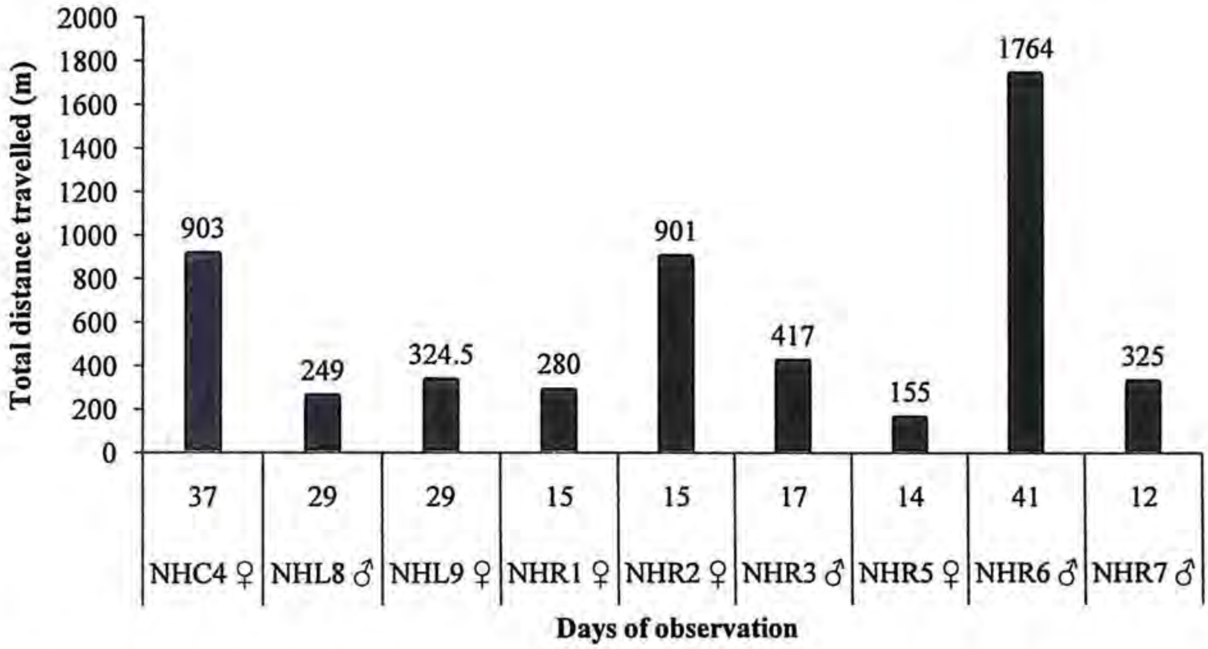

Figure 24 Total distance moved by tagged *Duttaphrynus melanostictus* with increasing observations

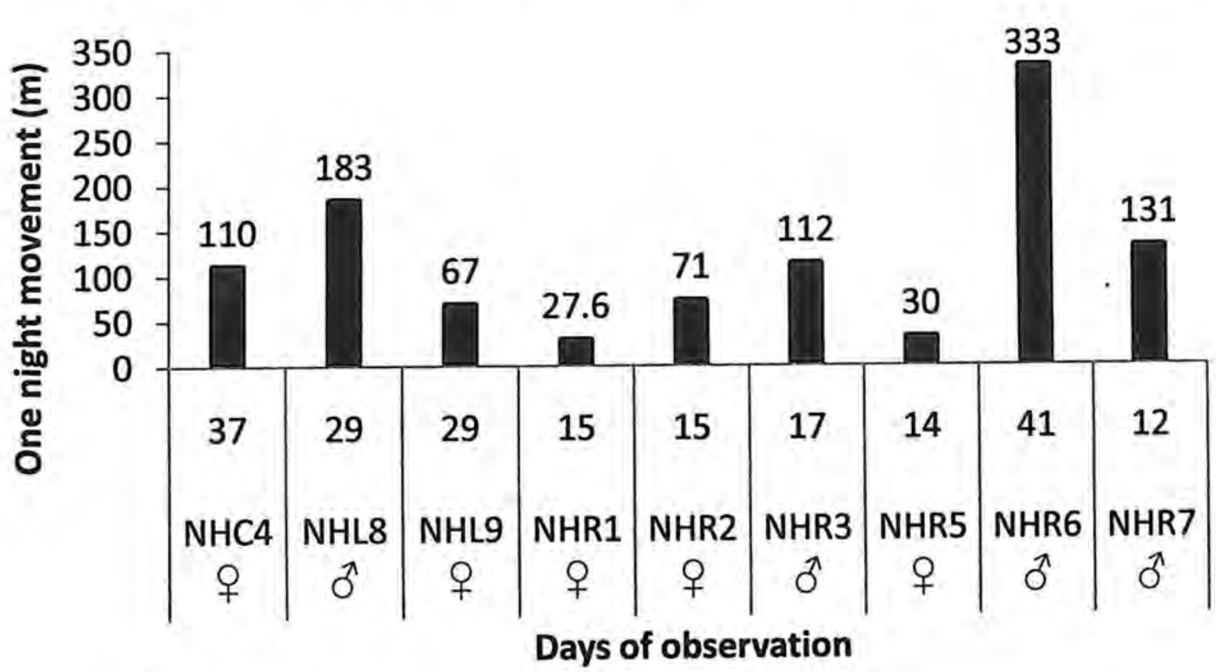

Figure 25 One night displacement made by tagged *Duttaphrunus melanostictus*

8.1.5 Orientation of toads with road

To calculate the angle of the toad with respect to the road, following formula was used:

Sin θ= anti sin (ds-de/dm)

ds= Distance to start point (of the NH)
de= distance to end point (of the NH)
dm= distance moved by the toad

The calculated angles were then plotted to see the trend of the positive and negative angles of the toads with respect to the NH. Examination of the distribution of angular distances from the road is represented in the graph. The toads at 0-150 m from the NH showed strong peaks at angles between 15 to 25, -15 to -25 and -25 to -35. Positive readings indicate movement towards the road and negative values indicate movement away from the road. But if considered the orientation preference for towards or away, there are more observations for toads moving away from the NH at 0-150 m.

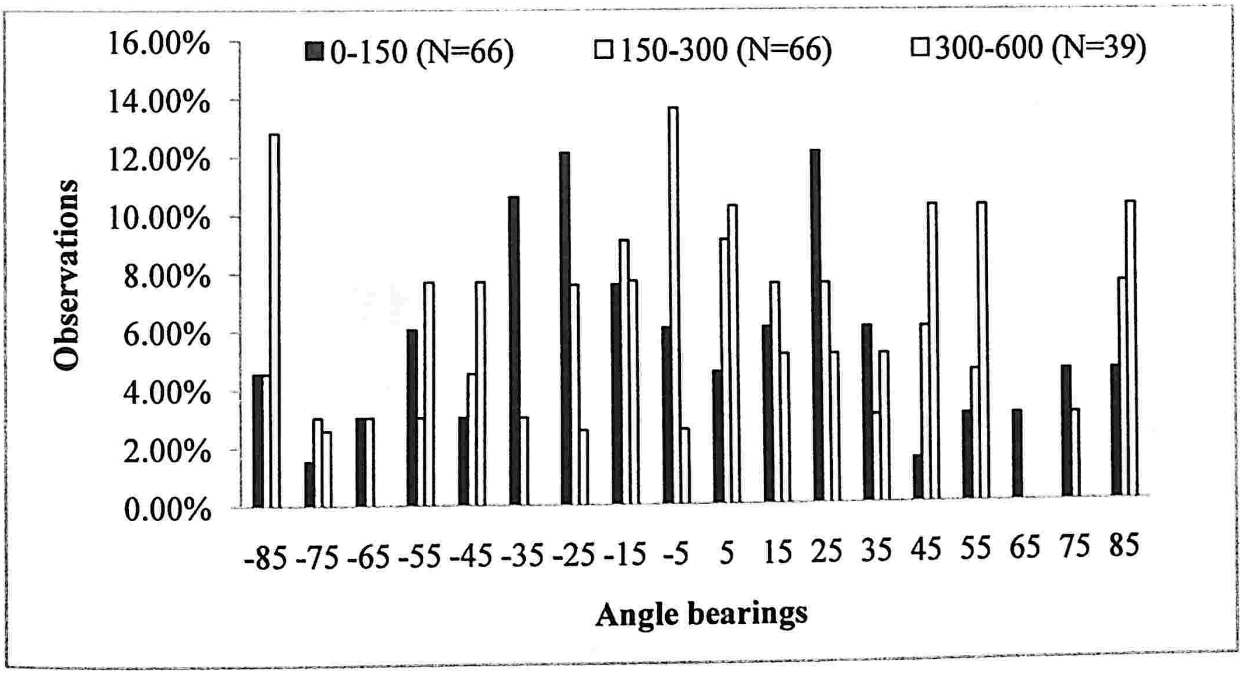

Figure 76 Distribution of angular bearings of toads at different distance classes from NH

Thus, it may be speculated that the movement of the toads is oriented away from the NH. However, for the individuals at 150-300 m showed strong orientation between 0 to -5 which indicates that the road may not affecting the orientation of the animals. The toads at 300 to 600 m show random movement with respect to NH which may be understood as no effect from the road.

However, to test if there is any significant difference between the positive and negative angles in different distance classes, a Chi-square test was done. The results of Chi-square test (Chi-square value: 1.19; Df: 2; P value: 0.55) showed no significant difference in the positive and negative angles at different distance classes (0-150m, 150-300m and 300-600m). A graph representing the percentage of angles towards and away at different distance classes is shown below.

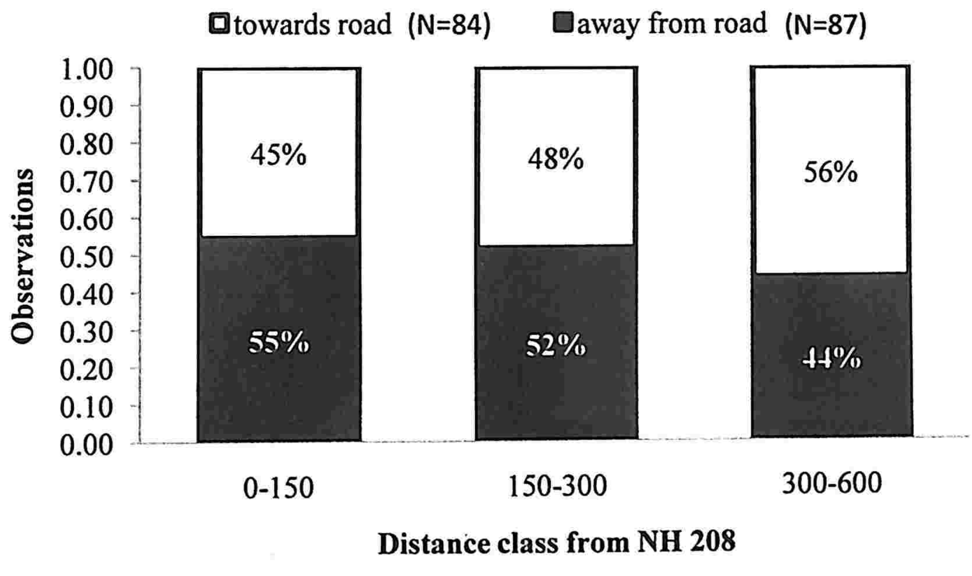

Figure 86 Percentage of orientation (towards and away) at different distance classes from NH

The movement of the toads did not show significant orientation with respect to the NH. However, more robust data may give further insight to the speculations.

8.2 Objective II

8.2.1 Recaptures and abundance estimation

I marked a total of 519 individuals of *Duttaphrynus melanostictus* across the three study sites. For first study site 182, second site 101 and for third site 236 individuals were marked during the study period over a period of 23 days for each site.

The capture probabilities across the three sites decreased by 30-50% thus the data across three plots was pooled and assigned plot IDs as groups. I tested if capture/recapture parameters differed between plots while simultaneously testing for time, behaviour, heterogeneity and their interactive effects. The best model is the one that accounts for time and heterogeneity effects with parameter estimates varying between plots. Abundance estimated by this model for the three plots is shown in Table 9.

Table 9 Population abundance estimates of Mark recapture plots

MRC PLOTS	Mt+1	N-hat	SE
Plot 3	236	365.94	46.85
Plot 2	102	125.59	9.68
Plot 1	180	228.97	14.09

The result shows that population abundance of *Duttaphrynus melanostictus* increases with the increase in distance from the road (NH).

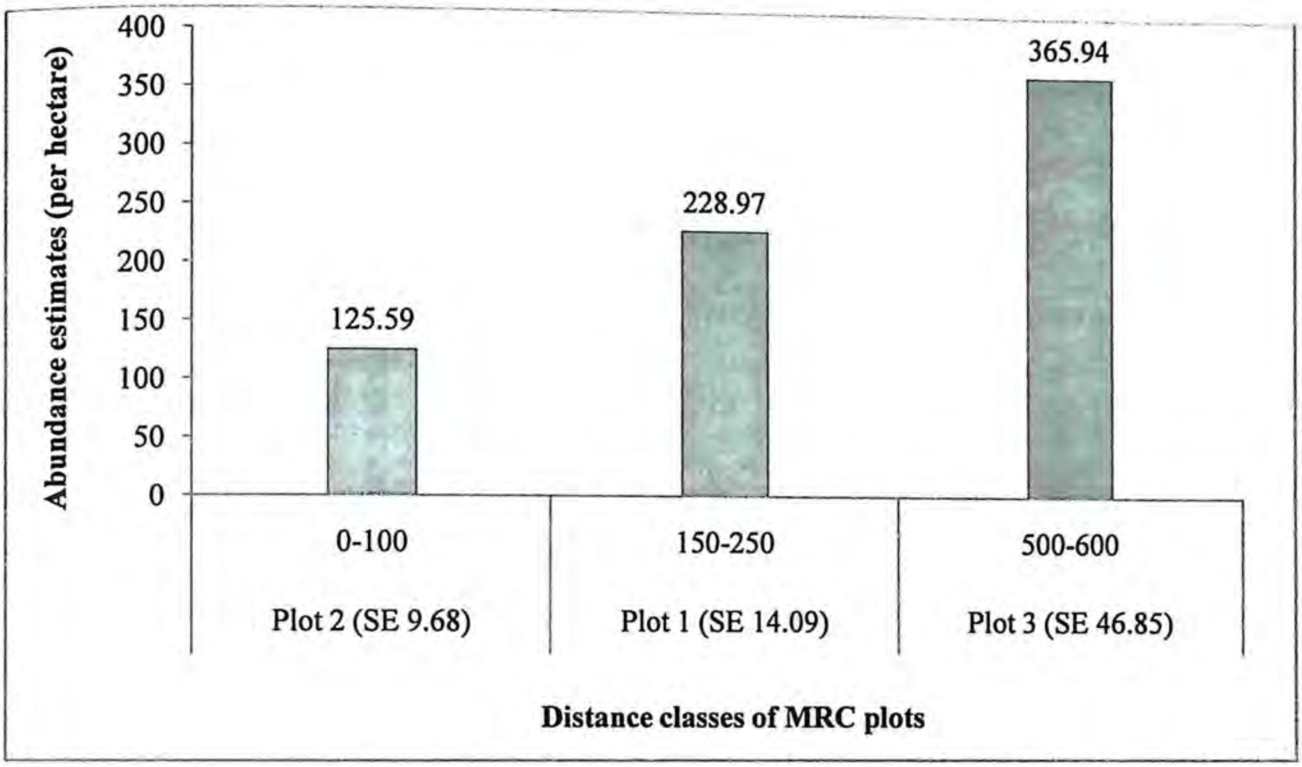

Figure 28 Population abundance of *Duttaphrynus melanostictus* shows an increasing trend at distance classes away from NH 208.

8.3 TRACKING HISTORY

Total nine individuals, 5 adult females (size ranging from size 93 to 100 mm; weight 100-200 g) and 4 adult males (size ranging from 80- 93 mm; weight 51 to 60 g) were tracked starting from February 1 to April 9, 2015. Summary of the tracking history is provided below.

NHR1 ♀ and NHR3 ♂

One adult female (NHR1 ♀: SVL 93.68 mm; weight 141 g) and one adult male (NHR3 ♂:SVL 80.89 mm; weight 58 g) were tagged 134 m inside the NH 208 near a building undergoing construction. This particular site had a village bar which was also a cause of pollution in the area. Since the weather conditions were too dry, the individuals were found using the water tank area at the construction site. Due to continuous supply of water and presence of stagnant water outside the construction area, congregations of more than 15-20 individuals were observed in the construction site at night hours during the study period. The presence of other species such as *Polypedates maculatus, Spherotheca rolandae, Hoplobatrachus crassus* was also recorded in the water tank area. Encounters of *Hemidactylus triedrus* (Termite hill gecko), *Hemidactylus* cf. *brookii* and saw scaled viper were quite often which reduced considerably as the weather became dry. Both the male and female had separate hideouts in day time. Only in several occasions they were found together hiding under vegetation cover along with other individuals. The NHR1 ♀ used to take shelter in the vegetation patches in day time and prefer to move out in the open during night hours. Whereas the male preferred to stay under granite pile in day hours and move out in the open in the evening.

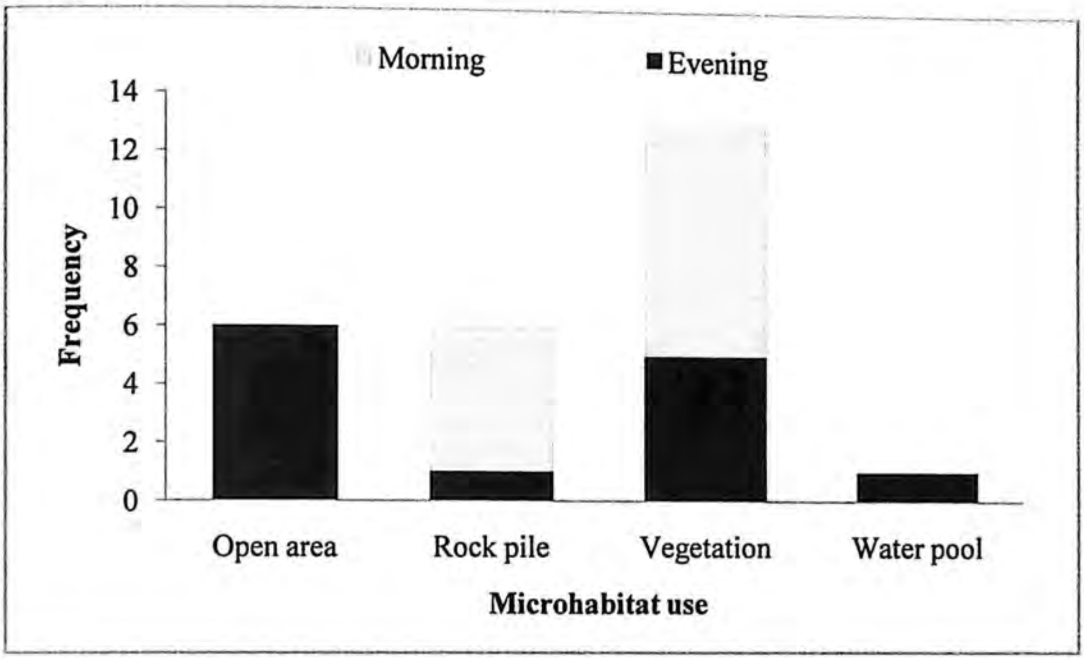

Figure 29 Microhabitat selection of NHR 1 Female during morning and evening hours

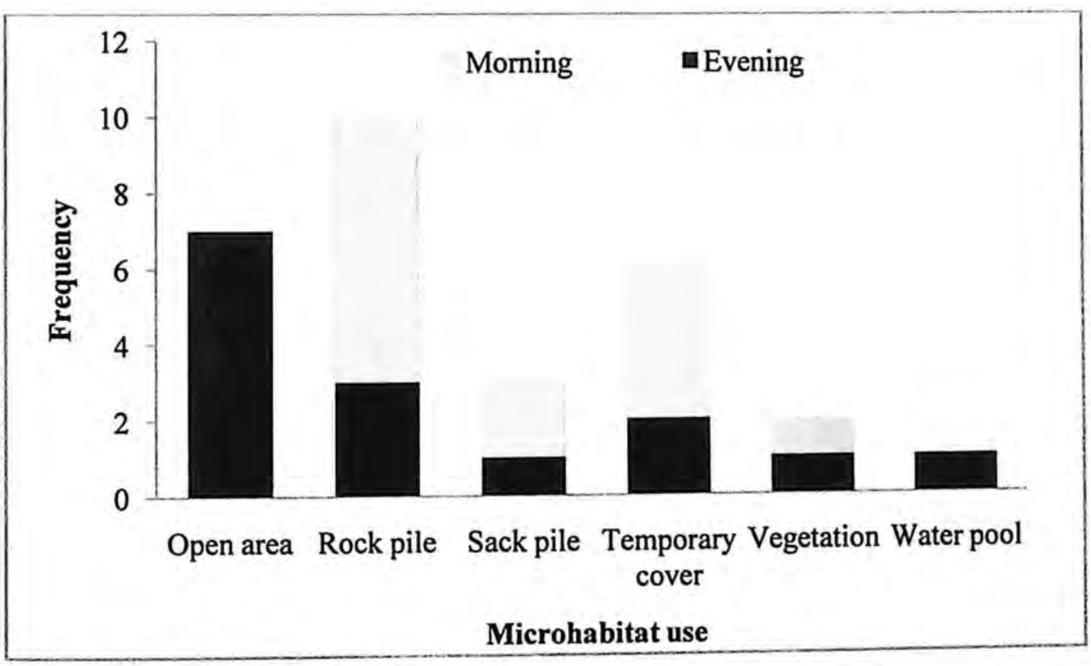

Figure 30 Microhabitat selection of NHR 3 Male during morning and evening hours

NHR 2 ♀

The second adult female (NHR 2 ♀: SVL 100.1 mm; weight 200 g) was tagged 219 m inside of the NH 208 in Krishnan kovil village. This place was not very far from the bar area but still it served different microhabitats to the individual due to presence of a temporary pond (length 4.3 m; width 2.9 m) and open scrub area to move around at night. The female was captured from a temporary water pond on February 1, 2015 in the evening and released the next day on February 2, 2015 after attaching the transmitter (Frequency 1.542). The animal was not tracked on the day of release so as to not stress it and get accustomed with the transmitter. From the relocations made over a period of 15 days, it was observed that the female used either vegetation cover to hide in the day light hours or adjust herself comfortably inside a hole (depth=18 cm; diameter=13 cm) in the ground in an open scrub area with dominant vegetation of *Acacia* and *Calotropis* and *Ziziphus*. In evening hours she was mostly found sitting in the water pool along with other individuals of the same species. This female showed a maximum movement of 71 m in one night. However, total distance recorded for the female in 15 days of tracking is 901.2 m.

NHC 4 ♀

The fourth individual was an adult female (NHC 4 ♀: SVL 95.86; weight 100 g) captured on February 2, 2015 (N 9.57271° E 77.69137°) in village area; Krishnan kovil. This site had hills on one side with rocky habitat, open scrub area with *Acasia*, *Prosopis*, *Calotropis*, *Ossimum*, *Zizypus* as major vegetation and human settlements coming up at a rapid pace. Although not very far from the NH 208, this location served best as control site because of its geographic location behind the hills and almost no disturbance from village metal road or national highway. This individual was tracked for a period of 37 days. Out of 59 locations obtained during the study period, 24 locations (40.67%) were from a motor pump area in the backyard of the house. The location of this motor pump was vertical (height from the ground= 80 cm) and was one of the places where the animal used to spend most of its time mostly during daylight hours. In evening the female preferred to move out in the open scrub area with low level vegetation or was found in the home gardens of the habitations. The animal showed a maximum linear movement of 50 m till 26 February, 2015. On 27 February, 2015 the study site received the first showers in past 3 months and the animal showed a linear movement of 110 m which was the maximum displacement in 37 days of tracking for this female. On March 5, 2015 during the morning tracking the transmitter gave no signal and the individual was nowhere to be found. After a strenuous search of 2 hours the signal was located from among huge rocks in the hills. This distance was 180 m from the previous night location of the toad. Even after extensive search for the next few days there was no evidence of where the female had vanished while the transmitter still gave very strong signals from the rocks. Only on 7 March, 2015, during the evening tracking, a dead body of an animal was procured from a narrow space between two huge rocks. The dead body was identified as a toad from the dorsal side which was still intact. It showed a clear evidence of predation, mostly by a raptor since the animal was eaten completely from the ventral side. The dead body was later identified as the NHC 4 ♀ using the SVL and the faint wart patterns. This female was tracked for the maximum number of days (37 days) out of all the 9 toads and gave total distance travelled as 903.4 m out of 58 locations taken during the study period.

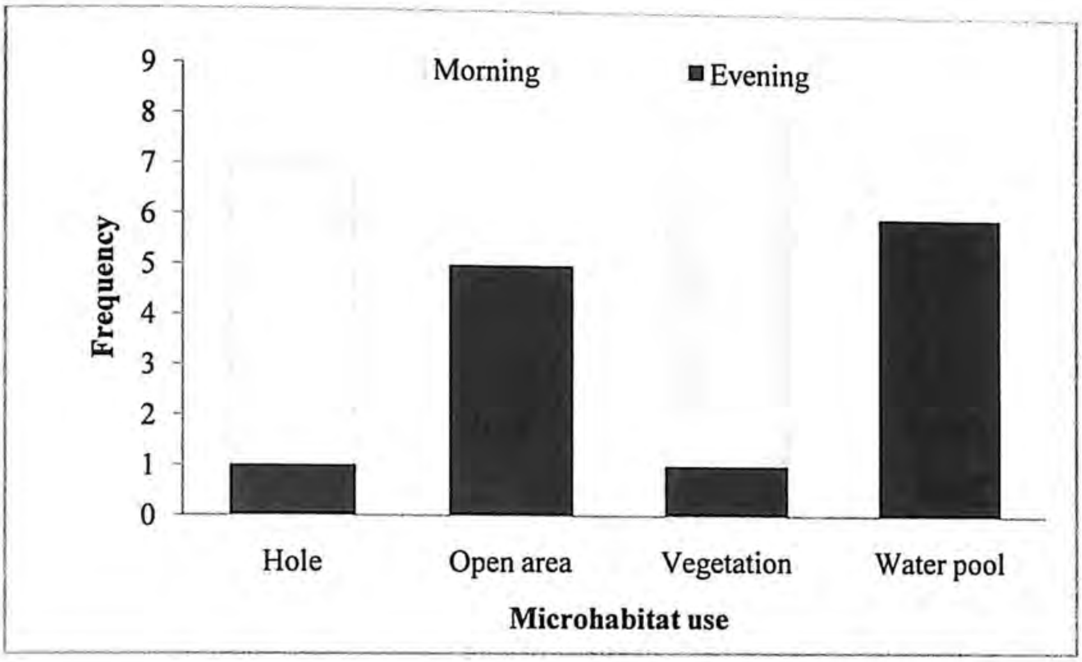

Figure 31 Microhabitat use for NHR 2 Female during day and night hours

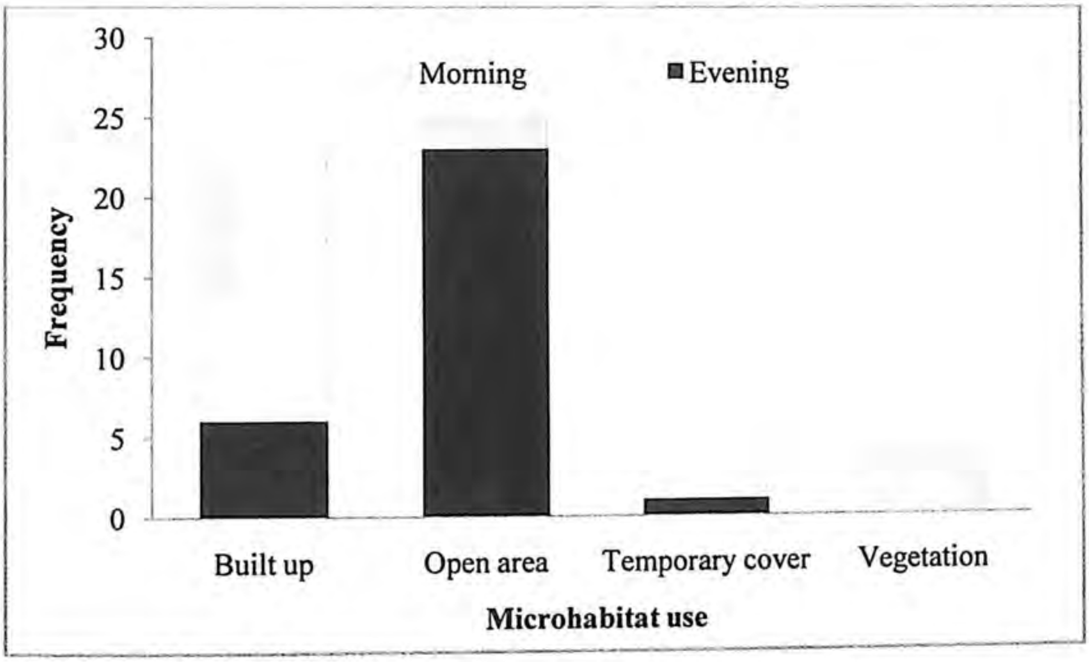

Figure 32 Microhabitat use of NHC 4 Female during morning and evening hours

NHR 5♀

The fifth individual tagged was an adult female (NHR 5♀: weight 100 g) captured in Krishnan kovil village on February 2, 2015. The animal was caught moving around in an open area in low level vegetation outside a house. Although the tracking days differed for every individual, this individual showed the least movement of all the nine tagged individuals. In 14 days tracking period, the animal showed total movement of 115 m and a maximum movement of 30 m in one night. This female used to move around in the human habitations in the village in evening hours and in day time she used to take shelter inside a chicken shed under a plastic sac. There were other individuals of different age class occupying the same microhabitat.

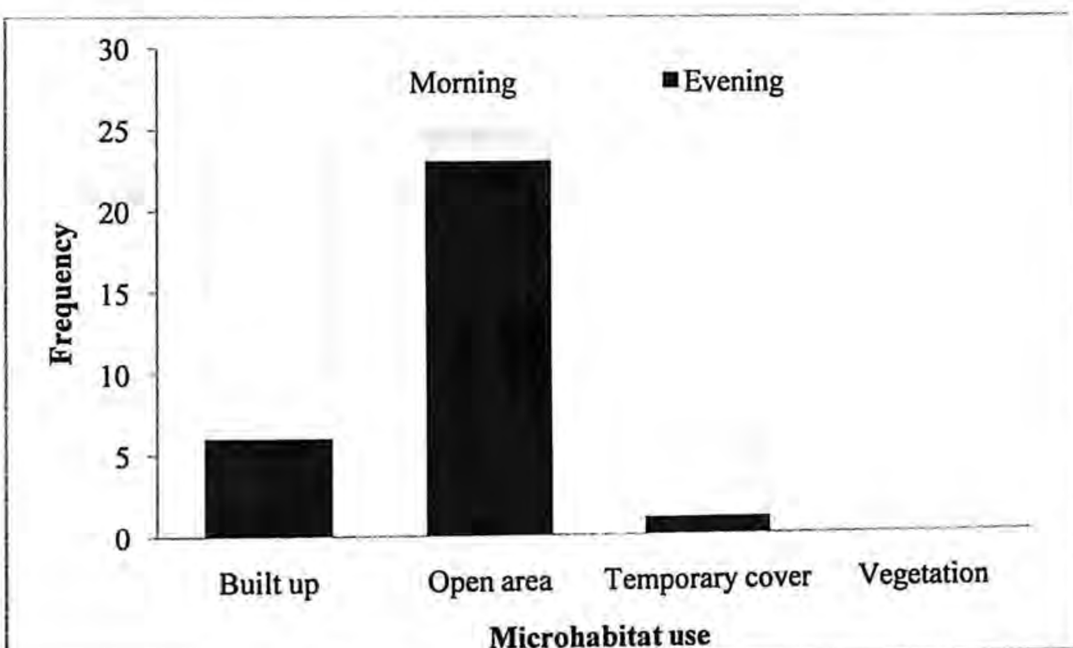

Figure 33 Microhabitat use of NHR 5 female during morning and evening hours

NHR 6 ♂

The male (NHR 6 ♂: SVL 93.33 mm; weight 60 g) was captured from Krishnan kovil village (N 9.56540^0 E 77.69274^0) from the backyard of a house on 28 February, 2015. A lot of individuals were encountered because of the good weather. The male was measured, weighed and tagged immediately after the capture in the field site and released. The male was found using a granite pile in the backyard of a house as its hide out in day hours and move around the human settlements and open area with low level ground vegetation to move at night. Although the capture site was 283 m inside the NH 208 but at the same time it was only 24 m away from the village metal road. The male showed strong site fidelity for the granite rock pile. On March 4, 2015 it rained heavily at night and the male crossed the village metal road and moved 276 m to the opposite side of the road in Thendral Nagar. On 15 march, 2015 in the evening the male was found in amplexus. For the next two days the male was located moving in Thendral Nagar for next 2 days and returned to the same granite pile on 7 March, 2015 travelling 268 m back to the same spot. After moving small stretches in Krishnan kovil village the animal again decided to move long distance on 19 March, 2015. This time the male covered a linear distance of 333 m and was located from a sewage drain in Thendral Nagar. After this the male continued to give locations from the sewage drain till April 9, 2015 which was the last date for tracking the individual. The reason for animal travelling this far could not be understood since the study period was short but the evidences of toad callings at night from the sewage drain points that it may be for breeding.

NHR 7 ♂

One male (NHR 7 ♂: SVL 83.45 mm; weight 51 g) was captured from along the NH 208 from behind a hotel area at a distance of 44 m from the NH 208. The hotel had a parking area and an open space behind the hotel with vegetation of 10-40 cm was tracked for a period of 12 days. The animal was tracked for a period of 12 days in which it used temporary cover like cement sheet, plastic sheet etc to use in the Day time and used to move out in the open in the parking area behind the hotel at night. After continuous tracking of 5 days the animal went missing and no signal was recorded after March 5, 2015 in that entire area. The area opposite to the NH 208 was also checked for signals if the male toad had crossed the national highway.

NHL 8 ♂ and NHL 9 ♀

One male and a female (NHL 8 ♂ and NHL 9 ♀) were tagged along the NH 208 at a distance of 72 m and 112 m. The male and the female individuals were captured on 11 March, 2015 and then were tracked up to April 9, 2015. Both male and the female toad showed negligible or limited movements. On March 23, 2015 the male who was continuously showing similar location from inside a house moved 183 m to a 20 m deep quarry. Interestingly this movement was parallel to the NH 208 and not across it. At night lot of toad callings were recorded. It clearly showed the instinct of the toads to congregate to breed. The female was found to spend most of her time in a narrow space between 2 houses. This narrow passage was 50 cm in width and 12 m in length. This area used to receive daily sewage from both these houses and thus was comparatively cooler than any other place. Female was observed to take shelter inside the PVC pipe from where the water used to drip into this passage. From 27 March, 2015 the female started giving locations other than this sewage area and was found to move towards the direction of the quarry. On 31

March, 2015 the female was giving signal from the quarry, the same area where the male was present. Subsequently, there were a lot of toads recorded moving into this 20 m deep quarry and congregating.

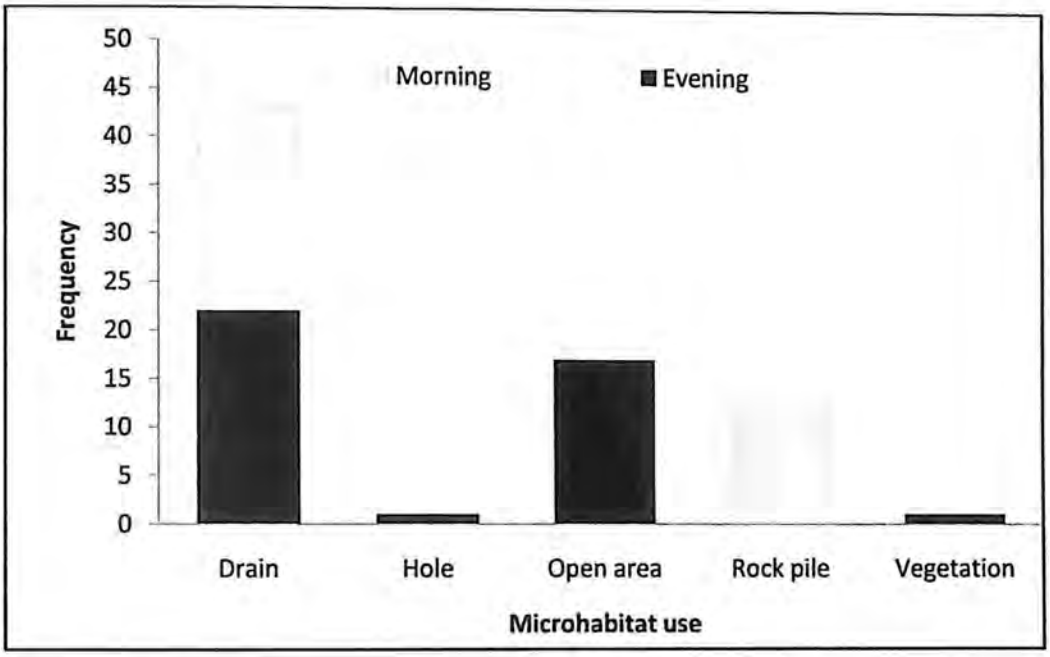

Figure 34 Microhabitat use for NHR 6 Male during morning and evening hours

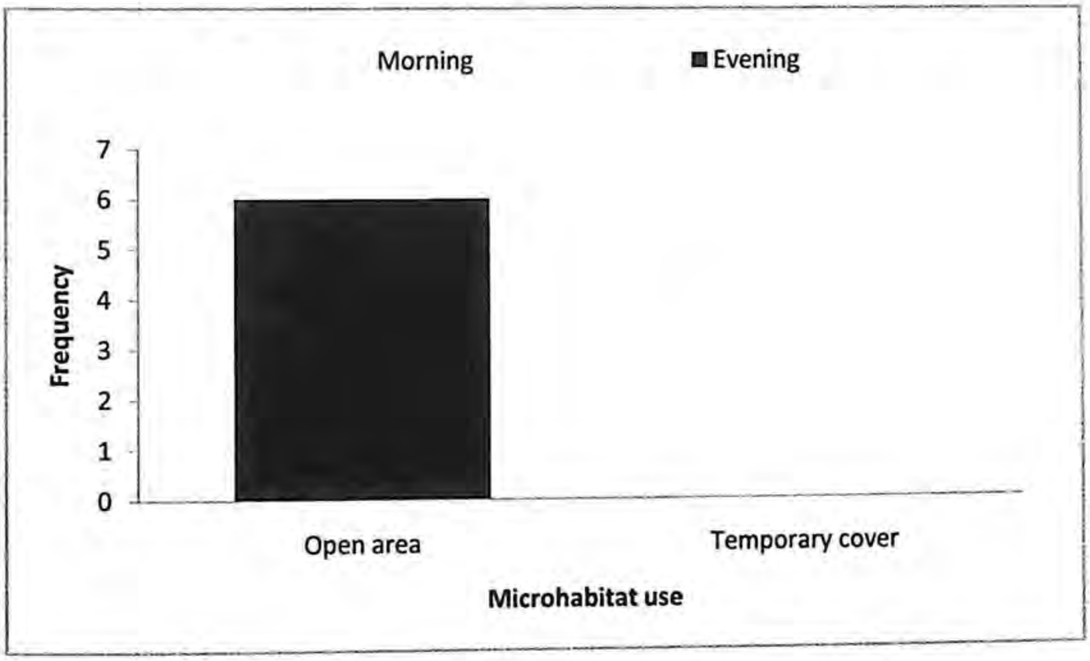

Figure 35 Microhabitat use of NHR 7 Male during morning and evening hours for the study period

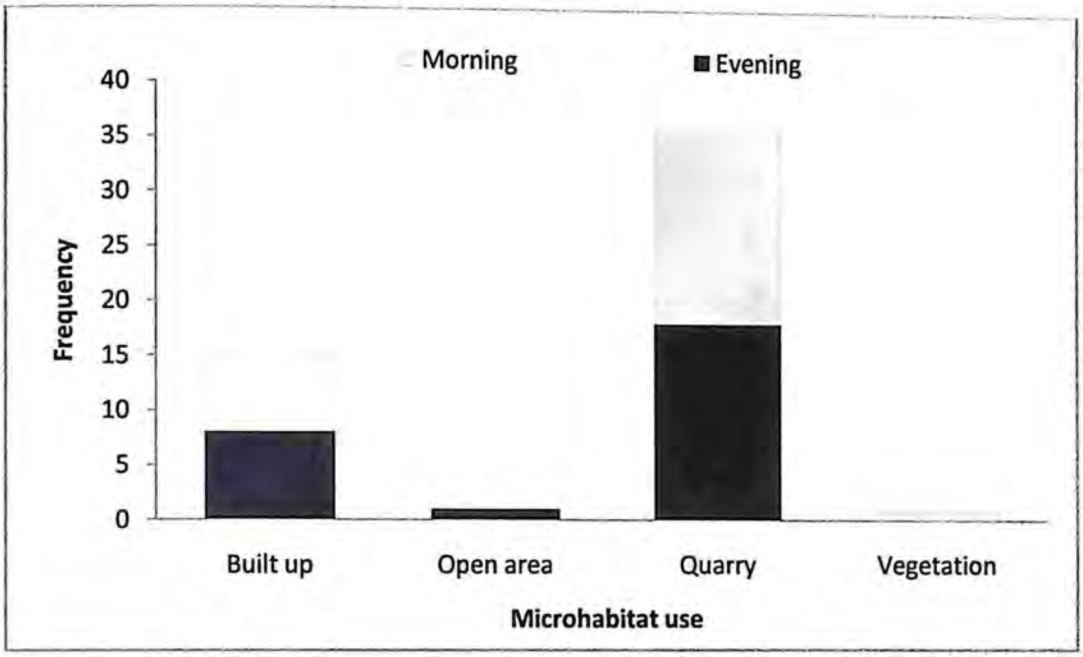

Figure 36 Microhabitat use of NHL 8 M during morning and evening hours

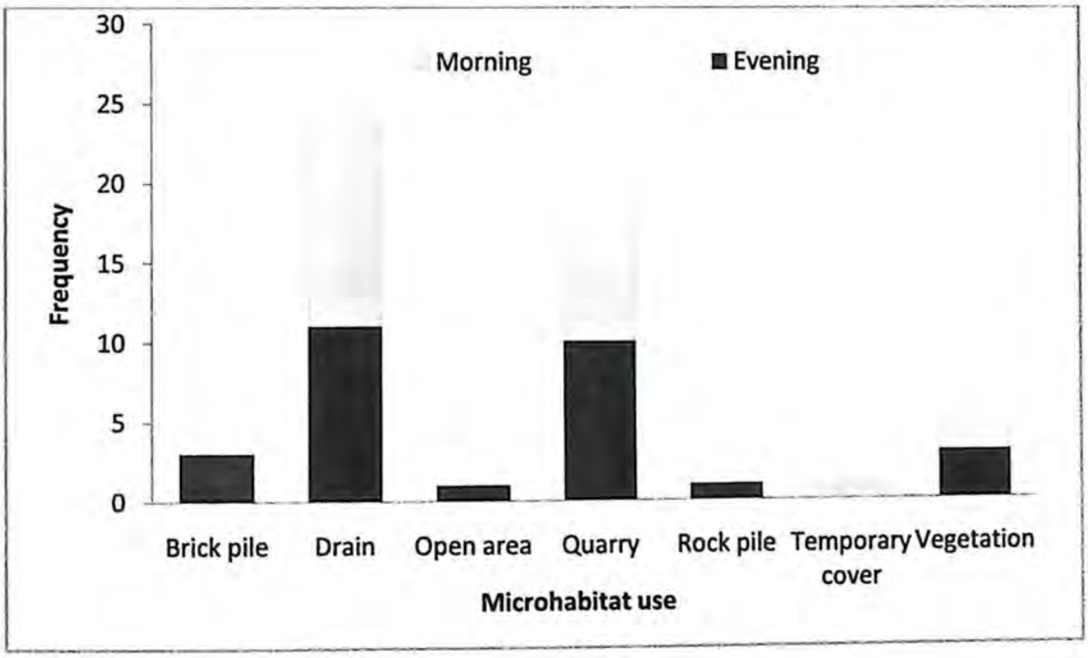

Figure 37 Microhabitat use of NHL 9 Female during morning and evening hours for the study period

Table 10 Description of various microhabitat used by tagged toads

Microhabitat use by tagged *Duttaphrynus melanostictus*	
Open area	Kitchen garden, gravel roads, low level vegetation patches, construction area
Water pool	Stagnant water, temporary water body
Vegetation cover	Vegetation or bush of various height (15-60cm); thick or sparse
Hole	Natural depression in ground
Rock pile	Granite or brick pile
Built up	Man-made structures like motor pump tunnel, space under houses, chicken shed
Temporary cover	Plastic cover, wooden stack, garbage dump
Drain	Sewage drain (big or small)
Quarry	A place, typically a large, deep pit, from which stone or other materials are or have been extracted

9. DISCUSSION

9.1 Tracking and Orientation

Brown et al (2006) have found that amphibians do orient themselves with respect to road and use road as corridor for dispersal. I did not find strong correlation between orientations of toad with road. However field observations during this study showed that movement of toads close to the NH was oriented either along or away from the road. It was also observed that toads near roads showed fewer displacements compared to toads away from road. Radio telemetry data showed that toads along the NH never tried to cross the NH, but there were crossings recorded for village metal road. This implies that the toads are probably avoiding the NH. This is in concordance with the findings that road avoidance in herpetofauna is widespread (Mader, 1984, Trombulak and Frissell, 2000, Develey and Stouffer, 2001, Epps et al 2005). Considering Indian amphibians, there was no information on the movement of amphibian's species. Thus, this study is the first that reported distance moved by individual toads based on radiotelemetry.

9.2 Home range

Home range is considered to be the area in which an individual carries out its normal activities (Duellman and Trueb, 1986). Although most of the amphibians are sedentary and has small home range, however, some species such as *Bufo marinus* have considerable home range size. (see Duellman and Trueb, 1986). Such information are lacking in India.

For the nine individuals that I tracked, the maximum home range observed (Minimum convex polygon 47557.10 sq m; Kernel density estimates 4262.92 sq m) was for adult male NHR 6 ♂ who was tracked for a period of 41 days. However home range between males and females differed since males showed high vagility than female. The maximum home range estimates among the females were of NHL9♀ (Minimum convex polygon 5076.01; Kernel density estimates 3460.27). The home range estimations may not be the true representations or under estimations of the actual home range since the study period was short and the number of locations per individual was less. Many studies have provided distances moved by amphibians during their daily activities, but they have not quantified home ranges as such (Heusser 1968, Pyburn 1958). This study thus provides an insight of the average home range and distance moved by toads in Indian context.

9.3 Site fidelity

Strong site fidelity was observed in both males and females. Many species of amphibians exhibit a high degree of site fidelity to the home range (Duellman & Trueb 1986). In some cases long distance displacements have resulted in individuals returning to exactly the same place where they had been living. Similar case was observed for NHR 6 ♂ who made a displacement of 276 m on opposite side of the village metal road and after 3 days returned back to the same place where it was using the most. Strong site fidelity was shown by females as well (NHR 2 ♀, NHC 4 ♀, NHL 9 ♀). There was no difference in response to site fidelity on individuals near and away from the road.

9.4 Microhabitat use

Radiotelemetry is the best means to know the microhabitat use by cryptic animals such as amphibians. Habitat selection leads to life time fitness by influencing reproduction and survival (Fretwell & Lucas 1970). The movement pattern and habitat use of species are important in identifying those species at most risk of extinction in human modified landscape (Hanski & Zhang, Travis & Dytham 1999).

Microhabitat used by nine radio tracked toads was recorded each time the animal was located in field in day and evening. Microhabitats were broadly classified into water pool (temporary water body), vegetation (20-50 cm high thick or sparse vegetation), hole (on ground), rock pile (granite, brick or crevices) , built up (motorpump, cemented spaces in house), temporary cover (wooden stack, sack, garbage dump), drain (sweage or household drain) , quarry (left out pit with accumulated water) and open area (any area with or without ground vegetation<20, gravel roads, garden) .

NHR 1 ♀ for the first adult female which was tagged on January 31, 2015, 134 m away from the NH, the preferred micro habitats were rock pile, vegetation, water pool or open area. The percentage of microhabitat use observed during the study period is open area-23 %, rock pile-23%, vegetation-50%, water pool-38%

NHR 2 ♀ Out of 26 locations the female was located 7 times (26.9%) hiding in the hole and 6 times (23%) in the pond, thus showing very high site fidelity for these two micro habitats.

NHR 3 ♂ the microhabitat used were open area (24.1%), rock pile (34.4%), sand pile (10.3%), Temporary cover (20.6%), Vegetation (6.8%), water pool (3.44%).

NHC 4 ♀, the microhabitat used were built up (40.6%), open area (42.3), temporary cover (10.16%), vegetation (6.7). NHC4 M This individual was tracked for a period of 37 days. Out of 59 locations obtained during the study period, 24 locations (40.67%) were from a motor pump area in the backyard of the house.

NHR 5 ♀, the microhabitat used were built up (68%), open area (24%), vegetation (8%)

NHR 6 ♂, the microhabitats used were drain (53.75%), hole (3.75%), open area (21.25%), rock pile (16.25%), vegetation (5%).

NHR 7 ♂, open area (54.5%) and temporary cover (45.4%) were recorded since the transmitter on the animal stopped giving signal after 5 days.

NHL 8 ♂, the microhabitats used were built up (28.3%), open area (1.88%), quarry (67.9), and vegetation (1.88%)

NHL 9 ♀, the microhabitats used were brick pile (8.77%), drain (42.1%), open area (1.75%), quarry (35%), rock pile (1.75%), temporary cover (1.75%) and vegetation (8.77%)

9.5 Mark recapture Study

Initial experimentation with marking of individuals (VIE tags) failed which took a considerable amount of time and effort to standardize the method to be employed in the study. After trials were done with VIE tags, toe tipping was chosen as the method for marking amphibians. Toe tipping has been one of the oldest, reliable and cost effective method for mark recapture in amphibians (Heyer et al 1993). Amphibians have a strong limb regeneration capacity (Dulleman & Trueb 1986). Hence the method does not hamper the movement and behavior of the animal (Heyer et al 1994). Toe tipping, thus proved reliable method during the study and showed accuracy of 98.11%. So far, studies in India have mostly looked into systematic and taxonomy of amphibians. This study however is a new initiative beyond taxonomy to understand the behavior and spatial ecology of amphibians in a day to day modifying landscape.

Mark recapture study showed significant representation of abundance estimates of toads across the three plots. The abundance estimates for plots; Plot 3 > Plot 1 > Plot 2, showed an increasing trend at increasing distances from the NH. This probably indicates that related traffic density might be exerting a negative effect on anuran population. Few studies have shown that this kind of trend has already been established in earlier studies (Rosen & Lowe 1994, Fahrig et al 1995). However, other related factors such as pollutants in road run off, exhaust emission, vibration, and noise may also affect anuran densities either by causing direct mortality or interrupting anuran behavior (Buchanan 1993).

Jaeger et al. (2005) predicted that the effect of roads on population persistence depends on the interaction between the behavioural response and mechanism in which roads are likely to affect the species. It has also been observed that species that behaviourally avoid road will not suffer direct mortality, but important habitats on the other side will remain inaccessible (Ford and Fahrig 2008). In the long term, both mortality and avoidance may lead to reduced gene flow among populations separated by roads. This, in turn, can decrease fitness and further reduce population viability.

I also had opportunistic observation of hourly variation of traffic volume on NH from 1 April to 7 April. In a stretch of 200 m, I observed 16.4 vehicles/ minute passing thorugh the NH in morning hours between 7:00 am to 8:00 am. In evening hours the traffic count was done at peak hours of volume was higher. It was estimated to be 18.58 vehicles/ minute. However, the traffic volume may increase during late night hours. Such traffic volume may be a significant barrier for slow moving animals such as toads.

Bouchard et al (2009) found that frogs took longer to move near roads with more traffic and that their movement was quickest in areas without roads nearby. Frogs tended to deviate more from a straight-line course when they were released near roads than compared with control areas. On very low traffic roads (10.86 mean vehicles per hour), 94% of frogs crossed the road successfully, whereas at higher traffic roads (58.29 mean vehicles per hour) 72% were successful. Frog's inability to avoid going onto roads and their slow movement combine to make them particularly vulnerable to road mortality, which likely explains the strong negative effects of roads on frog population abundance.

The result shows that abundance of toads increased with increase in the distance from road. However, this does not mean that frogs are safe from dangerous consequences of road. As Hoskin and Goosem (2010) found that road kill is rather not related to decrease in frog abundance near road but other factor such as traffic noise as frog near road known to have auditory masking effect on frogs call (Parris et al. 2009). It was also observed that sub adults or juveniles are relatively abundant near the road than away from it, pattern that could be generated by road mortality or via competition based on habitat quality. Road kills could skew body size in the observed direction because frogs living closer to the road have a greater chance mortality, resulting in potentially shorter life spans and potentially skewing the population toward younger, and therefore probably smaller (Halliday and Verrell 1988, Wells 2007) individuals near the road. However, in my study site, how exactly the road affects body size is still a research question.

10. LIMITATIONS

10.1 Environmental constraints

The study site, Krishnan kovil has been undergoing serious water crisis due to monsoon failure past four consecutive years. The site represents the eastern slopes of Western Ghats and thus is a dry habitat with scrub vegetation. Northeast monsoons are a common phenomenon during September to December. But except few showers in the month of March, there were no rains recorded during the four months of study period (December to April).

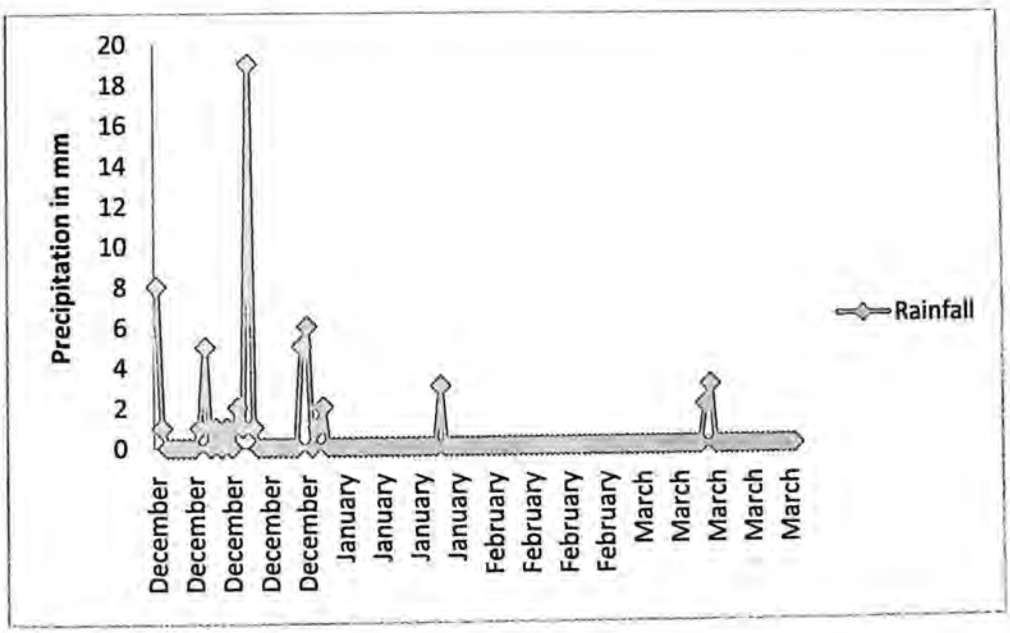

Figure 38 Rainfall during the intensive study period (Dec 2014 to March 2015)

For amphibians environmental parameters are of utmost importance that influences their movement and behavior. Due to the hot and humid weather conditions, the data collected on movement pattern was skewed.

10.2 Technical shortcomings

The telemetry study being a new Indian context, the Initial experimentation led to the loss of tags and the toads. The harness did not work properly in the initial days since the animals were taking it off. Thus, it took a lot of time to initiate the telemetry study.

The main challenge being faced was marking the toads on their rough warty skin which made the penetration of injecting syringe containing the VIE tag a real tuff task. The smudging of colours was observed on the dorsal side which made the individual identification ambiguous. It was observed that although the animals were doing fine but the silicone mixture did not solidify and was found to be moving around in the belly of the toads. Thus, the option to put VIE tags on the toads was dropped and other methods were given a thought. Due to the availability of PIT tags, it was the next possible option to mark the individuals. But the feasibility to use huge number of PIT tags and the cost involved with it strained the methodology and the duration of the study.

10.3 Logistic issues

Apart from the environmental and technical shortcomings faced during the study period, one of the major limitations for the study was fixing the logistics. The place and the language being absolutely new to me, made communication and work difficult. In the absence of a field assistant working at nights made the work challenging.

11. FUTURE DIRECTION

This is just the beginning. I will continue this research and gather robust amphibian movement data for a larger explanatory context that could help guide new avenues of research such as dispersal, migration and orientation of toad with respect to linear intrusions. I also intend to record a lifetime movement of a toad. Understanding such responses to habitat features during dispersal will aid in developing realistic, predictive models of amphibian movement that can be used to further conservation and management efforts and will also add to the knowledge about how movement mechanisms are related to genetic structuring and population persistence in an altered landscapes.

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Appendix 1 GPS locations of Intensive study area

Roads	Location ID	Distance	Latitude	Longitide
NH-208	NH 1	105	9.565278	77.689134
	NH 2	60	9.565966	77.689604
	NH 3	10	9.56642	77.689911
	NH 4	151	9.56678	77.69028
	NH 5	147	9.56745	77.6916
	NH 6	469	9.56797	77.69296
	NH 7	151	9.561492	77.696952
	NH 8	165	9.57039	77.69858
	NH 9	End	9.57119	77.9981
VR Kunnur	KUN 1	53	9.56642	77.689911
	KUN 2	139	9.56691	77.69025
	KUN 3	209	9.56804	77.69095
	KUN 4	98	9.56931	77.69231
	KUN 5	99	9.56996	77.69271
	KUN 6	102	9.57078	77.69318
	KUN 7	69	9.57147	77.69373
	KUN 8	73	9.57188	77.69419
	KUN 9	98	9.57246	77.69456
	KUN 10	End	9.5733	77.69479
VR Sivakasi	SIV 1	48	9.56642	77.689911
	SIV 2	78	9.566227	77.680375
	SIV 3	58	9.566151	77.690999
	SIV 4	73	9.565868	77.691496
	SIV 5	26	9.565478	77.691959
	SIV 6	77	9.56535	77.69205
	SIV 7	End	9.5651	77.69283

Appendix 2 Traffic volume data

Days	Morning	Evening	April	NH_mor/min	NH_eve/min
1	863	1140	1	14.3	19
2	1062	1212	2	17.7	20.2
3	907	1055	3	15.1	17.5
4	919	1264	4	15.3	21.06
5	1021	1273	5	17	21.2
6	1036	995	6	17.2	16.5
7	1078	1049	7	17.96	17.4
8	1016	948	8	17	15.8
			Total	131.56	148.66
			Vehicles per minute	16.445	18.5825

Appendix 3 Model selection table for Mark capture recapture study

Model	AICc	Delta AICc	AICc Weights	Model Likelihood	Num. Par	Deviance
Model-time-heterogeneity-plot	2587.0511	0	0.93073	1	33	4456.696
Model-time-behaviour-heterogeneity-plot	2592.2543	5.2032	0.06902	0.0742	47	4433.002
Model-time-behaviour-heterogeneity	2603.5195	16.4684	0.00025	0.0003	16	4507.8308
Model-time-plot	2627.0588	40.0077	0	0	15	4533.395
Model-time-behaviour-plot	2627.8316	40.7805	0	0	24	4515.8865
Model-behaviour-heterogeneity-plot	2628.5254	41.4743	0	0	14	4536.885
Model-behaviour-heterogeneity	2645.7625	58.7114	0	0	5	4572.262
Model-time	2646.3059	59.2548	0	0	5	4572.8055
Model-time-behaviour	2651.456	64.4049	0	0	8	4571.923
Model-time-heterogeneity	2712.9117	125.8606	0	0	11	4627.3321
Model-behaviour-plot	2766.2297	179.1786	0	0	6	4690.7199
Model-behaviour	2770.6823	183.6312	0	0	2	4703.2005
Model-heterogeneity-plot	3002.1871	415.136	0	0	9	4920.64
Model-heterogeneity	3006.5824	419.5313	0	0	3	4937.0959
Model-plot	3032.484	445.4329	0	0	3	4962.9974
Model-null	3038.6837	451.6326	0	0	1	4973.2049