ECOLOGY, BREEDING BIOLOGY AND CONSERVATION STRATEGY OF HIMALAYAN NEWT, *TYLOTOTRITON VERRUCOSUS* ANDERSON IN SENAPATI DISTRICT, MANIPUR

by

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INTRODUCTION OF THE SUBJECT ANIMAL

Himalayan Newt, *Tylototriton verrucosus* Anderson is a tailed amphibian species belonging to the order Caudata or Urodela. It is a beautifully coloured animal which in all respects qualifies to be considered as an ornamental tetrapod. This is the only caudate species reported in India from hilly pockets of Arunachal Pradesh, Darjeeling (west-Bengal), Ukhrul (Manipur), Meghalaya and Sikkim. In the current century, data is updated only for Newts from Darjeeling and Ukhrul districts. In the present work they could be successfully located in 10 habitats located in different hilly pockets of Senapati district, Manipur. In The Wild life (Protection) Act 1972, Schedule-I, *Tylototriton verrucosus* Anderson has been listed as an endangered species and in Wildlife (Protection) Act, Manipur, Rule 74 it has been declared as a protected species. Therefore, no animal was sacrificed nor any injury was inflicted to them in order to satisfy experimental requirements during the course of present study. Instead, wherever necessary, we relied on animals found dead or injured beyond recovery in or around their habitat on account of various anthropogenic activities and/or agricultural practices by the people in the area.

(Hoidina Lucy)

DECLARATION

I, Hoidina Lucy, hereby declare that the subject matter of thesis is the record of work done by me, that the content of this thesis did not form the basis of award of any previous degree to me or to the best of my knowledge to anybody else, and that the thesis has not been submitted by me for any research degree in any other University/Institute.

This is being submitted to the Nagaland University for the degree of Doctor of Philosophy in Zoology.

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PREFACE

Seasons repeat their unrelenting rhythms. Proximate factors such as light, temperature, humidity, precipitation, alterations in landscape, and other microclimatic environments vary according with each cyclical change and in turn, impose deep impact on the living beings. Living organisms for their successful survival tend to adapt to natural rhythms. The adaptations occur at all levels of organism including morphological, metabolic, and different behavioural attributes. This enables the organisms to utilize predictive information to effect temporal adjustments of events that demand sufficient time to accrue and that must culminate precisely when optimal climatic conditions appear in the habitats.

Amphibians represent the group of vertebrates that naturally bridge the gap in aquatic and terrestrial environments, and hence, are ought to exhibit immense physiological adaptations evolved in them in due course of time. The underlying mechanisms leading to successful survival of this group of vertebrates under vast array of habitats betraying extremely difficult environmental pursuits are not yet fully understood. It is however, pertinent that vast majority of amphibians exhibit cyclic mode of reproduction and similarly, other morphological and physiological adaptations associated with timed and/or opportunistic breeding also exhibit cyclical patterns, *e.g.* sex specific enlargement of thumb-pad, crocking of voice, colour change, courtship, amplexus and mating *etc*.. To cope with the requirements of reproductive events, a tendency to acquire changes in metabolic strategy of the animals become more pertinent since migration, moult and reproduction are highly energy demanding phenomena which provide limited opportunity for physiological and metabolic preparations. The most interesting characteristic features which this group of animals feature are hibernation (winter- sleep) and aestivation (summer-sleep). During hibernation, animals do not consume energy resources and hence, physiological activities are maintained at bare minimum utilizing stored energy in form of fat, proteins and carbohydrates prior to their entry in hibernation. In majority of species, this stage is also characterized by simultaneous development of gametes seemingly to exploit the opportunity of improved climatic conditions for shedding gametes soon after the hibernation. Cyclical breeding patterns are however, less pronounced or absent in the species inhabiting lower latitudes and significant proportion of individuals in a population may attempt reproduction throughout the

year. Obviously, breeding patterns in amphibians vary depending upon climatic conditions in specific habitats.

Himalayan Newt, *Tylototriton verrucosus* Anderson is the only caudate species found in the Indian sub-continent. The species is reported to have been living since the age of dinosaurs. It is regarded as the missing link between the Reptiles and the Amphibians. Throughout the stretch of its occurrence, it emerges from its hideouts following the onset of monsoon rains. It may be presumed that in the process of familiarizing to diverse habitats, Himalayan Newts might have succeeded in adopting strategies to enable them to exploit a vast array of food resources present in the ecological niche they occupy. The presumption that Himalayan Newts might occur in areas which current records exhibit, prompted us to take up present research work on the occurrence, assessment of habitat quality, food, feeding and moulting patterns, breeding behaviour, threat perception, survival prospects and conservation strategy in the Senapati district, Manipur of the north-eastern state of country. The dissertation has been divided into five chapters as follows :

(**i**). Chapter–I deals with occurrence, distribution pattern and assessment of habitat quality.

(**ii**). Chapter-II embodies the results on determining taxonomical characters of the polychromatic forms of the Himalayan Newts that co-exist with *Tylototriton verrucosus* Anderson.

(**iii**). Chapter-III describes the food, feeding patterns and moulting behaviour in the Himalayan Newt.

(**iv**). Chapter-IV deals with reproductive behaviour: Courtship, mating and egg laying.

(**v**). Chapter-V describes the threat perception, survival prospects and conservation strategy of the Himalayan Newts in mountainous pockets of the Senapati district, Manipur.

CONTENTS

GENERAL INTRODUCTION

In the Animal Kingdom, the Class : Amphibia represents animals which have survival potential in both aquatic and terrestrial environments. This class of tetrapods has 7,450 species spread into 03 orders, 546 genera and 75 families. One of the orders, the order **'Caudata or Urodela'** incorporates tailed-animals who can easily be mistaken for a lizard. Animals of this order are commonly referred as ***'Salamandrids'*** which include Newts and Salamanders. Newts and Salamanders are largely inhabitants of temperate regions with a few exceptions. Currently, in the world, Newts and Salamanders are spread into 10 families, 68 genera and 680 species (AmphibiaWeb, 2015). Of the 10 families of Caudates, the family 'Salamandridae' incorporates 112 species spread into 21 genera. One of these genera, the genus ***'Tylototriton'*** represents 22 species of Newts : *Tylototriton anguliceps, Tylototriton asperrimus, Tylototriton broadoridgus, Tylototriton daweishanensis, Tylototriton hainanensis, Tylototriton kweichowensis, Tylototriton lizhenchangi, Tylototriton panhai, Tylototriton pseudoverrucosus, Tylototriton shanjing*, *Tylototriton taliangensis, Tylototriton uyenoi, Tylototriton verrucosus, Tylototriton vietnamensis, Tylototriton wenxianensis, Tylototriton yangi, Tylototriton ziegleri, Tylototriton broadoridgus, Tylototriton liuyangensis, Tylototriton notialis, Tylototriton shanorum,* and *Tylototriton podichthys*. Further, in some species several intra-specific variations have been reported, for instance *Tylototriton anguliceps* has 8 variants; *Tylototriton daweishanensis* has 2 variants; *Tylototriton kweichowensis* has 24 variants*; Tylototriton lizhenchangi* has 6 variants*; Tylototriton panhai* has *5 variants; Tylototriton wenxianensis* has 2 variants and *Tylototriton verrucousus* has 19 variants including one called *T. pseudoverrucosus* (AmphibiaWeb, 2015).

It is not very easy to distinguish Newts and Salamanders since they possess many common characters, for instance, most Salamandrids have a long, slender, flexible body and a long-tail. The eye lids are movable. Lungs are present both in juvenile and adult forms. Larvae have feathery external gills. Further, many Salamandrids have well developed skin glands which often are large and prominent on the head (Parotid or paraoid glands). All Salamandrids have toxic and distasteful alkaloids as skin gland secretions which help them deter predatory animals. It is generally, agreed view that Newts form the species that spend a prolonged period each year living in water and become temporarily adapted to life in water. There is further line of demarcation within Salamandrids with regard to true Salamanders and Newts. Currently, there are 112 species of true Salamanders and Newts spread all over northern hemisphere – Europe, Asia, northern tip of Africa and north-America

(AmphibiaWeb, 2015). True Salamanders and Newts differ from other Salamandrids by the lack of rib or costal groove along the sides of the body, and usually have rough skin, except in aquatic phase, in which the skin becomes smooth, thin and slimy and serves as a route for gaseous exchange between body and the surrounding water.

Some populations of certain species of Newts become paedomorphic, meaning they become sexually mature adults while retaining a number of larval features, such as external gills and a large finned-tail. Paedomorphic adults do not become terrestrial and remain in water throughout life. Paedomorphosis has been reported in some European (*Trituruus)* species and in three north-American Newt (*Notophthalmus*) species. Why some populations of these Newt species become paedomorphic is not known.

The origin of Salamandrids is traced back to late Cretaceous or early Paleocene in Europe and later dispersed to Asia and North-America. The oldest fossil records of Salamandrids come from the Cenozoic in Europe. Amongst living Salamanders, the Himalayan Newt, *Tylototriton verrucosus* Anderson is considered as one of the most primitive species (Das, 1987). This Newt species commonly known as Himalayan Salamander, Red-knobby Newt, Himalayan knobby Newt, Crocodile Newt, Alligator Newt, Burmese Crocodile Newt, is a living fossil which has lived the age of the dinosaurs. It is also said to be the missing link between the Reptiles and the Amphibians (IUCN, 2015). This Newt species has acquired many vernaculars depending on the area of its occurrence : Garo (Nepali), Longling (Nepali), Panichapro (Nepali), Panikukur (Nepali), Lengva (Thankhul), Hangoi mamei Panba (Manipuri), Kalapuina mubila (Maram). This Newt species was first described by John Anderson in 1871, who discovered this species in flooded rice fields near the small Chinese town of Nantin, Momien and Hotha valleys, in western Yunnan Province, Peoples' Republic of China (Nussbaum *et al*, 1995; Wangyal and Gurung, 2012). Subsequently, presence of this species was noted in south-Asia and south-east Asia: Eastern Nepal (Schleich and Kästle, 2002; Shrestha, 1984, 1989, 1994) through the Kachin and Shan hills of Myanamar (Gyi, 1969) to western-Yunnan (Longchuan county and its vicinity), China (Zhao and Adler, 1993), scattered mountainous regions of northern Thiland (Taylor, 1962; Wongratna, 1984; Chan-Ard *et al*, 1999; Chan-Ard , 2003; Nabhitabhata *et al,* 2000, Okinawa in Japan (Zhao *et al,* 1988; Pomchote *et al*, 2008) and Lai Chau and Lao Cai Provinces in north-western Vietnam (IUCN, 2015). Its presence has recently been confirmed in Bhutan (Wangyal and Gurung, 2012). It most likely also occurs in Laos province, although at present there is no record (IUCN, 2015). In southern part of its range it is generally upland species inhabiting at

elevation between 1000-3000 metres at sea level. In the northern parts of its range, it is reported from low hills below 1000 metres at sea level (AmphibiaWeb, 2015). In the Indian sub-continent, although presence of *Tylototriton verrucosus* Anderson was noted at the beginning of 20th century (Annandale, 1907, 1908), it fetched attention of wild life researchers in 1964 when presence of this species was located at Jorepokhri, 19 *km* from Darjeeling town. In due course of time, many habitats of this species were spotted in Darjeeling hills of west-Bengal. At present, as many as 49 wetlands and 37 permanent breeding habitats of *Tylototriton verrucosus* have been spotted at different altitudes and cold climate of the eastern Himalayan region of Darjeeling-hills of west-Bengal (Chaudhuri, 1966; Dasgupta, 1984, 1990; Kuzmin *et al,* 1994; Roy and Mushahidunnabi, 2000, 2001; Seglie *et al*, 2003; Nag and Vasudevan, 2014). Presence of this species has also been reported in Sikkim (Sparreboom, 1999; AmphibiaWeb, 2015), Arunachal Pradesh (Mansukhani *et al*, 1976), isolated populations in Manipur (Selim, 2001) and Meghalaya (Das, 1984). Although updates on Its existence in Sikkim, Arunachal Pradesh and Meghalaya is awaited, it probably occurs more widely than current records suggest (Seglie *et al*, 2003; AmphibiaWeb, 2015; IUCN, 2015).

Tylototriton verrucosus Anderson is a fairly robust species of Newt and has survival potential in a large variety of habitats. It inhabits mainly paddy fields, tea-gardens, rain puddles, decaying wood-logs, forest organic-litters, rat holes, rock crevices, medows covering the shores of mountain ponds and lakes, lotic and lentinc water bodies and wet-lands at sites where mountain forests exist or previously existed (Nag and Vasudevan, 2014; Amphibia Web, 2015). Its terrestrial habitat is largely moist forest, with animals generally living close to fresh water bodies. The animal is reported to be most active during monsoon season when it emerges from its hibernacula, feeds actively and performs breeding activities. By October/November following decline in water temperature below 10^{oC} it leaves aquatic habitat to enter into hibernation. During the dry summer period animals show a tendency to enter into aestivation (IUCN, 2015). Current literature survey suggests the lack of precise information of life history, morphological features and distribution pattern of *Tylototriton verrucosus* Anderson on the global scenario. To elucidate all these aspects of *Tylototriton verrucosus* Anderson*,* extensive surveys need to be attempted into discrete hilly pockets in and around the habitats where presence of this species is known or anecdotally reported. John Anderson (1871) published Taxonomic descriptions of *Tylototriton verrucosus* Anderson assigning it a uniform blackish-brown colour pattern. He did not designate a type

series and only later presented a specimen to the British Museum of Natural History which is registered as a *syntype*. The colour pattern of this specimen does not match the original description of *Tylototriton verrucosus* Anderson made by John Anderson in 1871, as it has a vivid orange Cranial crest, Paratoid glands, vertebral crest, dorso-lateral warts, four limbs and a tail. Taking into account, the apparent differences in the colour morphs, and taxonomic details Nussbaum *et al* (1995) described the *syntype* as *Tylototriton shanjing*. The oversight between *Tylototriton verrucosus* Anderson and *Tylototriton shanjing* was attributed to the type locations of the described specimens from the two species. All *Tylototriton verrucosus* Anderson populations originally described from Yunnan province, China with the exception of a small range in extreme western-Yunnan were opined to be considered as *Tylototriton shanjing*. The remaining range of *Tylototriton verrucosus* Anderson is large, encompassing several countries as producing many variants or sub-species and possibly independent species. Till date, it could not be possible to define clear boundary in the distribution of *Tylototriton verrucosus* Anderson and *Tylototriton shanjing* (Young and Rao, 2008; IUCN, 2015). The issue of *Tylototriton verrucosus* Anderson and *Tylototriton shanjing* as two separate species came up once again when Zhang *et al* (2007), based on similarity in phylogeny of mitochondrial Cytochrome *b* gene suggested that *Tylototriton verrucosus* Anderson and *Tylototriton shanjing* should belong to the same species. This study was marred with difficulty that only one *Tylototriton verrucosus* Anderson specimen was included in the study against 39 samples from *Tylototriton shanjing*. Later, using *Tylototriton verrucosus* Anderson from localities different from Zhang *et al* (2007), Stuart *et al* (2010) found relatively large mitochondrial genetic divergence between *Tylototriton verrucosus* Anderson and *Tylototriton shanjing,* and thus supporting the validity of both the species. In due course of time based on specimens collected from different habitats it was realized that *Tylototriton verrucosus* Anderson has several polychromatic populations having moderate to severe differences in morphometric characteristics and thus represent great geographic variations. It is not yet understood whether these polychromatic populations represent geographic variants of a single species or distinct species?. This requirement warrants determination of the molecular phylogeny of this species across the habitats of its presence.

Himalayan Newt, *Tylototriton verrucosus* Anderson is a philopatric predatory species and shows limited locomotory capacities (Joly and Miaud, 1989). Throughout the stretch of its presence, it emerges from its hideouts following the onset of monsoon rains. Newts have tremendous capacity to locate their food even in complete darkness owing to their keen

perceptions for the smell of food. Looking grossly at the diversity of food and feeding habits of *Tylototriton verrucosus* Anderson, it may be presumed that in the process of familiarizing to diverse habitats, Himalayan Newts might have adapted strategies that could enable them s to exploit a vast array of food-resources present in the ecological niche it occupies. Data largely derived from captive feeding experiments and stomach content analysis in free living populations denote large variations in food preferences of *Tylototriton verrucosus* Anderson during developmental stages of the larvae, seasons and habitat occupancy (Dasgupta, 1988, 1996). The adult food mainly consists of larval and adult forms of insects, larvae of frogs, molluscs, cannibalized eggs and vegetal materials (Kuzmin *et al*, 1994; Dasgupta, 1996; Anders *et al*, 1998; Devi, 2005). These reports have made valuable contributions to the understanding of feeding ecology of the Himalayan Newt but data are limited to the Newt populations from Darjeeling district of the west-Bengal. Practically, nothing is known of food and feeding habit of *Tylotorriton verrucosus* Anderson from other known localities in the Indian sub-continent (Mansukhani *et al*, 1976; Das, 1984; Selim, 2001). Elsewhere, particularly from Europe (Germany), mostly animal hobbyists have published data on food preferences and feeding behaviour of *Tylototriton verrucosus* Anderson maintained under captive conditions (Staniszewski, 2000; Caudata Culture, 2006). Newts are reported to be greedy eaters in the terrarium and in general, females consume a lot more food as compared with males. Further, food objects smaller than 4-5 *mm* in size are not recognized by the adult Newts (Staniszewski, 2000; IUCN, 2015).

Under aquatic conditions Newts are known to shed their skin very often to acquire smooth, slimy and thin texture that presumably facilitates gaseous exchange between body of the Newts and the surrounding medium (Dasgupta, 1996; Caudata Culture, 2006). However, practically nothing is known of moulting pattern and the duration of the moult in the Himalayan Newt (AmphibiaWeb, 2015).

Several reports describe breeding behaviour (Annandale, 1908; Boulenger, 1920; Chaudhury, 1966; Thorn, 1969; Thant-Shima *et al*, 1979; Dasgupta, 1983; AmphibiaWeb, 2015), hibernation (Basnet, 2001; Nag and Vasudevan, 2014), Parental care (Dasgupta, 1984) and biology of eggs and larvae (Annandale, 1907, Smith, 1924; Dasgupta, 1988; Shrestha, 1989) of the Himalayan Newt. Throughout the stretch of its presence breeding activities in *Tylototriton verrucosus* Anderson is limited to monsoon months and in some habitats it may continue up to September. Current literature describe great inconsistency on breeding patterns

and associated behavioural attributes which Newts display during the breeding season. One of the most contentious aspects deals with the mode of transfer of spermatophores by male into the female during courtship display. It has been argued that vent to vent amplexus between male and female is mandatory for spermatophore transfer into the female. Alternatively, transfer of spermatophore in the female occurs during a circular dance during which cloaca of both male and female remains opened and male adopting circular path goes on depositing spermatophores in form of bolus and the female following the same circular path sucks spermatophores and store them into spermatheca to acquire internal fertilization which may take several hours (Roy and Mushahidunnabi (2001); Caudata Cultere, 2006; AmphibiaWeb, 2015; IUCN, 2015). Though anecdotally described, some records suggest both, vent to vent amplexus and circular dance as mandatory mechanism for spermatophore transfer by male into the female (Thant-Shima *et al*; 1979; Sparreboom, and Yunke, 2010). The species is secretive at nature and performs breeding activities only during night time (Roy and Mushahidunnabi (2001); AmphibiaWeb, 2015; IUCN, 2015). The secretive nature of the Himalayan Newt features daunting task to the behavioural biologists to candidly observe, record and elucidate different aspects of breeding behaviour in their natural habitat. Further, studies describing reproductive behaviour of the Himalayan Newts have either been made outside the Indian sub-continent or remain confined to Newt populations inhabiting Darjeeling hills of west-Bengal (Sparreboom and Yunke, 2010; AmphibiaWeb, 2015; IUCN, 2015). The last report to describe reproductive biology of the Himalayan Newt was made by Roy and Mushahidunnabi (2001) from Darjeeling hills, west-Bengal. Through carefully planned experiments, these authors successfully demonstrated courtship, mating and egg-laying in wild-caught Newts planted to laboratory conditions during the breeding season. Fertilization is internal and the female has been reported to spawn more than once in a breeding season (Shrestha, 1989; AmphibiaWeb, 2015; IUCN, 2015). Fecundity is reported to have positive correlation with the body size of the females (Roy and Mushahidunnabi, 2001). A mature female depending on size and bulk of the body may deposit 26-300 eggs during single spawn (AmphibiaWeb, 2015; IUCN, 2015). Eggs are entrapped in white jelly. The size of freshly laid eggs along with jelly measures from 6-10 *mm* and are laid inside water. Eggs are glued to the rocks, leaf-blades, stems and twigs of plants inside water and rarely outside the water (Shrestha, 1989; AmphibiaWeb, 2015; IUCN, 2015). At a about 25^{oC} eggs hatch between 12-14 days. Larvae mature before onset of winter and leave the aquatic habitat. In certain habitats breeding is delayed up to August- September and in these habitats larvae over-winter and become mature during following spring season (Nag and Vasudevan,

2014; AmphibiaWeb, 2015; IUCN, 2015). So far, there is no report to describe different aspects of reproductive behaviour of *Tylototriton verrucosus* Anderson based exclusively on field investigations in its natural habitat.

The scientific interest and potential value of the Himalayan Newt, *Tylototriton verrucosus* Anderson may be realized in that this species is considered a living fossil which has lived the age of the dinosaurs. It is also regarded as the missing link between the Reptiles and the Amphibians (IUCN, 2015). Once the species was considered to have been wiped out from the face of the earth but in the 19th century it was found to be present in cold and hilly pockets of the eastern Himalayan region (Anderson, 1871). The unique feature of this species in remaining selective to the cold and temperate type climate in hilly pockets at various altitudes, exclusively nocturnal and secretive habit, and prolonged period of hibernation (> 6 months in a calendar year) have probably been the reasons to catch late attention of researchers and conservation biologists. It is the only Salamander species known from the Indian sub-continent (Seglie *et al*, 2003; AmphibiaWeb, 2015). Its presence in paddy fields and selective exploitation of insect-fauna as food has rendered it the status of natural scavenger and friends of farmers. Retention of larval tail throughout life imparts interesting feature to this species. The unique features of larvae, attractive colour patterns in adults, robustness and easy survival in the glass aquarium has drawn the interest of animal hobbyists as a pet particularly from Europe and thus the species acquires the status of ornamental amphibians. In India, Himalayan Newt has been reported to serve the source of traditional medicine (Selim, 2001; AmphibiWeb, 2015).

The Red List Category of the IUCN has placed *Tylototrtiton verrucosus* Anderson in the category of a least concern species in view of its wide distribution, tolerance to a broad range of habitats, presumed large populations and assumed slow decline to qualify for listing into threatened category (AmphibianWeb, 2015; IUCN, 2015). However, though limited, publications made during the past one decade point to rapid decline in the population density of the Himalayan Newt throughout the entire range of its occurrence (Seglie *et al*, 2003; Pomchote *et al*, 2008; Nag and Vasudevan, 2014; AmphibiaWeb, 2015; IUCN, 2015). The habitats located near human settlements are worst affected owing to habitat loss from infrastructure development and following slash and burn method of cultivation particularly in the south-east Asia. Pollution of water resources from agrochemicals, domestic detergents, chemical fishing, keeping live-stock near ponds and extraction of water for irrigation of paddy and vegetable crops are reported to have negative consequence on the Newt population

(Selim 2001; Roy and Mushahidunnabi, 2001; Seglie *et al*, 2003; AmphibiaWeb, 2015). Further, infrastructure development clubbed with promoting tourism and creation of recreation centres around places that form potential habitats of Newts are adversely affecting the survival and growth of Newt populations. Himalayan Newt is considered a bad omen and thus killed in Myanmar in certain areas and it is often used as bait for fishing (Gyi, 1969). The demand for Himalayan Newt as a pet in the International market has posed a threat of over collection and trade and thus challenging the very existence of this species in many pockets (AmphibiaWeb, 2015; IUCN, 2015). In Ukhrul district of the Manipur state, north-eastern India, Himalayan Newt is harvested for use as traditional medicines and is also consumed by the local people and has thus led to rapid decline of this species making it critically endangered (Selim, 2001). Further, while it is reported that *Tylototriton verrucosus* Anderson attains sexual maturity at the age of 3-4 years and has maximum longevity of 10-11 years, there is paucity of information on Newt mortality resulting due to microbial/epidemic infections in their natural habitat. The alarming decline in *Tylototriton verrucosus* Anderson populations throughout its presence has prompted Governmental agencies to enact Laws to prohibit Himalayan Newts from collection and trafficking (AmphibiaWeb, 2015; IUCN, 2015). In The Wild life (Protection) Act 1972, Schedule-I, *Tylototriton verrucosus* Anderson has been listed as an endangered species and in Wildlife (Protection) Act, Manipur, Rule 74 it has been declared as a protected species. These acts protect *Tylototriton verrucosus* Anderson from collection and trafficking but do not have a bearing on protection of their habitats.

From the foregoing literature, it is obvious that efforts need to be made to trace the presence of *Tylototriton verrucosus* Anderson in areas other than places they have been currently reported from. In this regard, a clue was had in the publication of Selim (2001) wherein author has clearly mentioned about plentiful availability of Himalayan Newts in Mao area of the Senapati district, Manipur state in the early eighties where they are hardly seen now. Relying at this source of information, a survey was launched in discrete hilly regions of the Senapati district to trace the possible occurrence of *Tylototriton verrucosus* Anderson in this part of the country. Further, owing to publications that describe variations in morphological features and colour patterns of *Tylototriton verrucosus* Anderson depending upon geographical regions and the habitat occupancy, a protocol was determined to keep the track of this requirement in Senapati district, Manipur. An area covering approximately 300 km^2 was surveyed continuously for more than 6 years (2009-2015) during the months of May and June to spot and map habitat locations and possible occurrence of polychromatic forms of

Himalayan Newts in such habitats. Due focus was placed on the presence of food resources in their habitats, feeding patterns, skin-shedding and prevalence of microbial infections. Exhaustive efforts were made to monitor breeding behaviour of the Himalayan Newts in their natural habitats as and when opportunities existed. Increasing and/or decreasing trends in population size were made depending on numbers of adult Newts encountered in specific habitats during the survey period. The dissertation has been divided into five chapters as follows :

(i) Chapter-I deals with distribution, habitat quality and its' possible impact on increasing and/or decreasing population trends in adult Newts.

(ii) Chapter-II describes morphological feature and different colour variants of *Tylototriton verrucosus* Anderson collected from specific habitats located in Senapati district, Manipur.

(iii) Chapter-III includes findings on availability of food resources in habitats, and feeding and moulting behaviour of Newts. Quantification and types of food resources consumed in free living populations and selective feeding under captive conditions were analyzed in both the sexes of Newts from May to October.

(iv) Chapter-IV deals with findings on courtship, mating, and egg laying behaviour of Himalayan Newts in their natural habitats. This chapter also describes behaviour of stud male towards his female in the presence of alien males .

(v) Chapter-V is dedicated to the description of threat, survival prospects and conservation strategy of Newts. In this part of the thesis due analysis has been made of different activities which are likely to have negative influence on the habitat quality and survival prospects of the Himalayan Newt in Senapati district, Manipur.

CHAPTER – I

OCCURRENCE, DISTRIBUTION AND HABITAT ECOLOGY OF HIMALAYAN NEWT, *TYLOTOTRITON VERRUCOSUS* ANDERSON IN SENAPATI DISTRICT, MANIPUR, NORTH-EASTERN INDIA

INTRODUCTION

Himalayan Newt, *Tylototriton verrucosus* Anderson is a tailed amphibian which is considered as one of the most primitive species amongst living salamanders (Das, 1987). It is the only caudate species known from the Indian sub-continent. The species acquires several English names : Himalayan salamander, Red-Knobby Newt, Himalayan Knobby Newt, Crocodile Newt, Alligator Newt and Burmese Crocodile Newt *etc.*. Besides, it has several vernaculars depending upon the area of its occurrence and the people living around the place *viz*. Garo (Nepali), Longling (Nepali), Panikukur (Nepali), Panichapro (Nepali), Lengva (Tankhul), Hangoi mamei Panba (Manipuri) and Kalapuina mubila (Maram) (Global Amphibian Assessment Report, 2000; AmphibiaWeb, 2015; IUCN, 2015). Distribution of *Tylototriton verrucosus* Anderson has been recorded in south-Asia and south-east Asia: eastern Nepal (Schleich and Kästle, 2002; Shrestha, 1984, 1989, 1994) through the Kachin and Shan hills of Myanamar (Gyi, 1969) to western-Yunnan (Longchuan county and its vicinity), China (Zhao and Adler, 1993), scattered mountainous regions of northern Thailand (Taylor, 1962; Wongratna, 1984; Chan-Ard *et al*, 1999; Nabhitabhata *et al,* 2000; Chan-Ard , 2003; Pomchote *et al*, 2008), Okinava in Japan (Zhao *et al,* 1988; Pomchote *et al*, 2008), Lai Chau and Lao Cai Provinces in north-western Vietnam and Bhutan (Wangyal and Gurung, 2012; IUCN, 2015). It most likely also occurs in Laos province, although at present there is no record (IUCN, 2015). In India, *Tylototriton verrucosus* Anderson is reported from Darjeeling-hills of west-Bengal (Chaudhuri, 1966; Dasgupta, 1984, 1990; Kuzmin *et al,* 1994; Roy and Mushahidunnabi, 2001; Seglie *et al*, 2003; Nag and Vasudevan, 2014), Sikkim (Sparreboom and Yunke, 2010; AmphibiaWeb, 2015), Arunachal Pradesh (Mansukhani *et al*, 1976), Ukhrul district, Manipur (Selim, 2001) and Meghalaya (Das, 1984). In the IUCN Red-list, this species figures under the category of least concern assuming its prevalence in areas more than what the current records suggest (Global

Amphibian Assessment Report, 2006; AmphibiaWeb, 2015; IUCN, 2015). Selim (2001) has described *Tylototriton verrucosus* Anderson as a critically endangered species in Ukhrul district, Manipur. In the very report, the author has mentioned at its possible presence in Senapati district of the Manipur, though to our knowledge, till date there is no documented report to support this claim.

Himalayan Newt has been reported to occupy a large variety of habitats : paddy fields, tea-gardens, rain puddles, decaying wood-logs, forest organic-litters, rat holes, rock crevices, medows covering the shores of mountain ponds and lakes, lotic and lentinc water bodies and wet-lands at sites where mountain forests exist or previously existed (Nag and Vasudevan, 2014; Amphibia Web, 2015). Its terrestrial habitat constitutes moist forest, with animals generally living close to fresh water bodies (AmphibiaWeb, 2015; IUCN, 2015). In the present study, efforts were made to explore the possibility of the occurrence of *Tylototriton verrucosus* Anderson in discrete mountainous range of the Senapati district along with quality assessment of the water bodies that made its aquatic habitat.

MATERIALS AND METHODS

Between 2009 and 2013, every year from May to June, approximately 300 Kilometre^2 mountainous area of Senapati district, Manipur state was surveyed to trace the possible occurrence of *Tylototriton verrucosus* Anderson. A time constrained visual encounter search technique (Campbell and Christman, 1982; Corn and Bury, 1989) was used because of uneven topography of the land. The places where the species was encountered were used for distribution mapping (Fig. 1). Survey team of two to three persons was used for scouring stream premises, vicinity of perennial and seasonal water bodies, paddy fields and forest floor using electric lamps during night time. Light objects on the floor were gently turned, observed and replaced without much disturbance in search of Newts. When encountered, they were captured by hand and were subjected to the recording of body weight and morphometric measurements. Newts captured in a particular habitat were stocked in all glass aquaria for three days and after recording morphometric measurements they were released in their natural habitat.

Mesuring Index

A plastic ruler was used for recording the total length and the snout to vent length (SVL) to the nearest millimetre (*mm*). SVL was recorded from the tip of the snout to the part of vent making cloacal opening following the method described by Cockran and Thomas (1996). Tail-length was calculated by subtracting SVL from the total length. Body weight of individual Newt was recorded using small plastic bag and a small branded spring balance to the nearest of 100 milligrams (*mg*).

Latitude and longitude at places of Newt habitats were recorded using GPS MAP 76CSX from Garmin Company.

Feeding and Maintenance

During the recording of morphometric data, Newts were maintained in glass aquaria (3.5' x 3' x 2.5' in size) with water levels approximately at 10 *cm,* grass from ponds, stone heaps and floating wood pieces. Newts were fed with pieces of live and soaked dry-fish and earth worms *ad libitum*. In each habitat Newts were observed for at least three consecutive days and nights between 15-30th of May and June every year from 2009-2013. May and June months were opted for survey owing to the fact that in this region, north-eastern monsoon becomes active during May/June and if present, Newts are expected to emerge from their hideouts to perform breeding activities.

Sexing of Newts

Sexing was done based on the swollen cloaca with longitudinal slit in males and small rounded conical cloacal opening in females (Plate-I). Besides, males had rounded belly and secreted while milt on slight pressure on their belly. Females had relatively large distended belly. Towards the end of the study general health of the Newts was checked and they were released in their natural habitat.

The identification references used in the field include Smith (1943), Daniels, (2005), Yang and Rao (2008), Ahmed *et al* (2009), Fei *et al* (2010), and Vasudevan and Sondhi (2010).

During the survey period maximum and minimum air and water temperature of the habitats were regularly maintained. Water samples from habitats of Newts were collected and

analyzed for measurements of dissolved Oxygen content (DO_2), pH, total alkalinity, free carbon dioxide (CO_2) using Merck New Spectroquant test kit (Table 1).

Records of plants and amphibian species encountered within 100 meter^2 range of Newt habitats were also maintained.

RESULTS

During the survey period from May 2009 – June 2013 following 10 habitats of Himalayan Newts were encountered (Plate- II-VI).

1. **Tingbajot** : This habitat is located at forest edge, approximately 3 *km* north-west to Maram Centre on National Highway-39 (NH-39) at an elevation of 1754 metre at sea level (Lat. 25^o 26.032' N; Long. 94^o 05.411'E). The habitat consisted of a perennial pond (~55' x 10'; water level during rainy season variee from 20-30 *cm*). The pond was extended with approximately 100 metre^2 area of paddy fields. During the observation period, habitat was found well fringed with plants like *Sagittaria sagittifolia, and different species of Polygonum, Commelina, Euphatonium, Osbeckia, Impatiens, Fagopyrum, Astemisia, Bidenspilosa* and *Alteranthaceae. Hyla annectans, Rhacophorus maximus* and *Bufo melanostictus* were other amphibian species encountered during the survey. Air temperature was recorded at a maximum of 30.5^{oC} and minimum of 12^{oC} respectively. Water temperature ranged between a maximum of 28^{oC} and a minimum of 11^{oC}. Dissolved Oxygen Content (DO_2) was measured at 6.5 *mg/litre;* pH, 6.5; alkalinity, 33 and free Carbon Dioxide (CO_2), 2.99 *mg/litre.* Relative humidity was maximum at 91% and minimum at 68% respectively. In random survey between May 2009 and June 2013, 72% less number of Newts were encountered in this habitat (Plate- IIa, Table - 2).

2. **Kambadui** : This habitat is located inside a forest, at about 9.5 *km* north-west to Maram Centre on NH-39 at an elevation of 1818 metres at sea level (Lat. 25^o 26.042'N; Long. 94^o 05.055'E). The pond is located in the basement of hills (~ 30' x 9.5' in size) and received water seeping at the hill-base. The pond is cemented from three sides and during lean period (November-March) it serves as a source of water for domestic uses for the people from nearby settlement. During the rainy season water level in this pond varied from 20-25 *cm*. At a stone throwing distance another small pond exists at the forest edge which received water

overflowing from the cemented pond. Dominant plant species in this habitat consisted of *Alterenthera sessilis, Euphatonium odaratum, Rotala rotundifolia, Dryopteris flexis, Cynodon doctylus* and *Polygonum species. Hyla annectans, Rhacophorus maximus* and *Bufo melanostictus* were other amphibian species encountered during the survey. Air temperature was recorded at a maximum of 32.3^{oC} and minimum of 12^{oC} respectively. Water temperature varied between a maximum of 30^{oC} and minimum of 12^{oC}. DO_2 was recorded at 7.2 *mg/litre;* pH, 6.6; alkalinity, 35 and free CO_2, 3 *mg/litre*. Maximum and minimum relative humidity varied between a maximum of 90% and 68% respectively. During periodic survey between May 2009 and June 2013, 75% less number of Newts were encountered in this habitat (Plate-IIb, Table- 2).

3. Lakpiidui : This spot is located at about 7.5 *km* north-west to Maram Centre on NH-39 at an elevation of 1832 metres at sea level (Lat. 25^{o} 25.628'N; Long 94^{o} 04.924'E). A perennial pond (~ 26' x 16.5', water level in rainy season varied from 20-25 *cm*) located at the forest edge was found to harbour Newts. Vegetation consisted of different species of *Drymania, Musaenda, Polygonum, Urena lobata* and *Galengsoga paniflora.* Amphibian species like *Hyla annectans, Rhacophorus maximus* and *Bufo melanostictus* made other amphibian species encountered within 100 meter^2 area of the pond. The pond was filled up in July, 2011 for developmental activities. Meanwhile another small pond has been created in the area. Air temperature was recorded at a maximum of 31.3^{oC} and minimum of 13^{oC} respectively. Water temperature varied with a maximum of 30^{oC} and minimum of 12^{oC} respectively. DO_2 was measured at 7 *mg/litre;* pH, 6.7; alkalinity, 36 and free CO_2, 2.9 *mg/litre*. Maximum and minimum relative humidity varied at a maximum of 90% and minimum of 67% respectively. Observations made during 2009-2011 revealed this habitat supporting very small number of Newts (Plate-IIIa, Table 2).

4. Salumdui : This habitat is located under forest cover at about 7.5 *km* north-west to Maram Centre on NH-39 at an elevation of 1820 metre at sea level (Lat. 25^{o} 25.616'N; Long 94^{o} 04.913'E). The habitat consisted of a perennial water body (~ 11.5' x 5.5'; water depth in rainy season varied from 24-30 *cm*). *Alterenthera sessilis, Sagittaria sagittifolia* and different species of *Polygonum* dominated the aquatic habitat. *Hyla annectans, Rhacophorus maximus* and *Bufo melanostictus* were other amphibian species to be encountered during the survey. Air temperature varied at a maximum of 30^{oC} and minimum of 12^{oC}. Water temperature was recorded at a maximum of 28.3^{oC} and minimum of 11^{oC}. DO_2 was at 6.9 *mg/litre;* pH, 6.6;

alkalinity, 38 and free CO_2, 3.1 *mg/litre*. Relative humidity varied between a maximum of 89% and minimum of 68% respectively. Between May 2009-June 2013, 100% increase in number of Newts was registered in this habitat (Plate-IIIb, Table- 2).

5. Pongtuijai : This habitat is located at the forest edge at about 7.5 *km* north-west to Maram Centre on NH-39 at an elevation of 1825 metre at sea level (Lat. 25^o 25.637'N; Long 94^o 04.888'E). The habitat consisted of a seasonal water body (~ 36' x 25'; water level during rainy season varies between 20-25 *cm*) which becomes flooded during rainy season and dries up in winter (January/February). Plant species in this habitat consisted of *Alterenthera sessilis, Epatorium odaratum, Rotala rotundifolia, Dryopteris flexis, Cynodon doctylus* and *Polygonum species*. *Hyla annectans, Rhacophorus maximus* and *Bufo melanostictus* were other amphibian species encountered during the survey. Air temperature was recorded at a maximum of 31.2^{oC} and minimum of 12.5^{oC}. Water temperature varied at a maximum of 29.2^{oC} and minimum of 12^{oC} respectively. DO_2 was at 7.2 *mg/litre;* pH, 6.8; alkalinity, 37 and free CO_2, 3 *mg/litre.* Relative humidity varied between a maximum of 90% and a minimum of 67% respectively. Results show 125% increase in number of Newts encountered in this habitat between May 2009 and June 2013 (Plate-IVa, Table-2).

6. N'rekpiidui : This spot is located at the forest edge at about 8 *km* north-west to Maram Centre on NH-39 at an elevation of 1820 metres at sea level (Lat. 25^o 25.630'N; Long 94^o 04.829'E). Habitat consisted of a perennial water body (~ 26' x 15'; water depth in rainy season varied between 20-25 *cm*). During monsoon months, the habitat gets extended by approximately 100 metre^2 paddy fields. Aquatic vegetation consisted of *Cynodon doctylus*, different species of *Galengsoga, Fagopyrum,* and *Polygonum. Hyla annectus, Rhacophorus maximus* were other amphibian species encountered during the survey period. Air temperature was measured at a maximum of 30.5^{oC} and a minimum of 12^{oC}. Water temperature varied at a maximum of 28.5^{oC} and minimum of 11.5^{oC} respectively. DO_2 was recorded at 7.5 *mg/litre;* pH, 6.7; alkalinity, 36 and free CO_2, 3.0 *mg/litre*. Relative humidity varied between a maximum of 90% and a minimum of 66% respectively. Results show 40% decline in number of Newts encountered in this habitat during survey between May 2009 and June 2013 (Plate-IVb, Table- 2).

7. Apoubam Karii : This habitat consisted of a perennial stream located in deep terrain at about 4 *km* north-west to Senapati market on NH-39 at an elevation of 1332 metres at sea

level (Lat. 25^{o} 17.639'N; Long. 94^{o} 01.075'E). Plants like *Quercus lamellose* and different species of *Calocasia, Musaenda etc*. were found fringing stream banks. At places stream banks are extended with shallow water areas where water depth in rainy season remained below 30 *cm*. *Rana leibigii, Euphlyctis cynophlyctis* and *Megophrys wuliangshanensis* were frequently encountered in the habitat during survey. Air temperature was recorded at a maximum of 31^{oC} and minimum of 11.8^{oC} respectively. Maximum and minimum temperature of water varied between 28.8^{oC} and 12^{oC} respectively. DO_2 was measured at 7.2 *mg/litre;* pH, 6.8; alkalinity, 38 and free CO_2, 2.9 *mg/litre.* Maximum and minimum humidity varied at 89% and 67% respectively. Results exhibit 50% decline in number of Newts encountered in this habitat between May 2009 to June 2013 (Plate-Va, Table- 2).

8. N'tingliibam : This habitat is located at the forest edge at about 8 *km* north-west to Senapati market on NH-39 at an elevation of 1337 metres at sea level (Lat. 25^{o} 18.549'N; Long 94^{o} 00.659'E). Habitat consisted of a perennial water body (~ 16' x 10'; water level in rainy season variedbetween 20-25 *cm*) which gets extended with more than 100 metre^2 paddy fields during the rainy season. Plants like *Quercus lamellose* and different species of *Calocasia, Musaenda etc*. formed aquatic vegetation. *Rana leibigii, Euphlyctis cynophlyctis* and *Megophrys wuliangshanensis* were other amphibian species encountered during survey. Air temperature was recorded at a maximum of 30.5^{oC} and minimum of 12^{oC} respectively. Water temperature varied at a maximum of 28.2^{oC} and minimum of 12.1^{oC}. DO_2 was measured at 7.5 *mg/litre;* pH, 6.7; alkalinity, 36 and free CO_2, 2.9 *mg/litre*. Relative humidity varied between a maximum of 91% and minimum of 69% respectively. Results reveal 100% decline in number of Newts in this habitat encountered between May 2009 and June 2013 (Plate-Vb, Table- 2).

9. Apoukijang : This habitat is located in Koubru hills at about 30 *km* north-west to T. Khullen on NH-39. The place is at an elevation of 2411 metres at sea level (Lat. 25^{o} 03.397'N; Long 93^{o} 52.238'E). The habitat formed a perennial water body well fringed with plants like *Sagittaria sagittifolia, and different species of Polygonum, Commelina, Euphatonium, Osbeckia, impatiens, Fagopyrum, Astemisia, Bidenspilosa* and *Alteranthaceae. Hyla annectans, Rhacophorus maximus* and *Bufo melanostictus* were other amphibian species encountered during the survey. Air temperature was recorded at a maximum of 28.^{oC} and minimum of 12.3^{oC}. Water temperature varied between a maximum of 26.3^{oC} and minimum of 11.5^{oC} respectively. DO_2 was at 7.8 *mg/litre;* pH, 6.8; alkalinity, 58 and free CO_2, 2.8

mg/litre. Relative humidity varied between a maximum of 90% and minimum of 67% respectively. Survey results showed 50% increase in number of Newts encountered in this habitat between May 2009 and June 2013 (Plate-VI, Table - 2).

10. N'Jailong : This habitat is located at about 17 *km* north-west to Kongpokpi on NH-39 at an elevation of 1568 metres at sea level (Lat. 25^{o} 15.058'N; Long 93^{o} 55.919'E). Habitat consisted of a perennial water pond (~ 25' x 20'; water level during rainy season varied between 30-40 *cm*). Plants like *Sagittaria sagittifolia, and different species of Polygonum, Commelina, Euphatonium, Osbeckia, Impatiens, Fagopyrum, Astomisia, Bidenspilosa* and *Alteranthaceae* were found in the pond. *Hyla annectans, Rhacophorus maximus* and *Bufo melanostictus* formed other amphibian species encountered during the survey. Water and air temperature were recorded at a maximum of 30.5^{oC} and minimum of 13.3^{oC}. Water temperature varied between a maximum of 29.8^{oC} and minimum of 12.8^{oC} respectively. DO_2 was at 7 *mg/litre;* pH, 6.7; alkalinity, 37 and free CO_2, 2.9 *mg/litre*. Relative humidity varied between a maximum of 92% and minimum of 69% respectively. Survey results showed 60% decline in number of Newts in this habitat encountered between May 2009 and June 2013 (Plate-VIb, Table - 2).

DISCUSSION

In the only report published by K. Selim in 2001 on the occurrence of *Tylototriton verrucosus* Anderson in Ukhrul district of the Manipur state, the author had emphasized at plentiful availability of the Himalayan Newt in the Mao area adjacent to Nagaland state during eighties, where now a days they are hardly seen. During the present survey we had thoroughly examined Mao and adjoining areas for the possible occurrence of *Tylototriton verrucosus* Anderson but we could not come across any Newt-habitat in this part of the Manipur state. Our efforts continued and we were extremely happy on the evening of 9^{th} of May 2009 when we saw a Newt emerging from its hideout in Kambadui habitat (Plate-VIIa). Subsequently, in the last week of July 2009 when environmental temperature exceeded 30^{oC}, Newts were observed aestivating by taking shelter in holes existing at the side walls of aquatic water bodies or under rock crevices which received adventitious roots from trees bordering Newt habitat (Plate-VIIb, VIIIa,b). These observations are in agreement with the suggestion that with temperature rising above 25^{oC} Himalayan Newts exhibit a tendency to

undergo aestivation (Caudata Culture- species entry, 2000). Published reports advocate in favour of a temperature range of 15-23^{oC} most suitable for performing feeding and breeding activities in the Himalayan Newts and at temperature below 10^{oC} they enter into hibernation (Caudata Culture-species entry, 2000). Further, in the present study, progressive decline in number of Newts encountered from May 2009-June 2013 in 7 of the 10 habitats may denote shrinkage of Newt populations with alarming pace. It may be argued that present data included only adults of Newts who had visited water bodies for performing breeding activities and thus may not necessarily reflect at the factual trend since sexually immature individuals do not migrate to water bodies and remain confined to their terrestrial habitats. This may not however, be the case since during survey, areas around aquatic habitats and forest floor were thoroughly searched for day and night to find young ones of Newts but they were hardly seen. Further, water bodies observed during the survey period were found having few young Newts which were not counted for maintaining present records. These Newts might have visited water ponds for finding food resources which during monsoon month are easily available in water bodies than within their terrestrial habitats. Owing to the observation that declining trends in the number of Newts were pertinent only in habitats which were located near human settlements and in one way or the other received profound disturbances. it may be opined that human interferences with habitats have negative influences on Newt populations. Indiscriminate exploitation of forest resources, use of land that form potential breeding habitats of Newts for agriculture and extension activities, grazing and keeping of live stocks in or around Newt habitats and pollution of pond water with chemical detergents have all been discussed to have adverse influences on Newt populations (Caudata Culture, species entry, 2000; Seglie *et al*, 2003; AmphibiaWeb, 2015). It could not be ascertained if declining trends in the number of Newts in 7 habitats located near human settlements were also impacted by deliberate collection for consumption as practiced by the local people in Ukhrul district, Manipur state (Selim, 2001). Three habitats : Pontuijai, Salumdui and Apoukijang witnessing increase in number of Newts encountered during survey period were located at elevations above 1820 metres at sea level with practically no human disturbances.

Present observations support the view that moderate alterations in habitat ecology do not have severe adverse effects on the survival of *Tylototriton verrucosus* Anderson as the species could comfortably survive and breed in water bodies at normal concentrations of DO_2 and moderately sub-normal levels of pH, alkalinity and free CO_2. The fact that the Himalayan Newt is found in a wide range of habitats like tea garden, terrestrial meadows formed at

shores of mountains and lakes, swamps, ponds, streams, wet-lands and decomposing forest litters *etc.*, advocate in favour of this view (Seglie, 2002). The obvious depletion in population size of *T. verrucosus* Anderson in Senapati district may therefore, be accounted to habitat loss due to anthropogenic activities and drainage of pond water for irrigation of seasonal vegetables and paddy crops, keeping of live stocks in and around Newt habitats as discussed in Chapter- V. In Lakpiidui habitat no Newts could be seen during 2012 and 2013 because the only pond available in this place was filled for developmental activities by the end of 2011, although a new pond was created in the year 2013. Tingbajot and N'tinglibam habitats were carved gradually between 2009 and 2013 to develop paddy fields and the ponds were frequently drained to irrigate vegetables and paddy crops. Further, most of the habitats near human habitations are in continuous use for bathing and washing clothes by local people. All these features might add significantly to the destruction of habitat and consequent decline in Newt populations.

The number of male Newts exceeding the number of females in most of the habitats may be due to the fact that males arrive first to the breeding ponds (Spareboom, 1999) and they make more frequent exit from water to fend for themselves while females arrive to the breeding ponds late and remain engaged in egg laying and discharging parental care to the eggs and larvae (Dasgupta, 1984). Number of males exceeding the number of female Newts has also been reported from other habitats (Wangyal and Gurung, 2012). Extensive search is on to look forward for the presence of *T. verrucosus* Anderson in the remaining parts of mountainous hills of Manipur and adjoining hills in the Nagaland State to make factual assessment of distribution and population size of *Tylototriton verrucosus* Anderson in this part of the country. At the same time, data need to be updated from Sikkim, Arunachal Pradesh and Meghalaya where presence of this species has been reported or argued (Mansukhani *et al*, 1976; Das, 1984). This would help in valuing the factual status of this caudate in the Indian territory.

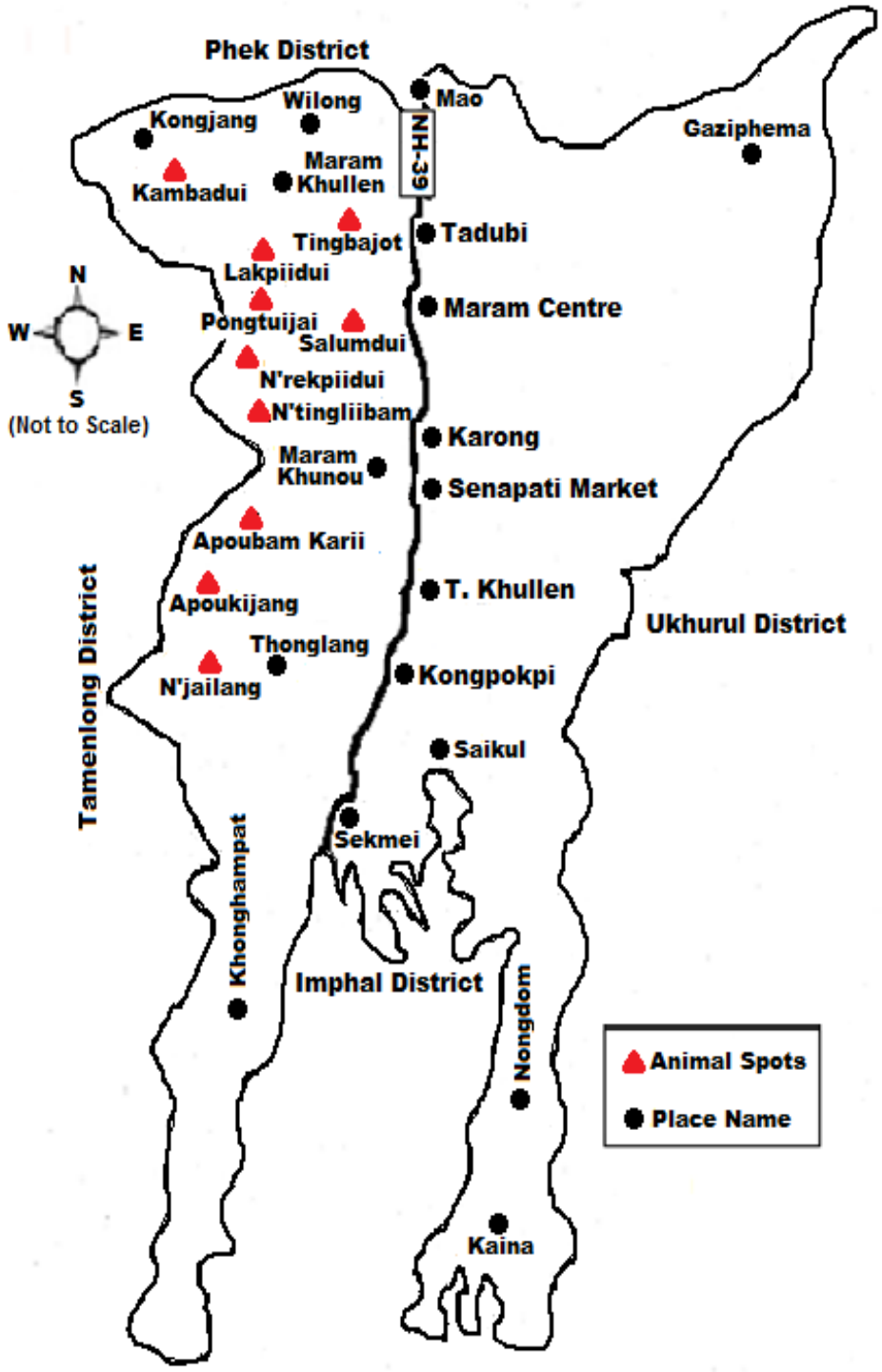

Plate – I: Map showing distribution habitats of *Tylototriton verrucosus* in Senapati District, Manipur.

Sexual dimorphism in *Tylototriton verrucosus A*nderson

(In the breeding season male has a longitudinal cloacal slit and female has a rimmed conical cloacal aperture (shown by arrows)

Plate – II: **a**.Tingbajot habitat is located at forest edge, approximately 3 km north – west to Maram Centre on NH-39 at an elevation of 1754 metre at sea level (Lat. 25^{o} 26.032' N; Long. 94^{o} 05.411'E).
b. Kambadui habitat is located in forest at about 9.5 km north - west to Maram Centre on NH-39 at an elevation of 1818 metres at sea level (Lat. 25^{o} 26.042'N; 26.042'N; Long. 94^{o} 05.055'E).

Plate – III : **a**. Lakpiidui habitat is located at about 7.5 km north-west to Maram Centre on NH-39 at an elevation of 1832 metres at sea level (Lat. 25o 25.628'N; Long. 94o 04.924'E). **b**. Salumdui habitat is located under forest cover at about 7.5 km north-west to Maram Centre on NH-39 at an elevation of 1820 metre at sea level (Lat. 25^{o} 25.616'N; Long 94^{o} 04.913'E).

Plate – IV : **a**. Pongtuijai habitat is located at the forest edge about 7.5 km north-West to Maram Centre on NH-39 at an elevation of 1825 metre at sea level (Lat. 25^{o} 25.637'N; Long 94^{o} 04.888'E). **b**. N'rekpiidui habitat is located at forest edge at about 8 km north-west to Maram Centre on NH-39 at an elevation of 1820 metres at sea level (Lat. 25^{o} 25.630'N; Long 94^{o} 04.829'E).

Plate – V: a. Apoubam Karii habitat is a perennial stream located in deep terrain at about 4 *km* north-west to Senapati market on NH-39 at an elevation of 1332 metres at sea level (Lat. 25° 17.639'N; Long. 94° 01.075'E).
b. N'tingliibam habitat is located at the forest edge at about *km* north-west to Senapati market on NH-39 at an elevation of 1337 metres at sea level Lat. 25° 18.549'N; Long 94° 00.659'E).

Plate – VI: **a**. N'Jailong habitat is located at about 17 km north-west to Kongpokpi on NH-39 at an elevation of 1568 metres at sea level (Lat. 25^{o} 15.058' N; Long 93^{o} 55.919'E).
b. Apoukijang habitat is located in Koubru hills (Meteis call it Kobru and Maram Tribe names it Apoukijang) at about 30 *km* north- west to T. Khullen on NH-39. The place has an elevation of 2411 metres at sea level (Lat. 25^{o} 03.397'N; Long 93^{o} 52.238'E).

Plate – VII: a. A Newt emerging from its hideout on 9.5.2009 in Tingbajot habitat,Senapati district, Manipur.
b. A Newt in search of a place for aestivation in Kambadui habitat on 28th July 2009.

Plate – VIII: a. A Newt aestivating in a hole in side wall of an aquatic water body at Kambadui habitat on 28.7.2009.
b. A Newt aestivating under the rock penetrated by tree roots in Tingbajot habitat on 30.7.2009.

Table-1. Ecological Parameters of different Habitats of the Himalayan Newt, *Tylototriton* 2009 *verrucosus* Anderson in Senapati district, Manipur recorded during the survey periods from May – June 2013.

Habitat	Air temperature		Water Temperature		DO_2	Free CO_2	pH	*Alkalinity*	Relative humidity (%)	
	Maximum	Minimum	Maximum	Minimum					Maximum	Minimum
Tingbajot	30.5^{oC}	12.0^{oC}	28.0^{oC}	11.0^{oC}	6.5	2.99	6.5	33	91	68
Kambadui	32.3^{oC}	12.0^{oC}	30.0^{oC}	12.0^{oC}	7.2	3.0	6.6	35	90	68
Lakpiidui	31.3^{oC}	13.0^{oC}	30.0^{oC}	12.0^{oC}	7.0	2.9	6.7	36	90	67
Salumdui	30.0^{oC}	12.0^{oC}	28.3^{oC}	11.0^{oC}	6.9	3.1	6.6	38	89	68

DO_2, dissolved Oxygen content; CO_2, free Carbon Dioxide

Table- 2. Number of Himalayan Newt, *Tylototriton verrucosus* Anderson encountered in different habitats of Senapati district during the survey period from May 2009 to June 2013.

Habitats	2009	2010	2011	2012	2013
Tingbajot	50 ♂ *(27)*, ♀ *(23)*	35 ♂ *(20)*, ♀ *(15)*	20 ♂ *(11)*, ♀ *(9)*	15 ♂ *(8)*, ♀ *(7)*	14 ♂ *(8)*, ♀ *(6)*
Kambadui	80 ♂ *(45)*, ♀ *(35)*	70 ♂ *(40)*, ♀ *(30)*	50 ♂ *(28)*, ♀ *(22)*	35 ♂ *(19)*, ♀ *(16)*	20 ♂ *(11)*, ♀ *(9)*
Lakpiidui	08 ♂ *(04)*, ♀ *(4)*	05 ♂ *(3)*, ♀ *(2)*	03 ♂ *(02)*, ♀ *(01)*	-	-
Salumdui	10 ♂ *(5)*, ♀ *(5)*	08 ♂ *(4)*, ♀ *(4)*	05 ♂ *(03)*, ♀ *(02)*	32 ♂ (17), ♀ (15)	20 ♂ (11), ♀ (09)
Pongtuijai	06 ♂ *(3)*, ♀ *(3)*	04 ♂ *(2)*, ♀ *(2)*	02 ♂ *(01)*, ♀ *(01)*	10 ♂ *(06)*, ♀ *(04)*	15 ♂ *(08)*, ♀ *(07)*
N'rekpiidui	20 ♂ *(12)*, ♀ *(8)*	18 ♂ *(10)*, ♀ *(8)*	10 ♂ *(06)*, ♀ *(04)*	15 ♂ *(08)*, ♀ *(07)*	12 ♂ *(07)*, ♀ *(05)*
Apoubam Karii	20 ♂ *(11)*, ♀ *(9)*	19 ♂ *(10)*, ♀ *(09)*	15 ♂ *(08)*, ♀ *(07)*	17 ♂ *(09)*, ♀ *(08)*	10 ♂ *(05)*, ♀ *(05)*
N'tingliibam	20 ♂ *(12)*, ♀ *(8)*	15 ♂ *(08)*, ♀ *(7)*	15 ♂ *(8)*, ♀ *(7)*	15 ♂ *(08)*, ♀ *(07)*	10 ♂ *(06)*, ♀ *(04)*
N'jailong	05 ♂ *(3)*, ♀ *(2)*	03 ♂ *(02)* ♀ *(01)*	04 ♂ *(02)*, ♀ *(02)*	05 ♂ *(03)*, ♀ *(02)*	02 ♂ *(01)*, ♀ *(01)*
Apoukijang	20 ♂ *(12)*, ♀ *(08)*	25 ♂ *(14)* ♀ *(11)*	30 ♂ *(16)*, ♀ *(14)*	30 ♂ *(17)*, ♀ *(13*	30 ♂ *(16)*, ♀ *(14)*

CHAPTER – II

MORPHOLOGY AND POLYCHROMATIC FORMS OF THE HIMALAYAN NEWT, *TYLOTOTRITON VERRUCOSSUS* IN SENAPATI DISTRICT, MANIPUR

INTRODUCTION

Newts, the tailed amphibians, belong to the family "Salamandridae", which includes 10 families, 68 genera and 680 species spread over north America and Eurasia (Frost,1985; Stuart and Papenfuss, 2002; Pomchote *et al*, 2008; AmphiaWeb, 2015). The five genera in Asia are *Cynops, Echinotriton, Pachytriton, Paramesotriton* and *Tylototriton* (Stuart and Papenfuss, 2002). *Tylotriton* has been reported from China Nepal, India, Myanmar, Bhutan, Thailand, Vietnam and Japan (Zhao *et al*, 1988; Wangyal and Gurung, 2012; AmphibWeb, 2015; IUCN, 2015). Currently, genus *Tylototriton* includes 22 species of Newts with many intra-species variations (AmphibWeb, 2015).

John Anderson (1871) established the genus '***Tylototriton'*** based on a specimen of the Himalayan Newt, *Tylototriton verrucosus* Anderson from Nantin, Momien and Hotha valleys, in western Yunnan Province, Peoples' Republic of China. The species was described as "Uniform blackish brown, paler on the lips, snout, chin, throat, under surface of the limbs brownish-olive tinge, under surface of the tail dull orange-yellow fading to light-brown on the sides". Later, Anderson submitted a specimen (BMNH 1874.6.1.3) in the British Museum from Nantin valley which is currently registered as a *syntype* of originally described specimen. A thorough examination of the collections housed in Museum as *Tylototriton verrucosus* Anderson revealed presence of some specimens coloured differently bearing varying taxonomic details as compared with *Tylototriton verrucosus* Anderson originally described by Anderson as a Salamander with uniformly blackish–brown dorsum. These differently coloured specimens were designated as separate species to be referred as *Tylototriton shanjing* (Nussbaum *et al*, 1995). In due course of time several geographic variants with distinct colour morphology were reported form across known localities of its distribution (Sparreboom and Yunke, 2010; AmphibiaWeb, 2015; IUCN, 2015). Currently, *Tylototriton verrucosus* Anderson species is known to incorporate 19 different forms including '*pseudo-verrucosus'* (AmphibWeb, 2015).

In India, *Tylototriton verrucosus* has been reported from Arunachal Pradesh (Mansukhani *et al*, 1976), Darjeeling (Roy and Mushahidunnabi, 2000, 2001; Seglie *et al*, 2003; Nag and Vasudevan, 2014), Manipur (Selim, 2001), Meghalaya (Das, 1984) and Sikkim (Sparreboom and Yunke, 2010). To our knowledge from all these habitats no geographic variations in morphological features or polychromatic forms of *Tylototriton verrucosus* Anderson have been described. In the present work, attempt has been focussed at the study of general morphological feature and possible colour variations in *Tylototriton verrucosus* Anderson in different habitats located in hilly pockets of Senapati district, Manipur.

MATERIALS AND METHODS

Between May 2009 and June 2015, 10 habitats of the Himalayan Newt located between altitudes : 1332 – 2411 metre at sea level; latitudes : 25^{o} 03.397'N - 25^{o} 26.042'N; Longitudes : 93^{o} 52.238'E - 94^{o} 05.055'E were periodically surveyed to locate Newts *vis á vis* shorting them out into different colour morphs as and when encountered. Six of the 10 habitats located nearby human settlements : Tingbajot, Kambadui, Lakpiidui, Salumdui, Pongtuijai, and N'rekpiidui were accessed frequently between May and August 2013-2015 in the search for polychromatic forms of the Himalayan Newt. Whenever encountered, Newts were picked up by hand or dip net. After the body weights of Newts were individually recorded, colour patterns noted, morphometric measurements determined, photographed, they were released in their natural habitat.

The sex of each specimen was determined by external examination of the cloaca. Following characters for each specimen were recorded : snout to vent length (SVL), tail length, head length, head width, inter-orbital distance between anterior angles of eyes, internarial distance, eye-naris distance, length of cranial crest, width of cranial crest at posterior corner of right eye, boss length (mid-dorsal ridge on the skull), width of mid-dorsal crest (vertebral ridge) at level of the 5^{th} pair of lateral warts, length of 5^{th} wart of right side, height of 5^{th} wart of right side, number of body warts on right side including the wart above the groin, number of tail warts (behind the groin) on right side, lengths of the extended fore limbs, lengths of the extended hind limbs in a total number of 516 Newts encountered between 2009 and 2015. Majority had colour patterns as originally described by Anderson (1871). Owing to very small number of colour variants encountered during survey, it could

not be possible to analyze data for statistical inferences. Therefore, present study has exclusive bearing on taking the cognizance of some probable neo-types which co-exist with *Tylototriton verrucosus* Anderson originally described from Nantin, Momien and Hotha valleys, in western Yunnan Province, Peoples' Republic of China (Nussbaum *et al*, 1995).

A plastic ruler was used for recording the total length and the snout to vent length (SVL) to the nearest of a millimetre (*mm*). SVL was measured from the tip of the snout to the anterior edge of the cloacal opening following the method of Cockran and Thomas (1996) and the tail-length was calculated by subtracting SVL from the total length. All other morphometric measurements were made using Helios dial callipers (0.1 *mm* precision). Body weight of individual Newt was recorded using small plastic bag and a small branded spring balance to the nearest of 100 milligrams (*mg)*.

GPS MAP 76CSX from Garmin Company was used for recording latitude and longitude of the habitats. Sexing was done based on the longitudinal slit in male and small rounded conical cloacal aperture in the female. Besides, males had rounded belly while females had relatively large distended belly during the observation period.

The identification references used in the field include Smith (1943), Daniels, (2005), Yang and Rao (2008), Ahmed *et al* (2009), Fei *et al* (2010), and Vasudevan and Sondhi (2010).

RESULTS

General Morphology of the Himalayan Newt, *Tylototriton verrucosus* : Most of the Newts had oval or flat heads, with strongly developed cranial crest and cranial boss. Paired nostrils were located closer to the tip of the broad and rounded snout. Eyes were moderate in size, with large, granular upper eye lids. A prominent glandular ridge ran from the frontal area of the head up to the vent (Plate-IXa,b). Dorso-lateral rows of 14 nodules were placed on each side of body and anterior part of the tail. Fore-limbs had four fingers and the hind-limbs had 5 fingers. Fingers and toes were not webbed. Skin of body and tail were granular which became more prominent during terrestrial life. Paratoids were large and distinct, slightly depressed and distinctly coloured. A tail little shorter than snout-vent length, compressed laterally, with well developed fin folds was present. Base colour was uniformly black or dark brown on dorsum, sides and venter. Tail and soles of limbs were lighter brown

than the body. Underside of the tail was lighter yellow or orange in colour. Tongue was small thick and free from sides. Vomerine teeth occurred in continuous series lying parallel anteriorly and diverging posteriorly (Plate-X). These details were in conformity with original description of Anderson (1871) and were also prominent in most of the specimens studied from Nepal, Darjeeling, Myanmar and Yunnan Province, China (Thant-Shima *et al*, 1979; Kuzmin *et al*, 1994; Ander *et al*, 1998).

Morphometric description of different colour forms of *Tylototriton verrucosus* :

1. ***Chocolate-brown forms*** : Only 3 specimens could be encountered from Kambadui habitat on 27th of August 2014. All the 3 specimens were male weighting between 16.8, 17.9 and 18.3*g*. Base consisted of uniformly ash colour with dorsal ridge and upper margin of tail chocolate-brown (Plate-IXa) to light yellow (Plates-XIc; XIIa). Cloaca, soles, limb digits and lower margin of tail were yellow (Plates-XIb,d). Paratoids shape and colour differed in all the three forms varying from granular chocolate colour in type 1 to thin curved brownish structures in type 2 and 3 (Plates-Xia,c; XIIa). SVL were 70.4, 83.2 and 78.5*mm* respectively (Plates-Xia,c; XIIa). Tail length varied from 84.5, 73.4, and 71.8*mm* in type1, 2 and 3 respectively. Head lengths were 17.3, 18.4 and 16.8 *mm*. Head widths were recorded at 16.5, 18.3 and 16.6*mm* in type 1, 2 and 3 respectively. Inter-orbital distances between anterior angles of eyes were at 9.9, 10.2 and 9.8*mm*. Eye-naris distances were recorded at 5.6, 5.8, and 5.7*mm*. Length of cranial crests varied between 16.1 in type1, 16.9, type 2 and 16.1*mm* in type 3. Widths of cranial crest at posterior corner of right eye differed between 3.2, 3.6 and 3.4*mm* in forms 1, 2 and 3 respectively. Boss length was recorded at 4.3, 4.7, 4.6 *mm*. Width of mid-dorsal crest (vertebral ridge) at level of the 5th pair of lateral warts differed at 2.5, 4.7 and 4.6*mm* in type 1, 2, and 3. Length of 5th wart of right side in type 1, 2 and 3 were recorded at 2.2, 2.5 and 4*mm* respectively. Height of 5th wart of right side varied at 2, 2 and 3.8*mm*. Number of body warts on right side, including the wart above the groin were at 14, 14 and 13 in type 1, 2 and 3 respectively (Plates-XIa,c; XII a) Numbers of tail warts (behind the groin) on right side in type 1-3 were 3 each. Lengths of the extended fore limbs were at 23.5mm each. Lengths of extended hind limbs were at 23.6*mm* each (Plates-XIb,d; XIIb).

2. ***Chocolate colour form*** : Single available specimen was collected from Kambadui habitat on 19th of June 2011. It was a male with 20.2*g* at weight. Body had dark chocolate colour base. Cloaca, soles, fingers, warts and ventral margin of the tail were with faded yellow colour (Plate-XIIc). SVL was recorded at 72.3 *mm,* and tail length at 83.4*mm*. Head length

was 18.5*mm*. Head width was recorded at 17.8*mm*. Inter-orbital distances between anterior angles of eyes were at 10.1*mm*. Eye-naris distance was recorded at 5.6*mm*. Length of cranial crest measured 16.1*mm*. Width of cranial crest at posterior corner of right eye was 3.6*mm*. Boss length was recorded at 5.0*mm*. Width of mid-dorsal crest (vertebral ridge) at level of the 5th pair of lateral warts measured 4.5*mm*. Length of 5th wart of right side was 4.6*mm.* Height of 5th wart of right side measured at 4.8*mm*. Number of body warts on right side, including the wart above the groin was 15 (Plate-XIIc). Number of tail warts (behind the groin) on right side was 3. Lengths of the extended fore limbs were at 22.5*mm* each. Lengths of the extended hind limbs were at 22.6*mm* each (Plate-XIId).

3. Sand elm colour forms : Two closely related colour forms were found in Tingbajot habitat on 10th of July 2011. Specimens weighed at 19.6 and 18.7*g* respectively (Plates-XIIIa, c). Both the forms had base colouration as sand elm with cranial crest, mid-dorsal ridge and side rows of warts, soles, fingers and ventral edge of the tails in olive yellow colour (Plates-XIII-a,d). SVL was recorded at 78.3 and 76.4*mm* and tail length at 73.4*mm* in type 1 and 72.2*mm* in type 2 respectively. Head lengths were recorded at 18.6*mm* in form 1 and 18.2*mm* in form 2. Head width varied from 18.0 *mm* and 17.8*mm* in type 1 and 2 respectively. Inter-orbital distances between anterior angles of eyes for type 1 and 2 were at 9.9*mm* and 9.8 *mm*. Eye-naris distances were recorded at 5.6 and 5.4*mm* respectively. Cranial crest lengths were recorded at 16.2 and 16.3*mm*. Cranial crest widths at posterior corner of right eye were 3.7 and 3.8*mm*. Boss length was recorded at 3.6 and 3.9*mm* in type 1 and 2 respectively. Width of mid-dorsal crest (vertebral ridge) at level of the 5th pair of lateral warts measured at 5.5 and 5.3*mm*. Length of 5th wart of right side was 4.6 and 4.7*mm*. Height of 5th wart of right side varied at 4.2 and 4.6*mm*. Number of body warts on right side, including the wart above the groin were 14 each (Plates-XIIIa,d). Number of tail warts (behind the groin) on right side was 2 each. Length of the extended fore limbs were at 24.0 and 23.5*mm* in form 1 and 2respectively. Length of the extended hind limb were at 24.0 and 23.6mm (Plates-XIIIb,d).

4. Sky-blue colour: Single male was encountered in Tingbajot habitat on 18th of June 2014. The specimen weighed at 18.9*g*. Body base colouration was sky blue with mid-dorsal ridge, side warts, cranial crests, and upper margin of the tail in olive-yellow colour. Soles, fingers and lower tail margin were yellow. SVL measured at 78.4 *mm,* and tail length at 72.8*mm.* Head length was 19.5*mm*. Head width measured at 18.6*mm*. Inter-orbital distance between

anterior angles of eyes was at 10.0*mm*. Eye-naris distance was recorded at 5.5*mm*. Length of cranial crest was measured at 15.5*mm*. Width of cranial crest at posterior corner of right eye was 3.4*mm*. Boss length was recorded at 4.0*mm*. Width of mid-dorsal crest (vertebral ridge) at level of the 5th pair of lateral warts measured 4.5*mm*. Length of 5th wart of right side was 4.0*mm*. Height of 5th wart of right side varied at 4.0*mm*. Number of body warts on right side, including the wart above the groin was 14 (Plate-XIVa). Number of tail warts (behind the groin) on right side was 3. Lengths of the extended fore limbs were at 24.0*mm* each. Lengths of extended hind limbs were at 24.0*mm* each (Plate-XIV-b).

5. Concrete elm colour form **:** Single specimen was collected from Kambadui habitat on 15th July, 2012. It weighed at 18.9*g***.** Base colour was concrete elm. Cranial crests, paratoids, soles, fingers, cloaca and the tail all brown in colour. SVL measured at 79.2 *mm,* and tail length at 73.5*mm.* Head length was 18.5*mm*. Head width measured at 18.2*mm*. Inter-orbital distance between anterior angles of eyes was at 9.8*mm*. Eye-naris distance was recorded at 5.4*mm*. Length of cranial crest was measured at 15.2*mm*. Width of cranial crest at posterior corner of right eye was 3.5*mm*. Boss length was recorded at 3.0*mm*. Width of mid-dorsal crest (vertebral ridge) at level of the 5th pair of lateral warts measured 3.5*mm*. Length of 5th wart of right side was 3.8*mm*. Height of 5th wart of right side varied at 3.7*mm*. Number of body warts on right side, including the wart above the groin was 15 (Plate-XIVc). Number of tail warts (behind the groin) on right side was 3. Lengths of the extended fore limbs were at 24.0*mm* each. Lengths of extended hind limbs were at 24.0*mm* each (Plate-XIVd).

6. Burgundy Colour forms**:** Two specimens, one female and one male were encountered during survey in Tingbajot habitat on 19th June 2011. Specimens weighed at 19.3*g* (male) and 22.6g (female). Both had black body base, dorsal side darker in female and ventral side darker in the male (Plate-XVa-d). Cranial crests, paratoids, mid-dorsal ridge, side rows of warts and dorsal side of limbs in male were more colourful as compared with female (Plate-XVa,c). Soles, cloaca and ventral tail margins were yellow in colour. SVL was recorded at 80.2 *mm* in female and 86.4*mm* in male. Tail lengths varied at 73.2*mm* in female and 72.2*mm* in male specimen. Head lengths were recorded at 18.6*mm* in female and 20.2*mm* in male. Head width varied from 18.0 *mm* and 16.8*mm* in female and male specimens respectively. Inter-orbital distances between anterior angles of eyes for female and male were at 10.0*mm* and 10.2 *mm* respectively. Eye-naris distances were recorded at 5.6*mm* and 5.9*mm* respectively. Cranial crest lengths were recorded at 16.1*mm* in female and 18.3*mm* in male specimen. Cranial crest widths at posterior corner of right eye were 3.7*mm* and 3.4*mm*. Boss

length was recorded at 3.6*mm* and 3.1*mm* in female and male respectively. Width of mid-dorsal crest (vertebral ridge) at level of the 5th pair of lateral warts measured at 5.3*mm* and 5.5*mm*. Length of 5th wart of right side was 4.0*mm* and 4.1*mm*. Height of 5th wart of right side varied at 4.2*mm* and 4.0*mm*. Number of body warts on right side, including the wart above the groin were 14 in female and 13 in male (Plate-XVa,c). Number of tail warts (behind the groin) on right side was 3 each. Lengths of the extended fore limbs were at 24.0*mm* and 23.7*mm* in female and male respectively. Lengths of extended hind limbs were at 24.0*mm* in female and 23.7mm in male (Plate-XVb,d).

7. Brown-black colour forms: Two specimens were collected from Tinbajot habitat, on 27th of July 2012. Both were male weighing at 18.7 and 18.9*g* respectively. Body base colour was black, (Plate-XVIa,c). Cranial crests, paratoids, mid-dorsal ridge, dorsal side of limbs blackish-brown. Ventral sides of toes, cloaca and ventral tail margins were yellow (Plate-XVIa) or brown (Plate-XVIc) in colour. SVL was recorded at 86.2 and 78.4*mm* respectively (Plates-XVIa,c). Tail lengths varied at 73.2*mm* and 74.4*mm.* Head lengths were recorded at 19.6*mm* and 18.2*mm*. Head width varied at 19.0 and 17.8*mm* respectively. Inter-orbital distances between anterior angles of eyes were at 10.0 *mm* and 9.8 *mm*. Eye-naris distances were recorded at 5.4*mm* and 5.2*mm* respectively. Cranial crest lengths were recorded at 16.2*mm* and 15.3*mm*. Cranial crest widths at posterior corner of right eye were 3.8*mm* and 3.0*mm*. Boss length was recorded at 3.8*mm* and 3.6*mm* respectively. Width of mid-dorsal crest (vertebral ridge) at level of the 5th pair of lateral warts measured 5.3*mm* and 5.2*mm*. Length of 5th wart of right side was 4.0*mm* and 3.5*mm*. Height of 5th wart of right side varied at 4.2*mm* and 3.0*mm*. Number of body warts on right side, including the wart above the groin were 13 (Plate-XVIa) and 14 (Plate-XVIc). Number of tail warts (behind the groin) on right side was 3 each. Lengths of the extended fore limbs were at 24.0*mm* and 23.9*mm* respectively. Lengths of the extended hind limbs were at 24.0*mm* each (Plate-XVIb,d).

8. Navy blue Colour form: It was a male specimen collected from Kambadui habitat on 27th of June, 2015 weighing at 20.2g. Body base colouration was Navy blue. But for soles, fingers, cloaca and ventral tail margin having faded yellow tinge, colour of dorsal body parts was brown mixed with blue (Plate-XVIIa). Ventral body side was Navy blue in colour (Plate-XVIIb). SVL measured at 75.2 *mm,* and tail length at 74.5*mm.* Head length was 18.5*mm*. Head width measured at 18.2*mm*. Inter-orbital distance between anterior angles of eyes was

at 9.9*mm*. Eye-naris distance was recorded at 5.3*mm*. Length of cranial crest was measured at 15.3*mm*. Width of cranial crest at posterior corner of right eye was 4.4*mm*. Boss length was recorded at 4.2*mm*. Width of mid-dorsal crest (vertebral ridge) at level of the 5th pair of lateral warts measured 3.5*mm*. Length of 5th wart of right side was 3.8*mm*. Height of 5th wart of right side varied at 3.2*mm*. Number of body warts on right side, including the wart above the groin was 14 (Plate-XVIIa). Number of tail warts (behind the groin) on right side was 2. Length of extended fore limbs were at 23.5*mm* each. Lengths of extended hind limbs were at 24.0*mm* each (Plate-XVIIb).

9. Caramel colour forms: Two male specimens weighing at 19.6 and 20.8*g* respectively were collected from Tingbajot habitat on 29th of June, 2014. Body base colouration were caramel. Cranial crests, soles, fingers, cloaca and ventral tail margin were brownish or faded yellow colour (Plates-XVIIc, XVIIIa). SVL measured at 75.3 and 79.2*mm* respectively. Tail lengths varied at 74.5 and 72.5*mm.* Head lengths were at 18.4 and 18.8*mm*. Head widths measured at 18.2 and 18.5*mm*. Inter-orbital distances between anterior angles of eyes were at 9.9 and 10.2*mm*. Eye-naris distance was recorded at 5.1 and 5.6*mm*. Lengths of cranial crest were measured at 15.3 and 16.3 *mm*. Widths of cranial crest at posterior corner of right eyes were 4.4 and 4.0*mm*. Boss lengths were recorded at 4.6 and 5.5*mm*. Widths of mid-dorsal crest (vertebral ridge) at level of the 5th pair of lateral warts measured at 3.8 and 3.6*mm*. Lengths of 5th wart of right side were at 3.5 and 3.7 *mm*. Height of 5th wart of right side varied at 3.3 and 3.6*mm*. Numbers of body warts on right side, including the wart above the groin were 14 (Plates-XVIIc, XVIIIa). Numbers of tail warts (behind the groin) on right side were 3 each. Lengths of extended fore limbs were at 23.6 and 23.8*mm*. Lengths of extended hind limbs were at 23.6 and 24.0*mm* respectively (Plates-XVIId, XVIIIb).

10. Dark brown-black forms **:** Two specimens, both male weighing at 19.8 and 20.6*g* respectively were encountered in Kambadui habitat on 10th of August, 2015. Base colour was uniform black on both sides. Cranial crests, soles, cloaca and ventral tail margins were yellow to orange in colour (Plates-XVIIIc, XIXa). SVL measured at 75.2 and 74.4*mm;* tail lengths at 75.5 and 76.5*mm* respectively. Head lengths were at 18.5 and 18.8 *mm*. Head width measured at 18.2 and 18.6 *mm*. Inter-orbital distances between anterior angles of eyes were at 9.8 and 8.9*mm* respectively. Eye-naris distances were recorded at 4.8 and 4.9*mm*. Lengths of cranial crests were measured at 15.1 and 15.3*mm*. Widths of cranial crest at posterior corner of right eye were 4.3 and 4.2 *mm*. Boss lengths were recorded at 4.7 and 4.8*mm*. Width of mid-dorsal crest (vertebral ridge) at level of the 5th pair of lateral warts measured at 4.0 and 5.0*mm*

respectively. Lengths of 5th wart of right side were at 3.8 and 3.1*mm*. Height of 5th wart of right side varied at 3.2 and 3.3*mm*. Numbers of body warts on right side, including the wart above the groin were at 12 and 14 respectively (Plates- XVIIIc, XIXa). Number of tail warts (behind the groin) on right side was 2 each. Lengths of extended fore and hind limbs were at 24.0*mm* each (Plates- XVIIId, XIXb).

11. Cola colour forms : Four number, all male Newts were collected from Kambdui habitat on 10th of July 2015, weighing at 19.4, 18.3, 20.2 and 19.6*g* respectively (Plates-XIXc,XXa,c,XXIa). Body base colouration were coca colour, cranial crests coca colour to dark-brown. Toes, fingers, cloaca and ventral margin of tail were dark-brown to yellow in colour (Plates-XIXd-XXb,cd, XXIb,). SVL measured at 73.2*,* 74.5, 80.4 and 80.6 *mm* respectively. Tail lengths varied at 76.5, 72.3, 69.8 and 75.5*mm* respectively. Head lengths varied at 18.4, 18.2, 18.5 and 18.6 *mm*. Head widths measured 18.2,18.0, 18.3 and 18.5 *mm*. Inter-orbital distances between anterior angles of eyes were at 9.8*,* 10.0*,* 9.7 and 9.9*mm* respectively. Eye-naris distances were recorded at 4.7, 4.8, 4.8 and 4.9*mm*. Length of cranial crests measured at 15.2, 15.3, 15.2 and 15.3*mm*. Cranial crest width at posterior corner of right eyes were at 4.3*,* 4.2, 4.3 and 4.2 *mm*. Boss length was recorded at 4.7*,* 4.6, 4.7 and 4.8*mm*. Widths of mid-dorsal crest (vertebral ridge) at level of the 5th pair of lateral warts measured 5.0, 5.1, 4.8 and 5.0*mm* respectively. Lengths of 5th wart of right side were at 3.8, 3.7, 3.0 and 3.1*mm*. Height of 5th wart of right side varied at 3.2*,* 3.2, 3.0 and 3.3*mm*. Numbers of body warts on right side, including the wart above the groin were at 13, 14, 14 and 14 respectively (Plates-XIXc, XXa,c). Numbers of tail warts (behind the groin) on right side were at 3, 4, 4 and 4 respectively. Lengths of the extended fore and hind limbs were at 23.6*mm* each (Plates-XIXd, XXb,d, XXIb).

12. Off-black colour forms : Six specimens weighing at 19.2,20.1,21,3,17.8, 19.8, and 20.6*g* were collected from Tingbajot, Kambadui, lakpiidui, N'rekpiidui, Solumdui and N'tingliibam habitats. All exhibited common morphological features with black body base, under surface of toes, fingers and cloaca yellowish in colour, and lower tail margins with faded yellow or brownish colour patterns (Plates-XXIc,d XXIIa-d, XXIVa,b,d). SVL measured at 80.5mm (Plate-XXIc), 75.8*mm* (Plate-XXId), 79.5*mm* (Plate-XXIIa), 75.8*mm* (Plate-XXIIb), 76.5*mm* (Plate-XXIIc), and 79.6*mm* (Plate-XXIId). Tail length varied from 63.8, 73.4, 76.7, 69.8, 71.7 and 69.8*mm* respectively (Plates- XXIc,d, XXIIa-d). Head lengths were measured at 18.8, 19.10, 20.2, 19.2, 18.2 and 19.8*mm* (Plates- XXIc,d, XXIIa-d). Head width measured at 18.2, 18.8, 19.9, 18.8, 18.0 and 18.5*mm*. Inter-orbital distances between anterior angles of eyes

were at 9.8, 104, 10.5, 10.2, 9.3, and 10.4*mm* respectively *(*Plates XXIc,d, XXIIa-d). Eye-naris distances were recorded at 5.7, 5.8, 5.9, 5.5, 5.2 and 5.8 *mm*. Lengths of cranial crests were measured at 16.5, 16.9, 16.6, 16.2, 15.3, and 16.8*mm* respectively (Plates XXIc,d, XXIIa-d). Cranial crests width at posterior corner of right eyes were 4.2, 5.4, 5.6, 4.5, 4.3, and 5.6*mm*. Boss lengths were recorded at 4.5, 4.2, 5.4, 6.2, 4.3, and 4.5*mm*. Mid-dorsal line crest widths at level of the 5th pair of lateral warts were measured 4.8, 5.6, 4.5, 4.3, 4.2, and 4.5*mm*. Lengths of 5th wart of right side were at 5.0, 3.8, 3.7, 4.1, 3.2, and 5.1*mm*. Height of 5th wart of right side varied at 4.5, 3.5, 3.4, 4.3, 4.1, and 4.6*mm*. Numbers of body warts on right side, including the wart above the groin were at 14, 13,13,14,14, and 13 respectively. (Plates- XXIc,d, XXIIa-d). Numbers of tail warts (behind the groin) on right side were 3 in each case. Lengths of the extended fore and hind limbs were at 24 *mm* each.

13. Jet-black colour forms: Two specimens, both male weighing at 21.3 and 18.7*g* respectively, were encountered in Tingbajot habitat on 15th of June, 2015. Specimens exhibited jet-black colour all over the body but for under surface of toes, fingers, cloaca, paratoids and ventral margin of tail which were faded yellow to brown in colour (Plate-XXIIIa-d). SVL measured at 69.2, and 75.6 *mm,* and tail lengths at 74.5 and 72.4*mm*. Head was oval or elongated and measured 16.4, and 19.6*mm* at length (Plate-XXIIIa,c). Head widths were measured at 16.3 and 18.4*mm* respectively. Inter-orbital distance between anterior angles of eyes was at 8.2 and 8.8*mm*. Eye-naris distances were recorded at 5.0, and 5.4*mm*. Lengths of cranial crest were measured at 14.3 and 16.4*mm*. Widths of cranial crest at posterior corner of right eye were 3.0 and 3.4*mm*. Boss lengths were recorded at 4.5 and 4.6*mm* respectively (Plate-XXIIIa,c). Widths of mid-dorsal crest (vertebral ridge) at level of the 5th pair of lateral warts were measured at 3.7 and 3.6mm. Lengths of 5th wart of right side were at 2.7 and 3.2*mm*. Height of 5th wart of right sides varied at 3.0 and 3.1*mm*. Numbers of body warts on right side, including the warts above the groin were at 14 and 16 respectively (Plate-XXIIIa,c). Numbers of tail warts (behind the groin) on right side were 3 in each. Lengths of the extended fore limbs were at 23.8 and 23.7*mm* each. Lengths of the extended hind limbs were at 23.8 and 23.9*mm* (Plate-XXIIIb,d).

14. Dark-brown form : A single male of this colour variant weighing at 18.7*g* was collected on 16th of August, 2014. The specimen exhibited dark-brown colour all over the body with toes, fingers, cranial crest, cloaca and ventral tail margin in faded brown colour (Plate-XXIVc). SVL was measured at 85.6 *mm,* and tail length at 69.6*mm*. Head length measured at 18.8*mm*. Head width was 18.5*mm*. Inter-orbital distance between anterior angles of eyes was

at 10.3*mm*. Eye-naris distance was recorded at 5.4*mm*. Length of cranial crest was measured at 16.3*mm*. Width of cranial crest at posterior corner of right eye was 3.2*mm*. Boss length was recorded at 4.8*mm*. Width of mid-dorsal crest (vertebral ridge) at level of the 5th pair of lateral warts measured 4.1mm. Length of 5th wart of right side was 4.2*mm*. Height of 5th wart of right side varied at 3.6*mm*. Number of body warts on right side, including the wart above the groin was 14 (Plate-XXIVc). Number of tail warts (behind the groin) on right side was 3. Length of the extended fore limb were at 23.8*mm* each. Lengths of the extended hind limbs were at 24.0*mm.*

15. Brown colour forms: Three Newts weighing at 19.8, 20.3 and 21.4*g* collected from Tingbajot habitat on 17th of July, 2012 exhibited base body colour as off-black with head, oval (Plate-XXVa) or elongated (Plate=XXVb,c). Cranium, mid-dorsal ridge as dark-brown, side warts, faded yellow to dark-brown, and tail with light to dark-brown in colour (Plate-XVa-c). Under side of toes, fingers and ventral margin of tail were light-yellow in colour. SVL were measured at 72.6, 85.6, and 76.8 *mm,* and tail lengths at 79.8, 68.9, and 68.8*mm.* Head lengths were recorded at 14.6, 18.4, and 19.8*mm*. Head widths were at 14.5, 17.8 and 18.9*mm*. Inter-orbital distance between anterior angles of eyes was at 8.3, 10,2and 10.3*mm*. Eye-naris distance was recorded at 4.4, 5.2 and 5.4*mm*. Length of cranial crest was measured at 14.3, 16.8 and 17.2*mm*. Widths of cranial crest at posterior corner of right eyes were at 3.2, 3.4 and 3.5*mm* respectively (Plate-XXVa-c). Boss length was recorded at 5.2, 5.3 and 5.4*mm*. Widths of mid-dorsal crest (vertebral ridge) at level of the 5th pair of lateral warts were measured at 4.2, 4.2 and 4.3mm. Length of 5th wart of right side was 3.4, 3.5 and 3.4*mm*. Height of 5th wart of right side varied at 3.1, 3.3 and 3.4*mm*. Numbers of body warts on right side, including the wart above the groin were 14 in each (Plate-XXVa-c). Numbers of tail warts (behind the groin) on right side were 3 each. Length of the extended fore limb were at 23.8, 24.0 and 24.0*mm*. Lengths of the extended hind limbs were at 24.0*mm* each.

16.Light cola colour : A single specimen weighing 20*g* was encountered on 17th of June, 2009 in Lakpiidui habitat. Base body colour of this specimen consisted of light cola colour with cranial crest, mid-dorsal ridge, warts at sides, tail and tail fins dark-brown. Undersides of toes and finger-tips were olive-yellow (Plate-XXVd). SVL was measured at 80.6 *mm,* and tail length at 72.2*mm.* Head length was measured at 16.8*mm*. Head width was 16.5*mm*. Inter-orbital distance between anterior angles of eyes was at 10.1*mm*. Eye-naris distance was recorded at 5.3*mm*. Length of cranial crest was measured at 14.3*mm*. Width of cranial crest at posterior corner of right eye was 5.2*mm*. Boss length was recorded at 5.8*mm*. Width of mid-

dorsal crest (vertebral ridge) at level of the 5th pair of lateral warts measured 4.8mm. Length of 5th wart of right side was 5.2*mm*. Height of 5th wart of right side varied at 4.6*mm*. Number of body warts on right side, including the wart above the groin was 14 (Plate-XXVd). Number of tail warts (behind the groin) on right side was 3. Length of the extended fore limb were at 23.8*mm* each. Lengths of the extended hind limbs were at 24.0*mm* (Plate-XXVd).

17. Dark-purple colour form : Single male specimen with body weight at 19.8*g* was collected at Kambadui habitat on 17th of June 2009. The body base colouration was dark purple with underside toes, fingers and lower margin of tail were faded yellow in colour (Plate-XXVIa). SVL was measured at 84.6 *mm,* and tail length at 65.2*mm.* Head length was measured at 20.8*mm*. Head width was 16.5*mm*. Inter-orbital distance between anterior angles of eyes was at 10.8*mm*. Eye-naris distance was recorded at 5.5*mm*. Length of cranial crest was measured at 15.3*mm*. Width of cranial crest at posterior corner of right eye was 5.1*mm*. Boss length was recorded at 5.2*mm*. Width of mid-dorsal crest (vertebral ridge) at level of the 5th pair of lateral warts measured 5.6*mm*. Length of 5th wart of right side was 3.7*mm*. Height of 5th wart of right side varied at 3.6*mm*. Number of body warts on right side, including the wart above the groin was 13 (Plate-XXVIa). Number of tail warts (behind the groin) on right side was 3. Length of the extended fore limb were at 24.2*mm* each. Lengths of the extended hind limbs were at 23.5*mm* (Plate-XXVIa).

18. Orange colour forms : Three male specimens collected from Kambadui habitat, on 15th may 2011 weighed 20.3, 19.2 and 19.4*g* respectively. Body base colourations were black with light-orange or dark-orange, cranial crest, mid-dorsal ridge, side warts, toes, fingers and tail regions (Plate-XXVIb-d). Crowns were well developed in all the 3 forms giving the feature of Emperor Newt, *T. shanjing*. SVL were measured at 83.3, 85.4, 82.2*mm*, with tail measuring at 72.3, 74.6, 69.6*mm* respectively. Head lengths were measured at 20.0*,* 20.8 and 19.7*mm*. Head widths were at 16.5, 18.2 and 17.8*mm*. Inter-orbital distances between anterior angles of eyes were at 10.2, 10.5 and 9.8*mm*. Eye-naris distances were recorded at 5.2, 5.4 and 5.0*mm*. Lengths of cranial crests were measured at 17.3, 18.4 and 16.9*mm*. Widths of cranial crests at posterior corner of right eye were 4.9, 5.2 and 5.6*mm*. Boss lengths were recorded at 6.2, 5.6 and 4.3*mm*. Widths of mid-dorsal crests (vertebral ridge) at level of the 5th pair of lateral warts were measured at 5.6, 5.4 and 5.8*mm* respectively. Lengths of 5th wart of right side were at 3.7, 3.8 and 3.9*mm*. Height of 5th wart of right side varied at 3.2, 3.6 and 3.5*mm*. Numbers of body warts on right side, including the wart above the groin were at 14, 12 and 14 respectively (Plate-XXVIb-d). Numbers of tail warts (behind the groin) on right

side were 3 each. Lengths of the extended fore limb were at 24.2, 24.2 and 23.6*mm*. Lengths of the extended hind limbs were at 23.5, 23.5 and 23.4*mm* respectively (Plate-XXVIb-d).

19. Pink colour form : Single pink colour specimen weighing 19.3*g* was collected from Tingbajot habitat on 29th May 2012. Practically the whole body was pinkish with soles and finger tips acquiring faded yellowish tinge. SVL was measured at 78.6 *mm,* and tail length at 68.2*mm.* Head length was measured at 18.8*mm*. Head width was 18.5*mm*. Inter-orbital distance between anterior angles of eyes was at 10.6*mm*. Eye-naris distance was recorded at 5.6*mm*. Length of cranial crest was measured at 15.4*mm*. Width of cranial crest at posterior corner of right eye was 4.8*mm*. Boss length was recorded at 5.6*mm*. Width of mid-dorsal crest (vertebral ridge) at level of the 5th pair of lateral warts measured 5.2*mm*. Length of 5th wart of right side was 3.5*mm*. Height of 5th wart of right side varied at 4.2*mm*. Number of body warts on right side, including the wart above the groin was 14 (Plate-XXVIIa). Number of tail warts (behind the groin) on right side was 2. Length of the extended fore limb were at 23.2*mm*. Length of the extended hind limbs was 23.5*mm* (Plate-XXVIIa).

DISCUSSION

Present observations clearly demonstrate the presence of as many as 19 polychromatic variants of Himalayan Newt, *Tylototriton verrucosus* Anderson in Tingbajot, Kambadui and Lakpiidui habitats of Senapati district, Manipur. This observation while supporting the possibility of finding many more colour variants in these habitats does not rule out the possibility of their presence in the remaining 7 habitats located in discrete hilly pockets of Senapati district (Chapter-I). In fact, only Tingbajot Kambadui and Lakpiidui habitats were thoroughly accessed owing to their location near human settlements at Maram. Secretive nature of the animals and distant locations of the habitats in discrete hilly pockets of the Senapati district compelled to limit our attempt for having frequent access to the remaining 7 habitats. This is the first authentic report to show the presence of polychromatic forms of the Himalayan Newt with their specific locations in the Indian sub-continent. Other reports published from India (Chaudhuri, 1966; Dasgupta, 1984, 1990; Kuzmin *et al,* 1994; Roy and Mushahidunnabi, 2000, 2001; Selim, 2001; Seglie *et al*, 2003; Nag and Vasudevan, 2014) while making notes on their distribution pattern and presumed population size (Seglie *et al*, 2003) placed no focus on availability of different colour morphs. Further, most of the specimens encountered in Senapati district, Manipur during the survey period from May 2009

- August 2015 exhibited the base colour features as originally described by John Anderson (1871).

The genus '*Tylototriton'* represents 22 species of Newts : *Tylototriton anguliceps, Tylototriton asperrimus, Tylototriton broadoridgus, Tylototriton daweishanensis, Tylototriton hainanensis, Tylototriton kweichowensis, Tylototriton lizhenchangi, Tylototriton panhai, Tylototriton pseudoverrucosus, Tylototriton shanjing*, *Tylototriton taliangensis, Tylototriton uyenoi, Tylototriton verrucosus, Tylototriton vietnamensis, Tylototriton wenxianensis, Tylototriton yangi, Tylototriton ziegleri, Tylototriton broadoridgus, Tylototriton liuyangensis, Tylototriton notialis, Tylototriton shanorum,* and *Tylototriton podichthys.* Further, some of the species exhibit many intra-specific variations : *Tylototriton anguliceps* with 8; *Tylototriton daweishanensis* with 2; *Tylototriton kweichowensis* with 24*; Tylototriton lizhenchangi* with 6*; Tylototriton panhai* with *5; Tylototriton wenxianensis* with 2 and *Tylototriton verrucousus* with 19 variants (Amphibiaweb, 2015). In the present study, occurrence of many colour forms with some of them exhibiting morphological features and colour patterns closer to *Tylototriton shanjing* and sharing habitats with forms described by Anderson (1871) may denote common ancestry of *T. Shanjing* and *T. verrucosus* Anderson. From the western border of China heading towards Myanmar, enormous distribution area of *Tylototriton verrucosus* Anderson overlap with *T. shanjing* and beyond Myanmar area extending towards India and Nepal is occupied predominantly by *Tylototriton verrucosus* Anderson. So far, in these regions, there is no known sympatric occurrence of the two species. Bulk of evidence though based on Newts collected from International pet market advocate in favour of *T. shanjing* and *T. verrucosus* Anderson being separate species (Nussbaum *et al*, 1995; Frost, 2008; Mahoney and Vrendenburg, 2008). It has been suggested that at least two forms of T. Shanjing exist which cannot be differentiated morphologically with the present knowledge. Both correspond to the appearance that at present *Tylototriton shanjing* (kursiv) *senso stricto* shows. Very clear differences result in the observations of the mating behaviour of two forms of *T. shanjing* (Mudrack, 1969, 1972, 2005; Rehberg, 1986). One form shows amplexus as *Tylototriton verrucosus* Anderson which takes place obligatorily in water. The other one mates in a circular dancing on land without amplexus. Mudrack (1969, 1972) was first to report amplexus and some years later with other individuals the circular dance (Mudrack, 2005). Rehberg, however (1986) only described the circular dance. Rehberg (1986) expressed the suspicion that one could have observed two different species, which would not be distinguishable macroscopically. With present means it

does not seem possible to differentiate both forms phenotypically. Another possibility could be that the amplexus may be exhibited by hybrids of *T. shanjing* with *Tylototriton verrucosus* Anderson that might have accidently occurred in the terrarium resulting the outcome of *Tylototriton verrucosus* senso stricto and *Tylototriton shanjing* senso lato. On the other hand *Tylototriton shanjing* of the circular dance form will not mate with *Tylototriton shanjing* of the amplexus form. This obviously presses a need for launching search for the species border between these two forms. Mudrack (2005) reported very valuable information from an accurately well known habitat of *Tylototriton shanjing* from Garfong village, Jingdong County, Yunnan Province. This is the distribution area of *Tylototriton shanjing* senso stricto. These animals exhibited circular dance during transfer of spermatophores in females during breeding season. It may thus be possible that *Tylototriton verrucosus* Anderson developed a cryptic form, which lives synoptically with *Tylototriton shanjing*. Both forms look alike to a great extent but so far, their factual status is not very clear. The possible hypothetical assumption may be that there is one "true Mandarin salamander" looking alike *Tylototriton shanjing* senso stricto and one false Emperor Newt which is a colouring variant of *Tylototriton verrucosus* Anderson. Both forms are macroscopically not distinguishable. It remains to be established whether different colour forms encountered in the present study have any ancestral linkage with so called false Emperor Newt.

The differing colour patterns observed in the present study may not be attributed to seasonal changes in colour patterns developed specially by the male Newts during the breeding season as suggested by some studies (Sparreboom and Yunke, 2010; AmphiaWeb, 2015). The observed colour patterns were seen lasting until October when Newts were undergoing preparations for entering into hibernation. Further, no courtship or mating behaviour could be seen between individuals of different colour morphs. Encounter with as many as 19 polychromatic forms of *Tylototriton verrucosus* Anderson during the course of present study thus might denote the existence of many sub-species or even full-fledged species. At this stage, it would probably not be proper to be too speculative assume them belonging to a different species. it would perhaps be more appropriate to designate them as *Tylototriton verrucosus* complex pending procurement of genetic details to trace their molecular phylogeny.

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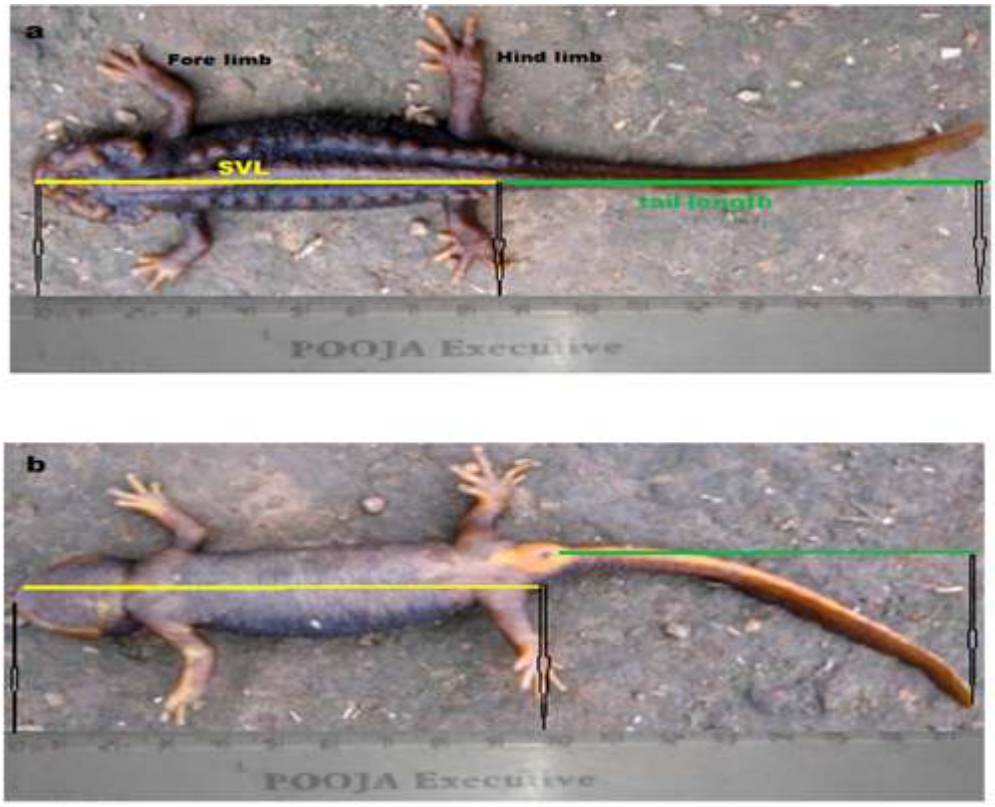

Plate IX

a. Dorsal view of the *Tylototriton verrucosus* Anderson encountered in Lakpiidui habitat of the Senapati district, Manipur on 19th June 2009.

b. Ventral view of the *Tylototriton verrucosus* Anderson encountered in Lakpiidui habitat of the Senapati district, Manipur on 19th June 2009.

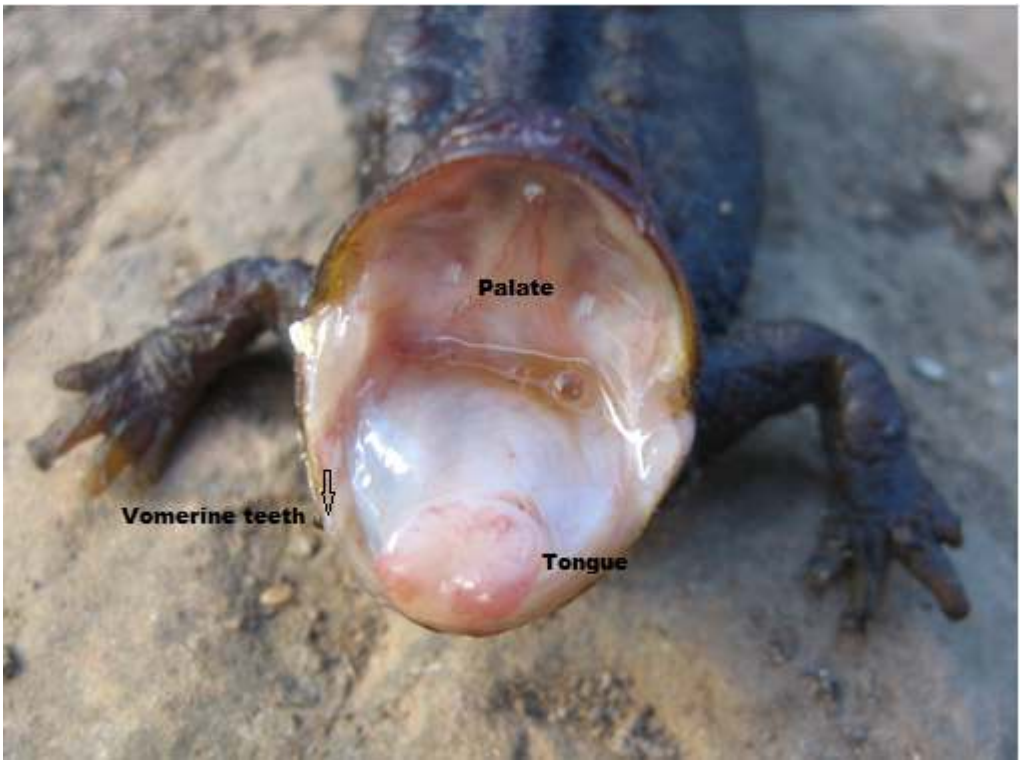

Plate – X

Mouth parts of the Himalayan Newt, *Tylototriton verrucosus* clicked on 15th of July 2015. Newt was collected from Kambadui habitat in Senapati district, Manipur and released back in its natural habitat after taking the photograph.

Plate-XI: a. Dorsal view of Chocolate-brown colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Kambadui habitat, Senapati district, Manipur on 14th August 2014. **b**. Ventral view of Chocolate-brown colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Kambadui habitat, Senapati district, Manipur on 14th August 2014.
c. Dorsal view of Chocolate-brown colour form of Himalayan Newt, T*ylototriton verrucosus* collected from Kambadui habitat, Senapati district, Manipur on 14th August 2014.
d. Ventral view of Chocolate-brown colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Kambadui habitat, Senapati district, Manipur on 14th August 2014.

Plate-XII : a. Dorsal view of Chocolate-brown colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Kambadui habitat, Senapati district, Manipur on 14th August 2014.**b**. Ventral view of Chocolate-brown colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Kambadui habitat, Senapati district, Manipur on 14th August 2014.**c**. Dorsal view of Chocolate colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Kambadui habitat, Senapati district, Manipur on 19th of June 2011.**d.** Ventral view of Chocolate colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Kambadui habitat, Senapati district, Manipur on 19th of June 2011.

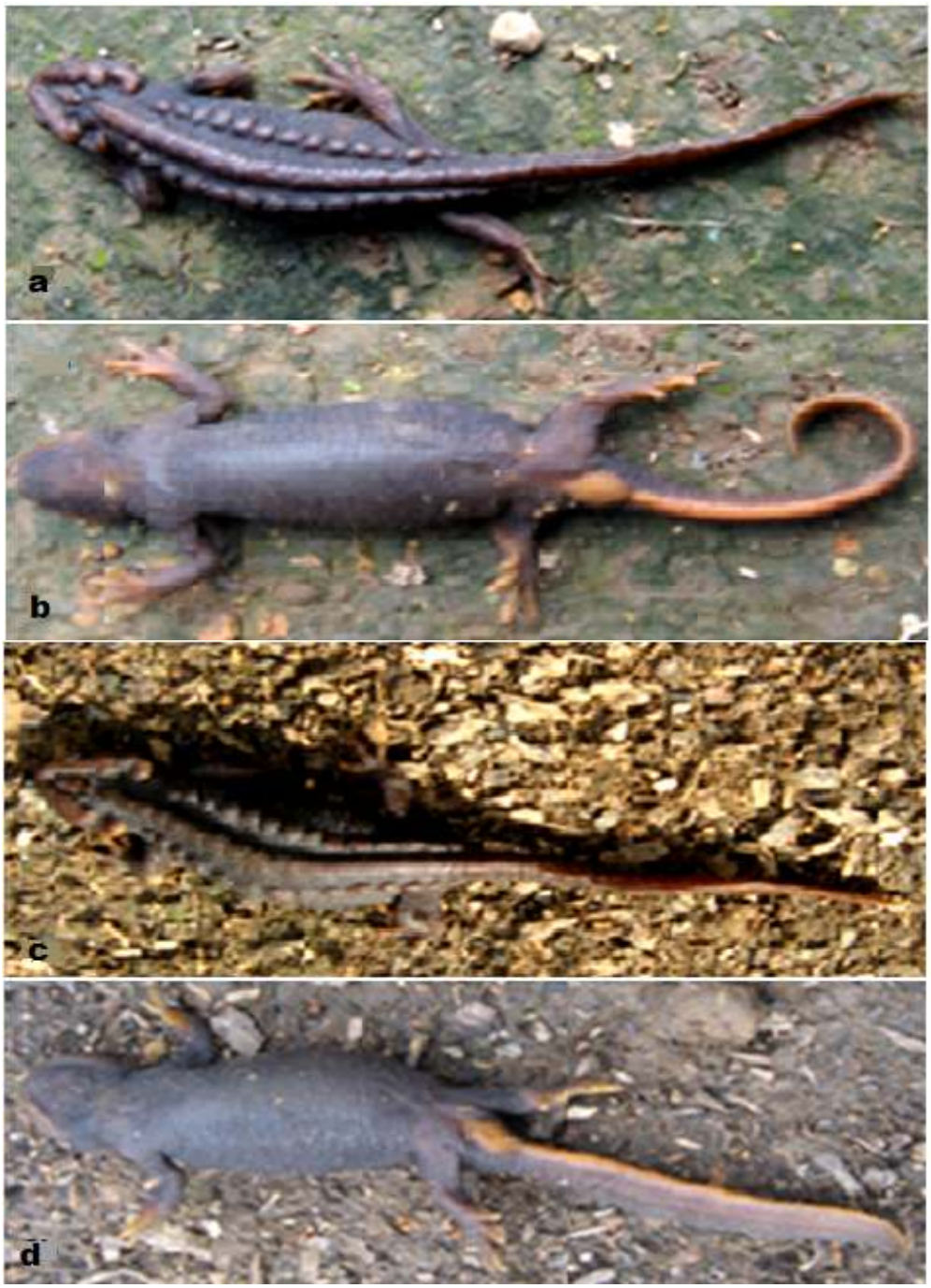

Plate-XIII: a. Dorsal view of Sand elm colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Tingbajit habitat, Senapati district, Manipur on 10th of July 2011.**b**. Ventral view of Sand elm colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Tingbajot habitat, Senapati district, Manipur on 10th of July 2011.**c**. Dorsal view of Sand elm colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Tingbajit habitat, Senapati district, Manipur on 10th of July 2011.**d**. Ventral view of Sand elm colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Tingbajot habitat, Senapati district, Manipur on 10th of July 2011

Plate-XIV:a. Dorsal view of Sky blue colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Tingbajit habitat, Senapati district, Manipur on 18th of June 2014.**b**. Ventral view of Sky-blue colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Tingbajot habitat, Senapati district, Manipur on 18th of June 2014.**c**. Dorsal view of Concrete elm colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Kambadui habitat, Senapati district, Manipur on 15th of July 2012.**d**. Ventral view of Concrete elm colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Tingbajot habitat, Senapati district, Manipur on 15th of July 2012.

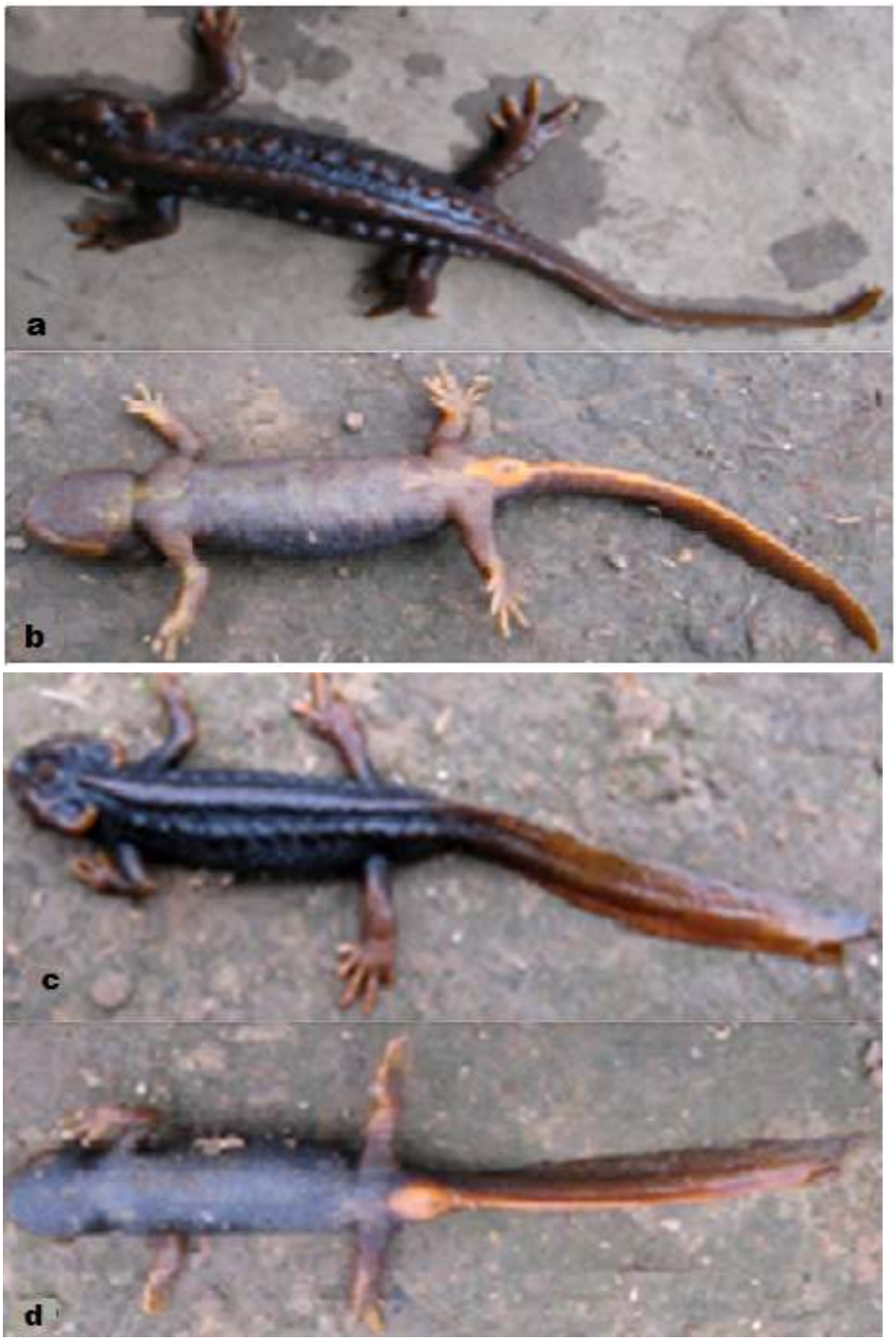

Plate-XV: a. Dorsal view of Burgundy colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Tingbajot habitat, Senapati district, Manipur on 19th of June 2011.**b**. Ventral view of Burgundy colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Tingbajot habitat, Senapati district, Manipur on 19th of June 2011.**c**. Dorsal view of Burgundy Colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Tingbajot habitat, Senapati district, Manipur on 19th of June 2011.**d**. Ventral view of Burgundy Colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Tingbajot habitat, Senapati district, Manipur on 19th of June 2011.

Plate- XVI: a. Dorsal view of Brown-black colour form of Himalayan Newt, T*ylototriton verrucosus* collected from Tingbajot habitat, Senapati district, Manipur on 27th of July 2012.**b**. Ventral view of Brown-black colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Tingbajot habitat, Senapati district, Manipur on 27th of July 2012.**c**. Dorsal view of Brown-black colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Tingbajot habitat, Senapati district, Manipur on 27th of July 2012.**d**. Ventrall view of Brown -black colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Tingbajot habitat, Senapati district, Manipur on 27th of July 2012.

Plate- XVII: a. Dorsal view of Navy-blue colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Kambadui habitat, Senapati district, Manipur on 27th of June 2015.**b**. Ventral view of Navy-blue colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Kambadui habitat, Senapati district, Manipur on 27th of June 2015.**c**. Dorsal view of Caramel colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Tingbajot habitat, Senapati district, Manipur on 29th of June 2014.**d**. Ventral view of Caramel colour form of Himalayan Newt*, Tylototriton verrucosus* collected from Kambadui habitat, Senapati district, Manipur on 29th of June 2014.

Plate- XVIII: a. Dorsal view of Caramel colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Tingbajot habitat, Senapati district, Manipur on 29th of June 2014. **b.** Ventral view of Caramel colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Tingbajot habitat, Senapati district, Manipur on 29th of June 2014. **c**. Dorsal view of dark brown -black form of Himalayan Newt, *Tylototriton verrucosus* collected from Tingbajot habitat, Senapati district, Manipur on 29th of June 2014.
d. Ventral view of dark brown-black form of Himalayan Newt, *Tylototriton verrucosus* collected from Tingbajot habitat, Senapati district, Manipur on 29th June 2014.

Plate- XIX: a. Dorsal view of dark brown-black form of Himalayan Newt, *Tylototriton verrucosus* collected from Tingbajot habitat, Senapati district, Manipur on 29th of June 2014.**b**. Dorsal view of dark brown-black form of Himalayan Newt, *Tylototriton verrucosus* collected from Tingbajot habitat, Senapati district, Manipur on 29th of June 2014.**c**.Dorsal view of Coca- colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Tingbajot habitat, Senapati district, Manipur on 10th of June 2014.**d**.Ventral view of Coca- colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Tingbajot habitat, Senapati district, Manipur on 10th of July 2014.

Plate – XX: a. Dorsal view of Coca- colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Tingbajot habitat, Senapati district, Manipur on 10th of June 2014.**b.** Ventral view of Coca- colour form of Himalayan Newt*, Tylototriton verrucosus* collected from Tingbajot habitat, Senapati district, Manipur on 10th of July 2014.**c**. Dorsal view of Coca- colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Tingbajot habitat, Senapati district, Manipur on 10th of June 2014.**d**. Ventral view of Coca- colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Tingbajot habitat, Senapati district, Manipur on 10th of July 2014.

Plate – XXI: a. Dorsal view of Coca- colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Tingbajot habitat, Senapati district, Manipur on 10th of June 2014.**b**. Ventral view of Coca- colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Tingbajot habitat, Senapati district, Manipur on 10th of July 2014.**c**. Dorsal view of off-black colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Tingbajot habitat, Senapati district, Manipur on 10th of June 2014.**d.** ventral view of off-black colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Tingbajot habitat, Senapati district, Manipuron 10th of June 2014.

Plate – XXII: a. Dorsal view of off-black colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Tingbajot habitat, Senapati district, Manipur on 10th of June 2014.**b**.Dorsal view of off-black colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Tingbajot habitat, Senapati district, Manipur on 10th of June 2014.**c**. Dorsal view of off-black colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Tingbajot habitat, Senapati district, Manipur on 10th of June 2014.**d**. Dorsal view of off-black colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Tingbajot habitat, Senapati district, Manipur on 10th of June 2014.

Plate – XXIII: a.Dorsal view of jet-black colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Tingbajot habitat, Senapati district, Manipur on 15th June 2015.**b**.Ventral view of jet-black colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Tingbajot habitat, Senapati district, Manipur on 15th June 2015.**c**. Dorsal view of jet-black colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Tingbajot habitat, Senapati district, Manipur on 15th June 2015.**d.** Dorsal view of jet-black colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Tingbajot habitat, Senapati district, Manipur on 15th June 2015.

Plate – XXIV:**a.** Dorsal view of off-black colour formof Himalayan Newt, *Tylototriton verrucosus* collected from Tingbajot habitat, Senapati district, Manipuron 10th of June 2014.**b**. Dorsal view of off-black colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Tingbajot habitat, Senapati district, Manipur on 16th of August 2014.**c**. Dorsal view of dark-brown colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Tingbajot habitat, Senapati district, Manipur on 10th of June 2014.d. Dorsal view of off-black colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Tingbajot habitat, Senapati district, Manipur on 16th of August 2014.

Plate – XXV:a. Dorsal view of brown colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Tingbajot habitat, Senapati district, Manipur on 17th of July 2012.**b.** Dorsal view of brown colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Tingbajot habitat, Senapati district, Manipur on 17th of July 2012. **c**. Dorsal view of brown colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Tingbajot habitat, Senapati district, Manipur on 17th of July 2012. **d.** Dorsal view of Light cola colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Lakpiidui habitat, Senapati district, Manipur on 17th of June 2012.

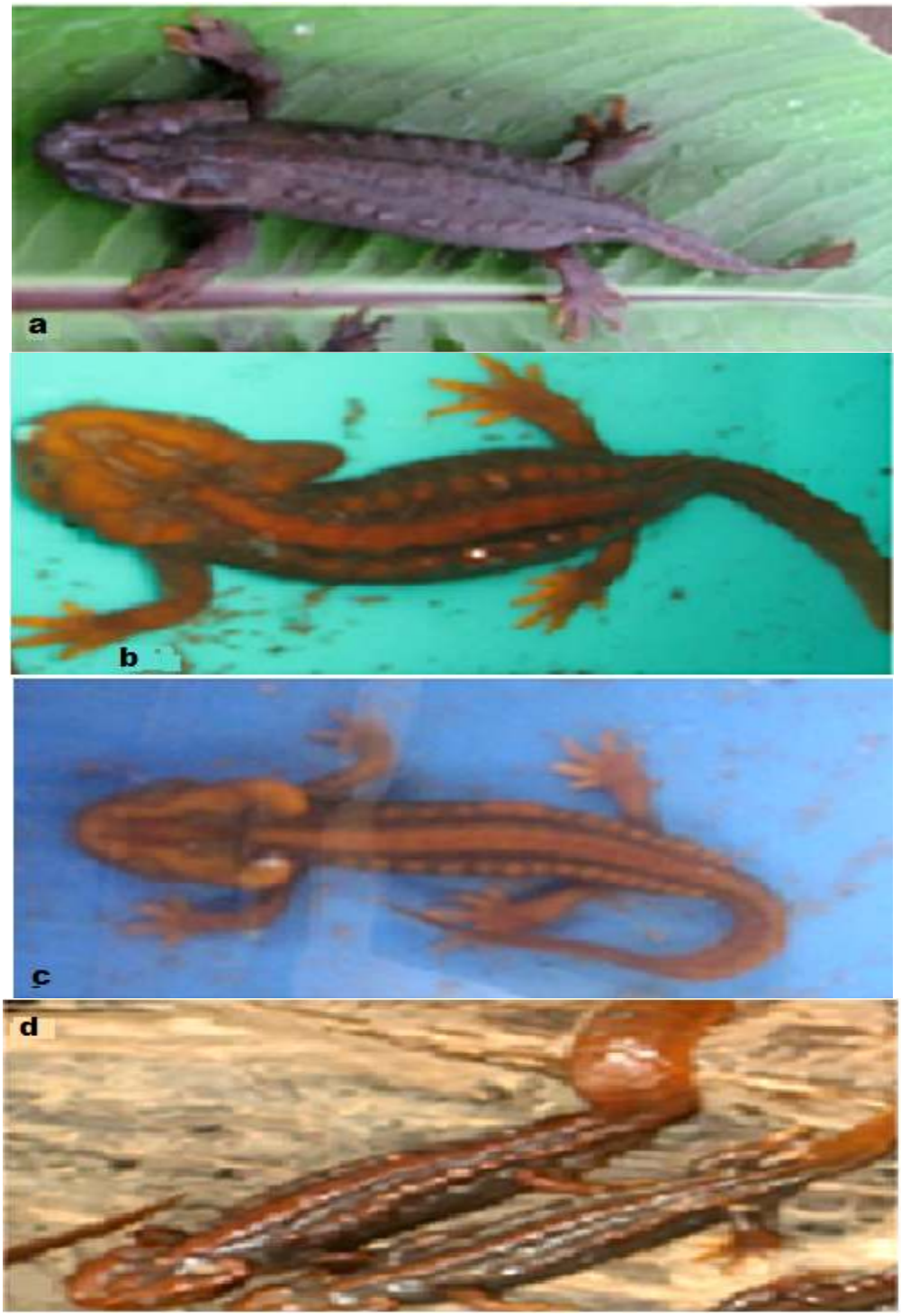

Plate – XXVI:a. Dorsal view of dark purple colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Tingbajot habitat, Senapati district, Manipur on 17th of June 2009.**b**. Dorsal view of orange colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Tingbajot habitat, Senapati district, Manipur on 17th of June 2009. **c.** Dorsal view of orange colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Tingbajot habitat, Senapati district, Manipur on 17th of June 2009. **d.** Dorsal view of dark orange form of Himalayan Newt, *Tylototriton verrucosus* collected from Tingbajot habitat, Senapati district, Manipur on 17th of June 2009.

Plate – XXVII: Dorsal view of pink colour form of Himalayan Newt, *Tylototriton verrucosus* collected from Tingbajot habitat, Senapati district, Manipur on 29th of May 2012.

CHAPTER – III

FOOD, FEEDING AND SKIN-SHEDDING IN THE HIMALAYAN NEWT, *TYLOTOTRITON VERRUCOSUS* IN SENAPATI DISTRICT, MANIPUR

INTRODUCTION

Reproduction, moult and migration pose great energy requirements in the annual life span of animals. Many species evolve metabolic strategies to help ensure temporal adjustments in the expression of such energetically exhaustive physiological events. Alternatively, species tend to widen the scope of food preferences and acquire tendency to exploit quality and/or quantity food resources available in their habitat. To cope with energy requirements of overlapping reproduction and moult, many predatory species adapt strategies to help maximize cannibalistic propensities particularly when food resources deplete in the environment. At the same time, preys tend to evolve newer strategies to enable them escape or resist predation (Ferrer and Zimmer, 2013). Himalayan Newt, *Tylototriton verrucosus* Anderson is a philopatric predatory species which shows limited locomotory capacities (Joly and Miaud, 1989). Throughout the stretch of its presence, it emerges from its hideouts following first monsoon shower. During monsoon months it remains most active, feeds voraciously, performs breeding activities and enters into hibernation by October/November (Shrestha, 1989, 1994; Sparreboom, 1999; Roy and Mushahidunnabi, 2001; Selim, 2001; Seglie *et al*, 2003; Wangyal and Gurung, 2012; AmphibiaWeb, 2015). The species exhibits changes in food preference during different developmental stages of the larvae, seasons and food resources available in the habitat (Dasgupta, 1988, 1996). Adults of *Tylototriton verrucosus* Anderson consume a taxonomically diverse prey assemblage, primarily including insects and their larvae, worms, snails, bivalves and other small invertebrates and switch over to cannibalism when acute depletion of food resources occurs in the habitat (Kuzmin *et al*, 1994; Dasgupta, 1996; Anders *et al*, 1998; Devi, 2005; Ferrer and Zimmer, 2013). Reportedly, Himalayan Newts prey on earthworms as one of their preferred food (Kerby and Kats, 1998; Sparreboom, 1999; Staniszewski, 2000). However, there is no report on predator-prey interactions during feeding on earthworms. Further, practically nothing is known about

moulting behaviour of the Himalayan Newt. In the present study efforts have been made to determine seasonal changes in quality and/or quantity food intake in Newt populations living free and those kept in captivity. Further, skin-shedding behaviour and predator-prey interactions were elucidated in normal and moulting Newts under captive conditions at Maram, Senapati district, Manipur.

MATERIALS AND METHODS

Experiments were performed on the Newts caught from Kambadui habitat located in the hill-forest of the Senapati district at about 9 *km* north-west to Maram Centre on National Highway-39 (Lat. 25^{o} 26.042' N; Long. 94^{o} 5.055' E; elevation, 1818 *m* a.s.l.). The habitat forms a perennial water body well fringed with different types of aquatic vegetation.

A set of four experiments were made :

Experiment-I. This experiment was made to determine seasonal variations in qualitative and/or quantitative food intake of adult Newts. Every month, starting from the 3rd week of May up to September, 2013, 10 male and 10 female Newts were caught either by hand or using dip-net between 8-10 *am*. In September, Newts were trapped from within 100 metre^{2} area of the pond. Food consumed by Newts was collected by stomach flushing following the technique as described by Dasgupta (1996) with slight modification. Broad end of a glass pipette filled with 0.6% saline was slowly inserted into the stomach of Newts through the oral route. Saline was pumped into the stomach by gently squeezing rubber bulb fitted on the other end of the pipette. The process was repeated three times for every individual Newt. Displaced stomach contents were separately collected in a Petri-dish. Food items stuck in the oral cavity and/or by the sides of the pipette were collected with the help of blunt forceps. Food collected from individual Newt was fixed in 5% formalin solution and stored separately into 20 *ml* glass vials for further analysis. The efficiency of this method could not be ascertained by simultaneous sacrifice of Newts to compare findings since killing of Newts is prohibited in India. Stomach flushing has been accepted as a reliable method for gut-content analysis in amphibian species (Bulakov and Voronin, 1976; Pisarenko and Voronin, 1976), and Newts are reported not to suffer from any ill-effect after such practice (Griffith, 1986; Joly, 1987; Dasgupta, 1996).

Stomach contents from individual Newt were separated, soaked on filter paper and weighed on an electronic balance with a sensitivity of 100 milligram (*mg*). Newts were released in their habitat immediately after stomach flushing. Data were expressed as gram (*g*)

for the whole food content and % wet-weight of different food types recovered during stomach flushing.

Experiment-II. This experiment was performed to assess seasonal food intake of adult *Tylototriton verrucosus* Anderson under captive conditions. During the 2nd week of May 2013, 6 male and 6 female Newts were caught from the Kambadui habitat. They were separately housed in glass aquaria (2' x 1.5' x 1.5' in size) and acclimatized for a week to ambient temperature and daylength at Maram (near Kambadui habitat). Each aquarium was filled with pond water up to 6 *cm. A* platform consisting of stone heaps and projecting 1 *cm* above water level was staged at one of the corners of each aquarium. Newts were fed with average size live earthworms *ad libitum* on alternate days between 5.30 to 6.0 *pm.* This feeding protocol was kept owing to the observation that peak foraging timing in Newts coincides with dusk. Further, feeding on daily basis renders captive Newts reluctant in accepting food (our unpublished data). On 23rd of every month, starting from May up to October 2013, earthworm consumed by individual Newt was recorded. Wet-weight of earthworms was recorded prior to their dropping in each aquarium. Unconsumed earthworms were collected after 30 minutes and were weighed on electronic balance to the nearest of 100 *mg*. Individual consumption was calculated after subtracting the weight of unconsumed earthworms from the total weight dropped in a particular aquarium. Care was taken that no earthworm hides under the stone heaps placed inside the aquarium. Selection of earthworm for feeding was based on the report that it is one of the preferred natural food of the Himalayan Newt (Staniszewski, 2000). Data were expressed as food consumed in *g/kg* body weight of the Newt. Following completion of data recording in October 2013, Newts were released in their natural habitat.

Experiment III- This experiment aimed at recording strategic preying of adult *Tylototriton verrucosus* Anderson on live earthworms, one of the most preferred natural food of the Himalayan Newt (Staniszewski, 2000). On 25th June, 2013, aquarium water was reduced to approximately 1 *cm*. At 5.30 *pm* every individual Newt was presented with live earthworm (10-11 *mm* in size) and their preying behaviour was observed, recorded and photographed. This observation was repeated for four consecutive days. On 29th June, Newts were arranged in 6 pairs, each aquarium having a male and a female. At 5.30 *pm* pairs were presented with live earthworms. Inter-sex competition in worm-preying behaviour was observed, recorded and filmed.

Experiment IV- This experiment was made to study preying behaviour of Newts at different stages of skin-shedding. On 15th July 2013, majority of Newts kept in captivity were found shedding their skin. Newts, at different stages of moulting were presented with live earthworms and their preying behaviours were observed, recorded and photographed.

Records on pH, alkalinity, free CO_2 and DO_2 of aquarium water were kept using Merck New Spectroquant test kit. GPS MAP 76CSX from Garmin Company was used for recording latitude and longitude of the habitats. Sexing of Newts was done based on long and swollen cloacal slit in males and distended belly and rimmed conical shape cloaca in females (Chapter-I, Fig. 2). Species identification references followed was based on description of Anderson (1871), Daniels (2005), Yang and Rao (2008), Ahmed *et al* (2009), Fei *et al* (2010) and Vasudevan and Sondhi (2010).

Data were analysed using Student's '*t*' test and one way independent measures of Analysis of Variance as deemed fit (Bruning and Kintz, 1977).

RESULTS

Seasonal variation in Stomach-contents **in free living Newts :** In either sexes of Newts, food recovered from stomach flushing differed significantly during breeding (May-July) and post-breeding periods (August-September) (Table-3). Food contents had different sizes of insects (Diptera, Lepidoptera, Isoptera, Odonata, Amphipoda), and their larvae, gastropods (8-15 *mm*), bivalves (8-18 *mm*), earthworms, cannibalized eggs and vegetal matter. Insects and their larvae in the diet varied between 13.41 ± 0.99 – 19.19 ± 4.45 % in males and 4.70 ± 1.40 – 15.37 ± 4.00 % in female Newts across the season (Table-3). During June and July insects and their larvae in the food of females were significantly less compared with males (Table-3). Gastropods ranged between 27.79 ± 1.46 – 34.67 ± 1.21 % in males and 20.08 ± 2.79 – 33.87 ± 1.80 % in the diet of females across the season. Bivalves varied between 32.44 ± 2.93 – 39.93 ± 1.31 % in males and 32.00 ± 2.41 % in the diet of females (Table-3). Vegetal matter ranged between 9.78 ± 1.02 – 15.47 ± 1.56 % in males and 12.18 ± 0.73 – 18.98 ± 2.14 % in females. Earthworms were present in the diet of males during July and August and in females during August only (Table-3). Newt eggs were present during June and July in the food of females only (Table-3).

Seasonal change in food consumption of Captibve Newts : No apparent change in body weight and/or length of Newts occurred between May and October, 2013. One way Analysis of Variance revealed a significant alteration in food intake in both the sexes of Newts (male : $F_{5,30} = 6.55$, $p<0.05$; female : $F_{5,30} = 10.80$, $p<0.05$) across the season. Food consumptions in both the sexes of Newts were significantly more during May-September as compared with October (post-breeding season) month value. Further, in breeding season, voluntary food intake in females was significantly ($p<0.05$) more compared with male (Table-4; Fig.2*)*. Positive correlation (male, r=0.63, r^2=0.3969; female, r=0.58, r^2=0.3364) was observed between average temperature (22.15 ± 0.45^{oC}, range : 20.73-21.94^{oC}) across months. Minimum average temperature recorded during different months (14.17 ± 0.83 ^{oC}, range : 11-17^{oC}) had positive correlation (male, r=0.43, r^2= 0.1849; female, 0.76, r^2= 0.5776) between temperature and food intake. Maximum average temperature (range: 31-33^{oC}) depicted negative correlation (male, r= - 0.72; female, r= - 0.72) between temperature and food intake (Table-5). Further, positive correlation was observed between average rainfall (127.17 ± 30.76 *mm* (range : 25-227*mm*) and food consumption in both the sexes of Newts (male, r = 0.69, r^2= 0.4761; female, r=0.61, r^2= 0.3721). A negative correlation (male, - 0.25; female, - 0.48) was observed between average humidity (range: 72.90-77.00%) across months and food consumption. During the study period maximum precipitation was recorded at *227 mm* in August and minimum at *25 mm* in October. Temperature variation was recorded at a maximum of *33^{oC}* in September and minimum of *12^{oC}* in October. Maximum and minimum humidity was recorded at *84%* and *55%* respectively. DO_2 of aquarium water was at 7.6-8.0 *mg/litre;* pH, 6.8-6.9; Alkalinity, 58-62 and free CO_2, 2.8-3.0 *mg/litre*. Water and air temperature were at 21 ± 0.12^{oC} and 26 ± 0.32^{oC} respectively.

Preying strategy **:** Following dropping of earthworms in aquaria, Newts positioned themselves concentrating at the head region of the crawling worm (Plate-XXVIIIa). Ten of the twelve Newts attacked worms at places beyond clitellum towards tail region (Plate-XXVIIIb). These Newts could comfortably catch worms and smoothly swallowed them within 5.0 ± 0.34 minutes (range 4.5-5.5 minutes) without much resistance from the prey (Plate-XXVIIIc). The remainder two Newts attempted catching earthworms at or closer to their mouth. In such cases immediate coiling of the worms occurred to wrap around the mouth and/or head of Newts (Plate-XXVIIId). The resistance exhibited during coiling was strong enough to compel Newts desist grabbing them. After about 5 minutes these Newts managed to catch worms behind clitellum and swallowed them within 5.3±0.23 minutes.

When worms were dropped in aquaria having male and female pair, Newts caught the worms randomly without choice for specific place of catching and in such cases, no noticeable resistance from worms were observed. Further, female Newt was noticed trying to grab the whole worm leaving no scope for the male to share and this feature was found consistent in all the 6 pairs of Newts included in the present study. Following the catch of worms, within few minutes, Newts were observed actively secreting saliva and with the increase in salivation uncoiling of earthworms occurred making it easy to swallow (Plate-XXXa).

Skin-shedding behaviour *:* In aquatic condition Newts were observed shedding skin very often and in some as many as twice a month. Some Newts were observed shedding skin in June while busy performing breeding activies. However, intense moulting in majority of Newts clustered around August/September. Prior to the onset of moult Newts were seen becoming sluggish with their body covered with thin dead-skin coat (Plate- XXIXa). Within 5-6 hours Newts were seen scratching areas around eyes and nose with fore-limbs to set them free from skin coat (Plate-XXIXb). In the next 5-6 hours shedding skin was observed progressing backwards, captured hind-arms and tail regions overnight and within 2-3 days moulting process was complete (Plate-XXIXc-g)

Food consumption during moult : Newts covered with shedding skin coat remained dormant for at least 5-6 hours during which they showed no interest to morsel of food given to them (Plate- XXXb). Once the moulting skin scrolled off the head region feeding resumed but remained restricted to acceptance of only the chopped-off worms (Plate-XXXc-e). Normal feeding on earthworms resumed only after 2-3 days following the start of moult (Plate-XXXg).

DISCUSSION

In india, *Tylototriton verrucosus* Anderson is reported from Darjeeling-hills of west-Bengal (Dasgupta, 1990; Roy and Mushahidunnabi, 2001; Seglie *et al*, 2003), Arunachal Pradesh (Mansukhani *et al*, 1976), Sikkim (Frost, 1985; Sparreboom, 1999; AmphibiaWeb, 2015), Meghalaya (Das, 1984) and Manipur (Selim, 2001). However, reports on feeding ecology of captive and/or free living Newts are limited to Darjeeling populations (Kuzmin *et al*, 1994; Dasgupta, 1984, 1996; Devi, 2005). This is the first report to describe food and feeding habit of the Himalayan Newt from hills of Senapati district, Manipur. The observation that in both the sexes of *T. verrucosus* Anderson, stomach contents were

significantly higher during breeding season as compared to post-breeding season, suggests seasonal variation in food consumption of this Newt-species. These results are in agreement with the finding of Dasgupta (1996) who reported high stomach-contents in breeding season compared with post-breeding season in Himalayan Newts at Beltar river and Namthing lake of Darjeeling district, west-Bengal. This view gains further support from the observation that in captive Newts voluntary food-intake dropped significantly during October as compared with May-September months. Interestingly, despite apparent changes in seasonal consumption of food in both the sexes, Himalayan Newts regardless did not exhibit significant alterations in their body weight. Obviously, *T. verrucosus* Anderson has no mechanisms to economize its energy resources across the season. Lack of such a mechanism might necessitate action oriented changes in food consumption of the Himalayan Newts. It is noteworthy that Himalayan Newts do not seem to accumulate proteins and/or lipids as energy resources to meet calorie requirements for survival and simultaneous reproductive preparations during hibernation. The energy cost posed on this account probably comes from the taxonomically heterogeneous assemblage of food consumed towards the end of breeding season. The observation that in the month of October gut contents recovered from a free living Newt contained muscles released from digested shells of snails and bivalves advocates in favour of such a supposition (Plate-.XXXI-XXXII).

Positive correlation between temperature range of 11-23^{oC} and voluntary food intake is in conformity with findings of animal hobbyists from Europe who have reported a temperature range of 18-25^{oC} as good regime for feeding and breeding activities in the Himalayan Newt (Caudata Culture, 2000). It is interesting to note that a temperature regime in excess of 30^{oC} finds negative correlation with food consumption in *Tylototriton verrucosus* Anderson. It is likely that temperature regimes below 10^{oC} and above 30^{oC} serve as environmental stressor and a consequent drop in voluntary food intake (Caudata Culture, 2000). The observation that Himalayan Newts undergo aestivation at temperature rising above 30^{oC} supports this view (Chapter-I). Further, a positive correlation between rainfall and food consumption might denote action oriented effects following precipitation combined with moderate temperature. This supposition musters support from the fact that increased food consumption and intensified breeding activities coinciding with precipitation and moderate temperature regime. The fact that positive influence of rainfall on voluntary food intake dissipates with temperature rising above 30^{oC} advocates in favour of such a supposition. The Correlation coefficient (r) between average humidity and food intake though negative, values in both the

sexes were closer to zero and thus do not support a significant role of humidity in influencing food intake of Newts. Further, consumption of significantly more food by females compared with males is in agreement with report from other workers (Caudata Culture, 2000) and may denote sex-specific food requirements of the species under captive conditions.

The diet of adult Newts is reported to contain both, aquatic and terrestrial elements. Reports, largely from Newt-habitats in Darjeeling hills describe Lumbricidae, larval Diptera, larval Coleoptera, larval Lepidoptera, larval Odonata, adult dytiscid beetles, larval Rhacophoridae, molluscs, cannibalized eggs and larvae of anurans in varying proportions to form natural food of the Himalayan Newt (Kuzmin *et al*, 1994; Dasgupta, 1996; Devi, 2005). In the present study only larval Diptera, adult and larval forms of Lepidoptera, Isoptera, larval Odonata and Amphipoda (millipedes) were present in the stomach contents (Table-3, Plate-XXXIIa,d) Absence of other arthropods in food intake of Newts in Kambadui habitat of Senapati district might denote that ecological conditions in this habitat do not support flourishing of other insect fauna. Conversely, presence of appreciable quantity of gastropods and bivalves in stomach-contents of Newts throughout the season might denote suitability of habitats for flourishing of gastropods and bivalves which make prime food resources of Newts in this habitat. Presence of different sizes of snails and bivalves in stomach contents of Newts throughout the study period lends support to this assumption. Presence of Newt eggs in the stomach of females only, denotes that in the Himalayan Newt oophagy is sex-specific. Similar observation is reported in *T. verrucosus* Anderson from Darjeeling hills (Dasgupta, 1996). Sex-specific oophagy has also been described in other Newt species (Marshall *et al*, 1990). It is interesting to note that during June and July females preferred Newt eggs over insect food. Significantly low percentage of insects in stomach contents of females compared with male Newts during June-July supports this view. During the breeding season female Newts guard their clutches and young ones and do not forage actively while males have frequent exit from water to fend for themselves (Dasgupta, 1984). It may therefore, be possible that attending females indiscriminately consume Newt eggs as an important source of energy. Presence of earthworms in the stomach of males during July-August and in females during August only suggests that males had exit from the pond during July-August and females came out only during August. Presence of vegetal matter in the stomach contents of both the sexes of Newts is in conformity with other reports (Kuzmin *et al*, 1994; Dasgupta, 1996; Devi, 2005) and may constitute as deliberate intake as roughage.

Observations show that dropping of earthworms in aquaria prompted Newts position themselves gazing at the head region of the crawling worms. Ten of the twelve Newts included in the present study attacked worms at places beyond clitellum towards tail region. In successful encounters Newts managed to catch and smoothly swallow the whole worm within 5.0 ± 0.34 minutes without any noticeable resistance from the prey. The remaining two Newts attempted catching earthworms at or closer to their mouth. In resistance, worms instantly twisted and wrapped around mouth and head of Newts. The quantum of resistance was much pronounced and had forced Newts to abort preying attempt. Presumably, these two Newts had no prior experience of preying on earthworms. The fact that within 5 minutes of aborting preying efforts these Newts like others picked up worms from behind clitellum and managed to swallow them within 5.3±0.23 minutes, strengthens this view. No error by these Newts in grabbing worms for following three days further advocates in favour of such a supposition. Predator-prey interaction became obscure amidst competition between sexes of Newts to grab major chunk of the prey. Following dropping of worm in the aquarium with male and female together, Newts prompted to catch the worm at different places of the body leaving no scope for the prey to exhibit any resistance. Further, female was seen resorting desperate efforts to grab the whole worm and this feature was consistent in all the 6 pairs of Newts included in the present study. This finding is in conformity with reports of Dasgupta (1996) and Staniszewski, (2000) that in captivity, females of Himalayan Newt consume food more aggressively as compared with males. This finding suggests that when Newts are to be maintained in captivity special care should be taken for feeding males lest they remain unfed or poorly nourished.

Interestingly, during the process of swallowing the prey, Newts were seen profusely secreting saliva which led to uncoiling of earthworms and thus making them easy to swallow. It is probable that in the Himalayan Newt, salivary mucus contained chemical ingredients which paralyse prey rendering them easy to swallow. It looks convincing when viewed together with Newts with simple vomerine teeth managing consumption of heterogeneous assemblage of food including larger insects, small fishes, amphibian larvae, molluscs, worms and young crabs (Kuzmin *et al*, 1994; Dasgupta, 1996; Devi, 2005; Ferrer and Zimmer, 2007). It is reported that *Taricha* Newts secrete toxic alkaloid like *Tetrodotoxin* which serves as sodium channel blocker in nerves and muscles and at very low concentration it may lead to the death of a wide spectrum of predators and/or preys (Ferrer and Zimmer, 2013). This possibility remains to be explored in the Himalayan Newt.

Present observations do not suggest possibility of definite moulting pattern in *Tylototriton verrucosus* Anderson as under aquatic condition Newts were found shedding skin very often; in some as frequent as twice a month. Data exhibit moulting becoming more prominent during July/August when majority of Newts were found undergoing moderate to heavy skin-shedding. Beginning of moult was characterized by Newts becoming dormant for 5.6 ± 00.89 hours followed by scratching of areas around eyes and nose with fore-limbs to set them free from shedding skin. In another 5.4 ± 0.65 hours shedding skin progressed backwards and overnight it captured hind-limbs and tail-regions. In next 2.3 ± 0.46 days Newts succeeded in renewing their whole skin. In fully moulted Newts skin became thin, smooth, and slimy probably to support gaseous exchange between body and the aquatic media. Further, predatory behaviour of Newts was greatly influenced by the stages of skin-shedding. Preying of worms was totally suspended during the dormancy period prior to start of moult. Once the head region became free from shedding skin, Newts began accepting food only as chopped-off worms. Normal predation resumed only after Newts had successfully renewed their skin.

It may thus be concluded that in the Himalayan Newt food consumption and feeding patterns vary depending upon season, sexual stage and moult intensity. In Kambadui habitat of the Senapati district, Manipur, gastropods and bivalves constitute prime food resources of the Himalayan Newt. Further, flourishing of habitat specific food resources and seasonality in food consumption of Newts may be influenced by physical factors and the reproductive state. Owing to the fact that in the Himalayan Newt, *Tylototriton verrucosus*, Anderson preying behaviour differs during different stages of the moult and that females are more aggressive consumers than males, it may be suggested that attempts inclined towards conservation of this critically endangered ornamental amphibian species through captive breeding (Sparreboom, 1999; AmphibiaWeb, 2015), must involve ways and means to ensure that males are being properly nourished.

Plate –XXVIII: a. A Newt positioning itself to an earthworm dropped in the aquarium. Strategic catching of an earthworm away from the head region as it minimizes resistance from the prey.**b**. A Newt awallowing the prey.**c**.Catching earthworm at or around the mouth results in instant coiling to wrap around the mouth and head of the Newt. **e,f.** Paired as male and female, Newts struggle to grab major chunk of the the prey.

Plate-XXIX : a. A Newt under preparation for shedding skin (5.6 ± 00.89 hours).**b**. A moulting Newt scratches skin around nose and mouth to set them free (5.4 ± 0.65 hours). **c,d,e.** During moult Newts accept only chopped-off worms. **f,** A Newt on the verge of completion of moult. **g**.As the moult completes, active feeding begins (2.3 ± 0.46 days).

Plate-XXX: a. Newts secrete salavary mucus to help them swallow earthworm (shown By arrow) by causing uncoiling of earthworm.
b. Preying on earthworm is suspened during active moult.
c.Newts reluctantly accept pieces of earthworm during mid-moulting period.
d,e,f. Newts on the verge of completion of skin-shedding;

Plate-XXXI: a. Bivalves , Moth larva and Glochidium larvae recovered from the gut of an adult Newt killed on 27th July 2012 during the process of weeding of paddy fields at Tingbajot, Senapati district, Manipur.**b**. Bivalves and Glochidium larvae recovered from the gut of an adult Newtkilled on 15th June 2011 during the process of ploughing of Paddy fields at Tingbajot, Senapati district, Manipur. **c**. Snails in advance stages of digestion as recovered from the gut of an adult Newt killed on 27th October 2009 during the process of harvesting paddy crops at Tingbajot, Senapati district, Manipur.**d**. Half digested Snails recovered from the gut of an adult Newt killed on 27th July 2013 during the process of weeding of paddy fields at Tingbajot, Senapati district, Manipur.**e**. Bivalves and Glochidium larvae recovered from the gut of an adult male Newt killed on 27th July 2014 during the process of weeding of paddy fields at Tingbajot, Senapati district, Manipur. **f**.Digested Snails and Newt eggs recovered from the gut of an adult female killed on 25th July 2011 during the process of making earthen ridge for draining pond water to irrigate vegetable crops.

Plate-XXXII: a. Snails and Millipedes recovered from the gut of an adult Newt killed on 2oth July 2011 during the process of weeding of paddy fields at Tingbajot, Senapati district, Manipur.

b. Bivalves and Newt eggs recovered from the gut of an adult female Newt killed on 15th July 2014 during the process of ploughing of Paddy fields at Tingbajot, Senapati district, Manipur.

c.Insect legs and bivalve muscles recovered from the gut of an adult male Newt killed on 25th July 2009 during the process of raising earthen ridge by the side of a pond at Tingbajot, Senapati district, Manipur.

d. An insect present in the gut content of an adult Newt killed on 10th June 2013 during the process of ploughing paddy fields at Tingbajot, Senapati district, Manipur.

Table- 3. Diversity of food items in Stomach Contents of wild caught Himalayan Newts, *Tylototriton verrucosus* Anderson at Kambadui habitat in Senapati district, Manipur*.

Months	Sex	SC *(g)*	I + IL *(%)*	GD *(%)*	BV *(%)*	VM *(%)*	EW *(%)*	NE *(%)*
May	M	2.13±0.04[a]	15.89±1.09	34.40±1.42	39.93±1.31	9.78±1.02		
	F	2.20±0.06[d]	15.27±1.10	33.87±1.80	38.18±2.25	12.66±1.67		
June	M	2.07±0.05[a]	13.41±0.99	34.67±1.21	39.03±1.57	12.36±1.57		-
	F	2.05±0.05[c]	4.70±1.40[h]	32.75±1.76	41.14±1.24	12.18±0.73		10.09±1.97
July	M	2.05±0.04[a]	17.15±3.34	31.08±1.69	35.99±3.14	15.47±1.56		-
	F	2.20±0.07[c]	7.28±3.17	20.08±2.79[g]	27.73±2.51	12.47±0.99		32.43±6.81
August	M	1.94±0.06	1.94±0.06	27.79±1.46	32.44±2.93	13.01±1.32	5.69±1.59	
	F	1.97±0.04	1.97±0.04	33.80±1.96[g]	32.00±2.41	15.38±1.72	3.60±0.54	
September	M	1.86±0.05	19,14±1.90	30.57±1.06	37.16±2.34	13.12±1.40		
	F	1.86±0.03	15.37±4.00	29.11±0.70	32.82±0.93	18.94±2.14		

*Values are expressed as Mean ± SEM; n=10 in each group.
SC, Stomach Contents; M, Male; F, Female; I+IL, Insects plus Insect larvae; GD, Gastropods; BV, Bivalves; VM, Vegetal Matter; EW,Earthworms; NE, Newt Eggs.

a,b differ from September month value in male at $p<0.05$ and < 0.01 respectively.
c,d differ from September month value in female at $p<0.05$ and < 0.01 respectively.
g,h differ from corresponding month value in male at $p<0.05$ and < 0.01 respectively.

Table 4. Seasonal change in Food intake of the Himalayan Newt, *Tylototriton verrucosus* Anderson maintained in Natural lighting at Ambient Temperature at Maram, Senapati district, Manipur*.

Months	Sex	Body weight *(g)*	Total Length *(mm)*	Food consumed *(g/kg b.wt.)*
May	Male	21.10 ± 0.82	153.00 ± 1.71	83.43 ± 5.78[a]
	Female	22.63 ± 0.74	157.50 ± 1.78	99.16 ± 5.62[d]
June	Male	20.77 ± 0.87	154.67 ± 1.41	86.28 ± 4.22[a]
	Female	22.50 ± 0.53	157.50 ± 1.78	110.06 ± 5.67[d]
July	Male	20.92 ± 0.94	156.67 ± 0.61	92.24 ± 4.82[a]
	Female	22.25 ± 0.47	157.50 ± 1.28	126.11 ± 5.17[e,f]
August	Male	21.07 ± 0.95	156.33 ± 1.17	99.00 ± 5.52[a]
	Female	22.50 ± 0.52	157.50 ± 1.29	114.46 ± 4.92[d]
September	Male	21.31 ± 1.01	156.33 ± 1.16	97.84 ± 5.80[a]
	Female	22.70 ± 0.45	157.50 ± 1.36	116.69 ± 2.36[e,f]
October	Male	21.16 ± 0.99	155.00 ± 1.10	56.79 ± 9.95
	Female	22.50 ± 0.43	155.33 ± 1.31	74.51 ± 7.89

*Values are mean ± SEM; N=6 in each group.
d differs from October month values in either sexes at $p<0.05$ level (Student's *t* test).
e differs from value of female during June at $p<0.05$ level (Student's *t* test).

Male, $F(5,30) = 6.55$, $p<0.05$ (One way independent measures of ANOVA).
Female, $F(5,30) = 10.80$, $p<0.01$ (One way independent measures of ANOVA).

Table – 5: Correlation Coefficient between Physical Factors and voluntary Food intake of the Himalayan Newt, *Tylototriton verrucosus* Anderson in Kambadui habitat of Senapati district, Manipur

Physical Parameters	Sex	Coefficient of Correlation (r)	r^2
Temperature			
14.17 ± 0.83 (range :	Male	0.43	0.1849
11- 17^{oC})	Female	0.76	0.5776
22.15 ± 0.45^{oC} (range	Male	0.63	0.3969
: 20.73-23.15^{oC})	Female	0.58	0.3364
	Male	-0.52	-
31.50 ± 0.08 (range : 31-33^{oC})	Female	-0.51	-
Rainfall			
127.17 ± 30.76 *mm*	Male	0.69	0.4761
(range : 25-227*mm*)	Female	0.61	0.3721
Humidity			
74.72 ± 1.56% (range	Male	-0.48	-
: 72.90 - 77%)	Female	-0.25	-

CHAPTER – IV

COURTSHIP, MATING AND EGG LAYING IN HIMALAYAN NEWT, *TYLOTOTRITON VERRUCOSUS* ANDERSON IN SENAPATI DISTRICT, MANIPUR, NORTH-EASTERN INDIA

INTRODUCTION

Himalayan Newt, *Tylototriton verrucosus* Anderson is the only caudate species found in the Indian sub-continent. This Newt species inhabits cold climates in mountainous regions of eastern Himalaya, emerges from its hibernacula following first monsoon rains (Shrestha, 1989; 1994; Selim, 2001; Seglie *et al*, 2003; Wangyal and Gurung, 2012; AmphibiaWeb, 2015; IUCN, 2015). During monsoon season, it feeds actively and performs breeding activities (Roy and Mushahidunnabi, 2001; Deuti and Hegde, 2007). Several reports describe breeding behaviour (Annandale, 1908; Boulenger, 1920; Chaudhury, 1966; Thorn, 1969; Thant-Shima *et al*, 1979; Dasgupta, 1983; AmphibiaWeb, 2015), hibernation (Basnet, 2001; Nag and Vasudevan, 2014), Parental care (Dasgupta, 1984) and biology of eggs and larvae (Annandale, 1907, Smith, 1924; Dasgupta, 1988; Shrestha, 1989) of the Himalayan Newt. Majority publications on different behavioural aspects of this Newt species are based on anecdotal observations and exhibit inconsistency at description. The secretive nature of the Himalayan Newt features daunting task to the behavioural biologists to candidly observe, record and elucidate different aspects of breeding behaviour particularly in their natural habitat. Further, studies describing reproductive behaviour of the Himalayan Newt have either been made outside the Indian sub-continent or remain confined to Newt populations inhabiting Darjeeling hills of west-Bengal (Sparreboom and Yunke, 2010; AmphibiaWeb, 2015; IUCN, 2015). The last report to describe reproductive biology of the Himalayan Newt was made by Roy and Mushahidunnabi (2001) from Darjeeling hills of west-Bengal. Through carefully planned experiments, these authors successfully demonstrated courtship, mating and egg-laying in wild-caught Newts planted to laboratory conditions during the breeding season. In the present study efforts were made to study anatomical features of the reproductive system and to record and elucidate different aspects of reproductive behaviour *viz*. courtship, mating and egg laying in the Himalayan Newt, *Tylototriton verrucosus* Anderson in their natural habitat in Senapati district, Manipur of the North-eastern India.

Study site

Two perennial water bodies **'Tingbajot'** and **'Kambadui'** were selected for the study. Tingbajot is located at the forest edge, approximately 3 *km* north-west to the Maram Centre on National highway-39 (NH-39) at an elevation of 1754 metre at sea level (Lat. 25^{o} 26.032' N; Long. 94^{o} 05.411'E). The habitat consisted of a pond (~ 55' x 10'; water level during rainy season varied between 20-30 *cm*) which was extended with approximately 100 metre^{2} paddy fields on its northern side. The pond was well fringed with plants like *Sagittaria sagittifolia*, and different species of *Polygonum, Commelina, Eupatonium, Osbeckia, Impatiens, Fagopyrum, Astemisia, Bidenspilosa* and *Alteranthaceae*.

Kambadui habitat is located in the forest at about 9.5 *km* north-west to the Maram Centre on NH-39 at an elevation of 1818 metre at sea level (Lat. 25^{o} 26.042'N; Long. 94^{o} 05.055'E). The pond is located in the basement of hills (~ 30' x 9.5' in size) and during lean period (November-March) it serves as a source of water for the people from nearby settlement. During the rainy season level of water in the pond varied between 20-25 *cm*. The habitat was well fringed with different plant species like *Alteranthera sessilis, Ephatonium odaratum, Rotala rotundifolia, Dryopteris flexis, Cynodon doctylus* and *Polygonum species.*

MATERIALS AND METHODS

During the survey of discrete mountainous hills of Senapati district from May 2009 to June 2015, on several occasions Newts were found dead or injured around their habitats or nearby paddy fields. These Newts suffered casualties owing to construction of earthen ridges to prevent back flow of water during drainage of ponds to irrigate paddy fields and/or vegetable crops. Casualties were also noticed during the process of development of water bodies into cultivable land and during the process of weeding and weaning of paddy crops. Dead Newts encountered due to developmental activities were preserved in 5% formalin solution for further study and those injured were brought to the laboratory and treated to cope with their injuries. One injured gravid female was collected on 21st of June 2012 from Tingbajot habitat. Same night, this female died half way of egg laying. It was dissected to study anatomical details of the reproductive apparatus. Anatomy of reproductive system of male Newt was studied from a specimen preserved in 5% formalin on 5th July 2010.

Between 11th- 20th June 2014, Newt habitats at Tingbajot and Kambadui were examined from 15.00 *hrs* to 6.00 *hrs* for the presence of *Tylototriton verrucosus* Anderson. During night, electrical torch and emergency lamps were used to monitor the movement of Newts and their display of different reproductive behavioural activities. Since Newts have been reported to prefer shallow water areas to perform their reproductive activities (Sparreboom 1999; AmphibiaWeb, 2015), due focus was placed on such sites of the ponds. Newts engaged in breeding activities were observed and photographed using digital camera (Canon Power Shot A550). Four pairs of Newts, two pairs each from Tingbajot and Kambadui habitats were observed, recorded and photographed for different components of breeding behaviour. Further, despite every possible care certain aspects of the breeding behaviour which were seen during the observation period but could not be clicked owing to varying depths of ponds, inside vegetation and movement of Newts in the dark. To cope with such missing links, four pairs of sexually mature Newts were caught by dip-net and were maintained in captivity in different male : female combinations using all glass aquaria (3.5' x 3' x 2.5' in size) placed at Maram, nearby Kambadui habitat. Display of reproductive behavioural attributes of Newts kept in pair and/or in different male : female combinations were monitored during and after mating was over. Reproductive behavioural attributes were recorded and photographed with due care. Glass aquaria were filled with pond water at about 8 *cm.* Stone heaps and green grass from ponds were arranged inside aquaria. Newts were fed with pieces of live and soaked dry-fish and earthworms *ad libitum* and remained in good health.

Observations were made on eggs laying behaviour of Newts both, in captivity and in their natural habitats. Events were observed, recorded and photographed. Newts were weighed on electronic balance and eggs laid in the captivity were counted manually. Correlation between body weight and clutch size was established by calculating Correlation Coefficient as described by Bruning and Kintz (1977).

Dissolved Oxygen content (DO_2), pH, alkalinity and free carbon dioxide (CO_2) of pond and aquarium water were measured using Merck New Spectroquant test kit. Water and air temperature and humidity were regularly measured using thermometer and hygrometer from Merck. Data on rainfall was collected from meteorological department at Maram.

Sexes were identified based on cloacal aperture which appeared as a longitudinal slit in males and rimmed conical opening in females. During the breeding season males had

streamline body with cylindrical belly and females exhibited relatively more prominent distended belly.

The identification references used include Anderson (1871), Yang and Rao (2008), Ahmed *et al* (2009), Fei *et al* (2010), and Vasudevan and Sondhi (2010).

RESULTS

Anatomical features of Reproductive system: The male reproductive system consisted of paired bi-lobed testes, one each on the left and right sides acquiring supra-renal placements. Well developed vasdeferens began from lower lobe of the testis and running down ended directly into the cloacal aperture (Plate-XXXIII). Two numbers of ovaries, left and right were placed in supra-renal positions. Fallopian tubes, both left and right were well developed and contained fully ripe eggs ready for their exit (Plate-XXXIII). Freshly recruited batch of developing oocytes were present in both, left and right ovaries (Plate-XXXIII).

Display of Reproductive Behaviour : During the breeding season male Newt was noticed producing *twak twak twak* acoustic stimuli, though not very pronounced but clearly audible (Plate-XXXIVa). Courtship behaviour commenced with gravid female approaching male and start nosing on his belly (Plate-XXXIVd). After few seconds female was observed nosing at the tip of the tail and lifting his tail with her nose she moved away (Plate-XXXIVe). The enticed male followed her (Plate-XXXIVf). Timing differed in individual pair but on the average the entire sequence of events was completed within 13.25±1.38 minutes (range: 10-16 minutes). The excited male was observed frequently nosing on different parts of the body of female (Plate-XXXIVg-i). Amidst nosing, the male was seen making desperate attempts to slip under the body of the female. After struggling for about 10-15 seconds, male managed to slip under the body of the female to establish vent to vent contact. Timing differed in individual pair but on the average it took 14.50±2.10 seconds (range: 10-15 seconds). During vent to vent contact male was found holding female interlocking her limbs from below (Plate-XXXV-j). Timing of amplexus differed between pairs and on the average lasted for about 58.57±12.09 minutes (range: 24-78 minutes) after which pairs separated, swam freely for about 5 minutes, settled at the bottom of the pond and then started making circular movements turning their faces towards each other (Plate-XXXVk). Circular nuptial movement occurred for 28.75±2.84 minutes (range: 23-35 minutes). During circular

movement cloaca of both the sexes remained open. Male Newt continued releasing white coloured spermatophore bolus during the circular movement and the female following the same path was found sucking spermatophores in her cloaca. Accidentally missed spermatophore bolus were found diffusing in the water (Plate-XXXVk,l). Egg laying was seen approximately after two hours of spermatophore entry into the cloaca of females and was observed continuing beyond six hours. Eggs were deposited one by one with clutch size varying from 30 to 180 eggs. In natural ponds, after the mating was over, females were seen moving through submerged vegetation for eggs deposition. Eggs were found glued to submerged rocks, stones, plant stems, submerged woods and dead tree leaves lying in the pond water (Plate-XXXVIa,b,d,e). No eggs were found deposited outside the water. In the captivity, yolky eggs wrapped in white jelly (8-10 *mm* in size along with jelly) were found settled at the bottom of the aquarium (Plate XXXVIc).

During June, captive Newts exhibited courtship behaviour. Introduction of alien males in the aquarium housing a courting pair led the stud male to promptly slip under the body of the female and holding fore-limbs trying to drag her away from alien males (Plate-XXXVm). The tendency of male in monopolizing his female partner was observed continuing even many hours after mating was over and females were already in the process of depositing eggs (Plate-XXXVn).

DO_2, pH, alkalinity and free CO_2 of pond and aquarium water ranged between 7.2 - 8.5 *mg/litre;* 6.6-6.8; 46-59 and 2.8-3.0 *mg/litre* respectively. Average water and air temperature during the study period were at 18.50 ± 0.62^{oC} (range: 17-20^{oC}) and 21.10 ± 0.72^{OC} (range : 20.30-21.98^{oC}) respectively. Average precipitation and relative humidity were at 213.00 ± 33.35 *mm* (range : 100 - 268 *mm*) and 84.24 ± 0.73% (range : 83.10 – 86.23 %) respectively.

Data on body weight and clutch size exhibited positive correlation ($r=0.81$; $r^2=0.6561$).

DISCUSSION

Observations show that in the Himalayan Newt courtship behaviour clicked with male producing slow but apparently distinct *twak twak twak* acoustic stimuli. Similar observation is reported by Shrestha (1989) during the breeding season of Himalayan Newts native to Dhankuta and Maipokhari habitats of the western-Nepal. Further, though based on only four

pairs of Newts, observations suggest a leading role of the female in triggering the start of reproductive behaviour. Courtship display accentuated with gravid female making advancement towards sexually mature male and indulging in seducing him by frequent nosing at his tail and belly followed by intent lifting of his tail by her nose and then leap forward leaving the exited male follow her. Consistency at this aspect of reproductive behaviour in all the four pairs of Newts under present observation denotes that this aspect of courtship display constitutes the most crucial step in breeding biology of the Himalayan Newt since in essence, it helps in establishing pair bonding as a mandatory requirement linked to the reproductive success and thus have survival value for the species. Present observation clashes with some reports wherein males have been suggested to have liberty in choosing his female partner from amidst many females approaching him as his probable mating partner (Shrestha, 1989; AmphibiaWeb, 2015). This is the first documented evidence to show pair-bond formation during courtship display in naturally breeding populations of *Tylototriton verrucosus* Anderson. Earlier reports on breeding biology of the Himalayan Newt made no mention of this important physiological aspect probably owing to limitations in acquiring data from animals acclimatized to laboratory conditions (Boulenger, 1920; Thorn, 1969; Thant-Shima *et al,* 1979; Roy and Mushahidunnabi, 2001; AmphibiaWeb, 2015).

In the present study courtship behaviour was observed becoming intensified progressively with excited male beginning rapidly nosing his female partner all over her body from all probable sides. The height of this behaviour became noticeable with male resorting desperate attempts to slip under the body of the female and succeeding within 10-15 seconds in establishing vent to vent contact interlocking her limbs from below. Amplexus as an important component of courtship behaviour of the Himalayan Newt has also been described by other workers (Boulenger, 1920; Thorn, 1969; Thant-Shima *et al,* 1979; Roy and Mushahidunnabi, 2001). The observation that spermatophore transfer in the female through circular movement following amplexus, is in conformity with the observations reported in Newts from Myanmar (Thant-Shima *et al*, 1979). Present observations thus support circular nuptial dance as a predominant strategy of spermatophore transfer into females of *Tylototriton verrucosus* Anderson (Shrestha, 1989; Sparreboom, 1999; Roy and Mushahidunnabi, 2001; AmphibiaWeb, 2015, IUCN, 2015). Observations of the present study refute the contention that circular nuptial dance as a mode of spermatophore transfer in females is limited only to the Emperor Newt, *Tylototriton shanjing* (Nussubaum *et al*, 1995; AmphibiaWeb, 2015, IUCN, 2015). Further, the observations that females began egg

deposition one by one after about two hours of spermatophore entry in her cloaca and the laying continued over several hours agree with many other reports made in the Himalayan Newt (Shrestha, 1989; Roy and Mushahidunnabi, 2001; AmphibiaWeb, 2015, IUCN, 2015).

Observations of the present study suggest that in *Tylototriton verrucosus* Anderson pair-bonding established at the onset of courtship behaviour is retained throughout the breeding season. Such a supposition stems from the finding that once the pair-bond was established, the stud male was observed defending his female partner from alien males introduced in the aquarium. Interestingly, male continued exhibiting the tendency of monopolizing his female even several hours after the mating was over and female was already in the process of laying eggs. Whether defending behaviour which the male was observed bestowing towards his female partner is circumstantial and occurs only in the presence of alien males or it betrays the monogamous trait of the species is difficult to speculate owing to limitations in ascertaining this fact in the natural habitat. The literature on breeding biology of the Himalayan Newt is marred with rather too many speculations on this account. It has been suggested that sexually mature male Newt mates with several females and a female mates and spawns more than once during a breeding season (Shrestha, 1989; AmphibiaWeb, 2015; IUCN, 2015). This assumption finds its base in the observation that sexually mature female of *Tylototriton verrucosus* Anderson exhibits asynchronous development of ova *i.e*. both, developing and developed ova are nested in the same ovary. It is presumed that developed ones are fertilized and released early and the developing ova mature and form the second clutch size during later part of a breeding season (Shrestha, 1989; AmphibiaWeb, 2015; IUCN, 2015). This assumption has been adopted to explain spawning period in the Himalayan Newt extending from early May-June till September leaving larvae born late in the breeding season to overwinter in some habitats (Nag and Vasudevan, 2014). However, present observations do not favour this contention owing to the species exhibiting behavioural traits falling in the support of its monogamous nature. Himalayan Newts are confined to the hilly pockets where seasonal fluctuations in photo-thermal cues more or less constitute the feature of temperate climate. In females of many amphibian species, recruitment of complement oocytes begins sometimes during summer months, oocytes grow synchronously to reach final size before hibernation and are retained in this stage until spring and/or early summer months before photo-thermal stimuli induce them to make their escape by way of spawning (Jɸrgensen *et al*, 1979; Jɸrgensen, 1981, 1984). In the present case, histological details of the ovary of the Himalayan Newt during the process of spawning in

June reveals the presence of fully mature and freshly recruited batch of oocytes (Plate-XXXIII). Freshly recruited oocytes during May-June were destined to develop and get discharged in the following breeding season. It thus seems proper to opine that like many other amphibian species inhabiting temperate latitudes (Jϕrgensen *et al*, 1979; Jϕrgensen, 1981, 1984; Saidapur, 1989), the Himalayan Newt, *Tylotoriton verrucosus* Anderson may be considered as a monogamous species.

It is thus obvious that in the Himalayan Newt, *Tylototriton verrucosus* Anderson courtship, mating and egg-laying constitute components of elaborate display of behavioural attributes. Onset of courtship behaviour is triggered by gravid female by way of enticing the male. Aroused male intensifies the courtship display, establishes vent to vent amplexus and consequently succeeds in transferring spermatophores in the female cloaca through well organized circular dance. Fertilization of eggs is exclusively internal. Further, male has a tendency to monopolize his female and this behavioural attribute continues operating even several hours after mating when females are already in the process of laying eggs. All the behavioural traits observed in the present study advocate in favour of the species adopting monogamous feature contrary to what have been speculated.

Plate-XXXIII : Anatomical features of male (left) and female (right) Himalayan Newt,*Tylototriton verrucosus* Anderson.
Male: **1**. Upper lobe of left Testis; **2**. Lower lobe of left Testis; **3.** Left Vasdeferens'
Female: **1**, Oviduct; **2**, Mature egg in Oviduct; **3**.Newly recruited oocytes in Left ovary; **4**. Cloacal opening for exit of fertilized eggs.

Note : Anatomy of male was studied in formalin preserved animal found dead near paddy fields in Tingbajot habitat on 5th July 2010. Study of reproductive system of female is based on an injured gravid female found on 21st of June 2012 near paddy fields at Tingbajot. Same night, it died during oviposition.

Plate- XXXIV : a. A male Newt producing Twak Twak Twak acoustic stimuli during breeding season. **b**. A female progressing towards a male Newt to find a mate.**c**. A female Newt approaching a male during the process of pair formation.**d.** A female nosing at the belly of a male Newt to start with courtship. **e.** A female lifting the tail of a male Newt by her nose. **f.** Aroused male chasing a female Newt. **g**. Excited male begins nosing at the tail of a female Newt. **h.** A male nosing at the belly of a female Newt. **i.** A male nosing at other side of the belly of female Newt.

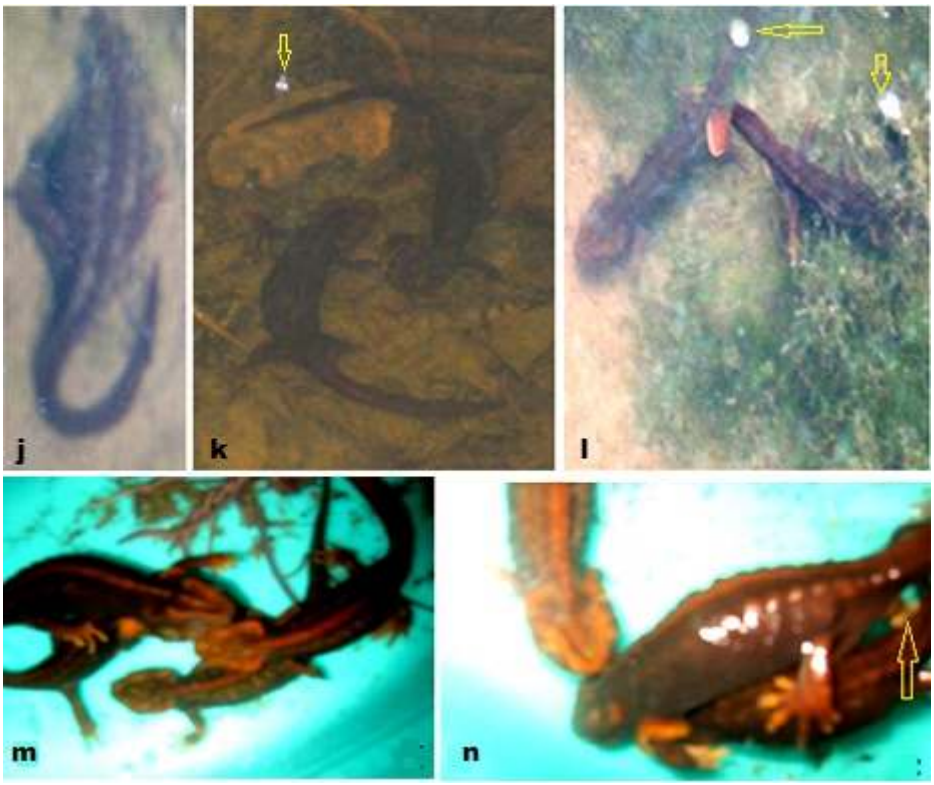

Plate-XXXV : j. A male Newt establishing vent to vent contact with female interlocking her limbs from below the body of the female.**k**. A pair engaged in circular movement following amplexus for transfer of spermatophores into female. **l.** A pair of Newt separating after circular dance.**m**. A stud male wrestling to drag away his female from alien males introduced in the aquarium.**n.** Tendency of monopolizing female by the stud male continues even several hours after mating when female starts laying eggs.

Arrows show spermatophore bolus missed by the female during circular movement

Plate-XXXVI: a. Feshly laid eggs glued to submerged rock and plant stem. **b**. Eggs glued to a boulder lying inside Newt pond. **c,d.** Eggs glued to the bottom of the aquarium.**e** Newt eggs attached to a dry tree twig falling inside pond water. **f.** Newt eggs adhering to a dry tree leaf falling inside aquatic habitat of Newts.

CHAPTER – V

TREAT, SURVIVAL AND CONSERVATION PROSPECTS OF HIMALAYAN NEWT, *TYLOTOTRITON VERRUCOSUS* ANDERSON IN SENAPATI DISTRICT, MANIPUR

INTRODUCTION

Himalayan Newt, *Tylototriton verrucosus* Anderson is a philopatric salamander species that exhibits limited locomotor capacities (Joly and Miaud, 1989), as a result they become highly susceptible to environmental alterations. The geographical confinement of this species covers an area exceeding 260, 364 Km^2 and ranges from India (west-Bengal (Chaudhuri, 1966; Dasgupta, 1984, 1990; Kuzmin *et al,* 1994; Roy and Mushahidunnabi, 2001; Seglie *et al*, 2003; Nag and Vasudevan, 2014), Sikkim (IUCN, 2015), Arunachal Pradesh (Mansukhani *et al*, 1976), Manipur Selim, 2001), Meghalaya (Das, 1964), Eastern – Nepal (Schleich and Kästle, 2002; Shrestha, 1984, 1989) through the Kachin and Shan hills of Myanmar (Gyi, 1969) to western Yunnan (Longchuan county and its vicinity), China (Zhao and Adler, 1993), scattered mountains in northern and north-eastern Thailand (Taylor, 1962; Wongratna, 1984; Chan-Ard, 2003; Chan-Ard *et al*, 1999; Nabhitabhata *et al,* 2000), Bhutan (Wangyal and Gurung, 2012), Okinava in Japan (Zhao *et al,* 1988; Pomchote *et al*, 2008) and Lai Chau and Lao Cai Provinces in north-western Vietnam (IUCN, 2015). It most likely also occurs in Laos province, although at present there is no record (IUCN, 2015).

Throughout the entire range of its occurrence, the current population status of the Himalayan Newt is precisely not known. Detailed ecology of the habitat is reported from eastern Nepal (Shrestha, 1989, 1994) and population structure has been reported for few habitats in the Darjeeling district, west-Bengal (Seglie *et al*, 2003). In IUCN Red data book, the Himalayan Newt has been assigned a category of least concern species owing to its presumed wider distribution than what current records exhibit (AmphibiaWeb, 2015; IUCN, 2015). Ironically, in many known areas of its occurrence, the species is becoming vulnerable and has succeeded in catching the attention of the governmental and non-governmental agencies across many countries to help sustain their comfortable survival. In many countries, the species is now protected by legislation (Humprey and Bain, 1990; Seglie *et al*, 2003;

IUCN, 2015). Habitat destruction owing to infrastructure development, promotion of tourism sector and creation of picnic/recreation spots around Newt habitats are likely to have adverse impacts on the quality of habitats and a consequent decline in population size of Himalayan Newts. Increased human demand for forest resources, slash and burn method of cultivation particularly in the south-east Asia are reported to have negative consequences on the terrestrial habitat of this Newt species. Water pollution resulting from agrochemicals, domestic detergents, chemical-fishing methods and extraction of water for irrigation of crops and for domestic uses have negative impacts on quality of habitats (Seglie *et al*, 2003). In some parts of India, Himalayan Newts are reported to be harvested for medicinal use by local healers and even for direct human consumption (Selim, 2001). In certain parts of Myanmar the species is considered a bad omen and thus killed intentionally (Gyi, 1969). Further, in Myanmar it is used as bait for catching large fishes. Himalayan Newt is in high demand in the international pet market owing to its ornamental features and ease at their rearing in captivity (AmphibiaWeb, 2015; IUCN, 2015). Further, all the features that add to the vulnerability of the species appear to be area and habitat specific. In the present study efforts have been made to identify threat perceptions and the survival prospects of the Himalayan Newt, *Tylototriton verrucosus* Anderson in habitats located in the hilly pockets of Senapati district, Manipur of the north-eastern India.

MATERIALS AND METHODS

During the survey of Newt habitats in Senapati district, Manipur from May 2009 to June 2013, we came across many dead Newts and their bones scattered in Tingbajot and Kambadui habitats (Plate-XXXVII). Further, in these habitats a good number of Newts were found suffering from necrotic tissue infections spread over different parts of the body. Study was extended further up to June 2015 and 4 habitats : Tingbajot, Kambadui, Lakpiidui and N'rekpiidui were thoroughly examined for the presence of diseased Newts. These habitats were selected owing to their location closer to human settlements and face frequent disturbances resulting from human activities in and around habitats and a consequent decline in the prevalence of Newt populations (Chapter-I).

Newts infected with necrotic tissue infections in these habitats were collected and were maintained in captivity at Maram using plastic tubs with water levels approximately at 6 *cm*. Infected Newts were treated with 5% Potassium permanganate ($KMNO^4$) solution soaked in

cotton and applied directly on the infected areas daily in morning and evening hours. Five minutes after the application of $KMNO^4$ Newts were placed back in plastic tubs. The frequency and duration of this treatment depended on the severity of the infection, recovery progress from aliments and complete recovery. In severely infected Newts antibiotic injections (Ciprofloxacin, 10 *mg*/*kg* body; Lark Lab. India, *Ltd.*) were given intramuscularly on alternate thigh muscles daily between 8 to 8.30 *a.m.* Simultaneously, Topical cream containing Ciprofloxacin, Fluocinolone Acetonide and Clotrimazole (Ciprolar-FC, Lark Lab. India *Ltd.*) was applied on infected areas to cope with ailments and inflammation resulting due to bacterial and fungal infections. Newts were provided with chopped fish and earthworms *ad libitum*.

Anthropogenic and agricultural activities that might cause or add to the vulnerability of Newt habitats in Senapati district were identified and possible remedial strategies for sustaining the existence of the Himalayan Newt species in this part of the country have been discussed.

RESULTS

Disease Prevalence and Newt Mortality: Evidence of epidemic diseases leading to tissue necrosis and heavy casualties of Newts was found in all the habitats under study. Newts in Tingbajot and Kambadui habitats were the worst sufferer and most of the skeleton recovered during survey came from these habitats (Plate-XXXVII). Between May 2009 and June 2015, 45 Newts (20 in Tingbajot, 15 in Kambadui and 5 each in Lakpiidui and N'rekpiidui habitats) were found having tissue necrotic infections spread over areas around neck, head, lower jaw, hind limbs, cloaca and tail (Plate-XXXVIIIa-h). Only 7 of them exhibiting mild infections could be successfully cured and reintroduced in their habitats. The remaining 38 had severe infections around head and cloacal regions and succumbed to their injuries during treatment.

Casualty due to agricultural and domestic activities : Heavy casualty of Newts was seen during the development of water bodies to acquire land for agricultural practices. During the survey period in Tingbajot habitat alone 15 Newts were killed during ploughing of land and erecting earthen ridges to prevent back flow of water during drainage of pond for growing paddy seedlings and to irrigate paddy and vegetable crops (Plate-XXXIX-a-c). Live stocks grazing near spawning ponds were found to inflict heavy casualties on adults and young ones

of Newts during the course of their movement (Plate-XXXIXd-f). This problem was aggravated further by keeping buffalos in small ponds forming potential breeding habitats of Newts. During June/July many young and adult Newts were found succumbing to suffocation under muddy water and injury inflicted on them by buffalo sitting inside shallow ponds (Plate-XXXIXd). Further, peoples' taking bath and washing clothes in small ponds located near human habitations specially during the lean period (October-March) was found instrumental in rendering detergent pollution to water bodies that formed aquatic homes of Newts (Table-XXXXa,b). In the month of July 2014, children were found enjoying catching Newts from Lakpiidui habitat for the fun sake and innocently killing them during play (Plate-XXXXd). Although practiced at relatively low scale slash and burn method of cultivation in and around Maram resulted destruction of terrestrial homes of Newts in Senapati district, Manipur.

DISCUSSION

Present observations suggest prevalence of tissue necrotic infections in Himalayan Newts inhabiting Tingbajot, Kambadui, Lakpiidui and N'rekpiidui habitats of Senapati district, Manipur. Tissue necrotic infections leading to the loss of infected body parts and/or resulting casualty have been described in the Emperor Newt, *Tylototriton shanjing* maintained under captive conditions (Staniszewski, 2000). To our knowledge, this is the first report to describe tissue necrotic infections in natural populations of the Himalayan Newt, *Tylototriton verrucosus* Anderson in Senapati district, Manipur. The etiology of the disease is not yet fully understood but in every probability it might result due to bacterial infections. The fact that infected Newts responded well to the treatment using broad spectrum antibiotics advocates in favour of such a supposition. Further, it seems likely that treatments for tissue necrotic infections are relatively easy at the beginning of the infection. Once manifested deeply, curing such infections becomes rather too difficult. The observation that only 7 of the 45 infected Newts suffering from mild infections could be fully cured with antibiotic and antifungal remedies leaving remaining 37 to succumb to their sore injuries caused by deepen infections, lends support to this suggestion. The severity of the problem calls for an immediate heed to be paid to establish etiology of the disease and develop sound specific line of treatments to cope with this dreaded menace. Recovery of plenty of Newt skeleton from around their habitats further emphasizes the seriousness of the problem and demands

everything that could be done to help ensure survival of Himalayan Newts in Senapati district, Manipur, the north-eastern part of the country.

Tingbajot and N'tinglibam habitats located at forest-edge were carved gradually between 2009 and 2015 to develop paddy fields and the ponds were frequently drained to irrigate vegetables and paddy crops. By June 2015, most of the spawning areas around Tingbajot habitat was ploughed to convert them into paddy fields leaving Newts remain confined to a small fish pond established in close proximity of paddy fields. Pesticides used for eradicating agricultural pests find their way to this fish pond as surface run-off during monsoon season and thus make the habitat quality worsen. Rearing of common carp, *Cyprinus carpio* in the pond further ceases survival opportunities of Newt - spawn and tender larvae in this habitat. Common carp is well known to prey on eggs and larvae of Himalayan Newts (Caudata Cultute, 2000; AmphibiaWeb, 2015). Further, though practiced at a relatively low scale, slash and burn method of cultivation in the area becomes instrumental in damaging terrestrial homes of the Himalayan Newt. Slash and burn cultivation practices clubbed together with setting fire on forests particularly in southeast Asia are known to have negative consequences on terrestrial habitats of Newts (Caudata Culture, 2000; AmphibiaWeb, 2015; IUCN, 2015). Further, small water bodies located nearby human settlements are in continuous use for taking bath and washing clothes during post-monsoon periods and thus add to detergent pollution and deterioration of habitat quality beyond tolerance limits of eggs, and young larvae born in every monsoon season. Small and shallow water bodies amidst forest that from shelter and potential breeding grounds of Newts are frequently used by local people for buffalo keeping which do not only make water very dirty but also lead to great mortality of both adult and young Newts during breeding season. Further, children are fond of catching Newts while taking bath in smaller water bodies, and inflict casualties while playing with them. All these features add significantly to the destruction of habitat and consequent decline in Newt populations in habitats located nearby human settlements in Senapati district, Manipur.

Himalayan Newt*, Tylototriton verrucosus* Anderson is the only recorded species of the order Caudata in the Indian subcontinent. In the Wild life (Protection) Act 1972, Schedule-I, Himalayan Newt species has been listed as an endangered species and in Wildlife (Protection) Act, Manipur, Rule 74, it has been declared a protected species. These acts protect *Tylototriton verrucosus* Anderson from collection and trafficking but do not have a

bearing on protection of their habitats. To conclude, we endorse the view of Seglie *et al* (2003) to include *Tylototriton verrucosus* Anderson in the IUCN Red List to focus the attention of National and International organizations on the conservation of this species. At the same time efforts need to be made to protect specific habitats wherein Newts stand to alarming vulnerability bearing evidence of rapid decline in their population size. Tingbajot, Kambadui, Lakpiidui, N'rekpiidui habitats of the Himalayan Newts in Senapati district, Manipur need to be paid immediate protection in consultation with people and local authorities. At the same time, establishment of a Newt disease diagnosis and treatment centre need to be persuaded in the district. As such, Senapati district is burdened with the history of rapid shrinkage in distribution areas of the Himalayan Newts (Selim, 2001).

Plate-XXXVII. Skeleton of Himalayan Newts recovered from Tingbajot and Kambadui habitats during 2009-2015

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Plate –XXXVIII :a. A Newt bearing necrotic infections on neck region near right paratoid and tip of the tail (shown by an arrows).
b. Necrotic infections in paratoid region and tip of the tail showing improvement after treatment.
c. A severely infected Newt on mid dorsal line behind head and tip of the tail (marked by arrows).
d. A Newt losing its tail due to necrotic infection (marked by arrow).
e. A Newt bearing necrotic infection in the lower jaw (marked by arrow)
f. A Newt bearing necrotic infection on ventral side of right thigh.
g. A Newt showing mild infection behind fore limb;
h. A necrotic infection on dorsal side of hind limb base.

Plate-XXXIX : a. At Tingajot development of a water body into paddy field results casualty of a Newts. **b.** Newt killed during erection of earthen ridge to help prevent the back flow of water during drainage of pond to irrigate paddy fields. **c.** A spawning ground of Newt at Tingbajot is being developed into a paddy field to grow paddy seedlings.**d**. Buffalo sitting in pond water during June/July inflicts heavy casualty on young and adult Newts due to suffocation and injury inflicted on them during buffalo sitting. **e,f**. Cattle grazing at sides of Newt habitats during June/July causes mortality of Newts during their movements.

Plate-XXXX : a. Lakpiidui habitat of Newts fetches detergent pollution due to taking bath and washing clothes by the female folk.
b. Kambadui habitat of Newts suffers from detergent pollution on account of Men taking bath and washing clothes.
c. Children enjoy taking bath in Solumdui habitat during monsoon months coinciding with spawning season of Newts that hinders them from visiting the spawning ground.
d. Children are fond of collecting Newts as playing objects and kill them for fun.

SUMMARY AND CONCLUSIONS

The research work embodied in the present Ph.D. thesis presents first hand information on the occurrence, distribution and habitat ecology of the Himalayan Newt, *Tylototriton verrucosus* Anderson in Senapati district, Manipur state of the north-eastern India. In daunting task carried out from May 2009 to August 2015, thorough exploration of approximately 300 square kilometre contiguous mountainous stretch of the Senapati district, Manipur was made to find possible existence of *Tylototriton verrucosus* Anderson in this part of the country. During survey almost all the water bodies falling within survey–zone were thoroughly examined for the presence of Himalayan Newts and wherever encountered, the place was considered for distribution mapping. Newts which exhibited distinctly different colour patterns and taxonomical characters as compared with originally described colour form by John Anderson (1871) from Nantin, Momien, and Hotha valleys in western Yunnan Province, People's Republic of China were considered as colour variants within populations of Himalayan Newts. Heterogeneous assemblage of food, consumed, feeding habits and/or skin-shedding behaviour of the Himalayan Newt were analysed in free living and captive conditions by implementing appropriate experimental protocols. Reproductive strategy adopted during courtship display, mating and egg-laying behaviours of Newts were studied under natural and captive conditions. Finally, Newt mortality resulting due to disease prevalence, anthropogenic and agricultural practices in and around Newt habitats led us to evaluate threat perception, survival probability and suggest conservation strategy of the Himalayan Newt to help them survive in Senapati district, Manipur.

Results show that :

1.*Tylototriton verrucosus* Anderson exists in 10 habitats : Tingbajot, Kambadui, Lakpiidui, Solumdui, Pongtuijai, N'rekpiidui, Apoubam Karii, N'tingliibam, N'jailong and Apoukijang, lying at varying altitudes of 1332 to 2411 meters a.s.l. between latitudes : 25^{o} 03.397'N - 25^{o}26.032' N; and longitudes: 93^{o} 52.238'E- 94^{o}05.411'E. Habitats were found well fringed with different species of plants : *Alterenthera sessilis, Cynodon doctylun, Dryopteris flexis, Ephatorium odaratum, Galengsoga paniflora, Quercus lamellose, Rotala rotundifolia, Sagittaria sagittifolia, Urena lobata,* and different species of *Polygonum, Commelina, Euphatonium, Osbeckia, Impatiens, Fagopyrum, Astemisia, Bidenspilosa, Alteranthaceae,*

Drymania, Musaenda, Odaratum, Calocasia,. Amphibian species viz. *Hyla annectans, Rhacophorus maximus, Bufo melanostictus, Rana leibigii, Euphlyctis cynophlyctis* and *Megophrys wuliangshanensis* were encountered within 100metre^2 area of Newt habitats. Moderate alterations in habitat quality did not seem to have serious negative influences on the survival of Newts as during the survey period from May 2009-August 2015, they were found living and shedding gametes during monsoon months in all the 10 aquatic habitats with levels of free CO_2, pH, alkalinity below their prescribed levels (*free CO_2 2.6-3.2 mg/litre, pH 6.5-6.8; alkalinity 30-58*). Three habitats lying above 1820 metres altitude and relatively free from human interferences, exhibited 50 to 125% increase in Newt populations encountered during May 2009-June 2013. Remaining seven of the 10 habitats had varying degrees of human interferences and showed a steep decline in number of adult Newts encountered during this period. Further, at water temperature in excess of 25^{oC}, Newts were observed undergoing aestivation by entering into holes existing in the side walls of their aquatic habitats are into rock crevices ramified with adventitious roots of trees bordering Newt habitats.

2. Data on different colour types of the Himalayan Newt, *Tylototriton verrucosus* Anderson revealed the presence of as many as 19 polychromatic forms : chocolate-brown colour, chocolate colour, sand elm colour, sky-blue colour, concrete elm colour, burgundy colour, brown- black colour, navy blue colour, caramel colour, dark brown-black colour, cola colour, off-black colour, jet-black colour, dark-brown colour, brown colour, light cola colour, and dark-purple colour forms spread over Tingbjot, Kambadui and Lakpiidui habitats. Numbers of such polychromatic forms were well within single digits and thus limited the scope of statistical analysis and inferences despite marked differences in their taxonomical details compared with original Newt type as described by John Anderson (1871). Further, in our collection, most of the differently coloured Newts happened to be male. Marked differences in colour patterns might not be due to sex specific colour patterns developed by males during the breeding season as some females present amidst sampled specimens exhibited colour morphs corresponding to their male counterparts. Owning to the fact that mating remained restricted within sexes of specific colour forms, it may be suggested that they might acquire the status of a sub-species or possibly different species. However, such a supposition presses the need for evaluating their molecular phylogeny taking into account the analysis of mitochondrial *Cytochrome b* gene analysis. Until then, it would perhaps be more proper to

assign them the category of individuals belonging to *Tylototriton verrucosus* Anderson complex.

3. Data derived from stomach flushing and gut content analysis of free living Newts revealed seasonal variation in food intake with maximum food consumption during monsoon months coinciding with the breeding season and minimum during post-breeding period. Food constituents consisted of Diptera, Lepidoptera, Isoptera, Odonata, Amphipoda, and their larvae, Gastropods (8-15*mm*), Bivalves (8-18 *mm*), Earthworms, cannibalized Newt-eggs and vegetal matter in varying proportions across the season. Seasonal variations in insects and their larvae ranged between 13.41– 19.19 % in males and 4.70–15.37 % in females; gastropods between 27.79–34.67 % in males and 20.08 – 33.87 % in females; bivalves between 31.99 – 39.93 % in males and 27.73 -41.14 % in females and vegetal matter between 9.78 – 15.47 % in males and 12.18 – 18.98 % in females. Earthworms appeared in food contents of males in July and August and in females during August only. Newt eggs were present during June and July in the food of females only. During June and July insects and their larvae in females were significantly less compared with males.

Seasonal variation in calorie intake in both the sexes of Newts was found to persist under captive conditions (male: $F_{5,30} = 6.55$, $p<0.05$; female: $F_{5,30} = 10.80$, $p<0.05$). In captivity females appeared to be more greedy consumers and during breeding season they consumed significantly ($p<0.05$) more food as compared with males. Positive correlation (male, $r=0.63$, $r^2=0.3969$; female, $r=0.58$, $r^2=0.3364$) was observed between average temperature (22.15 ± 0.45^{oC}, range: $20.73\text{-}21.94^{oC}$) across months. Minimum average temperature recorded during different months ($14.17 \pm 0.83\ ^{oC}$, range : $11\text{-}17^{oC}$) had positive correlation (male, $r=0.43$, $r^2= 0.1849$; female, 0.76, $r^2= 0.5776$) between temperature and food intake. Maximum average temperature (range: $31\text{-}33^{oC}$) depicted negative correlation (male, r= - 0.72; female, r= - 0.72) between temperature and food intake. Further, positive correlation was observed between average rainfall (127.17 ± 30.76 *mm* (range : 25-227*mm*) and food consumption in both the sexes of Newts (male, $r = 0.69$, $r^2= 0.4761$; female, $r=0.61$, $r^2= 0.3721$). A negative correlation (male, - 0.25; female, - 0.48) was observed between average humidity (range: 72.90-77.00%) across months and food consumption. Further, Newts were found to be very strategic in preying on live earthworms. In aquatic habitats, Newts were observed undergoing skin-shedding very frequently to acquire thin, smooth and slimy skin probably to facilitate gaseous exchange between body and the surrounding medium. Food intake was minimum or ceased completely depending upon intensity of moult. It is suggested that in *Tylototriton*

verrucosus Anderson food and feeding patterns vary across a season and maximum food intake coinciding breeding season may be linked to the action oriented energy requirements of the species. Availability of food in the habitat and seasonal variations in food intake may be influenced by physical factors and sexual stages of Newts. Further, in habitats located at Senapati district, Manipur, gastropods and bivalves constituted prime food resources of the Himalayan Newt across the season.

4. The male reproductive system consisted of paired bi-lobed testes, one each lying on left and right sides, supra-renal in placements. Well developed vasdeferens began from lower lobe of the testis and running down ended directly into the cloacal aperture. Two numbers of ovaries, left and right were placed in supra-renal positions. Fallopian tubes, both left and right were well developed and contained fully ripe eggs ready for their exit. Freshly recruited batch of developing oocytes were present in both, left and right ovaries.

Onset of breeding season was marked by male producing *twak twak twak* acoustic stimuli, though not very pronounced but clearly audible. Courtship behaviour commenced with gravid female approaching male and start nosing at his belly. After few seconds female was observed nosing at the tip of the tail and lifting his tail with her nose she leaped forward. The enticed male followed her. Timing differed in individual pair but on the average the entire sequence of events was completed within 13.25±1.38 minutes (range: 10-16 minutes). The excited male was observed frequently nosing on different parts of the body of the female. Amidst nosing, the male was seen making desperate attempts to slip under the body of the female. After struggling for about 10-15 seconds, male managed to slip under the body of the female to establish vent to vent contact. Timing differed in individual pair but on the average it took 14.50±2.10 seconds (range: 10-15 seconds). During vent to vent contact male was found holding female interlocking her limbs from below. Timing of amplexus differed between pairs and on the average lasted for about 58.57±12.09 minutes (range: 24-78 minutes) after which pairs separated, swam freely for about 5 minutes, settled at the bottom of pond and then started making circular movements turning their faces towards each other. Circular nuptial movement occurred for 28.75±2.84 minutes (range: 23-35 minutes). During circular movement cloaca of both the sexes remained open. Male Newt continued releasing white coloured spermatophore bolus during the circular movement and the female following the same path was found sucking spermatophores in her cloaca. Egg laying was seen approximately after two hours of spermatophore entry into the cloaca of female and was

observed continuing beyond six hours. Eggs were deposited one by one with clutch size varying from 30 to 180 eggs. Further, the size of the clutch had positive correlation with the body weight of the females (r=0.85). In natural ponds, after the mating was over, females were seen moving through submerged vegetation for eggs deposition. Eggs were found glued to submerged rocks, stones, plant stems, submerged woods and dead tree leaves lying in the pond water. No eggs were found deposited outside the water. In the captivity, yolky eggs wrapped in white jelly (8-10 *mm* in size along with jelly) were found settled at the bottom of the aquarium.

During June, captive Newts exhibited courtship behaviour. Introduction of alien males in the aquarium housing a courting pair led the stud male to promptly slip under the body of the female and holding fore-limbs trying to drag her away from alien males. The tendency of male in monopolizing his female partner was observed continuing even many hours after mating was over and females were already in the process of depositing eggs. It is suggested that in the Himalayan Newt, *Tylototriton verrucosus* Anderson courtship, mating and egg-laying constitute components of elaborate display of behavioural attributes. Onset of courtship behaviour is triggered by female by way of enticing the male. Aroused male intensifies the courtship display, establishes vent to vent amplexus and consequently succeeds in transferring spermatophores in the female cloaca through well organized circular dance. Fertilization of eggs is exclusively internal. Further, male has a tendency to monopolize its female and this behavioural attribute continues operating even several hours after mating when females are in the process of laying eggs. All the behavioural traits observed in the present study advocate in favour of the species adopting monogamous feature contrary to what have been speculated.

5. Evidence of epidemic diseases leading to tissue necrosis and casualties of Newts was noticed in all the aquatic habitats of their occurrence. Newts in Tingbajot, and Kambadui habitats were the worst sufferer as during the survey period, plenty of Newt skeleton was recovered from these habitats. Between May 2009 and June 2015, 45 Newts (20 in Tingbajot, 15 in Kambadui and 5 each in Lakpiidui and N'rekpiidui habitats) were found suffering on account of tissue necrotic infections spread over areas around neck, head, lower jaw, hind limbs, cloaca and tail. Only 7 of them exhibiting mild infections could be cured and reintroduced in their habitats. The remaining 38 had severe infections around head and cloacal regions and succumbed to their injuries during treatment.

Heavy casualty of Newts was seen during the development of water bodies to acquire land for agricultural practices. During the survey period in Tingbajot habitat alone 15 Newts were killed during ploughing of land and erecting earthen ridges to prevent back flow of water during drainage of pond for growing paddy seedlings and to irrigate paddy and vegetable crops. Live stocks grazing at spawning grounds were found to inflict heavy casualties of adults and young ones of Newts during the course of their movements. The problem was further aggravated by keeping buffalos in ponds forming potential breeding habitats of Newts. During June/July many young and adult Newts were found dead owing to suffocation under muddy water and injury inflicted on them by buffalo sitting inside shallow ponds. Further, peoples' taking bath and washing clothes in small ponds located near human habitations especially during the lean period (October-March) was found instrumental in rendering detergent pollution to water bodies that formed aquatic homes of Newts. In the month of July 2014, children were found enjoying catching Newts from Lakpiidui habitat for the fun sake and innocently killing them during their play.

In the Wild life (Protection) Act 1972, Schedule-I, Himalayan Newt species has been listed as an endangered species and in Wildlife (Protection) Act, Manipur, Rule 74, it has been declared a protected species. These acts protect *Tylototriton verrucosus* Anderson from collection and trafficking but do not prevent destruction of their habitats. To conclude, we endorse the view of Seglie *et al* (2003) to include *Tylototriton verrucosus* Anderson in the IUCN Red List to invite the attention of National and International organizations on the conservation of this species. Owing to vulnerability of Newts on various accounts and declining trend in their number, Tingbajot, Kambadui, Lakpiidui, and N'rekpiidui habitats in Senapati district, Manipur need to be paid immediate protection in consultation with people and local authorities. At the same time, establishment of a Newt disease diagnosis and treatment centre need to be pursued in the district. As such, Senapati district is burdened with the history of rapid shrinkage in distribution area of the Himalayan Newts (Selim, 2001).

REFERENCES

Ahmed M.F., Das A. and Dutta S.K. (2009). *Amphibians and Reptiles of Northeast India: A Photographic Guide*. Aaranyak, Society for Biodiversity Conservation. 50 Samanwoy Path, Survey, Beltola, Guwahati, Assam, India, pp168.

AmphibiaWeb (2015). Information on Amphibian Biology and Conservation. *(www.amphibiaweb.org*, last accessed on 15.11.2015).

Anderson J. **(**1871). Description of new genus of newts from western Yunnan. *Proc. Zoological Society, London*. **1871**: 423-425.

Anders C.C., Schleich H.H. and Shah K.B. (1998). Contributions to the biology of *Tylototriton verrucosus* Anderson 1871 from east Nepal (Amphibia : Caudata, Salamandridae). Contributions to the Herpetology of South Asia (Nepal, India), *Fuhlrott-Museum, Wuppertal*, **4**: 1-26.

Annandale N. (1907). Eggs of *Tylototriton verrucosus* Anderson. *Rec. Ind. Mus.* **1** : 278-279.

Annandale N. (1908). ''Breeding habits of *Tylototriton verrucosus*.'' *Records of the Indian Museum*. **2**: 305-306.

Basnet D.B. (2001). Observation on hibernation behaviour of *Tylototriton verrucosus* Anderson (Himalayan newt). *Indian J. Env. Ecoplannin,* **5**(3): 505-510.

Beaver O. (1982). Occurrence of salamander on Doi Suthep. *Science Society of Thailand,* **36**:398-400.

Boulenger G.A. (1920). Observations sur un Batracien Urodèle d'Asie, *Tylototriton verrucosus* Anderso. *Bulletin of the Zoological Society of France, Paris*, **45**:98-99.

Bruning J.L. and Kintz B.L. (1977). *Computational handbook of Statistics*. Scott, Foreman and Company, USA.

Bulakov V.L. (1976). Method of food investigation in live amphibians. *Voropsy stepnogo Lesovedeniya i okhrany Prirody, Dnepropetrovsk,* **6**: 146-156.

Campbell H.W. and Christman (1982). *Field techniques for herpetofaunal community analysis*, pp. 193–200. In: Scott, N.J. Jr. (ed.). *Herpetological Communities*. Wildlife Research Report 13, U.S. Department of the Interior, Fish and Wildlife Service.

Caudata Culture (2006). Species entry, Tylototriton verrucosus, Nate Nelson and Ed Kowalski, eds. Pp 1-6.

Caudata Culture Species entry (2000). *Tylototriton verrucosus*, Nate Nelson and Ed. Kowalski. eds. Pp 1-6. *http:/www.caudata.org/cc/species/tylototriton/T. verrucosus. Shtmlshtml,* downloaded in 2009.

Caudata Culture (2006). Species entry, Tylototriton verrucosus, Nate Nelson and Ed Kowalski, eds. Pp 1-6.

Chan-Ard T (2003). A photographic guide to amphibians in Thailand, Darnsutha Press, Bankok, pp176.

Chan-Ard T., Grossmann W., Gumprecht A., and Schulz K. D. (1999). Amphibians and Reptiles of Peninsular Malaysia and Thailand. An Illustrated Checklist. Wuerselen: Bushmaster Publications.

Chaudhuri, S.K**.** (1966). Studies on *Tylototriton verrucosus* (Hymalayan newt) found in Darjeeling. *J. Bengal, Natl. Hist. Soc.,* **35**: 32-36.

Cockran, C. and Thomas C. (1996): Amphibians of Oregon, Washington and British Columbia. *A field Identification Guide, Lone Pine Pub., Edmonton*, 175pp.

Corn P.S. and Bury R.P. (1989). Logging in western Oregon: responses of headwater habitats and stream amphibians. *Forest Ecology and Management,* **29**: 39–57.

Daniels R.J.R. (2005). *Amphibians of Peninsular India*. University Press (India) Private Limited. 3-5-819 Hyderguda, Hyderabad, 169Das I. (1984). Occurrence of the Himalayan salamander (*Tylototriton verrucosus*) in Shillong. *Hamadryad,* **9**: 11.

Das I. (1987). Natural history of the Indian salamander, herpetofauna News, **9**:3.

Dasgupta R. (1983). Observations on the Himalayan newt. *J. Bengal Nat. Hist. Soc. New Ser*, **2**(2): 59-60.

Dasgupta R. (1984). ''Parental care in the Himalayan Newt". *J. Bengal Nat.Hist. Soc*, **3**(2): 106-109.

Dasgupta R. (1988). Young of the Himalayan newt. *J. Bengal Nat. Hist. Soc.* (N.S.), **7**(1): 3-18.

Dasgupta R. (1990).''Distribution and conservation problems of the Himalayan Newt (*Tylototriton verrucosus*) in the Darjeeling Himalayas.'' *Hamadryad*, **15** (1): 13-15.

Dasgupta R. (1996). Feeding ecology of the adult Himalayan Salamander, *Tylototriton verrucosus* Anderson. 1871. *Herpetozoa,* **9** (1/2): 19-29.

Devi N.T. (2005). *The food of the Himalayan Newt (Tylototriton verrucosus*): A preliminary study. *J. Bombay Nat. Hist. Soc*., **102**(2): 166-168.

Deuti K. and Hedge V.D. (2007). Handbook on Himalayan Salamander-*Nature Books India*: New Delhi, India.

Ferrer R.P. and Zimmer R.K.(2007). Chemosensory reception, behavioral expression and ecological interactions at multiple trophic levels. *Journal of experimental Biology, **210**: 1776-1785.*

Fei L., Ye C. and Jiang J. (2010). *Coloured Atlas of Chinese Amphibians*. Sichuan Publishing Group. Sichuan Publishing House of Science and Technology, China, 517pp.

Ferrer R.P. and Zimmer R.K. (2013). Molecules of keystone significance: crucial agents in Ecology and Resource Management. *Bioscience*, **63**(6) : 428-438.

Frost D.R. (1985). Amphibian Species of the World. A Taxonomic and Geographic Reference. Allen Press, Inc., and Associations of Systematic Collections, Lawrence, (iv)+v+732 pp.

FROST D. R. (2008). Amphibian Species of the World. an Online Reference. Version 5.2 (15 July, 2008). Electronic Database accessible at http://research.amnh.org/ herpetology/amphibia/index.php. American Museum of Natural History, New York, USA.

Global Amphibian Assessment-Detailed Report (2000). *Tylototriton verrucosus*-Himalayan Salamander, Red-knobby Newt. http://www.globalamphibians.org /servlet/GAA?searchName=Tylototriton+verrucosus, downloaded in 2009.

Griffiths R.A. (1986). Feeding niche overlap and food selection in smooth and palmate newts, *Triturus vulgaris and T. helveticus* at a pond in mid Wales. *J. Animal Ecology*, **55:** 201-214.

Gyi K. M. (1969). The occurrence of *Tylototriton verrucosus* Anderson (1871) at Taunggyi, Burma. *Union of Burma J. Life Sci*., **1969 :** 23-26.

Humphrey S.R. and Bain J.R. (1990). Endangered animals of Thailand. Gainesville:Sandhill Crane Press, Florida.

IUCN (2015).IUCN Red List of Threatened Species version 2015. *www.iucnredlist.org* /details/ 59487/0, last accessed on 18.12.2015.

Joly P. (1987)**.** Le regime alimentaire des amphibiens: methods d'etude. Alytes, **6** (1-2): 1-17.

Joly P. and Miaud C. (1989). Fidelity to breeding site in the Alpine newt, Triturus alpestris. *Behav. Proc*. **19**: 47-56.

Jϕrgensen C.B. (1981). Ovarian cycle in a Temperate-zone frog, *Rana temporaria*, with special reference to factors determining number and size of eggs. *J. Zool. Soc. London*, **195**:444-458.

Jϕrgensen C.B. (1984). Ovarian functional patterns in Baltic and Mediterranean populations of a Temperate-zone anuran, the Toad, *Bufo viridis*. *Oikos*, **44**:309-321.

Jϕrgensen C.B., Larsen L.O. and Lofts B. *(*1979). Annual cycles of fat bodies and gonads in the toad, *Bufo, bufo* (L) compared with cycles in other Temperate-zone anurans. *Biolgiske Skrifter*, **22**:1-37.

Kerby J.L. and Kats L.B. (1998). Modified interactions between salamander life stages caused by wild-fire induced sedimentation. *Ecology*, **79**:740-745.

Kuzmin S.L., Dasgupta R. and Smirina E.M. (1994). Ecology of the Himalayan newt (*Tylototriton verrucosus*) in Darjeeling Himalayas, India. *Russian Journal of Herpetology*, **1**: 69-76.

Mahoney, M. and Vredenburg V. (2008): *Tylototriton shanjing* in: AmphibiaWeb: Information on amphibian biology and conservation. [web application]. 2008. Berkeley, California: AmphibiaWeb. Available: http://amphibiaweb.org/. (Accessed: Dec 22, 2008

Mansukhani M.R., Julka J.M. and Sarkar A.K. (1976). On occurrence of Himalayan newt, *Tylotriton verrucosus* Anderson from Arunachal Pradesh, India. *News Letter, Zoological Survey of India*, **2**(6): 243-245.

Marshall C.J., Doyale L.S. and Kaplan R.H. (1990). Intraspecific and sex specific oophagy in a salamander and frog; Reproductive convergence of *Taricha torosa* and *Bombina orientalis, Herpetologica*, **64** (4): 395-399.

Mudrack W. (1969): *Tylototriton verrucosus* ANDERSON, 1871, ein seltener Molch aus Asien. Aqua Terra. **6**: 134-136.

Mudrack W. (1972): Ein seltener Krokodilmolch – *Tylototriton verrucosus*. *Vom Ei zum Jungtier. Aquarien Mag*., **6** (10): 406-409.

Mudrack W. (2005). Nachzucht von Krokodilmolchen, *Tylototriton shanjing*. Amphibia, **4**(1):23–25.

Nabhitabhata J., Chan-ard T. and Chuayngern Y. (2000). *Checklist of amphibians and reptiles in Thailand*. Office of Environmental Policy and Planning, Thailand. p152.

Nag S. and Vasudevan K. (2014). Observations on overwintering of *Tylototriton verrucosus* (Cauata: Salamandridae) in Darjeeling, Himalaya, India*. Salamandra* **50**(4): 245-248.

Nussbaum R.A., Brodie E.D. and Yang D. (1995). A taxonomic review of Tylotriton verrucosus Anderson (Amphibia : Caudata: salamandridae). *Herpetologica*, **51**:257-268.

Pisarenko S. and Voronin A.A. (1976). Method for feeding study of live amphibians. *Ekologiya, Swerdlovsk*. **2**: 106.

Pomchote P., Pariyanonth P., and Khonsue W. (2008). Two distinctive color patterns of the Himalayan Newt, *Tylototriton verrucosus* (Urodela:Salamandridae) found in Thailand and its implication on geographic segregation. *The Nat. Hist. Journal of Chulalongkorn University*, **8**(1): 35-43.

Rehberg F. (1986): Haltung und Zucht des Krokodilmolches, *Tylototriton verrucosus*. *Herpetofauna.* **8** (45): 11-17.

Roy D. and Mushahidunnabi Md. (2000). Crocodile salamander of the eastern Himalayas. *ZOO's Print Journal*. **15**: 29-31.

Roy D. and Mushahidunnabi Md. (2001). Courtship, mating and egg-laying in *Tylotriton verrucosus* from the Darjeeling district of the Eastern Himalaya. *Current Science*. **81:** 693-695.

Saidapur S.K. (1989). Reproductive cycles of Amphibians; in Reproductive cycles of Indian vertebrates (S.K. saidapur, ed.), Allied publ., New Delhi, pp165-223.

Schleich H. H. and Kästle W. (2002). Amphibians and Reptiles of Nepal. Biology, Systematics, *Field Guide*. Ruggell: A.R.G. Gartner Verlag K.G.

Seglie D. (2002). *Tylototriton verrucosus*, an Endangered Species of India. DAPTF Report. 1-43.

Seglie D., Roy D., Giacoma C. and Mushahidunnabi M. (2003). Distribution and conservation of the Himalayan Newt (*Tylototriton verrucosus*, Urodela, Salamandridae) in the Darjeeling District, West Bengal (India). *Russian Journal of Herpetology*. **10**: 157-162.

Selim K. (2001). Notes on *Tylototriton verrucosus* Anderson. A critically endangered newt from Manipur. *J. of Bombay Natl. Hist. Soc*., **98** (2): 291.

Shrestha T.K. (1984). On the distribution and habitat of the Himalayan newt, *Tylototriton verrucosus* Anderson in the eastern Nepal. *J. Bombay Hist. Natl. Soc*. **81**: 485-487.

Shrestha T.K. (1989). Ecological aspects of the life history of the Himalayan newt, *Tylototriton verrucosus* (Anderson) with reference to conservation and management. *J. Bombay Natl. Hist. Soc*., **86** (3): 333-338.

Shrestha T.K. (1994). "Habitat ecology of the Mai Pokhary wetlands in management plan for survival of the Himalayan newt, *Tylotriton verrucosus*," in W.M. Mitsh (ed.), *Global wetlands. Old World and New,* Elsevier, Amsterdam-Lausanne-New York-Oxford-Shannon-Tokyo, pp 857-862.

Smith M.A. (1924). The tadpole of *Tylototriton verrucosus* (Anderson). Records of the Indian Museum, **26**: 309-312.

Smith M.A. (1943). The fauna of British India, Ceylon and Burma, including the whole of the Indo - Chinese region*. Reptilia and Amphibia. Vol. III. Serpentes*. *Taylor and Francis, London, i-xii+583pp+1 map.*

Sparreboom, M. (1999**).** Haltung und Nachzucht von Tylototriton verrucosus. *Elaphe*. **7**: 20-24.

Sparreboom M. and Yunke, W. (2010). Haltung und Nachzucht von *Tylototriton verrucosus*. *Elaphe*. **7**: 20-24.

Staniszewski M.S. (2000). The Mandarin Salamander FAQ. http:www.kingsnake.com/amphibian/mandarin/mandarin.html, downloaded on 1.1.2000.

Stuart B.L. and Papenfuss T.J. (2002). A new salamander of the genus *Paramesotrion* (caudate: Salamandandridae) from Laos. *Journal of Herpetology*, **36**: 145-148.

Stuart B.L., Phimmachak S., Sivongxay N., and Robichaud W.G. (2010). A new species in tylototriton spperrimus group (Caudata;salamandridae) from central Laos. Zootaxa, **2650**: 19-32.

Taylor, E.H. (1962). "The Amphibian Fauna of Thiland", *Univ. of Konsas Sci. Bull*. **43**(8): 265-299.

Thant-Shima M., Nakamura A., Shimazu H., Murao S., and Sugiyma T. (1979). Possible usefulness of Burmese Newt, *Tylototriton verrucosus* Anderson, in teratogenesis studies. *Kobe Journal of Medical Sciences,* **25:** 193-204.

Thorn, R. (1969). Les salamandres d'Europe, d'Asie et d'Afrique du Nord. Paris: Lechevalier.

Vasudevan K. and Sondhi S. (2010). *Amphibians and Reptiles of Uttarakhand, India.* Wildlife Institute of India, Chandrabani 18, Dehradun, Uttarakhand, India, 94*pp*.

Wangyal J. T. and Gurung D.B. (2012). Distribution of Himalayan Newts, *Tylototriton verrucosus* in the Punakha-Wangdue Valley, Bhutan. *Journal of Threatened Taxa*; **4** (13): 3218-3222.

Wongratana, T. (1984). Range extension of Crocodile salamander, (*Tylototriton verrucosus*) to Phu Luang, Thailand. *Natural History Bulletin of the Siam Society*. **32**: 107-110.

Yang D. and Rao D. (2008). *Amphibia and Reptilia of Yunnan*. Yunnan Publishing Group Corporation, Yunnan Science and Technology Press, Kunming (in Chinese), 152*pp*.

Zhang M**.,** Rao D., Yu G. and Yang J. (2007). The validity of red knobby newt (*Tylototriton shanjing*) species status based on mitochondrial cyt b gene. *Zoological Research*. **28**: 430-436.

Zhao E., Hu Q., Jiang Y. and Yang Y. (1988). Studies of Chinese salamanders. Society for the study of Amphibians and Reptiles, Ohio, p 67.

Zhao E. and Adler K. **(**1993). *Herpetology of China.* Society for the Study of Amphibians and Reptiles, Oxford, Ohio.