

Research into the ecology of the Keeled Box Turtle (*Cuora mouhotii*) in Vietnam



Keeled Box Turtle (McCormack, 2010)

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A research study on the time budget, daily activity patterns, the influence of rainfall on activity and daily movement patterns of the Keeled Box Turtle (*Cuora mouhotii*) in Vietnam

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Summary

Nowadays almost half of the world's population of freshwater turtles and tortoises are threatened with extinction in the wild. Particularly in Asia the population decline is severe and referred to as the "Asian Turtle Crisis". Turtles are thought to be keystone species and therefore their disappearance could have catastrophic effects on their ecosystems. The study species Keeled Box Turtle (*Cuora mouhotii*) is one of the endangered species of Vietnam and nearly nothing is known about its ecology. The aim of this research was to provide information on the ecology of the species to enhance the current knowledge for future conservation purposes. The study was divided into three parts: a survey on the daily activity patterns and time budget, an experiment on the influence of rainfall on the behaviour, and a survey on the daily movement patterns. All data were collected from July to September 2010 in the Cuc Phuong National Park in Vietnam.

The study site of the survey on daily activity and behavior and the experiment on the influence of rainfall were carried out at the same study site, an enclosure at the Turtle Conservation Centre (TCC) at the entrance of the Cuc Phuong National Park in Vietnam. The study population for both research parts consisted of four adult captive turtles housed at the TCC. The turtles were observed in shifts of two hours length over a period of 24 hours. While using the method of focal animal sampling fifteen behavior types and their duration were recorded continuously. The results of the survey show that the turtles spent most of their time *resting*, followed by the behaviours *under litter* and *observing*. The standard deviations found indicate big differences in behavior shown between the four individuals. The daily activity was significantly related to daytime and peaks of activity were shown from 6am-10am and 8pm-10pm whereas no activity at all was shown from 10pm-2pm. The *inactive* behavior of the turtles throughout a major part of the day was expected as well as the peaks of activity in the mornings due to former literature study of research that was done on other species of turtles.

The experiment on the influence of rainfall on the behavior of the turtles was set up by using sprinkler devices to create artificial rain as a treatment. The method used was a before-and-after study and focal animal sampling was used while observing the turtles in shifts of two hours throughout the day (12h period). The same behavioural data were recorded as for the survey on daily activity and time budget during the treatment with *artificial rain* for later comparison between the behavior shown during *artificial rainfall* and *without it*. A significant difference was found for the mean percentage of time spent for the two conditions *no rainfall* and *artificial rainfall* for the behavior types *resting* and *locomotion*. The mean percentage of *resting* was significantly higher *without rainfall* whereas *locomotion* was shown more often during the treatment with *artificial rainfall*. The percentages also show that the turtles spent a higher amount of time being active during observations with *artificial rainfall* than *without rainfall*. However, this difference in activity was not significant.

The survey on the daily movement was carried out with five individuals of the wild population at the park center (Bong) of the Cuc Phuong National Park. To follow a turtle's track over 24 hours exactly, thread-trailing was used, whereby the spooling device was attached to the carapace of the turtle. The position of the thread was recorded by using reference trees to calculate the coordinates of the places where the turtle changed direction. Additionally, the location of pathways and service facilities at the park center were recorded with a GPS device. A map was created which shows the thread-trailed path of the one turtle that was successfully spooled. That turtle crossed a pathway and moved at least 99m within the period of 24 hours in which it was being thread-trailed. The

results suggest that the pathways and the main road form an immediate danger to the turtles because they cross them as expected prior to the study.

It is recommended to conduct further behavioural research to enhance the knowledge of the study species. Based on this, conservation strategies for the management of in situ as well as ex situ populations could be developed. The gained results display activity peaks throughout the day as well as during rainfall and it is assumed that the turtles are more vulnerable to being poached at these times. Therefore turtle conservation organisations should consider this when developing their strategies. Furthermore, turtles passing the small pathways and especially the main road leading to the park centre are threatened to be picked up. Controlling and advising visitors frequently could reduce the numbers of turtles being collected. More spooling data of different individuals of Keeled Box Turtles should be obtained to map the turtles' movement within a year, for example, to determine when they move to other parts of the forest and how often they are in danger of encountering humans on roads or pathways. The future results of movement patterns and therefore turtles' home ranges can be implemented in conservation management of protected areas.

Abbreviations

ANOVA	Analysis of Variance
ATCN	Asian Turtle Conservation Network
CITES	Convention on International Trade in Endangered Species of Wild Fauna and Flora
DNA	Deoxyribonucleic acid
ENV	Education for Nature Vietnam
GIS	Geographical Information System
GPS	Global Positioning System
IUCN	International Union of Conservation of Nature
LSD	Least Significant Difference
MARD	Ministry of Agriculture and Rural Development
PASW	Predictive Analytics Software
SPSS	Statistical Package of the Social Sciences
TCC	Turtle Conservation Center
TCM	Traditional Chinese Medicine

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1. Introduction

According to the 2000 International Union for Conservation of Nature (IUCN) Red List of Threatened Species almost half of approximately 300 freshwater turtles and tortoises worldwide are listed as threatened and possibly face extinction in the wild (Turtle Conservation Fund, 2002). Habitat loss or degradation as well as the extensive consumption and pet trade are among the most significant threats. Some estimates put the number of turtles traded annually at ten million (Asian Turtle Network, 2006). Further Salzberg (1998) explains that almost all of these animals are wild caught and considering the low reproduction rate of most species that this exploitation of turtles and tortoises is not sustainable and extinctions of some species in the wild can be expected within the next decade (Gibbons et al., 2000). Since the convertibility of the Chinese currency in 1989 China's turtle imports increased dramatically (Cheung and Dudgeon, 2006) and today China is the largest consumer of turtles (Gibbons et al., 2000) with a minimum estimate of 13,000t annual traded, live turtles (van Dijk, 2000). The country's developing economy and the increasing affluence generated a demand for expensive food and medicine made out of turtles (Asian Turtle Network, 2006). Particularly for Asian freshwater turtles and tortoises, which are harvested as food source and for traditional Chinese medicine (TCM), the situation is very acute and described by conservationists as the "Asian turtle crisis" (Cheung & Dudgeon, 2006). As populations decline and the international pet trade of turtles becomes more popular (Asian Turtle Trade Working Group, 1999), the amount of animals traded has decreased although the value of each individual turtle has increased (Lau et al., 2000) providing incentives for further exploitation.

In 1992 the World Conservation Monitoring Center declared Vietnam as one of the 16 most biological diverse countries characterised by high biodiversity and endemism in flora and fauna (Dang Thi An and Chu Thi Thu Ha, 2005). Furthermore Vietnam is considered one of the most important hot spots for turtle diversity in Asia with 27 different native species of tortoise and freshwater turtles which are all threatened by hunting and the unsustainable trade to meet the demand from consumers in China (ENV, 2010). Only about 10% of turtles on the Vietnamese market are consumed by the domestic demand (Lehr, 1997) but with some species selling for \$1,000 on the Chinese market (Environmental News Network, 1999) and an average annual Vietnamese household income of \$1,052 (U.S. State Department, 2010) there is an obvious incentive to trade turtles.

Evidence suggests, that over the past 15 years wild populations of most turtle species in Vietnam have declined, leaving fragmented and degraded populations (ENV, 2010). The 2010 IUCN Red List states that of the 27 turtle species in Vietnam, 96.3% are threatened with extinction (see Appendix I, Table 3). Turtles and tortoises are major biodiversity components of the ecosystems they inhabit, often serving as keystone species from which other animals and plants benefit. They represent major resources of their environments and participate in the web of interacting and co-dependent species that constitute a healthy functioning ecosystem (Turtle Conservation Fund, 2002). Keystone species are often defined as species that support many other species or species that change the

physical structure of the environment, creating shelter for other species (Simberloff, 1997), like the gopher tortoise *Gopherus polyphemus* whose burrows are inhabited by up to 332 other species (Jackson and Milstrey, 1989).

The knowledge of the natural history of most Asian turtles is limited (Thirakhupt and van Dijk, 1994; Moll and Moll, 2004) but data on geographic distribution for example, could indicate possible consequences of turtle removal from ecosystems (Cheung and Dudgeon, 2006). Long-term or large-scale consequences of turtle species loss might include significant ecological impacts such as changes in energy flow, nutrient cycling and food-web structure (Cheung & Dudgeon, 2006). Nonetheless, Peterson (1998) suggests that the resilience of an ecosystem 'is generated by diverse, but overlapping, function within a scale and by apparently redundant species that operate at different scales, thereby reinforcing function across scales.' Already Darwin (1859) proposed that an area is more ecologically stable if it is occupied by a large number of species and MacArthur (1955) formalized this idea by proposing that the addition of species to an ecosystem increases the number of ecological functions present, and that this increase stabilizes an ecosystem.

The Keeled Box Turtle (*Cuora mouhotii*), the studied species in this research, is one of Vietnam's turtles that is affected by the Asian turtle crisis and currently listed by the IUCN as endangered (IUCN, 2010) as well as protected from international trade under CITES as an Appendix II species (CITES, 2010). Although there is a lack of knowledge about the turtles' ecology, a previous study by McCormack (2005) has shown a direct connection between seasonal activity patterns of *Cuora mouhotii* and their availability on illegal trade markets. During hibernation there were fewer individuals for sale on markets, thus allowing the conclusion that turtles prefer hibernation dens where they are not easily found and are well camouflaged (McCormack, 2005).

Almost nothing about the ecology of *C. mouhotii* is known but if competent conservation management guidelines are to be formulated, an increased understanding of habitat requirements and behaviour of the species is required (Moll, 1996, Wone and Beauchamp, 2003 cited in McCormack, 2005).

The Turtle Conservation Center (TCC), based at the Cuc Phuong National Park, is supporting this research, since they are dedicated to the rescue and conservation of chelonians and have identified six of Vietnam's 27 turtle species as priorities for developing captive breeding and assurance populations and reintroduction programs, one of them being the Keeled Box Turtle.

The Turtle Conservation and Ecology Project and the Cuc Phuong National Park's Turtle Conservation Center (TCC), were established by Fauna and Flora International in 1998, focusing on tortoises and freshwater turtles from illegal trade (The New York Turtle and Tortoise Society, 2010). In 1999, the turtle project was formally integrated into the Cuc Phuong National Park conservation actions and became the first official tortoise and freshwater turtle conservation program in Vietnam, officially endorsed from the MARD (The New York Turtle and Tortoise Society, 2010). The management of the TCC was transferred to the National Park Management in 2001 and is nowadays the flagship of turtle conservation in Asia (Asian Turtle Conservation Network, 2010).

A thorough knowledge of the ecology of *C.mouhotii* has benefits not only for current wild populations but also for any future reintroduction program or translocation e.g. selection of optimum release sites, time of year for release etc., as McCormack (2005) emphasised. Previous research on the Keeled Box Turtles by McCormack (2005) has identified long-term home ranges of wild individuals of Keeled Box Turtles in Cuc Phuong and their seasonal movement. However, the time budget, the daily activity patterns, the effect of rainfall on activity and behaviour and movement patterns (direction and distance) of the study species are unknown yet and were researched in this following study.

Aim of the research

The aim of this research was to provide information on the daily activity patterns and time budget of captive individuals of Keeled Box Turtles as well as the influence of rainfall on their activity and behavior. This information was collected with the purpose of enhancing the knowledge on the ecology of the species which is useful for the implementation of future conservation management and the understanding of the protection needs of the endangered species in Vietnam.

Furthermore the aim was to investigate the daily movement patterns of wild individuals of Keeled Box Turtles in the Cuc Phuong National Park in Vietnam. The purpose of this research was also to enhance the knowledge on the ecology of the species and the collection of this information is especially important regarding the future conservation management of the study species in the Cuc Phuong National Park in Vietnam. The results can indicate the distance and directions of turtles' daily movement and therefore, conclusions can be drawn about the potentially dangerous interferences caused by the main road and the human-made pathways, used by the staff of the national park and the tourists, with the turtles' habitat.

The research was carried out to answer the following questions:

1. *What is the time budget and daily activity pattern of the Keeled Box Turtle?*
2. *What influence does rainfall have on the activity of the Keeled Box Turtle?*
3. *What are the daily movement patterns of wild individuals of Keeled Box Turtles in the Cuc Phuong National Park in Vietnam?*

In order to answer the research questions this research was divided into three parts.

The first part consists of a study about the daily activity and time budgets of captive Keeled Box Turtles at the TCC. It is useful to investigate if there are certain patterns in the activity of the turtles during a 24 hour period to gain more understanding of the ecology of the species. Certain behaviour types might be linked to a particular time of the day and besides providing basic ecological knowledge, it can provide helpful information for conservation actions. If, for example, it would turn out that the observed turtles spent most of their time hiding or resting sheltered under logs this would indicate the importance of dead wood (logs lying on the forest ground) for the species, which would need to be considered in management plans for the conservation of the habitat of the species.

The second part of the conducted research also provides further information on the species' ecology. McCormack (2005) explains in his study, that 'carapace temperature and

rainfall each had highly significant effects on activity level in *C.mouhotii* although it remains unclear whether one of these factors is more influential than the other'. In order to detect if rainfall has an influence on the activity and behaviour shown by the study species, a controlled situation was created. An experiment using the treatment "rainfall" (in that case artificial rain by using sprinklers) has been set up and behaviour and activity of the captive population exposed to that treatment were recorded, analysed and compared to the recorded data without the application of the treatment.

To protect the species and enhance habitat conservation planning for the Keeled Box Turtle population in the Cuc Phuong National Park it is important to know how the tortoises use their habitat while moving around. Chelonians may use their habitats in non-random patterns, utilising some areas more frequently or at other times than other areas (Dodd et al., 1994; Nieuwolt, 1996; Lue and Chen, 1999; Smith et al., 1999). Turtles have been observed crossing the major road that divides the Cuc Phuong National Park. This is not only dangerous because of traffic but also because tourists and guides often illegally pick up turtles and take them back to their hotel or home as pets (McCormack, 2010). In order to gain more information on the daily movement patterns of the species a study about the daily movement of wild individuals at Cuc Phuong National Park was carried out and is dealt with in the third part. The method used to collect data on the movement was thread-trailing. Many researchers have used thread-trailing in order to gather data on the movement of tortoises (i.e., Hailey & Coulson, 1996; Loehr, 2002; Marlow & Tollestrup, 1982) and in contrast to radio-tracking, thread-trailing can reveal details about daily movements, thus where and how far the animals travel (Loehr, 2002). Because a continuous record of the exact route taken is available, spooling permits the study of certain aspects of spacing behaviour, predator avoidance, and foraging behaviour not readily answered with radio telemetry, which can only provide a series of spot readings of animal location (Boonstra and Craine, 1985).

The research took place from July to September 2010 since the studied species *Cuora mouhotii* is supposed to be most active at that time of the year (McCormack, 2010).

2. Study area and study species

2.1 Study area

The research was carried out at the Cuc Phuong National Park in the Socialist Republic of Vietnam. The Cuc Phuong National Park, approximately 22 000 hectares large and situated 120 km southwest of Hanoi, is the first National Park of Vietnam, established in 1962 (Footprint, 2010). The National Park is located between the boundary intersections of the provinces Ninh Binh, Hoa Binh and Thanh Hoa (see Figure 1 and 2), between 105029' E to 105044' E longitude and between 20014' N and 20024' N latitude (Cuong, 2007).

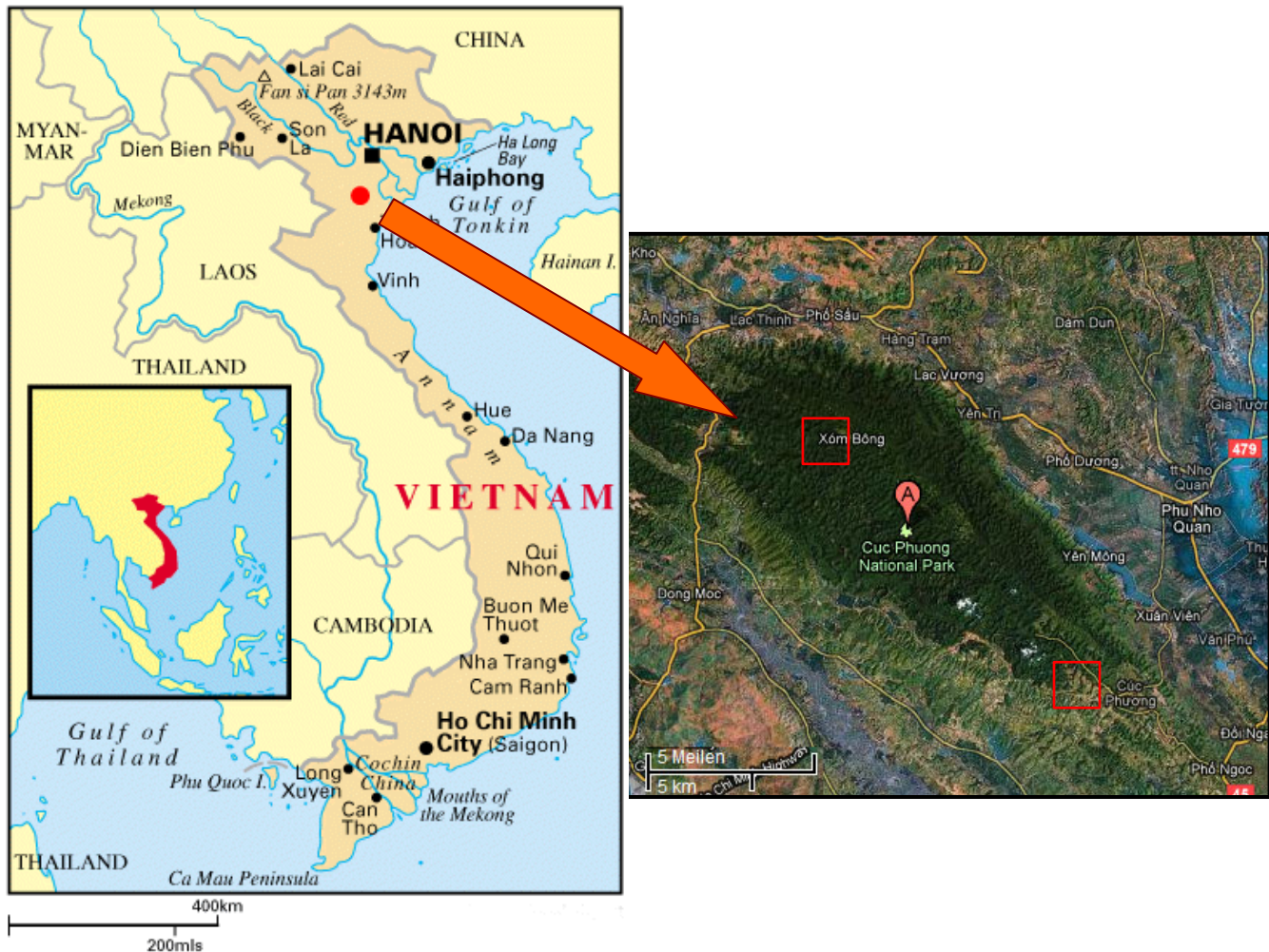


Figure 1 and 2: Location of Cuc Phuong National Park (Holiday Hotel, 2010 and Google Maps, 2010) and the approximate location of the study sites at the park entrance and the park centre (Bong area).

The area is managed as a National Park according to the IUCN Protected area Management Category II (Vietnam Open Tour, 2005). Responsible for the management of National Parks and protected areas within Vietnam is The Ministry of Agriculture and Rural Development (MARD) (McCormack, 2005).

Directly in charge of the protection of this area and the enforcement of wildlife protection laws such as Decree 18/48 is the Forestry Protection Department (FPD), since the MARD is only in charge of the management of protected areas (McCormack, 2005).

Biogeographically, Cuc Phuong National Park is classified as a Thaiindian Monsoon Forest with an altitude varying from 150-637m (Vietnam Open Tours, 2005).

The climate in the north of Vietnam can be classified as monsoonal with a hot rainy season (May-September) and a warm dry season from October to March (Central Intelligence Agency, 2010). At Cuc Phuong National Park, seasonal moist sub-tropical conditions can be found (Vietnam Open Tours, 2005). The mean annual temperature is 21°C with an average winter temperature of 9°C and a maximum of 35°C, respectively 0.5°C as minimum (Vietnam Open Tours, 2005).

Rainfall on an average of 224 days a year causes a mean annual rainfall of 2.100mm with the heaviest rainfall in July and August (Vietnam Open Tours, 2005).

The predominant soils in the forest area are generally very shallow with fast turnovers and ground rocks, which absorb mainly all surface water and lead to just a number of seasonal water courses (Vietnam Open Tours, 2005).

The 22 200ha rainforest, mainly evergreen, broadleaf tropical rainforest, is located on two limestone karsts mountain ranges (Soejarto and Kadushin, 2001) and hosts a wide variety of wildlife and is also known for its cultural heritage (Cuc Phuong National Park, 2006).

Within the park, a broad flat valley between the limestone hills, wide at the western end but narrow at the east end can be found (Vietnam Open Tours, 2005). South and west of the park, the bordering land is relatively flat, of a lower altitude and densely populated, whereas the north-west borders are forested limestone hills leading to the main mountain range of the Annamite Mountains (Vietnam Open Tours, 2005).

Especially in the flatter parts of the valley, a multi-layered canopy rain forest with trees up to a height of 70m and a large variety of ferns, orchids, lianas and cauliflory are present (Vietnam Open Tours, 2005). Forest areas on the karst crests are in general more specialised and less tall, comprising evergreen, semi-evergreen and deciduous species (Vietnam Open Tours, 2005).

These characteristics form the habitat for many species, currently including 97 mammal species, 17 amphibian species, 11 fish species, 300 bird species, 36 reptilian species, about 2000 vascular plant species and some thousand species of insects, many listed in the Vietnam Red Data book and the IUCN Red List (Viet Nam Travel, 2010).

These days Cuc Phuong National Park is a popular tourist destination with about 60.000 domestic and international visitors throughout the year (Adventure Tours Vietnam, 2010). On one hand, the high amount of tourists is a potential risk for some species, since they are disturbed by the visitors or even collected and taken out of the protected area. On the other hand, the National Park has a high potential value for raising awareness of nature and environmental issues among the public.

Several conservation and research centres are based in Cuc Phuong: Endangered Primate Rescue Center (EPRC), Small Carnivore Conservation and the Turtle Conservation Center (TCC) (Cuc Phuong National Park, 2006). All of these centres have strong support and connections to world-leading conservation organisations and a large number of zoos

around the world support their work with expertise, researchers and funding. These conservation centres are based at the park entrance (Cuc Phuong National Park, 2006).

The research was conducted at two different study sites, the so called “Bong Substation” in the centre of the park and the Turtle Conservation Center at the entrance of the park: both sites are described in detail in the chapters 3-5.

2.2 Study species

The Keeled Box Turtle belongs to the order of the *Testudines* and the family *Geoemydidae* and, depending on the taxonomist, is often treated under the Genus *Pyxidea*, as *Pyxidea mouhotii* as well (IUCN, 2010).

This terrestrial chelonian is also known as Jagged-shelled Turtle or Keel-backed Terrapin; native to China, India, Laos, Myanmar and Vietnam, is listed as an endangered species on the IUCN Red List (IUCN, 2010).

Two subspecies of *Cuora mouhotii*, *C. mouhotii obsti* and *C. mouhotii mouhotii*, have been described so far (McCormack, 2005). The subspecies *C. mouhotii mouhotii* is native to Cuc Phuong National Park (McCormack, 2005) and the object of this study.

Three large keels (see image 1) on the upper carapace gave this terrestrial box turtle its name (Wildscreen ARKive, 2010). Adult turtles are about 18-21 cm long and the brownish colour ranges from tan to mahogany to dark brown, with a yellowish-brownish plastron (see image 2) and an upper carapace, which is serrated at the rear and sometimes at the front as well (Rogner, 2008). The front of adult's plastron has a singular hinge and the head is usually brown with unclear dark fine lines, a short snout and a hooked and strong upper jaw (Wildscreen ARKive, 2010). Brown legs and sharp claws are common in both sexes, but males have usually thicker tails and a different eye colour than females (ETI BioInformatics, 2010).



Image 1: Keeled Box Turtle (*Cuora mouhotii*) (Devender, 2010).



Image 2: Plastron (Sheldon, 2010).

The Keeled Box Turtle was observed to be omnivorous in captivity (Rogner, 2008). In a previous research carried out by McCormack (2005), snails and mushrooms were identified to be the major food items of the observed wild individuals at Cuc Phuong. Furthermore, local people reported that *C. mouhotii* is regularly observed foraging under the tree species *Allospondias lakonensis* which fruits in August and September (McCormack, 2005).

The preferred habitats of the Keeled Box Turtle are moist forest areas (Rogner, 2008) and Meier (2010) explains that, at least in captivity, they like to spend time in water puddles but are not observed swimming, diving or entering deep water.

Captive *C.mouhotii* have been observed at the TCC and were most active during heavy rain (McCormack, 2005). Furthermore, the only research carried out on that species from McCormack (2005) showed, that they were most active during the wet season, home ranges in a time period of eight weeks reaching an average size from 0.52ha compared to just 2.1m² during the cold dry season and that the general activity drops significantly if temperature falls below 20°C.

Up to now, little is known about the preferred day times for activity, daily movement and behaviour patterns of the study species and existing information is often not conform. According to Rogner (2008), the turtles often dig themselves in during the day or during dry periods, are more active from dusk till dawn and like to spend time in water puddles, even though they are not able to swim. Other scientists point out, that this species has a strict terrestrial life and just gets wet during rain (ETI BioInformatics, 2010).

July and August are the months where the turtles are most active, mainly with foraging since the mating season (main month is May) is already over (McCormack, 2010).

3. Part I – Survey on the time budget and daily activity pattern

The first part of the research study consisted of a survey on the daily activity pattern and time budget of the Keeled Box Turtle (*Cuora mouhotii*).

3.1 Material and Methods

3.1.1 Study site

The survey was carried out with individuals from the captive population at the TCC (Turtle Conservation Centre).

The TCC is located at the entrance of the Cuc Phuong National Park in Vietnam and covers about 2000m², including enclosures, aquatic tanks, breeding and holding facilities for more than 600 individuals of turtles, laboratory, hatchery and incubation room, office and feeding kitchen (Asian Turtle Conservation Network, 2010).

The enclosure holding the captive population used for the survey is a fenced rectangle, 4,70m wide and 13,8m long with an area of about 65m². A 1m wide service pathway is situated along side the enclosure, separated from the enclosure by a 0,5m high fence. The enclosure and the service pathway are enclosed by a fence on each side. A sketch of the enclosure can be found in Appendix II.

The enclosure represents the natural environment of that area, since the fence was just reared around a natural piece of land. Besides the naturally occurring plants, rocks, trees and leaf litter piles, the enclosure was furnished with an artificial water pond and some extra hay/leaf litter stacks (see Appendix II).

3.1.2 Study population

The individuals of this sample shared the enclosure described in chapter 3.1.1 Study site. In total the enclosure accommodated six turtles of which four were used during this survey. Reasons for the use of only four individuals were mainly the restricted capacity of time and man power of this research. Furthermore the number of individual turtles within the enclosure was stated as five or six by the staff of the TCC, and the sixth individual was only found coincidentally during the second week of data collection and therefore was not included in the study population. The fifth turtle was found together with the other four during the pilot study, due to its looks- it was much bigger and of different shape and colour than the other four individuals- it was decided to not use it during the survey because the researchers and the TCC staff were not absolutely sure if this individual belonged to the same sub species as the four others.

The four individuals used during the study were all adults but the exact age of them is unknown. Unfortunately the sexes of the individual turtles are also unknown because a precise sex determination is not possible without DNA-sexing methods. Due to the finding of fertilized eggs within the enclosure last season and during the pilot study it was anticipated that both sexes are present within the enclosure, but it is unsure if this is also true for the study population.

The animals got fed every morning around 9am by the TCC staff. Food items included different chopped fruits and vegetable like bananas, apples and tomatoes.

3.1.3 Study design

Prior to the actual survey a pilot study was conducted at the TCC with the four captive individuals of Keeled Box Turtles described in the chapter 3.1.2 Study population.

The captive turtles were used to check if the in advance defined behaviour patterns of the turtles are clearly distinguishable. Furthermore, a Kappa value of 0.89, an almost perfect strength of agreement beyond chance, was reached (Landis & Koch, 1977).

The distance to the animals at which they can be clearly seen as well as the type of behaviour they are performing without altering their behaviour through researchers' presence was also determined during the pilot study by approaching the tortoises up to different distances and just trying which distance is best suited for the purpose of this research. It turned out to be best to keep at least a distance of 1m to the animal and between the researchers in order to prevent disturbance but the high density of vegetation made it impossible to clearly determine the type of behaviour if the distance to the tortoise was bigger than 1,5m. The four individuals of the study population were marked and numbered with nail polish on the top of their carapace in order to be able to distinguish them reliably even from out a distance. This was important to minimize the stress experienced by the animals during close encounters and to make sure that no turtle was observed twice while another one not at all, for example. The nail colours used in this research are developed for horn (human nails) and were therefore not harmful in any way for the animals (Meier, 2010).

The research method used was an observational cross-sectional study.

The approach conducted for the data collection was to observe the captive individuals in multiple shifts of two hours length daily with a two hours break in between each shift, to achieve more random data. The shifts were divided over a period of 24h.

The shifts of two hours were divided among the observers and individuals in order to make sure that every individual study object was observed for the same amount of time at similar times of the day by different observers. Every two hours shift of observation was carried out at least once for every turtle which provided data on at least one full day (24h period) for every individual of the study population. During the former mentioned observation shifts one individual of the study population was observed by one person. In order to gather the same amount of data on every individual, a time table of shifts has been developed in advance to the survey (Appendix III).

An advantage of this method of data collection is that two individuals were simultaneously observed under the same external conditions. Therefore, differences in behaviour patterns shown by the observed individuals should have other reasons than possible external conditions.

3.1.4 Data collection

The data collection was carried out on 15 days between the 16th of August and the 8th of September 2010 during the hot rainy season.

The categories on which the data were selected were especially created to suit the purpose of this study. Eleven different patterns of behaviour, further described in the ethogram in Table 1, were identified prior to the data collection and were recorded during the survey: *feeding, drinking, agonistic, locomotion, social activities, resting, basking, investigating, observing, under litter* and *not observed*.

The behaviour patterns *agonistic* and *locomotion* were further subdivided into three categories whereas the behaviour patterns *social activities* was subdivided into four categories in order to gather as precise data on their behaviour as possible. Most of these behaviour patterns were selected after literature research (see Table 2) and with the support of Mr. Meier of the Internationales Zentrum für Schildkrötenschutz Allwetterzoo Münster (International Turtle Conservation Center, Münster Zoo, Germany), because they summarize the main behaviours shown by this species which are performed to ensure their survival.

The two patterns *under litter* and *not observed* are used in case the observed animal is not visible and therefore it is not clear what it is doing, or when the animal was not observed at all.

During the pilot study and with the support of the TCC staff and Mr. McCormack (Asian Turtle Program Coordinator, Cleveland Zoo) two behaviour patterns were added/modified. The behaviour *locomotion climbing* was added, since parts of this species' natural habitat, rocky rainforest areas, demands climbing skills and turtles were observed climbing up rocks. Additionally, the behaviour *basking* was expanded to basking either in sun or rain, since individuals of Keeled Box Turtles were observed basking in rain, compared to other reptiles, who are known to sun bask.

Another advantage of the patterns of behaviour as they are defined in this study is that they are clearly distinguishable in the research species even by inexperienced observers.

Table 1: Description of the behaviour patterns, codes and definitions used for the Keeled Box Turtle in this study (McCormack, 2005; Kiester & Moskovis, 1987).

Behaviour patterns	Code	Definition
Feeding	F	Collecting and consuming food
Drinking	D	Consuming water
Agonistic (behaviour associated with conflict/disturbance)	Afi	Fighting
	Ae	Escaping
	Afr	Freezing
Locomotion	Ls	Walking slow
	Lf	Walking fast
	Lc	Climbing
	Ls	Swimming
Social activities	Sf	Following
	Sc	Courtship
	Scop	Copulating
	Scha	Chasing
Resting	St	Standing, no forward movement
Basking	B	Exposing itself to the warmth of the sun or to rain
Investigating	I	Exploring social, biological and physical environment
Observing	O	Watching/smelling environment
Under litter	UI	Not or barely visible under litter
Not observed	No	No behaviour observed

Furthermore the date, the number of the observed turtle and the number of the sample, the name of the observer, the observation shift, the time of the day as well as the external weather condition *rain/no rain* were recorded (see data sheet Appendix IV).

The date, the number of the turtle, the sample number, the observers name and the shift number, are important in order to keep an overview of the data sampling. Due to the research aim of collecting information on the activity patterns and time budget over a period of 24h there were twelve observation shifts, each shift consisting of a time period of two hours. Shift 1 started at 0:00am and in intervals of two hours a new shift began, so that the last shift, shift 12, was from 10:00pm to 0:00am.

The sampling method carried out during data collection of this survey was focal animal sampling and as a recording rule continuous sampling was used to record the behaviour patterns and their duration shown by the individuals at all times. Therefore the time, behaviour patterns performed by the turtle and weather condition listed in the data sheet in Appendix IV were recorded continuously. The date, the number of the observed turtle and the number of the sample, the name of the observer and the observation shift were recorded just at the beginning of each observation session because this information did not change during one observation shift.

During the night shifts of observation a flash light was used. The darker the light the lesser the disturbance of the turtles is supposed to be (Meier, 2010). Therefore red lights created by covering the torch with red plastic or paper or red lamps were used.

3.1.5 Data preparation and analysis

Time budget

In order to prepare the data set for the analysis, the original data set recorded in Excel Microsoft Office 2003 (see template Appendix V) had to be transformed into an SPSS file using the program PASW Statistics 17.0. For representing the data clearly, within the SPSS file all behaviour types defined prior to the survey (see Table 1 in 3.1.4 Data collection) which were not shown at all during these observation periods, *agonistic behaviour fighting, locomotion swimming, social activity chasing, basking* and *not observed*, were deleted from the data set, instead of taken into account with values of zero. The remaining 14 behaviour types were grouped together into the following eight classes: *resting, observing, investigating, foraging (feeding and drinking), agonistic (escaping, freezing), locomotion (slow, fast, climbing), social activity (following, courtship, copulating)* and *under litter*.

The percentages each turtle spent with certain behaviour types over a period of 24 hours were calculated to carry out statistical analysis. The means with standard errors, standard deviations, minimum and maximum of each behaviour type for all turtles together were calculated for the time budget.

Daily activity

The data set for analysing the daily time budget was also used for answering the second part of the first research question, daily activity. The remaining 14 behaviour types were grouped in the same way as mentioned above, in the paragraph *Time budget*. Furthermore, the behaviour types were grouped into two classes: *active (observing, investigating, foraging, agonistic, locomotion, social activity)* and *not active (resting,*

under litter). The percentages each turtle expressed one of the eight behaviour types and performed behaviour grouped in the behaviour classes *active/not active* for each of the twelve shifts were calculated. The values for each behaviour type over the different shifts were summed up for all turtles and averaged.

Furthermore, it was tested if the mean percentages turtles showed certain behaviour depended on the time of day. It was chosen to conduct the following tests only with the percentages for *active* behaviour, consequential that missing percentages up to 100 % are *inactive* behaviour.

The percentages of all behaviour types shown by each of the turtles per shift were arcsine square root transformed. This is appropriate for observations that are given in percentages prior to further statistical testing (Hill et al., 2005). After the transformation the repeated measures ANOVA was carried out.

Furthermore, a post hoc test was conducted for comparing the main effects within the twelve shifts, using the LSD (Least Significant Difference) test.

3.2 Results

Time budget

The mean amount of time \pm standard errors for all four turtles in percentage per 24 hours spent with the eight different behaviours, *resting*, *observing*, *investigating*, *foraging*, *agonistic*, *locomotion*, *social activity* and *under litter*, as well as the standard deviations, maximum and minimum for each case are given in Table 2.

Table 2: Total time budget over 24 hours. Means (\pm S.E.M.), minimum, maximum and standard deviation for all 4 turtles (N=4).

Behaviour	Percentage mean \pm S.E.M.	Percentage minimum	Percentage maximum	Percentage standard deviation
Resting	57.99 \pm 11.45	28.72	80.06	22.89
Under litter	20.96 \pm 10.22	7.22	51.22	20.44
Observing	17.92 \pm 5.40	12.11	34.10	10.79
Foraging	1.23 \pm 1.09	0.00	4.49	2.18
Locomotion	0.78 \pm 0.43	0.00	1.86	0.87
Social activity	0.67 \pm 0.41	0.00	1.67	0.82
Investigating	0.43 \pm 0.17	0.00	0.83	0.34
Agonistic	0.02 \pm 0.02	0.00	0.06	0.03

The turtles spent most of the time *resting* ($\bar{x} \pm$ S.E.M.; 57.99% \pm 11.45%), followed by the behaviours *under litter* (20.96% \pm 10.22%) and *observing* (17.92% \pm 5.40%). Low mean percentages (0.02% \pm 0.02% to 1.23% \pm 1.09%) are displayed for the behaviours *foraging*, *locomotion*, *social activity*, *investigating* and *agonistic* behaviour.

The standard deviations indicate the big differences in behaviour shown between the four observed individuals. Whereas the behaviours *resting*, *under litter* and *observing* were shown to different degrees (minimum 7.22%, maximum 80.06 %) by all four turtles, the remaining behaviours were not displayed by all individuals.

But even under consideration of the standard deviations, the behaviour types *resting*, *under litter* and *observing* are dominant during a 24 hours' time period (sum mean percentages 96.87%). Considerably less time (3.13%) was spent on all other behaviours.

Daily activity

Fig. 3 shows the distribution of active behaviour across the day, divided into the different shifts.

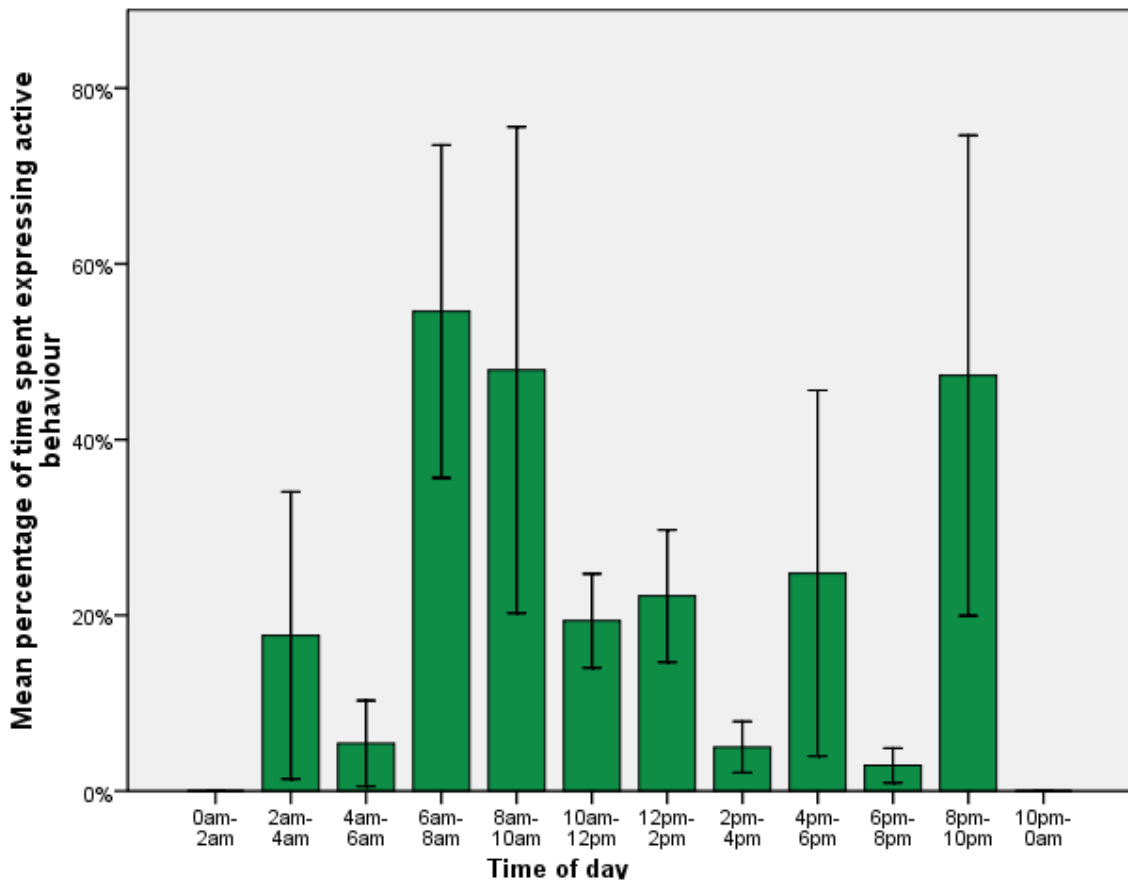


Fig. 3: Mean percentages (\pm S.E.M.) of active behaviour of all turtles in each shift.

The arcsine values revealed that the daily activity of the four turtles was significantly related to daytime (Repeated Measures ANOVA, $F_{11,33}=2.465$, $p=0.022$). Therefore, *active* behaviour differed significantly between some of the shifts.

From 10:00pm to 2:00am (Shift 12 and 1) only *non active* behaviour was shown. In contrast, peaks in activity were shown from 6am-8am ($\bar{x} \pm$ S.E.M; $54.58\% \pm 18.95\%$), 8am-10am ($47.92\% \pm 27.68\%$) and 8pm-10pm ($47.29\% \pm 27.32\%$). In the remaining times of the day, *active* behaviour was displayed between averaged $2.92\% \pm 1.97\%$ of the two hours shift time (6pm-8pm) and $24.79\% \pm 20.83\%$ (4pm-6pm).

Active behaviour differed significantly between 6am-8am compared to 10pm-6am and 2pm-4pm. Furthermore, clear differences were revealed from 0am-2am (*no activity* at all) to 10am-2pm and between 10am-12pm to 2pm-4pm and 10pm-2am (again, *no activity* at all). Last, the shift from 12pm-2pm differed from the ones 2pm-4pm and 10pm-2am (*no activity* at all).

The daily activity patterns divided into the eight different behaviour classes over the twelve different shifts are presented in Fig. 4.

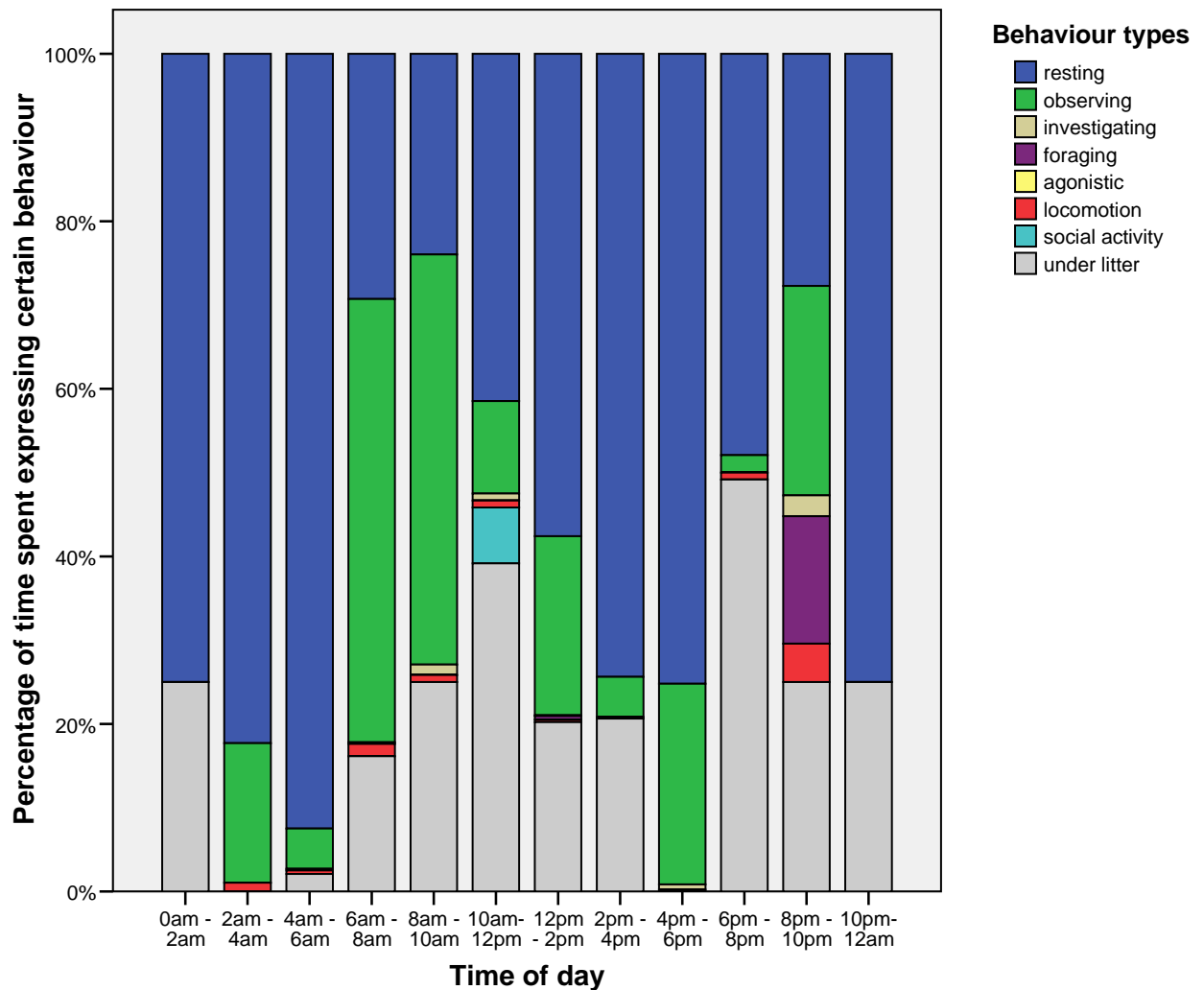


Fig. 4: Averaged percentage of time all turtles expressed certain behaviours per shift.

During the times of day with the highest percentages of *active* behaviour (6am-10am, 8pm-10pm), the behaviour type *observing* is averagely shown between 25% and 52.92% of each two hours interval. Furthermore, 15.21% of the time between 8pm-10pm was spent on *foraging*, 4.58% on *locomotion* and 2.50% on *investigating*. *Social activity* percentages were highest from 10am-12 pm with 6.67%. The averaged time spent *resting* reached from 23.96 % (8am-10am) up to 92.50% (4am-6am).

3.3 Discussion

The daily time budget of the Keeled Box Turtles at the TCC shows that the activity periods are low, *inactive* behaviour is displayed for a major amount of the day. This result confirms the outcomes of studies on African tortoise species (Keswich et al., 2006, and Hailey and Coulson, 1999). These studies were carried out on wild individuals, what was originally planned for the survey in this research as well. This survey was supposed to be conducted with wild individuals at the Cuc Phuong National Park. However, during the pilot study this turned out to be impossible due to a lack of time and man power. Furthermore, locating these wild individuals took too long and sometimes they were hidden under logs and dense vegetation and therefore greater parts of the vegetation had to be cut to find them. If this would have been done a couple of times a day over

some weeks, it would have had major impacts on the habitat and caused disturbance to the individuals. In addition, a turtle being observed in the wild seemed to be disturbed and did not display any behaviour except observing the researchers until the observation shift was over. This was also the case in other box turtle studies, carried out by Dodd (2002). Therefore it was decided to use the captive population at the TCC for this survey.

All individuals of the study population shared the same enclosure, which could have had influencing effects on each others behaviour. A fifth individual, which was not part of the sample, was assumed to influence the others, since it was bigger and stronger and was observed chasing some of the individuals of the studied population. Furthermore, the exact age and the sexes of the individuals are unknown, so no conclusion can be drawn about these factors having effects on the daily behaviour and time budget. However, former studies on differences between sexes in activity patterns revealed that there were no differences in the amount of time spent *resting* (Liu, 2009).

The possible influence of the staff of the TCC, visitors and regular procedures during the day like feeding on the turtles' behaviour was not taken into consideration.

However, as Jiang (2001) explains, rigid elements of behaviour will be performed in captivity as well. According to Meier (2010), bright light and a close distance to the observed individual could prevent the turtles from behaving naturally. Although red filters for the flashlights were used during night observations and the observers kept as much distance as possible to still be able to determine the behaviour types, it cannot be excluded that these factors had effects on the displayed behaviour. Nevertheless, as Hailey & Coulson (1999) point out, it is necessary to observe turtles for the whole period of 24 hours to get a real time budget. Even though effects of the captive environment cannot be excluded, it should be taken into consideration that the enclosures at the TCC display the natural habitat of the species very well.

However, the factors which are most affecting the reliability of the results of the study are the small sample size, the large differences in behaviour shown between the individuals and the small amount of observation hours. Furthermore the fact that the observations took only place in just one season of the year, from mid August to the beginning of September, and external conditions like temperature, weather and humidity were not taken into consideration.

The large spread in durations of behaviour types performed by the individual turtles was reflected in the high standard deviations. In order to achieve statistically significant results a sample size of at least 15 individuals (sample size calculations based on the results for the behaviour type *resting*, considering a relative precision from $p=20\%$) would be suitable.

The highest mean percentages of all four turtles for *resting*, *observing* and *under litter* were expected.

Furthermore, the data were analyzed in a way that considered only the length of time a behaviour type was carried out instead of its frequency. Consequently, some behavior types, even if shown very frequently, only form small percentages in the results because of their short duration. *Locomotion*, for example, was carried out frequently by the study

population, but only for a short amount of time which results in a low percentage of that behaviour type.

Surprisingly, the behaviour *basking* was not shown at all during the observations, which could indicate, that this species does not bask in the sun as observed in other species (Keswick et al., 2006), but instead, as suggested by McCormack (2010), in the rain, a condition that was not given during these observation periods.

For the daily activity, *active* and *not active* behaviour in the different shifts were recorded. Significant relations between the activity and day time were achieved. Peaks in activity occurred in the morning from 6am-10am and in the evening from 8pm-10pm. Peaks in daily activity in the morning, in that case from 10am-12pm were also observed by Keswick et al. (2005). Furthermore, Liu et al. (2009) found out, that another Asian turtle species showed the highest level of activity between 7am-1pm and 7pm-3am. Even though the activity peaks in this survey are located around the same activity peaks recorded by Liu et al. (2009), differences are given, since the Keeled Box Turtles showed no sign of activity from 10pm-2am.

However, due to the grouping of the behaviour type *under litter* to *not active* behaviour, the period of *inactive* behaviour observed could possibly be too high. According to the TCC staff, the turtles often rest when being under leaf litter, but this cannot be said with certainty, since it was not possible to determine the exact behaviour of the turtle while it was situated under litter. Based on these results, it cannot be confirmed, that the species is most active during this season (McCormack, 2010), since there is no data for comparison with other seasons.

Roger (2008) supposed that this species is mainly *active* during dusk, night and dawn. Even though activity peaks could be observed after dusk (8pm-10pm) and during dawn (6am-8am), there was also a higher activity level compared to the other times of day from 8am-10am. Displaying *active* behaviour throughout the day was also observed in the study from Hailey & Coulson (1999) and was shown from the Keeled Box Turtles throughout the day and night, except from 10pm-2am.

Even though significant differences in behaviour could be recorded between the different shifts, the large differences between each sample show that the individuals of the study population displayed very distinctive behaviours. The reason for this individuality is not clear. Furthermore, their slow metabolic rate (Sample, 2004) might be the reason for them to wait and observe till a food source comes up or slowly start looking for it. The low metabolic rate is also maintained by avoiding fast movement when possible and instead of energy intensive fighting or escaping the species can just retreat in its carapace if harassed by anything. Therefore it is possible that the turtles just move if an opportunity comes along or they are really in need of energy intake which means that they never established defined activity patterns connected to certain times of day. However, feeding times at the TCC are around 9am and do not seem to have an effect on turtles foraging behaviour, since that was observed mainly in the period 8pm-10pm.

The data were analyzed using the repeated measures ANOVA. However, the Mauchly's Test of Sphericity could not be carried out to check if the assumptions for conducting the

Repeated Measures ANOVA were met, since not enough degrees of freedom were left to calculate the significance value. Nevertheless, with sphericity assumed, a significant relation of *active* behaviour and day time was indicated. Therefore a post hoc test was conducted. For these data, the LSD (Least significant difference) test was preferred over the Bonferroni test. Even though the LSD test does not control the Type I-errors, it guarantees to not lose statistical power. The Bonferroni would have taken Type I errors into account, but at the same time a lack of statistical power could be expected and differences between means might have been rejected (Field, 2009).

Even though considering the small sample size and the high variation between the individuals, the results display activity tendencies, which could be confirmed with a larger sample size and more observation hours.

4. Part II – Experiment on the influence of rainfall on activity

The second part of the research study consisted of an experiment on the influence of rainfall on the activity of the Keeled Box Turtles (*Cuora mouhotii*).

4.1 Material and Methods

4.1.1 Study site

The experiment was carried out at the same study site, an enclosure at the TCC, as the survey on the activity patterns and time budget. Further information on it can be found in chapter 3.1.1 Study site and an additional outline of the enclosure in Appendix II.

4.1.2 Study population

The study population consisted of the same sample population of four captive individuals of the subspecies *C.mouhotii mouhotii* that was used during the survey on activity patterns and time budgets. More detailed information on the study population can therefore be found in the chapter 3.1.2 Study population.

4.1.3 Study design

Prior to the actual experiment a pilot study was conducted at the TCC with the study population. The aspects of the pilot study, which were similar to the pilot study conducted for the survey on the activity patterns and time budget of the Keeled Box Turtle, are the determination of the Kappa value between the researchers, the investigation of the most appropriate distance kept to the animals during the observations and the marking and numbering of the study population. These aspects of the pilot study are described in more detail in chapter 3.1.3 Study design. Another aspect of the pilot study was to test the range and functioning of the sprinkler device prior to the observations.

The study was set up as an experiment where the artificial rainfall created by the use of the sprinkler device was used as a treatment. Two sprinkler devices were installed at both ends of the ceiling of the enclosure which sprinkled the water in circles. The whole area of the enclosure was getting wet as a consequence of the sprinkling and the water for the treatment came out of the tap of the TCC. The turtles of the study population were already used to the treatment with the sprinkler devices because these were used earlier on to keep the soil within the enclosure humid according to the requirements of the turtles.

Due to the lack of a control group the experiment can not be called a true experiment and is rather defined as a quasi-experiment.

The research method used during the experiment was a before-and-after study and focal animal sampling was used. The approach for the data collection was to observe the captive individuals in multiple shifts of two hours length with a two hours break in between each shift, to achieve more random data. The observation shifts during which the sprinkler device was turned on were carried out throughout the whole day (12h period). The data that were used for comparison without the use of the sprinkler device and without natural rainfall were gained from the data that were originally collected and

used for the survey on activity patterns and time budget of the Keeled Box Turtle. By comparing the collected data of the turtles during the same shifts with and without the use of the sprinkler device or natural rainfall it is possible to answer the second research question if there is a difference in activity and behaviour of the study population between those two conditions.

The shifts of two hours were divided among the observers and individuals in order to make sure that every individual study object was observed for the same amount of time at similar times of the day by different observers.

Every two hours shift of observation was aimed to be carried out at least once for every turtle which provided data on at least a half day (12h period) for every individual of the study population. During the former mentioned observation shifts one individual of the study population was observed by one person. In order to gather the same amount of data on every individual while using the sprinkler device, a time table of shifts has been developed and the observations were carried out accordingly (Appendix III). An advantage of this method of data collection is that the researchers were simultaneously watching two turtles under the same conditions of humidity and temperature which could influence the behaviour of the animals.

4.1.4 Data collection

The data collection of the experiment was carried out on 19 days between the 16th of August and the 8th of September 2010 during the hot rainy season.

The patterns of behaviour that were recorded during the experiment are similar to the ones that were recorded for the survey on the activity patterns and time budget and can be found described in detail and shown in an ethogram in chapter 3.1.4 Data collection.

Recording those behaviour types makes it possible to determine if there is a difference in the behaviour carried out by the study population during the treatment of *artificial rainfall* compared to when there is *no rainfall*, neither natural nor artificial.

Furthermore the same variables were recorded as for the survey on the time budget and activity patterns in part 1 of the study except for the condition *artificial rainfall* or *no rainfall* (see Appendix VI).

The sampling method carried out during data collection of this survey was focal animal sampling and as a recording rule continuous sampling was used to record the behaviour types and their duration shown by the individuals at all times. Therefore the time and behaviour type performed by the turtle were recorded continuously as well as the condition (*artificial rainfall/no rainfall*). The date, the number of the observed turtle and the number of the sample, the name of the observer and the observation shift were recorded just at the beginning of each observation session because this information did not change during one observation shift.

4.1.5 Data preparation and analysis

In order to prepare the data set for the analysis, the original data set recorded in Excel Microsoft Office 2003 (see template, Appendix V) had to be transformed into an SPSS file using the program PASW Statistics 17.0. Within the SPSS file, the data set was prepared as described in chapter 3.1.5 Data preparation and analysis.

Contrary to the observations made in Part I - Survey on the time budget and daily activity, *basking* was observed during *rainfall*, so the values of this behaviour type were not deleted from the dataset and used for the analysis. The behaviour types were grouped in nine, respectively two (*active/not active*) classes. The behaviour type *basking* was counted as *active* behaviour.

The percentages each turtle performed a certain behaviour type and in the behaviour classes *active/not active* for the two conditions *artificial/natural rainfall* and *no rainfall* were calculated.

As the same individuals of turtles were observed during the treatment with *artificial rain* and during *no rain* (neither artificial nor natural) the samples were considered to be related. After the transformation of the values as described in 3.1.5 Data preparation and analysis, the Paired Samples T-Test was carried out to compare the arcsine values for each of the nine behaviour types for both conditions *rain* or *no rain*.

Furthermore, the Paired Sample T-Test was carried out to compare the transformed values of turtles being *active* either in *rain* or *no rain* conditions.

4.2 Results

Fig. 5 gives the mean percentages of all turtles (N=4) together for each of the nine behaviour types for both conditions *artificial rainfall* (blue) and *no rainfall* (yellow).

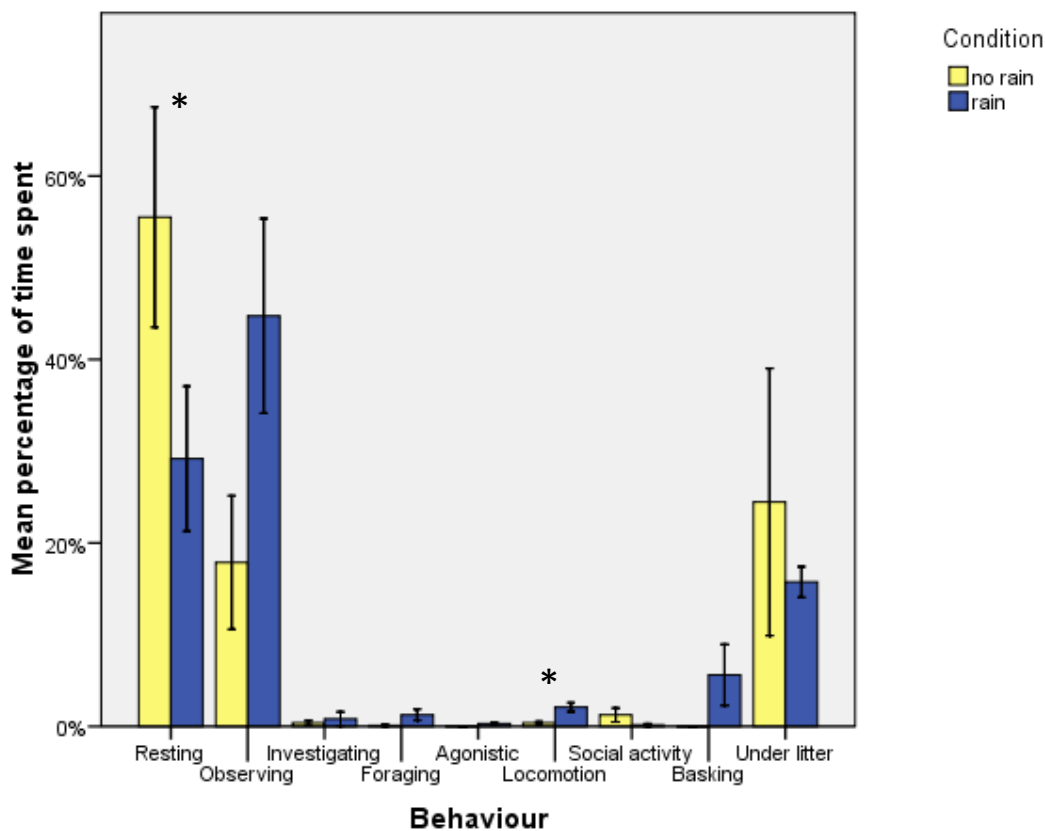


Fig. 5: Mean (\pm SEM) percentages for each behaviour type recorded with *artificial rainfall* (blue) and *without rainfall* (yellow) (n=4). Star symbol showing the behaviours with significant differences between the two conditions.

As Fig. 5 displays, the behaviour types *resting*, *observing* and *under litter* are the behaviour types that were shown for the largest amount of time by the study population and therefore have the highest mean percentages. Furthermore the graph shows that the difference in percentage of time spent while performing certain behaviour during *artificial rainfall* and *without rainfall* is the highest within the behaviours *resting* ($\bar{x} \pm$ S.E.M; rain: 29.18% \pm 7.90% / no rain: 55.51% \pm 11.99%) , *observing* (rain: 44.76% \pm 10.60% / no rain: 17.88% \pm 7.27%), *locomotion* (rain: 2.12% \pm 0.49% / no rain: 0.38% \pm 0.17%), *basking* (rain: 5.63% \pm 3.33% / no rain: 0.00% \pm 0.00%) and *under litter* (rain: 15.75% \pm 1.66% / no rain: 24.46% \pm 14.56%).

The comparison of the arcsine values for the behaviour types showed a significant difference in the mean percentage of time spent for the two conditions *no rainfall* and *artificial rainfall* in *resting* with $p=0.043$ (Paired Sample T-Test, $t_3=3.379$) and in *locomotion* with $p=0.001$ (Paired Sample T-Test, $t_3=-12.346$).

Fig. 6 gives the mean percentages of all turtles (N=4) together for the behaviour class *active* for both conditions *artificial rainfall* (blue) and *no rainfall* (yellow).

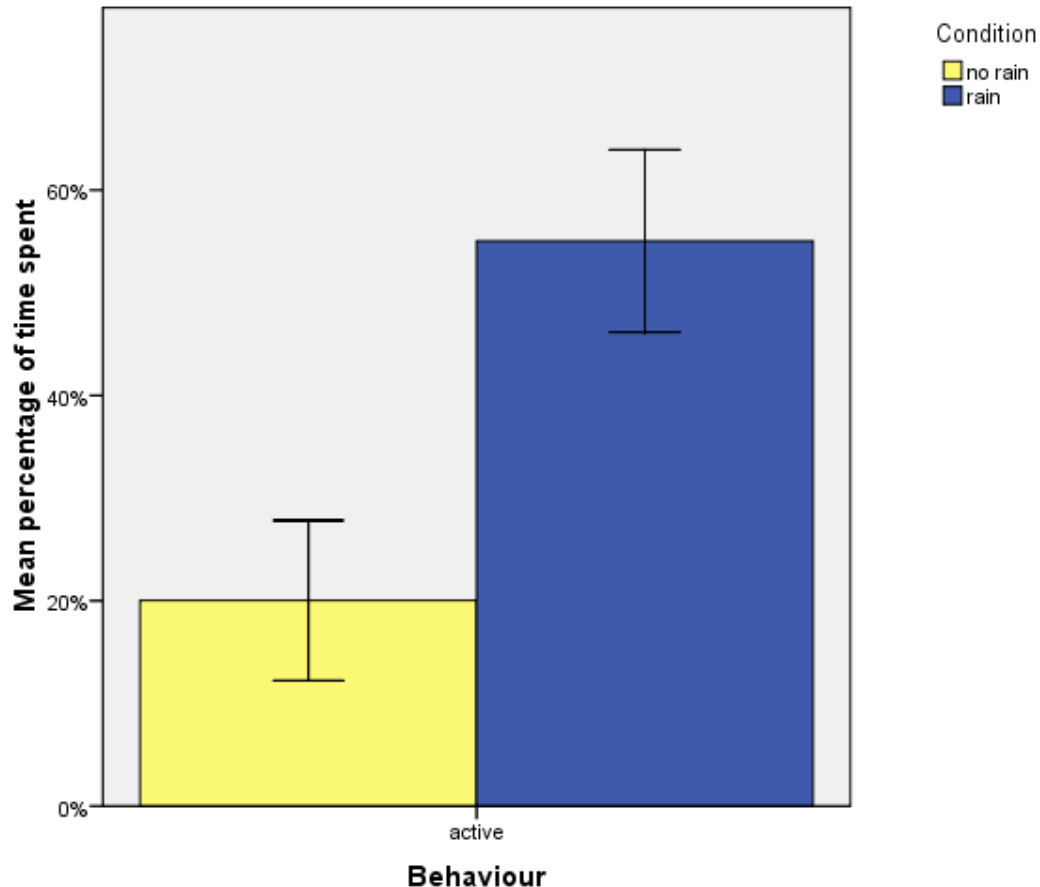


Fig. 6: Mean (\pm SEM) percentages for *active* behaviour recorded with *artificial rainfall* and *without rainfall* (n=4).

In Fig.6 it can be seen that in general the turtles of the study population spent a higher amount of time with *active* behaviour during observations with *artificial rainfall* (55.05 % \pm 8.89%) than *without rainfall* (20.03% \pm 7.81%)

This observation indicates that in general the study population was more *active* during the application of *artificial rainfall* than it was *without rainfall*.

However, the comparison of the arcsine values for each of the behaviour classes *active* (Paired Sample T-Test, $t_3=-2.265$) and *not active* (Paired Sample T-Test, $t_3=2.268$) with both of the conditions *rain* and *no rain* showed no significant differences ($p=0.108$ for both cases) in the amount of time spent with *active* or *not active* behaviour.

4.3 Discussion

The highest mean percentages of all four turtles for *resting*, *observing* and *under litter* were expected for these behaviour types due to the former literature study on the ecology of the study species and other tortoise species. The differences in behaviour shown during the treatment with *artificial rainfall* in comparison *without rainfall* were partly expected due to the hypothesis that the study species is more active during rainfall than without (McCormack, 2010). The significant differences for the behaviour types *resting* (significantly lower during the treatment) and *locomotion* (significantly higher during the treatment) confirm the hypothesis that this species is more *active* during rain.

Furthermore, the behaviour *observing* had a definitely higher percentage of time spent during the application of the treatment which was not really foreseen prior to the experiment but could be founded in the theory that various species of land snails, one of the turtle's favourite food sources, are also more active during rainfall and while moving easier to spot for the turtles (Iglesias et al., 1996). Furthermore, the high percentage of *basking* shown during the treatment in comparison with not being expressed at all *without rainfall* was rather surprising because tortoise species in general spend a large amount of time with sun basking (Keswick et al., 2006). However, Liu et al. (2009) discovered that their observed species, an Asian turtle species living partly terrestrial, did not sun bask for a considerable time of the day.

Anyway, due to the absence of similar studies on the study species or other rain basking turtles in south-east Asia the following possible explanations are just based on assumptions. The function of the rain basking could as well as for sun basking be thermoregulation. It is possible that the body temperature of the turtles is rather high during the hot summer months during which the research took place. The turtles could therefore use basking in the rain as a measure to lower their body temperature during the hot rainy season. Another reason for the basking behaviour shown could be that the turtles just use the rainfall to moisture their skin.

Resting and *under litter* were both grouped as *not active* behaviours and in both the mean percentage of shown behaviour is lower during *artificial rainfall*, which was expected. *Locomotion* was accounted as an *active* behaviour and it was also anticipated that the percentage of this behaviour shown was higher during *artificial rainfall*. However, comparing the values for *active* behaviour with and without the treatment revealed no significant differences. Due to the large differences between the turtles and the high standard deviations even though there are considerably high differences in percentages for observation with or without treatment, significant differences were expected but not achieved. The reason for that is a negative correlation between *active* behaviour with and without treatment. This means that turtles, which were more *active* than other turtles with the treatment *rain* are relative less *active* than the other turtles *without rain*. Again, the small sample size and the fact that the individuals showed very distinctive behaviour resulting in large standard deviations and their possible influence on each other as well as the small amount of observation hours could be a reason for that. Furthermore, the individuals and the study site were the same as used for the survey on time budget and daily activity and therefore, the results of this experiment face the same possible influences, assumptions and aspects as mentioned in 3.3 Discussion. Besides, access to the sprinkler device was limited due to a shortage of water in the tank of the TCC and the danger of flooding the enclosure of the study population. The fact that the observations with *artificial rainfall* and *without rainfall* were not collected in turns every other day within the same weeks could also have had an influence on the statistical outcome, as well as the lack of a control group. For statistical testing, the Paired Sample T test was conducted, but maybe due to the influence that the turtles had on each other, the samples were no independent samples of the population.

Although the possible influences on the results have to be taken into account and no significant differences were achieved considering *active* behaviour *with* and *without rain*, the results indicate, that the turtles were in general more *active* during *rain* than *without rain*. Therefore it is likely, that the observations made before by the staff of the TCC and McCormack's suggestion (2010) about a higher level of activity during rain, are true and are another characteristic of this species' ecology.

5. Part III – Survey on the daily movement patterns

The third part of the research study consisted of a survey on the daily movement of the study species which aimed to answer the third research question.

5.1 Material and Methods

5.1.1 Study site

The survey on the daily movement of the Keeled Box Turtles was carried out with individuals of the wild population at the park centre (Bong area) of the Cuc Phuong National Park (see Chapter 2.1 Study area). The habitat of the turtles lies between service areas that are highly frequented by tourists and a main road leading through their habitat (see Figure 7 in 5.2 Results). On the right of Figure 7 in 5.2 Results the map on the top right shows the part of the Bong area (park centre) where the thread-trailing took place, but it displays also an overview of the other environmental features: buildings, pathways, service areas and the main road. The forest between the accommodation and restaurant area is connected by smaller pathways. These pathways are also used as nature trails with labeled trees for tourists.

The map below shows the Cuc Phuong National Park and its close environment. The Bong area where the data was collected is marked with a red point and the main road is displayed as well. Further you can see on this particular map that the National Park is an 'island' of forest, surrounded by agricultural land and settlements.

5.1.2 Study population

During the pilot study five individuals of wild Keeled Box Turtles which already have been followed by using a GPS transponder during a former study were located, of which four were marked and weighed to be part of this study (see Appendix VII). The fifth turtle was known to be located within a cave for already a year at that point without ever changing location. It was therefore assumed that this individual either died in the cave or lost its transmitter in it. Either way, it was decided to exclude the individual in question from the study population. However, an additional turtle with a transmitter was found by chance during a telemetry search for another turtle, and added to the study population.

The five individuals used during the study on movement were all adults but their exact age and sex are unknown.

5.1.3 Study design

In order to exactly follow a turtles' track, with every change of direction, the thread-trailing method was used for this study. This method would also allow investigating where turtles are hiding or how often they cross a certain pathway.

For a detailed building plan of the spooling device used during this research, see Appendix VIII. Other researchers have used trailing devices which made up to 6% of the body weight of the turtles (Stott, 1987) and it is believed, that the threshold is about 10 % of the body weight (McCormack, 2005). The spools that were built for this study weighed approximately 40g which is definitely lower than 10% of the weight of any individual of the study population (weights of turtles see Appendix VII).

In the pilot study it was tested if the thread-trailing devices are applicable by applying them on the carapace of an Asian Leaf Turtle (*Cyclemys dentate*) in the TCC. This individual was chosen because it was more accustomed to being handled by humans. Therefore the testing of the spooling device was less stressful for this particular animal than it would have been for one of the captive Keeled Box Turtles. This species is similar in size and shape to the study species and can therefore be just as useful in determining if the device is working properly.

The devices were also applied on the study population to test the practicability within the vegetation of the study site and the feasibility of following the thread with a GPS device. The lower-back part of the carapace was chosen to attach the spools because not being the highest point it is more unlikely that the turtle will scrape off the device under a tree log for example. The normal activities of the turtles are not detectably altered by the attachment of the trailers or the handling and marking (Stickel, 1950, McCormack, 2010). However, in the pilot study it became clear that the study area, the habitat of the turtles, is an area which is highly frequented by tourists, who also walk through the habitat on paths. It was important to collect geographical data on the location of the buildings, pathways and other areas used by tourists because this information together with the coordinate data which results out of the thread-trailing would allow recommendations about conservation efforts in the park center.

The spooling devices were attached to the carapaces of the individuals of the study population after locating them with radio telemetry. Marine glue was preferred to attach the trailing devices because the high humidity and possible restricted locomotion of the turtle made duct tape an unfavourable option. In order for the marine glue to dry on the carapace the turtles were handled approximately one hour when the devices were attached. The animal was released at the location of capture, with the thread tied to vegetation nearby. During day light, the trail of the turtle was checked every two hours to ensure that the turtles are not tangled up in the thread, ran out of spool or crossed paths that are frequently used by tourists who could possibly follow the thread and find the turtles. The spools were exchanged if necessary simply by unscrewing the cap of the trailing device, with minimal disturbance to the turtle (Longepierre et al., 2001).

5.1.4 Data collection

Unfortunately GPS tracking could not be used to record the exact track (every point of direction change) of an individual because of the dense canopy in study area, cloudiness and the short distances between points of direction change. Instead, reference trees were used to calculate the GPS coordinates of every point where the direction of movement had changed. For this, trees near the thread track that were standing quite open were located. Several reference trees were used for measuring the turtle track because dependent on the location of the point that was going to be measured, a reference tree that was nearby would allow more accurate measurements of the turtles' track than one further away. The criterion for choosing a tree was that the two researchers involved in this data collection method would be able to establish a straight line between the reference tree and the point of direction change with a measuring tape, without vegetation blocking the line. The average of ten GPS waypoint coordinates (Garmin GPS60) recorded at the location of those trees was taken because this is supposed to increase the accuracy up to $\pm 1\text{m}$ or even less (Ha, 2010). Following the track of the turtle, at every point the direction changed, the distance and angles of that point

(of direction change) to the nearest reference tree were measured. The angles were measured from both directions by lining up a compass on the measuring tape, one person pointing in the direction of the reference tree and one person pointing at the point of direction change. Ensuring that the measurements were correct and the measuring tape line 'running' straight, it was checked if the angles combined exceeded $180^\circ \pm 4^\circ$. The degrees/angles taken would also indicate what formula to use for the calculation of the coordinates (see Chapter 5.1.5 Data preparation and analysis).

The distance the turtle had moved was recorded by measuring the thread of the spool after 24 hours with a measuring tape.

Due to time and personnel constraints it was not possible to spool all turtles at the same time to avoid differing weather conditions and temperature. However, it was tried to spool all five turtles and the attempts were repeated as often as possible.

The location of the buildings and paths was recorded by using the tracking program of a GPS device from Garmin (GPS60), taking one point each second (Hradsky, 2010). According to McCormack (2010), the accuracy of the GPS signal is approximately $\pm 5\text{m}$, which is suitable for habitat mapping. In the pilot study it became clear though that an accuracy of $\pm 5\text{m}$ is only realistic with good weather conditions (clear sky) and that the actual accuracy is $\pm 15\text{m}$ most of the times.

5.1.5 Data preparation and analysis

In order to analyze the data on the daily movement of the Keeled Box Turtle the coordinates of the spooling track had to be calculated by using the reference tree method. For the procedural method see the ATCN (Asian Turtle Conservation Network) document in Appendix IX. To display the calculated coordinates of the spooling track and the coordinates of the pathways and service areas recorded with the GPS device, the ATCN provided geographical layers for the ArcMap GIS and mapping software system version 9.3.1. Those layers provided the basic information like land use and roads on which the collected data were displayed (Flowchart Appendix X). The tracks and points representing the pathways and service facilities of the park center that were recorded with the GPS device were transferred to the MapSource Garmin Trip & Waypoint Manager (Version 4.00) software. Changing the format of the file containing the coordinates was necessary in order to display the data in ArcMap. The file with the coordinates of the tracks and points was then transformed into a GPS Exchange Format (gpx) by using the MapSource program. The calculated coordinates (Decimal degrees - WGS84) from the spooling were entered by hand into the MapSource program and this file was then also transformed into a gpx format.

In the ArcMap program the extensions tool 'Data Interoperability' was activated to allow the transfer of the GPS and calculated coordinates. In the ArcMap program the geographical layers Land use, Traffic, Boundaries and Elevation were used as a map basis to project the spooling track coordinates of the points of direction change on to. The coordinates of the pathways and service buildings were also added to give a better idea of the Bong area. Displaying this data is also necessary to show the frequency of potential human encounters on roads or near buildings.

5.2 Results

In Figure 7 the distance and direction moved over a period of 24 hours by turtle 2 is displayed in the main large scale map. Of all individuals of the study population, it was only possible to obtain one, complete data set. This particular turtle moved at least 99m in 24 hours, crossing one of the pathways that connect the restaurant area with the accommodation area. During day light it was checked every two hours if the turtle ran out of spool but before nightfall the spool was exchanged anyway to ensure that the turtle will not run out of thread overnight. Unfortunately, the turtle moved more than 50m during the night and ran out of spool anyway. The yellow point on the map represents the starting point of the spooling and the light blue point represents the end point of the spooling, where the turtle had run out of spool (after covering a distance of 64m). However, the turtle was then located by radio telemetry and the pink point represents its final location after 24 hours. The distance between the light blue and the pink point represents another approximately 35m (linear distance) that the turtle had moved after running out of thread. The small map on the right shows the spooling location in Bong and to visualize the entire 24hours spooling tack in this map, the light blue and pink point are connected by a dotted line.

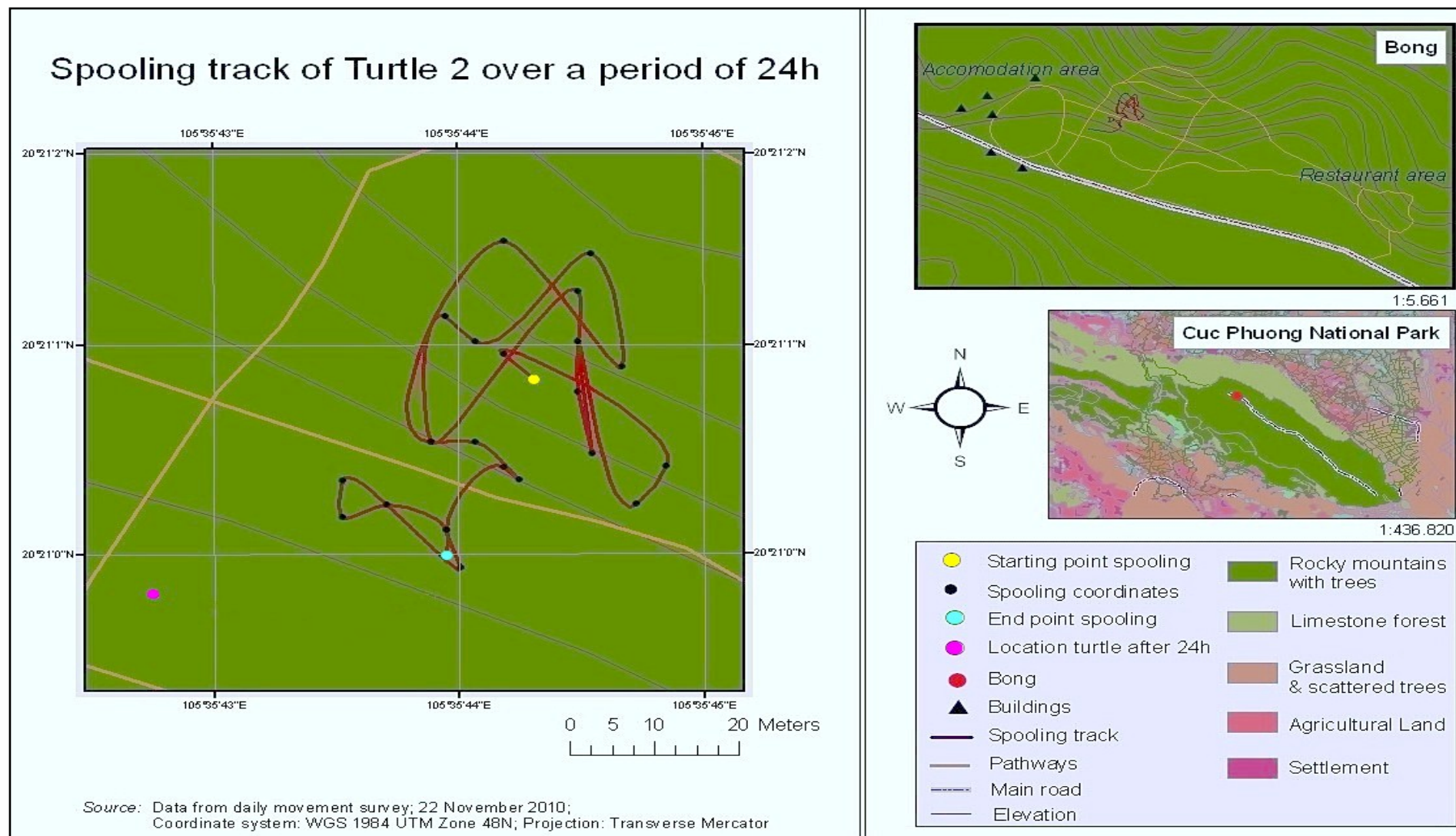


Fig 7: Direction and distance moved over a period of 24 hours by Turtle 2 of the study population in the park center (Bong) of the Cuc Phuong National Park.

5.3 Discussion

The results of the data collection on the daily movement of the turtles (see 5.2 Results) show that it is more than likely for a turtle to cross a pathway or road at least once during a period of 24 hours. Slow-moving species, such as turtles, are expected to be especially affected by road mortality (Smith and Dodd, 2003) and in this case study the detection by tourists is another valid mortality risk. It was expected that the individuals of the study population would cross a pathway during the 24h data collection. However, it was not expected that the turtle moved at least 99m because during the entire research time in the park centre, the turtles were always located in the same area. This suggests, that the turtles may have a well-defined home range or territory that they might only leave periodically (seasonal movements for reproduction or over wintering). However, they seem to move a lot within these home ranges/territories and it is assumed they know where to find hiding places, food etc. Another explanation for this is that although some turtles may remain in one location for days or even weeks, others are active nearly every day and travel considerable distances within a twenty-four-hour period (Dodd, 2002). In this context, the individuality of turtles has to be taken into account. Allard (1949) records what he terms 'selfish behavior' of turtles but Dodd (2002) explains that this behavior should rather be termed as 'single-minded' or 'determined'.

Like the results of this study, many authors using thread trailers have shown that turtles meander through their habitat, crisscrossing their own paths, turning often, and wandering around certain objects as trees (Stickel 1950, Metcalf and Metcalf, 1970). However, although not observed in this result, routes of turtles appear direct at other times, as if the turtle was moving to a specific location. Previous research has also shown that turtles use the same pathways in their habitat repeatedly (Dodd, 2002) and utilize their home range (Madden, 1975) by tending to use core areas for day-to-day activities.

The results of the movement of turtle 2 could also not be representative for the study species in general because the thread could not be checked every 2 hours during the night and therefore the turtle ran out of thread before the 24 hours of data collection were completed. Although it cannot be said after how many hours the turtle ran out of thread, it was possible to record the exact track of the turtle up to 64m. However, after locating the turtle again and measuring the distance from the end of the thread to the new location (air-line distance) of the turtle (after 24 hours exactly), it was assumed that the turtle had moved at least 99m in total. Nevertheless, this distance is only estimated because the turtle could have traveled a greater distance before settling in that one particular last location.

It is likely that the spooling data that was collected during this study is slightly biased because it is possible, that the reason for the turtle's movement was the disturbance of it by the researchers. Although thread trailing is a traditional field method for the study of spatial patterns, other researchers say that the method only provides partial, often discontinuous, information and generally involve some interference with normal behaviors (Dall'antonia et al., 2001). Therefore it has to be assumed that the long handling time of one hour while attaching the spool and the checking of the thread every two hours, influences the turtle in a way where it increases its movement. This would

mean that the collected data is not representative for the species' movement under undisturbed circumstances. However, once the thread trailing device is attached to the carapace of the turtle, spools can be easily changed, often within a minute and the turtle is relatively undisturbed.

During the survey on the daily movement of the turtles it was attempted to spool every turtle as often as possible for a period of 24 hours. Unfortunately, it was only possible to attain this data once from one turtle. There are many steps involved when collecting this data and although the data represents the movement of a turtle within 24 hours, a minimum of 48 hours is required to actually collect the data. For once, it was sometimes difficult because the telemetry device, which was used to locate the turtles, broke down a lot due to high humidity and age of the device. Also, the individuals of the study population were quite far apart, so it was not possible to easily switch between turtles. Furthermore, the habitat of the turtles is very dense and difficult to access. It was observed that they preferred 'hiding' in places where they could be located but not reached (within big, hollow logs). This behavior was not expected but it is very typical for the Keeled Box Turtle because generally, they live very secluded and hide most of the time (Aqualognews, 1998). It is therefore assumed, that the wild turtles were disturbed too often during data collection.

If the turtles could be reached it was often very difficult to attach the spool to the carapace because even if it was not raining that day, the high humidity made the carapace too wet for the marine glue to stick to it. The turtle had to be handled for approximately one hour to make sure the spool was not going to come off immediately but often it was observed that after releasing the turtle, the spool got scraped off under a tree log or other vegetation because the turtle was trying to escape and hide. This is a common problem with smaller tortoises that move a lot in densely structured habitats (Loehr, 2004).

Another problem during data collection was the monitoring of the spooling in case the turtle ran out of thread. Although the spool was checked every two hours, this was not possible at night because it was quite easy to get disoriented in the dark, to move in the difficult terrain and the venomous fauna of the jungle. Another reason for monitoring the spooling data collection was the potential detection of the thread and/or turtle by humans. If the turtle crossed a pathway only once during the 24 hour period it would have been easy for tourists to follow the thread and find the turtle.

Recording the turtle track with the GPS tracking program was not possible because of the dense canopy and an overcast sky. The accuracy for taking coordinates with the GPS device was often over 35m and with all the direction changes and the relatively small distance that the turtle moved it was only possible to record the coordinates by using the reference tree method. This method takes a lot of time of at least two persons and is difficult because it involves taking a lot of measurements. There is also the chance of small miscalculations of the coordinates because after taking the measurements, there are many calculations which have to be carried out (see Appendix IX).

A general problem with this data collection was also that the weather was often too severe to go out into the jungle to spool a turtle. During a thunderstorm, for example, it was dangerous to remain in the jungle because of falling branches, fruits etc. Another concern during the data collection was the destruction of the habitat of the turtle. Because the vegetation was so dense and inaccessible, it was obvious where a path had

been cleared in order to locate the turtle. This is a potential threat to the turtle because tourists will be more likely to investigate this path, find the turtle and potentially harm it or take it with them. For this reason, some areas around trees, where turtles were known to hide under, were not cleared in order to protect them.

6. Conclusion

What is the time budget and daily activity pattern of the Keeled Box Turtle?

The Keeled Box Turtles at the TCC spent a mean average of $57.99\% \pm 11.45\%$ of their time *resting*. *Under litter* and *observing* accounted for $20.96\% \pm 10.22\%$ and $17.92\% \pm 5.40\%$ respectively of their daily time budget. Only $3.13\% \pm 2.12\%$ of their time, they expressed the remaining behaviours *foraging*, *locomotion*, *social activity*, *investigating* and *agonistic* behaviour. The behaviour *basking* was not displayed at all during these observation periods. The data revealed large differences between the behaviours of the four individuals, which can be seen considering the minimum and maximum percentages for each behaviour type. The behaviour *resting* was for example performed between 28.72% and 80.06% of the time; other behaviours like *foraging* would not be expressed by all individuals, percentages of time spent ranging from 0% up to 4.49%.

The daily activity patterns revealed a significant relation to the time of the day.

No active behaviour at all was shown from 10:00 pm to 2:00am. In contrast, peaks in activity were shown from 6am-8am ($54.58\% \pm 18.95\%$), 8am-10am ($47.92\% \pm 27.68\%$) and 8pm-10pm ($47.29\% \pm 27.32\%$).

During the peaks in activity in the morning and evening, especially *observing* behaviour (up to 52.92%) was shown. Furthermore, the averaged percentages of time all turtles expressed the behaviour types *foraging*, *locomotion* and *investigating* is during one of the peaks of activity from 8pm-10pm. *Social activity* behaviour was shown longest from 10am-12pm. The averaged time spent *resting* reached from 23.96% from 8am-10am up to 92.50% in the early morning (4am-6am).

What influence does rainfall have on the activity of the Keeled Box Turtle?

The results of the experiment indicate that the Keeled Box Turtles are more *active* during *rainfall* even if the difference in activity could not be proven as statistically significant. During *rain*, the turtles performed $55.05\% \pm 8.89\%$ *active* behaviour, whereas only $20.03\% \pm 7.81\%$ of the time was spent with *active* behaviour *without rain*.

Furthermore the difference in mean percentages of the behaviour types *resting* and *locomotion* shown during *artificial rainfall* and *without rainfall* were tested significant ($p=0.043$ and $p=0.001$). The mean percentage of *resting* during *rainfall* ($29.18\% \pm 7.90\%$) was significantly lower than *without rainfall* ($55.51\% \pm 11.99\%$) whereas the mean percentage of *locomotion* was significantly higher during *rainfall* ($2.12\% \pm 0.49\%$) than *without rain* ($0.38\% \pm 0.17\%$).

What are the daily movement patterns of wild individuals of Keeled Box Turtles in the Cuc Phuong National Park in Vietnam?

The collected data of one individual shows, that the thread-tailed turtle walked a distance of at least 99m. Also, it is likely for turtles to cross the pathways leading through their habitat, at least once in a period of 24 hours.

7. Recommendations

To achieve more knowledge and significant results for the time budget and daily activity patterns of Keeled Box Turtles it is recommended to conduct behaviour surveys with larger sample sizes, because the individuals in this study showed very distinctive durations of behaviour types, which lead to high standard deviations. Therefore, a follow up survey with a larger sample size as well as more observations, using the same recording methods which proved to be suitable for the aim of the survey, is necessary to draw stronger conclusions about the species' time budget and daily activity. The findings could be used for developing conservation strategies for the management of in situ as well as ex situ populations. Research on time budget and activity patterns can help to improve husbandry guidelines and therefore enhance the breeding success of assurance populations in captivity. Besides, the additional ecological knowledge could be helpful for educating the local communities on the importance and intrinsic value of the species.

For in situ research, due to the dense vegetation, it is recommended to verify if the turtles are not disturbed by observers being close to them. Furthermore, behaviour studies on wild individuals should be carried out to eliminate possible influencing factors caused by the captive environment. Although the method is very time consuming, the observations of wild individuals could be conducted by habituating a study population of wild Keeled Box Turtles to the presence of observers prior to the survey.

In order to locate more individuals which can be added to a study population, turtle detection dogs could be used. This is actually a common way for poachers in Vietnam to find turtles but Timothy McCormack and the TCC recently started to use this knowledge and train dogs for turtle searches. Another option of improving the way to find wild turtles and conduct research on them would be the inclusion of former poachers into research activities. By doing so they would be provided with a steady income and their knowledge on the species could be more than valuable during field research.

The use of small electrical devices, even though those are rather expensive, like cameras attached to the carapace could be another method to successfully conduct behaviour research on wild individuals.

In order to gain more knowledge on the relation between rainfall and the activity or behaviour shown by the Keeled Box Turtle further experimental research should be carried out. The method used during the experiment of this study proved to be suitable for the most part and can therefore be used in further studies with some adaptations. It is recommended to use a sample size as large as possible to avoid having high standard deviations. Furthermore more hours of observational data over several 24 hours periods of each turtle of the study population should be collected. A control group should be used as a comparison to the group that receives the treatment during a follow up research in order to conduct a true experiment.

Besides, for further research on the behavior of this species it might be worth considering taking frequencies of behavior types instead of recording durations in order to test if that method leads to similar results.

The gained results display activity peaks throughout the day as well as during rainfall and it is assumed that the turtles are more vulnerable to being poached or collected by

humans at these times. Therefore conservation organisations that aim to protect the species should consider this information when developing their strategies.

The small pathways and especially the main road leading to the park centre are a considerable threat to the turtle population living there. The collection of turtles by humans remains the biggest threat to Asian turtles in general (Tisdell, 1986) and this is also the greatest danger to the turtles in the National Park. Controlling visitor busses frequently with a turtle search dog at the park exit is recommended to reduce the number of turtles being collected in the park.

During the study, turtles were found by tourists and also encountered on the main road where they are in immediate danger. More spooling data of different individuals of Keeled Box Turtles should be obtained to map the turtles' movement within a year, for example, to determine when they move to other parts of the forest and how often they are in danger of encountering humans on roads or pathways. Because even if the home range remains constant for most box turtle species, there may be slight shifts in habitat use which may occur weekly or monthly within a year or season (Stickel, 1950, 1989; Yahner, 1974; Madden, 1975; Strang, 1983). If turtles use a certain trail repeatedly, more effective conservation actions can be planned, like installing a certain barrier to prevent the turtle from crossing a road. Other research has found that drift fences, for example, reduced turtle road mortality (Aresco, 2005).

In case more spooling data is collected, researchers should determine more precisely when the turtle moved where by marking the thread. In addition to simply checking the proper functioning of the spool and the well-being of the turtle, the researchers could mark the location of the spool with tape that has the current time written on it, without disturbing the turtle too much. The future results of movement patterns and therefore turtles' home ranges can be implemented in conservation management of protected areas.

The results of the thread-trailing as well as encounters of individuals of the study species on the main road suggest that the turtles are crossing the pathways and road rather frequently. Furthermore, tourists found a turtle that had been run over by a vehicle. In this context, it is advised that the National Park authorities decide upon restricting the speed limit on the main road through the jungle to rather 20km/h than 40km/h. This would not only protect the turtles but also other endangered species that were observed on the main road. It is realistic to hide speeding cameras in the vegetation along the road that leads into the National Park and as the tourists enter and leave the National Park through the same gate, it would be easy to fine them for speeding. Eventually people would take speeding limits seriously because it was observed during the research study, that money is an important motivation for most Vietnamese people.

During the research it was also observed that tourists frequently leave the pathways to explore the forest. As a consequence, they leave noticeable trails that damage the vegetation and create open spaces. It is advised to educate tour guides about the potential risk to turtles if vegetation is cleared around their hiding places and that they should pass their knowledge on to the tourists. Additionally, appropriate signs which prohibit tourists to leave the pathways should be placed within the park. Another way of

reinforcing the obedience of the visitors of the park is to collect fines if they do not stay on the pathways.

The pathways connect the restaurant area with the accommodation area (see Figure 7 in 5.2 Results) and act as a nature trail for tourists. The problem arising from this is that tourists tend to carry their trash with them in the jungle and leave it there. On the side of the accommodation area where the pathways lead into the jungle, the National Park has already set up trash bins. It is advised that such trash bins are also set up at the beginning of the pathways in the restaurant area, where tourists are more likely to buy food and beverages packages. Also, the National Park could put up, at least, one sign, friendly reminding people not to leave their trash in the jungle. And again, if a friendly reminder does not work, fines can be taken if the rules are not obeyed.

8. References

8.1 Literature List

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8.2 Photograph and Map Credits

Devender, W.v., 2010. Image 1 Keeled Box Turtle (*Pyxidea mouhotii*). [Online] Available at: <http://jcvl.org/reptiles/families/geoemydidae.php> [Accessed 7 June 2010]

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All photographs in the appendices by courtesy of the authors.

8.3 Personal Communication

Ha, H.V., 2010. *Asian turtle program employee*. [conversation] (Personal communication, 19 August 2010)

Hradsky, B., 2010. *Wildlife Conservation and Science Department Manager*, Zoos Victoria, Australia (personal communication, email, 27 June 2010)

McCormack, T., 2010. *Program coordinator of the Asian Turtle Program of Cleveland Metroparks Zoo / Vietnam supervisor*. Contact through: Mr. C. Banks., Zoos Victoria. [phone call, conversation] (Personal communication 21 May 2010; 3 June 2010 and frequently during data collection in Vietnam)

Meier, E., 2010. *Program coordinator International Turtle Conservation Center*, Allwetterzoo Münster, Germany. Contact through: Dr. M. Raffel, In-situ curator of the Allwetterzoo Muenster, Germany [conversation] (Personal Communication, 26 June 2010)

Appendix I Threatened Turtles and tortoises worldwide and in Vietnam

Table 3: International Union for Conservation of Nature 2008 Red List of threatened turtles and tortoises worldwide and 2010 Red List of threatened turtles and tortoises in Vietnam.

THREATENED TURTLES AND TORTOISES WORLDWIDE IUCN 2008			THREATENED TURTLES AND TORTOISES IN VIETNAM IUCN 2010	
	N	%	N	%
Extinct	7	3,3%	0	0,0%
Extinct in the wild	1	0,5%	0	0,0%
Subtotal <i>Extinct</i>	8	3,8%	0	0,0%
Critically Endangered	31	14,6%	4	14,8%
Endangered	42	19,8%	13	48,1%
Vulnerable	59	27,8%	9	33,3%
Subtotal <i>Threatened</i>	132	62,3%	26	96,3%
Lower Risk/conservation dependent	1	0,5%	0	0,0%
Lower Risk/near threatened	42	19,8%	1	3,7%
Lower Risk/least concern	19	9,0%	0	0,0%
Data Deficient	10	4,7%	0	0,0%
Subtotal <i>Lower Risk</i>	72	34,0%	1	3,7%
NUMBER OF SPECIES	212	100,0%	27	100,0%

Source: Vié, 2009; IUCN, 2010

Appendix II Study site - Enclosure at the TCC



Images 3 and 4: Enclosure and service pathway and enclosure insight at the TCC.

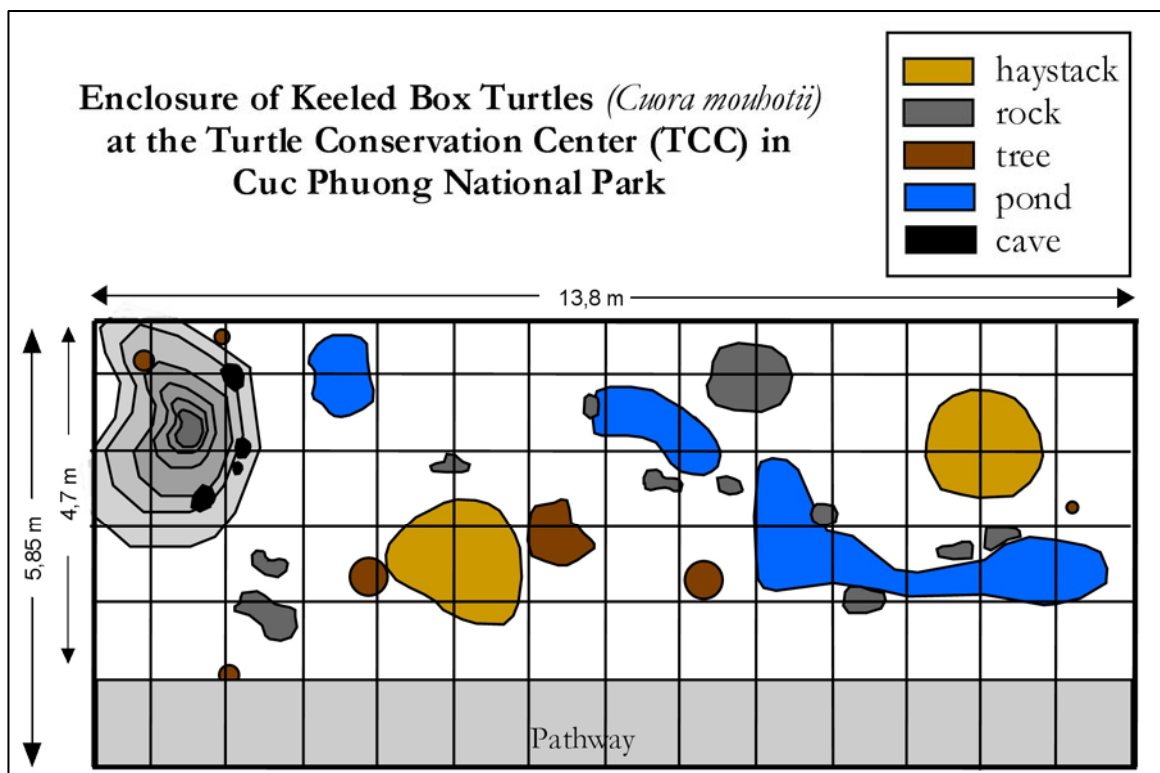


Figure 8: Enclosure where the observations took place.

Appendix III Time table observation shifts

		TIME	12	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
	SHIFT		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	
16. Aug	Sandra																									
	Sarah																									
17. Aug	Sandra																									
	Sarah																									
18. Aug	Sandra																									
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	Sarah																									
07. Sep	Sandra																									
	Sarah																									
08. Sep	Sandra																									
	Sarah																									

	Turtle 1		Turtle 1 with sprinkler
	Turtle 2		Turtle 2 with sprinkler
	Turtle 3		Turtle 3 with sprinkler
	Turtle 4		Turtle 4 with sprinkler

Appendix IV Data sheet survey for time budget and daily activity

Keeled Box Turtle *observation*

Sample #:

Turtle:

Date:		Time start: Time end:		Student:	
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Condition:

Rainfall=1

No rainfall=0

Behaviour types	Code	Definition
Feeding	F	Collecting and consuming food
Drinking	D	Consuming water
Agonistic (behaviour associated with conflict/disturbance)	Afi	Fighting
	Ae	Escaping
	Afr	Freezing
Locomotion	Ls	Walking slow
	Lf	Walking fast
	Lc	Climbing
	Ls	Swimming
Social activities	Sf	Following
	Sc	Courtship
	Scop	Copulating
	Scha	Chasing
Resting	St	Standing, no forward movement
Basking	B	Exposing itself to the warmth of the sun or to rain
Investigating	I	Exploring social, biological and physical environment
Observing	O	Watching/smelling environment
Under litter	UI	Not or barely visible under litter
Not observed	No	No behaviour observed

Time	Behaviour	Condition (1/0)

Appendix V Excel data sheet template

Turtle	
Number	

[illegible]

Appendix VI Data sheet experiment on the influence of rainfall on activity

Keeled Box Turtle observation

Sample #:

Turtle:

Date:		Time start: Time end:		Student:	
-------	--	--------------------------	--	----------	--

Condition:

artificial rainfall=1

no rainfall=0

Behaviour types	Code	Definition
Feeding	F	Collecting and consuming food
Drinking	D	Consuming water
Agonistic (behaviour associated with conflict/disturbance)	Afi	Fighting
	Ae	Escaping
	Afr	Freezing
Locomotion	Ls	Walking slow
	Lf	Walking fast
	Lc	Climbing
	Ls	Swimming
Social activities	Sf	Following
	Sc	Courtship
	Scop	Copulating
	Scha	Chasing
Resting	St	Standing, no forward movement
Basking	B	Exposing itself to the warmth of the sun or to rain
Investigating	I	Exploring social, biological and physical environment
Observing	O	Watching/smelling environment
Under litter	UI	Not or barely visible under litter
Not observed	No	No behaviour observed

Time	Behaviour	Condition (1/0)

Appendix VII Study population for daily movement

Table 4: Short characteristics of the wild individuals.

Turtle Number	GPS frequency	Colour type/name	Mark location	Weight (g)	Characteristics	Rough Location
1	8	Shining Star	Right middle	706	Red eyes, short tail, not afraid or aggressive	Cobra garden, jungle right side
2	14	Just in case	Left middle	546	Red eyes, vocalising while handling	Jungle left side, close to Turtle 4, often under a log
3	7	Speedy	Left middle	521	Red eyes, spare battery beside transponder on carapace, no aggressive behaviour	Far away. 5 km from Bong along the road towards park entrance
4	9/10	Pearl	Left middle	494	Red eyes, tries biting, prefers staying under a log when approaching	Jungle left side, close to Turtle 2, often under a log
5	5	Golden Bronze	Left middle	544	Transponder secured with tape around carapace. First time head and legs inside carapace, second localisation trying to bite	Bamboo Garden, Jungle right side

Note: Weight includes transponder. Rough locations are self-named and describe certain areas in the Bong area.

Turtle 1

Images 5 and 6: Turtle 1.



Turtle 2

Images 7 and 8: Turtle 2.



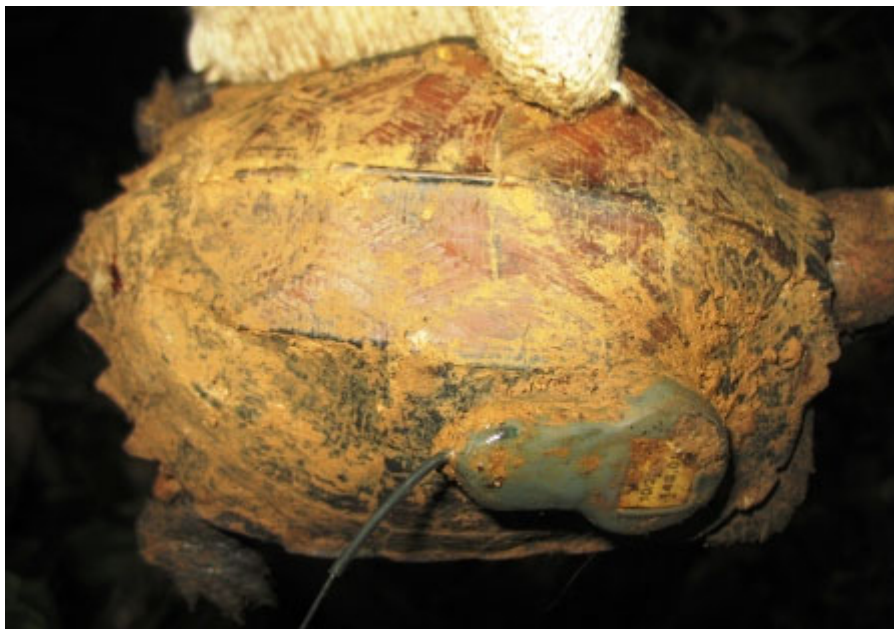
Turtle 3

Images 9 and 10: Turtle 2.



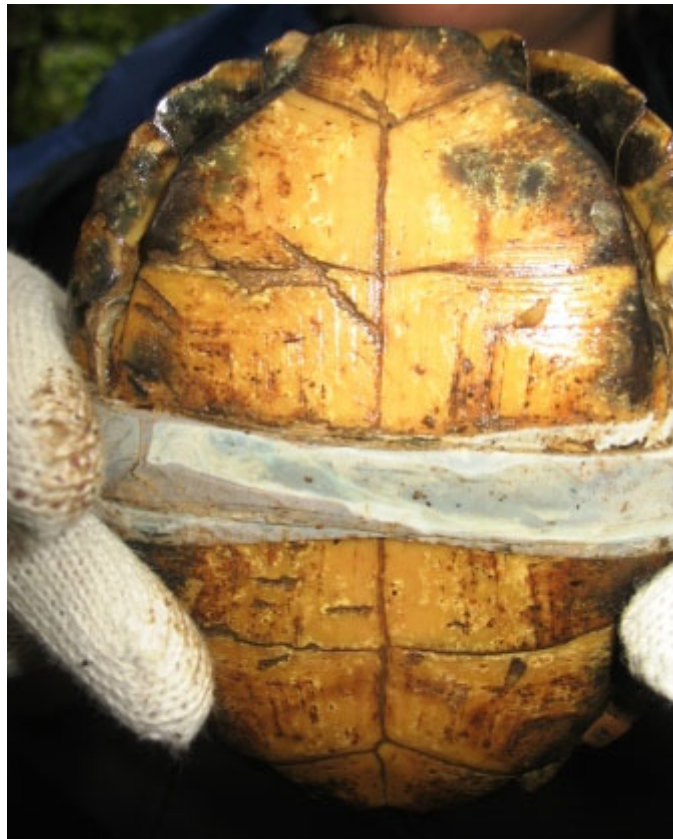
Turtle 4

Image 11: Turtle 4.






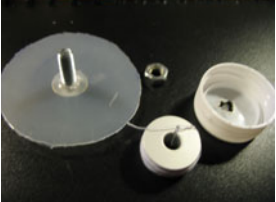

Turtle 5

Images 12 and 13: Turtle 5.



Appendix VIII Spool building

How to build a spool to record daily movement of the Keeled Box Turtle.
Constructed with the help and advice of Mr. McCormack.

	<p>Material (to build one spool):</p> <ul style="list-style-type: none"> • Bottle cap (diam. ca. 5 cm, height 2cm) • Plastic lid of potato chips container (diam. ca. 15 cm – for example ‘Pringles’) • Spool of thread • 1 screw nut • 1 screw (length of screw depends on height of bottle cap) • 1 washers that fit the screw • 1 thread spool (part of a sewing machine) • Scissors • Pocket knife
	<p>1. Cut of the border of the lid of the potato chips to create a platform on which to screw the cap on later. Cut a hole in the middle of the plastic lid just large enough for the screw to fit through. Put a washer on the screw and push the screw through the hole in the lid.</p>
	<p>2. Punch a hole through the middle of the bottle cap just large enough for the screw to fit through. Also, cut a tiny opening on the side of the bottle cap for the thread to fit through later on. Spool the thread on to the thread spool.</p>
	<p>3. Place the spool with the thread on the plastic bottom with the screw sticking out. Then, to cover the spool, place the bottle cap on the spool but do not forget to pull the end of the tread through the tiny hole on the bottle cap side. Finally, to fix the bottle cap, screw on the screw nut (but do not screw it on too tight because otherwise the thread might not run off the spool).</p>
	<p>4. Attach the spool to the back part of carapace with suitable glue (in humid areas use marine glue but also duck tape for security) or duck tape (if duck tape is used be sure it does not influence the turtles mobility). If possible, position it on the carapace so that it is not the highest point of carapace. This way the spool does not get scraped off when the turtle crawls under trees.</p>

Appendix IX Coordinates calculation method (ATCN, 2008)

Rules for working out new co-ordinates for turtle locations with a known straight line distance and point A and B bearings.

Below is an example in which Point A was the previous location of the turtle and point B is its new location, the turtle has moved south east with a known straight line distance (Line AB), the two bearings at point A and B are also known.

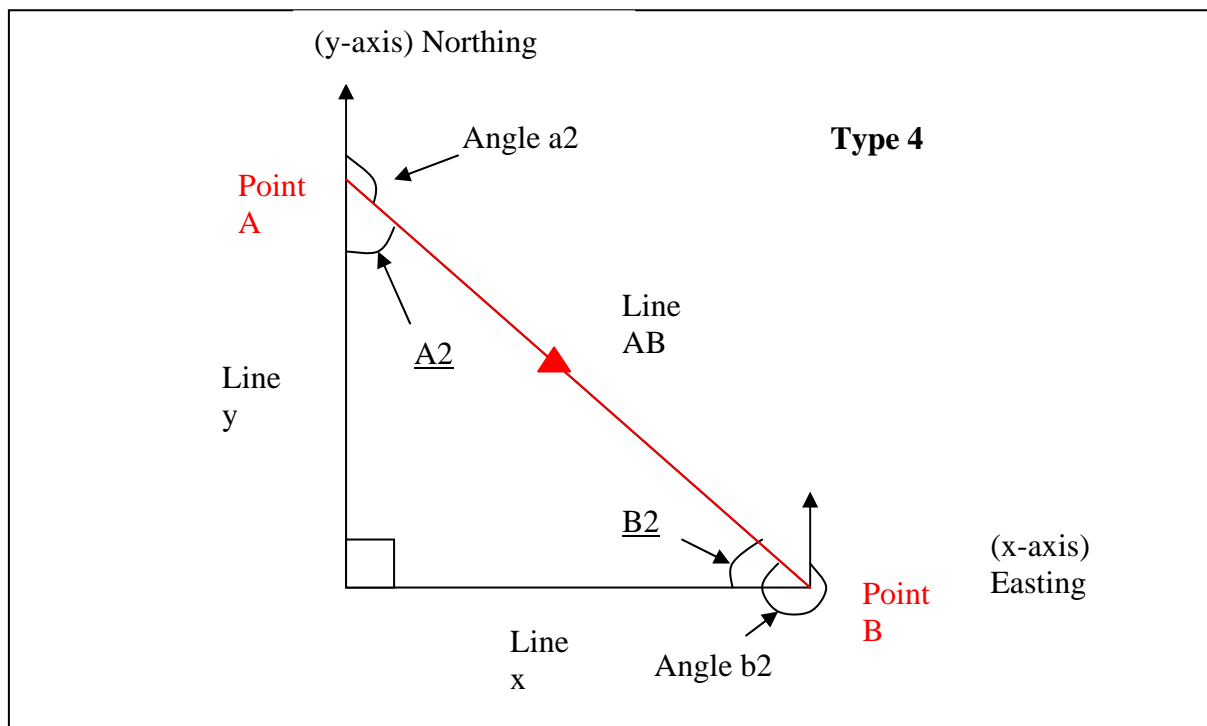
To find out the new co-ordinate for the angle at point B we can subtract the distance of the line y from the GPS Northing value (in metres) for the co-ordinate at point A.

Northing Co-ordinate for Point B = (Northing Co-ordinate @ Point A) – distance y

Similarly for the new Easting value at point B the distance of line x can be added to the known Easting co-ordinate value at point A.

Easting Co-ordinate for Point B = (Easting Co-ordinate @ Point A) + (distance x)

Note: for both new Easting and northing values there are four types of line 'direction' which need to be considered to decide if the lines of x and y should be added to or subtracted from the known GPS co-ordinates at Point A. These are discussed later after this first example which is a Type 4 line direction (South-easterly direction). Also the direction type of the line effects the method used to calculate the angles of 'a' and 'b' inside the right angled triangle formed.



x-axis is also representative of latitude (horizontal lines), which are called Eastings when North of the equator.

y-axis is representative of longitude (vertical lines), which are Northings when east of Greenwich London & west when west of Greenwich.

The Sine rule is used to find both values for lines x and y, the values of the internal angles A2 and B2 need finding first.

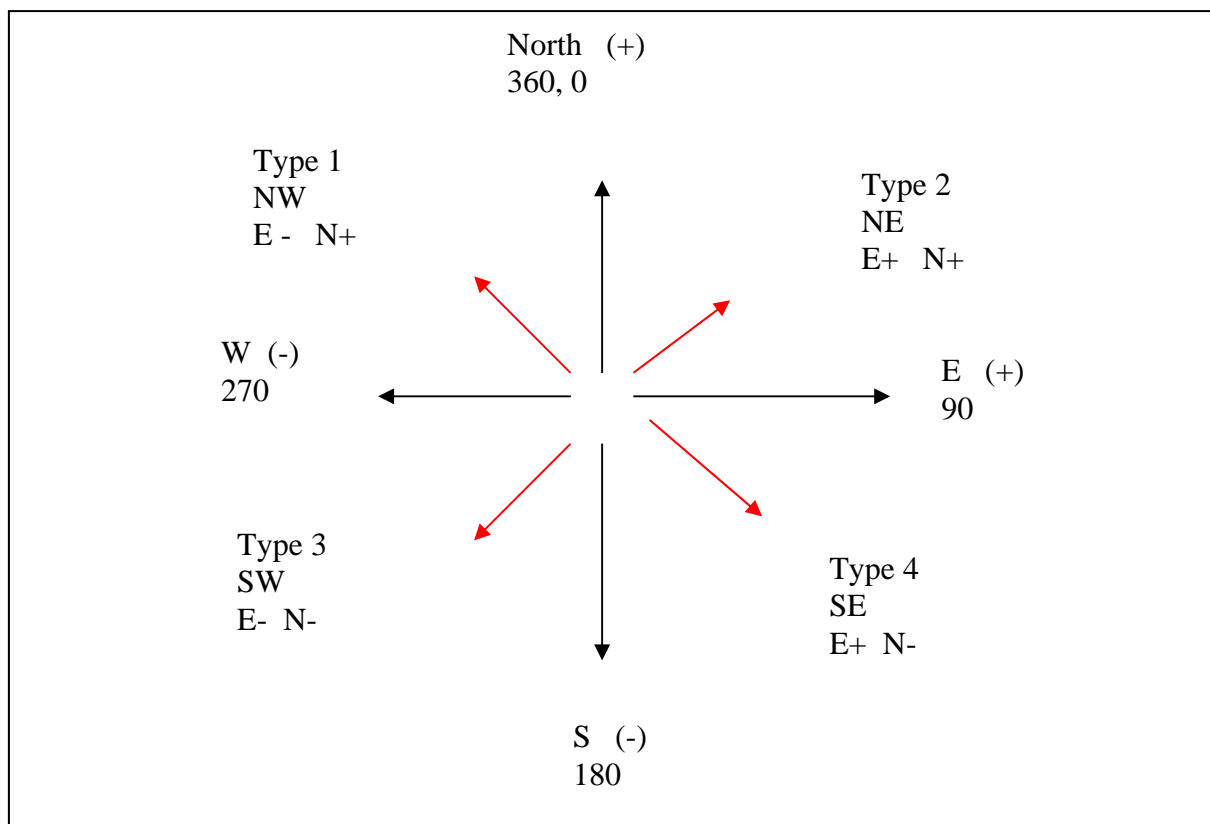
$$\underline{A2} = 180 - (\text{Angle } a2)$$

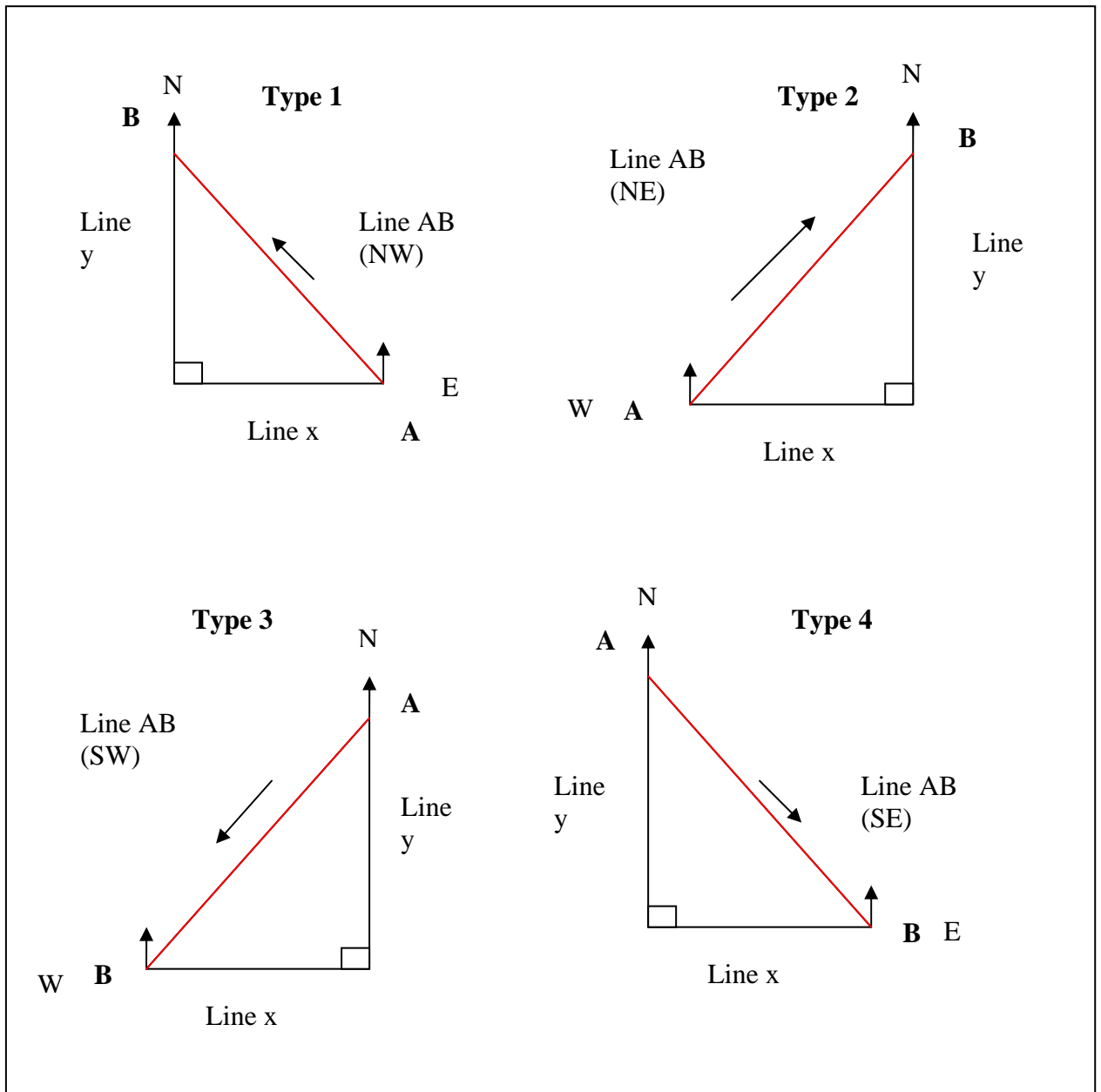
$$\underline{B2} = (\text{Angle } b2) - 270$$

See notes later how the calculations for internal angles A2 and B2 changes dependant on line AB direction type.

Then to calculate the distance of line x = $AB \sin \underline{A2}$

To calculate the distance of line y = $AB \sin \underline{B2}$





For the different type of AB lines the following rules apply.

Type 1.

A line with a northwest direction.

$$x = AB \sin (180-b_2) \quad (-)$$

$$y = AB \sin (a_2-270) \quad (+)$$

For point B's Easting co-ordinate subtract the value of x (-x) from the easting coordinate for point A.

For point B' Northing co-ordinate add the value of y (+y) to the northing coordinate for point A.

Type 2.

A line with a Northeast direction.

$$x = AB \sin (b_2-180) \quad (+)$$

$$y = AC \sin (90-a_2) \quad (+)$$

For points B's Easting co-ordinate add the value of x (+x) to the easting coordinate for point A.

For point B's Northing co-ordinate add the value of y (+y) to the northing coordinate for point A

Type 3.

A line with a Southwest direction

$$x = AB \sin (a_2-180) \quad (-)$$

$$y = AB \sin (90-b_2) \quad (-)$$

For point B's Easting co-ordinate subtract the value of x (-x) from the easting coordinate for point A.

For point B's Northing co-ordinate subtract the value of y (-y) from the northing coordinate for point A.

Type 4

A line with a southeast direction.

$$x = AB \sin (180-a_2) \quad (+)$$

$$y = AB \sin (b_2-270) \quad (-)$$

For point B's Easting co-ordinate add the value of x (+x) to the easting coordinate for point A.

For point B's Northing co-ordinate subtract the value of y (-y) from the northing coordinate for point A.

Table of how to calculate distance of x and y. Then how to use this along with the know coordinates of point A to calculate the new Easting and Northing coordinates for point B.

	Type 1	Type 2	Type 3	Type 4
x	=ABSIN(180-b ₂)	=ABSIN(b ₂ -180)	=ABSIN(a ₂ -180)	=ABSIN(180-a ₂)
y	=ABSIN(a ₂ -270)	=ABSIN(90-a ₂)	=ABSIN(90-b ₂)	=ABSIN(b ₂ -270)
Easting	Point a1-x	Point a1+x	Point a1-x	Point a1+x
Northing	Point a1+y	Point a1+y	Point a1-y	Point a1-y

For lines that fall perfectly on the horizontal or vertical.

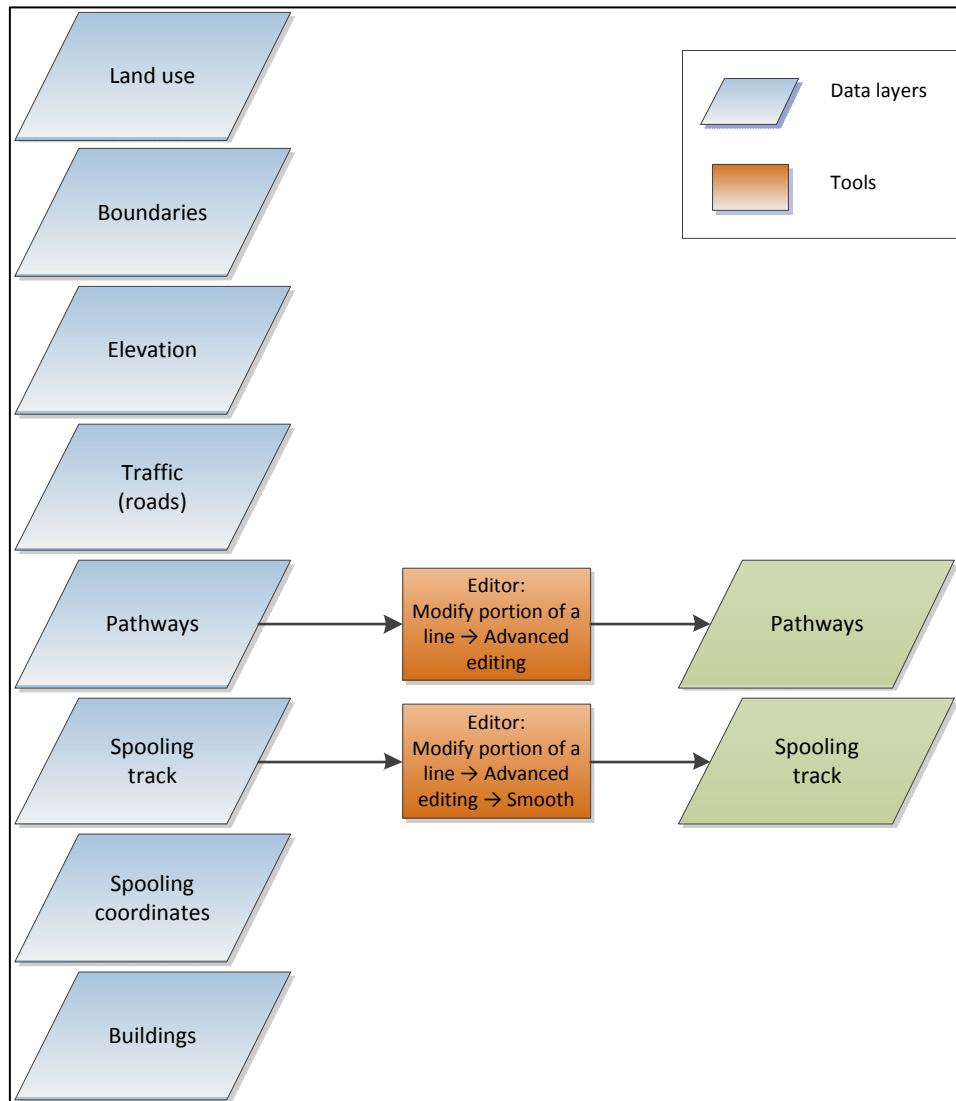


Fig.9: GIS Project Flow Chart - Flowchart of steps executed in ArcMap (tools applied to layers).

Steps

1. Insert data layers *Land use*, *Boundaries*, *Elevation*, *Traffic*, *Pathways* and *Buildings* in ArcMap.
2. Activate extension 'Data interoperability' in ArcMap
3. Insert gpx files *Spooling coordinates* & *Spooling track*
4. Export gpx file data in the same coordinate system as data frame → Output: shapefile
5. Data layer *Pathways* → Tools 'Editor' → Modify portion of a line → Advanced editing → Trim
6. Data layer *Spooling track* → Tools 'Editor' → Modify portion of a line → Advanced editing → Smooth
7. Data layer *Spooling coordinates* → 'Layer properties' → 'Symbology' → Add values of 'name' → change color of individual coordinate points