Chapter 6: Discussion
DISCUSSION

The physiography of Assam, its climatic conditions and wide variety of resources ranging from hill stream to beels, all in combination support a species rich turtle fauna. During this investigation the species *Kachuga sylhetensis* has been located in three new habitat localities viz. Biswanath ghat (26°50'N latitude and 93°21'E longitude), Gomirighat (26°53'N to 3°38'E), Kuruwa ghat (26°9'N to 91°45'E), on the other hand Nameri National Park (Jia Bharali river 26°55'N to 27°0'N), Gomirighat (92°40'E to 93°06'E) and in Kulshi river (26°42'N to 92°48'E) the endangered species has been relocated. However, no live individual could be recorded in Kulshi river, yet the availability of shell is an indication of its occurrence. The habitats of *Kachuga sylhetensis* are fast flowing stream to ox-bow lacks in plains with an extensive growth of vegetation were recorded in this investigation which could be confirmed with the records of other workers (Das, 1988; Choudhury, 1995).

Occurrence of *Kachuga sylhetensis* from the Khasi and Garo Hill areas of Assam of India and Bangladesh was earlier described by Jerdon (1870). This particular species is very timid and fully aquatic inhabitant in either stagnant or slow running water in the hilly terrain with plenty of aquatic vegetation. The first record on the availability of this species on the north bank of the river Brahmaputra was reported from a perennial stream within the Manas National Park (Sarma, 1988). However, availability of this species in various locations of the Northeastern region has been reported (Tikadar and Sharma, 1985, Das, 1988, Bhupathy and Choudhury 1995, Choudhury 1995, Choudhury et al. 1997, Pawar and Choudhury, 2000, Praschag and Fachbach 2001, Praschag and Gemel 2002). The reports and records and identification of new locations during the earlier works and in the present study suggest that Assam provides a very congenial habitat for the turtle species in particular and the freshwater chelonians in general.

Analysis of zoogeography of *Kachuga sp.* provides information and ranges of their natural distribution and boundaries that helps to find out the endemic status of the species. As such the distribution and identification of new localities may significantly
offer an endemic status to this particular group of reptile. Endemism constitutes an important feature in the formulation of conservation strategies. Therefore, distribution pattern of this species and the habitats support this view. The rich habitat that supports 447 species of reptiles found in India including 25 species listed in threatened categories, of which 19 species of turtles and tortoises have been recorded in the North East India (Murthy, 1994). This observation could be supported by the catch report of 136 number of individuals during this study (male 81 and female 55) and it supports the habitat suitability of this region (Table E4). Further, the count of 50 male juvenile and 20 female juvenile during this study confirms the suitability of this region as one of the proper breeding ground.

*Kachuga sylhetensis* was earlier reported to be restricted to the hill streams of Northeastern India and Bengal (Das, 1990). It has also been recorded from Assam, Arunachal Pradesh, Nagaland and Meghalaya (Bhupathy *et al.*, 1994). Presently it has been reported from Mizoram (Choudhury *et al.*, 2000). However, the population status about this species is not known. The major and serious threat of this animal (Schedule I) is mostly the habitat loss and human consumption (Choudhury *et al.*, 2000)

Habitat destruction and over exploitation are the most important factors for the decline of many terrapin in Malaysia, Thailand and India (Tikadar and Sharma 1985, Rao 1985). In this context, Das (1997) emphasised that the Indian population of *Kachuga sylhetensis* suffered a 90% decrease in the last decade and IUCN justifiably declared this species as endangered (IUCN, 2006). Further, Das (1997) reported that the species belongs to the ten most-threatened chelonians of the Oriental region. All other species of the genus *Kachuga* are also decreasing not only in India, but also in Nepal and Bangladesh (Ernst *et al.*, 1997; Sarker and Hossain, 1997), perhaps due to the anthropogenic interference, mostly the removal of sand from the nesting ground. Other general reasons for the decline of the species are illegal slaughtering for meat, egg perdition, water pollution and human disturbances (Gupta, 2000). But present investigation considers that there may be increase in numbers of *Kachuga sylhetensis* in
Spotting and occurrence of various species of turtles were recorded during this investigation and suggested the richness of the turtle/tortoise diversity of this region (List 1). However, the interrelationship of these species group could not be explained with the help of the available findings of the investigation. The potentiality of the chelonian diversity could well be confirmed from the works of Choudhury et al. (2000).

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<th>List 1: Different chelonian species observed in Darrang, Udalguri and Sonitpur Districts.</th>
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<tr>
<td>The present study has also confirmed the presence of following 10 species in different survey sites of Darrang, Sonitpur and Udalguri district.</td>
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<tr>
<td>1. Malayan box turtle (<em>Coura amboinensis</em>)</td>
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<td>2. Asian leaf turtle (<em>Cyclemys dentata</em>)</td>
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<td>3. Spotted pond turtle (<em>Geoclemys hemiltoni</em>)</td>
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<td>4. Indian peacock soft shell turtle (<em>Aspidertes hurum</em>)</td>
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<td>5. Crowned river turtle (<em>Hardella thurjii</em>)</td>
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<td>6. Brown roofed turtle (<em>Kachuga smithi</em>)</td>
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<td>7. Indian roofed turtle (<em>Kachuga tecta</em>)</td>
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<td>8. Indian tent turtle (<em>Kachuga tentoria</em>)</td>
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<td>9. Indian soft shell turtle (<em>Aspideretes gangeticus</em>)</td>
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<td>10. Indian black turtle (<em>Malanochelys tricarinata</em>)</td>
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Poska phukhuri near Barampur, Dighirpar phukhuri near Arjuntal, Marasuti of Mangaldoi river near Karimchowka, Beel near Shiv temple of Kuruwa ghat, Beel near Ganesh Kuwari of Dumuni chowki, Khalihoi beel, Rowmari, Chereng and Mowamari beel, Dova near Areng of Darrang district.

Dhansirighat, Mara-dhansirighat, Pond of Rawta charali, Kachubeel, beel near Orang National Park, Namkhala bazaar area of Udalguri district.

Nameri National Park, beel near Beseria village, near Gabharu river, Gahigaon wetland, Gohpur wetland of Sonitpur district of Assam.
Diversity of the *Kachuga species* in this region had already been cited (List 1). Moll and Vijaya (1986) recognized 7 species and 10 subspecies of *Kachuga*, along with the examination of museum specimens of *K. sylhetensis* in Cachar district of Assam and Garo hills of Meghalaya. Das (1988) described the chelonian fauna of the Northeastern states of India, which comprises at least 17 species, belonging to 3 families. Further Das (1990-91) reported distributional records for 10 species of chelonia, based on collection from the NE states. Choudhury (1988) recorded the collection of two roofed turtles of the genus *Kachuga* from the state of Assam, viz. *Kachuga smithi* from Disang river and *Kachuga tentoria* from Fulai-Dighali beel in Pani- Diing area of Sibsagar district. Choudhury (1993) enlisted 8 species of turtle from Dibru- Saikhowa Wild life Sanctuary, Assam along with the significant range of extension for new locality record for *Kachuga sylhetensis* in Sadiya subdivision of Tinsukia district of Assam. Further, Choudhury (1995) confirmed the existence of Kachuga species in the Brahmaputra river and in Lakhimpur district, Assam. Moreover, Bhupathy *et al.* (1994) carried out the studies in Assam and Arunachal and confirmed the occurrence of 14 species of chelonia from Assam and 8 species from Arunachal Pradesh. Further, Praschag and Fachbach (2001) reported *Kachuga sylhetensis* from the Nameli National Park of Assam India, and reconfirmed by the present study. Choudhury *et al.* (1997) recorded four species of *Kachuga* where they had described the distribution and food habits of *Kachuga sylhetensis* in Assam, which are in agreement with the present findings. The present findings suggest the food preference for Spirogyra (20.5%; Table E2).

The richness and the coexistence of various species of turtles could be judged from the observations made by various workers. Praschag and Fachbach (2001) suggested that the black soft shell turtles in Jia Bharali river live in synotypically with the *Kachuga sylhetensis* and *Cyclemys oldhani*. Gupta and Guha (2002) in an attempt emphasized the distribution pattern of chelonians and draw an inference for rich habitat sites that supports for turtle diversity. The external morphology observed is fully in agreement with the findings of Das (1995) and Choudhury *et al.*, (2000). In both the dead and live specimen it was observed that the morphological characters i.e. Straight Carapace
Length (7.6–8.9 cm in dead; 6.2–11.5 cm in live), Curved Carapace Length (8.8–10.4 cm in dead; 8.5–12.1 cm in live), Straight Carapace Width (5.4–6.6 cm in dead; 5.3–9.1 cm in live), Shell Height (3.8–4.6 cm in dead; 3.4–5.9 cm in live) and body weight (36.4–54.2 g in dead; 35.6–195.0 g in live). Body weights were found more in female than in male, whereas the anatomical character was found to be insignificantly different in male and female (Table E4). However, the female is bigger in size perhaps for carrying the eggs. Males and females were sexually dimorphic with the most obvious difference in tail. The tails of females and of juveniles of both sexes are relatively small extending over the marginal scuts, when they withdrawn beneath the marginal to lie against the body. When the tail is extended, the anterior margin of the cloacal opening lies well within the margin of the carapace. The tail of the adult male is longer and much more muscular.

The reproductive biology of the *Kachuga species* is not at all known. During this investigation, nests and eggs of *Kachuga sylhetensis* were observed. The egg laying started during the first part of October and reported to be continued up to the 1st week of December of a year, during which there is a fall of temperature in the winter season (Table E7). It is an established fact that the seasonally changing length of daylight is the most crucial factor in determining the onset of breeding since the pineal is known to be the major site of regulation of the exact circannual photoperiodicity (Miura, 1987). In the same animals photoperiodism may be one of the most temperature sensitive factors in the physiological responses. Hence changes in atmospheric temperature are also essential factors in determining the time of breeding and so is the case of rainfall (2.22–319.32 mm in a year; Table E8), which regulates the reproductive capacity. However, it is difficult to explain the role of rainfall and air temperature as abiotic factors extend enough impact on the egg laying incubation as well as on hatchings. The eggs are somewhat oval and elongated in shape and the incubation period is reported to be 6 to 8 months against 4-5 months described by Tikdar and Sharma (1985). The female *K. sylhetensis* digs a hole of 10-12 cm and lays eggs 6-8 numbers (Plate - SV). The mother covers the hole and used to come back to the water body. No apparent parental care was
noticed for hatching. It was really impossible to ascertain the incubation periods in nature due to fluctuations of environmental conditions. It has reported that saw-shelled turtle eggs were artificially incubated at 28°C hatched between 54 and 62 days, however there are records for 43-123 days for eastern snake turtle. Thus, it has been attempted for the first time to study about the reproductive biology of *K. sylhetensis*, though fragmentary in nature. The hatchlings are getting out from the eggs while there is rain and thunder and that too in the morning hours, as reported by local inhabitant of the habitat area. The moment hatchlings are coming out as observed, they are used to move towards the water body and hatching out from the eggs perhaps be associated with abundance of rainfall for their defence that they can save themselves from the predators. Rest part of the reproductive behaviour is not known. However, the ecological relationship in basking (Plate – SIV.b) and nesting site and reproductive adoption have been reported only in *Chitra indica* (Bhaduria *et al*., 1989). The rise of body temperature in the pre-monsoon season may perhaps one of the major attributors.

Das (1991) reported reproductive biology of spotted pond turtle (*Geoclemys hamiltonii*) of N.E. India, Pakistan, and Bangladesh while Bhupathy and Choudhury (1994) attempted to study the reproductive biology of *K. sylhetensis*, of Kaziranga National Park and Orang National Park.

The basking habit of *K. sylhetensis* was observed during this investigation in Nameri national Park as a part of their budget activity and growth. They are diurnally active, with peaks of activity in the morning and in a bright sunny day of the winter season. Although active throughout the year, they have seasonal activity, which might be related to environmental temperature. Growth also correlates well with the annual cycles of day length and ambient temperature (Priest and Franklin, 2002). Growth seems to cease in winter, even though they do remain active and used to feed. Growth rates are poorly correlated with the size, therefore, it has been difficult to calculate a satisfactory relationship between the size and age. But it is assumed that, in general, juveniles grow much faster than adults, and females grow faster than males.
The fundamental property of living organism apart from the capacity of "Self Replication" is the ubiquitous phenomenon of rhythms. The time span of rhythmic processes ranges from seconds i.e. the firing of an isolated neuron to periods of one year (seasonal rhythms) and rhythmic phenomena persist under constant environmental conditions. However, these rhythms are very much unknown in *K. sylhetensis*. That the observation made during this study was the basking in the morning hours during winter continued up to the month of February of a year. The basking habit as observed (Plate-SIV.b), may be correlated with the bright morning sunlight in "Nameri National Park" perhaps be effected by the endocrine system (Roelfsema, 1987).

The relationship between the maternal traits (i.e. nest site choice, lay date and nest depth) and the fitness-related attributes of offspring (hatchling sex and embryonic survival) in the riverine turtle *Carettochelys insculpta* has recently been addressed (Doody *et al.*, 2004). The principal determinant of hatchling of sex in 140 nests was lay date apparently related to the magnitude of the previous-wet seasons. Clutch laid earlier in the season, produced mainly males while late clutch were mostly female due to seasonal increase in air temperature.

The habitat availability observed during this investigation is bound to govern by the physicochemical factors. During this study period 3 new habitat spots were located and the influence of various physiochemical parameters was noted.

Transparency is one of the important parameters that affects growth and development of aquatic animals including turtles, which indicates the amount of biotic community in water. Since these communities interfere with the passage of light into the water, hence it becomes a limiting factor in the productivity of hill stream river, beels, ponds etc. It is also an indicator of eutrophication since the transparency of water body is affected by biotic community like algae (Karez *et al.*, 1994).

Several workers confirmed that 30 cm or less transparency, due to suspended particles, eroded soil, slit etc. may prevent growth of plankton in water, while more than 70 cm
indicates less productivity of water (Benerjee 1967; Paraschag and Gemal, 2002). Bayly et al., (1975) recorded that the brown colour of water severely limits penetration of light, which together with low concentration of inorganic ions restrict photosynthesis and presence of poorly developed phytoplanktons. Hence the low productivity of water body may affect the productivity of turtle as the turtles are deprived of their natural food in such water body. These variations were within the normal range in the water of Jia Bharali river, Biswanath ghat, Gamirighat, Kuruwa ghat and river Kulshi. The water of those riverine habitat areas was not turbid and sufficient turtle food organisms were present (Table E2). Thus the findings on transparency in Jia Bharali at Nameri National Park may support the concept of ideal habitat for *K. sylhetensis* in particular (45.5 cm to 63.5 cm).

The variation of transparency within the seasons, i.e. pre-monsoon, post-monsoon and winter were within the limit of normal range (45.5 to 72.5 cm; Table E9) and with no or little turbidity as well as sufficient food organisms support these places as ideal breeding ground for this species. But the overall records in relation to transparency seem to insignificantly differ from one another thereby extends an indirect support to other sites as habitat.

Eilers et al. (1984) had studied the distribution of the aquatic insects and planktons with respect to pH while Woodcock et al. (2005) suggested that competitive dominance of a taxa was responsible for lower richness in low pH wetlands, whereas competition was weaker in high pH wetlands, making coexistence possible which confirms our investigation that the water quality of the study sites is within the normal range showing more planktons which is very much a positive indicator of *Kachuga sp.* abundance. The water bodies with low pH and high accumulation of organic matters of terrestrial origin are not conducive for bacterial degradation, so that particulate and dissolved humid compounds are metabolized very slowly and prevent the growth of plankton (Wetzel, 1975). The pH variation observed in this investigation was between 6.5 to 7.6 and interestingly the winter season in all the study sites showed pH in the alkaline side. Reports showed that even with the dominance of humic acid and low pH (4.0 – 6.0) the
Fraser island short neck turtle showed no significant change of numbers of the species in lakes (Wetzel, 1975). Therefore, it is very much speculative that the range variation of pH may not have direct impact.

The free Carbon dioxide range of this investigation in the water of the study sites was found within the normal range (4.4 to 11.4mg/L; Table E10), which has a positive effect on the growth of planktons and thus increases the *Kachuga* population which was earlier suggested (Smith and Griffiths, 2000) and that decreases in CO₂ affinity beyond the rate of assimilation, could be attributed to increased diffusion limitation.

Atmospheric factors which affect the reproductive capacity of animals include not only the physical factors light and heat; but also chemical and biological parameters. Among the chemical factors, besides humidity or water vapour; CO₂ is also known to fluctuate seasonally as recorded in this investigation, owing to the changing photosynthesis of water plants including the phytoplankton. Earlier Rodhe (1969) emphasized the importance of the rate of Carbon fixation in aquatic habitats to denote their relative place on a trophic scale. The changing pattern of CO₂ of this investigation (Table – E10) showed no effect on the reproductive capacity of animals which was proved by Lloyd *et al.* (1958). Recent observations made by Etchberger *et al.* (2005) show that higher CO₂ lengthens the total incubation time and thus mimics the longer incubation time produced by low incubation temperature should increase the tendency to produce males in *Trachenys seripta*, while total incubation time was significantly lengthened with elevated CO₂, sex ratio was altered in favour of females.

The dissolved Oxygen (DO) having a relation with reference to species abundance, may be confirmed with the findings of following workers, Kramer (2005) studied the behavioural responses of fish to reduced level of dissolved Oxygen and the risk of predation varying with the Oxygen availability and the type of behavioural response. Recently, Myers and Halfman (2007) discussed the essentiality of dissolved Oxygen in aquatic life where dissolved Oxygen can extend impact on many physical and biological factors in water body such as lake. The DO in this investigation ranges between 7.4 –
14.8mg/L and it is the highest data obtained in Jia Bharali habitat site. The higher amount of DO (Table E10) might be associated with abundance of *K. sylhetensis*, which was also observed by Praschag, P. and Fachbach (2001). Thus the proposition that with the presence of high DO the growth of food spectrums is more. Therefore, Jia Bharali has been presented to be an ideal home for *K. sylhetensis*. It is also interesting to note that except Jia Bharali river the DO of other habitat sites showed depleted level of DO. The possible explanation may be associated with little or no disturbance in the protected areas. However, the aquatic Oxygen level had no effect on the dive duration of *Emydura macquarii* (Priest and Franklin, 2002) and no such observation was possible in this investigation.

The Nitrate level has an influence on aquatic life. And this investigation shows low Nitrate level (0.10 – 0.15 mg) in Jia Bharali river with more *Kachuga sylhetensis* compared to Kuruwa ghat (0.19 – 0.22 mg; Table – E11) where more Nitrate and less number of *Kachuga sylhetensis* is evident. Our investigation is also in accordance with the report of Canadian Council of Ministers of the Environment (2005), where it has been put forward that excessive Nitrate may result an indirect toxic effect to other aquatic organisms. Excessive levels of Nitrate are also directly harmful to aquatic animals.

The present investigation shows that Jia Bharali river contains maximum amount of Phosphate (Table E11, 0.140 mg/L) with highest number of *Kachuga sylhetensis* (male 30 female 20). The presence of higher number of individuals of this species in this area might be indirectly associated with high amount of Phosphate, since phosphorus is required for the growth of holophytic plants that are consumed by the *Kachuga* species as well as Phosphorus is required for the algal growth (Schwoerbel, 1987). The range variation of Phosphate i.e. within 0.1 to 0.28mg/L may support rich vegetation in these study area, but needs sufficient details.
Murphy (2007) has measured alkalinity ($A_t$) as the buffering capacity of water, or the capacity of bases to neutralize acids. He has further measured the importance of $A_t$ in determining a stream's ability to neutralize acidic pollution. $A_t$ does not refer to pH, but instead refers to the ability of water to resist change in pH. The presence of buffering materials helps to neutralize acids as they are added to the water. Recently, Murphy (2007) has further stated that the water with low alkalinity is very susceptible to changes in pH and the buffering capacity of the water. The range variation for $A_t$ observed in this investigation was between 23.5 to 40.5mg/L perhaps be suggested for good buffering progress of the habitat water.

Stimson (2004) has summarized the available toxicity data of Chlorides on freshwater plants and animal species. For aquatic animal, acute effect (short term effects of high concentration), and chronic effects (long term effects) were documented. The acute effects were observed at the concentration between 870 to 1070 ppm, which support our data (Table E11; 8.3 – 18.7mg/L), which falls within the normal range. Further De (1996) has studied the concentration of Chloride in natural water generally bears a strong correlation with the Sodium content and conductivity. However, impact of Chloride on the habitat analysis could not be explained.

Wurts (2004) has emphasized on the importance of water hardness in relation to fish culture and has further measured the quantity of divalent ions (such as Calcium, Magnesium and/or Irons) in water and their presence in water for fish culture. Wurts (2004) attempted to project the relationship between the water hardness and the growth of phytoplankton for the growth of fish. Possibly the herbivorous turtle's growth may be indirectly associated with the water hardness.

Water hardness levels up to 200 ppm have been considered to be of salutary value in freshwater conditions. Water hardness has a neutralizing effect on toxicants. At the same time high levels of water hardness have been found to retard breeding, affects the hatching process of organisms especially at juvenile stages beyond a certain limit. (Manigandavelu, 2006). Water hardness measured (20.8 – 40.5mg/L) in this
investigation has been found to support *Kachuga sylhetensis* abundance, particularly in Jia Bharali river.

The living creatures are influenced by temperature and rainfall as well as many other factors like nutrients, disease producing microbes and microorganisms, where viability is dependent on the air movement, temperature and humidity (Bouma, 1987). Moreover, the fertility of animals shows a pronounced seasonality with temperature and light affecting the production of sex hormones (Anderson and Cox, 1974). The Greeks were the first to describe that the atmosphere and its changes affect the functioning of the animal system, which has been acclaimed only in the twentieth century. In the fresh water river, ponds and lakes, the temperature never falls below 0°C and in oceans never falls below – 2.5°C. The temperature of hot spring may reach up to 100°C. Temperature of the air near the land surface is 17°C higher in the daytime than at night. In deserts this difference may be as much as 40°C. But in water bodies like a deep lake and river the difference between day and night temperature is usually less than 1°C. The maximum diurnal change in temperature in marine environment is about 4°C. The temperature changes in the river, lakes and ocean are much less and much slower than in terrestrial environment. Temperature is one of the most important factors in a given habitat. It has an universal influence and is frequently a limiting for the growth, development, reproduction, distribution and migration of turtle. The temperature also has effects on the life cycle of animal though literature is scanty on the turtles life history. The highest relative humidity measured in this investigation was 88.50% in Jia Bharali area during monsoon (Table E9). Though it is not known about the effect of relative humidity as such, yet, it has enormous impact on the habitat site. Kearney and Porter (2004) have emphasised the importance of relative humidity along with temperature in relation to niche in distribution of a nocturnal lizard, *Heteronotia binoei*, across entire Australian Continent. These workers further suggested that effect of mild raise in relative humidity and temperature of the soil may effect the egg development of the organism.
Analysis of the gut content of *Kachuga sylhetensis* showed that the algae groups are the dominant constituents of the food spectrum (37.6% and 40%; Table E2) representing 60–62% vegetation, while it has preference for zooplankton up to 38–40%. Algae has achieved importance as preferred food for protein for this species as well as for other aquatic vertebrates (Choudhuri and Sarkar, 2003). The present study on *K. sylhetensis* suggests a qualitative account of the food spectrum on which they depend. The species prefers *chlorophycae* (green algae), *cynophycae* (blue algae), *pistia* (barpuni), *ipomea* (kalmau), *lemna* (sarupuni), *cynodon grass* (dubari), *enhydra* (helonchi), *daphnia*, *cyclopes*, *crustaceans larva*, small fishes (minnows), earthworms and snails (Plate - E2). The records of the length of the alimentary canal suggest omnivorous habit of the species (Table E5), which has been suggested for the first time in this investigation.

This finding suggests the omnivorous habit of *K. sylhetensis* and is in agreement with the observations of Das (1995a). Auffenberg and Khan (1991) recorded the omnivorous nature of *K. smithi* and suggested that while the species is omnivorous, flesh is the preferred food. However, the present findings are not in agreement with the records of Minton (1966) that insects were commonly taken. Minton's observation was based entirely on the behaviour of captive but without stomach analysis. Afferberg and Khan (1991) concluded that *K. sylhetensis* is by and large a herbivore *Kachuga sp*. It is also a known fact that the pattern of food habits differs in the stages of development that it may be more carnivore during juvenile period. In view of the general need for proteinaceous diet, Choudhuri *et al.* (1997) described that in captivity *K. sylhetensis* is omnivorous and largely nocturnal although some feeding takes place during early morning. In captivity the food includes are pistia, eichhornia, stem of utricularia, leaves of salvania, inner soft part of ipomea, molluscs, aquatic insects, prawns and fishes (Das, 1995). Sarma (1988) observed this species in captivity that accepted small freshwater fishes and earthworm and thus it appears to be a favoured item in its diet in captivity and 3 to 4 earthworms are consumed at a time.

Choudhuri and Sarkar (2003) studied the microorganisms, which comprise a substantial proportion of food for fish fauna, avifauna, turtles and tortoises. Zooplankton
community plays an important role in the aquatic food chain and also contributes significantly to the secondary productivity of freshwater ecosystem (Saikia and Das, 2003), which also helps in biomonitoring the fresh water ecosystem (Sinha, 2001). Earlier Goswami (2004) has studied zooplankton in monitoring freshwater pollution by adopting appropriate diversity measures of species.

Earlier workers (Hynes, 1950) by way of killing method recorded the stomach content of *K. sylhetensis* and they were mainly plant materials and there was no demonstrable food preference of male and female. However, the stomach contains the greatest amount of food in May, August and October, but less in August as noted in this investigation. During this investigation, it has been observed that there is diminution of food in the stomach during July perhaps due to the reduced attention of the feeding, since this month seems to be the courtship and breeding period, however, these data are not available for *K. sylhetensis*. There is a distinct shift in diet with ages, with small juvenile’s feeding almost exclusively on crustacean larvae while the larger turns to be more vegetation. This observation could well be supported by observations on *Emydura kreffleri* (Georges, 1982) and the diet of the mature male and female of the same size does not differ appreciably.

Atomic absorption spectrophotometer is one of the most advanced methods for quantitative determination of low concentration of element in biological materials. The significant variation of element profiles viz. Ca, Fe, Mn, Se and Zn in water and soil (Table 12 and 13) as well as in the gut of *K. sylhetensis* (Table 14) has been reported for the first time in this investigation.

The macro and micro levels of certain metals have influences on the turtle population concentration of certain elements. Fe, Mn, Zn, Se and Ca in habitat water (Table E12) and soil of *K. sylhetensis* (Table E13) along with their evaluation in tissues (Table E14) have been highlighted for the first time in this investigation. The role of elements in organisms life both at trace level and macro levels has been evaluated at various level (Rastogi, 1977).
For example, the survival of some organism is more likely to be dependent on the availability of Fe rather than Mn; as with the Mn, acquisition of Fe is quite difficult at alkaline pH. Further, it has been inferred that the alkaline environment would selectively suppress the growth of some organism by interfering with the Fe uptake (Weinberg, 1984).

The result of certain element quantification for water shows that Se is dominating in all the seasons (2.22 - 0.72 ppm) followed by Ca (0.92 - 0.01 ppm) and Fe (0.58 - 0.01 ppm). Investigation of mineral requirement in *Kachuga* is probably the most untouched area of research. Minerals are not synthesized within, although they are essential elements and affect various metabolic processes (House, 1967). According to Ponta (2002), the admitted values in the surface water are 50 (Cu), 30 (Zn) and 100 – 800 (Mn) mg/L.

However, workers related to the accumulation of heavy in *Laminaira digitata* was ascertained (Bryan, 1969). Considerably high amount of Cu concentration 50.27g/ wet weight was mentioned. Cadmiun concentration decreased with the increase of carapace length. Juvenile green turtles in the pelagic ocean are likely feed on zooplankton, while adult coastal inhabiting green turtles mainly feed on seagrasses and seaweeds. Cd is lower in zooplankton. The specific accumulation of Cd found in the green turtle seems to be related to their feeding habit, which is a shift from carnivorous to herbivorous at different growth stages, but remain untouched in this investigation.

The soil properties are dependent on climate, living organisms topography and on the parent mineral material (Lag, 1987). In a climate with high precipitation, relatively large quantities of soluble materials are removed from the soil. The topography is heavily dissected resulting the run of the greater amount of water from the surfaces, which cause erosion, an evident phenomenon in the Brahmaputra valley of Assam. Thus the organisms are in interaction with the soil depending on it as well as influence it.
Changes in pH during soil formation influences the possibilities of the plants to take up nutrients. Many essential elements will be more readily available when pH decreases. These are however, exceptions for example; plants have better supply of molybdenum and selenium with increasing pH (Shamberger, 1976).

Soil pH profoundly influences availability of mineral elements, including physiologically important trace metals (Verlecar and Desai 2004). For example the Mn concentration is especially critical for species of some microbes. Consistently at least 100 times more of this metal than the quantity of 0.1 μM needed for vegetative growth is required by the cells of certain microbes (Bacillus) for depression of synthesis of secondary metabolites. Although, certain types of soil generally vary in Mn concentration from 200 to as much as 10,000 μM, yet in some other soils it is very much short due to the formation of Manganese dioxide variation of Mn between season as well as habitat site which has been found significant (P<0.05; Table E12).

The same trend was also followed for soil where Se with 2.23ppm was found in more throughout the study period followed by Ca (1.02 – 1.92 μg/g) and Mn (0.99 – 1.09 μg/g). Highest amount of Se was observed in Biswanath ghat and the lowest was recorded in Jia Bharali river observations on the soil. Se concentration from 2.01 to 2.32μg/g (Table E13) is very much suggestive of its presence in soil within tolerance limit. Significant variation both between the habitat site and between the seasons is also evident. The threshold level of selenium (4 ppm) in soil above which it affects aquatic life was earlier recorded (Garrett and Inman, 1984; Finley, 1985).

Fe was detected more in intestine than in liver and stomach. Regerand et al. (2004) has demonstrated that iron altered the value of the cholesterol-to-phospholipid molar ratio. In the absence of lethal effects, this was indicative of attempts to switch over to adaptational biochemical mechanisms to stabilize cellular structures. Perhaps more Fe (mean value) in water (0.09±0.05 and 0.34±0.02; Table 12 E.a) and soil (0.18±0.01 and
0.53±0.02; Table 13) may have some deterring effect on the abundance. Figure D1 is very suggestive to draw an inference with the availability of the species and the Fe content.

Fig D1: Comparison between the Fe concentration and abundance of the species

Ca was found to be more in intestine than in liver and stomach. FAO (1980) has studied the importance of Ca, as natural diets are rich for most aquatic species and are also capable of extracting dissolved Calcium directly from their aquatic environment. The
aquatic species are shown to efficiently extract Calcium from running water containing 5 ppm of the mineral element. Higher amount of Ca (mean value) concentration in water (0.82 µg/g; Table E12.b), soil (1.92µg/g; Table E13, Figure D2) with higher abundance of *K. sylhtensis* in Jia Bharali habitat site demands further study.

![Graph showing comparison between Mn concentration and abundance of species](image)

**Fig D3: Comparison between the Mn concentration and abundance of the species**

Higher Mn was found in stomach compared to intestine and liver. Ponta *et al.* (2002) compared the Cu concentration to the upper limit of tolerance for aquatic organisms (15 mg/L) and there is no risk, since the acceptable level of alkaline salts and dissolved Oxygen acts against the Cu toxicity. In the case of Mn and Zn, even when the admitted levels are exceeded, their content in water is almost equal (Mn) or far (Zn) from the upper tolerated value and there is no risk for aquatic organisms. Problems arise only in relation with the use of water in agriculture in these areas. The present value of the Mn in water (Table E12.c) and soil (Table E13) showed that lower Mn in water might be correlated with abundance (Figure D3). However, Mn difference in soil samples differed insignificantly. But the higher quantum of Mn in stomach and intestine may be associated with the process of digestion of plant materials.
Se is an essential trace element in animal diet, but it is toxic at concentrations only slightly above the required dietary levels. Habitat soil Se concentration has been assumed to be in the normal range (2.01 – 2.32 μg/g).

Though the role of Se is not known on this species, yet Se was added to animal feed to counteract with the Se deficient disease in New Zealand. Thus Se has been proved to be essential for growth in animals, but perhaps prevents the cardiovascular disease in man (Masironi, 1987). Figure D4 demonstrates higher Se concentration in water and soil with more number of *K. sylhensis* in Jia Bharali river. However, the relationship could not be argued with the help of presently available literature. The figure D1 suggests a possible relationship of Se with the habitat sites as well as with the *K. sylhetensis*.

![Relation between abundance of species and Se conc.](image)

**Fig D4: Comparison between the Se concentration and abundance of the species**

Zn is another essential element, which at elevated concentration may be toxic (Eisler, 1993). Although the tissue residues are not reliable indicators of Zn contamination, it interacts with numerous other elements as well as demonstrates its accumulation, metabolism and toxicity (Eisler, 1993). Figure D5 demonstrates a lesser amount of Zn concentration in water (Table E12.e) however, soil Zn concentration is in reverse order,
with higher abundance of *K. sylhensis* and it is difficult to infer logical inference from this investigation.

Fig D5: Comparison between the Zn concentration and abundance of the species

In the present investigation detailed histological studies of duodenum, stomach, liver and small intestine have been carried out, which show some morphological variation than in other vertebrates.

The alimentary canal observed for *K. sylhetensis* (Plate 2) is meant for herbivorous approach. Unlike *Callagur* or *Morenia* there is adaptive modification in the gut structure of *Kachuga* (Bauer, 2003). The intestine was long (Kardong, 2002), caecum was blunt and small and the hindgut was 2.7 cm long. These characters reflect the herbivorous nature of the species. However, the foregut is used to digest the carnivore components as has been suggested (Bauer, 2003).

Identification of cellular changes however may be difficult by light microscopy alone and hence ultra structural studies using scanning and transmission electron microscope appear to be important for better understanding (Bloom and Fawcett, 1968).
Regional distribution of endocrine cells was studied in the gastrointestinal tract of the freshwater turtle (Tarakci et al., 2002). *Mauremys caspica caspica* by immunohistochemistry using antisera against serotonin, somatostatin, gastrin, insulin, substance P, glucagon calcitonin gene related peptide (CGRP) were observed by (Tarakci et al., 2002). The immunoreactive cells were located in the gastric glands of stomach regions and in the intestinal epithelium with variable frequencies. Most of the immunoreactive cells in the intestine were spherical or spindle-like in shape (open type cells), while round cells (closed type cells) were occasionally found in the stomach. Serotonin – immunoreactive cells were most commonly found in the pylorus and duodenum. Gastrin- immunoreactive cells were restricted to the ileum and rectum at low frequencies. Insulin- immunoreactive cells were detected from pyloric to rectum.

Presence of the goblet cells as observed in the stomach, duodenum and intestine (Plate E4, E5, E6, E8, E9) are distributed in the columnar epithelial cells. These cells are characterized by the invagination of apical border forming a deep cavity that confines the nucleus to the basal region. The goblet cells are very prominent, are mostly associated with the mucous secretion (Plate E5) and presence of villi shows for efficient absorption (Plate E5.4): Surface structure in the duodenum is also observed and suggests the efficient absorption of food matters, perhaps the lamina propria region (Plate E8.4) supports the action of pancreatic. Hepatic duct and common bile duct are present. Liver cells are glandular and secrete bile. A complex network of capillaries traverses the largest gland. Hepatic artery, hepatic portal vein are seen. Kuffer's cells are seen in the liver (Plate E7, E8, E9).

Since *K. sylhetensis*, basically a herbivorous has to overcome certain physiological problem. That the potential difference maintained across the surface membrane of most of animal cells results from a high internal and low external concentration of K\(^+\) ion. Since in plant materials the concentration of K\(^+\) is very high and that of low Na\(^+\) ions, thus the group of animals perhaps prevent the K\(^+\) level in the body fluid for equilibrating with that of their food (Smith and Smith, 1989).
Gastric cell (Plate E4 and E8) observed by TEM and SEM suggests the digestion for protein and are connected with blood vascular system. The cells of gastric glands have power of forming, out of the material supplied to them by the blood, the gastric juice, by which proteins are digested. Jeon et al. (1986) described the electronic microscopic structure of the alimentary canal of pond tortoises. Sadhana and Belsare (1987) have studied the simple method of preparation of gastrointestinal tract tissues for scanning electron microscopy. The liver cells are polyhedral in shape with spherical and deep staining nuclei. The pancreas exhibits exocrine and endocrine cells. The stomach and the intestine have greater concentration of mucous and goblet cells certainly are in agreement with the present observation. Tyrosine containing proteins are almost uniformly distributed in different parts of digestive tracts. Triglycerides are found in greater concentration in the ileum. The activity of alkaline and acid Phosphates is mostly found in ileum and liver. The cellulose activity is more in ileum than in esophagus and pancreas.

Muniz (1991) have studied the immunohistochemical localization of Insulin like material in antral gastric mucous and intestinal epithelial cells of turtle- Chrysemys dorbignyi and Phrynaps hilarii. Further Muniz (1991) studied the gastrointestinal cells from sections of the stomach and intestine of the C. dorbigni and P. hilarii. In both the species the concentration of cells is positive for insulin. The localization of insulin like material in gastrointestinal mucosal cell of turtle is an unusual finding among vertebrates, because the insulin containing cells migrate from the mucosal epithelium of the intestine early in vertebrate evolution to the acinar pancreas. The chemical nature and the physiological role of the gastrointestinal insulin in Kachuga species remain unsolved. Brunner’s glands are not observed in the duodenum of Kachuga species (Kardong, 2002). Thus the present findings suggest that the adult K. sylhetensis is herbivorous in nature.

Turtles and tortoises are well adapted even to the natural threats by way of surviving for 200 million years (Rao, 1987). However, the massive environmental changes caused by
anthropogenic destruction of turtles natural habitats, direct killing and even their removal from the habitat may result in their extinction.

Survey and habitat analysis of the study are in clear view that the *K. sylhetensis* in particular and the turtle group in general are losing vast areas of their original habitats, since the human convert wetlands, forests and grasslands to agricultural fields, grazing land and villages and pollution from farms and urban areas have degraded many turtle habitats.

The pet trade, which affects mainly small terrestrial and semi aquatic species, is another threat to turtles. Though no known trade about the *K. sylhetensis* is known yet there are unconfirmed reports of trade involving this beautiful serrated species (Das, 1991). It may be one of the major threats for this species. Compared to other wildlife turtles are particularly vulnerable to harm from direct exploitation, its slow pace of reproduction, however, normally compensated by the long lives of mature adult. Thus, it is difficult for the population to recover after the killing of mature adult. Moreover, the killing or consumption of big sized turtle/tortoise, which is happened to be female, an alarming situation on the imbalance sex ratio has already been emerged. During the survey, it was noticed that consumption of turtle eggs, high mortality of hatchlings are the major attributing factors in the process of population decline. However, the population dynamics of not only of this species, but also for other freshwater species is not known.

Therefore, it is the need of the hour to protect the habitat to conserve this oldest survived animal.