Chapter – I

Introduction

Turtle belongs to the order Chelonii or Testudines characterised by a special bony shell developed from their ribs. "Turtle" may either prefer to the order as a whole, or to particular. Turtles and tortoise have been existed since 300 million years ago and long before most of the popularly recognized dinosaurs. Their survival to the present is a story of successful adaption and resiliency. Although they have overcome many catastrophes, they are now facing the most serious threat to their existence due to human greed. Turtles are currently one of the most threatened vertebrate groups, and many species worldwide have experienced severe declines in recent years, linked to a wide array of contributing factors, including habitat destruction, road mortality, nest predators and direct exploitation for food and the pet trade.

The Northeastern region of India, (22°-30° N and 89°-97° E) covers 2,62,379 sq.km. and is one of the richest biomes of the world, high in endemism of rare species which are now under constant threat. This is the transition zone between the Indian, Indo-Chinese and Indo-Malayan biogeographic regions and a meeting place of the Mountains of Himalaya and Peninsular India and is the habitat zone for much of India’s flora and fauna. The Northeast is among the 34 Hot Spots of the world, identified in India, the other being the Western Ghats. Northeast India comprises of the contiguous Seven Sister States Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Tripura, and the Himalayan state of Sikkim where the State of Assam has extensive flood plains.
The Chelonia form a well-defined group of tetrapods which is mainly characterized by the development of a shell and the derivation of a stegocephalian skull. A saltatory formation of a chelonian shell in the geological record, led to the migration of the limb girdles to positions inside the rib cage, has caused a big interest to the questions connected with the formation of the shell and origin of the group.

Fresh water turtles of India inhabit different water bodies ranging from shallow ponds to deep lakes and rivers and are divided into two broad categories namely hard shell turtle (Emydid turtle) and soft shell turtle (Trionychid turtle). The hard shell turtles representing 16 species have thick and hard carapace and plastron, while, six species of soft the shell turtles are covered with soft fleshy carapace and plastron. Turtles lack teeth having short-neck and use the tough edges of jaws to tear their food. The long necked turtles are ambush feeders. The intake of food is temperature dependent, with most turtles ceasing to feed below 15° C. However, many of the workers relating to freshwater turtle species have made their studies on taxonomical and distribution aspects (Smith 1933; Pritchard, 1979; Daniel, 1983; Das, 1985; Moll, 1984).

Aquatic substrate plays an important role as a habitat determinant of many of the river turtles (Fuselier and Edds, 1994, Das 1995), which is in fact itself the physical characteristics of the substrate like sandy soil etc. Thus the Brahmaputra river provides a very suitable habitat to many of the turtle species and supports a wide range of turtle diversity in its tributaries, swamps, beels etc. and these water bodies are often responded to large seasonal changes in precipitation and are to experiencing an ever changing array of ecological conditions including habitat (Welcomme, 1979). Many of the turtle species of such an ecosystem have responded
at morphological, ecological and behavioral changes to cope up with changes (Moll and Moll, 2004). Interestingly, the big waters like Brahmaputra and its vast water bodies often provide habitat for variety of turtle species ranging from small to large forms. Examples of small to moderate size species which have diversified into numerous forms are successful in fresh water habitats including the genus *Pangshura* in Asian rivers (Moll and Moll, 2004).

The optimum habitat for a turtle species in a given ecosystem is often governed by (a) Search for habitats supplying seasonally available foods (b) suitable nesting locations by adult females (c) mate seeking by adult males (d) migration to hibernation and aestivation site (Gibbons *et al.*, 1990). Recent findings showed that a group of population of *Myuchelys georgesi*, a freshwater turtle species whose population dynamics is influenced by habitat change and predation (Blamires and Spencer, 2013) and how do the animals responds to change in the environment and the type of habitat they used to avail (Morris, 2003, 2005). However, such information in terms of *Pangshura* ecology is not known.

Habitat structure, physico chemical parameters in terms of both the abiotic and biotic factors and their interactions are the major environmental factors that determine the aquatic resources and its integrity (Karr, 1991). Habitat structure has been determined as the major determinant in distribution and abundance of a species. Further several approaches have been proposed to judiciously used to assessing the status of ecosystem with measures and indicates of physiological properties and community functional and structural attributes (Scheidegger and Bain, 1995). The use of physicochemical properties as means of ecosystem structure has the longest history and has been the mainstay of regulatory program.
Linkage on the onset of breeding season and the timing of the reproductive eventualities of many animals including turtle are often associated with the weather and climate. The nesting and courtship in birds (Whitehead and Saalfield, 2000) and the turtles (Burke et al., 1994; Wilson et al., 1999) has been stated to be stimulated by rainfall. Influence of habitat site temperature affects the onset of nesting and its intervals in sea turtles are well understood (Solow et al., 2002). However, the link between the weather as well as climatic factors and the reproductive events are least understood in many of the freshwater species. Interestingly, many of the authors noted a interrelationship between the nesting behavior of freshwater turtles and the weather factors. The nesting timing of snapping turtle (*Chelydra serpentine*) could be well correlated with the spring water temperature in Quebec (Obbard and Brooks, 1987). Similarly the onset of the nest formation of *Chrysemys picta* is associated with the ambient temperature in summer.

Turtles are cold-blooded animal, which means that they cannot raise or lower their body temperature independent to that of the environment. Their body temperature reflects the temperature of the habitat. Cold-blooded animals do not have the ability to raise or lower their temperature by increasing or decreasing their metabolism and are therefore, extremely susceptible to surrounding temperatures. Many of the relationship between the ecological requirements (including biotic and abiotic factors) and distribution of turtle species must be optimally associated, though such an understanding are lacking. Light intensity, air, soil and water temperature have constantly been associated with the optimal habitat conditions of *Pangshura* species. The role of Relative humidity (RH) influence the growth, developments of the common snapping turtle, *Chelydra serpentine* (Obbard and Brooks, 1987). Increased
water availability during incubation appears to increase the rate of mobilization of nutrients from the yolk and their subsequent assimilation into tissue (Packard et al., 1987, 1988; Packard 1991), to increase the amount of water contained within the body of the embryo (Packard et al., 1988; Finkler, 1997).

On warm or hot days, turtles may leave the water and bask, usually stretching their hind legs out behind them to attain maximum contact with a warm surface, and will retreat into the water to cool down. The relationship between the ambient temperature and the basking habit of turtle is very critical in terms of thermoregulatory effect (Moll and Legler, 1971; Vogt 1980). A darker carapace will heat up more quickly than other light coloured turtle and will be able to reach a higher temperature. By increasing their body temperature, there is also increase in the rate of physiological processes (Crawford et al., 1983; Hammond et al., 1988), such as metabolic rate (Bennet, 1982), rate of digestion, and digestion efficiency (Kepenis and McManus, 1974; Avery et al., 1993; Koper and Brooks, 2000). Terrestrial basking or basking on sand or twig is common in freshwater turtles. Basking activity is one of the most conspicuous daily behavior exhibited by aquatic emydid turtles. Early studies suggested that the primary physiological role of basking in turtles was to regulate body temperatures, as well as condition the skin and shell (Cagle 1950; Boyer 1965; Shealy 1976).

pH of water plays significant role that may affects turtle in various ways in its own habitat. The effect of pH is highly variable in its influence from group to group. However, no information is available which directly or indirectly influences the life of fresh water turtle. In captive breeding of turtle, the acidic range of pH have been found to be effective (Gurley, 2003).
Alkalinity (AT) is closely related to the acid neutralizing capacity of a solution. Alkalinity is important for aquatic organisms because it protects or buffers against pH changes and keeps the pH fairly constant.

Hardness of water refers to high mineral content. Hard water contains high levels of metal ions, mainly Ca and Mg in the form of carbonates and also includes various metals as well as bicarbonate and sulphates. Thus the water hardness has significant influence in all phases of freshwater life, but very little consideration has been given to the quality aspects of water in view of water hardness (Wurts, 2003).

Water clarity refers to the transparency of water. Turbidity of water is due to the presence of suspended materials such as clay, silt, organic and inorganic matter and other microscopic organisms. The clarity of water is reduces due to scattering of light by suspended particulate matter. Transparency of water is important in determining the light penetration within a given water body, is important for photosynthetic activities of plants. As the light penetration decreases, the photosynthetic activity of plants reaches a point, where the photosynthesis is equal to respiration. At this point, CO$_2$ uptake is equal to O$_2$ release resulting in reductions in numbers of submersed aquatic macrophytes, aquatic invertebrates, decline in some fish populations due to food resources (Schueler, 1997).

Carbon dioxide is derived primarily from the atmosphere, with additional sources from plant and animal respiration and carbonate minerals. The fundamental effect of CO$_2$ in aquatic environment is the part that it plays in the photosynthesis of green plants. Moderately increase level of CO$_2$ in water bodies accelerated the rate of photosynthesis.
One of the most important abiotic components of freshwater ecosystems is the dissolved oxygen present in the water. Most natural water systems require 5-6 ppm of dissolved oxygen to support a diverse population. If oxygen levels are too low, organisms may not be able to survive. Dissolved oxygen and temperature are two of the fundamental variables in freshwater ecology. The DO concentration within a waterbody can experience large daily fluctuations. Aquatic plants and algae produce oxygen as a by product of photosynthesis by day, but at night they consume oxygen through respiration.

Phosphorus is a highly reactive natural element. In nature, phosphorus always reacts with other elements to form many compounds. Phosphates are produced when phosphorus is combined with oxygen. A range of elements, such as calcium and hydrogen may also be present within phosphate compounds. Phosphorus plays significant role in cell respiration and as a composition of DNA. The living tissues of all organisms contain significant amount of phosphates.

Nitrate ions are important parts of an ecosystem. Plants require a greater quantity of nitrogen than any other nutrient. Plants can absorb nitrogen in the forms of ammonia and nitrate. High nitrate levels in freshwater or marine ecosystems can be directly harmful to aquatic animals and may cause immune impairment, growth deficiencies or death. High nitrate level contribute to eutrophication in aquatic ecosystems. Nitrate fertilizers cause algae to grow in water. Nitrate pollution can lead to algal blooms. The excess of algae reduces sunlight penetration in water which in turn reduces photosynthesis and fall of dissolved oxygen. As the algae die, their decomposition feeds bacteria, which further deplete the dissolved oxygen supply, to the point where aquatic organisms can no longer survive.
Chlorides are present in aquatic ecosystem, and are essential elements of life. Chloride ions in the environment can come from sodium chloride or from other chloride salts such as potassium chloride, calcium chloride and magnesium chloride. High chloride concentrations in freshwater can harm aquatic organisms by interfering with osmoregulation, the biological process by which they maintain the proper concentration of salt and other solutes in their body fluids. Difficulty with osmoregulation can hinder survival, growth, and reproduction.

The concentration of various elements in the freshwater turtle species is not known. Many of the elements like K, Ca, Mg, Zn, Se, Mn and Fe have proved its essentiality in various biochemical processes. Metal concentration in certain algal species indirectly controls the food chain of turtle species (Karez et al., 1994). However, no information is available on the elemental status of the Pangshura habitat.

Complex variation associated with body form is one of the most difficult types of variation to quantify and the methods used to assess it are collectively reffered as morphometrics (Cadrin, 2005).These methods are dealing with the quantification of shape variation within and among species usually to address developmental and evolutionary questions relating to shape change during growth across experimental treatments or among different population (Rohlf and Marcus, 1993). Several workers (De Vries et al., 2002; Palma and Andrade, 2002) regarded the multivariate analysis of a set of phenotypic characters as powerful tool for the determination of morphological relationship between the two group of population of a species.

The age and sexual maturity determination in turtle species is very difficult. Though, interests have been generated to understand the long survivability of turtle group, yet
no specific method has been evolved. However, many of the workers usually emphasizes on capture and recapture methods. Yet, the scute annuli have been found to be useful to determine the age of certain turtle species (Germano and Bury, 1998). Therefore the ring documentation, produced annually could well be important attribution in turtle xerontology (Galbraith and Brooks, 1987; Brooks et al., 1997).

The feeding ecology of many of the fresh water turtle species are least known, particularly the Pangshura group remain almost untouched. However, the study of Chelonian diets could explain the habitat choice, variations in morphological characters (Plummer and Farrar, 1981; Rhodin et al., 1984) and the riparian vegetation composition (Moll and Jansen, 1995). Seasonality on the food availability has led to the dietary changes in turtle species (Schoener, 1971), for example *Chelodina rugusa* feeds maximum on the nymphs of odonata species during rainy seasons and more upon fishes during dry seasons (Kennon and Tory, 1996). Further, the seasonal change also influence upon the food habit of the fresh water turtle species. Interestingly, rainy season exert influence towards carnivore while the dry season influence towards herbivore effects (Clark and Gibbons 1969; Ottonello et al., 2005). Recently Brasil et al., (2011) observed the feeding ecology of *Acanthochelys spixii* in the Cerrado of central Brazil and recorded that there was no ontogenic shift in diet composition.

The genus *Pangshura* exhibits omnivorous habit (Baruah et al., 2013), though details are not known. However, ontogenic shift from carnivorous juvenile to relatively herbivorous adult in fresh water turtle species are not uncommon (Georges, 1982; Allanson and Georges, 1999). Perhaps, due to the low metabolic activity for adults, have tendency towards herbivory, while the juvenile need more
protein to sustained their rapid growth (Pough, 2004). In fact the adults use to ingest small prey probably due to their size (Souza and Abe, 1998).

The relationship between the sexual variations in diet composition could be resulted from sexual dimorphism in terms of body size (Pough, 2004). Freshwater adult female turtles are often larger than their male counterpart having differential habitat use (Plummer and Farrar, 1981) and the energetics for female is more during reproductive period (Ford and Moll, 2004; Macip-Rios et al., 2010), since the females of *Podocnemis unifilis* consume more fish and mollusks to meet the Ca requirement against their herbivore male (Fachin-Teran et al., 1995).

The specific reproductive characteristics of fresh water turtle population are poorly known, yet influenced by the myriad of environmental influences. Turtle of smaller size usually lay spherical to elongated eggs (Moll, 1980). Sexual differentiation in many chelonians depends on the temperature prevailing during incubation of the eggs (Pieau, 1971). All turtles lay eggs and bury them in soil or sand, but some lay eggs on the open ground. Turtles do not incubate their eggs. The eggs are incubated by environmental temperature. Sea turtles and Snapping turtles lay dozens to hundreds of round eggs in a single clutch. Many turtle species are capable of producing more than one clutch of eggs per year. The sex of most species of turtle is determined by environmental factors. Incubation requires several months during which time the turtle embryo grows from a few cells to a full formed organism capable of independent existence. During this process energy stored in the egg by the female is transformed into embryonic tissue. The developing embryo is coupled to the nesting beach through the exchange of the O₂, CO₂, H₂O and heat that is either required or produced by the energy transformation
process. Chelonians can build their nests in shady areas, or open areas with higher exposure to solar radiation (Ewert 1979).

Most egg-laying amniotes produce eggshell of calcium carbonate in the form of calcite (CaCO$_3$); however, turtles build their eggs out of aragonite (CaCO$_3$ + Mg). Simultaneously, organic material is deposited with the calcium carbonate. Pore canals of the eggshell permit gas exchange between the embryo and the environment. Based on the physical properties of the eggshell, eggs can be divided into three general categories: soft, flexible, or rigid. Rigidity of eggshell is determined by the proportion of inorganic to organic matter. The eggshell of amniotic egg has three layers, the outer layer or cuticle layer, the middle layer and the inner fibrous layer. For instance, the eggshell of *Chitra chitra* has three layers, which are the outer calcareous sheet (previously unseen), a middle crystalline layer, and an inner fibrous layer that aragonite form of CaCO$_3$. The basic compositions of reptile eggshells are O, C, Mg, Ca, Si, Al, Na, S and, K. The eggshell serves as a protective barrier between the embryo and environment. It plays significant role in the exchange of gas, moisture, and heat (Sahoo *et al.*, 1996a, b). It also protects the embryo from any mechanical injury and serves as a substrate for rotational movements of the embryo. Most importantly, it serves as a reservoir for most of the essential elements for the development of the embryo, with calcium being the most needed element (Sahoo *et al.*, 1996a, b, 1998; Mahanty and Sahoo, 1999; Kitimasak *et al.*, 2003).

Selection of a nest site is one of the last components of the reproductive investment of female freshwater turtles that determines the biophysical conditions necessary for successful embryo development. In addition, location of the nest determines the
suite of environmental cues available to hatchlings as they emerge from nests and initiate orientation and dispersal. (Noble and Breslau 1938; Anderson 1958; Iverson et al. 2009). Freshwater turtles often nested near the foraging and basking sites and the females normally use to stay near the nesting sites (Graham and Graham, 1991; Plummer et al., 1997) against sea turtles. Further, the timing of nesting is extremely uniform in temperate zone turtles and extremely variable in tropical fresh water turtle species (Moll and Moll, 2004), though nothing has been known for Pangshura species. The influence of environmental parameters like temperature, rainfall, and light intensity are the real controllers of nest timings (Tuker et al., 1997) and in tropical geographical variations influence nest (Kuchling, 1999).

Information regarding the life history of freshwater turtle is extremely poor except the emergence of neonates after hatching and before emergence from the nest chamber on to the surface (Moll and Moll, 2004). However, earlier many authors (Bleakney, 1963; Depari, 1996) recorded the emergence from the nest chamber proto-cooperative in response to temperature gradient and only known for sea turtles (Carr and Hirth, 1961), but such phenomenon is not known for freshwater turtles including Pangshura species. Interestingly Tuker (1999) found that slider hatchlings, Trachemys scripta elegans over wintered in central Illinois nest emerged when soil temperature gradient increases.

Therefore, an attempt has been made to determine the incubation period, hatching timing and influence of other abiotic factors on the emergence of hatchlings from the incubated eggs of Pangshura tentoria. Attempt has been made to evaluate the effects of certain environmental cues on the habitat and reproductive biology of
freshwater turtle species *Pangshura tentoria* (Gray, 1834). The following objectives have been targeted to understand the ecobiology of the species:

(A) (i) The habitat ecology in terms of the habitat search and influence of climatic parameters upon them.

(ii) The assessment of the physicochemical parameters of the habitat site (soil and water)

(iii) Food availability in the habitat site and Ca assessment in them

(B) (i) Morphometric variation(s) in terms of body size of adult male, female and juvenile

(ii) Variation in the gut length and structure to correlate with the food types

(C) Breeding biology to evaluate the

(i) Sexual dimorphism, if any

(ii) Sexual maturity in terms of age determination

(iii) Nesting and egg laying

(iv) Basking habit in relation to egg laying and egg hatching

(D) Egg and egg shell

(i) Egg shell analysis for Ca estimation along with some other elements

(ii) Phopholipid quantification in yolk as a source of energy

The mentioned aim(s) and subsequent objective(s) has facilitated to hypothesize that the biology of the species is/are controlled by environmental cues and Ca abundance.