*Journal of Applied and Natural Science* 5 (1): 118-124 (2013)

JANS

# Biochemical indices to monitor health status with respect to reproductive cycle of *Melanochelys trijuga*

**Vidya R. Chandavar[1]*, N. Raghu[1], R. Lalitha[1] and Prakash R. Naik[2]**

[1]Molecular Endocrinology Laboratory, Department of Zoology, Yuvaraja's College, University of Mysore, Mysore-570005, INDIA

[2]Endocrinology Laboratory, Department of Studies in Zoology, University of Mysore, Manasagangorti, Mysore-570006, INDIA

*Corresponding author: E-mail: vidyachandavar@yahoo.co.uk

*Received: December 13, 2012; Revised received: February 20, 2013; Accepted: March 15, 2013*

**Abstract:** Blood analysis can provide information regarding the physiological condition of an individual animal or population health as a whole. The present investigation analyzed clinical laboratory data of soluble organic substances of plasma namely triglycerides, cholesterol, HDL, serum glutamic pyruvic transaminase (SGPT/ aspartate amionotransferase) and albumin of Asian pond turtle *Melanochelys trijuga*. Using multiple samples measures of analysis and variance was determined. *M. trijuga* exhibited variation in biochemical parameters with respect to reproductive cycle. Biochemical profiles of cholesterol, HDL, LDL, SGPT and albumin values found to be high in preparative period and minimum in recrudescent period of the reproductive cycle. This indicates that the preparative period is the stage where the animals prepare for the forth coming reproductive period. The level of triglycerides and cholesterol varied independently. All the parameters fluctuated with in normal range. The data may be useful to evaluate the health status of the turtle for veterinary care and conservation.

**Keywords:** Albumin, Biochemical parameters, Reproductive cycle, Triglyceride, Turtle

## INTRODUCTION

Indian black turtle *Melanochelys trijuga* of family Geoemydidae lives in fresh water bodies, often come to land for basking and feeding on vegetation and smaller insects. It is a medium sized turtle of northern, north-eastern and peninsular India, Sri Lanka, Myanmar, Nepal, Bangladesh, Thailand and possibly Pakistan (Das and Bhupathy, 2009).

Blood biochemical parameters represent a valuable diagnostic tool for monitoring the health status of free ranging wildlife (Metin *et al.,* 2006). Plasma biochemical ranges for the European pond turtle *Emys orbicularis* was published (Metin *et al.,* 2006). Physiological blood parameters in loggerhead sea turtle (*Carreta carreta*) have been validated by Gelli *et al.* (2008). Chaffin *et al.* (2008) made health assessment in free ranging Alligator snapping turtle (*Macrochelys temminckii*). In 2009, Deem *et al.* compared blood values in foraging, nesting and stranded loggerhead turtles (*Carreta carreta*). Standard biochemical parameters were determined to evaluate the health status in sea turtle *C. caretta* (Delgado *et al.,* 2011). Recently, Perrault *et al.* (2012) correlated maternal biochemical parameters with hatching success in sea turtles *Dermochelys coriacea*. Although various studies have contributed much to the understanding of the turtle hematology, turtles are known to exhibit an extremely diverse physiological adaptation to their respective ecology, which results in differences in physiological parameters within species itself. Hence, investigating blood biochemical profile helps in better understanding of how the internal biochemical environment plays a role in aiding the turtles to adjust their physiological conditions to the respective environment in which they dwell. Normal ranges of blood values for *M. trijuga* are lacking in the literature. The species *M. trijuga* although is in no immediate danger in India, but it is exploited for food and is under population decline (Das and Bhupathy, 2009). The purpose of this study was to provide a basic plasma profile of the turtle *M. trijuga* in order to monitor the health status with respect to its reproductive cycle.

## MATERIALS AND METHODS

**Animals:** Permission to collect blood samples from *M. trijuga* was obtained from – Principal Chief Conservator of Forests (wildlife) and Chief Wildlife Warden, Bangalore-Karnataka State vide letter no.: D/WL/CR-149/2009-10. The animals were collected during 2010 from Bellur (Latitude 12$^{o}$272 N; Longitude, 74$^{o}$00' to 78$^{o}$202 E; *Altitude* 1046 *Meters above sea Level*) 117 kms from Mysore city. Minimum of two active live adult turtles were collected on the spot with the help of local fisherman. "Guidelines for Care and Use of Animals in Scientific

ISSN : 0974-9411 (Print), 2231-5209 (Online) 

Research" were followed (Anonymous, 2000). As the animals were collected from the natural habitat, they were weighed and measured from notch to tip as cited in Deem *et al.* (2009).

**Sample collection:** One ml of blood sample from two live animals (without sacrificing or harming the animal) was obtained from femoral vein (Rohilla and Tiwari, 2008) every month irrespective of the sex using 22 gauge needle (BD) and dispensed directly into vacutainer tubes (BD 367884) containing lithium heparin as anticoagulant to avoid red cell hemolysis. Soon after the collection of blood samples the animals were medicated and relocated to their habitat. Blood in tubes were immediately stored in a portable cooler until processing in the laboratory 24 hour after capture to avoid enzyme degradation. The serum was separated from whole blood by centrifugation at 3000 rpm for 10 min and serum biochemical parameters were determined. The estimation was conducted over a period of 12 months from January to December 2010, to find any influence of seasonal changes on physiological conditions of the animals, every month between $10^{th}$ to $20^{th}$ day.

**Biochemical analysis:** Serum biochemical parameters were determined using blank and by averaging the values of minimum of five samples from each animal for each parameter every month (total ten readings per month per parameter). Biochemical indices of serum components were measured using Semi Bioauto Analyser from Swemed Diagnostics with appropriate kits supplied by the manufacturer of instrument and by following well-known methods. The triglycerides (Fossati and Lorenzo,1982); cholesterol (Friedewald *et al.,* 1972); HDL-cholesterol and albumin (Teitz, 1976) and serum glutamic pyruvic transaminase (SGPT /aspartate amionotransferase) were measured in fresh samples. LDL-cholesterol concentrations were calculated using Friedewald formula.

**Statistical analysis:** The results for different periods, irrespective of the sexes were analyzed using analysis of variance (ANOVA). Where ever the variance value was found to be significant at 5%, Duncan's Multiple Range Test (DMRT) was applied. Statistical presentation was organized using STATISTICAL PRESENTATION SYSTEM SOFTWARE (SPSS), Windows version 10.0, 1999, New York. SPSS Inc.

Correlation and regression analysis were made between the parameters, but only similar or related components like, lipid components- cholesterol, triglyceride, HDL and LDL; serum proteins-albumin and SGPT were considered.

## RESULTS

The annual seasonal cycle of reproduction was reported earlier (Chandavar and Naik, 2008; 2012) and it was distinguished in to three separate periods namely preparative, reproductive and recrudescent. Different periods of reproductive cycles were assigned by careful observation of the status of the gonad during two successive cycles of reproduction (Chandavar and Naik, 2008). Initiation of gonad activity occurred in both the sexes during preparative period. This period fall between March to June and corresponded with summer season (Table 1). The animals were active and voracious eaters during this period.

The peak of gonad activity occurred in reproductive period in both the sexes, which falls between July to August. These are the months of monsoon with longer days. In males the testis attained its largest size only between July and August after which it regressed very soon. Mating occurred during these months. The females showed the presence of eggs in the oviduct. This condition prevailed much earlier than July. They started laying eggs in clutches by the end of August and continued till October.

Recrudescent period falls between September to February and corresponded with winter when nights are longer with shorter days. During this period gonads showed degeneration in both sexes. The change ultimately resulted in loss of reproductive ability. *M. trijuga* usually bask under sun during this period or remain submerged in water, very few animals were seen hiding in the loose soil for a short period in a day.

**Table 1.** Biochemical parameters of *M. trijuga* during annual cycle of reproduction.

| | Preparative | Reproductive | Recrudescent |
|---|---|---|---|
| Months | March-June | July-Aug | Sept-Feb |
| Season | Summer/Spring | Summer/Rainy | Winter |
| Animal weight (g) | $2400 \pm 0.048^{a}$ | $2480 \pm 0.035^{b}$ | $2480 \pm 0.01^{b}$ |
| Cholesterol (mg/dl) | $170.62 \pm 35.79^{b}$ | $173.85 \pm 48.47^{b}$ | $138.57 \pm 6.13^{a}$ |
| Triglyceride (mg/dl) | $94.35 \pm 10.30^{a}$ | $146.33 \pm 16.57^{c}$ | $117.65 \pm 10.95^{b}$ |
| HDL (mg/dl) | $45.32 \pm 1.64^{a}$ | $47.94 \pm 1.01^{b}$ | $42.41 \pm 3.48^{a}$ |
| LDL (mg/dl) | $103.05 \pm 29.23^{c}$ | $96.63 \pm 50.79^{b}$ | $71.99 \pm 9.98^{a}$ |
| Albumin (g/dl) | $1.66 \pm 0.73$ | $1.85 \pm 0.04$ | $1.37 \pm 0.44$ |
| SGPT (IU/L) | $3.3 \pm 1.85^{b}$ | $1.47 \pm 0.31^{a}$ | $1.70 \pm 0.36^{a}$ |

Note: Mean with same letters is not significantly different

**Table 2.** Correlation between biochemical parameters in *M. trijuga* at 'r' table value 0.576.

| Parameters | Highest correlation | Negative correlation | Least correlation | Significance of ' r 'at 0.05 level, df-12-2=10 |
|---|---|---|---|---|
| Triglyceride | - | HDL, Cholesterol, LDL | - | - |
| Cholesterol | LDL, HDL | Triglyceride | - | Significant (r=0.97 and 0.71) |
| HDL | Cholesterol | Triglyceride | - | Significant (r=0.71) |
| LDL | Cholesterol | Triglyceride | - | Significant (r=0.97) |
| Albumin | - | - | SGPT | Significant (r=0.64) |
| SGPT | - | - | Albumin | Significant (r=0.67) |

Triglycerides ranged from 81.62±23.73 mg/dl in preparative period (April) to 158.05±19.65 mg/dl in reproductive period (August) (Fig. 1). Cholesterol ranged from 130±11.11 mg/dL in recrudescent period (November) to 224.11±16.52 mg/dl in preparative period (June) (Fig. 2). HDL ranged maximum of 56.8±1.67 mg/dl in preparative period (June) to minimum of 37.4±0.64 mg/dl in recrudescent period (January) (Fig. 3). LDL was found to be maximum of 146.31±17.0 mg/dl in preparative period (June) to minimum of 59.51±14.31 mg/dl in recrudescent

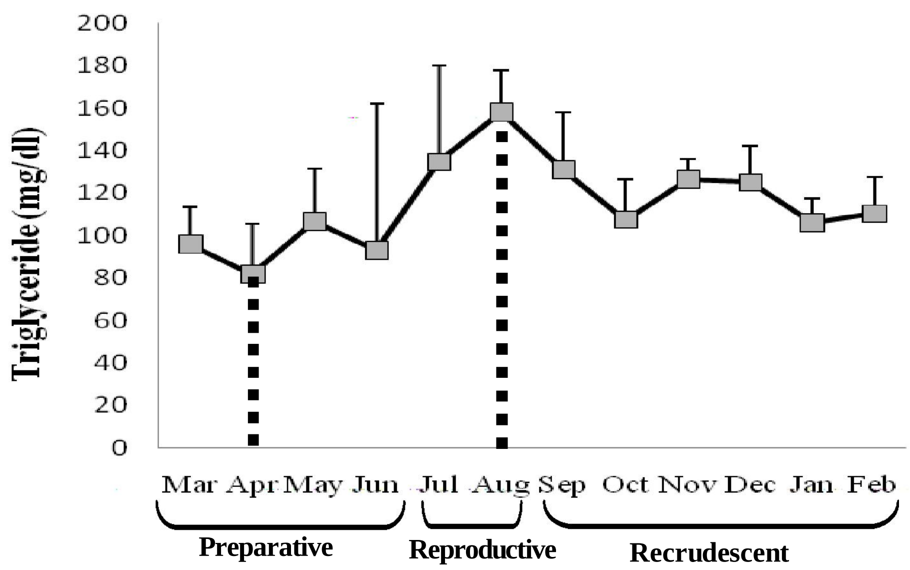


Fig. 1

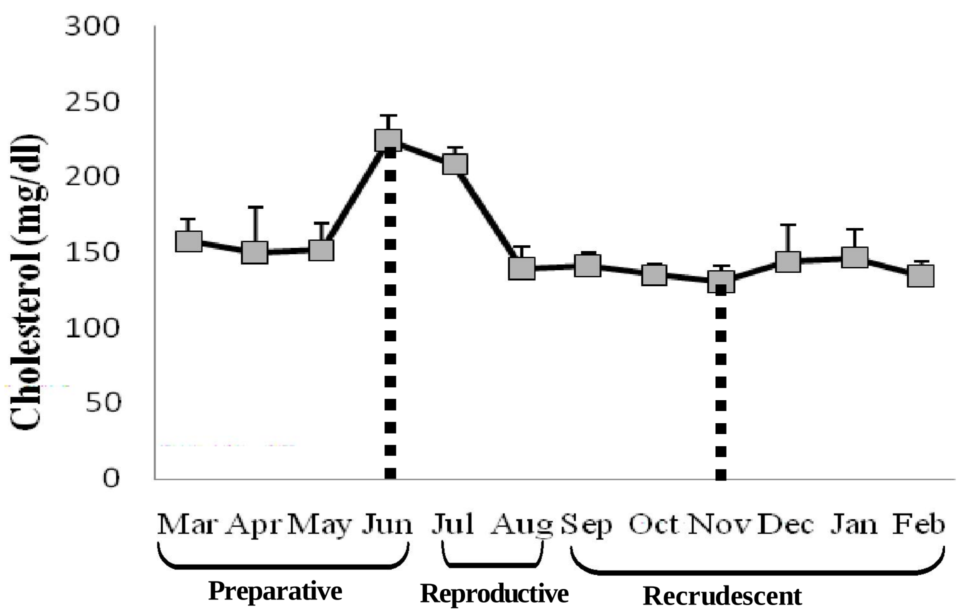


Fig. 2

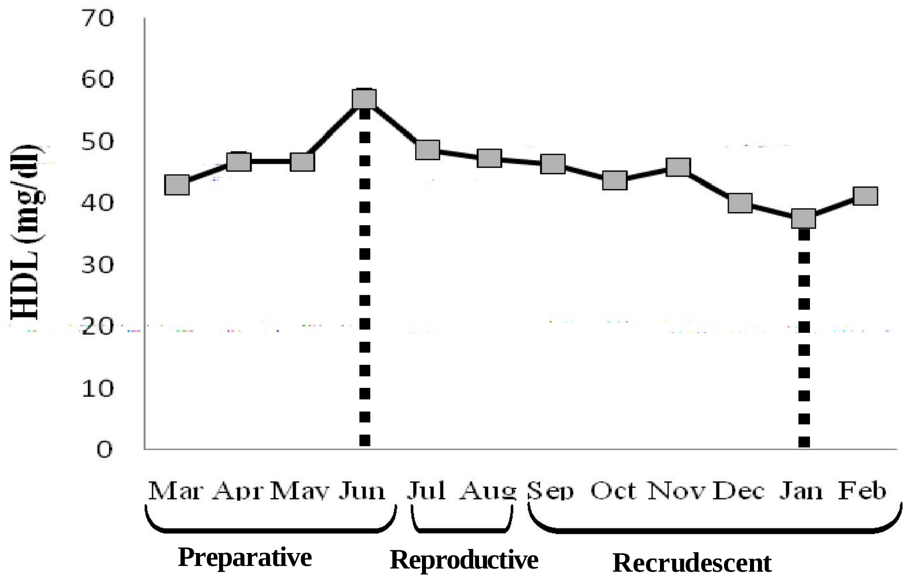


**Fig. 3**

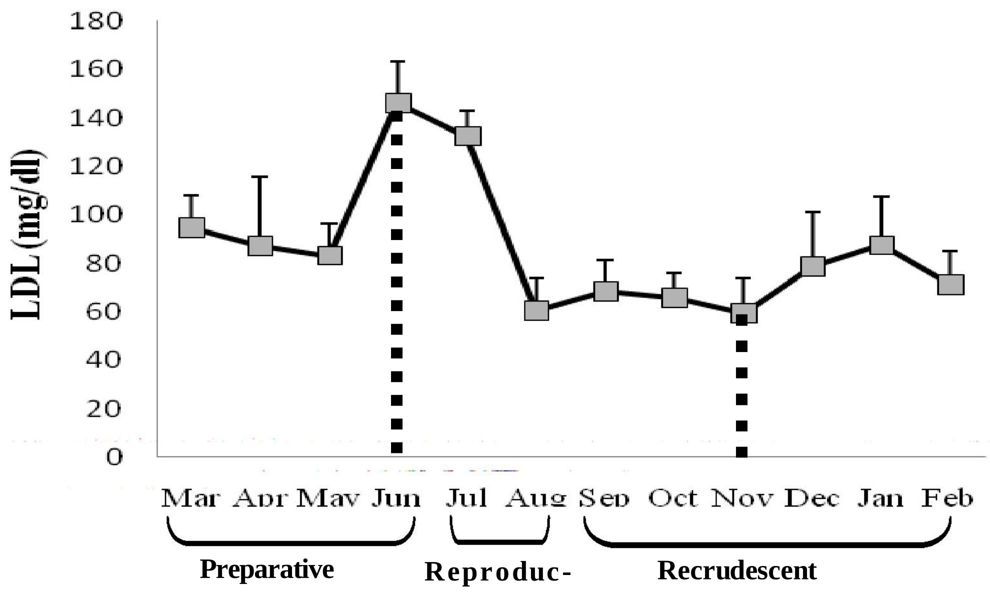


**Fig. 4**

period (November) (Fig. 4). The value of albumin measured between 0.79±0.28 g/dl in recrudescent period (October) to 2.5±0.35 g/dl in preparative period (June) (Fig. 5). The values of SGPT ranged from 1.06 ±0.33 IU/L in recrudescent period (November) to 5.79±2.19 IU/L in preparative period (June) (Fig. 6).

Averaged values (Table 1) represent orders of magnitude rather than fixed levels as the range of variability for most constituents is great even between individuals of a single population sample.

## DISCUSSION

Daily events in the life of a reptile as well as seasonal metabolic cycles influence the blood levels of a number of substances. Blood collection is common in sea turtle studies and is often used in population genetics (Perrault *et al.,* 2012). Blood serum harbors several vital biochemical components which are both organic and inorganic. Plasma of reptiles like that of all vertebrates constituting 60-80% of blood volume is a colorless or straw colored fluid, containing a great variety of different substances with most being present in trace quantities but still with significant influence on animals physiological behavior. There is no difference between females and males in terms of glucose, triglyceride, urea and total protein in *Mauremys caspica,* a turtle (Metin *et al.,* 2008).

Triglycerides circulate in blood as complexes with lipoproteins. In *M. trijuga* triglycerides measured on an average value of 114.66±21.10 mg/dl. In *C. mydas* triglyceride ranged from 43 to 413 mg/dl (Bolten and Bjorndal, 1992). Triglycerides in alligator snapping turtle *Macrochelys temminckii* measured 48.7±7.0 mg/dl (Chaffin *et al.,* 2008). The values ranged from 5.5 to 104.4 mg/dL in controls and 11.6 to 75.5 mg/dL in malnourished

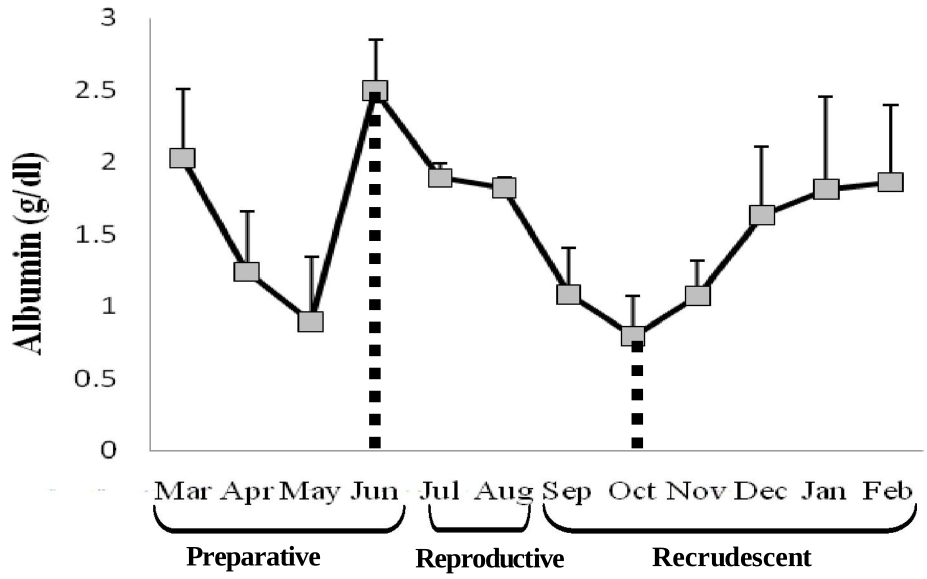


**Fig. 5**

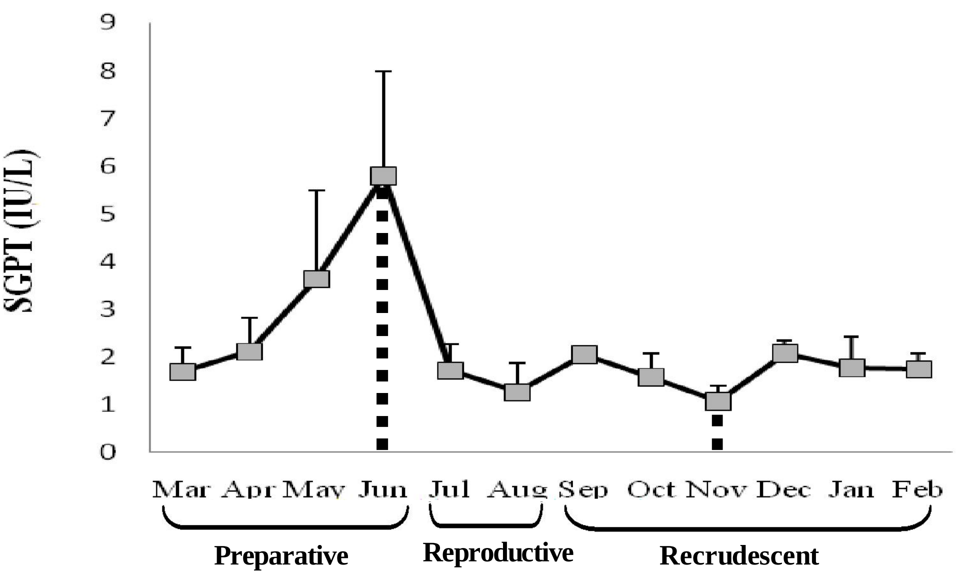


**Fig. 6**

**Figs. 1-6.** *Showing seasonal variation in plasma profile of M. trijuga: 1. Triglyceride (mg/dl). 2. Cholesterol (mg/dl). 3. High density lipid (mg/dl), 4. Low density lipid (mg/dl), 5. Albumin (g/dl), and 6. Serum glutamic pyruvic transaminase (IU/L).*

*Podocnemis expansa* (Dias *et al.,* 2009). Pre-prandial and post-prandial triglyceride values in juvenile *Lepidochelys kempii* were found to be 348 mg/dl and 354 mg/dl respectively; and in juvenile *Chelonia mydas,* the values were 151 mg/dl and 154 mg/dl respectively (Anderson *et al.,* 2011). The values in the present investigation indicated that they were not malnourished instead fed well and were in healthy ecosystem.

Cholesterol on an average measured 155.13±29.67 mg/dl in *M. trijuga*. In *C. mydas* it ranged from 73 to 365 mg/dl (Bolten and Bjorndal, 1992). In *M. temminckii,* the cholesterol was observed to be 57.2±3.4mg/dl (Chaffin *et al.,* 2008). In controls of *Podocnemis expansa,* the cholesterol ranged from 22.2 to123.1 mg/dl with an average of 62.7 ± 24.3 mg/dl (Dias *et al.,* 2009). Average cholesterol values in juvenile *L. kempii* before and after food were 198 mg/dl and 202 mg/dl respectively- and in juvenile *C. mydas,* the values were 207 mg/dl and 216 mg/dl respectively (Anderson *et al.,* 2011). The present value of cholesterol was found to be higher than in *P. expansa* and *M. temminckii* but lower than in *C. mydas.* The level of cholesterol and triglycerides varied independently and negatively correlated (Table 2). Total cholesterol averages 50 mg% in *Alligator mississippiensis.* Averages as low as 69 mg% and as high as 480 mg% are reported in different species of emydine turtles (Dessauer, 1970). Liver samples showed maximum glycogen mass, both white and brown fat were abundant in the abdomen in preparative period of spring season, where as in recrudescent period liver glycogen and abdominal fat were at minimum in *M. trijuga* (Chandavar and Naik, 2012). Fat levels change slowly during starvation and cold torpor in the turtle *Chrysemys picta* (Crawford, 1994). Total fatty acids decreased only 89 mg/dL in turtles fasted 6 to 8 weeks. In *M. trijuga* on an average triglyceride and cholesterol was comparatively higher in reproductive period of rainy season (Table 1). The radiated tortoise, *Geochelone radiata* had significantly higher triglyceride in winter and higher cholesterol in summer (Zaias *et al.,* 2006). Increased triglyceride at the end of reproductive period is to cope up with forth coming winter season in *M. trijuga*.

HDL on an average measured 45.2±0.49 mg/dl in *M. trijuga*. It was found to be positively correlated with cholesterol and negatively correlated with triglyceride (Table 2). High HDL cholesterol values are associated with both increased longevity and low incidence of morbidity and mortality. LDL cholesterol has a causal role in the development of cardiovascular disease (Sandhu *et al.,* 2008).

Albumin is a major component of plasma proteins, which has major effect on total protein concentration. It contributes to the amino acid pool. It transports a wide range of substances in blood like bilirubin, fatty acid, calcium, magnesium etc., High concentration of albumin with high charge densities are found in active species with high metabolic rates and in those living in dry, hot environment for example lizards and other desert reptiles. Albumin of the turtles *Clemmys japonica* has the same isoelectric point as human albumin (Dessauer, 1970). Albumin in *C. mydas* measured 1.5 g/dl (Bolten and Bjorndal, 1992). In *M. temminckii* albumin measured 0.75±0.028 g/dl (Chaffin *et al.,* 2008). Pre-prandial and post-prandial albumin in juvenile *L. kempii* was found to be 1.5 g/dl and 1.6 g/dl respectively; and in juvenile *C. mydas* the values were 1.9 mg/dl and 2.1g/dl respectively (Anderson *et al.,* 2011).The value of albumin in *M. trijuga* on an average was 1.55±0.52 g/dl (Table 1). Albumin concentrations of the turtles used for this study were within the range of loggerhead sea turtle *C. caretta* (0.6 ± 0.8 g/dL) (Bolten *et al.,* 1992) and African side neck turtle *Pelusios sinuatus* (1.07±0.71 g/dL to 1.18±0.13 g/dL) (Omonona *et al.,* 2011) (Fig. 4) therefore, these turtles were considered healthy.

RBC's are rich in SGPT therefore lithium heparin was used as an anticoagulant to avoid hemolysis and sample was stored in cooler. In a field situation in which a centrifuge is unavailable, samples can be stored in a portable cooler up to 24 hr without appreciable change in biochemical analytes (Eisenhawer *et al.,* 2008). An average value of SGPT in *M. trijuga* was 2.19±1.29 IU/L. SGPT in *C. mydas* measured 6 U/L (Bolten and Bjorndal, 1992). In juvenile *L. kempii*, ALT (SGPT) value was found to be 6 U/L both before and after food (Anderson *et al.,* 2011). In leatherback turtle, the range was 5 to 20 IU/L (Perrault *et al.,* 2012). Normal level of SGPT ranges 5 to 40 IU/L in other animals. Elevated levels of SGPT (ALT) often suggest the existence of medical problems such as viral infection, liver damage etc. Drugs and toxic substances which are detoxified by the liver cells can result in elevated level of this enzyme. The values indicate that the animals were neither infected nor damaged or had hemolyzed RBC's in the estimated sample.

It was concluded that in *M. trijuga* the cholesterol exhibited highest correlation with HDL and LDL; albumin and SGPT varied independently (Table 2) but all these parameters were found to be maximum in June (Figs. 2-5) that corresponded with preparative period. Only triglycerides were found to be maximum in August (Fig. 1) which corresponded with reproductive period. All the parameters fluctuated within normal range, therefore the turtles of Bellur were considered normal and healthy.

## ACKNOWLEDGEMENTS

Corresponding author acknowledges for financial support from University Grants Commission, New Delhi. (Grant: F. No. 38-269/2009 (SR) Dated: 25-12-2009). Raghu. N acknowledges for the award of UGC fellowship sanctioned to the first author. Lalitha. R acknowledges Department of Science and Technology for INSPIRE fellowship.

## REFERENCES

Anderson, E.T., Minter, L.J., Clarke, III E.O., Mroch, III R.M., Beasley, J.F. and Harms, C.A. (2011). The effects of feeding on hematological and plasma biochemical proûles in Green (*Chelonia mydas*) and Kemp's Ridley (*Lepidochelys kempii*) sea turtles. SAGE-Hindawi Access to Research Veterinary Medicine International Volume 2011, Article ID 890829, 7 pages doi:10.4061/2011/890829.

Anonymous. (2000). Guidelines for care and use of animals in scientific research. New Delhi: Indian National Science Academy.

Bolten, A.B. and Bjorndal, K.A. (1992).Blood profiles for a wild population of green turtles (*Chelonia mydas*) in the Southern Bahamas: Size- specific relationships. *Journal of Wildlife Diseases*, 28(3):407-413.

Bolten, A.B., Jacobson, E.R. and Bjorndal, K.A. (1992). Effects of anticoagulant and auto analyzer on blood biochemical values of loggerhead sea turtle. *American Journal of Vetternary Research*, 53:2224-2227.

Chandavar, V.R. and Naik, P.R. (2008). Immunocytochemical localisation of pancreatic endocrine cell and reproductive cycle in the turtle, *Melanochelys trijuga*. *Journal of Biosciences*, 33(2):239-247.

Chandavar, V.R. and Naik, P.R. (2012).Variation in thyroid activity with respect to annual seasonal cycle of reproduction in turtle *Melanochelys trijuga*. *International Journal of Life Sciences & Pharma Research*, 2(3):126-134.

Chaffin, K., Norton, T. M., Gilardi, K., Poppenga, R., Jensen, J.B., Moler, P., Cray, C., Dierenfeld, E.S., Chen, T., Oliva, M., Origgi, F.C., Gibbs, S., Mazzaro, L. and Mazet, J. (2008). Health assessment of free-ranging alligator snapping turtles (*Macrochelys temmincikii*) in Georgia and Florida. *Journal of Wildlife Diseases*, 44(3):670-686.

Crawford, K.M. (1994).Patterns of energy substrate utilisation in overwintering painted turtles, *Chrysemys picta*. *Comparative Biochemistry & Physiology*, 495-502.

Das, I. and Bhupathy, S. (2009). *Melanochelys trijuga* (Schweigger 1812) Indian Black Turtle. Conservation Biology of freshwater Turtles and Tortoises. Chelonian Research foundation. 8th December, 038.1.

Deem, S.L., Norton, T.M., Mitchell, M., Segars, Al., Alleman, A.R., Cray, C., Poppenga, R.H., Dodd, M. and Karesh, W.B. (2009). Comparison of blood values in foraging, nesting, and stranded Loggerhead turtles (*Caretta caretta*) along the coast of Georgia, USA. *Journal of Wildlife Diseases*, 45(1):41-56.

Delgado, C., Valente, A., Quaresma, I., Costa, M. and Dellinger, T. (2011). Blood biochemistry reference values for wild juvenile loggerhead sea turtles (*Caretta caretta*) from Madeira archipelago. *Journal of Wildlife Diseases*, 523-529.

Dessauer, H.C. (1970). Blood chemistry of reptiles: physiological and evolutionary aspects. *In:* Gans C, ed. Biology of the reptilian. volume III. Academic Press, New York.1-54.

Dias, M.T., Oliveira-Junior, A.A., Silva, M.G., Marcon, J.L. and Barcellos, J.F.M. (2009). Comparative hematological and biochemical analysis of giant turtles from the Amazon farmed in poor and normal nutritional conditions. *Veterinarski arhiv*, 79(6):601-610.

Eisenhawer, E., Courtney, C.H., Raskin, R.E. and Jacobson, E. (2008). Relationship between separation times of plasma from heparinised whole blood on plasma biochemical analytes of loggerhead sea turtles (*Caretta caretta*)[abstract]. *Journal of Zoo and Wildlife Medicine*, 208-215.

Fossati, P. and Lorenzo, P. (1982). Serum Triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Journal of Clinical Chemistry*, 2077.

Friedewald, W.T., Levy, R.I. and Fredrickson, D.S. (1972). Estimation of the concentration of low density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. *Journal of Clinical Chemistry*, 18:499-502.

Gelli, D., Ferrari, V., Zanella, A., Arena, P., Pozzi, L., Nannarelli, S., Vaccaro, C., Bernardini, D., Romagnoli, S. (2008). Establishing physiological blood parameters in the loggerhead sea turtle (*Carette Caretta*). European *Journal of Wildlife Research*, doi:10.1007/s10344-008-0214-7.

Metin, K., Turkozan, O., Kargin, F., Basnmoglu, K.Y., Taskavak, E. and Koca, S. (2006). Blood cell morphology and plasma biochemistry of the captive european pond turtle *Emys orbicularis*. *Acta Veterinaria Brno*, 75:49-55.

Metin, K., Basnmoglu, K.Y., Kargnn, K.F., Koca, S. and Türkozan, O. (2008). Blood cell morphology and plasma biochemistry of captive *Mauremys caspica* (Gmelin,1774) and *Mauremys rivulata* (Valenciennes, 1833). *Acta Veterinaria Brno*, 77:163-174.

Ommonona, A.O., Olukole ,S.G. and Fushe, F.A. (2011). Haematology and serum biochemical parameters in free - ranging African side neck turtle (*Pelusios sinuatus*) in Ibadan,Nigeria. *Acta Herpetolgica*, 6(2):267-274.

Perrault, J.R., Miller, D.L., Eads, E., Johnson, C., Merrill, A. et al. (2012). Maternal health status correlates with nest success of leatherback sea Turtles (*Dermochelys coriacea*) from Florida.;PLoS ONE 7(2): e31841. doi:10.1371/journal.pone.0031841

Rohilla, M.S. and Tiwari, P.K. (2008). Simple method of blood sampling from Indian freshwater turtles for genetic studies. *Acta Herpetologica*, 3(1):65-69.

Sandhu, M.S., Waterworth, D.M., Debenham, S.L., Wheeler, E., Papadakis, K., Zhao, J.H. (2008). LDL-cholesterol concentrations: a genome-wide association study. *Lancet*, 371:483-491.

Teitz, N. (1976). Fundamentals of Clinical Chemistry. W. B Saunders Co, Philadelphia. 335-337.

Zaias, J., Norton, T., Fickel, A., Spratt, J., Altman, N.H. and Cray, C. (2006). Biochemical and hematologic values for 18 clinically healthy radiated tortoises(Geochelone radiata) on St Catherines Island,Georgia. *Veternary Clinical Pathology*, 35(2):321-325.