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Emphasizing Lissemys punctata Health Status with reference to Hematological, Biochemical, and Water Quality Parameters of two Different Water Bodies

Manikyam Giddakindha[1] and Swathi Gunduluru*

[1]Department of Zoology, Sri Venkateswara University, Tirupati, Andhra Pradesh, India
****Department of Zoology, Government Degree and PG College, Nagari, Andhra Pradesh, India***

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ABSTRACT

By studying turtles enable us to gain better understanding of many species living in water and impact of the environment on their health. The purpose of the study is to analyse *Lissemys punctata*, also known as the Indian flapshell turtle, basic health in comparison of two diverse areas of water bodies. The water bodies selected were 120 km apart as in Narayanavanam mandal, near to human activities and other in natural environment, Somala mandal, Andhra Pradesh. We had observed 24 turtles, 12 each from the water sources in two mandals. We had collected blood samples from the turtles and analyzed for haematological and biochemical parameters. We had also collected water samples in the ponds and analyzed for water quality parameters. Our findings revealed that turtles haematological and biochemical parameters of Narayanavanam mandal water bodies showed significant stress when compared with other group of Somala mandal, this was justified by water quality parameters also. This clearly indicates that other aquatic organisms, which share these types of ecosystems, may also be facing similar environmental stress. In general, we conclude that there must be taken some conservative measures much quicker to ensure the preservation of biodiversity in ponds situated near to human settlements. In addition, our data on haematological and blood biochemical parameters will benefit future endeavours to study *Lissemys punctata*, especially where the availability of data is scarce.

Key words: Lissemys punctata, *Water quality parameters, Hematological parameters, Biochemical parameters, Conservation.*

Introduction

Reptiles are most interesting living creatures which dwell in both aquatic and terrestrial environment, (Swimmer, 2000) yet are first true land animals. They play a crucial role in ecosystem, functioning their role in so many ways (Bohm *et al.*, 2013). Turtles are indispensable for the preservation of biodiversity and ecological equilibrium in diverse habitats. Their habitat was disappearing due to climate change (Hawkes *et al.*, 2007; Chaloupka *et al.*, 2008) as high temperatures (Fong, 2010) and irregular rainfall etc. They are now facing a conflict with humans as their habitat was encroached by human settlements. They are also increasingly vulnerable to hunting and poaching near to human settlements.

Lissemys punctata, also known as the Indian flapshell turtle, is a species of freshwater turtle found in South Asia. In this study we examined this turtle, its overall health by assessing their hematological, biochemical parameters and compared water quality parameters in those places. Analyzing these parameters of the Indian flapshell turtle, may be helpful for obtaining its information about the

(*Assistant Professor)

health, physiology and to detect any environmental stress and are vital in conservation of this species (Hofer and East, 2012; Muñoz-Pérez *et al.*, 2017; Ley-Quiñónez *et al.*, 2017). Therefore, studying these parameters of *Lissemys punctata* is crucial for understanding their physiological and health requirements and implementing effective conservation and the preservation of their habitats and also their residing water bodies.

Materials and Method

Area of the study: The study was carried out in two different locations, namely Narayanavanam (Fig.1) (13.426917, 79.568750) and Somala (Fig. 2) (13.463904, 78.775609) of Andhra Pradesh. Turtles were sampled from January to December in 2021 and 2023.

Fig. 1. Narayanavanam water body

Fig. 2. Somala water body

Sample collection

The turtles were initially identified based on their key characteristics and were manually handled without the use of anesthesia. Only male turtles weighing over 750g were selected for the study. Blood samples were collected in accordance with standard protocols with help of veterinary doctor. All the samples were collected in their vicinity only. 2 ml sample of blood was collected using a 23g × 1" hypodermic needle and a 3 ml syringe. The samples were stored at -20 ^{o}C for further analysis of hematological and biochemical parameters. We had also collected water in those water bodies for water quality parameters.

Water quality parameters

Water reaction (PH), Electrical Conductivity, Carbonates, Bicarbonates, Chlorides, Sulphates, Calcium, Magnesium, Sodium, Potassium, Boron and Total Dissolved Salts (TDS) of the water samples were analyzed

Biochemical and hematological parameters analysis

Total cholesterol (TC), triglycerides, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, very low-density lipoprotein (VLDL) cholesterol, TC/HDL, LDL/HDL, blood glucose, hemoglobin, RBC, WBC, heterophils, lymphocytes, eosinophils, monocytes, platelet count, hematocrit (PCV), mean corpuscular (cell) volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), as well as serum electrolytes such as sodium, potassium, and chloride were analysed in the samples. Additionally, renal function tests such as blood urea and serum creatinine, and liver function tests such as total bilirubin, direct bilirubin, indirect bilirubin, AST (Aspartate transaminase), ALT (alanine aminotransferase), alkaline phosphatase, total proteins, albumin, and globin were estimated in blood samples.

Statistical analysis

Results are presented as mean ± SEM. t- test was used to compare differences between means in more than two groups. A probability value of < 0.001 was considered to be statistically signiûcant.

Results

The key characteristics features studied to identify *Lissemys punctata* in our study

The shell of *Lissemys punctata* is oval in shape, and

30±8 centimeters in length. The carapace is brown or black in color with a series of small, yellowish spots or lines. The plastron is of yellow or cream in color. The head and neck relatively large, and they are brown or black in color with yellow or cream stripes or spots. The skin is dark brown or black with yellow or cream spots. The skin on the neck and legs can have a rough texture, and there may be small, sharp projections on the front legs. The eyes are large and located on the sides of the head. They have a brown or black iris and a circular pupil.

Water quality parameters

In Table 1, the levels of Water reaction (PH), Electrical Conductivity, Carbonates, Bicarbonates, Chlorides, Sulphates, Calcium, Magnesium, Sodium, Potassium, and Total Dissolved Salts (TDS) of the Narayanavanam water samples comparatively more than Somala water samples. The levels of Boron were in reverse.

Hematological parameters

In Table 2, Blood glucose (mg/dl), RBC (million / Cumm), WBC (cells), Heterophils (%), Lymphocytes (%), Eosinophils (%), Monocytes (%), Platelet Count (Lakhs/Cumm), Hemotocrit (Pcv) (%), MCV (fl), MCH (pg), MCHC (g/dl) of the Narayanavanam turtles samples were significantly more than Somala turtles samples, except Hemoglobin (g/dl) which were more in Somala mandal turtle samples.

Biochemical parameters

In table 3, Total cholesterol (mg/dl), Triglycerides (mg/dl), LDL (mg/dl), VLDL (mg/dl), TC/HDL (mg/dl), LDL/HDL (mg/dl) of the Narayanavanam turtles samples were more than Somala turtles samples, except HDL (mg/dl) which were significantly more in Somala mandal turtles samples.

Serum electrolytes, kidney and liver function tests

In table 4, Serum electrolytes like K (mmol/L), Na (mmol/L), Cl (mmol/L),

Renal Function test like Blood urea (mg/dl), Serum creatinine (mg/dl),

Liver Function test like Total Biluribin (mg/dl), Direct Bilurubin (mg/dl), In-direct Bilurubin (mg/dl), AST (IU/L), ALT (IU/L), Alkaline phosphatase (IU/L), Total proteins (g/dl), Albumin (g/dl), Globin (g/dl) which were significantly more in Somala mandal turtles samples.

Discussion

Turtles have played significant roles in all diasporas with ecological significance linking both aquatic and terrestrial ecosystems. Hence studying them is of greater use to conserve the biodiversity. Turtles, like other reptiles, have a unique blood biochemistry that allows them to function in their specific environment. Here we had analyzed some of the key characteristics of turtle hematological and blood biochemical parameters. We had also analyzed the water quality parameters in those different water bodies to see the relation between their health and their stress conditions.

The water quality parameters have various influences on turtles like PH, Electrical Conductivity (EC) (Tchobanoglous, 2003) and dissolved salts are indicators of contamination of water or its quality. High levels of chlorides and sulphates can indicate pollution or contamination of water sources, the data sup-

Table 1. Water quality parameters in Narayanavanam and Somala water bodies

S.No	Parameters	Narayanavanam water	Somala water
1	Water reaction (Ph)	8.8	7.8
2	Electrical Conductivity	1.06mmhos/cm	0.77 mmhos/cm
3	Carbonate	0.8 m.eq./l	Nill m.eq./l
4	Bicarbonate	1.4 m.eq./l	1.1 m.eq./l
5	Chloride	2.4 m.eq./lt	2.1 m.eq./l
6	Sulphate	14.10 m.eq./l	7.165 m.eq./l
7	Calcium	14.71 m.eq./l	5.466 m.eq./l
8	Magnesium	21.88 m.eq./l	11.14 m.eq./l
9	Sodium	3.98 m.eq./l	2.86 m.eq./l
10	Potassium	3.545 m.eq./l	3.081 m.eq./l
11	Boron	0.092 m.eq./l	0.101 m.eq./l
12	Total Dissolved Salts (TDS)	357 mg/l	298 mg/l

ports this in Narayanavam water bodies these levels are high compared to Somala water bodies. High TDS levels can indicate pollution or the presence of harmful chemicals, which can negatively impact turtles' health (Table 1). In general we can see the relationship between water quality parameters and turtle health (Aguirre *et al*., 1994; Van Houtan *et al.*, 2010; Santos *et al*., 2011, Boyle, 2017). Therefore, maintaining suitable water quality is crucial for the health and survival of turtles in their natural habitats. Overall human activity is playing a major role in negative impact of water quality (Seminoff *et al*.,. 2015, Mazaris *et al.,* 2017) Conservation efforts often include monitoring and improving water quality in turtle habitats to support their populations.

Hematological parameters

As per results in table 2, there is significant increase in blood glucose, hemoglobin, RBC, WBC, heterophils, lymphocytes, eosinophils, monocytes, platelet count, hemotocrit (PCV), MCV, MCH, and MCHC levels in turtles living near human settlements i.e., in Narayanavam water bodies. This may indicate that they are experiencing some sort of stress or inflammation in their bodies (Omonona *et al.,* 2011). These changes in blood parameters may be attributed to pathogens, or other environmental stress causing agents that are present in their habitat as it reflects the water quality parameters (Table 2). Turtles living near human settlements may experience chronic stress due to factors such as noise pollution, habitat fragmentation, and changes in their natural environment. This chronic stress can cause an increase in the production of certain hormones such as cortisol in repitiles (Duncan *et al*., 2022), which can lead to elevated blood glucose levels. High blood glucose levels (Table 2) in turtles near human settlements could be attributed to various factors such as they have access to different types of food sources, which can also affect their blood glucose levels. Lastly, exposure to pollutants or chemicals commonly found near human settlements, such as pesticides or heavy metals (da Silva 2016), can lead to changes in glucose metabolism and contribute to elevated blood glucose levels.

Blood Biochemical parameters

There is significant increase in total cholesterol and LDL levels (Table 3) in turtles in Narayanavanam mandal could be due to several factors such as water pollution. A decrease in triglycerides and HDL levels (Table 3) in turtles living near human settlement may indicate a change in their diet and environment; these studies evaluate the health status of turtle (Swimmer, 2000). The decrease in triglycerides and HDL levels in turtles near human settlements may suggest that they are being exposed to more Environmental contaminants that are affecting their overall health (da Silva *et al.*, 2016, da Silva and Bianchini, 2019). This could have negative consequences for their health and wellbeing, as well as the health of other organisms in the ecosystem.

Serum electrolytes, kidney and liver function tests

There is significant increase in serum electrolytes (Table 4) in turtles residing in near to human settle-

Table 2. Hematological parameters of turtles in Narayanavanam and Somala

S. No.	Parameters	In Narayanavanam (Mean± SEM)	In Somala (Mean ± SEM)	t-value
1	Blood glucose (mg/dl)	219 ±0.9	124±0.6	85.96*
2	Hemoglobin (g/dl)	4.2 ±0.45	4.6 ±0.06	0.39
3	RBC (million /Cumm)	0.33 ±0.08	1.2 ±0.005	10.02*
4	WBC (cells)	34,900 ±3.02	2,500±2.48	8316.81*
5	Heterophils (%)	9.5 ±0.3	0.85 ±0.06	22.78*
6	Lymphocytes (%)	78.1 ±0.93	82.4±1.18	2.8373*
7	Eosinophils (%)	10.4±0.41	8.1 ±0.32	4.34*
8	Monocytes (%)	2.0 ±0.15	1.0 ±0.08	6.4*
9	Platelet Count (Lakhs/Cumm)	0.98 ±0.05	0.45± 0.02	82.10*
10	Hemotocrit (Pcv) (%)	22 ±0.6	15.0±0.63	7.9*
11	MCV (fl)	67.6±0.61	45.0±0.99	19.41*
12	MCH (pg)	16.3 ±1.2	15.2±0.61	0.80
13	MCHC (g/dl)	20.90 ±0.28	18.2 ±0.19	7.87*

Significant P value is less than 0.001; #P value equals 0.2108, not statistically significant ments, may indicate the presence of pollutants or contaminants in their environment. High levels of electrolytes such as sodium, potassium, and chloride could be due to exposure to pollutants in the water or food sources, or due to chronic stress caused by habitat destruction or other human activities. These changes in electrolyte levels may also affect the overall health and physiology of the turtles, and can serve as an indicator of environmental stress. High levels of blood urea and serum creatinine (Table 4) in turtles may indicate kidney dysfunction or damage (Camila *et al.*, 2022). These parameters are commonly used to assess renal function, and elevated levels can be a sign of kidney disease or impaired kidney function. Human activities such as pollution (Table 1), habitat destruction, can have negative impacts on the health of turtles and their habitats, which may lead to renal dysfunction or damage (Milton and Lutz, 2003).

If the AST and ALT levels are high (Table 4) in turtles, which may indicate liver damage or injury. This can be caused by various factors such as exposure to pollutants or toxins in the environment, infections, or other diseases. Elevated levels of these enzymes in the blood can indicate liver cell damage, as they are normally found in the liver and released into the blood when liver cells are damaged or destroyed. Therefore, high AST and ALT levels (Table 4) in turtles may suggest that there are environmental stressors or other health issues impacting the liver function of these animals. The increase in alkaline phosphatase levels may indicate liver damage or stress, while the increase in total protein levels could be a response to inflammation or infection. High levels of albumin and globulin may also suggest inflammation or immune system activation (Table 4) (Labrada-Martagón *et al.*, 2010; de Deus Santos *et al.*, 2015). In general all the parameters had

Table 3. Biochemical parameters of turtles in Narayanavanam and Somala

S. No.	Parameters	In Narayanavanam (Mean±SEM)	In Somala (Mean±SEM)	t-value
1	Total cholesterol (mg/dl)	88 ±0.93	75 ±0.89	10.04*
2	Triglycerides (mg/dl)	550 ±2.5	280 ±3.2	65.02*
3	HDL (mg/dl)	25 ±3.2	45 ±2.1	18.10*
4	LDL (mg/dl)	30 ±1.01	10 ±0.6	16.90*
5	VLDL (mg/dl)	38 ±0.3	20 ±1.4	11.89*
6	TC/HDL (mg/dl)	3.55± 0.28	1.5 ± 0.20	5.83*
7	LDL/HDL (mg/dl)	1.3 ±0.05	0.23 ±0.05	131.04*

*Significant P value is less than 0.001

Serum electrolytes, kidney and liver function tests

Table 4. Serum electrolytes, kidney and liver function tests of turtles in Narayanavanam and Somala

S. No.	Parameters		In Narayanavanam (Mean±SEM)	In Somala (Mean±SEM)	t-value
1	Serum electrolytes	K (mmol/L)	13.9±0.3	10.5 ±0.2	7.61*
		Na(mmol/L)	200 ±2.34	175.0 ±1.53	8.92*
		Cl(mmol/L)	140 ±0.93	135 ±0.70	4.27*
2	Renal Function test	Blood urea (mg/dl)	80 ±1.7	16 ±0.9	31.35*
		Serum creatinine (mg/dl)	0.5 ±0.05	0.2 ±0.02	12.60*
3	Liver Function test	Total Biluribin (mg/dl)	1.10 ±0.3	0.65 ±0.2	11.12*
		Direct Bilurubin (mg/dl)	0.30 ±0.005	0.18 ±0.017	6.57*
		In-direct Bilurubin (mg/dl)	0.80 ±0.02	0.47 ±0.02	80.83*
		AST (IU/L)	180 ±2.7	145 ±1.8	10.71*
		ALT(IU/L)	14.0 ±2.3	9.5 ±1.7	15.04*
		Alkaline phosphatase(IU/L)	483 ±2.0	200 ±1.8	101.76*
		Total proteins(g/dl)	12.5±0.32	8.0 ±0.41	8.58*
		Albumin(g/dl)	8.0 ±0.2	5.0 ±0.2	9.93*
		Globin(g/dl)	4.5 ±0.05	3.0 ±0.17	8.21*

*Significant P value is less than 0.001

been changed indicating that these turtles are under constant stress (Aguirre and Balazs, 2000; Foley *et al.,* 2005; Santos *et al*., 2015). These results may reflect the impact of anthropogenic interactions with the environment (Andreani *et al*., 2014, Kelly *et al.*, 2015, Stacy *et al*., 2017).

Conservation strategies

Conservation of turtles involves a variety of methods aimed at protecting and preserving their populations like installing fences, nesting sites and ensure the water is free of pollutants like pesticides, industrial wastes and is well oxygenated are some common methods in our observation that may preserve them.

Conclusion

The study of turtles not only enriches our comprehension of the intricate tapestry of life on Earth but also sheds light on pressing scientific and conservation concerns. *Lissemys punctata,* like numerous other turtle species, confronts threats stemming from habitat degradation. The outcomes of our investigation imply that their blood biochemistry experiences deterioration when they inhabit regions proximate to human settlements, possibly attributable to a multitude of stressors. Consequently, further research should be conducted to elucidate these stress factors. In light of its ecological and conservation significance, it becomes imperative to institute conservation measures aimed at safeguarding these species. This underscores the paramount importance of preserving both the species and its environment.

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Conflict of Interest No conflict of interest

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