



Effects of Thermal Stress on Behavioural Patterns of Legally Protected Indian Soft-shell Turtle *Lissemys punctata*

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Lissemys punctata, the Indian fresh-water soft-shelled turtle is a species under threat which has been classified as a Schedule-I animal under Wildlife Protection Act 1972 and in CITES is marked as 'Vulnerable'. A high demand for its' meat being rich in protein and considered to be a delicacy results in its illegal sale in international and local markets till date though it is prohibited by law. The study aims to investigate the effects of high temperature on the physiology of freshwater turtle, *L. punctata* and to observe their coping abilities with respect to wide fluctuations of environmental

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temperature. In the present study, adult soft-shelled turtles were rescued, collected from local market of Doltala in North 24 Parganas, West Bengal and kept in the garden with diverse plant and grass varieties and an adjacent water body. An artificial culture conditions were created in the garden with different soil types and the turtles were fed with food and water *ad libitum*. Our method is based on the principle- 'study of ecology of turtle in captivity'. In this study feeding, breeding and nesting behaviour including habitat preference of *Lissemys punctata* from the month of February 2023 to May 2024 were observed. Behavioural changes and reduction in food intake were observed which shows adaptability to thermal stress by regulating Corticosterone Releasing Factor and Adrenocorticotrophic Hormone (CRF-ACTH) axis. The study emphasizes the significance of freshwater turtles as vital part of food web and ecosystem while identifying key threats including environmental fluctuations, effects of pollution and human activities and proposes necessary conservation strategies. Existing laws and regulations are reviewed to devise appropriate methods for protection of freshwater turtles and argues that most efforts have focussed on protection of sea-turtles which need to be replicated for freshwater turtles through use of different methods like Captive Husbandry, promotion of Citizen Science, usage of genetic markers (mitochondrial markers) and advanced technology.

Keywords: Soft-shell turtles; *Lissemys punctata*; behavioural patterns; thermal stress and conservation.

1. INTRODUCTION

In the mythologies of many ancient cultures including Hinduism we find the mention of turtles. Besides its ecological significance in their natural system, they have contributed towards development of artistic, cultural and spiritual values. Turtles can be classified into 'marine turtles' and 'freshwater turtles'. Freshwater turtles face severe threats and challenges such as illegal wildlife trade and smuggling, residential and commercial development projects besides other human interventions such as agriculture, aquaculture, hunting and trapping. This necessitates government measures, national and international laws and regulations and their effective implementation by enforcement agencies to protect and conserve different species of freshwater turtles. Twenty-nine species of tortoises and freshwater turtles have been located throughout India and West Bengal remains a major hotspot for illegal trade of turtles for meat. The freshwater softshell turtle *Lissemys punctata* (Bonnaterre, 1789), also known as Indian Flapshell Turtle (Family Trionychidae) are distributed throughout India and also in other South Asian countries like Myanmar, Nepal, Pakistan and Bangladesh [1]. *Lissemys punctata* is a relatively small sized softshell turtle whose length of the carapace varies from 250 to 350 mm. The carapace is olive-green in colour and with yellow spots on the carapace [2,3]. They are omnivorous and distributed in diverse types of natural aquatic habitats, such as marshes, salt marshes, lakes, ponds, rice fields, rivers and streams. It is also found in lagoons of brackish

water (Sunderban delta region) in the coastal parts of eastern India and is quite prevalent in irrigation canals as well as in the stagnant waters of ponds, tanks and rice fields. *Lissemys punctata andersoni*, sub species of Indian flapshell turtle is also found in the hilly areas of Darjeeling in West Bengal indicating that they can survive in various types of environment [4]. *Lissemys punctata* can survive for a long-time during periods of drought by aestivating in suitable sites on land, but they prefer to stay in shallow waters [5-8]. They are also called as opportunistic omnivores as they feed on variety of foods.

Softshell turtles have fleshy shells adapted for swimming. The soft shell helps the turtle to move faster on the land and also protect them from predators. Femoral flaps and nasal septal ridges are present. Turtles can close their shell (femoral flaps and moveable anterior plastral lobe) in a way by which they can conceal completely their limbs, neck and head within the shell to give protection from predators and to avoid desiccation.

According to the Wildlife Protection Act, 1972 of India, *L. punctata* is listed under Schedule-I which covers endangered animal species that need the maximum level of protection against threats. *L. punctata* is mentioned in CITES Appendices 2 (see Table 1) and is marked in IUCN as Vulnerable [9]. Yet, *Lissemys punctata*, faces a wide range of threats as it is very much used and traded in international markets and also in local markets for its meat and medicinal

products [10-12]. In West Bengal overexploitation of *L. punctata* for the local market as well as for international trade is observed since early 1980s till date [13-15]. It has been reported that from coastal regions of Odisha, *L. punctata* comes to West Bengal for trading and is still being sold in local fish markets in West Bengal on regular basis even today [16].

Lissemys punctata can survive in the drought for a long time by the process of aestivation on land and can withstand severe stresses [17,18]. Temperature is a very important factor for organisms because many physiological processes which are vital for the organisms, are dependent on temperature. Extreme high or low

temperatures can both be lethal for the softshell turtles.

Animals can be divided into two groups regarding the heat source of the body such as endotherms and ectotherms. Turtles are dependent on the environment for the heat source necessary for life processes, so they are ectotherms. As the ectotherms are with little insulation, so their body temperature fluctuates parallelly with the environmental temperature.

The Table 2 charts the legal and conservation status of *Lissemys punctata* under various Acts and lists, geographical distribution, key threats to its population and its ecological role.



Fig. 1. Soft-shelled turtle (*Lissemys punctata*)



Fig. 2. Smuggled Soft-shell Turtles being seized by the Wildlife Crime Control Bureau (WCCB)

Source: Hindustan Times (7 March 2020)

Table 1. CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) Appendices

Fauna	Appendix I	Appendix II	Appendix III
<i>Reptiles</i>	105 spp. (incl. 7 popns) + 4 sspp	870 spp. (incl. 6 popns)	215 spp. (incl. 1 popn) + 8 sspp.
Family: Trionychidae Softshell turtles		<i>Lissemys punctata</i>	

Appendices I, II and III valid from 25 May 2024. The abbreviation “spp.” is used to denote all species of a higher taxon, “ssp.” is used to denote subspecies and “popns” for populations (Supplementary 1).

Table 2. Species description and ecological threat

<i>Lissemys punctata</i>-Status	<ul style="list-style-type: none"> ○ According to the Wildlife Protection Act, 1972, it is classified under Schedule 1 Part II and their smuggling is a non-bailable offence. ○ According to Red List of Threatened species of IUCN, it is marked as Vulnerable
Geographic distribution	<ul style="list-style-type: none"> ○ India, Myanmar, Nepal, Pakistan and Bangladesh
Reasons for turtle smuggling	<ul style="list-style-type: none"> ○ Turtles have sexual arousing properties ○ They are used in animal feed ○ Their skins are used to produce leather ○ Their blood is used for creating potions ○ They are used as bait in fishing ○ They are hunted for their meat and medicinal values.
Threats	<ul style="list-style-type: none"> ○ Human activities leading to disturbances i.e. unethical. ○ Different types of developmental projects – infrastructural, residential etc. ○ Fishing activities ○ Modifications to naturally occurring systems, such as dam construction and water management ○ Pollution from agricultural and forestry effluents.
Ecological role	<ul style="list-style-type: none"> ○ In aquatic (stagnant water) ecosystem such as ponds and rivers, they act as scavengers and thus help the aquatic systems to be free from pollution ○ They also act as predators. They feed on aquatic insects, crustaceans and fish, and thus they help to keep such populations under control in the habitat.

Objective of the Study: In the present study, we observed the feeding behavior and habitat preferences of softshell turtle. The study also looks at the effect of thermal stress on behavioural physiology. The breeding and nesting habits with their preferred habitats were also studied from the month of February 2023 to May 2024. As *Lissemys punctata* is a vulnerable species and also enlisted under Schedule-I animals under Wild Life Protection Act, 1972 so doing any harm or killing of the animals is strictly prohibited. All the findings were based on visual observations.

2. MATERIALS AND METHODS

Adult soft-shelled turtles (10) were rescued and collected from local market of Doltala in North 24 Parganas, West Bengal. They were kept in the garden with an adjacent water body. Turtles were fed with Tubifex and shrimp, mollusk such as small snails, boiled rice, bread, biscuit and water *ad libitum*. The garden had a variety of plants including big, medium and small trees, bushes and grasses. The soil was loamy and the soil was prepared sandy in another part of the garden. An artificial culture conditions were created in the garden to make observations based on the principle- 'study of ecology of turtle in captivity'.

3. FINDINGS AND RESULTS

3.1 Feeding Behaviour

Lissemys punctata is an opportunistic omnivore. They consume fish, crustaceans, mollusks, earthworms and insects. They also consumed rice, biscuit, bread and aquatic plants. The turtle used to browse in the bottom layer of the shallow water bodies with extended neck in search of prey.

3.2 Basking and Browsing Behaviour

We made our observations daily in the early morning and afternoon so that they can be spotted easily. In the months of February, March and at the beginning of April they used to lie exposed to get the warmth of the sunlight which is known as basking. The duration time of basking gradually declined with the increase in the environmental temperature as days passed. As per our observations, we found the turtle would bask in the early morning and afternoon, but also during the noon especially when the weather is cloudy. Turtles preferred logs and

solid surfaces as their basking place. Turtles also move overland which is known as browsing behaviour. Turtles do not prefer deep waters, rather they prefer shallow area of the water which have less aquatic vegetation, so that longer periods can be spent in the mud in a shallowly buried condition.

3.3 Thermal Stress and Aestivation

As the environmental temperature rises (38 degree Celsius to 40 degree Celsius) in the month of May and June, perceptible behavioural changes were observed in them. Increase of temperature caused certain unusual behavioural manifestations, such as running activity, they were found to extend their necks and kicking their legs with buccopharyngeal movements, gasping and eventually exhibiting restlessness. Less consumption of food was also observed. Additionally, some of the turtles showed oedema in the eyelids, hyperemia of the buccal cavity (evaluated from reddish appearance of buccal cavity due to profuse blood supply) and enhanced mucus secretion dripped out from their mouth (frothing) after exposure to high environmental temperatures. This however was no longer observed after decrease in environmental temperatures. In summer, the turtle preferred to be in burrowing condition in a suitable site for aestivation in moist soil.

3.4 Breeding and Nesting Behavior

The reproductive cycle of *L. punctata* is very much related with rainfall and especially heavy rains. During rainy season, turtles eat excessively. Browsing activity is also very much evident during this period. They browse mainly in the bottom of the shallow waterbodies. To find a prey they extend their neck first downward and then upward. As this type of neck movement is not possible in land, so always waterbody is preferred for catchment of the prey.

In our study, it has been observed that mating season of *L. punctata* is from the month of May to the month of July. They prefer mating in water areas. The males swim around the female in a circular manner with head and limbs in an extended condition and bobbing or nodding of its head is also observed. Occasionally, they show signals by patting or biting at the frontal edge of the female's carapace. The females would respond in a similar way by bobbing her head in return. Aquatic mating is observed among the softshell turtles.

In the present study, we observed that *L. punctata* has shown to build the nests from the month of June to the month of August. The nests were made by the female turtles. They made the nest by digging holes with the sharp claws of their hind limbs in moist and muddy soil. They selected spaces under shades or fully open area as their nesting sites. During our study period, we found four nests were made in September. The nests were 8-10 cm deep. The average clutch size was 7-14 eggs. Female turtles lay their eggs in burrows made by them and immediately after laying of eggs, they cover the space with the soil. The hatchlings emerged in May after an incubation period of approximately 235-250 days.

3.5 Hibernation

As the environmental temperature declines and winter approaches, the turtles started finding suitable space for hibernation in burrowing condition.

4. DISCUSSION

Unusual behavioural manifestations of turtles exposed to high ambient temperature is index of stress. Several steroidogenic enzymes are known to be stimulated at higher temperature in ectothermic vertebrates [19,20]. High temperature acts as stress that primarily affects the adrenal or interrenal gland and causes interrenal gland hyperactivity. Therefore, in the present study, parallel changes in the interrenal gland of turtle may reflect its stimulation including steroidogenesis [21]. Catecholamines are also known to influence adrenocortical steroidogenesis. Catecho-lamines such as norepinephrine and epinephrine levels were increased following exposure to high ambient temperature in turtles [22]. Thus, an involvement of catecholamines in inducing corticoid synthesis through activation of steroid synthesizing enzyme following high ambient temperature cannot be ruled out. It is known that adrenocortical activity is regulated by CRF-ACTH axis [23].

5. EFFECTIVE STRATEGIES AND SUSTAINABLE SOLUTIONS FOR TURTLE CONSERVATION

A multi-faceted approach is necessary for the conservation of the turtle populations.

- The nesting sites should be protected by several ways to reduce illegal hunting and poaching.

- Creating awareness about the ecological role of turtle, protection and conservation measures and building support for turtle protection initiatives can be done by educational campaigns and outreach programmes. Long-term initiatives need to be taken.
- The impact of human activity must be identified particularly on juvenile turtle populations such as fishing activities which causes the death of juvenile turtles as they are too young to withstand commercial nets.
- Tourism and different types of developmental projects of the river bank and sea beach causes destruction of nesting sites. Development projects should consider its potential ecological impact on turtle habitats to prevent damage.
- Molecular markers such as Mitochondrial markers are now used as a tool for conservation of turtles. The population genetic variation, as well as the evolutionary history of the animal species can be revealed by cytochrome b gene sequencing which provides information for conservation [24]. Mitochondrial 16S rRNA have been used for species identification of *Lissemys punctata*. Cytb and D-loop sequences were also used to locate the intraspecific variations which will be helpful for conservation and to prevent crimes against wildlife animals [25]. The relationship between turtle species can be estimated by genotype comparison of polymorphic loci using molecular markers such as Cytb gene [26].
- New technological methods such as Artificial Intelligence and robotic exploration should be used for providing data regarding the study of their behavior and habitats.
- Laws and different regulations related to the conservation and protection of Turtles should be implemented properly.
- Promoting Captive Husbandry and turtle farming can be a significant conservation strategy. *Lissemys punctata* can survive in captivity and the species is compliant to successful captive breeding where artificial incubation and hatching of eggs is done.
- To make aware the local populations, communities and fishermen about the usefulness of turtle conservation, different programmes should be arranged. This can be facilitated through promotion of *Citizen science research* that encourages direct

involvement and participation of general public, local communities, amateur or non-professional participants in science and inter-disciplinary fields to aid in collection and classification of data, educating and spreading awareness about scientific processes and the importance for communities to participate in turtle conservation strategies through different approaches.

- To stop trading of turtle eggs and turtle meat in the wholesale and local market, awareness about turtle conservation needs to be promoted.
- Infrastructural development projects such as Highway construction and several others are spoiling the different habitats and nesting sites of turtles. Therefore, environmental impact assessment (EIA) and proper plans should be made to save the turtles before sanctioning and implementing infrastructural development projects.
- Collaboration between government, non-governmental organizations, environmental groups and networking with citizen science initiatives and organizations are necessary to create awareness about turtle conservation, monitoring, raising funds and to support law enforcement agencies to prevent illegal hunting and trading of softshell freshwater turtles.

6. LAWS AND REGULATIONS FOR CONSERVATION OF TURTLES

There are international laws and national laws for protection and conservation of turtle. But significant focus has been on protecting the marine or sea-turtles as because of their biological characteristics and migration patterns, they are especially susceptible to risks of incidental capture. The *Law of Sea Convention* (UNCLOS III) 1982 has codified customary international law that prohibits states from hunting sea turtles to extinction in the high seas and lays responsibility on the states for not to endanger living resources by over-exploitation in their Exclusive Economic Zone (EEZ). The *Convention on International Trade in Endangered Species of Wild Fauna and Flora* (CITES) is an agreement that aims to regulate the movement of certain wild animals and plant species across international borders and has stronger provisions that gives extensive protection for sea turtles, in the form of international trade restrictions by including them

in the Appendices of CITES. The *Bonn Convention* (CMS) also includes provisions on protection of sea turtles. The *Convention on Biological Diversity* contains provisions regarding habitat protection mechanisms to conserve biological diversity on both national and regional levels.

The Wildlife (Protection) Act, 1972 (Schedule I) include provisions for protection of 11 turtle species in India and their international trade is also regulated under the CITES which India has signed in 1976. In the 19th meeting of the Conference of Parties to the CITES (CoP19) held in Panama City between 14th-25th November 2022, India in the CoP19 agenda had put forward a proposal to amend the appendices and to include a particular endangered species of freshwater turtle, risking extinction, that is native to India and Bangladesh, called the red-crowned roofed turtle (*Batagur kachuga*) under the Appendix 1 (transferring it from Appendix 2) of the Convention on International Trade in Endangered Species (CITES) to ensure its better protection which received wide support from the parties at COP19 and was adopted [27]. Operation Save Kurma was launched by the Wildlife Crime Control Bureau (WCCB) to prevent poaching, transportation and illegal trade of live turtles and tortoises. KURMA-tracking India Turtles is a mobile application for engaging citizen scientists in monitoring and conservation of Indian turtles that has over 1300 plus registered users and 90 plus assistance facilities [28]. It is a citizen science tool that aims to educate general public on turtle, has a digital field guide to easily identify turtle species, upload turtle observation, locate nearby assistance facilities through geotagged maps and to directly connect to experts who can offer rescue assistance. Some other initiatives by India include 'Operation Turtshield- I' and 'Operation Turtshield-II' to prevent wildlife crimes which have also been appreciated. To reduce the illegal turtle trade, the Wildlife Conservation Society - Turtle Survival Alliance - India team has been using ex-situ conservation measures and focusses on strengthening capacity building of enforcement agencies.

The major threats to freshwater turtles have been smuggling for their meat or as pets, poaching and habitat destruction due to pollution, development and infrastructure projects. Besides overharvesting for illegal consumption, illegal international trade continues to be a major threat. A major initiative developed and produced by a

global NGO working on turtle conservation TRAFFIC, (India branch office) with Turtle Survival Alliance (TSA)-India, is "The Identification (ID) Cards: Tortoises and Freshwater Turtles of India", to aid and support law enforcement agencies for wildlife protection. It will help to identify the different species of tortoises and freshwater turtles' that are illegally traded and provide information on their legal and conservation status, geographical distribution, morphological features and significant threats [29].

7. CONCLUSION

The softshell turtle *Lissemys punctata* found in various types of habitats is under threat due to over-exploitation, illegal hunting and poaching, habitat destruction due to urbanization, pollution and unethical human interference. Conservation of this species is very much needed as *Lissemys punctata* plays an important role in ecosystem particularly for sustainable development of the aquatic ecosystem. Turtles act as consumers of plants and other animals as well as they are preyed upon by other animals (turtle eggs and juveniles) and are an important part of food-web of the ecosystem. Fresh water turtles are scavengers and turtle scavenging can remove fish carcasses from water much faster than natural decomposition. They can improve the quality of water such as pH and are a vital component of the ecosystem.

Behavioural studies is essential to observe adaptability in freshwater turtles to the variations in environmental temperatures. Heat stress is very common in tropical and sub-tropical areas due to global warming. Heat stress is mainly counteracted by physiological and behavioural responses to maintain body's homeostasis (eustasis). This study observes that hyperthermia causes reduced feeding and induces some behavioural changes which is related to hormonal disorders, mainly adrenocorticoids secretion by activating Hypothalamo-pituitary-adrenocortical (HPA) axis through feedback mechanism. Adrenocorticoid secretion is further regulated by adrenomedullary hormones and/or cytokines coming from the adrenal medulla. The present study looks at the impact of stress on turtles, its ability to withstand stress and adaptability to changes in environmental conditions. It is seen that turtles can withstand fluctuations in temperature and we observed changes in its feeding, basking, burrowing, and breeding behavior across seasons.

Turtles are consumed by some people as a source of protein and are seen as a delicacy and because of its high demand, it is still being covertly and illicitly sold in the local fish markets in India. Implementation of laws and regulation to prevent illegal trade of freshwater turtles are critical. Due to industrial pollution and urbanization, habitat fragmentation is major threat to the turtles. Habitat loss and nest destruction through unethical human activities such as people unlawfully occupying bank of rivers and streams for agriculture purposes and the bleaching of the water bodies to increase fish production is also creating a lot of pressure on this species. Hence there is an urgent need of conservation of this species through fundraising, creating awareness among local people and communities, further development and implementation of laws for protection of freshwater turtles and through use of genetic tools and modern technology including AI tools in biodiversity and conservation strategies. This study is a small step towards the conservation of this species by helping to develop our understanding of their behavioral patterns, ecology and reviewing sustainable conservation measures that need to be taken.

Turtles are ideal models for research in the field of longevity as they survive in a wide variety of environmental fluctuations and can endure different kinds of stresses such as temperature, dehydration and pollutants. Citizen science is a significant tool for data collection for biodiversity documentation and conservation and it enables involvement of the local people to participate in the study. Volunteers can collect reliable, long-term and geographically widespread data. It not only can become an important resource for increasing knowledge and skill development, but also influences participants' behaviour and attitude towards environmental issues. Citizen science programs can help to create an informed and educated public that knows how to find sustainable solutions to address the effects of climate change and other global issues and also care about contributing to and participating in such solutions to turtle protection. Citizen Science tools like KURMA can help in easy identification, creating a digital bank of observational reports on turtles, maintain records on temporal and spatial coverage to turtle's distribution and threats, aid authorities to prevent illegal activities and wildlife crimes and offer accessible rescue assistance. Further research on citizen science projects is necessary to understand its applications and effectiveness in

turtle conservation strategies with the help of public participation. Captive Husbandry is also a major area of extensive research to create favourable conditions for artificial incubation and hatching of eggs which is an important measure for protection and conservation of the turtle species.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

DELCLARATION

We, Dr. Pounami Basu and Dr. Prajna Paramita Basu declare that the submitted research paper is our original work and no part of it has been published anywhere else in the past.

SUPPLEMENTARY MATERIALS

Supplementary materials available in this link: <https://mbimph.com/index.php/UPJOZ/libraryFiles/downloadPublic/24>

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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