Mysterious deaths of aquatic reptiles, with special emphasis on the Indian Flap-shelled turtle *Lissemys punctata* (Bonnaterre, 1789) from Gujarat State, India

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Abstract
The freshwater ecosystems in Gujarat State, in the western part of India, are important habitats for various freshwater turtles. Due to the lack of information on survival and the morality of the freshwater turtles, we present new information on threats to the Indian Flap-shelled turtle *Lissemys punctata* (Bonnaterre) and species mass mortalities observed in some water bodies of the Gujarat State. Approximately 1071 specimens of *L. punctata* and three specimens of the Ganges Soft-shelled turtle *Nilssonia gangetica* (Cuvier), along with large numbers of many other species of reptiles and fishes were recorded dead within a span of five years from January 2016 to December 2020. In some of the cases, the circumstantial evidence suggests that entanglement and trapping in fish nets could have been the reason, but large number of the deaths remained mysterious. This mysterious sudden death of aquatic reptiles in various water bodies of the state is of serious concern. We also discuss the possible explanation for these mysterious deaths.

Key words: Freshwater turtles, Gujarat, reptiles, Testudines, unnatural deaths

Introduction
The current scenario of freshwater ecosystem in India is miserable (Sandilyan, 2016; Chandra et al., 2017; Gopi et al., 2017). These alarming situations could lead to extinction or population depletion of many inhabiting species, especially the freshwater turtle fauna. Somewhat similar scenarios have been observed in the Gujarat State, especially in urban wetlands (Vyas, 2015) and to some extent, in rural ponds. The state’s turtle fauna contains ten species of turtles belongs to six families, including four native and one invasive exotic turtle species inhabiting fresh water (Patel and Vyas, 2019). The Indian Black turtle *Melanochelys trijuga* (Schweigger), a freshwater turtle species, once was uncommon in the Dang forests and rivers of south Gujarat is locally extinct now (Daniel and Shull, 1963; Vyas and Patel, 1990; Vyas, 2007). Here, we have presented recent information on threats and current scenarios of *Lissemys punctata* and its mass mortalities observed in some of the water bodies of the Gujarat State.

Material and Methods

Study Area
Gujarat State (Fig. 1) (20°.21’-24°.80’ N and 68°.16’-74°.76’ E) lies in the western corner of India. The state is bio-geographically and climatically further divided into five sub-regions; South, Central, North Gujarat,
Saurashtra, and Kutch. This study area is in Central Gujarat, a sub-region that contains ten districts including fast developing and densely populated areas (see Patel and Vyas, 2019).

**Study Taxa**

The Indian Flap-shelled turtle *Lissemys punctata* is a member of the family Trionychidae. It is one of the commonest and most widely distributed species in India. *Lissemys punctata* is also widely distributed in Pakistan, Nepal, Bangladesh, and Myanmar. There are introduced and established populations found in freshwater habitats of the Andaman and Nicobar Islands group (Bhupathy et al., 2014). This turtle species is quite adaptable and found in all types of water bodies, including large rivers, lakes, tanks, ponds, swamps and man-made water bodies, including in water-logged agricultural fields, irrigation canals, temporary puddles in roadside ditches, and urban sewage canals (Bhupathy et al., 2014). The species is legally protected as Schedule I, under the Indian Wildlife Protection Act-1972 and Appendix II of CITES (Bhupathy et al., 2014). There are three subspecies: the Southern Indian Flap-shelled Turtle *L. p. punctata*, Central Indian Flap-shelled Turtle *L. p. vittata* (Peters, 1854), and the Spotted Northern Indian Flap-shelled Turtle *L. p. andersoni* (Webb, 1980). All three subspecies of *Lissemys punctata* are distinct in morphological and behavioral traits, but the distribution range limits within India are unclear. The Gujarat State population considered *L. p. vittata* (Praschag et al., 2011; Bhupathy et al., 2014).

In Gujarat, *Lissemys punctata* is the most common and abundant species, found in various types of water bodies, including the large rivers, large man-made water reservoirs, small puddles to open drainages and urban sewages (Patel and Vyas, 2019).

**Figure 1**: Spatial distribution of the dead specimens of the Indian Flap-shelled turtle *Lissemys punctata* observations in Gujarat state, India. (1) Vadgam, Anand, (2) Kumbharwada, Bhavangar, (3) Narmada Main Canal, Gandhinagar, (4) Gotri Pond, Vadodara, (5) Rudramata Dam, Kutch, (6) Vadhera, Amreli, (7) Gorasar, Kutch, (8) Timbi Reservoir, Vadodara, (9) Petli, Kheda, (10) Bhokhan, Vadodara, (11) Kamala Nagar, Vadodara, (12) Bakrol, Anand.
Data Collection

We collected the turtle mortality data from the intensive study area, and further extended it to Gujarat State. The data were gathered within the last five years from January 2016 to December 2020 and were assembled from various sources, including organization like Voluntary Nature Conservancy, Anand, Vasundhara Nature Club, Junagadh and other NGOs (who actively work for reptile conservation), forest department, print and electronic media. In addition, regular visits, at an approximate interval of 2 months, were made to specific sites for the collection of information on death, size, health, photographic records and use of water bodies, in order to understand the circumstances of death. For those observations collected by authors, post-mortem were not done. Moreover, we did not assess the water quality at the observed sites.

Results

A total mortality of 1071+ specimens of Flap-shelled turtle Lissemys punctata, along with 2 specimens of the Mugger crocodile Crocodylus palustris (Lesson), 3 specimens of the Ganges Soft-shelled turtle Nilssonia gangetica, 4 Checkered keelback snake Fowlea piscator (Schneider) and large numbers of fish fauna were recorded from 12 wetlands, within five years period (details mentioned in Table 1; Figs. 1 and 2). The cause of these mysterious and sudden deaths of four taxa of aquatic reptiles in various water bodies of the state are not yet known.

All these incidents occurred in two types of wetlands, including four in the natural village ponds and eight in man-made water bodies. The man-made water bodies are further categorized as 2 dams, 1 irrigation canal, 3 urban ponds and 2 sewage ponds. Overall, the dead turtles were found in all parts of the state, except in south Gujarat (Fig. 1). High numbers of dead turtles were recorded from remote rural areas compared to urban ponds and 2 sewage ponds. Overall, the dead turtles were found in all parts of the state, except in south Gujarat (Fig. 1). High numbers of dead turtles were recorded from remote rural areas compared to urban areas / cities.

The details of each water body and chronology of the records of the deaths of turtles along with other details are given in Table 2. High mass mortality with 700 turtles in three instances was noted during the year 2016 and low numbers of mortality with 8+ turtles was recorded during 2017 and 2018 (Table 3). Such number of reptiles’ death within five years is a not too alarming, but in three cases where mysterious mass deaths of the flap-shelled turtles are concerned, is of most serious investigation, especially the sudden death of 200–350 turtles in the water bodies of Vadgam, Gorasar and Bhavnagar City (Fig. 3). The first two water bodies are located in remote rural parts of Gujarat and the last one is on the edges of cities.

In six cases, nearly 60 adult Lissemys punctata turtles were found dead after being entangled and trapped in the fishing nets. Fishing is practiced regularly in the study region, and turtles and crocodiles being caught in nets is not uncommon. For the remaining 1011 turtles, causes of death could not be identified. In the absence of veterinary facilities, postmortems could not be performed, and the cause of death remains a mystery.

The major numbers of mysterious turtle is match with r morphological characters of L. punctata as olive green color carapace without marking or with indistinct black speckled marks along with black marks on the head (Fig. 4).

Discussion

In the early 1980s, every year 50000−70000 Lissemys punctata were sold at the Howrah Market, Kolkata (Das, 1991). Whitaker (1997) stated that not only live adult turtles were sold, even their eggs are also heavily exploited for food and medicine. This is the most common turtle species in the meat trade. Habitat loss was a major concern (Choudhury et al., 2000). The recent Turtle Survival Alliance (TSA) survey indicated about 70,000 specimens of L. punctata were poached just in the year 2018, through international shipment from India (TSA, 2019). Despite being a protected species under Schedule I, the Indian Wildlife Protection Act, 1972, and its trade is indicated about 70,000 specimens of L. punctata as olive green color carapace without marking or with indistinct black speckled marks along with black marks on the head (Fig. 4).

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Table 1: Summary of the reptilian species, numbers of individuals found dead in the study area and status of the species in the IUCN Red List of Threatened Species.

| No. | Common English Name | Scientific Name (Author) | Found at No. Water body | No. Death | IWPA-1972 | Status | IUCN Red List | RAGS |
|-----|---------------------|--------------------------|------------------------|-----------|-----------|--------|----------------|------|
| 1   | Indian Flap Shell Turtle | Lissemys punctata (Bonnaterre) | 12 | 1071 | Schedule-I | Least Concern | Ab   |
| 2   | Mugger Crocodile | Crocodylus palustris Lesson | 1 | 2 | Schedule-I | Vulnerable | Co   |
| 3   | Ganges Soft Shell Turtle | Nilssonia gangetica (Cuvier) | 2 | 3 | Schedule-I | Vulnerable | Co   |
| 4   | Checkered Keel Back Snake | Fowlea piscator (Schneider) | 1 | 4 | Schedule-IV | Least Concern | Ab   |
| 5   | Unidentified Fish Fauna | 9 | Abundance | --- | --- | --- | --- | --- |

IWPA-1972= Indian Wildlife Protection Act- 1972, RAGS= Relative Abundance in State, Ab-Abundant, Co-Common.
Figure 2: Types of the reptilian species found dead during the observation period (January 2016 to December 2020), (A); Indian Flap-shelled turtle *Lissemys punctata*, (B); Mugger crocodile *Crocodylus palustris* (C); Ganges Soft-shelled turtle *Nilssonia gangetica*, (D) Checkered keelback *Fowlea piscator*.

Table 2: Details and chronology of the dead specimens of *Lissemys punctata* found at various location of Gujarat State, India.

| No | Date       | Location                          | GPS Locations                  | Type of water body / Use          | No. of dead turtles | Remarks                                      |
|----|------------|-----------------------------------|--------------------------------|----------------------------------|---------------------|----------------------------------------------|
| 1  | 2 May 2016 | Vadgam, Khambhat, Anand           | 22°19'16.92"N; 72°23'52.94"E   | Village Pond Communal Use        | 350+                | Numbers of fishes died; Mysterious Death (0.20 sq. km) |
| 2  | 2 July 2016| Kumbharwada, Bhavangar City       | 21°46'48.66"N                 | Urban Sewage Pond                | 200+                | Mysterious Death                            |
| 3  | 20 Nov. 2016| Narmada Main Canal, Nr. Gandhinagar | 23°8'58.54"N; 72°34'14.14"E    | Irrigation Canal                 | 150+                | Mysterious Death                            |
| 4  | 25 Oct. 2017| Gotri Pond, Vadodara City         | 22°18'51.95"N; 73°08'03.18"E   | Urban City Pound; Scenic Beauty and Fishing activity | 8+                  | Numbers of fishes died; Two *Nilssonia gangetica*; Fishing activates |
| 5  | 17 July 2018| Rudramata Dam, Bhuj-Kutch         | 23°22'17.68"N; 69°42'45.34"E   | Portable Water Supply for Nr. Bhuj City | 5+                  | Mysterious Death, Few fishes died; Fishing activates |
| 6  | 1 Nov. 2018 | Vadhera, Jafirabad, Amreli        | 20°51'10.47"N; 71°18'38.15"E   | Village Pond Communal and Fishing activities | 3+                  | Trapped in abandoned fish-net               |
| 7  | 29 May 2019 | Gorasar, Gogadar, Rapar, Kutch   | 23°23'14.08"N; 70°48'22.25"E   | Village Pond                     | 300+                | Water level reduced, Numbers of fishes died, 14 adults turtle rescued (1 sq. km) |
| 8  | 23 Juneti/mbi Reservoir, Nr. Vadodara City | 22°18'48.71"N; 73°17'0.15"E | Irrigation Tank Communal and Fishing activities | 6+                  | Four *Fowlea piscator*; Numbers of fishes died; snakes Trapped in Fish-net |
| 9  | 4 July 2019 | Petli Village, Kheda              | 23°35'46.39"N; 72°45'10.33"E   | Village Pond Communal and Fishing activities | 2+                  | Two juveniles’ *Crocodylus palustris*; Fishing activities; Trapped in fish-net, Discharging Urban Sewage One *Nilssonia gangetica*, Few fishes died |
| 10 | 9 May 2020  | Bhokhan Pound, Padara, Vadodara  | 22°14'19.23"N; 73°4'33.81"E   | Town Pound Urban Sewage          | 8+                  | Discharging Urban Sewage; One *Nilssonia gangetica*, Few fishes died |
| 11 | 26 June 2020| Kamala Nagar Pound, Vadodara City | 22°19'12.64"N; 73°15'45.23"E   | Urban City Pound Scenic Beauty  | 36+                 | Few fishes died                              |
| 12 | 2 Sep 2020  | Bakrol, Nr. Anand City,           | 22°33'43.74"N; 72°54'52.95"E   | Urban City Pound Scenic Beauty  | 3+                  | Discharging Urban Sewages Numbers of fishes died |

Total death of *Lissemys punctata* = 1071+
Table 3: Year wise records of the Indian Flap-shelled turtle *Lissemys punctata* deaths in Gujarat state.

| No | Year | No of Water bodies | Total Number of Turtle Death |
|----|------|--------------------|-----------------------------|
| 5  | 2016 | 3                  | 700+                        |
| 4  | 2017 | 1                  | 8+                          |
| 3  | 2018 | 2                  | 8+                          |
| 2  | 2019 | 3                  | 308+                        |
| 1  | 2020 | 3                  | 47+                         |
|    |      |                    | 12                          | 1071+                       |

Figure 3: Mass mortality (350 + individuals) of the Indian Flap-shelled turtle *Lissemys punctata* observed at Vadgam Village, Anand, Gujarat State, India.

The sudden or mysterious death of 1071+ specimens of *L. punctata* within a five-year span points out that some vigilance and proactive conservation measure is needed to investigate such phenomena. Because earlier a similar case was noted with the mass death of *L. punctata* in the state, when on 19th November 2006 about 150 adult specimens of *L. punctata* were found dead floating in the main Narmada Canal, near Jaspur (23°10’18.75”N; 72°30’16.15”E; a.s.l./elevation 65 m), Ahmedabad (Reuters, 2006). Few of them were sent for post-mortem but until today, the report remains mysterious. However, large numbers of sudden mysterious death of *L. punctata* in the state is a big question and further subject of serious investigation on these cases, somewhere the authority failed to investigate properly and did not take any serious action. However, the following could be the possible causes behind the large numbers of mysterious death of the turtle species, based on our experiences: 1) Some unknown disease contamination in the species; 2) Illegal release of some industrial toxic influent in the water body; and 3) It could be that some culprits discarded the dead turtles as the result of illegal activities such as collection of certain specific body parts alone from the turtles.

Frequently published reports about the deaths of sea turtle like Olive Ridley turtles in newspapers and journals indicate that the unnatural death of turtles is a regular occurrence (Pandav et al., 1997; Murthy and Ramana Murthy, 2011; Das and Mandal, 2019). Casualties take place when these turtles are caught in trawl-fishing nets as incidental catch.
While there are many reported incidents of mass mortality of sea turtles, we could not find much published literature or news about mass mortality of freshwater turtles. One such example of mass mortality of freshwater turtle was from Alappuzha district, Kerala, where turtle carcasses were found from various parts of the districts (TNN, 2018). The reasons of the death could not be ascertain because of the decayed condition of the bodies. In the absence of information about similar incidents, it is difficult to corroborate the reasons of such mass mortalities.

We have a gloomy experience with the most critical endangered Indian crocodilian known as the Gharial *Gavialis gangeticus* (Gmelin, 1789). From 2007 to 2008, over 113 Gharials were found dead all measuring specific sizes (1.6 to 3.5 m) in a 25 km long river stretch of Chambal and Yamuna confluence area of the National Chambal Sanctuary, India (Whitaker et al., 2008). It was the worst tragedy in the history of conservation of aquatic reptiles (Webb, 2008). In addition, we do not know who could have been responsible and which factors caused the damage to the kidneys (some unidentified nephrotoxin) for mass mortalities of *G. gangeticus*. Until date, the mass death of *G. gangeticus* is an unsolved puzzle. There are two possibilities, an unknown disease or contamination from an industrial toxic influent in the water body. In Gujarat, 25 adults Ganges Soft-shelled turtle, *Nilssonia gangetica*, were found dead in a single urban water body at Ratan talav, Bharuch. This is also a case of mysterious deaths (Vyas, 2015).

We cannot deny the possibility of illegal activity where some culprits discarded the dead turtles, as dead turtles were found in Narmada canal in 2006 and 2016. The great number of dead turtles found at Vadgam, Anand District and Gorasar, Kutch District, Gujarat may be due to some unknown disease contamination in the population or illegal releasing of industrial toxic effluents in the water body, because both cases occurred in some remote areas of the state (see: Pande and Datla, 2016). In the Gujarat State pollutants such as industrial hazardous waste or toxic effluent were spilt into water bodies and agricultural lands and several aggrieved farmers brought a public interest lawsuit to compel disciplinary action from the court (CSE, 2009). The rivers and waterways of the state have been often unfit for drinking or irrigation, mainly because of industrial processes (Pande and Datla, 2016). In 2007, the federal government identified five of the most polluted rivers in the country, of which three were in Gujarat (CPCB, 2007). A study shows that there is constant deterioration of quality of surface water resources due to discharge of treated or partially treated effluents from the industries (Vyas, 2010; Bansal, 2018; Vyas et al., 2020). Thus, all above incidences and its references support that all three probabilities had caused the sudden mass deaths of the species in the state. It is an important alarm call to the authorities for awakening and to be actively vigilant about fresh water bodies.

**Figure 4:** Dead Indian Flap-shelled turtle *Lissemys punctata* observed at the Rudramata Dam, Bhuj, Gujarat State, (A); habitat of the site, (B); ventral side of a dead individual, (C); dorsal side of a dead individual.
The Indian turtle fauna is facing various major to minor anthropogenic threats, including loss of habitat, alteration of habitat, sand mining, pollution and trade. A recently recorded invasive, *Trachemys scripta elegans*, has been reported from the entire Gujarat state (Vyas, 2015; 2020) and other reports of such invasion are also known, from across the country (Kiruba-Sankar et al., 2018) adding new horizons of threats to the important turtle fauna of India (Vyas, 2021). The turtle fauna is under tremendous pressure from anthropogenic threats, especially recent development and upcoming various industrial projects in the state, including large scale irrigation expanses of canal networks, transportation and communication networks of road and railways, riverside development. Moreover, major water bodies are being linked to each other with the linking of the main irrigation canal of the Narmada Sarovar Project. Although all these future developments and projects will boost the state economy, they will definitely result in the loss of natural resources. It may cause great environmental disasters in the future if the state government does not take a proper look at the issue and take positive steps toward creating a balance between development and nature conservation.

Although awareness campaigns in the form of “turtle walks” and community education programmes in fishing villages are organized every year by Green Mercy (a local NGO and a member of the Turtle Action Group), involvement of all stakeholders in sea turtle conservation on a larger scale is vital for securing the long term survival of the species and their coastal nesting habitats in the region.

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**Conflict of interest**

All the authors declare that there are no conflicting issues related to this research article.

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