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Article

In-situ and ex-situ hatching of olive ridley turtles in Cox's Bazar Coast for conservation

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Abstract: The remarkable mass nesting behavior of the olive ridley turtle (*Lepidochelys olivacea*) is well-known. In the present study, the hatching success of *L. olivacea* at Sonarpara beach was investigated, for the nesting periods 2023–2024. In the in-situ hatchery, the hatching percentage was recorded high (80%). A total of 73 eggs were hatched out of 91 eggs after 52 days of incubation. In the ex-situ hatchery, the hatching percentage was recorded low (74%). A total of 1070 eggs were hatched out of 1445 after 45–66 days of incubation. A significant difference in hatching percentage was found between the two hatching methods ($p < 0.05$). Temperature and humidity are the most influential parameters that regulate the hatching of olive ridley. In terms of in-situ study, the surface sand temperature ranged from 36 °C–42 °C, while the air temperature was recorded between 34 °C–37 °C. In the ex-situ hatchery, the surface sand temperature ranged between 19.2 °C–34.3 °C. In the in-situ hatchery, the average sand humidity was recorded 79.9%, and air humidity was recorded 83.68%. The average sand humidity was recorded 73.7% in the ex-situ hatchery, and air humidity was recorded 72.9%. The hatching percentage was recorded high when sand humidity was high in the in-situ hatchery and the hatching rate was found low when the sand humidity was recorded low in the ex-situ hatchery. The present findings designate that there is a positive relation between sand humidity and hatching success. This study also suggests intensifying long-term scientific studies along the whole coast to conserve this vulnerable turtle.

Keywords: olive ridley; hatching; incubation; temperature; conservation

1. Introduction

Sea turtles are essential to a healthy ocean because of their direct influence on other species. Additionally, they play an important role in the main interactions that shape the dynamics and structure of various marine ecosystems. As a result, sea turtles serve as consumers, competitors, hunters, prey, and hosts or carriers of other creatures [1,2]. Removing a keystone species from an area affects the natural order, which affects other plants and animals in different ways [3]. Olive ridleys have a high degree of behavioral flexibility worldwide. They can travel through oceanic seas and eat surface-living things, or they can remain in coastal shallow areas and graze on invertebrates [4]. Many olive ridley turtles exhibit migratory behavior, which is an effective tactic for their opportunistic hunt for patchily scattered prey. The reason olive ridleys are the most common sea turtles may be due to their adaptability, which may enable them to handle erratic shifts in dynamic habitats [5].

An estimated 800,000 female olive ridley sea turtles nest annually throughout the world, making them the most common sea turtle [6]. They can be found worldwide in tropical areas of the Atlantic, Pacific, and Indian oceans. It is widely distributed across more than 80 nations. Their populations have drastically decreased in recent decades; despite they are the most prevalent and abundant of the seven species. The International Union for Conservation of Nature (IUCN) has listed olive ridleys as a vulnerable species for more than ten years due to a 30%–50% decrease in their populations worldwide. The main causes of the loss are bycatch, illegal trade, destruction of natural habitat, animal and human predation, pollution, and climate change [7].

Hatching (ex-situ & in-situ) of olive ridley is a very important way to conserve their population worldwide. Understanding how physical factors like temperature affect the nest environment is crucial to comprehending sea turtle sex determination and embryonic development [8]. Higher female-to-male hatchling sex ratios are the result of higher incubation temperatures. Only females are produced by incubation temperatures more than 30 ℃, according to earlier research on olive ridley turtles in Gahirmatha, India [9]. Male sea turtles are often produced at lower incubation temperatures (25 ℃–28 ℃), while females are produced at higher temperatures (30 ℃–32 ℃) [10]. The middle third of the incubation phase corresponds with the Thermo-Sensitive Period. In the olive ridley, the regulated male-promoting temperature (MPT) is 26 ℃ ± 0.5 ℃, whereas the female-promoting temperature (FPT) is 33 ℃ ± 0.1 ℃ [11,12]. According to Sandoval Espinoza [13], MPT and FPT in the field have been estimated to be 27.03 and 32.86 ℃, respectively. For Mexican populations in the field, the crucial temperature—the temperature at which equal numbers of males and females are produced—has been determined to be 29.95 ℃ [13]. Nevertheless, this value may differ between populations or even within the same population. Humidity is crucial; according to reports, hatching success is increased by humidity levels above 69% [14].

The status of olive ridley sea turtles in Bangladesh are concerning due to various environmental and anthropogenic threats. They are considered critically vulnerable at the national level due to the combination of high mortality rates, reduced nesting sites, and habitat degradation. Sea turtles are found along the Bay of Bengal, particularly on St. Martin’s Island and Cox’s Bazar beaches, which are key nesting sites. These turtles utilize the sandy beaches for laying eggs, but their nesting activities have significantly declined in recent years. Some efforts like focusing on relocating eggs in protected hatcheries to ensure hatchling survival are needed. Many non-governmental organizations (NGOs) collect eggs and put them in the hatcheries until they hatch. After the hatching of hatchlings, they are released into the ocean. They have been conducting these activities for a long time. They are doing these activities to conserve this species. It is very important to conduct scientific research to know the hatching parameters, and which factors are responsible for hatching success. It also requires knowing the hatching environment and factors that regulate hatching success. This turtle is decreasing day by day due to many factors [6]. Conservation of this turtle is very important to balance the marine ecosystem. Ex-situ and in-situ hatching (with regular monitoring) are crucial to increase this turtle population. To conserve the olive ridley turtle and marine ecosystem, the present study was conducted to find out the in-situ and ex-situ hatching percentage of olive ridley turtles in Cox’s Bazar coast.

2. Materials and methods

2.1. Study area

The in-situ and ex-situ experiment was conducted on Sonarpara beach, Cox's Bazar coast, Bangladesh (**Figure 1**). The study area is the location where olive ridleys naturally nest and lay eggs. There is a fishing community along the coast. The boats of fishermen are anchored on the beach when they come back from fishing. They also repair their boats and nets on the beach. They process their fish on the beach and catch shrimp fry on the coast. Fry catchers use a Set Bang Net for catching fry. The beach is also a tourist attraction for the tourists. Tourists and local people drive CNG vehicles, motorcycles, and cars on the beach. There is an arrangement of swings for tourists. Plastic materials (chip packets, biscuit packets, polythene, etc.) are very common on the beach. Local people and tourists also play football on the south side of the beach. On the north side of the beach, there is a sand dune area that is calmer and quieter than the south side. Most of the turtles lay their eggs on the north side rather than the south side due to less disturbance.

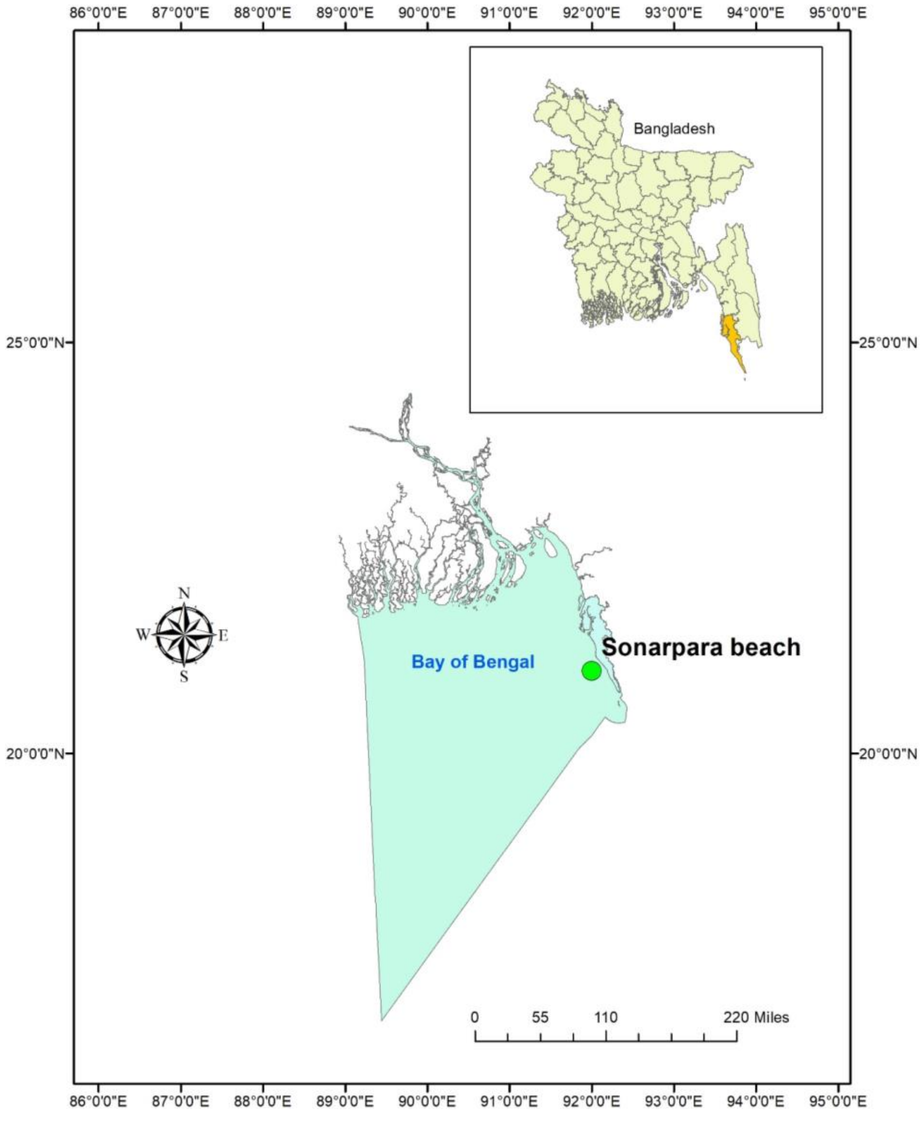


Figure 1. Map showing the experimental hatching sites of olive ridley in Cox's Bazar coast.

2.2. Experimental setup

2.2.1. In-situ

A hatchery was constructed on the beach in 2023. The hatchery was made beyond the highest high tide level (Distance 23 m) where the turtle had naturally laid eggs. The size of the hatchery was 5 m × 5 m. Wood, bamboo, rope, and plastic nets were used to build the hatchery. A caretaker cum security was appointed for regular data taking and security. The turtle laid eggs on 4 March 2023 and the eggs were incubated for 52 days. All the eggs hatched into hatchlings on 26 April 2023. The depth of the nest was recorded as 46 cm.

2.2.2. Ex-situ

Two ex-situ turtle hatcheries were set up on the beach in 2024 (**Figure 2**). Both the hatcheries were constructed with few customizations. The hatchery was customized with piled-up (31 cm) of sand collected from the beach to protect it from the highest high tide or inundation. The hatchery was constructed beyond the highest high tide level (Distance 25 m). The size of each hatchery was 5 m × 5 m. A total of 14 turtle nests (total eggs = 1445) were incubated at different times in the hatchery depending on the availability (**Table 1**). Turtle eggs were incubated in both customized hatchery 1 (n = 7 turtle eggs = 736 eggs) and customized hatchery 2 (n = 7 turtle eggs = 709 eggs). The turtle eggs were incubated in the nests (60.96–76.2 cm depth) for 45–66 days in the hatchery.

(A)

(B)

(C)

Figure 2. (A) construction of hatchery; **(B)** construction of hatchery; **(C)** measuring distance between high tide line and hatchery.

Table 1. The number of turtles and turtle eggs incubated at different times in the hatchery.

Number of Turtle	Date	Eggs
01	31.01.2024	125
01	09.02.2024	132
01	26.02.2024	101
03	27.02.2024	293
02	28.02.2024	168
01	29.02.2024	108
02	02.03.2024	217
01	05.03.2024	87
01	06.03.2024	107
01	07.03.2024	107
Total = 14		Total eggs = 1445

Wood, bamboo, rope, plastic net, and a sandbag were used to construct the hatchery. A caretaker cum security was appointed for regular data taking and security. In the nesting hole of the turtle, 1st sand was used that was collected from the site where the turtle laid their eggs (original site of laying eggs).

2.3. Turtle eggs collection for ex-situ hatchery

Eggs were collected from the beach where turtles naturally lay their eggs. The research team and caretaker searched for eggs at night. Caretakers were also trained to collect eggs from the natural environment. After locating the natural nest, clutch information (e.g., date, time, location, number of eggs) was recorded in a data sheet. Eggs were collected when the turtle returned to the ocean. Eggs were not removed during or before the turtle finished nesting. No olive ridley turtles were disturbed or harmed during egg collection. To avoid contamination, hand gloves were used to collect eggs from the nest. Eggs were carefully removed one at a time, and they were placed in the same vertical configuration without being rotated. A clean plastic container filled with soft sand from the nesting beach was used to carry the collected eggs. Immediately after collection, eggs were transferred to the ex-situ hatchery.

2.4. Determination of nest temperature, pH, and humidity

The sand temperature was monitored using the HI-98331 Groline Direct Soil Conductivity (EC) & Temperature Tester (Made in the USA). The air temperature was measured using a G. H. Zeal glass thermometer (made in the UK). Humidity was measured using the Smart Sensor Digital Hygrometer Humidity and Temperature Meter AR867 (Made in China). Sand pH was determined using a Takemura Soil pH Meter DM-13 (made in Japan). The data-taking activities in hatcheries are shown in **Figure 3**.

(a) **(b)** **(c)** **(d)**

Figure 3. **(a)** taking sand temperature data; **(b)** recording data in the datasheet; **(c)** monitoring the natural hatching ground; **(d)** taking temperature and humidity data.

2.5. Statistical analysis

One-way ANOVA was executed to know the substantial difference in hatching percentage between the two hatching methods. For this, SPSS v.22 was used to find the results. To execute the analysis, Tukey was selected as Equal Variance Assumed and Tumhane's T2 was checked as Equal Variance Not Assumed. The significance level was set at 0.05 in post hoc multiple comparisons.

3. Results and discussion

3.1. Hatching success

The most prevalent species of marine turtle in the world is the olive ridley. In the present study, two studies (in-situ & ex-situ) were conducted on the Sonarpara beach. The percentage of eggs that produced live hatchlings, including live hatchlings found during nest excavation, was used to determine hatching success. In the in-situ hatchery, a total of 91 eggs were incubated for 52 days. Among 91 eggs, 73 eggs were hatched and 18 eggs were not hatched. Hatching success was recorded as high (80%) in in-situ hatchery conditions. In the ex-situ hatchery, a total of 1445 eggs were incubated, and 1070 were found hatched, whereas 376 eggs were not hatched. The hatching success was recorded as lower (74%) in ex-situ hatchery conditions than in-situ hatching (**Table 2**). The significant difference in hatching percentages was recorded in hatching methods (in-situ & ex-situ) (F = 60.84; p = 0.004).

Table 2. Hatching percentage of olive ridley in ex-situ and in-situ hatchery and comparison with international findings.

Incubation Method	Success (%)	Region	Sample Size (nests)	References
In-situ	80	Cox's Bazar coast, Bangladesh	1	Present study
Ex-situ	74	Cox's Bazar coast, Bangladesh	14	Present study
Ex-situ	85	Batu Hiu Beach, Indonesia	1	[15]
Ex-situ	77.4	Boca de Tomates, Mexico	13	[16]
Ex-situ	59.8–93.6	Pacific coast, Guatemala	5	[17]
Ex-situ	60.68	Bantul, Indonesia	-	[18]
Ex-situ	74.7	Nayarit, Mexico	2571	[19]
In-situ	0	Indonesia	30	[20]
Ex-situ	54.2–73.6	Indonesia	109	[21]
In-situ	14.81	Ostional Beach, Costa Rica	5	[22]
In-situ	67.9	Sinaloa, Mexico	1071	[23]
Ex-situ	64.1	Sinaloa, Mexico	926	[23]
Ex-situ	46.9	Sinaloa, Mexico	969	[23]
In-situ	66	Jalisco, Mexico	ND	[24]
Ex-situ	59	Jalisco, Mexico	130	[24]
In-situ	73.7	Baja California Sur, Mexico	45	[25]

The hatching success rate on the Orissa coast at Rushikulya Rookery Beach ranges from 63.79 to 100%, with an average of 89.64% [26]. With over 300 individuals nesting per season (2016–2017) and a hatching success rate of at least 75%, olive ridley sea turtles are the dominant species at Ramnagar along the N–E coast of the North Andaman Islands [27]. According to Mohanty et al. [26], the majority of newborn turtles (average 95.63%) can leave their nests and enter the ocean. da Silva et al. [28] reported almost similar findings in Brazil that included the data from 1991/1992 to 2002/2003. In the in-situ hatchery, a total of 91 eggs (eggs from one turtle) were incubated for 52 days whereas it was 45–66 days in ex-situ hatchery. Calvianto et al. [29] recorded an average hatching percentage of 48% at an incubation period of 48 days in the Serangan Village, Bali Province, Indonesia.

3.2. Factors that regulate turtle hatching

Temperature and humidity are the most influential parameters that regulate the hatching of olive ridley. In terms of the in-situ hatchery, the surface sand temperature ranged from 36 ℃–42 ℃, while the air temperature was recorded between 34 ℃–37 ℃ (**Table 3**). Successful nests had an average incubation temperature of less than 35 ℃ in the Pacific Coast of Central America [30]. For East Pacific olive ridleys, 30.24 ℃ is the key temperature—theoretically, this temperature produces both sexes in equal proportion [30]. Turtle nesting in San Narciso, Zambales, Philippines, is favored by temperatures between 27 ℃ to 33 ℃ throughout the nesting months of October through March [31]. According to Maulany et al. [20], hatchlings' emergence success and locomotor performance decreased when their nest temperatures were higher than 34 ℃ for at least three days in a row during incubation in the hatchery. Over 50 days of measurement, the seminatural nests on the Bantul beaches had daily temperature changes ranging from 24.3 ℃ to 31 ℃ [18].

Table 3. Hatching parameters data collected from the Sonarpara beach nest (in-situ) during the study.

Sl. No.	Date	Name of Parameters				
		Sand Surface Temperature (℃)	**Sand pH**	**Sand Humidity (%)**	**Air Temperature (℃)**	**Air Humidity (%)**
1	21.03.23	41	7.2	82	37	84
2	28.03.23	40	7.15	80.5	36	83.7
3	05.04.23	39	7.10	79	35	82
4	12.04.23	36	7.05	75	37	89
5	19.04.23	38	7.09	78	34	81
6	26.0423	42	7.20	83	37	85
7	30.04.23	41.5	7.16	82	36	81

In ex-situ hatchery, the surface sand temperature ranged between 19.2 ℃–34.3 ℃ (**Table 4**). Only Orissa's olive ridley population is reported to benefit from India's critical temperature of ~29.2 ℃ [32]. Morales-Mérida et al. [33] recorded that the temperature of four nests on Guatemalan beaches was from 30.154 ℃ to 37.935 ℃. In Costa Rica and Java (Indonesia), lethal temperature limits of 34 ℃ or 35 ℃ were suggested for *L. olivacea* [22]. According to Valverde et al. [22], olive ridley hatchlings were not produced on Ostional Beach in Costa Rica when the mean incubation temperature was higher than 35 ℃.

Table 4. Hatching parameters data collected from the Sonarpara beach nest (ex-situ) during the study.

Sl. No.	Date	Name of Parameters					
		Sand Surface Temperature (℃)	**Sand pH**	**Sand Humidity (%)**	**Air Temperature (℃)**	**Air Humidity (%)**	**Day/Night**
1	01.03.2024	28.8	7.1	73	26	71	D
2	02.03.2024	27	7.0	76	22	70	D
3	03.03.2024	28	7.2	79	24	73	N
4	04.03.2024	29.8	7.1	71	25	69	N
5	05.03.2024	33.01	7.05	58	32	60	D
6	06.03.2024	33.02	7.06	59	32	61	D
7	21.03.2024	19.2	7.12	92	19	88	N
8	22.03.2024	19.3	7.06	91	20	89	N
9	24.03.2024	34.2	7.05	70	32.5	75	D
10	25.03.2024	34.3	7.10	68	32	73	D

According to Howard [34], certain olive ridley embryos, however, have been known to endure temperatures above 37 ℃ for brief periods if the average incubation temperature was lower than 35 ℃. There was found a temperature-dependent sex ratio in olive ridley turtles. Temperatures above 35 ℃ accelerated embryonic growth in flatback turtles (*N. depressus*) and did not decrease hatching success in nests in the Gulf of Carpentaria, Australia [35]. All male hatchlings emerged at a temperature of 26 ℃ while all females were produced at a temperature of 29 ℃ [18]. The findings indicate that high-temperature nests produce more female hatchlings than male [36]. The few hatchlings that were hatched were most likely females because incubation temperatures of 32 ℃ and higher that were reported during the gonadal thermosensitive period were higher than the mean crucial temperature of 30.5 ℃ [22].

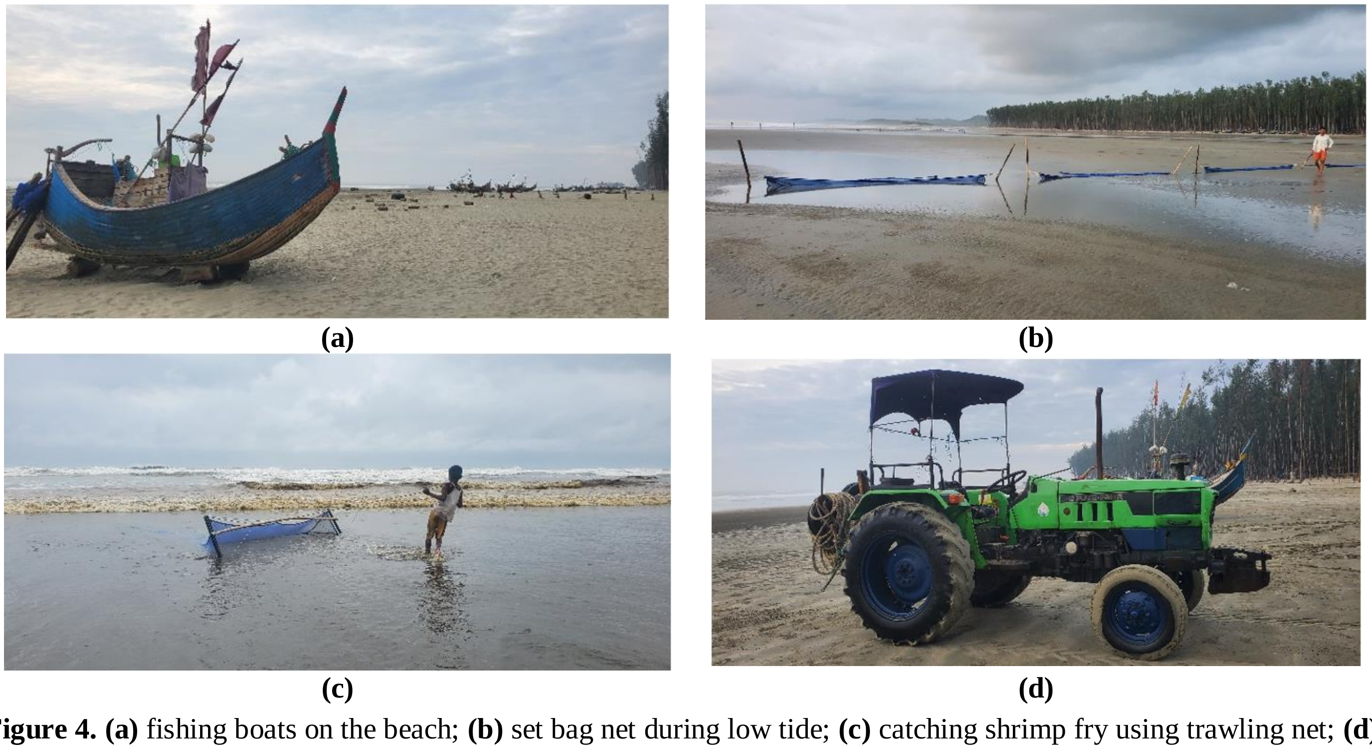
High relative humidity and precipitation caused sand temperatures to drop, whereas higher air temperatures caused sand temperatures to rise. There was a negative correlation between sand temperatures and relative humidity (effect size: −0.03, standard error: 0.01) and a positive correlation with air temperatures (effect size: 0.47, standard error: 0.04) [37]. The sand humidity in the hatchery ranged from 2% to 14% in Batu Hiu Beach, Pangandaran, West Java, Indonesia, with an average daily humidity of 10% ± 0.22%. The air humidity within the hatchery ranged from 56% to 94%, with a daily average of 85% ± 0.05% [15]. In in-situ hatchery conditions, the average air temperature was recorded 36 ℃ ± 5.31 (**Table 3**). The average air temperature was 26 ℃ in ex-situ hatchery conditions (**Table 4**).

The average pH level of the nest was 7 on beaches in Bantul, Java, Indonesia, supporting the hatchling embryology process [18]. In the present study, an average pH = 7.12 ± 0.05 was recorded in the in-situ hatchery (**Table 3**), while it was recorded as 7.1 in the ex-situ hatchery (**Table 4**).

A positive correlation was found between the percentage of relative sand humidity and hatching success in the incubation of treatments for *Eretmochelys imbricata*. Hatching success was recorded 93.3% when the sand humidity was 100%. The success rate decreased (73.3%) when the humidity decreased (75%). The success percentage came to 0% when the humidity was recorded 30% [38]. The average incubation time for high-moisture clutches was 58.33 days, which was longer than the average incubation time for low-moisture clutches, which was 54.17 days. High moisture concentrations shortened the incubation period, lowered the nest temperature, and had a slight impact on the hatchling shape. However, they did not influence *Chelonia mydas*'s hatching success, embryonic death stage, crawling speed, or first swimming performance [39]. In the present study, in the in-situ hatchery, the average sand humidity was recorded 79.9%, and air humidity was recorded 83.68% (**Table 3**). The average sand humidity was recorded 73.7% in the ex-situ hatchery, and air humidity was recorded 72.9% (**Table 4**). The present findings also proved that there is a positive relation between sand humidity and hatching success. The hatching percentage was recorded high when sand humidity was high in the in-situ hatchery and the hatching rate was found low when the sand humidity was recorded low in the ex-situ hatchery (**Table 2**).

3.3. Anthropogenic pressure in the experimented beach

There is pressure from fishing and fishing activities on the beach (**Figure 4**). A fishing community is located very close to the beach. Some of the fishermen go fishing with small- and medium-sized engine boats. Some catch shrimp fry using a trawling net (locally toling net or tana jhal) or a set bag net (locally Behingi jhal) on the coast and sell these fries to local hatcheries. Very recently, there are oil-operated tractors for easy management of boats. This is also very harmful to the turtle and other small biodiversity. Repairing torn nets and boats on the beach is also very common.

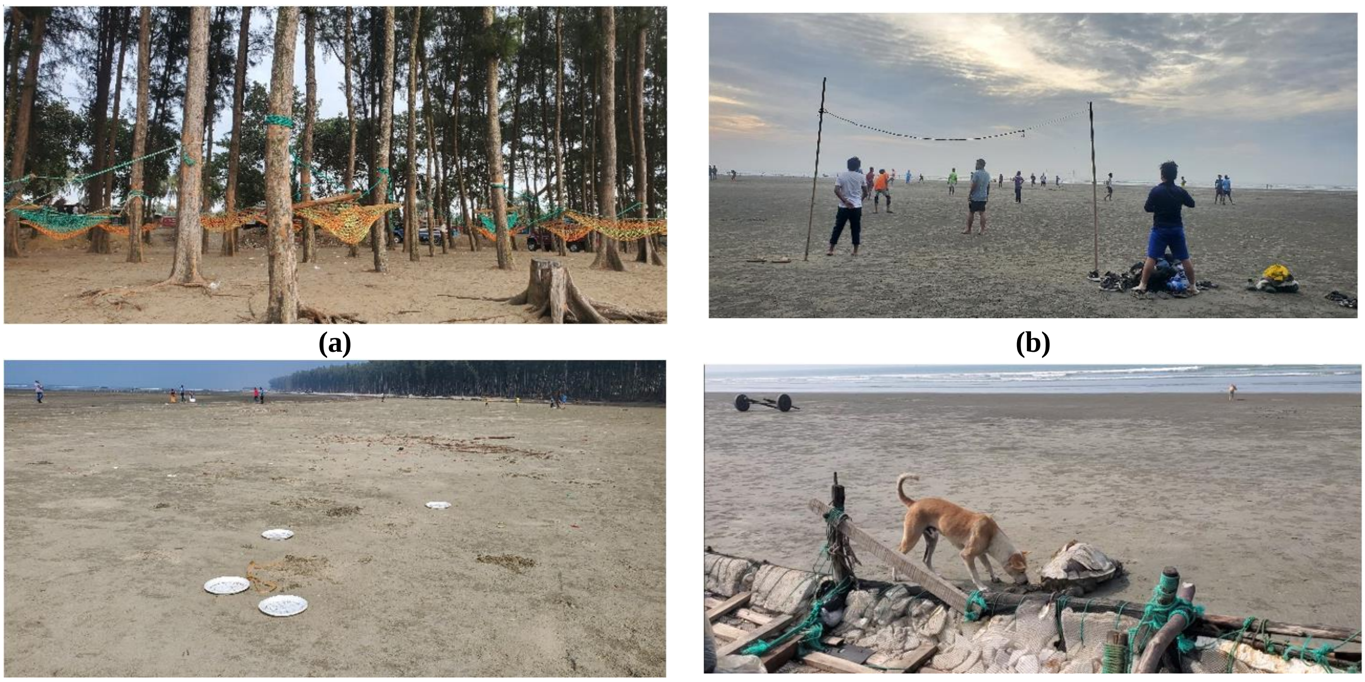


(a) (b)

(c) (d)

Figure 4. (a) fishing boats on the beach; **(b)** set bag net during low tide; **(c)** catching shrimp fry using trawling net; **(d)** tractor on the beach after carrying boat.

Tourism is another important issue for this beach. This sector is gaining popularity on this beach day by day. There is a rope-made swing for tourists (**Figure 5**). Sometimes, tourists ride motorcycles on the beach and in cars. Both local people and tourists play football on the beach. Plastics are another big problem that is produced by both local people and tourists. The dog is a very serious issue since they attack turtles when they come to the beach to lay their eggs (**Figure 5**).



(a) **(b)** **(c)** **(d)**

Figure 5. (a) swing for tourists along the coast; **(b)** local people playing football on the beach; **(c)** one-time plastic plates on the beach discarded by tourists; **(d)** dog biting olive ridley turtle on the beach.

Moreover, turtles were caught as bycatch in fishermen's nets when they fished on the coast and offshore. Every year, a lot of olive ridleys die due to anthropogenic and natural causes when they come to the beach to lay eggs. In 2024, a total of 99 dead turtles were recorded from the Cox's Bazar coast (**Table 5**). The collection of this information was very labor-intensive and difficult.

Table 5. Number of dead turtles encountered during January 2024 to March 2024.

Sl. No.	Date/Month/Year	Total number of dead turtles
1	02/01/2024	2
2	05/01/2024	1
3	07/01/2024	1
4	09/01/2024	2
5	14/01/2024	3
6	17/01/2024	1
7	21/01/2024	2
8	25/01/2024	2
9	28/01/2024	2

Table 5. (*Continued*).

Sl. No.	Date/Month/Year	Total number of dead turtles
10	31/01/2024	1
11	03/02/2024	2
12	04/02/2024	2
13	10/02/2024–26/02/2024	70
14	29/02/2024	1
15	02/03/2024	1
16	04/03/2024	1
17	07/03/2024	2
18	10/03/2024	2
19	13/03/2024	1
Total = 99 dead turtles		

Conservation activities are being done by government organizations and NGOs. Both types of organizations, collect eggs and hatch them in their hatchery. After hatching, hatchlings were released to sea. A total of 29,887 eggs were collected from 243 turtles in 2024 (**Figure 6**).

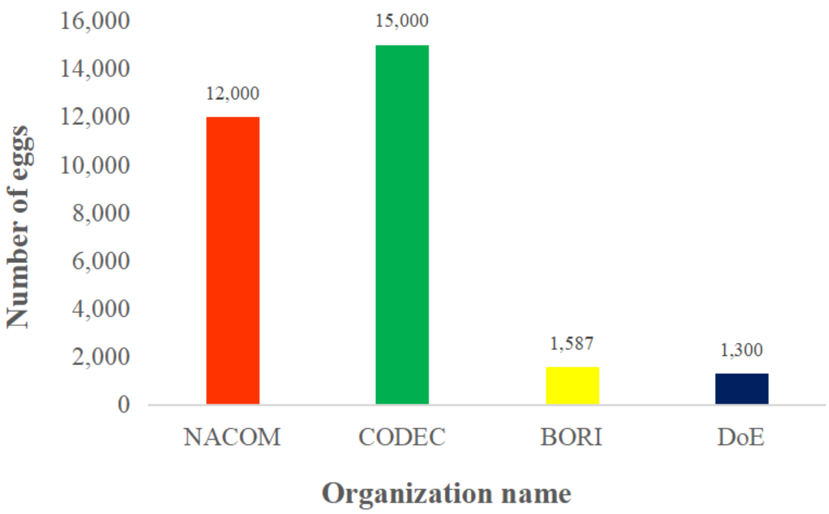


Figure 6. Number of eggs collected by different organizations in 2024.

3.4. Recommendations

Conservation measures can stabilize fragile populations and prevent the extinction of animals that are at risk [40]. The following recommendations should be followed for the best scientific outputs and better conservation of the olive ridley:

1) Temperature, humidity, and pH should be monitored regularly.

2) Nests should be incubated at natural depths to avoid effects on hatchling fitness and locomotion.

3) Hatching can be done in different places to compare the hatching rate percentages.

4) Sex (male and female) determination after hatching is very crucial.

5) Strict monitoring should be ensured to prohibit egg collection.

6) Tourism should be limited or regulated in turtle-hatching areas.
7) Awareness needs to increase among local communities and fishermen.
8) The government and NGOs should work together to conserve the turtles.
9) Conservation should be conducted based on scientific data.

4. Conclusion

The IUCN has categorized the olive ridley as vulnerable. National Oceanic and Atmospheric Administration (NOAA) Fisheries stated that though they are found worldwide, they are protected by the Endangered Species Act. The present study was conducted to improve the hatching percentage of this vulnerable species. The hatching percentage was recorded good in the in-situ hatchery than in the ex-situ hatchery. Temperature was the most controlling factor in hatching, and a positive relation was found between sand humidity and hatching success. The turtle is a very important component of the marine ecosystem. So, it urges more extensive research in the forthcoming future. Regrettably, this turtle quantity is declining day by day. Only a few NGOs are working on the conservation of this turtle species. Different universities and research institutions should emphasize turtle research. The government should focus on this valuable component of the ecosystem and formulate a comprehensive plan to conserve it scientifically.

Author contributions: Conceptualization, MSB and MTI; methodology, MSB and MTI; software, MSB; investigation, MSB and MTI; writing—original draft preparation, MSB; writing—review and editing, MSB; visualization, MSB; supervision, MSB and MTI. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest: The authors declare no conflict of interest.

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