Hatchability Success Rate of Olive Ridley Sea Turtle (*Lepidochelys olivacea*) Eggs Using the INTAN BOX Incubation Device on Pulau Santen Beach, Banyuwangi

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ABSTRACT

The natural hatching success rate of olive ridley sea turtle (*Lepidochelys olivacea*) eggs on Pulau Santen Beach is often hindered by predator disturbances, temperature fluctuations caused by human activities, and unfavorable environmental conditions, with a success rate of only 50–70%. This study aimed to analyze the hatching success rate of olive ridley sea turtle eggs using the INTAN BOX incubation device on Pulau Santen Beach, Banyuwangi. A quantitative descriptive method was employed to observe 365 eggs relocated from four nests to the INTAN BOX over an incubation period of 50–60 days. Parameters observed included temperature, humidity, and hatching success rates. Results indicated an average hatching success rate of 86%, with individual nest success rates ranging from 83% to 89%. These rates are significantly higher than the 50–70% success rate achieved through natural hatching. The INTAN BOX provides a stable environment, protects eggs from predators, and optimizes temperature and humidity conditions, thereby enhancing hatching success. Additionally, the device enables the management of hatchling sex ratios through temperature-dependent sex determination (TSD), which is critical for sea turtle population conservation. This study concluded that the INTAN BOX is an effective innovation for olive ridley turtle conservation while also supporting local community empowerment around Pulau Santen Beach.

Keywords: *Lepidochelys olivacea*, INTAN BOX, sea turtle conservation, hatching success rate, Pulau Santen Beach

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INTRODUCTION

The olive ridley sea turtle (*Lepidochelys olivacea*) is one of the seven sea turtle species globally, classified under Appendix I by the Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES), which prohibits its international trade (CITES, 2022). Additionally, the species is listed as Vulnerable on the Red List of the International Union for Conservation of Nature (IUCN, 2022).

In Banyuwangi, particularly on Pulau Santen Beach, various environmental issues threaten the hatching success of sea turtle eggs. Conservation efforts in this area face significant challenges due to its popularity as a crowded tourist destination, contrary to the natural nesting preferences of sea turtles for quiet locations free from human disturbance, light, and noise (Yudhana *et al.,* 2023). Soil and water pollution from human activities increase pathogenic bacteria on the beach (Azaria *et al*., 2014), adversely affecting egg hatchability, especially during the nesting season (Al-Bahry *et al*., 2011). Other threats to olive ridley turtle populations include illegal hunting, incidental capture in fishing activities, habitat destruction from urbanization, and climate change, which affects sand temperatures critical for egg incubation (Putra *et al*., 2020).

One key aspect of sea turtle conservation is improving egg hatching success rates, which serve as indicators of reproductive success. In natural hatching conditions, success rates are often influenced by factors such as predation, suboptimal environmental conditions, and human disturbances (Wicaksono, 2018). Previous studies have reported natural hatching success rates ranging from 50% to 70%, with key influencing factors including temperature, humidity, and nest quality (Yulianto & Sari, 2019).

Effective conservation efforts are needed to protect and enhance olive ridley turtle populations. One strategy is relocating eggs from natural nesting sites to controlled artificial incubators. The *Banyuwangi Sea Turtle Foundation* (BSTF), in collaboration with the Faculty of Health Sciences, Medicine, and Natural Sciences of Universitas Airlangga, has developed an innovative technology called the INTAN BOX. This device serves as an artificial incubator that eliminates the need for sand while maintaining optimal temperature and humidity for egg incubation (Haditanojo, 2024). Proper temperature regulation in the INTAN BOX not only increases hatching success but also enables sex ratio management, as incubation temperature influences the sex determination of sea turtles. In sea turtles, sex is determined by temperature during the middle third of incubation, with higher temperatures favoring females and lower temperatures favoring males within a thermal tolerance range of 25–35°C (Ackerman, 1997). For loggerhead turtles (*Caretta caretta*), incubation temperatures below approximately 29.0°C produce males, while temperatures closer to 25°C result in more males within the thermal tolerance range (Yntema & Mrosovsky, 1979; Ackerman, 1997).

The INTAN BOX stabilizes incubation temperatures to support hatching success and sex determination through the temperature-dependent sex determination (TSD) mechanism. With integrated temperature and humidity sensors, the INTAN BOX minimizes thermal fluctuations commonly experienced in natural environments, enabling the management of sex ratios for conservation purposes, such as supporting female populations in threatened areas.

Innovative technologies like the INTAN BOX offer practical solutions to address the challenges threatening olive ridley turtle egg hatching success. This study aims to analyze the hatching success rate of olive ridley sea turtle (*Lepidochelys olivacea*) eggs using the INTAN BOX incubation device on Pulau Santen Beach, Banyuwangi.

METHODS

This study employed a quantitative descriptive method to describe the hatching success rate of Olive Ridley Sea Turtle (*Lepidochelys olivacea*) eggs at Pulau Santen Beach, Banyuwangi, using the INTAN BOX incubation tool. The descriptive method aimed to provide a detailed depiction of the sea turtle egg hatching phenomenon through the collection, analysis, and presentation of data obtained during the incubation period. The study was conducted over a 60-day period, aligning with the Olive Ridley Sea Turtle nesting season, from July to September 2024, using the INTAN BOX incubator at Pulau Santen Beach, Banyuwangi. The observed parameters included the total number of eggs, the number of hatched eggs, and the number of unhatched eggs.

The study population consisted of all Olive Ridley Sea Turtle eggs found on Pulau Santen Beach during the nesting period, totaling 365 eggs from four nests that were relocated into the incubator. All eggs served as the research samples. Egg collection was carried out immediately after the female turtles laid their eggs on the beach to prevent threats from predators and other external factors that could damage the eggs. The egg collection technique included mapping the potential nesting locations before the turtles laid their eggs, coordinating with local residents and the *Banyuwangi Sea Turtle Foundation* (BSTF), collecting eggs between 03:00 AM and 05:00 AM WIB, separating eggs from different nests, documenting each egg batch (including finder's name, egg count, date, and time found), and placing the eggs into plastic bags before transferring them to the INTAN BOX.

The procedure for operating the INTAN BOX, as conducted by the Airlangga University team, involved several steps. First, viable eggs were separated from non-viable ones. The eggshells were sprayed with water to prevent bacterial contamination, given the sterile conditions of the INTAN BOX. Eggs were grouped based on the collected data and carefully transferred into specialized hatching tubes within the INTAN BOX to facilitate monitoring. The tubes were then arranged within the INTAN BOX, and the temperature and humidity were adjusted to approximately 29°C to mimic the natural environment of the eggs. Seawater was added to the control box of the INTAN BOX to stabilize the temperature amidst environmental changes. The eggs were monitored daily over 90 days, the typical hatching duration for Olive Ridley Sea Turtle eggs.

After hatching, the eggs were carefully removed from the INTAN BOX, and yolk remnants were filtered using a separate container. Seawater in the control box was replenished three times daily to maintain optimal humidity conditions inside the INTAN BOX throughout the 60-day incubation period until hatching was completed. The operational workflow of the INTAN BOX is illustrated in Figure 1.

The eggs from each nest were grouped based on the collection time and placed in the INTAN BOX, which was programmed to maintain a stable temperature between 28°C and 32°C, the optimal range for sea turtle egg hatching (Yulianto & Sari, 2019). Humidity within the INTAN BOX was also regulated to avoid being too high or too low, with an optimal temperature range of 27.8°C to 29°C and a humidity level of 80%. This was achieved using the INTAN BOX control system to prevent egg damage.

During the incubation period, daily observations were conducted to record egg development and environmental conditions within the INTAN BOX. These observations included monitoring temperature, humidity, and the status of the eggs-whether they successfully hatched or not. After an incubation period of approximately 50 to 60 days, hatching results were documented. The number of successfully hatched eggs was compared to the number of unhatched eggs, and the success rate was calculated.

Data collection involved gathering documents from previous studies conducted without the INTAN BOX incubation tool. Field observation data were analyzed, and calculations, as well as data tabulation regarding the hatching success rate of Olive Ridley Sea Turtle eggs, were performed using the formula (Sari & Ilyosa, 2020) as follows: Hatchling rate (%)= [total number of hatched eggs x total number of eggs^{-1}] x 100%.

RESULT AND DISCUSSION

The percentage of hatching success for olive ridley turtle eggs in each nest using the INTAN BOX at Pulau Santen Beach is presented in Table 1. The data include the total number of eggs, the number of hatched eggs, and the hatching percentage for each nest.

The percentage of hatchling success for olive ridley sea turtle eggs using the INTAN BOX is presented in Table 1. The data include the total number of eggs, the number of eggs hatched, the number of unhatched eggs, the hatching percentage per nest, temperature, and humidity.

The results shown in the table indicate that four nests were studied, containing a total of 365 eggs. Of these, 314 eggs successfully hatched, while 51 eggs failed to hatch. This equates to an overall hatching success rate of 86%. A detailed analysis of hatching rates per nest reveals some variation. In Nest 1, the hatching success rate reached 89%, with 109 eggs laid and 97 successfully hatched. For Nest 2, out of 81 eggs, 68 hatched, resulting in an 84% success rate. Nest 3 contained 55 eggs, with 46 hatching, yielding an 83% success rate. Finally, Nest 4 showed an 86% success rate, with 120 eggs laid and 103 hatched.

The incubation period for olive ridley sea turtle eggs typically range from 45 to 60 days, depending on environmental temperature and humidity (Putra *et al.*, 2020). In this study, the incubation period using the INTAN BOX fell within this normal range, averaging approximately 50 days. The INTAN BOX provides more stable conditions for the eggs, including controlled temperature and protection from external disturbances. These factors may accelerate hatching and improve success rates.

The hatching percentage using the INTAN BOX at Pulau Santen Beach was higher compared to previous studies without the use of this incubation tool. Hatching success rates in natural environments can vary significantly depending on environmental factors and predator threats. A prior study by Samosir *et al.* (2018) on olive ridley sea turtle egg hatching using semi-natural nests at Boom Beach, Banyuwangi, reported a 0% success rate. Additionally, Wicaksono (2018) reported that without incubation tools, the hatching success rate for sea turtle eggs generally ranged between 50–70%, depending on nest conditions, climate, and threats such as crabs and predatory birds. Based on these findings, the use of the INTAN BOX has been proven to significantly enhance hatching success rates compared to natural hatching methods in the wild.

A previous study conducted by Ayuningtyas *et al.* (2019) mentioned that the success of sea turtle egg hatching is influenced by fungal infections found on the turtle's carapace, neck, eyes, and legs, as well as on the eggshells of unhatched olive ridley turtle eggs at the TCEC Bali. The pathogen fungus *Trichoderma sp.* was found on the unhatched eggs. The most commonly found fungi on the olive ridley turtle hatchlings' bodies, particularly on the neck, were *Fusarium sp.*, with 4 colonies identified. The dominant fungi on the unhatched olive ridley turtle eggshells were *Aspergillus sp.*, with 2 colonies identified. Of the 15 fungal samples identified, *Aspergillus* and *Fusarium sp.* each had 5 colonies, and only one sample showed no fungal growth on the unhatched eggs.

Hatching success is influenced by both biotic and abiotic factors. Biotic factors refer to all living organisms that interact and affect biological processes. In the context of sea turtle egg hatching, these biotic factors are crucial and can influence the success of hatchlings (baby turtles) in emerging and reaching the ocean. Some important biotic factors include predators and microbial contamination in the nests. On the other hand, abiotic factors refer to the physical and chemical environmental factors that are non-living but significantly affect the life of organisms, including turtles. In terms of sea turtle egg hatching, abiotic factors play an essential role in the success of hatching and the survival of hatchlings. Key abiotic factors influencing sea turtle egg hatching include sand temperature, sand humidity, nest depth, and predation on turtle eggs.

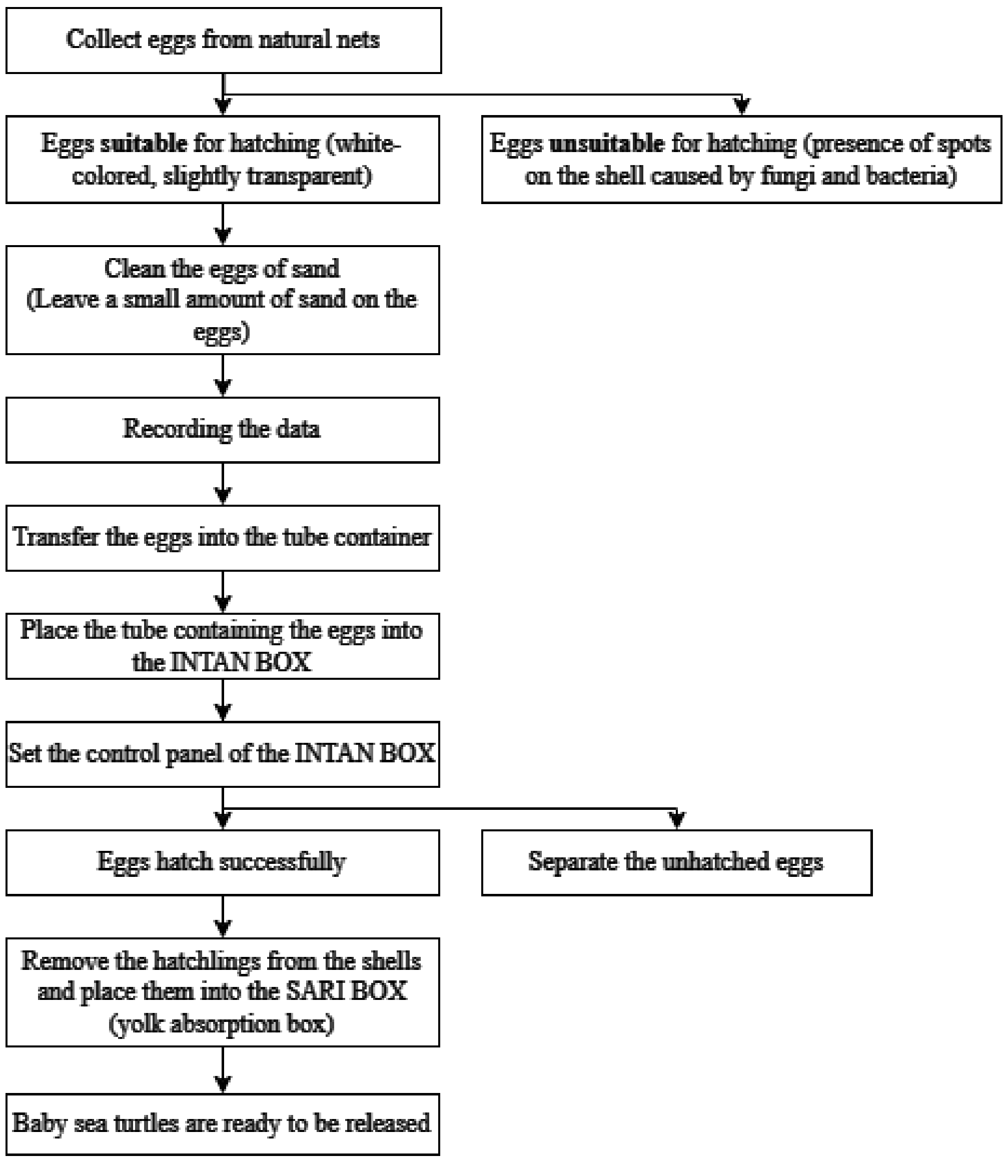

Figure 1. The procedure for egg relocation to the working mechanism of INTAN BOX.

Table 1. Hatching Data of Sea Turtle Eggs at Pulau Santen Beach using INTAN BOX

Nest	Number of eggs (pieces)	Hatched (pieces)	Unhatched (pieces)	Hatching Success Rate per Nest (%)	Temperature (°C)	Humidity (%)
1	109	97	12	89	29,0	80
2	81	68	13	84	27,2	80
3	55	46	9	83	27,6	80
4	120	103	17	86	28,7	80
	365	314	51	85,5	28,1	80

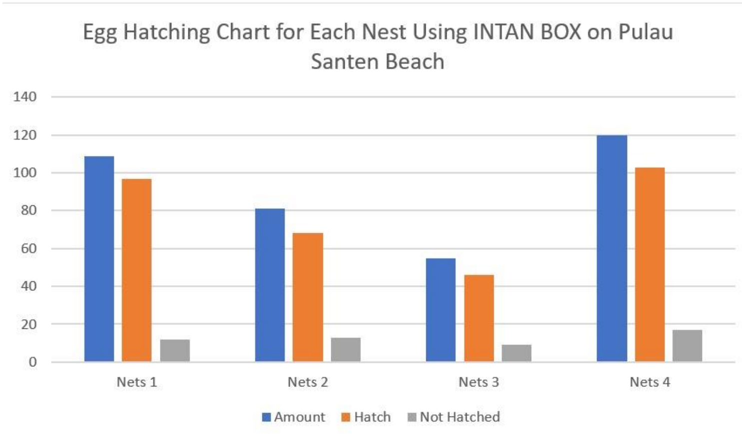

Figure 2. Hatching Rate per Nest Using the INTAN BOX at Pulau Santen Beach.

Figure 3. Research documentation (A) Condition of successfully hatched hatchlings; (B) Condition of unhatched eggs with cloudy yellowish shells; (C) INTAN BOX incubation device; (D) Release of hatchlings hatched using the INTAN BOX; (E) INTAN BOX inauguration at Pulau Santen Beach; (F) Community education on the INTAN BOX mechanism.

Other factors that can reduce sea turtle egg hatching success include microbial contamination. Microbial contamination is a serious threat to the successful hatching of turtle eggs. Ageng *et al.* (2017) noted that hatching success is influenced by microbial contamination on the eggs. Their study, which conducted microbiological analysis, confirmed the presence of contaminating microorganisms such as bacteria, fungi, coliforms, *Enterobacter*, and *Salmonella-Shigella* in all samples. The highest number of microorganisms was found in egg samples from semi-natural nests that failed to hatch (TB). The presence of microorganisms can originate from both the female turtles and the nesting environment or the nest itself, all of which influence egg hatching success. In relatively low quantities, microorganisms may not pose a threat to the egg embryos, but when present in high numbers, they can cause eggs to fail to hatch or result in embryo mortality.

The success of sea turtle egg hatching is influenced by the presence of various bacteria that can be pathogenic. Mauboy *et al.* (2022) stated that bacteria from the genera *Salmonella*, *Clostridium*, and *Shigella* were found in cloacal fluid samples, while bacteria such as *Bacillus*, *Micrococcus*, *Klebsiella*, and *Salmonella* were detected on the eggshells of unhatched eggs. Although some of these bacteria are considered normal flora in the body and environment, they possess pathogenic potential and can contribute to low egg hatching success rates. In an effort to address this threat, Praja *et al.* (2021) found that the cloacal fluid of olive ridley sea turtles (*Lepidochelys olivacea*) has antimicrobial properties that effectively inhibit the growth of bacteria such as *Escherichia coli*, *Salmonella spp.*, and *Bacillus spp.* on natural nest sand. These findings emphasize the important role of cloacal fluid in protecting embryos from microbial contamination during the early stages of incubation. By understanding this natural mechanism, conservation efforts such as the use of innovative incubation tools, including the INTAN BOX at Pulau Santen Beach, can be optimized to improve egg hatching success rates.

In addition to microorganisms, Sari *et al.* (2020) highlighted that the success of sea turtle egg hatching is also significantly affected by temperature. The average hatching success rate was found to range from 69% to 93.3%, which corresponds to an average nest temperature between 25.68 and 29.7°C. This temperature range is close to the normal temperature for sea turtle egg incubation. It can be concluded that the optimal temperature for sea turtle egg hatching is 29°C. A decrease in the sand temperature would result in reduced evaporation of moisture from the sand, preventing excessive evaporation.

Humidity also plays a role in the success or failure of turtle egg hatching. Humidity in the turtle nest is closely related to the moisture content of the sand. High moisture content in the sand leads to high humidity levels within the nest. This condition can cause some of the incubated eggs to rot and fail to hatch (Sari *et al.*, 2020).

In addition, the pH level in each sea turtle nest also influences the success or failure of egg hatching. A study conducted by Sari *et al.* (2020) reported that the average pH at each depth ranged from 6.3 to 6.6. A pH of 6.5 to 6.7 is considered neutral, and the variation in pH levels across different nests has been identified as a factor contributing to the failure of sea turtle egg hatching.

Yudhana *et al.* (2023) reported that at Pulau Santen Beach, the turtle conservation program through local community education has proven effective in raising awareness and encouraging community participation in the preservation of coastal ecosystems and wildlife. One of the key steps taken was the relocation of turtle eggs from natural nests to semi-natural nests designed to protect the eggs from tourist disturbances and damage to natural habitats. This program demonstrates that collaboration between conservation organizations, educational institutions, and local communities is crucial to the success of conservation efforts, not only increasing hatching success rates but also promoting the local economy through the development of conservation-based edutourism.

The use of the INTAN BOX in community service activities at Pulau Santen Beach further strengthens these conservation efforts. In addition to helping preserve turtle populations, the presence of this tool provides economic benefits by attracting tourists who are interested in witnessing the hatching and release of hatchlings. This innovation is expected to encourage the independence of local communities in conservation efforts, allowing them to care for and save hatchlings on their own, from nesting to releasing them back into their natural habitat. As a result, this activity not only supports sea turtle conservation but also enhances the involvement and well-being of the local community around Pulau Santen Beach.

CONCLUSION

The use of the INTAN BOX at Pulau Santen Beach has shown positive results in increasing the hatching success rate of olive ridley sea turtle eggs, reaching 86%. This is higher compared to natural hatching, which ranges from 50% to 70%. Environmental control within the INTAN BOX, including temperature and humidity, as well as protection from predators and human disturbances, are key factors contributing to this success. However, there are still risks related to the physiological condition of the eggs and humidity management.

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Author Contribution

AY, JDES, RNP, ADH, DUN, RAF, and BM: conceived and designed the study, analyzed the data, and drafted the manuscript, contributed to the data collection, analysis, and manuscript editing, assisted in data collection and interpretation, contributed to the fieldwork and manuscript revision. SIP and HK: supported the data analysis and manuscript preparation. S, RIS, and M: provided technical support and supervised the study. WH, BS, and GS: contributed to the final manuscript review and provided resources for the research. All authors approved the final version of the manuscript.

Competing Interest

The authors declare that have no financial, personal, or professional conflicts of interest that could have influenced the content or outcomes of this study.

Ethical Approval

This study did not involve the use of experimental animals or treatments. All data were obtained through noninvasive methods and complied with applicable regulations. Therefore, ethical clearance was not required.

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