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# Hatcheries efficiency for hawksbill sea turtle (*Eretmochelys imbricata*) conservation in the Persian Gulf: Over a decade monitoring of emergence success and incubation period

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#### ABSTRACT

Conservation of the critically endangered hawksbill sea turtles (Eretmochelys imbricata) is an important global concern that requires attention. There is currently a lot of debate surrounding the use of hatcheries as a technique for ex-situ conservation. Over the past decade, hatchery sites were established with the aim of reducing the embryonic mortality and increasing the number of hatchlings on Kish Island. Out of a total of 415 hawksbill turtle nests, 216 nests were monitored in their original location (in-situ) whereas the remaining nests were relocated to a hatchery site (ex-situ) due to several threats, including feral predators, coastal development, ecotourism pressure, and waves of monsoon storms. The aim of this study is to investigate the effectiveness of a hatchery site in the emergence success and incubation period compared to natural nests by analyzing the data from thirteen nesting seasons (2010-2022). In most years, the emergence success rate in ex-situ conditions has been significantly higher than *in-situ* (P < 0.05). Furthermore, a notable variation in the incubation period was noticed among various ex-situ years (P < 0.05), whereas it was not observed in in-situ. The incubation period in the *ex-situ* condition (57.38  $\pm$  7.39 days) was 3.68 days shorter than the *in-situ* nests (61.25  $\pm$  8.46 days). Delays in transferring eggs to the hatchery site from distant shores were found to be linked with a decrease in emergence success, 47.02% vs. 58.18% (P < 0.05). As a result, nest relocation in a minimum time, could significantly enhance turtle hatchlings' emergence success and survival. On the other hand, reducing the incubation period due to *ex-situ* method maybe an important challenge in the future. Further research should be done to investigate the influence of nest relocation on morphological, physiological, immunological, and biometric offspring features, as well as on sex ratio.

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# 1. Introduction

The hawksbill turtle (*Eretmochelys imbricate*; Linnaeus, 1766) is one of seven extant species of sea turtles. Because of their unique diet, hawksbill turtles are often found in coral reef habitats that have a lot of sponges (Meylan, 1988). Their life cycle is complicated and involves many migrations. It being with laying and hatching of eggs and ends with maturity of the hatchlings and reproduction (Hayes, 2015). This species breeds in tropical and subtropical marine ecosystems, which can be found in the Atlantic, Indian, and Pacific Oceans. In 1996, hawksbill turtles were added to the IUCN Red List as critically endangered, following several decades of decline caused by extensive hunting (Meylan and Donnelly, 1999). Their global populations reduction

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https://doi.org/10.1016/j.rsma.2023.103053 2352-4855/© 2023 Elsevier B.V. All rights reserved. have continued to decrease due to anthropogenic threats such as incidental catch, loss of habitat, water pollution, egg poaching, and the illegal trade of tortoiseshell (Siqueira et al., 2021; Mast et al., 2005).

After mating, females lay their eggs on sandy beaches. One of the threats, the egg clutches may be placed very close to the tide line and damaged by waves. Additionally, the eggs are often consumed by local people and other natural predators such as ants, ghost crabs, feral omnivorous and carnivorous, and monitor lizards (Rusli et al., 2015; Ahmad et al., 2004; Ekanayake et al., 2002; Hewavisenthi, 1993). When animals face different threats over time, these negative threats have a cumulative effect on their survival, making them vulnerable to the risk of extinction. Therefore, conservation efforts that act to reduce high levels of embryonic mortality help boost hatchling production.

In general, there are two different techniques proposed for conserving eggs on nesting beaches. The first, known as *in-situ*,





involves carefully monitoring the nests and allowing the eggs to hatch naturally in their original location. The second, called exsitu, involves moving the threatened eggs to artificial nests in a protected area, commonly referred to as a hatchery (Rusli et al., 2015). Hatchery site management plays a significant role in ex*situ* conservation techniques. Some sea turtle populations around the world may have been successfully conserved as a result of the protection of eggs in structures like hatcheries (Phillott and Kale, 2017; Mazaris et al., 2017). As an example, the relocation of clutches to the hatchery site within the Sea Turtle Natural Reserve (STNR) on Boa Vista Island, Cabo Verde has been beneficial for preserving loggerhead turtles population particularly in cases where the natural hatching success rate is very low (Martins et al., 2021). Furthermore, a well-managed hatchery is a useful tool for hawksbill sea turtle's conservation in Cousine Island, Seychelles, and it provides evidence of the hatchery's success in increasing the recruitment of hatchlings (Evans et al., 2022).

Although there is evidence to support the importance of hatchery sites, their effectiveness has long been a subject of debate in sustainable conservation (Mrosovsky, 1983; Mrosovsky and Yntema, 1980; Pritchard, 1980). If the proper conditions are not established, using hatcheries for conservation purposes may end up being ineffective and could end up causing more harm than good (Hewavisenthi, 1993). Even if the best arrangements are followed for egg catching, transportation, hatching, storage, and release of hatchlings in ex-situ conservation, there could be a decline in newborn production (Revuelta et al., 2015; Pintus et al., 2009; Eckert and Eckert, 1990; Wyneken et al., 1988; Limpus et al., 1979) and/or fitness (Rusli et al., 2015; Maulany et al., 2012b: Pilcher and Enderby, 2001), or skewed sex ratios (Sari and Kaska, 2017; Revuelta et al., 2015; Maulany et al., 2012a; Sieg et al., 2011; van de Merwe et al., 2005), compared to in-situ. Relocating activities can lead to changes in physical conditions, such as nest temperature, that typically affect the sex ratio, incubation duration, nest success, and mortality rate (Robledo-Avila et al., 2022; van Lohuizen et al., 2016; Matsuzawa et al., 2002; Spotila et al., 1987). Moreover, there are several other studies with varying outcomes that demonstrate *ex-situ* incubation reduced the growth of the brain and gonadal development, as well as body size and locomotor performance in newborn turtles (Tanabe et al., 2021; Herrera-Vargas et al., 2017). Robledo-Avila et al. (2022) reported contradictory findings, observing increased body size, improved immune system, and higher hatching and emergence success rates in Lepidochelys olivacea turtle hatchlings following ex-situ conservation (Robledo-Avila et al., 2022).

Hawksbill nests are conserved in the north of the Persian Gulf as well as around the world. The conservation efforts were first began on Qeshm Island due to the prevention of egg poaching by local people in 2002. Previously, local communities believed that the eggs had health benefits for the elderly individual and their rheumatic diseases. In recent years, concerns about hawksbills conservation have increased due to various factors on Qeshm Island. These include the pressure of ecotourism and walking on nesting beaches, the presence of feral dogs, as well as natural predators such as red foxes, mongooses, and crabs. Humans and feral dogs are present even during the nesting season of hawksbill turtles, although there is more control at this time. Environmental volunteers try to protect the nests during the critical months, but this does not always seem to be enough. After Qeshm Island, conservation efforts began on Kish Island in 2006. Hawksbill nests have been conserved on the island for over a decade (16 years). It is done in two main methods with a slight difference in action. In the *in-situ* method, the nesting area is fenced off to prevent both humans and predators' entry. Thus, the eggs are hatched in their original places without relocation. Sometimes, the hawksbill nests were placed on the tide line and destroyed by sea waves and monsoon storms. To prevent this, in *ex-situ* conservation, a suitable area on the nesting beach that is at a safe distance from the high tide line is selected, and the threatened eggs are carefully transferred to artificial nests called the hatchery site. Depending on the conditions, the hatchery may be fenced off or not. Also, high-risk nests are moved from other nesting beaches to the hatchery site due to ecotourism pressure or flooding caused by sea storms. In recent years, similar the Qeshm Island, the presence of feral dogs and sea storms have made *ex-situ* conservation a serious and inevitable method from the point of view of government experts in Kish Island.

In addition to the two nesting areas mentioned earlier, *ex-situ* conservation has been continuously carried out in Nayband Bay. Moreover, a similar method has also been used on Nakhiloo and Hendurabi Islands in some years. In 2016, Pazira et al. examined the success of hawksbill turtle nests through both *in-situ* and *ex-situ* incubation on Nakhiloo Island. The study results showed that nest relocation has been a successful strategy that increased the number of hatchlings and nest success, when compared to *in-situ* conservation. Also just a study was conducted on Qeshm Island that compared the emergence success of two different hatchery sites (Mahtabi et al., 2019).

Relocation of eggs and *ex-situ* conservation has continued as a routine operation on Kish Island and some other beaches for several years. Meanwhile, nesting beaches in Kish Island have a special importance in terms of protection, fencing and predator control for continuous studies. However, no research has been done on the effectiveness and efficiency of this method with regard to the success of nests on Kish Island. Therefore, the aim of this study were: (i) evaluating contemporary *ex-situ* and *in-situ* conservation operations based on successive yearly emergence rate over one decade, (ii) investigating the effects of relocation on emergence success and incubation duration (potential risk for skewed sex ratios), (iii) studying how the length of time and distance impacts the success of emergence.

#### 2. Materials and methods

#### 2.1. Study site

Kish Island is one of the relatively large islands (with an area of 91.5 km<sup>2</sup>) in the north of the Persian Gulf. It annually attracts both local and foreign tourists due to the presence of coral reefs that grow at depths of 5–20 m. The coast of the island has a gentle slope and is covered with fine sands, which makes it an ideal nesting spot for hawksbill turtles (Hesni et al., 2016). About 3 ha from the south coast of Kish Island is fenced off and protected and known as Turtle Protected Beach, where the hatchery site is established. Other nesting beaches (10 locations) on Kish Island are not as well-protected and are used by female turtles randomly for nesting. Only a small proportion (less than 28.14%) of total nests are located outside of Turtle Protected Beach (Fig. 1).

# 2.2. Data collection

The study was conducted following the relevant guidelines, and it was approved by the appropriate institutional animal ethics committee of Tarbiat Modares University. In addition, the monitoring and data collection of hatchery sites were carried out in accordance with the Department of Environment (DoE) regulations at the Kish Free Zone Organization (KFZO). Special care was taken not to disturb the turtles' natural habitat during the process.

From 2010 to 2022, a total of 415 nests of hawksbill turtles were continuously monitored on Kish Island during the reproductive season from the beginning of March to the end July. Over

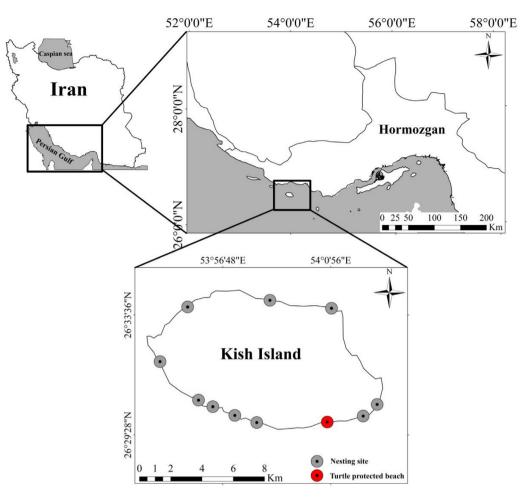


Fig. 1. Study area of hawksbill nesting beach on Kish Island. The turtle protected beach as a main place for nesting and hatchery site (red) and other nesting beach (gray) are shown by the circle points. . (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the course of 13 years, the annual mean number of nests was approximately 31.92  $\pm$  17.15 (M  $\pm$  SD). Despite the decreasing trend, all the nests were monitored. During the turtle nesting period, historical records such as the algae patterns left by waves washing ashore were used to determine the position of the high tide mark. Then, the nests at risk were identified not only through the rangers' experience but also by observing the signs left by the tides. The hawksbill nesting activity is mostly nocturnal because the island is a tourist destination. In order to identify threatened nests using turtle crawl marks, park rangers patrolled the beach, two hours before and after high tide. Once the rangers discovered egg laying in a high-risk location, they carefully collected the entire clutch after the end of oviposition. These activities were performed in a non-invasive way after the female left the beach. In cases where more than 8 h, had elapsed since egg laying, the staff did not relocate the nests due to embryo development. The complete clutch of eggs was placed into large plastic buckets without any turning or shaking. Then the eggs were covered with a thin layer of wet sand and transported to the hatchery site on turtle protected beach. Egg translocation took more than 15 min for the farthest beach by vehicle, and 5 min for the nearest one by walking. Finally, the clutches were buried in artificial nests at the same depth as the previous nests. These nests with  $25 \sim 30$  cm diameters were excavated at a distance of 75 cm between the nest centers. The minimum distance between the edges of two nests was about 45 cm. The area of the hatchery site is approximately 100  $m^2$ , and up to 50 nests can be transferred to it annually. Both natural and artificial nests were labeled by a small placard and details such as the laying date, number of eggs, and location recorded by collector immediately. The nests were monitored and protected until the emergence of hatchlings (Fig. 2). During the years of this study, there were no interventions aside from relocating nests from high-risk areas to the hatchery site. As a result, any hatchlings that were unable to leave the nest were recorded as fatalities.

After the emergence of the last hatchlings completely, the nests were checked for the number of unfertilized eggs, eggshells and dead hatchlings. These parameters counted and subtracted to calculate exact emergence success (emerged hatchlings/ total number of egg laid). While being monitored, six of the *in-situ* nests were impacted by storms and were completely destroyed (ie., all eggs washed away). These nests, along with other ones that did not have any hatchlings, were categorized as "unsuccess-ful nests" during the analysis of emergence success. We defined incubation period as the number of days between the date of laying and the date of first observed emergence from the nest.

#### 2.3. Data analysis

The open source statistical software package R version 4.2.3 was used to analyze the dataset. The chi-square test was used to compare impact of the relocation on unsuccessful nest rate changes (i.e., no emergence of hatchlings). The generalized linear models (GLMs) were applied to determine the effect of the year, nest relocation on the incubation period and emergence success in both *in-situ* and *ex-situ* approach too. To compare emergence

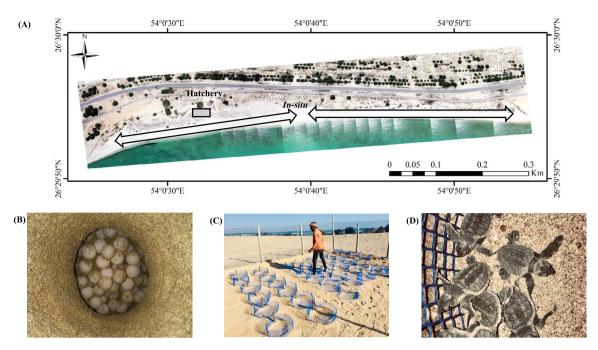


Fig. 2. Location of the study site and nesting conditions. (A) Aerial photograph of turtle protected nesting beaches in Kish Island; *ex-situ* (Gray rectangle) and *in-situ* nests (Double sided flash). (B) Artificial nests. (C) Hatchery site (D) Hawksbill sea turtle hatchlings emerging from nest.

#### Table 1

Chi-square  $(\chi^2)$  test of independence result to compare the number of unsuccessful nests in the *in-situ* and *ex-situ* conservation (N: number of nests, UN: unsuccessful nests).

	Ν	UN	Df	Chi-square	P-value
In-situ Ex-situ	216 199	57 15	1	9.529	<0.01

success between *in-situ* and *ex-situ* nests for each year we used the Tukey test.

#### 3. Results

#### 3.1. Emergence success

### 3.1.1. In-situ vs. ex-situ unsuccessful nests

About 26.39% and 7.53% of the monitored *in-situ* (N = 216) and *ex-situ* (N = 199) nests showed no emergence of hatchlings, respectively. In comparison to *ex-situ* nests, the *in-situ* nests had approximately 3.5 times more unsuccessful nests. The *p*-value of the chi-square ( $\chi$ 2) test of independence showed that there was a significant difference in the proportion of unsuccessful nests between the two conservation methods (P < 0.01) (Table 1).

#### 3.1.2. In-situ vs. ex-situ emergence success

The generalized linear models were used to compare the effects of two conservation methods as treatments, year, and interactions on the emergence success of 415 nests. The annual emergence success rates in both *in-situ* and *ex-situ* conservation are presented in Fig. 3A. Out of the total 13-year study period, there was a significant difference in 8 years. In 7 years, the success rate of emergence in *ex-situ* conditions has been significantly higher than *in-situ*. (P < 0.05) (Fig. 3A).

Although the medians were close, the difference between the means was significant. The mean of *ex-situ* emergence success ( $M_{ex-situ} = 63.90\%$ ) was significantly higher than *in-situ* ( $M_{in-situ} = 50.70\%$ ) (Fig. 4A). There was a significant difference (P < 0.05) in the emergence success between the two treatments (Table 2).

As a criterion, emergence success above 50%, was recorded in 61.83% and 55.41% for *ex-situ* and *in-situ* nests respectively. On average, the *ex-situ* nests showed 3.42 time more hatchlings than the *in-situ*, and the analysis confirmed that relocation of the nests facilitated the emergence of hatchlings.The effect of year was more significant (P < 0.001), although the interaction of year and protective treatments was not significant (Table 2).

#### 3.1.3. Distance from hatchery site

Almost half (47.95%) of all hawksbill turtle nests on Kish Island were relocated to a hatchery site from 2010 to 2022. Only 28.14% (n = 56) were moved from areas outside of the Turtle Protected Beach. The remaining nests (n = 143) that were translocated to the hatchery site, came from the Turtle Protected Beach itself. On average, nests that were moved from distant shores had a lower likelihood of producing successful hatchlings, 47.02% vs. 58.18% (P < 0.05).

# 3.2. Incubation period

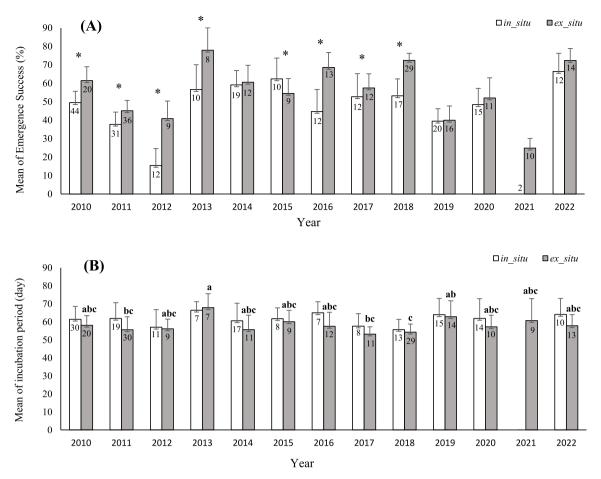
The annual incubation period rates in both *in-situ* and *ex-situ* conservation are presented in Fig. 3B. The duration of the *in-situ* incubation period was consistent across all years, while for *ex-situ* conservation it showed different sub-sets for five years.

The incubation period was not significantly affected by different years (Table 2). The average annual incubation periods for *in-situ* nest (n = 159) and *ex-situ* deposited nest (n = 184) were  $61.25 \pm 0.67$  and  $57.38 \pm 0.54$  (Mean  $\pm$  SE) respectively. Relocated nests experienced a shorter incubation period than *insitu* nests. Hence, there was a meaningful impact of treatments on the incubation period (P < 0.001) (Table 2, Fig. 4B). The average incubation period of *ex-situ* nests was reduced by approximately 3.86 days compared to *in-situ*.

#### 4. Discussion

#### 4.1. Emergence success

This study evaluated the success rates of hawksbill sea turtle nests that were relocated to a hatchery site (*ex-situ*) versus those



**Fig. 3.** (A) Mean of emergence success of hawksbill sea turtle, and (B) Mean of incubation period from 2010 to 2022. Error bars are based on SE and number inside bars are sample sizes. Stars on the bars represent the significant difference (p-value < 0.05). Based on the findings of Tukey's test, there was no difference observed for 8 years (abc) out of the total of 13 years.

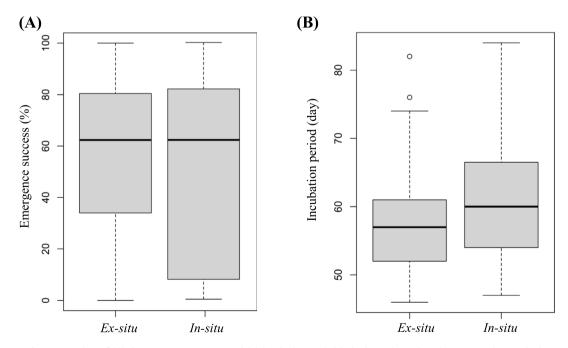


Fig. 4. Box plots of (A) the emergence success and (B) incubation period in both Ex-situ and In-situ conservation methods.

left in their original location (*in-situ*) on Kish Island during a 13-year period. The results indicate a significant increase in the percent of emergence success at the hatchery site, supporting

the use of relocation as an effective conservation strategy. Emergence success was significantly influenced by relocated as well as year, with inter-annual variation observed when comparing to

#### Table 2

Results from generalized linear effects model of year, treatments (*in-situ* and *ex-situ*), and their interactions on emergence success (%) with a binomial and incubation period with Poisson family distribution. Bold values indicate statistical significance.

		Year (Y)		Treatments (T)		Y×T	
	Df	F	P-value	F	P-value	F	P-value
Emergence success	1	1706.90	<0.001	120.02	<0.050	249.98	0.81
	Df	χ2	P-value	χ2	P-value	χ2	P-value
Incubation period	1	46.07	<0.001	17.79	<0.001	7.47	0.76

*in-situ* conservation. Despite variations in *in-situ* treatment, it was generally more consistent than *ex-situ* treatment (Fig. 3A). Environmental and anthropogenic changes could potentially impact the inter-annual variation (Gane et al., 2020; McElroy et al., 2015). Although the process of relocation is standardized, differences in technique and mistakes made by individuals may explain the observed variation (McElroy et al., 2015). During the years mentioned in our study, it appears that natural nests had more similar conditions in inter-annual variation. This may be attributed to the lack of human intervention from 2010 until now.

Several environmental threats especially flooding can impact in-situ nests. During hawksbill nesting, some nests are located close to the tide line in Kish Island. 20% of natural nests were excavated at a distance of 0-5 m from the tidal line (Nasiri et al., 2022a). There is considerable evidence that *in-situ* nests may also be impacted by tidal, storm and coastal flooding (Shaver, 2020). In present study 57 nests out of 216 were completely unsuccessful in the *in-situ* condition (Emergence Success = 0%) (Table 1). Few unsuccessful in-situ nests (6 from 57 nests) were flooded due to sea storms, while no hatchery nests were affected during the study period because they were located 30 m away from the sea. While the area did not show any noticeable presence of predators such as foxes, dogs, mongooses, and crabs, it is possible that the remaining *in-situ* unsuccessful nests (n = 51)due to the female turtle's inability to choose an optimal location, which may have been caused by environmental stress. In ex-situ conservation, hatchery location is very important. It should be at least one vertical meter above the highest tide line and away from tidal creeks, creeks and river mouths to reduce the possibility of flooding and fetal mortality (Ahmad et al., 2004; Maulany et al., 2012a). Then, the better hatchery site conditions, such as suitable distance from the tide line, may have contributed to the higher success rates of emerging from *ex-situ* nests.

In the general comparison of emergence success between *exsitu* and *in-situ* treatments, the analysis showed a significant increase ( $\chi^2$  = 120.02, df = 1, P < 0.05) in the emergence success of relocated clutches compared to those left *in-situ* (Table 2. Fig. 4A). A similar study showed the statistical analysis indicated that the hawksbill turtle nests in the hatchery had a significantly higher rate of emergence success compared to those that were left in their natural location (p < 0.05), which is consistent with our results (Table 3) (Evans et al., 2022). This finding indicates the efficiency of relocating clutches to a hatchery site as a conservation tool.

Table 3 displays a comparison between *in-situ* and *ex-situ* treatment methods for hawksbill turtles across various coastal regions worldwide. The maximum *in-situ* and *ex-situ* hatching success was 86% in Qatar and 91% in Venezuela respectively. In some studies, the hatching success was examined in both *in-situ* and *ex-situ* methods. In the mentioned studies such as Brazil, Seychelles, and Nakhiloo Island *ex-situ* hatching success was found to be considerably higher than *in-situ* (Table 3). In many cases, hatching success is higher than emergence success because some newborns are unable to leave the nest, resulting in their death. There are various reasons why sea turtle hatchings may face difficulty in reaching the sand surface after hatching,

including: the compaction or density of the sand by foot traffic (Lutcavage, 2017), and human-made obstacles such as beach debris within the sand column which can preventing them from leaving the egg chamber (Nelms et al., 2016). Previous studies have already shown one potential restoration strategy to enhance sea turtle nesting could be the effective removal of significant debris (Fujisaki and Lamont, 2016). Emergence success for the Persian Gulf hawksbill ranged from 63.90% (present study) to 59.92% in Qeshm Island in *ex-situ* conservation (Mahtabi et al., 2019).

The relocation of nests can also result in embryo mortality during egg transfer, reducing hatching and emergence success (Ware et al., 2021; Ware and Fuentes, 2018). In the present study, it was found that increased time and distance spent transferring eggs to the hatchery site significantly reduced emergence success. It seems that, in addition to the stress caused by the vibrations of egg transport, pauses and wasted time are negative factors. Although attempts are often made to complete a transfer operation within 15 min, sometimes the rapid delivery of eggs to the hatchery site can result in human error, which reduces the quality of the transfer. Furthermore, minimizing the distance between the nesting beach and the hatchery can have a notable impact on transport time and the likelihood of embryonic death. While the duration of the transfer is certainly an important factor, the choice of an appropriate container for transportation can also have a significant impact. Then, another method, such as using Styrofoam boxes instead of plastic buckets, can be more effective in reducing vibrations and absorbing minor shocks (Ahmad et al., 2004; Mortimer, 1999).

#### 4.2. Incubation period

The study of two conservation methods on the south coast of Kish showed that relocating the eggs reduced incubation period by more than 3.86 days. Evidence on the coast of Mexico showed that the relocation of nests affects the maximum–minimum sand temperature, moisture, and incubation period. The average incubation period for hatchlings from *ex-situ* clutches was 0.8 days shorter than *in-situ*. Also, the standard deviation was more than 3 times higher in *ex-situ* (Unda-Díaz et al., 2022).

The incubation duration was considered as a criterion for evaluating the mean nest temperature. The changes in the incubation duration are affected by nest temperature (Noble et al., 2018). Some previous studies have shown the correlation between incubation period and nest temperature in both in-situ and ex-situ conditions (Tuttle and Rostal, 2010). Thus, it can be used as a relative indicator to examine the temperature difference between *in-situ* and *ex-situ* nests. The average incubation duration for hatchery nests in Kish Island was about 3.86 days fewer than *in-situ*, with statistically significant difference. However, it seems that a significant reduction in the incubation duration for 199 nests on Kish Island indicates a higher average temperature in hatchery sites than the initial laying site. Our observations showed that the difference in hatchery site elevation in the study area is at least one meter higher than the average elevation in *in-situ* nest. Also, we calculated the average distance of natural

#### Table 3

Hatching and emergence success reported for hawksbill turtle's nesting area from several locations in the world.

Location (District, Beach/Village)	Ex-situ/ In-situ	Nesting Year/s	Nests &/or # Eggs Collected	Hatching Success (HS)/ Emergence Success (ES) (M $\pm$ SD)	Sources
Present study	Ex-situ	2010-2022	199	63.90 (ES)	
Qeshm Island (Iran)	Ex-situ	2016	102	59.92 $\pm$ 29.1 (ES)	Mahtabi et al. (2019)
Nakhiloo Island (Iran)	Ex-situ	2011	19	55 (-) (HS)	Pazira et al. (2016)
Setiu, Terengganu (Malaysia)	Ex-situ	2009–2012		73.9 (-) (HS)	Abd Mutalib and Fadzly (2015)
Bahia- Sergipe- Espirito Santo (Brazil)	Ex-situ	2001-2002	44	57.43 (-) (HS)	Chalacatepec (2006)
Cipara Beach (Venezuela)	Ex-situ	2000	3	91.2 (-) (HS)	Médicci et al. (2006)
Malacca (Malaysia)	Ex-situ	-	-	$32.3 \pm 25.0$ (HS) $30.3 \pm 23.3$ (ES)	Hoh (2015)
Cousine Island, Seychelles	Ex-situ	2017-2021	272	84.6 $\pm$ 15.3 (HS) 78.5 $\pm$ 20.8 (ES)	Evans et al. (2022)
Present study	In-situ	2010-2022	216	50.70 (ES)	
Nakhiloo Island (Iran)	In-situ	2011	19	44 (-) (HS)	Pazira et al. (2016)
Kish Island (Iran)	In-situ	2009-2012	-	76 (-) (HS)	Hesni et al. (2016)
Shidvar Island (Iran)	In-situ	2012	35	79 (-) (HS)	Zare et al. (2012)
Big Giftun Island (Egypt)	In-situ	2001-2008	11	$67~\pm~13~(HS)$	Hanafy (2012)
Qaru and Umm Al Maradim (Kuwait)	In-situ	2010-2013	16	$58 \pm 26 (HS)$ $41.2 \pm 30.5 (ES)$	Rees et al. (2020)
Fuwairit Beach (Qatar)	In-situ	2005	22	$73 \pm 20 \ (\text{HS})$	Pilcher (2006)
Ras Laffan Industrial City, (Qatar)	In-situ	2001-2002	17	86 $\pm$ 15 (HS)	Tayab and Quiton (2003)
Bahia- Sergipe- Espirito Santo (Brazil)	In-situ	2001-2002	594	53.79 (-) (HS)	Marcovaldi et al. (2006)
(United Arab Emirates)	In-situ	1998-2003	-	68.1 (-) (HS)	Al-Ghais (2006)
Cousine Island (Seychelles)	In-situ	2004-2014	1031	60.1 (HS)	Gane et al. (2020)
Cousine Island (Seychelles)	In-situ	2017-2021	153	$58.2 \pm 34.5$ (HS) $55.6 \pm 34.4$ (ES)	Evans et al. (2022)

nests from the tide line for 50 nests. The distance was found to be 9.71  $\pm$  5.04 m (M  $\pm$  SD) (Nasiri et al., 2022a), whereas the distance observed for established hatchery sites was not less than 30 m in any of the years. Increasing the hatchery site elevation and distance from the sea are associated with a decrease in humidity and cooler winds. The extent of these effects needs to be investigated further. On the other hand, the close proximity of the artificial nests in a hatchery site can have an impact on the gas levels and temperature within the nests, potentially leading to a shorter incubation period (Ackerman, 1977; Wallace et al., 2004). In the present study, the artificial nests were excavated at a distance of 75 cm between the nest centers. The minimum distance between the edges of two nests was about 45 cm. The reason for the reduction of incubation duration in this study could be due to the higher temperature in the points selected as the hatchery site and the close proximity of artificial nests.

When the temperature is raised or lowered, the incubation duration is decreased or increased respectively (Noble et al., 2018). The length of the incubation period can be used as a reliable indicator, with 10% accuracy, to determine the sex ratio of offspring. Thus, determining the length of incubation can be considered an indicator to evaluate sex ratio (Mrosovsky et al., 1999). The sex ratios of hatchlings can be skewed if the temperatures in a hatching area differ from those on the natural beach (Phillott and Shanker, 2018). But this issue should be confirmed by determining the sex of hatchlings in other ways. Since the emergence success in ex-situ sites was greater than in-situ sites, we can conclude that the increase in temperature was not enough to cause significant death in the nests (Nasiri et al., 2022b). However, it may interfere with the temperature-depended sex determination (TDS) of turtles and increase the sex ratio skew to feminization. This means that relocations and temperature changes can have some effects on sex ratio, even if it does not look like an important event. Global warming may be increasing the number of female turtles by warming nest temperatures. With *in-situ* nests already warming, the fact that *ex-situ* temperatures are higher than *in-situ* temperatures is alarming, with potential exacerbation towards global feminization.

The research did not focus on studying the relationship between humidity or sand moisture and temperature. Instead, it is hypothesized that the frequency of seawater inundation may be a factor in the different incubation periods observed between in-situ and ex-situ nests. Humidity, like a buffer, may control the temperature fluctuations of the nests and have a secondary effect on the incubation duration (Tezak et al., 2020). In-situ nests, which have an average distance of 9 m and are closer to the sea, are more likely to be inundated and cooled compared to hatchery sites which are approximately 30 m away. In addition to temperature changes, relocation of nests in ex-situ conservation can also change the humidity inside the nests. Therefore, decreasing humidity can be another reason for reducing the incubation duration. The average distance of *in-situ* nests from the sea is much less than the distance of hatchery sites from the sea, because one of the reason for choosing a site is a safe distance from the sea and prevention of flooding of nests. Due to the distance from the sea and the reduction of the spray effect of sea waves, it is expected that the sand moisture at hatchery sites be lower than in in-situ nests. This factor can be a reason for the acceleration of the embryonic development's duration inside the eggs, which ultimately shortens the incubation duration.

#### 5. Conclusion

The present study confirms previous findings and suggests that establishing hatchery sites could improve the emergence success and survival of newborns. However, the distance from hatcheries and the time required for transfer are crucial factors. Therefore, reducing the distance and time seems necessary. As a consequence, it is suggested that several hatcheries be established instead of a single protected beach to avoid the negative effects of egg transfer. Regarding the length of the incubation period, there is often evidence of a reduction in hatcheries, resulting in an increase in nest temperature and a sex ratio bias towards feminization. However, in some cases, there has been a rise in the number of male embryos in hatcheries. A more detailed examination of incubation period duration, hatchery temperature, and feminization are important topics for further study. Finally, further research is required to determine the effect of ex-situ conservation on offspring's gonadal development, morphological and physiological features, as well as immunological and biometric characteristics.

## **CRediT authorship contribution statement**

Zohreh Nasiri: Analyzed the data, Writing of the manuscript. Maryam Mohammadi: Data collection. Seyed Ali Jebeli: Data collection. Mehdi Gholamalifard: Review & editing. Seyed Mahmoud Ghasempouri: Review, Editing, Supervision.

#### **Declaration of competing interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Data availability

Data will be made available on request.

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