

Studying the incubation temperature of nesting population of olive ridley turtles (*Lepidochelys olivacea*) in coast of Maharashtra.

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By

Sumedha Korgaonkar

PhD scholar

Wildlife Institute of India, Dehradun.

Project Supervisor

Dr K Sivakumar

Scientist F & Head of department of
endangered species management

Wildlife Institute of India, Dehradun.

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EXECUTIVE SUMMARY:

In recent times the consequence of climate change is profoundly observed both at a global and local level. The species resilience and vulnerability should be ascertained with a long term monitoring programme for robust conservation efforts. In Maharashtra Olive ridley turtle conservation is been implemented for almost two-decade with fair success. Analysis of nesting data from 2002 onwards clearly shows a major shift in the peak nesting season from Dec / Jan to Feb / March viz: from winter nesting to summer nesting. In sea turtles temperature plays a significant role in sex determination, embryonic development and growth, hatching and emergence and locomotory movements of hatchlings. Global warming increasing the ambient temperature coupled with shift in nesting season extending in summer is a major threat to this nesting population. The threat is in the form of increase in the female-biased sex ratio of the population, decrease in hatching and emergence success, and development of small hatchlings which might be having defect in locomotory moments reducing their survival rate in open waters.

To mitigate the effect of climate change the project aims to implement a long term temperature monitoring programme so that conservation efforts can be improvised for it to succeed. A customised temperature data logger was installed on seven sites during nesting season. Preliminary results shows increase mortality of embryo decreasing the hatching success due to high temperature. At some sites emergence success is negatively affected due to increase temperature and hardness of sand. Post TSD period it is required to keep the nest temperature within tolerance limit of 28°C till 32°C by appropriate techniques.

ACKNOWLEDGEMENT

I'm grateful to my PhD supervisor and mentor Dr K Sivakumar in giving me an opportunity to work on sea turtles. He has been a vital support for me to work on a larger scale. We both are thankful to Shri N Vasudevan APCCF Mangrove cell & Director, Mangrove and Marine Biodiversity Conservation Foundation of Maharashtra, for selecting this project for funding. I'm grateful to Dr Manas Manjrekar for trusting my ideas, initiating the process and helping me in all the administrative work making the working of the project easy.

The project is not a one-man show but is the collective effort of the local beach managers and locals who had actively helped us in the execution of the project. Shri Pradeep Dingankar of Gaokhadi beach needs special mention. His enthusiasm, prompt action and full cooperation have helped us collect most of important data related to turtle nesting. Working with him makes things very easy and comfortable on field. Shri Rakesh Patil of Gaokhadi, Shri Ajinkya Kelaskar of Anjarla, Shri Dattaram Vanarkar of Dabhol, Shri Sagar Maladkar of Tambaldeg also contributed majorly for this project. Ajinkya and Dattabhau taking time out from their regular schedule assisted me in post-hatching data collection and their efforts are highly appreciated. I'm grateful to Shri Suhas Toraskar of Vayangani, Shri Pravin Todankar and Shri Deepak Mahajan of Kolthare and Shri Mangesh Padwal of Divegar for their cooperation in this project and look forward to their continued support. I am grateful to Shri Vaibhav Phadke, Shri Marathe, Shri Omkar Ranade for their voluntary participation providing logistic support in installing the datalogger at its initial phase.

Lastly, I would like to thank PCCF (WL) of the Maharashtra state forest department for granting me permission to work on Schedule I species. I especially thank Shri V K Surve DFO Chiplun – Ratnagiri for his cooperation and patience.

This project won't have been complete without the technology developed by Shri Kishor Jambhekar of Syslab Automation Pvt Ltd Pune who has customised it for sea turtle research. Very patiently and carefully he understood my requirement and worked on the instrument on a priority basis. I must mention his efforts in providing validation service for the instruments after the field use.

Sumedha Korgaonkar
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List of Acronyms

IUCN: International Union for Conservation of Nature.
ICCB: International Congress for Conservation Biology.
mAH : Milli ampere-hour.
RTD : Resistance thermometer.
WDT : Watchdog timer.
RTC : Real-time clock.
GSM : Global system for mobile communication.
IP 65 : Enclosure rated as dust-tight and protected against water.
SCB : Society for Conservation Biology.
TSD : Thermosensitive period for sex determination.

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INTRODUCTION:

The most populous among sea turtles are the olive ridley considered, vulnerable by IUCN and is protected as Scheduled I species by Wildlife Protection Act 1972 of India. The east coast of India has both mass nesting and solitary nesting population whereas the west coast has only solitary nesting. Both the populations are geo-physically isolated forming Indian Ocean east and Indian Ocean west population. Maharashtra on the west coast of India has only olive ridley turtles nesting sites. For two decades, a lot of conservation efforts by the state forest department involving local community is implemented with fair success. The current project focused on using modern technology which will help in improvisation of conservation management in a present scenario of climate change. The use of customised digital temperature data logger is most important to understand the sex ratio of the released hatchlings and analyse the effect of temperature on hatching and emergence success over a long period of time. It is expected to contribute to mitigating the effect of extreme temperature on developing eggs thereby increasing the hatching and emergence success ratio. The report provides the objective, methodology, analysis, and outcome of the project with suggestions for future conservation management.

OBJECTIVES OF THE PROJECT:

1. To determine the incubation temperature of the nesting population of olive ridley turtles in the coast of Maharashtra.
2. To evaluate the effect of the temperature on the hatching and emergence success rate.

STUDY SITES:

Proposed Sites

Site No	Geographical locations	Latitude	Longitude
1.	Diveagar	18.16673° N	72.98218° E
2.	Anjarle	17.8568° N	73.0966° E
3.	Kolthare	17.65204° N	73.13324° E
4.	Dabhol	17.58463° N	73.17392° E
5.	Gaokhedi (SCB project)	16.80438° N	73.31700° E
6.	Madban	16.5955° N	73.3429° E
7.	Vaigani vengurla	15.87235° N	73.60780° E

Actual Sites: Due to very low nesting this season in Madban, the site was changed and the data logger was installed at Tambaldeg (16.3010° N, 73.4018° E). All the other proposed sites remained the same

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ACTIVITIES:

Sr.No	Activity	Timeline	
		Planned	Actual
1.	Lab prototype of customised temperature data logger ready with the vendor	Jan 2019	Jan 2019
2.	Ordering of the data loggers after receiving the sanctioned amount	By 23 rd Feb 2019	22 nd Feb 2019
3.	Receiving the data logger and installing it on study sites with data collection of one day.	By 15 th March 2019	9 th – 15 th March
4.	Submission of 1 st interim report	By 20 th March	26 th March
5.	Data collection and charging of batteries on all sites	March end / April 1 st week	March, April, May
6.	Data collection and charging of batteries on all sites	April end / May 1 st week	
7.	Final data collection (as per availability of nest on various sites) and data logger handing over to Mangrove Foundation.	May end / 1 st week of June	May end
8.	Validation of data logger by the vendor for quality assurance.	–	June and July
9.	Presentation of research work in International conference (ICCB 2019) held in Malaysia.	–	20 th June – 25 th June.
10.	Data analysis and report submission	June end/ 1 st week of July	July – Sept 2019

METHODOLOGY:

Procurement of customised Data Logger: A temperature data logger used in industries was modified & customized by Mr Kishor Jambhekar of Syslab Automation Pvt Ltd (<https://syslab.co.in>) for its optimized use in field conditions.

The technical specifications are as follows:

1. Battery capacity = 3.7 V/ 8000 mAh
 2. Battery life (minimum) = 30 days
 3. Inbuilt Battery Charging Circuitry = YES
 4. Battery Charging Source = 5 Volts source (power bank)
 5. No. of Analog Interfaces available = 8 RTD (PT 100 sensors)
 6. Temperature measurement accuracy = $\pm 0.1^{\circ}\text{C}$
 7. Data storage source & its capacity = 8 GB micro SD card
 8. External WDT & RTC provision facility = yes
 9. Data log timing interval = 1 min to 20 mins. It can be set as per requirement.
 10. Data can be transfer to the laptop from the unit in Excel files.
 11. Warranty is for one year for any manufacturing defect.
- The same device is compatible with the GSM interface facility by which data can be received through the cloud by using mobile SIM.
 - The same device is compatible with a solar panel for charging the batteries.
 - The device is encased in IP 65 box which is resistant to corrosion.

Installation of Datalogger: All six data loggers were procured and installed on sites as per the given work plan. The data loggers were installed at Diveagar in Raigad district, Anjarla, Kolthare, Dabhol in Ratnagiri district, Tambaldeg and Vayangani in Sindhudurg district. Madban site was changed due to very low nesting and instead, Tambaldeg was selected as one of the study sites. The 7th data logger funded by SCB was installed at Gaokhadi in Ratnagiri district. On five sites, the datalogger unit was further placed in thermacol box. At some places, they are buried in sand and other sites just kept inside the hatchery. At Tambaldeg and Vayangani the unit was placed on sand without any thermocol box.

Data collection: The beach managers were instructed about the installation and use of the data logger. They were told to put sensors only in the freshly relocated nest and not inside an existing nest. On all the sites one of the sensors was placed half a feet beneath the sand, to record the temperature of sand without eggs (**control**). The second sensor was placed exposed to air or placed

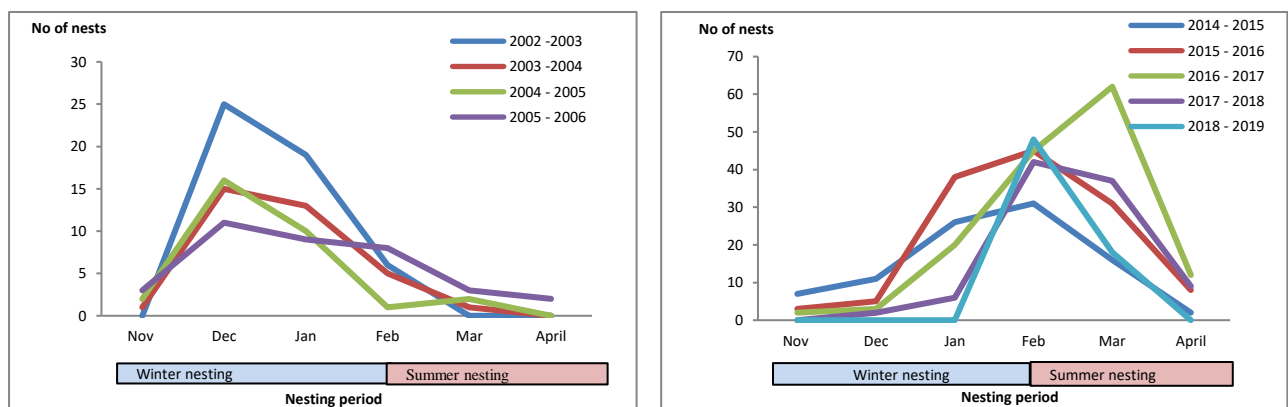
on the upper layer of sand to get the surrounding air/ upper sand temperature and other six sensors were free to be placed in the middle of the nest.

Post hatching and after the release of hatchlings, the unhatched eggs were collected from Anjarla, Dabhol and Gaokhadi from the nest with and without sensors. Development stage at mortality was identified as per Crastz (1982).

RESULTS:

Peak nesting season.

The data of peak nesting season from 2002 to 2006 and 2014 to 2019 shows a major shift of peak nesting from winter to the summer season. Nesting after 15 February results in incubation period extending to April and early May. The incubation period coincides with high ambient temperature.



Graph 1: Shift in the average peak nesting period in Maharashtra over a period of 17 years from winter nesting to summer nesting. Data source: Sahyadri Nisarga Mitra (SNM) report and Maharashtra State Forest Department.

Temperature Sex Determination (TSD) period of incubation

The TSD period (Thermo-sensitive period) for olive ridley turtle is from 10th day till 21st day of the 50 days of incubation period where the sex is determined. In olive ridley turtles, only males are developed when the temperature is $\leq 28^{\circ}\text{C}$ and only females developed when the temperature is above 31.5°C (Davenport, 1997). The pivotal temperature where both males and females developed in 1: 1 ratio is 29.5°C for this species. For analysis, TSD period of those nests having sensors for the complete incubation period of 50 days were considered.

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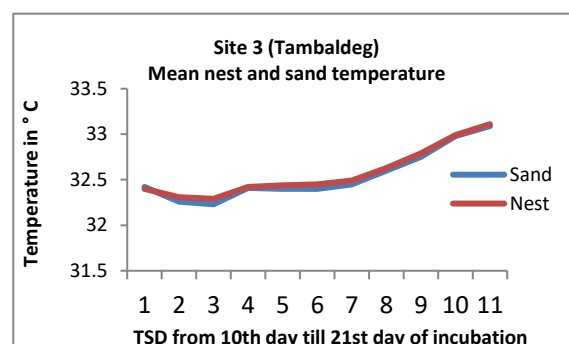
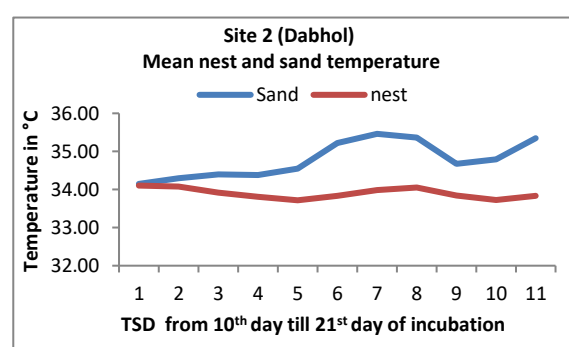
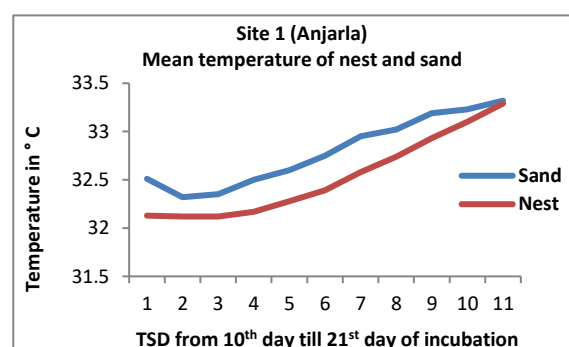
	Site1: Anjarla		Site2: Dabhol		Site3: Tambaldeg	
Day	Temperature ½ feet inside sand in °C (Control)	The temperature of nest ½ feet inside in °C	Temperature ½ feet inside sand in °C (Control)	The temperature of nest ½ feet inside in °C	Temperature ½ feet inside sand in °C (Control)	The temperature of nest ½ feet inside in °C
(TSD Period)						
10 th	32.5	32.1	34.15	34.1	32.42	32.4
11 th	32.3	32.1	34.3	34.07	32.26	32.30
12 th	32.4	32.1	34.4	33.92	32.23	32.29
13 th	32.5	32.2	34.38	33.81	32.41	32.42
14 th	32.6	32.3	34.55	33.72	32.4	32.44
15 th	32.8	32.4	35.22	33.83	32.4	32.45
16 th	32.9	32.6	35.46	33.99	32.45	32.49
17 th	33	32.8	35.36	34.05	32.6	32.63
18 th	33.2	32.9	34.68	33.84	32.75	32.79
19 th	33.2	33.1	34.79	33.73	32.98	32.99
20 th	33.3	33.3	35.35	33.83	33.09	33.11
21 st	33.5	33.5	35.19	33.98	33.06	33.05
Mean	32.8	32.6	34.82	33.9	32.58	32.61
Max	33.5	33.5	35.46	34.1	33.09	33.11
Min	32.3	32.1	34.15	33.72	32.23	32.23

Table 1: Comparative data of 3 sites during the TSD period of 10th day till the 21st day of incubation.

At site 1 a difference of 0.1°C – 0.2°C was observed between the control and the nest with eggs. This difference is insignificant. Both the temperatures showed a gradual increase from 10th day till 21st day with min temperature of 32.1°C and a maximum temperature of 33.5°C. In site 2 ie Dabhol a difference of 1°C is observed between the temperature of control and nest. The temperature of the nest is constant at 34°C throughout the TSD period but that of control shows variation.

Insignificant variation was seen in temperature of the sand and of nest throughout the TSD period in site 3. The increase in the temperature is gradual from 32.2°C to 33°C.

Graph 2: a) Site 1: The temperature of the control and nest increases correspondingly in the TSD period. B) Site 2: Temperature of the nest is considerably constant at 34°C but that of control shows variation. C) Site 3: The temperature of control and nest is absolutely the same with a small increase of 0.8 °C throughout the TSD period.

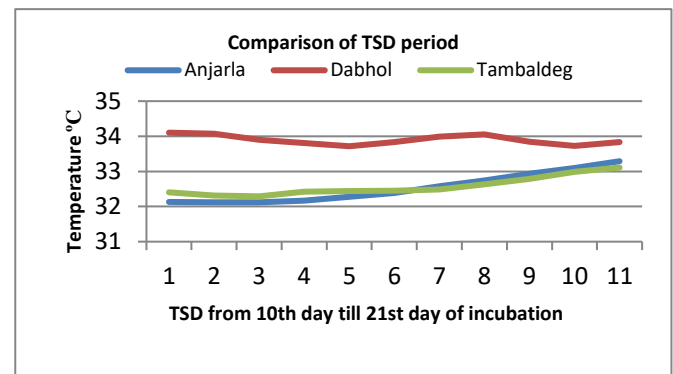


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The ambient upper sand temperature on all the 3 sites shows huge variation with a min temperature of 25 °C to max temperature of 50 °C throughout the incubation period

Predicting the Sex Ratio

The temperature during the TSD period differs among sites. Avg temperature during TSD at site 1 & 3 is well below 33°C while in site 2 it is above 33.8°C. The sex of the developing hatchling can be predicted as females at all the sites.



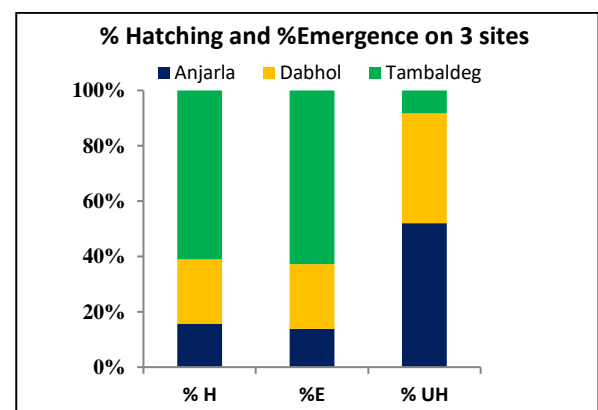
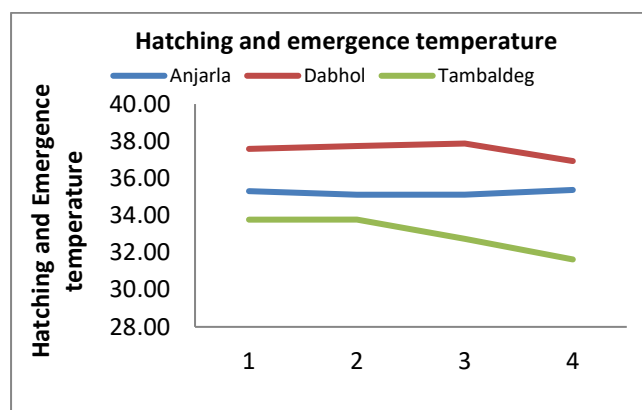
Graph 3: Prediction of sex ratio from the TSD period.

Hatching and Emergence temperature: The hatchlings hatch in the nest 3 - 4 days prior to emergence from the nest. The hatching and emergence temperature of the nest in Dabhol is between 37°C to 38°C which might be resulting in a high percentage of unhatched eggs and less hatching and emergence success (33%). Similarly, on-site 1 ie Anjarla the temperature is above 35°C and the % hatching and emergence success is also less (22%).

	Anjarla	Dabhol	Tambaldeg
Nest No	8	20	8
Date of Nesting	27-Mar	20-Mar	26-Mar
No of eggs	89	136	95
Date of hatching	13-May	07-May	14-May
No of			
# Live hatchlings released	17	44	82
# Dead hatchlings	3	1	nil
# Piped dead	nil	nil	nil
# Unpiped dead but developed	61	1	
# Unfertilized / early mortality	8	20	
% Hatching success	22%	33%	86%
% Emergence success	19%	32%	86%
% unhatched eggs	88%	67%	14%

Table 2: Nesting data of the nests with sensors on 3 sites.

whereas at site 3 ie Tambaldeg the temperature is below 34°C and drops to 31.5°C at the time of emergence. Hatching and emergence of success are observed to be 86%.



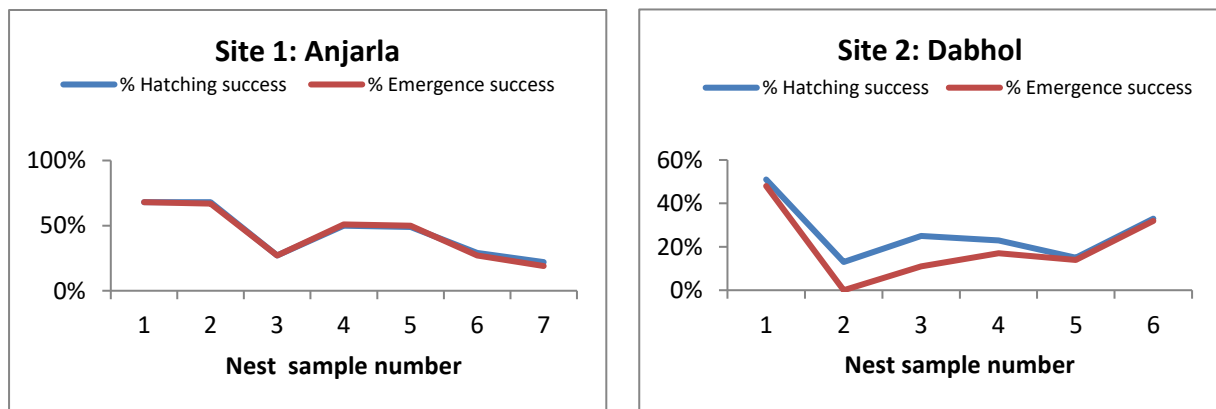
Graph 4a: Temperature of the nest on the last four days of incubation on 3 sites considered together as hatching and emergence temperature. Graph 4b: Comparison of hatching and emergence success in percentage on all the 3 nesting sites. H is hatching, E is emergence and UH is unhatched.

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Additional data of hatching and emergence were collected from site 1 and 2: Post hatching and release of hatchlings, the nest were opened and nest examined for empty shells, dead hatchlings, piped dead, un piped dead but developed, unfertilized/early mortality.

	Site 1: Anjarla							Site2: Dabhol					
Sample nest No	1	2	3	4	5	6	7	1	2	3	4	5	6
Date of Nesting	26-Jan	15-Feb	17-Feb	27-Feb	13-Mar	14-Mar	27-Mar	25-Jan	17-Feb	22-Feb	25-Feb	01 Mar	20-Mar
No of eggs	104	112	83	132	64	113	89	88	88	108	131	143	136
Date of hatching	17-Mar	06-Apr	08-Apr	18-Apr	02-May	03-May	13-May	30-Mar	-	20-Apr	20-Apr	21-Apr	07-May
No of													
# Live hatchlings released	71	75	23	68	32	31	17	43	nil	12	23	21	44
# Empty shells	70	75	21	68	-	-	-	46	14	27	27	23	45
# Dead hatchlings	nil	1	nil	1	1	2	3	2	12	15	8	1	1
# Piped dead	nil	nil	nil	nil	nil	nil	nil	7	nil	1	1	94	nil
# Unpipid dead but developed	20	18	22	52	15	69	61	31	52	79	84	15	71
#Unfertilized / early mortality	11	17	40	7	16	11	8	5	24	1	15	12	20
% Hatching success	68%	68%	27%	50%	49%	29%	22%	51%	13%	25%	23%	15%	33%
%Emergence success	68%	67%	27%	51%	50%	27%	19%	48%	0%	11%	17%	14%	32%

Table 3: Percentage Hatching and Emergence success of nests from site 1 and 2 without sensors.



Graph 5a &b: Percentage hatching and emergence success of nest on site 1 and 2 not having temperature sensors in them.

The nesting period peaked after mid-Feb resulting into incubation period extending in summers till mid-May. At site 1 the hatching & emergence success show a marked decline as the incubation period progressed. At site 2 the hatching and emergence dropped drastically. The precautionary steps were taken later for the nests hatching during late April and early May at the end of incubation period in both the sites thus increasing the emergence rate. The nest sample no 2 and 3 in Dabhol showed no emergence even after

the completion of the incubation period hence it was opened for examination. Dead hatchlings were found trapped in nest. The sand inside the nest has become hard trapping the hatchlings. The proportion of dead hatchlings in Dabhol was found to be very high. Dead hatchlings were very well developed. With this other nests were opened at both the sites two days prior to getting depression and/or completion of incubation period of 50 days. This rescued the trapped hatchling increasing the hatching success. The stage of development at mortality of un piped dead hatchlings were from 26 to 28 viz: development days of 33 to 40 days (Crastz, 1982).

Working on temperature data logger:

The field testing of data logger was verified in the lab for any hardware damage by the vendor Shri Kishor Jambhekar. Except for two units having faulty parts, all the units functioned well. The parts were replaced. IP65 box encasing the data logger gives adequate protection so that it could be directly installed above or below sand. Styrofoam box is not required and plastic box should be avoided.

DISCUSSION AND CONCLUSION:

Climate change has a profound and direct effect on incubation temperature of sea turtles. The incubation temperature not only influences the embryonic development, incubation period, growth rate, sex ratio, hatching success but also the size and locomotor performance of hatchling (Booth et al. 2013). Lower incubation temperature $< 28^{\circ}\text{C}$ produces bigger size hatchlings which are mostly males whereas higher incubation temperature $> 32^{\circ}\text{C}$ produces smaller size females (Booth et al. 2004; Burgess et al. 2006; Booth et al. 2008). The hatchlings developed in the optimal incubation temperature range of 28°C to 32°C shows good locomotory skills, increasing its survival rate in the open waters (Booth. D, 2017). The global air temperature which has increased in last two decades coupled with the shift in the peak nesting period extending in summer season in Maharashtra is a major concern. Together it would result in female-biased sex ratios, low emergence rate and poor hatchling locomotor movement. Post TSD period ie after 23 days of incubation, the temperature of the nest could be maintained at optimal range by shading which has shown to be effective as climate mitigating technique (Hill et al. 2015). This should be restricted to sites and nests showing exceeding temperature.

The temperature study from three sites shows variation in temperature range during the emergence period resulting in varying emergence success. This might be due to the type of sand present on the beach. Most of the nesting sites in Maharashtra have big or small estuaries depositing varying placer minerals on the adjoining beaches. The colour of the sand is responsible for its temperature; dark colour sand absorbs more heat than lighter colour sand (Mortimer, 1990). The water holding capacity of the sand is because of the composition of sand, with the percentage of silt and clay present. Optimum moisture is required for high hatching success from incubating eggs (McGehee, 1990). Moisture and temperature are interrelated and shows a combined effect on incubation process. At early stages of development temperature plays a major role whereas at later stage during growth moisture may have a profound effect due to increased rate of metabolism which utilizes egg yolk (Sifuentes-Romero et. al, 2018). Moisture has a cooling effect on eggs and keeps them hydrated. Metabolic moisture of the nest might be keeping the temperature of nest constant and much lower than ambient air temperature (above 40°C) and upper sand temperature (reaching 50°C) during midday at study sites. Though this natural phenomenon is advantageous during development it is observed that it affects negatively on study sites. Increase in moisture makes the sand hard at some

places reducing the gaseous exchange in developing embryo and trapping the hatchlings during emergence. This results in reducing the hatching and emergence rate. The unhatched eggs show growth restricted at 32 stages ie 40 days of incubation resulting in high mortality rate. This is not observed uniformly on all study sites possibly due to variation in sand composition.

The project could not collect complete information about incubation temperature due to delayed installation of the data logger and brief nesting period in last season. It was successful in testing the functioning of customised data logger in the field conditions, especially during harsh summer. This project has the potential to be implemented in phase 2 and 3 for the next two seasons. Phase two would have advance GSM facility and complete incubation temperature data for the season. Based on data received in phase 2, phase three will focus on developing an analytical software that can be easily used by a technical person.

FUTURE SCOPE:

- 1) Temperature data logger with GSM facility should be deployed on the sites at the start of the season (ie: early Dec). It will facilitate the real-time data transfer through cloud facilitating regular monitoring without disturbing the data logger on the field. In case of data showing extreme temperatures, immediate actions can be taken. (see suggestions)
- 2) Solar panel for battery charging can be used as a power source.

SUGGESTIONS:

The following suggestions are meant for the future project and can be implemented with continued use of data logger having GSM facility. The technical person handling and monitoring the data logger should follow these suggestions. In case of interventions needed for mitigating the effect of temperature the technical person should convey them to the beach managers.

1. No mitigation efforts are needed when the nest shows an optimum temperature range of 28°C – 32°C. The nest needs to be maintained at this temperature during summer.
2. Any kind of manipulation of temperature during TSD period ie 10th day to 23rd day of incubation should be strictly avoided. This would affect the sex determination process.
3. Efforts should be made to reduce the temperature of the nest as soon as the temperature crosses 33°C. It should be done after the 23rd day of incubation. This can be achieved by putting a shade on the hatchery or on the specific nest. Alternatively, above the nest an inverted cane basket having wet jute gunny bag over it can be used (This is done at Madban by the beach manager Shri Shyamsunder Gavankar with fair success).
4. Post hatching nest should be opened to see the stages of mortality. The observations need to be recorded for later analysis. Appropriate training needs to be given to beach managers for the same in an easy and simple format.

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Picture 1: a) Data logger with 8 sensors encased in IP 65 box b) Encased data logger inside Styrofoam box before installation on field.



Picture 2: Installation of data logger at a) Dabhol b) Vayangani c) Gaokhadi d) Tambaldeg e) Kolthare.

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Figure 3: a) The empty shells from nest b) Un-hatched eggs c) Piped dead hatchling d) Un-piped dead hatchling stage 26 -30 of incubation e) Dead hatchlings.

Report on ‘Studying the incubation temperature of nesting population of olive ridley turtles (*Lepidochelys olivacea*) in the coast of Maharashtra’.

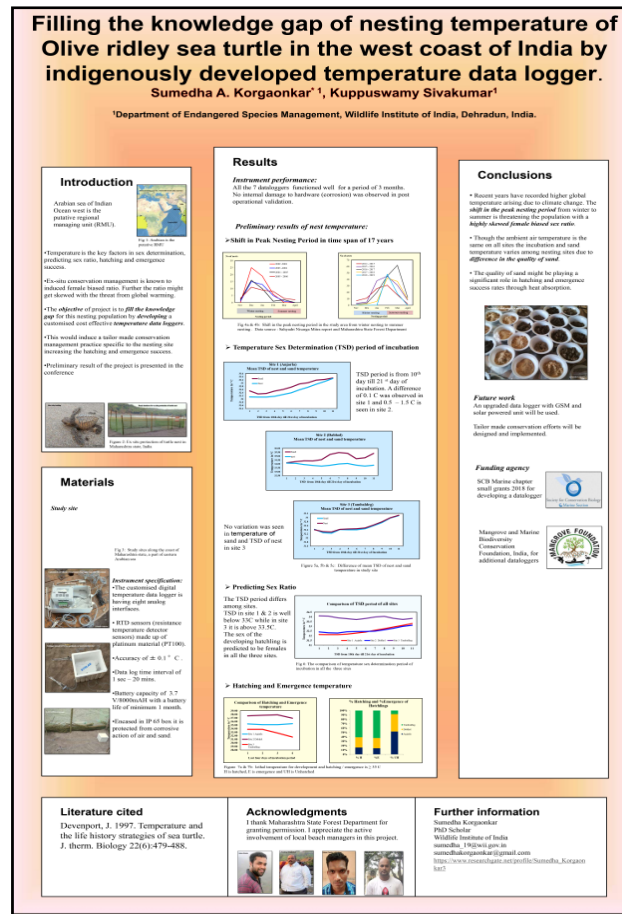


Figure 4: The project work was presented as a Poster in International Congress for Conservation Biology 2019 (ICCB 2019) held in Malaysia on 22nd July 2019.



Figure 5: Local beach managers who has actively participated a) Dattabhau Vanarkar (Dabhol)
b) Sagar maladkar (Tambaldeg) c) Pradeep Dingankar (Gaokhadi – SCB project) d) Ajinkya
Keluskar (Anjarla).