GALAPAGOS WHALE SHARK PROJECT

FIELDWORK REPORT 2019

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PARTICIPATING ORGANISATIONS:

[1]DIRECCION DEL PARQUE NACIONAL GALÁPAGOS,
[2]MARINE MEGAFAUNA FOUNDATION,
[3]GALAPAGOS CONSERVATION TRUST,
[4]UNIVERSIDAD SAN FRANCISCO DE QUITO,
[5]GALAPAGOS WHALE SHARK PROJECT,
[6]GEORGIA AQUARIUM
[7]PLANETERRA

Figure 1: Darwin Arch by Darwin Island, dive and study site. Photo ©Jonathan R. Green 2018

GALAPAGOS MARINE RESERVE

With the creation of the Galapagos Marine Reserve, (GMR) extending 40 nautical miles from the outermost point of the peripheral islands a large area of the north east Pacific provides protection to a diverse and thriving marine community that includes many of the Galapagos endemic species as well as resident native and migratory fauna, including the world's largest fish, the whale shark, Rhincodon typus.

An ocean traveller the whale shark is found between the latitudes of 40° north and 45° south in all the oceans and mostly associated with tropical, sub-tropical and temperate waters, (Ryan et al. 2017) Whale sharks feed predominantly by filter feeding on a wide variety of planktonic (microscopic) organisms but have been observed lunge feeding on nektonic (larger free swimming) prey such as schooling fishes, small crustaceans, and occasionally tuna and squid. Whale sharks are ovoviviparous with eggs hatching within the female's uteri and the female giving birth to live young. Whale sharks in the GMR are seasonal with highest recorded numbers during the months of July – October. (Hearn et al. 2013) Very little is known about their biology and ecology, and their movements, particularly in the Eastern Tropical Pacific.

The Galapagos Whale Shark Project began in 2011 with a series of field trips to study the movements of whale sharks within the GMR and with satellite tagging to track their movements on a local and regional scale. Early data showed that over 99% of all sightings in the Galapagos were of adult females. (Acuña et al 2014). The study site is Darwin Arch near Darwin Island at the furthest north of the Galapagos Archipelago. (See Figures 1 & 2)

Figure 2: Map of Galapagos Marine Reserve with baseline and 40 nm limit and Bathymetric map of Darwin Island. (Cesar Peñaherrera P. CDF)

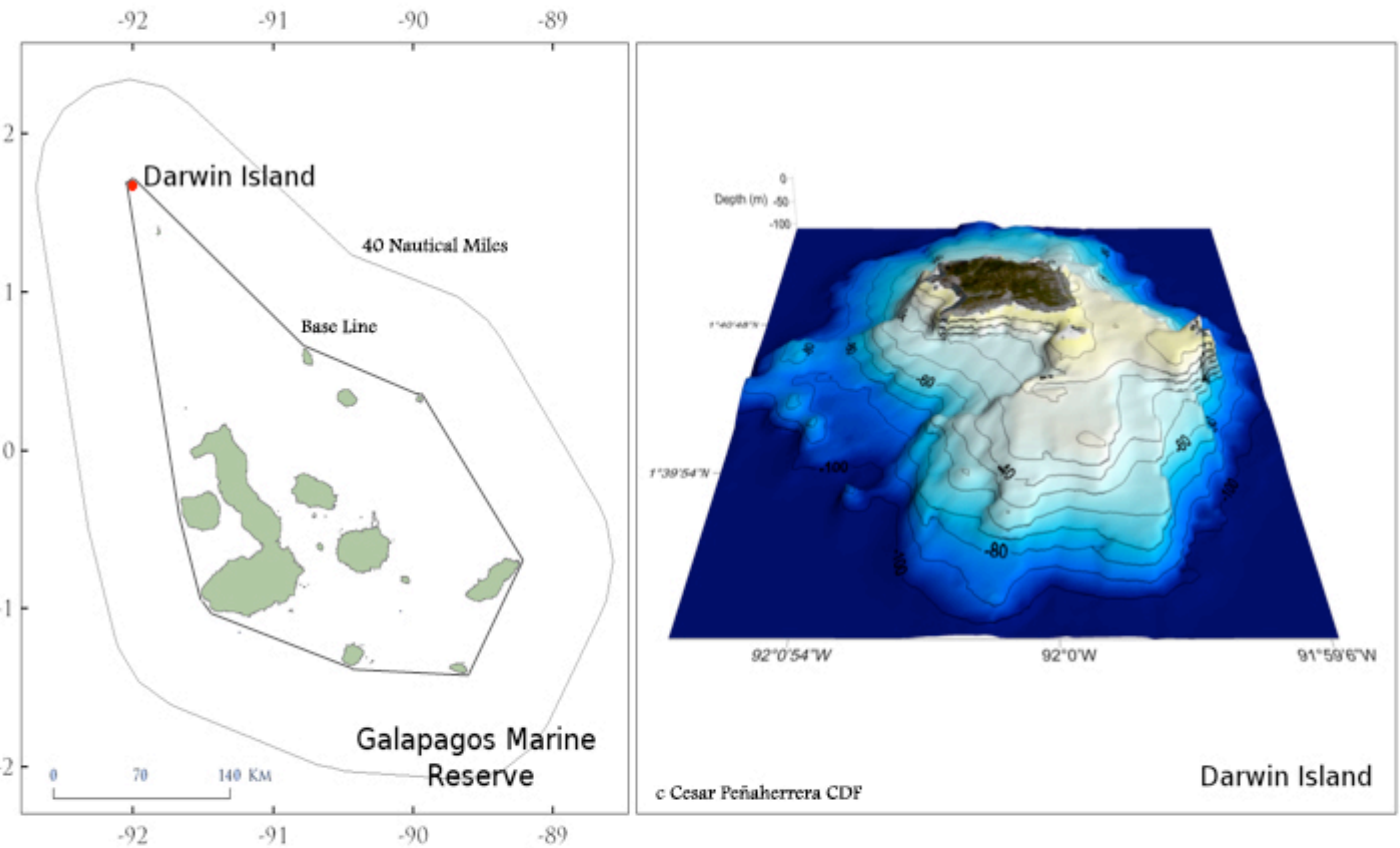

Figure 3: Diver tagging a whale shark with Hybrid miniPAT / SPOT6 257 tag. Photo © Simon J. Pierce 2019

OBJECTIVES

This season we are continuing with the fin mount tag deployments but modifying the mount to hold two tags, the SPOT6 257 and the miniPAT (see Figures 4 & 5). This is in order to attempt less invasive techniques of tag attachment and to try to replicate the transmission and retention successes of last season. Last year we attached the SPOT6 tags by fin mount but the miniPAT tags were still being deployed by compressed air spear guns. Although this did not create any apparent stress to the animal and over 90% showed zero reaction to the implantation of a small dart with tag, our aim is to reduce not only possible impact but also the perceived impact when using spear guns.

We are attempting to correlate data about diving behaviour, (with the miniPATs) with position relative to the ocean floor, (with the SPOT6 257s) to determine if diving and surface movements are related to benthic features and geological

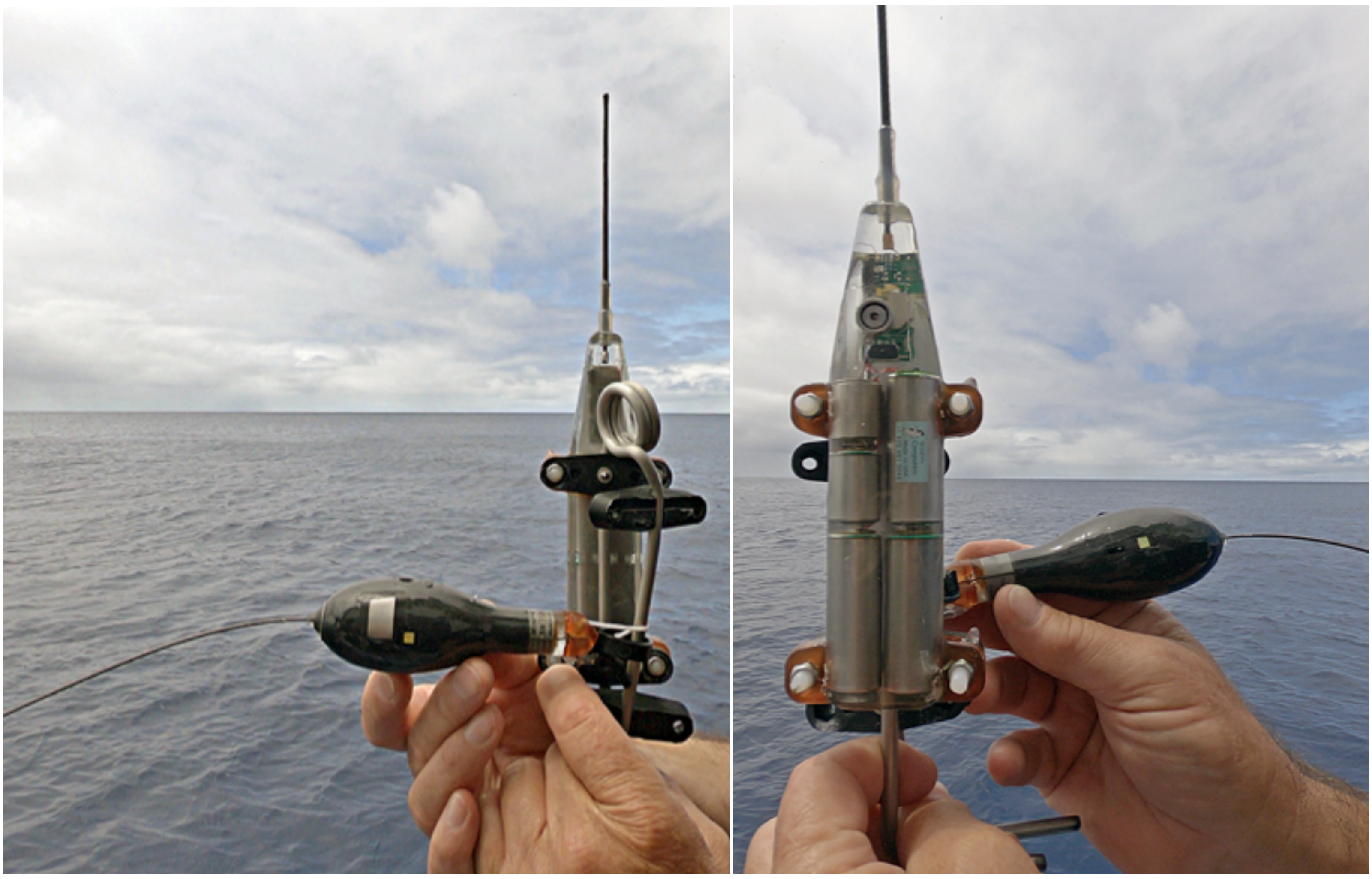

Figure 4 (left) & 5 (right):
Hybrid SPOT6 / 257 & miniPAT satellite tags on single fin mounted clamp. Photo: ©Sofia M. Green 2019

structures such as fracture zones, faults, fissures, seamounts or even plate margins.

However both tags have depth limitations that either causes their premature release or they are crushed and stop transmitting. For the miniPAT the release depth is set at 1700m and the SPOT6 257 the maximum depth is approximately 1850m. Certainly beyond 2000m it is highly unlikely that either tag will survive being crushed and cease transmissions and data storage.

In order to collect data we are assisted this season by Dr Al Dove from the Georgia Aquarium who, in collaboration with Thom Maughan with iSea Solutions who have developed a “DEEP” tag V2. Deployment of these tags was first tried on whale sharks at St Helena in the Atlantic Ocean but without results or any data returned. We hope the second version will provide data about exactly how deep this species is diving. The DEEP V2 tag is tested to 4500m and it is hoped that it could provide information to a depth of 6000m.

Figure 6: Hybrid miniPAT / SPOT6 257 tag deployed on the fin. Photo ©Simon J. Pierce 2019

SATELLITE TAGS SETTINGS

The SPOT6 type 257 has an estimated battery life of 1500 days or 4.1 years. This potentially gives us a better opportunity of tracking regional movements and maybe even recording their return to the GMR. Last season in 2018 one tag #175950 (aka Oceana), reported for a period of over eight months, (the longest confirmed track thus far for this project). The last recorded transmission was May 24th after over 250 days of track data. The SPOT6 track is limited to surface data so the track only represents straight-line movements between each transmission.

The miniPATs record and archive depth data, which provides the information for better understanding of diving behaviour. The incorporated light sensors register light changes throughout the day, dawn and dusk and midday light time and intensity, which provide a location. The location does not have the same accuracy as the Argos

Satellite data from the SPOT6, but by creating a hybrid fin mount combining both SPOT and miniPAT, we will be able to compare position and accurately recreate the track against the ocean floor and benthic geological features.

Both miniPATs and SPOTs record temperature at depth with greater accuracy from the miniPAT tags. The double tagging will allow us to correlate temperature data from prior SPOT deployments to estimate depth.

The four DEEP V2 tag are programmed for 10 day, 20 day, 30 day and 40 day deployments respectively. Post release they transmit first the deepest depth recorded during diving and then begin to upload all the data set from 5-minute recordings of time, temperature and depth. (See figure 7)

Figure 7: “DEEP” V2 tag. Photo: ©Jenny Waack 2019

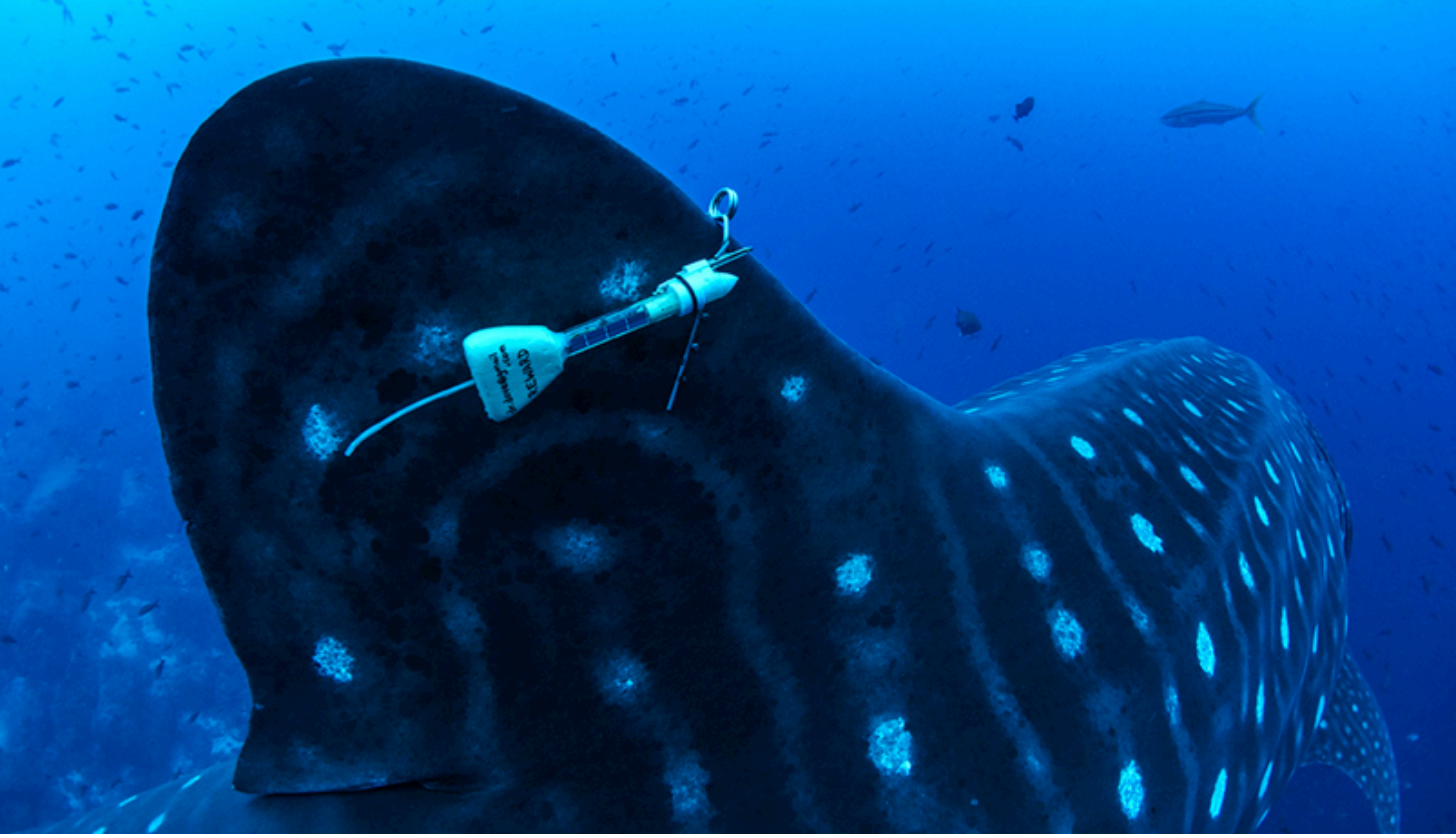

Figure 8: DEEP V2 tag deployed on the fin. Photo ©Simon J. Pierce 2019

The hybrid tags were set with identical temperature bins, (data storage parameters, see Figure 9), again for correlation but the miniPAT maximum deployment is set for 6 months whereas the SPOTs could potentially transmit for a much longer period. Once the data has been correlated then it may be possible to more accurately estimate depth when we only have the temperature bins from the SPOT tags.

Histogram Bin Limits

Bin #	1	2	3	4	5	6	7	8	9	10	11	12
Temp.	3.0 °C	5.0 °C	7.0 °C	10.0 °C	14.0 °C	18.0 °C	21.0 °C	24.0 °C	27.0 °C	30.0 °C	33.0 °C	45.0°C
Depth	10.0 m	20.0 m	50.0 m	100.0 m	200.0 m	300.0 m	500.0 m	750.0 m	1000.0 m	1250.0 m	1500.0 m	2,000m

Figure 9: Temperature bins for both of the tags, SPOT6 and miniPAT used for the hybrid fin mount.

Figure 10: Whale Shark close to the surface. Photo ©Jenny Waack 2019

SATELLITE TAGS DEPLOYMENT

Environmental conditions have been optimum for this field trip with variable north south currents but no more than 4 knots strength. Most days we have been able to find the divide in the current that has allowed the team to hang off the wall and boulder slope, staggering the divers both horizontally and vertically in order to maximise sightings.

We have deployed tags each day averaging 3 tags daily, a single DEEP V2 and the double hybrid SPOT / miniPAT, (see Figure 6). All four DEEP V2 tags were deployed in the first 4 days of the trip, (see Figure 8).

By Day 5 we had also attached all the hybrid tags so began with the blood draw whilst continuing photo ID.

Figure 11: Blood draw from the pelvic fin. Photo ©Simon J. Pierce 2019.

BLOOD DRAW

Last field season we successfully collected blood from 7 individuals, 6 females and 1 male. (See Galapagos Whale Shark field report Green et al. 2018) These samples were analysed on board for basic data, partial pressure of O2, CO2, pH and Lactic Acid. The latter can be used to determine stress levels in an individual and importantly has shown that after tagging and other procedures the lactic acid levels do not show any stress in the animal, (see Figure 12).

Figure 12: Results of hormonal analysis of blood samples from the 7 individual whale sharks sampled in 2018. Dr Ryo Nozu

	A	B	C	D	E	F	G	H	I	J	K
1	TubeNum	ID	Sex	Status	SamplingDate	TL(m, Estimate)	Testosterone(pg/ml)	Estradiol(pg/ml)	Progesterone(pg/ml)	Remarks	
2	2	2018.Rt10	Female	Mature	2018.09.17	11	2.896	312.108	LDL	LDL:lower detection limit	
3	3	2018.Rt11	Female	Mature	2018.09.17	11	7.576	173.548	LDL	LDL:lower detection limit	
4	4	2018.Rt14	Female	Mature	2018.09.19	11	10.228	480.644	LDL	LDL:lower detection limit	
5	5	2018.Rt19	Female	Mature	2018.09.20	10~11	12.332	190.928	7.644		
6	6	2018.Rt26	Female	Mature	2018.09.24	NA	19.028	505.428	15.052		
7	NA	2017.01	Female	Mature	NA	NA	19.392	158.592	18.976		
8	NA	2017.02	Male	Mature	NA	NA	3141.2	153.872	60.076		

Posterior analysis through electrophoresis by Dr Ryo Nozu, from the Okinawa Churashima Foundation of hormone levels proved inconclusive for signs of pregnancy, (see Figure 13).

Dr Nozu writes in his report “Blood samples provide us with various physiological information. For example, sex steroid hormones concentration in the blood is considered to be useful for estimating reproductive status of individuals. There are several reports that increase in estradiol (E2) are often correlated with follicular development, and progesterone (P4) is involved in ovulation and/or pregnancy. On the other hand, for the whale shark, such information is insufficient, especially there is no information about sex hormones in mature individuals. In the recent field research, we collected the blood samples from 7 “presumed mature” individuals (Male; 1, Female; 6) and measured the levels of sex steroid hormones including E2, P4 and Testosterone (T) for the first time in the world. From the present

Figure 13: On board analysis with FUSO i-STAT. Photo ©Jonathan R. Green 2018

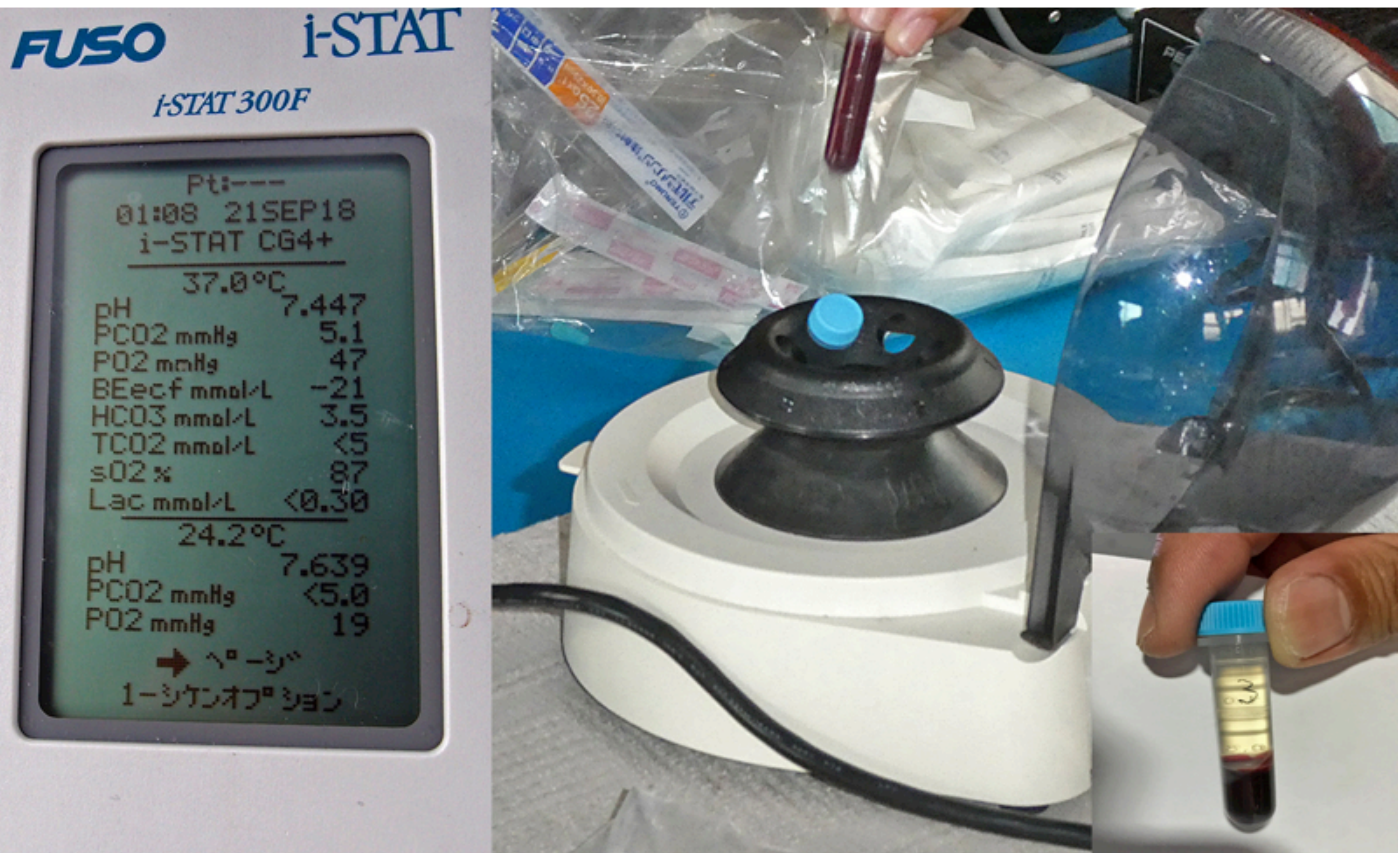

Figure 14: Dr Alastair Dove carrying out blood draw from the pectoral fin. Photo ©Simon J. Pierce

results, we could provide the comparison information about sex steroid hormones levels in the whale shark. In the future, it is important to compare the blood samples obtained from individuals of various body lengths or having each developmental stage of follicles identified by ultrasonography".

Although the analysis and subsequent data did not show evidence of pregnancy or associated parameters that might indicate the reproductive state, except that they might have "recently birthed" we are again attempting blood draw this season and next season but at differnet times of the year in order to compare the results. As whale sharks begin arriving in significant numbers in June, reach a peak in August – October and decline late November to December, it may be that we find a difference in hormonal levels next season as we will be in the field two months earlier at the end of June, beginning of July.

As with last season we will store the plasma and whole blood, after centrifuging, in the freezer for later analysis. Attempts to draw blood were made on both pelvic and pectoral fins with successful draws from both. (See figures 11 & 14). A total of four samples were collected, two from pectoral and two from pelvic fins.

Immediately after collection the blood was taken back to the Queen Mabel for pre-analysis processing. The blood was added to an anticoagulant to allow time for centrifuging without clotting. This allowed the separation of the sample into whole blood and plasma and the plasma is subsequently analysed for the presence and amounts of three hormones, testosterone, progesterone and oestrogen. On board the plasma and whole blood were stored in separate vials in the freezer prior to their transport to the USFQ. The sample was also tested on board for partial pressure of oxygen and carbon dioxide, lactic acid and pH to understand the physical condition of sharks.

Figure 15: Diver documenting the project work for future outreach and education. Photo ©Sofia Green 2019

Figure 16: A tagged and curious individual approaches a diver in the blue . Photo © Simon J. Pierce 2019

SATELLITE TRACKS OF SPOT6 257 & MINIPAT RESULTS FROM 2018

Satellite tracks SPOT6 257 results from 2018:

2 of the SPOT tags gave significant tracks over a period of just over 8 months from the date of tagging. Neither of these were double taggings with miniPATs so we only have the data from the surface transmissions. (See Figure 17)

Tags #175950 and #175952 both headed towards the continental shelf dropoff and the Chile Peru Trench. However from their respective tracks it appears they are travelling via different routes but without sub surface data to correlate we simply have straight line tracks.

For #175950 the track remains close to Darwin Arch for a period of 15 days and then disappears for a period of 3 months. Her surface track shows a distance of approximately 750nm but the whale shark may have travelled much further. Until we are able to determine with any accuracy the routes that the whale sharks are following, the areas where they are feeding and, the "Holy Grail", where they are mating and birthing, we cannot hope to create Marine Protected Areas that

Figure 17: Tags #175950 and #175952 showing their respective tracks from Darwin Arch to the Peru shelf dropoff.

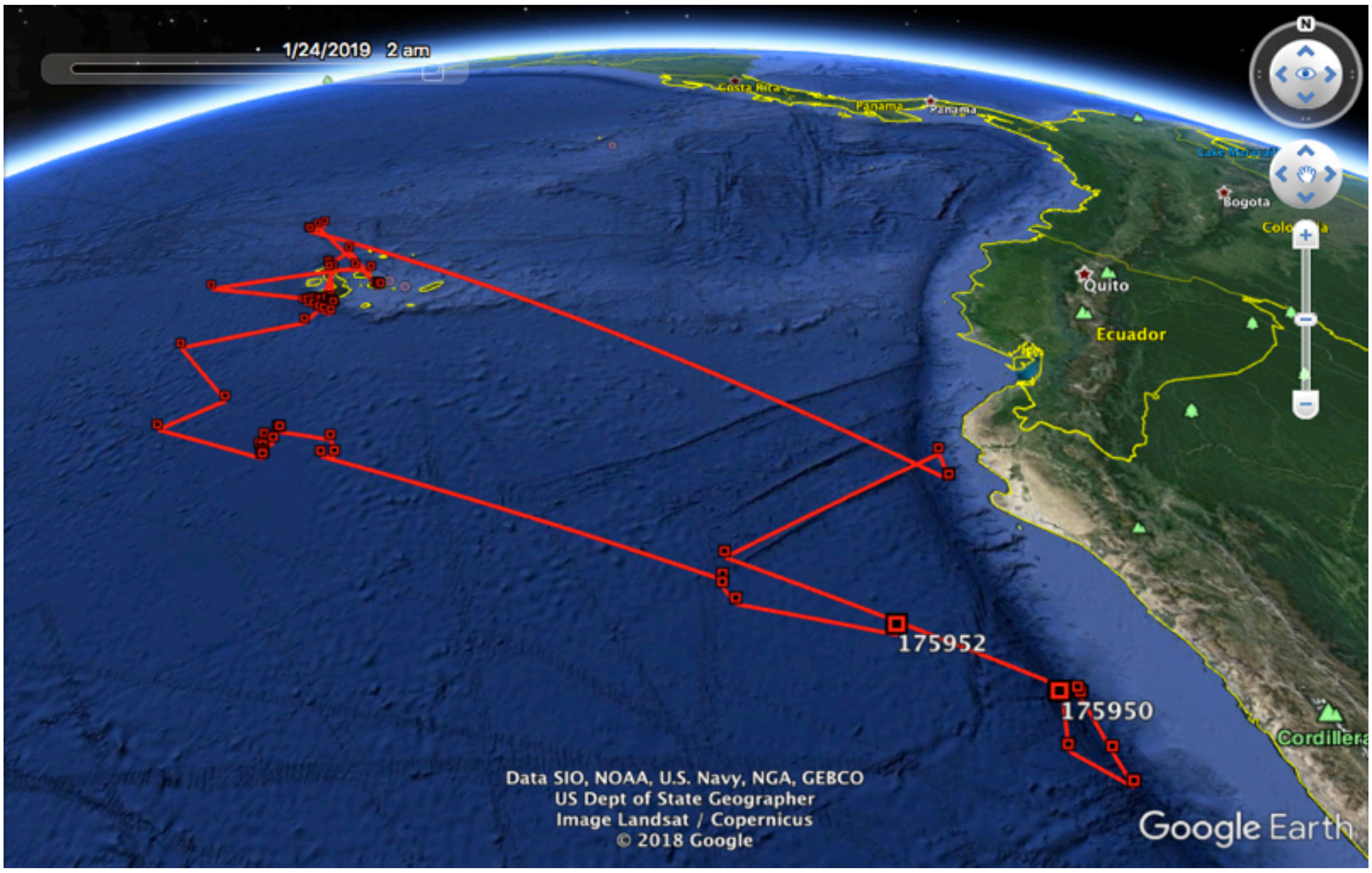

Figure 18: Tag #175950 showing the detailed movements along the Chile / Peru Trench and Peru coastal drop off.

realistically afford the protection this species and so many others so urgently need.

The last transmission from #175950 toward the end of May, 2019. Despite the fact that over four months have transpired since that date at the time of writing this report, the tags remain active in the hope that the individuals are simply travelling sub surface and might transmit at a later date.

Satellite tag miniPAT results 2018:

Of the 7 miniPAT tags deployed we received transmissions from 4, (see Figures 19 - 22).

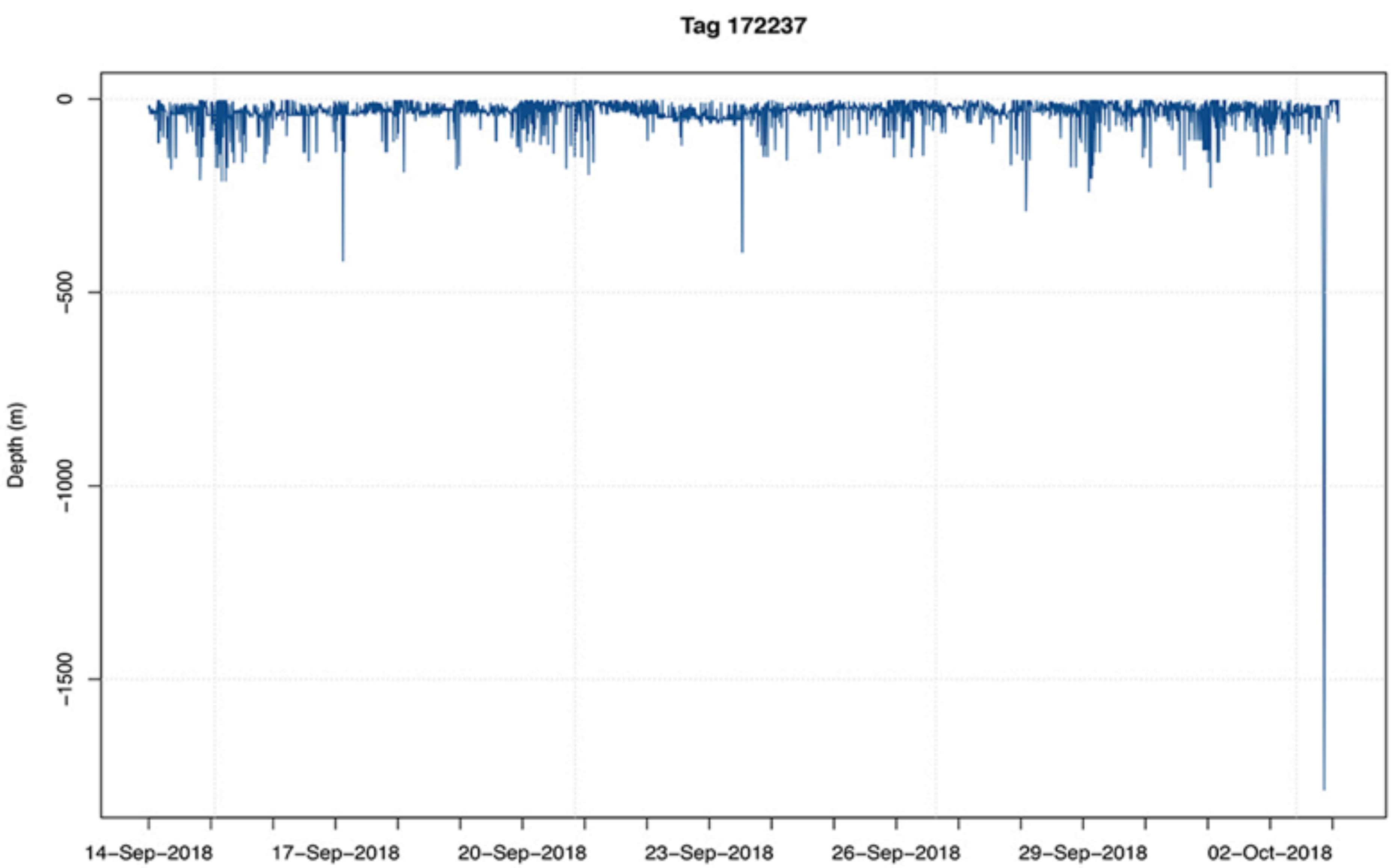

Figure 19: Tag #172237 released early due to a deep dive to 1896m. Tag deployment time 22 days.

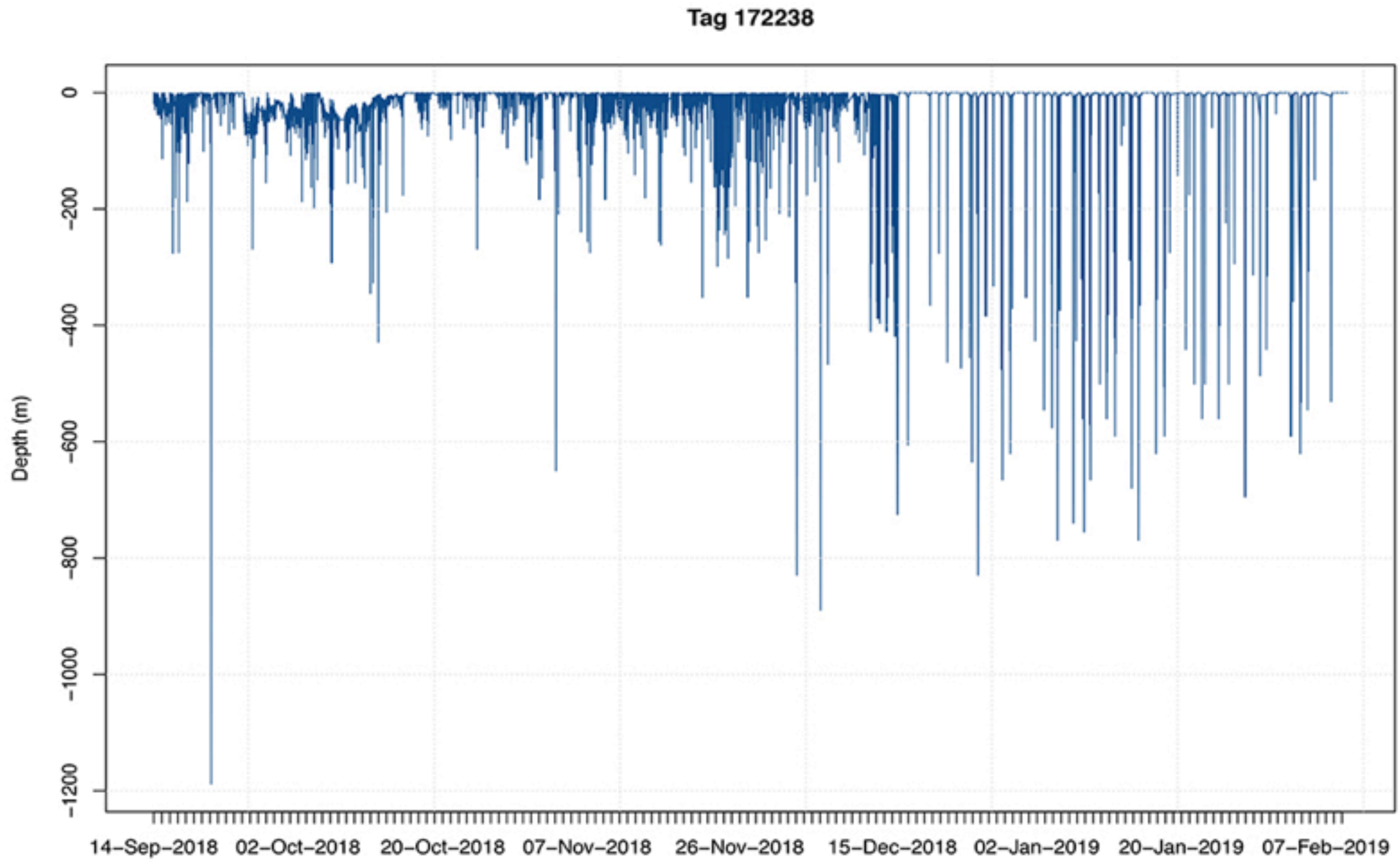

Figure 20: Tag #172238 released due to set release date. Total deployment 152 days. Max depth reached 1272m.

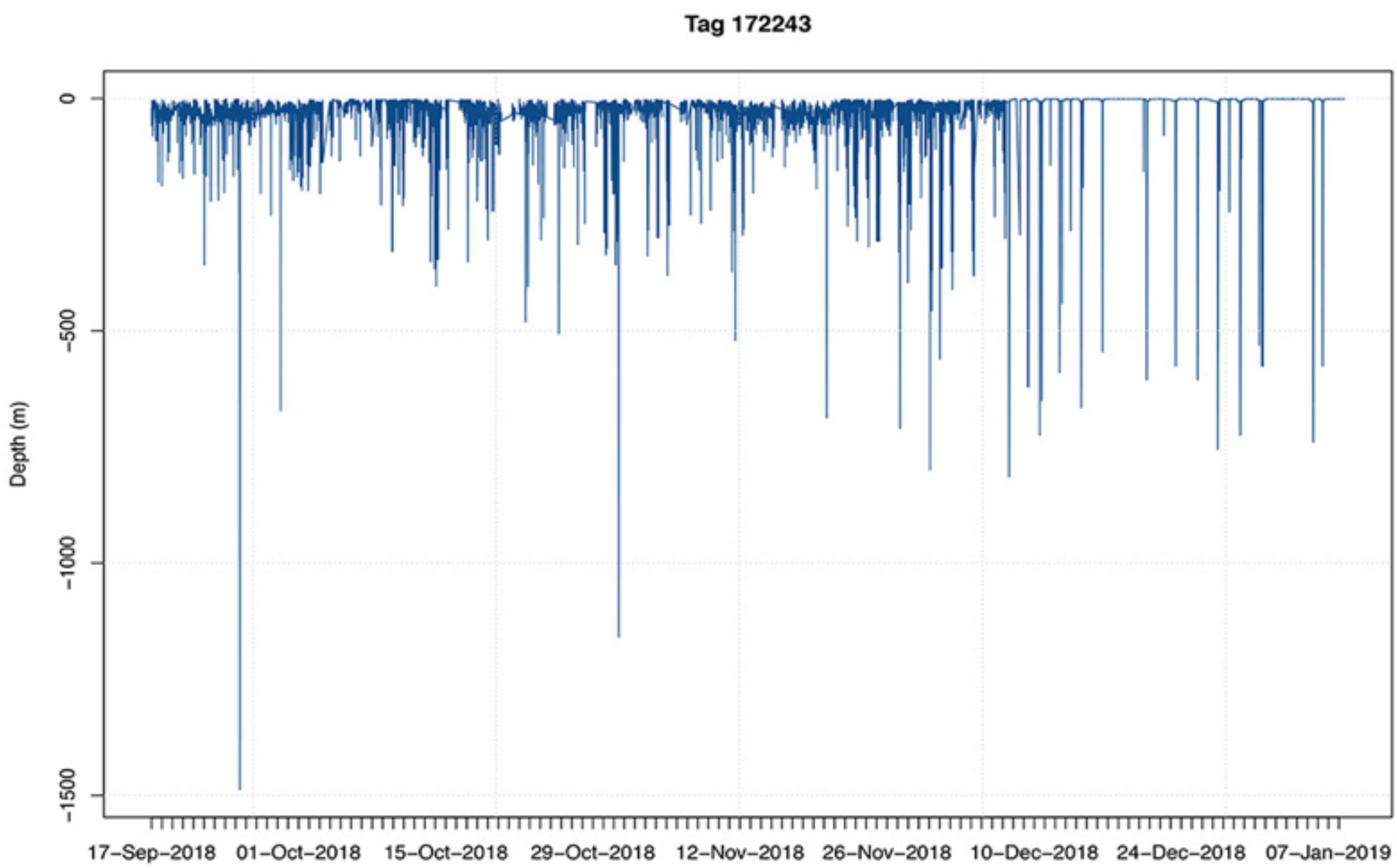

Figure 21: Tag #172243 premature, reasons unknown. Max depth 1592m Tag deployment time 117 days.

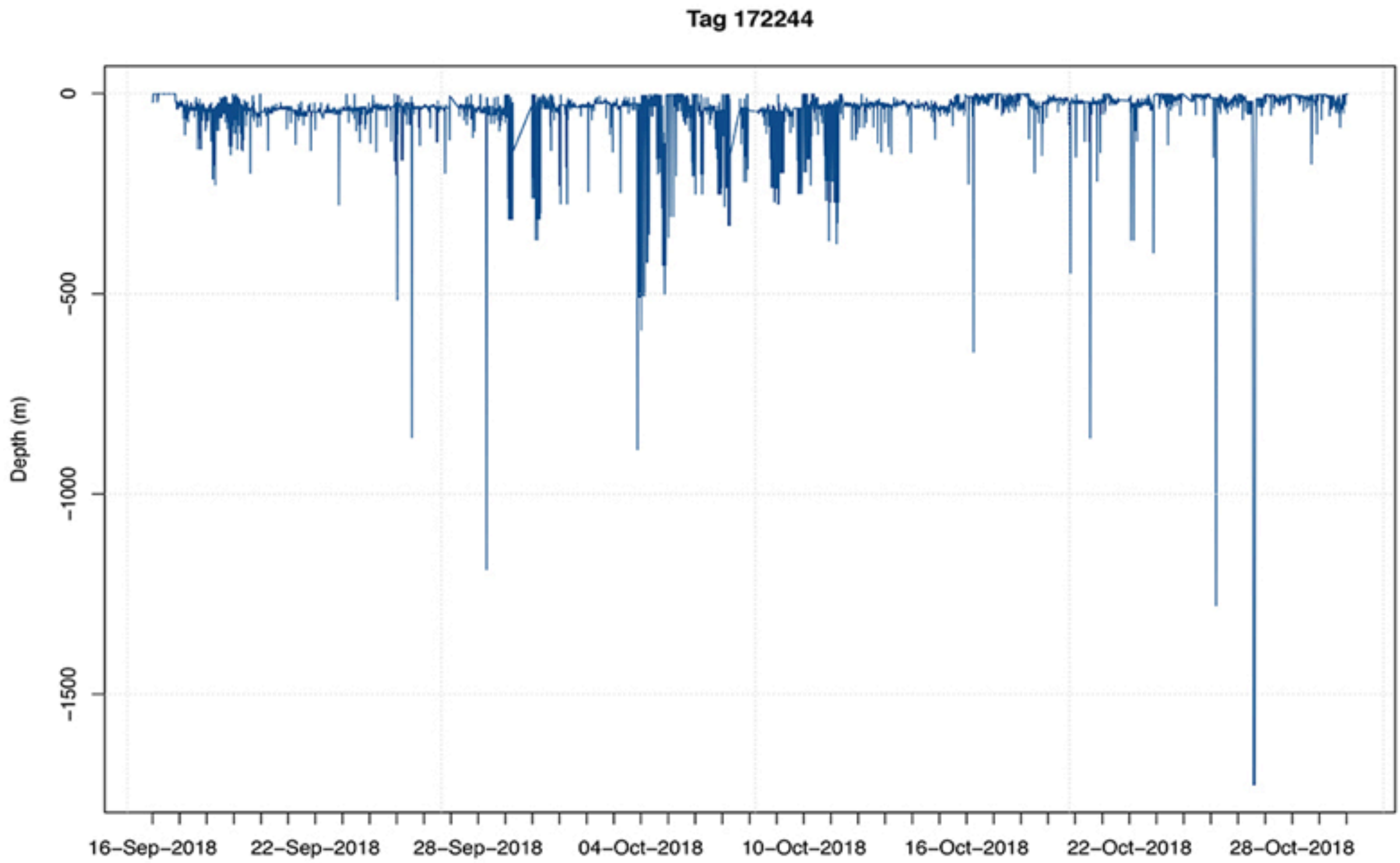

Figure 22: Tag #172244 Premature release, deep dive. Max depth 1839m. Tag deployment 47 days.

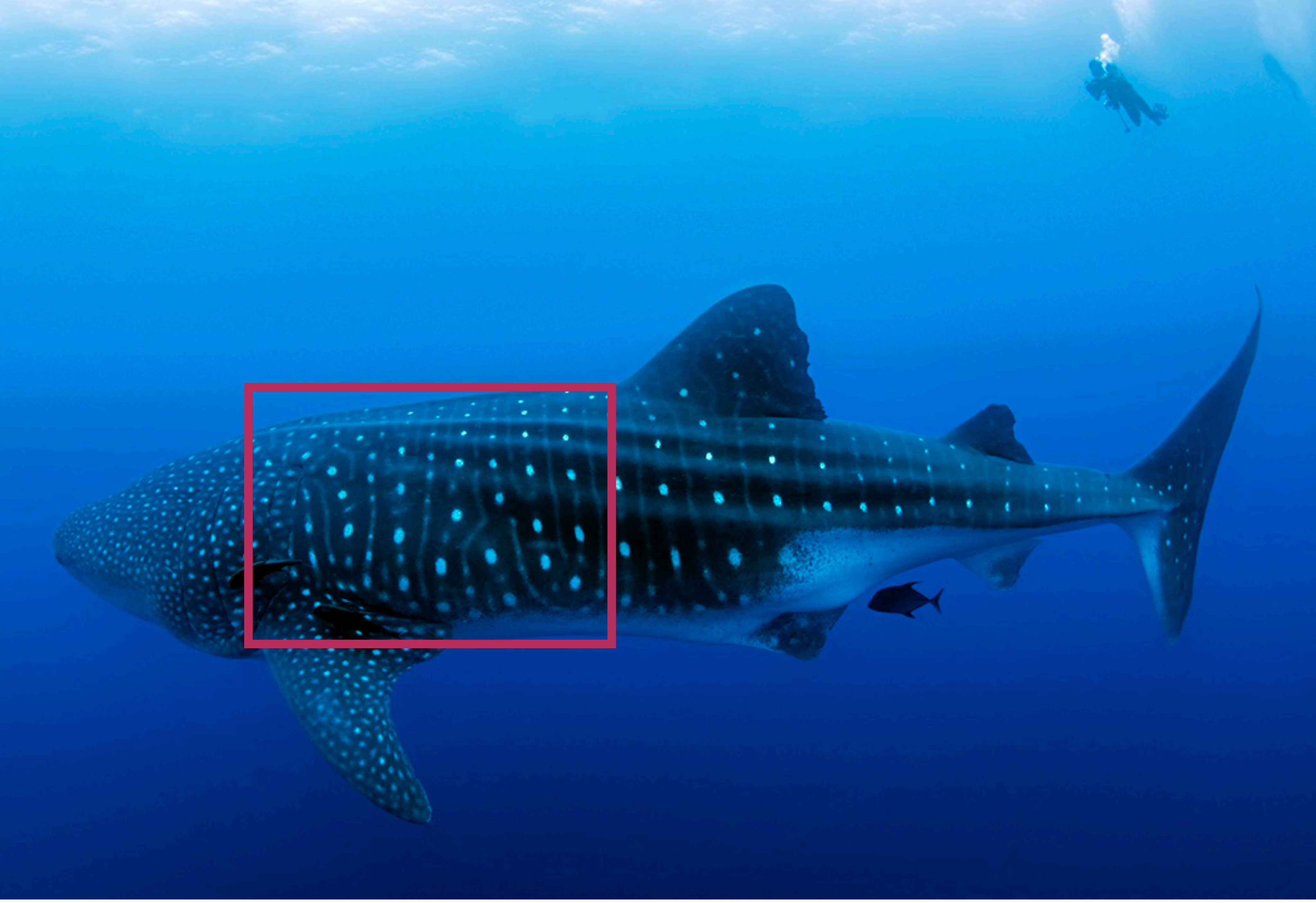

Figure 23: The area shown in red is used for mapping the unique spot patterns for identification. Photo ©Jonathan R. Green 2018

PHOTO IDENTIFICATION

A total of 33 individual whale sharks were photographed and the data submitted to the Wild book for Sharks, https://www.whaleshark.org/ global database, for identification. (See Appendix #1).

As with all previous fieldwork we try to obtain both left and right flank images from the fifth gill slit to the anterior base of the dorsal fin, with priority on the left side. (See Figure 23). The purpose of photo identification is to study site fidelity, how long they stay in the area of Darwin Arch and the frequency with which they return to this area. Also any previous or future sightings give data of the sharks' movements over a period of time. This data may be used to infer behaviour and identify areas of specific need such as feeding, breeding and birthing.

In Galapagos we now have a total of over 550 individual whale sharks identified, as the next generation of dive masters have been actively supporting the program and submitting IDs to Sofia Green. Special thanks to Dive Master and Guide Paolo Tobar who in particular has been consistently submitting images.

Figure 24: Divers taking photo-ID of a whale shark. Photo ©Jonathan R. Green 2017

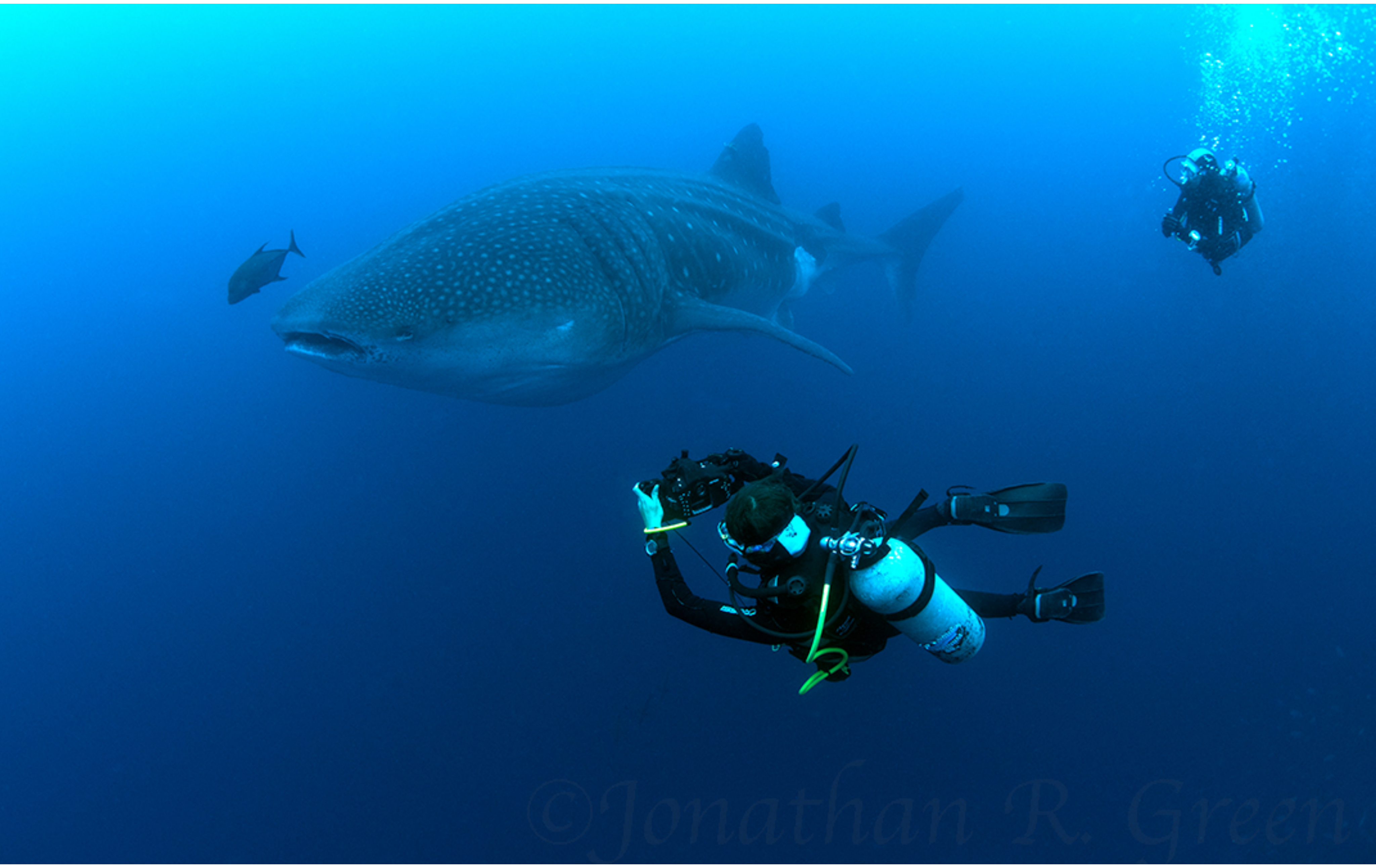

Figure 25: Satellite tag data shows that the sharks are travelling great distances across the oceans. Photo ©Jenny Waack 2019

PRIMARY RESULTS

The tagging method proved extremely efficient and safe both for tagger and the whale shark as the use of a single fin mount for a hybrid double tag ensures each individual shark is enabled to give us the detailed data needed for analysing diving behaviour in association with benthic features, habitat use and temperature.

On our return to port we were able to check on the SPOT tag transmissions on the Argos platform and all 5 tags have transmitted. This is now the second consecutive year that we have had 100% transmission rate from these tags. Furthermore none of the miniPATs on the hybrid fin mount have transmitted so we do not have any premature releases to date. (See Figure 26)

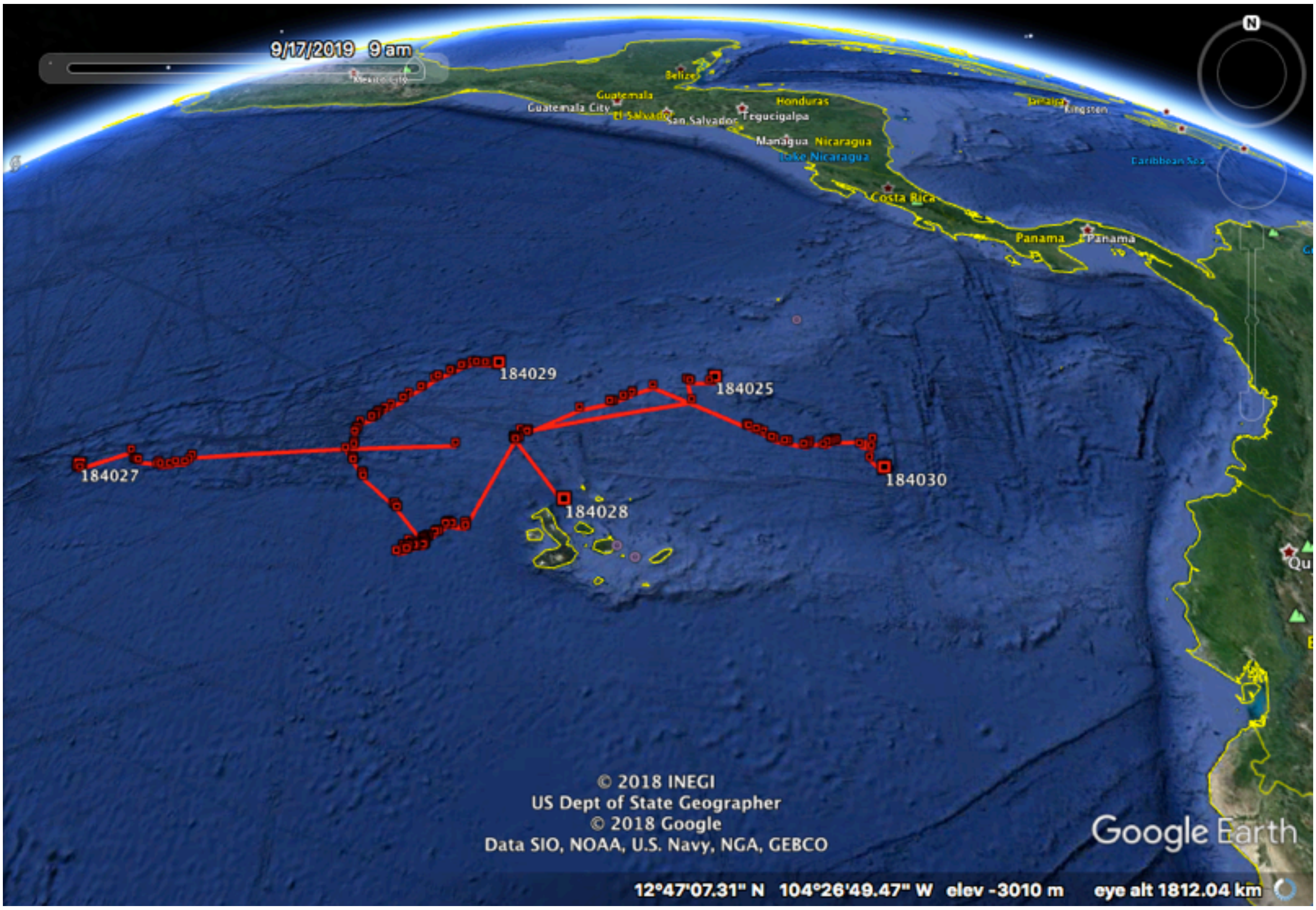

Figure 26: Tracks of the 5 SPOT6 tags since the date of tagging giving regional perspective (see Appendix #1).

Figure 27: The diver next to the shark gives good perspective of overall length and size of the abdomen. Photo: ©Sofia Green 2019

CONCLUSIONS & FURTHER WORK

As we move towards longer term data with the fin mount method we need to investigate tag options that are made to work at depths of up to 5-6000m. The miniPAT data shows premature release as the maximum depth is reached often long before the scheduled time release. In order to be able to study in detail their dive behaviour, it is imperative that we are able to record the data throughout their entire diving range. As none of the hardware currently available has this capability we will be hoping to team up with research and investigation companies that have an interest in this field.

Data sets from both tag types last season show that a double tagging is necessary in order to build up an accurate profile of both horizontal and vertical movements through time. This data can then be used to look at habitat usage and compare their movements to benthic features. By including a 3D accelerometer we then might be able to determine diving techniques, such as gliding, vertical free fall, circular movement and return to surface after deep diving strategies.

The success in blood draw is also leading to fieldwork that will be aimed at studying the baseline health of the population of whale sharks in the Galapagos compared to other populations where this study has been undertaken. We also hope to incorporate this with the Georgia Aquarium team who have carried out this work previously and include a Nano plastics analysis of the blood.

Given the success of the hybrid SPOT / miniPAT fin mount deployment we will continue with this combination until a viable alternative is developed.

With the data we obtained from the blood samples taken last season in September we are now planning to carry out the next field trip at the end of June until mid July. This, with the intention of comparing hormonal levels and diving behaviour early and late season to determine if there are significant differences.

ACKNOWLEDGMENTS & THANKS

Photo: Darwin's Arch, Galapagos ©Jonathan R. Green 2018

Figure 28: Whale shark emerges through a school of fish. Photo: ©Jonathan R. Green 2019

As always a huge thanks to our partners the Galapagos National Park Service for their continued support, collaboration and participation in the field.

Also to all our donors who maintain the faith with our project and progress over the years.

Last but not least once again to Eduardo "Viko" Rosero and the Queen Mabel crew who put in long hours in frequently tough climatic conditions to help us succeed.

Figure 29: Captain „Viko" and the Queen Mabel. Photo: ©Jonathan R. Green 2018

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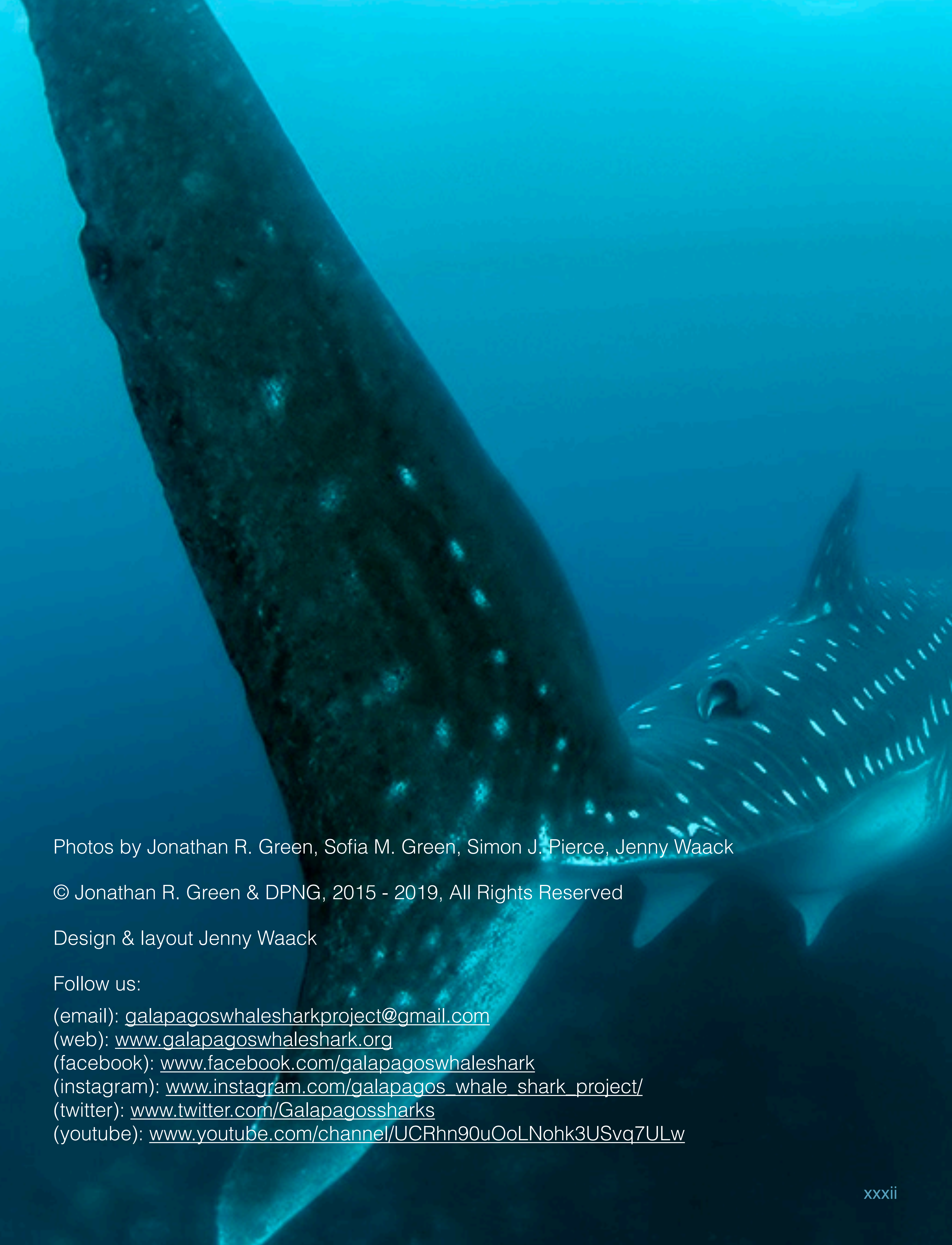
APPENDIX

Photo: Whale Shark dwarfs the Hammerhead sharks at Darwin's Arch ©Jonathan R. Green 2018

# *Rhinodon typus*	Gender	SPOT #	miniPAT #	Deep Tag #	Date # Dive of the day	Photo ID	Blood Samples	Biopsy	Total Length (meters)
1	F	0	0	195395	01/09/2019 # 1	GD 010919-1	0	0	11,5
2	F	0	0	0	01/09/2019 # 1	No photo ID	0	0	12
3	F	184029	184036	0	01/09/2019 # 2	GD 010919-2	0	0	10
4	F	0	0	0	02/09/2019 # 1	GD 020919-1	0	0	0
5	F	0	0	195396	02/09/2019 #1	GD 020919-2	0	0	10,5
6	F	184028	184034	0	02/09/2019 # 3	GD 020919-3	0	0	10
7	F	0	0	0	02/09/2019 # 3	GD 020919-4	0	0	10
8	F	0	0	195397	03/09/2019 # 1	GD 030919-1	0	0	11,5
9	F	0	0	0	03/09/2019 # 1	GD 030919-2	0	0	12
10	F	0	0	0	03/09/2019 # 1	GD 030919-3	0	0	5
11	F	184030	184035	0	03/09/2019 # 2	GD 030919-4	0	0	11
12	M	0	0	0	04/09/2019 # 1 04/09/2019 # 2	No photo ID	0	0	7
13	F	184025	184032	0	04/09/2019 # 1 04/09/2019 # 2	GD 040919-1	0	0	11
14	F	0	0	0	04/09/2019 # 1	GD040919-2	0	0	10
15	F	0	0	195398	04/09/19 #2	GD040919-3	0	0	11
16	F	0	0	0	04/09/2019 #2	GD040919-4	0	0	5
17	F	0	0	0	04/09/19 #3	GD 040919-5	0	0	10

# *Rhinodon typus*	Gender	SPOT #	miniPAT #	Deep Tag #	Date # Dive of the day	Photo ID	Blood Samples	Biopsy	Total Length (meters)
18	F	0	0	0	04/09/19 #3	GD 040919-6	0	0	8
19	F	0	0	0	05/09/19 #1	GD 050919-1	0	0	11
20	F	184027	184037	0	05/09/19 #1	GD 050919-2	0	0	7
21	F	0	0	0	05/09/19 #2	GD 050919-3	0	0	10
22	F	0	0	0	05/09/19 #2	GD 050919-4	0	0	10
					06/09/19 #2				
23	F	0	0	0	05/09/19 #2	GD 050919-5	0	0	10
24	F	0	0	0	05/09/19 #2	GD 050919-6	0	0	10
25	F	0	0	0	06/09/19 #1	GD 060919-1	0	0	10
					06/09/19 #3				
					07/09/19 # 1				
					07/09/19 # 2		1		
26	F	0	0	0	06/09/19 #1	GD 060919-2	0	0	12
27	F	0	0	0	06/09/19 #1	GD 060919-3	1	0	11
					06/09/19 #2				
					06/09/19 #3				
28	F	0	0	0	06/09/19 #2	GD 060919-4	0	0	11

# *Rhinodon typus*	Gender	SPOT #	miniPAT #	Deep Tag #	Date # Dive of the day	Photo ID	Blood Samples	Biopsy	Total Length (meters)
29	F	0	0	0	07/09/19 # 1	GD 070919-1	0	0	12
30	F	0	0	0	07/09/19 # 1	GD 070919-2	1	0	10
31	F	0	0	0	07/09/19 # 2	GD 070919-3	0	0	4
32	F	0	0	0	07/09/19 # 3	GD 070919-4	0	0	10
33	F	0	0	0	08/09/19 # 2	GD 080919-1	1	0	11
34	F	0	0	0	09/09/19 # 1	GD 090919-1	0	0	10
35	F	0	0	0	09/09/19 # 2	GD 090919-2	0	0	12
TOTAL	1M/34F	5	5	4		33	4	0	10,0



Photos by Jonathan R. Green, Sofia M. Green, Simon J. Pierce, Jenny Waack

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