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Marine and Freshwater Research

Supplementary Material

An integrated approach for assessing the survival of discarded sandbar sharks, *Carcharhinus plumbeus*, captured in scientific longlines

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Methods

Adrenocorticotropic hormone (ACTH)

The percentage intra-assay coefficient of variation (CV) was obtained as the average of the individual CVs for each duplicate sample. Individual CVs were calculated as the standard deviation of each duplicate sample, divided by the duplicate mean and multiplied by 100. The intra-assay coefficient of variation for the assay was 7.15%. The accuracy of the assay expressed as the percentage error between the assay-determined value for the assay standards and the assigned value for those standards was 1.20% (±1.62 s.e.). The detection limit of the assay (80% B/B_0) was 72 pg mL^{-1}. No cross-reactivity between fish ACTH and other plasma components has been reported by the manufacturer, however, some cross-reactivity may still exist.

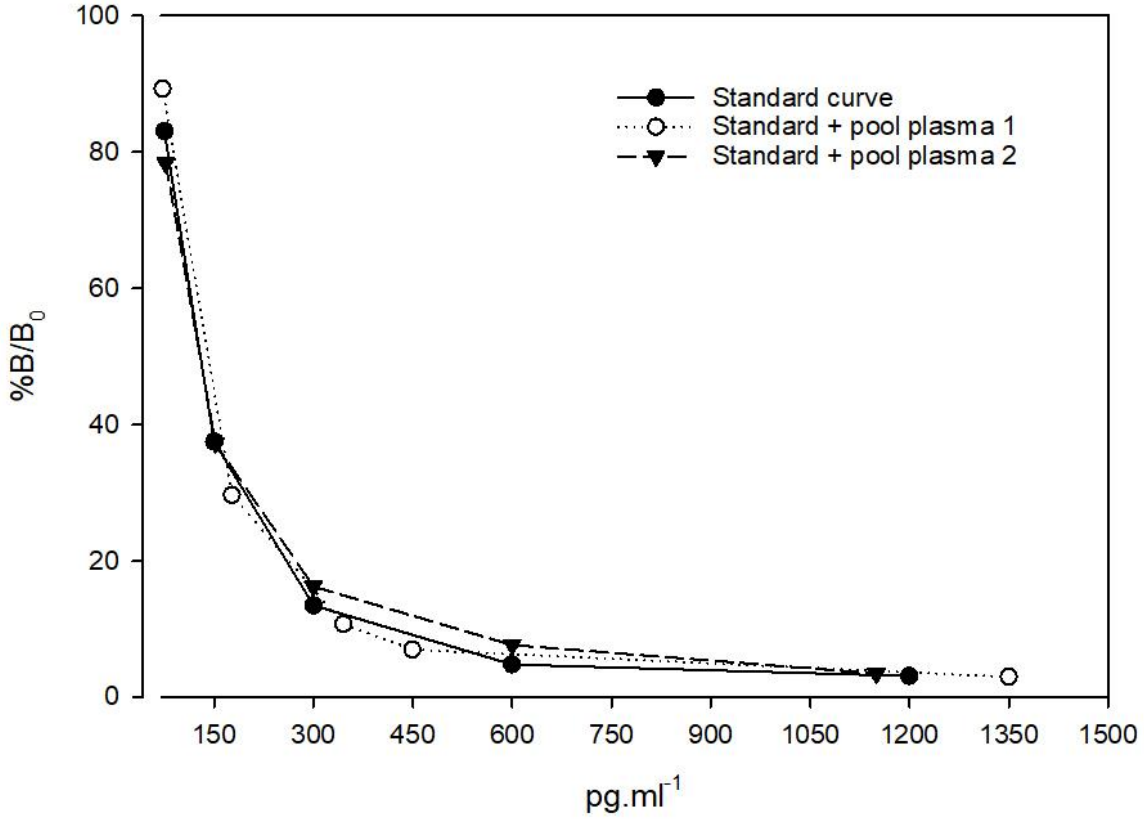

Figure S1. Percentage bound (B/B_0) of the ACTH standard curve and two new standard curves spiked with two different pools of plasma samples. Good parallelism is distinguished between the three curves.

Glucocorticosteroids

Plasma samples (200 µL) were added to a glass tube and diluted 1:5 with diethyl ether, vortexed for 30 s, and frozen at –20 °C for 1 h to separate the phases. After 1 h, the top diethyl ether layer was transferred to a clean glass test tube. This step was repeated twice to maximise the recovery of the steroid from the sample. The diethyl ether phase was evaporated by nitrogen and reconstituted with 100 µL of ELISA buffer (fractions were twice concentrated due to the general low values of GCs reported in elasmobranchs). For the assay, 50 µL of each reconstituted sample was assayed in duplicate in a single kit. The absorbance of each well was measured with a microplate reader at a wavelength of 410 nm and converted to picograms per millilitre by a four parameters logistic curve and linearised using a logit transformation. The recovery of spiked GCs from sandbar sharks' plasma was 87%. Validation of the assay kit was done by comparing serial dilution of plasma samples with the assay standards. The standard curve was made following the manufacturer's instructions consisting of eight points ranging from 5000 to 8.2 pg mL^{-1} and the serial dilutions were made by preparing two pools of 200-µL plasma samples, each constructed by combining 25 µL from eight individuals, and diluted 1:2.5, 1:5, 1:7.5, 1:10, 1:12.5, 1:15, 1:17.5, 1:20 with Elisa buffer (Figure S2). The intra-assay CV was determined as above (2.2.1) and was 9%. The accuracy of the assay was 1.09 % (±1.54 s.e.) and the detection limit was 30 pg mL^{-1}.

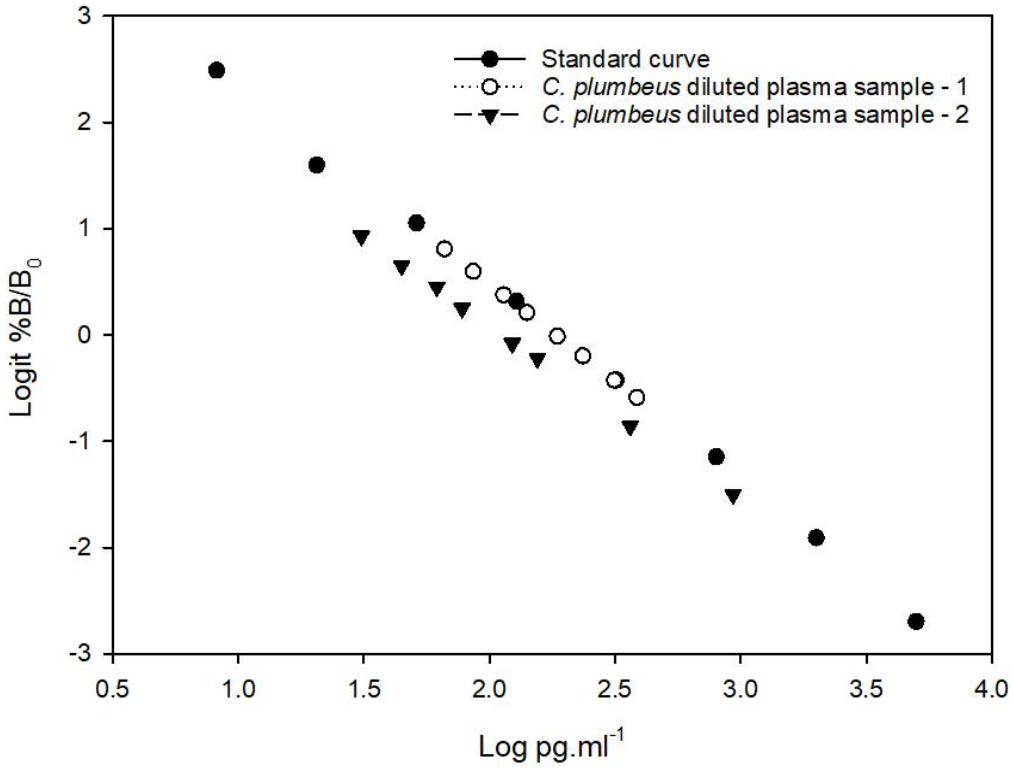

Figure S2: Percentage bound (B/B_0) of the total GCs standard curve and two different diluted 1:2.5 pools of plasma samples. Good parallelism is distinguished between the three curves.

Results

Table S1. Morphometrics, blood chemistry and time on hook (TOH) from five premature released tags in the survivorship pop-up transmitting tag (sPAT) day and night depth (m) analysis (Figure 6).

Tag number	FL (cm)	Sex	Hook time (min)	Release condition	ACTH (pg mL^{-1})	GCs (pg mL^{-1})	Whole-blood lactate (mmol L^{-1})
200441	127	F	189	1	105.01	350.29	22.9
200444	140	M	222	1	62.64	406.31	≥ 25
200447	152	F	103	1	71.87	374.2	17.5
200450	134	F	125	2	83.02	743.01	≥ 25
200455	124	M	184	1	66.2	593.08	≥ 25

High values were given a value of ≥25.

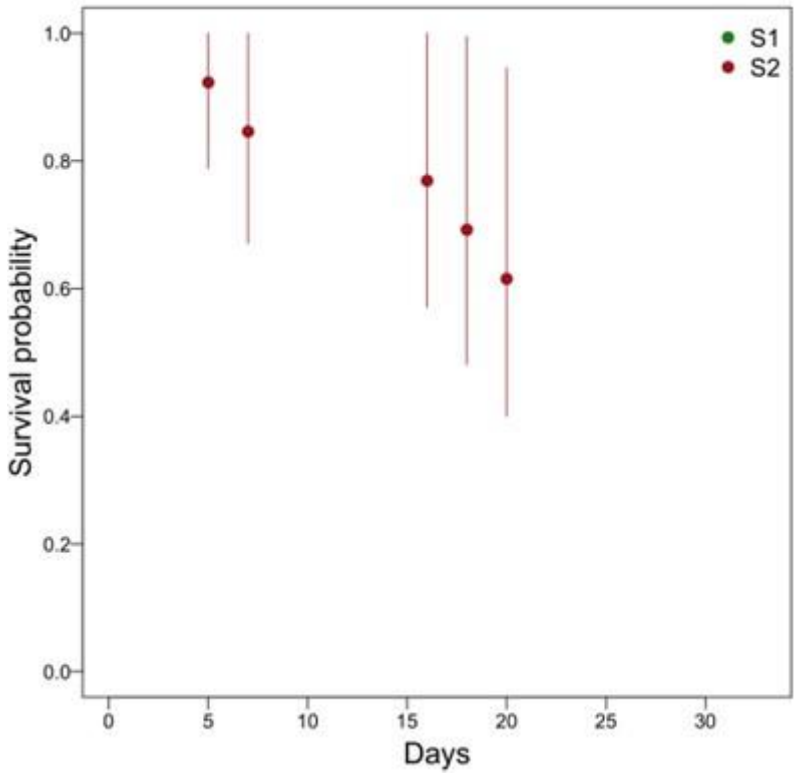

Fig S3. Kaplan–Meier survival plot illustrating median survival probability over time (days) for 13 sandbar sharks tagged and released with sPAT tags. Where S1 is categorised as death (sinker) and S2 is categorised as death (premature tag release). The vertical lines are the 95 % CI.

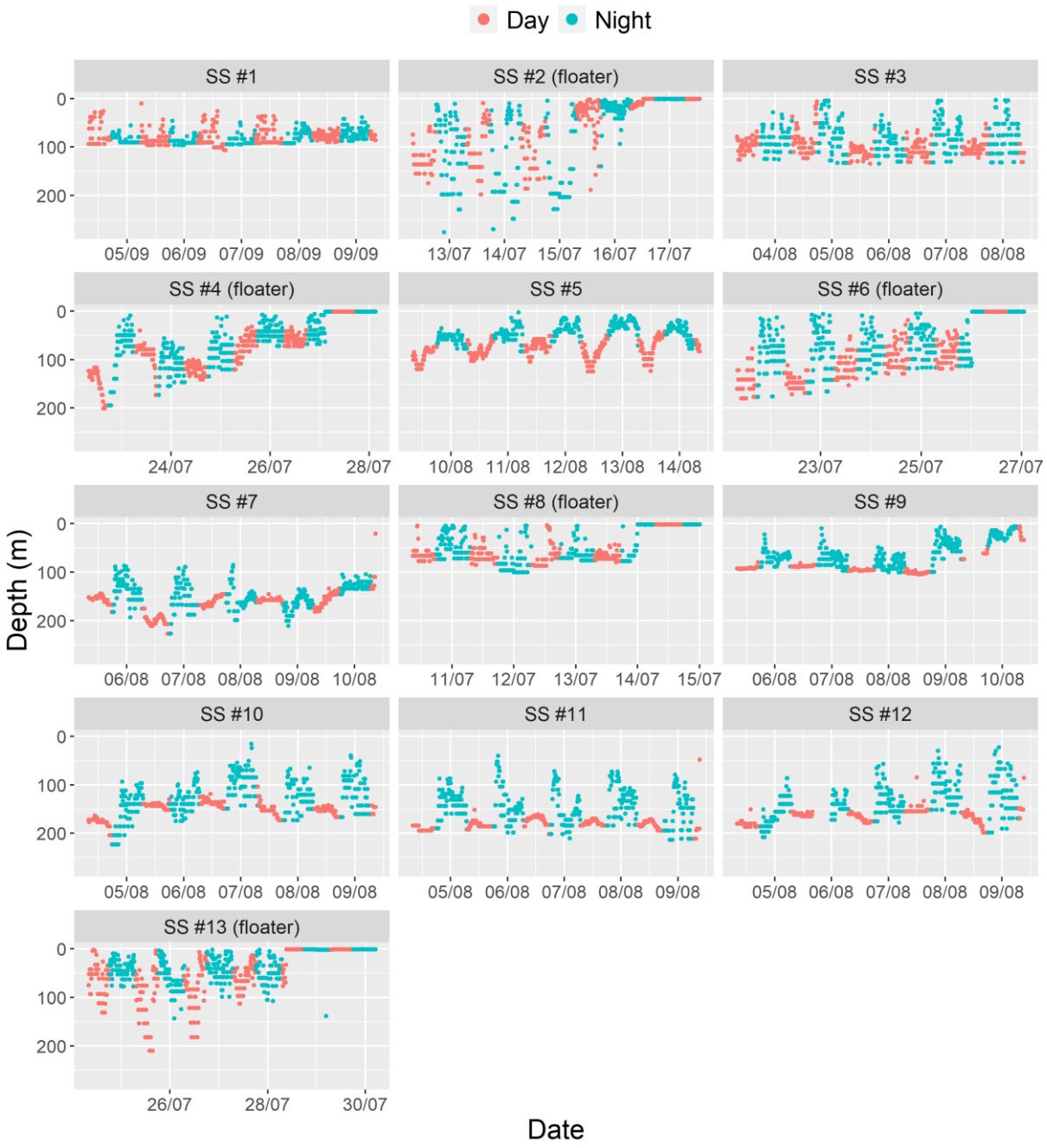

Fig S4. Temporal patterns in depth for the last five days of deployment for satellite-tagged sharks.

Depth profiles and vertical behaviour

TOH ranged 0:56 to 4:07 hours but did not result in mortality. These individuals showed a variety of depth profiles during the full tag deployment, ranging from the surface (0 m) to 307 m. The depth record of 1200 m (tag number SS #11) is likely to be a tag malfunction. Some samples showed large vertical behaviour, such as 200433 ranging from 10 m (depth of tag activation) to 106.9 m (mean: 80.56 m, ±0.14) and SS #10 ranging from 10 m to 223 m (mean: 137.52 m, ±0.21) (Fig. S5). There was no significant difference in mean range in depth among sharks (d.f. = 1, P = 0.295).

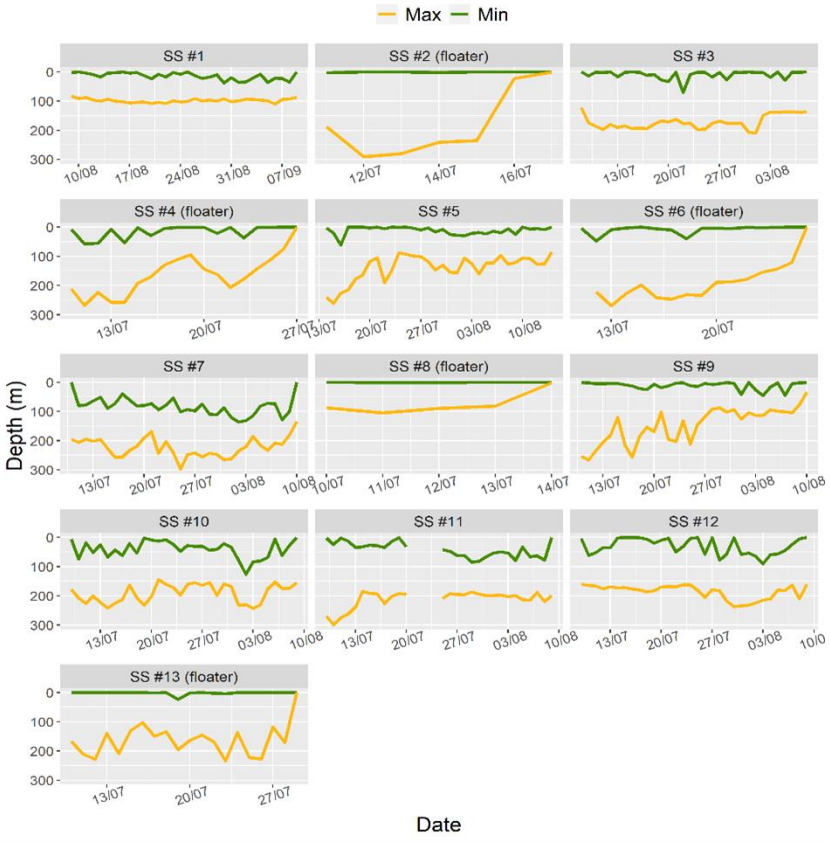

Fig S5. Temporal patterns in depth for the full 30-day deployment.