Supporting Material for:

Acoustic telemetry and network analysis reveal space-use of multiple reef predators and enhance MPA design

by

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Supplementary Methods

Study site

D'Arros Island (S 05°24', E 53°17') is a small sand cay (~1.6 km^2) situated on a patch reef (~3.6 km^2) in the Amirantes chain of islands of the Republic of Seychelles, western Indian Ocean (Fig. S1). Just over one kilometre east of D'Arros, separated by a channel of 60–70 m depth, is St Joseph Atoll (~22 km^2; S 05°25', E 53°20'). St Joseph Atoll has 16 small islands atop an uninterrupted reef flat that encloses a shallow (3–9 m), access-restricted lagoon of ~5 km^2. The flats surrounding St Joseph lagoon are largely exposed at low tide, causing temporary isolation of the lagoon from the outer reef. Up to 2 m of water covers the flats at high tide. The lagoon is predominantly sand bottomed with numerous large coral outcrops that rise to the surface, with patches of seagrass *Thallasodendron sp.* along the flats and some mangroves *Rhyzophora mucronata* fringing the islands [1]. The reefs surrounding D'Arros and St Joseph have reasonable coral cover and slope steeply from near the surface to 20–25 m depth. These reefs give way to the Amirantes plateau, which varies between 15–60 m depth and stretches 155 km from north to south. The plateau is predominantly covered by patches of seagrass and sandy reef rubble, with occasional patches of high coral cover. The plateau is surrounded by very deep water, with the edges descending from 30–60 m to over 1,000 m deep within a few hundred metres.

Animal telemetry

Between August 2012 and March 2015 a total of 116 sharks of five different species (blacktip reef *Carcharhinus melanopterus*, sicklefin lemon *Negaprion acutidens*, grey reef *Carcharhinus amblyrhynchos*, tawny nurse *Nebrius ferrugineus*, silvertip shark *Carcharhinus albimarginatus*) and 25 hawksbill turtles *Eretmochelys imbricata* were tagged with acoustic transmitters (either V13 180 s nominal delay or V16 120 s nominal delay, Vemco Ltd, Bedford, Canada). Sharks were caught either on research longlines, hand lines, or by hand whilst using SCUBA, and an acoustic transmitter was surgically implanted into each individual shark's abdominal cavity while it was in tonic immobility alongside the research vessel. The small incision was closed with three sutures (Ethibond Excel 4 x 75 cm non-absorbable coated, Ethicon Inc., Somerville, USA). Measurements were taken for each shark (precaudal length, fork length, total length), sex recorded, tissue sample for genetic analysis, and a number ID with contact details was attached for recapture purposes. Turtles were hand-captured using standard methods [2], and had the acoustic transmitter attached using a cable tie and epoxy resin to the 2nd rear marginal scute (right). Sharks and turtles were tracked using an array of 88 acoustic receivers (VR2W, Vemco Ltd) (Fig. S1). All field work was approved by, and conducted with the knowledge of, the Ministry of Environment, Energy, and Climate Change, Seychelles. The animal handling and tagging methods were performed in accordance with the approved guidelines of the University of Plymouth, UK.

The array was installed in stages for logistical reasons. Initially 50 receivers were installed around D'Arros and St Joseph between August and November 2012, 25 in the immediate vicinity of the islands covering lagoon and coastal reef habitats, and another 25 spread across the surrounding plateau up to 15 km away, covering plateau and drop-off habitats. In October 2013 a further 10 receivers were added so there was at least one at each of the other islands across the whole Amirantes plateau. In November 2013, 10 more receivers were installed along the reef flats of D'Arros and St Joseph to monitor their use during the high tide and in August 2014 a further 18 receivers. Given the staggered deployment of the array over time, only a subset of the detection records were used for analysis in the present study to avoid biases caused by the developing array design. Firstly, only receivers that had been deployed for over two years were included in this study, reducing the working array for analysis to all receivers installed up until November 2013 (n = 70 in total; 35 coastal to D'Arros and St Joseph and 35 across the plateau). Three of these receivers experienced failure causing gaps in their detections records and were omitted from subsequent analysis. Secondly, track data before November 2013 were discarded so that we only considered track data when all 67 receivers were active, reducing the effective sample size to 110 animals (86 sharks and 24 turtles).

All downloaded detections were imported into a Microsoft Access (Microsoft Corporation, Redmond, USA) database, which assigned transmitter detections (pings) to the appropriate sharks and receiver locations, and filtered out any pings that did not match an active tag or receiver (i.e. false positives). Receiver clock-drift time corrections were also made during the import process, being calculated from the difference between the receiver and PC clock at the time of download, assuming linear drift. Tags were detected within ~150 m of the receiver, as determined by range testing: mean range 165 m ± 33 (SD).

Network analysis

Network analysis was used to determine both where sharks and turtles spent more time and how they moved through the array [3]. Each receiver location was treated as a node within the network, with node strength weighted according to the number of detections at that location. Any pair of subsequent pings that occurred between different nodes was treated as a connection between those nodes, with connection strength weighted by the number of times that specific pairing occurred. In this way matrices were constructed that detailed the connections between receivers and the detections at each receiver, allowing networks to be constructed and graphed to visualise movements and occupancy throughout the array for each species.

Due to the different ping frequencies of the V13 and V16 tags (180 s vs. 120 s nominal delays), the node and connection strengths of V13 networks were increased by 50% to account for the decreased probably of detection compared to the V16 networks. All network maps were produced using ArcGIS (ESRI Inc., CA, USA), with bathymetry data obtained from the U.S. Department of Commerce, National Oceanic and Atmospheric Administration (NOAA): 2-minute Gridded Global Relief Data (ETOPO2v2). The satellite image of D'Arros and St Joseph was acquired from LAND INFO Worldwide Mapping, LLC, and includes material Copyright © DigitalGlobe (Longmont, Colorado).

Several network metrics were used to describe each network: occupancy (or node strength) was computed from the number of detections occurring at each node and provided a measure of how much time individuals spent at each receiver location. Connectivity (or node centrality) is calculated from the total number of connections made to that node, i.e. the proportion of other nodes to which there is a connection. Transit (or node betweenness) represents the total number of paths to pass through that node and is computed by counting pings occurring at a receiver where the prior and subsequent pings for that individual occur at a different receiver. Transit therefore measures the extent to which a node is part of a corridor of movement as opposed to an area of occupancy. Node density is the proportion of total available nodes actually used in the network, measuring the extent of the array occupied, and edge density is the proportion of total available connections actually formed within the network, providing a measure of mobility within the network.

To test whether the observed movement networks were different from random, random networks were generated and their node metrics were tested against those of the real tracks using Wilcoxon matched-pairs signed rank tests (SigmaPlot, Systat Software, San Jose, CA). For node and edge density, the values produced by the random networks were tested against the real network values as the population mean in one-sample signed rank tests. Random networks were constructed as follows: for a given set of detections (i.e. for a single animal), the node and connection matrix was first constructed as normal to provide the observed data. For each randomisation, the first ping at the first receiver was kept, and then a swim distance was calculated based on the time between detections and a 1 m s^{-1} swim speed. Receivers were then selected at random until two were found within range of the swim distance. The closer of the two was then selected as the next receiver in the random track. If no receiver was found in range after 100 random selections then no move was deemed to occur and the current receiver was assigned (i.e. the animal was deemed not to have moved). This was repeated for the duration of the track, producing a random walk through the array with steps constrained by the observed detection intervals. This was repeated 100 times for each track, to provide mean random network metrics to test against the observed real track metrics.

Each receiver location was designated a habitat type: lagoon (habitat within St Joseph Atoll, including the flats), coastal reef (sloped reefs bordering islands), plateau (flat-bottomed areas of patchy reef rubble and seagrass beds) or drop-off (the edge of the plateau, before it drops to hundreds of metres). To reveal differences in space use between habitats for each species, node metrics were grouped according to habitat type and had their values compared to those of the same habitat type in the random networks. This was achieved by calculating a randomisation index:

$$Rnd_i = \frac{O_m - R_m}{R_m} \times 100$$

Where O_m is the observed and R_m the random metric. Mean values were then plotted for each node metric in each habitat type, according to species. For each individual a residency index was calculated, representing the percentage of days during its track that it was detected within the array:

$$Res_i = \frac{D_d}{D_{al}} \times 100$$

Where D_d is days detected and D_{al} is days at liberty.

Grid occupancy analysis

The data were further used to evaluate the potential efficacy of two MPA designs. Each design had its boundary radius restricted to 1 km as this matches the current best in Seychelles for the UNESCO World Heritage Site of Aldabra Atoll [4]. The first MPA model, the null MPA, matches the Aldabra designation, with the boundary being formed by 1 km from the beach at MHW. The second proposed MPA keeps the same boundary radius of 1 km, but instead measures it from the edge of the reef flat at the lowest astronomical tide. Due to the extensive reef flats at D'Arros and St Joseph, that are exposed at low tide and can exceed 1 km width, this forces the boundary to include all of the lagoon and coastal reefs, some of which remain exposed in the null MPA. The smaller null MPA encompassed an area of approximately 42.3 km^2, while the larger proposed MPA covers approximately 64.9 km^2 (~50% increase in area).

The potential efficacy of both MPAs was determined using a grid occupancy analysis. In order to account for bias that may stem from the uneven distribution of acoustic receivers, each track was interpolated across all gaps shorter than 24 hrs (longer gaps were ignored to limit erroneous interpolation). The array was then divided into 0.5 km grid squares, and the number of days each individual occurred within each grid square was summed. Using the boundaries of the null and proposed MPAs, it was then possible to sum the number of days each individual would have spent within the boundaries of each, based on which grid cells were in which MPA. The number of days inside/outside was then used to calculate the proportion of each individual's recorded array occupancy that was inside each MPA. Proportion of time inside each MPA was then plotted using box plots, to see how much time each species spent within each MPA. The significance of differences in time spent inside each MPA was tested for each species using Wilcoxon matched-pairs signed rank tests, with Monte Carlo p values calculated after 10,000 permutations (IBM SPSS Statistics, IBM Corp. USA).

Supplementary Results

Over the course of the study (August 2012 to November 2015) 141 acoustic transmitters were deployed on five different shark species and one turtle species, providing a total of 75,911 tracking days. However, to accommodate the staggered deployment of acoustic receivers (see Methods for details), the study period was restricted to November 2013 to November 2015 and the effective sample to 110 tagged individuals: blacktip reef (*n* = 25), grey reef (*n* = 22), sicklefin lemon (*n* = 20), tawny nurse (*n* = 6), silvertip sharks (*n* = 13), and hawksbill turtle (*n* = 24), providing over 50,477 tracking days (Table 1). A range of juveniles and adults was tagged for each species, apart from silvertip sharks and hawksbill turtles, all of which were juvenile. Mean track duration across all sharks (*n* = 86) was 484 days ± 265 (SD), with 64.0% of tracks lasting more than a year. Mean turtle track (*n* = 24) duration was 368 days ± 210 (SD), with 62.5% of tracks lasting more than a year. All shark species showed a bias towards females amongst tagged individuals, with grey reef sharks displaying the largest disparity of six females for every male tagged. As all turtles were juvenile, sex determination was not undertaken as it was not relevant to the study's objective and can only be achieved through costly and potentially invasive procedures (laparoscopy and blood sampling).

Species-specific habitat use

All metrics of the real networks of all species were statistically different from those generated by the random networks (Tables S1 and S2). Blacktip reef sharks displayed very restricted movements, with 99.8% of all detections occurring within the confines of St Joseph Atoll

(Fig. 2), residency that is reflected by their moderate node density (0.52). Blacktip reef sharks displayed very high occupancy of lagoon habitats compared to random networks (Fig. 3). Even within the atoll, blacktip reef shark movements were largely focused on the eastern end of the lagoon, consistent with their very low edge density of 0.09, compared to the mean random network edge density of 0.72. There was very limited movement between D'Arros and St Joseph across the deep channel, with little time spent on the coastal reefs. When around D'Arros, blacktip reef sharks appeared to spend the majority of their time on the more expansive reef flat to the west. Some blacktip reef sharks were only detected infrequently by the subset of receivers used in this study. However, evidence from newer receivers not included in the present analysis (see Methods) reveals that these individuals spent the majority of their time in pools along the atoll flats. These individuals were therefore within lagoon habitat but outside the range of this study's acoustic monitoring array (Fig. S1).

Broadly, the sicklefin lemon sharks showed a similar pattern to the blacktip reef sharks, with 98.8% of all detections occurring within the atoll (Fig. 2). Moreover, comparison of node metrics by habitat type revealed elevated occupancy of atoll habitats in real networks compared to random networks, with other habitats being used less frequently (Fig. S2). However, the sicklefin lemon shark network shows greater movement throughout the atoll, particularly around the deep lagoon perimeter where it borders the flats. Lemon shark movements also connect more frequently to the coastal reefs outside the atoll and, most notably, several individuals were recorded making wider movements across the Amirantes plateau, including to Desnoeufs Island 94 km south of D'Arros. This is reflected in their higher node density of 0.84, along with a higher edge density of 0.15, revealing much greater use of the array. One tagged lemon shark was also caught by fishermen at Marie-Louise 80 km south of D'Arros, while another was caught at Bird Island, 300 km away across deep water (>1,000 m). Two lemon sharks were also recorded by a receiver at Marie-Louise, but this location was one of the three receivers excluded from the present analysis due to incomplete temporal coverage. All lemon sharks recorded moving away from the islands and across the plateau (n = 9) were ≥ 177 cm total length, whereas those smaller remained exclusively within the confines of the atoll and its coastal reefs.

Despite similar node and edge densities to blacktip reef sharks (0.45 and 0.04 respectively), grey reef shark movements differed significantly to blacktip reef and sicklefin lemon sharks in that no detections occurred within the atoll (Fig. 2). Instead, grey reef sharks were largely recorded along the coastal reefs (62.1% of detections), with 30.4% of detections also occurring along the drop-off. This is emphasised by the comparison of node metrics by habitat type between real and random networks, which show elevated occupancy of drop-off and coastal reef habitats in real versus random sharks (Fig. 3). Coastal reef areas involved more patrolling movements, indicated by high transit values for those receivers, whereas drop-off use was more focused and had low transit values. Grey reef movements also produced fragmented networks, with some tagged nearer the drop-off not being recorded on the coastal reefs of D'Arros and *vice versa*. One tagged grey reef shark is known to have been caught by fishermen on the reefs of D'Arros.

Although fewer individuals were tracked, the tawny nurse sharks displayed a range of movements similar to the lemon sharks (Fig. 2), reflected by similar node and edge densities (0.76 and 0.12 respectively). The majority of nurse shark detections (70.0%) occurred within the atoll, with regular movement throughout. Almost all (98.1%) of nurse shark detections within the lagoon were from individuals <200 cm (n = 3), whereas 84.0% of all nurse shark detections outside the lagoon were from individuals >200 cm (n = 3). These larger nurse sharks frequently circumnavigated D'Arros and travelled more widely across the plateau, particularly spending time at a sandy patch several kilometres south of the islands. Chance encounters during underwater visual surveys have also revealed large aggregations (50+ individuals) of adult nurse sharks of both sexes along both the eastern and western drop-offs of the Amirantes. The high use of the atoll is apparent in the comparison between real and random habitat use, where tawny nurse sharks occupied the lagoon more often than random sharks, but also the disparity for other habitats was smaller compared to other species (Fig. S2).

Silvertip sharks showed the most restricted movements (node density 0.13, edge density 0.01), producing fragmented networks that almost exclusively associate with the drop-off (96.5% of all silvertip detections were along the drop-offs (Fig. 2)). This is again reflected in the real vs. random network comparison, which showed that real silvertip sharks occupied drop-off habitats much more than random sharks, even transiting along the drop-offs more than random sharks did (Fig. S2), revealing significant patrolling behaviour. All tagged silvertip sharks were small juveniles, one of which still had a healing umbilical scar (this shark was 78 cm total length). Four of the 19 tagged silvertip sharks are known to have been caught by fishermen at their original tagging location, which is reflected by their low mean time at liberty (Table 1).

Similar to blacktip reef sharks, hawksbill turtles also displayed movements largely restricted to the atoll, with 99.0% of all detections occurring in lagoon habitats (Fig. 2). Hawksbill movement was more focused, however, with comparatively few node (receiver) connections made (edge density was only 0.03, node density 0.46, more similar to grey reef sharks). Hawksbill turtles also displayed very high occupancy of lagoon habitats compared to random networks (Fig. 3). Individuals predominantly remained very close to where they were tagged, with very restricted dispersal between the islands and onto the plateau.

Apart from silvertip sharks along the drop-offs, all real networks displayed lower connectivity in all habitats than random networks for all species, suggesting that all tracked individuals displayed more directed movement between nodes than their random counterparts (Fig. S2). This is also consistent with the universally low edge densities for all species, which are significantly lower than their random counterparts (Table S2). The large standard error bars on positive results in Fig. S2 reveal large variation even within habitat type, showing highly focused use of particular areas within a habitat, e.g. the eastern lagoon for blacktip reef sharks, and patches of high coral cover near the drop-off for grey reef sharks.

MPA Use

Grid occupancy revealed that, overall, the proposed, larger MPA increased coverage of predator movements by 33.8% ±150.3 (SD), although there was considerable variation between species. Analysis revealed that 89.9% of the blacktip reef shark tracks occurred within the boundaries of the null (smaller) MPA, compared to 98.7% occurring within the proposed (larger) MPA (Fig. 4; Table S3). Lemon sharks received a similar increase in coverage from the larger MPA, with 83.5% of recorded tracks occurring within the null MPA versus 96.5% for the proposed MPA (Fig. 4; Table S3). Larger lemon sharks spent more time outside both MPAs than smaller individuals, attributable to their wider movements.

Grey reef sharks overall received very poor coverage from both MPAs, but still received a significant increase in coverage from the larger MPA (26.6% of time in the smaller versus

32.8% inside the larger; Fig. 4; Table S3). Predominantly larger individuals along drop-offs receive no benefit. Smaller individuals receive high coverage from the larger MPA but very little from the smaller – attributable to their frequent movements along the northern coastal reefs (Fig. 1), which are barely covered by the smaller MPA. This drives the apparent large increase in MPA coverage for grey reef sharks evident in Fig. 4 (although the median remains low): two of the smallest grey reef sharks (79 cm and 99 cm) had their coverage more than double from ~47% to ~98%.

Nurse sharks also receive a significant increase in coverage from the small MPA to the larger MPA (from 63.7% to 82.9%; Table S3), but larger individuals still frequently travel outside across the plateau. Silvertip sharks spend very little time in either MPA (2.7% and 4.0%), with no significant difference between the two (Table S3), as movements are largely focused along the offshore drop-offs (Fig. 2). Hawksbill turtles received similar coverage from the smaller MPA (84.9%) to blacktip reef sharks, and had significantly higher coverage from the larger MPA (99.1%, Fig. 4; Table S3).

MPA management

An early form of the habitat use and MPA results presented here were communicated to the Ministry of Environment, Energy and Climate Change, Seychelles, in order to demonstrate the importance of the habitat provided by D'Arros and St Joseph, and to indicate the likely effectiveness of the larger MPA for protecting sharks. The results in part contributed to the Seychelles government formally adopting the larger MPA and declaring D'Arros and St Joseph a Special Reserve (International Union for the Conservation of Nature, IUCN, Category 1a) with a no-take zone extending 1 km from the low tide mark, effective from 14/07/2014 [5]. An implementation plan was also agreed where the Save Our Seas Foundation would also provide facilities (e.g. a patrol boat) to help enforce the protection. In response to this management outcome at D'Arros and St Joseph, there has also been a proposal by the Ministry of Environment, Energy and Climate Change to extend the 400 m MPA of Aride Island on the Mahe plateau to 1 km.

Figure S1: Distribution of acoustic receivers (*n* = 67) around D'Arros and St Joseph (a), the surrounding plateau (b) and across the Amirantes (c). Receiver locations marked with Θ. Maps created in ArcGIS, using satellite imagery from LAND INFO Worldwide Mapping and ETOPO2v2 bathymetry data.

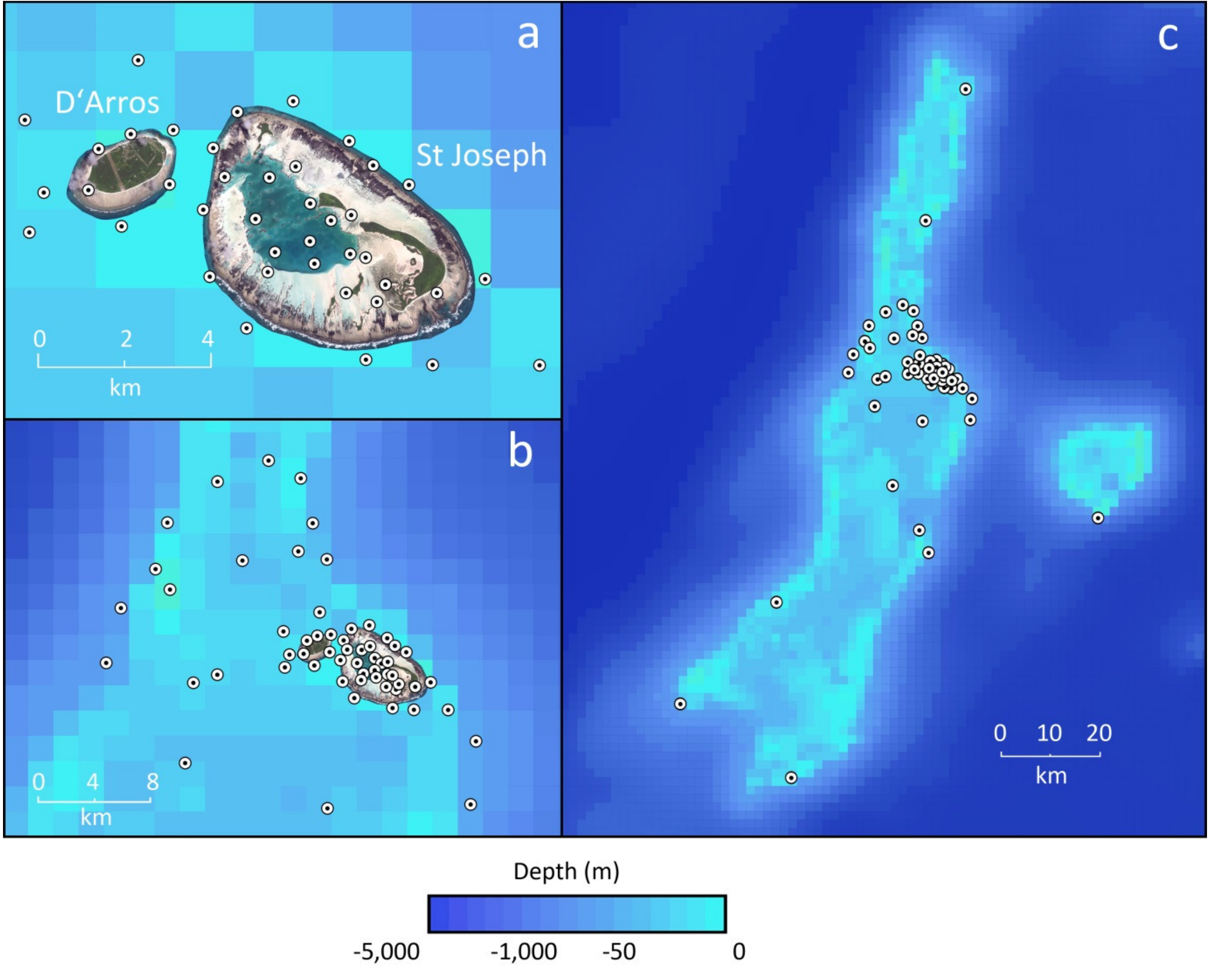

Figure S2: Charts showing, for each species, the mean percentage difference between the actual node metric and those from the randomly generated networks (n = 100 per species), with nodes grouped by habitat type. BT = blacktip reef, LM = lemon, GR = grey reef, TN = tawny nurse, ST = silvertip, HB = hawksbill. Positive deviations denote where actual metric values were higher for that habitat than random, and vice versa. Please note the different scales on the *y*-axis. Error bars represent the standard error of the mean.

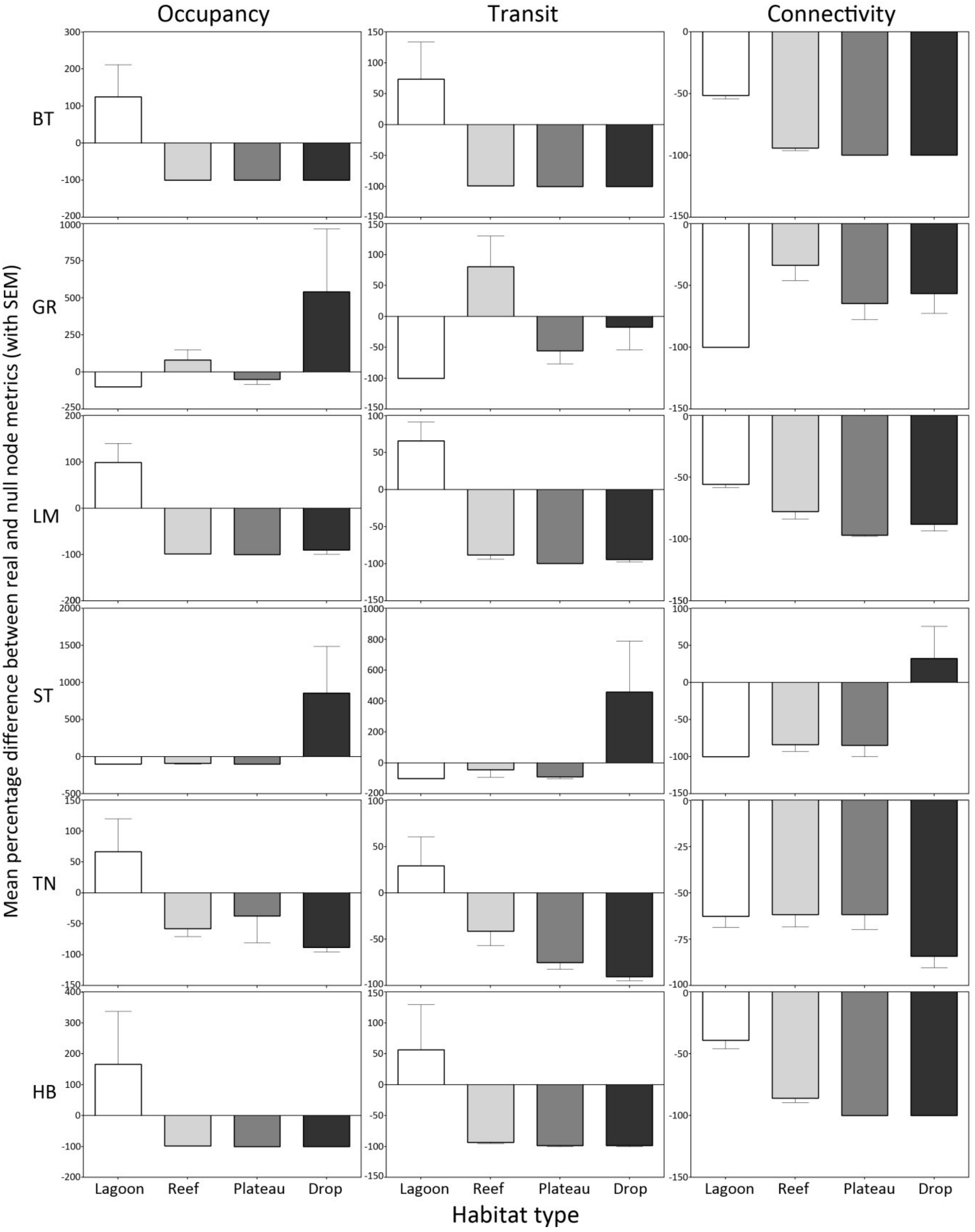

Table S1: Results of Wilcoxon signed rank tests comparing node metrics (strength, betweenness, centrality) between real and randomly generated networks. BT = blacktip reef, LM = lemon, GR = grey reef, TN = tawny nurse, ST = silvertip, HB = hawksbill.

Species	Metric	*n*	Z	p
BT	Occupancy	67	4.304	<0.001
BT	Transit	67	4.623	<0.001
BT	Connectivity	67	7.115	<0.001
GR	Occupancy	67	2.942	0.003
GR	Transit	67	2.53	0.012
GR	Connectivity	67	5.36	<0.001
LM	Occupancy	67	3.098	0.002
LM	Transit	67	3.198	0.001
LM	Connectivity	67	7.102	<0.001
ST	Occupancy	67	5.959	<0.001
ST	Transit	67	5.485	<0.001
ST	Connectivity	67	5.578	<0.001
TN	Occupancy	67	2.624	0.009
TN	Transit	67	2.561	0.011
TN	Connectivity	67	7.009	<0.001
HB	Occupancy	67	5.041	<0.001
HB	Transit	67	5.516	<0.001
HB	Connectivity	67	6.965	<0.001

Table S2: Results of one-sample signed rank tests comparing the node and edge densities of the randomly generated networks to those of the real networks. BT = blacktip reef, LM = lemon, GR = grey reef, TN = tawny nurse, ST = silvertip, HB = hawksbill.

Species	Density	Actual	Random (mean)	*n*	Z	p
BT	Node	0.52	0.99	100	8.843	<0.001
BT	Edge	0.09	0.72	100	8.682	<0.001
GR	Node	0.45	0.98	100	8.762	<0.001
GR	Edge	0.04	0.54	100	8.682	<0.001
LM	Node	0.84	0.99	100	8.836	<0.001
LM	Edge	0.15	0.72	100	8.683	<0.001
ST	Node	0.13	0.93	100	8.727	<0.001
ST	Edge	0.01	0.21	100	8.683	<0.001
TN	Node	0.76	0.97	100	8.762	<0.001
TN	Edge	0.12	0.52	100	8.683	<0.001
HB	Node	0.46	0.98	100	8.772	<0.001
HB	Edge	0.03	0.59	100	8.682	<0.001

Table S3: Results of Wilcoxon matched-pairs signed rank tests comparing the time spent inside the two different MPAs, with Monte Carlo p values calculated after 10,000 permutations.

Species	*n*	Z	p
Blacktip	25	4.015	<0.001
Grey	22	2.521	0.006
Lemon	20	3.621	<0.001
Nurse	6	2.201	0.019
Silvertip	13	1.826	0.073
Hawksbill	24	2.805	0.001

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