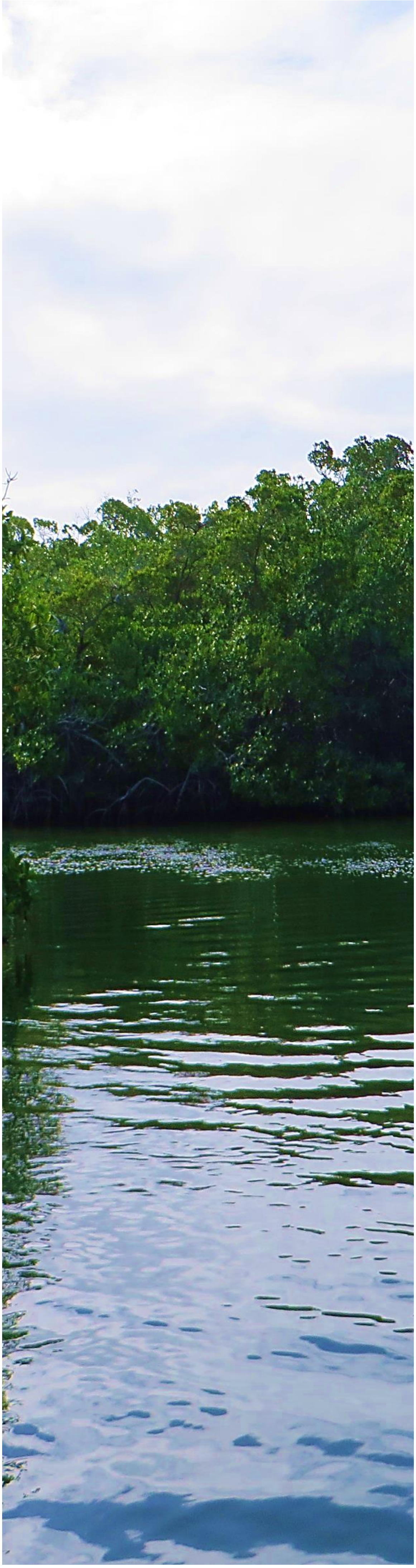

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Manatees of Bacalar Chico Marine Reserve and National Park, Belize: project summary 2014

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Front page: Journey through the mangroves, by Winnie Courtene-Jones (2014)

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Executive Summary

Often referred to as 'sea cows', the manatee is a large herbivorous marine mammal of the Order Sirenia, which includes both manatees and dugongs. There are three species of manatee: the South American manatee, found in the Amazon Basin, West African manatee, found in West Africa, and the West Indian manatee, found in the Caribbean Sea and Gulf of Mexico.

Manatee populations are affected by environmental fluctuations, natural disasters and disease, as well as anthropogenic threats. These threats pose a major risk to the survival of this species and as such all three species of manatees are listed as Vulnerable on the IUCN Red List of Threatened Species. The Antillean manatee (*Trichechus manatus manatus*), a subspecies of the West Indian manatee, has been identified as a priority protected species of regional concern across its extant range, which includes Western and Southern Gulf of Mexico, Central America, and NW South America west of the Lesser Antilles.

The Antillean manatee is present year-round in Bacalar Chico Marine Reserve and National Park (BCMRNP), located in Ambergris Caye, Belize. There is already substantial development on southern Ambergris Caye and further development is planned for areas adjacent to and nested within BCMRNP, presenting potential threats to manatees through development-related impacts, such as habitat degradation, pollution, and increased boat traffic.

Blue Ventures' Bacalar Chico Manatee Monitoring Programme aims to provide information on the abundance of manatees using the marine reserve, their spatial and temporal variations in distribution, habitat preferences and use patterns. During the first year of data collection (2014), a total of ten individual manatees were observed during surveys. The results of the data show that there is no statistical difference in manatee sightings per unit effort (SPUE) or manatee abundance between seasons. However, it is evident that specific areas within the mangroves had higher manatee sighting frequency, where they were observed year-round, indicating that these areas of mangrove are important sites for the species. Salinity and species composition of benthic vegetation also did not have a significant effect on manatee abundance as far as can be determined by the 2014 data. It is recommended that these transects continue to be surveyed for a further four years producing a five year data set, which will provide further clarification on seasonal differences and habitat use trends. Once key areas have been identified for the Antillean manatee in BCMRNP, local management strategies can be implemented to ensure that BCMRNP remains a stronghold for the Antillean manatee.

1. Introduction

1.1. Ecological importance of manatees

Manatees are large herbivorous aquatic mammals from the order Sirenia and Family Trichechidae. There are three species of manatee: the South American Manatee (*Trichechus inunguis*), the African Manatee (*T. senegalensis*), and West Indian Manatee (*T. manatus*). The West Indian Manatee is further divided into two subspecies, the Florida Manatee (*T. m. latirostris*), and the Antillean Manatee (*T. m. manatus*). The latter of these two subspecies, found in Caribbean and Atlantic waters, is the focus of our research.

In addition to being an indicator species for ecosystem health, Sirenians play a significant ecological role affecting seagrass meadow communities and productivity (Aragones and Marsh, 2000; Aragones et al., 2012; Perry and Dennison, 1999). It is suggested that, as large herbivorous mammals with high energetic requirements, intensive grazing by Sirenians upon seagrass meadows results in rapid nutrient cycling and soil aeration, thereby encouraging the growth of fast-growing seagrass species with shorter life cycles. While the majority of studies have focused on dugongs (Family Dugongidae), it is considered that intensive grazing by manatee causes similar effects (Aragones et al., 2012). Primarily due to the species' feeding ecology, manatees have been termed an 'umbrella species'[1] (Marsh and Morales-Vela, 2012), whereby their conservation supports the protection of the complex underlying community (Zacharias and Roff, 2001; Simberloff, 1998).

1.2. Status of the Antillean manatee in Belize

Worldwide, manatee populations are affected by environmental fluctuations, natural disasters and anthropogenic threats, with the highest contributors to manatee mortality being watercraft collisions, habitat loss/degradation and poaching (Quintana-Rizzo and Reynolds III, 2008). All three species of manatee are listed as Vulnerable on the IUCN Red List of Threatened Species, and the Antillean subspecies has been considered Endangered since 2007 (www.iucnredlist.org).

The Antillean manatee (Figure 1) is found in 19 countries throughout the Caribbean, Central and South America (Lefebvre et al., 2001), and is the species most commonly found in Belize. Their distribution within their geographic range is patchy with very low abundance, with merely 100-500 individuals recorded in most countries (UNEP, 2010). The Antillean manatee has been identified as a "priority species of regional concern"' (UNEP, 1995), requiring protection due to increasing threats and pressures on an already shrinking population (Auil, 2004; Auil Gomez, 2011; Mignucci-Giannoni *et al.*, 2000).

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[1] Umbrella species are defined as "non-migratory species that demonstrate fidelity to particular set of habitats, are specialists rather than generalists, do not thrive in disturbed or anthropogenic habitats, require large areas of relatively natural habitat and will cause limited change in community or habitat structure if removed" (Zacharias and Roff, 2001)

Figure 1- Antillean manatee sighted in Bacalar Chico Marine Reserve, 2013.

Belize is generally considered to be one of the last strongholds for the Antillean manatee in the Caribbean (O'Shea and Salisbury, 1991), attributed to the wide availability of suitable habitats – seagrass beds, lagoons, rivers and mangrove cayes (Auil, 1998; Quintana-Rizzo and Reynolds III, 2008; Spieglberger and Ganslosser, 2005). Although present along the entire Belizean coastline, areas with consistently high manatee sightings are Corozal Bay, the Belize River and Belize City area (including the Drowned Cayes), Southern Lagoon, Placencia Lagoon and Port Honduras Marine Reserve (Morales-Vela et al., 2000, Figure 2).

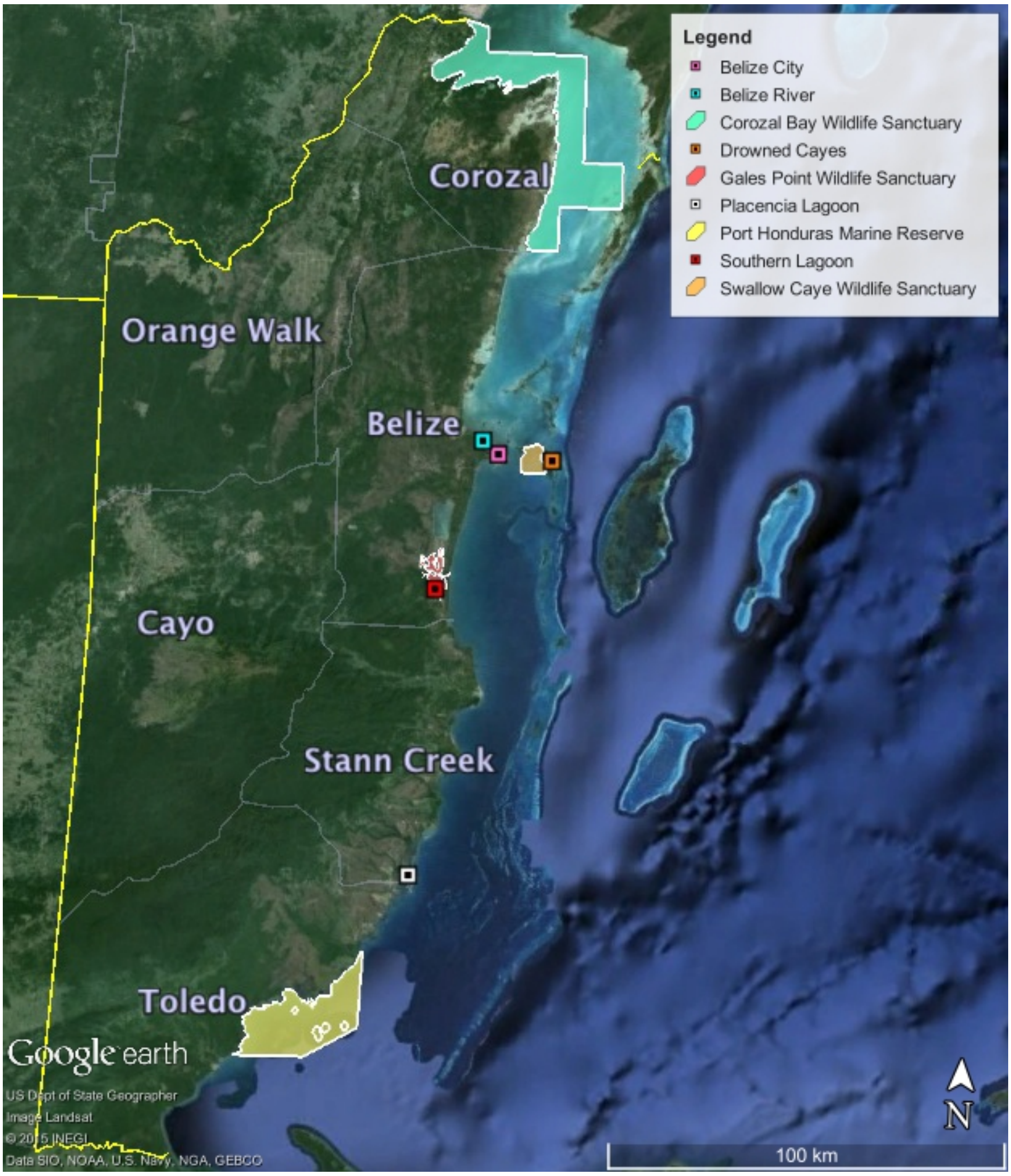

Figure 2- Areas of importance for manatees in Belize.

Aerial survey data collected between 1997-2004 estimated manatee populations in Belize to be approximately 1000 individuals (Auil, 2004), however at this time it was not known if the population was stable or slowly declining. Discrepancies within studies and a lack of later comparable research have made it difficult to draw any conclusions about the status of manatee populations in Belize (Auil, 2004). It is clear that establishing a realistic population size for the Antillean manatee presents a number of challenges. Recommendations to address these problems include establishing site-specific long-term monitoring programmes for manatee within different coastal ecosystems (Auil, 2004).

1.3 Bacalar Chico Marine Reserve and National Park

Bacalar Chico Marine Reserve and National Park (BCMRNP) is one of seven marine protected areas that form the Belize Barrier Reef World Heritage Site. The area covers 60km^2 and approximately 15,530 acres of marine habitat and encompassing coral reefs, seagrass beds, mangroves and coastal lagoons. It is situated at the northernmost tip of Ambergris Caye and includes the northern tip of the Belize Barrier Reef System. A protected area since 1996, BCMNRP shares its northern border with *Arrecife de Xcalak*, a marine protected area in Mexico (Walker and Walker, 2011). The characteristic limestone scenery that comprises the National Park forms numerous creeks and lagoon systems. Noted for its rich biodiversity, the area is home to a number of ecologically and commercially valuable species (Walker and Walker, 2011).

Opportunistic sightings of manatees in BCMRNP have been recorded by Blue Ventures (BV) since 2010. These sightings logs confirm a year-round presence of manatees in backreef, seagrass, mangrove and lagoon areas of BCMRNP (Chapman, 2012), and it is also believed that individuals may move between Bacalar Chico, Corozal Bay Wildlife Sanctuary and into Mexico (Castelblanco-Martínes et al., 2012). BV's opportunistic sightings data from 2010-2013 also showed seasonal differences in manatee sighting frequency, with a significantly higher frequency of manatees observed in lagoon and mangrove areas from November to February (Jenko *et al.*, 2014). The drivers behind these seasonal differences are not fully understood. In 2013, BV developed a manatee monitoring programme to address knowledge gaps related to manatee distribution and movements in BCMRNP.

1.4 Programme aims

BV's manatee monitoring programme aims to provide information on the abundance of manatees within BCMRNP, and their spatial and temporal variation in distribution. Using these data, BV aims to inform best practice for tourism and development activities, guiding management of BCMRNP through:

- identifying local manatee hotspots
- describing behaviour of manatees and relevant habitat associations
- establishing a photo-identification database of individuals using the area

A photo-identification database of individuals will further provide information on Antillean manatee life history traits such as maturation, reproductive rates, site fidelity, and adult survival, as well as connectivity of manatee areas both within BCMRNP and with other regions.

2. Methods

Four survey routes were chosen based on manatee sighting areas from BV's opportunistic sightings data from 2010-2013 (Figure 3) encompassing exposed and sheltered mangrove channels, lagoons, seagrass and backreef areas. Manatee sightings were recorded along survey routes (while the boat was moving) as well as at stationary observation points. Habitat monitoring points were sampled independently.

Commencing in January 2014, baseline data on manatee sightings (Section 2.2) and habitat type (Section 2.3) were collected once per season (*Norte*: November to February; Dry: March to June; Wet: July to October) for one year along each route. Surveys were performed between 09:00 and 12:30 and in Beaufort Sea State 3 or less to standardise sighting probability. Substrate was composed of mud, rock, or sand, combinations of which were recorded as 'silt' during the *Norte* season, but clarified in subsequent seasons.

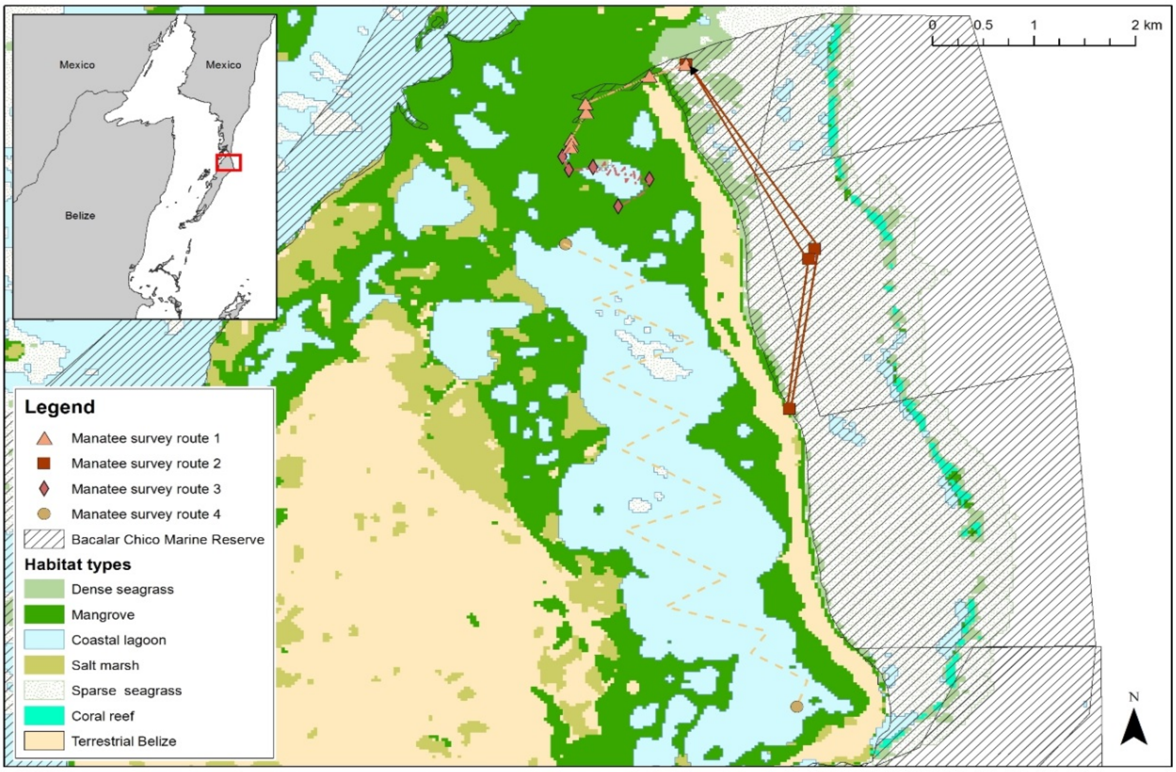

Figure 3- Map of BV's four manatee transect Routes in BCMRNP.

2.1 Description of survey routes

See Appendix 4: Manatee mapping Routes and point details (BCDC)

Route 1: 'Manatee Hole'

Boca Bacalar Chico – 1A – 1B – 1C – 1D – 1E – Lovers Tunnel

Route 1 begins at Boca Bacalar Chico, where the mangrove channel system meets the reef side of BCMRNP, and has five observation points (1A-E, Table 1, Figure 4) and three habitat monitoring points (1A, between 1B and 1C, and 1D). This Route surveys a distance of approximately 1500m. The main vegetation at each of these sites is red mangrove (*Rhizophora mangle*).

Table 1- Manatee observation points and habitat descriptions for Route 1.

Observation Point	Name	Description
1A	Mangrove Mouth	Lies in a channel approximately 80m wide and 2m deep. Represents the entrance from sea to mangroves on the Belize-Mexico border.
1B	Manatee Hole	Features a sinkhole approximately 25m wide and 3m deep and is a known manatee resting area.
1C	N/A	Positioned towards the end of Manatee Hole.
1D	Turtle Pond	Small lagoon, 30m wide, 50m long and 4m deep, which is connected to Manatee Hole.
1E	Teacup Lagoon	Connected to Turtle Pond. This lagoon is approximately 20m wide, 20m long and approximately 2.5m deep.

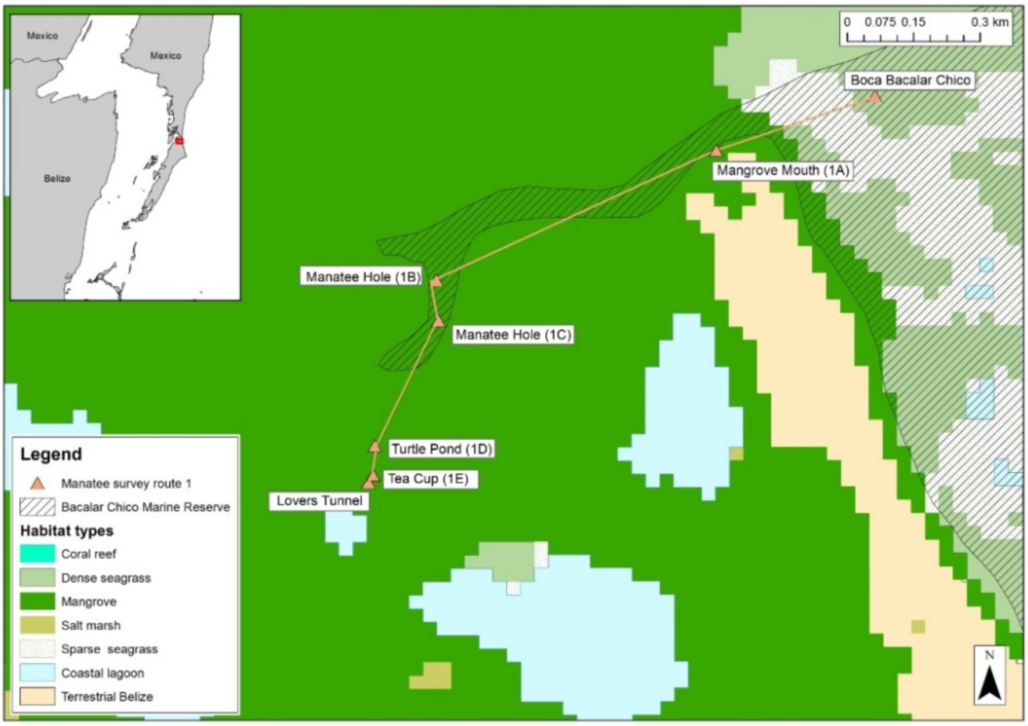

Figure 4- Manatee transect Route 1, "Manatee Hole", in BCMRNP.

Route 2: 'Barracuda Patch'

BCDC – 2A – Boca Bacalar Chico – 2B – BCDC

This transect route surveys a distance of approximately 8000m. Starting at Bacalar Chico Dive Camp (BCDC), this route traverses seagrass beds within the lagoon behind the barrier reef, travelling to and from Boca Bacalar Chico via a patch reef called Barracuda Patch, where manatees are often sighted opportunistically. There are two observation points (2A, 2B) and one habitat monitoring points (haphazardly on seagrass bed around 2A and 2B), with 2A being the northern mooring buoy at Barracuda Patch, and 2B being the southern mooring buoy (Figure 5). Barracuda Patch is approximately 3.5m deep.

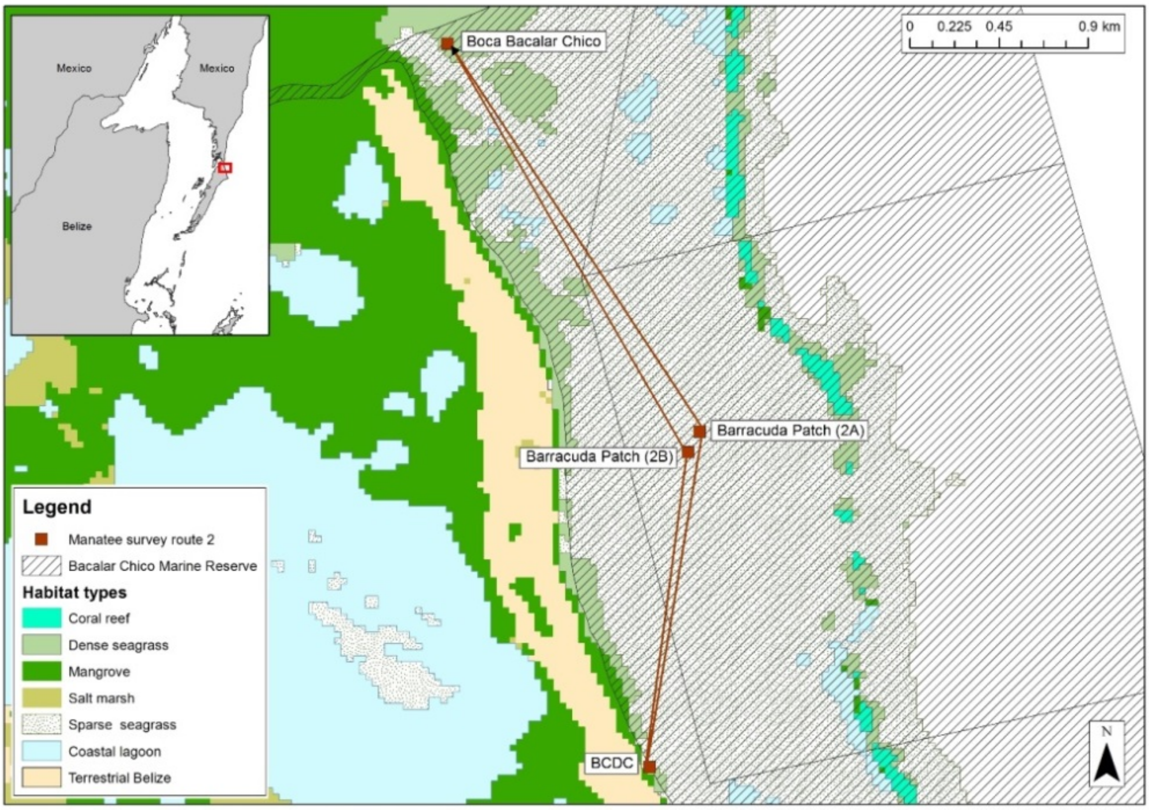

Figure 5- Manatee transect Route 2, "Barracuda Patch", in BCMRNP.

Route 3: 'Dusk lagoon'

Lovers Tunnel – 3A – 3B – 3C – 3D – 3E

Route 3 continues on from the end point of Route 1 within the mangrove system and covers a distance of approximately 1500 m. This transect has five observation points (3A-E, Table 2) and two habitat monitoring points haphazardly located within Crocodile and Dusk lagoons (Figure 6). Point 3A is at the entrance and 3B is at the far side of Crocodile Lagoon. Crocodile Lagoon is a wide (±100 m), shallow (<1 m) lagoon. Points 3C, 3D and 3E are all in Dusk Lagoon, which is 250m long, 200m wide, and shallow (±1m) lagoon. Mangrove islands are scattered throughout Dusk Lagoon. At both of these sites the main vegetation is red mangrove.

Table 2- Manatee observation points and habitat descriptions for Route 3.

Observation Point	Name	Description
3A	Crocodile Lagoon	located at the entrance of *Crocodile Lagoon*, a wide (±100m), shallow (<1m) lagoon connecting *Lovers Tunnel* and *Dusk Lagoon*. Mangrove canopy height ±10m.
3B	Crocodile Lagoon	Positioned at far side of lagoon, near channel entrance to Dusk Lagoon.
3C	Dusk Lagoon	At the entrance to Dusk Lagoon, which is a 250m long, 200m wide, and shallow (±1m) lagoon.
3D	Dusk Lagoon	Furthest point in the lagoon from the inlet entrance
3E	Dusk Lagoon	Positioned at South end of lagoon, roughly in the middle of the lagoon length. Route 3 end point.

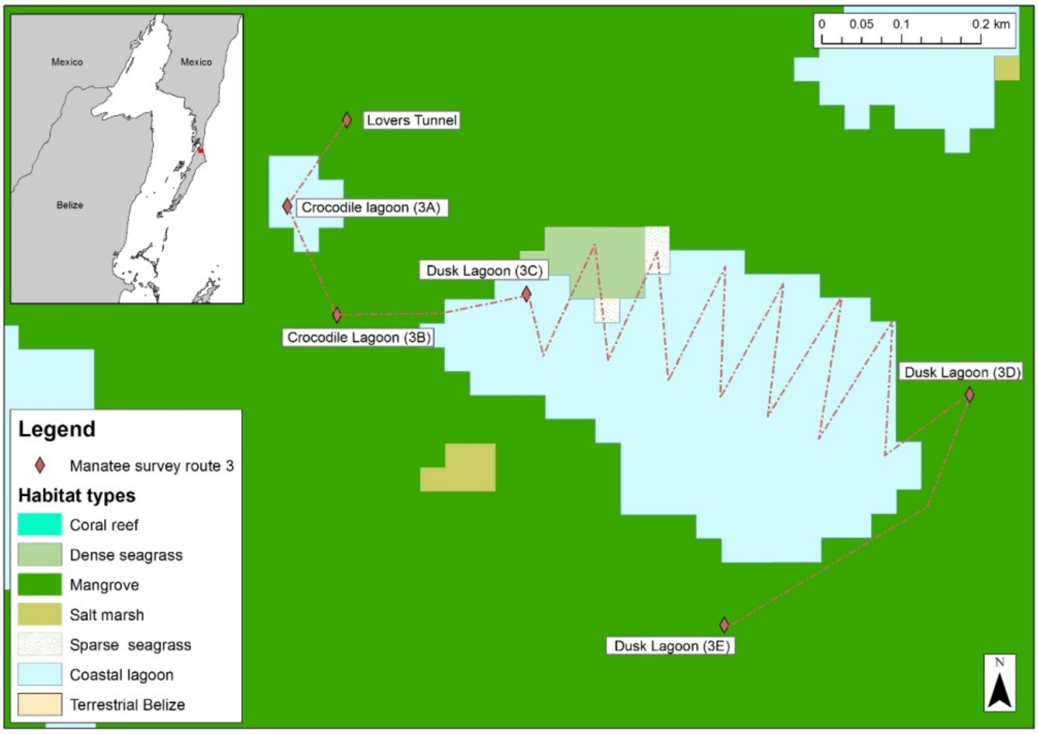

Figure 6- Manatee transect Route 3, "Dusk Lagoon", in BCMRNP.

Route 4: Cantena Lagoon: Cantena North – Cantena South

Cantena Lagoon is a large (3km long by 400m-1km wide) and shallow (0.5-2m) coastal lagoon, predominantly surrounded by red mangrove, with small mangrove islands scattered within the lagoon. The straight-line distance between Cantena North and South is approximately 5300 m, though surveys cover a distance in excess of this. The three habitat monitoring points are located at the north, middle, and south of the lagoon (Figure 7), and there are no observation points.

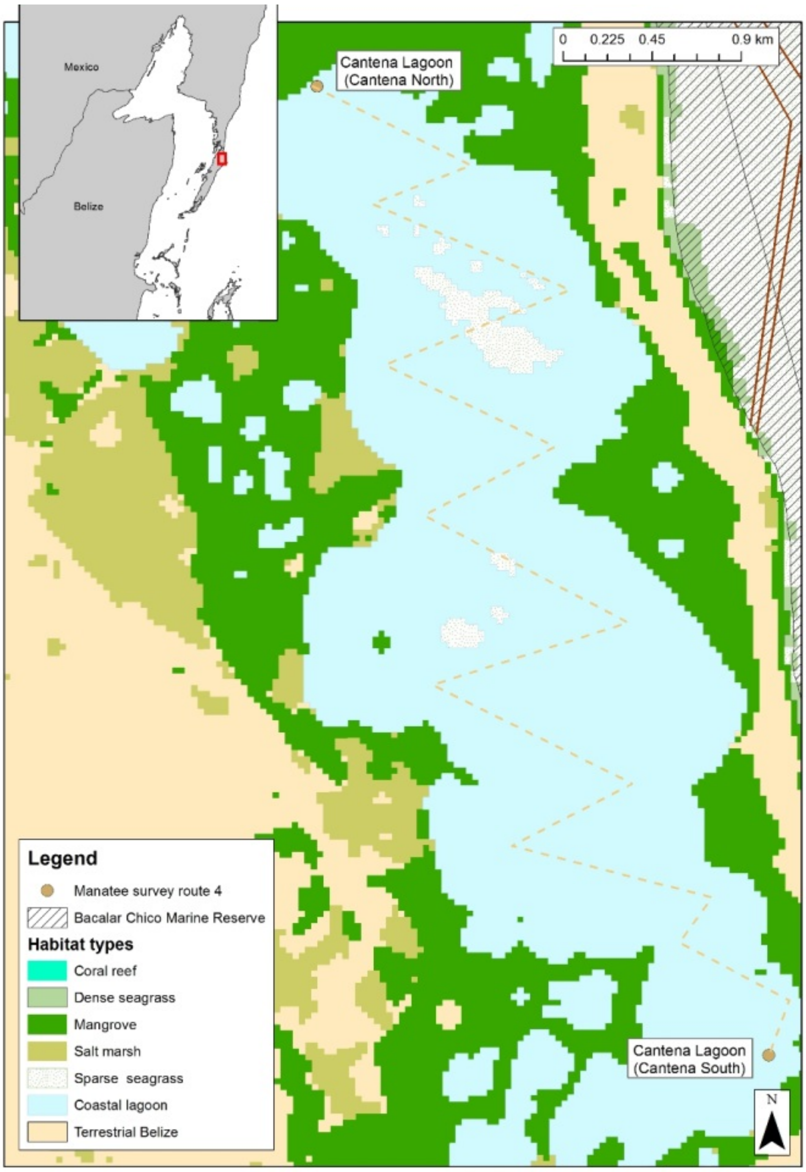

Figure 7- Manatee transect Route 4, "Cantena Lagoon", in BCMRNP.

2.2 Manatee surveys

Manatee sightings were recorded along survey routes as outlined. The boat followed a zig-zag pattern on the outward journey and a straight line route on the return journey between marked GPS points. When the channel or lagoon was narrow/small enough to see well from one side to another then straight line routes were used. Manatee sightings were recorded on both outward and return journeys, and the total journey time was recorded. Twenty-minute scans were performed at each observation point, and the start and end time of these was also recorded.

Manatee sighting data included records of:

1. Minimum number of individuals (the number of manatees sighted for which there is 100% certainty they are different individuals).
2. Best estimate of the number of manatees present.
3. Behaviour: feeding, resting, socialising, social travel, travelling, milling, sexual, boat interaction, mixed, undetermined.
4. Manatee reaction to boat(s): avoidance, attraction, none.
5. Estimated size in cm. All manatees were size estimated and placed into size categories (N. Castelblanco, pers. comm.): adult (≥270 cm), juvenile (<270 cm), calf (≤140 cm and in close association with another manatee, breathing simultaneously).

Additionally, salinity (ppt), depth (m), and turbidity (m), were recorded at each point using a refractometer, depth measure (weight with string) and Secchi disk, respectively. If the conditions upon sighting were suitable, attempts were made to collect videos and/or photos from the boat for later photo-identification of all individuals. If any manatee was sighted between observation points these same details were recorded, along with the location marked with a GPS.

See Appendix 1: Detailed method of manatee surveys.

2.3 Habitat monitoring

After checking that no manatees were present, a 0.5 m^2 quadrat was haphazardly thrown from the boat five times at each of the habitat monitoring points. An exact GPS coordinate and photograph was taken for each quadrat, in order to maintain a record of and verify estimates of vegetation cover. A snorkeler recorded the main substrate type and estimated the total percentage vegetation cover by species. Environmental data, salinity (ppt), depth (m) and turbidity (m), was recorded from the boat at each monitoring point.

Appendix 2: Detailed method of habitat monitoring.

2.4 Data Analysis

Sighting per unit effort (SPUE) of manatees along transect routes was calculated using best estimate of number of manatees sighted/boat/hour spent surveying. Number of observers was not taken into account as the methodology ensured a minimum of four surveyors so as to have a full panoramic view of the survey area, with each observer covering a 90° angle searching for manatees. Therefore, more surveyors would not necessarily increase the likelihood of spotting a manatee (N. Castelblanco, pers. comm.).

Sample sizes of SPUE data were too small for normality testing, therefore non-parametric Kruskal-Wallis (K-W) ANOVA tests were carried out to compare SPUE values using PRISM 5 for Mac OS X, version 5.0b. Manatee sighting abundance data also did not meet criteria for parametric testing (D'Agostino & Pearson omnibus normality test, $P < 0.05$), so Kruskal-Wallis (test statistic H) was also used to determine statistical differences in manatee sighting abundance along transect routes seasonally. Salinity data met the criteria for parametric testing (D'Agostino & Pearson, omnibus K2 normality test, $P < 0.05$), and comparisons were tested using a 2-way ANOVA. A 2-tailed Mann Whitney U (M-W) test (test statistic Z) was used for non-parametric comparisons with sample sizes of less than 3.

Mean percentage cover of each species of vegetation was calculated at monitoring points for each season. Not all habitat cover data met criteria for parametric testing (D'Agostino & Pearson omnibus normality test, $P < 0.05$) so Kruskal-Wallis (K-W) ANOVA was carried out to identify changes in vegetation cover between sites and seasons. In instances where less than three groups were available for comparison, a 2-tailed Mann-Whitney U test was used for analysis. Furthermore, a canonical correspondence analysis (CCA) was used to investigate the relationship between environmental variables (depth, salinity and mean percentage vegetation cover) and response variables (percentage cover of *Thalassia testudinum*, *Syringodium filiforme,* and *Halodule sp.*, and manatee sighting frequency).

See Appendix 5: Statistical Values of Non-Significant Comparisons.

3. Results

3.1 Manatee surveys

In total, ten manatees were sighted during twelve surveys, with one being sighted along a transect route and nine observed during focused manatee scans. Eight manatees were adults and two were juveniles. The majority (6) of manatees displayed milling behaviour and the remainder displayed resting (2) and mixed (2) behaviours, comprising of milling, resting and social interactions (Figure 8).

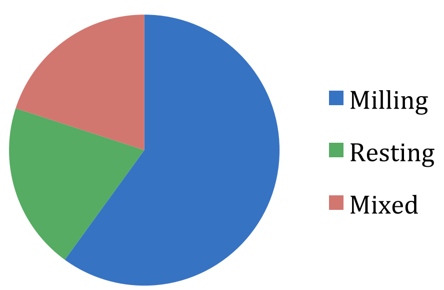

Figure 8- Observed behaviour of Antillean manatee during 2014 surveys.

Mean SPUE was calculated seasonally for each route, and then averaged to find the annual mean by route (Figure 9). Route 1 had the highest mean SPUE (1.52) followed by Route 2 (1.43). Route 3 and 4 had no sightings and therefore an SPUE of 0. There was no significant difference in mean SPUE between routes or between seasons overall. Further analysis was performed to compare only Routes 1 and 2, where mean SPUE >0, and there was also no significant difference in SPUE between these routes. There was no significant difference in manatee SPUE between seasons for all routes (Figure 9).

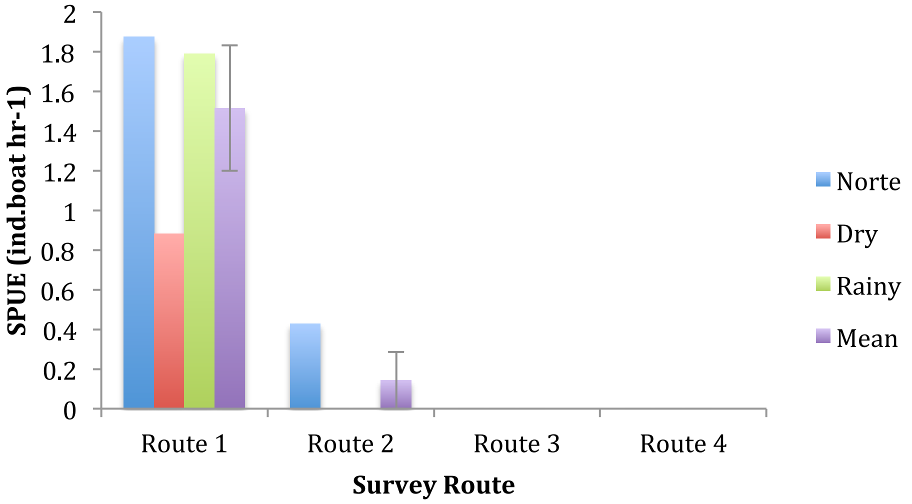

Figure 9- Seasonal manatee sightings per unit effort (SPUE) with mean (+/- standard error) for each transect route.

When examining each of the transect routes further to identify location hotspots for manatee, it was found that there was no significant difference in mean sighting abundance between observation points, or between seasons (Figure 10). Opportunistic sightings were not included in these analyses as they are not associated with any observation point. There was also no significant difference in manatee sightings between observation points along Route 1 or Route 2.

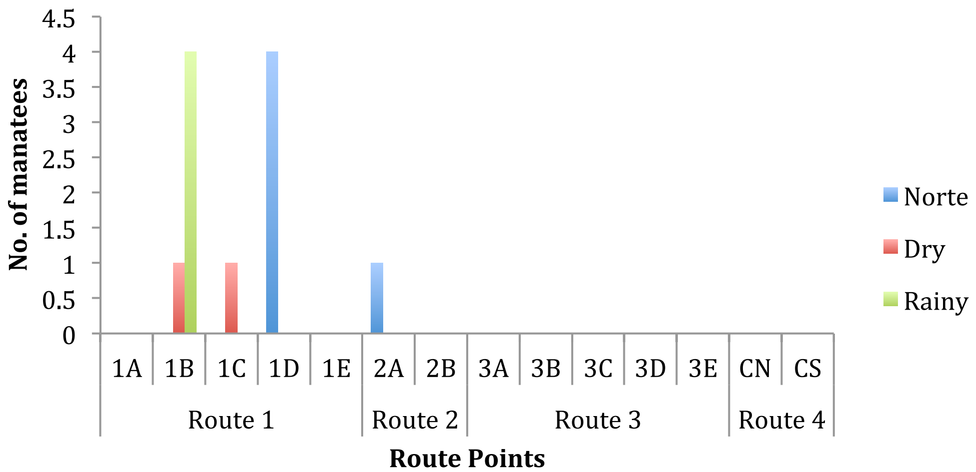

Figure 10- Total sighting abundance of manatee at each observation point seasonally.

Salinity measured at all observation points varied significantly between seasons (F=16.59, df=2, P<.0001) (Figure 11). At sheltered lagoons, such as Cantena South and Crocodile Lagoon (3A, 3B), salinity reached its highest during the dry season (37 ppt) and its lowest during the *Norte* season (13 ppt). For more exposed areas, such as Manatee Hole (1B), Turtle Pond (1D) and Barracuda Patch (2A), salinity was lowest during the wet season (23ppt, 15ppt, and 15ppt, respectively). There was no significant difference in mean salinity values between observation points.

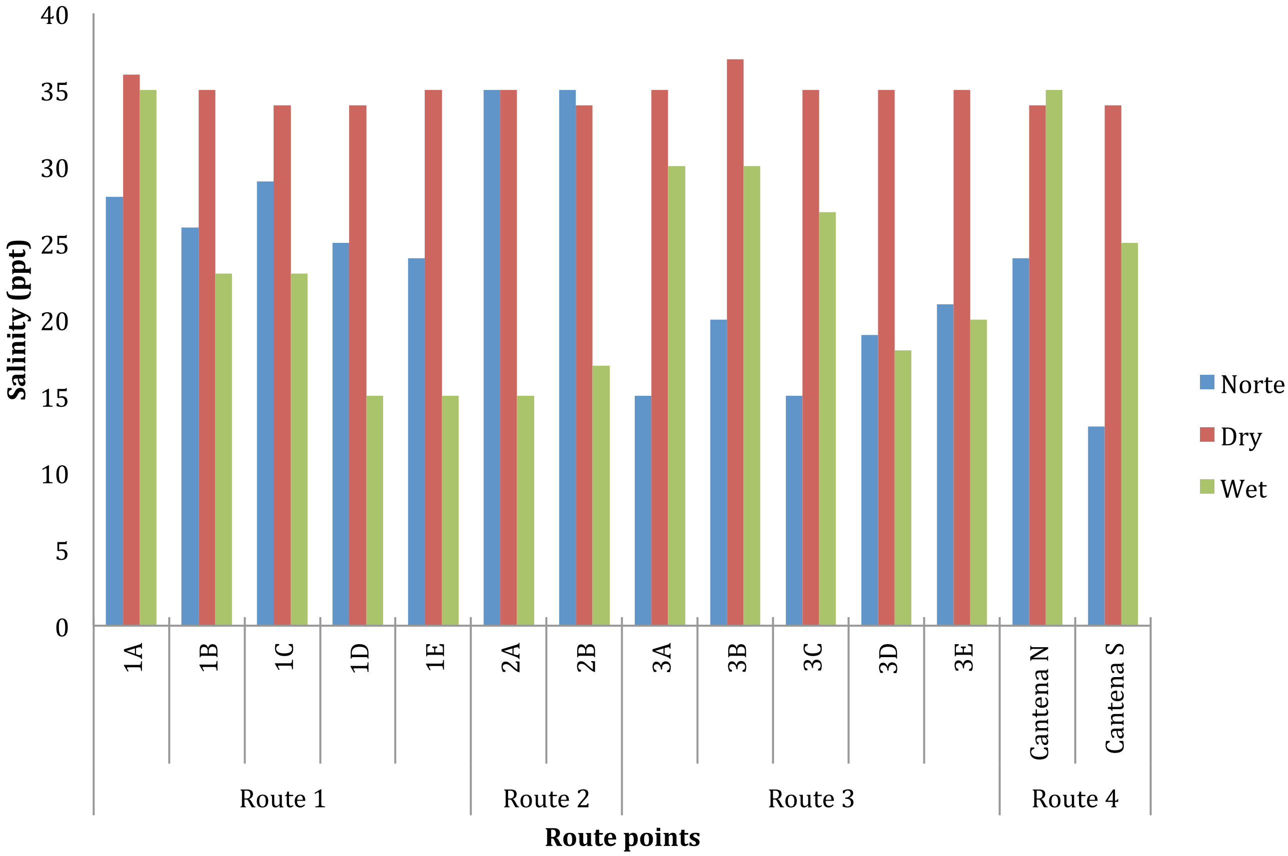

Figure 11- Salinity (ppt) for each of the monitoring points seasonally.

3.2 Habitat monitoring

A total of 20 different vegetation species were identified in Bacalar Chico in 2014 (see Appendix 3: Vegetation recorded on habitat monitoring surveys). Substrate was also recorded (see evaluation section for further clarification). Data from each of the 5 transects was averaged to find mean vegetation cover by observation point for each season. Mean vegetation cover and percent species composition at each monitoring site did not differ significantly between seasons or observation points. The most abundant vegetation type overall was cyanobacteria, followed by *Thalasia testudinim* (turtle grass). Halimeda, Penicillus, *T. testudinum*, Batophora, and Halodule (shoal grass) were present along each route at least once per season (Figure 12).

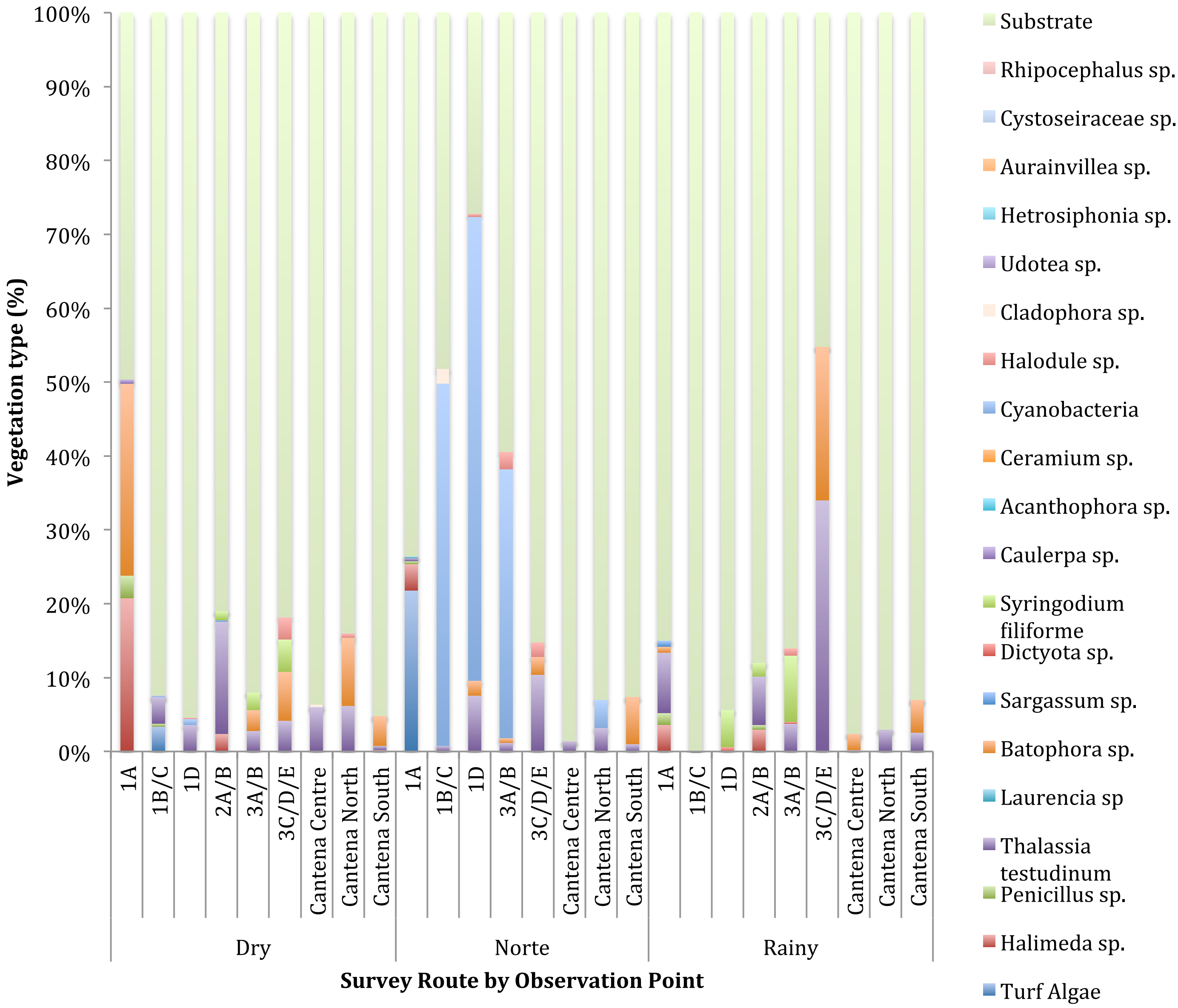

Figure 12- Benthic composition for all monitoring sites by season.

The main substrate type was recorded as silt at point 1B and 1D in the *Norte* season, however was subsequently recorded as mud during the other seasons. Species composition along Route 1 did not vary significantly between observation points or between seasons. Cyanobacteria was the most common vegetation species recorded along the route overall, though present only during *Norte* and dry seasons. *T. testudinum* was the only vegetation species present at all observation points along this route (Figure 13).

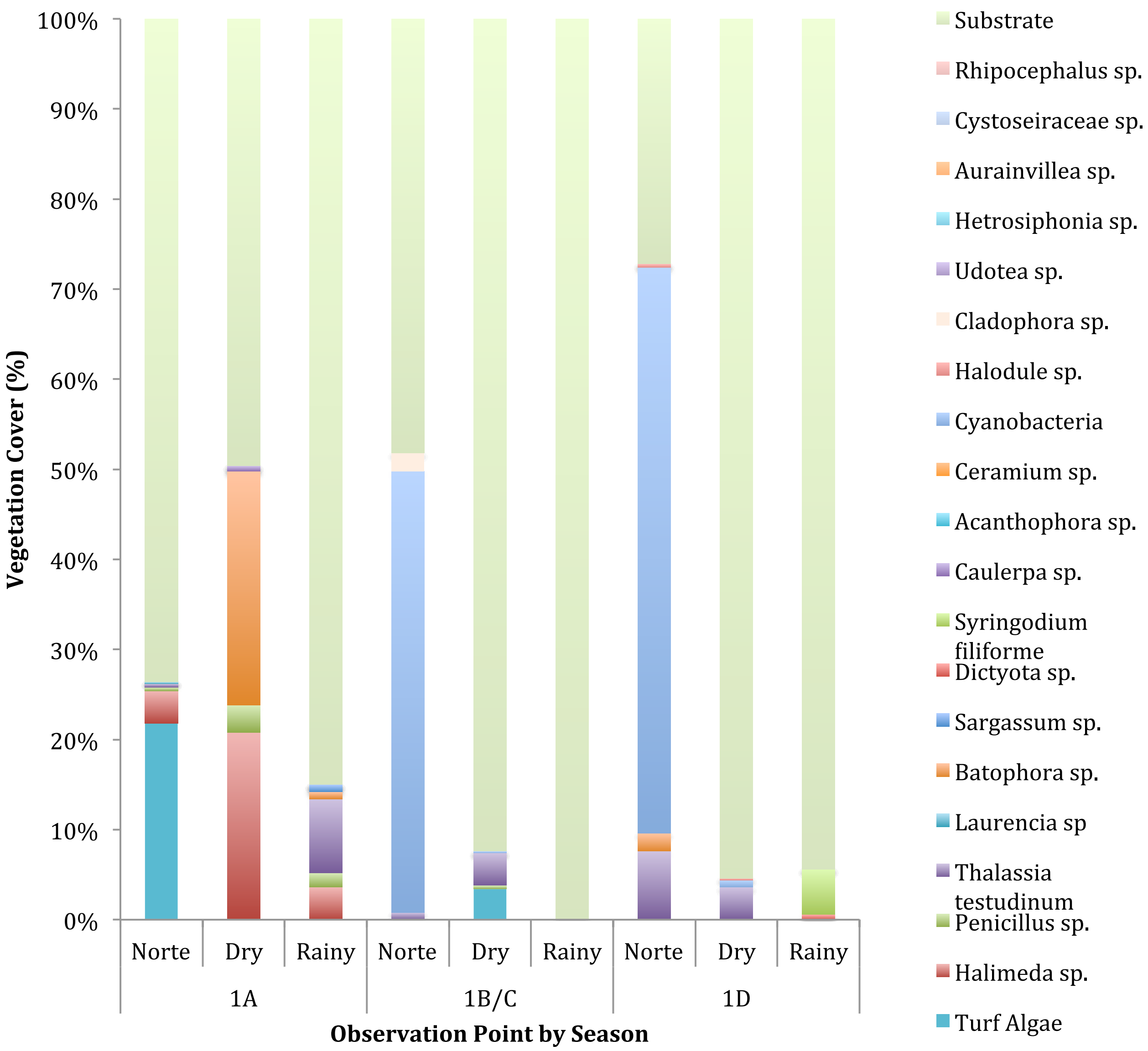

Figure 13- Mean benthic composition for Route 1 (Manatee Hole) observation points seasonally

(NB substrate may be sand, mud or silt – see note in explanation).

The main substrate type recorded at Route 2 was sand. No data was collected in the *Norte* season along Route 2. Percent species composition did not vary significantly between the rainy and dry seasons along this route. *T. testudinum* was the most abundant vegetation type along this route, and was present during both seasons along with Halimeda and *Syringodium filiforme* (manatee grass) (Figure 14).

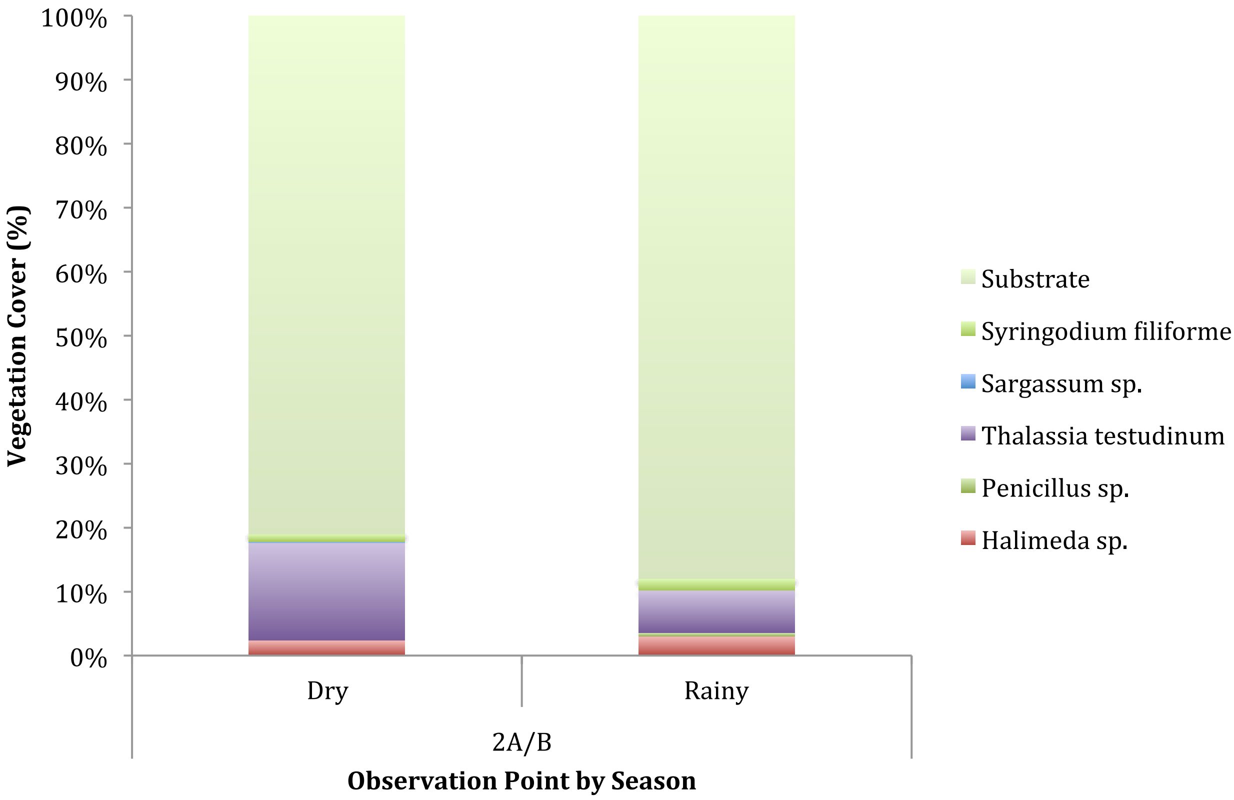

Figure 14- Mean benthic composition for Route 2 (Barracuda Patch) for dry and rainy seasons.

(NB substrate may be sand, mud or silt – see note in explanation).

The main substrate recorded along Route 3 was mud. Mean species composition also did not vary significantly between observation points or between seasons along Route 3. *T. testudinum* was the most abundant vegetation type recorded along this route, and was seen during all seasons at both observation points. Batophora, *S. filiforme* and Halodule were also seen at both observation points (Figure 15).

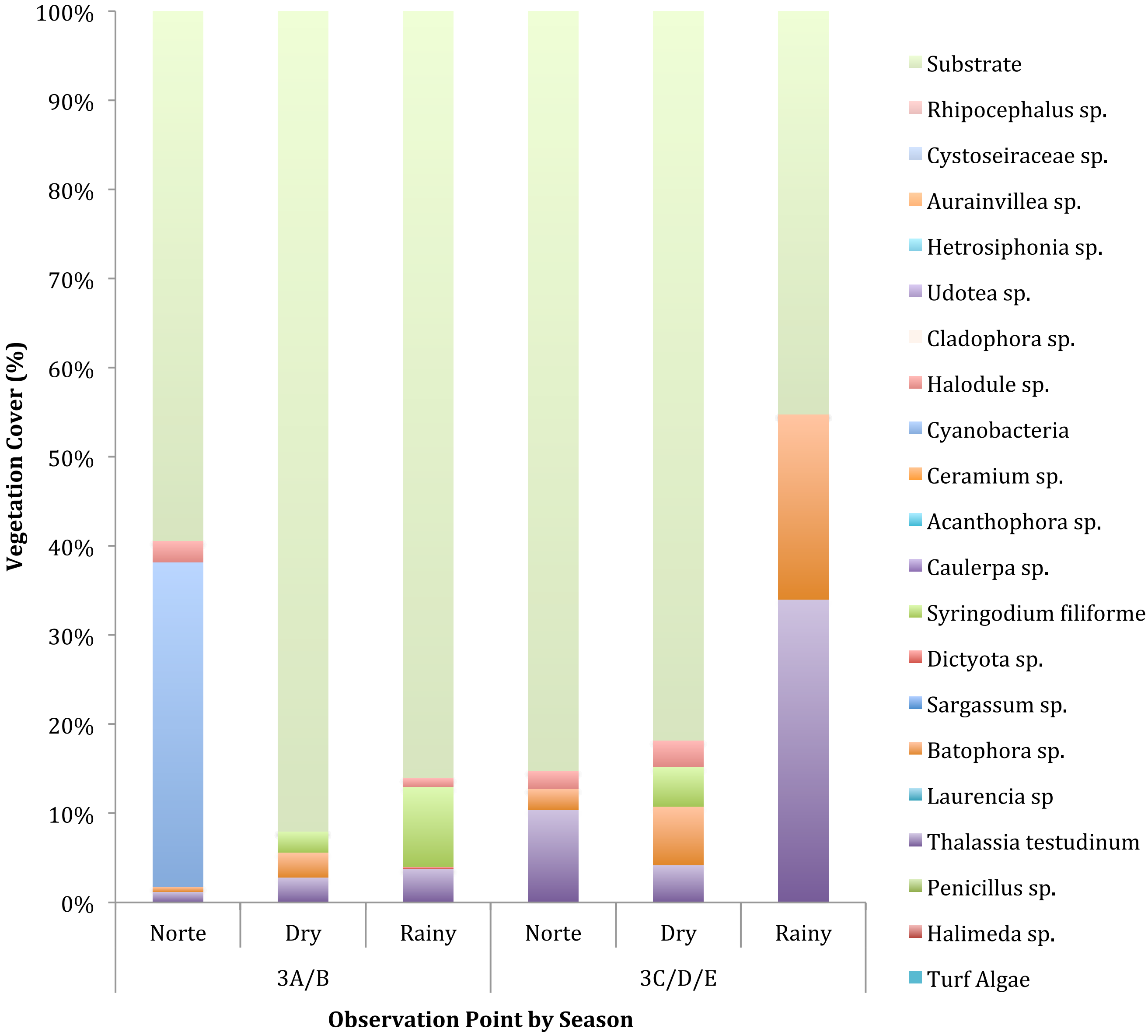

Figure 15- Mean benthic composition for Route 3 (Dusk Lagoon) observation points seasonally.

(NB substrate may be sand, mud or silt – see note in explanation).

Substrate type along Route 4 was recorded as silt at all three locations during the *Norte* season, but as mud in the dry and wet seasons. Species composition along Route 4 did not vary significantly between observations points or between seasons. Batophora was the most commonly recorded species, followed by *T. testudinum.* These were the only two species recorded at all observation points, and were observed at least once during each season (Figure 16).

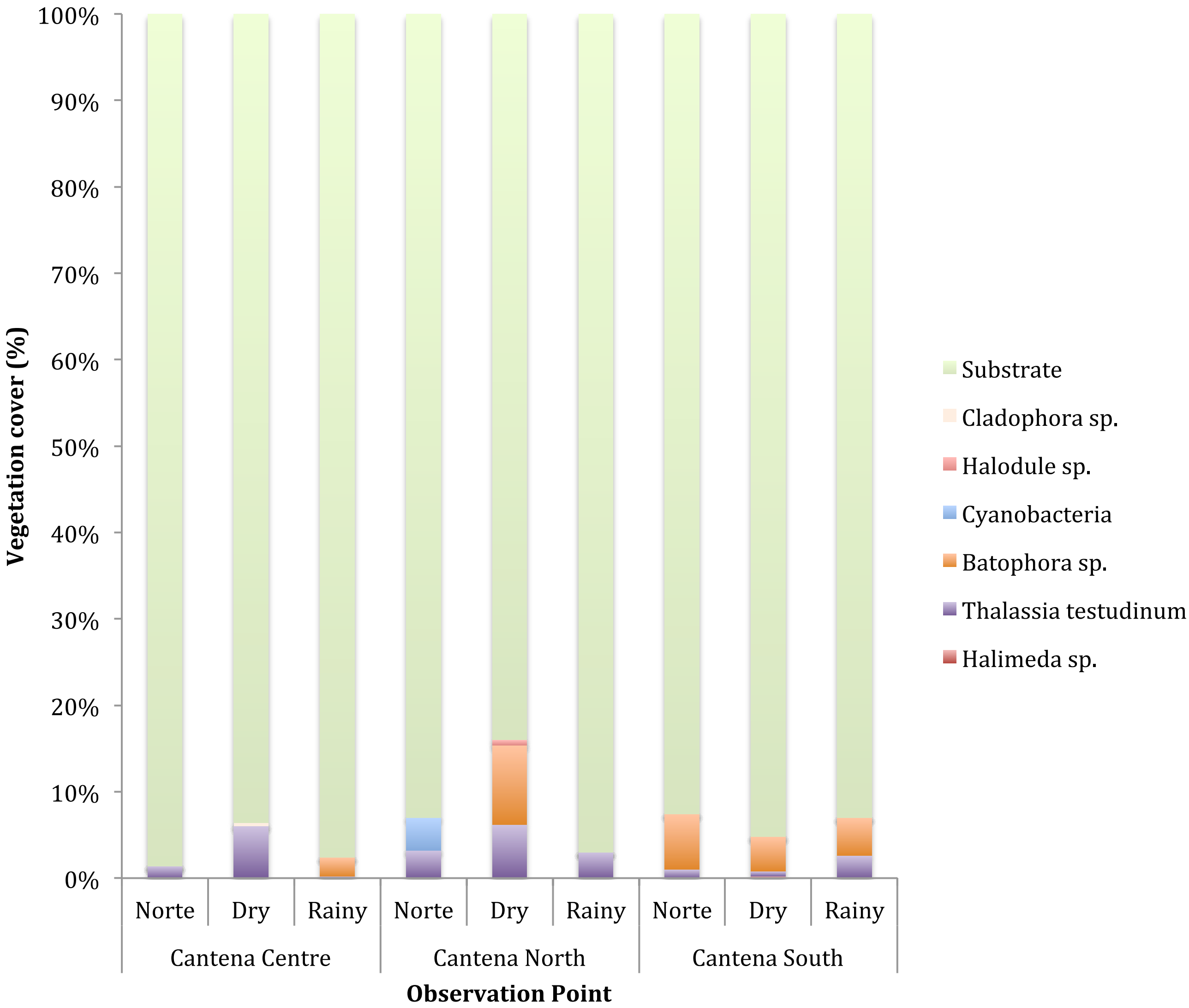

Figure 16- Mean benthic composition for Route 4 (Cantena Lagoon) observation points seasonally.

(NB substrate may be sand, mud or silt – see note in explanation).

A Canonical Correspondence Analysis (CCA) was carried out to assess the relationship between response variables (mean manatee sighting abundance, mean percentage seagrass cover including *T. testudinum*, *S. filiforme* and *Halodule* sp.) and environmental variables (mean percentage vegetation cover, depth and salinity) (Figure 17). Higher percentage cover of *T. testudinum* occurred at lower salinity. Manatee sighting abundance was associated more closely with depth than the other environmental variables of salinity of mean percentage vegetation cover. Results show that observation points where manatee were sighted, such as points 1B, 1D, and 2A, clustered around *T. testudinum* percentage cover and depth, identifying these factors as important for manatee sightings.

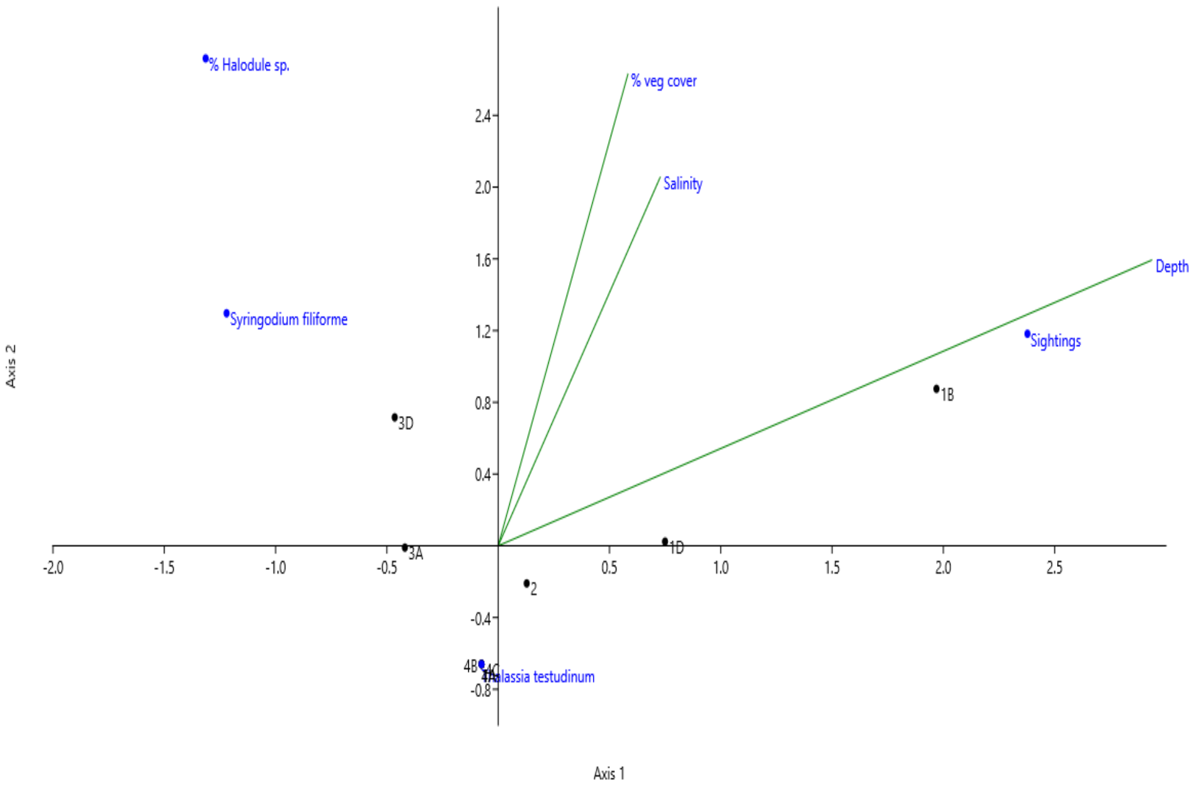

Figure 17- CCA results showing relationships between environmental and response variables.

Annual point and route-specific mean vegetation cover was then compared. The habitat monitoring point 1A had the highest recorded mean vegetation cover throughout the year (30.6%). Route 1 also contained the highest maximum vegetation cover (72.8%) measured at point 1D during the Norte season. Furthermore, Route 1 also had the highest overall mean vegetation cover (26.02%), followed by Route 3 (25.07%), and then Route 2 (15.5%). Route 4 had the lowest mean vegetation cover throughout the year (6.16%). Mean percentage of vegetation cover did not differ significantly between observation points (Figure 18) or between routes annually (Figure 19).

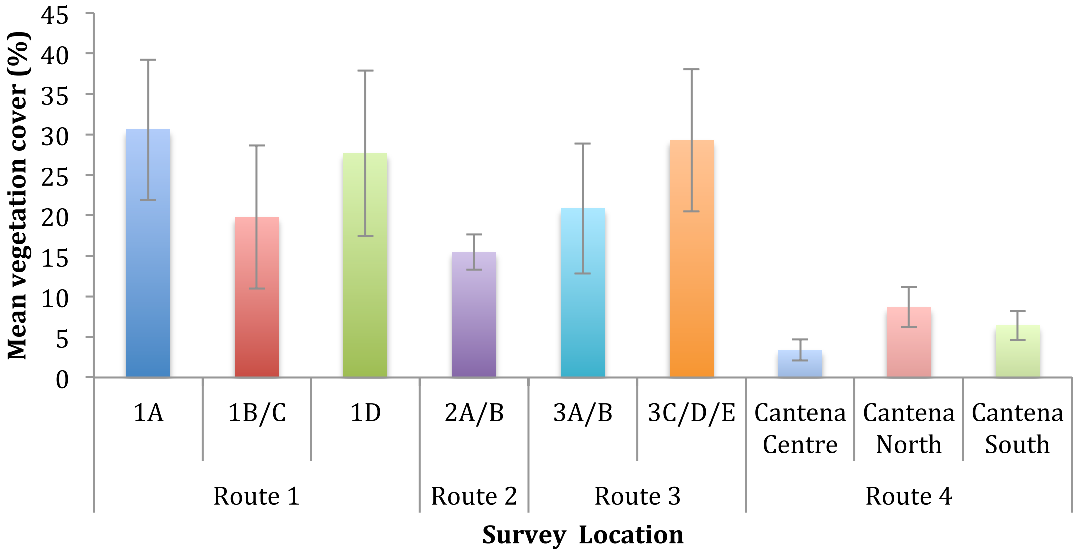

Figure 18- Mean percentage vegetation cover (+/- standard error) for habitat monitoring points at BCMRNP in 2014.

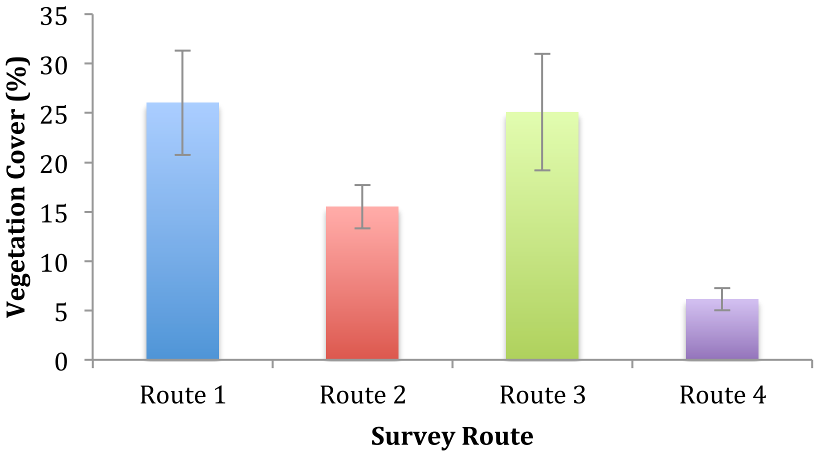

Figure 19 - Mean percentage vegetation cover (+/- standard error) for each survey Route at BCMRNP in 2014.

4. Discussion

While the habitat use patterns of the Antillean manatee are not well understood, a few studies have shown sighting frequency to vary with season (Self-Sullivan, 2003; Auil, 2004). A study by Auil in 2004 in Belize found a higher than expected probability of sighting manatees in river habitats during the dry season and a reverse distribution during the wet season. An earlier study (Self-Sullivan *et al*. 2003) identified seasonal differences in the sighting frequency of manatees within Belize's Drowned Cayes, with significantly higher numbers of manatees observed in the summer months (June-November). Anecdotal evidence from local fishers and BV's opportunistic sightings in BCMRNP in 2013, suggest that manatees may use the mangroves of BCMRNP to shelter from strong winds in the *Norte* season (Jenko *et al*., 2014). However, results from the current study did not show manatee sighting frequency or habitat to vary with season. This is likely due to the small sample size (n=10) and short survey period (one year). A better understanding of manatee habitat use and seasonality would be gained through ongoing monitoring of the BCMRNP area and a larger sample size.

Considering that only ten manatees were sighted along two routes (1 and 2) during dedicated manatee surveys in BCMRNP in 2014, seasonal use patterns cannot be confirmed from the results of this study. However, data suggest a spatial preference by manatees for habitat along Route 1. Observation points along this Route, particularly points 1B, 1C, and 1D, were areas which manatees frequented during all three seasons. This may be due to their proximity to high vegetation areas and a resting hole (points 1B and 1C), as well as a coastal lagoon (point 1D), though further research will be needed to test this theory. Indeed, La Commare *et al.* (2008) identified seagrass habitat and resting holes to be the most important variables related to manatee sightings in the Drowned Cayes.

Manatee sighting frequency has been shown to be associated with specific habitat characteristics, such as warm waters (above 22°C) and availability of freshwater (Auil, 2004; Gibson, 1995; Rathburn *et al.*, 1990). The current study found manatee sighting abundance was associated with water depth, wherein sighting abundance was higher in deeper areas, and percentage cover of *Thalassia testudinum*, suggesting they may prefer deeper habitats with more vegetation. Previous studies have found the primary diet of manatees consists of seagrasses such as *T. testudinum*, *Syringodium filiforme* and *Halodule* sp. (Alves-Stanley et al. 2010; Ledder 1986; Lefebvre et al. 2000; Mignucci-Giannoni 1998). While these results are preliminary, subsequent data on manatee habitat preferences will be important in identifying hotspots and informing management, especially as northern Ambergris Caye continues to develop.

Understanding manatee habitat use and any potential seasonal trends needs further long-term monitoring. The protocol outlined in this study should be followed for at least another four years to provide a larger five-year data set, from which trends can be inferred. Monitoring programmes such as this are crucial for protection of manatees and their habitat through providing scientific advice on best management actions for areas where manatees are found. These may include communication and enforcement of wildlife laws, such as the Belizean law against touching or following manatees (CZMAI), introduction of 'no wake zones' in high manatee-use areas to avoid manatee disturbance and collisions and ensure necessary protection of habitats crucial for the survival of the species such as feeding and nursing grounds. Long-term studies would also provide a baseline to monitor potential impacts of ongoing development on Ambergris Caye.

This report has presented the key findings of the first year of BV's manatee monitoring programme, working towards providing information on the abundance trends of manatee in BCMRNP and their spatial and temporal variation in distribution. A working method is outlined with results of the first year of monitoring, adding to the knowledge base on status of Belize's manatees.

Evaluation

During this first year of data collection, substrate type classification was not defined, resulting in inconsistency in results: 'silt' was recorded at all three locations during the *Norte* season, replaced by 'mud' in the dry and wet seasons. As of 2015, clear definitions should be followed, according to the following definitions in Coastal and Marine Ecological Classification Standard (CMECS) (CMECS, 2014):

- **Sand** is a surface layer which shows no trace of gravel and contains >90% sand (particles 0.0625mm to <2mm in diameter)
- **Mud** contains no trace of gravel and is comprised of 90% of mud (particles 0.0625mm) and the remained (<10%) is composed of sand particles
- **Silt** is a surface layer which has no trace of gravel and contains <10% sand, and the remaining mixture is 67% or more silt

Currently, BV uses a manual refractometer (model RHSN 10ATC) to measure salinity during manatee surveys, with an accuracy of +/- 0.10% (1ppt). However, we have expressed doubt about the accuracy and reliability of the refractometer, as the readings seem inconsistent and fluctuate widely. Furthermore, the range of salinity recorded at Barracuda Patch (15-23 ppt) seem unlikely to be true considering that this is a patch reef in the lagoon of the Belize barrier reef system, and the average salinity of seawater is 35 ppt (Weyl, 1966). The results from SACD's Water Quality Monitoring programme show that salinity was 36 ppt at Barracuda Patch at all depths in *Norte* 2013, 36 ppt in the dry season 2013 at Barracuda Patch at all depths, 35 ppt at all depths in rainy season 2013, and back to 36 at all depths for *Norte* 2014 (J. Chapman, pers. comm.). BV does not currently have equipment to measure water temperature, pH or dissolved oxygen content. A YSI Pro Plus Quattro with dissolved oxygen, salinity and temperature probes would allow more accurate and reliable data collection that could be coupled with manatee surveys, and therefore determine the role of temperature and salinity in manatee sightings more specifically.

More repetitions within one year would also give more power to statistical analyses from which to draw conclusions about manatee populations in the BCMRNP. Specifically, analyses for Route 2 are of quite low power due to small sample sizes, few repetitions, and few observation points along this route. Since the 2014 data collection effort, it has been emphasized that all observations be made at *least* once per season, and indeed data could be further strengthened with more frequent surveys. Furthermore, the 2014 manatee mapping methodology stipulates that surveys be performed between 0900 and 1230. Comparing surveys conducted at different times of day would provide insight into the temporal distribution of manatees in the area. This information could be useful in collecting larger sample sizes without increasing frequency of survey collection. Nonetheless, the data collected and shown here will serve as a basis for comparison from which to monitor change in manatee population and habitat in the reserve.

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Appendix 1: Detailed method of manatee surveys

Equipment:

- Slate
- Pencil
- Binoculars
- GPS
- Refractometer
- Depth meter
- Thermometer
- Secchi disk
- Underwater camera mounted on a pole

Method:

1. A minimum of 4 observers (including the lead scientist and a scribe) are needed. Each observer covers a 90° angle searching for manatees.
2. Surveys are performed along each Route between 0900 and 1230.
3. The boat follows the Route in a zigzag pattern on the outward journey, turning at narrow angles of 45° and a straight line Route on the return journey between the marked GPS points, unless the channel or lagoon is narrow/small enough to see well from one side to another.
4. If a manatee is observed at any time during the survey, record:
    a. Minimum number of individuals present at that point (i.e. the number of manatees the observer is 100% confident are different individuals)
    b. Best estimate of the number of individuals at that point (may be the same or greater than the minimum number of individuals)
    c. Location (mark using the GPS if between points)
    d. If possible, size of the individual(s) in meters
    e. Behaviour – choose from categories below:
        i. Feeding
        ii. Resting
        iii. Travelling
        iv. Socialising
        v. Social travel
        vi. Sexual
        vii. Milling (moving around aimlessly)
        viii. Boat interaction
        ix. Mixed (specify)
        x. Undetermined
5. All boat traffic is recorded along the Route, noting the type of boat (tourist, fisheries, etc.) and, if present, the manatee's behavioural response to the boat is recorded.

Behavioural categories:

- **Avoidance**: manatee swims away from boat / leaves area
- **Attraction**: manatee moves towards the boat / comes to the surface to investigate the boat
- **No response**: manatee carries on with behaviour observed before the boat interaction.

6. Each Route includes a different number of "observation points" (permanent, stationary monitoring locations). Upon arrival at an observation point, the captain turns off the engine and secures the boat using a wooden pole, not anchor.
7. Twenty-minute manatee scans are performed at these "observation points" with noise kept to a minimum.
    a. Record:
        i. Start and end time of point observation.

ii. Minimum number of individuals observed during the 20-minute scan
iii. Best estimate of individuals
iv. Location (use point codes)
v. Size of the individual(s) in meters, when possible
vi. Behaviour (in categories, as above)
vii. Environmental data:
    - Salinity (ppt)
    - Depth (m)
    - Turbidity (m)

8. If the conditions upon sighting are suitable, an attempt to collect videos and/or photos for later photo-identification of all individuals (seen opportunistically or during scans) is made. The camera is mounted to a stick and video taken from the boat. At no time will anyone deliberately enter the water in the presence of a manatee, in accordance with Belizean law.

Appendix 2: Detailed method of habitat monitoring

Equipment:

- Slate & pencil
- 0.5x0.5m quadrat made of PVC pipes divided into quarters with string
- Mask and snorkel
- GPS
- Thermometer
- Refractometer
- Depth meter
- Secchi disk
- Marker pen
- Camera
- Zip lock bags

Methods:

1. Between four and six people (including lead scientist and a scribe) are needed.
2. At each habitat monitoring area captain turns off the engine and the boat is allowed to float around the monitoring area.
3. The quadrat is haphazardly thrown from the boat 5 times.
4. GPS coordinates of the exact location within the monitoring area where the quadrat has landed are taken.
5. The snorkeler enters the water with a slate and camera
    a. Photograph the quadrat
    b. Record the main substrate type (Sand, mud, rock)
    c. Record the percentage vegetation cover by species.

Example: There is 50% vegetation cover consisting of three species. The percentage of these three species must add up to 50% (Thalassia 20%, Acetabularia 5%, Syringodium 20%).

6. If any new species are found or snorkelers cannot ID them in water, samples should be taken and put in a zip lock bag to be identified upon return to camp using (Littler et al. 1989, Marine Plants of the Caribbean). It is sufficient to identify all macro algae specimens to genus level.
7. From the boat, collect environmental data
    1. Salinity (ppt)
    2. Depth (m)
    3. Turbidity (m)
8. Upon returning from the survey photographs are added to the hard drive library, unknown specimens are identified.

Appendix 3: Vegetation recorded on habitat monitoring surveys

Thalassia testudinum

Syringodium filiforme

Halodule sp.

Batophora sp.

Turbinaria sp.

Sargassum sp.

Padina sp.

Stypopodium sp.

Turf Algae

Penicillus sp.

Halimeda sp.

Laurencia sp

Cyanobacteria

Cladophora sp.

Aurainvillea sp.

Hetrosiphonia sp.

Udotea sp.

Rhipocephalus sp.

Caulerpa sp.

Dictyota sp.

Appendix 4: Manatee mapping Routes and point details (BCDC)

Route	Point	GPS E (Latitude)	GPS N (Longitude)	Point details for manatee survey	Point details for habitat survey
1	Boca Bacalar Chico	410611, 18.186382	2010891, -87.845242	Route 1 start point	
	Mangrove Mouth (1A)	0410269, 18.185274	2010770, -87.848470	Observation Point	Habitat monitoring point (5 quadrats haphazardly thrown from this location)
	Manatee Hole (1B)	0409666, 18.182646	2010482, -87.854159	Observation Point (next to the hole)	Habitat monitoring point around and in between the two points (5 quadrats haphazardly thrown from these locations)
	Manatee Hole (1C)	0409670, 18.181824	2010391, -87.854117	Observation Point (towards the end of the channel)	
	Turtle Pond (1D)	0409532, 18.179287	2010111, -87.855410	Observation Point	Habitat monitoring point (5 quadrats haphazardly thrown from this location)
	Tea Cup (1E)	0409528, 18.178690	2010045, -87.855445	Observation Point	
	Lovers Tunnel	0409518, 18.178536	2010028, -87.855538	Route 1 end point	
2	BCDC	0411564, 18.153595	2007259, -87.836075	Route 2 start point	
	Barracuda Patch N (2A)	0411811, 18.168807	2008941, -87.833812	Observation point (2nd buoy coming from BCDC)	Observation point (haphazardly between and around the 2 mooring buoys)
	Boca Bacalar Chico	0410611, 18.186382	2010891, -87.845242	Route 2 turn around point	

	Barracuda Patch S (2B)	0411755, 18.167865	2008837, -87.834337	Observation point (1st buoy coming from BCDC)	
	BCDC	00411564, 18.153595	2007259, -87.836075	Route 2 end point	
3	Lovers Tunel	0409518, 18.178536	2010028, -87.855538	Route 3 start point	
	Crocodile Lagoon (3A)	0409446, 18.177566	2009921, -87.856214	Observation Point	Habitat monitoring point around and in between the two points (5 quadrats haphazardly thrown from these locations)
	Crocodile Lagoon (3B)	0409505, 18.176340	2009785, -87.855651	Observation Point	
	Dusk Lagoon (3C)	0409732, 18.176575	2009810, -87.853506	Observation Point (at the inlet entrance)	Habitat monitoring point around and in between the two points (5 quadrats haphazardly thrown from these locations)
	Dusk Lagoon (3D)	0410262, 18.175440	2009682, -87.848489	Observation point (at the furthest part of the lagoon from the inlet entrance)	
	Dusk Lagoon (3E)	0409967, 18.172843	2009396, -87.851266	Observation point (on the south side of the lagoon, roughly in the middle of the lagoon length)/Route 3 end point	
4	Cantena Lagoon North	0409472, 18.169280	2009004, -87.855928	Route 4 start/end point	Habitat monitoring point (5 quadrats haphazardly thrown)
	Cantena Lagoon Centre	No GPS point set, roughly middle of lagoon		N/A	Habitat monitoring point (5 quadrats haphazardly thrown)

	Cantena Lagoon South	0411621, 18.125227	2004120, -87.835402	Route 4 turn around point	Habitat monitoring point (5 quadrats haphazardly thrown)

black coordinates= NAD 27 Central, UTM
green coordinates = WGS 84 decimal degrees

Appendix 5: Statistical values of non-significant comparisons

Comparison	Test Performed	Test Value	P Value	df
SPUE between Routes	K-W	H=3.00	0.392	3
SPUE between Seasons	K-W	H=.6671	0.716	2
SPUE between Routes 1 & 2	M-W	W=0	0.077	1
Mean manatee sighting abundance between observation points	K-W	H=13	0.448	13
Mean manatee sighting abundance between seasons	K-W	H=.02311	0.989	2
Manatee sighting abundance between Route 1 observation points	K-W	H=.5062	0.281	4
Manatee sighting abundance between Route 2 observation points	WSR	W=1	1	1
Salinity by observation point	2-way ANOVA	F=1.31	0.27	13
Mean vegetation cover (%) between seasons	K-W	H=.9723	0.615	2
Mean vegetation cover (%) between observation points	K-W	H=4.404	0.819	8
Species composition (%) along Route 1 by observation point	K-W	H=.9123	0.634	2
Species composition (%) along Route 1 by season	K-W	H=.9044	0.636	2
Species composition (%)	M-W	Z=219	0.973	1

along Route 2 by season				
Species composition (%) along Route 3 by observation point	M-W	Z=205	0.636	1
Species composition (%) along Route 3 by season	K-W	H=.1332	0.936	2
Species composition (%) along Route 4 by observation point	K-W	H=.2070	0.902	2
Species composition (%) along Route 4 by season	K-W	H=1.011	0.603	2
Vegetation cover (%) by observation point (annual)	K-W	H=8.000	0.434	8
Vegetation cover (%) by route (annual)	K-W	H=3.000	0.392	3