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Population estimates of Antillean manatees in Puerto Rico: an analytical framework for aerial surveys using multi-pass removal sampling

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Effective management of the threatened Antillean manatee (*Trichechus manatus manatus*) in Puerto Rico requires reliable estimates of population size. Estimates are needed to assess population responses to management actions, and whether recovery objectives have been met. Aerial surveys have been conducted since 1976, but none adjusted for imperfect detection. We summarize surveys since 1976, report on current distribution, and provide population estimates after accounting for apparent detection probability for surveys between June 2010 and March 2014. Estimates in areas of high concentration (hotspots) averaged 317 ± 101, three times higher than unadjusted counts (104 ± 0.56). Adjusted estimates in three areas outside hotspots also differed markedly from counts (75 ± 9.89 versus 19.5 ± 3.5). Average minimum island-wide estimate was 386 ± 89, similar to the maximum estimate of 360 suggested in 2005, but fewer than the 700 recently suggested by the Puerto Rico Manatee Conservation Center. Manatees were more widespread than previously understood. Improving estimates, locally or island-wide, will require stratifying the island differently and greater knowledge about factors affecting detection probability. Sharing our protocol with partners in nearby islands (e.g., Cuba, Jamaica, Hispaniola), whose populations share genetic make-up, would contribute to enhanced regional conservation through better population estimates and tracking range expansion.

El manejo efectivo del manatí antillano amenazado en Puerto Rico requiere estimados de tamaños de poblaciónes confiables. Dichas estimaciones poblacionales son necesarias para evaluar las respuestas a las acciones de manejo, y para determinar si los objetivos de recuperación han sido alcanzados. Se han realizado censos aéreos desde 1976, pero ninguno de ellos han sido ajustados para detecciones imperfectas. Aquí resumimos los censos desde 1976, actualizamos la distribución, y reportamos los primeros estimados poblacionales ajustados para la probabilidad de detección aparente en los censos de Junio 2010 a Marzo 2014. Las estimaciones poblacionales en áreas de mayor concentración del manatí promedió 317 ± 103, tres veces más abundante que los conteos sin ajuste (104 ± 0.56). Las estimaciones poblacionales en tres áreas fuera de las áreas de mayor concentración del manatí también fueron marcadamente diferentes (75 ± 9.89 vs 19.5 ± 3.5). El estimado mínimo poblacional en la isla entera fue de 386 ± 89, similar al estimado máximo de 360 sugerido en el año 2005, pero menor a los 700 sugeridos recientemente por el Centro de Conservación de Manatíes de Puerto Rico. Documentamos que el manatí tiene una distribución más amplia de lo que se sabía con anterioridad. El mejoramiento de los estimados poblacionales locales o a nivel de isla requerirá que se estratifique a la isla en forma diferente y que se investiguen los factores que influencian a la probabilidad de detección. Compartir protocolos como este con colaboradores de islas vecinas (por. ej., Cuba, Jamaica, Española), cuyas poblaciones de manatíes comparten material genético, contribuiría a la conservación regional mediante mejores estimaciones poblacionales y monitoreo de la expansión de su ámbito doméstico.

Key words: aerial survey, Antillean manatee, detection probability, population size, Puerto Rico, removal method, repeated counts

The Antillean manatee (*Trichechus manatus manatus*), a subspecies of the West Indian manatee (Domning and Hayek 1986), was listed as a federally endangered species in Puerto Rico in 1970 (35 Federal Register, 18319, 2 December 1970), but down-listed as part of the re-classification of the West Indian manatee as threatened in the United States (United States Fish and Wildlife Service 2007, 2017). Since its initial listing, federal and territorial agencies, as well as non-governmental organizations, have implemented various recovery activities that include mortality assessments (Mignucci-Giannoni et al. 2000; Bonde et al. 2012), rescue and rehabilitation efforts (Adimey et al. 2012), environmental education, community outreach, regulatory efforts, habitat mapping, and monitoring (United States Fish and Wildlife Service 2007). Education and regulatory efforts have targeted the public, particularly recreational boaters to minimize incidental watercraft collisions, the most common cause of mortality in the island (Mignucci-Giannoni et al. 2000; Bonde et al. 2012). Habitat mapping has been used to identify potential manatee protection areas that maximize habitat suitability and minimize watercraft collisions (Drew et al. 2013).

The manatee population in Puerto Rico has been monitored since 1976 with most surveys conducted from single-engine, high-wing airplanes (Powell et al. 1981; Rathbun et al. 1985; Freeman and Quintero 1990; J. P. Zegarra, USFWS Caribbean Field Office, pers. obs.). Descriptive statistics of aerial surveys since 1976 are summarized in Table 1. Total counts from 1976 to 2009 ranged from eight (Freeman and Quintero 1990) to 125 (2002; J. P. Zegarra, USFWS Caribbean Ecological Services Field Office, pers. obs.). Mignucci-Giannoni (2005) conducted an island-wide helicopter survey reporting a count of 116 and 21 calves, and speculated that the total population in Puerto Rico ranged from 150 to 360. More recently, Mignucci-Giannoni et al. (2018) summarized helicopter surveys along the southern coast of Puerto Rico from 2001 to 2015. Counts in the southern region of the island averaged 61.7 ± 35.9 (n = 9) and ranged from 16 to 134. After expressing counts on a per unit effort basis (hours), Mignucci-Giannoni et al. (2018) also indicated that the population size along the southern coast of Puerto Rico was increasing, particularly after 2007. Surveys from both aerial platforms, coupled with radiotelemetry (Slone et al. 2006), have highlighted several locations along the coast where manatee presence and activity are consistently higher. Slone et al. (2006) and Mignucci-Giannoni et al. (2018) suggested that these patterns were likely associated with low wave action, available food, and sources of fresh water for drinking.

Reliable estimates of population size are critical for effective management of manatee populations (United States Fish and Wildlife Service 2007; Martin et al. 2015; Alves et al. 2016). Estimates provide a basis to assess population responses to management (recovery) actions, and thus, gauge whether recovery objectives have been met. Moreover, if data are available over multiple years, it is also possible to reliably estimate other population metrics such as trends and observed growth rates (Caughley 1977; Lefebvre et al. 1995; Langtimm et al. 2011). Demographic inferences from available counts, however, have serious limitations due to several unique factors that result in imperfect detection of individuals (Lefebvre et al. 1995; Pollock et al. 2006; Langtimm et al. 2011; Martin et al. 2014, 2015). First, manatees occur over a large geographic area. A frame bias might be introduced if the survey area is smaller than the area manatees occupy and not all individuals are available to be seen (Lefebvre et al. 1995; Reynolds et al. 2012). Second, manatees in the survey area may spend a portion of their time at depths that make them impossible to be observed. Finally, observers, even in ideal conditions, are likely to miss some individuals at the surface during a survey. This phenomenon introduces perception bias. Imperfect detection from all sources affects the estimation of population size as observers invariably count a fraction of detectable individuals while surveying. Not adjusting for detection probability could lead to biased population estimates and lead to unreliable population parameters (Pollock et al. 2006; Langtimm et al. 2011).

Table 1.—Summary of aerial surveys of Antillean manatees (*Trichechus manatus manatus*) conducted in coastal waters and adjacent islands of Puerto Rico since 1976.

Dates	Number of surveys	Platform	Effort-hours	Lowest count	Highest count	% calves	Manatees/effort-hour	Calves/effort-hour	Abundance estimate	Source
1976–1979	10	Airplane	71.5	11	51	6.4	3.1	0.2	None	Powell et al. (1981)
1984–1985	12	Airplane	57.4	20	62	7.6	9.1	0.7	None	Rathbun et al. (1985)
1989–1990	10	Airplane	41.1	8	28	14.1	4.7	0.5	None	Freeman and Quintero (1990)
1992–2009	27	Airplane	156.6	22	125	7.1	11.8	0.8	~100[a]	USFWS, unpubl. data
2005	1	Helicopter	9.5	116	116	18.1	12.2	2.2	150–360	Mignucci-Giannoni (2005)
2001–2015	33	Helicopter	70.5	16	134	12.4	13.0	1.6	None	Mignucci-Giannoni et al. (2018)[b]
2010–2014	7	Airplane	84.7	76	194	10.3	11.3	1.2	386[c]	This study

[a] Reported by Slone et al. (2006).
[b] Just south coast.
[c] Average minimum population size = point estimate at hotspots + point estimate at three areas in non-hotspot zone + count in remaining non-hotspot zone.

The detection process for manatees is often thought of as being a function of two probabilities: availability and perception probabilities (Lefebvre et al. 1995; Pollock et al. 2004; Martin et al. 2015). Availability probability is the probability (fraction of the time) that an individual is close enough to the surface to be detected. Perception probability is the probability that observers see an animal given that it is available. Telemetry, animal models, mark-recapture, and depth recorders are some of the methods that have been used to ascertain diving behavior and estimate the fraction of time animals are available for detection (Marsh and Sinclair 1989; Marsh and Rathbun 1990; Pollock et al. 2006; Edwards et al. 2009). However, the estimation of availability and perception probabilities for a wide variety of species has a long history and has been a central focus in the biometric literature (Williams et al. 2002). In the aerial survey literature, the majority of survey designs for estimating detection probability are based on some form of replication. This replication is broken into two classes: 1) independent or dependent observers as replicates of a sampling unit, and 2) repeated counts or removal sampling. In the independent observer designs, the aircraft is modified to isolate observers so that the behavior of one observer does not influence the sightings made by the other (Pollock et al. 2006; Langtimm et al. 2011). In a dependent observer design, a primary observer looks for animals, while a secondary observer notes the individuals both see but also those missed by the primary observer (Nichols et al. 2000). Either of these designs is often coupled with distance sampling (Buckland et al. 2001, 2010; Borchers et al. 2006).

In this study, we surveyed the entire coastline of Puerto Rico using a multi-pass removal sampling scheme to estimate population size (Royle and Nichols 2003; Dorazio et al. 2005; Kery et al. 2005; Langtimm et al. 2011). This approach was adopted for several reasons. First, airplanes were not fitted with the appropriate partitions to collect data under an independent observer's protocol (Pollock et al. 2006). Second, personnel roles and positions (e.g., observers, recorder) followed the arrangement used in the past to ensure count data continuity. Third, the use of transects was not suited for the small and irregularly shaped embayment or sampling units in Puerto Rico. In a repeated count survey, sample units are sampled repeatedly, and during each repeat, the total number of individuals is recorded (Royle and Nichols 2003; Kery et al. 2005). Additionally, the number of new individuals is counted with every pass in each survey unit, the removal portion of the sampling scheme. This sampling scheme is analogous to the time-of-detection method (Alldredge et al. 2007; Nichols et al. 2009), whose aim is to partition availability and detection probabilities when surveying avian species. Apparent detection probability is defined as the product of the perception and availability probabilities. In other words, apparent detection is the probability of manatees being available for detection and being detected during a pass or repeat count.

Herein, our objective was to provide federal and territorial agencies with a sampling design to reliably estimate population size as stipulated in the species recovery plan (Rathbun and Possardt 1986). Adjusted estimates were obtained from five island-wide surveys conducted using a high-wing airplane between June 2010 and March 2014. In concert with historical survey protocols, we initially focused in areas of high manatee concentrations (henceforth termed hotspot zones), but extended the multi-pass removal sampling protocol to three areas outside of hotspots during the last two surveys. We summarized descriptive statistics of all prior aerial survey counts conducted since 1976, provide minimum island-wise population estimates, and report on the current distribution of manatees. We make recommendations on how to improve future surveys, and discuss the conservation implications of results.

Materials and Methods

Study area and survey protocol.—The study area encompassed the entire coast of the island of Puerto Rico, including offshore cays and islands (Isla de Culebra, Isla de Vieques, Caja de Muerto), but excluding Isla Desecheo and Isla de Mona (Fig. 1). Manatees have never been seen in the latter two islands, perhaps because they are far off the coastline of Puerto Rico and have no freshwater streams. We stratified surveys into two zones. The first zone was designated as "hotspots." These were embayments, shallow coastal areas, and areas near river mouths, where higher numbers had been consistently recorded in the past in Puerto Rico (Mignucci-Giannoni 1989, 2005; Slone et al. 2006; United States Fish and Wildlife Service 2007). These were: puerto Medio Mundo, Ensenada Honda, bahía de Algodones, puerto Patillas, bahía de Jobos, bahía de Rincón (Salinas), bahía de Tallaboa, bahía de Guayanilla, bahía de Guánica, and punta Guanajibo (Fig. 1). For each of these hotspots, boundaries were specified with the help of local experts such that each had a specific survey area. The remainder of the coast of Puerto Rico was designated as the non-hotspot zone, and within it some areas were sampled opportunistically as air traffic restrictions permitted (e.g., Parguera–September 2011).

Seven surveys were conducted between June 2010 and March 2014, following an adaptation of general aerial survey protocols for manatees (Reynolds et al. 2012). We provide summary statistics for the seven surveys, but used only five of the surveys to estimate population numbers adjusted by apparent detection probability. We excluded the survey of March 2012 because > 40% of the coastline could not be surveyed due to inclement weather and sea conditions. We also excluded the survey of December 2013 due to difficulties reconciling input data for modeling. Flights maintained an altitude of 152.3 m ± 5.2 ($\overline{X} \pm SD$) and airspeed of 146.9 km/h ± 11.4. The flight crew was divided into four roles: pilot, co-pilot–observer, recorder, and observer. These roles and the personnel assigned to them did not change throughout this work. The co-pilot–observer job was to direct the route, communicate with the pilot, and look for manatees on the frontal right side of the aircraft. The other observer's role was to look for manatees on the left side (behind pilot). Behind the co-pilot–observer sat the recorder, whose job was to record all manatee detections and plot manatee

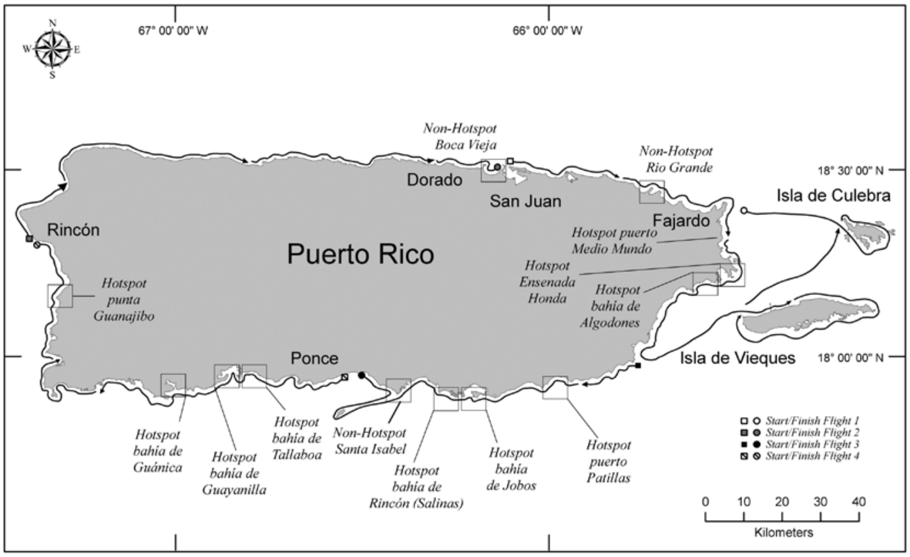

Fig. 1.—Map of Puerto Rico depicting hotspot survey zones (squares) along the coast of the island, and the flight routes followed during multiple days to complete surveys. The remainder of the coast was considered the non-hotspot zone. Three areas within the non-hotspot zone were sampled in January 2013 and March 2014 using the same sampling scheme as in hotspots. Starting and ending point of each flight is indicated with symbols.

locations on a nautical chart. The recorder also kept track of "new" manatees on every pass in coordination with observers. Generally, an entire survey was a 2- to 4-day event needed to safely and efficiently (e.g., due to observer fatigue) survey 700 km of coast line (i.e., main island, adjacent islands and cays). Flight dates were selected to maximize good visibility over the entire length of the flight survey extent. Good visibility included both the sea state and water turbidity (e.g., flights after strong rain events were avoided). The island was surveyed in a clockwise direction following pre-selected routes, but with different starting points depending on weather and pending coverage of coastal zones (Fig. 1). Upon entering a zone, the recorder indicated to observers that the multi-pass removal sampling was to start. For a relatively fixed period of time (~ 5 min, hereafter a "pass"), observers looked for manatees within the boundaries of each hotspot flying straight as much as possible, depending on the shape of the contours of the coastline and shape of sampling zones. At the end of the pass, a new pass began with active searching within the hotspot again. The recorder would note all previously undetected manatees as well as the pass number (i.e., second, third, etc.). This process continued until the target number of passes had been completed (four or six). Our estimates of apparent detection probabilities are, therefore, expressed on a 5-min (i.e., pass) basis. We highlight two important advantages of our protocol. First, 5 min encompassed the known diving time (2–3 min) of Antillean manatees (Hartman 1979). Second, four to six passes meant that zones were sampled for 20–30 min, a period that should accommodate longer dives (e.g., foraging). Manatee counts throughout the non-hotspot zone were conducted in the same way as historical counts, that is, we simply counted manatees. For surveys conducted in January 2013 and March 2014, we extended the multi-pass removal sampling approach to three areas within the non-hotspot zone (Fig. 1). These areas had attributes deemed attractive to manatees (e.g., embayment, proximity to fresh water). Adjusted estimates were restricted to only hotspot zones between June 2010 and September 2011. For January 2013 and March 2014, estimates included hotspot zones and the aforementioned areas within the non-hotspot zone. For an island-wide estimate, we report a minimum population size. This estimate is defined as the sum of the detection-adjusted point estimate in hotspot zones + total count outside multi-pass removal zones (non-hotspot zone) for surveys between June 2010 and September 2011. For surveys in January 2013 and March 2014, the minimum estimate is defined as the sum of the detection-adjusted point estimate in hotspot zones + total count outside multi-pass removal zones (non-hotspot zone) + the detection-adjusted point estimate in three non-hotspot areas.

Modeling.—We used a Bayesian statistical model to estimate the population size within hotspots and three areas within the non-hotspot zone. Let N_i represents the number of manatees in survey location i, with $x_{i,j}$ indicating the number of manatees in location i first detected in pass j, $i = 1, \ldots, I$, $j = 1, \ldots, J_i$. Then $x_{i,j}|N_{i,}\ x_{i,1},\ xi_{,2},\ x_{i,j-1,j-1,}\ d_{i,j} \sim Binom\left(N_i - \sum_{k-1}^{j-1} x_{i,k},\ p_{i,j}\right)$, where $p_{i,j} = d_{i,j} * \prod_{k=1}^{j-1}(1 - d_{i,k})$ is the apparent probability of being available for detection and detected within the duration of a pass. The priors for the N_i were selected as $DiscUniform$ $\left(\sum_{k=1}^{j} x_{i,j},\ \sum_{k=1}^{j} x_{i,j} + K\right)$ where $DiscUniform$ is the discrete uniform distribution, K is an arbitrary constant selected to be large enough to be well outside any estimates, but not so large as to make computation excessively slow. For hotspots, we use a K of 35. Several approaches were considered to add additional structure to $p_{i,j}$, including hierarchical priors and use of covariates; however, none of these approaches yielded better than a fixed detection probability across all survey locations. The prior for $d_{i,j} = d \sim beta(1,1)$. For surveys where hotspot and non-hotspots were surveyed with multiple passes, we note how the Bayesian removal sampling allows for additional flexibility. Several innovations could occur but here we highlight one: allowing each repetition zone to have its own detection probability. As before, let $N_{i(h)}$ represent the total number of manatees in unit $i_{(h)}$, where i is the ith repetition zone in type $h \in 1 \ldots N_h$, where h represents repetition zone type (here, hotspot or non-hotspot, $h \in 1, 2$). The apparent detection probability in unit i of type h, $d_{i(h)} \sim Beta(\alpha_h, \beta_h)$, where $\alpha_h, \beta_h \sim Uniform(0, 1{,}000)$. Then $p_{i(h),j} = d_{i(h)} \prod_{k=1}^{j-1} (1 - d_{i,h})$ where j represents the pass number, $j = 1, \ldots, Ni_{(h)}$. Then $x_{i(h),j} \mid N_{i(h)}, x_{i(h)},1, \ldots x_{i(h),j-1} \sim Binom\left(N_{i(h)} - \prod_{k=1}^{j-1} x_{i(h),k}, p_{i(h),j}\right)$. For the MCMC computation, we used PyMC2 (Patil et al. 2010). A single chain was run for 20,000,000 iterations, with a burn-in of 100,000 thinning to every 20th observation. We summarized parameter estimates for population size and apparent detection probability as $\overline{X} \pm SD$ and report their model-based 95% credible confidence intervals (*CCI*s) for location-specific estimates. We provide the Python code used to analyze data (Supplementary Data SD1), and detailed results for each survey (Supplementary Data SD2 and SD3). Results include the effective detection probability, E, $\left(1 - (1 - d)^{J_i}\right)$ or the probability that a manatee within a location i was detected at least once within four passes. We also report the point estimate (± *SD*) and 95% *CCI*s for location-specific estimates. Coefficients of variation were derived using *SD* model-based values (Supplementary Data SD2 and SD3).

Results

Seven surveys were flown between June 2010 and March 2014 with an average survey effort-hours of 12.1 ± 1.5 (Table 2). The total number of manatee sightings per survey averaged 96.9 ± 29.7 and the total number of manatees observed per survey averaged 138 ± 39.4 (min 76, max 194). Of the total number of manatees counted, 10.0% were calves, with an average of 13.9 ± 4.8 mother–calf pairs sighted per survey (Table 2). The number of manatees observed per effort-hour varied between 8.4 and 15.9, with an average of 11.3 ± 2.9 for the entire study. Manatees per effort-hour during this study were similar to counts made from helicopter between 2005 and 2018 (Table 1).

Manatees were sighted from the municipality of Dorado on the north-central coast, clockwise around the island, throughout its south coast, to just south of punta Ensenada in the municipality of Rincón on Puerto Rico's west coast (Fig. 2). Manatees were sighted off isla de Culebra, isla de Vieques, and Caja de Muertos, as well as off many of the coastal cays of the east and south coast. A marked gap of sightings occurred from Rincón clockwise to Dorado on the north coast, from Fajardo to Culebra (La Cordillera island chain), and in the municipality of Maunabo on the southern corner of the island. Concentrations of manatees were repeatedly found in Rio Grande, Ceiba and Naguabo, Patillas, Guayama and Salinas, Santa Isabel, Peñuelas and Guayanilla, Guánica, and between Cabo Rojo and Mayagüez (Fig. 2).

We used data from five surveys to estimate population numbers in hotspot zones and three non-hotspot zones, after adjusting for apparent detection probability (Table 3). Estimates in hotspot zones averaged 317 ± 101, fluctuating between 214 (March 2014) and 494 (September 2011). Estimates from three areas within the non-hotspot zone averaged 75 ± 9.89 (Table 3). Apparent detection probability averaged 0.08 in hotspot zones, whereas it averaged 0.09 in three areas within the non-hotspot zone. Coefficients of variation for point estimates in hotspots ranged between 9% and 20% and between 22% and 28% for three areas within non-hotspot zone. Coefficients of variation for apparent detection probability in hotspots ranged between 14% and 50% (Supplementary Data SD2) and in three areas within the non-hotspot zone between 44% and 64% (Supplementary Data SD3). The average adjusted estimate per hotspot zone was three times higher than the average total unadjusted count (Fig. 3). Unadjusted counts averaged 104.60 ± 49.56, fluctuating between 80 and 194. The minimum island-wide population estimate averaged 386 ± 89 manatees (range 312–535; Table 3).

Discussion

We report on the current distribution of manatees and estimate numbers of manatees in coastal Puerto Rico, accounting for apparent detection probability. Manatee distribution during the study followed the general historical distribution described by Powell et al. (1981), Rathbun et al. (1985), Mignucci-Giannoni (1989), and Freeman and Quintero (1990), in which manatees

Table 2.—Summary statistics of seven Antillean manatee (*Trichechus manatus manatus*) surveys conducted in coastal waters and adjacent islands of Puerto Rico, 2010–2014. M–C = mother–calf.

Date	Effort-hours	Sightings	Total number of manatees/survey	M–C pairs	Manatees per effort-hour
Jun. 2010	12.17	80	120	9	9.9
Oct. 2010	12.63	98	138	10	10.9
Sept. 2011	11.18	128	178	17	15.9
Mar. 2012	9.06	55	76	12	8.4
Jan. 2013	12.94	103	142	14	11.0
Dec. 2013	13.33	139	194	23	14.6
Mar. 2014	13.38	75	118	12	8.8
Total	84.7	678	966	97	
Average	12.1	96.9	138.0	13.9	11.3
SD	1.5	29.7	39.4	4.8	2.9

are found from the town of Dorado eastwards along the east (including Vieques Island) and south coasts, and up the west coast reaching the Guanajibo River between Cabo Rojo and Mayagüez. The new surveys recorded the previously unreported use of Isla de Culebra and Rincón, as well as use of Caja de Muertos and the offshore cays of La Parguera, bahía de Tallaboa, and bahía de Jobos. However, the study did not yield manatee sightings north of Rincón and east towards Vega Alta, and in San Juan and Carolina, locations where recent sightings have been recorded (i.e., Aguadilla, Isabela, Camuy, Hatillo; A. A. Mignucci-Giannoni, Puerto Rico Manatee Conservation Center, pers. obs.). The lack of sightings in San Juan and Carolina, particularly bahía de San Juan, laguna del Condado, río Puerto Nuevo, and laguna La Torrecilla, areas where frequent land-based manatee sightings have been made (A. A. Mignucci-Giannoni, Puerto Rico Manatee Conservation Center, pers. obs.), was likely due to difficult access to these areas because they were within the landing approach to an international and a regional airport. The distribution and area-specific concentrations observed during the study (Ceiba, Patillas, Guayama, Salinas, Guayanilla, Guánica, and Cabo Rojo–Mayagüez), validate the selection of hotspots as the most important areas for manatees, except for Rio Grande's Ensenada Comezón and punta Petrona in Santa Isabel, which should be considered hotspots in future studies. This study, and recent records (A. A. Mignucci-Giannoni, Puerto Rico Manatee Conservation Center, pers. obs.), suggest that manatees are more widespread than previously thought, perhaps expanding their distribution and exploring new areas previously unreported for the species in Puerto Rico. A similar pattern of range expansion as source population increases has been observed for the Florida manatee (Reid 2000; Bonde and Lefebvre 2001; Fertl et al. 2005; Mellilo-Sweeting et al. 2011). The extent of this natural dispersal as the population of manatees in Puerto Rico expands would need to be corroborated as well as evidence of reproduction and calving.

Our adjusted estimates of population size in hotspot zones, areas considered of importance for manatees in Puerto Rico, were, on average, three times higher than unadjusted counts in the same zones. Similarly, adjusted estimates were nearly four times higher than unadjusted counts in three areas within the non-hotspot zone of the island in January 2013 and March 2014. Differences underscore the importance of adjusting for imperfect detection, as ignoring it might lead to biased

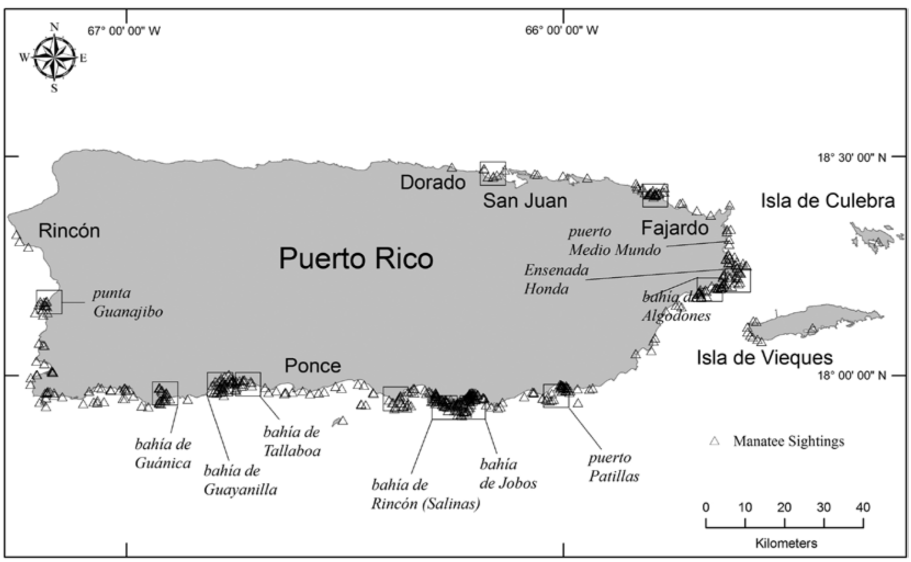

Fig. 2.—Map of Puerto Rico depicting distribution of observed Antillean manatees (*Trichechus manatus manatus*; triangles) during seven aerial surveys relative to hotspot zones (squares). Surveys were conducted between June 2010 and March 2014.

Table 3.—Parameter estimates of population size (*n*, 95% credible confidence intervals [*CCI*s]) and apparent detection probability (*p*, 95% *CCIs*) of Antillean manatees (*Trichechus manatus manatus*) derived from aerial surveys conducted in hotspot (HS) and non-hotspot (NHS) zone along coastal Puerto Rico. Estimates of three areas within the NHS zone were derived from surveys in January 2013 and March 2014. Minimum island-wide estimates between June 2010 and September 2011 were defined as the sum of the adjusted point estimate at HS + non-adjusted count in the NHS; for those in January 2013 and March 2014, the minimum was defined as the adjusted point estimate at HS + non-adjusted count in NHS + adjusted point estimate at three areas within the non-hotspot zone. Empty rows indicate "not estimable."

Survey date	Parameter estimates (*CCI*s)				
	n_{HS}	p_{HS}	n_{NHS}	p_{NHS}	$Minimum_{Island\text{-}wide}$
Jun. 2010	347.60 (257, 432)	0.05 (0.04, 0.07)			387.6 (297, 472)
Oct. 2010	271.50 (171, 372)	0.09 (0.05, 0.15)			322.5 (222, 423)
Sep. 2011	479.20 (394, 562)	0.07 (0.05, 0.08)			535.0 (450, 618)
Jan. 2013	276.40 (199, 348)	0.07 (0.03, 0.27	68 (32, 102)	0.10 (0.03, 0.27)	374.0 (286, 455)
Mar. 2014	214.30 (146, 287)	0.14 (0.05, 0.28)	82 (45, 112)	0.09 (0.03, 0.18)	312.0 (233, 395)

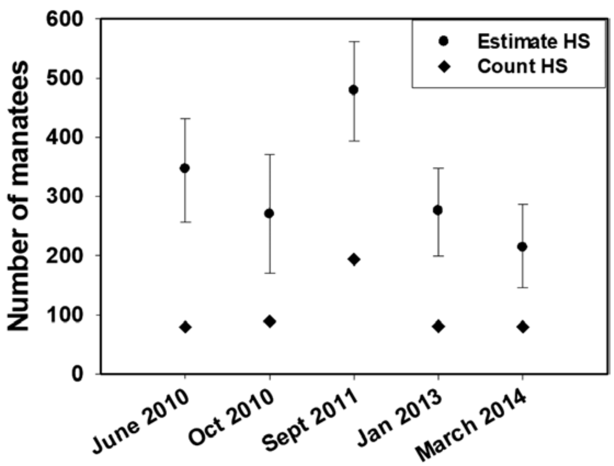

Fig. 3.—Estimated number (95% *CCI*s) and total unadjusted count of Antillean manatees (*Trichechus manatus manatus*) in hotspot (HS) zones in coastal Puerto Rico obtained from five aerial surveys conducted between June 2010 and March 2014.

estimates and spurious inference about population status and responses to recovery actions (Pollock et al. 2006; Langtimm et al. 2011). Adjusting for detection probability is also paramount to model where species occur rather than where species are detected, essential for distribution studies (Guillera-Arroita et al. 2014). Lahoz-Monfort et al. (2014) demonstrated that disregarding imperfect detection could compromise the identification of optimal habitat for a species and misguide spatial prioritization of habitat.

Initially, we implemented multi-pass removal sampling only in hotspot zones, but as observers gained experience, we extended the scheme to three areas in the non-hotspot zone that featured attributes attractive to manatees (e.g., embayment, fresh water). Extending the multi-pass removal sampling to areas outside of hotspots can be viewed as the first step toward a more comprehensive sampling scheme that obviates the use of ad hoc (unadjusted) or minimum population estimates, or requires the assumption of equal detection probability between hotspots and non-hotspots to assess island-wide population status. The latter assumption is difficult to meet without additional information because it is possible that manatees behave differently (e.g., diving times) in different zones (e.g., foraging versus movement between areas of high concentrations). The airplane and observer role setup that we employed did not allow the estimation of observer-specific detection probabilities. Researchers wishing to assess observer-specific differences could adopt a double-observer sampling approach (Cook and Jaconson 1979; Nichols et al. 2000, 2009; Koneff et al. 2008). Inferentially, an independent observer scheme is stronger (Nichols et al. 2000), and the setup and protocol followed by Pollock et al. (2006) is a good example of how independent observers contribute toward the estimation of detection probability and population estimate. The alternative is a dependent observer scheme, requiring that the primary and secondary observers be seated on the right side, behind each other, and engaging in frequent in-flight communication to reconcile counts. We encourage readers to review Koneff et al. (2008) on the effects of observer roles on population estimation using airplanes. We stress that the double-observer sampling scheme, by itself, does not explicitly yield estimates of or accounts for availability. It is analogous to distance sampling in that, by itself, it provides an estimate of individuals (e.g., manatees) at a snapshot in time. A multi-method approach needs to be considered to account for availability (Alldredge et al. 2006; Pollock et al. 2006; Nichols et al. 2009; Martin et al. 2015).

Mignucci-Giannoni et al. (2018) used helicopters to conduct island-wide (2005) and southern-coast (2001–2015) surveys. One of the main advantages of this platform is that it offers observers the option to be stationary over an area of interest. Adopting this platform, coupled with the sampling approached outlined herein, would likely improve detection probability, but costs might be prohibitive (~US$1,600/h for helicopters versus ~US$180/h for aircraft). The use of drones might be an alternative, and has been proven to work to survey a small area (Hodgson et al. 2013; Ruíz and Sabater 2018), but it is likely not a practical approach to conduct island-wide surveys. In light of these constraints, island-wide surveys will likely continue to be conducted with high-wing airplanes.

We offer the following recommendations to improve the reliability of surveys from high-wing airplanes, although they are also applicable to helicopters. First, apparent detection probabilities in hotspots and three areas within non-hotspots were low (< 0.15), and those within areas within the non-hotspot zone exhibited greater variability. Improvements in detection probability in either zone could be gained from studies of factors that impinge upon detection probability (e.g., depth preferences, turbidity, diving times in different sampling strata—sensu Pollock et al. 2006). Findings would help improve survey design, but also identify covariates that could be incorporated into estimation models to strengthen the inferential power of results, not only about population estimates, but also about distribution and habitat quality (Guillera-Arroita et al. 2014; Lahoz-Monfort et al. 2014). Possible covariates could include sea conditions, proximity to fresh water, depth, and presence of seagrass as in Mignucci-Giannoni et al. (2018).

Second, we believe the rationale behind how Puerto Rico's coastal zone is stratified should be revised to more aptly generate data for island-wide estimates (sensu Martin et al. 2015 in Florida). One possibility would be to divide the island's coastal zone into smaller sampling units, perhaps using the average size of current hotspot zones as an initial guideline. To minimize costs, selected units could get a random number of samples (e.g., 1, 3, 5), thus, ensuring that all units get at least one pass (i.e., 5-min coverage). Historical survey data and this study strongly suggest that manatees are less abundant in the northern coast of the island. Thus, consideration could also be given to proportionally allocating the one-pass sampling efforts. Importantly, the proposed stratification would minimize the prospect of misidentifying "hotspots," which could carry the consequence of inducing a frame or coverage bias (Lefebvre et al. 1995; Nichols et al. 2009). This prospect was underscored by estimates from three areas within the non-hotspot zone that were comparable to many hotspot zones (e.g., Rio Grande's Ensenada Comezón). Misidentifying hotspots might also preclude extending some recovery actions to other areas where it might be warranted (e.g., watercraft education). It should be noted that Martin et al. (2014) advanced methodology that generates estimates of occupancy and upper estimates of the number of manatees in areas where they are not expected to occur (i.e., non-hotspot zone). We believe that it is possible to adapt data collected using the multi-pass removal sampling outlined in this work for modeling as suggested by Martin et al. (2014) if deemed necessary. Lastly, standardizing survey schedules cannot be overemphasized. Scheduling should allow a reasonable time window (e.g., 1–2 weeks) within which to conduct surveys under suitable weather conditions, in sequential days, and using natural geographical barriers in the Puerto Rico population (i.e., northwest coast and Maunabo) to minimize potential effects of movements by manatees between sampling strata. The number of sampling units and times surveys should be replicated should be dictated by ecological and recovery questions and objectives. A suitable survey design might require pilot work to assess sample size needs to estimate relevant demographic metrics like trends and population growth (Caughley 1977; Lefebvre et al. 1995).

Our sampling approach accommodated to the manner in which historical surveys were conducted, but within those constraints, we incorporated a sampling scheme that yields reliable estimates of population size. The sampling framework is suitable for areas that receive different types of use by manatees (hotspots versus non-hotspots), and areas deemed too small for the practical application of flight transects. It can also be adapted for other marine species (e.g., sea turtles, dolphins). We note that our minimum island-wide estimates were similar to the upper estimate of 360 suggested by Mignucci-Giannoni (2005), but below the 532 island-wide estimate suggested by United States Fish and Wildlife Service (2017), or the 700 estimate recently suggested by the Puerto Rico Manatee Conservation Center (A. A. Mignucci-Giannoni, pers. obs.). We believe that the uncertainty regarding the population size of Antillean manatees in Puerto Rico can be resolved by following the recommendations outlined in this study. Our sampling framework provides a stronger basis to set revised population objectives and re-estimate other important conservation and demographic parameters (e.g., effective population size, growth rates, trends—Caughley 1977; Langtimm et al. 2011; Hunter et al. 2012). On a larger geographic scale, the survey protocol outlined herein could be shared with conservation partners in nearby Caribbean islands (i.e., Cuba, Jamaica, Hispaniola), especially if they face similar sampling constraints as those in this study. Manatee numbers in those islands are believed to be lower than those in Puerto Rico (Self-Sullivan and Mignucci-Giannoni 2008), but their shared genetic make-up implies some degree of evolutionary and historical gene flow between populations (García-Rodríguez et al. 1998; Vianna et al. 2006; Alvarez-Alemán et al. 2018). Monitoring approaches that yield more accurate population estimates on each of the West Indies islands, but also help determine if populations are growing and their distribution expanding, are of major conservation value to foster the survival of the species.

Supplementary Data

Supplementary data are available at *Journal of Mammalogy* online.

Supplementary Data SD1.—Python code for population estimation of Antillean manatees (*Trichechus manatus manatus*) in Puerto Rico.

Supplementary Data SD2.—Summary statistics of parameter estimates ($\overline{X} \pm SD$, *CV*, and 95% credible confidence intervals) for aerial surveys of Antillean manatees (*Trichechus manatus manatus*) conducted in Puerto Rico in June 2010, October 2010 and September 2011.

Supplementary Data SD3.—Summary statistics of parameter estimates ($\overline{X} \pm SD$, *CV*, and 95% credible confidence intervals) for aerial surveys of Antillean manatees (*Trichechus manatus manatus*) conducted in Puerto Rico in January 2013 and March 2014).

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