Density and abundance estimate of Antillean manatees (*Trichechus manatus*) within a landlocked lake in Southeastern

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Manatees are threatened along their range. In México, this species is listed as endangered. Manatee conservation strategies require density or occupancy estimates, especially in areas where species face survival risks. On turbid waters, like those found in rivers and lakes of the Southern Gulf of México coast, visual methods used to detect and count manatees underestimate actual numbers. Our goal was to estimate the density and abundance (N) of Antillean manatees in a small State Natural Protected Area, where a manatee population of unknown size inhabits. We performed line transects using a side scan sonar to detect animals and mark-recapture in the isolated population of manatees within Laguna de las Ilusiones, México, a landlocked lake that excludes transit. Using distance sampling from 14 boat trips, estimates of density and abundance were 15.5 manatees km² and 27 \pm 5 manatees in the lake (CV \approx 16.6%). With MARK, from six capture events and 19 individual encounter records, the abundance estimated was 24 manatees (CV \approx 16 to 24%). Previous number of manatees were based only on visual surveys, which reported at least seven manatees. Density is lower than other similar studies along narrow waterways in important areas in México and other Central and South America countries. Studying this endangered subspecies is limited by cryptic habits, turbid waters, poor funding, and low densities, making density or abundance estimates difficult. However, within particular areas and established monitoring areas, these methods could be useful to generate baselines for conservation strategies.

El manatí está amenazado en todo su área de distribución, en México se encuentra enlistado en peligro de extinción. Para implementar estrategias de conservación del manatí se requiere de estimaciones de densidad u ocupación, especialmente en áreas en las que enfrentan riesgos. En aguas turbias, como en ríos y lagunas de la costa del sur del Golfo de México, los métodos visuales de detección y conteo muy probablemente subestiman los números. Se estimó la densidad y abundancia de manatí antillano en una pequeña Área Natural Protegida Estatal donde habita una población de número desconocido de manatíes. Se usaron transectos lineales empleando un sonar de barrido lateral de imágenes para detectar animales y modelos de captura-recaptura de una población aislada de manatíes en Laguna de las llusiones, México, un cuerpo de agua cerrado que impide la entrada y salida de manatíes. Usando muestreo por distancias de 14 navegaciones estimamos una densidad y abundancia de 15.5 manatíes por km⁻² y 27 ± 5 manatíes habitando la laguna. Utilizando datos de recapturas con MARK, con seis eventos de captura y 19 historias de encuentro individual, la abundancia estimada fue de 24 manatíes (CV 16 a 24 %). Estudios previos en la laguna reportaban al menos siete animales. La densidad estimada es menor que la encontrada en otros estudios con métodos similares, pero realizados sobre cursos de agua estrechos, en zonas importantes en México y en otros países de Centro y Sudamérica. Los estudios con esta subespecie amenazada se dificultan por los hábitos crípticos de los animales, aguas turbias y escasez de financiamiento; aunado a las bajas densidades, haciendo difícil la estimación de la densidad y abundancia, sin embargo, dentro de áreas específicas y en zonas establecidas de monitoreo, estos métodos pueden ser útiles para generar una línea base para estrategias de conservación.

Keywords: Distance sampling; freshwater environment; mark-recapture; side scan sonar; turbid waters.

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Introduction

The West Indian manatee (*Trichechus manatus*) is distributed from the Southeastern United States to Northeastern Brazil, including the Major Antilles and Trinidad and Tobago (Lefebvre et al. 2001). This species is considered vulnerable by the International Union for Conservation of Nature, IUCN and the Convention on International Trade in Endangered Species of Wild Fauna and Flora, CITES. *Trichechus manatus manatus*, the Antillean subspecies, is distributed outside the United States and listed as Endangered due to the lack of data on its status. This subspecies has been listed as a conservation priority species in México (SEMARNAT 2018) and in the Wider Caribbean region (UNEP 2010).

Among the key recommendations in the Regional Management Plan for the West Indian Manatee (UNEP 2010) is the assessment of manatee status and distribution, including abundance and population trend estimations whenever possible.

Abundance estimates are prone to difficulties when working with Sirenians, given the high variability in environmental conditions and site occupancy (Marsh et al. 2011). For the Antillean manatee, especially within freshwater inland systems, given the complexity of its habitat, high water turbidity in most areas, and its elusive behavior, aerial surveys yield poor results in manatee counts and density estimates. Interviews and opportunistic sightings

are alternatives to aerial surveys, but they are biased and yield irreproducible information. New sighting methods include side scan sonar on boat platforms, which can become acceptable if standardized procedures and data interpretation improve (<u>UNEP 2010</u>).

The use of Side Scan Imaging Sonar beams (SSI) has been explored to detect and count manatees on turbid or dark waters with promising results (González-Socoloske et al. 2009; González-Socoloske and Olivera-Gómez 2012; Arévalo-González et al. 2014; Puc-Carrasco et al. 2016, 2017; Guzmán and Condit 2017; Castelblanco-Martínez et al. 2017). This sonar has also been used to detect other aquatic fauna (Davy and Fenton 2013; Flowers and Hightower 2013).

The capture and tagging of Antillean manatees had been conducted in specific regions in México, mainly for health assessment purposes, but also to monitor their movements and preferred areas (e. g., Castelblanco-Martínez et al. 2013; Morales-López et al. 2012; Aragón-Martínez et al. 2014). Capture information can complement interviews and aerial and boat surveys, to draw distribution and abundance information. Along with line transects on aerial and boat platforms, mark-recapture models (Otis et al. 1978; White et al. 1999) are reliable and well-established methods for estimating abundances when constrained by specific conditions, like those of closed populations or long-term population follow-up. However, the high cost and the logistic demands required to obtain reliable results from large and cryptic species, such as manatees, limit their use in developing countries.

Most of the studies for the Antillean manatees have logistic and funding limitations, producing minimum counts on distribution surveys (e. q. Morales-Vela et al. 2000). There are a few studies that have dealt with area-specific abundance or density estimates of Antillean manatees (La Commare et al. 2012; Guzmán and Condit 2017; Puc-Carrasco et al. 2017) or abundance over extended regions (e. g. De Olivera-Alves et al. 2015; Collazo et al. 2019).

The Grijalva-Usumacinta River basin is assumed to have México's densest manatee population. Conservation actions in this region must have a high impact on the country's species (UNEP 2010; SEMARNAT 2018). However, density and abundance estimates are constrained by the high turbidity of its interior waters. Counts to estimate relative abundance on specific waterways provide few insights into the whole population of this region (Puc-Carrasco et al. 2016, 2017). Combining Mark-recapture, distance sampling and interviews could produce better estimates. The lake of Laguna de las Ilusiones is located within the Grijalva-Usumacinta Rivers basin and presents conditions usually found in most of the basin's water bodies. This lake is currently landlocked and hosts an isolated manatee population (Pablo-Rodríguez and Olivera-Gómez 2012), which brings the opportunity to explore and standardize methods to estimate population density. In this study, we explore the estimation of the abundance of manatees in Laguna de las

Ilusiones through boat line transects, using sonar sightings and through data on manatee mark-recapture procedures performed for telemetry and health assessment purposes. This research is useful to highlight limitations of methods and variance associated with detection. Once accepted and standardized, these methods could be readily replicated in other wetlands.

Materials and methods

Study area. Laguna de las Ilusiones (17° 59.7′ N, -92° 56.3′ W; hereafter LI) is a lake covering 189.1 ha with a 41 km perimeter and a depth mostly less than 3.5 m (Ricárdez-de la Cruz et al. 2016). LI hosts an isolated manatee population in the state of Tabasco in Southeastern México (Figure 1; Pablo-Rodríguez and Olivera-Gómez 2012). LI, like most of the lakes in the Mexican Gulf plain, is a quaternary fluvial and lacustrine alluvium deposit from the Grijalva River, that has a meandric origin from contact with the Mezcalapa river. In the past, LI was connected to the Mezcalapa river by the "EI Espejo" stream; however, a dam was built at the mouth of the creek, blocking water flow. Currently, the water input to LI is mostly through rainfall. In LI, the submerged vegetation is nearly absent, and the floating plants, mostly Pistia stratiotes and Eicchcornia crassipes, are present but scarce because they are actively controlled. Bank vegetation is the primary food source for manatees (Ponce-García et al. 2017), composed mostly of grasses, other associated plant species, and isolated trees.

LI was declared a State Natural Protected Area in 1995, but the growth of Villahermosa city has resulted in a highly urbanized lake perimeter (Pablo-Rodríguez and Olivera-Gómez 2012; Ramírez-Jiménez et al. 2017). Fishing in LI is prohibited; however, small artisanal fishing remains. The boat traffic in the lake is highly reduced, and usually the manatee population remains undisturbed. Preliminary limited surveys in LI reported the presence of at least seven manatees on the lake (Pablo-Rodríguez and Olivera-Gómez 2012).

Abundance and density estimate by boat trips. Between April and June 2011, we conducted 14 boat trips (ca. 3 h each) in LI during daylight hours along an 18 km zigzaglike fixed transect, with a minimum distance of 20 m to the banks (Figure 1). During all the surveys, the boat path never changed. We used a 1.3 m length aluminum boat with a 15 HP four-stroke outboard engine (Honda, BJ 15, Japan), with a sustained speed between 2.5 and 7.0 km/h. During the surveys, we monitored the manatees observed with a side scan imaging sonar (Humminbird 987c SI, Eufaula, Alabama, USA). Two observers participated in the boat trips, one was focused on the sonar, and the other piloted the boat path and took notes. The observers were trained to detect manatees with the sonar. The sonar was operated at 450 kHz, and the beam had an 85° detection angle on the water column and was set to detect manatees within 20 m on each side of the boat. Screen images of all suspected manatees in the sonar were saved to verify later, using the procedure described by Gonzalez-Socoloske et al. (2009).

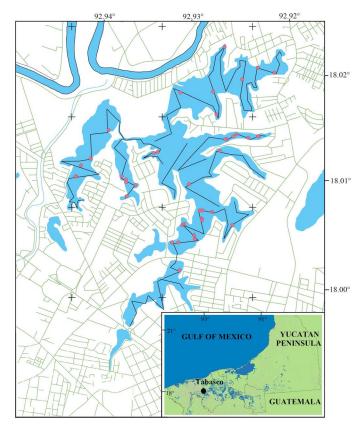


Figure 1. Study area in the lake of "Laguna de las Ilusiones", Villahermosa, Tabasco, México. The solid black line marks the boat route followed in the manatee surveys from April to June 2011. Red points indicate the location of detections with the Side Scan Imaging Sonar.

Coordinates and manatee group size were taken from all the sonar sightings. The perpendicular distances were estimated directly on the saved screen images. In the case of multiple individuals, the distance to the approximate geometric center of the group was estimated. Sighting depths were obtained directly from the sonar, and distance to the nearest bank was calculated on the polygon map of the lake using the software QGIS version 3.12.3-Bucuresti (QGIS 2021). We used the perpendicular distance to sighted individuals, with depth and distance to the nearest bank as covariates, to fit data distributions to several typical models using DISTANCE package (Miller 2022) in R (vers. 4.1.2). We pooled the distance of the 14 surveys to improve the fitting of the detection functions. Best models were selected using the Akaike Information Criteria for small samples (AICc) (Thomas et al. 2010), when the sample size is small or the number of parameters is large relative to sample size, AICc is recomended over AIC (Strindberg 2012). The estimated probability of detection was used to calculate the effective width of transects. Encounter rate was calculated manually for each survey, dividing the number of manatees sighted in a survey by the accumulated line kilometers traveled and then, density was obtained multiplying the encounter rate by the effective transect width obtained earlier in DISTANCE. Finally, the mean and standard error among all surveys were taken as the estimated parameter,

and abundance (N) was calculated for the lake using the proportion of area sampled to the most recent calculation of the water surface area of the of LI made by Ricardez-de la Cruz et al. (2016).

Mark-recapture models. We used data from six capture events in LI conducted during the ten-year period between July 2006 and June 2016, with 19 individual encounter histories. Of the 19 individuals, three died at some moment during the ten-year period. Manatees were captured for radio tagging and health assessment (e. g., Morales-López et al. 2012; Aragón-Martínez et al. 2014; Ramírez-Jiménez et al. 2017). Captures were done with purse seine nets, and manatees were pulled up to the boat and towed carefully to a nearby lake shore where they were measured, biological samples obtained, and equipped with a standard VHF telemetry system (Morales-López et al. 2012; Ramírez-Jiménez et al. 2017). After the procedure, the animals were taken back to the capture site. All captures were conducted under federal permits (SGPA/DGVS/04060/06, 01103/07, 01754/09, 04675/10, 00646/16). A subcutaneous PIT tag (AVID, Norco, California, USA) was implanted in all the captured individuals in the dorsal area between the scapula and the occipital condyles, following Bonde et al. (1983). Manatee's recaptures were confirmed with a manual PIT tag reader (AVID, Norco, California, USA).

Mark-recapture data were stored in a binary matrix, where the rows are individuals and the different sampling events are columns, additional columns were added for sex and length at first capture event, the first for grouping and the last as a continuous covariate. This matrix was entered into the MARK software 8.2 (White and Burnham 1999). There is no individual migration to or from LI, but new individuals were born, and others died along the tenyear interval of sampling used for capture histories, reason because we used the Jolly-Seber approach, CJS model, for open populations in MARK with the POPAN formulation to explore variation when estimating manatee abundance. An average of nearly one manatee dies in the lake per year; activities of cleaning the lake and people frequenting the shores ensure that practically all carcasses are found.

Parameters set in the models were: phi (probability of passing over to the next capture event), p (probability of capture), pent (probability of entrance), and N (population number). MARK estimates of parameters, CV (coefficient of variation), and CI (confidence intervals) were computed from the best-fit models. In the structure of models in MARK, sex of individuals was incorporated as grouping variable, as well as one continuous covariate: length at first capture. The simple model was that with all parameters set as constant (phi{.}p{.}pent{.}N{.}) and variants of it are those where parameters are a function of time(t), group (g) or covariate (cov). Models in MARK were compared using Akaike's Information Criterion for small samples, AICc, and model likelihood (Burnham and Anderson 2002) and the best models were selected.

Results

Abundance and density estimate by boat trip. We detected an overall of 59 manatees in 45 groups. The mean number of manatees detected per survey was 4.2 ± 2.7 , with a maximum number of manatees per survey of nine and a minimum of one. We detected single manatees on 80 % of the sonar sightings, pairs were 13 %, and the rest were groups of three and four individuals. The mean depth where the manatees were detected in sonar sightings was 2.1 ± 0.6 m.

The best model for the distance function was the Half Normal key function (Figure 2). We observed that the likelihood ratio between the best model and the next was 0.9, and the transect width was truncated to 15.2 m, as it was the maximum distance for an observed manatee. The density and abundance estimates using the Distance detection function were 15.546 \pm 2.575 manatees km⁻² (CV = 16.6%) and 27.1 \pm 4.5 manatees in the lake.

Mark-recapture models. Table 1 shows the best two models that converged in MARK. In the best model, *phi* and *pent* were set as constant over time; *p* dependent on covariate (length at first capture encounter), and *N* was set to depend on group (gender). The abundance estimate from the best model was 24 manatees (CI 20 to 42; Table 1).

Discussion

There was no previous estimation of the density nor the abundance of manatees in the LI protected area; reports only gave the maximum number of manatees sighted along navigations in the lake (Pablo-Rodríguez and Olivera-Gómez 2012). Our results will serve as a baseline for management purposes in this lake, 19 manatees were captured in LI from 2006 to 2016, and 16 remained with pit tags, and an estimate of 22 to 32 individuals in the lake are likely. The density of 15.5 manatees km⁻² is lower than that reported in a hotspot area in the lower basin of the Usumacinta River (21.7 manatees km⁻², Puc-Carrasco et al. 2017). Compared with LI, where manatees are confined to a small but rounded

Table 1. Mark models, parameters, and estimates from six capture events and 19 individual encounter histories of manatees in Laguna de las Ilusiones, Tabasco, México. A is the best model and B is the second-best model.

Model	N	S.E.	CI95 %	del- taAICc	likeli- hood
A) phi{.} p{.,cov} pent{.} N{g} phi= 0.997 ± 0.003, p = 0.381 ± 0.088, pent = 0.999	Females 17 Males 7 Total 24	2.78 1.74	14 - 27 6 - 15 20 - 42	0.000	1.000
B) phi{} p{.,cov} pent{} N{} phi = 0.997 ± 0.003, p = 0.381 ± 0.087, pent = 0.999	Total 15	1.70	14 - 21	0.642	0.725

phi = survival probabilities between successive occasions, p = capture probability, pent = probability of entrance, N = population number, g = grouping variable (gender), cov = covariate (length at first capture encounter).

area, surveys performed in other sites within Grijalva- Usumacinta River basin were done over stretch water courses, not much wider than the sonar reaching width increasing manatee encounter probability along the transect, as we noted that in this cases manatees behave remaining in the bottom or swimming parallel to path of the water course, and probably had an effect on the density calculation.

Even though this study estimates are helpful, there are several concerns about the proper method to gather information about population density or abundance in this kind of shallow environment with low water transparency and complex contours and with the assumptions the method requires, because we observed that manatees tend to be distributed towards some embayment and not following a desirable random dispersal.

Manatees fairly unreacted to boat approach, and this is part of the problem with collisions (<u>Lima et al. 2015</u>). Manatees react more when boat pass less than 15 m from the animal, if a deeper area is near, usually manatees heading toward it (<u>Miksis-Olds et al. 2007</u>) but heading, or reactions depend much on the activity level of the animal (<u>Rycyk et</u>

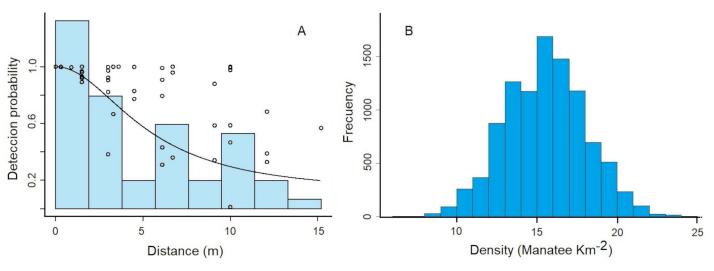


Figure 2. (A) Best fitted detection function (solid line) for manatee sightings from boat surveys, estimated in Distance in R from surveys conducted in the lake "Laguna de las Ilusiones" in Tabasco, México, between April and June 2011. Lake detpth and distance to the nearest bank were used as covariates. (B) Distribution of densities in the lake using 10,000 bootstrap sample means from the observed 14 survey mean densities of manatees km².

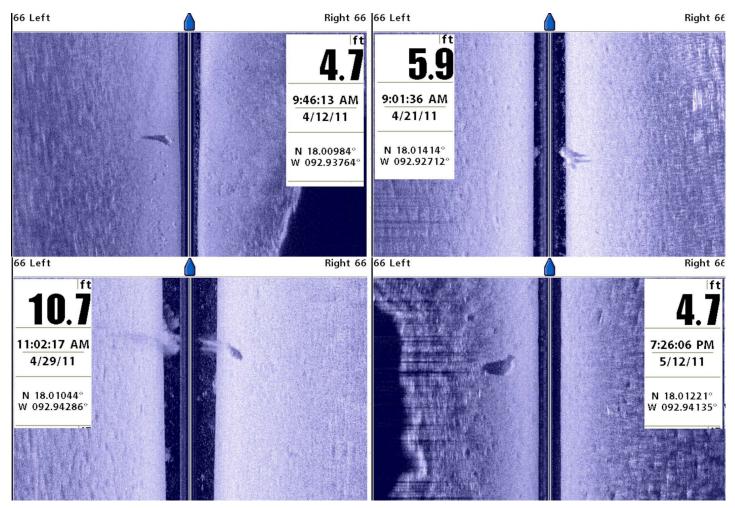


Figure 3. Examples of manatees sighted using side scan sonar (screen savings) on different dates along this study.

al. 2018). When manatees detected the boat, we observed two behavioral responses: the first, usually swimming away from the boat axis, but occasionally parallel or towards the ship; the second, remaining passive at the bottom of the lake. An observer using the visual method would be unable to detect manatees that adopted the passive behavior because the animals would not leave the distinctive sediment plumes or bubbles associated with swimming; sonar observers were able to detect these passive manatees, this being the main advantage of the method; boat speed must assure that manatees along the transect remain on it before the sonar pass. The relative position of manatees with respect to the boat and sonar beam axis could result in variations in the strength and quality of the object represented in the sonar screen.

Availability bias could also explain variation (Packard et al. 1985; Walker et al. 2000; González-Socoloske et al. 2009). Puc-Carrasco et al. (2016) estimated a correction factor of 20 % opportunistically during their sonar survey in a nearby area, based on a two-observers' procedure. Manatee's differential daily and seasonal use of the study site, and being different from a random distribution, would also account as a source of variation (Ramírez-Jiménez et al. 2017), considering strata will serve to lower this effect, but it is difficult to implement them in a small area.

Estimates with mark-recapture and line transects produced close numerical results and a similar coefficient of variation. However, distance sampling is preferable because it is cheaper and less time-consuming. Recent studies obtained reliable results using sonar (e. g., Puc-Carrasco et al. 2017; Guzmán and Condit 2017). The side scan sonar also shows advantages over other methods when estimating group size and discriminating small calves from larger individuals when they are together (González-Socoloske and Olivera-Gómez 2012). However, combining methods could bring insights into low-density populations (Gerrodette et al. 2011).

Capture-recapture model assumptions are difficult to meet with small groups of animals; compared to distance sampling, mark-recapture is known to be more sensitive to assumption violations (Thomas et al. 2002). The survival probabilities between occasions in the different models were high, 0.997 monthly and 0.965 yearly, in full accord with the mean number of deaths in the lake, almost one individual per year in data from 1984 to 2016.

Including the gender of the captured animals as a group improves the mark-recapture models. The model that did not account for gender produced an estimated 15 animals: a number smaller than the total of the tagged manatees alive at the last capture event. The length at first capture of each individual as a covariate contributed to the variance in capture probabilities, with young animals being more likely to be trapped than older ones along this study. The mark-recapture model was helpful in LI but is hard to replicate in other localities in the region. This method is expensive and laborious given that the low visibility environment restricted the use of individualized permanent marks such as those derived from boat collisions (Beck and Reid 1995), necessitating the capture of individuals to insert PIT tags. This approach could be considered as a long-term project for well-suited areas and with the proper funding.

It is a logistic and funding challenge to design surveys for the manatee's inland populations in México and the rest of the range of Antillean manatees. Still, appropriate index localities, using available methods, could lead to viable regional conservation strategies, and the estimate of density could be a better parameter for comparison of overabundance.

The group of isolated manatees in LI is currently under high pressure due to the rapid growth of the Villahermosa urban area. Threats associated with urban development include the presence of pathogens such as *Leptospira* spp. (Aragón-Martínez et al. 2014), and genetic problems resulting from an isolated inbreeding group (Gómez-Carrasco et al. 2018). The undisputable presence of a couple of dozen animals at the time of this study should be considered in a management plan for this lake.

Acknowledgments

FPG received a scholarship from Consejo Nacional de Ciencia y Tecnología for the Master of Science degree in Environmental Sciences at Universidad Juárez Autónoma de Tabasco. Part of the study was conducted under project number UJAT-2008-C04-48 from the PFICA Fund. Secretaría de Recursos Naturales y Protección Ambiental of Tabasco (SERNAPAM) provided logistic support for this study.

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Associated editor: Juan Pablo Gallo-Reynoso Submitted: November 3, 2022; Reviewed: January 4 2023 Accepted: March 27, 2023; Published on line: April 24, 2023