El Colegio de la Frontera Sur

Variación en las características vocales de los manatíes antillano (*Trichechus manatus manatus*) y de la Florida (*T. m. latirostris*) en tres zonas geográficamente aisladas.

TESIS

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Por

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Las personas abajo firmantes, miembros del jurado examinador de: Jessica Dayanh Reyes Arias hacemos constar que hemos revisado y aprobado la tesis titulada: Variación en las características vocales de los manatíes antillano (*Trichechus manatus manatus*) y de la Florida (*T. m. latirostris*) en tres zonas geográficamente aisladas. Para obtener el grado de **Maestra en Ciencias en Recursos Naturales y Desarrollo Rural.**

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Resumen

El manatí americano o manatí de las Indias Occidentales (*Trichechus manatus*) cuenta con dos subespecies descritas: el manatí antillano (*Trichechus manatus manatus*) y el manatí de la Florida (*Trichechus manatus latirostris*), las cuales han sido ampliamente estudiadas con diferentes enfoques biológicos. En general, se conocen las características acústicas de ambas subespecies, el repertorio vocal se compone de aproximadamente cinco tipos de vocalizaciones discretas con estructura y nombre onomatopéyico definido. Sin embargo, la variación geográfica en el comportamiento vocal de los manatíes es en gran parte desconocida. El presente trabajo tuvo como objetivo comparar las características vocales de las dos subespecies de manatí distribuidas en tres grupos: dos grupos de manatíes antillanos en Belice y México y un grupo de manatí de la Florida, con el fin de determinar si sus vocalizaciones varían entre subespecies y regiones geográficas. Se obtuvieron 3859 registros de vocalizaciones y se midieron seis parámetros (Frecuencia máxima y mínima, duración, centro de frecuencia, pico de frecuencia y frecuencia delta) a partir de la frecuencia fundamental de cada una de ellas. Nuestros resultados revelaron que los tres grupos de manatí presentan similitudes y diferencias en sus características vocales, los análisis estadísticos mostraron que los tres grupos estudiados tuvieron valores promedio diferentes en todos los parámetros de frecuencia y duración medidos, sugiriendo una posible diferenciación geográfica. Sin embargo, debido a la incapacidad de identificar a los individuos emisores de las vocalizaciones durante las grabaciones, no está claro si esta variación está relacionada con otros factores diferentes al aislamiento geográfico, tales como la edad, el sexo, la variación intergrupal o las condiciones ambientales propias de cada sitio de estudio. Esta investigación destaca la importancia del conocimiento de las características vocales de los manatíes y su aplicabilidad en los programas de monitoreo acústico pasivo, que busquen aumentar el conocimiento de ambas subespecies en favor de su conservación.

Palabras clave

Vocalizaciones, bioacústica, subespecies, variación geográfica, monitoreo, manatíes.

Capítulo I:

Introducción

El estudio de las vocalizaciones de los mamíferos marinos ha permitido conocer y analizar características poblacionales, comportamentales, acústicas y ecológicas de muchas especies de cetáceos (Rendell y Whitehead 2005), pinnípedos (Bjørgesæter et al. 2004; Risch et al. 2007), entre otros mamíferos marinos (Booth et al. 2020). Recientemente han aumentado los esfuerzos investigativos que buscan estudiar y analizar las características acústicas de los manatíes (Umeed et al. 2017; Brady et al. 2020; Ramos et al. 2020), muchos de estos, con el objetivo de aportar conocimiento que pueda ser aplicado al desarrollo de herramientas metodológicas de bajo costo y fácil realización, en favor de su conservación y monitoreo a través del reconocimiento automatizado de las señales acústicas (Sousa-Lima et al. 2013; Castro et al. 2016; Merchan et al. 2019; Merchan et al. 2020).

El manatí americano (*Trichechus manatus*) también llamado manatí de las Indias Occidentales habita en sistemas ribereños y costeros del Atlántico occidental (Domning y Hayek 1986; Marsh y Lefebvre 1994; Marsh et al. 2012). Se reconocen dos subespecies con base en diferencias craneométricas (Domning y Hayek 1986): *T. m. latirostris* que se distribuye en la península de Florida hasta el norte del Golfo de México y *T. m. manatus* que habita desde el sur del Golfo de México, pasando por el Mar Caribe hasta el norte de Brasil (Hartman 1979; Powell y Rathbun 1984; Domning y Hayek 1986; Deutsch et al. 2008; Marsh et al. 2012). Estas dos subespecies varían genética y morfológicamente (Domning y Hayek 1986; Nourisson et al. 2011; Hunter et al. 2012), dado que se encuentran aisladas geográficamente por grandes extensiones de agua fría y profunda (Domning y Hayek 1986). Ambas subespecies están presentes en la lista de especies amenazadas de la IUCN desde hace más de una década (Deutsch et al. 2008; Deutsch 2008; Self-Sullivan y Mignucci-Giannoni 2008), por lo cual, muchos de los esfuerzos de investigación actuales se han centrado en la conservación de la especie, que constituye una parte representativa de la biodiversidad de los ecosistemas acuáticos americanos (Daniel-Renteria et al. 2010).

El repertorio vocal del manatí ha sido ampliamente descrito y analizado (Sousa-Lima et al. 2002; Mann et al. 2006; O'Shea y Poché 2006; Sousa-Lima et al. 2008). Se sabe que los manatíes cuentan con un sistema de comunicación basado en vocalizaciones y señales táctiles, usado en contextos de cuidado parental, reproducción e interacciones sociales (Sousa-Lima et al. 2002; O'Shea y Poché 2006; Sousa-Lima et al. 2008; Umeed et al. 2017). Los manatíes tienen la capacidad de producir señales acústicas complejas de una sola nota con armónicos múltiples, diferentes contornos, modulaciones de frecuencia y otros elementos relacionados no armónicos ni lineales (Alicea-Pou 2001; Mann et al. 2006; O'Shea y Poché 2006; Berta et al. 2015; Umeed et al. 2017). Sus características vocales varían con respecto al sexo y la edad (Sousa-Lima et al. 2008; Umeed et al. 2017), y su repertorio vocal está compuesto por cinco o seis tipos de llamadas discretas (Alicea-Pou 2001; O'Shea y Poché 2006; Umeed et al. 2017; Brady et al. 2020), con un rango de frecuencia entre los 0.5 – 5.0 kHz y una duración promedio entre los 0.2 - 0.5 s (Schevill y Watkins 1965; Steel 1982; Nowacek et al. 2003; Sousa-Lima et al. 2008).

Estudios previos enfocados en la variabilidad geográfica y taxonómica de los manatíes, han reportado similitudes y distribuciones superpuestas en la mayoría de los parámetros medidos (Alicea-Pou 2001; Nowacek et al. 2003), a excepción de los parámetros relacionados con el tiempo; sin embargo, en el caso de los resultados obtenidos por Alicea-Pou (2001), estas investigaciones han sido dirigidas a pequeños grupos de animales de dos zonas geográficas aisladas. La presente investigación tuvo como objetivo principal comparar las características vocales de dos grupos aislados de manatíes antillanos (Belice en estado silvestre y México en cautiverio) y un tercer grupo de manatíes de la Florida en estado silvestre. Utilizamos análisis estadísticos multivariados para determinar si existen variaciones entre estos tres grupos con respecto a su origen geográfico y clasificación taxonómica, tomando en cuenta seis parámetros acústicos, medidos a partir de la frecuencia fundamental de cada sonido.

La metodología y resultados obtenidos se detallan en el capítulo II. En el capítulo III (Conclusiones) se presenta una síntesis de los resultados obtenidos y se enfatiza la importancia de esta clase de estudios, que no solo aumenta el conocimiento vocal propio de la especie, sino que apunta a ser útil para la mejora y desarrollo de técnicas de identificación automatizada, que busquen facilitar la implementación de programas de conservación, monitoreo y conteo de individuos en poblaciones silvestres, usando como entrenamiento rangos de frecuencia específicos por distribución geográfica.

Capítulo II

Vocal variation of the two subspecies of the West Indian Manatee in three geographically isolated regions.

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Geographic variation in the vocal behavior of manatees has been reported but is largely unexplored. Vocalizations of West Indian manatees (*Trichechus manatus*) from Belize, Mexico, and Florida were analyzed and compared to determine if calls varied between subspecies and geographic region. Six parameters were measured from the fundamental frequency of 3,859 manatee calls obtained from wild and captive animals. Discriminant function and cluster analysis revealed similarities and differences in measured frequency values and duration of calls among the three groups. Overall, findings suggest that the acoustics parameters of the West Indian manatee differ between different study groups and geographic origin. Due to the inability to identify manatees producing the vocalization during recordings, it is unclear if variation is related to age, gender, individual variation, environmental conditions, and other factors. Our findings highlight the need for further study of the vocal behavior of manatees throughout their range and provides critical information needed for improved automated detection of manatee calls for wildlife monitoring.

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I. INTRODUCTION

Marine mammals produce many kinds of sounds for communication. However, while our understanding of manatee vocalizations' acoustics characteristics has increased (Umeed *et al*., 2017; Brady *et al*., 2020; Merchan *et al.*, 2020; Ramos *et al*., 2020), how the vocal repertoire varies by region or taxa is still poorly understood. Understanding these differences is essential to studies implementing passive acoustic monitoring to detect, count, and census marine mammals (Rendell and Whitehead, 2005; Risch *et al.,* 2007; See Booth *et al.,* for a review).

The West Indian manatee is distributed in riparian and coastal systems of the Western Atlantic, Caribbean Sea, Gulf of Mexico, and south to Brazil (Marsh *et al.,* 2012; Steel, 1982; Deutsch *et al*., 2008). The two recognized subspecies of the West Indian manatee—the Florida manatee (*T. m. latirostris*) and the Antillean manatee (*T. m. manatus*)—are listed as threatened by the IUCN (Deutsch, 2008; Self-Sullivan and Mignucci-Giannoni, 2008; Deutsch *et al.*, 2008). Florida manatees range from the Florida peninsula to the north of the Gulf of Mexico (Powell and Rathbun, 1984; Domning and Hayek, 1986) and migrate seasonally in the winter to warm water refuges (Laist and Reynolds, 2005). The Antillean manatee inhabits shallow and warm waters from the Caribbean Sea to Brazil, experiencing less dramatic seasonal shifts (Alicea-Pou, 2001; Gonzalez-Socoloske and Oliveira-Gómez, 2012). These subspecies are geographically isolated by large expanses of cold and deep water and differ in morphology and genetic origins (Domning and Hayek, 1986; Nourisson *et al.*, 2011).

Manatees are known to produce a variety of vocalizations with different structural properties and acoustic characteristics (Alicea-Pou, 2001; Berta *et al.*, 2015). These sounds play a fundamental role in their communication, particularly, in maintaining contact between mothers and their calves, and in social interactions (O'Shea and Poché, 2006; Steel, 1982, Hartman, 1979; Umeed, 2017; Miksis-Old and Tyak, 2009). Manatees have a relatively small vocal repertoire composed only of five or six types of discrete call structures (Alicea-Pou, 2001; O'Shea and Poché, 2006; Umeed *et al.,* 2017; Brady *et al.,* 2020). The frequency and duration range reported for vocalizations of West Indian manatee is between 0.5 - 5.0 kHz and 0.2 - 0.5 s, respectively (Sousa-Lima *et al.,* 2008; Nowacek *et al.,* 2003; Steel, 1982). Few studies have focused on geographic variability in manatee vocalizations (e.g., Alicea-Pou, 2001; Nowacek *et al.,* 2003). However, these previous studies were based on small groups of manatees and between two geographic locations and have reported similarities and overlapping distributions in most of the parameters measured, and only Alicea-Pou (2001) reported variation in the time-related parameters.

The aim of this study was to compare the vocal characteristics of two subspecies of West Indian manatees: two isolated populations of Antillean manatees (one group in the wild and one group in captivity) and one Florida manatees in the wild. Multiple statistical methods were used to determine if the frequency and temporal parameters of vocalizations varied between the three groups of different geographical origin and subspecies classification.

II. MATERIALS AND METHODS

A. Sampling and study sites

Recordings of wild manatees in Belize and Florida and captive manatees in Mexico were analyzed for this study (Fig. 1). In Quintana Roo, Mexico (hereafter "Mexico"), 28 Antillean manatees (13 females, 15 males) of all age categories (calf, juvenile, and adults) were recorded in captivity. Animals were maintained in seawater pools isolated by a mesh or wall in six facilities with a variable depth between 2 and 3 m. Manatee vocalizations were recorded during one week in March, one week in July and three weeks between September and October in 2019, using an omnidirectional SQ26-8 hydrophone (linear frequency range 0.02–50 kHz, sensitivity of -169 dB re 1V/µPa) attached to a Tascam-DR05 digital recorder, recording at a 96 kHz sample rate (24 bit) to WAV format. The hydrophone was suspended at one end of the pool at an average depth of 1 m.

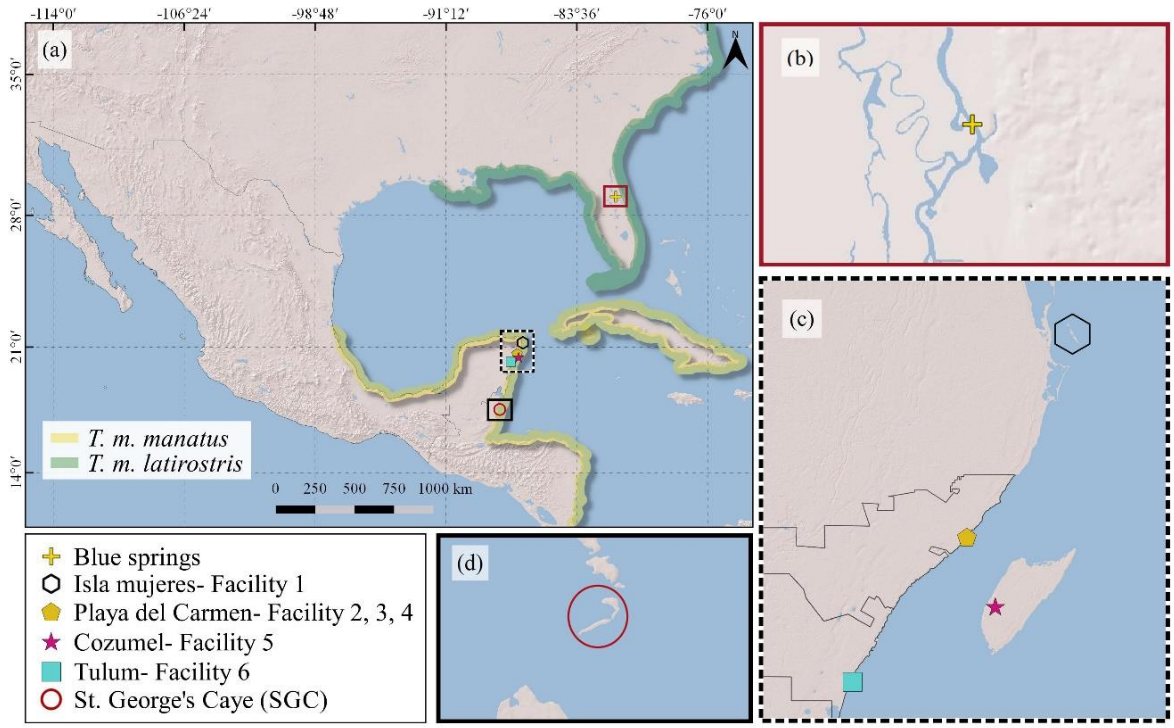

FIG. 1. (Color online). Recording locations of West Indian manatee (*Trichechus manatus*) vocalizations. **(a)** Distribution of Florida manatee (*T. m. latirostris*) and Antillean manatee (*T. m. manatus*). **(b)** Location where manatees were recorded in the wild in Blue Springs, near the St. Johns River in Florida. **(c)** Locations where manatees were recorded in captivity in Quintana Roo, Mexico. **(d)** Location where manatees were recorded in the wild at SGC near the barrier reef in Belize.

Vocalizations of wild Antillean manatees in Belize (hereafter “Belize”) were recorded at St. George’s Cave, a small crescent-shaped island located 9.5 km east of mainland Belize and 2.5 km west of the Belize Barrier Reef. The site is surrounded by expansive seagrass flats, sand patches, deep channels, and resting holes. The area is regularly inhabited by manatees of all ages and sex classes (Ramos *et al.,* 2017). Recordings were made during one week in July 2017 and 2.5 weeks in January 2018 using a SoundTrap 300 HF (Ocean Instruments, New Zealand). The recorder sampled at a frequency of 288 kHz (16-bit, flat frequency response: 0.02–150 kHz ±2 dB, clip level: 172 dB re: 1 µPa) with the preamplifier gain on. The device was anchored to the seafloor with a cinderblock and suspended in the water column at a depth of 1 m above the seafloor in water 1.5 m deep.

Florida manatees were recorded at Blue Springs, Florida (hereafter "Florida"), a warm freshwater refuge utilized by manatees when the ambient water temperature drops below 20°C in the winter. Recordings were made on January 3, 2010, and were obtained using an omnidirectional SQ26-08 hydrophone (linear frequency range: 0.02–50 kHz; sensitivity: -169 dB re 1V/µPa, 48 kHz) attached to an M–Audio MicroTrack 24/96 recorder (48 kHz sample rate; in 16 bit). The hydrophone was suspended at a depth of 1 m below the surface in water that was 1.5 m deep.

B. Acoustic analysis

Recordings were visually inspected using Raven 1.5 software (Center for Conservation Bioacoustics, 2014). For quantitative measurements, only non-overlapping calls with a signal-to-noise ratio (SNR) ≥6 dB, with clear and identifiable parameters were analyzed. Spectrograms for the group of Mexico were calculated with a time resolution of 2.67 ms and a frequency resolution of 93.75 Hz for recordings at a sample rate of 192 kHz (DFT: 1024; Hanning window: 50% overlap) for the group of Florida with a time resolution of 5.33 ms and a frequency resolution of 46.8 Hz for recordings at a sample rate of 96 kHz (DFT: 1024; Hanning window: 50% overlap), and for the group of Belize with a time resolution of 9.81 ms and a frequency resolution of 70.31 Hz at a sample rate of 576 kHz (DFT: 4096; Hanning window; 50% overlap).

Six parameters were characterized from the fundamental frequency (the first harmonic) of each vocalization: maximum and minimum frequency (measured from the power spectrum with a 10 dB threshold), duration (measured from the waveform), peak frequency (frequency at which peak power occurs within the selection, Charif *et al.,* 2010), delta frequency (value of the maximum frequency minus the minimum frequency) and center frequency (the frequency that divides the sound into two frequency intervals of equal energy, Charif *et al.,* 2010).

The range of the number of wild Antillean manatees vocalizing in recordings from Belize (range: 17–60 individuals) was estimated according to sightings of manatees detected during drone flights each season at SGC from 2017 to 2018 in previous studies (Landeo-Yauri *et al.,* 2020; Ramos *et al.,* 2018; Ramos *et al.,* 2017). The numbers of wild Florida manatees were counted (n = 120 animals) during a visual survey at the beginning of the recording session. In captivity and the wild, it was not possible to identify the calling animal or determine how many calls individuals produced relative to others during any given recording. Therefore, each call was considered an independent event.

C. Statistical analysis

To compare vocal characteristics between groups, frequency histograms were constructed for each of the measured parameters. To analyze the relationships of these parameters across manatee groups (Florida, Belize, and Mexico), the permutational analysis of variance (PERMANOVA) on Bray-Curtis dissimilarities coupled with a Dunn's *post hoc* test was used. A Kruskal-Wallis test was conducted for groups that showed significant differences for each variable.

When significant differences were found, the parameters that most contributed to these differences were identified using the SIMPER analysis technique, which provides the percentage contribution of each variable differentiated between groups (Clarke, 1993). The SIMPER technique was followed by discriminant analysis (DA) to find the linear combination of the independent variables that discriminated between groups. Half of the vocalizations recorded from captive manatees in Mexico came from one pool with four individuals, leading to high pseudo-replication of representation for each individual animal. Therefore, statistics were performed in pairs (DA and PERMANOVA) between: 1) wild manatee calls in Belize and Florida where more individual manatee were present, and 2) calls from wild manatees in Belize and captive manatees in Mexico that belong to the same subspecies. The statistical test was performed using the software PAST 3.25 (Hammer *et al.,* 2001).

Cluster analysis was performed to determine if the vocalizations from the different groups fell into similar or different clusters based on the measured parameters. Since the parameters were correlated, a principal component analysis (PCA) was first performed using the R package *Psych* (Revelle, 2014). The first component explained 98% of the variance in the data. Using the values from the first component of the PCA a model-based, cluster analysis was implemented using the package *MClust* (Fraley *et al.,* 2012*)*. Following the methodology of Hedwig *et al.* (2014) and Brady *et al.* (2020), calls were grouped into 1–20 clusters; 10 models were run for each number of clusters, totaling 200 cluster solutions. The Bayesian Information Criterion (BIC) was used to select the best model. Finally, the percentage of calls from each group in the best cluster solution was calculated as a function of the total number of calls measured and the total number of calls obtained from each group.

III. RESULTS

A. Acoustic recordings

Analysis of acoustic parameters included a total of 15 h of recording time from Florida, 116 h from Belize, and 34.6 h from Mexico. This resulted in 3,859 calls of good quality (i.e., SNR ≥6 dB) including 2,007 calls from wild Florida manatees, 769 calls from wild Antillean manatees in Belize, and 1,083 calls from captive manatees in Mexico. Representative samples of calls from each of the three groups are shown in Fig. 2.

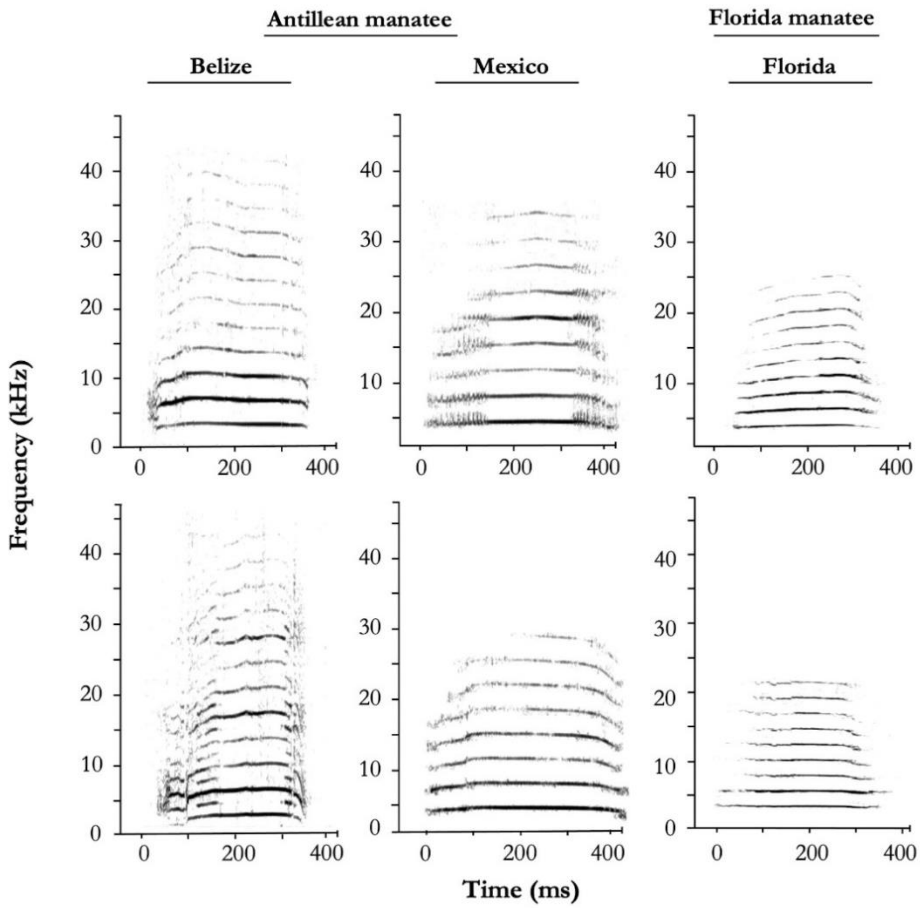

FIG. 2. Spectrograms of calls of three groups of West Indian manatees. Spectrograms parameters are 1024 samples of FFT for calls from Mexico and Florida and 512 samples of FFT for Belize, 80% overlap, and Hanning window.

B. Comparisons between groups

All three groups shared fundamental frequency ranges between 0.5 and 5 kHz and 0.05 to 0.5 s in duration. The mean and standard error (SE) of all measured parameters for the three groups are in Table I. The PERMANOVA analysis coupled with a Dunn's *post hoc* test indicated statistical differences among the three groups ($p < 0.01$) and in pairwise comparisons (Belize-Florida, Florida-Mexico, and Mexico-Belize) ($p < 0.01$) in all measured frequency and temporal parameters (Fig. 3). Kruskal-Wallis analysis revealed all acoustic parameters had significant differences between sample medians of the three groups of manatees ($p < 0.05$).

Table I. Mean of measured parameters and standard error (SE) for the three groups of West Indian Manatee.

Group	Minimum frequency (kHz)	Maximum frequency (kHz)	Duration (s)	Peak frequency (kHz)	Delta frequency (kHz)	Center frequency (kHz)
Belize	3834.5 ± 56.6	4495.3 ± 57.6	0.126 ± 2×10^{-3}	4178.0 ± 57.4	660.7 ± 17.3	4179.0 ± 57.1
Florida	2789.1 ± 25.0	3189.1 ± 25.5	0.199 ± 1×10^{-3}	2986.6 ± 25.3	400.0 ± 4.3	2990.3 ± 25.1
Mexico	3309.0 ± 37.7	4285.1 ± 40.0	0.220 ± 2×10^{-3}	3775.0 ± 39.2	976.2 ± 14.3	3794.6 ± 38.2

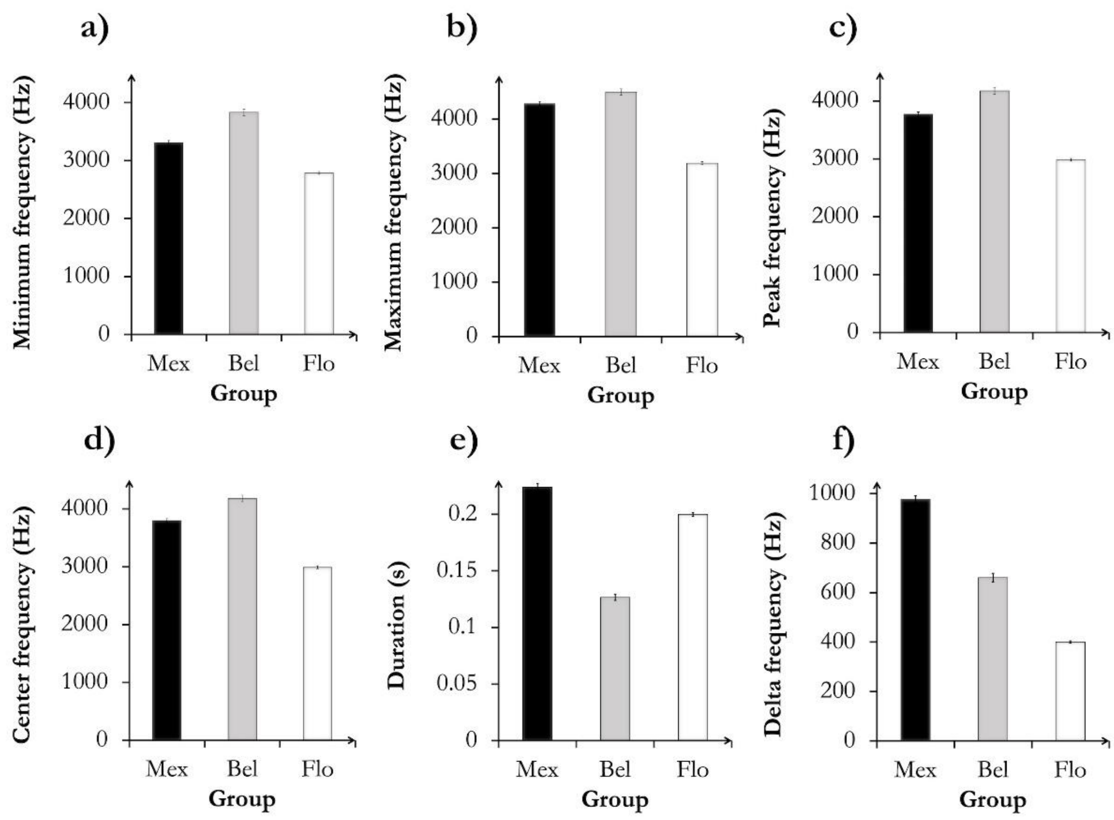

FIG. 3. The mean and standard error of minimum frequency, mean maximum frequency, mean peak frequency, mean center frequency, mean duration, and mean delta frequency

of vocalizations of three geographically distinct manatee groups. Bars in each graph represent the standard error (SE), and the different letters indicate significant differences among groups (PERMANOVA, followed by Dunn's *post hoc* tests, $p < 0.01$).

SIMPER analysis revealed maximum frequency (23.4%), minimum frequency (22.8%), peak frequency (23.2%), and center frequency (23.0%) contributed most to the separation of the three groups. The mean values of minimum (2789.2 ± 25.0) and maximum frequencies (3189.2 ± 25.5) of Florida manatee were generally lower compared to the two Antillean manatee groups. Manatees from Belize had the highest mean values of minimum (3834.5 ± 56.6) and maximum frequencies (4495.3 ± 57.6). Captive manatees from Mexico displayed intermediate values of mean minimum (3309.0 ± 37.7) and maximum frequencies (4285.1 ± 40.0) (Fig. 3). The discriminant analysis revealed differences between groups based on maximum and minimum frequency (Fig. 4).

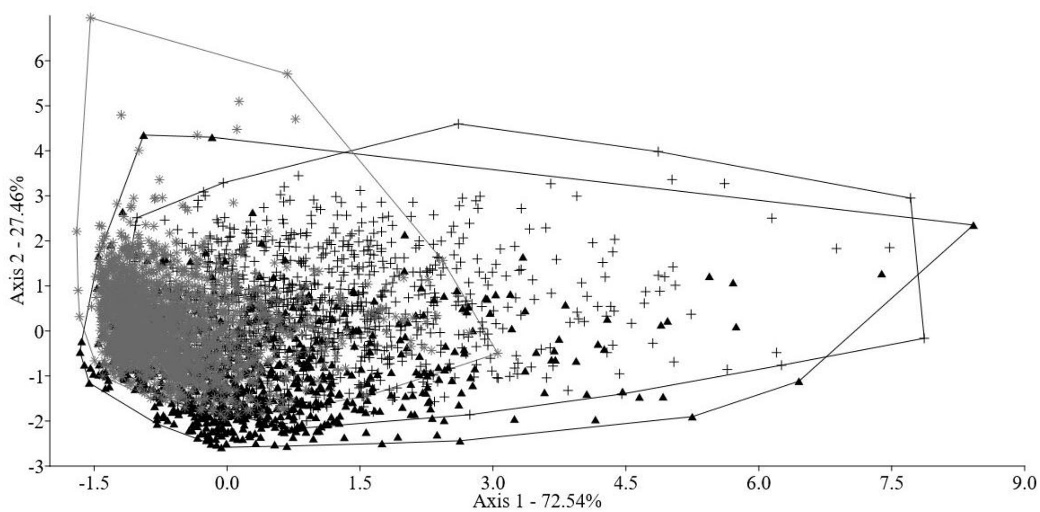

FIG. 4. Discriminant linear analysis of all vocal parameters measured in the three groups of West Indian manatees. Convex hull represents the set smallest of data that contains all points of all parameters in each group. Mexico (**+**), Florida (∗), and Belize (▲).

For the cluster analysis, the best model chosen by the BIC was a 16-cluster solution. Clusters within this solution suggested similarities and differences in vocalizations between the three groups. Six out of the 16 clusters accounted for 63.6% of all calls analyzed. These six clusters (1, 2, 4, 5, 8, and 12) included 56.8% of all calls from the Belize group, 52.4% of all calls from the Mexico group, and 72.2% out of all calls from the Florida group. However, most calls from each geographic site fell into clusters other than the six main clusters. Calls were grouped as follows: 68% of calls from Belize were in clusters 2, 5, 6, 8, and 12; 80% of calls from Mexico were in clusters 2, 5, 7, 11, and 16. Florida has 83% of the total number of calls in clusters 1, 2, 4, 8, 10, 12, and 15 (Table II).

Table II. Percent and number of individual calls of West Indian manatee that fell into each of 16 clusters according to cluster analysis.

Cluster	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Belize	1.95% (15)	11.18% (86)	0% (0)	6.37% (49)	13.65% (105)	19.89% (153)	0.52% (4)	15.60% (120)	3.25% (25)	3.90% (30)	2.34% (18)	8.06% (62)	6.24% (48)	0.39% (3)	3.51% (27)	3.12% (24)
Florida	20.37% (409)	12.70% (255)	6.82% (137)	14.34% (288)	4.43% (89)	1.04% (21)	0.64% (13)	7.37% (148)	0.24% (5)	7.42% (149)	3.28% (66)	12.95% (260)	0.09% (2)	0.74% (15)	7.47% (150)	0% (0)
Mexico	0.09% (1)	17.61% (191)	0% (0)	1.66% (18)	26.29% (285)	2.49% (27)	16.42% (178)	6.54% (71)	4.24% (46)	0.55% (6)	12.28% (133)	0.18% (2)	3.41% (37)	0.92% (10)	0.09% (1)	7.10% (77)
Total	11.01% (425)	13.78% (532)	3.54% (137)	9.19% (355)	12.40% (479)	5.20% (201)	5.05% (195)	8.78% (339)	1.96% (76)	4.79% (185)	5.62% (217)	8.39% (324)	2.25% (87)	0.72% (28)	4.61% (178)	2.61% (101)

IV. DISCUSSION

In this study we analyzed 3,859 vocalizations of West Indian manatee's two subspecies from the wild (Florida and Belize), and in captivity (Mexico). Six parameters were measured from the fundamental frequency to determine if there were differences between geographic locations and subspecies. Whereas the calls of each group had similar frequency ranges (0.5 and 5 kHz) and duration 0.05 - 0.5 s, our statistical analyses revealed differences in all measured vocal parameters. These findings suggest there are vocal differences at the subspecies level; however, multiple factors that could have contributed to this variation.

Similarity in vocal features was supported by the cluster analysis which showed an overlap of 68% of the total number of calls of the three groups. However, our cluster and discriminant analyses revealed differences between the three groups. West Indian manatees have similar vocal tract anatomies (Grossman *et al.*, 2014; Landrau-Giovannetti *et al.*, 2014) and cranial morphology (Barros *et al.,* 2016), which are directly related to vocal range capabilities (Grossman *et al.*, 2014; Landrau-Giovannetti *et al*., 2014). Previous studies identified few differences in comparing Antillean and Florida manatee calls (Nowacek *et al.,* 2003; Alicea-Pou, 2001). However, in our study, statistical analyses revealed differences between the three groups, which could be attributed to factors such as individuality, gender, or geographic separation, since the animal's identity emitting the vocalization is unknown.

Variation in the vocal parameters and production rate of individual manatees' calls could have contributed to differences found across the three groups. In a prior study on Antillean manatees, individuals were distinguishable by variations in the minimum frequency, maximum frequency, mean frequency, and signal duration (Sousa-Lima *et al.,* 2002). These individual characteristics can cause sounds to be grouped into a single cluster due to their similarity if an individual's calls are over-represented in the sample. The cluster analysis showed that 43% Mexico total calls were grouped within clusters 2, 5, 7, 11, and 16 and came from four individuals in a single facility (mother and calf, and two adult males). This may have influenced the differences observed from the groups from Mexico.

Temporal and frequency parameters differ within the gender and age of manatees (Sousa-Lima *et al*., 2008; Umeed *et al.,* 2017). Studies upon age-related differences in mammals' vocalizations have shown that vocalizations of younger animals tend to have higher fundamental frequencies and shorter duration than adults (Morton, 1977). Manatee calves tend to vocalize 2 to 3 times more frequently than adults and produce calls with higher fundamental frequencies and shorter duration (Sousa-Lima *et al.*, 2002; O'Shea and Poché, 2006; Sousa-Lima *et al.,* 2008). The higher frequency values recorded for the group from Mexico may result from the calls emitted from younger individuals. Since the group of four individuals representing 43% of all calls from Mexico had a calf, and the other groups consisted of 5 calves and subadults, calf vocalizations may have been more prevalent in recordings relative to adults. Several studies have reported that females tend to have higher values of maximum and minimum fundamental frequency but lower mean peak frequencies concerning males (Sousa-Lima *et al.*, 2002; Sousa-Lima *et al.,* 2008). Furthermore, female calls are longer in mean note duration. compared to males (Sousa-Lima *et al.,* 2008; Umeed *et al.,* 2017). These differences may account for the variation observed between Mexico and Belize.

The variations in the vocal repertoire of potentially isolated but related groups may be due to geographical isolation. Previous studies identified genetic (Nourisson *et al.,* 2011; Vianna *et al.,* 2006) and morphological differences between Antillean and Florida manatee subspecies (Domning and Hayek, 1986; Barros *et al.,* 2016). We found differences in the vocal characteristics of calls between the subspecies; frequencies parameters had higher mean values in manatee calls recorded in Belize and Mexico than manatee calls from Florida. These differences were also evident with the cluster analysis (most vocalizations of each group were grouped into different clusters) and are supported by previous research. Ramos *et al.* (2020) found that the mean fundamental frequency of calls produced by wild Antillean manatees in Belize was lower than those reported for other populations of the subspecies from Central and South America but higher than the ones reported from Puerto Rico.

Other factors that were not explored in this study may account for differences in vocal characteristics. It has been reported that many animals adjust the frequency and temporal content of vocalizations to optimize the propagation of signals in their respective habitat (Ey and Fisher, 2009; Miksis-Olds and Tyack, 2009; Morisaka *et al.,* 2005). Studies of geographic variability in some marine mammal species' vocalizations suggest that different environmental conditions (e.g., noise levels and water depth and temperature) can influence regional variation in vocal parameters (Risch *et al.,* 2007; Baron *et al.,* 2007; Bjørgesæter *et al.,* 2004). Although all three sites were shallow-water habitats, Florida animals were recorded in a freshwater refuge where boats were not allowed to enter. Groups from Mexico and Belize were exposed to anthropogenic activities from boats in Belize and their captive environment in Mexico. In the captive recordings from Mexico, tourist's presence in the water, vocalizations and continuous echolocation sounds produced by dolphins in nearby tanks and the captive environment's acoustics may have influenced manatee vocal behavior.

Our findings provide important information for future studies implementing automated detection of calls using supervised learning to count individuals and monitor manatee populations (Castro *et al*., 2016). Passive acoustic monitoring provides valuable information for monitoring cryptic and endangered marine species through non-invasive and relatively low-cost monitoring (Booth *et al.,* 2020; Sousa-Lima *et al.,* 2013; Erbe and King, 2008). This methodology has been used to estimate the presence and density of several of marine mammal species since it can generate important sample sizes in different spatial extensions and types of ecosystems than can document population characteristics and changes in the phenology of marine mammals in relation to the environmental change (Marcoux *et al.,* 2016; Booth *et al.,* 2020).

V. CONCLUSIONS

Our study provides novel insights into the differences in the West Indian manatee's vocal characteristics, and differences observed may be due to individuality, environmental noise, age, gender, and geographic separation. The variability of manatee vocalizations between isolated groups and subspecies highlights the importance of studying their acoustic behavior in a different region. Generalizing our findings to other manatee populations is limited by each group's different recording conditions and the small numbers of animals recorded in the captivity. More research efforts are needed to verify these possibilities and investigate the reasons for these differences, addressing the present study's main limitations. Findings on the variation in acoustic characteristics of manatee vocalizations in Florida and the Western Caribbean will facilitate improved acoustic monitoring of manatees and understanding of how environmental and evolutionary factors shape their vocal behavior.

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Capítulo III. Conclusiones

- Se obtuvieron 3859 vocalizaciones de buena calidad en la relación señal / ruido (SNR ≥ 6), 2007 vocalizaciones correspondientes a manatíes de la Florida en estado silvestre, 769 correspondientes a manatíes de Belice en estado silvestre y 1083 provenientes del grupo de manatíes de México en cautiverio.

- Los tres grupos de estudio comparten características en los rangos de frecuencia y duración, ya reportados para la especie (entre los 0.5 - 5.0 kHz y 0.05 - 0.5 s, respectivamente).

- Las similitudes evidenciadas entre los tres grupos de estudio pueden deberse a que a pesar del aislamiento geográfico, siguen siendo la misma especie compartiendo características anatómicas en el tracto vocal y morfología craneal, las cuales están directamente relacionadas con las capacidades vocales (Alicea-Pou 2001; Grossman et al. 2014; Landrau-Giovannetti et al. 2014; Barros et al. 2016).

- Los análisis estadísticos evidenciaron diferencias significativas en todos los parámetros de medición fuera de los rangos de frecuencia y tiempo compartidos (Figuras 3 y 4).

- Los parámetros que más contribuyeron a la diferenciación fueron: la frecuencia máxima y mínima, pico de frecuencia y frecuencia central.

- Los valores medios de frecuencias fundamentales del grupo de Florida fueron en general más bajos que los valores obtenidos para los grupos de Belice y México.

- El mejor arreglo de conglomerados separó el total de las llamadas en 16 clústeres. Se evidenció una fuerte agrupación de vocalizaciones en seis conglomerados con el 63.6% del total de las vocalizaciones analizadas. Sin embargo, la mayoría de las vocalizaciones propias de cada grupo integraba, además, clústeres diferentes a los seis que contienen la mayoría de las llamadas totales.

- En este estudio se evidencian variaciones en los rangos de frecuencia y duración alcanzados para todos los parámetros medidos en los tres grupos, las cuales pueden estar relacionadas con factores individuales, ambientales, taxonómicos o geográficos.

- Las características individuales pueden causar que los sonidos se agrupen en clústeres independientes, cuando determinados individuos vocalizan frecuentemente y son más representados en la muestra. Los altos rangos de frecuencia obtenidos para el grupo de México pueden deberse a que dentro de los individuos grabados había al menos seis individuos juveniles, los cuales emiten sonidos con valores de frecuencia fundamental altos y vocalizan más frecuentemente en comparación con los adultos.

- Los mamíferos marinos tienden a ajustar sus valores de frecuencia fundamental para optimizar la propagación de las llamadas en presencia de ruido. Los grupos de Belice y México se encontraban expuestos a altos niveles de ruido. En el caso del grupo de México, las grabaciones presentaron contantemente vocalizaciones de delfines y ruidos antropogénicos provenientes de la presencia continua de turistas. En el caso de Belice se identificó la presencia de ruidos provenientes de actividades antropogénicas de turismo y pesca. Sin embargo, no podemos atribuir las variaciones identificadas directamente al ruido ambiental.

- Es necesario implementar más estudios dirigidos a estudiar las variaciones con respecto a factores de diferenciación no integrados en este estudio, tales como el efecto del cautiverio, el aprendizaje vocal y el ruido antropogénico, entre otros.

- Este estudio hace un aporte al conocimiento sobre las características vocales de ambas subespecies de manatí. Los rangos de frecuencia obtenidos pueden ser de utilidad para la mejora y desarrollo de metodologías de identificación automatizada que contribuya al monitoreo poblacional y conteo de individuos en vida silvestre, usando como entrenamiento características vocales y rangos de frecuencia específicos por subespecie o por distribución geográfica.

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