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Original Research Article

Sharing the space: Review of humpback whale occurrence in the Amazonian Equatorial Coast

Nathali G. Ristau ^{a, b, f, *}, Cristiane C.A. Martins ^c, Ricardo Luvizotto-Santos ^{b, d}, Deisi Balensiefer ^e, Glauca Sousa ^e, Miriam Marmontel ^f, Izeni P. Farias ^g^a Instituto Amares – Pesquisa e Conservação de Ecossistemas Aquáticos, MA, Brazil^b Programa de Pós-Graduação em Biodiversidade e Conservação, Departamento de Ciências Biológicas, Universidade Federal do Maranhão, São Luís, MA, Brazil^c Tryphon Océans, Tadoussac, Quebec, Canada^d Instituto de Ciências do Mar, Universidade Federal do Maranhão, São Luís, MA, Brazil^e Centro Nacional de Pesquisa e Conservação de Mamíferos Aquáticos, Instituto Chico Mendes de Conservação da Biodiversidade (CMA/ICMBio), Brazil^f Grupo de Pesquisa em Mamíferos Aquáticos Amazônicos, Instituto de Desenvolvimento Sustentável Mamirauá (IDSMA), Tefé, AM, Brazil^g Laboratório de Evolução e Genética Animal (LEGAL), Departamento de Genética, Instituto de Ciências Biológicas, Universidade Federal do Amazonas, Manaus, AM, Brazil

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ABSTRACT

Humpback whales have a cosmopolitan distribution and undertake annual migrations between low and high latitudes where breeding and feeding takes place, respectively. In Brazil, the main breeding area encompasses the Abrolhos Bank at the Eastern Brazilian Coast and the feeding area of this population is located in South Georgia and South Sandwich Islands. Here, two recent records of humpback whales are reported for Maranhão State, Amazonian Equatorial Coast (AEC), one of which involves a newborn calf with remains of the umbilical cord. Additionally, an in-depth review of the stranding and observation records for this area is presented. Phylogenetic analysis placed the stranded animals within the two most common haplogroups reported for the Southern Hemisphere breeding grounds (CD and IJ). A possible extension of the species range or the recolonization of a historical breeding area along the Brazilian coast is discussed. Presented results provide evidence of distribution overlap between the humpback whale populations of the northern and southern Atlantic Ocean which will require the development of conservation strategies among neighboring countries and underline the need to develop management strategies that will allow sustainable management of the Amazonian Equatorial Coast.

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1. Introduction

The humpback whale, *Megaptera novaengliae* (Borowski, 1781), is a cosmopolitan species present in all oceans (Clapham and Mead, 1999). The species is currently considered monotypic, but there is considerable evidence to support at least three subspecies - North Atlantic, North Pacific and Southern Hemisphere (including Arabian Sea) - based on coloration,

* Corresponding author. Instituto Amares – Pesquisa e Conservação de Ecossistemas Aquáticos, MA, Brazil.

E-mail address: institutoamares@yahoo.com.br (N.G. Ristau).

reproduction, migratory behavior, diet and genetics (Bettridge et al., 2015). The species is characterized by large seasonal migrations, from low to high latitudes, between breeding and feeding areas (Clapham and Mead, 1999; Clapham, 2018). Most reproductive activity occurs in the tropical or subtropical regions, while feeding is mainly observed in high latitudes (Kellogg, 1929). In the tropics, the species is generally observed in coastal waters within the 200 m isobath (Zerbini et al., 2006). The species, which was widely exploited by the whaling trade until the twentieth century drastically reducing its populations (Gambell, 1973; Tønnessen and Johnsen, 1982; Best, 1994), has shown worldwide recovery levels (e.g. Brandão et al., 2000; Clapham et al., 2001; Zerbini et al., 2010; Cooke, 2018).

In the Southern Hemisphere, they have been classified by the International Whaling Commission on reproductive stocks, from A to G (IWC, 2001). The "A" stock, located in the western South Atlantic (IWC, 1998, 2005), has as its main reproductive site at Abrolhos Bank at Eastern Brazilian coast, where the species has been studied since 1990 (Engel, 1996; Siciliano, 1997; Martins et al., 2001; Morete et al., 2003; Andriolo et al., 2006, 2010a,b; Wedekin et al., 2010). Its main feeding area is on the east of the Scottish Sea, around South Georgia and South Sandwich islands (Stevick et al., 2006; Zerbini et al., 2006, 2011; Engel et al., 2008; Engel and Martin, 2009).

Despite evidence of recovery of the breeding stock "A" (Bortolotto et al., 2017; Zerbini et al., 2019), the species is not evenly distributed along the Brazilian coast ($\sim 5^{\circ}$ – 23° S) (Andriolo et al., 2010a,b). It shows a strong preference for the Abrolhos Bank (e.g. Martins et al., 2013) and some authors point to the reoccupation of historical reproductive areas (Zerbini et al., 2004; Rossi-Santos et al., 2008), and possibly the colonization of new areas along the coast (Andriolo et al., 2010a). The main period of occurrence in the Abrolhos Bank is from July to November (Martins et al., 2001; Morete et al., 2003), but early and late observations have already been recorded (Bernadete Barbosa, personal communication). Observations have been increasingly common in the northeastern region of Brazil, but there is little or no systematic effort in the northern limit of the population range ($\sim 5^{\circ}$ S) (Andriolo et al., 2010a,b).

In the North Atlantic, the West Indies constitutes the main breeding area for the species (e.g. Katona and Beard, 1990; Stevick et al., 1999). Animals breeding in the West Indies during the boreal winter (November to April) migrate north to specific feeding sites (Stevick et al., 2006). Limited or little systematic effort in the southern limit of the West Indies breeding area results in a gap of information between the Amazonian Equatorial Coast (AEC) and the Caribbean.

The objective of this study is to review the occurrence of humpback whales off the AEC through a literature review and the analysis of two recent stranding records at the coast of Maranhão State. Efforts to study marine mammals in Maranhão State are recent, and until now four species of mysticets have been recorded: the humpback whale, the Bryde's whale (*Balaenoptera edeni*) (Magalhães et al., 2008a), the common minke whale (*Balaenoptera acutorostrata*) (Magalhães et al., 2008b), and the Antarctic minke whale (*Balaenoptera bonaerensis*) (AMARES, unpublished data). Here, we report the recent occurrence of an adult and a newborn specimen of humpback whales in isolated stranding events on the coast of Maranhão State, northeastern Brazil. By using molecular analysis, this paper suggests the potential population stocks to which those two individual whales belong and discuss a possible distribution extension or recolonization of this equatorial region of the Atlantic Ocean.

2. Materials and methods

2.1. Study area

The Maranhão State ($05^{\circ}04'49.50''$ S; $45^{\circ}36'2.56''$ W) is located on the border between the northern and northeastern Brazilian regions and is at the center of the Amazonian Equatorial Coast (AEC) (Fig. 3). It has approximately 640 km of coastline constituted mainly by mangroves, dunes, and sandy beaches (Palma, 1979; Stride, 1992). Its hydrography is under the influence of the South Equatorial Current, driven by prevailing trade winds throughout the year. This region is marked by the strong influence of macro tides, which amplitude may reach 7.5–8 m in some areas during syzygy tides (BRASIL, 1972). The AEC covers five Brazilian States: Amapá, Pará, Maranhão, Piauí and Ceará and in it is present the largest continuum of mangroves in the world (Nascimento et al., 2013).

2.2. Data collection

Due to the lack of systematic studies of marine mammals in the AEC an in-depth review of the available literature was performed including national and Latin American conference books (Society of Latin American Specialists in Aquatic Mammals - SOLAMAC). Besides, research groups working within the limits of the study area were consulted in order to obtain the most updated list of observations and stranding records of the species in the area.

In Maranhão, cetacean stranding records were obtained opportunistically through notifications by fishermen, community members or local media. Following reports of the strandings, researchers went to the local site for data collection. Data were collected following standard protocols (Geraci and Lounsbury, 2005; Jefferson et al., 2008). Geraci and Lounsbury (2005) characterize the state of animals in codes ranging from 1 to 5, where: 1 - live animals; 2 - fresh carcass; 3 - initial decomposition; 4 - advanced decomposition; 5 - mummified. For each stranding event the following information was collected: geographical coordinates (Garmin EtreX Vista HCX), decomposition status, probable taxonomic identification, complete biometry, and evidences of human interaction (e.g. gillnet marks, propeller marks). Lack of infrastructure and remoteness of stranding locations prevented complete necropsies. Tissue samples were collected and preserved in absolute alcohol for

later molecular analysis. The samples were labelled following the standard protocol of the Laboratory of Evolution and Animal Genetics (LEGAL–UFAM).

The present study conforms to published American Society of Mammalogists guidelines for the use of wild mammals in research.

2.3. mtDNA data

In order to verify to which mitochondrial DNA (mtDNA) groups the sampled individuals belong, according to the classification by Baker et al. (1993) and Olavarría et al. (2007), two samples were submitted to a molecular analysis. The performed DNA extraction used the phenol-chloroform method, following the standard protocol (Sambrook et al., 1989). PCR amplification of mitochondrial DNA control region was performed using the primers LTRO and HDH6 (Banguera-Hinestroza et al., 2003) in reaction with 15 µL of final volume containing 3.7 µL of ddH₂O, 2 µL of MgCl₂ (25 mM), 2 µL of dNTPs (10 mM), 1.5 µL of 10× PCR buffer (100 mM Tris-HCl, 500 mM KCl), 1.5 µL of each primer (2 µM), 0.8 µL of Taq DNA Polymerase (1 U/µL), 1 µL of BSA (20 ng/µL) and 1.5 µL of DNA (concentration varied between 50 ng and 100 ng). The PCR reactions consisted of 35 denaturation cycles at 93 °C for 100 s, primer annealing at 55 °C for 40 s, and primer extension at 72 °C for 90 s, followed by a final extension at 72 °C for 5 min.

Sequencing reactions were performed using the Big Dye terminator kit (Life Technologies) following the manufacturer's instructions. The products of sequencing reactions were submitted to an ABI3130XL sequencer. The sequences generated were edited in the program BioEdit (Hall, 1999) and aligned with the dataset of mtDNA control regions deposited in GenBank (N = 723), which included sequences of the following studies: Palsbøll et al. (1995), Baker et al. (1998), Witteveen et al. (2004), Hatch et al. (2006), Olavarría et al. (2007), Engel et al. (2008), Jackson et al. (2009), Rosenbaum et al. (2009), Valsecchi et al. (2010) and Jackson et al. (2014). The dataset represents the entire known geographic distribution of the species and all the defined haplogroups. The maximum likelihood criterion was implemented in the program Treefinder (Jobb et al., 2004) under the model HKY + G (Hasegawa et al., 1985) using the species *Balaenoptera physalus* (GenBank accession number FJ832130) and *Balaenoptera musculus* (GenBank accession number DQ145102) as outgroups.

3. Results

3.1. Stranding records

Here, two new records of stranded humpback whales were added to the coast of Maranhão State (Figs. 1 and 3). In October 2010, an incomplete carcass of an adult Mysticeti (identification number: MTnid 01) stranded on the Outeiro Beach, Cedral (02°00'30.30" S; 44°30'34.41" W). As reported by residents, the animal was found without the head and fluke (Fig. 1a and b). Fluke fragments of this specimen were later found on Araoca Beach, Guimarães (02°03'56.19" S; 44°30'45.09" W). The carcass was classified as code "5", according to Geraci and Lounsbury (2005), and was later identified as *Megaptera novaeangliae* through molecular analysis Blast system for species identification (Ristau, 2012). The second stranding event (identification number: Mno 01) occurred in September 2011, in the central portion of the state coastline. It was a dead newborn, which stranded on Mangue Seco Beach (02°27'5.40" S; 44°09'39.49" W), São Luís Island, Maranhão (Fig. 1c; d). The animal was a male, code "3" (Geraci and Lounsbury, 2005), measured 4.6 m (total length), and had umbilical cord remnants. The lungs were found externalized in the oral cavity (Fig. 1c). There were marks of cuts and fishing nets on the dorsal and ventral regions of the carcass, especially on the caudal peduncle (Fig. 1d). No epibionts were recorded.

3.2. Genetic analysis

The topology of the maximum likelihood tree of phylogenetic relationships of the two specimens sampled in the present study is shown in Fig. 2. According to the haplogroup classifications suggested by Baker et al. (1993) and Olavarría et al. (2007), the specimen Mno01 belongs to the clade CD and the specimen MTnid 01 to the clade IJ, the two most common haplogroups reported for the Southern Hemisphere breeding grounds (Olavarría et al., 2007). While our samples belong to two globally distributed clades, Rosenbaum et al. (2009) demonstrated a significant degree of population structure between all ocean basins breeding stock using the mtDNA control region. Thus while mitochondrial DNA is unsuitable for performing assignment tests, it is safe to assume that the two individuals belong to the stock A.

3.3. Literature review of the species within the Amazonian Equatorial Coast

A total of thirteen strandings and three sightings were found during the literature review in the AEC and neighboring countries which compose the equatorial coast of South America (Table 1). All the records found in the literature and the strandings here reported are presented in Fig. 3. The oldest record of a humpback whale at the AEC was of a single stranding in the 1980s, which was formally reported by Magalhães et al. (2008a). Afterwards, three standing events were reported to the coast of Ceará: a male calf in November 1997 (Furtado-Neto et al., 1998), an adult of unidentified sex and a female calf, both in 2001 (Meirelles et al., 2009). In November 2002, a pair of adult humpback whales was observed of the coast of Maranhão at a depth of 50 m, during a biota monitoring activity of a seismic prospecting enterprise in the blocks PA-MA 1–2 (00°32'S;



Fig. 1. (a) and (b): Carcass of a humpback whale stranded in advanced decomposition state in Cedral, state of Maranhão, Amazonian Equatorial Coast; (c) and (d): A newborn humpback whale stranded on São Luis Island, Maranhão State, with umbilical cord remnants and protruded male genital organ.

44°17'W) (Ramos et al., 2010). In January 2003, an adult female stranded at Pedra do Sal beach, Parnaíba, Piauí State. The animal was identified to the species level using the morphology of the pectoral fins (Severo et al., 2004). Meirelles et al. (2009) reported two strandings recorded in 2004 along Ceará State coastline: a male calf stranded in April and an adult of unknown sex recorded in October. Additionally, two stranding records were reported to Piauí and Maranhão States. A scapula was found by locals at Carnaubinha beach (Luís Correa, Piauí State) in 2005, and a carcass with no skull registered in Rio Novo beach (Tutóia, Maranhão State) in 2009. The size of the animal was estimated at 13 m (Costa et al., 2017). The most northerly record of the species along the AEC was recorded in 2008 at Peruquara Beach, Quatipuru, Pará State (Pretto et al., 2009). The animal measured around 10 m and was classified as code “4” (Geraci and Lounsbury, 2005).

In addition to AEC, eventual records of humpback whales have been reported for other countries in northern South America (Table 1). In September 2009, one individual of unknown sex and maturity stranded in Guyana (Kalamandeen and Chesney, 2013). Vines et al. (2010) reported a probable sighting of two humpback whales unknown sex and maturity, in October 2009 the coast of French Guyana. More recently (April 20, 2013), two individuals (adult and calf) were sighted by crew members of a small fishing boat in Suriname coast, with photographic records (Boer and De Willems, 2015).

4. Discussion

The records presented here are outside the traditionally known breeding areas of humpback whales in the South and North Atlantic suggesting the use of the AEC by individuals from both hemispheres. The results here presented corroborates with Boer and De Willems (2015). They have suggested an extension of the southern limit of the West Indies breeding area, based on the observation off the coast of Suriname. Presented information support the extension north of 5°S to the breeding Stock “A” and extension south to the West Indian breeding stock.

Stranded aquatic mammals are mostly found at an advanced decomposition stage, which alters diagnostic characters and even makes species identification unreliable (Geraci and Lounsbury, 2005). Molecular tools as used here, allow specimens identification to the species level (Baker et al., 1996; Cipriano and Palumbi, 1999; Dalebout et al., 1998) even for carcasses at an advanced decomposition stage (Sholl et al., 2013). The higher temperatures of the tropical region speed up the decomposition process. As the AEC is located outside the traditional distribution range of the stock A, the use of genetics was necessary in order to identify the probable origin of these individuals. The two stranded individuals reported in the present study, belong

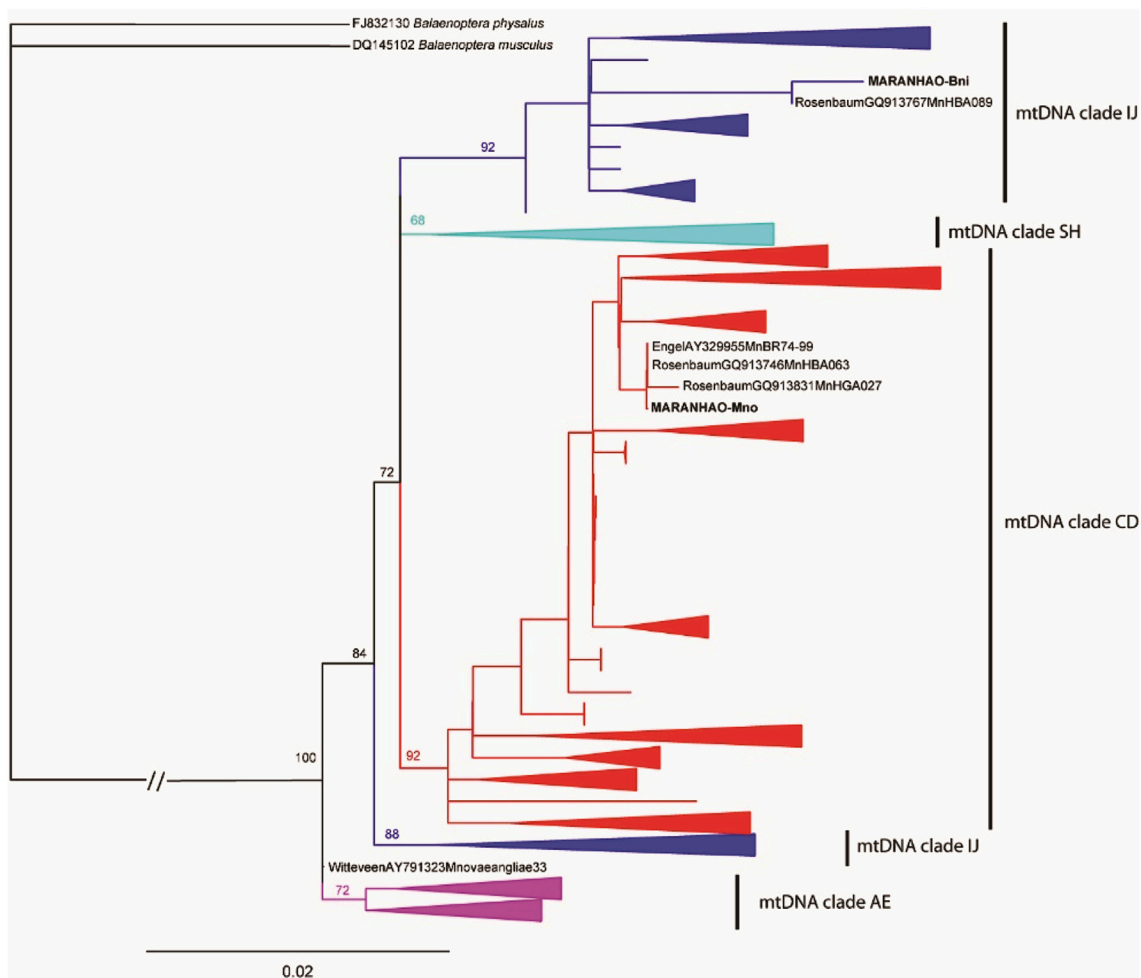


Fig. 2. Maximum likelihood tree showing the phylogenetic relationships between the individuals MTnid 01 and Mno 01 and the clades CD, IJ, AE, and SH, following previous publications (Baker et al., 1993; Olavarría et al., 2007).

to the most frequent haplogroups according to the studies by Olavarría et al. (2007) and Engel et al. (2008). Both stranding events occurred during the reproductive season of humpback whales off the Brazilian coast, and thus most likely belong to the breeding stock A (Rosenbaum et al., 2009 Fig. 1). However, to definitely assign individuals to breeding stocks, a public microsatellite or SNP database with samples of individuals from all known breeding groups needs to be created. The molecular analysis here presented does not allow us to confirm with 100% certainty that the stranded animals belong to the breeding stock A, but this is the most likely scenario. Darling and Sousa-Lima (2005) found some degree of acoustic interaction between individuals from stock A and B, however, there is little chance that the currents would transport a carcass from Western Africa to the AEC.

The injuries found in the newborn calf suggesting interaction with fishing gears are rarely visible in animals stranded in the tropics, which are usually too decomposed to allow marks identification. However, they bring our attention to the risk of entanglement in the region. There is a large local fishing fleet in the region and the fleets of neighbor states which also use this coast (Almeida et al., 2010) may threaten the species due to the risk of entanglement in fishing nets (Siciliano, 1986, 1994; Pizzorno et al., 1998; Bettridge et al., 2015). In addition, the heavy traffic of large vessels in the vicinity of the Itaqui Harbour and Ponta da Madeira Terminal in São Luís, Maranhão (02°33'31"S; 44°21'30"W), might represent a threat to individuals using this area. As suggested by Andriolo et al. (2010b), the increasing presence of humpback whales along the Brazilian Coast concomitant with an intensification of human activities in the coastal area may result in a high number of entanglement episodes and of collision with large vessels.

The stranding of the newborn occurred in September, the month during which humpback whales abundance peaks in the Abrolhos Bank (Martins et al., 2001; Morete et al., 2003), and is also the month with the highest concentration of mother and calf groups in the Abrolhos Bank (Martins et al., 2001). It is widely accepted that stranding records do not provide precise information about cetacean distribution as tides and prevailing winds may carry the carcasses to unusual areas (Raga et al.,

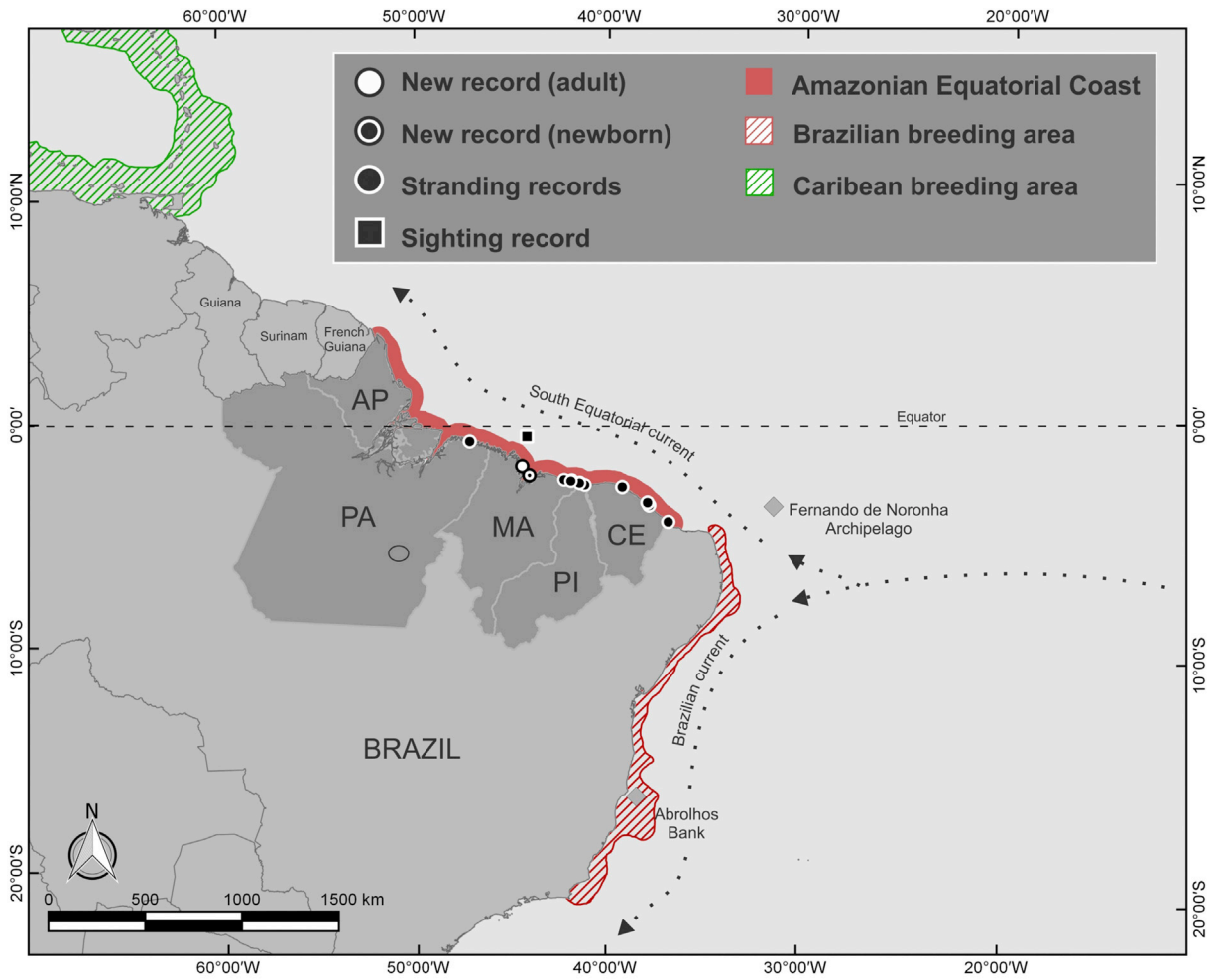


Fig. 3. Map of the Amazonian Equatorial Coast and respectively Brazilian states: Amapá (AP), Pará (PA), Maranhão (MA), Piauí (PI) and Ceará (CE) with the location of humpback whale records found in the literature review and here reported.

Table 1

Humpback whales records in the Equatorial Atlantic (-5° S; 7° N) showing its location, type, date, number of individuals (N ind), sex, maturity (when available) and reference.

	Local		Record type	Month	Year	N ind	Sex and Maturity	Reference
	Country	Emplacement						
Northern Hemisphere	French	–	Sighting ^a	October	2009	2	Not identified	Vines et al. (2010)
	Guyana	–	Stranding	September	2009	1	Not identified	Kalamandeen and Chesney (2013)
	Guyana	–	Stranding	September	2009	1	Not identified	Kalamandeen and Chesney (2013)
AEC BRAZIL	Suriname	–	Sighting	April	2013	2	Adult and calf	Boer et al., 2015
	PA	Peruquara Beach	Stranding	October	2008	1	Not identified	Pretto et al. (2009)
	MA	Pará-Maranhão Sedimental basin	Sighting	November	2002	2	Adult	Ramos et al. (2010)
		Oceânica Beach, Caju Island	Stranding	–	80's	1	Adult	Magalhães et al., 2008
		Outeiro Beach		October	2010	1	Adult	This paper
		Araçagy Beach		January	2011	1	Male/Neonate	
	PI	Pedra do Sal Beach	Stranding	September	2003	1	Female/Adult	Severo et al. (2004)
	CE	–	Stranding	November	1997	1	Male/calf	Furtado-Neto et al. (1998)
		–		August	2001	1	Adult	Meirelles et al. (2009)
		–		September	2001	1	Female/calf	
	–		April	2004	1	Male/calf		
	–		October	2004	1	Adult		

PA – Pará State.

MA – Maranhão State.

PI – Piauí State.

CE – Ceará State.

^a Observation unconfirmed (sighting without pictures by local fisherman).

1991). However, the stranding of a fresh newborn calf with umbilical cord remnants ratifies not only that the coastal area of Maranhão State is being used during the breeding season, but that it is also being used as a nursing area.

Siciliano (1997) was the first to suggest the recolonization of historical sites along the Brazilian coast by humpback whales. Although the recolonization of the coast of Maranhão by humpback whales should be considered, the lack of knowledge on the species range before the whaling period precludes a conclusion. The Breeding Stock "A" has recently be considered as recovered from the hunting period (Zerbini et al., 2019) and the population presents high rates of annual growth (~7.4% according to Ward et al., 2006). Thus, the increasing records of the specie on the AEC indicate that the animals are probably looking for new areas, as suggested by Andriolo et al. (2010a), and expanding their breeding range. Recent studies reported a higher number of smaller individuals in areas outside of the Abrolhos Bank, suggesting that younger animals would be responsible for searching and settling down in new areas of the breeding sites (Del Vecchio et al., 2015), though the low recapture rates in the Abrolhos Bank still does not allow greater inferences concerning these aspects (Baracho-Neto et al., 2012).

The range extension or recolonization of the Breeding Stock "A" is not only restricted to the AEC. The records from Guyana and French Guyana occurred during the wintering period in the Southern Hemisphere (September and October) (Vines et al., 2010; Kalamandeen and Chesney, 2013). Additionally, in the Marine Mammal Atlas published recently by the Museum of French Natural History, rare observations of humpback whales were reported on Guyana's continental shelf in September and November (Counihan et al., 2012), and the authors suggested that the animals belong to the Southern Hemisphere (SH) breeding population (Counihan et al., 2012). On the other hand, the record from Suriname occurred during the wintering period of the Northern Hemisphere (April 20th). Boer and De Willems (2015) suggest that the individuals belong to the Northern Hemisphere (NH) breeding grounds. Other records outside the typical period of occurrence of humpback whales along the Brazilian coast have been recorded in Ceará and Sergipe States, both in April (Meirelles et al., 2009; Petrobras, 2015). The most probable scenario for the records from April is that these animals belong to the West Indies breeding population. Therefore, that would suggest that not only the breeding Stock "A" is expanding its range in the NH but also that the NH humpback whales are expanding their breeding range in the SH. In other words, that would mean that with the species recovery in both Hemispheres, SH and NH populations are sharing the same area in different periods of the year. A similar behavior has already been observed in the Pacific Equatorial region. Rasmussen et al. (2007) have previously observed trans-equatorial movements of humpback whales in the Pacific Ocean. South Pacific humpback whales were observed as far north as 11° N off Costa Rica, in an area also used by the North Pacific population during the opposite winter season. They have shown that the temperature regime at the wintering areas probably constitutes a major selective force driving humpback whale habitat use around the tropics (Rasmussen et al., 2007). Individual movements of animals belonging to the breeding stocks F (Cook Islands and French Polynesia) and G (Ecuador, Colombia, Panama and Costa Rica) to the equatorial region in the Pacific, demonstrate that such movements are biologically possible (Stevick et al., 2004; Olavarría et al., 2007; Nauser et al., 2010). A less plausible scenario to explain the records of April would be an anticipation of humpback whales migration from austral feeding areas. Here, we provided other information to support the distribution overlap between the humpback whale populations from both hemispheres of the Atlantic Ocean around the AEC that will require the development of conservation strategies among the neighboring countries. Besides, it underlines the need to develop management strategies that will allow human activities and an increasing whale presence to share the same portion of the AEC.

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Ethical approval

The present study conform to published American Society of Mammalogists guideline for the use of wild mammals in research.

Sampling and field studies

The samples were collected in collaboration with the Center for Aquatic Mammals/ICMBio – Ministério do Meio Ambiente (MMA) – Brazilian environmental agency with attribution to the rescue of marine mammals strandings.

Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.gecco.2019.e00854>.

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