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Advances in the knowledge of the biology and conservation of the Amazonian manatee (*Trichechus inunguis*)

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Abstract

The last relevant work that reviewed the biological and conservation aspects of the Amazonian manatee (*Trichechus inunguis*) was published almost 30 years ago. In these three decades, scientific knowledge about the species has advanced substantially. This review article updates knowledge about the various biological and conservation aspects of Amazonian manatee, summarizing the information present in the literature. Topics such as morphology, habitat, distribution, population aspects, food and feedings habits, reproduction, behavior, health, threats, status, and conservation efforts were addressed. The main actions already carried out, as well as the future strategies needed for the conservation of the Amazonian manatee, are also highlighted.

Keywords:
biological aspects, conservation efforts, research, sirenian, threats

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Introduction

The Amazonian manatee (*Trichechus inunguis*), endemic to the Amazon basin, is one of the four living sirenian species, but the only one that inhabits freshwater environments exclusively. As other sirenians, the Amazonian manatee is classified as Vulnerable by the IUCN based on a suspected population decline, increases in incidental calf mortality in recent years, and loss of habitat (Marmontel et al., 2016).

The biological aspects of the Amazonian manatee have been described since its first description made by Natterer in 1883. A review of the knowledge on biological aspects of the species, threats, and conservation efforts was published 100 years later (Rosas, 1994) demonstrating differences with other sirenians including evolutionary adaptations to the Amazonian environment. That review also pointed at large gaps in several topics of the biological aspects of the species. Since then, several studies on morphology, physiology, feeding habits, health, behavior, habitat, conservation issues, and others have been carried out and after almost 30 years, we herein present a review of the recent advances in the knowledge of the biology of the Amazonian manatee and the current efforts for its conservation.

Morphology

The Amazonian manatee is the smallest living sirenian, reaching up to 2.75 m in length and weighing up to 420 kg (Amaral et al., 2010a). The range of body length and weight at birth was estimated as 85 – 105 cm and 10 – 18 kg, respectively (Best, 1984; Amaral et al., 2010a) and there are no differences in body size or even in cranial characters between the sexes (Domning & Hayek, 1986; Amaral et al., 2010a; Valdevino, 2016; Barros et al., 2017; Valdevino et al., 2021). Growth curves were estimated for captive (Colares, 2002; Albuquerque Junior, 2003) and free-ranging individuals (Vergara-Parente et al., 2010), and the growth rates observed, despite methodological differences among studies, were less than 0.4 cm/year. An allometric pattern of growth is observed in the skull and other bones (Domning & Hayek, 1986; Valdevino, 2016; Barros et al., 2017; Valdevino et al., 2021). Therefore, osteological measurements can be used on body length estimations for this species (Valdevino et al., 2021). Additionally, Brum (2013) demonstrates the potential use of the flipper radiographic evaluation on age estimation of juvenile and young adult Amazonian manatees. Barros (2014) described the 2n = 56 chromosomes of the Amazonian manatee with the karyotype formula (10m + 22 or 24sm + 20 or 22a + XX/XY). The X chromosome is submetacentric and Y is acrocentric. Heteromorphism and polymorphism were also observed by the author, suggesting a recent chromosomal evolution in this species.

Besides the morphological aspects previously compiled by Rosas (1994), studies regarding digestive tract and food acquisition, sound production and reproduction were carried out in the last decades. Being an herbivorous species, the Amazonian manatee, as the other sirenian species, manipulates the food using an elaborate facial musculature and the perioral bristles, directing the plants to the mouth (Marshall et al., 2003). The tongue is muscular and firmly fixed in the oral cavity, showing numerous mechanical papillae distributed over the dorsum and foliate papillae (with the presence of taste buds) distributed on the root (Nascimento et al., 2022). The gastrointestinal tract was described by Beddard (1897), Mitchell (1905) and Colares (1994), showing a singular morphology. The stomach is relatively small and has a noticeable appendix formation (cardiac gland). The large intestine is almost as long as the small intestine, with two diverticula on the cecum. The long gastrointestinal tract, its particular morphology and the low metabolic rate (Gallivan & Best, 1980) allow a long gut transit time of approximately five days (Barbosa et al., 2013) improving the nutritional use of the food. The mechanism of sound production was investigated by Landrau-Giovanneti et al. (2014) who suggested the vocalization production is due to the existence of rudimentary vocal folds in addition to the vibration of the laryngeal cartilages and the nasal region previously suggested.

The reproductive tract is similar to other sirenians. Females have a bicornuate uterus with the horns projected to the hypogastric fossae and ovaries with approximately 6 cm length (Rodrigues et al., 2008). The placenta is classified as zonary and endotheliochorial, showing a four-chambered allantoic sac similar to other afrotherians (Carter et al., 2008). In males, the testes are ovoid, flattened ventrally in shape, with approximately 7.5 cm of length and located intracavitary caudolateral to the kidneys (R.S. Amaral, unpub. data). The males have a distinguished vesicular gland, a nonpronunciated prostate and a musculocavernous penis (Chávez-Pérez et al., 2018; Araújo & Amaral, 2020). The morphological aspects of the spermatozoa were also described for the species: spermatozoa measure approximately 60 μm with an oval flattened head and a long midpiece with mitochondrial helix, similar to other sirenians (Amaral et al., 2010b). Both sexes have mammary glands with one teat located on the axillary region, under each flipper, being more developed on adult females (Rodrigues et al., 2014). The milk has high fat content (8.76 – 19.73%), apparent absence of carbohydrates, and variation on its content throughout the lactation period (Barbosa, 2011; 2016).

Habitat, Distribution and Populational aspects

The original extension of the distribution of the Amazonian manatee and the areas in which the species could be extirpated are not well known. The early reports on the species are from the European explorers as Friar Gaspar de Carvajal who, in 1542, accompanied Francisco de Orellana's expedition from the Napo River in Ecuador to the Amazon estuary and wrote about the use of the manatee skin and meat by the natives. One hundred years later, the missionary Cristóbal de Acuña, traveling in the same area, reported a high abundance of the species and that its exploitation was mainly for subsistence (da Silva, 2022).

Amazonian manatees inhabit most basins of the main rivers in the Amazon region. The species occurs in white, black and clearwater rivers and is present in areas of *igapó* (black and clearwater floodplain forest) and *várzea* (whitewater floodplain forest), rivers and lakes (Junk & da Silva, 1997; da Silva et al., 2019). They can be found in almost every tributary of the Amazon River, in Colombia, Peru, Ecuador, and Brazil (Bonvicino et al., 2020), and are mainly limited by troubled waters in rivers with rapids and falls (Best, 1984).

The occurrence of Amazonian manatee among these rivers is illustrated on Fig 1. In Brazil, Amazonian manatees are distributed along the tributaries and lakes of the Amazon River as Solimões, Juruá and Purus (da Silva et al., 2019; Brum et al., 2021). Although the Amazonian manatee occurs in the Tocantins, Xingu, Tapajós and Madeira rivers in Brazil, no records of the species exist upriver from the falls in these rivers. In Peru, Amazonian manatees were recorded in the Loreto Department, and in the Pacaya-Samiria Reserve, in Peru, including the rivers Marañón, Pastaza, Ucayali, Huallaga, Purus, Tapiche, Yarapa, Yanayacu-Pucate, Puinahua and Iracahua. Amazonian manatees have also been registered at the Nanay, Orosa, Yavarí, Yaguas, and Putumayo Rivers, and in the Reserva Comunal Tamshiyacu-Tahuayo (Reeves et al., 1996; Silva et al., 2015; Marmontel et al., 2016).

Amazonian manatees have been reported in the Negro and Putumayo-Içá basins, which have areas within four Amazonian countries (Brazil, Colombia, Peru and Ecuador). In Ecuador, the majority of records for the species are from the extreme northeastern portion of the country in the hydrographic systems of Aguarico and Cuyabeno, including the Lagartococha River (Denkinger, 2010; Utreras et al., 2013; Brice, 2014; Ruano et al., 2021). Amazonian manatees were also recorded in the Yasuní

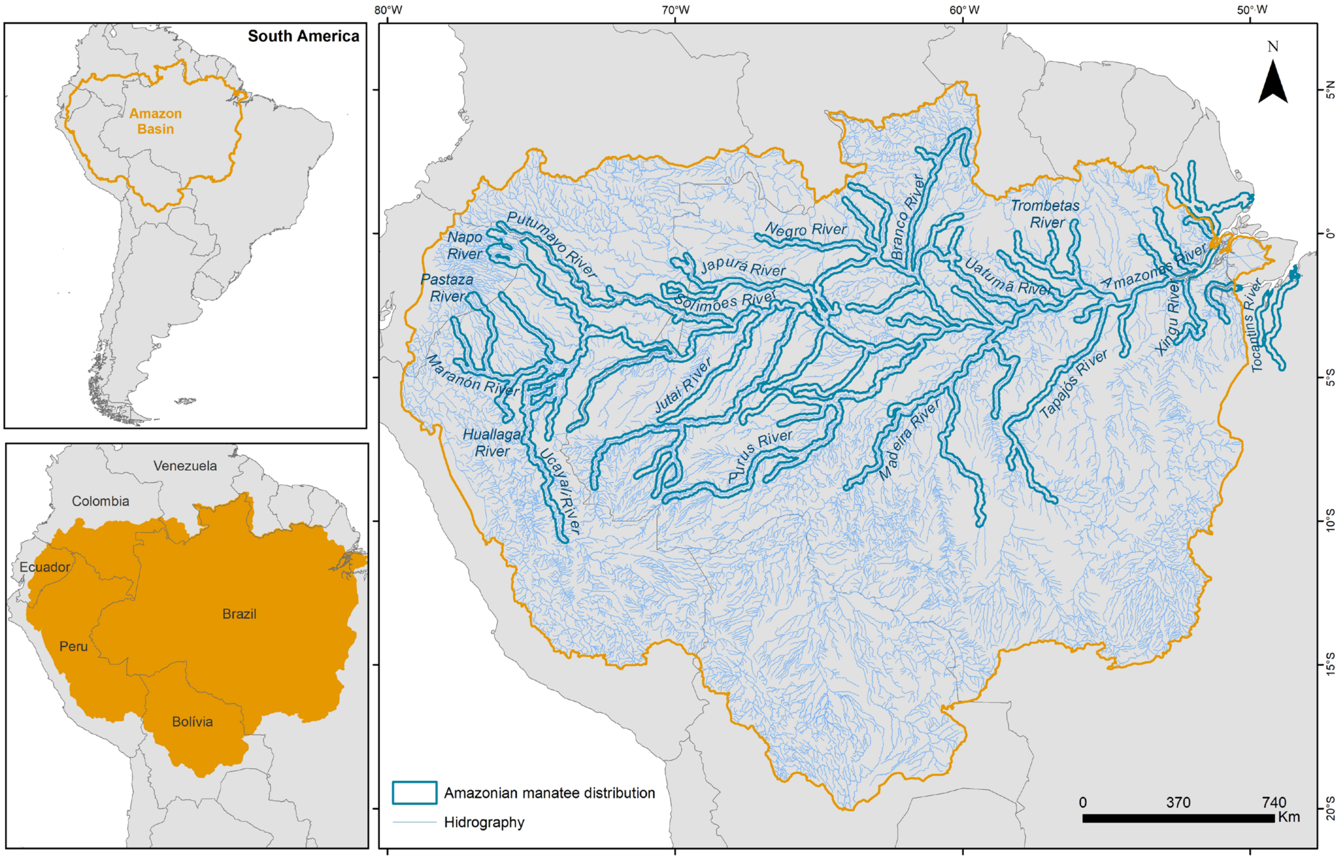

Figure 1. Distribution map of Amazonian manatee (*Trichechus inunguis*). Spatial data from IUCN (2016).

River, including the Jatuncocha and Tambococha lagoons, Añangu River and Cacaya River (Brice, 2014; Ruano et al., 2021). Evidences of Amazonian manatee presence exist for the Tiputini River and for the Gueppi River, tributary of the Putumayo (Utreras et al., 2011). There are historical records for the species at the Napo River (Timm et al., 1986), and Utreras et al. (2011) report its presence in the Challuacocha and Yuturi systems. In Colombia the species can be found in the Amazon River, Putumayo River (below Puerto Arica), Caquetá River (below the rapids of Córdoba), and in the lower Apaporis River (mainly in the Taraira lake) (Trujillo et al., 2006). The presence of the species in Guyana was mentioned by Bertram and Bertram (1963) and documented by a rescue done by INPA (National Institute of Amazonian Research – INPA, Brazil) in the Takutu River border of Brazil and Guyana (V.M.F. da Silva, unpub. data). The occurrence of hybrids of the two species of manatee (*T. inunguis* and *T. manatus manatus*) in the sympatric area at the mouth of the Amazonas River and up to French Guiana has already been reported (Vianna et al., 2006; Lima et al., 2019; Vilaça et al., 2019; Luna et al., 2021a; Noronha et al., 2022).

Recently, some studies applied or suggested different and innovative methodologies to estimate the occurrence of Amazonian manatees. Souza et al. (2021b) utilized a combination of visual effort for individual observation and indirect evidence (feces and feeding records) based mainly on the skills of experienced former manatee hunters in identifying the presence of the animals, while Brice (2014) and Ruano et al. (2021) used a fish finder side-scan sonar to detect Amazonian manatees' presence in the evaluated area. Additionally, Hunter et al. (2018) suggested the use of eDNA as a tool for estimating the occurrence of Amazonian manatee, as promising results have been obtained with *T. manatus* in the wild (Lozano-Mojica & Caballero, 2021). Although these techniques have been shown to be useful to detect the presence of manatees, an efficient method to estimate density and abundance has not yet been developed; the low number of detections over a wide and variable area remains a challenge for the development of abundance and density estimates. New technologies such as passive acoustic monitoring and the use of multi-beam sonar are also being tested for this purpose (Gonzalez-Socoloske et al., 2022; Gonzalez-Socoloske & Olivera-Gómez, 2023).

Therefore, the Amazonian manatee population size remains unknown due to the species' cryptic behavior, and the complexity and dimensions of the Amazon region. From all historical accounts, the species was abundant, well adapted and widespread in the rivers and lakes of the region. Historical records reveal the indiscriminate exploitation of this species, reducing the population to a point where it became commercially extinct (Domning, 1982; Best, 1984; Antunes et al., 2016). Previous studies have found a high genetic diversity across the current Amazonian manatee distribution, without any evidence of population fragmentation, despite the historical reduction by hunting. These results suggest that the Amazonian manatee's most likely behaves as a panmictic population (Cantanhede et al., 2005; Vianna et al., 2006). However, Satizábal et al. (2012) observed a low but significant cluster structure among Amazonian manatee populations, suggesting the existence of two large populations, a Peruvian Amazonian rivers population, and a Colombian and Brazilian Amazonian rivers population.

Foods and feedings habits

The Amazonian manatee is an exclusively herbivorous species, non-ruminant, with a high digestive efficiency (44 - 68%) (Best, 1981). Apparently, the species is opportunistic, consuming a great variety of aquatic and semiaquatic plants (Colares & Colares, 2002; Guterres et al., 2008; Franzini et al., 2013; Guterres-Pazin et al., 2014; Crema et al., 2019a; Carvalho et al., 2022). Over 49 species of plants have been recorded as food for Amazonian manatees, most belonging to the families Poaceae and Azollaceae (*e.g. Hymenachne amplexicaulis, Oryza grandiglumis, Paspalum repens, Azolla caroliniana, and Limnobium spongia*) (Guterres-Pazin et al., 2014). These records were generated from interviews at local communities (Guterres et al., 2008; Franzini et al., 2013; Crema et al., 2019b) and from stomach and fecal samples (Colares & Colares, 2002; Guterres-Pazin et al., 2014). Leaves and fruits of riverine trees (*e.g. Cecropia latiloba, Elaeoluma glabrescens*) also were reported as components of the Amazonian manatee's diet (Guterres-Pazin et al., 2014). The species can also consume, although incidentally, small invertebrates associated with roots and leaves of aquatic macrophytes and algae (Best, 1981; Colares & Colares, 2002; Guterres-Pazin et al., 2012). Colares and Colares (2002) and Guterres-Pazin et al. (2012) assumed the Amazonian manatee is not an efficient seed disperser, since the seeds observed in the stomach contents and fecal samples were broken.

Recently, two main studies were published focusing on the stable isotope analyses of manatees' teeth and bones (Crema et al., 2019a; Carvalho et al., 2022). The samples were obtained from different biological collections encompassing manatees from distinct Amazonian environments such as *várzeas, igapós* and estuaries. Carvalho et al. (2022) showed that where C4 plants are abundant, mainly *várzeas*, they are consumed in a significant proportion for all age classes. C3 aquatic plants were the item that most contributed to the diet of the adult Amazonian manatees in these flooded areas, possibly due to the high diversity of C3 species and availability during all year. Crema et al. (2019a) observed different isotopic values according to age class, and suggested mother-calf pairs have different food preferences or habitat use compared to other age classes in *igapós.*

Longevity and Reproduction

The longevity of the Amazonian manatee is still unknown. From animals deposited in museums and scientific collections, the oldest free-ranging individual recorded was 36 years old (Vergara-Parente et al., 2010). In captivity, the longest-lived individual died at age 63 in a Zoobotanical Garden in Belém, Brazil. Therefore, despite the existence of more robust information from free-ranging animals, the longevity is estimated at 60 years.

Captive male and female *T. inunguis* apparently reach sexual maturity at the same body size (approximately 180 cm and 120 kg), but the onset of puberty in females (~3 years) occurs earlier than in males (~6 years) (Amaral et al., 2018). The estrous cycle lasts approximately 40 days, showing some similarity on the hormonal dynamics with elephants (Amaral et al., 2014). Gestation lasts around 12 months (Amaral et al., 2015a), with each pregnancy producing one calf that is nursed by the mother for about two years (Best, 1984; da Silva et al., 2000). The species shows reproductive seasonality, with the copulation and births occurring during the rising-water period of rivers, the time when food availability is greatest (Best, 1982). Reproductive seasonality also is reported in captivity (Amaral et al., 2015b) suggesting an environmental trigger other than food availability on the control of reproductive seasonality in this species.

The Amazonian manatee has a promiscuous mating system. The first reports of Amazonian manatee sexual behavior are found in the writings of Alexandre Rodrigues Ferreira, a Brazilian naturalist who traveled the Brazilian Amazon between the years 1756 and 1815. In 1944, Pereira summarized more detailed information: reproduction could take place at different times of the year, although he believed to be more frequent in the flooded season, when there are greater depths for their movements; about 15 to 30 manatees could be involved in the sexual activity. The reports of this vigorous and promiscuous behavior are also present in interviews of local communities (Calvimontes & Marmontel, 2006). Recently, direct observations of Amazonian manatee mating herds were reported in Brazil (middle-Solimões River and Pará River estuary) and Ecuador (Reserva de Producción de Fauna Cuyabeno) (Carvalho et al., 2017; Emin-Lima et al., 2021). All those observations reported an active interaction among males disputing for a position next to the receptive female.

Behavior

Vocalizations in Amazonian manatees consist of one to four notes with fundamental frequency ranging from 1.07 to 8 kHz (Sousa-Lima et al., 2002). The individuals show vocal signatures and the vocalization patterns are different between sexes and age classes. Calves and lactating females show higher vocalization rates than other age classes. This high vocalization rate seems to be important in the maintenance of proximity between mother and calf. Additionally, mother-calf pairs show similarity in vocalization patterns, suggesting a process of vocal learning and a vocal strategy for mother-calf recognition (Sousa-Lima et al., 2002; Dantas, 2009).

Amazonian manatee movements and habitat use follow the seasonal variations in the hydrological cycle in the Amazon (Best, 1984). Arraut et al. (2010) reported the monitoring of 10 individuals using VHF telemetry between 1994 and 2006 in the Mamirauá and Amanã Sustainable Development reserves, which constitutes the only long-term dataset on Amazonian manatee movements in the wild. Authors showed a migratory route between a floodplain lake (Mamirauá Reserve) and a *ria* (drowned river valley) lake (Amanã Reserve). During the high-water season, the animals stay in *várzea* lakes in association with macrophytes. During low-water, the drastic reduction in aquatic space in the *várzea* leads to the risk of their habitat drying out and promotes the migration of the animals to deep-water lakes for protection from predators such as caimans, jaguars and humans. This migratory behavior apparently is intrinsic to the species, occurring in other parts of the Amazon (Pereira, 1944; Kendall et al., 2004; Landeo-Yauri et al., 2017; Crema et al., 2019a). However, distances of such seasonal migrations will depend on the animals' habitat spatial configuration (Deutsch et al., 2022b). Therefore, it is difficult to extrapolate the routes and distances of migration to all species' range due to the heterogeneity of habitats, degree of anthropogenic impact (historical and current hunting) and

Table 1. Hematological values of captive immature Amazonian manatees (*Trichechus inunguis*).

		Sousa et al. (2016)						Maduro et al. (2020)		
	Units	Immature males	Mean	SD	Immature females	Mean	SD	Immature males and females	Mean	SD
Hct	%	8	37.2	3.76	12	38.12	5.66	10	36.21	1.92
Hemoglobin	g/dL	8	12.3	1.13	12	12.67	1.89	10	11.82	0.69
RBC count	106/µ	8	4.38	0.25	12	4.46	0.41	-	-	-
MCV	fL	8	84.7	3.78	12	85.73	4.77	-	-	-
MCH	pg	8	28	1.07	12	28.48	1.6	-	-	-
MCHC	%	8	33.04	0.31	12	33.2	0.07	-	-	-
WBC count	103/µL	8	8,300	1,717	12	9,643	1,887	-	-	-
Heterophils	%	8	60	3	12	54	6	-	-	-
Heterophils	103/µL	8	4,995	1,154	12	5,136	1,049	-	-	-
Lymphocytes	%	8	35	3	12	41	5	-	-	-
Lymphocytes	103/µL	8	2,943	684	12	3,994	1,106	-	-	-
Monocytes	%	8	2	1	12	2	1	-	-	-
Monocytes	103/µL	8	141	71	12	182	140	-	-	-
Eosinophils	%	8	3	1	12	4	2	-	-	-
Eosinophils	103/µL	8	221	103	12	336	267	-	-	-

Hct = hematocrit; RBC = red blood cell; MCV = mean cell volume; MCH = mean cell hemoglobin; MCHC = mean cell hemoglobin concentration; WBC = white blood cell; SD = standard deviation

habitat alteration (Arraut et al., 2017). In Piagaçu-Purus Reserve, lower Purus River, Brazil, manatees additionally occupy floodplain lakes during the low-water season (Souza et al., 2021b). In this case, other effects such as the largest distance to the *ria* lake and appropriate conditions (*e.g.* larger lakes and reduced risk posed by humans) can maintain the animals in the *várzea* lakes during the dry season (Souza et al., 2021b). In the Negro River basin, Crema et al. (2019b), through interviews with local people, also reported the migratory behavior of the species, determined by the hydrological cycle. Through isotopic analysis, Crema et al. (2019a) reported that, in Rio Negro, lactating females can be induced to migrate to floodplain areas to protect themselves and supplement their nutritional needs. In the Tapajós River, in the lower Amazon region, the authors reported that manatees might not perform seasonal migrations, as the *igapós* offer habitats and abundant plant nutrition during all phases of the flood pulse, but do not rule out the possibility of migration in this region.

Daily patterns in movements and habitat use have not been documented in Amazonian manatees, but the little information that exists is mostly from captive released animals (Deutsch et al., 2022a). Kikuchi et al. (2012) recorded the swimming behavior of two rehabilitated-and-released Amazonian manatees in 3D, showing a maximum dive depth of 10.8 m in the wild. The first radio-marked Amazonian manatee was a juvenile male that moved 2.6 km/day after release (Montgomery et al.,1981). The animal was tracked for 20 days and moved at a similar rate by day and night. According to Landeo-Yauri et al. (2017), Amazonian manatees spend most of their time resting or performing short displacements. Usually the displacements are about 3 km/day, but the animals are able to move greater distances mainly in the dry season (Souza et al., 2018).

Health

Most of the information regarding health aspects of the Amazonian manatee was obtained from animals maintained in captivity. The first observations on the blood parameters and functional properties dated from the seventies (Farmer et al., 1979). From then onwards the knowledge has grown substantially, thus contributing to a better management of the animals in captivity and increasing the survivorship rates of rescued orphaned calves.

The establishment of baseline values is of primary importance for an efficient monitoring of physiological and health aspects of free-ranging and captive individuals. So far, there is no available detailed data regarding the state of wild animals. However, some important data was generated over the years from animals in captivity in relation to body condition, baseline blood chemistry and hematological values, and urinary parameters (Colares et al., 1992, 2000; Rosas et al., 1999; Amaral et al., 2010a; Pantoja et al., 2010; Mello et al., 2011; Carmo et al., 2013; Sousa et al., 2016; Maduro et al., 2020). Hematological values were determined mostly for captive immature individuals (Table 1). One exception to this is the measurement of the hematocrit and hemoglobin concentration of five wild individuals sampled at the Mamirauá Sustainable Development Reserve as part of capture-release procedures for the attachment of radio-transmitters (Rosas et al., 1999). The hemoglobin concentration of the three sampled males (mean = 12.2 g/dL) and the two females (mean = 11.2 g/dL) were within the concentration range found for captive individuals (Table 1). The mean hematocrit value of 36% is also similar to what is observed in healthy captive individuals (Rosas et al., 1999). It is important to note that environmental conditions such as season of the year and diet can significantly modify the

Table 2. Serum chemistry values and diet of captive Amazonian manatees (*Trichechus inunguis*) maintained in two different rehabilitation institutions from Pará and Amazonas states in Brazil.

		Mello et al. (2011)			Sousa et al. (2016)			Maduro et al. (2020)		
Analyte	Unit	Calves, juveniles and adults	Mean	SD	Juveniles	Mean	SD	Calves	Mean	SD
Albumin	g/dL	21	3.4	0.4	20	4.23	0.46	10	2.65	0.15
Total Protein	g/dL	24	6.9	0.5	20	6.12	0.51	10	6.69	0.53
Calcium	mg/dL	24	13.2	2	20	10.08	0.45	10	10.17	0.25
Magnesium	mg/dL	20	6.8	1.1	-	-	-	10	3.06	0.34
Iron	µg/dL	13	303	69	-	-	-	-	-	-
Glucose	mg/dL	21	46	13	15	43.63	17.8	10	36.7	12.9
Cholesterol	mg/dL	21	209	75	20	310.13	115.12	10	180.1	35.57
Triglycerides	mg/dL	21	135	53	20	127.98	42.72	10	65.2	22.09
ALP	IU/L	19	75	27	20	98.73	47.85	-	-	-
ALT	IU/L	18	12	8	-	-	-	-	-	-
Amylase	IU/L	21	1,413	223	-	-	-	-	-	-
AST	IU/L	21	15	7	20	6.02	2.41	-	-	-
CK	IU/L	11	157	85	15	112.53	77.7	-	-	-
GGT	IU/L	21	47	9	19	25.24	9.53	-	-	-
LDH	IU/L	20	166	49	-	-	-	-	-	-
Total Bilirubin	mg/dL	21	0.43	0.47	-	-	-	-	-	-
Creatinine	mg/dL	22	2.3	0.1	17	1.34	1.25	-	-	-
Urea	mg/dL	21	41	13	19	30.21	28.4	-	-	-
Sodium	mmol/L	-	-	-	-	-	-	10	148.16	1.15
Uric acid	mg/dL	22	1.1	0.3	-	-	-	-	-	-
Phosphate	mg/dL	-	-	-	20	6.5	1.17	10	5.95	0.74
Diet		The diet of adults consisted of carrots, lettuce, paragrass (*Brachiaria mutica*), and water-hyacinth (*Eichhornia crassipes*). Calves were fed a milk substitute made from powdered cow milk, canola oil, and premix of amino acids, vitamins, and minerals.			Lactose-free milk with vegetable oil four times a day; and *ad libitum* native aquatic and semi-aquatic plants: *Pistia stratiotes, Utricularia breviscapa, Phyllanthus fluitans, Paspalum repens, Salvinia minima.*			Milk substitute composed by water, whole milk powder, canola oil, soybean meal, rolled oats, cornflour, and premix of amino acids, vitamins, and minerals, four times per day.		

ALP = alkaline phosphatase; ALT = alanine transaminase; AST = aspartate transaminase; CK = creatine kinase; GGT = gamma-glutamyl transpeptidase; LDH = lactate dehydrogenase; IU = international units; SD = standard deviation.

blood parameters of Amazonian manatees (Colares et al., 2000; Maduro et al., 2020). Also, the management/handling of the individuals, the enclosure type and natural expected variation such as among different age classes may be a source of variation in blood values (Silva et al., 2009; Mendonça et al., 2020). Some variances of the serum chemistry values can be observed among individuals kept in different institutions (Table 2). Therefore, it is important to keep an institutional database of blood parameters from healthy animals for proper interpretation of exam results and the subsequent decision-making.

The most common clinical diseases of rescued calves are related to the gastrointestinal tract. The clinical signs are varied/miscellaneous and unspecific. They may include diarrhea, constipation, cramping, hyporexia, restless swimming, tenesmus, and signs of dehydration (Lazzarini et al., 2014; Davis & Walsh, 2018). Guerra-Neto et al. (2016) reported the first case of necrotizing enterocolitis with pneumatosis intestinalis in an Amazonian manatee calf. Many rescued calves arrive in poor body condition, underweight and frequently hypoglycemic and dehydrated (Davis & Walsh, 2018). Lesions caused by anthropogenic factors or interactions with other species are not uncommon. Incidental injuries and wounds caused by the interaction of the manatees with fishing nets vary from superficial lesions in the skin to severe strangulation of limbs. Lesions related to agonistic interaction with botos (*Inia geoffrensis*) and tucuxis (*Sotalia fluviatilis*) were also recorded (Vergara-Parente et al., 2004; da Silva et al., 2022). Fungal skin lesions have been reported in manatee calves under rehabilitation (Mok & Best, 1979), but with natural resolution. Temporary skin warts related to papillomavirus have been observed in some individuals under management-related stress (Gravena et al., 2012).

Captive and free-ranging Amazonian manatees have tested positive for *Toxoplasma gondii* and *Leptospira* spp. in seroprevalence surveys; however, no animals showed clinical symptoms of these diseases (Mathews et al., 2012; Mathews Delgado et al., 2015; Rodrigues et al., 2022). *Salmonella* spp. infection has been observed in captive animals even in apparently healthy individuals (Oliveira et al., 2018). However, a fatal case of salmonellosis was reported in an Amazonian manatee (Correa-Neto et al., 2021). Therefore, isolation and genetic identification of bacteria with subsequent antibiograms can be a very useful tool for an efficient treatment against pathogenic bacteria. A recent case of salmonellosis in a calf rescued in Almeirim, Brazil, was successfully solved at INPA after pathogen isolation and antibiogram test, with the oral and intramuscular administration of Enrofloxacin (10%) during seven days in combination with gastric intubation of fluids (sodium chloride and Glicopan Pet®) and pain management with dipyrone (D.M.D. Mello, unpub. data). Reisfeld et al. (2018) also reported the treatment of *Mycobacterium fortuitum* and *M. abscessus* isolated from a persistent cutaneous lesion in the rostrum of an adult male Amazonian manatee with oral administration of ciprofloxacin and clarithromycin (6 mg/kg), once daily for two months based on the antibiogram test in combination to other supportive drugs.

A systematic investigation to characterize the yeast microbiota of captive Amazonian manatees detected at least 12 different species of *Candida* in their natural cavities (oral cavity, nostrils, genital opening, and rectum), and the occurring rates seemed to be proportional to the number of individuals kept in each pool (Sidrim et al., 2015). Investigation of these microorganisms' resistance to antifungal susceptibility tests revealed that *C. albicans, C. tropicalis*, and *C. guilliermondii* were resistant to itraconazole and fluconazole (Sidrim et al., 2016).

Different species of protozoans have been reported in captive and wild Amazonian manatees. *Eimeria trichechi* was first described in 1983 from fecal samples of captive asymptomatic animals (Lainson et al., 1983). *Cryptosporidium* spp. and *Giardia* sp. were detected in fecal samples collected from wild and captive Amazonian manatees, suggesting a contamination by domestic wastewater without treatment systems, feces of domestic and wild animals and/or indirectly through contaminated water or food in captive individuals (Borges et al., 2007; 2011; 2017; 2018).

Conservation

Threats

Amazonian manatees were exploited since the pre-colonial period mainly for their meat and hide, but it was the commercial exploitation, at the beginning for their meat and later also for hide, that reduced their populations leading the species to extinction risk (Domning, 1982; Best, 1984; Antunes et al., 2016; Marmontel et al., 2016). Although protected by laws in most countries along its distributional area, the hunting of Amazonian manatee is a deeply embedded cultural practice and remains widespread throughout its range (Brum et al., 2021). Both indigenous and riverine communities conduct this practice, and the illegal trade of the meat still persists in local markets (Hamada-Fearnside et al., 2018; El Bizri et al., 2019). The incidental entanglement of calves in fishing nets, and several habitat alterations are also threats to the species.

Considering the broad distribution of the species in the Amazon region, the information available on the extension of the illegal hunting is anecdotal and the incidental entanglements in fishing nets are not quantified. The only information available is from the calves rescued from fishermen and brought to rehabilitation centers, where around 10-15 calves are received annually in Brazilian centers. This information however is underestimated since a small proportion of incidentally caught calves are reported.

Habitat modifications affect the aquatic environment on which the survival of species depends. Habitat alteration due to deforestation and modification of the river and lake banks and floodplain areas, pollution and contamination by agricultural residues and pesticides, heavy metals from the mercury used in the illegal gold mining, deforestation and forest burn, hydrocarbons and oils spills, and climate change may directly affect manatees' main source of food. Silva Junior et al. (2022) observed high mercury levels and other toxic metals in tissues of captive Amazonian manatees fed with macrophytes which were grown in locations with a history of gold mining activity and mercury contamination.

Another potential threat caused by environmental change is the construction of dams on Amazonian rivers. Arraut et al. (2017) indicate the risks of habitat modification due to hydroelectric dams' construction, affecting the food availability, exposing the animals during the dry season and fragmenting populations; therefore, increasing the probability of manatee mortality.

Although the effects of climate change on *T. inunguis* are difficult to quantify (Brum et al., 2021), Marsh et al. (2017) suggested that extreme droughts might affect the survival of Amazonian manatees. Additionally, Marsh et al. (2022) evaluated the possible impacts of each climate change consequence on sirenians, including Amazonian manatees. An impact already observed, for example, was during the severe drought of 2010, when several hundred manatees became trapped in a section of the lower Purus River; similar reports occurred in several localities, with estimated thousands of individuals killed throughout the Brazilian Amazon (Brum et al., 2021).

Status

The Amazonian manatee is protected by national and international laws and agreements throughout its entire distribution range. In Brazil, legislation regarding hunting and the general use of the Amazonian manatee has existed since 1967 (Law no. 5.197 of 3 January 1967). Hunting of manatees has an uncertain legal status in Brazil. Although in principle it is banned, it is allowed by law if it is done for subsistence of traditional communities. This ambiguity has historically been subject to erroneous interpretation by law enforcement personnel (Antunes et al., 2019; Brum et al., 2021). Colombia and Peru have bans on the hunting and exploitation of Amazonian manatee (Colombian law no. 2811 of 18 December 1974 and Peruvian law no. 158-77-AG of 31 March 1977). In 1973, the species was included in Appendix I of the Convention of International Trade of Endangered Species (Rosas, 1994). These laws provide important and necessary protection for the species, but the lack of human and financial resources of environmental management agencies in Amazonian countries severely limit the potential effectiveness of that legislation through poor enforcement, which has led to widespread non-compliance (Brum et al., 2021).

Amazonian countries have developed National Action Plans (NAPs) as conservation tools to assess the main threats and research actions that are required to develop a strategy to protect the species. However, their potential effectiveness has been limited by absence of government funding to carry out the proposed actions (Brum et al., 2021). Amazonian manatees have been recorded in at least two Protected Areas in Ecuador, three in Colombia, five in Peru and 53 in Brazil (Andrade et al., 2011; Utreras et al., 2011; Castelblanco-Martínez et al., 2016; Cossios Meza, 2018). Unfortunately, hunting continues even within Protected Areas (Marmontel et al., 2016). Although no information on its abundance is available, the main threats to the species fatally represent a risk to its persistence in the long-term. Nevertheless, *T. inunguis* is still categorized as Vulnerable under criteria A3cd in the IUCN Red List (Marmontel et al., 2016), based on an expected declining population under growing threats.

Conservation efforts

Faced with the main threats to the Amazonian manatee, several institutions have promoted numerous actions to recover their natural populations as well as to increase awareness of the society about the importance of these animals for the Amazonian freshwater ecosystem.

Rescue and captive rehabilitation - As a result of human negative impacts on the Amazonian manatee population, a Rescue and Rehabilitation Program of orphaned calves victim of poaching or incidental capture in fishing nets has been promoted by institutions in Brazil, Colombia and Peru, contributing significantly for conservation and management of the species. The large experience of teams in the management of the animals and the expanded knowledge about the biology and physiology of the species in the last decades have improved the success of the calves' survival at facilities. As a result, there are some records of births in captivity (da Silva et al., 1998; Carter et al., 2008).

Captive animals are an important source of biological information, helping to understand the species biology, including the rehabilitation process of the rescued calves, and also the support to promote educational and *in situ* conservation actions for the species. The final goal is to return all apt animals (low-level domestication, good health and pathogen-free) to the wild, to promote the conservation of the species in the long-term.

Release of captive manatees in the wild - In the last two decades, the success of management of manatees in captivity increased considerably, promoting the release efforts of rehabilitated manatees. About 100 individuals were released into the wild since 2000. This activity has been conducted with intense educational campaigns by several institutions as Mamirauá Institute (Marmontel & Petta, 2000; Marmontel, 2015), INPA and AMPA (Friends of the Manatee Association – AMPA) (Souza et al., 2018), ZOOUNAMA, and Aquatic Mammals Center (CMA/ICMBio) (Luna et al., 2021b) in Brazil; Amazon Rescue Center in Peru (Sánchez-Babilonia, 2014; Landeo-Yauri et al., 2017); and Omacha Foundation in Colombia (Kendall et al., 2014). Most released individuals have been monitored by VHF telemetry, with support of local field assistants (Souza et al., 2018).

Post-release monitoring has shown that most of the released animals were successful (Landeo-Yauri et al., 2017; Souza et al., 2018), including the confirmation of a manatee pregnancy, 18 months after being released into the wild (Souza et al., 2021a). Despite the evidence of success in this release, the recovery of the Amazonian manatee cannot rest solely on the efforts of rehabilitation programs (Landeo-Yauri et al., 2017). Post-release monitoring offers an opportunity for the development of ecological and behavioral studies of the species in the wild, and also in the construction of awareness strategies of riverine communities of the manatee release sites. At this moment, about 150 Amazonian manatees are undergoing rehabilitation in authorized captive facilities in Brazil, Colombia and Peru, most of them with a large potential group ready to release (Souza et al., 2022). Faced with this situation, the strengthening of Amazonian manatee release programs by the governments of these countries is strategic and extremely necessary to ensure this important conservation approach of the natural populations of the species.

Environmental Education - The engagement of the riverine population and other stakeholders, such as researchers and local authorities, is essential for the success of the Amazonian manatee conservation. The environmental education programs are promoted by several non-governmental institutions and undertaken in public schools of rural areas of the numerous municipalities in the zone of influence of these institutions to explain the importance of protecting water resources and their aquatic fauna, including manatees. Educational materials such as videos, brochures, and books for children as well as puzzles and games are very important tools to interact and diffuse information on the conservation programs and protection of the animals.

The interaction with residents of local communities has expanded the contact and collaboration network, boosting the rescue of orphaned manatees, and promoting the immediate release of healthy calves during incidental entanglements in fishing nets by fishermen (Kendall, 2013; Marmontel et al., 2016; da Silva et al., 2019). Additionally, the engagement of riverine populations is crucial to the maintenance and success of the release programs; their participation in monitoring released animals, especially former manatee hunters, generates a sense of relationship and responsibility for the conservation of manatees at the release sites.

Conclusions

The knowledge of the Amazonian manatee biological aspects has significantly increased over the last 30 years. The access to individuals in captivity was one of the main factors for this improvement. However, the difficulties imposed by the Amazonian habitat and the cryptic behavior of the species still hamper advances in research on free-ranging animals. Efforts should be made toward studies regarding population estimates and the impacts of direct and indirect threats on manatee abundance.

Regarding threats, despite the protection efforts, the risks are still present. Therefore, the reinforcement of protective measures and the establishment of additional ones, such as the creation of protected areas, are necessary to reduce those impacts. Additionally, the involvement of the local community is mandatory, because hunting still persists and it impacts the whole conservation chain, removing adult animals from the wild and introducing new calves on the rehabilitation-release system. Thus, the continuity of environmental education programs and the engagement of local people on the scientific and conservation activities are key for the conservation of Amazonian manatee.

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