Conservation of Amazonian manatees in Brazil with emphasis on reproductive aspects

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Abstract

Amazonian manatees, *Trichechus inunguis*, are classified as vulnerable, both under the IUCN Red Data List and the Brazilian List of Threatened Species. Although the main factor leading to the present status – commercial hunting - has been removed, subsistence hunting persists, and the depressed numbers of animals, associated with a low reproductive rate common to all sirenians, continue to worry scientists about the future of the species. Recent efforts to improve chances of recovery include the establishment of protected areas for the species, rescue of accidentally-captured calves, recovery of illegally-held specimens, and captive breeding. Two research and conservation efforts in Brazil have succeeded in breeding and raising manatees in captivity.

The Mamirauá Institute for Sustainable Development has been working with manatees in two large protected areas (Mamirauá and Amanã Sustainable Development Reserves) in the western Brazilian Amazon for the past 17 years. The emphasis of the research is on the natural setting, working with the native population of manatees. Over this period we have captured and VHF-tracked over 12 manatees at different times, which has allowed us to document migratory routes between the 2 Reserves. In order to understand population dynamics of Amazonian manatees, we have conducted age-estimation studies of hunted specimens and experiments to attempt to estimate present abundance of the species. Ecological studies include the description of their diet through the examination of free-floating feces collected in lakes and rivers, parasitological studies and anatomical and morphological research. We have collected reproductive tracts from culled animals and carcasses in order to examine stages of maturation and infer reproductive seasonality. We are currently conducting a study of reproductive hormones from wild animals, based on hormones extracted from feces.

The work with the local human population includes documenting the level of hunting still current, and the use given to the animals. Young calves tend to get entangled in fishing nets, representing an additional cause of mortality. Rescued animals are usually rehabilitated in two major centers in urban settings, but since 2007 the Mamiraua Institute is conducting a pilot work, raising orphan calves in a semi-captive situation, with the help of local communities.

The work of the small research group is expanded with the participation of co-investigators from the local communities, who take part in the field activities, share their knowledge and experiences with researchers, and receive training and information, all in a rich exchange situation.

Introduction

The Amazon Basin is a unique environment. It drains over 6 million km^2 of 9 countries, including almost 50% of Brazil´s territory. There are three types of water: clear (greenish and nutrient-poor), black (dark-tea colored and acidic) and white (murky and sediment- and nutrient-laden). Temperatures are quite stable during the year, ranging in the 20's Celsius. There are only two recognizable seasons: wet and dry. However, the changes between those seasons may be dramatic - water levels may rise and fall up to 15 m every year. In this water world, all life – human, animal or plant – depends on and has to adapt to the ebb and flow regime, and the Amazonian manatee is no exception. Life and death of manatees - birthing, finding food, meeting mates, encountering shelter from hunters - all is intricately woven with the cycle of the waters.

Amazonian Manatee

The smallest representative of an order of aquatic herbivores (Sirenia) comprising 2 other living species of manatees and the dugong, all tropical or sub-tropical in distribution, *Trichechus inunguis* is the only species restricted to freshwater. Member of the first group of aquatic mammals to have a representative go extinct, Amazonian manatees have been hunted for centuries, which has granted them the status of "vulnerable" by the IUCN, and category I protection under CITES. Endemic to the Amazon basin, the species occurs from the headwaters of rivers in Ecuador, Peru and Colombia to the Marajó Island at the mouth of the Amazon River (Domning 1981) in Brazil. Under Brazilian law, it is considered vulnerable to extinction and protected from harassment and culling, however subsistence hunting goes on throughout the region. Brazil also harbors and protects the Antillean manatee *Trichechus manatus manatus*, which occurs along the country's Northeastern coast, and is considered endangered of extinction.

Like other sirenians, Amazonian manatees are known for a low reproductive rate, which adds to the vulnerability associated with an endemic species under hunting pressure. Considered semi-social, the mother-calf is the only long-term stable bond, as opposed to temporary feeding and mating aggregations. That may render them more susceptible to mortality, as hunters catch calves to serve as bait to facilitate harpooning the female. Hunters are also said to prefer females for their greater amount of fat, although it is questionable whether they are able to distinguish sexes in the wild.

Mamirauá - the Reserves, the Institute and the Research Group

Mamirauá and Amanã sustainable development reserves, located on western Brazilian Amazon, were established in 1990 and 1996, respectively. Both are managed by the Mamirauá Institute for Sustainable Development with the aim of conserving biodiversity with the participation of the local inhabitants. Mamirauá Reserve is totally comprised of floodplains, whereas Amanã Reserve is mainly highlands, with some proportion of flooded areas. Together, they protect over 3.4 million hectares of tropical rainforest. The Mamirauá Institute works with the social aspects of the areas and prioritizes research on elements of the biodiversity that represent resources for the local population; together with the people, the Institute

attempts to find alternate economic activities to raw exploitation of the natural resources. Examples of this are the management for pirarucu fish, community forest management, ecotourism and experimental caiman management. Some species, on the other hand, cannot be productively managed – that is the case of the aquatic mammals, including the Amazonian manatee.

Mamirauá´s Aquatic Mammals Research Group has been conducting research on and around the area of the Mamirauá and Amanã reserves since 1993. Working with all 5 species of freshwater mammals (manatees, pink and tucuxi dolphins, giant-river otter and Neotropical otter), all studies are focused towards the wild populations, working both with free-ranging animals and biological material from carcasses.

Over 20 manatees have been captured in the wild with the main purpose of fitting belt-mounted transmitters with a unique VHF frequency that could be manually monitored to follow the animals´ movements and habitat use. While being handled, blood was collected for genetic and baseline health studies, and the animal was marked for further re-identification. The monitoring effort resulted in documenting migratory routes that manatees use annually. The work has shown that manatees move from the aquatic plant-rich floodplain areas they use during the wet season (Mamirauá Reserve and portions of Amanã Reserve), to seek refuge in deep, blackwater Amanã Lake during the dry season (Marmontel et al., in prep). A habitat study based on satellite images and modeling has been able to mathematically confirm the importance of the water level – and its associated plant growth – to manatee dynamics (Arraut et al. 2009). Efforts have also been invested in trying to answer one of the most sought-after questions about manatees: how many there are. Examination and documentation of "comedias", or patches of vegetation with signs of manatee browsing, a community-level counting of animals, and the use of sonar have been experimented with in order to provide a manatee abundance index in the region. The hunting activity has been monitored over the years, and documented in detail for the Amanã Reserve during 2002-2008 (Calvimontes 2009); hunting is almost exclusively done by older men, with the use of harpoons and mostly geared to subsistence; unfortunately, culling numbers do not seem to have decreased in the last decade.

Handling of captured animals and donation of specimens by the local population have allowed the building of a unique collection of manatee bones (especially skulls), as well as soft tissue samples for biological analysis. An age-estimation study of the animals hunted between 1980 and 2006 has shown that animals of all ages are being taken; in contrast, the most affected age class among coastal manatees is that of calves, that strand on beaches due to separation from mothers (Vergara-Parente 2009). Sixty-nine plants potentially eaten by manatees have been identified with the aid of local people, and 50 have already been confirmed through the examination of feces from captured specimens and found free-floating in the water channels (Guterres and Marmontel 2008). Parasitological analyses of the same material identified the presence of Cryptosporidium (Borges et al. 2007) in manatee feces. Examination of internal organs has detected the presence of Chiorchis in the intestines. Finally, blood and muscle samples have allowed genetic research leading to the conclusion that manatees in the Amazon still present a high genetic diversity (Vianna et al. 2006). Nevertheless, the same study confirmed the presence of hybridization between Amazonian and Antillean manatees around the mouth of the Amazon area, as first predicted by Domning (1981).

In the past few years, the number of projects with Amazonian manatees has increased. Three groups stand out as conducting long-term research and conservation projects with the species, in the state of Amazonas: the National Institute for Amazonian Research (INPA), and the Center for the Research and Preservation of Aquatic Mammals (CPPMA), both working mostly with captive animals; and the Mamirauá Institute for Sustainable Development, focusing its studies on the wild population.

Manatee Reproduction

There are still a lot of unknowns about the reproductive biology of Amazonian manatees due to the difficulty of obtaining fresh material from wild animals. Most of what has been documented is derived from captive animals, which may not reflect accurately what happens in the natural environment. A smaller body of material has been generated from carcasses of culled animals to which researchers had access. Most of the culling takes place in remote places, and locals tend to flense the carcass rapidly and utilize most of it for various purposes. Naturally dead animals are rare to be found, given the fast rate of decomposition under the tropical sun and the action of scavengers. At this point, what is now known about Amazonian manatee reproductive biology is assumed to be similar to its much-better studied relative, the Florida manatee (see O´Shea et al. 1995).

1: Anatomy

Information on reproductive anatomy of *Trichechus inunguis* is limited to a study with 3 female specimens: a stillborn fetus, a 6-month-old calf and a 6-year-old adult (Rodrigues et al. 2008). Results agree with the general mammalian pattern described at the anatomical and histological level for the Florida manatee based on 275 female reproductive tracts (Marmontel 1988). The two oval-shaped ovaries, enveloped by a peritoneal bursa, are located in the caudal end of the abdominal cavity. Numerous follicles, of varying sizes, mostly concentrated on the cranial pole, were only observed in the large specimen; diameter of the largest mature follicles ranged between 2.7 and 8.6 mm. Similar to what occurs in Florida manatees (Marmontel 1988) and dugongs (Marsh et al. 1984), *T. inunguis* may be polyovular. As in the above species, superovulation would result in multiple corpora lutea producing enough progesterone to support a pregnancy and stimulate endometrial growth. The presence of 13 Graafian follicles, a hemorrhagic body, whitish scars and histologically-confirmed corpora albicantia in the ovaries, and yet no embryo or placental scar in the uterine horns of an adult female sharing a pool with male manatees and with intact hymen, suggest that infertile cycles before the first pregnancy and even before the first copulation may also occur in *T. inunguis* (Rodrigues et al. 2003, 2008). Dugongs have been documented as polyestrous (Marsh et al. 1984). Infertile estrous cycles may occur before pregnancy in Florida manatees (Hartman 1979), but evidence of a polyestral cycle is not confirmed by multiple corpora lutea in similar stage of development in ovaries (Marmontel 1988).

2: Biology and physiology

Sexual maturity

There are no hard data on the age or length at sexual maturation of Amazonian manatees, but maturity is expected to ensue between 5 and 10 years (Rosas 1994) old. Two females captive at CPPMA had their first calves between 7,5 and 8,5 years of age; one captive female at INPA had not yet reproduced at 10 years of age (do Nascimento 2004, Carter et al. 2008). The oldest Antillean manatee in captivity in Brazil had its first calf at the amazing age of 36; 10 ½ years later it gave birth to its second calf.

Seasonality

Pereira (1954), in a very early book on manatees, already mentioned there was no rigorous season when manatees reproduced, but that they could mate at any time of the year. Additionally, he wrote that the fact that females are seen with calves both in summer and winter gave rise to the comparison of reproductive function with that of bovines, an interpretation that still persists for amazonids. However, the author pointed out that sexual activity was more frequent during the period of high water, as the copulation movements required large spaces, considerable depth and, possibly, temperatures cooler than summer´s.

Florida manatees and dugongs have a diffusely seasonal reproduction, due to an ability to reduce this activity when the food basis is depressed. A seasonal reproductive cycle has also been suggested for the Amazonian manatee (Best 1982), based on the influence of the hydrological cycle of the rivers of the Amazon basin on food availability. The conspicuous seasonal cycle of the waters in the Amazon drainage system, with variation amplitudes of 10-15 m, exerts a profound effect on macrophyte production. Dry and mineral matter, ethereal extract, protein and energy levels in macrophytes during the wet season are markedly different from those of the dry season (do Nascimento 2004).

Births have been reported throughout the year, but birthing seems to occur more often during the floods (December-June) with a peak in February-May (Best 1983). Coinciding birthing with high productivity of aquatic plants ensures appropriate energetic supply for females in the final portion of gestation and during the first lactational phase. Young manatee calves, like other sirenians (Marsh 1999) may start nibbling on plants at a few weeks of age, so having tender shoots available would be desirable. Seasonality was also suggested by results of captive studies where, despite several years in captivity, follicular activity coincided with the high water level (do Nascimento 2004).

In his 1983 paper, Best reports an apparent dry season-associated fasting episode starting the end of August till the first week of March. Best adds that the dry season may lead to emaciation and stall the reproductive activity. Pimentel (1998) measured testosterone metabolites in feces of 3 captive Amazonian manatee and suggested that ambient temperature and precipitation are intimately related factors that may influence reproductive activity. Alternative matrices to measure androgens (besides blood) through radioimmunoassay are urine or saliva (Amaral, 2008). Seasonality has been suggested for Florida manatees based on histology of testes, linking season of the year with degree of spermatogenesis (Hernandez et al., 1995).

Levels of steroid hormone metabolites present in urine or feces have been used in longitudinal monitoring

of ovarian function and non-invasive diagnostics by radioimmunoessays of gestation in several species of wildlife (Lasley and Kirkpatrick, 1991). Based on 4 adult females monitored over a year, do Nascimento (2004) concluded that concentrations of fecal estrogen are highest during the high water period. Although not statistically significant, fecal progestin levels rose on the final third of the high-water period to the initial third of rising water period, which may be due to the presence of persistent corpora lutea (do Nascimento 2004).

Estrous periods last 5 to 8 days (average 6,4±0,45 days) and the estrous cycles range from 20-27 days (average 22,49±1,26 days) (do Nascimento, 2004).

All of the above information on hormones and reproductive cycles are derived from captive studies. We have now available over 200 samples of feces from wild animals, found free floating in the Amanã and Mamirauá Reserves; this sample size is being expanded in an attempt to document the reproductive cycle of the native population.

Behavior

Reproductive behavior is also believed to be closely linked to the hydrological cycle of the Amazonian region (Best 1982). Assuming a pregnancy length of roughly a year, copulation is likely to take place as the water level starts to rise as well.

Pereira (1954) mentioned the local fishermen´s expression "cavalgação" (horseback riding) referring to the period when manatees were found in "lotes" (herds) of 15-30 chasing one or more females; the fittest (the "tuchaua", or Indian chief) might drive the other males away. According to the same account, mating would take place perpendicular to the water, in a belly-to-belly or side-by-side position. As a result of harassment, it was said not to be uncommon for the female to die of exhaustion, or to seek refuge by the margin, where it would become more susceptible of being hunted (Pereira, 1954). Females in captivity copulate by lying horizontally on the surface, and the male hugging her obliquely from underneath; several males may sequentially copulate with the same female (d´Affonseca Neto and Vergara-Parente, 2006).

Gestation period and interbirth interval

Gestation period in captivity has been confirmed as 11-12 months , based on plasmatic progesterone concentrations (do Nascimento et al., 2002, do Nascimento, 2004). Clear signs of gestation are weight gain, increase in abdominal diameter and vulva enlargement but ultrasound equipment has been used to confirm pregnancies (d´Affonseca and Vergara-Parente, 2006). In captivity, a minimum 2-year lactational anestrous has been documented, interbirth intervals corresponding to approximately 3 years (d´Affonseca and Vergara-Parente, 2006, Rodrigues et al. 2008). A female brought into captivity at INPA as an adult had its first captive offspring 18 years later (do Nascimento, 2004).

Birth length, sex ratio, litter size

Amazonian manatees are uniparous (Husar, 1977; Best, 1984; Timm et al., 1986; do Nascimento et al.,

2002), but twinning may occur (Pereira, 1954). The rescue of a male and female pair of young calves from a fishing net close to the São Félix Indian village (Amazonas state) in June 2006 suggests they may have been twins. Twinning occurs in 4% of the births in Florida manatees (Marmontel, 1995). Female twins were born to a pair of captive Antillean manatees held in captivity at the Aquatic Mammal Center, in Itamaracá (PE), northeastern Brazil in 1997. One case of adoption of two orphaned calves was documented in the INPA pools (Rosas et al. 2001); calves have also been adopted at Center for Aquatic Mammals.

Newborn Amazonian manatees measure between 85 and 105 cm of total length, and weigh from 10 to 15 kg (Best, 1984; do Nascimento, 2003). Birthing presentation is caudal (d´Affonseca and Vergara-Parente, 2006).

Longevity, reproduction and senescence

The oldest Amazonian manatee documented so far was a 36-year old animal (Vergara-Parente, 2009); longevity for Florida manatees is estimated at over 60 years (Marmontel et al., 1996). Senescence has not been documented in manatees: the oldest lactating Florida manatee female was accidentally killed at 39 (Marmontel, 1995). A 46-year-old Antillean manatee gave birth at the Aquatic Mammal Center in 2007.

Captive breeding

Brazilian institutions have been successful at breeding manatees in captivity. The first Amazonian manatee captive-birth took place at INPA in 1998 (da Silva et al., 1988), to a 25-year old female that had been in captivity since age 1. Mating and birthing conditions are believed to have substantially improved after the introduction of a more natural and varied diet as well as increasing the size of the pools. Since then, 5 others have been born (1 of which was stillborn) (Carter et al., 2008). At Center for Research and Protection of Aquatic Mammals (CPPMA), 60 km from Manaus, 3 have been born in captivity so far. On the coast, at the Aquatic Mammal Center 6 calves have been born in captivity, including one of a second generation (born to one of the female twins born at the facility).

Orphaned calves and the community-level rehabilitation center

Every year, a considerable number of orphaned calves is rescued, either donated by locals, rescued from inadequate captive settings or confiscated by the environmental agency from powerful businessmen who enjoy having manatees as pets in their private ponds. The situation has been growing in magnitude, as the use of fishing nets becomes more and more extensive throughout the region. Although most adults are able to break free, calves are not strong enough to escape, and either drown or are removed and taken into captivity. This situation becomes especially worrisome during episodes of extreme droughts, such as in 2005, when the amount of nets set increases significantly. At the end of 2010, INPA and CPPMA together held over 70 animals in captivity, operating at or close to maximum capacity. Several of these animals have grown mature and remain in captivity, mainly because of the difficulty of returning large bulky animals to distant locations, and the fear that those animals will become easy targets for hunters. As an alternative to

these urban captive settings, the Mamirauá Institute has proposed and been approved as a conservation rehab center, with the intent of conducting it in an enclosure in the natural setting, and with the support and collaboration of local people, acting as co-investigators and stewards of wildlife. The center started operation in the Amanã Reserve in July 2007, when a calf was brought in for rehabilitation. It was officially established upon approval of the Environmental Agency, in January 2008 and just in February 2009 received its 2nd guest. Today the Center cares for 7 orphan calves; two of those are planned to be released in early 2011, during the wet season.

3: Conservation considerations

A large number of calves has been saved from being killed or dying in the wild in the last 30 years, being rehabilitated in oceanaria at INPA and CPPMA. Nevertheless, despite these successes, these animals should be reintegrated to the wild population to become part of the genetic pool and produce new generations in the wild. Return of the animals to the wild, to a place as close as possible to their original capture site, and as soon as possible, are the priorities. Meanwhile, we are conducting a very intense awareness program, to involve the local people as much as possible in the animals´ rehabilitation and have them build responsibility, not only for these individuals in particular, but with the species in general that is a symbol of the Amanã Reserve.

A word of caution is in order relative to genetics, given the possibility of differences among populations along the distribution area. Housing together breeding animals of very distant locations should be avoided, and release sites should be carefully evaluated. All animals brought into captivity should be evaluated to identify possible hybrids (Vianna et al., 2006).

Man and nature may play critical roles in the manatees´ future. Increases in human population may increase the hunting pressure over a species in which survivorship is a critical factor in population maintenance (Marmontel et al., 1997; Eberhardt and O´Shea, 1995). Habitat alterations may impact manatee food sources, negatively affecting their reproductive parameters and growth. Finally, the global changes in climate may affect the ebb and flow pattern on which manatee biology depends.

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