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Case Report

Behavioral patterns of a manatee in semi-captivity: implications for its adaptation to the wild

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Abstract

Rehabilitation of orphaned endangered Antillean manatees (*Trichechus manatus manatus*) enhances *in situ* conservation. We investigated the behavior of a five year-old male manatee rescued in Quintana Roo (Mexico) in relation to its failed rehabilitation. This is a unique case of a semi-captive manatee in the Caribbean, and the first endeavor to release a rehabilitated orphan in Mexico. Through 134 hours of direct and *ad libitum* observations, we described the manatee's behavior and assessed his behavioral time budget. The frequency of states was determined by instantaneous sampling, while the frequency of events was defined by the number of events per time unit. We designed an ethogram of 105 behaviors (56 states and 49 events), distributed in six behavioral categories. Compared with previous catalogs designed for manatees, the subject displayed 43 new behaviors (24 states and 19 events). The manatee showed indications of a daily rhythm; the animal consistently performed displacement behaviors in daytime hours, while engaged in comfort behaviors mainly at night. The use of space depended on the behavioral category and the time of day. The manatee showed dietary preference for the food provided by the caretakers, and virtually no consumption of native aquatic plants. This inadequate feeding behavior, along with a strong attachment with people, made the individual completely dependent on human care. Therefore, despite being free to explore natural areas, the animal remained close to the facilities after release. Future recommendations on the management of rescued manatees are discussed.
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Keywords: Trichechus manatus manatus, ethology, release, management, rehabilitation, diet

Introduction

The Antillean manatee (*Trichechus manatus manatus*)*,* a subspecies of the West Indian manatee, is considered "Endangered" by the Red Data Book of IUCN (17). The main threats to manatees are related to anthropogenic activities such as hunting, habitat loss, environmental contamination, and collision with boats leading to injuries and fatalities (25). Another impact on the natural populations are orphaned calves that are left fending for themselves after injury or death of the mother (6). One of the best strategies for species conservation is through recovery and management plans, including the successful rehabilitation and release of orphans. Approximately 70 West Indian manatees are being rehabilitated in captivity, while another 100 remain permanently held (1). Evaluating the success of rehabilitation programs requires health assessments and behavioral documentation.

In 2003, Mexican authorities rescued an orphaned male Antillean manatee. After failed attempts to release the weaned manatee into the wild, it was kept in semi-captive conditions and used to promote public awareness and species education (1). This represents the first attempt of rehabilitation and release of a manatee in Mexico and a unique case of an Antillean manatee held in a semi-captive environment. The present work aims to describe the behavior of this individual in order to provide possible causes for his dependence on human care and unsuccessful release.

Methods

History of the observed manatee:

The subject of this study was a male orphaned manatee rescued in 2003, at Guerrero Lagoon (18° 41' 25" N, 88° 15' 31" W), Quintana Roo, Mexico. The area is a freshwater aquifer, with salinities ranging between 3 and 4 psu which discharges into Chetumal Bay (7). This larger, interconnected system of coastal habitats, is considered to be a traditional use site for manatees (22). At the first stage of the recovery (September 2003 – May 2004), the calf remained in a plastic pool at El Colegio de la Frontera Sur (Ecosur). During the second phase (May 2004 – May 2005), the 1-2 year-old manatee was transferred to an enclosure in Guerrero Lagoon with the following objectives: (i) to familiarize the animal with his natural habitat, (ii) to

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facilitate the interaction of this individual with wild manatees, and (iii) to teach him to forage on natural aquatic vegetation (24). In July 2005, at approximately 2 years old, the manatee was tagged with a VHF radio and released near the enclosure. However, the animal followed people, including its caretakers, and preferred to stay near the pens. Despite the manatee's relocation by boat to the end of the lagoon (approx. 5 km), he returned to the release area within a few hours (24). After several days, the manatee was relocated to a pen in a closed channel designated for soft-release (a release strategy involving pre-release conditioning and training) with the intention of increasing its chances of survival and minimizing negative effects during readaptation to the wild. Soft released animals are kept within a shelter at the release location to allow acclimation to the new environment before they are completely independent and responsible for their own welfare. The individual was kept in the closed channel from July to September 2005, but was found stranded on several occasions, requiring caretakers to rescue and care for him. By mid-September, the animal was completely released. Five months later, it was seen in an emaciated state, displayed hyperkeratosis and a weight loss of 30 kg (33% of total weight) (24). In 2006, the 2.5 year old manatee was declared unfit for survival without human care and has since been maintained at the Center of Care and Rehabilitation of Aquatic Mammals (CARMA) (24). At the time of this study (August 2008 - December 2009), the five to six year old manatee required food provisioning from caretakers at the facility, but could move freely between captivity and the wild. Provisioned food consisted of fruits and vegetables such as apples (*Malus domestica*), pears (*Pyrus* sp.), squash (*Cucurbita maxima)*, carrots (*Daucuscarota* sp.), swiss chard (*Beta vulgaris L.)*, beetroot (*Beta vulgaris*), and lettuce (*Lactuca sativa*).

Behavior recording:

We conducted behavior observations from an observation platform, 2 x 5 m in surface area and 3 m in height. The selected observation method was 'instantaneous sampling', in which the observation time was divided in intervals (19). Each observation session lasted 30 minutes, with behavioral assessments occurring every minute. Observed behaviors were grouped into the following categories: breathing, displacement, feeding, elimination, comfort, and interaction with objects. The behaviors were organized, described, and codified in a catalog based on previous ethograms (8,15,28). Graphics of the cumulative number of behaviors were created in order to determine the sufficiency of observations. When the curve asymptote was reached, the possibility of observing a new behavior was considered low, and the catalog was considered complete.

The probability of finding a new behavior was also assessed by the formula:

$$\theta = 1 - \frac{N_1}{\ell}$$

Where N_1 is the number of behaviors observed once and ℓ is the total number of observed behaviors (19). To calculate the frequency of behaviors, 4 instantaneous samplings of 30 minutes each were carried out daily. Samples were equally distributed throughout the 24-hour period (day- and night- time) to detect temporal variation of the behavior. To facilitate detection during night sampling, a small plastic bottle equipped with two AA batteries and a small red LED light was attached to a belt on the manatee. To determine variations on space use within the enclosure, five imaginary quadrants were outlined inside the observation area (Figure 1a and 1b). The location of the manatee was identified concurrent with its associated behaviors. There were no differences in depth or any other condition between quadrants.

During each observation, details on the food offered and the animal's preference were annotated. Additionally, fecal samples were collected opportunistically and stored at room temperature in FAA (85% ethyl alcohol, 10% formalin, 5% glacial acetic acid). Fecal samples were observed under an optical microscope. Comparisons of plant epidermises with micro-histological descriptions and permanent collections of samples allowed for identification of consumed plants (5). Five glass slides per sample were examined under the microscope at 10X, 40X, and 100X magnifications. A total of 500 points was identified for each sample as recommended by Hurst and Beck (16). For each point, the plant fragment was identified using guides and a reference collection of plant tissues created from the provisioned food (16).

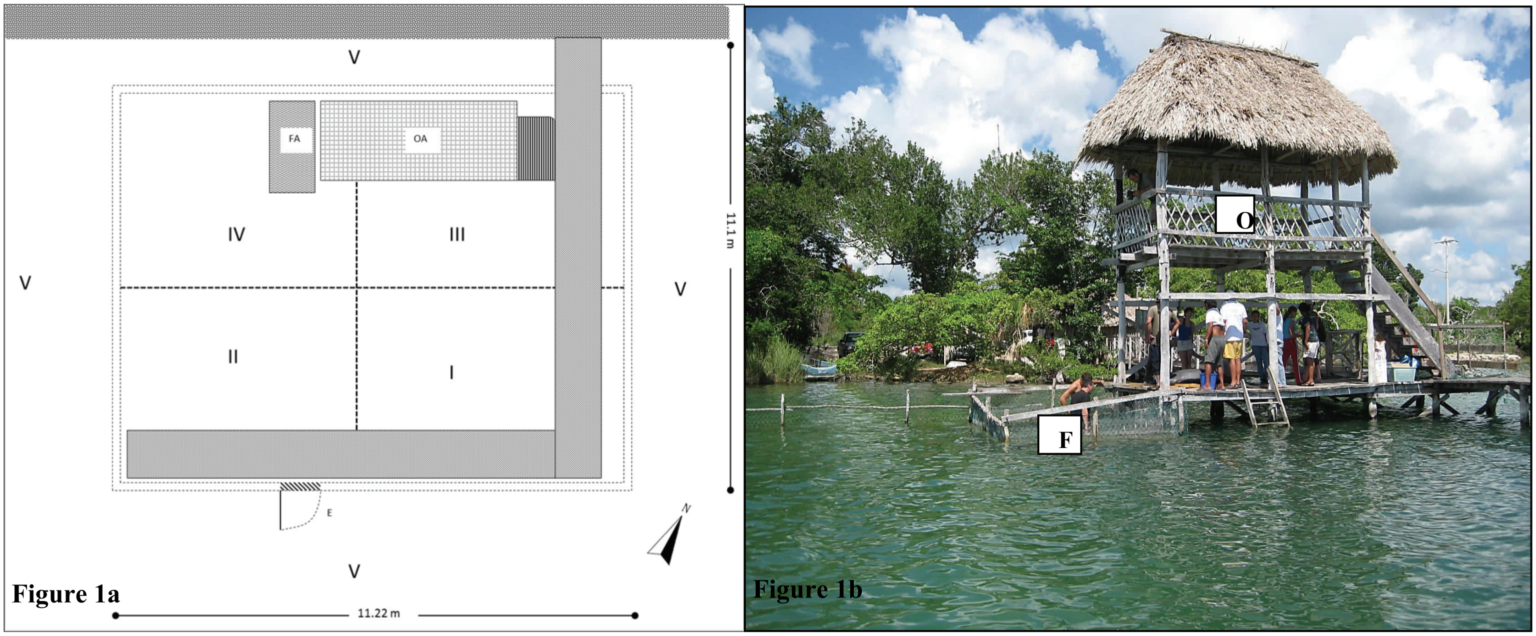


Figure 1: Facilities where the manatee was kept during the study. (a): Schematic view showing the location of the quadrants (I -V), limits of confinement (double dotted line), exit gate (E), feeding area (FA), and the observation area (OA). (b): Photo taken during a health assessment activity of the manatee, showing the feeding (F) and observation (O) areas. Reproduced with permission by Nataly Castelblanco.

Data Analysis:

Observed behaviors were constructed into a catalogue (19). Behaviors were classified as states or events: "events" were observations of relatively short duration, while "states", such as body postures, had relatively long durations (20). The frequency of occurrence, defined as the number of occurrences in time period, was calculated for behavioral events. The duration, in terms of proportion of time spent, was measured for behavioral states. The use of the space was explored as percentages of time in a determined quadrant. Differences in frequency of behavioral category and use of quadrants were tested with a Kruskal-Wallis one-way analysis of variance on ranks, followed by all pairwise multiple comparison procedure (Dunn's method). The Student's t-test was used to detect significant differences in the presence of the items found in fecal samples.

Results

A total of 151 observation sessions was completed, totaling 134 hours of effort distributed as follows: 36 hours in the morning (0500 - 1200); 49 hours in the afternoon (1300 - 1900) and 49 hours at night time (2000 - 0400). The calculated probability of finding a new behavior was low ($\theta = 0.91$), and therefore, we considered the invested effort adequate to guarantee a satisfactory sample of the individual's behavioral repertoire (Figure 2). A total of 108 behaviors was registered, of which 43 (24 states and 19 events) were not recorded in previous catalogues; therefore, those were considered 'new behaviors' (Table 1). During the 14% of observations in which the individual was undetected, the behavior was considered 'absent'. The frequency of occurrence was statistically different between categories (Kruskal-Wallis: $H = 59.335$, df=3, $P<0.001$). The most frequent categories were comfort (44.60%) and displacement (39.50%), while feeding and interaction with objects were least frequent at 8.00 and 7.70% respectively (Figure 3).

The use of the space was significantly dependent on the quadrant (Kruskal-Wallis: $H = 174.126$, df=4, $P<0.001$) (Figure 4). The manatee used quadrants V (45.00%) and III (30.00%) a greater proportion of time. Quadrants I, II, and IV were used less often. Quadrant III was preferred for displacement behaviors, while quadrant V was used mainly for resting (comfort behaviors) (Figure 5). A preference for quadrant III was observed during daytime hours, when the interaction with people and feeding events occurred. The animal used quadrant V during nighttime hours and stayed

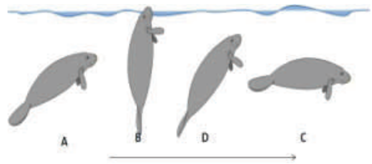
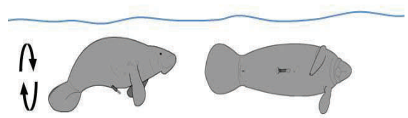
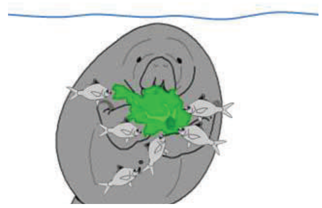
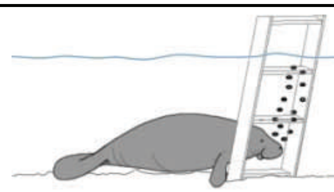
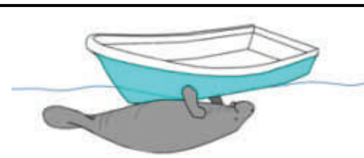
Category	Description	Example	Behaviors already reported[*]		New behaviors		Total of behaviors
			States	Events	States	Events	
Breathing	The manatee opens its nostrils for air at the moment of reaching the surface and closes them during submersion. Exhalation is followed by inhalation.	 "Vertical breathing" (198)	---	2	---	1	3
Displace-ment	Locomotive behaviors.	 "Spin 90°" (264)	14	15	4	5	38
Feeding	Includes foraging (searching for food) and grazing (consumption) behaviors.	 "Grazing + competition with fish" (380)	14	1	9	1	25
Elimination	Air expulsion.	 "Bubbles" (410)	---	6	---	1	7
Comfort	Relaxation periods.	 "Rubbing eye with fin" (571)	18	11	1	6	36
Interaction With objects	Contact with objects.	 "Playing with object" (674)	---	5	10	5	20
							Total=129

Table 1. Behavioral catalog for a West Indian manatee in semi-captivity. * Behaviors reported in the catalogs of Castelblanco-Martínez 2000, Charry 2002, and Mercadillo 2010. In parenthesis the code with name of the behavior in the example.

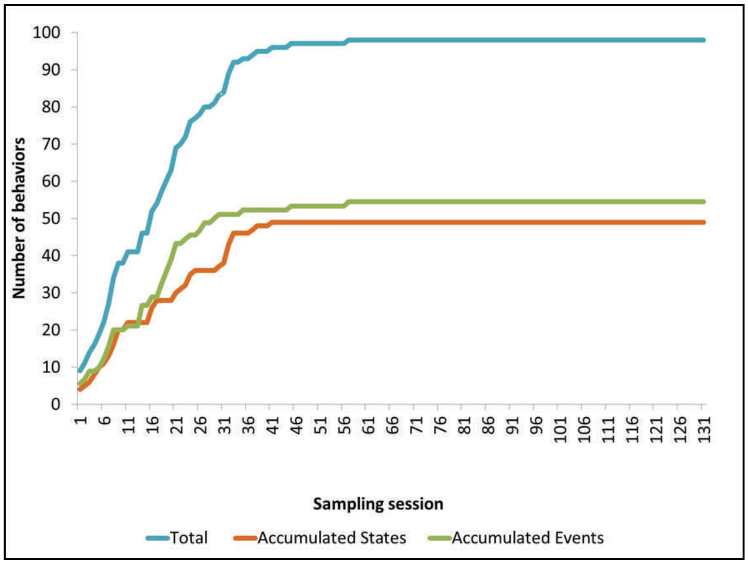


Figure 2: Cumulative curve of behaviors displayed by a semi-captive Antillean manatee.

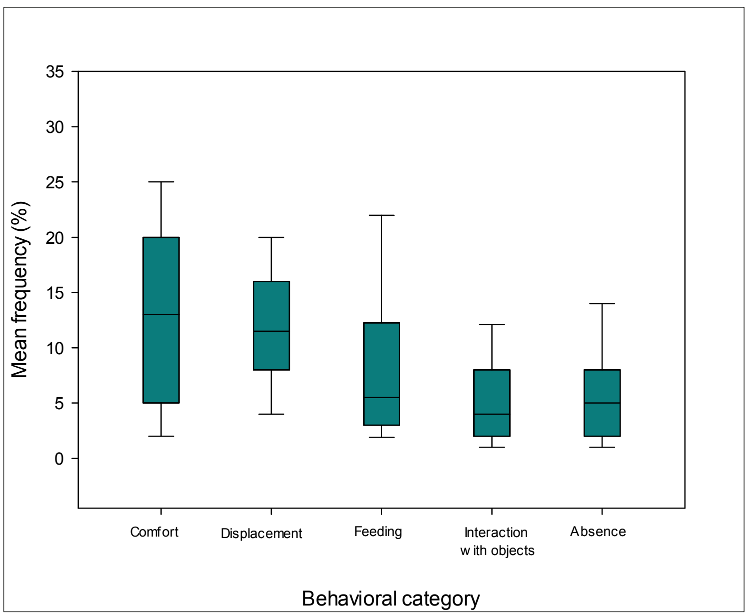


Figure 3: Frequency of behavioral categories displayed by a semi-captive Antillean manatee.

submerged at the bottom the majority of the time. The manatee showed a greater breathing and displacement frequency during daytime hours, while comfort behaviors were most often seen during the night (Figure 6).

Feeding behaviors occurred mainly during daytime observations (60.17%) and were recorded in areas where keepers and visitors were constantly present. The manatee showed preference for tubercles

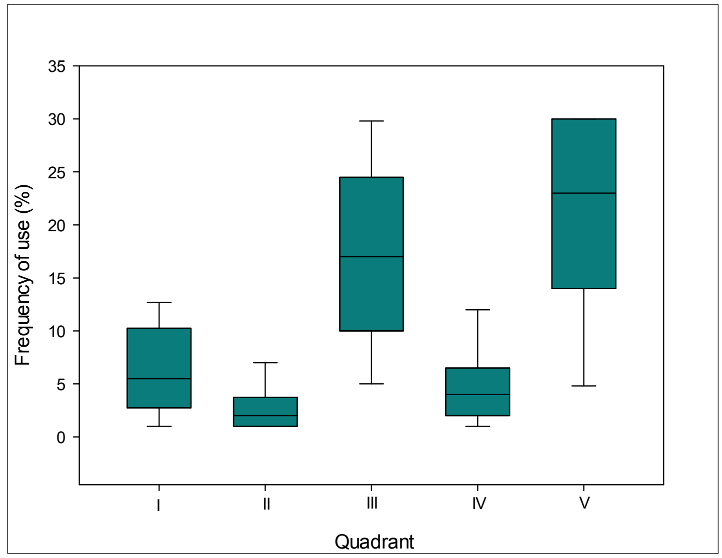


Figure 4: Occupancy of quadrants by a manatee in semi-captivity.

such as beetroot (30.19%), carrot (14.03%), and jicama (6.23%). Direct observations did not reveal consumption of any vegetation naturally occurring in the region. Fecal sample collection was difficult due to the presence of fish that opportunistically and efficiently consumed the excrement. However, we were able to collect and analyze three samples, which contained seven items offered by caregivers (Table 2). Lettuce was the most frequently consumed plant (31.44%), followed by beetroot and broccoli. The turtle grass (*Thalassia testudinum*) was the only species native found in the feces, representing 11.79% of the detected items. There were no significant differences between the frequency of items within fecal samples (T_c= 1.14, $P<0.05$).

Discussion

We consider our sampling effort sufficient at identifying new behavior since the probability of finding a new behavior is low (θ=0.91); similar to what was found in previous studies: 0.94 and 0.96 (9). The manatee's behavior was somewhat predictable, showing higher breathing rates during daylight hours (between 0600 and 1800). Breathing behavior is considered a response to the level of metabolic activity; behaviors requiring high energy investment (displacement, interaction with an object, and feeding) showed the same increased rhythm pattern, while low-energy activities, such as resting, were

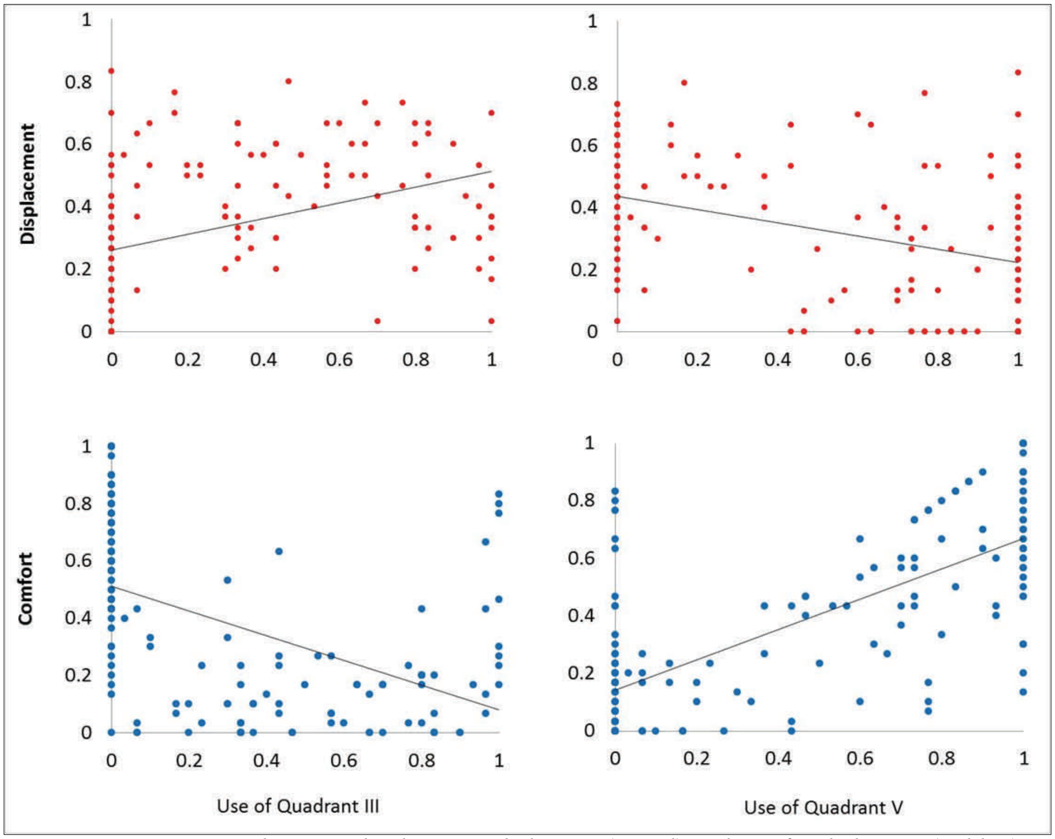


Figure 5: Comparison between displacement behavior (in red) and comfort behavior (in blue), and the relationship with the occupancy of the space.

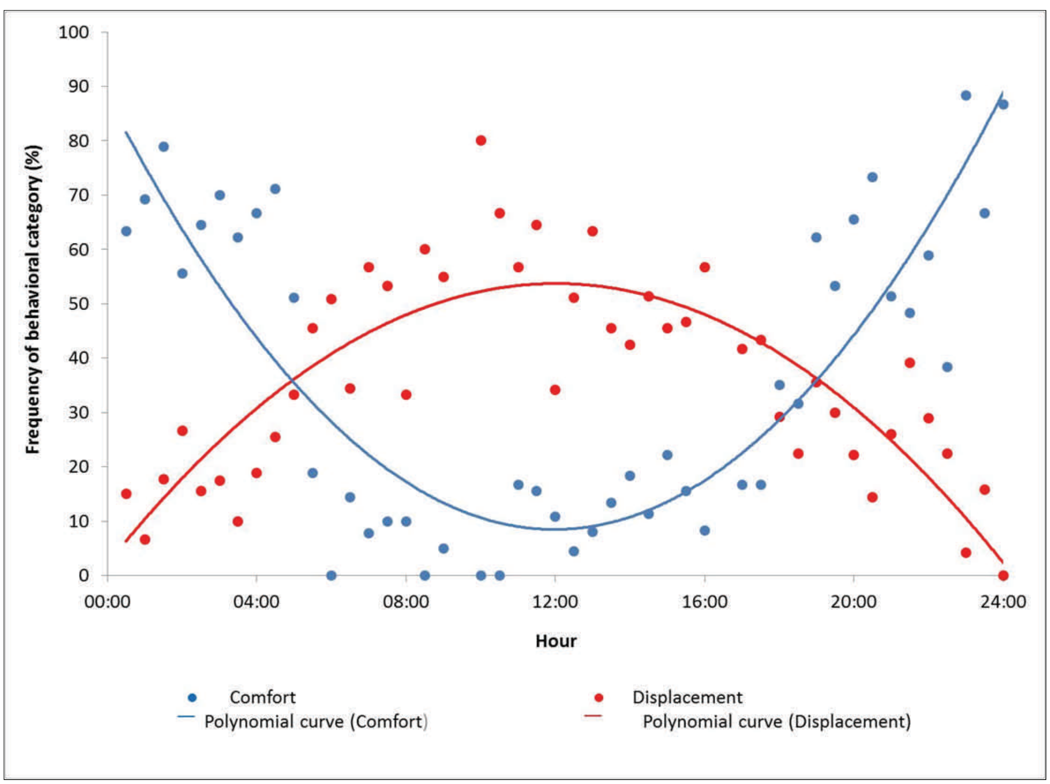


Figure 6: Daily variation of displacement and comfort activities displayed by a manatee in semi-captivity.

	Species	**Consumption observed directly**	**Average consumption observed in feces**
Artificial supply	Lettuce	Yes	31.44%
	Carrot	Yes	6.98%
	Apple	Yes	5.24%
	Pear	Yes	---
	Banana	Yes	---
	Beetroot	Yes	23.14%
	Broccoli	Yes	17.03%
	Cauliflower	Yes	---
	Chard	Yes	4.36%
Natural supply	*Thalassia testudinum*	No	11.79%
	Halodule sp.	No	---
	Batophora sp.	No	---
	Chara sp.	No	---
	Ruppia sp.	No	---

Table 2. Dietary preferences of a manatee in semi-captivity through direct observation and analysis of fecal samples.

were more frequent during the afternoon. In Florida and Puerto Rico, increased activity rate in the manatees' nocturnal behaviors seems to be quite common, however, it is unclear if wild manatees in the study area normally exhibit a daily rhythm of activities (10). Captive manatees have been observed to alter their activity rates from day to night, showing displacement and foraging behaviors during the day, and rest during the night (2,8,14,28). The authors speculate that the activity rhythm could be artificially influenced by their daytime feeding schedule. Conditioned responses to food provisioning are poorly understood for wild manatees outside of Florida, making it challenging to speculate on its effects on survival in other regions. Additionally, the occupancy of quadrants was also affected by the presence of visitors. The manatee actively sought human contact during the visits, which always took place during daylight hours.

According to previous studies, at least six species of plants typically found in the diet of manatees are naturally present in Guerrero Lagoon: *Rhizophora mangle, Thalassia testudinum, Halodule wrightii, Batophora* sp., *Chara* sp., and *Ruppia* sp. (4). However, the manatee was never observed consuming naturally occurring vegetation, and only one species (*Thalassia* sp.) was identified in his feces in low proportions. In the current investigation, we did not register any indication of coprophagy, however, previous studies have observed that some captive manatees eat their own feces (8,9,15). In this study, the manatee may not have had the opportunity to eat his own feces since fish rapidly consumed all that the animal passed. Previously studies were conducted in captivity, where the absence of fish allowed the animals access to consume their own feces. Therefore, it is possible that coprophagy in the wild occurs less frequently than in captivity where fishes are present.

Maintaining a captive manatees' natural behavior, including foraging, is key to the success of rehabilitation and release programs. A mortality analysis developed with released Antillean manatees in Brazil indicated that adaptation to a natural diet is important in the release process (23). Therefore, the ability to forage on local vegetation is vital for adult manatees and a dependency on human food provisioning compromises an individual's ability to survive on its own. Previous rehabilitation experience with other mammal species demonstrated the importance of providing naturally occurring vegetation during the pre-release stage to ensure the development of adequate foraging skills, as well as acclimating the animals to their future diet in the wild

(11,13,21,26). Furthermore, the need for a permanent food supply such as apples, pears, and lettuce, represents a significant cost for the managers and entities in charge.

Animals reared in captivity may form unnatural attachments to people because of the strong learning that sometimes occurs during sensitive, early periods in development. Our observations indicate that this individual was prone to search for human contact such as swimmers, canoes, visitors, and keepers, which led to dangerous situations for both the animal and the people involved. On one occasion, a fisherman wounded the manatee with his spear, causing a severe lesion on the animal's back. Although the cause of the attack is unknown, it is speculated that the fisherman was trying to avoid contact with the animal. While injuring a manatee is considered illegal, the affiliation of the manatee with people promotes human-related interactions that can harm both parties. Humans constitute a major threat to manatees in their natural environment through poaching, injuries from boat strikes, capture in fishing nets, and harassment. If manatees are habituated to humans and/or associate humans with rewards, they would most likely approach or at the very least not actively avoid them. The manatee in this study was most likely habituated to humans due to the regular presence of people during feeding episodes, health assessment procedures, and public visits. During the early stages of release, behavioral flexibility is critical to survival, as animals adapt their current behavioral strategies to meet the demands of the wild environment (27). Thus, dishabituation to people in the wild can be expected because of the substantial differences in context or following the simple passage of time (3). For example, post-release observations of tracked captive-reared manatees in Florida revealed an initial period of weeks or months in which the individuals did not travel far from the release site. However, over time they began to explore their surroundings and travel farther from the release site. If they do join local manatees, captive-reared animals seem to conform to their wild type behavior (12). In the case of the manatee in this study, attachments to humans in conjunction with a lack of normal learning experiences about the natural environment, particularly food resources, may adversely have affected the dishabituation process. Hand feeding represents a close temporal-spatial association and correlation between human presence and food consumption (3). Under such circumstances human presence is predictive of food, leading to a strong, excitatory association between humans and food rewards (3). For example, because food was not available at night, there was an extended period when a ¨no human, no food¨ association could be developed. In the long-term, these associations may also strongly modify a manatee's natural circadian rhythm.

There are several factors to consider within any rehabilitation or training plan. Animals must be able to successfully forage and process locally available food, locate freshwater, avoid predators, interact appropriately with conspecifics, find shelter, orient, and navigate in a complex environment (18). By the completion of this study, the manatee showed a satisfactory health status for release but displayed abnormal behaviors that could have jeopardized his successful release. The manatee demonstrated a total dependence for human care and strong habituation to humans, confirmed by: (i) the preference for food provided by humans over naturally occurring items, (ii) the presence of the manatee in the observation area despite the opportunity to leave and explore natural environments, and (iii) the high frequency of interaction behaviors with humans. Additionally, while anecdotally the manatee has been seen interacting with conspecifics, during this study there were no observed encounters between the individual and wild manatees.

The persistence of abnormal behaviors induced by captivity could reduce survivorship of released animals, as has been demonstrated for terrestrial mammals such as bears and orangutans (29). The possibility that management decisions can potentially impair the normal behavior of the manatees designated for release, suggesting that further protocols need to be applied in order to address the best ways to prevent such detrimental behavioral displays. The literature suggests that training can improve the behavioral skills of captive animals, and facilitate a potentially successful release (18). The preparation of animals prior to release should include training in behaviors that are likely to contribute to survival (e.g. feeding on natural vegetation) and extinguishment of undesirable behaviors (e.g. approaching or not avoiding humans). Manatees are completely herbivorous, semi-social, and apparently, do not have natural predators in this study area. Therefore, learning of hunting behaviors, highly complex social behaviors or predator avoidance behaviors might not be necessary for survival. During rehabilitation processes of manatees with intentions of release to the wild, it is highly recommended to feed them with the vegetation found within their natural habitat. Although this could imply some extra effort in the short/medium time, this investment will be compensated by the rapid and successful weaning of the animal towards natural vegetation. Also, special attention has to be given to the contact between a manatee in rehabilitation and humans. It is undesirable to create human-food associations that elicit a conditioning of manatee behavior to human presence.

Rehabilitation programs are established for several reasons, including conservation of the species, increasing public awareness, supporting local economies, and satisfying political concerns (1). The manatee in this study is a symbol for manatee conservation in the Laguna Guerrero community, and during the completion of this study, was considered a major attraction for visitors. However, rehabilitation has greater worth when it contributes to species conservation and population growth (1). Given the current degradation of manatee habitat, along with a sustained prevalence of various other threats to the species, most manatee populations seems destined to decline and the rehabilitation and release of rehabilitated orphans as a conservation strategy is becoming increasingly necessary (6). However, considerable infrastructure is needed for maintaining captive animals as technology for monitoring released individuals is essential to assess post-release success, as well as veterinary interventions that may be necessary during any phase of the release. In our case, the human and infrastructural resources were limited, making it difficult to take appropriate measures. Finally, it is important to recognize that rehabilitation efforts *per se* will not reverse the impact of human-related activities on manatee survival. Clear management plans, education programs, and basic research are also necessary to promote the species conservation.

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