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ARTÍCULO DE INVESTIGACIÓN/RESEARCH ARTICLE

INTERACTIONS BETWEEN CALVES OF AMAZONIAN MANATEES IN PERU: A STUDY CASE

Interacciones entre crías de manatí amazónico en Perú: un caso de estudio

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ABSTRACT

Trichechus inunguis is an endemic species of the Amazon, which inhabits mainly in lakes and calm rivers. The objective of this study case was to describe the social behaviour of two female-orphaned calves, of *T. inunguis* in captivity. They were kept in the same pool at the facilities of the Amazon Rescue Center (ARC, Iquitos, Peruvian Amazon). Between February and October 2011, the individuals were observed during day and night times, completing 352 hours of observation. Through *ad libitum* observation of the individuals, we developed a catalogue of social behaviors that includes descriptions of 93 behaviours, classified in eight behavioural categories.. The frequency of behaviours was assessed by instantaneous sampling (for states) and continuous recording (for events). Manatees displayed mainly social behaviours, and the most frequent interaction was the simultaneous starting of the same behaviour by both individuals. Most of social behaviours occurred during the day, but 'group resting' was recorded mostly at night. 'synchronized breathing' and 'group feeding' did not have temporary variation between days or between daytimes. Regarding the use of the space, the majority of interactions occurred in shaded places. This study revealed strong interactions between confined calves, suggesting that social activity may play an important role in their learning process.

Keywords: endangered species, ethology, social behaviour, *Trichechus inunguis*.

RESUMEN

Trichechus inunguis es una especie endémica del Amazonas que vive principalmente en lagos y ríos de aguas tranquilas. El objetivo de este estudio de caso fue describir el comportamiento social de dos crías hembras huérfanas de *T. inunguis* en cautiverio, mantenidas en el mismo estanque en el Centro de Rescate Amazónico (CREA, Iquitos, Amazonía peruana). Entre febrero y octubre de 2011, las crías fueron observadas durante el día y la noche, para un total de 352 horas de observación. Desarrollamos un catálogo de comportamientos social para los individuos mediante observación *ad libitum*. La frecuencia de comportamientos fue evaluada por muestreo instantáneo (para estados) y registro continuo (para eventos). Los manatíes exhibieron principalmente comportamientos sociales, y la interacción más frecuente fue el inicio simultáneo del mismo comportamiento por ambos individuos. La mayoría de los comportamientos sociales ocurrieron durante el día, pero 'descanso grupal' fue registrado mayormente en la noche. 'respiración sincronizada' y 'alimentación grupal' no tuvieron variación diaria entre días o entre horas del día. En lo que respecta al uso de espacio, la mayoría de interacciones ocurrió en lugares sombreados. Este estudio reveló fuertes interacciones entre crías en cautiverio, sugiriendo que la actividad social cumple un rol importante en los procesos de aprendizaje de los manatíes.

Palabras clave: comportamiento social, especie amenazada, etología, *Trichechus inunguis*.

INTRODUCTION

The Amazonian manatee *Trichechus inunguis* (Natterer, 1883) is the smallest manatee and exclusively inhabits freshwater environments. The species is endemic to the Amazon region, living along the Amazonian Basin mainly in calm rivers and lakes (Domning *et al.*, 1981; Montenegro-Paredes, 1994; Rosas, 1994). The Amazonian manatee is a non-ruminant herbivore that feeds on a wide variety of aquatic and semiaquatic plants (Guterres *et al.*, 2008). The species plays an important role in the ecosystem, returning more than half of the ingested material to the environment as feces and urine, and is therefore essential to primary production (Best, 1984; Timm *et al.*, 1986). The commercial exploitation (Domning, 1982), along with the slow rate of reproduction (Rodrigues *et al.*, 2008), have seriously reduced the Amazonian manatee population (IUCN, 2008). The species is currently considered Vulnerable, according to the Red List of Threatened Species of the International Union for Conservation of Nature (IUCN, 2015).

Amazonian manatees are difficult to observe in natural environments, due to their dark colour, long underwater times and solitary and cryptic behaviour. Also, the species' habitat is extensive and complex: manatees can easily camouflage in the turbid waters of the Amazon Basin rivers and abundant vegetation associated to the water bodies. Therefore, encounters with Amazon manatees in wild are very rare, and systematic observation of its natural behaviour is almost impossible. Keeping individuals in captivity with rehabilitation purposes provides a unique opportunity to develop ethological studies. Studies of manatee behaviour in captivity have provided useful information on training (Colbert *et al.*, 2001; Lima *et al.*, 2005), communication and auditory capabilities (Bullock *et al.*, 1980; Gerstein *et al.*, 1999; Mann *et al.*, 2005; Colbert *et al.*, 2008), visual acuity (Bauer *et al.*, 2003; Maple and Segura 2015), tactile discrimination (Marshall *et al.*, 1998) and learning behaviour (Gerstein, 1994), among others (Bauer *et al.*, 2010). The results of these studies are useful not only to evaluate the conditions of a particular individual, but also to open new insights on the manatee behaviour knowledge.

Illegal trade in wild animals is a global conservation and animal welfare concern (Daut *et al.*, 2004). Rehabilitation plans are often created to protect animal populations at risk of extinction, providing animals with a second chance to survive in the wild and contributing to the population's gene pool. Therefore, rehabilitation and reintroduction has a greater value when it contributes to species conservation and population growth (Adimey *et al.*, 2012). An adequate study of manatee behaviour might allow us to recognize stress behaviours and other abnormal conditions that would serve later to validate a possible reintroduction to the wild (Castelblanco-Martínez and Zerda-Ordóñez, 2000; Manire *et al.*, 2003; Normande *et al.*, 2015). Also, by observing the interaction behaviours between conspecifics and with

humans, researchers can infer if an animal will be able to survive in the wild, especially in areas where hunting pressure is a concern. Therefore the characterization of behavioural patterns could serve as a baseline for monitoring during rehabilitation for reintroduction process (Gomes *et al.*, 2008).

Solitary or transient social systems are predominant among manatees (Marmontel *et al.*, 1992), and therefore Amazonian manatees are considered semi-social or moderately social animals (Hartman, 1979; Reynolds III, 1981). It is widely accepted that the only readily identifiable social unit in sirenians is the cow-calf pair, with bonds persisting for 1–2 yrs (Anderson, 2002). 'Juvenile' or 'sub adult' manatees are weaned animals that are not fully grown (usually smaller than an adult), or sexually mature (Kunz *et al.*, 1996). 'Adults' are biologically capable of producing offspring, in other words males that present spermatogenesis (Hernández *et al.*, 1995) and females ovulating (Marmontel, 1995). In this study 'group behaviour' is understood as any interaction occurring between two or more individuals.

By observing and analysing two calves in captivity, we tested the following hypotheses: (i) Social behaviours are not important, in comparison with non-social behaviours, (ii) there is not a significant difference between categories of social behaviours, (iii) there is not a rhythm of social activities during the times of the day, and (iv) there is not a preference in the use of the space to develop social activities.

Therefore, the aim of this research was to identify and describe captive/orphan manatee social behaviour in order to gather baseline information that can be useful for decision making during manatee calves' rehabilitation. The specific objectives were: (1) To develop a catalog of social behaviour of *T. inunguis* individuals in order to obtain a descriptive baseline for orphan/captive manatee behaviour that can be used for further studies, (2) to infer the relevance of social activities for calves in rehabilitation in order to give advises for manatee management in captivity, and (3) to determine the temporal and spatial variation of social activities in order to define preferences of space and time to display social behaviours.

MATERIAL AND METHODS

Subjects

We studied the social behaviour of two orphan females of Amazonian manatees rescued at the Peruvian Amazon: (i) Nauta was 18-months-old and was rescued near Nauta town, Loreto Province on 14 March 2010, and (ii) Yuri was one-year-old and was rescued in Alto Amazonas Province on 26 November 2010. The rescued calves were transferred to individual quarantine ponds of the ARC facilities with the goal of rehabilitate them for further release. The dimensions of the quarantine ponds were 2.80 x 1.95 x 0.75 m, with an approximate capacity of 4.095 L. Once rehabilitated and habituated to bottle feeding, they were moved together to

directionality of social interactions, where ties reflect the initiator and recipient of the relationship (Wey *et al.*, 2008). The initiator of the behaviour was recorded as follows: Yuri initiating an interaction with Nauta (Y→Na), Nauta initiating an interaction with Yuri (Na→Y), both individuals initiating an interaction simultaneously (Y↔Na), or one of the manatees initiating an interaction with humans (Na→H or Y→H). Behaviours were considered to be synchronized when displayed by both individuals simultaneously, and could be initiated by any one of them or both. 'Events' were recorded by focal-animal sampling and continuous recording; the researcher recorded a complete account of all behaviour units of interests (bouts), obtaining frequencies of each behaviour.

With the goal of assessing the horizontal use of space, the pond was divided into eight equal imaginary quadrants (Fig. 1). The position of the dyad was registered every minute, i.e., simultaneously with the 'states' recording.

Data analysis

'States' frequency was represented in terms of percentages. For each sample period, budgets of the 'states' of social interaction were compiled by summing the total of points (i.e. each sample record) when a 'state' was recorded and dividing this sum by the total of observation points (i.e., 30). Therefore, the 'state' frequency was determined by the formula $f_s = (n_s/30) \times 100$; where s = the state and n = number of points when the states were recorded. Frequency rates of 'events' were calculated for each sampling period by dividing the total number of bouts by the number of minutes of observation ($f_e = \text{bouts}/30$). Budgets were also assessed separately for day and night samples to investigate the influence of the sunlight on social behaviour. The Friedman test (non-parametric alternative to the one-way ANOVA with repeated measures) was used to detect the difference between day (06:00 to 18:00) and night (18:00 to 06:00). Statistical analyses were done using PAST 3.x (Hammer *et al.*,

2001). Finally, we established social activity budgets for each quadrant in order to examine any potential space preference in which to perform social interactions. For this, the relative frequency of social interactions per time unit was measured for each quadrant. The normality assumption was verified with the Shapiro-Wilk test. Overall comparisons between groups of social behaviour were made with Kruskal-Wallis one-way analysis of variance on ranks; followed by a Tukey pair-wise multiple comparison procedure. The paired t-test was applied to compare two groups of data (for example, between shaded and non-shaded quadrants). When the data failed to show normality, a Wilcoxon Signed Rank test was used. In this point, the statistical analyses were done using SigmaPlot (Kornbrot, 2000).

RESULTS

We completed 352 hours of observation. The asymptotic cumulative curve of behaviours was obtained after 104 samples (a sample consisting of 30-min observation period), with a total of 93 social behaviours described (52 events and 41 states, see Arevalo-Sandi 2012). The value obtained of the coverage sample (θ) was 0.90. The majority (74 %) of state behaviours observed corresponded to social interactions. The catalog of social behaviours was divided into the following categories (codes in parenthesis): Synchronized breathing (100-199), synchronized displacement (200-299), tactile contact (300-399), affiliative (close-proximity behaviour that includes touching, grooming, and nuzzling; 400-499), agonistic (any social behaviour related to fighting, such as aggressive or submissive behaviours; 500-599), group feeding (600-699), group resting (700-799), and human interactions (800-899). See Table 1 for a detailed description of each category. The Kruskal-Wallis analysis of variance found a significant difference between social categories ($H=230.207$, $df=2$, $p<0.001$). The most common category was synchronized displacement (32 %), followed by group resting (25 %) and group feeding (18 %) (Fig. 2).

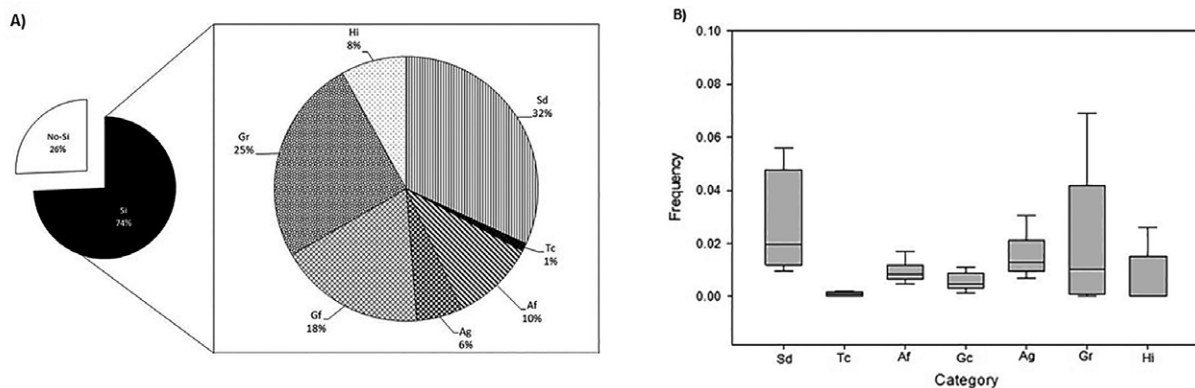


Figure 2. Frequencies of 'states' observed in a dyad of Amazonian manatee. A) Left pie: proportions of social (SI) and no-social (No-SI) behaviours. Right pie: Proportions of social behaviours, B) Sd=Synchronized displacement, Tc=Tactile contact, Af=Affiliative, Gf=Group feeding, Ag=Agonistic, Gr=Group resting, Hi=Human interactions.

Table 1. Catalogue of social behaviours of two Amazonian manatees calves in captivity in Peru.

Code	Category	Description	N° of events	N° of states	Total
Sb 100-199	Synchronized Breathing	Both manatees breathe at the same time. This behaviour is detected visually and sometimes listened.	1	0	1
Sd 200-299	Synchronized Displacement	Steady movement in one direction. Animals move either simultaneously, or one of them with reference or proximity to the other.	2	5	7
Tc 300-399	Tactile Contact	Physical interactions between the individuals. This may include embracing, rolling, touching or rubbing.	31	13	44
Af 400-499	Affiliative*	An individual tends to approach and remain near the other. We defined "affiliative" as any interaction that does not involve aggression.	10	11	21
Ag 500-599	Agonistic*	Interaction involving aggression: attack, threat, appeasement, and fight behaviour. <i>Syn.</i> Aggression.	4	1	5
Gf 600-699	Group Feeding	Simultaneous foraging on aquatic plants and/or simultaneous ingestion of water.	1	3	4
Gr 700-799	Group Resting	Both individuals rest/sleep at the bottom or surface for more than 10 seconds	0	3	3
Mi 800-899	Manatee-Human Interactions	The manatee approaches toward humans.	2	6	8
TOTAL OF BEHAVIOURS			51	42	93

*Adapted from Barrows (2000).

In most of the cases (68.7 %), the interaction was initiated simultaneously ($Y \leftrightarrow Na$) (Fig. 3). In this type of interaction, categories group resting and synchronized displacement showed the highest frequency. Yuri and Nauta played the initiator role in similar proportion (9.82 % and 9.68 % respectively). However, Nauta often initiated displacement conducts (synchronized displacement), while Yuri initiated mostly agonistic behaviours. Nauta was never observed starting agonistic interactions. Interactions with humans took place during bottle feeding, and also during the

presence of visitors. In all the cases, both manatees started interactions with humans with similar frequency (Yuri = 5.03 %, Nauta = 6.72 %).

Social interactions were observed during both day and night, but were significantly more frequent during the night (Wilcoxon test: $Z=4.286$, $p<0.001$). Also, the daily rhythm varied depending on the category (Fig. 4). The statistical analysis showed significant differences in behavioural frequency between day (6:00 to 18:00) and night (18:00 to 6:00) for the following categories: Synchronized displacement

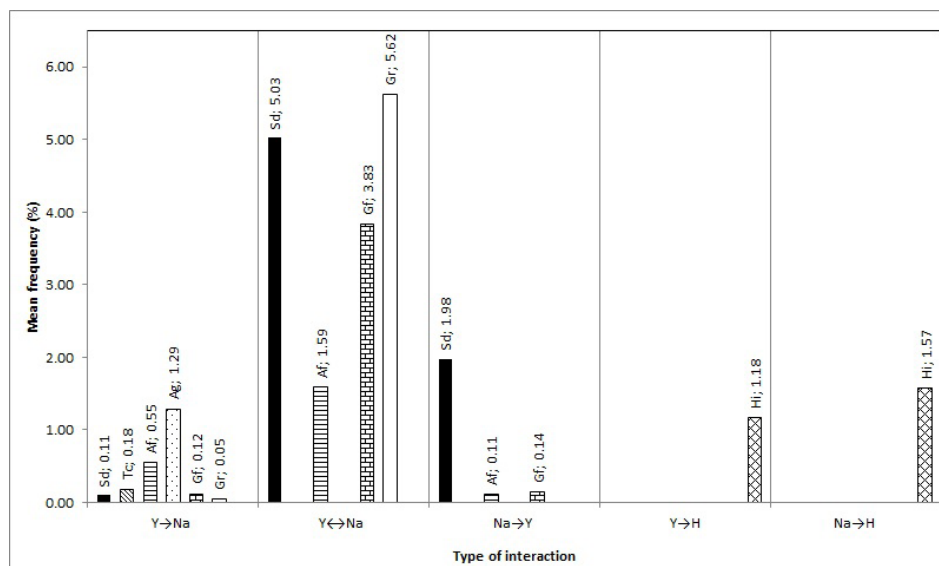


Figure 3. Mean frequencies of interactions according to the behavior initiator among manatees and humans. Only states are included. Y=Yuri, Na=Nauta, H=Humans. Arrows indicate the interaction direction; double arrow indicates a simultaneous interaction. Sd=Synchronized displacement, Tc=Tactile contact, Af=Affiliative, Ag=Agonistic, Gf=Group feeding, Gr=Group resting, Hi=Human interactions.

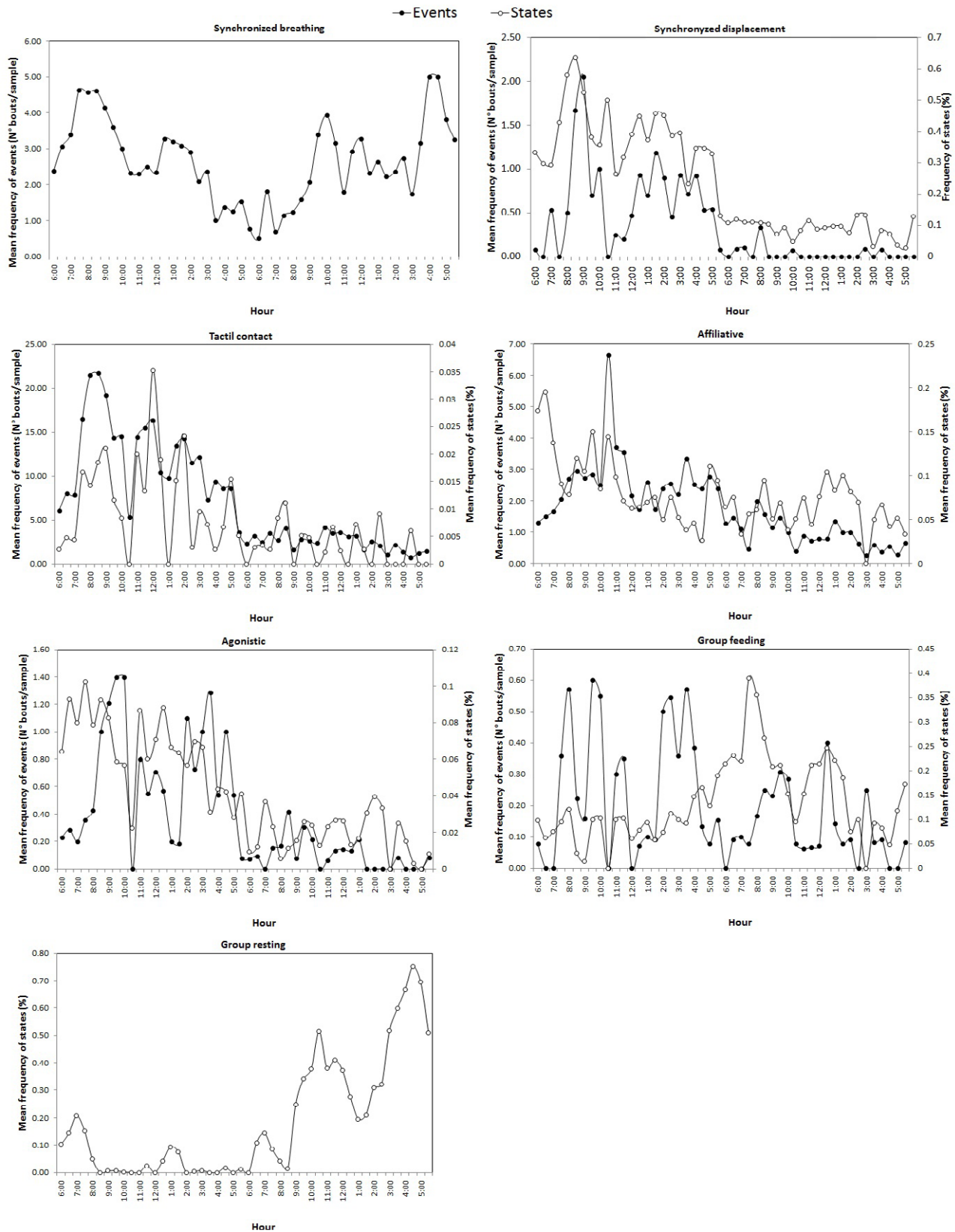


Figure 4. Activity daily rhythm of social behaviours observed in two female Amazonian manatees in captivity.

(Friedman test statistics= 16.66, $df=1$, $p<0.0001$), tactic contact (Friedman test statistics=24, $df=1$, $p<0.0001$), affiliative (Friedman test statistics=24, $df=1$, $p<0.0001$), agonistic (Friedman test statistics =15.04, $df=1$, $p<0.0001$), group resting (Friedman test statistics=0.66, $df=1$, $p=0.004$) and human interactions (Friedman test statistics=0.66, $df=1$, $p=0.00035$). No significant differences were found for synchronized breathing and group feeding.

The use of space-frequency (%) differed significantly between quadrants (Kruskal Wallis; $H=42.6$, $df=6$, $p<0.001$) (Fig. 5). In general, shaded quadrants particularly 2B, 3B and 4B, were preferred while displaying social behaviours (paired t-test: $t=4.45$, $df=47$, $p>0.001$).

DISCUSSION

Both individuals showed a normal growing rate compared with other Amazonian calves in captivity (Sousa Lima, 1999).

During the time of the study, Nauta grew from 50 to 62 kg (0.6 kg/week), while Yuri increased her weight from 28 kg to 40 (0.6 kg/week). As about the behaviours, the obtained sample coverage value ($\theta=0.90$) was slightly lower than those reported for *Trichechus manatus* ($\theta=0.91$; Mercadillo-Elguero et al., 2014, and for *T. inunguis* ($\theta=0.94$ and $\theta=0.96$; Castelblanco-Martínez, 2000 and Charry, 2002 respectively). However, we consider that the majority (90 %) of the probable social behaviours were included in the catalog. The total number of observed social behaviours was higher (74 %) than that obtained in other studies such as: Harper and Schulte (2005), Gomes *et al.* (2008), Holguin-Medina (2008) and Hénaut *et al.* (2010); probably due to the greater sampling effort conducted in the present study (Table 2).

The manatees observed in the mentioned studies (4 – 20 individuals) were calves, juveniles and adults, while manatees observed in the present study were just two orphaned calves

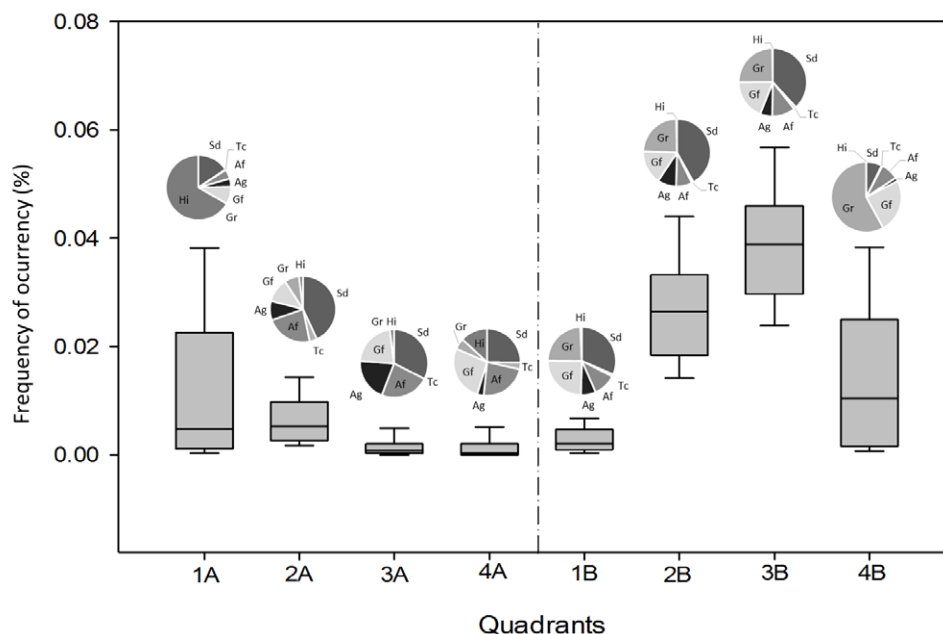


Figure 5. Space used by a dyad of Amazonian manatees in captivity to develop social behaviours. Quadrants names correspond to Figure 2, and those named with B were shaded areas. Sd = Synchronized displacement, Tc = Tactile contact, Af = Affiliative, Ag = Agonistic, Gf = Group feeding, Gr = Group resting, Hi = Human interactions.

Table 2. Social behaviours reported during budget activity assessments for manatees in captivity.

Reference	Species	Country	Number of individuals	Sex	Age class	Social Interactions (%)	Total hours sampled
Harper and Shulte, 2005	<i>T. m. l.</i>	USA	20	Females	All	NA	36
Gomes <i>et al.</i> , 2008	<i>T. m. m.</i>	Brazil	7	Males and females	Juveniles	< 3.57	84
Holguin-Medina, 2008	<i>T. m. m.</i>	Brazil	7	Males and females	Juveniles	36.61	240
Hénaut <i>et al.</i> , 2010	<i>T. m. m.</i>	Mexico	4	Males and females	All	31.62	136
Present study	<i>T. i.</i>	Peru	2	Females	Calves	74	352

T.m.l. = *Trichechus manatus latirostris*; *T.m.m.* = *T. m. manatus*; *T.i.* = *T. inunguis*. NA = Not available.

(suckling, bottle-fed individuals). Due to the fact that these individuals were still in the early stages of development, it is to be expected that social interaction will be actively searched for more often (Bekoff, 1972). Hénaut *et al.* (2010) observed that a calf with his mother presented the highest number of interactions in a pool with several adults. Activities that may reinforce the female-calf bond are nuzzling, mouthing and synchronous breathing (Reynolds III, 1981). Orphan manatee calves have been observed to intently look for physical proximity to other individuals, regardless of age or sex (Castelblanco-Martínez, per. obs.). In this study, most of the interactions were initiated by the youngest calf (Yuri), while Nauta was usually indifferent to Yuri. This suggests that younger manatees look more intensely for attention from older individuals. Like other large mammals, sirenian calves gain nutritional independence gradually as they shift from a milk diet to solid food (Langer, 2003). The weaning is divided in two phases: 1) when only milk is taken and 2) a mixed period: in which the milk intake is combined with the consumption of solid vegetation. The longer the mixed-feeding period, the more time is available for ontogenic developments, for instance, learning how to obtain food, avoiding predators, and establishing immune competence (Langer, 2003). That means that even when the calf is relatively independent from the mother in terms of nutritional intake, a long calving period is needed to reinforce behaviours that could be decisive for survival. Previous studies suggested that during inter-individual contact, the manatee calves learn and develop social skills and recognition abilities by being exposed to conspecifics and thus build a type of social map used when manatees come together in groups or when encountering isolated individuals in the wild (Hénaut *et al.*, 2010). Our findings reinforce the recommendation that, during rehabilitation processes, maintaining several manatee calves in the same pond might facilitate the creation of inter-individual bonds, which could be important for survival in wild if manatees are released together.

The synchronous surfacing (breathing) has been observed in wild Florida manatees (Hartman, 1979) and dugongs (Anderson, 1995). It is possible that it is an extension of behaviour learned at birth when a mother synchronizes her movements with those of her offspring. It has been suggested that breathing in synchronous fashion may help reduce an individual's chance of being captured by a predator (Hastie *et al.*, 2003). Synchronous surfacing ensures closer side-by-side contact between animals, decreasing the odds of accidental separation, a constant hazard in turbid waters, especially for a calf (Hartman, 1979). Synchronicity in cow-calf pairs is well known (Hartman, 1979), and since both individuals lost their mothers at an early age and are of similar age, our results suggest that these two individuals may have been considering each other "substitute mothers". Bonde (2009) presented some intriguing genetic evidence of wild Florida manatee suckling adopted calves. If adoption

in manatees is widespread, orphan calves prone to seek the company of an older conspecific must facilitate such adopting mechanism. Both studied manatees also searched intensively for human contact, as a response of feeding conditioning. This behaviour may be problematic in the future, but necessary during the bottle feeding period. For future plans of release, human contact must be limited to guarantee an adequate adaptation to the wild.

Even though social activities were recorded at any time, the manatees studied showed preference to rest simultaneously during the night. A previous study showed certain daily rhythm in the activities of a captive calf Amazonian manatee; with more display of breathing and displacement behaviours during daytime (Castelblanco-Martínez, 2000). However, it is not clear if the rhythm of activity is a consequence of the artificial bottle feeding schedule which occurs only during the day. The studied manatees showed predilection for indoor areas, maybe because these areas were perceived as safer. This behaviour would be useful in the natural habitat for a possible camouflage and thus avoid humans and possible predators, as mentioned by Charry (2002).

CONCLUSIONS

We present a study-case of captive/orphan Amazonian manatee social behaviour. Our study revealed strong interactions between the calves, suggesting that social activity may play an important role in the learning process of the individuals. However, caution is necessary in extrapolating results from our observations to natural behaviour. There are no studies on social behaviour of wild Amazonian manatees, and our findings should be only interpreted into the frame of particular circumstances, such as the confinement during a rehabilitation process. Despite of this, this work may represent the beginning of the collection of long-term baseline information about Amazonian manatees' behaviour in captivity. This might help implement adequate management in captivity in Peru and, consequently, increase the odds of a successful rehabilitation process.

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