

TRAINING OF ANTILLEAN MANATEE *TRICHECHUS MANATUS MANATUS* LINNAEUS, 1758 AS A MANAGEMENT TECHNIQUE FOR INDIVIDUAL WELFARE

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ABSTRACT: Behavioral training is a method used in institutions that keep captive animals to assist in husbandry and health assessment issues. It consists of training animals using positive reinforcement to perform behaviors that facilitate veterinary procedures without the use of physical restraint or drugs, thereby improving animal welfare. A female Antillean manatee (*Trichechus manatus manatus*), eight years old, weighing approximately 400kg and measuring 266cm, was the subject of this study. The training method used was operant conditioning with positive reinforcement, which encourages the animal to cooperate during veterinary inspections. The animal was trained that every time it performed a commanded behavior correctly, it would be rewarded with food and verbal praise. Furthermore, just prior to the moment of the reward a whistle was sounded; thus the animal associated this sound with the correct performance of the commanded behavior. To control the body position, the animal was trained by operant conditioning to touch a target. Our subject was trained in two stages, to perform necessary behaviors to collect biological samples (e.g. blood). Some of the possible factors influencing the training sessions were evaluated, such as the identity of the keeper, the impact of sounds and the number of days between training sessions. Only the identity of the keeper was found to influence training sessions. Our subject rapidly learned to express a number of commanded behavior patterns to assist its management in captivity. Therefore we consider this method to have been successful. We consider this method indispensable when managing large endangered species in captivity, as training reduces stress for the animal and reduces risk to its human caregivers. Finally, the application of this method will allow us to collect more biological data about this endangered species.

RESUMO: O treinamento comportamental é um método que vem sendo utilizado em instituições mantenedoras de animais em cativeiro. Consiste no desenvolvimento de um repertório comportamental, visando facilitar o acesso veterinário sem o uso de contenção física ou química, reduzindo o comprometimento do bem-estar animal. Uma fêmea de peixe-boi marinho (*Trichechus manatus manatus*), com oito anos de idade no momento do experimento, cerca de 400kg de peso e medindo 266cm de comprimento total foi o animal em estudo. Utilizou-se o condicionamento operante como técnica de treinamento, onde estimula-se, de forma positiva, a cooperação do animal durante o manejo veterinário. A cada vez que o animal executava o comportamento que lhe foi solicitado, recebia recompensas, como por exemplo alimentos e frases de incentivo. Simultaneamente, um apito era acionado para estabelecer uma associação entre comportamento e recompensa. Foi utilizado um alvo para orientar o animal a posicionar-se na forma desejada. A fêmea foi treinada, durante duas fases, a exibir comportamentos necessários para a coleta de amostras biológicas (por exemplo, sangue). Foram avaliados fatores de possível influência no treinamento do animal, como tratador; impactos sonoros e dias sem treinamento, verificando-se que apenas o tratador exerce influência. O animal em estudo rapidamente tornou-se capaz de expressar os comportamentos necessários para o manejo em cativeiro, sendo o método considerado bem sucedido. A utilização do condicionamento proporciona a coleta de dados de forma menos estressante que a habitual, sem gerar riscos tanto para o animal quanto para as pessoas envolvidas no procedimento. O uso correto da técnica de condicionamento contribui para ampliar as informações a respeito desta espécie em eminente risco de extinção e obter dados importantes para o conhecimento e conservação da mesma.

KEYWORDS: *Trichechus manatus*, management, training, operant conditioning

Introduction

Behavioral training is a method that is being used with a number of animal species, including aquatic mammals. It consists of developing a behavioral repertoire which has several possible applications in the management of the species. Usually in institutions holding animals in captivity, health assessment of these animals is undertaken by the application of physical and/or chemical restraint, both of which imply discomfort and risk to the animal (Grandin *et al.*, 1995).

Training is, therefore, a very important tool for all animal species, especially those threatened with extinction, where the risks associated with veterinary management can be avoided (Young and Cipreste, 2004). Training allows reduction of stress, both to the animal and the people involved in the procedure, therefore reducing the negative consequences to the animal's well-being or alterations in the biological samples collected (Sweeney, 1990).

The training method usually employed is Operant Conditioning (Chance, 1988), where an association is built

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between the behavior exhibited by the animal and the consequence for its performance. One of the tools employed, primary reinforcement, makes use of something that the animal apparently enjoys, e.g. foods that are not part of its basic diet (treats). A secondary reinforcer, such as a sound signal from a whistle or a 'clicker', acts by making the connection between the performance of the behavior and the reinforcement that is to come. Another common training tool is the 'target', which may be any object (most commonly a stick) used to indicate to the animal a commanded position or direction.

The conditioning of manatees is currently used with the Florida subspecies (*Trichechus manatus latirostris*) by Gerstein (1994), Colbert *et al.* (2001) and Martinez-Diaz *et al.* (2001)⁵.

Although the conditioning of aquatic mammals is allowed by law in Brazil, according to IBAMA's regulation n° 3 of 08 February 2002, the technique is still not widely used. The Antillean manatees, *Trichechus manatus manatus*, maintained in captivity at the National Center for Research, Conservation and Management of Aquatic Mammals - Aquatic Mammal Center/IBAMA (CMA/IBAMA) are physically and clinically examined through physical restraint. Prior to the procedure it is necessary to drain the tank, leaving the animal in the ventral position (Vergara, 2001). Physical restraint is then conducted by keepers (Bossart, 2001; Vergara, 2001); however, the animal is still able to create risks of trauma and stress, to itself and the personnel involved in management alike (Colbert *et al.*, 2001).

Given the results obtained by these researchers, and the concern with the captive species' welfare, especially those captive at CMA/IBAMA, and given that these animals will not be returned to their natural habitat, a training program for these manatees has been proposed. A pilot program was initiated by Paszkiewicz (2002)⁶, where a presumed pregnant female was the subject selected, as there was a desire to not use traditional restraining techniques since the stress involved and the compression of the abdomen caused by the animal's body weight might jeopardize gestation. During the first phase of training, the female was conditioned to perform behaviors necessary to routine veterinary procedures. Then a new phase was initiated, to include new behaviors and procedures, improve the performance of behaviors already trained, and examine whether there were factors influencing the animal's learning processes. In the present study we started training two other females to perform these behaviors for future veterinary procedures.

Material and Methods

Study animal

The animal used in this study was a female Antillean manatee called "Marbela", identification number 01S0112/11. At the time of this study the animal was eight years old, 266cm long and weighed approximately 400kg. Marbela was rescued in May 1994 having been found stranded on Pipa Beach, on the coast of Rio Grande do Norte state, at approximately one day of age.

Study site

This study was conducted at the National Center for Research, Conservation and Management of Aquatic Mammals - Aquatic Mammal Center/IBAMA (CMA/IBAMA), located on Itamaracá Island (07°41'47,6"S; 34°50'16,2"W), northern coast of the Pernambuco state. Data were collected between December 2002 and February 2003. Eight Antillean manatees, some rescued from inadequate captive settings and others found stranded on beaches, were maintained on site as part of a reproductive pool. The animals were maintained in three tanks in the public access area, two of which were 4m deep and had volume capacity of 348.8m³ and a third with a volume of 143.44m³ and 2m deep, all of which were fed with brackish water. Intermediate areas of lower depth and width, called "handling area", where the veterinary management procedures took place, connected the tanks.

Training

Training sessions took place in the handling area, without draining, approximately six times a week with an average duration of 30 minutes for each session. The door between the tank and the handling area was raised such that the animal could spontaneously move to the training area. In the case that this did not occur, the animal was attracted into the area with carrot pieces, tossed into the handling area. Carrot and apple pieces, foods appreciated by manatees but not part of their normal daily diet, were used as primary reinforcers. Other reinforcers consisted of other types of food items (mango, pineapple, cashew, etc), rubbing of the body with a brush and verbal praise, when the animal performed a particular commanded behavior despite external interferences or at the end of the training sessions. A whistle was used as a secondary reinforcement. A 22 x 17cm white plastic rectangular 'target', with a 11 x 8.5cm red rectangle in the middle, was fixed to the inner wall of the tank such that the animal associated it with the beginning of the session and for it to position itself correctly. The maintenance of a behavior was obtained by using reinforcements at

⁵ Martinez-Diaz, K., Perez-Lewis, M., Quijano-Rossy, A., Valentin-Narvaez, J.A. and Mignucci-Giannoni, A.A. (2001) *Training of medical behaviors in an orphan manatee to be re-introduced into wild*. Page 136 in Abstracts, XIV Biennial Conference on the Biology of Marine Mammals, November, Vancouver, Canada.

⁶ Paszkiewicz, E. (2002) *Relatório final do estágio curricular obrigatório*. Curso de Medicina Veterinária. Centro Universitário UniFMU. São Paulo.

variable time intervals. The opportunity to receive a positive reinforcement ceased, for a short period of time, when the animal left the stationary position (*i.e.* a 'time-out' was applied) (Young and Cipreste, 2004).

The female had been previously trained during the pilot study to approach, touch and stay close to the target, such as to present dorsum and venter. With these two positions, it was possible to further train behaviors already presented by the animal and to introduce new behaviors; the objective was to obtain cooperation in taking body measurements and body temperature, collecting blood, fecal and urine samples and performing ultrasonographic exams.

Biometry

The target was placed in front of the animal, leading to a position sideways to the border of the handling area and close to the water's surface. This allowed us to train the animal to accept total body length and curvilinear length measurements. Afterwards, the target was transferred to the lateral border of the tank and a manual loop signal performed. With the exposure of the abdomen it was possible to collect other measurements: length and maximum width of the tail; girth of the caudal peduncle; thorax girth at the level of the anal opening, genital opening, navel and posterior to the flipper; and width, length and girth of the flipper. Sometimes while taking these measurements it was necessary to have a person inside the tank, handling the measuring tape. The data were recorded on appropriate data sheets, identifying each one of the measured sites.

Ultrasonographic exams

The behavior of exposing the ventral surface by the animal allowed training for conducting ultrasonographic exams, with the objective of pregnancy diagnosis. The average duration of the performance of the behavior obtained during the pilot study (seven minutes) was considered insufficient for the completion of this procedure; therefore it was necessary to extend the average maintenance of the behavior to 15 minutes. Consequently, almost exclusive attention was given to the behavior of exposing the ventral surface by the animal during the training sessions. The manipulation of the animal in the ventro-lateral orientation, desensitization to the ultrasonographic transducer on the abdomen and desensitization to the presence of people inside and outside of the handling area helping with the exam, were also trained. The desensitization to the transducer was accomplished with the help of objects that exerted a similar pressure to that produced by the transducer. For the pregnancy diagnosis we used 3.5MHz and 5MHz linear transducers, connected to a portable ultrasonographic gear ALOKA SSD-500V.

Blood sampling

Training for blood collection commenced as soon as the animal positioned itself correctly with its abdomen exposed. Pressure was then applied with a 25 x 7mm disposable needle on the flipper. Prior to this, the animal was submitted to a desensitization phase using non-piercing objects, such as a pen's cap. The pressure with the needle was applied on the space between the radius and the ulna, where a venous plexus allows routine blood collection. Such pressure was gradually increased along the training period and one person always remained inside the tank assisting with the procedure.

Collection of fecal and urine samples

For this procedure it was necessary that the animal remained exposing its abdomen on the water's surface. Pressure was applied to the abdominal region close to the anal opening. One person remained inside the tank supporting the animal's back such that it did not submerge as pressure was applied. A plastic container was placed close to the anal opening, preventing the collected material from coming into contact with the tank's water.

To collect urine, the animal was maintained exposing its ventral region. A person outside of the water applied a continuous pressure, 5cm from the genital opening. An assistant, in the water, supported the animal's weight at the time pressure was exerted. A plastic container was placed close to the genital opening, preventing the entrance of water. The animal was trained to remain parallel to the water's surface, maintaining the genital opening without contact to the water.

Body temperature measurement

Initially the animal was desensitized to the measurement of body temperature, by using a 10cm long, 3mm diameter flexible plastic tube. While the animal remained exposing the ventral region, the tube was gradually inserted into the anus approximately 7cm deep. After the desensitizing phase, a digital thermometer with a flexible handle was used to measure body temperature.

All training sessions were measured with a stopwatch. All the data obtained, as well as factors related to the development of the training, were recorded in data sheets to monitor the animal's performance.

Statistical Analysis

In order to analyze whether factors such as keeper's identity, sound stimuli and days without training influenced the duration of the sessions or exposure of the ventral region of the animal, a statistical

analysis was performed. Non-parametric tests were applied: Kruskal-Wallis (H) when more than two categories were analysed, and Mann-Whitney U-test to compare two categories. The correlation between the duration of training with the dates when it was conducted, and the number of the session conducted, was evaluated through Spearman Rank Correlation (r_s). Statistical significance was accepted when $P < 0.05$.

Results

Training

The animal was conditioned for ventral and dorsal exposure as follows. When the target was placed in front of the animal, it positioned itself parallel to the side of the tank, exposing its back to the water's surface. When the target was transferred to the animal's side, it touched it immediately afterwards and a manual signal (of a loop) was used so that the animal turned around on its own body axis, therefore exposing the abdomen and presenting its flipper as support.

Thus, 53 training sessions were performed, totalling 1664 minutes, where three behaviors and seven procedures necessary during the veterinary management were trained; these varied during the sessions according to the animal's performance and collaboration (Table 1).

Biometry

The animal was easily conditioned to cooperate during collection of body measurements (Figure 1). A new measurement was added at each session until all measurements were obtained. Measurements were successfully taken in 100% of the sessions, after approximately two months of conditioning. All measurements were successfully taken, except for the measurement relative to the thorax girth posterior to the flipper, due to the animal's high sensitivity in this area.



Figure 1. Training for collecting body measurements of manatees in the *cambiamento* tank of the CMA/IBAMA, on Itamaracá Island, northern coast of the Pernambuco state, northeastern Brazil.

Ultrasonographic exams

The goal of having the animal remain 15 consecutive minutes exposing its venter (ventral surface) during the sessions was reached. The desensitizing to all necessary procedures during ultrasonographic exams was also obtained. During the exam, the animal remained calm with its ventral region exposed on the water's surface for approximately 30 minutes, which had not been previously achieved. The animal accepted manipulation of the transducer in the entire abdominal region. Special attention was given to the region close to the female's

Table 1. Number of sessions conducted with each of the procedures during the training period.

PROCEDURES	NUMBER OF SESSIONS
Desensitizing of flipper	18
Desensitizing of genital area	21
Desensitizing of the abdominal region	53
Desensitizing of the anal region (temperature)	19
Desensitizing of the anal region (feces collection)	12
Ventro-lateral manipulation	49
Biometry	20

genital opening, to facilitate gestational diagnosis. It was also possible to visualize and record the anatomic relationship of the following structures: thorax (structure and cardiac frequency), abdomen (hepatic parenchyma, gall bladder, urinary bladder, kidneys, gastric cavity, intestinal tract, uterus and ovaries) and body fat depth.

Collection of blood sample

Training for cooperation in this procedure was attempted at irregular intervals, due a certain discomfort caused by the needle penetrating the animal's flipper. In some sessions the animal apparently demonstrated such discomfort by terminating its venter-exposed position. This behavior was minimized by offering extra primary reinforcements at the very moment of needle introduction. The penetration of the sterile disposable needle in the flipper was gradually increased up to its totality during the sixth session of pectoral fin desensitization. Two complete penetrations were achieved, although with unsuccessful sampling.

Collection of fecal and urine samples

During training for fecal sample collection the animal became desensitized to the manipulation close to the anal region during the first sessions, possibly because this procedure is less invasive. The animal cooperated positively when some pressure around the anal region was applied, which allowed the collection of fecal samples ($n = 8$) for parasitological analyses.

In the case of training for urine collection, the female response to the desensitizing process was satisfactory. The procedure of pressing the region close to the

female's genital opening was successful during the first training sessions, however, the animal responded positively to the pressure (ejection of urine) in only one of the training sessions.

Recording of body temperature

The female accepted the insertion of the plastic tube into the anus during the first session. At no point the female seemed bothered with the object introduction. After the seventh session it was already possible to use the thermometer to obtain body temperature, and we recorded a variation between 33 and 35°C.

Analysis of the possible factors influencing training

The results obtained with the Kruskal-Wallis test showed that the different keepers involved in training sessions caused a significant difference in the total duration of sessions ($H = 15.26$; $DF = 3$; $P = 0.002$), with longest duration of sessions under caretaker II (Table 2).

Keepers did not act on their own, such as to influence the period the animal remained exposing the venter ($H = 3.36$; $DF = 3$; $P = 0.0340$). Average time of venter exposure by the animal was 535 seconds (Standard deviation = 230) during 20 training sessions assisted by keeper I, 683 seconds (Standard deviation = 405) in 18 sessions with keeper II and 434 seconds (Standard deviation = 177) during 5 sessions.

The intervals between sessions did not influence the total duration of training in a significant way ($H = 23.5$; $DF = 3$; $P = 0.503$) (Table 3) neither the permanence of the animal in venter exposition ($H = 2,53$; $DF = 3$; $P = 0,470$) (Table 4).

Table 2. Number of sessions, average and standard deviation for each keeper involved

KEEPER*	NUMBER OF SESSIONS	AVERAGE (SEC)	STANDARD DEVIATION
0	10	1452	339
I	20	1941	408
II	18	2085	582
III	5	1440	391

(*) Keeper 0 = absence of keeper during training; Keeper I, II and III = keepers who assisted during training

Table 3. Average length of training relative to the number of days without training

INTERVAL OF DAY*	NUMBER OF OCCURENCE	AVERAGE (SEC)	STANDARD DEVIATION
0	39	1783	499
1	5	1980	326
2	4	2257	1009
3	5	1920	342

(*) Interval 0 = absence of days of interruption of training sessions; Interval 1, 2 and 3 = number of days of interruption of training sessions.

The absence of sound stimuli in the environment (impact 0), as well as loud noises occasionally caused by the doors between the tanks and the handling area (impact 1), did not influence the duration of training in a significant way ($H = 2.06$; $DF = 2$; $P = 0.358$) (Table 5).

Significant correlations were observed between the duration of the training and the number of training days ($r_s = -0.680$; $N = 52$; $P = 0.000$), and between the duration of training and the number of sessions conducted ($r_s = -0.682$; $N = 52$; $P = 0.000$). Thus, there was a slight decrease in the total duration of the sessions across the dates of training sessions.

Discussion

One of the greatest concerns in institutions maintaining wildlife in captivity is the animals' welfare. Among the alternatives to avoid jeopardizing the well-being of the animal, behavioral training with the aim of cooperation during the veterinary management has been used as a tool that allows the physical and clinical inspection without involving risks and minimizing the stress caused to the animal and to the personnel involved in the procedure. In addition to facilitating veterinary procedures, behavioral training of manatees is instrumental to allow some types of biological research using captive animals, *e.g.*, brightness discrimination ability (Griebel and Schmidt, 1997) and underwater visual acuity (Bauer *et al.*, 2003).

The choice of behaviors that animals can be trained to perform depends on the species being trained (Young and Cipreste, 2004) as well as several other factors that influence the learning process (Sapolsky, 1996), *e.g.* genetics, temperament, history of life of

the animal (Grandin, 1997). Gerstein (1994) conducted studies on manatee cognition and verified that this species presents high learning capability. The training process conducted with the female manatee in this study was considered relatively easy, due to the fact that the animal had an extremely docile character and, mainly because all phases of the training were attained with success, verifying its learning ability. Such easiness may be due to the animal's habituation to captivity and to human contact during feeding and pool maintenance since early age.

In several of the training steps, the help of a person with experience in management was necessary. The caretakers are the best persons to assist during the procedure, since they maintain a direct contact with the animals. The quality of the interaction caretaker-animal often times can act directly in the development of learning (Hemsworth and Barnett, 2001) throughout the training process. The results obtained connecting the involvement of caretakers in the total duration of the sessions show that the identity of the keeper can influence animal's performance of trained behaviors, and possibly, the welfare of the animal. It was evident that the animal cooperates for a longer period of time when the caretaker shows interest in the procedure, and transmits care and reassurance to the animal. Hemsworth *et al.* (1993) also observed in their research that the personality, attitudes and behavior of the caretaker during training are directly associated to the behavior, performance and welfare of the animal. According to Young and Cipreste (2004), correctly selecting the people to help in animal management is a determinant factor in the success of a training program.

Sapolsky (1996) mentions the animal's memory as another factor that may influence learning. There were

Table 4. Average time of venter exposure related to the number of days without training

INTERVAL OF DAYS*	NUMBER OF OCCURRENCE	AVERAGE (SEC)	STANDARD DEVIATION
0	39	550	240
1	5	680	222
2	4	942	777
3	5	528	97

(*) Interval 0 = absence of days of interruption of training sessions, Interval 1, 2 and 3 = number of days of interruption of training sessions

Table 5. Impacts, number of occurrence, average and standard deviation

IMPACTS*	NUMBER OF OCCURRENCE	AVERAGE	STANDARD DEVIATION
0	42	1904	544
1	9	1680	418

(*) Impact 0 = absence of sound stimuli in the environment, Impact 1 = noises caused by doors between tanks and *cambiamento*.

days when the training was interrupted due, for example, to maintenance of the tanks; however, we found that these interruptions did not affect the learning process in the present study. This suggests that the female's memory facilitated the continuity of the learning process. Kastak and Schusterman (2002) also observed a great memory capacity of a specimen in California sea lion (*Zalophus californianus*) even after long periods in the absence of training.

One of the implications for a good level of animal learning is that the training be conducted in the absence of conditions that can cause stress (Young, 2002), such as psychological or physical conditions (Grandin, 1997). Studies conducted by Stillman *et al.* (1998) verified that stressful situations occurring previously to training sessions generated an increasing reduction in the animal's performance. A hypothesis at the beginning of this training experiment was that noise caused by people and doors in the tanks would be possible causes of stress. This hypothesis was not confirmed, since there was no variation in the duration of training under the absence or presence of sounds in the environment. It is suggested, therefore, that the absence in variation was due to the fact that the animal was habituated to sounds in the tank, as well as those caused by tourists during the visiting hours.

There was a reduction in the duration of sessions in relation to the number of days of training. The behaviors previously trained could be executed, during the sessions of training, in ever shorter periods of time. Occasional session took longer than expected only when we decided to train a new behavior or conduct biometry or ultrasonography. Luttrell *et al.* (1994) also observed the development of learning during the process of training. Given the short period of conditioning, few urine and feces samples, and no blood samples, were obtained, but this does not invalidate the method, which we considered adequate for the proposed management functions.

We conclude that training of Antillean manatees that are non-releaseable is important, since it allows for the collection of specific biological data from the animals in a less stressful manner than is usually conducted. The method also has applicability to other situations and species, and could be used, for example, in Amazonian manatees (*Trichechus inunguis*) kept in other Brazilian institutions. It is important to stress that the training sessions be carefully designed, so as to avoid possible development of abnormal behaviors due to poor application of training methods. The correct use of the technique of conditioning applied to Antillean manatees contributes to our knowledge regarding learning abilities of an endangered species, and allows for the collection of important biological data for the conservation of the species.

Acknowledgements

To CMA/IBAMA and Aquatic Mammals Foundation (FMA) for allowing the conduction of this work, specially

R. P. Lima and D. F. Castro; to C. Assunção, C. L. Parente, E. Monteiro-Filho, F. Trujillo, M. Marmontel and T. Young for suggestions and corrections to the manuscript; to C. Cipreste, E. Sanchez, J. C. Borges, K. Souza and M. C. Marcondes for collaborating in many ways; to the managers at CMA/IBAMA for the help during the training sessions. This work had the support of the National Environment Fund - Federal Government.

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Received 1 April 2004. Accepted 2 May 2005.