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## Journal of Veterinary Behavior

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## Research

Behavior and salivary cortisol of captive dolphins (*Tursiops truncatus*) kept in open and closed facilitiesCristian Ugaz<sup>a</sup>, Ricardo A. Valdez<sup>b</sup>, Marta C. Romano<sup>b</sup>, Francisco Galindo<sup>a,\*</sup><sup>a</sup> Departamento de Etología y Fauna Silvestre, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Ciudad Universitaria, México D.F., México<sup>b</sup> Departamento de Fisiología, Biofísica y Neurociencias, CINVESTAV, México D.F., México

## ARTICLE INFO

## Article history:

Received 10 May 2012

Received in revised form 8 August 2012

Accepted 25 October 2012

Available online 27 December 2012

## keywords:

dolphins  
behavior  
salivary cortisol  
welfare

## ABSTRACT

Few studies have been carried out on the welfare of captive bottlenose dolphins (*Tursiops truncatus*). Of these, most include information on animals kept in closed facilities or pools. The aim of this study was to assess the welfare of captive bottlenose dolphins in open and closed facilities by measuring states of individual behavior and salivary cortisol concentrations. A total of 23 bottlenose dolphins were studied in 4 different dolphinaria. Dolphinaria A and B have closed facilities, whereas dolphinaria C and D have open facilities. A total of 152 hours of behavioral observations were analyzed using a combination of behavior and scan sampling to obtain information on individual time budgets. Salivary cortisol concentrations were measured using radioimmunoassay in 96 and 180 saliva samples of dolphins kept in closed and open facilities, respectively. In general, the results found that dolphins kept in open facilities spent less time floating ( $P < 0.05$ ) and swimming in circular patterns than linear ones ( $P < 0.05$ ) compared with dolphins in closed facilities. Dolphins kept in open facilities also had lower salivary cortisol concentrations than dolphins kept in closed facilities ( $P < 0.05$ ). For this reason, we suggest that further research should include other welfare indicators such as reproductive function and health measurements to know more about the relationships between the design of pools and dolphin welfare.

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## Introduction

The behavior of any wild species is the result of many generations of natural selection and adaptation to the environment. The ability to cope with changes in the environment such as those related to captivity depends on a complex interplay of biological factors (Mason, 1990; Shepherdson, 1994; Carlstead, 1996; Poole, 1998). The welfare of many species of terrestrial mammals has been

studied (Carlstead, 1996), but little information is available on marine mammals in captivity (Pedernera-Romano et al., 2006; Luna, 2008). Bottlenose dolphins (*Tursiops truncatus*) in captivity are still used for entertainment and assisted therapy in several countries. It is known that these animals have to adapt to changes in their physical and social environments as a result of the design of the enclosures where they are kept, changes in food presentation, and also because of changes in their social structure (Pedernera-Romano et al., 2006).

It is well known that stress induces behavioral changes (Fowler, 1986; Dierauf, 1990; Waples and Gales, 2000) that could be related to prolonged adrenal activity and long-term welfare and health problems (Moberg, 1985; Broom and Johnson, 1993; Fowler, 1995; Chrousos et al., 1998; Sapolsky et al., 2000; Reeder and Kramer, 2005).

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Some studies carried out with bottlenose dolphins have been useful for understanding general aspects of their maintenance behaviors in captivity (Gygax, 1993; Sobel et al., 1994; Small and DeMaster, 1995; Bassos and Wells, 1996; Galhardo et al., 1996; St. Aubin and Dierauf, 2001; Sekiguchi and Kohshima, 2003; Ugaz et al., 2009). Fewer studies, however, have assessed adrenal function through measurements of plasma, serum, and salivary cortisol (Thomson and Geraci, 1986; St. Aubin, 1996; Ortiz and Worthy, 2000; St. Aubin, 2001; Suzuki et al., 2003; Pedernera-Romano et al., 2006; Noda et al., 2007). Salivary cortisol of dolphins has proven to be a noninvasive procedure of great value when repeated sampling is necessary as its collection is potentially stress free and practical (Pedernera-Romano et al., 2006). So far, most of this information has been obtained from dolphins kept in dolphinariums with closed pools. A recent study that compared the behavior of 10 dolphins that were kept in pools and moved to open sea enclosures showed that the behavior of dolphins varies according to the type of facilities (Ugaz et al., 2009). The results show that when dolphins were kept in closed facilities they spent less time swimming and more time floating than when in open facilities. Dolphins in closed facilities tend to swim in circles, whereas those in open facilities spend more time swimming in linear patterns. Furthermore, dolphins kept in closed facilities spent more time interacting socially than those in open facilities. For these reasons, the aim of this study was to compare individual and social behaviors, as well as salivary cortisol, of different groups of captive bottlenose dolphins kept in open and closed facilities. This information can enhance our knowledge of how the characteristics of open facilities for captive dolphins may influence their behavior and adrenal activity in comparison to closed pools.

## Methods

### Locations and animals

A total of 23 bottlenose dolphins were studied in 4 different dolphinariums.

Dolphinarium A and B have closed facilities, whereas dolphinarium C and D have open facilities (Table 1). A closed facility was defined as a pool with no access to the sea and with treated water, whereas an open facility was defined as a pen, usually designed as a dock, with access to the sea and seawater and contained within a fence.

The average age of the dolphins studied was 16.3 years old (age range, from 11 to 24 years) with 6 males and 17 females (Table 1). All individuals were adults and healthy at the time of the study. All were captured in the Gulf of Mexico, and they had an average of 9.5 years in captivity (range, from 8 to 15 years). All dolphins, both in closed and open facilities, were fed with the same species of fish: capelin (*Mallotus villosus*), Atlantic Herring (*Clupea harengus*), and squid (*Loligo patagonica*, *Loligo gahi*, or *Loligo vulgaris*). The aquariums were opened to the public and regularly hosted interactive swimming sessions. The 4 dolphinariums had 5 interactive sessions per day, each lasting 40 minutes to every 80 minutes. The first interactive

**Table 1**

Individual dolphins kept in the 4 dolphinariums studied

Dolphinarium	Facilities	ID	Sex	Age (y)	Time in captivity (y)
A	C	1	Female	12	+8
A	C	2	Male	18	+15
B	C	3	Female	12	+8
B	C	4	Female	14	+8
B	C	5	Female	14	+8
B	C	6	Male	15	+8
B	C	7	Male	18	+8
B	C	8	Male	17	+8
C	O	9	Male	18	+10
C	O	10	Female	14	+10
C	O	11	Female	12	+9
C	O	12	Female	11	+8
C	O	13	Female	16	+8
D	O	14	Male	16	+10
D	O	15	Female	24	+10
D	O	16	Female	18	+10
D	O	17	Female	17	+10
D	O	18	Female	16	+10
D	O	19	Female	18	+10
D	O	20	Female	16	+10
D	O	21	Female	18	+10
D	O	22	Female	18	+10
D	O	23	Female	22	+10

Facilities are marked as C = closed and O = opened.

program is at 9:30 AM and the last one at 15:00. The observations were not conducted during an interaction or performance session.

### Closed facilities

Dolphinarium A is a round pool with a conical bottom reaching a maximum depth of 5 m and with a capacity of 1964 m<sup>3</sup>. It is located in Mexico City at 2240 m above sea level. Dolphinarium B is a rectangular pool, with an average depth of 4 m, subdivided into a main area of 60 × 20 m and 4 small (5 × 10 m) holdings or pens, 2 at each end. It has total capacity of 5600 m<sup>3</sup> and is located on the Pacific coast of central Mexico.

### Open facilities

Dolphinarium C is located on the east coast of the Yucatan Peninsula. It is an artificial breakwater with access to the sea allowing the exchange of seawater and small fish and other marine species. It has a maximum depth of 3.5 m, a total volume of 6074 m<sup>3</sup>, and is subdivided into 2 areas of equal size with a dock separation and an oval rubble quay. Dolphinarium D is located in the north coast of the Yucatan Peninsula. It has 3 holdings or pens each of 33 × 10 m, separated by an open fence and with access to a central area of 100 × 35 m, with a maximum depth of 4 m, and an estimated total water capacity of 26,250 m<sup>3</sup> (Table 2).

**Table 2**

Size of the pools in each dolphinarium

Dolphinarium	Length (m)	Width (m)	Minimum depth (m)	Maximum depth (m)
A	25	25	3.5	5
B	70	20	4	4
C	75	30	3	3.5
D	100	45	3.5	4

**Table 3**Comparison of behaviors and salivary cortisol levels between dolphinarium (average  $\pm$  standard error)

Variable	Dolphinarium A	Dolphinarium B	Dolphinarium C	Dolphinarium D
TTS (%)	62.87a $\pm$ 13.97	71.76a $\pm$ 7.73	90.95b $\pm$ 2.96	93.98b $\pm$ 1.88
LS	21.73a $\pm$ 5.16	58.10b $\pm$ 10.49	66.39b $\pm$ 17.66	92.77ab $\pm$ 2.06
SS	10.61a $\pm$ 2.81	4.63b $\pm$ 2.72	3.34b $\pm$ 1.58	6.52ab $\pm$ 2.34
DS	11.12a $\pm$ 4.69	53.48b $\pm$ 12.38	63.05b $\pm$ 16.21	86.24ab $\pm$ 3.54
CS	41.14a $\pm$ 24.10	13.66b $\pm$ 5.04	24.56a $\pm$ 15.97	1.21ab $\pm$ 0.70
TTF (%)	37.13a $\pm$ 11.26	28.24a $\pm$ 7.73	9.05b $\pm$ 2.96	6.01b $\pm$ 1.87
Cortisol (nmol/L)	1.40a $\pm$ 1.10	0.65a $\pm$ 0.17	0.30ab $\pm$ 0.10	0.09b $\pm$ 0.02

Different letters (a, b, and ab) in each column represent statistical differences between groups.

TTS, total time swimming; LS, linear swimming; SS, superficial swimming; DS, deep swimming; CS, circular swimming; TTF, total time floating.

### Measurements of behavior

A behavioral catalog was adapted from Galhardo et al. (1996), Miguel (2004), and Singh (2005) using 48 hours of ad libitum sampling during day and night completing two 24-hour cycles. During this time, individuals were identified by their distinctive natural marks. Data were collected through direct observations using a combination of behavior and scan sampling (Martin and Bateson, 1993) over 48 hours divided in 2 cycles of 24 hours, distributed over 6 days, and divided into 4 hours every day. Observation periods were divided as follows: day 1, 0800–1200; day 2, 1200–1600; day 3, 1600–2000; day 4, 2000–2400; day 5, 2400–0400; and day 6, 0400–0800. In total, 152 hours of behavioral observations were analyzed, 58 hours in closed facilities, and 94 hours in open facilities. This information was used to obtain individual time budgets.

The behaviors observed and recorded were total time swimming (TTS) when a dolphin was moving and total time floating when the dolphin was static. TTS was divided into linear swimming (LS), when a dolphin swam alone in different directions covering the entire length of the pool, without a fixed circular pattern; and circular swimming (CS), when a dolphin swam in a circular pattern. LS was subdivided into deep swimming (DS), when animals swam in different directions with their dorsal fin always under the water and superficial swimming (SS), when dolphins swam in different directions with at least one part of the dorsal fin out of the water.

For each animal, the time spent in behaviors during the study was expressed as proportion of time calculated as follows: number of observations of a behavior divided by total minutes observed. The term time budget was defined as the summed proportions (total 100%) of these behaviors.

### Saliva samples and cortisol analysis

The dolphins that participated in this study were trained to emerge from the water and open their mouths voluntarily, thereby assuring a noninvasive procedure to obtain the samples. Saliva samples were obtained on the same day that observations were carried out and before the first meal of the day. This was always between 9:00 AM and 9:30 AM, 16 hours after the last activity with the trainer. Before saliva collection, all cotton swabs were washed with ether (which evaporated completely before use) to eliminate all organic residues. When taking the sample, the swab was introduced up to the base of the tongue and placed in a sterile plastic tube.

The swabs were then centrifuged at 3000 rpm, and the sample of saliva was stored at  $-20^{\circ}\text{C}$  until assayed. In total, 276 saliva samples were collected, 12 from each animal, 96 samples in closed facilities and 180 in open facilities. Radioimmunoassay kits of iodine-125 (Cort CT2; CIS Bio International, France) were described by Pedernera-Romano et al. (2006).

The kits have an affinity of 100% to serum, urine, and salivary cortisol, and cross-reaction with prednisolone (42%), fludrocortisone (12.1%), corticosterone (2.8%), prednisone (1.2%), cortisone (0.9%), and less than 1% with other similar metabolites. The intra- and interassay coefficients of variation were 9.01% and 10.9%, respectively.

### Statistical analysis

Data were analyzed using SAS, version 6.0 (Manufacturer SAS Instituted, Cary, North Carolina). Kolmogorov–Smirnov tests of normality were applied to variables of behavior, and the results were not significant ( $P > 0.05$ ). For this reason, an analysis of variance test was used to compare behavior and average salivary cortisol levels between all facilities.

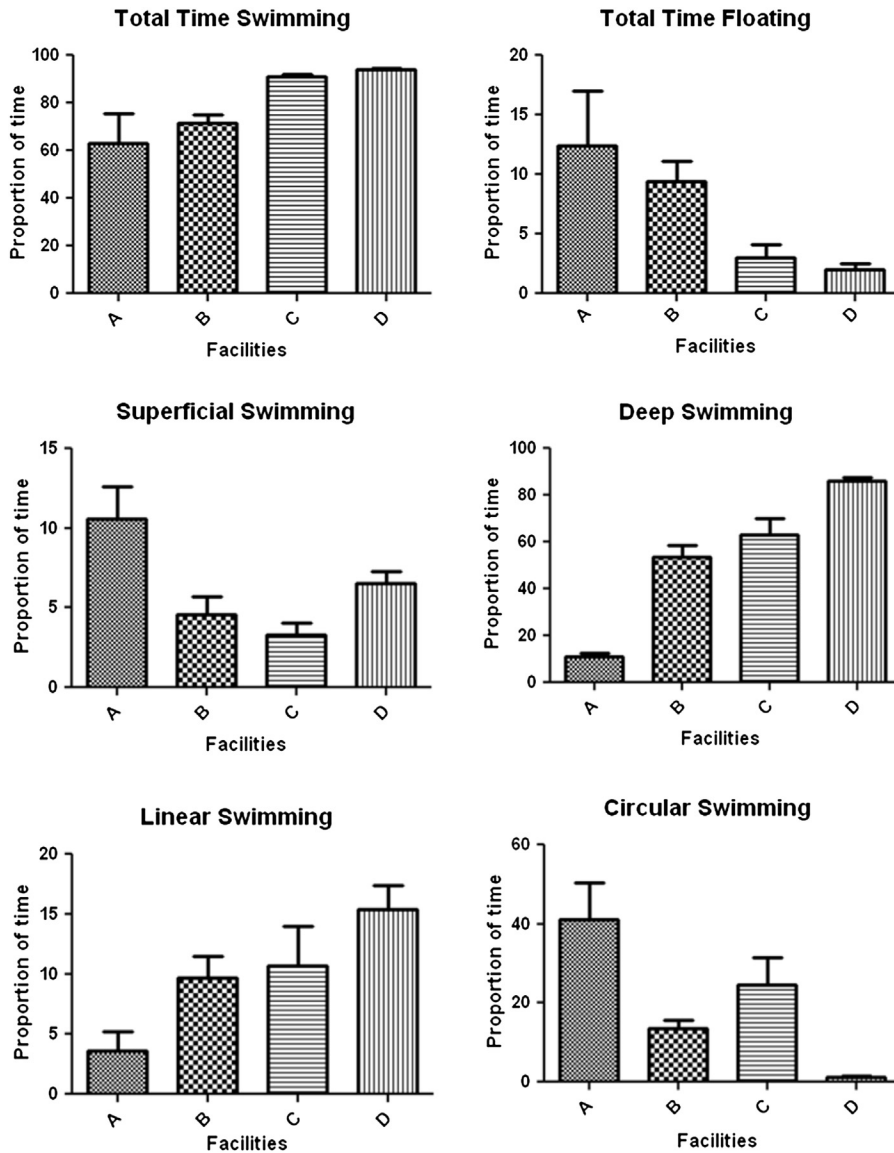
## Results

### Behavior

When behaviors were compared between groups, statistical differences were found (Table 3). The time swimming was higher, and the time floating was lower, in dolphinarium C and D ( $F = 27.37$ ,  $P < 0.001$ ) compared with dolphinarium A and B ( $F = 10.83$ ,  $P < 0.001$ , Table 3), respectively. There were also differences in the types of swimming: the time spent in LS was lower in dolphinarium A ( $F = 36.31$ ,  $P < 0.0001$ , Table 3) than in the other 3 groups, and the time spent in CS was lower in dolphinarium D ( $F = 17.46$ ,  $P < 0.001$ , Table 3). Finally, there were differences in the time spent in the 2 types of LS: the time in SS was higher in dolphinarium A ( $F = 5.45$ ,  $P < 0.01$ ), and the time in DS was lower compared with dolphinarium B, C, and D ( $F = 36.33$ ,  $P < 0.001$ , Table 3), respectively (Figure 1).

### Salivary cortisol

Salivary cortisol levels ranged from 0.0116 to 1.5109 nmol/L with an average value of 0.3293 nmol/L. When the average salivary cortisol values were compared between



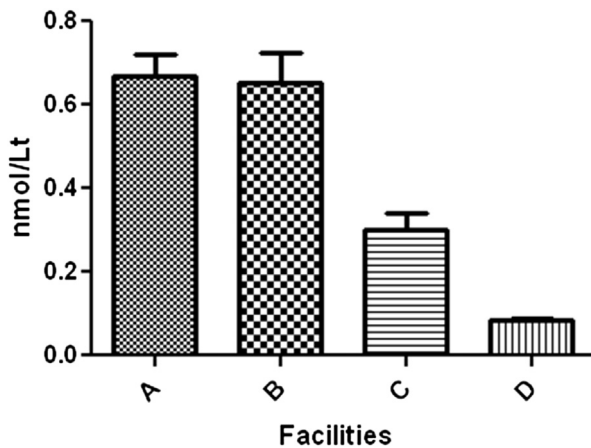
**Figure 1.** Average time budgets in the 4 groups of dolphins observed. Total time swimming ( $F = 27.37, P < 0.001$ ), total time floating ( $F = 10.83, P < 0.001$ ), linear swimming ( $F = 36.31, P < 0.0001$ ), circular swimming ( $F = 17.46, P < 0.001$ ), superficial swimming ( $F = 5.45, P < 0.01$ ), and deep swimming ( $F = 36.33, P < 0.001$ ).

groups, it was found that dolphins in dolphinarium A and B had higher values than those kept in dolphinarium C and D ( $F = 49.89, P < 0.001$ , Table 3; Figure 2).

### Discussion and conclusions

These results demonstrate that the enclosure type influences the behavior and salivary cortisol concentrations of captive bottlenose dolphins. Dolphins that were kept in open facilities were more active and spent more time swimming in a linear pattern than dolphins that were kept in closed facilities. Furthermore, dolphins in closed pools show higher salivary cortisol concentrations than those individuals housed in open pens.

Previous studies carried out with dolphins housed in pools or closed facilities have shown that these animals spent most of the time swimming in a continuous circular pattern (Gygax, 1993; Galhardo et al., 1996; Singh, 2005). These results, supported by the fact that dolphins housed in open facilities spent more time swimming in a linear pattern, suggest that the lack of space and depth in a pool, compared with an open pen, plays a role in this change of behavior. It is likely then that the shape and size of the pools could influence the type and intensity of the swimming patterns (Sobel et al., 1994). In this sense, it would also be important to further investigate the possible relationship of this swimming pattern with an abnormal behavior, as previously suggested (Gygax, 1993).



**Figure 2.** Average salivary cortisol concentrations in the 4 groups of dolphins. These values are calculated from 12 saliva samples in each dolphin.  $N = 2, 6, 5, 10$  in each dolphinarium, respectively ( $F = 49.89$ ,  $P < 0.001$ ).

Although we found that CS was less frequent in open than in closed facilities, there was also a difference in this behavior between open facilities. This could be attributed to one of the dolphins in dolphinarium C that showed this behavior more frequently close to the medical area, possibly related to having spent some time there soon before the start of this study and to the close presence of the trainers in that section of the pool.

Also, the fact that dolphins in closed facilities were less active supports previous studies (Bassos and Wells, 1996; Singh, 2005) that reported that dolphins spent a greater proportion of time at rest in smaller pools than in bigger ones. These results suggest that the occurrences of passive states of behavior in small and closed pools could be related to the quality of the space and how the animal perceives it.

Several authors have reported a CS pattern of bottlenose dolphins in captivity (Sobel et al., 1994; Bassos and Wells, 1996; Miguel, 2004; Singh, 2005). To know more about the implications of these behaviors, it is important to investigate how they relate to both external and internal stimuli, such as the design and size of a pool, water currents, and laterality behaviors (Gygax, 1993; Marino and Stowe, 1997; Sekiguchi and Kohshima, 2003; Stafne and Manger, 2004).

Considering the fact that most dolphins sampled for saliva collection in this study were trained to open their mouth voluntarily, the hormone concentrations obtained from the dolphins studied could be considered as non-stressed cortisol values. The results show, for the first time, salivary cortisol measurements of dolphins kept in open facilities with open access to seawater. The values obtained in this study from dolphins kept in closed facilities are similar to those found by Pedernera-Romano et al. (2006) for similar types of pools. However, the values of salivary cortisol in open facilities were lower. Carlstead (1996) argues that limited space, and inadequate environmental stimuli, among other factors, can be stressful. It could be argued that dolphins kept in open facilities are exposed to more diverse stimuli related to the space available and to

the fact that these facilities have access to the sea and, hence, increase swimming in slow linear patterns and have lower salivary cortisol levels. Although information on social interactions was not included in this analysis, it is important to consider in future studies a possible link between aggression and cortisol levels, especially in closed facilities.

In summary, this study demonstrates that dolphins kept in open facilities show different swimming behaviors and lower salivary cortisol concentrations than those kept in closed facilities. It should be noted that it is likely that dolphins in open facilities also have to cope with problems associated with that type of facilities. To better understand these relationships, we suggest that further research using other welfare indicators, apart from behavior and adrenal activity, could be carried out to assess the welfare of captive dolphins. Such welfare indicators could include reproductive function, through measurements of hormonal profiles, offspring survival, or semen quality, among others; as well as epidemiological studies, health records, and survival rates. This could also help to know more about the biological significance of many of the behavioral changes seen in this species when they are kept in captivity.

## Acknowledgments

The program Programa de Apoyo a los Productos de Investigación e Innovación Tecnológica de la Universidad Nacional Autónoma de México IN223106 supported this study. We are very grateful to the dolphinarium that participated in this study and acknowledge the support given by Dirección General de Asuntos del Personal Académico de la Universidad Nacional Autónoma de México to Dr. Francisco Galindo to conclude the analysis and writing of this article.

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