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# Non-invasive Multidisciplinary Approach to the Study of Reproduction and Calf Development in Bottlenose Dolphin (*Tursiops truncatus*): The Rimini Delfinario Experience

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Reproduction is a fundamental biological process that occurs only when all other vital needs are satisfied. In cetaceans reproduction takes place completely in water. From courtship and mating to calf weaning, every step of the reproductive process occurs under the water's surface. This complicates data acquisition in wild populations, making captive observations a useful complement to wild studies. By allowing close examination of phenomena, studies in captive environments are able to collect long-term data on known subjects, and sample, in detail, complete behavioural sequences while monitoring physiological or acoustic patterns. Studies of reproduction in bottlenose dolphins (*Tursiops truncatus*) were initiated at Rimini Delfinario (Italy) in 1995. Four bottlenose dolphin births (in 1995, 1997, 2003 and 2007) have occurred since the start of this research. Due to evidence suggesting that mother and calf associations are closest in the first year of the calf's life, mothers and calves were studied from birth to the end of the first year. Beginning in 1997, studies encompassed the behaviour and physiology of dolphin mothers during gestation. Here, we report results of interdisciplinary studies of reproductive processes in bottlenose dolphins, including aspects of behaviour, physiology, endocrinology, and acoustics. In an effort to reduce the potential for bias brought about by invasive sampling, we investigated methods of sampling expired air from the dolphin's blowhole as a means of monitoring steroid hormone levels. In summary, our research combines an interdisciplinary network with specialized professional alliances and offers a potentially crucial approach to the biological aspects of reproduction. At the same time, research findings presented here aim to help bridge the gap existing between captive and wild studies in favor of a common aim of conservation biology.

Since reproduction has to be linked with the process of gathering resources for both reproductive effort and continued survival, taking a very broad view, the "function" of marine mammals is to convert prey to offspring (Fedak, Wilson, & Pomeroy, 2002). Understanding the processes in cetacean reproduction has,

We thank Rimini Delfinario owners and staff, especially Carlotta Severi, Claudia Giglietti, Arata Deana and all the trainers for their precious help in blowhole sampling. A special thank to Daniela Silvia Pace, Francesca Triossi, Jorge San Juan Rodriguez, Silvia Garcia Martinez, Sergio Catacchio, Matteo Gamberoni, Roberta Viggiani, Danilo Matteuzzi, Manuel García Hartmann and all the students who valuably participated in the research. Acknowledgment also goes to Oltremare Training staff who collaborated in the blow sampling of the dolphin Blue. We are finally grate to Stan Kuczaj for his kind assistance, to the two anonymous reviewers who with their important suggestions definitely improved the paper and to Trevor Willis who kindly accepted to straighten the quality of writing. The endocrinology study was supported by Università di Bologna (FIL 2008). Correspondence concerning this article should be addressed to Tizzi Raffaella, Delfinario Rimini, Lungomare C. Tintori 2, 47921 Rimini, Italy. (raftizzi@tin.it).

however, been severely hampered by the difficulties inherent in observing fast moving and wide ranging animals that are entirely aquatic. What is known is pieced together from anatomical studies, whaling operations, live captures, individual identification, genetic analyses, and interspecies comparisons (Fedak et al., 2002; Whitehead & Mann, 2000). Since there are so many questions that have not or cannot be answered using wild marine mammals, captive animals are still a primary source of data for several fields such as physiology, acoustics and reproduction (Corkeron, 2002).

Since modern marine mammal studies combine aspects of all major branches of the physical and biological science, most scientists specialize concentrating on one or a few aspects of marine mammal science (Perrin, Würsig, & Thewissen, 2002). In this context, the general aim of the research carried out in Rimini Delfinario has been to analyse bottlenose dolphin (*Tursiops truncatus*) reproduction and calf development, combining aspects of physiology, ethology, and acoustics. Non-invasive techniques reduce the probability of introducing bias to results (especially when measuring stress responses) and help maintain the well-being of the subjects of the study. While the study of captive populations of cetaceans introduces its own biases (Gaskin, 1982; Mainardi, 1997), it also provides opportunities to conduct time-series of detailed behavioural and physiological observations that contribute to the understanding of their biology.

Pregnancy is an orderly, coordinated progression of biological and behavioural factors that lead animals to reproduce successfully. Weaknesses or disruptions at any point of the process can cause reproductive failure such as abortion, stillbirth or premature birth (Geraci & Lounsbury, 2002). This paper reports research from long term studies of hormones levels and respiratory rate in female *Tursiops truncatus* before, during and after pregnancy.

Hormones influence behaviour, and are also influenced by behaviour. Monitoring their levels can therefore provide insights into the mechanistic aspects of behaviour (Koren et al., 2002). Hormones such as progesterone (Ozharovskaya, 1990; Sawyer-Steffan, Kirby, & Gilmartin, 1983; Schroeder, 1990; Schroeder & Keller, 1989; West et al., 2000; Williamson, Gales, & Lister, 1990) and cortisol (Elsner, 2002; Medway, Geraci, & Klein, 1970; Mostl & Palme, 2002; St. Aubin, 2001; St. Aubin, Ridgway, Wells, & Rhinehart, 1996; Suzuki, Tobayama, Katsumata, Yoshioka, & Aida, 1998; Thompson & Geraci, 1986; Wasser, Monfort, Southers, & Wildt, 1994) in cetaceans are usually extracted from blood samples or from saliva, urine and feces. We hypothesized that an alternative source may be found in expired air from the blowhole, which can be collected non-invasively. Specific aims of this part of our research were a) to set up and validate the use of progesterone and cortisol dosage procedures using blowhole samples, b) to compare progesterone concentration during estrous, pregnancy and lactation, and c) to evaluate if non-invasive blowhole sampling could represent a reliable alternative to blood sampling for detecting stress cortisol responses. Cortisol concentration is a strong indicator of stress in many types of animals (Moberg, 2000; Schreck, 2000; Suzuki et al., 2003).

The first step in evaluating the reliability of blowhole expirations for sampling progesterone and cortisol was determining if they were significantly corresponded to blood serum levels.

Considering the crucial relationships existing between hormones and behaviour, with endocrine mechanisms able to affect the expression of behaviour and vice versa (Koren et al., 2002), the second step was to test for correlation between these two aspects.

The development of young dolphins is marked by a progressive gain of independence from the mother correlated with an expansion of the calf's behavioural repertoire (Miles & Herzing, 2003), determinant for both calf survivorship and adult skills acquisition. Observations of infant bottlenose dolphins indicated that motor coordination improves with age (Mann & Smuts, 1999), expanding their potential behavioural capacity.

According to Mann and Smuts (1999), the changes correlated with the infant development are grouped in two categories: a) changes in early physical characteristics; b) changes correlated with the development of muscular system, such as motor development, developmental changes in spatial relations and mother-infant proximity and maternal and infant activity budgets, including how mothers and infants coordinate their behaviour.

To secure the transition from dependence to independence, marine mammal offspring must acquire, for instance, necessary survival foraging skills, developed by learning or experience (Mann, 2002). This part of our research quantitatively investigated, on a weekly basis, when and in what circumstances the calves showed new behavioural elements carrying on this enhancing process.

As for the study carried out on acoustic behaviour, the aim was to study the possibility of using acoustic monitoring for assessing the changes in the physiological state of dolphins in three periods crucial for their own group: the pre-conception period, the pregnancy period and, the post-partum period.

In particular, regarding infant dolphins, the aims were to point out: a) the possible existence of an infant acoustic repertoire, not linked to external factors, including calves' mothers and their communities; b) the time necessary for a calf to produce, or learn to produce, well structured sonar signals; c) the role played by the mother in this developing or learning process.

## Method

### *Environment and subjects of the study*

The study was conducted in the Rimini Delfinario (Italy), in an open-air rounded pool (diameter 20 m; capacity 1300 $m^3$; area 310 $m^2$; max depth 5 m) with four underwater windows (80 × 80 cm) for visual observations below the surface. The facility, built in the 1960's, is characterized by a "one-pool" management system with no side pools. Since 1995 the dolphin group has consisted of eight bottlenose dolphins (Table 1). No individuals have been transferred from other facilities, but the size of the group was increased by four births in 1995, 1997, 2003, and 2007. Two individuals were moved to another aquarium in 2001 and 2002.

Since Rimini delphinarium houses only a few individuals, the sample size was increased for study of respiration and acoustic patterns in pregnant females and calves by including animals in three other facilities: Gardaland Palablù (Peschiera, VR, Italy), Aquatic World Delphinarium (Cattolica, RN, Italy), and Aqualand Dolphinarium (Tenerife, Spain).

**Table 1**
*Bottlenose dolphin community of Rimini Delfinario since 1995.*

| Name | Sex | Birth Date | Origin | Offspring |
|---|---|---|---|---|
| Speedy | Male | 1970 (presumed) | Adriatic Sea (Pescara, Italy) | Sole, Luna, Blue, Rocco, Lapo |
| Alfa | Female | 1979 (presumed) | Gulf of Mexico (Campeche) | Sole, Luna, Rocco, Lapo |
| Beta* | Female | 1981 (presumed) | Gulf of Mexico (Campeche) | Blue |
| Sole | Male | 03 May 1993 | Born at Rimini Delfinario | - |
| Luna | Female | 12 May 1995 | Born at Rimini Delfinario | - |
| Blue** | Female | 26 June 1997 | Born at Rimini Delfinario | - |
| Rocco | Male | 27 September 2003 | Born at Rimini Delfinario | - |
| Lapo | Male | 28 July 2007 | Born at Rimini Delfinario | - |

*Moved to other facility in the year 2001
**Moved to other facility in the year 2002

### *Measures of hormone concentration*

Fifteen samples of both blow and blood were simultaneously collected from the subject Blue between June 2005 and September 2008. Sampling was done opportunistically when blood sampling was planned for routine veterinary examinations. The dolphin was trained to blow into polypropylene sterile bottles with a screw closure with at least six expirations per sample collected. After collection, samples were stored at -20°C until assay at the laboratory. Progesterone concentration was determined by validated radioimmunoassay (RIA) (Seren, Leopold, & Bolelli, 1974; Tamanini, Giordano, Chiesa, & Seren, 1983). Cortisol was simultaneously sampled using the same procedures in 15 blow and blood samples collected from the subject Speedy, between September 2003 and June 2006.

Blow samples were used to monitor relative progesterone concentrations during the estrous cycle in three female bottlenose dolphins (Alfa, Blue and Luna). Sampling was done once a week, for approximately one year each from the year 2004 to 2007. To determine progesterone profile during pregnancy, the subject Alfa was studied for 52 weeks from August 2006 until the birth of her male calf Lapo on 28 July 2007. Blow sampling occurred every day throughout the entire gestation. The results are presented as mean weekly concentration of progesterone.

One month after the birth of her calf Lapo, blow sampling on Alfa was restarted, in order to determine the concentration of progesterone during lactation. Sampling occurred once a week from the end of August 2007 until January 2008 (19 weeks total).

Cortisol concentrations were sampled from the female Alfa for 52 weeks from August 2006, using the same methods employed for progesterone monitoring. The method required daily blow samplings throughout the entire period of gestation. The seven samples collected each week were averaged to provide the mean weekly concentration of cortisol.

### *Respiratory activity*

The rate of respiration in pregnant female dolphins was monitored on two days per week, for twelve weeks prior to parturition. Observations consisted of recording breathing rates within four time blocks in each day (0800-1059, 1100-1359, 1400-1659, 1700-1959). Within each block, a 15-

min period was randomly selected and the number of breaths recorded, as well as the apnea intervals – the exact time elapsing between two consecutive openings of the blowhole. We distinguished between "dive time" (immersion time lapse from a breath and the next one) and "roll interval" (surface time lapse from a breath and the next) (see Watson & Gaskin, 1983). The female Beta was monitored from April to 26 June 1997 when she gave birth to her first calf Blue. Alfa was studied in two different pregnancies, from July to 27 September 2003 when she delivered her calf Rocco and from May to 28 July 2007 until she gave birth to her calf Lapo.

Respiratory patterns were monitored in two male and two female healthy bottlenose dolphin calves (Luna, Blue, Rocco and Lapo, Table 1) to identify breathing trends and determine if such data could be used for monitoring the well-being of newborn dolphins. Each dolphin was observed for 15 min each hour in the 24 hr following birth. From day 2 to day 14, respiration was monitored in 15 min sessions randomly assigned within eight time intervals (0800-1059, 1100-1359, 1400-1659, 1700-1959, 2000-2259, 2300-0159, 0200-0459, 0500-0759). From the third week after birth until the 26th week, sampling was scaled back to four sessions during the day (0800-1059, 1100-1359, 1400-1659, 1700-1959).

### *Behaviour*

The behaviour of females was monitored throughout gestation. On two days per week, the day was divided into the same time blocks used for respiratory monitoring, and a one hour observation period was randomly selected within each block. The nulliparous female Beta was monitored from April to 26 June 1997 when she gave birth to her first calf Blue (12 weeks total). On the other hand, the multiparous Alfa was studied in two different pregnancies, from May to 27 September 2003 when she delivered her fourth calf Rocco (20 weeks total) and from August 2006 until she gave birth to her fifth calf Lapo on 28 July 2007 (52 weeks total).

Since 1995, a database containing a detailed classification of dolphin behaviours, both in the wild and in captivity, has been developed. In this study, a behavioural catalogue of 66 behavioural units was grouped into six main categories: “Locomotory and Postural behaviour,” “Social behaviour,” “Sexual behaviour,” “Aerial behaviour,” “Bubble behaviour” and “Play” (see Appendix 1 for details). All behaviours listed in the Appendix are distinguished as "events" (i.e., behaviour patterns of relatively short duration) or "states" (i.e., behaviour patterns of relatively long duration) (see Martin & Bateson, 1986). All states were measured in terms of both their duration and, since the onset of an activity can itself be scored as an event, their frequency. All events were recorded in terms of their frequency of occurrence per unit time.

Observations were conducted according to the "Individual follow protocol" defined by Mann (1999), with continuous recording sessions lasting one hour. Frequencies and durations of observed behaviours were recorded by means of a video camera and Observer 5.0 (Noldus) through the underwater windows, thereby limiting potential observer effects on animal behavior. The frequency of occurrence of each behavioural categories observed was averaged to a weekly value for analyses.

Behavioural data collection during the first year of calves’ lives follows the same methodology as for adult females and the same sampling design as for respiratory monitoring of calves, except that observation periods were 30 min in duration instead of 15 min, and sampling continued for 52 weeks instead of 26.

The behavioural catalogue for calves included 74 displays organized into seven main categories: “Locomotory and Postural behaviour,” “Social behaviour,” “Mother/calf related behaviour,” “Sexual behaviour,” “Aerial behaviour,” “Bubble behaviour” and “Play" (see Appendix 1 for details). Observations were carried out according to Individual Follow Protocol (Mann, 1999) with continuous recording sessions lasting 30 min. Occurrence, frequency and duration of observed behavioural elements were scored by means of a video camera and Observer 5.0 (Noldus) through underwater windows.

The Proximity Index (PI), as defined by Martin and Bateson (1986), assigned responsibility for maintenance of the bond between mother and calf. The two individuals may spend a great deal of time together. A measure of their relationship is the extent to which their proximity is due to the movements of one member of the dyad rather than the other. This is measured by counting the occasion on which one member of the dyad approaches or leaves the other while the other member remains still. The PI ranges from –1 to +1, which indicates complete responsibility of the mother or the calf, respectively, in maintaining spatial proximity. We follow Mann & Smuts (1998) in defining mother-infant associations as inferior to 2 m.

### *Acoustics*

The study was conducted from June 2002 to October 2003 during three different periods: the pre-conception period (P1: June 2002); the pregnancy period (P2: from April to September 2003); the postpartum period (P3: October and November 2003). At the time of the study, the bottlenose dolphin group at Rimini Delfinario included the adult female Alfa, the adult male Speedy and their two juvenile calves Sole (male) and Luna (female).

The signals emitted by the dolphins were collected once a month in 1 hour-long recording sessions. Signals were recorded by a Brüel and Kjær Type 8105 hydrophone, positioned 2 m below the surface and 3 m away from the pool wall, using the wide band (30 Hz – 300 kHz) channel of an analogical recorder and were analysed with a HP 54520-A digital oscilloscope. A skilled researcher observed both the monitor and the animals, paying particular attention to which of the dolphins was oriented towards the hydrophone. This observer recorded his comments into the low band (< 20 kHz) channel of the same recorder.

Because the narrow sound beam (≈ 10°) emitted from dolphin's melon in the rostral direction requires the dolphin to turn and lightly bend its head toward the hydrophone, it was possible to discriminate between signals that were emitted by different dolphins using the comments from the observer on the different dolphin postures at the moment of signal recording. Then the waveform and spectra of the signals assigned to individual dolphins were examined visually, to test whether the discrimination could be supported by differences in signal characteristics among presumed individual dolphins. Unidentified, uncertain and not well-shaped signals were discarded, and the remaining signals were processed using Matlab m-file language (Azzali, Garbati, & Impetuoso, 1998). For each session the following parameters, extracted by the set of signals of individual dolphins, were analysed:

1) the number of signals emitted by each dolphin, normalized to ten minutes: $N_{10}$ (N re 10 min). This parameter can be assumed as a measure of the acoustic activity of a dolphin.
2) the mean Gabor time width given by $T_G = \left[(t_2) - (t_1)^2\right]^{1/2}$;

   where $t_i$ are the time moments of waveform s(t): $t_i = \int_0^{\infty} t^i \frac{|s(t)|^2}{E} dt$ , and

   $E = \int_0^{+\infty} |s(t)|^2 \, dt$ is the energy in the waveform.
3) the mean Gabor bandwidth given by $B_G = \left[(f_2) - (f_1)^2\right]^{1/2}$;

   where $f_i$ are the frequency moments of the spectrum S(f): $f_i = \int_0^{\infty} f^i \frac{|S(f)|^2}{E} df$ ;
4) the mean barycentre of the frequency spectrum S(f), close to the power peak of the spectrum: $C_f$ (kHz).

The Gabor time width is linked to Gabor bandwidth: $T_G B_G \geq 1/4\pi$, with equality only if s(t) is a Gaussian pulse.

Infant dolphin acoustic signals were collected with the same recording system and methods. The subjects were the two calves Blue (born in June 1997) and Rocco (born in September 2003), and their respective mothers Beta and Alfa. In order to widen the sample size and to verify that external factors do not affect the parameters of calves' signals, two other newborns held in two different facilities were added (together with their mothers) as subjects of the research: Dafne (born in September 1992) and her mother Bonnie, housed in Aquatic World Delphinarium (Cattolica, RN, Italy), and Naù (born in October 2001) with her mother Betty, housed in Palablù of Gardaland (Verona, Italy). The data presented are based on weekly 1 hour long recording sessions carried out from birth throughout the first eight weeks after birth, and then every two weeks until the 15th week.

In each session, six adimensional statistical parameters were extracted from the set of signals emitted by each dolphin (four calves and their mothers): asymmetry coefficient ($X_1 = a_3$), kurtosis coefficient ($X_2 = a_4$), mean barycenter of the time domain waveform s(t) related to standard deviation ($X_3 = C_t / \sigma_t$), mean Gabor time width related to standard deviation ($X_4 = T_G / \sigma_T$), mean barycenter of the frequency spectrum S(f) related to standard deviation ($X_5 = C_f / \sigma_f$ ) and, mean Gabor bandwidth related to standard deviation ($X_6 = B_G / \sigma_F$). Each set of signals emitted by a dolphin during a session was represented as a data point in a space with six dimensions ($X_n$, n = 1, 2,… 6). Euclidean distances between each pair of data points were used as measures of dissimilarity (or similarity):

$$D_{i,j} = \left[ \sum_{n=1..6} (X_n^i - X_n^J)^2 \right]^{1/2},$$

where $X^i$ and $X^j$ are two different data points (e.g., two different dolphins or the same dolphin tested in two different periods), and $D_{i,j}$ is a measure of the similarity (or dissimilarity) for them (similarity = k/ $D_{i,j}$; dissimilarity = k. $D_{i,j}$ ). The nearest distances among a group of dolphins were calculated using hierarchical clustering method. Initially every data point is considered as a separate cluster. For assessing changes in the acoustic proprieties of calves and their mothers, the data were grouped in three periods and then processed. The three periods are: from the 3rd to the 8th postnatal week; from the 9th to the 12th postnatal week; from the 13th to the 15th postnatal week.

## Results

### *Hormonal levels*

Figure 1a reports progesterone concentrations measured in blood and blow while Figure 1b reveals the significant relationship existing between the two biological sources ($r = 0.55$, $p = 0.03$). Cortisol concentrations are reported in Figure 2a. Figure 2b shows the scatter plot underlining the significant relationship between blood and blow ($r = 0.89$, $p < 0.01$). Although the blow sampling expresses a relatively weak association with blood concentrations of progesterone with respect to cortisol, nonetheless, both correlations are statistically significant.

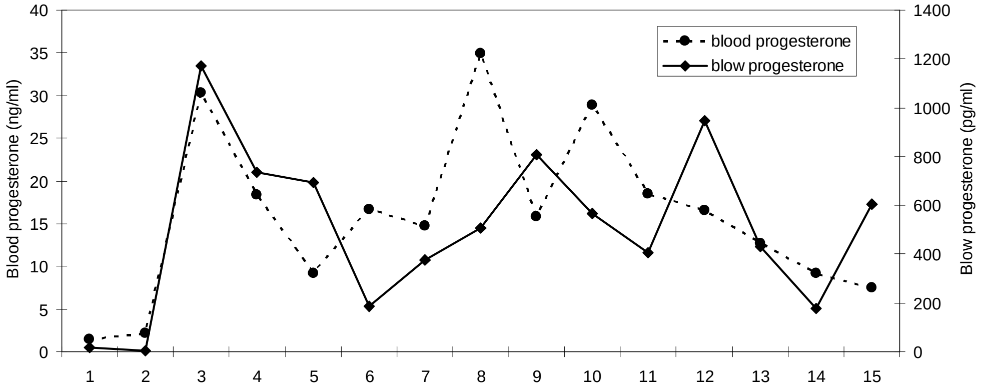

***Figure 1a.*** Progesterone concentrations simultaneously measured in blood and blow in the subject Blue.

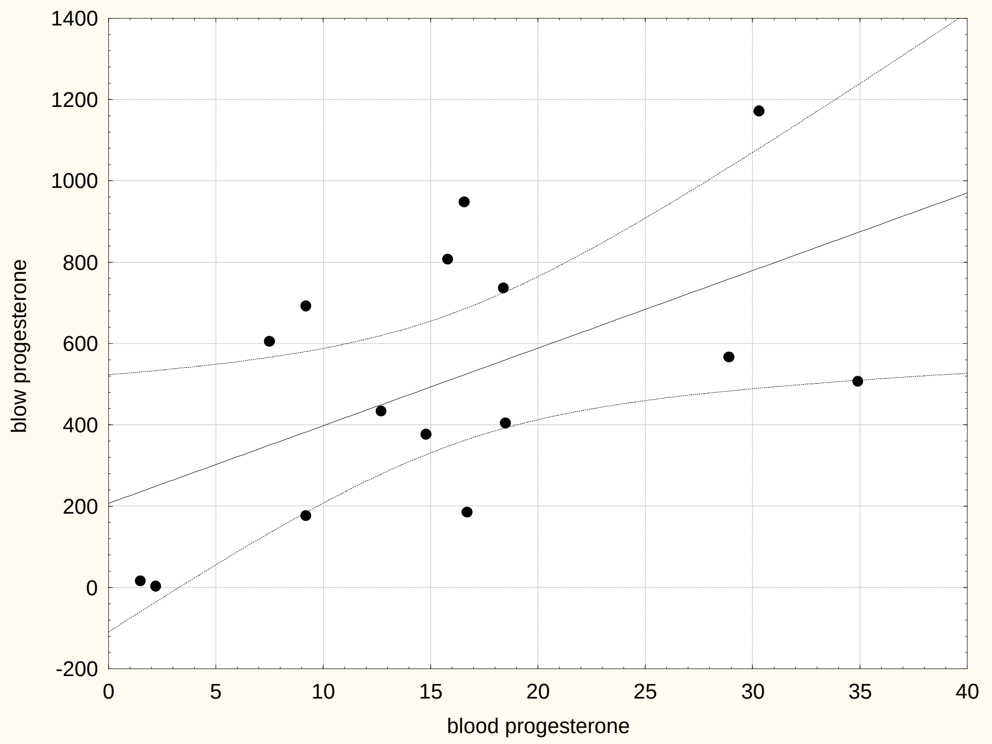

***Figure 1b.***Correlation between blood (ng/ml) and blow (pg/ml) progesterone levels in the subject Blue ($n$ = 15; y = 19.08x + 206.92; $r$ = 0.55, $p$ = 0.03). The dashed lines represent the 95% confidence interval for the mean**.**

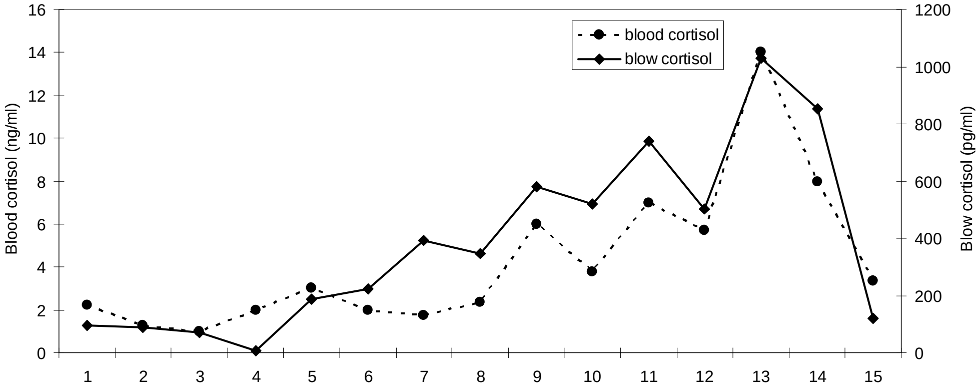

***Figure 2a.***Cortisol concentrations simultaneously measured in blood and blow in the subject Speedy.

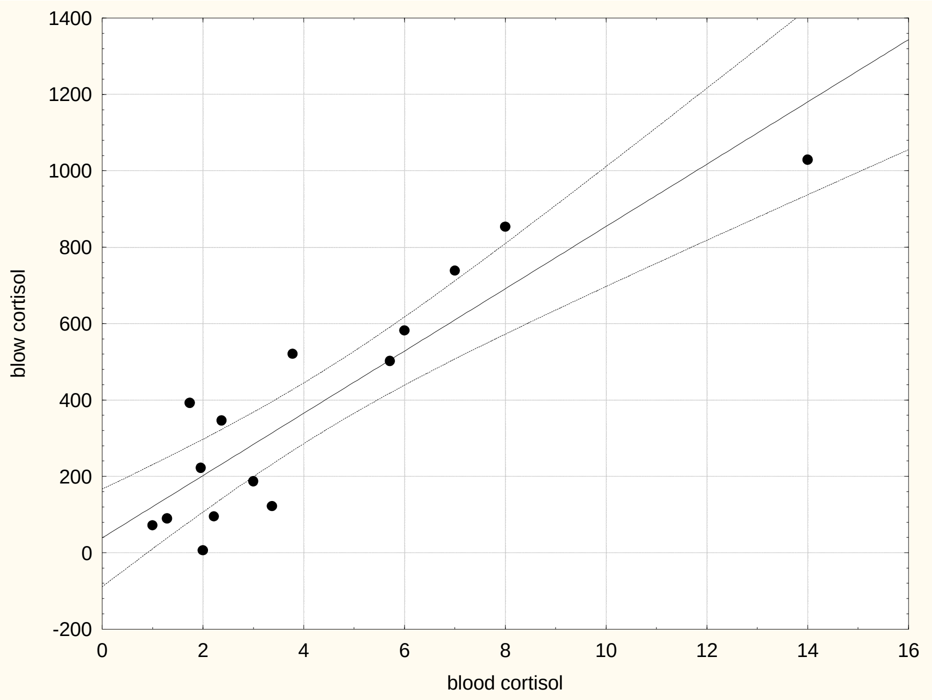

***Figure 2b.***Correlation between blood (ng/ml) and blow (pg/ml) cortisol levels in the subject Speedy (*n* = 15; y = 81.55x + 38.87; *r* = 0.09, *p* < 0.01). The dashed lines represent the 95% confidence interval for the mean**.**

The results shown in Figure 3 confirmed the seasonal nature of *Tursiops truncatus* estrous (Kirby & Ridgway, 1984; Robeck, 2000; Robeck, Curry, McBain, & Kramer, 1994), with ovulations occurring several times per year, in a prevalent period ranging from spring to mid-autumn. Progesterone concentrations revealed a mean value of 98.00 ± 95.04 pg/ml (range 18.74 – 574.83 pg/ml, *n* =

46) in Alfa, 69.66 ± 59.31 pg/ml (range 0.59 – 259.61 pg/ml, *n* = 55) in Blue, and 55.89 ± 72.26 pg/ml (range 2.38 – 380.39 pg/ml, *n* = 94) in Luna.

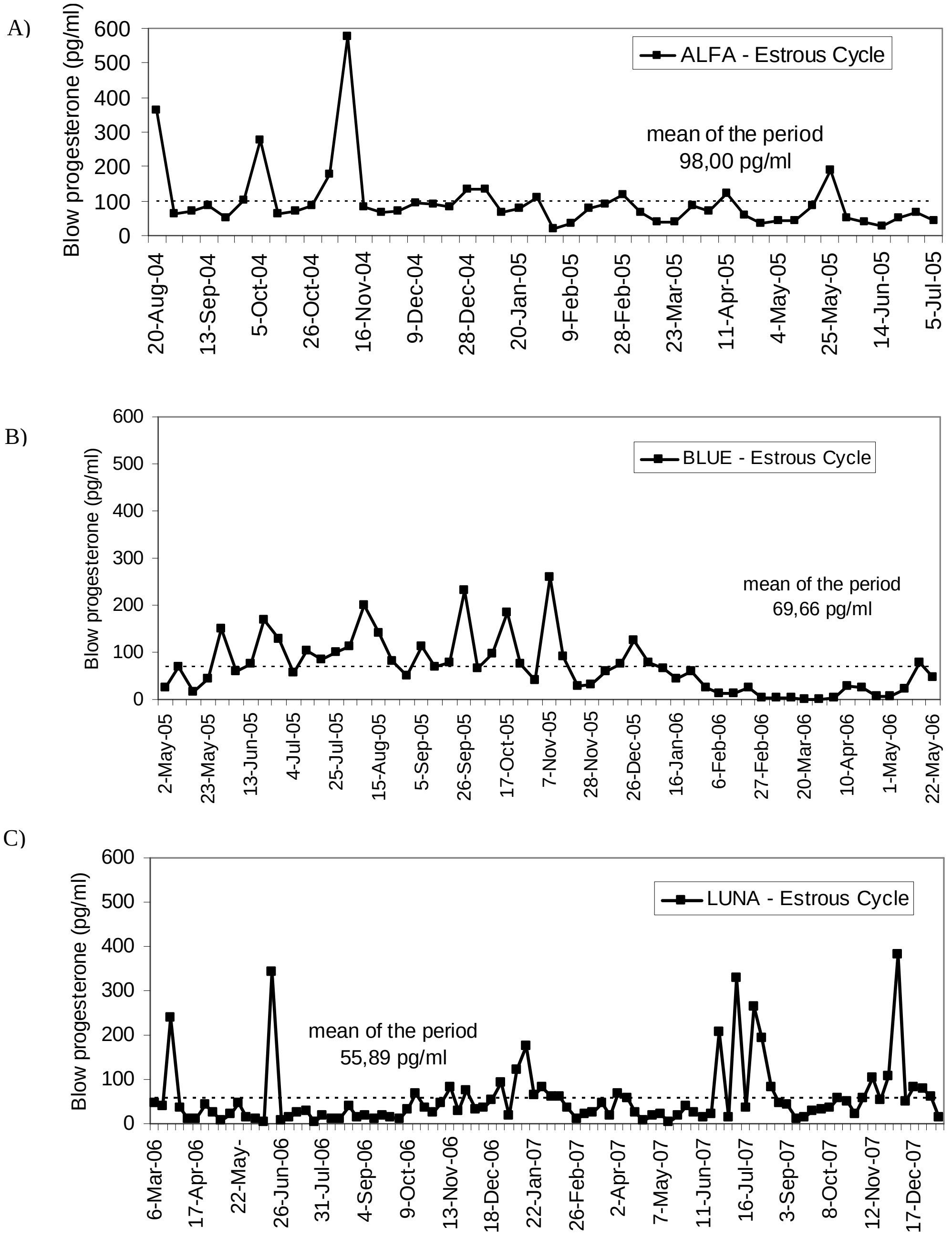

***Figure 3.*** Blow progesterone weekly levels in the three subject A) Alfa, B) Blue, and C) Luna during the estrous cycle monitoring when animals were not pregnant nor lactating.

By comparison to normal estrous (Fig. 3A), progesterone concentrations remained high during the 12 months of gestation (mean value: 201.73 ± 90.16 pg/ml, range 86.48 – 503.46 pg/ml, *n* = 52) (Fig. 4). However, as expected, during the final week, progesterone values gradually decreased as the birth date approached (trend line equation: y = -40.84x + 450.39; $R^2 = 0.75$), showing the lowest value the day before parturition (132.53 pg/ml) (Fig. 5).

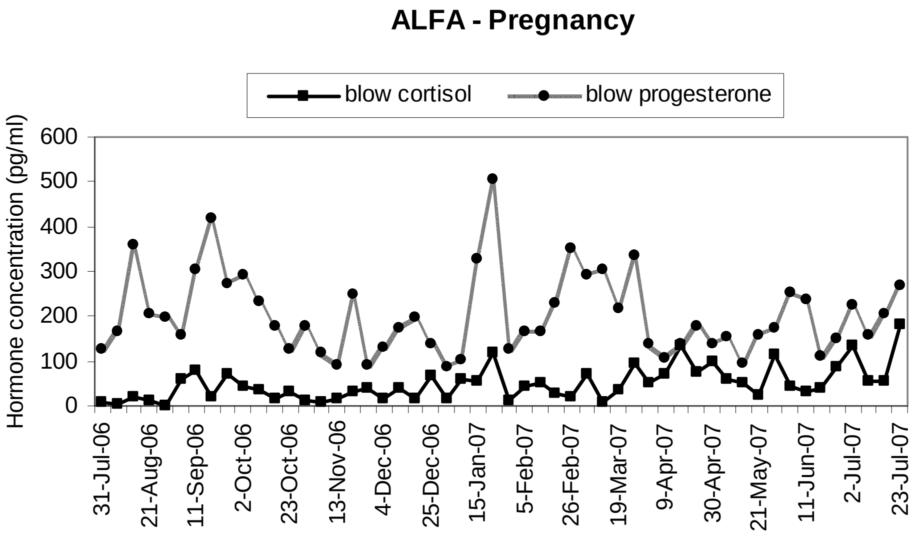

***Figure 4.*** Blow progesterone and cortisol weekly levels in the subject Alfa during the complete progression of her pregnancy in 2006-2007 (52 weeks total).

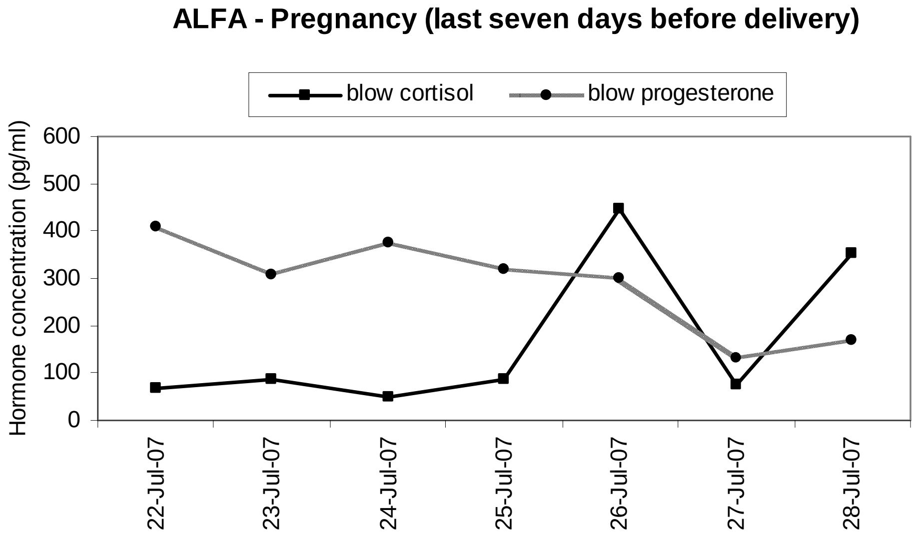

***Figure 5.*** Blow progesterone and cortisol levels in the subject Alfa during the last seven days of the pregnancy in 2006-2007. The delivery occurred in mid-afternoon on 28 July, about seven hours after the last blow sampling.

Dolphin progesterone revealed a short half-life and decreased sharply with expulsion of the fetus, as found by Schroeder & Keller (1989). Post-partum progesterone values, were lower than ever previously recorded in Alfa (mean value: 39.14 ± 33.28 pg/ml, range 0.57 – 112.42 pg/ml, *n* = 19) (Fig. 6).

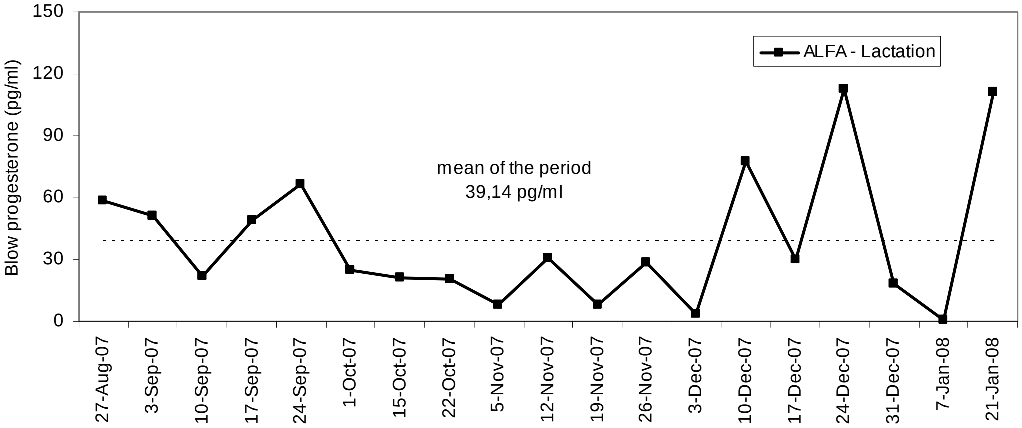

***Figure 6.*** Blow progesterone levels in the subject Alfa during the first six months of the nursing period of her calf Lapo
in 2007.

As reported in Figure 4, cortisol showed gradually increasing values (trend line equation: y = 1.3707x + 12.974; $R^2$= 0.2928), that rose from the low concentrations of the first period after conception (first week mean value: 8.08 pg/ml), up to the high mean of the last week preceding the delivery (166.42 ± 161.82 pg/ml). In particular, two days before the birth, cortisol showed its maximum peak of 446.23 pg/ml (Fig. 5), more than nine times the mean concentration of the pregnancy (49.30 ± 38.39 pg/ml, range 1.39–182.42 pg/ml, *n* = 52).

## *Respiration*

The results showed a similar trend in all the three examined pregnancies with both females Alfa and Beta gradually increasing their apnea's duration while approaching the delivery day. In particular, while the latter showed an apnea mean weekly value shifting from 26-27 to 34-35 s, in the older female Alfa the mean weekly apnea changed from 28 to 32-33 s in 2003 and from 25-26 up to 28-29 s in 2007.

The tendency of pregnancy to alter respiratory behaviour was reported by two other studies which applied the same methodology on captive bottlenose dolphins. Dallan and colleagues (2002) at Gardaland Palablù (Peschiera, VR, Italy), recorded a mean increase of apneas of 0.17 seconds per week. San Juan and Tizzi (2005), at Aqualand Dolphinarium (Tenerife, Spain), observed two females increasing their mean weekly apnea values respectively from 28 to 33-34 s (mean increase: 0.37 s per week) and from 30 to 33-34 (mean increase: 0.17 s per week), recording respiratory trends similar to the above reported results. On the other hand, a third subject at Aqualand Dolphinarium revealed nearly no changes in her respiratory habits (mean increase: 0.01 s per week) while a fourth one showed an even negative trend with decreasing values from 21-22 to 19-20 s (mean decrease: 0.39 s per week). However, the results of the last two cases appeared interesting since while the former prematurely expelled her fetus and did not positively conclude her gestation, the latter was known to be suffering from a respiratory disorder connected to asthma. Because the last two individuals were excluded for the reasons mentioned, the identical sampling procedures used in the different studies allowed us to apply an overall statistical analysis to the six successful and healthy pregnant bottlenose dolphins. A one-way Anova significantly confirmed how pregnancy development with time seems to affect females' respiratory rates, $F(11) = 2.15$, $p = 0.0320$ (see Fig. 7).

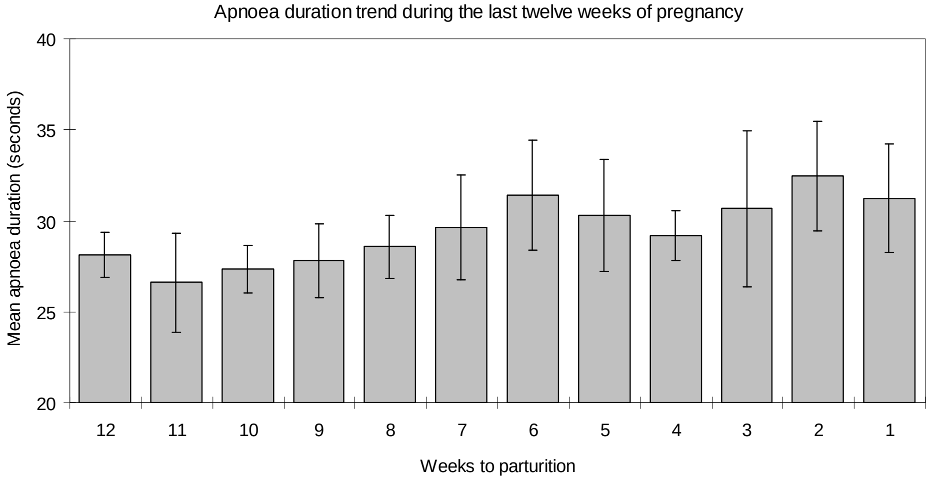

***Figure 7.*** Trend of the duration of apnea (mean ± *SD*) measured during the last twelve weeks of pregnancy in six bottlenose dolphin females who had successfully given birth to their own calves (Alfa 2003, 2007 and Beta 1997 housed in Rimini Delfinario; two females from Aqualand Dolphinarium, Tenerife, Spain, one female from Gardaland Palablù, Italy).

## *Behaviour*

Both females' behaviour changed significantly as the delivery time approached (Beta 1997: $F(11) = 3.50$, $p < 0.0001$; Alfa 2003: $F(11) = 3.47$, $p < 0.0001$; Alfa 2007: $F(11) = 6.09$, $p < 0.0001$). Since physiological responses interact with behavioural reactions and their combined study yields important insights on animals (Elsner, 2002), only behavioural results that revealed a significant correlation with hormonal profiles in terms of duration, frequency or both are herein reported.

During gestation, the female Alfa appeared to spend a considerable amount of time (> 60%) swimming with an almost constant speed and direction. However, three components of the swimming pattern were significantly correlated with progesterone: a) accelerations leading to a very fast swim ($r = -0.28$, $p < 0.05$), b) the act of proceeding through the water while maintaining an upside down position, with the belly to the surface ($r = 0.27$, $p < 0.05$), and c) swimming alone ($r = -0.33$, $p < 0.05$). Negative correlations between progesterone and typical social behaviours such as rubbing of the belly towards another individuals ($r = -0.29$, $p < 0.05$) and receiving contacts on her flank ($r = -0.29$, $p < 0.05$) were also observed. Finally, significant negative relationships with progesterone and the behaviour of exploring the pool ($r = -0.76$, $p < 0.05$) and with the emission of bubble streams ($r = -0.77$, $p < 0.05$) emerged in 2003.

Progesterone was negatively correlated with all the above-mentioned behaviours but one. Belly-up swimming — an action performed by the subject between 2 and 7 times per hour in the last twelve weeks of gestation, with single occurrences lasting about 8-10 s — increased in terms of duration with increasing progesterone. On the other hand, a decrease in progesterone concentrations appeared to coincide with an increase in the withdrawal of the pregnant female from other group members. Decrease progesterone coincided with a higher duration of solitary swimming (mean 22.6 ± 5.48 min/hr, range 3.57-33.7), and the solitary exploration of different parts of the pool (mean 0.21 ± 0.15 events/hr, range 0-0.44). However, one social interaction occurred with increased frequency only when progesterone levels went down: active rubbing interactions performed by Alfa with the belly — a rare behaviour that consists of repeated physical contacts of a very sensitive area of the body of the pregnant female rubbed towards other dolphins' bodies. This seemed to suggest a role of rubbing events for relieving discomforts of pregnancy, rather than social bonding purposes.

Episodes of both fast swimming and bubble stream emission appeared significantly reduced when progesterone levels were high, supporting the idea that progesterone may act to mitigate symptoms of anxiety (Frye, Petralia, & Rodhes, 2000; Picazo & Fernandez-Guasti, 1995). The frequency of fast swimming declined from peaks of at least one act per hour to zero (mean 0.36 ± 0.26 min/hr, range 0-1.0) while bubble streams, usually connected with whistle emissions (Dudzinski et al., 2003; Marten, Shariff, Psarakos, & White, 1996; Miles & Herzing, 2003; Pryor, 1990) and sometimes joined with excited vocalizations of the subject (Miles & Herzing, 2003), decreased from a maximum of one episode about every three hours to practically zero (mean 0.1 ± 0.11 events/hr, range 0-0.3).

Cortisol values were significantly and positively correlated with stationary positions such as resting at the surface and lying ($r = 0.70$, $p < 0.01$ and $r = 0.59$, $p < 0.05$, respectively) during Alfa’s pregnancy in 2007. In the first semester of pregnancy the average rest time was 12.4 ± 1.2 min $hr^{-1}$ (range 10.4-13.6 min $hr^{-1}$), while in the second it reached 16.6 ± 1.7 min $hr^{-1}$ (range 14.7-19.3 min $hr^{-1}$). While the mean duration of a single rest ranged between 42-46 s, in the last 4 months this state rose to 55 s per occurrence. Lying behaviour appeared almost only in the second half of gestation.

Two other behaviours previously suggested to be related to reproduction, and in particular to pregnancy (Tavolga & Essapian, 1957), had significant relationships with cortisol: flexions ($r = 0.68$, $p < 0.01$) and side arches ($r = 0.89$, $p < 0.01$). Almost completely absent in the first six months (mean frequency $hr^{-1} = 0.004 \pm 0.01$, range 0-0.03), side arches were exhibited in about one out of every two hours in the last five months before delivery. Similarly, flexions were initially quite rare (first semester: mean frequency $hr^{-1} = 0.18 \pm 0.05$, range 0.12-0.27) but they doubled their values in the second phase (second semester: mean frequency $hr^{-1} = 0.42 \pm 0.19$, range 0.23-0.67). These results pointed out that both displays involving a severe bending of the body are principally observed in the second part of pregnancy, gradually increasing in frequency while approaching parturition, $F(11) = 5.46$, $p = 0.0045$.

Dolphin calves tended to perform the longest duration of apnea soon after birth (Fig. 8) and breathing frequency increased with time. Mean apnea duration shifted from 28.09 ± 2.56 seconds (range: 24.72–30.84 s) in the first neonatal week to a predominant interval of 20-25 s in the following periods. In the early weeks, calves were usually in a strict breathing synchrony with the mother. Synchrony was defined as respiration performed by both mother and calf within a maximum range of 2 s. During the first two weeks of life, more than 80% of respirations were synchronous (first week: 91.2%; second week: 84.6%) (Fig. 9). The degree of breathing synchrony decreased with calf growth, $F(25) = 4.76$, $p < 0.0001$, $y = –1.8384x + 62.334$, $R^2 = 0.552$, such that at the end of the studied period, values of coordinated respiration between mother and infant had decreased to 20%.

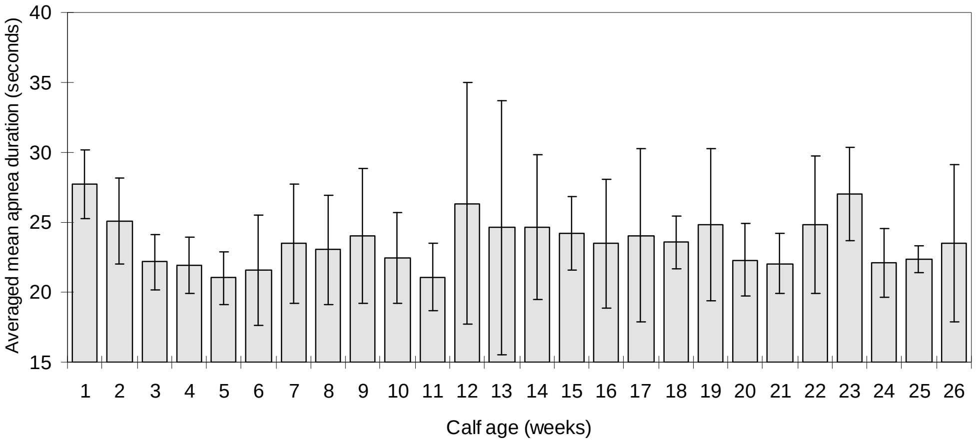

***Figure 8.*** Trend of the duration of apnea (mean ± *SD*) measured during the first twenty six weeks of life in four healthy bottlenose dolphin calves (Luna born in 1995, Blue 1997, Rocco 2003, and Lapo 2007).

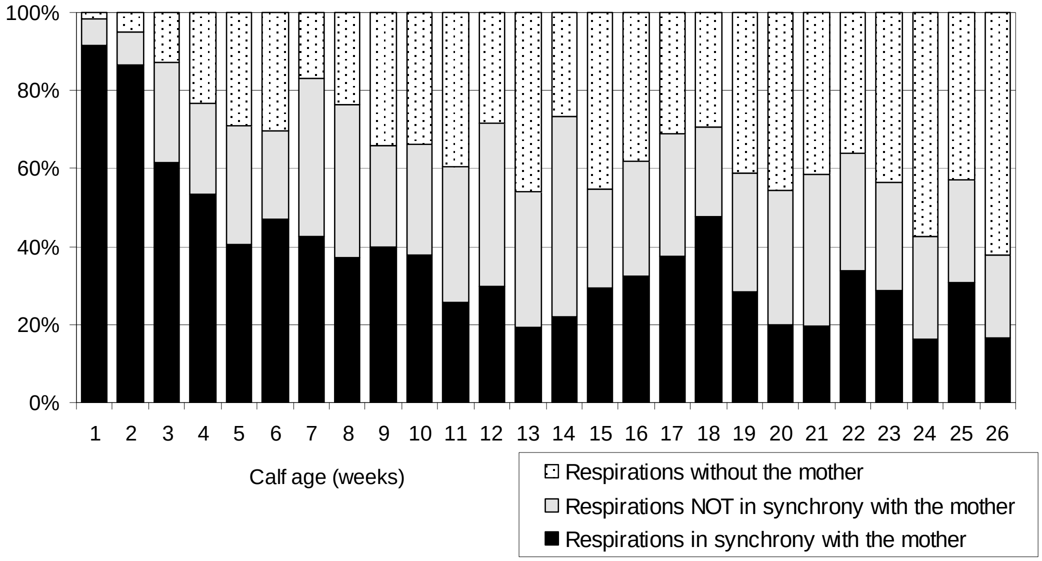

***Figure 9.*** Breathing synchrony with the mother in the four studied calves (Luna born in 1995, Blue 1997, Rocco 2003 and Lapo 2007) during the first twenty-six weeks of life.

In the calves there was no significant variations with time in the maximum duration of apneas, *F*(25) = 0.90, *p* = 60.37 (Fig. 10). With apneas of about two minutes (120.58 ± 11.00 s) performed during the first neonatal week, the studied calves exhibited average values never below 1 minute and 40 s throughout the semester.

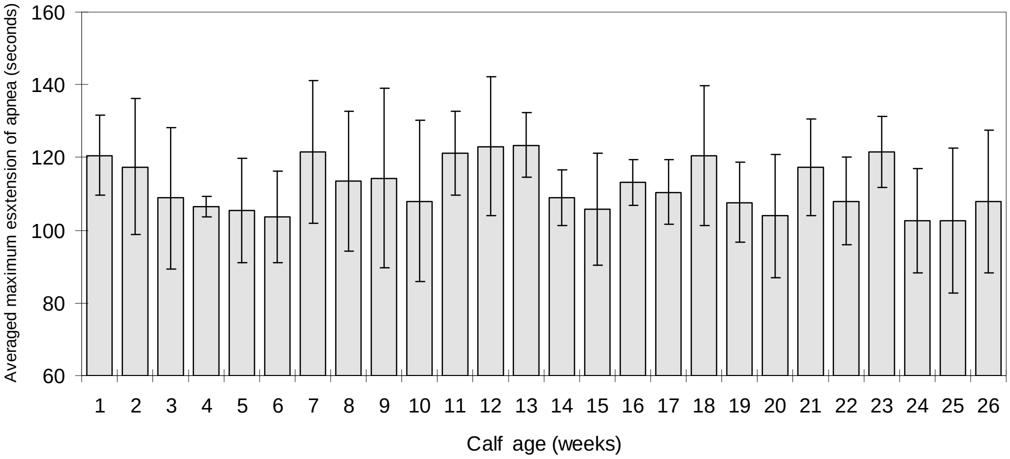

***Figure 10.*** Trend of the maximum extension of apneas (mean ± *SD*) measured during the first twenty six weeks of life in four healthy bottlenose dolphin calves (Luna born in 1995, Blue 1997, Rocco 2003 and Lapo 2007).

In the first year of life the calves appeared, as expected, to improve their behavioural repertoire with growth (Fig. 11). While no significant differences in development were pointed out between individuals, *F*(3) = 1.15, *p* = 0.33, the expansion with time of behavioural repertoire — i.e., the percentage of the 74 total display described in Appendix 1 — emerged as statistically significant, *F*(51) = 40.26, *p* < 0.0001.

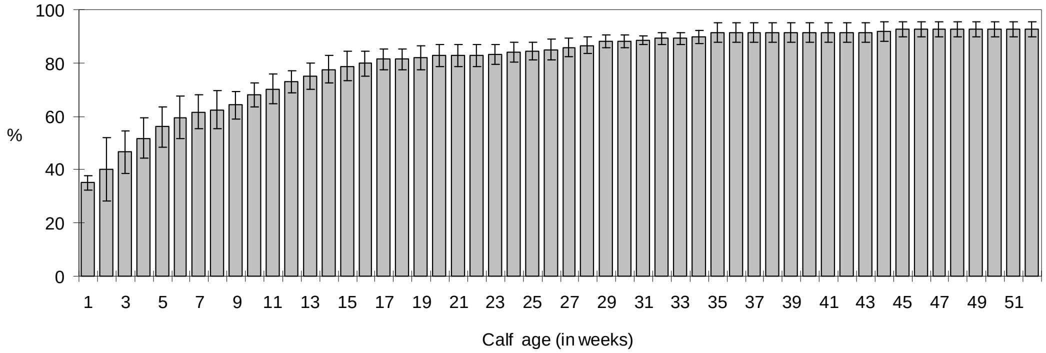

***Figure 11.*** Mean percentage of the total behavioural repertoire (74 displays presented in Appendix 1) expressed (mean ± *SD*) during the first fifty two weeks of life in four bottlenose dolphin calves (Luna born in 1995, Blue 1997, Rocco 2003, and Lapo 2007).

All infants started to perform not only displays concerning survivorship, such as correctly swimming and suckling, but added aerial and sexual behaviours in the first week of life. Leaping activity is a common characteristic of almost all Delphinids (Würsig & Würsig, 1979) with different groups exhibiting species-specific repertoires. In this study, bottlenose dolphin calves began to quasi-leap (Hui, 1989) only 2-3 days after birth. At an age of 2.7 ± 1.53 weeks of life, the newborns finally performed a complete leap with the entire body clearing the water. Genital rubbing (Herzing & Johnson, 1997; Mann & Smuts, 1998), exhibited within fifteen days of life (1.7 ± 1.15 weeks), was the first sexual activity seen.

Aggressive displays, such as direct open mouthing or rostrum hitting, emerged at 8-9 months of age, confirming that bottlenose dolphin infants were rarely involved in aggressive interactions with other members of the group during the first phase of life (Mann, 1997; Mann & Smuts, 1999). However, aggressive displays such as jaw-clap and head-jerk were never registered throughout the first year after the birth, with a clear difference with Mann and Smuts (1999) data, who reported that these behaviours did occur within the first month of life.

With the exception of bubble streaming, already emitted simultaneously with sound in the first week of life, behaviours related to the production of more complex bubbles were developed only later. Large round bubbles expelled from the blowhole were observed at 1.7 ± 1.15 weeks of age while smooth, stable rings of air that linger in the water for several seconds sometimes forming a single larger ring, were performed at 16.3 ± 11.7 weeks after birth.

The mother-calf pairs Beta-Blue (1997) and Alfa-Rocco (2003) maintained close contact during the first month of life, with over 90% of time spent together (Fig. 12). However, while the 46.75% of time was spent with the mother only, a higher proportion of time (49.60%) involved the presence of other dolphins. However, dolphins other than mothers escorted the newborns for less than two minutes per hour (2.79%) while newborns solitary swimming did not appear until the fourth week after birth, and then rarely (0.86%). During the first week infants swam rapidly with their mothers almost exclusively in echelon position (Mann & Smuts, 1999) receiving an useful hydrodynamic boost (Norris & Prescott, 1961). The calves adopted infant position only briefly, presumably just to nurse (Cockroft & Ross, 1990; Eastcott & Dickinson, 1987; Tavolga & Essapian, 1957). During the second week of life, the mothers started to tolerate further and longer separations and infants engaged in socializing and playing bouts either with their mothers or with other dolphins.

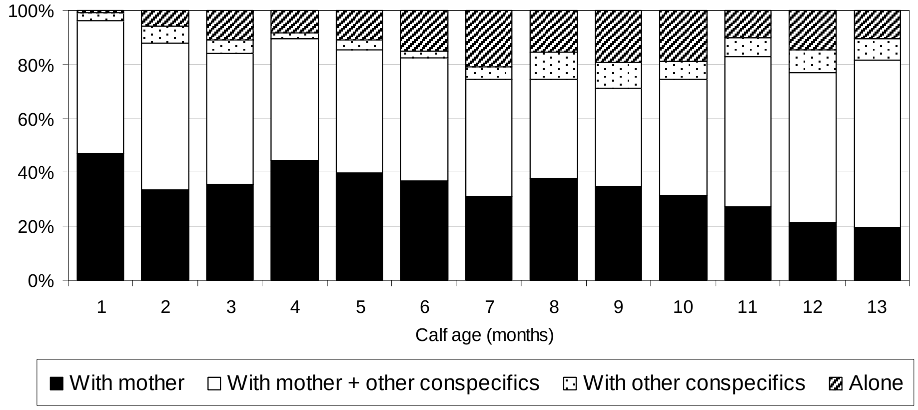

***Figure 12.*** Amount of time spent in association with the mother, with the mother together with other conspecifics, with other dolphins only, or alone measured in two mother calf pairs (Beta and Blue, 1997 and Alfa and Rocco, 2003) during the first thirteen months of calves' lives. (Since the study lasted exactly fifty-two weeks after the calves' birth, we considered a "month" as a group of four consecutive weeks).

In the neonatal phase, the Proximity Index had a negative value indicating that the mothers were primarily responsible for sustaining the maternal-calf bond (in the first month: PI = –0.261) (Fig. 13). At the end of the first year of life the time spent with the mother only (19.47%) dramatically declined with respect to the close bond at one month of age, in favour of longer periods shared with both mother and other dolphins (62.19%), with mates only (8.04%) and, finally, spent in a complete solitude (10.30%).

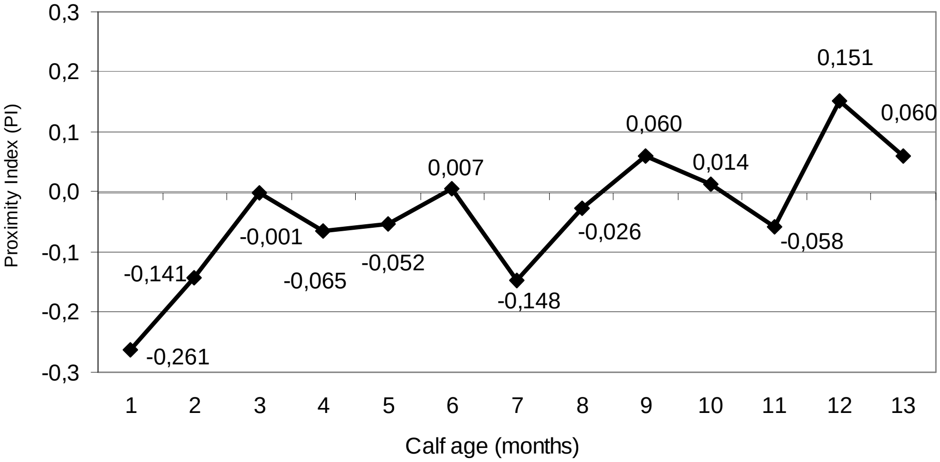

***Figure 13.*** Responsibility for proximity maintenance in two studied mother calf pairs (Beta and Blue, 1997 and Alfa and Rocco, 2003) during the first thirteen months of calves' lives. Proximity Index was calculated according to Martin & Bateson (1986). A negative PI value indicates responsibility for proximity assigned to the mother, while a positive PI value indicates responsibility of the calf.

### *Acoustics*

The female Alfa exhibited a dramatic reduction in the number of signals emitted during pregnancy relative to the acoustic activity exhibited prior to pregnancy, which appeared to be mimicked by the calves Sole and Luna (Fig. 14). The male Speedy, on the other hand, intensified his acoustic activity during gestation to a peak in June, which then declined as birth approached in September 2003 when almost no signals were produced by any of the dolphins (Fig. 14).

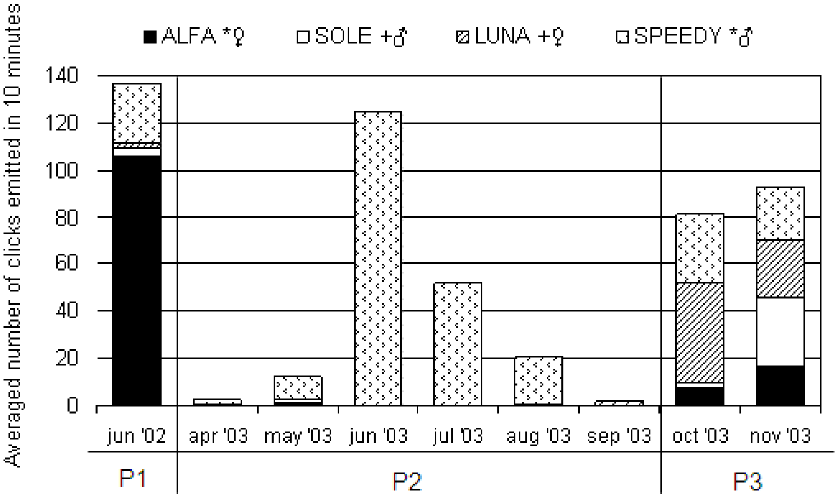

***Figure 14.*** Number of signals, normalized to ten minutes, emitted by each individual during the preconception-period (P1), the pregnancy (P2) and the post-partum period (P3) of Alfa in 2002-2003. (**: parents; +: offspring; ♂: male; ♀: female*).

The other parameters extracted from the signals emitted by the female showed a similar, if less dramatic trend, with the minimum values reached during pregnancy (P2) and increased again only after the calf birth (P3) (Table 2).

**Table 2**
*Parameters characterizing the signals emitted by Alfa during the preconception-period (P1), the pregnancy (P2) and the post-partum period (P3) in 2002-2003.*

| **Parameter** | **Preconception period (P1)** | **Pregnancy (P2)** | **Post-partum period (P3)** |
|---|---|---|---|
| Barycenter ($C_f$) | 128.80 ± 14.83 kHz | 84.09 ± 2.51 kHz | 99.44 ± 13.74 kHz |
| Bandwidth ($B_g$) | 55.84 ± 9.67 kHz | 35.24 ± 0.47 kHz | 52.83 ± 15.67 kHz |
| Duration (TG) | 6.0 ± 1.4 µs | 5.7 ± 0.046 µs | 6.9 ± 2.5 µs |
| Band Duration (TG* $B_g$) | 0.33 | 0.20 | 0.36 |

A dendrogram of acoustic dissimilarity among the eight dolphins during the 3rd to the 8th postnatal week shows two distinct clusters that separates the calves from the adults (Fig. 15). Acoustic similarity among calves is much greater than that among the mothers.

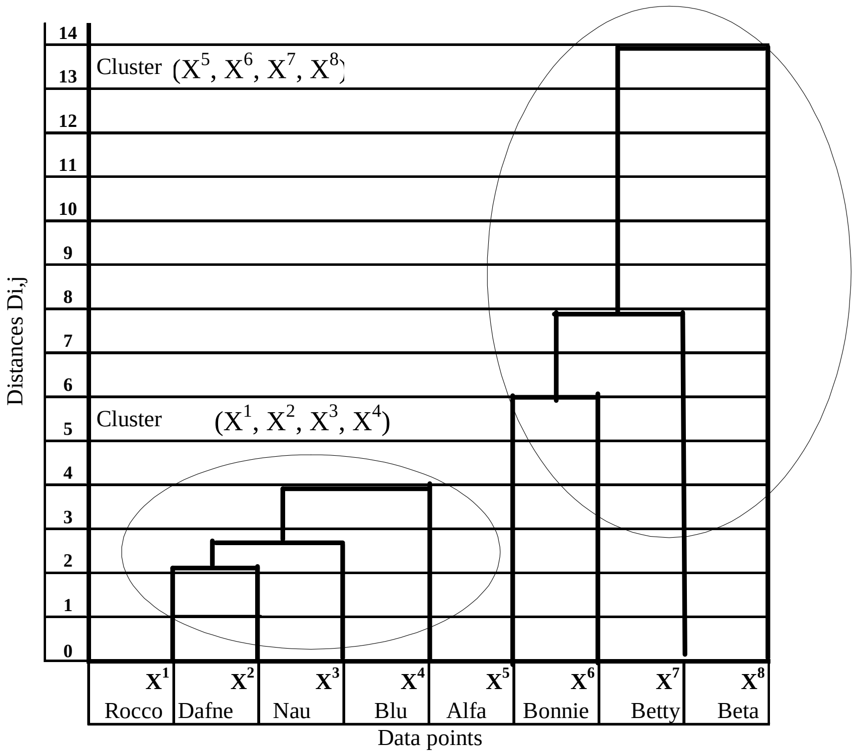

***Figure 15.*** Dendrogram for four calves, and their mothers during the 3rd to the 8th postnatal week. It shows two distinct clusters: the cluster of calves (X1, X2, X3, X4), that have dissimilarities between 2 and 4 and the cluster of their mother (X5, X6, X7, X8) that have dissimilarities between 6 and 14.

Table 3 reports the evolution of the mean dissimilarity of a calf versus all other calves in the three periods under examination. In the first period the mean dissimilarity was very low (from 2.7 of Naù to 3.4 of Blue). However, in the second period the dissimilarity increased more than three times as the acoustic repertoire evolved, and in the third period among-calf dissimilarities had increased to five times that of the first period. The acoustic dissimilarities among mothers remained constant in the three periods (from 7 of Bonnie to 10.2 of Beta).

**Table 3**

*Evolution of the acoustic dissimilarities of a calf versus the others in the three periods under examination (A). The dissimilarity of a mother versus other mothers remained constant in the entire period (B). (Data are expressed in arbitrary units) .*

## A)

| Dolphins | Rocco | | | Dafne | | | Naù | | | Blue | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | weeks | | | weeks | | | weeks | | | weeks | | |
| | 3-8 | 9-12 | 13-15 | 3-8 | 9-12 | 13-15 | 3-8 | 9-12 | 13-15 | 3-8 | 9-12 | 13-15 |
| Other calves (Dafne, Naù, Blue) | 2.90 | 10.12 | 15.30 | | | | | | | | | |
| Other calves (Rocco, Naù, Blue) | | | | 2.90 | 8.30 | 19.40 | | | | | | |
| Other calves (Rocco, Dafne, Blue) | | | | | | | 2.70 | 11.70 | 13.10 | | | |
| Other calves (Rocco, Dafne, Naù,) | | | | | | | | | | 3.40 | 10.35 | 13.10 |

## B)

| Dolphins | Alfa | Bonnie | Betty | Beta |
|---|---|---|---|---|
| | weeks | weeks | weeks | weeks |
| | 3-15 | 3-15 | 3-15 | 3-15 |
| Other mothers (Bonnie, Betty, Beta) | 7.80 | | | |
| Other mothers (Alfa, Betty, Beta) | | 7.00 | | |
| Other mothers (Alfa, Bonnie, Beta) | | | 9.70 | |
| Other mothers (Alfa, Bonnie, Betty) | | | | 10.20 |

Table 4 shows the changes in the dissimilarity of a calf versus his own mother in the three periods under examination. In the first phase the dissimilarities between mother and calf were on average almost three times greater than the dissimilarities among calves in the same period (see Table 3): they ranged from 5.4 (Rocco-Alfa) to 14 (Blue-Beta). In the second period calf mother dissimilarities decreased in average around 38%. They ranged from 2.8 (Rocco-Alfa) to 8.5 (Blue-Beta). Surprisingly, in the third period the dissimilarities increased markedly for all the calf-mother pairs, except for Blue and Beta. They ranged from 4.1 (Naù-Betty) to 18.2 (Dafne-Bonnie). On the contrary, the Blue-Beta dissimilarity decreased further to 2.8.

There was considerable variability in the time taken for calves to learn the use of sonar or develop their acoustic system. The infant Blue was the slowest: she emitted her first high frequency sounds only around the 13-15th post natal week, while Rocco, Dafne and Naù produced high frequency sounds from the 7th, 9th, and 11th week of age, respectively.

In Table 5 some features of a sample of the signals emitted by Blue before and after her 13th post natal week are reported and compared with the impulses of her mother Beta.

In the first twelve weeks of age Blue preferred to use low frequency sounds (min freq. 15 kHz; max freq. 28 kHz), with few cycles and only one peak around 25 kHz. Her mother Beta, however, generally presented a maximum power peak between 80-100 kHz, with 2-4 sub-peaks (harmonics) within a bandwidth from 35 to 120 kHz. Around the 13th and 15th week of age Blue produced more structured waveforms (bimodal). The main power of these signals fell between 40 and 80 kHz, with the frequency of maximum power between 40 and 75 kHz. In this period Beta lowered the maximum power peak of her signals to 45-75 kHz, within the bandwidth used by her calf Blue.

**Table 4**
*Changes of acoustic dissimilarity of a calf versus his/her own mother in the periods under examination. The mean dissimilarity over the entire period is given in bold type. (Data are expressed in arbitrary units).*

| Calves vs. mothers | Rocco | | | | Dafne | | | | Naù | | | | Blue | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | weeks | | | | weeks | | | | weeks | | | | weeks | | | |
| | 3-8 | 9-12 | 13-15 | **3-15** | 3-8 | 9-12 | 13-15 | **3-15** | 3-8 | 9-12 | 13-15 | **3-15** | 3-8 | 9-12 | 13-15 | **3-15** |
| | | | | | | | | | | | | | | | | |
| Mothers | | | | | | | | | | | | | | | | |
| Alfa | 5.40 | 2.80 | 7.80 | 5.30 | | | | | | | | | | | | |
| Bonnie | | | | | 6.10 | 4.20 | 18.20 | 6.3 | | | | | | | | |
| Betty | | | | | | | | | 6.20 | 4.10 | 4.80 | 5.00 | | | | |
| Beta | | | | | | | | | | | | | 14.00 | 8.50 | 28.80 | 8.90 |

**Table 5**
*Features of a sample of the signals emitted by Blue before and after the 13th post natal week compared with typical signals emitted by her mother Beta in the same periods.*

| Blue: 3-12 post natal weeks | | | | | Beta: 3-12 post partum weeks | | | | |
|---|---|---|---|---|---|---|---|---|---|
| No. | Min frequency (kHz) | Max frequency (kHz) | Freq. of Max power (kHz) | No. peaks in the spectrum | No. | Min frequency (kHz) | Max frequency (kHz) | Freq. of Max power (kHz) | No. peaks in the spectrum |
| 1 | 15 | 28 | 25 | 1 | 15 | 35-40 | 110-120 | 80-100 | 2-4 |
| 2 | 15 | 28 | 22 | 1 | | | | | |
| 3 | 15 | 28 | 22 | 3 | | | | | |
| **Blue: 13-15 post natal weeks** | | | | | **Beta: 13-15 post partum weeks** | | | | |
| No. | Min frequency (kHz) | Max frequency (kHz) | Freq. of Max power (kHz) | No. peaks in the spectrum | No. | Min frequency (kHz) | Max frequency (kHz) | Freq. of Max power (kHz) | No. peaks in the spectrum |
| 1 | 40 | 80 | 70 | 2 | 1 | 35 | 100 | 45 | 3 |
| 2 | 40 | 80 | 75 | 2 | 2 | 40 | 100 | 50 | 2 |
| 3 | 40 | 80 | 40 | 2 | 3 | 35 | 100 | 75 | 2 |
| 4 | 40 | 80 | 75 | 2 | 4 | 45 | 100 | 60 | 3 |

## Discussion

### *Hormones*

In particular, the dynamics of cortisol shifts during pregnancy, especially the increasing concentrations measured in the expectant mother in the last days before parturition, appeared analogous to other mammals under similar physiological conditions from saliva (Behringer et al., 2009) and blood (Bell, Wood, & Keller-Wood, 1991; Flisinska-Bojanowska, Komosa, & Janusz, 1991; Vivrette, 1994) sampling.

In this view, according to Hogg and colleagues (2005) and their studies on blow testosterone, the innovative technique based on blowhole sampling seemed to favour animal welfare. In fact, while the method supported the possibility to program, for even long periods, systematic and very frequent samples, the results appeared to provide reliable information on hormonal levels. The ability to detect measurable levels of hormones in the blow is probably due to their diving adaptations. In fact, as stated by Hogg and colleagues (2009) the cetacean blow should not be viewed simply as air and water, but as a matrix of organic material.

Moreover, while blood sampling is invasive and potentially painful which can cause stress to animals and in turn affect reproduction and reproductive hormone concentrations (Hogg, Vickers, & Rogers, 2005), blowhole process did not incur in the undesirable detriment of affecting the subjects with the stress of handling. Stress effects on hormones' concentration are well described by different authors (see Buholzer et al., 2004; Koren et al., 2002; St. Aubin et al., 1996).

Finally, according to Hogg and colleagues. (2009) worthwhile steps in steroid hormone concentration and profile knowledge, could increase the attempt to collect and process, with adapted methodologies, exhalations of wild cetaceans, especially whales (e.g., humpback whales, *Megaptera novaeangliae* and North Atlantic right whales, *Eubalaena glacialis*), whose breath in the form of a cloud of air with condensed water droplets, forms clearly visible sprays.

According to Ozharovskaya (1990), ovulations occurred at least 2-3 times per year, in a period ranging from the beginning of the spring to mid-autumn, with peaks during the summertime.

Similarly to what resulted for cortisol, blow progesterone concentrations fluctuated in response to maternal endocrine changes during pregnancy (Suzuki et al., 2003). To summarize, the results appeared similar to those obtained in the same species and in other mammals under similar physiological conditions from blood serum and milk (Atkinson, 1999; Katsumata, 2010; Schroeder & Keller, 1990; West et al., 2000), with a constant secretion of progesterone essential to maintaining the pregnancy. Schroeder and Keller (1989), in particular, used radioimmunoassay of serum progesterone to determine pregnancy and used as positive diagnosis of pregnancy increased progesterone levels ranging between 3-55 ng/ml, sustained over two months.

The period of anestrous occurring during the nursing season following the delivery was considered a suckling induced anestrous, due to suppression of hypothalamic-pituitary-ovarian function (Katsumata, 2010).

### ***Respiratory activity***

Little work has been done on respiratory changes that occur during pregnancy. Joseph and colleagues (1999) reported breathing was generally irregular during parturition, while Williams and colleagues (1992) suggested how the approach of the delivery seems to lead the females to save as much as energy possible in their movements, in order to maintain both cardiac and respiratory rhythm similar to the levels recorded during resting phases. Therefore, our hypothesis is that increases in mean dive time and the consequent low respiratory rate of the last weeks immediately before parturition seemed to be connected to a progressive tiredness related to the metabolic changes consistent with the pregnant females' conditions.

According to Mann and Smuts (1999), during the first days of life newborns were usually observed exhibiting typical chin-up surfacing, followed by jaw slapping down against the water surface. Moreover, it was not rare to note newborns remaining at the surface more than their mothers and sometimes breath more frequently.

These data underlined how, even from a respiratory point of view, in the youngest stages of life a biologically correct relationship with parents appears essential for the survival of calves and for the developmental changes leading them to maturity. In effect, according to Miles and Herzing (2003) coordination of activity including travelling, nursing, and feeding periods between the mother and her calf is critical to survival since the calf remains dependent on its mother for survival for an extensive period of time.

As stated by Mann and Smuts (1999), this decline in respiratory synchrony may reflect the fact that the infant has achieved respiration and diving competence sufficient to guide its own surfacing and diving patterns, independent of the mother.

### *Behaviour*

Our observations show that the activity levels of pregnant dolphins decreased as pregnancy proceeded, and flexions and arching behaviours generally appeared in the second half of the gestation period. The increase in resting and lying phases could be due to the pregnant dolphin getting gradually tired and heavy, or to try and save energy for delivery. As observed in other studies, a dramatic decrease of time dedicated to rest is expected when the birth of the calf takes place (Sekigushi & Koshima, 2003).

Body contractions did not appear as certain indication of imminent delivery, or at least it is not a behaviour indicative of imminent parturition in all individuals (Kaunitz, 2002; Lockwood, 2002). In the subject Alfa, an experienced female having had many viable pregnancies, flexions and arches seemed to remain constant for a long period (lasting several months) and the highest incidence was not necessarily in the last period before delivery. Increased cortisol levels might therefore be linked to general distress resulting from fetus mass and movements.

In agreement with Connor and colleagues (2000), Mann (1997) and Mann and Smuts (1999), genital rubbing presented very high frequencies since the early stages of life, in particular in males whose penis was clearly visible during rubbing. Selected targets were primarily the mother and immature individuals of the group. As for the part of the body of the partner preferentially rubbed on, males seemed to prefer rubbing their genitals on the convexity of conspecifics' pectoral fins while females usually chose wider zones such as head and back. Even if Connor and colleagues (2000) and Mann and Smuts (1998) included genital rubbing among socio-sexual behaviours, this behaviour exhibited by bottlenose dolphin calves did not appear functionally direct to reproduction but through repeated affiliative touches seemed to carry especially communicative and social bonding effects.

Among bottlenose dolphins one of the strongest mutual attractions is the mother-calf relationship (Caldwell & Caldwell, 1966; Scott, Wells, & Irvine, 1990). The affiliative bond may last for years until the offspring, after a complete weaning and autonomy acquisition, join sub-adult groups. According to Wells (1990), mother and calf spend about 5.4 years together, in a range between 2 and 11 years. In this context, learning to manage spatial proximity, in terms of separations and approaches with the mother, appear to be a crucial task for newborns and absolutely essential for their growth and survival. Our aim was to comparatively investigate association patterns in bottlenose dolphin mother-calf pairs during the first year of the calf's life through systematic observations, in order to: 1) measure the amount of time spent by the calves with their mother, with other conspecifics or alone; 2) assess the responsibility for proximity maintenance from mother to calf and vice versa. In an adaptive view, all these activities could hold a decisive value with newborns progressively learning control and coordination of movements and attaining swimming, breathing and, in general, behavioural competences.

Studies on captive dolphins recognized changes in behaviour related to increased independence from as early as two weeks (Chirighin, 1987; Cockroft & Ross, 1990; Reid, Mann, Weiner, & Hecker, 1995; Tavolga & Essapian, 1957) and during the first 10 weeks of development for free-ranging dolphins (Mann & Smuts, 1999). Maternal activity, however, did not change over time, except that mothers decreased their role in maintaining proximity to their infants from the first month to the second month of infants' lives (Mann, 1997; Mann & Smuts, 1999; Miles & Herzing, 2003). This tendency found a confirmation in PI recorded throughout the first year of life where a gradual shift in responsibility for proximity maintenance from parents to offspring was shown. At six months, PI was for the first time positive ($P = 0.01$) but at twelve months it showed its maximum value of 0.151 (trend line equation of PI: $y = 0.0197x - 0.1735$; $R^2 = 0.5239$).

In the second semester, swimming and socializing with individuals other than mother as well as solitary activities acquired more consistency, confirming the expected calf independence with age. According to Mann (1997), the degree of coordination and synchronization between parent and infant declined and mothers did not appear to restrict their calves such as they did before, although they were likely to influence the behaviour of their young.

In summary, with the worthwhile addition of a punctual quantification of the phenomenon, the results seemed perfectly agree with the parent-offspring conflict theory (Trivers, 1974), confirming how the mother-calf relationship results based on a complex and intense system balanced between attractive impulses that stabilize the pair and repulsive forces stretching it up to a separation.

### ***Acoustics***

The use of the orientation and the scanning movements of dolphin's rostrum, as a criterion to assign signals to an individual dolphin, is an uncertain method. However, if dolphins under observations are few (e.g., four in Rimini community), and the observer is skilled and remains the same during the entire research period, as it occurred in this experiment, then we can expect that on average the inaccuracies of the method are circumvented. The visual analysis of the recorded signals indicated that waveforms and spectra emitted, presumably by the same dolphin, can change considerably also during the same session. Therefore distinguishing dolphins from the features of their signals is perhaps a more difficult and uncertain task than using observer's comments. Only the adult male Speedy produced signals that for amplitude (by far the highest peak to peak source levels of the community) and shape, were clearly recognizable from those of the other three dolphins.

The research revealed how much pregnancy can consistently affect the acoustic activity of both the pregnant female and her group. In the last six months of pregnancy the signals emitted in a ten minute period decreased more than 100 times with respect to the preconception period, and only in the post-gestation phase began again increasing.

The preconception period could be considered a “silence” period for the Rimini community (mean $N_{10} < 0.25$), with the exception of the adult male Speedy whose signals became predominant and perhaps played a role of "sentinel" for the group.

The number of information-bearing parameters in the echoes increases with the width of the frequency band ($B_G$): broad bandwidths can have a high number of discernible highlights in the echoes than narrow bandwidths. Therefore, the parameter $B_G$ can be considered as an indicator of the expected information content of a returned echo. In the last six months of her pregnancy, Alfa decreased her $B_G$ by about 37% compared to the preconception period, and only in the post-gestation phase did she begin to increase the signal bandwidth and hence the potential information content of the related echoes.

The mean barycentre of the frequency spectrum ($C_f$), that is very near to the frequency of maximum power of the spectrum, represents the group of fundamental frequencies that give the signal its own characteristic “intonation”. This parameter, like $B_G$, decreased by about 35% in the preconception period respect both pre and post conception periods.

Similar behaviour, with small differences perhaps due to social factors, were found in two other studies on a pregnant female at Palablu Delphinarium of Gardaland (VR, Italy) in 2001 and 2005 (Biagiotti, Catacchio, Simoni, & Azzali, 2006; Ricciardo, Azzali, & Manoukian, 2003).

The dolphin calves’ very limited repertoire of high frequency sounds during their first month of life (Lindhard, 1988) was confirmed by the study. However, calves aged between 3-8 weeks began to evolve their own acoustic repertoire, largely independently of external and genetic factors, as the acoustic apparatus developed.

From the third month of life the acoustic dissimilarities among calves, even belonging to the same community (e.g., Rocco and Blue), began to increase very quickly. Moreover, bottlenose dolphins aged between 3 and 5 weeks, appeared to produce little modulated, poorly structured, low frequency sounds.

The time necessary to produce well shaped high frequency signals seemed to vary among individuals. The male Rocco needed around 7 weeks while the females Dafne, Naù and Blue required 9.11 and more than 13 weeks, respectively. The emission of signals from a calf should be related to its departures from his mother, however, it remains still unclear at what age calves intentionally produce signals, even poorly structured, to obtain information on their environment.

At first, the acoustic mother-calf dissimilarities decreased with calf growth, with the calf perhaps using the mother’s signals as a model for developing its own. Subsequent divergence in signal similarity may be indicating the calf is finding its own “voice” and decreasing dependence on the mother. This was not the case in all pairs, however. With Blue and Beta, the acoustic dissimilarities continued to rapidly decrease till the end of the observed period (15 weeks), suggesting how the mother seemed to persist for a longer time in providing echolocation models to her offspring. In addition, when Blue was able to emit her first high frequency signals, her mother appeared to adapt some features of her typical signals to those of Blue. A similar acoustic behaviour towards her offspring was observed in Naù's mother too. Very often, without any significant difference in different communities, the mother's acoustic emissions were immediately followed by those of his offspring, as if the mother would provide to his calf a standard acoustic signal as model to imitate. However, many questions still remains unsolved on how learning, experience and natural development affect and determine an efficient use of sonar system in calves.

## Conclusions

Discussions on captive studies, in cetaceans as well as in other animal taxa, recognize two principal groups of thought. Some scientists claim the minimal value of scientific data collected in an environment where animals live in confined conditions, in an extremely different way from natural habitat. Shape and size of the pools, together with socio-psychological implications seem the main factors leading individuals to consistent behavioural changes (Gaskin, 1982; Mainardi, 1997). On the other hand, some behavioural patterns in controlled environments can represent a first assumption to the comprehension of animal behaviour in wild. In this view, reproductive behaviour and reproductive physiology, the primary focus of Rimini Delfinario studies, appear to maintain their basic peculiarities also in captivity. Moreover, according to Odell and Robeck (2002), the studies on captive cetaceans provide a unique scientific resource that allow us to document, in great detail, these specific biological aspects. As for cetaceans, with the stringent difficulties generated by marine environment, controlled conditions can provide powerful means for observing long behavioural sequences and social relationships among known individuals (Samuel & Gifford,1997). Of course, results coming from captive research must find confirmation in the wild, with scientific data compared and integrated (Lehner, 1996; Mann, 2000; Samuels & Gifford, 1997) in a manner that recognizes how both types of research (i.e., laboratory and field) are necessary to fully describe the biology of a species.

Regarding the Rimini Delfinario scientific contribution, some reliable evidence about bottlenose dolphin pregnancy and calf development could originate from the systematic plan of observations. The intense sampling effort, severely carried out for years, and the specific attention paid in both avoiding human interference and equally balancing sampling sessions within different times of the day, were connected to the constant purpose to precisely quantify behavioural, physiological and acoustic parameters. At the same time, concerns with the low statistical power possibly resulting from limited sample sizes, were addressed by using study methods repeatedly applied at every new pregnancy and birth (or even exported) with overlapping settings, in different facilities.

The innovative methodology for obtaining hormone concentration profiles through dolphin blow exhalations, provided results comparable with other terrestrial and aquatic mammals, confirming how the new technique could represent a possible alternative to traditional methods, with new scenarios even in wild. Moreover, the integration with behavioural patterns not only underlined the consistent connection existing between hormones and behaviour but also identified the specific dyad hormone/behaviour involved and their development through time. On the other hand, individual characteristics emerged in behaviour or acoustics, underlining a sort of lack of common rules, appeared raising interesting considerations. Temperament disposition and experience, as well as social context, in fact affect behavioural style in many mammal species. In particular, as for reproduction, a costly component of life, individual differences potentially important in the adaptive sense may have important evolutionary implications.

Far from providing complete answers, the multidisciplinary approach presented here was meant to represent an integrated view that highlights the importance of non-invasive sampling. Since marine mammals scientists bring different perspectives coming from their specific backgrounds, a big effort in combining approaches and sharing data seemed strongly recommended. Integrative research and novel methods will surely provide additional insights into the knowledge of reproduction and calf development in bottlenose dolphin and other complex marine mammal species.

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# Appendix 1

# Behavioural Catalogue Utilized in Studies Including Behavioural Observations on Bottlenose Dolphins Housed in Rimini Delfinario

| # | Code | Type | **Behavioural Unit** | Description and Reference |
|---|---|---|---|---|
| | | | **Locomotory and Postural Behaviours** | |
| 1 | SWM | State | **Directional swim** | One dolphin moves in a dorsal fin up position making forward progress in a single general direction. Direction changes can occur, but usually are not erratic or rapid. Body is generally extended along the long axis and swim speed is relatively stable (Miles & Herzing, 2003). |
| 2 | BUP | State | **Belly up swim** | Forward progress with the belly upward (Müller, Boutière, Weaver, & Candelon, 1998). |
| 3 | SID | State | **Side swim** | Forward progress in a 90-degree rotation from the dorsal position, orienting one pectoral fin upward and the other downward (Müller et al., 1998). |
| 4 | SRD | State | **Random swim** | Dolphins showed frequent changes in heading that sometimes appeared as a transition behaviour between other behavioural states (Constantine, Brunton, & Tennis, 2004). |
| 5 | RST | State | **Rest** | The dolphins stayed still at the surface for a long time (5 s-55 min) keeping the blowhole and the tip of dorsal fin at the surface and bending the posterior part of the body down at an angle of about 45° (Sekiguchi & Kohshima, 2003). |
| 6 | LIE | State | **Lie** | Hanging (any position but vertical) in water column or lying flat on the seafloor (Dudzinski, 1996). |
| 7 | SPY | Event | **Spy hop** | Brief vertical or near-vertical elevation of the body and head-up exposure of the foresection followed by a sinking return to the water (Müller et al., 1998). |
| 8 | FKO | Event | **Fluke out** | A dolphin hung vertically in the water, head downward, the tail and the peduncle protruding above the water (Karczmarski, Thornton, & Cockroft, 1997). |
| 9 | VST | State | **Vertical stand** | Dolphin hangs/suspends itself vertically with its head up or down in mid-water column (Miles & Herzing, 2003). |
| 10 | APP | Event | **Approach** | A directional swim oriented towards the other dolphin, after swimming alone (Miles & Herzing, 2003). |
| 11 | LEA | Event | **Leave** | A directional swim oriented away from the other dolphin, after unidirectional swim or parallel swimming (Miles & Herzing, 2003). |
| 12 | EXP | State | **Exploratory behaviour** | Scanning perpendicular/horizontal relative to bottom (Herzing, 1996). |

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|---|---|---|---|---|
| 13 | OMT | Event | **Open and close mouth** | Brief opening and closing of mouth (Östman & Folkens, 1996). |
| 14 | VAR | Event | **Ventral arch** | Dolphin body aligns in a crescent shape usually head up and perpendicular to the sea floor. Head and flukes are bent towards each other, extending the back and compressing the belly region (Miles & Herzing, 2003). |
| 15 | DAR | Event | **Dorsal arch** | Head and flukes bent outwards extending the belly region and compressing the back (Miles & Herzing, 2003). |
| 16 | SAR | Event | **Side arch** | The dolphin lifted its head while arching its tail stock and twisting it to one side (Nelson & Lien, 1994). |
| 17 | FLX | Event | **Flexion** | The fluke and caudal peduncle moved ventrally and as the flexion reached its maximum, the tail approached an angle of 45 degrees from the horizontal. Often the head also bent ventrally. At the maximum the entire body formed an arch, and the muscles are taut. Then the animal relaxed and the tail was raised dorsally (Tavolga & Essapian, 1957). |
| 18 | PJR | Event | **Pectoral jerk** | Rapid, jerky movements back and forth with, generally extended, flippers (Östman & Folkens, 1996). |
| 19 | TRU | State | **Tank rub** | They often rub their bodies against an object, floor, the bottom, the side of the basin. When swimming on the side, belly in front of the wall, they keep a contact of their flippers with the wall (Sylvestre, 1985). |
| 20 | ROL | Event | **Roll** | The body is rotated through 360° on the longitudinal axis to either side of the dolphin (Renjun, Gewalt, Neurohr, & Winkler, 1994). |
| 21 | LOO | Event | **Loop** | The orca emerging out of the water at an acute angle, brings its ventral side to the surface. It then returns into the water describing a large loop (Martinez & Klinghammer, 1978). |
| 22 | SOM | Event | **Somersault** | Tail dorsally/ventrally over head in a somersault (Östman & Folkens, 1996). |
| 23 | STP | Event | **Stop** | Suddenly stops dead in water after swimming forward (Östman & Folkens, 1996). |
| | | | **Social Behaviours** | |
| 24 | CHA | State | **Chase** | Dolphin is rapidly following a dolphin in flee position, with its head orienting towards the tail of the lead dolphin, and also can involve swimming with dorsal fin down and belly up, i.e., inverted chasing (Miles & Herzing, 2003). |
| 25 | CNT | Event | **Contact** | Any form of body contact other than pounce, usually not forceful (Slooten, 1994). |
| 26 | RUB | Event | **Rub** | One individual actively rubs a body part on another individual (Connor, Wells, Mann, & Read, 2000). |

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| 27 | MOU | Event | **Mouthing** | Activity similar to nuzzling, but took place with the mouth open. At time one animal took a flipper, fluke, head or snout of the partner between the teeth and gently closed the mouth upon it, thereby holding the part (Tavolga & Essapian, 1957). |
| 28 | NBL | Event | **Nibbling** | Nibbling at another's body, usually at the flukes (von Streit, 1995). |
| 29 | BIT | Event | **Bite** | One animal draws the open jaws across another animal's body or extremities; often leaving parallel lines (Pryor, 1990). |
| 30 | DOM | Event | **Direct open mouth** | Opened mouth directed at another; often with abrupt vertical head movement (Samuels & Gifford, 1997). |
| 31 | JCL | Event | **Jaw clap** | Dolphin opens and shuts its jaws rapidly [...] once or consecutive times. A loud clapping sound is made (Miles & Herzing, 2003). |
| 32 | BTB | State | **Belly to belly swim** | One dolphin swims parallel to another, belly to belly, with or without contact and simply mirroring the other dolphin's movement (Miles & Herzing, 2003). |
| 33 | BND | State | **Bonding** | One dolphin rests its pectoral fin against the flank of another dolphin, behind the other dolphin's pectoral fin and below or just posterior to the dorsal fin (Connor,Mann, & Watson-Capps, 2006). |
| 34 | BGP | Event | **Beak-genital propulsion** | An animal invites a second animal to come up from below it and place the tip of its rostrum in its genital slit. The lower animal often turns partially on its side, sometimes supporting the horizontal flukes of the upper animal from its head to its outstretched pectoral flippers. In this position, the lower animal, its tail bent down slightly from the horizontal, propels the upper animal forward, only breaking from the pattern to rise for breaths of air. Sometimes it swims belly-up or dorsum-up during such propulsion (Johnson & Norris, 1994). |
| 35 | PUS | State | **Push** | Pushing body with its melon, rostrum, pectoral fins and body (Dudzinski et al., 2003). |
| 36 | SQU | Event | **Squeeze** | Swim in between 2 or more animals, pushing them apart (Östman & Folkens, 1996). |
| 37 | BSL | Event | **Body slam** | A charging dolphin slams into another with any part of its body other than its rostrum, peduncle and tail, fins and pecs (Connor, 1995). |
| 38 | HLD | Event | **Hold down** | Hold another on bottom, usually by lying over the other's body (Samuels & Gifford, 1997). |
| 39 | RHT | Event | **Rostrum hit** | One dolphins hits another dolphin with its rostrum (Dudzinski, 1996). |
| 40 | THT | Event | **Tail hit** | One dolphin uses its fluke to hit another dolphin's head or body, can be done mid-water column or at the water surface (Miles & Herzing, 2003). |

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| **Mother-Calf Related Behaviours** |  |  |  |  |
| 41 | BMP | Event | **Bump** | Before suckling, the calf usually started to swim underneath the mother, with its head touching the genital region (Kastelein, Dokter, & Zwart, 1990). |
| 42 | ASK | Event | **Suckling attempts** | When there wasn't cessation of infant body movements (Peddermos et al., 1992). |
| 43 | SKL | Event | **Suckling** | Is defined as being successful when the calf having inserted its lower jaw into the mother's urogenital groove and the upper jaw being in contact with the lateral skin of the mammary gland, stiffened its neck as if bracing, with a complementary cessation of tail flexing. In this posture, the calf was riding in the vortex created through the mother's momentum. This bracing of the body indicated the starting of milk intake (Peddermos et al., 1992). |
| 44 | MLE | Event | **Milk expulsion** | She flexed her peduncle forward ventrally and expelled a large cloud of milk (Connor & Smolker, 1990). |
| 45 | NUR | Event | **Nurturant behaviour** | When the infant is carried away from danger (Mann & Smuts, 1998). |
| 46 | PAS | Event | **Passing** | One dolphin suddenly accelerates and rapidly swims directly towards and then next to (< 1 m) the infant causing the infant to veer away from another animal and follow him (Mann & Smuts, 1998). |
| 47 | CLS | Event | **Clasp** | A mother swims belly-up at the surface with the calf between the pectoral fins (Mann & Smuts, 1999). |
| 48 | TOU | Event | **Touring** | When a dependent calf repeatedly approaches and leaves (within 2 m) from an adult or adolescent animal, or the baby remains at ≤ 2m from this animal while remaining ≥,5 m from the other (Connor, 1995). |
| **Sexual Behaviours** |  |  |  |  |
| 49 | BPR | Event | **Belly presentation** | One dolphin turning its ventral surface towards another. Done by swimming on its side next to or upside-down under the other individual and within two body lengths (Slooten, 1994). |
| 50 | GIN | Event | **Genital inspection** | Includes one dolphin inspecting the genital region of a second while producing a burst pulsed sound. No physical contact is observed (Dudzinski et al., 2003). |
| 51 | ERE | Event | **Erection** | Male showing penis out genital slit (Slooten, 1994). |
| 52 | GRD | Event | **Genital rub on dolphins** | Genital rubs (on conspecifics) (Herzing & Johnson, 1997). |
| 53 | GOO | Event | **Goose** | Rostro-genital contact in which one individual moves its rostrum into the genital area of another, gently or roughly (Connor et al., 2000). |

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| 54 | FMO | Event | **Fin/Fluke mount** | Slowly progressing animals often swam with the tip of the dorsal fin or flukes of one dolphin inserted into the genital slit of its partner (Saayman, Tayler, & Bower, 1973). |
| 55 | PIN | Event | **Penis insertion** | Penis inserted into blow hole/anus of other (male or female) (Östman & Folkens, 1996). |
|  |  |  |  | **Aerial Behaviours** |
| 58 | LEP | Event | **Leap** | Entire body clears the water (any height). Exit and enter head first with venter/dorsum/side facing down (Shane, 1990). |
| 59 | QLE | Event | **Quasi-leaps** | The beak was entering the water while the tail had not yet emerged but the middle of the body was clearly above the water's surface (Hui, 1989). |
| 60 | VSL | Event | **Ventral slap** | A dolphin raises itself out of the water to at least the dorsal fin or leaps clear of the water and then slaps its belly on the water surface. May be light or hard (Connor, 1995). |
| 61 | SSL | Event | **Side slap** | The dolphin comes out of the water and returns back to water on its side. Very often, only half or two-thirds of the body length is out of the water; the tail usually remains underwater (Bel’kovich, Ivanova, Yefremenkova, Kozarovitsky, & Kharitonov, 1991). |
| 62 | DSL | Event | **Dorsal slap** | The foresection is elevated above the surface with the ventrum uppermost and dropped backward, landing noisily on the dorsum. Occasionally, the animal will emerge in a dorsal or lateral position and rotate on the long axis before dropping back. The end of the tailstock may be kicked out from under the body before landing (Müller et al., 1998). |
| 63 | HSL | Event | **Head slap** | A dolphin, on its side or belly-up, raises its head out of the water and slaps the side of its head or the rostrum on the water surface. May be light or hard (Connor, 1995). |
| 64 | PSL | Event | **Pectoral fin slap** | Slapping the pectoral fin on the water surface (Mann & Smuts, 1999). |
| 65 | TSL | Event | **Tail slap** | Flukes raised above the surface and ventral/dorsal side slapped downward, usually making a loud, percussive sound (Shane, 1990). |
| 66 | TWK | Event | **Tail-Walk** | One dolphin raises itself out of the water by more than two thirds the length of the body, furiously working the tail to remain in an upright pose for approximately ten seconds (Bel’kovich et al., 1991). |
| 58 | LEP | Event | **Leap** | Entire body clears the water (any height). Exit and enter head first with venter/dorsum/side facing down (Shane, 1990). |

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|  |  |  |  | **Bubble Related Behaviours** |
| 67 | BUB | Event | **Bubble** | Dolphins [...] expel air from their blowholes, generating large, amorphous bubbles that rise quickly to the surface (Marten et al., 1996). |
| 68 | BUR | Event | **Bubble ring** | Dolphins can blow smooth, stable rings of air that linger in the water for several seconds.[...] Dolphins puff out bubbles from their blowholes that become halos of air that expand in radius while decreasing in thickness as they rise to the surface. Sometimes can be emitted two rings in succession, which fuse together into a single, large ring (Marten et al., 1996). |
| 69 | BUS | Event | **Bubble stream** | The animals also produced a column of small air bubbles coming from their blow holes (they were either blown all at once or gradually) (Delfour & Aulagnier, 1997). |
| 70 | TBR | Event | **Tail-made bubble ring** | An energic tail-slap against the surface with a concave body arch generated a bubble curtain on the top of the tail then the dolphin accomplished a decise tail up movement with a convex body arch generating a strong floating air ring (Pace, 2000). |
| 71 | BIN | State | **Bubble interest** | Dolphin shows interest for the air bubbles […] and/or follows them to the water surface (Pace, 2000). |
| 72 | BCN | State | **Bubble contact** | Dolphin bites/passes through/pushes the bubbles (derived from the ring rupture) or the ring (Pace, 2000). |
|  |  |  |  | **Play Behaviours** |
| 73 | PLO | State | **Play object** | Transporting an object by using the outside of the beak, the flippers or the melon, passing and slightly touching an object, balancing/dribbling/catching/throwing and catching/pushing or pulling an object whit the beak, pressing it under water /rolling it on the ground by using the beak or the body and holding it in the beak while swimming (Denkinger & von Fersen, 1995). |
| 74 | PLW | State | **Play water** | The orca opens its mouth and takes in a considerable amount of water then the Orca presses the water through its teeth and lips, the water forms a fountain-like spray (Martinez & Klinghammer, 1978). |

With the aim to provide a tool able to make the identification of behaviours easier and non-ambiguous and to support quantitative research, each distinctive activity included in the above Behavioural Catalogue for bottlenose dolphin (*Tursiops truncatus*), in a controlled environment, was complemented with relevant video sequences of it.

Since, in our view, conservation requires a real effort in combining and sharing materials and data on environment and species, the final step of our project consisted in making the behavioural catalogue easily available to interested people. Therefore, catalogue and motion pictures are now free for downloading at *www.delfinariorimini.it/behavioural_catalogues.htm*.