

## Short Note

### Variations in Interspecific Behavior Throughout the Estrous Cycle of a Killer Whale (*Orcinus orca*)

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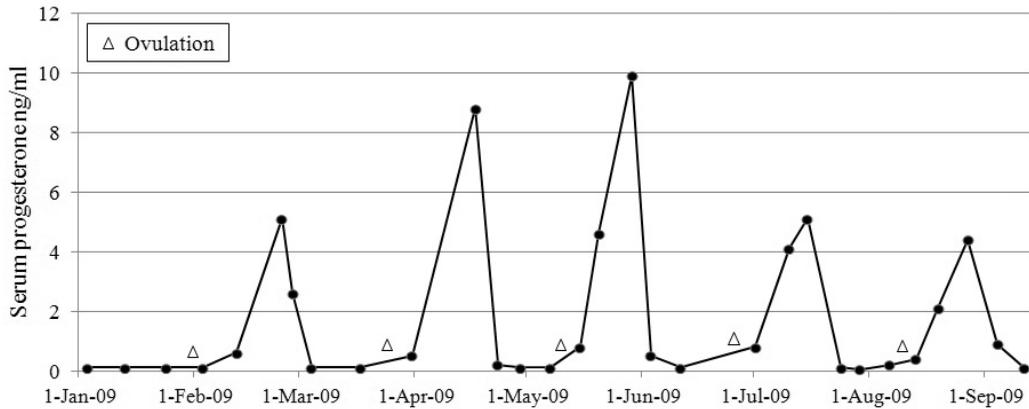
At Six Flags Discovery Kingdom in Vallejo, California, an aquarium-born, sexually mature, nulliparous female killer whale is occasionally housed with a sexually mature, male Atlantic bottlenose dolphin (*Tursiops truncatus*). The 18-y-old female killer whale is 5.0 m in length and weighs a mean of 2,131 kg, while the 24-y-old Atlantic bottlenose dolphin male (who has sired four dolphin calves) is approximately 3 m in length and weighs 190 kg. Both animals are kept in a series of four connected pools: a 7.6 m deep show pool, a 5.5 m deep middle oval pool, and two 4.6 m deep side pools. The 7.6 L of water are maintained at an average of 180° C. Although it is rare for these two delphinids (oceanic dolphins) to sexually associate in the wild (Jefferson et al., 1991), these pool mates frequently reciprocate tactile and sexual behaviors. Based on previous studies relating overt behavioral changes to the fluctuations of gonadal steroid hormones in mammals, it was hypothesized that the female's initiation of sociosexual behaviors would change throughout her estrous cycle.

All delphinids are polyestrous, spontaneous ovulators, and they breed throughout the year (Stewart & Stewart, 2002; Pomeroy, 2010). Previous studies on the reproductive endocrinology of captive killer whales have determined that there is a slight seasonal increase in birthing rates during the spring (Sawyer-Steffen et al., 1983; Walker et al., 1988; Robeck et al., 1993, 2004). On average, the estrous cycle in killer whales lasts 41 d and is comprised of a 17-d follicular phase and 21-d luteal phase (Robeck et al., 2004). The estrogen in female killer whales peaks on average 50 h before ovulation, while the LH surge, which enables ovulation to occur roughly 38 h later, occurs on average 12 h after the estrogen peak

(Robeck et al., 2004). After ovulation, increased progesterone production occurs for approximately 4 wks. During the luteal phase, progesterone concentrations remain elevated, while estrogen remains low. For nonpregnant killer whales, serum progesterone concentrations can range from 0.04 ± 13.7 ng/ml (Walker et al., 1988; Robeck et al., 1993, 2004; Duffield et al., 1995). Previous reproductive endocrinology studies have found that killer whales may exhibit long periods of anestrus (i.e., estrous cycle without ovulation) which can range from 3 to 24 mo (Robeck et al., 1993; Duffield et al., 1995).

In order to delineate the follicular and luteal phases of the female killer whale, blood serum progesterone concentrations and ovarian ultrasound images were analyzed. Blood samples were collected roughly every week and a half throughout the 8 mo which span this study. All samples were collected from an unrestrained animal trained to voluntarily present the fluke for venipuncture (i.e., Duffield et al., 1995). Using a 21 gauge needle, 12 cc of blood were extracted and subsequently stored in 5 ml plastic vials and kept in a -62.2° C freezer. Radioimmunoassays (RIA) (provided by IDEXX Laboratories, Inc<sup>®</sup>, Westbrook, ME, USA) of the serum were used to determine progesterone concentrations (Lasley, 1985; Whitten et al., 1998). Serum progesterone concentrations were considered elevated when concentration ranged from 0.8 ± 10 ng/ml; this delineated the luteal phase. The follicular phase was defined as when the progesterone decreased to a lower concentration of 0.04 ± 0.7 ng/ml (Robeck et al., 1993, 2004) (Figure 1).

Real-time B-mode ultrasound scans (Sonosite Titan<sup>®</sup> with a 2-5 MHz curvilinear transducer) were conducted once a week in order to monitor



**Figure 1.** Serum progesterone levels and ovulation dates during the study period

the growing follicles (Figure 2). During these scans, the subject was trained to lay calmly pool-side in both left and right lateral recumbent positions. Ovaries were visualized laterally in-between the rectus abdominus and hypaxialis lumborum muscles. Ovulation was determined based on follicle diameter size and lack of follicle presence in subsequent exams (Robeck et al., 2004). All procedures described were reviewed and approved by the Six Flags Inc.® Institutional Animal Care and Use Committee.

Thirty serum samples were analyzed for progesterone, and approximately 27 ultrasound scans were performed during the study period of 1 January through 11 September 2009. Five estrous cycles were recorded during the study with a mean length between ovulations of 47 d (Table 1). Sixteen serum progesterone samples were tested during the follicular phase with a mean level of  $0.159 \pm 0.123$  ng/ml. Developing and pre-ovulatory follicles were visualized during each cycle, with ovulation occurring when the follicle reached 3.5 to 4.0 cm in diameter (Figure 2a). Fourteen serum progesterone samples were tested during the luteal phases with a mean level of  $3.593 \pm 3.012$  ng/ml. Ovulated follicles and the corpus luteum were visualized during each cycle (Figure 2 b-d).

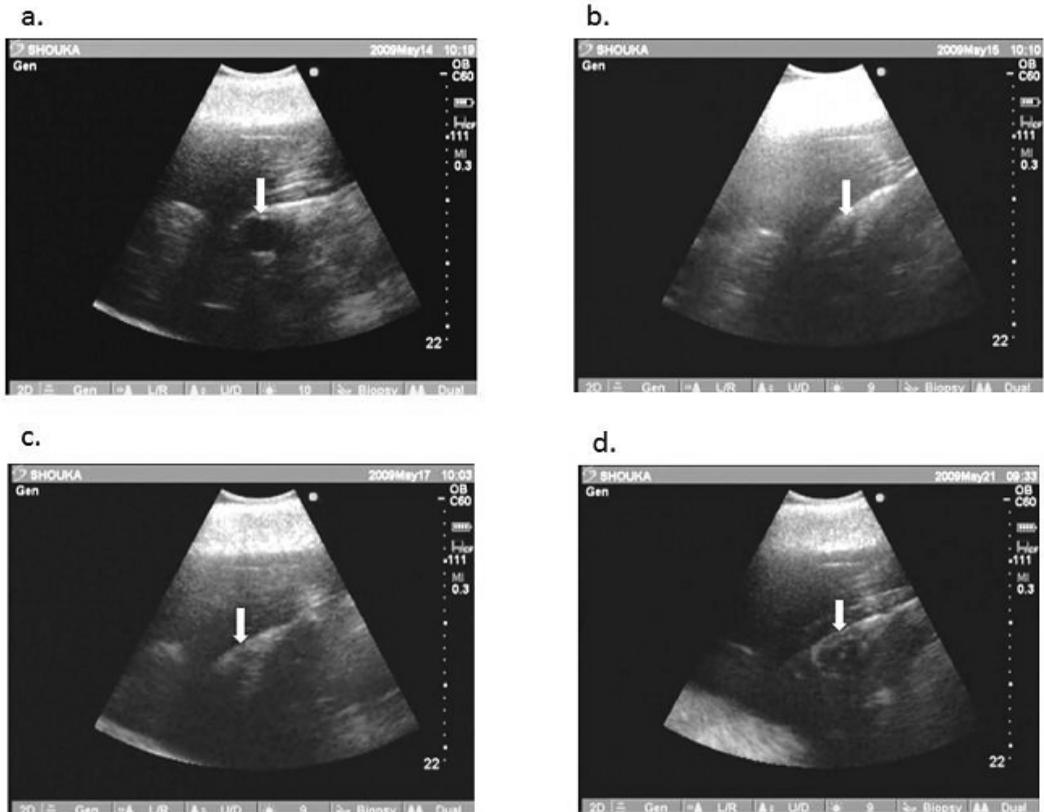
Behavioral footage was opportunistically filmed (Sony Handycam® 60× Optical Zoom 6.9 cm) by the animal care staff in continuous segments ranging from 10 to 30 min on varying days, anywhere between 0830 and 1400 h from 31 January to 21 September 2009. This resulted in approximately 30 min to 1 h of the subjects' interaction spread per week, for a total of 15 h and 15 min of video data. Given that all video was recorded without direct knowledge of the estrous cycle phase (in order to prevent bias in recording), and that the luteal phase is longer in duration than the follicular phase (Robeck et al., 2004), 21 video segments

were recorded during the luteal phase, while only 11 video segments were recorded during the follicular phase of five separate estrous cycles.

Video data was searched using an all-occurrence behavioral sampling method (Altmann, 1974) for key social behaviors: bite, chase, hit, mouth, pair swim, and tactile contact (see Table 2 for operational definitions), each instance of which was recorded in an Excel® database. When applicable, the initiator and recipient of each behavior, as well as the specific body parts involved, were recorded. In addition, the duration of each behavior, including resting behavior, during each segment was recorded in order to determine if her overall activity level changed throughout her estrous cycles.

To assess the reliability of behavioral measures, a second observer coded 42% of the total video data ( $n = 360$  min). The level of agreement between the two observers was high ( $\kappa = 0.85$ ) (Cohen, 1960). All coded behaviors were transformed into rates for each day filmed (i.e., the sum of behaviors observed divided by the number of minutes filmed for that day), as well as percent of time for that day (i.e., duration of behavior divided by total number of minutes filmed for that day). Descriptive statistics, and binomial and chi-square tests were all calculated using SPSS®, Version 15, for Windows. Effect sizes for differences in mean rates and durations were estimated using Cohen's  $d$  (Cohen, 1988).

The average rate of female-initiated tactile behavior, female to male chasing, and pair swimming was significantly higher during the luteal phase than the follicular phase (Table 3). Changing hormone concentrations did have a large effect ( $d = 1$ ) on the average rate of female chasing behavior and female-initiated tactile behavior ( $d = 0.47$ ). A statistically significant proportion (compared to the expected 72%,  $p < 0.001$ , binomial test) of female-initiated tactile contact occurred during the luteal phases of



**Figure 2.** Ovarian ultrasound; solid white arrows indicate ovarian activity (a) pre-ovulatory follicle, (b) following ovulation, (c) developing corpus luteum during the luteal phase, and (d) corpus luteum appearance during peak progesterone levels of the luteal phase.

**Table 1.** Five estrous cycles recorded based on weekly progesterone samples and ultrasound imaging

Cycle	Estimated ovulation	Luteal phase	Follicular phase	Cycle length (days)
1	31 Jan	1 Feb - 1 March	2-24 March	53
2	25 March	26 March - 22 April	23 April - 14 May	51
3	15 May	16 May - 7 June	8 June - 1 July	48
4	2 July	3-21 July	22 July - 8 Aug	38
5	9 Aug	10 Aug - 8 Sept	9-29 Sept	52

**Table 2.** Operational definitions for behavioral events recorded

Behavior	Operational definition
Bite	Opening and closing of jaw to make direct teeth to body contact
Chase	Active orienting to and swimming in pursuit of other subject
Hit	Forceful, direct body-to-body contact
Mouth	Gaping of jaws while orienting to body part of other subject
Pair swim	Subjects swim within 1 m of each other for at least 2 s; formations include side by side, echelon, follow, or push.
Regurgitation	Retrograde movement of food or fluid from the esophagus or stomach into the mouth (Lukas, 1999); attempts were deduced from protrusion of tongue by subject and undulating movement.
Rest	Subject remains at surface or underwater without active movement
Tactile contact	Brief or sustained body to body contact; specific body parts/regions and initiator/recipient identified.

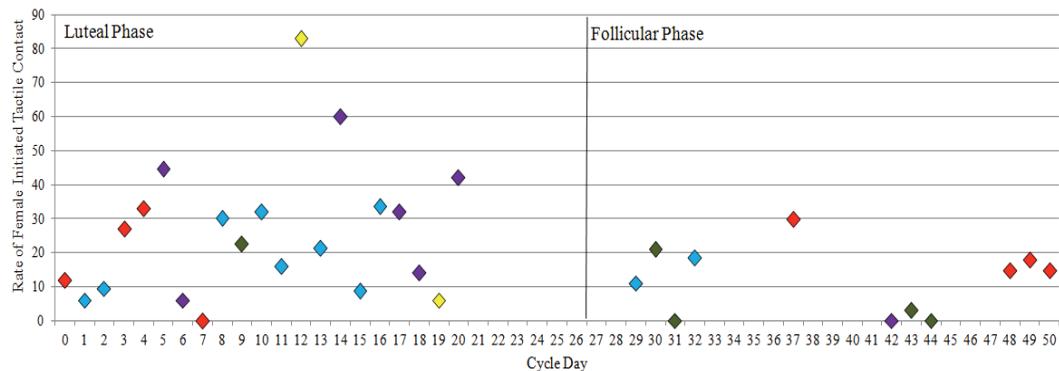
these five estrous cycles (Figure 3). In addition, the female initiated contact with her ventral side and genital region more often than expected by chance ( $\chi^2 [5, N = 96] = 20.1 p < 0.01$ ) during the luteal phases. Conversely, it appears that the estrous cycle phase had an extremely small effect ( $d = -0.08$ ) on the duration of female resting behavior, suggesting that the increase in socially proceptive behaviors is not a byproduct of an overall increase in general activity level due to a hormonal surge following ovulation. Instead, we theorize that the changes in the female's social behavior reflected her natural hormonal changes.

While examining artificial insemination techniques in ten female bottlenose dolphins, Robeck and colleagues (2005) reported that subjective

behavioral estrus was observed on average 12 to 48 h prior to ovulation. These behaviors included “displays of listing on the water surface, sinking and showing reduced responsiveness during standard training sessions” (p. 668). Previously, while examining methods to measure the killer whale estrous cycle, Robeck et al. (1993) also found that increasing estrogen during the follicular phase coincided with an increase in mating behavior. In fact, 75% of copulations observed for the six female killer whales studied occurred within 72 h of the preovulatory LH surge. Based on these observations, the fertile window for killer whales is estimated to be the 8 d preceding and 2 d following ovulation (Walker et al., 1988; Robeck et al., 1993).

**Table 3.** Average rate (#/h) and duration (% of time on tape) of female-initiated behaviors

Behaviors	Follicular phase (n = 4 h 21 min 30 s)	Luteal phase (n = 10 h 53 min 30 s)
Bite	n = 0	n = 0
Chase	n = 4 M = 0.54, SD = 1.3	n = 15 M = 2, SD = 4.7
Hit	n = 0	n = 0
Mouth	n = 0	n = 0
Regurgitate	n = 23, M = 2.5, SD = 5.5	n = 18, M = 2.5, SD = 6.9
Rest	n = 1 h 5 min 23 s M = 25%, SD = 24%	n = 2 h 30 min 18 s M = 23%, SD = 25%
Pair swim (rate)	n = 8 M = 2.3, SD = 3.3	n = 40 M = 3.2, SD = 5.3
Pair swim (duration)	n = 5 min 14 s M = 2%, SD = 4.3%	n = 22 min 13 s M = 3.4%, SD = 6.4%
Tactile (rate)	n = 12 M = 2.4, SD = 4	n = 48 M = 5.3, SD = 11.2
Tactile (duration)	n = 6 min 1 s M = 2.3%, SD = 2.1%	n = 29 min 24 s M = 4.5%, SD = 4.8%



**Figure 3.** Rate (per h) of female-initiated tactile contact throughout five separate estrous cycles (each colored marker represents a different cycle); the abscissa represents the day of each phase, with day 0 being ovulation.

There has only been one previous study that specifically examined reproductive hormones and behavioral correlates in delphinids. Wells (1984) investigated the possible correlation between the gonadal steroids in two male and two female captive Hawaiian spinner dolphins. The tactile behaviors examined in the study included genital-to-genital contact, rostrum-to-genital propulsion, other genital contact, non-genital contact, ventral presentations, and chases. The female-initiated propulsion of males (i.e., pushing their rostrums to the males' genital region) was found to be related to one female's estradiol concentrations ( $D = 0.82$ ,  $n = 8$ ,  $p < 0.01$ ), while mutual female-male ventral presentations were found to be related to the other female's estradiol concentrations ( $D = 0.67$ ,  $n = 5$ ,  $p < 0.05$ ). All other sexual behaviors were not significantly related to hormone concentrations and were, therefore, assumed to be more social than reproductive (Wells, 1984). A major shortcoming of this study was the very limited sample size; there was only one day of hormone samples from a single female which had concentrations of estradiol (i.e., above 90 pg/ml).

The female killer whale of the present study was expected to display an increase in social and tactile behaviors during the final days of her follicular phase (Walker et al., 1988; Robeck et al., 1993). However, there was not an increase in the key social behaviors recorded in the days preceding her assumed ovulation. The peak in all female-initiated social and tactile behavior occurred during the second week of her luteal phase, more than a week after the hypothesized fertile window. Research on female reproductive endocrinology has found that behaviors not related to copulation are also significantly affected by estrous cycle hormones. Female bottlenose dolphins have been shown to increase solitary swimming duration and solitary exploration of pool exhibit when progesterone concentrations are low (Tizzi et al., 2010). The female subject of the present study also showed a decrease in rate and duration of pair swimming when her progesterone concentrations were low.

Due to the great difficulty of distinguishing the social aspects of sexual behavior from its reproductive function, animal behaviorists often define these interactions as sociosexual (Hanby & Brown, 1974; Puente & Dewsbury, 1975). A behavior is considered playful if it is voluntary, spontaneous, and appears to be purposeless (Bekoff & Byers, 1981). Sociosexual behaviors merge aspects of play and mating without physical intercourse occurring. This grey area in courtship and copulatory behavior is especially evident in delphinids (oceanic dolphins) (Puente & Dewsbury, 1975). For example, all-male killer whale (*Orcinus orca*)

subgroups commonly engage in playful sexual behavior, such as physical rubbing of penile erections and nosing of genital areas (Osborne, 1986; Rose, 1992), an apparently purposeless behavior. These sociosexual behaviors are assumed to establish and reinforce strong social bonds in pods, and they may help adolescents, especially males, gain courtship skills.

A caveat to this study is its small sample size and rare interspecific pairing, meaning that the results of this study are not generalizable to all delphinids. Killer whales have been observed to attack or prey on at least 20 different species of cetaceans (i.e., whales, dolphins, and porpoises) (Jefferson et al., 1991). There are also, however, various accounts of amicable interactions between killer whales and other dolphins (Dall's porpoise [*Phocoenoides dalli*]: Jacobsen, 1986; White-beaked dolphin [*Lagenorhynchus albirostris*]: Evans, 1980; dusky dolphins [*L. obscurus*]: Hawke, 1989). In aquaria, killer whales often interact with dolphins, and many marine parks showcase interspecies performances. It is possible that prolonged exposure to other species results in social bonds between individuals of different species. For example, in 1973, Pryor reported that a trained male killer whale left his open ocean training facility and was observed socializing with Hawaiian spinner dolphins. On 15 May 1985, the first known offspring of a male false killer whale (*Pseudorca crassidens*) and a female Atlantic bottlenose dolphin was naturally bred and born at the Hawaii Sea Life Park (Carroll, 2010).

Mixed-species exhibits have become more common in zoos and aquariums in recent years (Ziegler, 2002). As a result, it is now clear that closely related species can coexist and provide social enrichment within the zoological setting (Heymann & Sicchar-Valdez, 1988; Thomas & Maruska, 1996; Wojciechowski, 2000). Despite the limitation of a sample size equal to one, this study did find overt behavioral changes in conjunction with cyclic hormone changes in a female killer whale. This suggests that future behavioral monitoring of captive animals may assist animal caretakers in identifying early signs of biological changes.

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