

Supplementary Information

Data

Jim McBain at SeaWorld San Diego assisted with the killer-whale observations, and Lev Mukhametov and the staff of the Utrish Dolphinarium assisted with the dolphin observations.

Methods

Two killer whale females (about 23 and 11 years old) lived in a pool complex (Shamu Stadium) at SeaWorld San Diego for many years. One female was caught when it was 1 year old and the other female was born in captivity. The observations were conducted during 4 months before parturition and then continued for 5 months and 1 year after parturition. During the observation period they were housed in a 6400 m³ (1,680,000 gallon) pool, which had a semicircular shape, with a maximum width of 60 m and a depth varying from 3 to 12 m, with an expansion into a 10 m x 15 m bay with a depth of 5 m. Four dolphin mothers and their calves, 2 non-pregnant females and one adult male were housed in 600 and 900 m³ rectangular pools at facilities in Russia. The group housing is similar to conditions in the wild, where cetaceans generally swim in pods. Three pairs of the dolphin females and calves were housed at the Gelendgick Dolphinarium (Russian Black Sea region) in a rectangular pool, 25m x 14m, with a depth of 1.5 m over half of the pool and a depth of 3 m over the other half and an abrupt transition from 1.5 to 3 m in the middle. The remaining female and calf pair was housed at the Utrish Marine Mammal Research Station (Russian Black Sea region) in a circular tank 13 m in diameter and 4.5 m in depth. Dolphins were kept in the pools for a minimum of 3 months before parturition.

One dolphin mother-calf pair was alone in the circular pool at Utrish during the first 2 postpartum months. The three other dolphin pairs were always housed together with 1-4 adult dolphins and one calf. The adult dolphins consisted of females and one male. We saw a significant reduction in sleep behavior in all postpartum females, and all calves showed the same pattern of minimal sleep behavior at birth. Killer whale mothers were never alone with their calves. One to three adult whales and occasionally one other calf were in the pool together with a mother and calf pair. The cetaceans swam in irregular patterns, generally around the perimeter of the pools, turning frequently to avoid walls, the bottom of the pool and the other animals. We never saw collisions between the postpartum cetaceans and the other occupants of the pool or the walls of the pool. It took 30-50 seconds for the killer whales and 20-30 seconds for the dolphins to complete one circuit of their pools.

Killer whales and dolphins were fed three times per day. Observations of eye state in dolphins were made by two observers placed at opposite sides of the pool when the dolphins surfaced to breathe. The nighttime illumination level at the pool was 500 lux during both baseline and post-parturition periods. Rest behavior was scored when dolphins and whales floated motionless at the surface or when killer whale mothers lay immobile on the bottom of the pool for at least 20 sec. Episodes of rest at the surface in killer whale females lasted up to 40 min, and episodes of rest at the bottom lasted up to 7 min. Such long episodes were rarely seen in the first few postpartum weeks. Twenty-four hour video observation was used to confirm visual observations.

Cortisol was measured by radioimmunoassay (RIA) in duplicate on 100 μ L aliquots of whale plasma. Each sample was mixed with 2 ml of acetate:hexane (3:2),

vigorously shaken in a vortex mixer for 60 sec and then flash frozen in a bath of dry ice and methanol. The organic phase was decanted directly into 12 x 75 mm disposable glass culture tubes and dried under filtered air in a water bath at 37°C. To each tube was added 500 µL of 0.1M phosphate-buffered saline (PBS), pH 7.0, 100 µL of antibody specific to cortisol (MP Biomedical, Cost Mesa, CA) diluted 1:3,000 in PBS, and 100 µL of tritiated cortisol (NEN, Boston), 10,000 cpm, in PBS. The tubes were shaken briefly on a vortex mixer then placed in a 4°C refrigerator overnight. The following morning, free cortisol was separated from the antibody-bound cortisol by the addition of 250 µL of a dextran:charcoal slurry and the mixture was allowed to sit for 30 min. Following centrifugation at 8°C for 15 min at 3000 rpm, the supernatant containing the bound fraction was decanted directly into glass scintillation vials, 5 ml scintillation fluid was added, and the radioactivity was measured in a liquid scintillation counter (Beckman LS 6500). Three different commercial human serum pools with known cortisol concentrations were extracted and run in the same assay as the whale samples, as internal controls. Prior to assaying the whale samples a number of validation steps were taken. A pool of whale plasma was diluted and run in the assay to test for parallelism to the standard curve, and recovery of steroid from plasma was tested by adding 10,000 cpm of tritiated cortisol to a pool of five plasma samples and extracting with the same solvent mix used for the experimental samples. The whale plasma dilution curve ran parallel to the standard curve, and recovery was $93 \pm 3 \%$, $n = 5$. Cortisol level was measured in 3 females during the last 6 months of their pregnancy. The average was calculated (2.6 ± 0.6 ng/ml, $n=3$). The cortisol level was also measured once in the same females during the first postpartum month and the values were averaged (3.5 ± 0.4 ng/ml, $n=3$).