Circulating Concentrations of Cortisol Encompassing Controlled Cessation of Suckling During Weaning Under Managed Care in Cow and Calf Bottlenose Dolphins (*Tursiops truncatus*)

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Abstract

The evaluation of circulating concentrations of cortisol associated with controlled cessation of suckling encompassing the weaning process in bottlenose dolphins (Tursiops truncatus) under managed care has fundamental and practical implications to enhance and improve management and welfare practices. This study involved five cow-calf pairs of which calves were 12 to 19 months at weaning and accustomed to a fish diet. Blood samples were collected from cows and calves between 0800 and 1100 h episodically for two weeks before weaning day (Day 0) and on Days 1, 3, 5, 8 12, 16, 20, and 31 post-weaning. Pre-weaning involved conditioning through positive reinforcement with respective trainers where cows moved freely from maternity to holding pens while calves remained in maternity pens. After 2 to 6 months, when cows and calves remained separated for at least several minutes without reuniting, weaning day was initiated, and gates between maternity and holding pens were closed. Physical separation resulted in cows and calves exhibiting different types and degrees of behavior dominated by increased vocalization and locomotor activities (rapid and erratic swimming, side jumping, breaching). While pre-weaning cortisol concentrations were at baseline and not different (p > p)0.1) between cows and calves, post-weaning concentrations on Day 1 increased in cows (p = 0.0045) and calves (p = 0.0001), reaching higher (p < 0.07)concentrations in calves than in cows. Thereafter, cortisol decreased to pre-weaning concentrations on Day 5 (p = 0.0031) in cows and Day 12 (p = 0.0417)in calves. While stress-like behavior post-weaning returned to pre-weaning conditions by Day 5 in cows and calves, cortisol remained slightly higher (p < 0.1) in calves than in cows until the study ended

on Day 31. Thus, although preliminary, the acute, temporal physiological and behavioral responses to the cessation of suckling encompassing weaning in dolphin cow–calf pairs are novel and provide a basis for future studies to comprehensively evaluate short- and long-term physiological and behavioral relationships associated with weaning in dolphins under managed care.

Key Words: cow-calf pairs, cortisol, cessation of suckling, weaning, bottlenose dolphin, *Tursiops truncatus*

Introduction

The bottlenose dolphin (Tursiops truncatus) is the predominant cetacean bred, born, and housed under managed care and often serves as a model species for less common species of aquarium-based cetaceans to support basic and applied research due, in part, to their accessibility, sociability, amenability to conditioning and training, and the abundance of historical data on this species. While dolphin cow-calf interactions involving growth, development, reproduction, and behavioral relationships have been studied in situ (Read et al., 1993; Mann & Smuts, 1999; Sargeant & Mann, 2009; Stanton et al., 2011; Blasi et al., 2020) and ex situ (Chirighin, 1987; Cornell et al., 1987; Peddemors et al., 1992; Sakai et al., 2013; von Streit et al., 2013), the physiological responses or adaptations encompassing artificial or controlled cessation of suckling associated with the weaning process have not been documented in cow-calf pairs under managed care.

Many definitions and models of weaning abound (Trivers, 1974; Martin, 1984, 1985, 1986; Counsilman & Lim, 1985; Lee, 1996). Generally, weaning is a progressive process involving the ontogeny of offspring from birth that leads from dependence on maternal care during infancy to independent juveniles and thriving adults. Encompassed within the weaning process, the termination or cessation of suckling is considered a milestone event. Although the transition from a liquid diet of maternal milk to solid food is progressive, the cessation of suckling in natural or controlled habitats can be gradual or abrupt and can occur for a variety of reasons in situ or ex situ. For many domestic (Enríquez et al., 2011; Lynch et al., 2019; Henry et al., 2020; Mikuš et al., 2020) or wild (Goo & Fugate, 1984; Wallis & Valentine, 2001; Li et al., 2022) mammals under managed care, cessation of suckling is done artificially or under controlled conditions to hasten a return to reproduction, enhance production, support offspring due to maternal refusal or death, advance athletic training and performance, or accommodate other behavioral or health-related concerns. Whatever the reasons, unexpected or controlled abrupt or gradual physical separation between dam and offspring during the weaning process will likely result in some degree of biological stress (Newberry & Swanson, 2008). Biological stress is a more contemporary version of the general adaptation syndrome previously proposed (Selye, 1936, 1973) where adaptive physiological responses to aversive stimuli aid in restoring homeostasis (Cannon, 1932). In general, mammals respond to acute biological stressors by way of integrated physiological processes that primarily involve the sympathetic nervous system and endocrine system via the hypothalamic-pituitaryadrenal axis (Moberg, 2000; Romero et al., 2009). Stimulated beyond a basal threshold to an acute state of homeostatic imbalance, these systems beneficially mobilize and direct sources of chemical energy to other major organs (Sapolsky et al., 2000; McEwen, 2001) to allow the animal to physiologically and behaviorally address the aversion, adapt, and regain homeostasis.

While in an acute state of homeostatic imbalance, the type and degree of physiological and behavioral changes have been quantitated and correlated to determine the status of animal well-being, with cortisol being the most common physiological indicator (Clarke & Boinski, 1995; Flow & Jacques, 1997; Waples & Gales, 2002). In marine mammals, the U.S. National Marine Fisheries Service conducted a comprehensive evaluation of tools currently used to assess biological stress and found no one tool was reliable within or among species (Curry, 1999; Pabst et al., 2002; St. Aubin, 2002). In bottlenose dolphins, attempts to correlate cortisol with behavioral characteristics have apparently not been successful (Thomson & Geraci, 1986; St. Aubin et al., 1996; Curry, 1999; Ortiz & Worthy,

2000; St. Aubin & Dierauf, 2001; Frohoff, 2004). Nonetheless, behavioral characteristics have been useful to assess stress in dolphins under managed care (Waples & Gales, 2002; Frohoff, 2004). Shortterm behavioral responses to biological stress have been postulated to include vocalizations, dive and surface swimming patterns, postures, loss of appetite, and social instability. While there is an extensive number of peer-reviewed publications on mammalian physiological and behavioral changes associated with the weaning process in various performance (horse: Henry et al., 2020), companion (dog: McMillan, 2017; Clark, 2021; cat: Martin, 1986; Ahola et al., 2017), and production (beef cattle: de Souza Teixeira et al., 2021; dairy cattle: Mikuš et al., 2020; sheep: Freitas-de-Melo et al., 2022; pigs: Campbell et al., 2013) animals, comparable peer-reviewed publications on physiological and behavioral responses to weaning in aquatic mammals under managed care are nil.

The study herein was primarily designed to quantitate the physiological response in circulating concentrations of cortisol encompassing the controlled, gradual cessation of suckling under managed care in bottlenose dolphin cow–calf pairs associated with the weaning process. Secondarily, evaluation of behavioral responses to cow–calf separation at weaning and thereafter were attempted in a qualitative manner.

Methods

Dolphins and Dolphin Management

The study involved postpartum, lactating bottlenose dolphin cows (n = 5) and related calves (n = 5). The first group of three cow–calf pairs were of lesser maturity (14, 18, and 18 y, respectively) and primiparous in relation to the second group of two cow–calf pairs which were of greater maturity (31 and 34 y, respectively) and polyparous (four and two births, respectively). Related calves from the first group were females that were 13.5 and 15 mo of age and a male at 19 mo of age at weaning. Both calves from the second group were females 12 mo of age at weaning.

Animals were housed and managed at Dolphin Discovery in Grand Cayman (19.32° N, 81.24° W) in an aquatic habitat that consisted of a semi-open enclosure with concrete pools separated by ropemesh barriers where natural seawater was constantly pumped in and exchanged at an estimated rate of 4.2 times a day. Estimated water volume was 9,309 m³ with depths ranging from 3 to 3.7 m. Lactating dolphin cows were initially prescribed an individual diet of imported frozen-thawed wild-caught fish (Atlantic herring [*Clupea haren-gus*], capelin [*Mallotus villosus*]), and squid (*llex illecebrosus*), which was provided periodically throughout the day by the trainers and served, in part, as positive reinforcement for training and conditioning. Beginning at approximately 6 mo of age, calves were gradually introduced to a prescribed diet of fish (e.g., Atlantic herring, capelin) which is in accord with calves beginning to catch fish in the wild as early as 4 mo of age (Leatherwood, 1977; Mann & Sargeant, 2003) and ingesting fish 3 to 5 mo of age under managed care (Ridgway & Benirschke, 1977; Cornell et al., 1987). Reportedly, fish represent a substantial part of a dolphin's diet by 9 to 12 mo of age (Essapian, 1953; Caldwell & Caldwell, 1972; Amundin, 1986; Chirighin, 1987; Cornell et al., 1987; Cockcroft & Ross, 1990). Subsequent to weaning, diets for each cow and calf were adjusted to control caloric intake.

Dolphins were housed and managed in compliance with the standards and guidelines of the Alliance of Marine Mammal Parks and Aquariums (AMMPA) (2010), U.S. Animal Welfare Act (AWA) (2019), and International Marine Animal Trainers' Association (IMATA) (2023). In addition, the study was approved and conducted in accord with the Institutional Animal Care and Use Committee (IACUC 15-8-023) at Ross University School of Veterinary Medicine in St. Kitts.

Weaning Process

Weaning was a gradual and staggered process for the groups of three and two cow–calf pairs such that the day of physical separation and cessation of suckling for the first group was initiated 10 d before the second group following pre-weaning conditioning.

While this study was not designed to critically evaluate cow-calf behavioral responses associated with weaning, an attempt was made to qualitatively assess the type and degree of responses at the time of physical separation and thereafter in accord with behaviors (e.g., vocalizations, dive and surfacing swimming patterns, postures, loss of appetite, social instability) previously postulated to be representative of short-term biological stress (Waples & Gales, 2002; Frohoff, 2004).

Pre-Weaning

Pre-weaning began with conditioning cow-calf pairs to voluntarily distance themselves for an extended period during daily training sessions by moving the cows from maternity pens to adjacent holding pens as shown in Figure 1. Cows of the three cow-calf group in Maternity Pen 2 (M2) were cued to voluntarily move to Holding Pen 2 (H2) for training sessions, while respective calves remained in M2 under corresponding training sessions. Initially, the cow training sessions lasted for seconds without cows breaking and returning to respective calves but, as the process continued multiple times per week, cows were expected to become more attentive, working with trainers in the absence of related calves without breaking for at least 3 min. Once achieved, trainers moved from H2 to a more distant holding pen, Holding Pen 3 (H3) (Figure 1), where the training or conditioning process was repeated. At all times during the training sessions, gates between pens remained open to allow cows or calves to voluntarily rejoin if they lost attentiveness and broke from the training sessions. Correspondingly, the pre-weaning process was repeated with cows of the two cow-calf pairs, except cows in Maternity Pen 1



Figure 1. Dolphin Discovery aquatic habitat in Grand Cayman showing the layout of maternity pens (M1 and M2), holding pens (H1, H2, H3, and H4), and the lagoon that consisted of concrete pools separated by rope-mesh barriers with a constant exchange of natural seawater and depths ranging from 3 to 3.7 m

(M1) were cued to voluntarily move to Holding Pen 1 (H1) and eventually to the more distant H2 (Figure 1), while related calves remained in M1 working with trainers.

Weaning

On the day of weaning (physical separation and cessation of suckling = Day 0), at approximately midday, cows of the three or two cow–calf pairs were cued to voluntarily swim from Maternity Pen M2 or M1 to Holding Pen H3 or H2, respectively (Figure 1), where trainers were stationed to begin their training sessions. As trainers started their sessions, the gates between the maternity and holding pens were closed, and the type and degree of behavioral responses were observed.

Post-Weaning

Subsequent to the day of weaning, individual diets were adjusted to decrease caloric intake for cows and increase intake for calves as lactation waned and suckling terminated, respectively. Depending on the degree of aversion to weaning and attentiveness to training, cows were eventually joined together with other adult dolphins in the lagoon, and calves were joined together in Maternity Pen M2 (Figure 1).

Blood Collection

Blood samples were collected periodically from cows and calves encompassing the day of weaning (Day 0). Preconditioned behavior previously done facilitated the voluntary collection of serial blood samples from animals during the first morning training sessions between 0800 and 1100 h. Trainers cued dolphins to voluntarily move into a dorsal-down or ventral-up position with flukes presented to the veterinarian. Pre-weaning samples were collected on various days for 2 wks; and post-weaning samples were collected on Days 1, 3, 5, 8, 12, 16, 20, and 31. Samples were not collected on the day of weaning due to the observation of stress-like behaviors and lack of attentiveness of animals to trainer cues. Blood was collected (approximately 10 mL) via venouspuncture of vessels of tail flukes using a 21-ga winged BD Vacutainer® Safety-Lok[™] Blood Collection Set attached to a Vacutainer® tube containing EDTA (BD Company, Franklin Lakes, NJ, USA). Subsequent to collection, samples were placed on crushed ice, transported to the laboratory, and centrifuged at approximately $1,350 \times g$ for 10 min. Thereafter, plasma (2 to 3 mL) was pipetted into Eppendorf cryotubes, labelled, and initially stored at -20°C.

Plasma samples from Grand Cayman were transported frozen to Ross University School of Veterinary Medicine where all samples were stored at -80°C until analysis. Export and import of dolphin blood plasma samples were in accord with the Convention on International Trade in Endangered Species (CITES), Appendix II.

Cortisol Analysis

Analysis of total concentrations of plasma cortisol and cortisol-like metabolites was conducted using a commercial enzyme immunoassay kit (#K003-H1/H5 Cortisol ELISA Kit; Arbor Assay, LLC, Ann Arbor, MI, USA), which was previously validated for use with dolphin plasma (Bergfelt et al., 2020). Briefly, a solvent:sample ratio of 5:1 (v/v)with 5 mL of diethyl ether and one mL of plasma were combined in a set of glass tubes, vortexed (approximately 1 min), set aside at ambient temperature for solvent:sample separation, and placed in a -80°C freezer for approximately 10 min. Subsequent to freezing, the solvent layer was decanted into a corresponding set of 2-mL conical Eppendorf tubes and evaporated using a Speedvac (CentriVap Centrifugal Vacuum Concentrator; Labconco Corp., Kansas City, MO, USA) for 2 to 3 h or until dry. Thereafter, extracted samples were resuspended initially with 5% v/v with 100% ethanol followed with an assay buffer to yield 1:1 dilution. If cortisol concentrations were near the maximum range of the standard reference curve (< 10%of specific binding), a 1:2 dilution was used in the re-analysis. Assay performance as assessed over three assays indicated intra- and inter-assay CVs of 5.6 and 7.9%, respectively, with a mean sensitivity of 45.64 pg/mL at 95% specific binding.

Statistical Analysis

Plasma concentrations of cortisol in cows and respective calves were analysed encompassing the day of weaning (Day 0) for Weeks -2 and -1 pre-weaning and Days 1, 3, 5, 8, 12, 16, 20, and 31 post-weaning. Initially, data were checked for outlying values using Dixon's outlier test and tested for normality by the Kolmogorov-Smirnov test and transformed to ranks. Statistical analyses were done using SAS/STAT[®] software (SAS, University Edition, Version 9.4M5; SAS Institute Inc., Cary, NC, USA). A proc mixed procedure with a repeated statement to minimize autocorrelation between sequential measurements and a spatial power statement were used to account for uneven days between samples. Main effects of group (cows and calves), time (weeks/days encompassing weaning), and interaction of groupby-time were determined. A significant effect of time was further analysed using one-way analysis of variance with a Least Significant Difference test to determine mean differences among weeks/ days within groups. A significant effect of group or group-by-time interaction was further analysed

using one-sided *t* tests to determine mean differences between groups within time. A probability of $p \le 0.05$ indicated that a difference was statistically significant, and a probability of p > 0.05 to $p \le 0.1$ indicated a tendency for significance. Unless otherwise indicated, data are presented as the mean ± SE.

Results

Pre-Weaning

The pre-weaning process associated with voluntary separation between cow–calf pairs during conditioning took approximately 6 mo for the first group of three cow–calf pairs and 2 mo for the second group of two cow–calf pairs.

Weaning

On the day of weaning (Day 0), cows were cued to voluntarily swim from Maternity Pen M2 or M1 to Holding Pen H3 or H2 to start midday training sessions (Figure 1). Upon entering the holding pens, the gates leading back to the maternity pens were closed. While the cows initially started their training session, they broke within 3 min from their sessions and swam back to the gates. Finding the gates closed and being unable to return to related calves, cows began exhibiting stress-like behaviors such as increased vocalization, rapid and erratic swimming, side jumping, breaching, and repeated tail slapping. Correspondingly, calves in M2 or M1 who were initially working with their trainers broke from their training sessions, swam to the gate areas, and began exhibiting similar stress-like behaviors. By the mid-afternoon training sessions, cows and calves continued to exhibit stress-like behaviors and exhibited poor attentiveness to trainers and loss of appetite. By late afternoon, the type and degree of stress-like behaviors appeared to be declining for some cows and calves, but they still continued to break before completing their training sessions.

Post-Weaning

On the day after weaning (Day 1), mid-morning training sessions with cows in the holding pens and calves in the maternity pens were initially attended with good appetite, but animals continued to break within minutes before completing their sessions, while type and degree of other stress-like behaviors appeared to decline. Over the next several days, weaning stress-like behaviors in cows and calves decreased, and appetites and attentiveness to training increased with longer sessions (20 to 30 min) such that by Day 5 postweaning, respective cows were cued to move to Holding Pens H4 and H3 (Figure 1). Over the following days, cows joined other adult dolphins

in the lagoon, while calves joined other calves in Maternity Pen M2. The lagoon and M2 were separated by a rope-mesh barrier, which allowed cows and calves more visual, auditory, and physical contact without suckling. Daily observations and training sessions continued until the study ended on Day 31.

Cortisol

Profiles of circulating concentrations of cortisol in dolphin cow–calf pairs encompassing weaning (Day 0) along with statistical results are shown in Figure 2. For approximately 2 wks pre-weaning, mean concentrations were at baseline and not different (p > 0.1) between cows and calves. However, from Week -1 to Day 1 encompassing weaning, mean cortisol concentrations increased in cows (p = 0.0045) and calves (p = 0.0001), reaching higher (p < 0.07) concentrations in calves than in cows.

Relative to Day 1 post-weaning in cows, mean cortisol concentrations decreased and were lower (p = 0.0401) on Day 3, which was followed by an additional decrease (p = 0.0031) to baseline on Day 5 (Figure 2). Thereafter, concentrations remained at baseline until Day 31.

Relative to Day 1 post-weaning in calves, mean cortisol concentrations decreased but not significantly (p > 0.1) and remained higher on Days 3 (p < 0.02), 5 (p < 0.03), and 8 (p < 0.04) compared to concentrations in cows (Figure 2). Thereafter, while cortisol decreased (p = 0.0417) to near baseline on Day 12, mean concentrations remained slightly higher (p < 0.1) in calves than in cows until Day 31.

Although not statistically analysed due, in part, to the small number of observations, a descriptive characterization of cortisol concentrations associated with the parity of cows encompassing weaning was done ad hoc. In the younger three primiparous vs two polyparous cows, mean (± SE) cortisol concentrations were $3,058.0 \pm 959.0$ vs $2,764.8 \pm$ 567.2 pg/mL, respectively, over the 2 wks of preweaning and 12,717.7 ± 4,970.9 pg/mL vs 6,273.0 ± 1,408.0 pg/mL, respectively, on Day 1 postweaning. Before and after weaning, the mean differences in cortisol were, respectively, about 10 and 50% higher in the less mature than the more mature cows. Correspondingly, an ad hoc evaluation was done to determine if the age among calves at weaning influenced changes in circulating concentrations of cortisol pre- and postweaning. Again, due in part to the small number of observations and small differences in the age ranges among calves (12 to 13.5 mo to 15 to 19 mo), there was no apparent pattern that cortisol was higher or lower among calves of different ages encompassing weaning.



Figure 2. Mean (\pm SE) plasma concentrations of cortisol associated with the cessation of suckling encompassing weaning in bottlenose dolphin (*Tursiops truncatus*) cow–calf pairs (n = 5). Weeks pre-weaning, day of weaning (Day 0), and days postweaning are presented with statistical results for main effects of group (cows vs calves), time (weeks/days encompassing weaning), and interaction of group-by-time. Asterisks (*p < 0.05 and **p < 0.1) indicate differences between cows (solid line) and calves (dashed line) within time, and superscripts (*p < 0.05 and *p < 0.05) indicate differences within cows and calves over time.

Discussion

In the present study, the five dolphin cow-calf pairs were conditioned or trained in advance of physical separation on the day of weaning to facilitate the cessation of suckling when calves reached 12 to 19 mo of age after the calves adapted to a fish diet that started at approximately 6 mo of age. The pre-weaning process involved daily training sessions where two or three calves remained in the maternity pen with trainers while cows were cued to work with their trainers in holding pens distant from the maternity pen with gates between pens remaining open during the sessions. Initially, cows or calves broke from training within seconds to rejoin one another. As the conditioning process continued, the time of separation between cow-calf pairs increased, eventually reaching several minutes, which prompted the trainers to move cows to a holding pen more distant from the maternity pen for training. Again, the daily process of conditioning separation between cowcalf pairs was repeated until cow-calf separation lasted for at least several minutes. Behaviorally, the pre-weaning process of conditioning took approximately 6 mo for the three cow-calf pairs

of which the cows were younger and primiparous compared to approximately 2 mo for the two cow-calf pairs of which the cows were older and polyparous. While the basis for an apparent 4 mo longer period of conditioning for the less mature and primiparous cows is not known and requires clarification, previous studies with production animals (Orihuela et al., 2021) and dolphins (von Streit et al., 2013) under managed care reported that adaptive, maternal experiences play a role in the care of offspring.

While the type and degree of behavioral characteristics associated with the weaning process were not the primary focus of this study, shortterm behavioral responses such as vocalizations, surface swimming patterns, postures, and appetite were considered based on their value as indicators of biological stress in dolphins under managed care (Waples & Gales, 2002; Frohoff, 2004). Throughout the pre-weaning process or about 2 wks before weaning and the cessation of suckling, there were no apparent behavioral characteristics indicative of biological stress. Correspondingly, physiological changes in circulating concentrations of cortisol were not statistically different and were considered basal within and between cows and calves in serial blood samples collected before the first morning training sessions during the 2-wk period prior to weaning.

On the day of weaning (Day 0), cows and calves started their morning training sessions in respective maternity and holding pens as was done during pre-weaning conditioning; however, when cows and calves were cued to move to their respective pens for training at midday, the gates between pens were closed. While cows and calves immediately swam to rejoin one another, the gated barrier prevented physical contact. Immediately, both cows and calves responded with behavioral characteristics indicative of biological stress such as increased vocalization, extremely rapid and erratic swimming, side jumping, breaching, and repeated tail slapping. By the late afternoon training sessions on Day 0, while various types and degrees of stress-like behaviors continued, there was an observable lack of attentiveness to trainers and reduced appetite, which may be attributed, in part, to the increased locomotor activities.

Due to the extent of biological stress on Day 0, blood samples were not collected on the day of weaning but were collected on Day 1 postweaning and episodically thereafter until Day 31. Circulating concentrations of cortisol were significantly higher on Day 1 compared to pre-weaning concentrations in cows and calves with a tendency for higher concentrations in calves than cows. Over the next several days, stress-like behaviors diminished by Day 5 in cows and calves with longer or attentive training sessions and improved appetites. Correspondingly, cortisol concentrations decreased by Day 3 in cows and calves and reached baseline or pre-weaning concentrations by Day 5 in cows and Day 12 in calves. Since behavioral and physiological indicators of postweaning stress had returned to pre-weaning conditions in cows by Day 5, they were moved to the lagoon to join other adult dolphins, while all calves were moved to Maternity Pen M2 for daily training sessions. Notably, the lagoon was adjacent to M2, which was separated by a rope-mesh barrier, thus allowing respective cow-calf pairs to more visually, auditorily, and physically interact without suckling. Although delayed relative to dolphin cows, perhaps the return of cortisol to pre-weaning concentrations in dolphin calves by Day 12 postweaning was hastened or facilitated by the capability of cows and calves to interact across the rope-mesh barrier. While the behavioral and physiological responses to weaning stress in cattle calves (Enríquez et al., 2011; de la Cruz-Cruz et al., 2021) and sheep lambs (Freitas-de-Melo et al., 2022) were not necessarily prevented or reduced when offspring had access to respective mothers across a fence-line barrier post-weaning, horse foals that

were allowed to see, hear, smell, and touch respective mares through a fence, but not suckle, resulted in fewer stress-like behaviors (McCall et al., 1985, 1987; Henry et al., 2020).

In the present study, while the acute increase in cortisol concentrations in dolphin calves at weaning returned to pre-weaning concentrations by Day 12 post-weaning, concentrations remained slightly higher compared to concentrations in cows until the end of the study on Day 31. While the temporal changes in cortisol encompassing weaning may represent an acute adaptive physiological response to the initial physical separation and loss of suckling in calves, perhaps the continued slight elevation of cortisol in calves post-weaning represents a chronic response in the absence of close contact with respective cows, which may be indicative that more time is required to fully adapt to the separation. Future studies are required to evaluate this concept of acute vs chronic physiological response in cortisol and the relationship to behavioral changes post-weaning as calves mature under managed care.

Although the number of animals was too small to statistically assess the effect of ages of cows and calves on cortisol encompassing weaning, a descriptive approach was applied. Averaged over the 2 wks of pre-weaning in relation to Day 1 post-weaning, the mean differences in cortisol were, respectfully, about 10 and 50% higher in less mature (14 to18 y) than in more mature (31 to 34 y) cows. In contrast, there was no apparent pattern that cortisol was higher or lower among calves of different ages (12 to 13.5 mo to 15 to 19 mo) encompassing weaning. Considering that parity and age are related, primiparous beef cows (Ungerfeld et al., 2011) and sheep (Dwyer, 2008) exhibited lesser degrees of weaning-related stress behavior (decreased vocal and locomotor activities) compared to polyparous animals. In piglets, plasma cortisol concentrations were higher in those weaned at 3 wks compared to those at 8 wks of age (Worsaae & Schmidt, 1980). While the present results in dolphin cows and calves does not appear to be consistent with those results in domestic production animals, the 4-mo delayed behavioral response to conditioning cow-calf separation during pre-weaning in less mature and primiparous dolphin cows appears consistent with higher cortisol in the inexperienced cows herein. Although limited, the results of the present study in dolphins with inference to those in domestic production animals are indicative of the need for more comprehensive studies with dolphin cowcalf pairs under managed care to clarify the potential effects of parity and age on the acute stress response associated with weaning.

While secondary to the primary physiological aspect of the present study, the qualitative nature

of the behavioral observations at weaning and immediately thereafter appeared comparable with those documented in numerous studies conducted in domesticated performance (horse) and production (cattle, sheep, swine) animals. In horses (Fazio et al., 2009; Wulf et al., 2018; Henry et al., 2020; Normando et al., 2022), cattle (Lynch et al., 2019; Orihuela & Galina, 2019), sheep (Freitasde-Melo et al., 2022), and swine (Merlot et al., 2004; Colson et al., 2012), increased vocalization and locomotor activities (walking, running, pacing) were the main behavioral characteristics consistently observed in association with physical separation of offspring from mothers during weaning. While there were various degrees of these types of stress-like behaviors in mothers and offspring depending on maturity or parity; complete, partial, or gradual separation; preconditioning; single or paired offspring; age or gender of offspring; and other factors in accord with the various study designs, these altered behaviors during weaning returned to pre-weaning conditions within 1 to 3 d post-weaning.

From the multitude of studies done in performance and production animals to better understand the weaning process with the intention of enhancing and improving management and animal welfare practices (Lynch et al., 2019; Orihuela & Galina, 2019), vocalization and locomotor activities were considered reliable behavioral indicators of weaning-related stress (Enríquez et al., 2011). In bottlenose dolphins, it was proposed that vocalizations may serve as an indicator of stress (Lilly, 1963). While one study (Esch et al., 2009) evaluated dolphin whistles as potential indicators of stress during capture-release sessions, a more recent study (Probert et al., 2021) evaluated vocal correlates of arousal in dolphins under managed care during daily routines not involving stressrelated activities. While clarification is required, present and previous results provide support that vocalizations of various degrees relative to conditions may be a major reliable behavioral indicator of the weaning process in dolphins under managed care.

In previous studies, attempts to correlate behavioral characteristics with cortisol concentrations apparently have not been successful in bottlenose dolphins (Thomson & Geraci, 1986; St. Aubin et al., 1996; Curry, 1999; Ortiz & Worthy, 2000; St. Aubin & Dierauf, 2001; Frohoff, 2004). Moreover, a comprehensive evaluation of tools (behavioral and physiological) currently used to assess biological stress in marine mammals found that no one tool was reliable within or among species (Curry, 1999; Pabst et al., 2002; St. Aubin, 2002). In contrast, the type and degree of physiological and behavioral responses associated with acute homeostatic imbalance in marine and other mammalian species have been quantitated and correlated to determine the status of animal well-being, with cortisol being the most common physiological indicator (Clarke & Boinski, 1995; Flow & Jacques, 1997; Waples & Gales, 2002). In the present study, the apparent positive relationship between the acute, temporal changes in cortisol and behavior encompassing weaning in dolphin cow–calf pairs requires a comprehensive evaluation with the intent to further enhance and improve management and animal welfare practices associated with the weaning process in dolphins under managed care.

Conclusion

Although the present study was preliminary, it is novel in its documenting of acute increases and decreases in circulating concentrations of cortisol encompassing the cessation of suckling associated with weaning in bottlenose dolphin cow-calf pairs under managed care. Secondarily, acute behavioral observations at weaning and immediately thereafter qualitatively indicated increases and decreases in vocalization and locomotor activities (vocalizing, rapid and erratic swimming, side jumping, breaching, repeated tail slapping) in both cows and calves. Although the cessation of suckling and physical separation of cow-calf pairs was preceded by a gradual controlled pre-weaning process of conditioning or training under a state of homeostatic balance, weaning and results immediately thereafter indicated an acute state of homeostatic imbalance or biological stress represented by physiological (cortisol) and behavioral (vocalization, locomotor activities) changes that accompanied an adaptive response and return to or near homeostasis by 5 or 12 d post-weaning. Future comprehensive studies are required to fully evaluate the physiological and behavioral responses at weaning and the subsequent adaptation in cows and calves, especially in regard to the latter as they grow and develop to maturity under managed care.

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