Fetal Survival of Common Bottlenose Dolphins (*Tursiops truncatus*) in Sarasota Bay, Florida

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Abstract

Reproductive success is an important aspect of dolphin population health as it is an indicator of the trajectory for the population into the future. Concerns about potential reproductive impacts of environmental contaminants have stimulated increased interest in measuring reproductive success in wild dolphin populations. One measure of reproductive success is the survival of fetuses to parturition. Pregnancy determination for wild dolphins, including differentiation of pregnancy stage, is possible during capture-release health assessments through application of diagnostic ultrasound to evaluate fetal development and viability, estimate gestational age, and measure anatomical structures. As a first step toward understanding reproductive success in utero, we combined pregnancy detections during health assessments with subsequent observational population monitoring to examine and evaluate pregnancy outcome for well-known, long-term resident common bottlenose dolphins (*Tursiops truncatus*) in Sarasota Bay, Florida. In total, 0.83 (95% CI = 0.52 to 0.99) of detected pregnancies were documented as resulting in live births. The use of ultrasound for systematic pregnancy determination provides a useful tool for measuring an important component of reproductive success. Application of this approach for conservation of wild populations benefits from the establishment of baseline values such as the estimates provided herein for the reference population of bottlenose dolphins residing in Sarasota Bay, Florida.

Key Words: pregnancy detection, fetal survival, ultrasound, bottlenose dolphin, *Tursiops truncatus*, reproductive success

Introduction

Reproductive success is an important aspect of dolphin population health as it is an indicator of the trajectory for the population into the future. Concerns about potential reproductive impacts of environmental contaminants have stimulated increased interest in measuring reproductive success in wild dolphin populations through nonlethal techniques (Schwacke et al., 2002; Wells et al., 2005; Hall et al., 2006; Yordy et al., 2010). In some cases—for example, resident populations of common bottlenose dolphins (Tursiops truncatus) in bays, sounds, and estuaries-well-established procedures exist for measuring the survival of calves during their first year of life and beyond. These include regular, systematic photographic identification surveys used to monitor identifiable mothers and their calves through time (e.g., Wells & Scott, 1990; Wells, 2003, 2014).

An equally important measure of reproductive success is the survival of fetuses to parturition, but this is more difficult to measure in free-ranging animals. Methods have been developed to identify pregnancies in free-ranging cetaceans, including ultrasound and measurement of progesterone concentrations in small blubber samples such as those collected through remote biopsy darting (Kellar et al., 2006). When blubber progesterone measurements are combined with follow-up

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photo-identification, pregnancy success can be evaluated. For example, Pérez et al. (2011) used biopsy darting to sample progesterone in the blubber of identifiable bottlenose dolphins and longfinned pilot whales (Globicephala melas) in the Strait of Gibralter and Gulf of Cadiz, and documented the appearance of newborn calves with two individuals of each species previously determined from hormone concentrations to be pregnant. However, while blubber progesterone appears to distinguish pregnancy status, it is unlikely to differentiate pregnancy stage (Kellar et al., 2006). Knowledge of pregnancy stage can help to focus field efforts to monitor for the appearance of a new calf or to guide field research efforts with regards to minimizing potential risk to the pregnancy.

Pregnancy determination, including differentiation of pregnancy stage, is possible through the application of diagnostic ultrasound to evaluate fetal development and viability, estimate gestational age, and measure anatomical structures (Williamson et al., 1990; Brook, 1994; Stone et al., 1999; Lacave et al., 2004; Smith et al., 2013). However, pregnancy determination via diagnostic ultrasound requires capture, examination, and release of individuals, which can be logistically complex and expensive, and is limited to zoological parks and other institutions where training is possible or to habitats where safe handling can be accomplished. Evaluation of the success of pregnancies requires close observational monitoring of the examined animals post-release. As a result, few data are available on the success of pregnancies for wild dolphin populations.

Opportunities to identify pregnancies through ultrasonic examinations have been provided with resident bottlenose dolphins in Sarasota Bay, Florida, during occasional capture-release efforts for health assessments (Wells et al., 2004). Beginning in 1989, portable ultrasound consoles were brought into the field when possible for health assessments, especially to check for pregnancies and to measure testis size (Wells, 2003, 2009, 2014). Early examinations were limited by availability of equipment and trained personnel, as well as image quality and resolution, but they have evolved into a core component of systematic health assessments since the late 1990s.

As a first step toward understanding reproductive success in free-ranging dolphins *in utero*, we combined pregnancy detections with ongoing observational population monitoring to examine and evaluate pregnancy outcomes. Specifically, we examined cases in which pregnancies were reported from ultrasonic examinations during health assessments of well-known resident Sarasota Bay bottlenose dolphins and identified which cases were observed to result in calves that survived long enough to be observed during photographic identification surveys. These analyses resulted in a minimum estimate for the proportion of observed fetuses reaching successful parturition.

Methods

Study Site and Animals

Field work occurred in and around Sarasota Bay (27.33° N, 82.57° W) along the central west coast of Florida, the site of ongoing bottlenose dolphin research since 1970 (Wells, 2003, 2009). The long-term resident community of approximately 160 bottlenose dolphins spans up to five concurrent generations (calf through great-great-grandmother), and as of 2014 includes individuals up to 64 y of age (Wells, 2014). More than 96% of the dolphins in the study area are identifiable, some have been observed in the area for four decades, and some individuals have been observed more than 1,450 times each. Seasonal photographic identification surveys through the range of the resident Sarasota Bay bottlenose dolphin community conducted during 1980 through 1992 were modified into monthly surveys in 1993, and these continue today. Such surveys have allowed monitoring of more than 120 mothers and documentation of up to 10 calves each.

Health Assessments and Ultrasonic Examinations

Health assessments of Sarasota Bay bottlenose dolphins were initiated in 1988 (Wells, 2009), and experimental incorporation of diagnostic ultrasound began the following year. Small groups of selected bottlenose dolphins are encircled with a 500-m-long seine net, typically in waters less than 2 m deep (Wells et al., 2004; Loughlin et al., 2010). Teams of biologists, veterinarians, and trained handlers carefully restrain individual dolphins for sampling and examination. Dolphins considered to have a high probability of pregnancy due to recent social patterns and reproductive histories were avoided for captures, but this was an imprecise indicator, and some pregnant dolphins were inadvertently caught. Ultrasound was used to determine pregnancy and guide handling relative to pregnancy stage. Ultrasound examinations occur either in the water off the stern of a specially designed 8.5-m-long veterinary examination vessel, or a dolphin is lifted aboard the vessel and placed on a shaded pad on deck for examination. Typically, about 10% of the resident community is sampled during a health assessment session.

Since the first use of ultrasound for life history studies and health assessment in 1989, ultrasound consoles have become more portable, imaging has improved in terms of quality and resolution, and sonographer experience with animals in human care and in the wild has improved the ability of veterinarians to interpret images. Over the years, several different ultrasound units have been used. Beginning in 1989, an Ausonics 2000 (Ausonics, Milwaukee, WI, USA) unit was used, followed by a GE Logiq Book (GE Healthcare, Waukesha, WI, USA) unit with a GE 3C-RS convex curvilinear transducer (2 to 5 MHz) in the early 2000s. In 2011 and 2012, a portable GE Voluson i (GE Healthcare) ultrasound unit with a 3D/4D transducer (RAB; 2 to 5 MHz) was used. The ultrasound unit was fitted with a personal video heads-up display (Z800, eMagin, Bellevue, WA, USA) to improve the ability of the sonographer to see the ultrasound image in bright sunlight. In 2013, a Sonosite M-Turbo unit (Sonosite, Bothell, WA, USA) with a convex curvilinear transducer (C60; 2 to 5MHz) was used and outfitted with video glasses (Cinemizer, Carl Zeiss, Oberkochen, Germany).

Techniques for determining pregnancy have evolved over the past 24 y with improvements in equipment. Blood samples for reproductive hormone concentration measurements have been collected since 1984 (Wells, 2000). Beginning in 1989, the focus of ultrasound was on pregnancy detection and, to a lesser extent, gestational aging. An early pregnancy was suspected if a vesicle was visualized within a fluid-filled uterine horn. If an embryo or fetus was detected, pregnancy was confirmed. The criteria and degree of certainty for a pregnancy diagnosis at the time of examination were considered insufficient to warrant further analysis of pregnancy data from the period prior to 1998, but the efforts served to help train the research team.

Beginning in 1998, more regular and systematic examination of ovaries was initiated. Currently, as part of the regular systematic ultrasound exam for each female, ovaries are examined for the presence or absence of ovarian structures, specifically follicles and corpora lutea (CL). If follicles are detected, the dominant follicle is identified and measured (Figure 1). If a CL is detected, it is also measured (Figure 2). Any evidence of pregnancy, including a CL or uterine fluid, warrants close inspection of the uterine horns for an embryonic vesicle or fetus. When a fetus is detected, the biparietal skull diameter (BPD) is measured (Figure 3), and the gestational age is estimated according to size (Stone et al., 1999; Lacave et al., 2004; Smith et al., 2013). The fetus is further evaluated for viability based on observation of a heartbeat and fetal movement (see video link on the Aquatic Mammals website: www.aquaticmammalsjournal.org/index.



Figure 1. Ovarian follicles: dorsal plane sonogram of the left ovary of an adult female bottlenose dolphin (*Tursiops truncatus*). Cranial is to the left of the image and caudal is to the right. The thin line delineates the borders of the ovary, and arrow heads are pointing to two distinct follicles.



Figure 2. Corpus luteum (CL): dorsal plane sonogram of the left ovary of an adult, pregnant, female bottlenose dolphin. Cranial is to the left of the image and caudal is to the right. The dotted line represents the diameter of the CL, which measured 3.1 cm.



Figure 3. Fetal skull: dorsal plane sonogram of the left uterine horn of an adult female bottlenose dolphin containing a fetus. Cranial is to the left of the image and caudal is to the right. The thin line depicts the fetal biparietal skull diameter, which measured 13.2 cm.



Figure 4. *Fetal heart rate:* motion (M) mode was used to measure the fetal heart rate of the bottlenose dolphin, determined to be 113 beats per minute (bpm).

php?option=com_content&view=article&id=695 &catid=48&Itemid=101). Motion (M) mode is utilized to measure fetal heart rate when possible (Figure 4). Basic organ definition is assessed and abnormalities are noted if found (Smith et al., 2013). Blood samples for reproductive hormone concentrations were also collected as further evidence of pregnancy.

Pregnancy success was scored when a new calf was observed swimming alongside its presumed mother within 1 y of the latest estimated due date following a pregnancy diagnosis. If a female diagnosed as pregnant was observed without a new calf during the period extending from at least 2 wks after her expected due date (to accommodate late deliveries) to 1 y after the latest estimated due date, then her pregnancy was considered unsuccessful. Calf size was considered to preclude the possibility of incorrectly scoring loss of the diagnosed pregnancy followed immediately by cycling and another pregnancy.

Results

During 1998 through 2013, 13 putative pregnancies were diagnosed via ultrasound for Sarasota Bay resident bottlenose dolphins, involving females ranging in age from 9 to 31 y (Table 1). All of the females were observed after the pregnancy diagnosis and after the time that the calf should have been born, based on an approximately 12.5 mo gestation period for bottlenose dolphins (O'Brien & Robeck, 2012; Smith et al., 2013). Serum progesterone concentrations elevated above baseline, potentially indicative of pregnancy (> 5 ng/ml), were found for all 12 of the cases for which hormone data were available (Table 1) (Sawyer-Steffan et al., 1983; O'Brien & Robeck, 2012; Smith et al., 2013).

Twelve of the 13 pregnancy diagnosis cases met the criteria for determination of success. In the anomalous case of FB01 (in 2003), following diagnosis of pregnancy, a premature (90 cm) fetus was recovered 5 d before the carcass of the mother was recovered nearby. The carcass of FB01 had a deep, fatal puncture wound of the external body wall that perforated the uterus, and she was

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								No. days	#					
		Age at						from	sightings				No.	
	Pregnancy	pregnancy		Pregnancy criteria				US to	in y	Latest	1st sighting	Last sighting	sightings	Calf
Mom ID	diagnosis date	diagnosis (y)	Pregnancy description	(fetus/no fetus; I viability assessment)	Progesterone (ng/ml)	Calf	First calf sighting	1st calf sighting	prior to diagnosis	anticipated due date	after due date	within 1 y of due date	in y past due date	survived 1st y
F163	4 June1998	27	"3rd trimester"	Noted on dataform, from ultrasound, no other data	9.45	F216	16 July 1998	42	39	4 June 1999	9 June 1999	30 May 2000	20	Yes
FB33	5 June 2001	19	"Very pregnant"	Noted on dataform, from ultrasound no other data	15.59	C336	5 July 2001	30	43	5 June 2002	21 June 2002	2 June 2003	36	No
FB01	5 June 2003	12	"Late 2nd trimester"	Aborted 90 cm, 7.5 kg calf recovered on 6 June	11.90	Premature	6 June 2003	1	20	5 June 2004	2002 11 June 2003	11 June 2003	N/A	N/A
F193	3 June 2005	21	"2nd trimester"	Fetus, 10.2 cm thorax, heartbeat visualized	14.21	F201	7 Nov 2005	157	4	3 June 2006	13 July 2006	3 May 2007	10	Yes
FB33	7 June 2006	24	"Very pregnant"	Noted on dataform, from ultrasound, no other data	11.79	C338	26 June 2006	19	43	7 June 2007	13 June 2007	6 June 2008	49	No
F175	6 May 2008	17	"Very pregnant"	Noted on dataform, from ultrasound, no other data	8.57	1753	11 June 2008	36	74	6 May 2009	13 May 2009	28 April 2010	33	Yes
F215	7 May 2009	6	"Early 3rd trimester"	 Noted on dataform, from ultrasound, no other data 	30.41	2151	6 Aug 2009	91	19	7 May 2010	20 May 2010	14 Oct 2010	L	Yes
F225	17 May 2010	N/A	"Very pregnant, 3rd trimester"	"3-4 weeks until full term"	35.36	None	N/A	N/A	13	17 May 2011	8 Sept 2011	10 April 2012	5	N/A
F113	19 May 2010	14	"Very pregnant, early 3rd trimester"	Noted on dataform, from ultrasound, no other data	12.91	F274	2 June 2010	14	20	19 July 2010	5 Aug 2010	19 July 2011	19	Yes
F113	20 July 2012	16	"∼3 months (1st trimester)"	Fetus, 2 cm skull, viability confirmed from movement	27.92	1134	8 May 2013	292	36	26 May 2013	11 June 2013	9 Dec 2013	27*	Yes
F155	7 May 2013	23	"3rd trimester"	Fetus, 9.9 cm skull, 108 bpm heart rate, frequent fetal movement, no defects detected	N/A	1556	27 Sept 2013	143	26	18 Aug 2013	4 Sept 2013	9 Dec 2013	10*	Yes
F137	9 May 2013	13	"3rd trimester, early"	 Fetus, 10.3 cm skull, 100 bpm heart rate, frequent fetal movement, no defects detected, good cord blood flow 	11.60	None	N/A	N/A	13	10 Aug 2013	9 Sept 2013	3 Dec 2013	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	N/A
FB33	10 May 2013	31	"Late 3rd trimester"	Fetus, 13.2 cm skull, 113 bpm heart rate, some fetal movement, no defects detected	10.90	C330	17 June 2013	38	24	27 May 2013	10 June 2013	9 Dec 2013	34*	Yes

* A full year had not yet passed since the due date at the time of writing.

without her late second trimester fetus. Because it cannot be determined if this pregnancy would have reached term in the absence of the injury, this anomalous case is not considered in the following analyses. Overall, new calves were seen with 10 of 12 (83%) of the females diagnosed as pregnant (Table 1).

Discussion

In total, 0.83 (95% CI = 0.52 to 0.99) of detected pregnancies were documented as resulting in live births. The pregnant females not seen with a calf, F225 (in 2010) and F137 (in 2013), were seen less frequently in Sarasota Bay than most of the other females, based on comparisons of the numbers of times they were recorded during the years immediately prior to, and after, their pregnancy diagnosis (Table 1). On average, females seen in 1998 through 2013 with calves following a pregnancy diagnosis were seen 2.7 ± 1.59 SD times/mo in the year prior to their diagnosis, and 2.3 + 1.24 SD times/mo after the latest estimated due date (n =10 females). In contrast, F225 and F137 were each seen only 1.1 times/mo prior to pregnancy detection. F225 was seen only 0.4 times/mo after the latest estimated due date, and F137 was seen only 0.8 times/mo. Given the infrequency of resightings, it is possible that their calves were born, and lost, before they could be observed.

Eight of the 10 calves (80%) resulting from documented pregnancies survived through the calendar year of their birth and, therefore, were considered to have been successfully recruited into the Sarasota Bay bottlenose dolphin population. This value compares favorably with the 81% firstyear survival reported by Wells & Scott (1990) for Sarasota Bay bottlenose dolphins. Thus, approximately 66% of documented pregnancies led to successful recruitment.

Factors impacting survivorship or reproduction are among the most important considerations for conservation. Direct measures of reproductive success are difficult to obtain for wild populations of small cetaceans. Both blubber progesterone concentrations and examinations with ultrasound can detect pregnancies, and when coupled with photo-identification monitoring, both approaches can assess the survival of fetuses to parturition. However, only ultrasound can provide data on fetal viability, estimated due date, and fetal condition relative to stage of pregnancy-parameters that are important for dolphin conservation. For example, estimated due date might inform field research plans by providing a window of time in which observations should be increased, or fetal condition relative to stage of pregnancy might provide insight into biotic and abiotic conditions affecting the health of the mother. The use of ultrasound for systematic pregnancy determination provides a useful tool for measuring an important component of reproductive success. Application of this approach for conservation of wild populations benefits from the establishment of baseline values such as the estimates provided here for the reference population of bottlenose dolphins residing in Sarasota Bay. Increased sample sizes and data from additional sites will increase the precision and potentially aid in understanding the factors that influence successful parturition.

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