

## WHISTLES AS POTENTIAL INDICATORS OF STRESS IN BOTTLENOSE DOLPHINS (*TURSIOPS TRUNCATUS*)

H. CARTER ESCH,\* LAELA S. SAYIGH, JAMES E. BLUM, AND RANDALL S. WELLS

Biology Department, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA (HCE, LSS)

University of North Carolina Wilmington, Wilmington, NC 28403, USA (LSS, JEB)

Chicago Zoological Society, c/o Mote Marine Laboratory, Sarasota, FL 34236, USA (RSW)

We examined the possibility that parameters of bottlenose dolphin signature whistles may serve as indicators of stress. Bottlenose dolphins (*Tursiops truncatus*) in Sarasota Bay, Florida, were recorded during brief capture–release events, which are potentially a source of short-term stress to these dolphins, although no effects of chronic or long-term stress have been observed over the 37+-year duration of the research. Whistles recorded during both brief capture–release and undisturbed, free-ranging conditions were examined to determine whether whistle parameters differ during capture–release versus undisturbed conditions; at the beginning of a capture–release session versus at the end of a session; during an individual’s 1st capture–release session versus later capture–release sessions; and when a mother is caught and released with a dependent calf versus without a dependent calf (i.e., she has no dependent calf at the time of capture–release). We examined a variety of acoustic parameters, including whistle rate, number of loops (repetitive elements), maximum and minimum frequency, and loop, interloop, and whistle duration. We found that whistle rate and number of loops were greater during brief capture–release events than during undisturbed conditions; number of loops decreased and loop duration increased over the duration of a capture–release session; whistle rates decreased with number of capture–release sessions; and females caught and released with dependent calves produced whistles with higher maximum frequencies and shorter interloop intervals than when they did not have dependent calves. Thus, whistles appear to have potential as noninvasive indicators of stress in bottlenose dolphins. Further research is warranted in this area, for example by correlating physiological indices to whistle rates under varying levels of stress. Reliable, noninvasive correlates of stress could be used to monitor dolphins in a variety of circumstances, such as during exposure to anthropogenic noise.

Key words: bottlenose dolphin, signature whistle, stress, *Tursiops truncatus*, welfare

The welfare of an animal, or its state in relation to how it copes with its environment, is often related to the stress that it experiences (Broom 1988). Stress has been defined as a substantial imbalance between demand and response capability, under conditions where failure to meet demand has important consequences (McGrath 1970). Stress can be either acute or chronic. Acute stress may have negative or positive physiological effects, whereas chronic stress typically has long-lasting, negative effects on immune function, reproduction, and growth, and may cumulatively have population-level impacts (reviewed in Nelson 2000).

The term “stress response” refers to a suite of physical and emotional changes that occur in response to a threat or stressor (Selye 1936). Stress hormones such as cortisol, aldosterone, and epinephrine function to increase heart rate, blood pressure, and respiratory rate to more efficiently circulate energy and oxygen to tissues (Sapolsky 1998). The role of cortisol as an indicator of stress has been well studied in selected mammals (e.g., humans, nonhuman primates, pigs [*Sus scrofa*], and cattle [*Bos taurus*]—Clarke 1991; Grandin 1997; Sapolsky 1987, 1993). Correlations often are made between cortisol levels and alternative indicators of stress such as heart rate or serum enzyme levels (Curry 1999). However, most physiological metrics for stress response require handling the subject, a process that may confer additional stress (e.g., harbor porpoises [*Phocoena phocoena*]—Desportes et al. 2007), and cortisol response to stress varies among species. Noninvasive assessment tools would eliminate the potentially confounding effects of invasive techniques (e.g., blood sampling and body core temperature measurements).

\* Correspondent: carter@whoi.edu

Establishing criteria by which to evaluate stress in dolphins was prioritized as a result of a 1997 amendment to the Marine Mammal Protection Act that created the International Dolphin Conservation Program Act (IDCP Act, United States Public Law 105-42). The IDCP Act mandated research aimed at determining how the stress of repeated encirclement and capture–release affects dolphins in the Eastern Tropical Pacific tuna purse-seine fishery. Thus, the National Marine Fisheries Service conducted a comprehensive evaluation of the tools that are currently used to assess stress response in marine mammals (Curry 1999; Pabst et al. 2002; St. Aubin 2002). Although no tool was deemed to be singularly reliable within or among species, the information provided a useful foundation on which to base further development of tools for stress-response assessment.

In bottlenose dolphins, attempts have been made to correlate stress hormones (e.g., cortisol) with behavioral state, but no consistent relationship has emerged (Curry 1999; Frohoff 2004; Ortiz and Worthy 2000; St. Aubin and Dierauf 2001; St. Aubin et al. 1996; Thomson and Geraci 1986; Waples and Gales 2002). Behavioral observations also have been used to assess stress in captive, unrestrained dolphins (e.g., Frohoff 2004; Waples and Gales 2002). Loss of appetite, social instability, and changes in respiratory rate, dive and surfacing patterns, postures, and vocalizations were postulated as short-term responses to stress (Frohoff 2004; Waples and Gales 2002). The current study aimed to assess the potential of using vocal rates or other vocal parameters as a noninvasive means to evaluate acute stress in bottlenose dolphins.

Vocal responses to stress are well documented in some domestic animals (e.g., pigs, cattle, and sheep [*Ovis aries*]), all of which share a common ancestry with cetaceans (reviewed by Thewissen [1994]). In particular, researchers have proposed that vocal responses to forced isolation could be a distress reaction that is context specific, an attempt to communicate with conspecifics, or both (Watts and Stookey 2000). Weary and Fraser (1995) determined that quantifiable differences existed in rate, intensity, and duration of piglet calls, and that these differences reflected various degrees of need. Watts and Stookey (1999) asserted that contemporary animal welfare research should aim to better understand the correlation (if any) between vocalizations of a distressed animal and its own perceived state of well-being. Vocalizations of a variety of species have been found to vary according to level of inferred stress (e.g., cattle: electric cattle prod [Grandin 1998] and branding [Watts and Stookey 1999]; domestic piglets: castration [Weary and Fraser 1995]; guinea pigs [*Cavia porcellus*]: isolation [Monticelli et al. 2004]; common marmoset [*Callithrix jacchus*]: isolation [Newman and Goedeking 1992]; and beluga whale [*Delphinapterus leucus*]: isolation [Castellote and Fossa 2006]) and measured response to stressors (e.g., cattle: heart rate [Watts and Stookey 2001]; rhesus macaques [*Macaca mulatta*]: cortisol [Bercovitch et al. 1995]; and squirrel monkey [*Saimiri sciureus*]: cortisol [Wiener et al. 1990]). Vocalization parameters that have been found to vary with stress include

vocal rate, intensity, frequency range, maximum frequency, minimum frequency, number of harmonics, and duration. In addition, Rahurkar et al. (2002) determined that 4 particular frequency bands in human speech were highly affected by stress, and could be used to efficiently determine stressed speech in humans.

Lilly (1963) was the 1st to propose that dolphin vocalizations may serve as indicators of stress. In particular, he suggested that dolphins produced a specific “distress whistle” to which other dolphins responded by offering aid. However, in a comprehensive study of whistles of more than 100 captive bottlenose dolphins, Caldwell et al. (1990) did not find evidence for distress whistles. Instead, they found that each dolphin produced a stereotyped, individually distinctive whistle, which they called a signature whistle. Caldwell et al. (1990) did find that certain parameters of signature whistles (e.g., number of loops and duration of loops) appeared to be closely related to the level of arousal of an individual dolphin; however, these differences were not consistent across individuals. Similarly, Janik et al. (1994) found that 9 of 14 signature whistle frequency and time parameters differed significantly between isolation and interaction conditions, supporting the existence of both identity- and context-related information in signature whistles. Increased whistle rates produced by captive dolphins during interactive programs may result from stress rather than “an attempt to communicate with people who are in the water with them,” as suggested by Akiyama and Ohta (2007:169).

The overall contour, or pattern of frequency changes over time, of signature whistles tends to be highly stable, despite changes that may occur in other whistle parameters (e.g., duration and maximum or minimum frequency—Caldwell et al. 1990; Janik et al. 1994; Sayigh et al. 1990). This contour stability makes signature whistles, as opposed to highly variable nonsignature whistles, ideal signals for the comparison of whistle features that might vary among contexts. Signature whistles may consist of a single element (or loop), or repeated loops (disconnected, connected, or both) that may be accompanied by an introductory or terminal loop or both (Caldwell et al. 1990; Sayigh et al. 1990, 2007). We evaluated whether whistle rate, loop number, loop and interloop duration, or frequency parameters (maximum or minimum frequency) could serve as potential indicators of acute (or short-term) stress in bottlenose dolphins. If vocal parameters are correlated with stress, they could provide a noninvasive metric for evaluating stress in dolphins.

Whistles recorded during a health-monitoring project in Sarasota Bay, Florida, were used for this study (Scott et al. 1990; Wells 1991, 2003; Wells et al. 2004). This project involves brief capture–release events, in which small groups of dolphins are encircled by a seine net in shallow water (<2 m deep), and then either held in the water or lifted onto the shaded, padded deck of a boat by stretcher. Onboard, dolphins are given a physical and ultrasonic health exam by experienced marine mammal veterinarians, who also monitor the condition of the animal throughout the procedures. The

dolphins are recorded, measured (lengths, girths, and blubber depth via ultrasound), weighed, sampled, freeze-branded, and photographed. Blood samples are drawn immediately after a dolphin is restrained and sometimes at the end of the exam period. This basic sampling regime has remained relatively unchanged over the 2 decades of the capture–release program for health monitoring (Wells et al. 2004). In addition to the basic sampling regime, additional procedures have been incorporated over the years of the project (e.g., thermoregulatory research and auditory evoked potential measurements). All efforts are made to complete the exam within 1 h.

Since 1998, cortisol values have been determined from blood samples collected during the bottlenose dolphin health-monitoring project in Sarasota Bay. Although these values are available, they were not used in the current study as a physiological indicator of stress to compare with changes in whistle parameters for multiple reasons. First, many of the recordings included in the current study were collected before cortisol values became available. Second, cortisol values fall within a narrow range for bottlenose dolphins, and fluctuations in these values are often subtle compared to those of other species (St. Aubin and Dierauf 2001). Third, cortisol response to stress in dolphins may not reach its peak until well after the health-monitoring exam is complete (St. Aubin and Dierauf 2001). Both Thompson and Geraci (1986) and St. Aubin et al. (1996) found that cortisol values in captured and handled bottlenose dolphins took at least 1–2 h to elevate significantly. Finally, the infrequently captured and released dolphins of Sarasota Bay did not exhibit a stronger glucocorticoid response to the stress of capture and handling than did frequently handled captive dolphins (Ortiz and Worthy 2000; St. Aubin et al. 1996). Overall, using the degree of cortisol elevation as an absolute measure of the stress a bottlenose dolphin is experiencing is considered unreliable (St. Aubin and Dierauf 2001).

Although there is no evidence that capture–release events have any long-term adverse impacts on members of the Sarasota dolphin community, they are potentially a source of short-term stress to the dolphins. Vocalizations recorded from dolphins of different ages and levels of capture–release experience were used to examine whether whistle parameters (whistle rate, number of loops, loop and interloop duration, and maximum and minimum frequencies) vary systematically in the following conditions: 1) during capture–release versus undisturbed conditions; 2) at the beginning of a capture–release session versus the end of a session; 3) during an individual's 1st capture–release session versus later capture–release sessions; and 4) when a female is caught and released with a dependent calf versus when the same female does not have a dependent calf (e.g., during an intercalf interval). If signature whistle parameters are influenced by stress, then we expect to see a difference between capture–release and undisturbed conditions, as outlined in condition 1. However, it is also likely that habituation (i.e., a decrease in response over time) will occur, both during the duration of a capture–release session, as addressed in condition 2, and over the

course of several capture–release sessions, as addressed in condition 3. Because temporary separation from a dependent calf could be stressful to a mother, we also examined whether whistles varied according to whether a mother was with a dependent calf or not, as outlined in condition 4.

## MATERIALS AND METHODS

The bottlenose dolphins of Sarasota Bay are long-term, year-round residents of the bay, spanning as many as 5 generations, and >90% are recognizable from dorsal fin features (Scott et al. 1990; Wells 1991, 2003). Shallow, sheltered inshore waters facilitate monitoring the individual residents throughout their lives. As part of the monitoring process, health assessments are performed on occasion, taking advantage of the shallow waters to safely capture, handle, and release the animals (Wells et al. 2004). Capture–release work is conducted under Scientific Research Permits issued by the National Marine Fisheries Service and with Institutional Animal Care and Use Committee approvals, and all methods satisfy the guidelines approved by the American Society of Mammalogists (Gannon et al. 2007). Recordings from brief capture–release events in Sarasota Bay, Florida (Scott et al. 1990; Wells 1991, 2003; Wells et al. 2004), have been collected over a period of 34 years (1975–2008), and many dolphins have been recorded multiple times (maximum = 15,  $\bar{X}$  = 3.3). Most animals are of known age, which is determined primarily from observing multiple generations of dolphins (Wells 2003), or in some cases by examination of tooth growth-layer groups (Hohn 1990; Hohn et al. 1989). Custom-built suction-cup hydrophones were placed directly on the head of each individual, allowing researchers to unequivocally identify the vocalizing dolphin (Tyack 1985). The hydrophones were not calibrated because amplitude values were not being measured. Whistles were recorded onto either Marantz PMD-430 (Marantz, Itasca, Illinois) or Sony TC-D5M stereocassette recorders (approximate frequency response 30–20,000 Hz; Sony Electronics Inc., New York, New York), Panasonic AG-6400 or AG-7400 videocassette recorders (approximate frequency response 20–32,000 Hz; Panasonic Corp. of North America, Secaucus, New Jersey), or a Sound Devices 744-T digital recorder (frequency response 10–48,000 Hz; Sound Devices, LLC, Reedsburg, Wisconsin), at a sampling rate of 96 kHz. The predominant whistle produced by an animal during a capture–release event is defined as its signature whistle. Because most dolphins in Sarasota Bay have been captured and released more than once, signature whistle identifications have been confirmed by reviewing multiple recordings for an individual animal.

Dolphins in Sarasota Bay also have been recorded under undisturbed conditions. Free-swimming, known individuals were recorded during approximately 145 h of boat-based observations during May–August 1994 and May–July 1995 as part of a study on vocal development (Cook et al. 2004). These animals were considered relatively undisturbed despite the presence of the research platform, based on the assumption

that Sarasota Bay dolphins are habituated to the consistently dense boat traffic in Sarasota Bay. Nowacek et al. (2001) determined that dolphins in the Sarasota Bay community are exposed to a vessel passing within 100 m every 6 min during daylight hours, and that dolphins in Sarasota Bay showed no change in interbreath interval (a proxy for disturbance in the study) when a single observation vessel (i.e., a research platform) was present, whereas the presence of multiple nonobservation vessels prompted changes in interbreath interval, heading, and swimming speed. Recordings were collected during observation sessions using a Panasonic AG-6400 hi-fi videocassette recorder (approximate frequency response 20–32,000 Hz; Panasonic Corp. of North America) and 2 weighted hydrophones that were towed while underway. Concurrent visual observations (e.g., group composition, interanimal distance, activity, location, and group size) were made at 5-min intervals. Activities were categorized as milling, traveling, feeding (or probable feeding), resting, and socializing (Cook et al. 2004). If at any point during the recording session animals appeared to be avoiding the boat (e.g., erratic surfacings or high-speed swimming), the observation session was terminated. Localization of the source of whistles was not possible; however, Cook et al. (2004) were able to match whistles of identified individuals to whistles produced by the same individuals during capture–release events. This data set of identified signature whistles produced during undisturbed conditions was used in the current study.

Whistle rates were determined by evaluating the number of signature whistles produced per minute. Using only signature whistles (i.e., no nonsignature or variant whistles) was essential for comparing whistle rates during capture–release and undisturbed conditions, because Cook et al. (2004) were not able to associate nonsignature whistles with a particular individual. Because signature whistles are stereotyped and repeatable, comparisons of signature-whistle parameters among studies are feasible, whereas comparisons of nonsignature whistles would be more difficult. In addition, nonsignature whistles occurred relatively rarely in capture–release recordings. Therefore, only signature whistles were considered for all components of this study.

**Recording selection.**—For this study, only recordings that began no more than 25 min after net deployment and 10 min after the animal was initially restrained were used. The logistics of a capture–release setting nearly always precluded the deployment of a hydrophone earlier than 10 min after initial restraint. Only continuous (uninterrupted) recordings were used, and the recording length used varied by research question. To evaluate changes in whistle rate during the course of a single capture–release, only continuous recordings of 40 min or longer were used. This was the longest recording time consistently available that provided a sufficient sample size. Recordings used to address the other 3 conditions (i.e., differences in whistle parameters between capture–release and undisturbed conditions, across multiple capture–release sessions for an individual dolphin, and when a mother is captured and released with a dependent calf versus when she does not

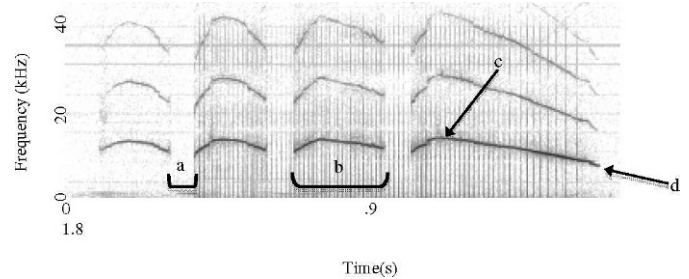
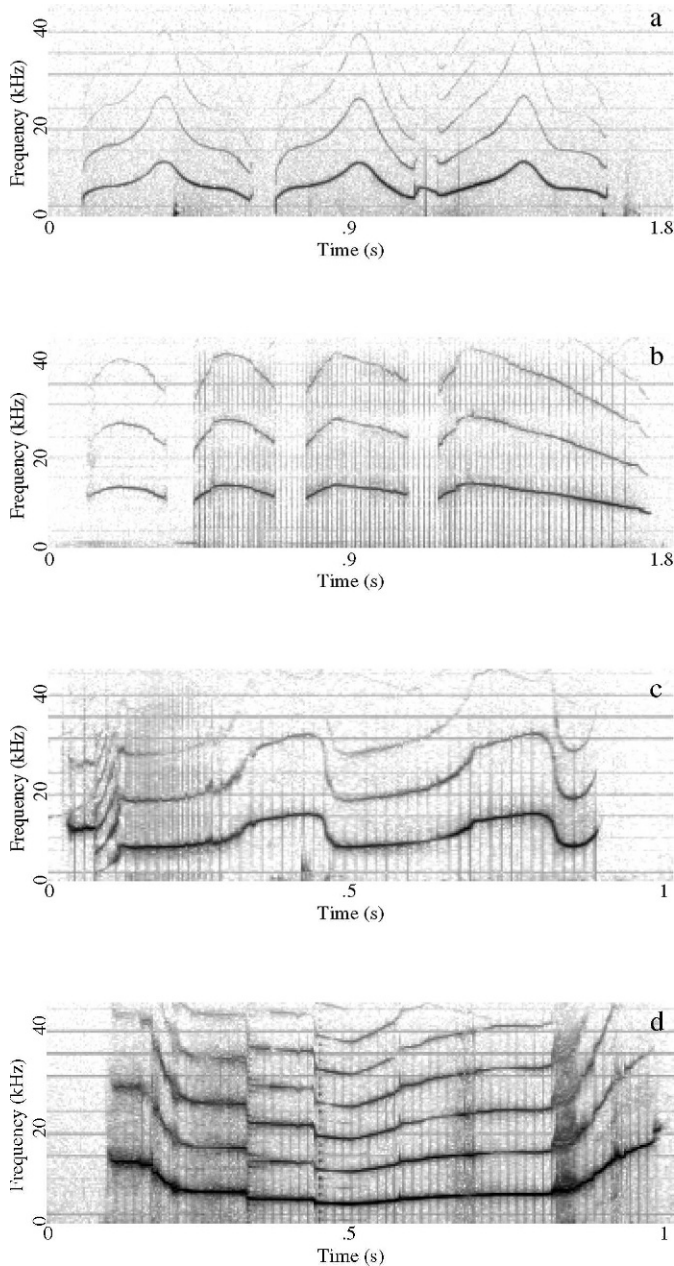


FIG. 1.—Example of measurements made for each of 20 whistles (recorded in Sarasota Bay, Florida) for each bottlenose dolphin (*Tursiops truncatus*). Specific measurements made for each whistle include a) interloop interval, b) loop duration, c) maximum frequency, and d) minimum frequency.

have a dependent calf) ranged from 20 to 30 min in length. Comparisons were made between recordings of equal length (e.g., a whistle rate calculated from a 25-min recording was compared to a whistle rate based on another 25-min recording).

Most recordings were prepared by digitizing the original analog reels (VHS and cassette) using a Sound Devices 744T digital recorder (Sound Devices, LLC; sampling frequency 96 kHz, 24-bit). This process produced digital files (.wav) that were then analyzed using Avisoft-SASLab Pro 3.2 (Raimund Specht, Berlin, Germany) or Signal/RTSD 3.0 (Engineering Design, Belmont, Massachusetts) sound analysis software. Each recording was manually reviewed, and every signature whistle was noted. Whistles were then randomly selected (using a random number table generated in Microsoft Excel [Microsoft Corporation, Redmond, Washington]) for parameter measurements. The number of whistles randomly selected was dependent on the research question being addressed (as described in more detail below). There were several cases in which the number of signature whistles produced was fewer than the number selected for sampling (e.g., only 3 signature whistles were produced during a dolphin's recording period, whereas 10 whistles was the target sample size). In these instances, comparisons of whistle parameters were based on means calculated from unequal sample sizes among dolphins. Measurements of time and frequency were made manually using the cursor functions in each acoustic analysis software system (see Fig. 1 for example).

We have recognized 4 categories of signature whistles (see examples in Fig. 2): 1) loops sometimes connected, sometimes not (perhaps affected by recording conditions), loops may vary in number or contour or both; 2) loops always disconnected, may vary in number or contour or both; 3) loops always connected, may vary in number or contour or both; and 4) no repetitive loop structure. Of the 205 dolphins represented in our database, 159 produced multilooped whistles (categories 1–3, above). For this study, whistles of 34 dolphins were analyzed, of which 30 were multilooped. Loop duration was measured for whistles with single loops (e.g., Fig. 2d; 4 of 34 signature whistles in our sample) and disconnected loops (e.g., Fig. 2b; 20 of 34 whistles). For whistles with multiple connected loops (e.g., Fig. 2c; 10 of 34 whistles), loop



**FIG. 2.**—Example of each of 4 signature whistle types produced by bottlenose dolphins (*Tursiops truncatus*) recorded in Sarasota Bay, Florida. a) Loops sometimes connected, sometimes not; b) loops always disconnected; c) loops always connected; and d) no repetitive loops structure.

duration was determined by dividing total whistle duration by the number of loops produced.

Wilcoxon signed-rank tests (JMP IN version 4.0.2; SAS Institute Inc., Belmont, California) were used to test whether whistle rates were higher in one context versus another (e.g., at the beginning versus at the end of a capture–release session) and to assess differences in mean parameter measurements. Significant results were those with  $P < 0.05$ .

In order to investigate potential relationships between whistle rate and age, sex, and capture–release number, gamma values ( $G$ ), associated 95% confidence intervals, and signif-

icance values were calculated. Optimized generalized linear models were developed, in which the natural log of whistle rate (whistles per minute) was modeled as a function of predictors including sex (which was used in all model fits), age, and a linear term for capture–release number. The best model from a set of all reasonable combinations of the predictor set was chosen. All of the recordings used in this study (total dolphins = 34; total recordings = 78) were included in these additional analyses.

Methods specific to each of the 4 conditions outlined in the introduction are described separately below.

*Do whistle parameters differ between capture–release and undisturbed conditions?*—A total of 20 recordings (2 from each of 10 dolphins, 7 males and 3 females) was analyzed. For each of the 10 dolphins, a recording made in undisturbed conditions and a recording from a 1st-time capture–release session were selected. The sample size was limited by the number of animals whose vocalizations were positively identified during undisturbed observations by Cook et al. (2004) and for whom there were recordings of sufficient length and quality. Whistle rates under undisturbed conditions were calculated from the 30 min following the 1st identification of an animal’s signature whistle during a recording session. Whistle rates during capture–release were based on the first 30 min of an animal’s 1st recording session. Twenty whistles were randomly selected from each recording and were subjected to the measurements outlined above. When 20 whistles were not available, means were based on the number of whistles produced. Of the 10 dolphins included in these analyses, 4 produced whistles with multiple connected loops and 6 produced multiple disconnected loops.

Most dolphins were younger when recorded during 1st capture–release than under undisturbed conditions. To address the potentially confounding effects of age on whistle rate, a regression analysis of age versus whistle rate for 1st capture–release was performed ( $n = 27$ ), and used to predict whistle rate by sex during 1st capture–release at the age of each dolphin when it was recorded under undisturbed conditions. The same analysis was conducted for dolphins recorded during undisturbed conditions, to determine if whistle rates varied with age for free-swimming dolphins.

*Do whistle parameters vary between the beginning and end of a capture–release session?*—A total of 20 recordings (1 from each of 20 dolphins, 9 males and 11 females) was analyzed, with a minimum uninterrupted recording length of 40 min for each (range = 40–136 min,  $\bar{X} = 64$  min). Whistle rates during the first 10 min of a session were compared to whistle rates during the last 10 min of a session. Ten whistles were randomly selected from each time period for parameter measurements. Of the 20 dolphins included in these analyses, 2 produced whistles with a single loop, 7 produced multiple connected loops, and 11 produced multiple disconnected loops.

*Do whistle parameters vary between an individual’s 1st capture–release session and later capture–release sessions?*—A total of 36 recordings (3 from each of 12 dolphins, 5 males

and 7 females) was used. Dolphins were included in this portion of the study if they had been recorded at least 5 times (49 of 205 dolphins in the recording library). Of the 49 dolphins that met this criterion, the individuals selected for this portion of the study had recordings of sufficient length (minimum = 20 min), quality (uninterrupted), and timing (initiated no more than 10 min after restraint). Recordings from the 1st, 3rd, and 5th capture–release session were selected for each dolphin. In 3 cases (FB33, FB51, and FB163), a sufficiently long recording was not available for 1 capture–release session, and an alternate was used (e.g., 1st, 4th, and 5th). Recordings ranged from 20 to 30 min in duration. In 7 cases the 1st recording was obtained when the subject was a juvenile (<6 years old for females and <10 years old for males—Wells and Scott 1999), and in 5 cases the 1st recording was obtained when the subject was an adult. Thus, we were able to compare changes in whistle rate with capture–release experience between adults and juveniles.

Whistle rates were calculated for each recording and compared among the 3 capture–release sessions for each animal. Twenty whistles were randomly selected from each recording for measurements. Of the 12 dolphins included in these analyses, 2 produced whistles with a single loop, 2 produced multiple connected loops, and 8 produced multiple disconnected loops.

*Do whistle parameters differ when a female is caught and released with a dependent calf versus when the same female does not have a dependent calf?*—Recordings from capture–release sessions for 8 individual females, when both with and without dependent calves, were analyzed. Recordings ranged from 20 to 30 min in duration. Whistle rates were calculated for each recording. Twenty whistles were randomly selected from each recording for parameter measurements. Of the 8 dolphins included in these analyses, 2 produced whistles with multiple connected loops, and 6 produced multiple disconnected loops.

## RESULTS

*Do whistle parameters differ between capture–release and undisturbed conditions?*—Whistle rate and number of loops were both significantly higher during capture–release than during undisturbed conditions (whistle rate:  $W = -27.5$ ,  $d.f. = 9$ ,  $P = 0.001$ ; number of loops:  $W = -16.5$ ,  $d.f. = 9$ ,  $P = 0.05$ ; Table 1). Predicted whistle rates were still significantly higher during 1st-time capture–release than during undisturbed conditions based on the regression analysis used to correct for potential age effects ( $W = -55$ ,  $d.f. = 9$ ,  $P = 0.005$ ; Table 1). No relationship was detected between age and whistle rate for dolphins recorded under undisturbed conditions ( $n = 10$ ,  $R^2 = 0.04$ ). No significant differences were found for loop or interloop duration, or maximum and minimum frequency.

*Do whistle parameters vary between the beginning and end of a capture–release session?*—For multilooped whistles, the number of loops produced in the first 10 min was significantly

**TABLE 1.**—Age, whistle rates, and mean (*SD*) number of loops produced during undisturbed observations (U) and 1st-time capture–release (C) sessions for each of 10 bottlenose dolphins (*Tursiops truncatus*) recorded in Sarasota Bay, Florida. Sex (M = male; F = female) is included in parentheses. Predicted whistle rates (determined using regression analysis) reflect predicted whistle rate values under capture–release conditions at the age at which dolphins were recorded under undisturbed conditions (i.e., 10.22 whistles/min predicted if FB2 was recorded during capture–release at age 4 years). Each recording period was 30 min.

Dolphin	Age (years)	Whistle rate (whistles/min)	Predicted whistle rate (whistles/min)	No. loops
FB2 (M)				
U	4	0.27		1.8 (0.8)
C	1	21.93	10.22	1.9 (0.6)
FB6 (M)				
U	10	0.1		2.3 (0.6)
C	1	10.37	2.02	3.4 (0.9)
FB7 (F)				
U	10	0.4		1.9 (0.4)
C	1	19.93	15.47	3.4 (0.9)
FB9 (F)				
U	10	1.1		3.1 (0.4)
C	1	16.43	15.45	2.1 (0.3)
FB10 (M)				
U	13	0.33		1.5 (0.7)
C	4	8.23	1.01	3.4 (0.7)
FB20 (M)				
U	5	0.17		3.2 (2.2)
C	2	6.03	8.99	2.3 (1.4)
FB92 (M)				
U	7	0.07		2.5 (0.7)
C	1	16.63	6.36	2.9 (0.6)
FB122 (M)				
U	3	0.57		2.5 (1.2)
C	2	21.4	11.62	2.4 (0.5)
FB131 (M)				
U	6	0.07		1 (0)
C	3	10.3	7.68	2.7 (0.5)
FB182 (M)				
U	7	0.07		2 (0)
C	4	12.13	6.36	3.8 (1.3)

higher than in the last 10 min of a capture–release session ( $W = -37.0$ ,  $d.f. = 17$ ,  $P = 0.04$ ; Table 2). When all whistles were considered (multiple connected and disconnected loops and single-loop whistles), loop duration was significantly shorter during the first 10 min of a session than during the last 10 min of a session ( $W = -0.54$ ,  $d.f. = 19$ ,  $P = 0.01$ ; Table 2). Whistle rate and interloop interval tended to decrease from the first 10 min to the last 10 min of a capture–release session, although these differences were not significant at a 0.05 level (whistle rate:  $W = -40.5$ ,  $d.f. = 19$ ,  $P = 0.06$ ; Table 2; interloop interval:  $W = -11.0$ ,  $d.f. = 10$ ,  $P = 0.08$ ). There were no significant differences in maximum or minimum frequency.

*Do whistle parameters vary between an individual's 1st capture–release session and later capture–release sessions?*—Whistle rate was significantly higher during an individual's 1st capture–release session than its 5th session ( $W = 50$ ,  $d.f. = 11$ ,  $P = 0.02$ ; Table 3). This significant decrease was

**TABLE 2.**—Contextual information, whistle rates, mean (*SD*) number of loops produced, and mean (*SD*) loop durations for the first 10 min and the last 10 min of a single capture–release recording for each of 20 bottlenose dolphins (*Tursiops truncatus*) recorded in Sarasota Bay, Florida. Sex (F = female; M = male) is included in parentheses.

Dolphin	Age (years)	Capture no.	Recording length (min)	Whistle rate (whistles/min)	Mean no. loops	Mean loop duration (s)
FB3 (F)	5	3	50			
First				9.3	5.0 (1.7)	0.44 (0.03)
Last				8.2	5.0 (1.1)	0.45 (0.02)
FB11 (F)	2.5	3	40			
First				13	3.5 (1.9)	0.32 (0.04)
Last				17.2	2.3 (0.5)	0.33 (0.03)
FB15 (F)	41	5	65			
First				9.2	2.3 (0.7)	0.32 (0.06)
Last				1.6	1.2 (0.4)	0.30 (0.04)
FB24 (M)	2	1	41			
First				1.9	1 (0)	0.94 (0.13)
Last				6.4	1 (0)	0.93 (0.11)
FB25 (F)	11	7	42			
First				13.2	1.8 (0.4)	0.51 (0.07)
Last				7	2 (0)	0.57 (0.09)
FB35 (F)	33	3	60			
First				5.4	1 (0)	0.87 (0.23)
Last				5.8	1(0)	0.98 (0.21)
FB38 (M)	20	8	71			
First				2.6	3.0 (0.8)	0.24 (0.02)
Last				4.3	2.2 (0.6)	0.28 (0.10)
FB48 (M)	29	3	125			
First				3.5	3.7 (1.3)	0.19 (0.07)
Last				1.1	3.8 (0.4)	0.21 (0.06)
FB54 (F)	24	9	84			
First				1.8	3.3 (1.4)	0.41 (0.06)
Last				6.5	2.1 (0.6)	0.39 (0.07)
FB55 (F)	16	12	48			
First				4.1	2.2 (0.6)	0.38 (0.05)
Last				3.7	2.5 (0.5)	0.44 (0.06)
FB67 (F)	21	4	40			
First				11.5	4.0 (0.7)	0.53 (0.02)
Last				5.9	3.1 (0.8)	0.58 (0.07)
FB71 (F)	29	8	50			
First				18.2	3.1 (0.3)	0.24 (0.02)
Last				3.3	3 (0)	0.25 (0.02)
FB84 (F)	32	6	68			
First				0.8	3 (0)	0.34 (0.09)
Last				1.7	2.3 (0.9)	0.33 (0.06)
FB92 (M)	1	1	40			
First				15.8	2.9 (0.6)	0.28 (0.05)
Last				15.6	2.6 (0.7)	0.31 (0.03)
FB101 (F)	10	3	77			
First				12.5	2.1 (0.7)	0.27 (0.06)
Last				2.7	2.6 (0.8)	0.36 (0.07)
FB118 (M)	2	1	136			
First				11.8	1.9 (0.8)	0.47 (0.07)
Last				4.2	2.8 (1.0)	0.42 (0.03)
FB122 (M)	2	1	103			
First				17.6	2.6 (0.5)	0.38 (0.04)
Last				20.5	1.9 (0.3)	0.43 (0.09)
FB140 (M)	7	1	40			
First				15.5	4.9 (1.5)	0.37 (0.08)
Last				4.7	4.9 (1.9)	0.37 (0.02)
FB166 (M)	4	1	42			
First				9	3 (0.7)	0.49 (0.06)

**TABLE 2.**—Continued.

Dolphin	Age (years)	Capture no.	Recording length (min)	Whistle rate (whistles/min)	Mean no. loops	Mean loop duration (s)
Last				5.7	2.2 (0.8)	0.47 (0.07)
FB186 (M)	3	1	61			
First				13.3	1.4 (0.5)	0.48 (0.11)
Last				14.7	1.5 (0.5)	0.55 (0.08)

**TABLE 3.**—Age, recording length, and whistle rates from 3 recording sessions for each of 12 bottlenose dolphins (*Tursiops truncatus*) recorded in Sarasota Bay, Florida. Sex (M = male; F = female) is included in parentheses.

Dolphin	Age (years)	Recording length (min)	Whistle rate (whistles/min)
FB6 (M)			
1st	1	30	10.37
3rd	3	30	8.8
5th	5	30	1.93
FB7 (F)			
1st	1	30	19.9
3rd	3	30	12.93
5th	5	30	2.93
FB9 (F)			
1st	1	25	16.72
3rd	3	25	8.36
5th	5	25	8.36
FB11 (F)			
1st	1	30	10.07
3rd	2.5	30	16.13
5th	3	30	14.93
FB32 (M)			
1st	4	25	1.56
3rd	6.5	25	0
5th	7.5	25	0
FB33 (F)			
1st	2	25	23.84
4th	5	25	6.36
5th	5.5	25	6.08
FB36 (M)			
1st	12	25	0
3rd	14.5	25	0.04
5th	16	25	1.68
FB38 (M)			
1st	10	25	1.08
3rd	12.5	25	0.56
5th	14	25	0
FB51 (F)			
1st	34	20	16.05
4th	46	20	5.6
7th	47	20	8.5
FB79 (F)			
1st	6	25	23.2
3rd	8	25	13.72
5th	9.5	25	5.4
FB118 (M)			
1st	2	25	18.84
3rd	7	25	0.04
5th	11	25	0.04
FB163 (F)			
1st	19	27	15.52
2nd	20	27	6.26
3rd	21	27	0.52

**TABLE 4.**—Contextual information, whistle rates, mean (*SD*) maximum frequencies, and mean (*SD*) interloop durations for recordings of 8 female bottlenose dolphins (*Tursiops truncatus*) caught and released in Sarasota Bay, Florida, when they had a dependent calf and when they did not have a dependent calf.

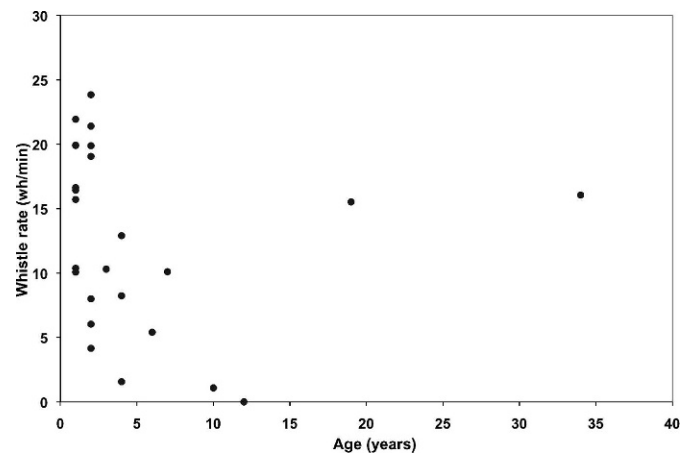
Dolphin	Age (years)	Capture no.	Recording length (min)	Whistle rate (whistles/min)	Mean frequency maximum (Hz)	Mean interloop duration (s)
<b>FB11</b>						
With calf	14	13	30	6.07	20,737 (1,867)	0.10 (0.01)
Without calf	3	5	30	14.93	18,678 (986)	0.18 (0.05)
<b>FB51</b>						
With calf	46	4	20	5.6	15,142 (1,741)	0.11 (0.03)
Without calf	47	7	20	8.5	14,997 (2,624)	0.17 (0.07)
<b>FB54</b>						
With calf	24	9	30	1.27	21,479 (2,754)	0.08 (0.02)
Without calf	17	6	30	1.83	17,546 (974)	0.12 (0.02)
<b>FB55</b>						
With calf	16	12	30	4.6	13,834 (2,560)	0.16 (0.01)
Without calf	7.5	11	30	4.3	14,581 (703)	0.19 (0.02)
<b>FB67</b>						
With calf	21	4	28	7.5	23,021 (2,040)	
Without calf	20	3	28	6.96	18,630 (963)	
<b>FB71</b>						
With calf	28	7	30	9.7	22,424 (3,805)	0.18 (0.04)
Without calf	29	8	30	7.03	20,358 (2,933)	0.22 (0.02)
<b>FB84</b>						
With calf	32	6	30	0.5	18,791 (1,343)	0.10 (0.02)
Without calf	43	7	30	1.13	17,908 (3,795)	0.11 (0.03)
<b>FB163</b>						
With calf	20	2	27	6.26	26,130 (1,908)	
Without calf	21	3	27	0.52	20,625 (2,123)	

preceded by a trend toward decreased whistle rate from an individual's 1st to 3rd capture–release session ( $W = 37.5$ ,  $d.f. = 11$ ,  $P = 0.07$ ; Table 3). The association between decreasing whistle rate with increasing capture–release experience was significant for juvenile animals only (juvenile:  $G = -0.65 \pm 0.34$  *SD*,  $P < 0.001$ ; adult:  $G = -0.31 \pm 0.51$  *SD*,  $P = 0.23$ ). There were no significant differences in maximum or minimum frequency, loop number, loop duration, or interloop duration across capture–release sessions.

*Do whistle parameters differ when a female is caught and released with a dependent calf versus when the same female does not have a dependent calf?*—Females captured and released with a dependent calf produced whistles with significantly higher maximum frequencies ( $W = -16.0$ ,  $d.f. = 7$ ,  $P = 0.01$ ; Table 4) and shorter interloop intervals ( $W = -10.50$ ,  $d.f. = 5$ ,  $P = 0.01$ ; Table 4) than the same females caught and released at times when they did not have dependent calves. There were no significant differences in whistle rate, loop number or duration, or minimum frequency.

Capture–release experience and age were both reliable predictors of whistle rate. Based on the generalized linear model, whistle rate decreased by 17.2% for each additional capture–release experience and by 4.2% with each year of age. However, the decrease in whistle rate with increasing capture–release experience was only significant for juvenile dolphins (juvenile:  $G = -0.65$ ,  $P < 0.001$ ; adult:  $G = -0.31$ ,  $P = 0.23$ ). In addition, whistle rate decreased more quickly with age for males than for females (males: Pearson  $r = -0.55$ ;

females: Pearson  $r = -0.40$ ). Overall, dolphins 2 years old and younger had significantly higher whistle rates than dolphins 3 years old or older during 1st capture–release recordings (Student's  $t$ -test,  $P = 0.005$ ) and over all capture–release recordings (Student's  $t$ -test,  $P < 0.0001$ ). However, when age and whistle rate were compared for 1st-time capture–release recordings only ( $n = 27$ ), 2 older dolphins had whistle rates similar to those of juveniles (Fig. 3).



**FIG. 3.**—Age versus whistle rate for 1st-time capture–release events of bottlenose dolphins (*Tursiops truncatus*) recorded in Sarasota Bay, Florida.



## DISCUSSION

Signature whistle rate appears to be the most promising potential vocal indicator of short-term stress in bottlenose dolphins. Signature whistle rate was significantly higher during capture–release than in undisturbed conditions, and higher during the 1st capture–release session than during subsequent sessions. A decrease in whistle rate throughout a single capture–release session (i.e., from the first 10 min to the last 10 min) also bordered on significance ( $P = 0.06$ ). Higher whistle rates during capture–release versus undisturbed conditions was expected to some degree, based on previous work by Cook et al. (2004), who found that signature whistles comprised 50% of the vocal repertoire of free-ranging bottlenose dolphins, as opposed to at least 90% of the vocal repertoire of temporarily restrained bottlenose dolphins (as described by Caldwell et al. [1990]). Watwood et al. (2005:1384) also measured higher production of signature whistles by dolphins in Sarasota Bay when temporarily restrained versus free-swimming, noting that the freely swimming individuals they observed “were most likely not experiencing the same level of disturbance and separation as the temporarily restrained animals.” The differences in signature whistle rates in our study ranged from 1 to 2 orders of magnitude, with mean whistle rates of 0.3 and 14.3 whistles/min during undisturbed and capture–release conditions, respectively. This difference could be even greater if any of the signature whistles identified in undisturbed recordings were imitations produced by a dolphin other than the individual with whom the signature whistle was associated (Janik 2000; Tyack 1986). Although it is assumed that dolphins in Sarasota Bay are relatively habituated to boat traffic, changes in interbreath interval, heading, swim speed, and whistle rate increases have been documented for dolphins approached by multiple boats (Buckstaff 2004; Nowacek et al. 2001). If boat traffic was a disturbance that induced increased whistle rates in the current study, then the actual difference in whistle rate between dolphins in truly undisturbed conditions and capture–release conditions would be even greater than that reported here. Although it is likely that the use of 1st-time capture–release recordings in these comparisons positively biased whistle rates calculated for capture–release conditions, age-corrected whistle rates predicted using regression analysis also were significantly higher during 1st-time capture–release than undisturbed conditions. In addition, whistle rates calculated for later sessions for several of these same individuals (FB6, FB7, and FB9; see Table 3) were all higher during capture–release conditions versus undisturbed conditions.

Loop number also was greater during capture–release versus undisturbed conditions, and in the first 10 min versus the last 10 min of the 1st capture–release session. These findings suggest that multilooped signature whistles may contain information about the signaler’s motivational state, as is the case with social signals of other species (Watts and Stookey 2001; Weary and Fraser 1995).

If the stress of capture–release contributed to the production of more whistles or loops, then habituation to the capture–

release context may have caused the decrease in whistle rate and loop number over the course of a single session. However, other (not mutually exclusive) factors could have caused the observed changes in whistle or loop production in the capture–release context. For example, increases could reflect an increased motivation to communicate rather than an increased stress level, and decreases over the course of a capture–release session could result from fatigue, rather than habituation. Alternatively, changes in whistle rate, loop number, or loop duration may in some way enhance communication with conspecifics; variations in these parameters might allow dolphins to communicate additional information while also conveying identity. Another factor that could have influenced the observed differences in whistle rate and loop number is the age of the animals. In the comparison between capture–release and undisturbed conditions, the 2 oldest animals were only 4 years old at the time of their capture–release recording, 1 animal was 3 years old, and 7 animals were <2 years old. In contrast, ages of the animals in the undisturbed recordings ranged from 3 to 13 years. However, despite the range in ages among dolphins recorded during undisturbed conditions, whistle rates were all very low (Table 1) and similar to whistle rates reported for free-ranging bottlenose dolphins by other studies (Buckstaff 2004; Cook et al. 2004; Jones and Sayigh 2002; Watwood et al. 2005). In addition, age-corrected whistle rates for 1st-time capture–release recordings were still significantly higher than whistle rates under undisturbed conditions. Finally, no relationship was detected between age and whistle rate for dolphins recorded under undisturbed conditions in this study.

When all 1st-time capture–releases were considered, dolphins that were 2 years old or younger when 1st captured and released whistled at significantly higher rates than dolphins that were 3 years or older at the time of 1st capture–release. A related factor possibly affecting differences in whistle rate between contexts was that 7 of the 10 animals were still dependent calves when captured and released, but only 1 (FB122) was still dependent on his mother during undisturbed observations. Because whistles are commonly used to maintain contact between mothers and calves (Sayigh et al. 1990), whistle rates may be higher in dependent than in independent calves.

The effect of experience on behavior during capture–release is quite evident among the Sarasota Bay dolphins. Inexperienced animals appear much more likely to attempt to escape from the net enclosure than those experienced with the capture–release process, and more often become briefly entangled as a result. Experienced dolphins tend to remain in the middle of the net corral and often are more easily restrained, without entanglement. It thus appears likely that dolphins recall previous capture–release experiences, and that this may influence their behavior in subsequent capture–releases. However, because very young dolphins (<2 years old) whistled at higher rates than older dolphins across all capture–release recordings, the decrease in whistle rate between capture–releases could be a result of maturation in

addition to capture–release experience. Because age and capture–release experience are significantly related to each other, it is difficult to tease apart the relative influence of these factors, and it is likely that both play a role. As discussed above, a related factor that could affect whistle rates of young dolphins is dependence on the mother. Of the 6 animals in the longitudinal study that were 2 years old or younger at the time of 1st capture–release, 5 were dependent calves. In many species, including bottlenose dolphins, young animals respond to isolation from their mother by vocalizing (e.g., piglets [Fraser 1995; Weary and Fraser 1995], guinea pigs [Monticelli et al. 2004], several rodent species [Okon 1971], cattle [Marchant-Forde et al. 2002], and bottlenose dolphins [Sayigh et al. 1990]). Therefore, the observed decrease in whistle rate with age may be related to a shift from dependence to independence in young dolphins. However, whistle rates were high for 2 older dolphins when only 1st-time capture–release recordings were considered (Fig. 3). Although the sample size of 1st-time capture–release recordings for older dolphins is small, this provides some evidence that whistle rates under 1st-time capture–release conditions can be as high in adult dolphins as in calves.

A final factor potentially influencing changes in whistle rate between capture–releases is sex. Of the 5 adult animals (females > 6 years old and males > 10 years old) included in the longitudinal data set, the mean whistle rate during the 1st capture–release of the 3 females was more than 30 times higher than the mean 1st capture–release whistle rate for both males. Females appear to maintain higher signature whistle rates than males across age classes and capture–release events, which may be related to their social structure. In Sarasota Bay, as bottlenose dolphin calves are weaned and reach independence, they leave their natal group and associate with other juveniles. Once sexual maturity is reached, females often interact with other females, including those from their natal group, whereas males usually travel alone, in pairs, or in very small groups (Scott et al. 1990). Therefore, females generally engage in more social interactions than males. Because signature whistles function as contact calls and in maintaining group cohesion (Caldwell and Caldwell 1965; Caldwell et al. 1990; Janik and Slater 1998; Sayigh et al. 2007), one would expect higher signature whistle production by females than by males. However, a larger sample size is needed to determine whether decreases in whistle rate across multiple capture–releases occur differentially in males and females and among age classes. Such sex and age differences are important to understand before whistle rates can potentially be used as indicators of stress.

Females caught and released with a dependent calf produced whistles with significantly higher maximum frequencies and significantly shorter interloop intervals than the same females caught and released at times when they had no dependent calves. Higher-frequency vocalizations are often produced in stressful situations. Pigs produce higher-frequency vocalizations in response to separation, handling, and pain (Weary et al. 1997). When isolated, guinea pig pups

(Monticelli et al. 2004) and squirrel monkey infants (Wiener et al. 1990) also produced calls with higher mean frequencies. Thus, the higher maximum frequencies observed in female bottlenose dolphins separated from their dependent calves could be related to the stressful nature of this situation. It is unlikely that the frequency difference is a product of maturation, because the mean age of females caught with a dependent calf (28 years) was similar to that when they were caught without a dependent calf (24 years). Of the 16 recordings analyzed, 15 were made when the female was mature (i.e., >6 years old). It also is unlikely that differences in maximum frequency are due to different amounts of previous capture–release experience, because females in both groups had similar amounts of experience (mean capture–release number with a dependent calf = 7.1; without a dependent calf = 6.3). A possible factor contributing to the observed difference in maximum frequency could be that higher-frequency sounds are easier to localize (Heffner and Heffner 1992), and thus may aid in the reunion of females and their calves when they are separated in the wild. Higher-frequency sounds attenuate more quickly in a marine environment, but may serve to broadcast location more effectively over short distances. Calves are likely to be close associates of adult females, minimizing the need to broadcast signals over long distances. Further research is needed into the abilities of dolphins to localize sounds of varying frequencies in a variety of environmental conditions in order to test this idea.

The shorter interloop intervals observed in the whistles of females caught and released with dependent calves resulted in faster repetitions of loops, but did not coincide with higher loop number. These faster repetitions may combine with higher maximum frequency to convey an alternative type of information than that conveyed by whistle rate or loop number. Female dolphins showed no significant difference in whistle rate or number of loops when with or without a dependent calf. Most of the females had extensive previous experience with capture–release, possibly resulting in reduced whistle rates independent of calf presence or absence. Of the 16 recording sessions analyzed, 11 were at least the 5th capture–release for that individual. For those dolphins that had been captured and released fewer than 5 times ( $n = 3$ ), the 2 recordings used were not separated by more than 1 year to control for other factors that might influence whistle rate between recordings, such as maturation and habituation. However, 14 of the 16 recordings were obtained when the subject was at least 14 years old, beyond the age at which female bottlenose dolphins are fully mature (Wells and Scott 1999). Thus, both age and capture–release experience may have influenced the lower whistle rates and loop numbers in these mature females.

Further experiments are necessary in order to tease apart alternative explanations for the observed changes in whistle rate and loop number. Both the fact that loop number did not change as a function of capture experience, and that not all dolphins produce multiloop whistles, indicate that loop

number may not be as useful an indicator of stress as whistle rate. However, loop number is worthy of further investigation, given that it was greater during capture–release versus undisturbed conditions, and in the first 10 min versus the last 10 min of a capture–release session. Although loop duration was significantly shorter during the first 10 min versus the last 10 min of a capture–release session, this parameter did not differ in the other conditions, thus limiting its overall usefulness as an indicator of stress. The same is true of changes in frequency and interloop duration of whistles, which occurred only in females caught and released with and without a dependent calf.

The results of this study indicate that although significant changes in whistle parameters relative to a dolphin's involvement in capture–release operations can be detected, there is no indication of any long-term adverse impact. If indeed changes are due to stress, our findings suggest that habituation occurs within a capture–release session, and from 1 session to the next. Examination of data on multigenerational residency and social structure stability (Wells 2003) also suggests that the Sarasota dolphin community is not experiencing any adverse long-term impacts from the capture–release program.

Future work should include comparisons of signature whistle rate and loop number in free-ranging dolphins in the presence and absence of potential sources of disturbance, such as jet-skis, predators, or swimmers participating in swim-with-dolphins programs. Buckstaff (2004) evaluated the influence of watercraft noise on the acoustic behavior of the resident bottlenose dolphins in Sarasota Bay, and determined that whistle rate (signature and nonsignature whistles per dolphin per minute) increased at the onset of a vessel approach and decreased once the vessel passed (onset of vessel approach = 1.4 whistles/dolphin/min, no vessels present = 0.7 whistles/dolphin/min). Watwood et al. (2005) evaluated signature and variant, or nonsignature, whistle production in male bottlenose dolphins in Sarasota Bay. Whistle dictionaries identifying whistle types produced by known individuals were constructed, and the rate of production of each whistle type was compared between capture–release and free-swimming conditions. Watwood et al. (2005) reported significantly lower signature whistle rates for individuals that were freely swimming versus temporarily restrained. The maximum reported whistle rate for free-swimming dolphins was 0.4 whistles/dolphin/min, comparable to the low whistle rate values reported in the current study. In a comparison of whistle production by dolphins in Sarasota Bay among multiple behavioral categories (e.g., feeding, milling, milling and traveling, resting, and socializing), Cook et al. (2004) determined an average whistle rate of 1.89 whistles/5 min, or 0.38 whistles/min (which is exactly in line with the findings of Watwood et al. [2005]). Finally, Jones and Sayigh (2002) compared bottlenose dolphin whistle production among 4 regions (Sarasota Bay and 3 regions in southeastern North Carolina) and across activities (e.g., feed, mill, mill and travel, social, travel, with boat, and with shrimp boat), and reported a

maximum whistle rate of approximately 1.2 whistles/dolphin/min. Although these studies included both signature and nonsignature whistles, all documented much lower whistle rates than occurred in capture–release recordings (Watwood et al. 2005; this study). Therefore, disturbance may induce an overall increase in whistle rate, as observed by Buckstaff (2004), as well as an increase in the proportion of whistles that are signature whistles. Signature whistle rates determined for captive bottlenose dolphins could serve as a baseline for comparison so that loop number and whistle rate could be monitored for dolphins in captive facilities that are undergoing construction or habitat alterations, or both before and after a hurricane or some other predicted weather event. The introduction of a new dolphin into a captive facility might create stress for both the new and established dolphin(s); whistle rate and loop number could therefore potentially provide information about the process of acclimation for newly acquired dolphins.

If the patterns in whistle parameters detected in this study persist once effects such as age and previous capture–release experience have been better quantified, these measures could be utilized in conjunction with physiological indicators to ground-truth their reliability as indicators of various types of stressors in bottlenose dolphins. Age-corrected signature whistle production was 1–2 orders of magnitude higher in dolphins during capture–release versus undisturbed conditions, evidence of a vocal response to short-term stress. The lack of relationship between age and whistle rate for freely swimming dolphins in this study suggests that decreases in whistle rate with increasing capture–release experience may reflect a behaviorally moderated response to the potential stress of capture–release, and that decreasing whistle rate is not simply the result of maturation. Although more work is needed, this study indicates that acoustic monitoring holds promise as a noninvasive means of assessing the impact of potentially stressful situations on bottlenose dolphins.

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