WHISTLES AS POTENTIAL INDICATORS OF STRESS IN
BOTTLENOSE DOLPHINS (*Tursiops truncatus*)

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Abstract

Stress has been defined as an environmental effect on an individual that overtaxes its functional abilities. The diversity of stress responses among marine mammals makes it difficult to develop a comprehensive diagnostic protocol to evaluate stress. The development of a relatively non-invasive tool with which to evaluate stress in bottlenose dolphins (*Tursiops truncatus*) could allow for assessments of animals that may be at risk, and assessment of free-ranging animals without capture-release. The goal of this study was to evaluate whether vocalizations, specifically signature whistles, could serve as possible indicators of acute (or short-term) stress in bottlenose dolphins. Recordings made during brief capture-release events and during focal follows of undisturbed animals in Sarasota Bay, FL, were used to address this question. Although there is no evidence that capture-release events have any long or short term adverse impacts on members of the Sarasota dolphin community, it is likely that they are a source of short-term stress to the dolphins. I asked the following questions:

Will whistle rates and number of loops (repetitive elements in whistles) be greater:

(1) during capture-release than during undisturbed focal follows?

(2) at the beginning of a capture-release session than at the end of a session?

(3) during an individual's first capture-release session than during later sessions?

(4) when a mother is caught and released with a dependent calf than without a dependent calf?

I also examined whether the duration of loops and/or inter-loop intervals, and maximum and minimum frequency of whistles change in any of the above contexts. Loop number was significantly higher during capture-release than during focal follows, and decreased significantly from the beginning to the end of an individual's capture-release session. Loop duration was

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significantly shorter at the beginning than at the end of an individual session. Whistle rate was also significantly higher during capture-release than during focal follows, and during a dolphin's first capture-release than during subsequent sessions. Females caught with a dependent calf produced whistles with significantly higher maximum frequencies and shorter inter-loop intervals than when caught and released without a dependent calf. Based on the results of this study, further research would be warranted on assessing the utility of signature whistle rate and loop number as behavioral indicators of short-term stress in bottlenose dolphins. These measures could potentially be utilized independently, or in conjunction with physiological indicators, in assessments of the impact of potentially stressful human activities on bottlenose dolphins.

ACKNOWLEDGEMENTS

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INTRODUCTION

Stress has been defined as an environmental effect on an individual that overtaxes its functional abilities, potentially reducing its fitness (Broom and Johnson 1993). 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 can be either acute or chronic. Acute stress may have positive physiological effects and can potentially increase survivorship, analogous to the way that strenuous exercise can be strengthening (St. Aubin and Dierauf 2001). However, chronic stress can in some cases have 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). The diversity of stress responses among marine mammals makes it difficult to develop a comprehensive diagnostic protocol to evaluate stress (St. Aubin and Dierauf 2001). Establishing criteria by which to evaluate stress in dolphins was prioritized as a result of a 1997 amendment to the Marine Mammal Protection Act (MMPA) that created the International Dolphin Conservation Program Act (IDCP Act, U.S. 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 (ETP) tuna purse-seine fishery. Repeated capture-release is a potential source of chronic stress for spotted (*Stenella attenuata*) and spinner dolphins (*Stenella longirostris*) in the ETP (Curry 1999), and policy makers have become concerned that this chronic stress may have detrimental effects on the reproductive success and overall success of these species. Thus, the National Marine Fisheries Service (NMFS) recently requested a review of the current knowledge of stress in

marine mammals, resulting in a comprehensive evaluation of the tools that are currently used to assess stress response in many marine mammals (not only *S. attenuata* and *S. longirostris*; e.g. Curry 1999; St. Aubin 2002; Pabst et al. 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.

Typically, stress hormone profiles are produced from blood samples drawn from restrained animals (stranded, temporary capture-release). 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, non-human primates, pigs, cattle; Grandin 1997; Sapolsky 1987, 1993; Clarke 1991). Correlations are often made between cortisol levels and alternative indicators of stress such as heart rate or serum enzyme levels (Curry 1999). In bottlenose dolphins (*Tursiops truncatus*), attempts have been made to correlate stress hormones with behavioral state, but no consistent relationship has emerged (Curry 1999; Frohoff 2004; St. Aubin and Dierauf 2001). Thomson and Geraci (1986) compared cortisol levels in extensively chased and calm-capture-release (minimally chased) dolphins at captive facilities in the Florida Keys. All of the dolphins evaluated in their study had been in captivity for at least one year, and had been handled frequently. A stress response was observed in response to capture-release and handling even under the calmest conditions. The authors reported that cortisol levels peaked shortly after the capture-release process was initiated, and thus showed little elevation in subsequent blood samples. In contrast, members of the resident population of bottlenose dolphins in Sarasota Bay, FL, and bottlenose dolphins captured and released in Beaufort, NC, in 1995 appeared to exhibit only modest elevations in cortisol in

response to capture-release (St. Aubin et al. 1996; Ortiz and Worthy 2000). Although cortisol is the most often analyzed stress response hormone, elevated levels of glucocorticoids (e.g. cortisol and corticosterone) can occur in non-stressful situations as well (Bercovitch et al. 1995). In addition, stress hormones require the acquisition of blood samples, a process that itself may confer stress. Thus, cortisol may not be a reliable indicator of stress response in cetaceans (St. Aubin and Dierauf 2001).

St. Aubin and Dierauf (2001) called for the development of new and creative diagnostic tests that could be used to consistently detect stress in marine mammals. The development of a passive means by which to evaluate stress in dolphins could allow for more efficient and comprehensive assessments of animals that may be at risk. For example, behavioral observations have been used to assess unrestrained dolphins (captive, free-swimming; Frohoff et al. 2004; Santurtun and Galindo 2002). Behaviors that were thought to be indicative of stress included submission to humans and/or other dolphins, agitation, or aggression (Frohoff et al. 2004). Loss of appetite, and changes in respiratory rate, dive and surfacing patterns, postures, and vocalizations have all been postulated as short term responses to stress in dolphins (Frohoff et al. 2004). My study aimed to assess the potential of using vocal rates and/or other vocal parameters as a non-invasive means to evaluate stress in bottlenose dolphins.

Vocal responses to stress are well documented, particularly for some domestic animals (e.g. pigs, cattle, and sheep), all of which share a common ancestry with cetaceans (reviewed in Thewissen 1994). 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. They determined that cattle (*Bos taurus*) vocalized significantly more while being branded than mock-branded, and that branded

cattle produced vocalizations with a greater frequency range and a higher maximum frequency. Vocal rates appeared to be most affected by severe stressors (e.g. branding), while remaining relatively insensitive to moderate stressors (e.g. restraint). This finding contrasts with cortisol and/or heart rate responses, which can elevate in response to normal handling (Watts and Stookey 1999; Thomson and Geraci 1986).

Watts and Stookey (1999) suggested that the type of vocalization (determined by measurement of physical parameters such as frequency or duration), in addition to the rate of vocalization, may communicate information about an animal's experience. In particular, they found that cattle produced sounds that were context specific, and that vocal rate could vary by individual as well as by context. The subjects were recorded while being processed by veterinary staff as well as while in isolation. Calls produced in isolation showed less variation in frequency and were shorter than those produced during handling (Watts and Stookey 2001). Weary et al. (1997) also found that an animal's vocal production may provide useful information about its behavioral state. Domestic piglets (*Sus scrofa domestica*) were found to vocalize more often when isolated, and used higher maximum frequencies and longer call durations when kept in a thermally stressful environment (14°C, well below thermoneutrality for young piglets). Hillman et al. (2004) found that domestic pigs increased production of high-frequency vocalizations when exposed to minor thermal stress. They did not, however, find any correlation between acoustic response and saliva cortisol concentrations. Hillman et al. (2004) proposed that an automatic acoustic monitoring system could be employed to monitor the impacts of climate on the welfare of pigs in livestock housing systems. Grandin (1998) used vocal response to monitor welfare issues in slaughterhouses and the improvements that resulted from modifications to slaughterhouse practices. Because vocal response is variable among

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individuals, Grandin (1998) used the proportion of cattle vocalizing, rather than an individual's vocal rate, as an indicator of the condition of the overall population in slaughterhouses. Following an initial assessment of vocal rate, Grandin made recommendations (e.g. reduction of voltage in electric cattle prods, loosening of restraint collars) aimed at reducing the level of discomfort for the cattle. Vocal rates decreased substantially following the slaughterhouses' compliance with Grandin's recommendations.

Other species also appear to vary vocal rate in response to stress. Bercovitch et al. (1995) found that female rhesus macaques (*Macaca mulatta*) were less likely to respond to moderate threats (e.g. mock capture-release, approach of a quasi-independent infant) with alarm calls when cortisol levels were suppressed (Bercovitch et al 1995). In addition, alarm calls produced by test subjects with suppressed cortisol levels were low intensity pant calls rather than high intensity shrill calls. Monticelli et al. (2004) measured whistle rate and whistle parameters (e.g. frequency maximum and minimum, duration, and number of harmonics) of guinea pig (*Cavia porcellus*) pups throughout a 15 minute isolation period. They found a significant decrease in whistle rate and whistle duration from the first 30 notes to the last 30 notes produced, presumably reflecting habituation to the isolation context. The guinea pig pups also increased the mean frequency of whistle notes from the beginning to the end of the isolation period. These changes are similar to those seen in isolated common marmoset (*Callithrix jacchus*) pups (Newman and Goedeking 1992). In contrast, Wiener et al. (1990) found that squirrel monkey (*Saimiri sciureus*) infants that were completely separated from the mother (versus maintaining visual contact with the mother) produced fewer vocalizations than when they were allowed visual contact with the mother. However, vocalizations that occurred during complete separation had higher mean frequencies than those produced when visual contact was allowed. Complete separation also

resulted in higher plasma cortisol levels in these squirrel monkey infants.

Rahurkar et al. (2002) examined characteristics of certain frequency bands in human speech and attempted to use those characteristics to distinguish between neutral and stressed speech. The study was based on prior research that used a Teager Energy Operator, a voice stress detection program used in both military and law enforcement applications. Rahurkar et al. (2002) hypothesized that certain frequency bands may be more sensitive to stress (for all speakers), and could therefore be used to assess stress in speech. Heart rate and blood pressure were evaluated to confirm that the subjects were experiencing a physiological response to the stressor, and the subjects' spoken responses to questions were recorded as the physiological readings were taken. The authors determined that four particular frequency bands were highly affected by stress. As a result, these bands were more heavily weighted during analysis by the detection scheme to more effectively and efficiently determine stressed speech in humans.

Lilly (1963) was the first to propose that dolphin vocalizations may serve as indicators of stress. In particular, he suggested that dolphins produced a specific whistle contour when stressed, which he called a "distress whistle", and to which other dolphins responded by offering aid. Frohoff et al. (2004) also claimed that the type of vocalizations produced by bottlenose dolphins appeared to vary in response to stressors (isolation, relocation, entanglement, etc.), as did vocal rate. Although Caldwell et al. (1990) did not find evidence for "distress whistles" (see below), they did find that certain parameters of dolphin whistles (e.g. number of loops, duration of loops) appeared to be closely related to the level of arousal, or behavioral stimulation, of an individual dolphin. If vocal parameters are correlated with stress, this could provide a reliable, non-invasive method by which to evaluate stress in dolphins. The goal of this study was to determine whether aspects of whistle production in bottlenose dolphins (as

described in more detail below) may serve as reliable indicators of acute (or short term) stress.

Bottlenose Dolphin Whistles

The variety of vocalizations bottlenose dolphins employ includes broad-band burst pulsed sounds, broad-band clicks used in echolocation, and narrow-band frequency modulated whistles (Caldwell et al. 1990; Herman and Tavolga 1980). For an individual dolphin, the third category includes highly stereotyped whistles as well as more variable contours (or variants; Tyack 1986; Sayigh et al. 1990). For nearly 40 years, the term "signature whistle" has been used to refer to highly stereotyped contours in a dolphin's repertoire. This term was the result of work by David and Melba Caldwell (Caldwell and Caldwell 1965; Caldwell et al. 1990), who observed that isolated, captive dolphins produced whistles that exhibited individually distinctive characteristics, which were hypothesized to transmit identity information. Approximately 90% of whistles produced by isolated animals, both in captivity and in the wild, have been found to be individually distinctive (Caldwell and Caldwell 1968, 1979, Caldwell et al. 1990; Sayigh et al. 1990), and approximately 50% of whistles produced by freely swimming wild bottlenose dolphins in Sarasota Bay, FL, are signature whistles (or probable signature whistles; Cook et al. 2004).

Signature whistles appear to play an important role in social interactions (Caldwell and Caldwell 1965; Caldwell et al. 1990) and in maintaining group cohesion (Janik and Slater 1998). The individually distinctive nature of signature whistles provides a mechanism for individual recognition among conspecifics, a function that has been demonstrated in playback experiments in which dolphins showed a stronger response to whistles of kin vs. familiar non-kin (Sayigh et al. 1999). Janik (2000) speculated that whistle matching, observed in wild bottlenose dolphins of

Moray Firth, Scotland, may allow individuals to address one another. Janik and Slater (1998) found that captive dolphins produced signature whistles only when separated from group members, and Smolker et al. (1993) found that mother-calf reunions in Shark Bay, Australia, were often preceded by whistling; both of these studies support the role of signature whistles in maintaining group cohesion.

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 (duration and maximum or minimum frequency; Caldwell et al. 1990; Sayigh et al. 1990). Signature whistles may consist of a single element (or loop), or repeated loops (disconnected and/or connected) which may be accompanied by an introductory and/or terminal loop (Figure 1, Caldwell et al. 1990; Sayigh et al. 1990). Duration changes often result from variable repetition of loops (Esch and Sayigh, in prep.). The number and duration of these loops has been suggested to vary according to a dolphin's level of arousal (Caldwell et al. 1990). Thus, I evaluated these parameters, in addition to whistle rate, inter-loop duration, and maximum or minimum frequency, as potential indicators of acute (or short term) stress in bottlenose dolphins.

Whistles recorded during a health monitoring project in Sarasota Bay, FL, 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 dolphins are encircled by a net, and then either held in the water or transported onto the shaded, padded deck of a boat by stretcher. Although there is no evidence that these capture-release events have any long or short term adverse impacts on members of the Sarasota dolphin community, it is likely that they are 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 address the following questions, which are described in
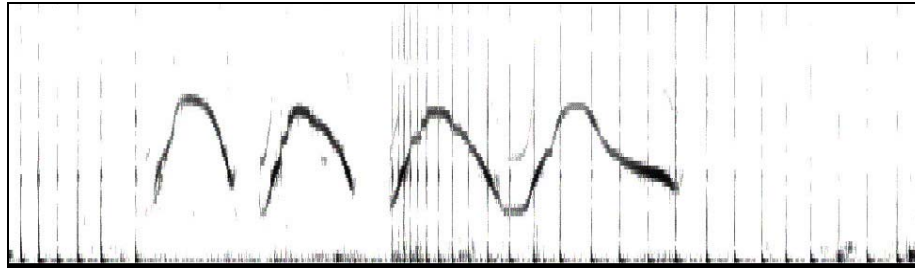
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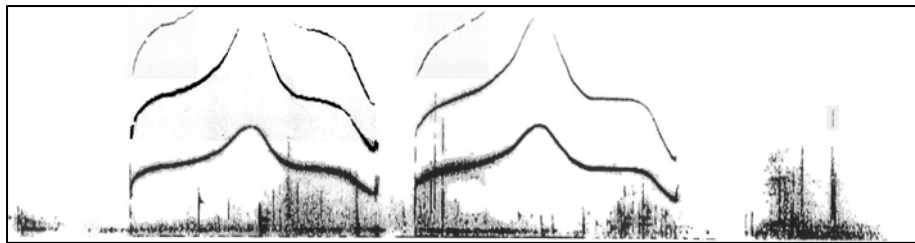
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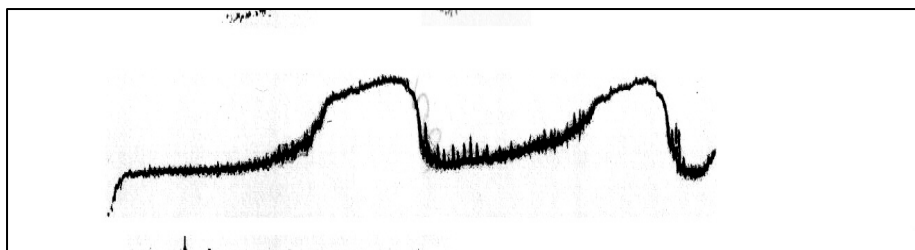
c. Loops always connected
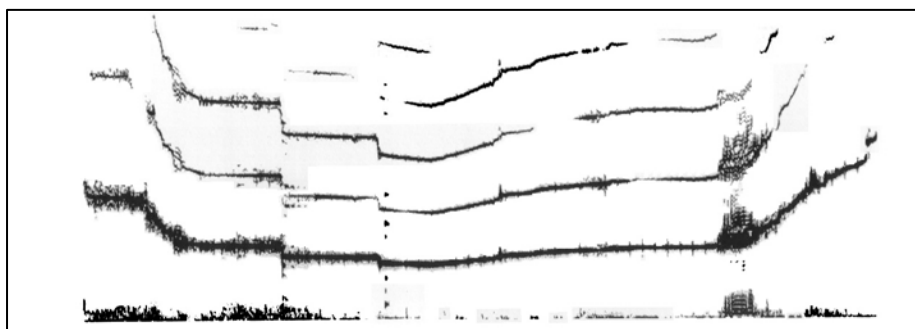
d. No repetitive loop structure

Figure 1. Example of each of four signature whistle types

more detail below: Will whistle rates and number of loops will be greater (1) during capture-release than during undisturbed focal follows; (2) at the beginning of a capture-release session than at the end of a session; (3) during an individual's first capture-release session than during later capture-release sessions; (4) when a mother is caught and released with a dependent calf than without a dependent calf? If signature whistle rates and loop number increase in stressful conditions, then these parameters would be higher during capture-release than during undisturbed focal follows, as outlined in research question (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 research question (2), and over the course of several capture-release sessions, as addressed in research question (3). Since temporary separation from a dependent calf could be stressful to a mother, I also examined whether mothers with dependent calves produced more whistles, as outlined in research question (4). In addition, I asked whether dolphins would produce shorter loops and inter-loop intervals in each of the contexts outlined above. Maximum and minimum frequencies were measured for all whistles to determine if these parameters vary by context.

METHODS

Recordings from brief capture-release events in Sarasota Bay, FL (Scott et al. 1990; Wells 1991, 2003; Wells et al. 2004), have been collected over a period of 30 years (1975-2005), and many dolphins have been recorded multiple times (minimum = 1, maximum = 15, mean = 3.3). Most animals are of known age, which is determined primarily from observing multiple generations of dolphins over the course of the study (Wells 2003), or in some cases by examination of tooth growth layer groups (Hohn et al. 1989; Hohn 1990). Recordings were

made with suction cup hydrophones placed directly on the head of each individual, allowing researchers to unequivocally identify the vocalizing dolphin. The predominant whistle produced by an animal during a capture-release event is defined as its signature whistle. The Sarasota Dolphin Community Signature Whistle Catalogue (Sayigh, unpublished data) currently contains signature whistles from 205 dolphins. Since most dolphins in Sarasota Bay have been captured and released more than once, signature whistle identification for an individual has been confirmed by reviewing multiple recordings for a single animal.

Dolphins in Sarasota Bay have also been recorded under undisturbed conditions. Free-swimming, known individuals were recorded during approximately 145 hours of focal follows conducted during May-August 1994 and May-July 1995 (Cook et al. 2004). Localization of the source of whistles recorded in these follows was not possible. However, Cook et al. (2004) were able to match whistles of identified individuals present during follows to whistles produced by the same individuals during capture-release events. This dataset of identified signature whistles produced during follows was used in the current study.

For each of the research questions being addressed, whistle rate was determined by evaluating the number of signature whistles produced per minute. Using only signature whistles (i.e., no variant whistles) was essential to addressing whether whistle rate will be higher in a capture-release setting than in undisturbed conditions, because Cook et al. (2004) were not able to associate variant (non-signature) whistles with a particular individual. Thus variants were excluded from the current analysis of individual whistle rate variations between capture-release and undisturbed conditions. To maintain consistency, only signature whistles were considered when determining whistle rate during capture-release for all four research questions. Using only signature whistles also increases the potential utility of this approach, if it were to be used in the

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future to assess stress levels of free-swimming dolphins. In many cases, signature whistles can be identified from recordings of free-swimming dolphins, but non-stereotyped variant whistles are more difficult to attribute to particular individuals.

Recording selection

The capture-release process begins with the deployment of a net around the animal(s), followed by restraint and a series of health assessment and sampling procedures during which dolphin vocalizations were recorded. For this study, only recordings that began no more than 25 minutes after capture-release and 10 minutes 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 this. The selection of recordings for analysis was limited by several criteria. First, continuous recordings were prioritized. Depending on the nature of the capture-release event (e.g., number and behavior of animal(s) caught), recordings were often interrupted due to the need to move an animal or move a hydrophone to another animal, or because of an animal's level of activity, which sometimes interfered with hydrophone attachment. In addition, acoustic playback experiments were sometimes conducted during recording sessions. Because these experiments may elicit a vocal response from the study animal, recordings containing acoustic playbacks were excluded. I used only continuous (uninterrupted) recordings, 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 minutes or longer were used. This was the longest recording time consistently available that also allowed for a sufficient sample size. Recordings used to address the other three research questions ranged from 20-30 minutes. Comparisons were made only between recordings of equal length. For instance, a

whistle rate calculated from a 25 minute recording was compared to a whistle rate based on another 25 minute recording.

Most recordings were prepared by digitizing the original analog reels (VHS and cassette) using a Sound Devices 744T digital recorder (sampling frequency 96kHz, 24-bit, Sound Devices, LLC, Reedsburg, WI, USA). This process produced digital files that were then analyzed using Avisoft-SASLab Pro 3.2 (Raimund Specht, Berlin, Germany) sound analysis software, although several recordings were analyzed using Signal/RTSD software (version 3.0, Engineering Design, Belmont, MA, USA). Each recording was manually reviewed, and every signature whistle was noted. For each recording, whistles were then randomly selected (using a random number table generated in MS-EXCEL) 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 less than the amount selected for sampling. In these instances, comparisons of whistle parameters were based on means calculated from unequal sample sizes. The physical parameters evaluated included maximum and minimum frequency, duration (loop and inter-loop when available), and the number of loops produced. Time and frequency measurements were made manually using the cursor functions in each acoustic analysis software system.

Sayigh (unpublished data) described four categories of signature whistles (see examples in Figure 1): 1. loops sometimes connected, sometimes not (perhaps affected by recording conditions), loops may vary in number and/or contour; 2. loops always disconnected, may vary in number and/or contour; 3. loops always connected, may vary in number and/or contour; 4. no repetitive loop structure. To address whether the number and duration of loops would differ between contexts (as outlined in the research questions), recordings of dolphins that were known

to produce multi-looped whistles (categories 1-3) were among those selected for analysis (30 of 34 animals). Of the 205 dolphins represented in the Sarasota Dolphin Community Signature Whistle Catalogue, 150 produce multi-looped whistles. However, loop duration was measured only for whistles with single loops (e.g. Figure 1d; 4 out of 30 whistles in my samples) and disconnected loops (e.g. Figure 1b; 20 out of 30 multilooped whistles in my sample). For whistles with multiple connected loops (e.g. Figure 1c; 10 out of 30 multilooped whistles in my sample), loop duration was determined by dividing total whistle duration by the number of loops produced.

Whistle rates and whistle selection

Whistle rate calculation and whistle selection were specific to each research question, as described below. Physical parameters (number of loops, loop and inter-loop duration, maximum and minimum frequency) were measured, and compared between contexts. Statistical analyses are described in the following section.

1. Will whistle rate and number of loops be greater during capture-release than in undisturbed conditions?

A total of 20 recordings (two from each of 10 dolphins, 7 male and 3 female) was analyzed. For each of the ten dolphins, a focal follow recording and a first time capture-release session were selected. The sample size was limited by the number of animals whose vocalizations were positively identified during focal follows by Cook et al. (2004). First capture-release sessions were used to standardize the analysis. Whistle rates during capture-release were based on the first 30 minutes of an animal's first recording session. Whistle rates under

undisturbed conditions were calculated from the 30 minutes following the first identification of

an animal's signature whistle during a focal follow. 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, four produced multiple connected loop whistles and six produced

multiple disconnected loop whistles.

2. Will whistle rates and number of loops be greater at the beginning of a capture-release session than at its end?

A total of 20 recordings (one from each of 20 dolphins, 9 male and 11 female) was

analyzed, with a minimum recording length of 40 minutes for each (range = 40 – 136 min, mean

= 64). Whistle rates during the first 10 minutes of a session were compared to whistle rates

during the last 10 minutes of a session. Ten whistles were randomly selected from each time

period for parameter measurements. Of the 20 dolphins included in these analyses, two

produced single loop whistles, seven produced multiple connected loop whistles, and 11

produced multiple disconnected loop whistles.

3. Will whistle rates and number of loops be greater during an individual's first capture-release session than during later capture-release sessions?

A total of 30 recordings (three from each of ten dolphins, 5 male and 5 female) was used.

Dolphins were included in this portion of the study if they had been recorded at least five times

(49 of 205 dolphins in the recording library). Recordings from the first, third and fifth capture-

release session were selected for each dolphin. In one case (FB33), the third capture-release

recording was not suitable for analysis, and the fourth capture-release recording was used

instead. The following criteria were developed in order to standardize the analyses: recordings

were 25-30 minutes in duration, they were not separated by more than five years, and the first

recording was obtained when the subject was a juvenile (<8 years old). However, in two cases,

three recordings from each of two mature animals were analyzed (FB36 and FB38), for the

purpose of comparison. These two individuals were 12 and 10 years old, respectively, at the

time of their first capture-release session.

Whistle rates were calculated for each recording and compared among the three capture-

release sessions for each animal. Twenty whistles were randomly selected from each recording

and measured. Of the 10 dolphins included in these analyses, two produced single loop whistles,

one produced multiple connected loop whistles, and seven produced multiple disconnected loop

whistles.

**4. Will whistle rates and number of loops be greater when a mother is caught and released
with a dependent calf than without a dependent calf?**

For each of eight individual females, two recordings were analyzed. The two recordings

sessions analyzed were collected when female was captured and released (1) with a dependent

calf and (2) without a dependent calf. Recordings ranged from 20-30 minutes in duration. First

time capture-release sessions were excluded from these analyses, as first time capture-releases

may influence whistle rates (refer to second research question). Whistle rates were calculated for

each recording. Twenty whistles were randomly selected from each recording for parameter

measurements. Of the eight dolphins included in these analyses, two produced multiple

connected loop whistles, and six produced multiple disconnected loop whistles.

Statistics

Wilcoxon signed-rank tests (JMPIN version 4.0.2) were used to determine whether whistle rates were higher in one context versus another (e.g. at the beginning versus at the end of a capture-release session). Significant results were those with $p < 0.05$. In addition, plots of whistle rate over the course of each recording were generated using SASLab to evaluate changes in whistle rate over time. Wilcoxon signed-rank tests were also used to assess differences in mean parameter measurements. Linear regressions and Spearman's correlation coefficients were calculated to investigate potential relationships between whistle rate and (a) age, (b) sex, (c) the number of conspecifics present during a capture-release session, and (d) capture-release number. All of the recordings used in this study (total dolphins = 34; total recordings = 76) were included in these additional analyses.

RESULTS

1. Will whistle rate and number of loops be greater during capture-release than in undisturbed conditions?

Whistle rate and number of loops were both significantly higher during capture- release than during focal follows (whistle rate: $p = 0.001$, $W = -27.5$, $df = 9$, Table 1, Figure 2; number of loops: $p = 0.05$, $W = -16.5$, $df = 9$, Table 2). No significant differences were found for loop and inter-loop duration, or maximum and minimum frequency.

Whistle rate was not correlated with the number of conspecifics a dolphin was in association with across all of the recordings (capture-releases: $n = 76$, Spearman's $r = -0.14$, $p = 0.20$, Figure 3; follows: $n = 10$, Spearman's $r = 0.045$, $p = 0.90$, Figure 4).

Table 1. Contextual information and whistle rates for focal follow (F) and first time capture-release (C) sessions for each of 10 dolphins (gender is in parentheses).

FB#	Age (yrs)	ID #'s of conspecifics present	Recording length (min)	Whistle rate (wh/min)
FB2 (M)				
F	4	34,59(mom),62,131	30	0.27
C	1	14,59(mom),94	30	21.93
FB6 (M)				
F	10	59	30	0.1
C	1	51&calf,71(mom)	30	10.37
FB7 (F)				
F	9	9,84(mom),65,90,122,c652	30	0.4
C	1	4,15,61,84(mom),90	30	19.93
FB9 (F)				
F	9	7,48,65,90,122,c652	30	1.1
C	1	10,63(mom),79	30	16.43
FB10 (M)				
F	13	Check	30	0.33
C	4	9,63(mom),79	30	8.23
FB20 (M)				
F	5	25,59,75,131	30	0.17
C	2	89(escaped)	30	6.03
FB92 (M)				
F	7	2,34,59,62	30	0.07
C	1	84(mom)	30	16.63
FB122 (M)				
F	3	48,90(mom)	30	0.57
C	2	90(mom)	30	21.4
FB131 (F)				
F	7	20,25,59(mom),75	30	0.07
C	3	44,183	30	10.3
FB182 (M)				
F	6	20,25,59,75,111,131	30	0.07
C	4	Alone	30	12.13

Figure 2. Comparison of changes in whistle rate between first capture-release and focal follow recordings for each of ten dolphins. The absence of a curve or a break in the curve indicates a whistle rate of 0 wh/min. (capture-release = black, follows = red)

Table 2. Mean (SD) physical parameter measurements for a) multiple disconnected loop and b) multiple connected loop whistles from follow (F) and first capture-release recordings (C).

FB#	Freq.max. (Hz)	Freq.min. (Hz)	Loop Duration (sec)	Inter-loop duration (sec)	# of loops	# of whistles
FB2						
F	15380 (1607)	6000 (825)	0.54 (.35)	0.04 (0)	1.8 (.8)	9
C	15849 (438)	6543 (851)	0.34 (.06)	0.08 (.03)	1.9 (.6)	20
FB6						
F	17386 (463)	7757 (458)	0.24 (.03)	0.16 (.02)	2.3 (.6)	3
C	17359 (779)	7094 (1175)	0.28 (.05)	0.17 (.03)	3.4 (.9)	20
FB7						
F	11429 (568)	4748 (316)	0.27 (.05)	0.13 (.02)	1.9 (.4)	11
C	14826 (1650)	4580 (671)	0.24 (.02)	0.13 (.02)	3.4 (.9)	20
FB9						
F	12261 (1122)	6605 (361)	0.35 (.09)	0.12 (.12)	3.1 (.4)	20
C	14512 (1520)	7381 (566)	0.48 (.07)	0.14 (.03)	2.1 (.3)	20
FB10						
F	13893 (1566)	5487 (680)	0.25 (.05)	0.15 (.02)	1.5 (.7)	10
C	13403 (529)	4269 (613)	0.34 (.05)	0.15 (.02)	3.4 (.7)	20
FB92						
F	7560 (134)	4780 (606)	0.31 (.04)	0.083 (.01)	2.5 (.7)	2
C	7364 (219)	4343 (516)	0.29 (.04)	0.08 (.02)	2.9 (.6)	20

a. Multiple disconnected loop whistles

FB#	Freq.max. (Hz)	Freq.min. (Hz)	Loop Duration (sec)	Inter-loop duration (sec)	# of loops	# of whistles
FB20						
F	17260 (4281)	5240 (329)	0.31 (.19)		3.2 (2.2)	5
C	11595 (1404)	5898 (488)	0.61 (.17)		2.3 (1.4)	20
FB122						
F	14876 (773)	6847 (565)	0.44 (.07)		2.5 (1.2)	17
C	14155 (344)	4535 (999)	0.38 (.04)		2.4 (.5)	20
FB131						
F	15500 (0)	5300 (0)	0.420		1 (0)	2
C	13910 (550)	4775 (308)	0.66 (.90)		2.7 (.5)	20
FB182						
F	14800 (283)	5800 (283)	0.329		2 (0)	2
C	16115 (1406)	4665 (644)	0.47 (.04)		3.8 (1.3)	20

b. Multiple connected loop whistles
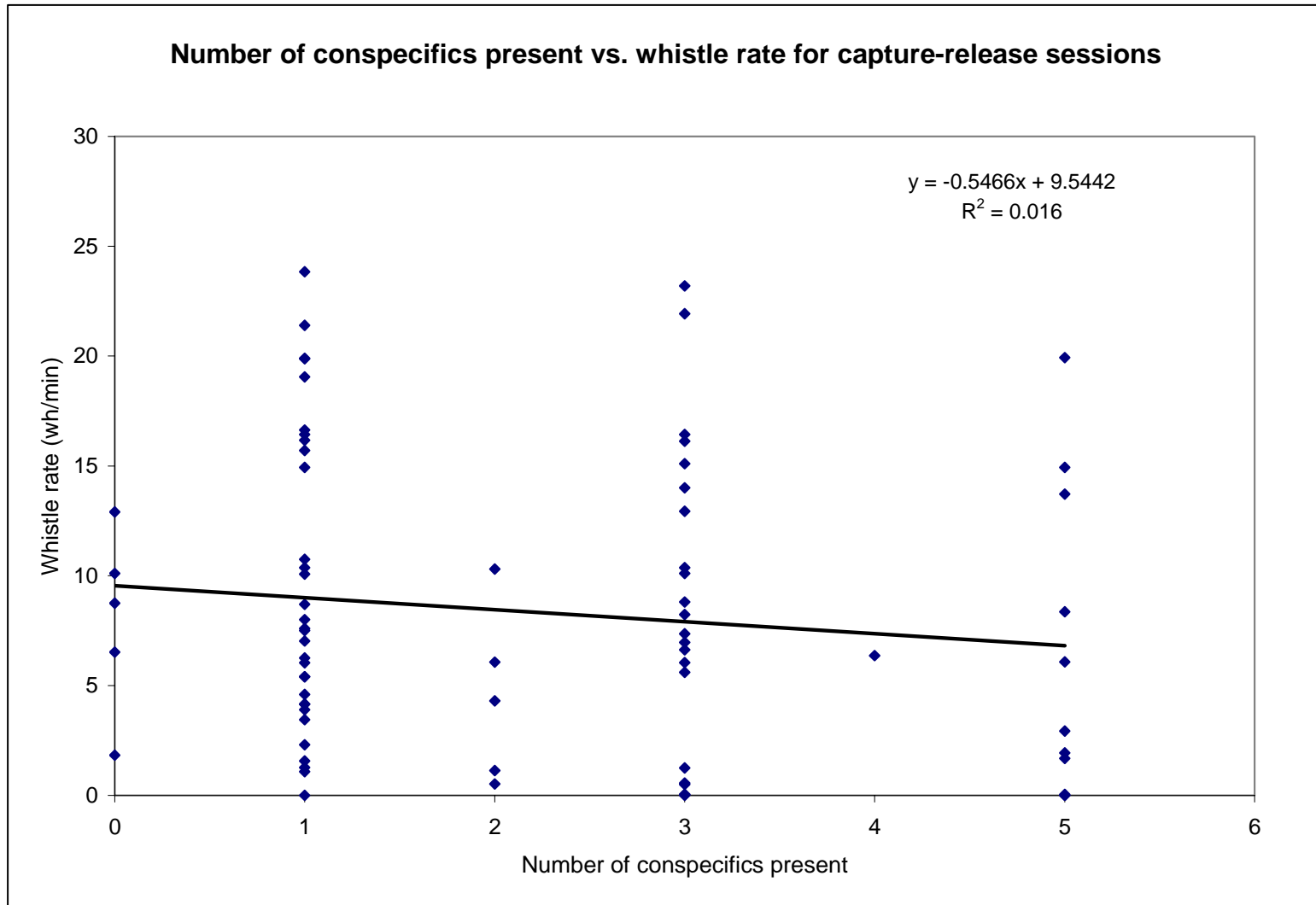
Figure 3. Number of conspecifics present versus whistle rate for all capture-release recordings (n = 76; Spearman's r = -0.14, p = 0.20).
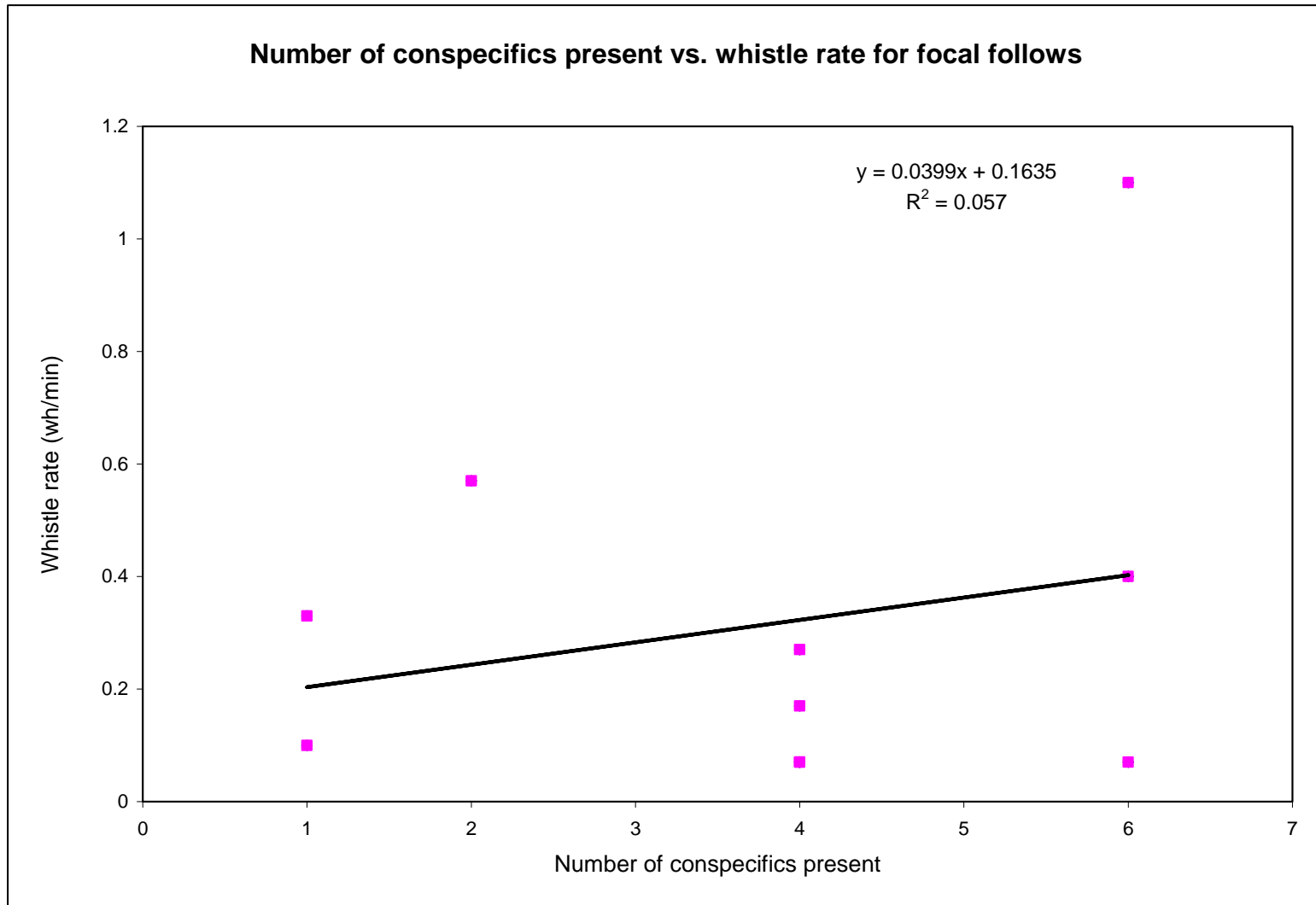
Figure 4. Number of conspecifics present versus whistle rate for all follow recordings (n = 10; Spearman's r = 0.045, p = 0.90).

2. Will whistle rates and number of loops be greater at the beginning of a capture-release session than at the end?

In multi-looped whistles, the number of loops produced in the first 10 minutes was significantly higher than in the last 10 minutes of a capture-release session (p = 0.04, W = -37.0, df = 19; Table 3). When all whistles were considered (multiple connected and disconnected loop, and single loop whistles), loop duration was significantly shorter during the first 10 minutes of a session than during the last 10 minutes of a session (p = 0.01, W = -0.54, df = 19). Whistle rate tended to decrease from the first 10 minutes to the last 10 minutes of a capture-release session, although this difference was also not significant (p = 0.06, W = -40.5, df = 19; Table 4, Figure 5). In addition, inter-loop interval showed a non-significant tendency to decrease from the first to the last 10 minutes (p = 0.08, W = -11.0, df = 10; Table 3). No significant differences in maximum frequency or minimum frequency were noted.

3. Will whistle rates and number of loops be greater during an individual's first capture-release session than during later capture-release sessions?

Whistle rate was significantly higher during an individual's first capture-release session than its third session (p = 0.03, W = -18.5, df = 9; Table 5, Figure 6), and continued to decrease from the third to the fifth capture-release, although this decrease was not significant (p = 0.06, W = -13.5, df = 9; Table 5). The most significant decrease was from the first to the fifth capture-release (p = 0.01, W = -20.5, df = 9; Table 5). When separated by age class, whistle rate appeared to be much higher in young dolphins at the time of their first capture-release. The mean whistle rate for the younger animals during first capture-release was 16.7 wh/min compared to 2.0 wh/min for the older animals (2-tailed t-test, p < 0.0006). When just the

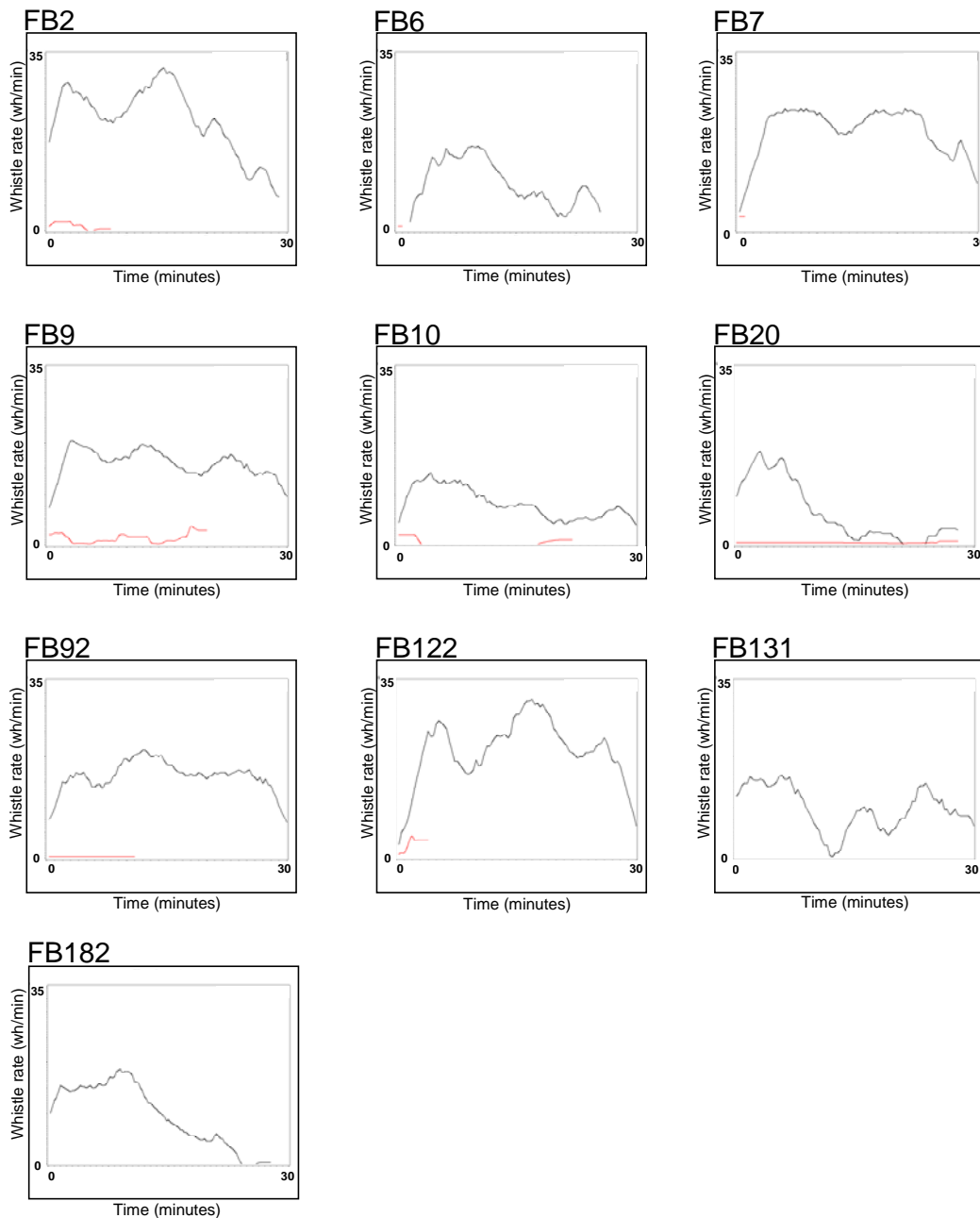Table 3. Mean (SD) physical parameter measurements for a) multiple disconnected loop and b) multiple connected loop and single loop whistles from the first and last 10 minutes of a single capture-release session.

FB#	Freq.max. (Hz)	Freq.min. (Hz)	Loop Duration (sec)	Inter-loop duration (sec)	# of loops	# of whistles
FB11						
First	20277 (1129)	6369 (1155)	0.32 (.04)	0.12 (.03)	3.5 (1.9)	10
Last	20939 (287)	7235 (468)	0.33 (.03)	0.15 (.01)	2.3 (.5)	10
FB15						
First	15687 (2383)	4517 (359)	0.32 (.06)	0.24 (.09)	2.3 (.7)	10
Last	14300 (457)	5175 (783)	0.30 (.04)	0.21 (.03)	1.2 (.4)	10
FB25						
First	21487 (4409)	7215 (208)	0.51 (.07)	0.07 (.02)	1.8 (.4)	10
Last	19548 (1859)	7416 (183)	0.57 (.09)	0.06 (.02)	2 (0)	10
FB48						
First	14186 (1487)	5209 (954)	0.19 (.07)	0.05 (.01)	3.7 (1.3)	10
Last	13376 (393)	6226 (926)	0.21 (.06)	0.05 (.01)	3.8 (.4)	10
FB54						
First	21274 (2765)	6241 (291)	0.41 (.06)	0.08 (.02)	3.3 (1.4)	10
Last	21083 (2806)	5949 (440)	0.39 (.07)	0.06 (.02)	2.1 (.6)	10
FB55						
First	12986 (2426)	4118 (1039)	0.38 (.05)	0.16 (.01)	2.2 (.6)	10
Last	15528 (1632)	3652 (572)	0.44 (.06)	0.16 (.01)	2.5 (.5)	10
FB71						
First	21052 (3197)	6403 (246)	0.24 (.02)	0.22 (.02)	3.1 (.3)	10
Last	21103 (2902)	6270 (351)	0.25 (.02)	0.23 (.01)	3 (0)	10
FB84						
First	17862 (361)	6756 (632)	0.34 (.09)	0.12 (.02)	3 (0)	3
Last	17469 (2121)	7469 (761)	0.33 (.06)	0.09 (.02)	2.3 (.9)	7
FB92						
First	7286 (239)	4359 (586)	0.28 (.05)	0.09 (.02)	2.9 (.6)	10
Last	7573 (229)	4192 (306)	0.31 (.03)	0.09 (.01)	2.6 (.7)	10
FB101						
First	14605 (4244)	4254 (902)	0.27 (.06)	0.22 (.07)	2.1 (.7)	10
Last	20520 (3632)	4027 (620)	0.36 (.07)	0.19 (.02)	2.6 (.8)	10
FB166						
First	11847 (252)	3809 (920)	0.49 (.06)	0.12 (.15)	3 (.7)	10
Last	11589 (389)	3639 (859)	0.47 (.07)	0.07 (.01)	2.2 (.8)	10

a. Multiple disconnected loop whistles

Table 3 cont.

FB#	Freq.max. (Hz)	Freq.min. (Hz)	Loop Duration (sec)	Inter-loop duration (sec)	# of loops	# of whistles
FB3						
First	26060 (1826)	13441 (304)	0.44 (.03)		5.0 (1.7)	10
Last	27843 (987)	13443 (601)	0.45 (.02)		5.0 (1.1)	10
FB24						
First	13929 (645)	5511 (1167)	0.94 (.13)		1 (0)	10
Last	13388 (982)	4900 (197)	0.93 (.11)		1 (0)	10
FB35						
First	14645 (1596)	5459 (675)	0.87 (.23)		1 (0)	10
Last	15487 (2302)	5396 (284)	0.98 (.21)		1 (0)	10
FB38						
First	15084 (1288)	5422 (263)	0.24 (.02)		3.0 (.8)	10
Last	14683 (495)	5214 (245)	0.28 (.10)		2.2 (.6)	10
FB 67						
First	21936 (1816)	5032 (191)	0.53 (.02)		4.0 (.7)	10
Last	22601 (2497)	5169 (411)	0.58 (.07)		3.1 (.8)	10
FB118						
First	17192 (986)	7078 (489)	0.47 (.07)		1.9 (.8)	10
Last	17907 (1544)	6375 (642)	0.42 (.03)		2.8 (1.0)	10
FB122						
First	13970 (353)	4210 (393)	0.38 (.04)		2.6 (.5)	10
Last	14590 (233)	4650 (1276)	0.43 (.09)		1.9 (.3)	10
FB140						
First	18560 (1829)	4003 (531)	0.37 (.08)		4.9 (1.5)	10
Last	18686 (440)	4179 (586)	0.37 (.02)		4.9 (1.9)	10
FB186						
First	18194 (2158)	5001 (448)	0.48 (.11)		1.4 (.5)	10
Last	20072 (1735)	4695 (575)	0.55 (.08)		1.5 (.5)	10

b. Multiple connected loop whistles and single loop whistles

Table 4. Contextual information and whistle rates for a single capture-release recording for each of 20 dolphins. Whistle rates were calculated for the first 10 minutes and the last 10 minutes of each recording.

FB#	Gender	Age (yrs)	Capture-release #	ID #'s of conspecifics present	Recording length (min)	Whistle rate (wh/min) (first/last)
3	F	5	3	alone	50	9.3/8.2
11	F	2.5	3	19,25,54	40	13/17.2
15	F	41	5	23	65	9.2/1.6
24	M	2	1	35	41	1.9/6.4
25	F	11	7	50,90,97	42	13.2/7
35	F	33	3	13&calf,93	60	5.4/5.8
38	M	20	8	17	71	2.6/4.3
48	M	29	3	26	125	3.5/1.1
54	F	24	9	118	84	1.8/6.5
55	F	16	12	218	48	4.1/3.7
67	F	21	4	65	40	11.5/5.9
71	F	29	8	33	50	18.2/3.3
84	F	32	6	50,90,92	68	0.8/1.7
92	M	1	1	84	40	15.8/15.6
101	F	10	3	196	77	12.5/2.7
118	M	2	1	54	136	11.8/4.2
122	M	2	1	90	103	17.6/20.5
140	M	7	1	alone	40	15.5/4.7
166	M	4	1	101,115,163	42	9/5.7
186	M	3	1	117,118,157	61	13.3/14.7

Figure 5. Changes in whistle rate during a single capture-release session for each of 20 dolphins.

Figure 5 cont.

FB84

FB92

FB101

FB118

FB122

FB140

FB166

FB186
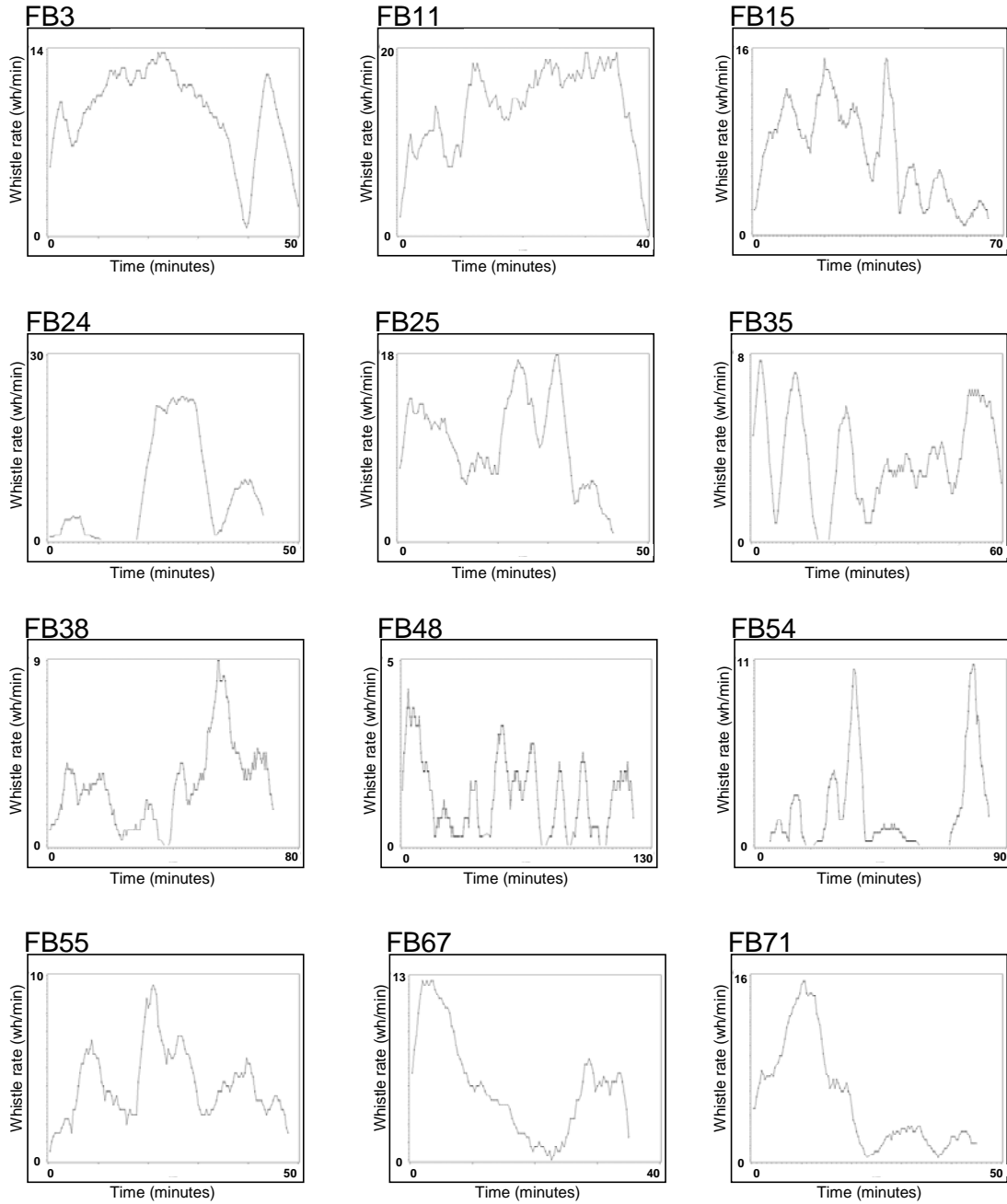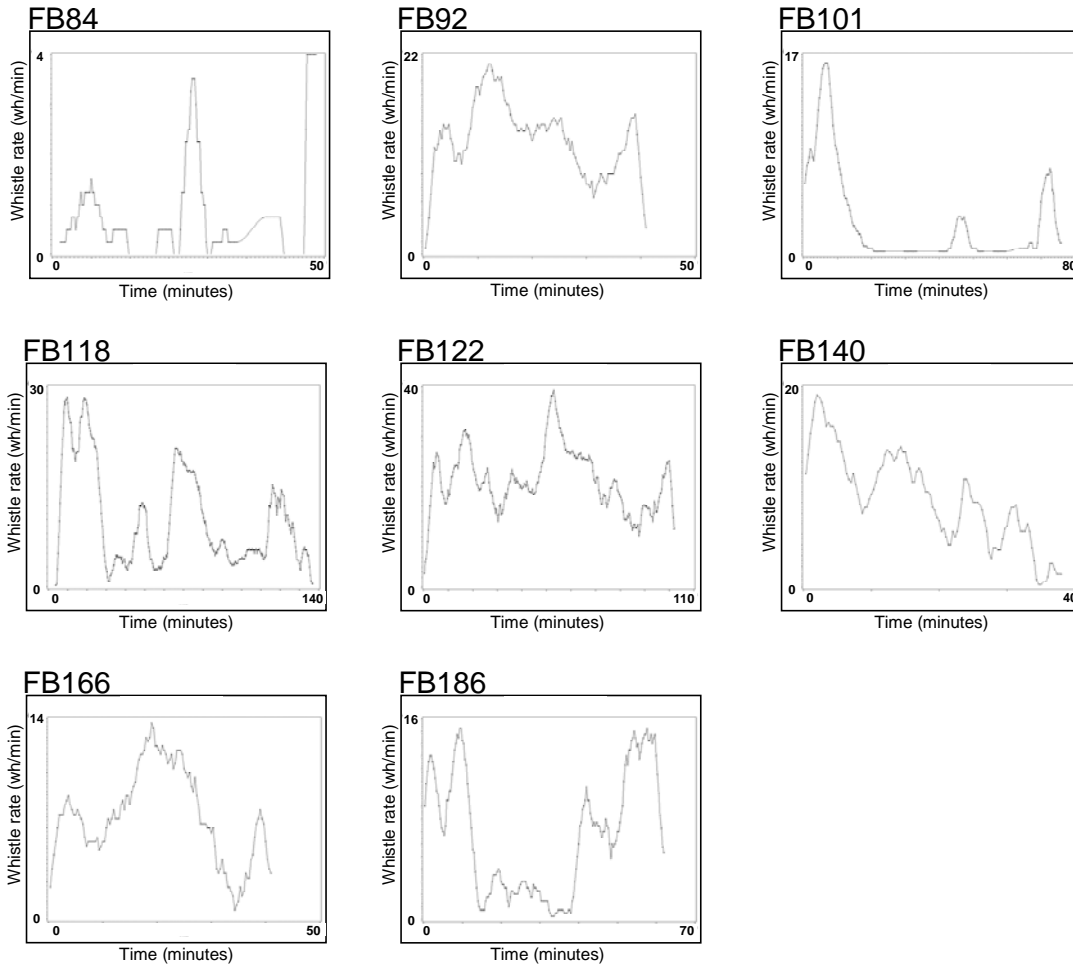
Table 5. Contextual information and whistle rates from three recording sessions for each of 10 dolphins (gender is in parentheses).

FB#	Age (yrs)	ID #'s of conspecifics present	Recording length (min)	Whistle rate (wh/min)
FB6 (M)				
1st	1	51,71(mom),&others	30	10.37
3rd	3	71	30	8.8
5th	5	alone	30	1.93
FB7 (F)				
1st	1	4,13,15,61,84(mom),90	30	19.9
3rd	3	62,84(mom),94	30	12.93
5th	5	9	30	2.93
FB9 (F)				
1st	1	10,63(mom),79	25	16.72
3rd	3	5,55,79	25	8.36
5th	5	7	25	8.36
FB11 (F)				
1st	1	alone (in gill net)	30	10.07
3rd	2.5	19(mom),25,54	30	16.13
5th	3	19,51	30	14.93
FB32 (M)				
1st	4	5(mom),27,36,94	25	1.56
3rd	6.5	27,33,51	25	0
5th	7.5	alone	25	0
FB33 (F)				
1st	2	51(mom),84&calf	25	23.84
4th	5	44	25	6.36
5th	5.5	alone	25	6.08
FB36 (M)				
1st	12	38	25	0
3rd	14.5	38,62,66,77	25	0.04
5th	16	5,38,55	25	1.68
FB38 (M)				
1st	10	36	25	1.08
3rd	12.5	alone	25	0.56
5th	14	no notes	25	0
FB79 (F)				
1st	6	9,10,63	25	23.2
3rd	8	37,52	25	13.72
5th	9.5	14,61,67,76	25	5.4
FB118 (M)				
1st	2	54(mom)	25	18.84
3rd	7	57,186,117	25	0.04
5th	11	79	25	0.04

Figure 6. Comparison of changes in whistle rate among three capture-release
recordings for each of nine dolphins. A figure was not produced for FB32,
who had a whistle rate of zero for more than one capture-release recording.
The absence of a curve or a break in the curve indicates a whistle rate of
0 wh/min (first = black, *third = red, fifth = green).
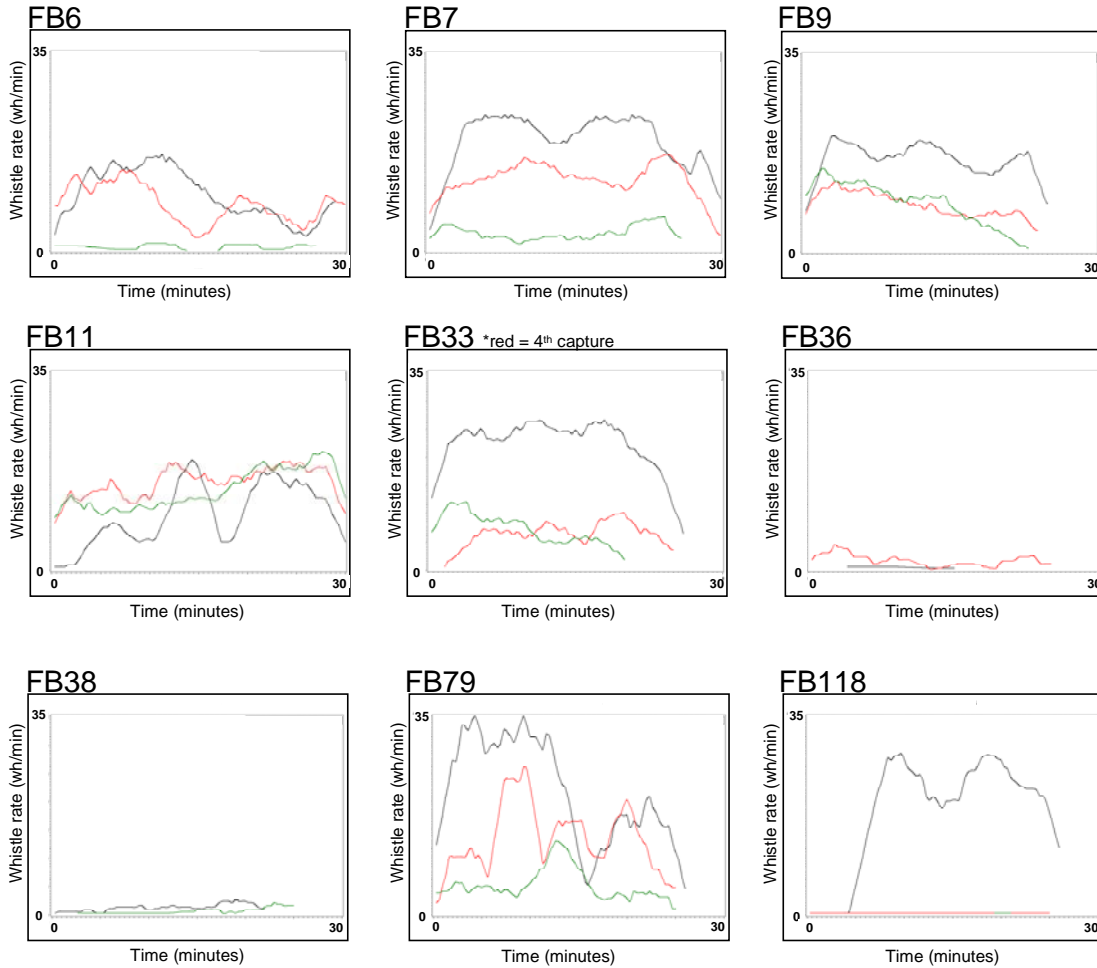
six younger animals were considered, whistle rate decreased significantly from the third to fifth capture-release (p = 0.04, W = -8.5, df = 5) and from the first to the fifth capture-release (p = 0.03, W = -9.5, df = 5), and whistle rates did not differ significantly between the sexes (2-tailed t-test, p = 0.70). There were no significant differences in whistle rate among any of the capture-release sessions for the four older animals (3 males and 1 female). However, whistle rates for the older males were very low in all capture-release sessions. The single older female's first capture-release whistle rate was more than six times the mean first capture-release whistle rate for the three males in the older class (23.2 wh/min vs. a mean of 0.88 wh/min). The female's whistle rate decreased substantially in subsequent capture-releases (first = 23.2 wh/min, third = 13.72 wh/min, fifth = 5.4 wh/min). Whistle rate was negatively correlated with age across all capture-release recordings used for this study (Spearman's r = -0.59, p < 0.0001; Figure 7). In addition, capture-release number was positively correlated with age (Spearman's r = 0.71, p < 0.0001; Figure 8), and negatively correlated with whistle rate (Spearman's r = -0.46, p < 0.0001; Figure 9). Whistle rate declined in males and females with increasing capture-release frequency (Spearman's r = -0.63, p = 0.0003 (males), r = -0.60, p < 0.0001 (females); Figure 10). However, whistle rate appeared to decline more quickly with age in males (Spearman's r = -0.74, p < 0.0001 (males), r = -0.65, p < 0.0001 (females); Figure 11). No significant differences in loop number, maximum and minimum frequency, or loop and inter-loop duration across capture-release sessions were detected. There were non-significant trends for minimum frequency to decrease from the first to the fifth capture-release (p = 0.07, W = -9.0, df = 6; Table 6) and for inter-loop interval to decrease from the first to the third capture-release (p = 0.06, W = -6.5, df = 4; Table 6).
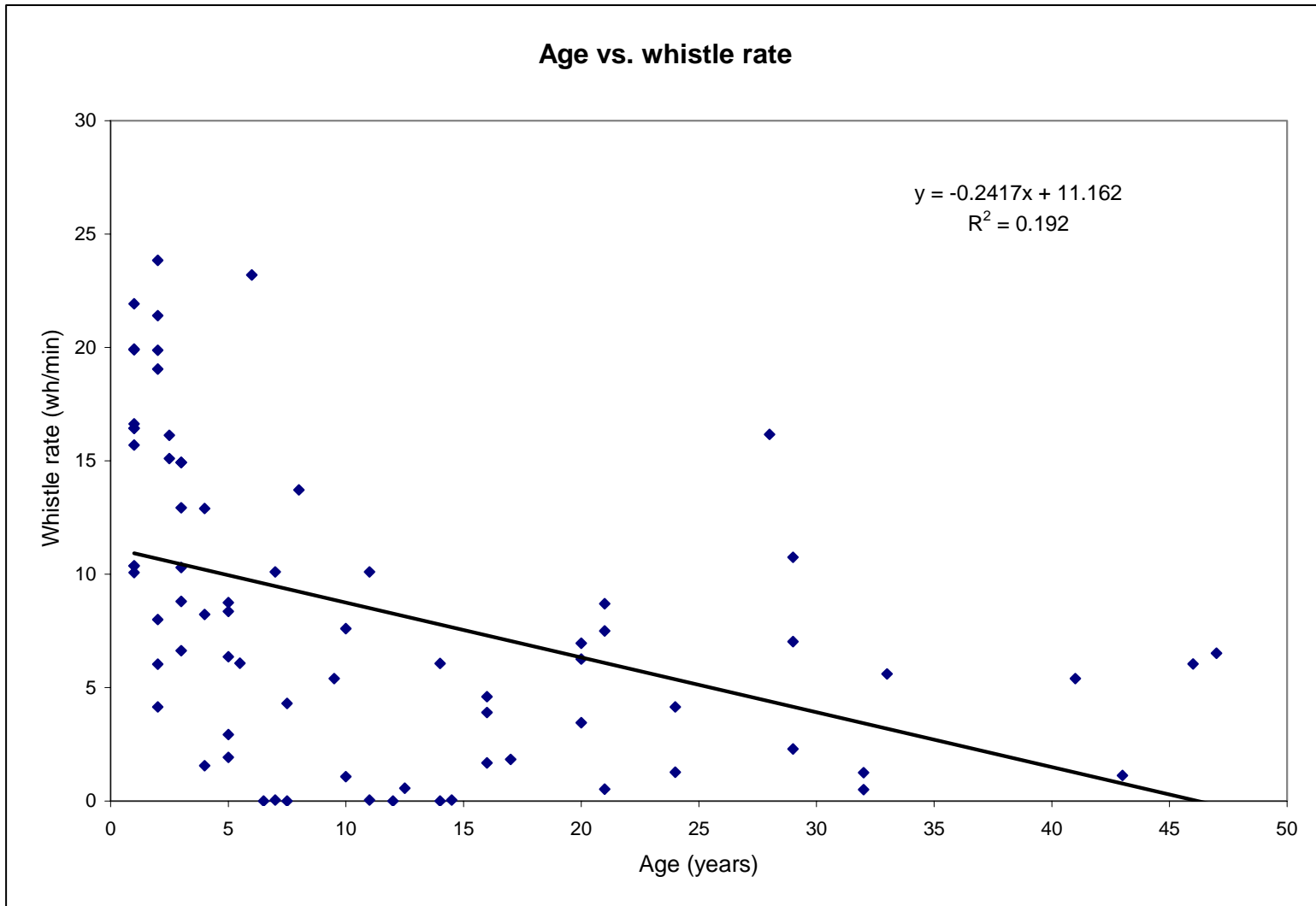
Figure 7. Age versus whistle rate for all dolphins (n = 34) across all capture-release recordings (n = 76; Spearman's r = -0.59, p < 0.0001).
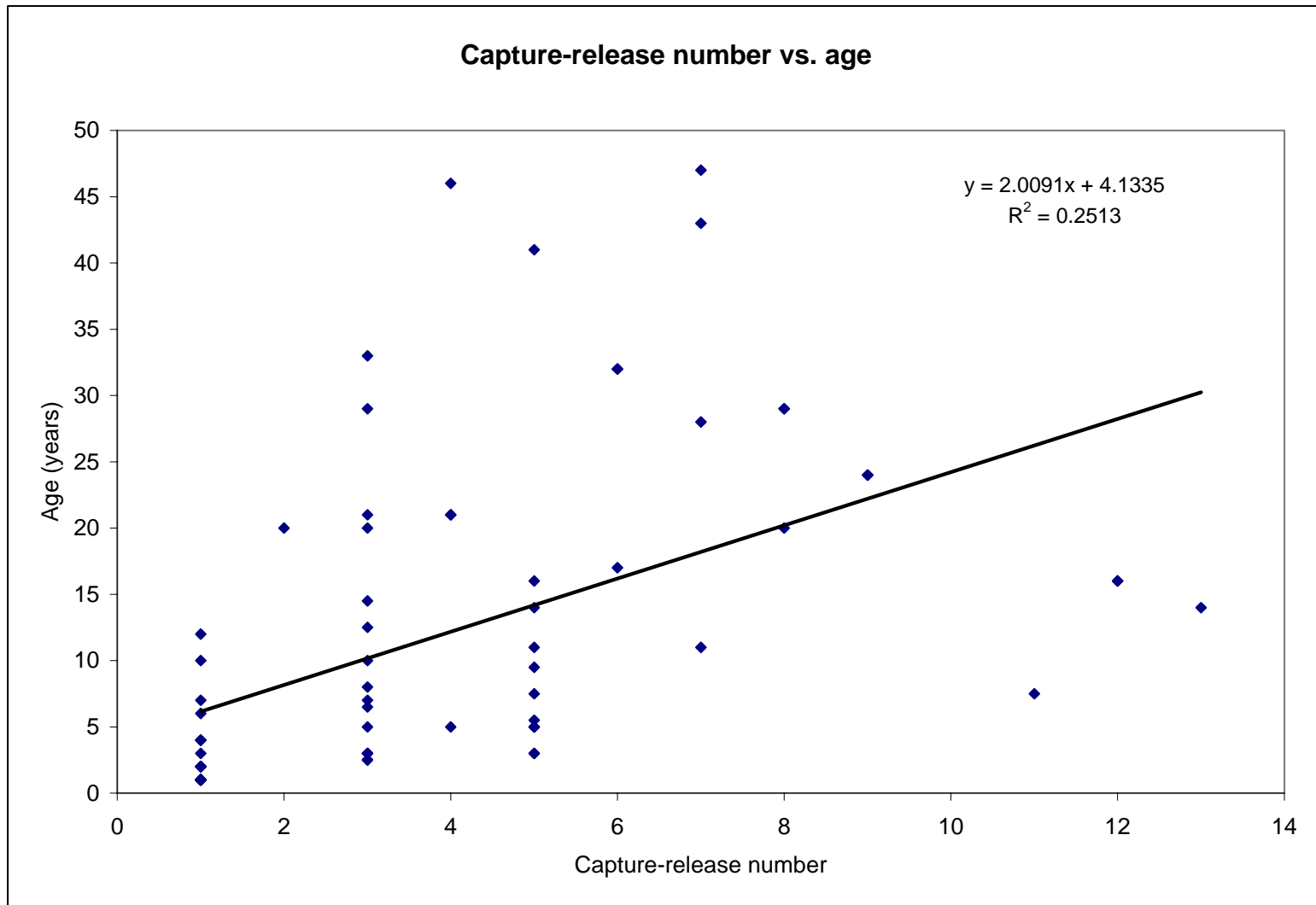
Figure 8. Capture-release number versus age for all dolphins (n = 34) across all capture-release recordings (n = 76; Spearman's r = 0.71, p < 0.0001).

Capture-release number vs. whistle rate

$y = -0.89x + 11.716$
$R^2 = 0.1645$
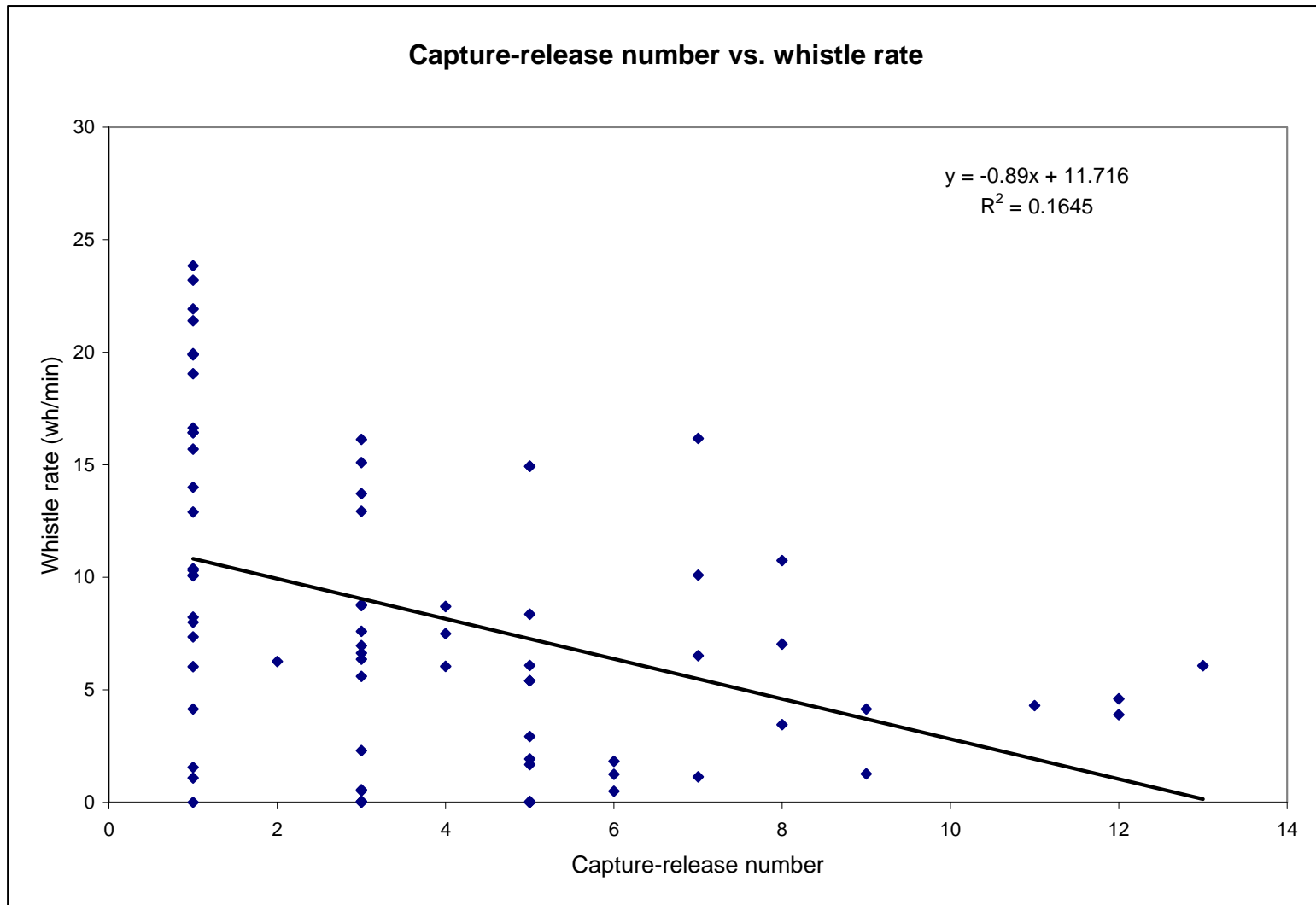
Figure 9. Capture-release number versus whistle rate for all dolphins (n = 34) across all capture-release recordings (n = 76; Spearman's r = -0.46, p < 0.0001).
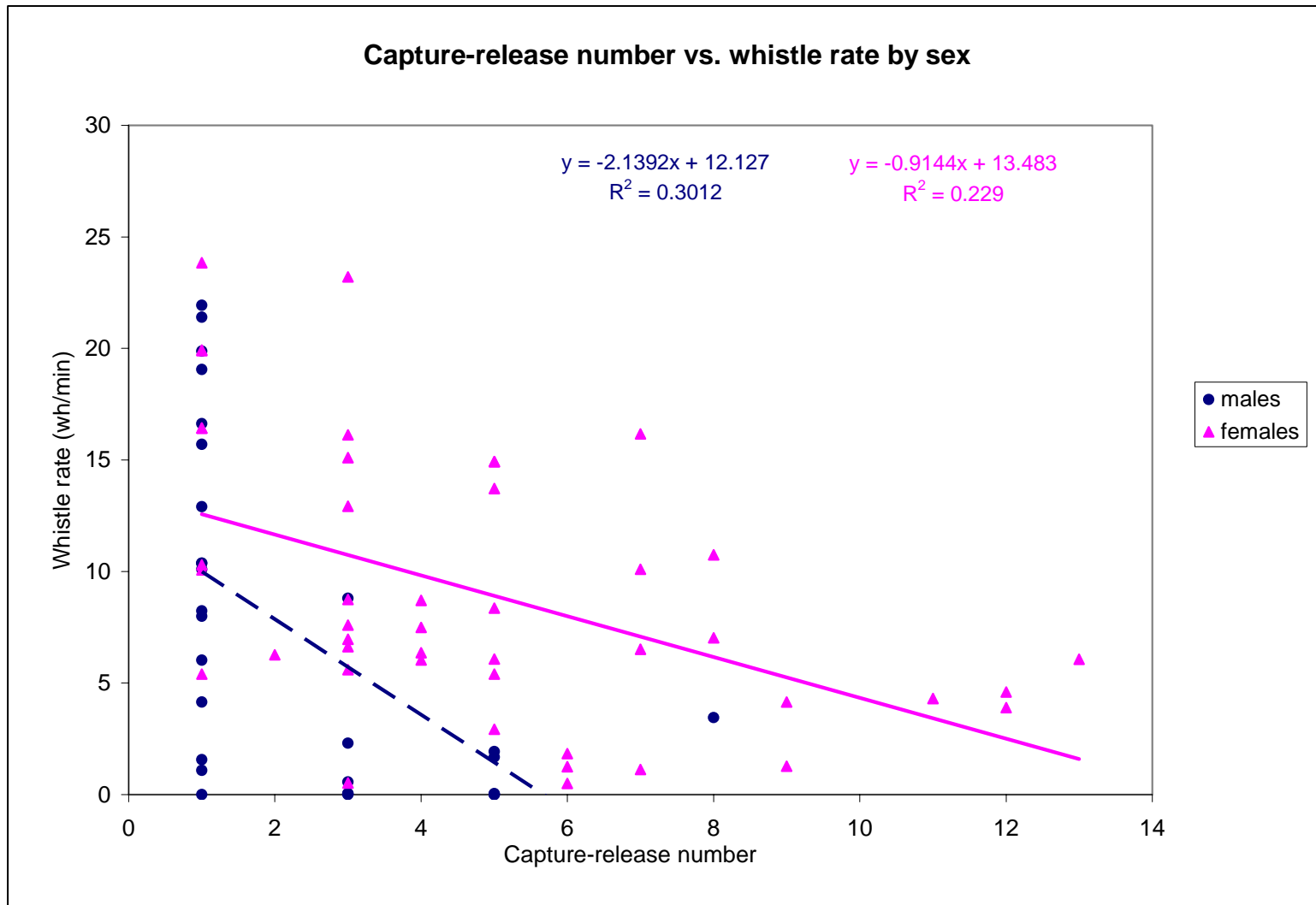
Figure 10. Capture-release number versus whistle rate for all capture-release recordings of males (n = 31; Spearman's r = -0.63, p = 0.0003) and females (n = 45; Spearman's r = -0.60, p < 0.0001).
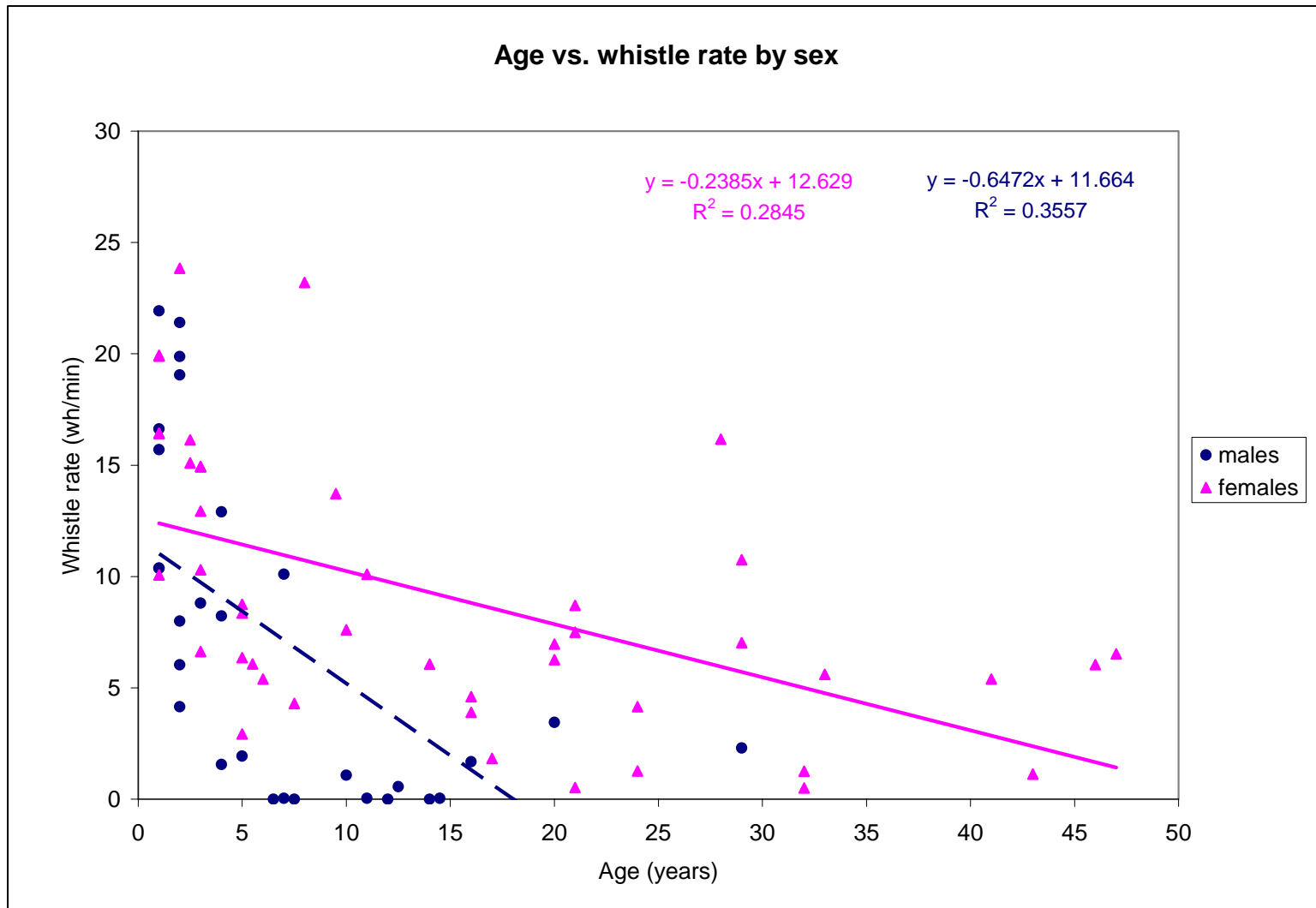
Figure 11. Age versus whistle rate for all capture-release recordings of males (n = 31; Spearman's, r = -0.74, p < 0.0001) and females (n = 45; Spearman's, r = -0.65, p < 0.0001).

Table 6. Mean (SD) physical parameter measurements for a) multiple disconnected loop and b) multiple connected loop and single loop whistles from multiple capture-release sessions.

FB#	Freq.max. (Hz)	Freq.min. (Hz)	Loop Duration (sec)	Inter-loop duration (sec)	# of loops	# of whistles
FB6						
1st	17359 (779)	7094 (1175)	0.28 (.05)	0.17 (.03)	3.4 (.9)	20
3rd	18509 (1511)	6451 (682)	0.32 (.04)	0.13 (.02)	2.4 (.5)	20
5th	18813 (2666)	6585 (682)	0.34 (.06)	0.09 (.03)	3.4 (1.6)	20
FB7						
1st	14826 (1650)	4580 (671)	0.24 (.02)	0.13 (.02)	3.4 (.9)	20
3rd	12582 (1115)	4012 (495)	0.22 (.02)	0.11 (.01)	2.9 (.9)	20
5th	12703 (673)	4897 (836)	0.26 (.05)	0.12 (.01)	3.3 (.6)	20
FB9						
1st	14512 (1520)	7381 (566)	0.48 (.07)	0.14 (.03)	2.1 (.31)	20
3rd	13688 (1029)	7448 (231)	0.48 (.04)	0.12 (.01)	2 (0)	20
5th	13398 (702)	7242 (326)	0.41 (.07)	0.12 (.02)	2.2 (.5)	20
FB11						
1st	17827 (1083)	7431 (499)	0.30 (.04)	0.34 (.19)	3.1 (1.3)	20
3rd	20363 (979)	6493 (963)	0.33 (.03)	0.12 (.03)	3.4 (1.5)	20
5th	18678 (986)	6303 (533)	0.37 (.04)	0.18 (.05)	3.2 (.8)	20
FB33						
1rt	17932 (672)	6755 (1023)	0.29 (.03)	0.15 (.02)	3.3 (1.4)	20
4th	16538 (910)	6259 (1169)	0.28 (.02)	0.16 (.02)	4 (.9)	20
5th	16326 (892)	6582 (1237)	0.29 (.03)	0.17 (.02)	3.1 (.6)	20
FB36						
1st						0
3rd	17050	3350	0.41	0.06	2.0	1
5th	17338 (1116)	3397 (419)	0.33 (.06)	0.03 (.01)	2.1 (.5)	20
FB38						
1st	15100 (719)	5367 (529)	0.27 (.04)	0.11 (.02)	2.1 (1.0)	20
3rd						0
5th	15255 (852)	5397 (649)	0.18 (.02)	0.14 (.03)	2.4 (.5)	14

a. Multiple disconnected loop whistles

Table 6, cont.

FB#	Freq.max. (Hz)	Freq.min. (Hz)	Loop Duration (sec)	Inter-loop duration (sec)	# of loops	# of whistles
FB32						
1st	16640 (791)	5080 (2000)	0.75 (.21)		1 (0)	20
3rd						0
5th						0
FB79						
1st	17375 (498)	3025 (434)	0.63 (.05)		1 (0)	20
3rd	17470 (807)	3605 (584)	0.64 (.09)		1 (0)	20
5th	15150 (2594)	2695 (397)	0.74 (.15)		1 (0)	20
FB118						
1st	16900 (804)	7216 (571)	0.53 (.14)		1.5 (.6)	6
3rd	16600	7400	0.34		1	1
5th	16800	7300	0.70		1	1

b. Multiple connected loop whistles and single loop whistles

4. Will whistle rates and number of loops will be greater when a mother is caught and released with a dependent calf than without a dependent calf?

Females captured and released with a dependent calf produced whistles with significantly higher maximum frequencies (p = 0.01, W = -16.0, df = 7; Table 7) and shorter inter-loop intervals (p = 0.01, W = -10.50, df = 5; Table 7) than the same females caught and released without calves. No significant differences in whistle rate (Table 8, Figure 12), loop number or duration, or minimum frequency were detected.

DISCUSSION

Whistle rate and loop number emerged from this study as the most promising potential vocal indicators of short-term stress in bottlenose dolphins. Dolphins produced a significantly higher number of loops during capture-release than during focal follows, and in the first 10 minutes than in the last 10 minutes of their first capture-release session. These findings suggest that multi-looped signature whistles may contain information about the signaler's motivational state, as is the case with social signals of other species (reviewed in Weary and Fraser 1995; Watts and Stooky 2001). There are several possible explanations for the changes in loop number observed in this study. First, if the stress of the capture-release context contributes to the production of more loops, as is indicated by the difference observed between capture-release and focal follow recordings, then habituation to the capture-release context may have caused the decrease in loop number over the course of a single session. However, increased loop number in the capture-release context may reflect an increased motivation to communicate rather than an increased stress level. Decreases in loop number over the course of a capture-

Table 7. Mean (SD) physical parameter measurements for a) multiple disconnected loop and b) multiple connected loop whistles from capture-releases with and without a dependent calf.

FB#	Freq.max. (Hz)	Freq.min. (Hz)	Loop Duration (sec)	Inter-loop duration (sec)	# of loops	# of whistles
FB11						
w/calf	20737 (1867)	5672 (736)	0.41 (.05)	0.10 (.01)	3 (.5)	20
w/out	18678 (986)	6303 (533)	0.37 (.04)	0.18 (.05)	3.2 (.8)	20
FB51						
w/calf	15142 (1741)	6505 (619)	0.27 (.13)	0.11 (.03)	3.3 (.6)	20
w/out	14997 (2624)	5940 (536)	0.28 (.12)	0.17 (.07)	4.6 (.7)	20
FB54						
w/calf	21479 (2754)	6270 (331)	0.40 (.05)	0.08 (.02)	3.2 (1.3)	20
w/out	17546 (974)	6917 (530)	0.40 (.04)	0.12 (.02)	2.1 (.4)	20
FB55						
w/calf	13834 (2560)	3993 (822)	0.39 (.05)	0.16 (.01)	2.2 (.6)	20
w/out	1458 (703)	4382 (655)	0.39 (.02)	0.19 (.02)	1.9 (.3)	20
FB71						
w/calf	22424 (3805)	6544 (463)	0.22 (.02)	0.18 (.04)	3.6 (1)	20
w/out	20358 (2933)	6408 (256)	0.24 (.02)	0.22 (.02)	3.1 (.3)	20
FB84						
w/calf	18791 (1343)	6912 (498)	0.35 (.08)	0.10 (.02)	3.2 (.6)	15
w/out	17908 (3795)	4819 (963)	0.39 (.11)	0.11 (.03)	2.5 (1.2)	15

a. Multiple disconnected loop whistles

FB#	Freq.max. (Hz)	Freq.min. (Hz)	Loop Duration (sec)	Inter-loop duration (sec)	# of loops	# of whistles
FB 67						
w/calf	23021 (2040)	4988 (190)	0.62 (.04)		3.3 (.8)	20
w/out	18630 (963)	4930 (198)	0.63 (.07)		3.1 (.8)	20
FB 163						
w/calf	26130 (1908)	3765 (290)	0.53 (.07)		3 (1)	20
w/out	20625 (2123)	4600 (294)	0.78 (.05)		1 (0)	4

b. Multiple connected loop whistles

Table 8. Contextual information and whistle rates for recordings of eight
females caught and released with and without a dependent calf

FB#	Age (yrs)	Capture-release #	ID #'s of Conspecifics present	Recording length (min)	Whistle rate (wh/min)
FB11					
with calf	14	13	19,51	30	6.07
w/out calf	3	5	146	30	14.93
FB51					
with calf	46	4	27,32,33	20	5.6
w/out calf	47	7	alone	20	8.5
FB54					
with calf	24	9	118	30	1.27
w/out calf	17	6	alone	30	1.83
FB55					
with calf	16	12	218	30	4.6
w/out calf	7.5	11	29,71	30	4.3
FB67					
with calf	21	4	65	28	7.5
w/out calf	20	3	14,66,79	28	6.96
FB71					
with calf	28	7	95	30	9.7
w/out calf	29	8	33	30	7.03
FB84					
with calf	32	6	50,90,92	30	0.5
w/out calf	43	7	5,33	30	1.13
FB163					
with calf	20	2	168	27	6.26
w/out calf	21	3	14,94	27	0.52

Figure 12. Comparison of changes in whistle rate between recordings of females caught and released with and without a dependent calf. The absence of a curve or a break in the curve indicates a whistle rate of 0 wh/min (without calf = black, with calf = red).
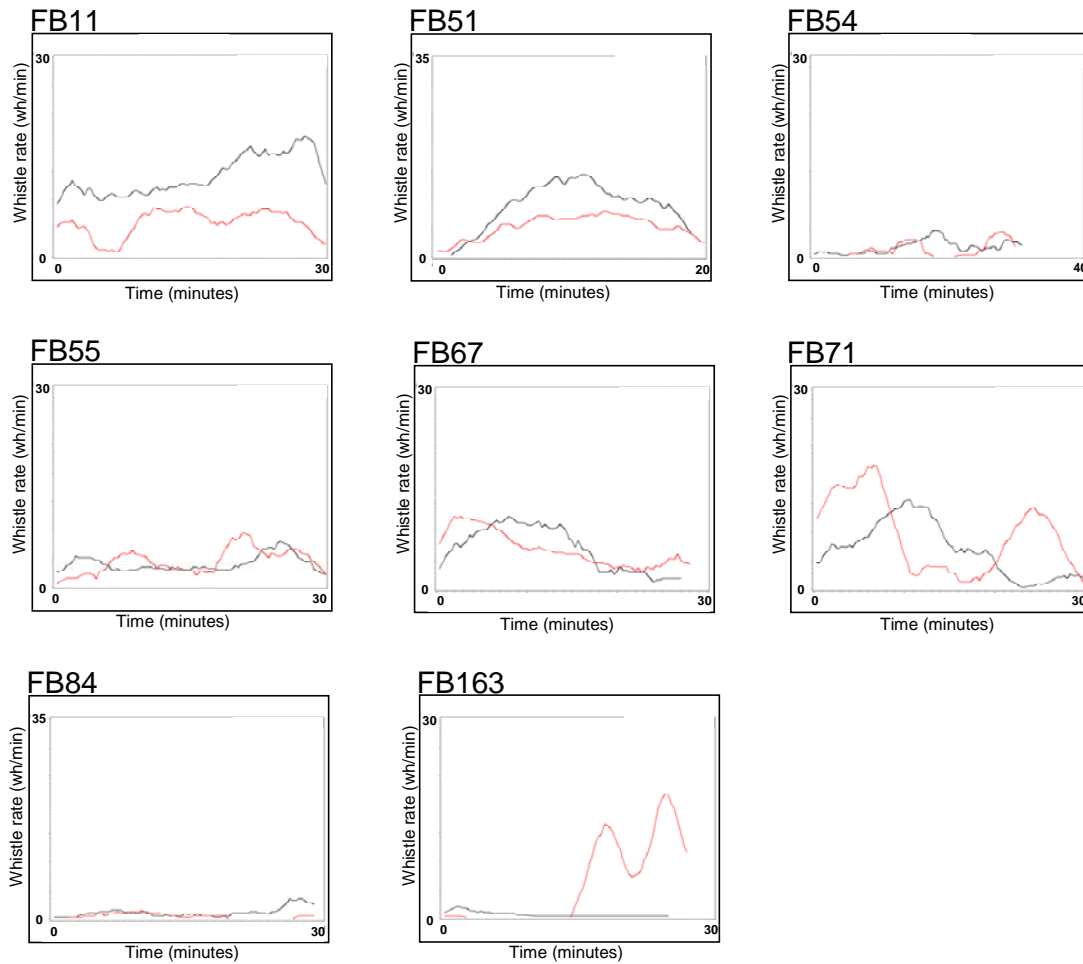
release session may be a result of fatigue, rather than habituation. Loop duration was also significantly shorter, or compressed, during the first 10 minutes of a session. Weary and Fraser (1995) determined that quantifiable differences existed in rate, intensity and duration of piglet calls, and that these differences reflected various degrees of need. In domestic animals, researchers have proposed that vocal responses to forced isolation could be due to a distress reaction that is context specific, an attempt to communicate with conspecifics, or both (Watts and Stooky 2000). It is not known whether variation in loop number and duration result from the potential stress of isolation or other aspects of the capture-release context, if they in some way enhance communication with conspecifics, or if they may serve both or alternative functions. Variation of loop number and duration might allow dolphins to communicate additional information, such as motivational state, while also conveying identity. The results from this study suggest that loop number, in particular, would be a useful parameter on which to focus future studies of the effects of stress on bottlenose dolphin vocalizations.

Signature whistle rate was also significantly lower during follows than during capture-releases. This finding was expected to some degree, based on prior 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 90% of the vocal repertoire of temporarily restrained bottlenose dolphins (as described by Caldwell et al. 1990). However, the differences in signature whistle rate found in this study ranged from one to two orders of magnitude, with mean whistle rates of 0.3 and 14.3 whistles per minute during follows and capture-releases respectively. Signature whistle imitation, or mimicry, has been observed in bottlenose dolphins (Tyack 1986; Janik 2000). It is possible that some of the signature whistles identified in focal follow recordings were imitations produced by a dolphin other than the individual with whom the

signature whistle is associated (based on the Sarasota Dolphin Community Signature Whistle Catalogue). If mimicry occurred in the focal follow recordings, then the actual rate of signature whistle production by an individual would be lower than the rate reported in the current study (Cook et al. 2004). Therefore, the difference between signature whistle rates in first time capture-release versus focal follow recordings would be even greater than that reported here.

Stress may have contributed to the higher whistle rates observed in the capture-release vs. focal follow contexts. Several additional or alternative factors may also have influenced this difference. First, whistle rates during focal follows were compared to first-time capture-release recordings for the same animals, which is the context in which the highest whistle rates occurred. Another factor may have been the ages of the animals. The two oldest animals in the sample were only four years old in their capture-release recording, and the remaining eight animals were between one and three years old. In contrast, ages of the animals during focal follows ranged from 3-13 years. Dolphins that were two years old or less when first capture-released whistled at significantly higher rates than dolphins that were four years or older at the time of first capture-release. Still another possible factor affecting differences in whistle rate between contexts was that seven of the ten animals were still dependent calves when capture-released, but only one (FB122) was still dependent on his mother during follows. Since whistles are commonly used to maintain contact between mothers and calves (Sayigh et al. 1990), whistle rates may be higher in dependent than independent calves. These factors are discussed in greater detail in the following paragraphs.

The findings of this project indicate that while significant patterns 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. In fact, findings suggest that habituation occurs

within a capture-release session, and from one session to the next. These data are in accordance with data on multi-generational residency and social structure stability (Wells 2003) along with increases in the numbers of dolphins regularly using Sarasota Bay (pers. comm. R.S. Wells) that suggest that the Sarasota dolphin community is not experiencing any adverse long-term impacts from the capture-release program. Bottlenose dolphins are capable of both associative (classical and operant conditioning) and non-associative (habituation and sensitization) learning (Herman 1968; Herman and Arbeit 1971; Herman et al. 2001).   Behavioral habituation is defined as learning to disregard stimuli that are without significance and is considered one of the simplest forms of non-associative learning (Cerbone and Sadile 1994). However, there is conflicting evidence regarding whether dolphins experience habituation to capture-release (Curry 1999; St. Aubin et al. 1996; Thomson and Geraci 1986). Thomson and Geraci (1986) determined that dolphins that had been handled in captivity for many years continued to produce a physiological stress response, even when capture-released under calm conditions. St. Aubin et al. (1996) suggested that the lower levels of cortisol measured in semi-domesticated dolphins may be a result of behavioral conditioning to medical husbandry procedures, while higher levels of cortisol in wild dolphins may be a result of the stress of the capture-release process. In the Eastern Tropical Pacific, where spinner and spotted dolphins are routinely capture-released in the tuna purse-seine fishery, dolphins appear to anticipate the backdown process (the sinking of the cork-line to release dolphins from the net) and subsequent release (Norris et al. 1978; Curry 1999). Dolphins were observed remaining stationary near the release area of the net prior to backdown (Santurtun and Galindo 2002). Once the net was lowered for release, the dolphins quickly moved out of the net. This shift from apparent passivity to high-energy movement might indicate that the dolphins were familiar with the chronology of capture-release, and therefore

avoided attempting to escape until backdown was initiated. Thus, this response is more likely a result of associative learning than habituation. Among bottlenose dolphins that are infrequently capture-released as part of a capture-release health assessment program conducted in Sarasota Bay, FL, inexperienced animals appear much more likely to attempt to escape from the net than those experienced with the capture-release process, and more often become entangled as a result (pers. comm. R. S. Wells). Experienced dolphins tend to remain in the middle of the net compass and often are more easily restrained. It thus appears likely that dolphins recall prior capture-release experiences, and that this may influence their behavior in subsequent capture-releases. However, young dolphins whistled at higher rates than older dolphins across all capture-release recordings; thus the decrease in whistle rate between capture-releases could be a result of maturation rather than capture-release experience. Since age and capture-release experience are significantly related to each other, it is difficult to tease apart the relative influence of these factors. A related factor that could affect whistle rates is dependency on the mother. Of the six animals in the present study that were two years old or less at the time of first capture-release, five were dependent calves and one was no longer a dependent calf. In many species, including bottlenose dolphins, young animals respond to isolation from their mother by vocalizing (e.g., piglets: Fraser 1975; Weary and Fraser 1995; guinea pigs: Monticelli et al. 2004; several rodent species: Okon 1971; cattle: Marchant-Forde et al. 2002; bottlenose dolphins: Sayigh et al. 1990).

A final factor potentially influencing changes in whistle rate between capture-releases is gender. Of the four older animals (>4 years old) included in the longitudinal data set, the female's first capture-release whistle rate was over six times higher than the mean first capture-release whistle rate for all three males. Females appeared to maintain higher signature whistle

rates than males across age class and capture-release events, which may be related to their social structure. In Sarasota Bay, as bottlenose dolphins 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, while males usually travel alone, in pairs, or in very small groups (Scott et al. 1990). Females, therefore, often engage in more social interactions than males. If signature whistles function as contact calls, and in maintaining group cohesion, one would expect higher signature whistle production in females than in 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 inter-loop intervals than those without 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 produce calls with higher mean frequencies. Thus, the higher maximum frequencies observed in females 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, since the mean age of females caught with a dependent calf (25 years) was similar to that of females caught without a dependent calf (23 years). Of the 20 recordings analyzed for this research question, 18 were made when the female was mature. It is also unlikely that differences in maximum frequency are due to different amounts of prior capture-

release experience, since 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. 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.

Surprisingly, the shorter inter-loop intervals produced by females with dependent calves did not coincide with higher loop number. Shorter inter-loop intervals result in faster repetitions of loops. 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. Many of the females had extensive prior 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 fifth capture-release for that individual. For those dolphins that had been capture-released less than five times (n = 3), the two recordings used were not separated by more than one year in order 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, the combined

effects of age and capture-release experience may have influenced the lower whistle rates in these mature females.

Whistle rate during the course of a single capture-release was highly variable. This variability may be related to the various activities in which the dolphin was engaged. These activities included movement of the animal (e.g. from water to boat), relocation closer to a conspecific, or a variety of sampling protocols. Anecdotal reports indicate that whistle rate often appears to decrease while the animal is on the boat (pers. comm. Laela Sayigh). On occasion, blubber biopsy samples are obtained for environmental contaminant analyses (Wells et al. 2005), or, more rarely, a tooth may be extracted for age determination (Hohn et al. 1989). Dolphins receive local anesthesia before either of these procedures are performed. White et al. (1995) measured the effect of local anesthetic on piglets' vocal responses to castration, and found that call rate was lower in anesthetized piglets. However, field notes accompanying acoustic recordings do not consistently report the activity in which dolphins were engaged during recordings, so it is difficult to correlate whistle rate with any specific sampling activity.

Inter-individual variability in vocal responses can also be affected by a variety of factors, including social rank and temperament. Subordinate pigs are far more stressed than dominant pigs as a result of transport (McGlone et al. 1993). Rank could be a factor influencing the higher whistle rates seen in young animals in this study, although it is not known if age is related to social rank in free-ranging dolphins. Temperament in cattle is a heritable trait that seems to impact vocal response to handling and isolation (Watts and Stooky 2001). Specifically, the authors found that cattle phenotype influenced an animal's proclivity to vocalize. In addition, Watts et al. (2001) determined that both genetic and age factors influenced the vocal rate of isolated bulls. Watts and Stooky (2001) found that an animal that vocalized during one trial was

more likely to vocalize in a second trial of the same experiment. Thus, individual variation can occur in vocal responses even if all individuals are exposed to the same treatment (Watts and Stooky 2001). Similar factors could contribute to the variability in whistle rates among bottlenose dolphins. Sayigh (1992) found a great deal of individual variability in whistle responses to playback experiments, and observed that some individuals tended to be more or less vocal than others, regardless of context. In particular, FB84 (dolphin #16 in Sayigh 1992) showed low whistle rates during playbacks experiments carried out from 1989-1991. In the current study, FB84 also had whistle rates that were substantially lower than those of most other females (including ones of comparable age and capture-release experience; Tables 4 and 8, Figures 5 and 12). This suggests that signature whistle rate in bottlenose dolphins may be affected by temperament, as was found by Watts and Stooky (2001) for cattle. Comparisons of whistle rates by the same individuals in a variety of contexts would provide insights into the effects of temperament on vocal production, as would comparisons of whistle production in different maternal lineages. Finally, inter-individual variability in whistle rates might have resulted from differences in the degree of prior capture-release experience among dolphins. The recordings analyzed to evaluate changes in whistle rate during a single capture release session ranged from the first to the twelfth session for an individual dolphin. If whistle rate decreases with increasing capture-release experience, dolphins with more extensive capture-release experience would have lower whistle rates at the beginning of a session than dolphins with less experience.

The general stability of most whistle parameters supports the existence of individually distinctive signature whistles. Loop duration was stable for each individual in all contexts except for within a single capture-release session. Minimum frequency was not significantly different

between contexts for any individual, although there was an overall tendency for it to decrease from the first to the fifth capture-release. Sayigh and Williams (unpublished data) observed a similar decrease in minimum frequency over time in female bottlenose dolphins, and speculated that it may be related to maturation. Maximum frequency and inter-loop interval were stable except when comparing whistles of females capture and released with or without a dependent calf. Physical features of whistles that remain relatively stable for an individual across contexts contribute to the individually distinctive nature of a signature whistle. Therefore, despite recent unsubstantiated suggestions to the contrary (McCowan and Reiss 1995, 2001), the results of this study indicate that bottlenose dolphins produce individually distinctive vocalizations, and that several parameters of signature whistles can vary with context.

Signature whistle rate and loop number have the potential to serve as indicators of stress in bottlenose dolphins and warrant further research. Since bottlenose dolphins have individually distinctive whistles, whistle rate and loop number could potentially be used to monitor the welfare of individual dolphins for which signature whistles have been identified (e.g. Sarasota Bay population, captive dolphins). Future work should include comparisons of loop number and signature whistle rate in free-ranging dolphins in the presence and absence potential sources of stress, such as jet skis, predators, or swimmers participating in swim-with-dolphins programs. Buckstaff (2004) found that signature whistle production increased as dolphins in Sarasota Bay, FL were approached by boats, supporting the results of the current study. Loop number and whistle rate could also be monitored for dolphins in captive facilities that are undergoing construction or habitat alterations. The introduction of a new dolphin into a captive facility might create stress for both the new and established dolphins. Whistle rate and loop number could therefore potentially provide information about the process of acclimation for newly

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acquired dolphins. These parameters could also be monitored in an outdoor captive facility both before and after a hurricane or some other predicted weather event.

If the patterns in whistle parameters detected in this study persist once effects such as age and prior capture-release experience have been quantified, these measures could be utilized in conjunction with physiological indicators to groundtruth their reliability as indicators of various types of stressors in bottlenose dolphins. Although more work is needed, this study indicates that acoustic monitoring holds promise as a non-invasive means of assessing the impact of potentially stressful situations on bottlenose dolphins.

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