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Vocal Response of Bottlenose Dolphins (*Tursiops truncatus*) to a Novel Stimulus

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VOCAL RESPONSE OF BOTTLENOSE DOLPHINS (*TURSIOPS TRUNCATUS*) TO
A NOVEL STIMULUS

by

Lindsey E. Johnson

A Thesis
Submitted to the Graduate School,
the College of Education and Human Sciences
and the School of Psychology
at The University of Southern Mississippi
in Partial Fulfillment of the Requirements
for the Degree of Master of Arts

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ABSTRACT

Bottlenose dolphins utilize acoustic signals as their primary mode of communication. Although some aspects of dolphin vocal behavior are well understood, less is known about vocalizations in different behavioral contexts and how these vocal behaviors may indicate habituation and sensitization. The focus of this study was to investigate how bottlenose dolphins respond vocally to a novel stimulus. Archival data from three populations of bottlenose dolphins ($N = 20$) living in a human-care facility were exposed to a novel apparatus (a mirror) for 10 trials, each lasting 20 minutes. Five of the trials presented the mirror covered with an opaque cloth whereas the other five trials left the mirror uncovered, exposing the reflective surface. Rates of two distinct vocal types (whistles and burst pulses) were calculated for each trial. Vocal rates were greater on the last (non-novel) trials versus novel trials—a pattern of sensitization to the novel stimulus. Vocal rates and the degree of sensitization were greater on reflective trials than non-reflective trials. Finally, vocal rates were greater for whistles than burst pulses overall, but sensitization was most apparent with burst pulses. This study identifies how the production of two types of vocalizations change in response to a novel stimulus of varying reflectivity over repeated exposures thereby providing a greater understanding of the contexts in which these utterances are used.

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Thank you to my current and past Marine Mammal Behavior and Cognition Lab mates. All of you are inspirations to me and I am so grateful that you all were with me through this journey. Finally, I would like to thank all of the Brain and Behavior Psychology graduate students for providing me with an invaluable support system during the creation of this thesis.

DEDICATION

I would like to dedicate this thesis to Charles Darwin Johnson, my brother. Thank you for showing me the way to be brave, joyful, and adventurous. Your strength and love of life touched so many lives and will never ever be forgotten. You are always with me, Brother.

Further, this thesis will also be dedicated to my parents, Carl and Beverly Johnson and oldest brother, Dr. Thor Johnson. I have a sincere appreciation for all of the love and support you all have given me throughout my life and time in graduate school. Without all of you, this would not have been possible.

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CHAPTER I - INTRODUCTION

In low visibility environments, cetaceans often rely on sound for communication, navigation, localizing prey, and alerting others to the presence of a predator (e.g., Herzing, 1996). Studies have shown that marine mammals, specifically bottlenose dolphins (*Tursiops truncatus*), have a varied and complex vocal repertoire involving patterns of whistles, burst pulse signals, and echolocation clicks (e.g., Bowles & Anderson, 2012; Caldwell & Caldwell, 1968; Connor & Smolker, 1996; Reiss, McCowan & Marino, 1997; Wood, 1953). Each sound type serves a specific function (e.g., Herzing); however, the role these vocalizations play when dolphins encounter novel stimuli is currently under investigated. Therefore, this study characterized the vocal responses of bottlenose dolphins ($N = 20$) in the context of a novel reflective and non-reflective mirror and how these responses changed over repeated exposures.

Behavioral Reactions to Novel Objects

Stimulus novelty is defined by an organism's lack of previous exposure to that specific stimulus (Corey, 1978). Non-human animals exhibit a range of responses to novel stimuli which fall into two general categories: Neophobic responses, which include aggressive/agonistic and avoidant behaviors, or neophilic responses, which include exploratory and orienting behaviors (Bowles & Anderson, 2012; Fu et al., 2013; Greenberg & Mettke-Hofmann, 2001). These responses have been found in numerous species including ravens (*Corvus corax*: Stowe et al., 2006), Sichuan snub-nosed monkeys (*Rhinopithecus roxellana*: Fu et al), garden warblers (*Sylvia borin*: Mettke-Hofmann, Rowe, Hayden, & Canoine, 2006), and marine mammals (Bowles & Anderson; Delfour & Herzing, 2013). Naturally, each of these species encounter novel

stimuli, and responses to those stimuli may impact survival (Fu et al.; Greenberg & Mettke-Hofmann). Novel objects are ambiguous and may afford costs and/or benefits. For instance, novel objects could threaten survival (Fu et al.; Stowe et al.), or be safe and even beneficial (Greenberg & Mettke-Hofmann). Stowe et al. reported that ravens weigh both costs and benefits when approaching novel food sources as evidenced by a reduced approach latency when alone versus being accompanied by a conspecific. The authors interpreted these findings as ravens being less willing to incur the risks of interacting with an unfamiliar stimulus if there is a chance a conspecific will take the risk instead. Now that some initial reactions to novel stimuli have been identified, the focus will turn to how those reactions change as an animal is repeatedly exposed to a stimulus.

When analyzing animal responses to novel stimuli, it is important to consider how responses change as animals gain experience with a stimulus (Fu et al., 2013; Groves & Thompson, 1970; Stow et al., 2006). For instance, the dual-process theory (Groves & Thompson) suggests that repeated exposures will produce behavioral plasticity as a result of the interaction of two processes: Habituation and sensitization. Habituation reflects the reduction of responding to novel stimuli with successive exposures, while sensitization reflects an increase in responding as the stimulus is repeatedly presented to the animal. These processes are argued to be independent (with the S-R neural pathway controlling habituation, and state systems, which influence the arousal level of the animal, controlling sensitization), yet both interact to produce the behavioral response of the organism. These responses can be displayed on a “habituation curve” that depicts the pattern of behavioral plasticity. Results from both the hindlimb flexion reflex test with cats and acoustic startle responses in rats indicate that intensity of the stimulus was the

factor with the most impact on sensitization. At low intensities, habituation curves reflect the predominance of the habituation process, but as intensities increase, sensitization increases and habituation is attenuated. Interestingly Groves and Thompson found that if an animal continues to be exposed to a sensitizing stimulus, even at the highest intensities, the sensitization process will eventually begin to decay or habituate.

Before addressing cetaceans' reactions to novelty, it is important to be familiar with how dolphins behave under baseline conditions. Dolphins live in a fission-fusion society that is socially driven. They can be seen associating with different individuals from day to day and interactions can range from tactile rubbing which strengthens social bonds, to sexual play, to physical fights that solidify the dominance hierarchy (e.g., Harvey, Dudzinski, & Kuczaj, 2017). In both wild and human-care populations, synchronous behavior, defined by two or more animals engaging in the same behavior, is common and serves a variety of functions including being an indicator of alliance unity, a signal of cooperation, and a way to reduce tension (Conner, Smolker, & Bejder, 2006).

When examining how marine mammals respond to novel stimuli, most of the literature has focused on non-vocal behavior. Some of the work in this area was conducted when research on marine mammals was in its nascent stages. Both McBride and Hebb (1948) and Caldwell, Haugen, and Caldwell (1962) described how dolphins became "excited" when a novel object (e.g., an inflatable float) was introduced in their enclosure. The dolphins in both studies swam in a tight formation very quickly around the far side of the habitat. These early reports were descriptive in nature and did not include a quantitative analysis of concurrent vocal behavior.

More recently, a systematic approach by Bowles and Anderson (2012), examined the effects of exposure to novel stimuli that resembled fishing gear on several species of marine mammals in human-care to analyze responses to potentially dangerous anthropogenic, man-made, stimuli. The behavioral responses exhibited by the marine mammals were variable and showed many interspecies and intraspecies differences. Specifically, during initial exposure, harbor seals (*Phoca vitulina*) showed neophobia but later, over additional exposures, the seals' avoidant behaviors diminished, and they began touching the objects with little indication of fear or aggression. Sea lions (*Zalophus californianus*) showed similar patterns to the harbor seals, though initial reactions were more aggressive, including open-mouth threats, chewing, and rapid flips. The sea lions' aggressive reactions also dissipated at a faster rate versus the harbor seals, returning to baseline swim patterns within 30 to 60 minutes of initial exposure while the seals interacted with the stimuli throughout the entirety of the study. Thus, the habituation process was more pronounced with the sea lions; even though the seals avoidant behaviors decreased, their behavioral patterns never returned to baseline.

In contrast, relative to harbor seals and sea lions in Bowles and Anderson (2012), cetaceans exhibited different behavioral patterns altogether. Dolphins made minimal contact with novel objects with the exception of Commerson's dolphins (*Cephalorhynchus commersonii*) who showed three instances of aggressive behavior towards the fishing gear which included charging and breaking through one of the net-like objects. The authors noted that the most common reaction for Commerson's dolphins and bottlenose dolphins was an increase in speed/activity and erratic swim patterns when exposed to the novel stimuli. This increase in activity following exposure to the fishing

gear reduced both slow synchronous swimming and frequency of affiliative behavior versus baseline. Dolphins often charged towards unfamiliar objects only veering away at the last moment. Additionally, the dolphins spent time circling around the stimuli in tight circles, apparently investigating the objects. According to the authors, this collection of behaviors has often been associated with agonistic contexts, suggesting a neophobic response. These responses described by Bowles and Anderson subsided relatively quickly and dolphins returned to their typical synchronous swimming pattern, a pattern consistent with habituation, though they swam in closer proximity to each other than baseline.

Sensitization in Bowles and Anderson's (2012) study was found, but only reported after the novel object was paired with a novel auditory stimulus, a pinger, which emitted a beep every four seconds. The pairing of the novel stimulus and the pinger was aversive and the dolphins spent greater time in a separate refuge pool away from the pinger as the experiment progressed. Further, the dolphins began avoiding previously habituated stimuli once that stimulus had been paired with the pinger. These patterns of responding are consistent with the dual-process theory in that behavioral plasticity varied based on the intensity of the stimulus. The additional auditory component changed the environmental input in such a way the dolphins' response strategy changed from habituation to sensitization.

Other studies have similarly examined dolphin behavioral responses to novel stimuli. Delfour and Herzing (2013) exposed human-habituated, free-ranging spotted dolphins (*Stenella frontalis*) to a mirror 14 times over the course of many years. The exposures were relatively infrequent and brief (e.g., nine years separating exposure 11 and 12; each exposure lasting between 46–416 s)—a variable schedule which has been

shown to reduce habituation (Kuczaj, Lacinak, Fad, Trone, Solangi, & Ramos, 2002). The dolphins most frequent behavioral response was to circle around the mirror, as opposed to simply swimming by it or orienting towards it. Aggressive behaviors were rare, though one male positioned himself directly in front of the stimulus and exhibited an aggressive stationary posture. A sex-related difference was also noted with more females coming in close proximity to the mirror than males. Overall, these wild dolphins were noted to be uninterested in the apparatus, despite the infrequent/variable presentation schedule and the mirror's reflective qualities.

Somewhat contradictory results were reported by Clark et al. (2013) when they introduced a novel underwater maze to two homogenous groups (one female and one male) of bottlenose dolphins in human care. Females ($N = 5$) did not approach the device and showed greater rates of synchronous swimming compared to baseline levels throughout the experiment despite specific training to desensitize them to the stimulus. Female behaviors showed no evidence of habituation as they never approached/explored the novel device and synchronous swimming rates remained elevated throughout the experiment. Increased rates of synchronous swimming contrast Bowles and Anderson (2012) who reported reduced synchronous swimming with the novel stimulus present in the dolphins' habitat, but more closely align with McBride & Hebb (1948) and Caldwell et al. (1962) who both noted that dolphins tend to respond to novel objects by swimming together in tightly packed groups. This difference could reflect differences in nature and complexity of the stimulus in each experiment, or population-based differences. Interestingly, Bowles and Anderson (2012) and Clark et al. (2013) both suggested the dolphins' behavioral response was indicative of neophobia.

In contrast to females, males ($N = 6$) in Clark et al. (2013) showed greater interest in the novel device. Three males approached the device in the first minute of exposure, and spent on average 12% of the time interacting with the maze—patterns that suggest neophilia and rate of habituation may be sex-based. However, Delfour and Herzing (2013) reported that females exhibited a higher rate of neophilic behaviors versus males with an underwater mirror which may suggest sex-related differences are not reliable or at least are moderated by the type of novel object dolphins are exposed to.

Although a fair number of studies have visually examined dolphin behavioral responses to novel stimuli (e.g. Bowles & Anderson, 2012; Clark et al., 2013; Delfour & Herzing, 2013), less is known about dolphin vocal responses. Acoustic data may have complemented pure behavioral responses (e.g., swim patterns and posturing), helping to elucidate the function of these vocals to gain a greater understanding of how dolphins respond to novel stimuli. Recently, Lopes et al. (2016) began to address these shortcomings incorporating a methodology that allowed for simultaneous collection of behavioral and acoustic data, though in their study, only visual, non-vocal behaviors were analyzed. A novel apparatus—a mirror attached to PVC pipes—was introduced into the habitat of three separate populations of bottlenose dolphins. The mirror was chosen as a novel stimulus due its inclusion in prior studies (Delfour & Herzing, 2013), and allowed for an examination of the effects of stimulus complexity by comparing behaviors when the mirror’s reflective surface was uncovered versus covered. When uncovered, the reflective mirror transmitted more visual information and therefore was visually more complex relative to when the mirror was covered by a black cloth. The complexity of a novel stimulus has been shown to influence the intensity and duration of both neophobic

and neophilic responses (Mettke-Hofmann et al., 2006). Lopes et al. reported that non-vocal responses were more aggressive when the mirror was reflective than non-reflective and that these aggressive responses (e.g., tail slap, jaw clap, open mouth, etc.) increased across successive exposures—a pattern indicative of sensitization. Despite these stark differences between reflective and non-reflective conditions, it is unclear whether these behaviors converge with vocal patterns that are also consistent with aggressive behaviors. The goal of my thesis was therefore to analyze the acoustic portion of the data collected in the Lopes et al. (2016) study to provide greater understanding of dolphin vocal responses to a novel stimulus.

In general, the literature suggests that dolphins exhibit a neophobic response to novel objects initially and progress into exploratory behaviors once they become habituated to the stimulus, though some results show clear deviations from this pattern (e.g., Clark et al., 2013; Lopes et al., 2016). These differences may be affected a variety of variables including sex, age, cohort size and make-up, and methodological differences. The existing literature also reveals a lack of clarity regarding how particular vocal responses vary with the amount of prior exposure to a stimulus, a topic which I will now discuss.

Vocalizations and Acoustic Reactions to Novel Stimuli in Dolphins

Cetacean vocal behavior is integral to communication and navigation and takes a variety of forms. Researchers have shown that vocalizations range from 2-10 different general vocal types (Conner & Smolker, 1996; Herzing, 1996). Despite this variability, the majority of researchers have agreed that vocal utterances should be grouped into three main types for the sake of maintaining consistency across the literature: Narrow-band

frequency-modulated whistles, broad-band burst-pulse signals, and broad-band echolocation clicks¹ (Eskelinen, Winship, Jones, Ames, & Kuczaj, 2016; Herzing, 2014; Reiss et al., 1997). My thesis aims to examine the two categories associated with social behavior (whistles and burst pulse signals, Eskelinen et al., 2016) as a basis for the acoustic analyses given the presence of novel objects may encourage social communication among conspecifics.

The most commonly studied vocal response is the narrow-band, frequency-modulated whistle (Herzing, 2014). Dolphins tend to emit one individually-specific whistle, termed a signature whistle, which accounts for approximately 80-100% of total whistles in isolation and 38-70% of total whistles in free range (Buckstaff, 2004; Janik & Sayigh, 2013; Watwood, Owen, Tyack, & Wells, 2005). Signature whistles are defined as learned acoustic signals containing information that identifies a specific individual. Young dolphins develop signature whistles throughout their first year of life (Sayigh, Tyack, Wells, & Scott, 1990) and once solidified, the signature whistle remains stable throughout the lifetime (Herzing, 1996; Janik & Sayigh; Sayigh et al.). Signature whistles have been correlated with multiple behavioral contexts, however a primary function of the whistle is broadcasting identity and location which is critical for group cohesion (Janik & Sayigh). Signature whistles have been shown to be important in mother-calf reunions, allowing each member of the pair the ability to request the others' presence (Herzing; King, Guarino, Keaton, Erb, & Jaakkola, 2016b; Kuczaj, Eskelinen, Jones, & Borger-Turner, 2015). Dolphins occasionally engage in vocal matching, copying a conspecifics signature whistle, which could be interpreted as an affiliative signal, serving to strengthen bonds between group members (Wood, 1953).

Dolphins are able to modify the production rate of their vocalizations, enabling information to be conveyed beyond the producers' identity. Janik and Sayigh report that free-ranging dolphins are generally silent, especially when travelling and in close proximity, though whistle rates, including both signature and non-signature whistles, range from 1–9.8 whistles/min, reaching their maximum when the dolphins terminate directional swimming and engage in social activities, emphasizing the importance of whistles in conspecific communication. Human-care populations have a similar range of whistle rates, 0–7.6 whistles/min during baseline conditions (Caldwell, Caldwell, & Tyack, 1990). Increased rates of signature whistles have also been correlated with stressful situations in dolphins living in both environments (Esch, Sayigh, Blum, & Wells, 2009; Janik & Sayigh, 2013). Dolphins frequently emit signature whistles repetitively when isolated, with whistle rates increasing as the distance from conspecifics increases (King et al., 2016b). Esch et al. reported that whistle rates and the number of repetitive loops during brief capture events increased during capture versus a baseline condition. These patterns were suggested to reflect increased stress levels as dolphins were extracted from their habitat.

Like whistles, cetaceans are also known to use pulsed signals for social communication (Connor & Smolker, 1996; Herzing, 1996; Overstrom, 1983; Ridgeway, 1983). Blomqvist and Amundin (2004) outline how one type of pulsed signal, the broadband burst pulse, is frequently emitted during aggressive interactions. They found that burst pulses increase in rate and intensity as aggressive behaviors intensify. The authors also noted that the burst pulses were only observed in aggressive or agonistic interactions and could potentially serve as an alternative to physical fights as a way to settle

dominance disputes. Other researchers have also associated burst pulses with agonistic contexts such as head-to-head altercations, males herding unreceptive females, sexual play, and discipline (Connor & Smolker, 1996; Herzing, 1996; Overstrom, 1983; Ridgeway, 1983). Burst pulses may be used for collaborative efforts as well. For instance, they were emitted when two dolphins were pulling on ropes attached to a cylindrical apparatus containing a reward (Eskelinen et al., 2016). When the ropes were simultaneously pulled in opposite directions, the apparatus would open, allowing the dolphins to access the reinforcement. While it is possible these burst pulses were used to aid in cooperation, others have noted this design could also represent a competitive/agonistic context (King, Allen, Connor, & Jaakkola, 2016a), suggesting the type of information communicated by these sounds is unclear.

Less is known about burst pulse production rates than whistle rates in both the wild and human care settings, although Connor and Smolker (1996) reported rates for a different pulsed signal, the low frequency “pop”. This vocalization was produced in bouts of 3–30 utterances at a rate of 6–12 per second in the context of male herding behavior. The authors determined this particular signal produced by male bottlenose dolphins in Shark Bay, Australia functioned as a threat to induce females to approach.

The focus will now turn to what is known about the vocal behavior of dolphins when they encounter novel stimuli. While McBride and Hebb (1948) reported increased whistle production when a novel object was presented, their study did not utilize a hydrophone to quantify vocal utterances. Bowles and Anderson (2012) did utilize hydrophones and showed that Commerson’s dolphins exhibited greater click rates in the presence of a novel stimulus, suggesting greater use of echolocation to gather information

about the unfamiliar object (see too Verboom & Kastelein, 1995). Bottlenose dolphins however, showed no change from baseline for most novel objects that were presented. One exception however, was when a gillnet was paired with the pinger. Here, bottlenose dolphins showed greater sound production rates over initial exposure at baseline when the pinger was inactive. Once the pinger was activated and began producing noise, dolphin vocal rates dropped below baseline levels. The authors suggested the addition of the auditory stimulus prompted the dolphins to perceive the object as a threat and the decline in vocalizations represented a defense mechanism to acoustically hide from a potential predator. To facilitate data analysis, Bowels and Anderson grouped acoustic behaviors into vocal bouts due to substantial overlap of the various vocal types when viewed on a spectrogram. These bouts were then reported as number of sounds per minute. Analyzing vocal responses by separating them into different types would provide a more fine-grained analysis of how utterances are used in the presence of novel objects. In particular, separation of whistle rates and burst pulses—two common vocal utterances in bottlenose dolphins, can provide important information about the complexity of communication patterns towards new objects.

Purpose of Study

Given the limited research on the vocal responses of bottlenose dolphins towards novel stimuli, the goal of the present study is to provide a comprehensive assessment of whistles and burst pulse signals—vocal responses that have previously been associated with stressful events. The study was designed to provide a reliable measure of vocal responses by using a relatively large sample size with a large range of ages ($N = 20$, dolphins ranging from calf to adult) over a series of repeated exposures (10 trials). In the

present study, the analyses examined the acoustic portion of data that were not reported by Lopes et al. (2016) and included whistle rates and burst pulses rates over repeated exposures to a reflective or non-reflective apparatus that was placed into three dolphin habitats.

There were several factors to consider when making predictions about the current project. Based on the literature (e.g., Bowles & Anderson, 2012; Wood, 1953), dolphins often display neophobic reactions, treating novel objects as a threat. Some studies reported that dolphins habituated to a novel stimulus after repeated exposures (e.g., Bowles & Anderson; Wood), however Lopes et al. (2016), reported that dolphins showed more aggressive/agonistic behaviors as the experiment progressed—a pattern of sensitization. Previous studies have shown that signature whistle rates increase in stressful situations (e.g., Esch et al., 2009; Janik & Sayigh, 2013), and therefore, one hypothesis (**H₁**) of the present study was that whistle rates would increase across successive exposures to the novel stimulus, with rates on the last trial being greater than rates on the novel trial, suggesting a pattern of sensitization. The same sensitization pattern was hypothesized (**H₂**) to occur for burst pulse rates as they were predicted to correspond with the behavioral indicators of aggression that were reported by Lopes et al. When considering stimulus reflectivity, both whistle rate and the rate of burst pulse signals were hypothesized (**H₃**) to be greater in the reflective condition versus the non-reflective condition. The reflectivity of the stimulus was expected to cause greater uncertainty and in turn, increase the aversive/aggressive response indicative of stress (i.e., more vocalizations). Overall whistle and burst pulse rates were also compared. Mean whistle rates were hypothesized (**H₄**) to be greater than mean burst pulse rates because

whistles are produced in a wide variety of situations (e.g., communication, stress, group cohesion), while burst pulses are only produced in isolated situations (e.g., aggression). These analyses could provide foundational evidence in support of using vocal rates as a measure of sensitization to anthropogenic stimuli in captive dolphin populations, which may have implications for their wild counterparts. The results of this study may serve to inform policy-making decisions that would enhance the protection of the species.

CHAPTER II - METHOD

Subjects

Data collection for this project took place at three managed-care marine mammal facilities in Key Largo, Florida: Dolphins Plus Bayside (Cove), Dolphins Plus Oceanside (North), and Island Dolphin Care (South) during September of 2012. The three populations used for this study, totaling 20 bottlenose dolphins, contained males and females of various age-classes ranging from calf to adult (Eskelinen, Winship, Borger-Turner, 2015; see Appendix). Subjects were housed in three separate enclosures that were connected to the ocean and contained natural seawater. The North and South enclosures were each comprised of 836 m² (mean depth 4m ± 1.2m) on a public canal adjacent to the Atlantic Ocean. Dolphins Plus Bayside consisted of a 1,000 m² (mean depth 6m ± 1.2m) single enclosure connected to the Florida Bay.

Apparatus

A mirror consisting of four PVC pipes (1 m²) framing a flat, reflective, acrylic surface, was used as the novel stimulus. A black cloth covered the reflective surface for half of the trials to determine if the reflective properties of the stimulus had any influence on habituation or sensitization. Four cameras were used to collect visual and audio data. Two cameras were affixed directly on the apparatus: A GoPro HERO on the top left-hand corner and a Power shot D10 directly in the middle of the reflective surface. A third camera, a Canon G9 Power Shot 12.1 mega-pixel was placed approximately 1 m below the surface and was stationed on a monopod to collect the high-quality audio files used in the present analyses. Finally, a Canon G12 Power Shot 12.1 mega-pixel was utilized

above the water to record surface behaviors. See Figure 1 for a visual representation of the apparatus (Lopes et al., 2016).

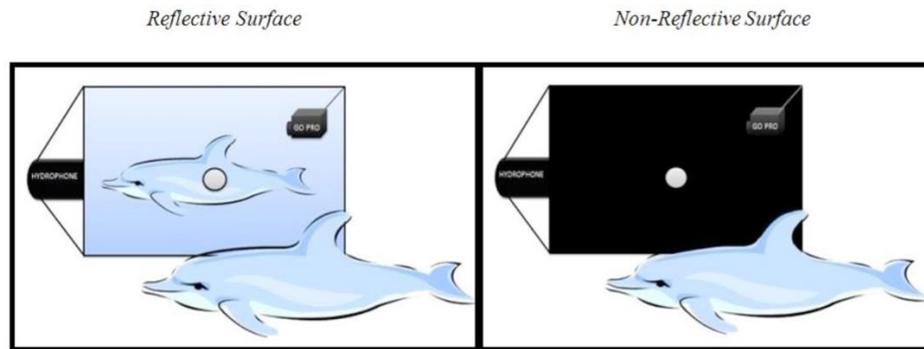


Figure 1. Visual representation of the apparatus shown in both the reflective and non-reflective conditions (Lopes et al., 2016).

Procedure

Each group of dolphins was exposed to the stimulus over 10 trials over a two-week period, each lasting 20 minutes. Experimental trials were conducted during the morning hours between 07:00hr and 12:00hr, during high tide, and between training sessions. No dolphins had prior exposure to the apparatus and it was stored out of the dolphins' sight between trials. Experimental trials were randomized to determine whether the stimulus would be reflective or non-reflective for each presentation. The presentation order varied across the 3 habitats, with 2 of the 3 groups of dolphins experiencing the non-reflective condition on their novel exposure (see Appendix). Pre-trial data was collected for 10 minutes before each exposure with an above-water recording. This type of recording did not collect data for an acoustic analysis, but the behavioral state of the dolphins in the habitat was captured with these observations.

After the pre-trial data were collected, the apparatus was introduced into the habitat and affixed to the edge of the enclosure, allowing the dolphins access to all sides

of the stimulus except for the back. The placement location was held constant across the 10 trials for each group. Audio and visual recordings were taken for the entirety of each 20-minute test trial.

Statistical Analyses

Raven Pro 1.4, a bioacoustics analysis program, was used to calculate overall rates for whistles and burst pulses for each trial. Whistles and burst pulses were classified and measured using operational definitions whose validity has previously been demonstrated in the literature (Eskelinen et. al, 2016; Herzing, 1996; see Appendix). The methodology employed to collect the data did not allow vocalizations to be localized to particular dolphins. Therefore, age and sex differences in vocal responses could not be examined. Further, logistical constraints (i.e., dolphins living together in separate habitats) restricted the researchers' ability to vary the condition each dolphin experienced for their novel exposure (i.e., reflective or non-reflective). Since the presentation order was yoked to habitat location, it was necessary to examine vocal responses pooled across the three lagoons. Therefore, the dolphins' habitat location was included as covariate in all of the analyses.

To calculate vocal production rates, an all-occurrence sampling methodology (Altmann, 1974) was utilized, meaning all the whistles and burst pulses that were emitted during the test trials were included in the analyses. For whistles, all individual whistles that met the operational definition (see Appendix) were identified and recorded in the software program to calculate their rate of occurrence. The primary dependent measure, mean whistle rate, was computed as the number of whistles per trial divided by the duration of the trial, then divided by the number of dolphins in the lagoon (mean whistle

rate⁻¹). A data point was created for each dolphin in the habitat by including the mean whistle rate⁻¹ (calculated for that particular trial and lagoon) one time for each dolphin present. Burst pulses were similarly recorded and tallied as whistle rates.

Lopes et al. (2016) conducted the behavioral analysis on the visual portion of the data, so the aim of the present study was to analyze the dolphins' vocal responses to the stimulus. Of particular interest were the vocal rates on the first (novel) exposure and the last (non-novel) exposure. Those rates were compared across reflectivity contexts to identify evidence suggestive of the dolphins' response strategy to the novel stimulus.

CHAPTER III - RESULTS

Auditory data were collected at three separate lagoon locations, these locations were analyzed as a covariate for all results reported to account for location-related influences on main effects and interactions reported². For all significant comparisons effect sizes were computed using partial-eta squared (η_p^2) for ANCOVAs and Cohen's *d* for *t*-tests.

Whistle Rates

The effect of trial number on whistle production was first analyzed with a repeated-measures ANCOVA that compared mean whistle rates across all 10 exposures collapsed across reflectivity conditions (see Figure 2). The lagoon habitat location was found to be a significant covariate, $F(1, 18) = 32.75, p < .001, \eta_p^2 = .65$, thus lagoon was covaried out of all subsequent whistle rate analyses. A significant effect of trial was found, $F(9, 162) = 14.24, p < .001, \eta_p^2 = .44$. Bonferroni adjusted pairwise comparisons yielded significant differences between the mean whistle rate for the novel exposure Trial 1 (1.02) and Trial 4 (2.12), Trial 1 and Trial 5 (1.87), Trial 1 and Trial 6 (1.82), and Trial 1 and Trial 7 (1.46). Descriptively, whistle rates were low on the novel exposure, Trial 1, and remained somewhat stable across the first 3 trials (Mean Rate Range = 1.02–0.78). A sensitization pattern was then found beginning at Trial 4 in which the peak mean whistle rate was recorded across all 10 trials. Whistle rates then gradually decreased across the remaining trials, possible evidence for habituation, with rates on the last three trials returning to the levels on the novel trial.

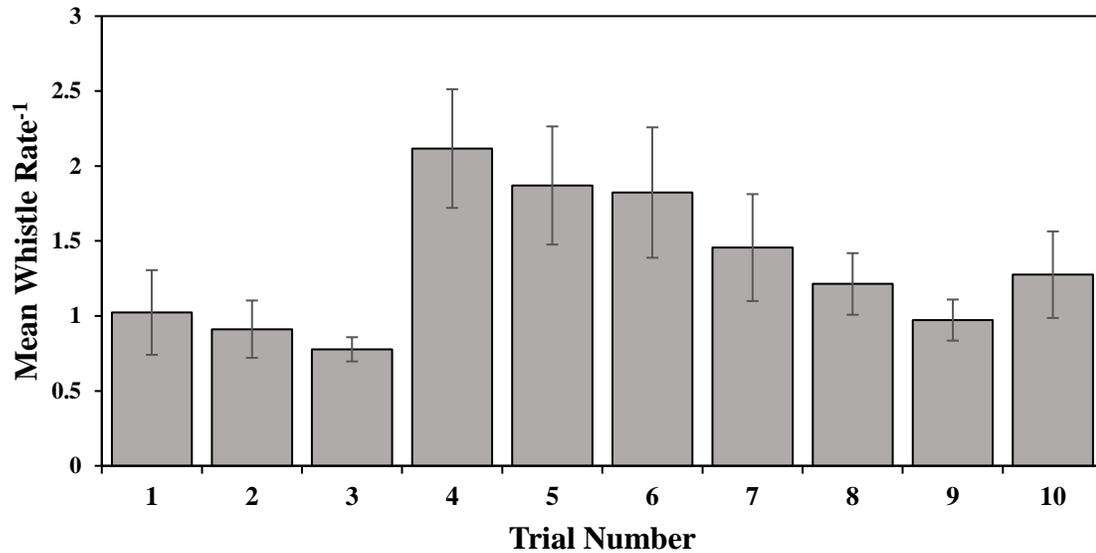


Figure 2. Mean whistle rates as a function of trial number collapsed across lagoon habitat. Mean whistle rate is defined as number of total whistles emitted in the trial divided by the trial’s duration (minutes), divided by the number of dolphins in the habitat (whistle rate⁻¹). Error bars represent 95% confidence intervals.

The next set of analyses examined the effects of reflectivity across trials. Mean whistle rates as a function of reflectivity and trial number are reported in Figure 3. A 2 (reflectivity) × 5 (trial) repeated-measures ANCOVA again indicated a significant covariate of lagoon, $F(1, 18) = 32.75, p < .001, \eta_p^2 = .65$. A main effect of trial number was found, $F(4, 72) = 83.88, p < .001, \eta_p^2 = .82$ which indicated a pattern of sensitization in which mean whistle rates on all non-novel trials (i.e., Trials 2–5) were significantly greater than the mean whistle rate for novel exposures to each condition (Trial 1 vs. Trial 2 (0.97 vs. 1.51), $t(19) = 11.75, p < .001, d = 1.11$; Trial 1 vs. Trial 3 (0.97 vs. 1.59), $t(19) = 13.06, p < .001, d = 1.03$; Trial 1 vs. Trial 4 (0.97 vs. 1.25), $t(19) = 2.76, p = .01, d = 0.58$; Trial 1 vs. Trial 5 (0.97 vs. 1.41), $t(19) = 9.50, p < .001, d = 0.75$). All comparisons held when analyzed with a Bonferroni adjustment. These results are consistent with **H₁**

which predicted that whistle rates would be greater on the last trial than on the first novel trial.

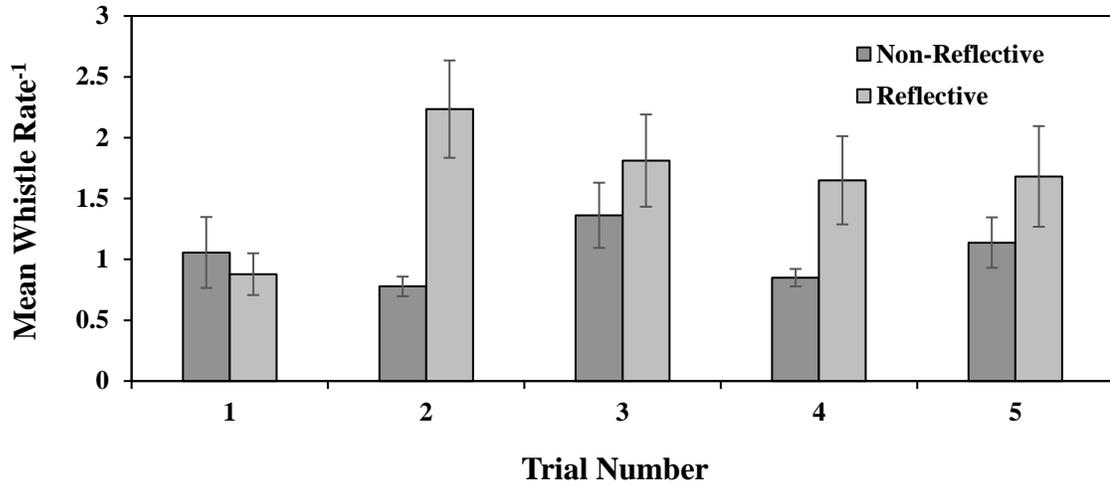


Figure 3. Mean whistle rates as a function of trial number and reflectivity condition. Mean whistle rate is defined as number of total whistles emitted in the trial divided by the trial's duration (minutes), divided by the number of dolphins in the habitat (whistle rate⁻¹). Error bars represent 95% confidence intervals.

While all non-novel trials had mean whistle rates that were significantly greater than the novel trial, there was some evidence for habituation when comparing rates on each trial with the rates of the trial that occurred directly before it. The difference between the mean whistle rates for Trials 2 and 3 as well as the difference between Trials 4 and 5 were not significant (Trial 2 vs. Trial 3 (1.51 vs. 1.59), $t(19) = 1.25$, $p < 0.23$, $d = 0.13$; Trial 4 vs. Trial 5 (1.25 vs. 1.41), $t(19) = 1.52$, $p = 0.15$, $d = 0.27$), but there was a significant decrease in mean whistle rates between Trials 3 and 4 (1.59 vs. 1.25), $t(19) = 2.8$, $p = .01$, $d = 0.57$. This comparison held when analyzed with a Bonferroni adjustment.

The ANCOVA also revealed a main effect of reflectivity ($F(1, 18) = 9.70$, $p < .01$, $\eta_p^2 = 0.35$), indicating greater overall whistle rates on reflective than non-reflective

trials (1.65 vs. 1.04). These main effects were qualified by a significant trial \times reflectivity interaction, $F(4, 72) = 11.34, p < .001, \eta_p^2 = 0.39$, which indicated that the increase in mean whistle rates from the novel trial to the non-novel trials was greater in the reflective than non-reflective condition. Post-hoc tests confirmed that for reflective trials, a robust increase in whistle rates occurred from novel to all non-novel reflective trials: Trial 1 vs. Trial 2 (0.88 vs. 2.23), $t(19) = 7.43, p < .001, d = 1.93$; Trial 1 vs. Trial 3 (0.88 vs. 1.81), $t(19) = 6.13, p < .001, d = 1.39$; Trial 1 vs. Trial 4 (0.88 vs. 1.65), $t(19) = 3.78, p = .001, d = 1.19$; Trial 1 vs. Trial 5 (0.88 vs. 1.68), $t(19) = 4.66, p < .001, d = 1.11$. For non-reflective trials however, whistle rates decreased from Trial 1 to Trial 2 (1.06 vs. 0.77), $t(19) = 2.05, p = .05, d = 0.57$, increased from Trial 1 to Trial 3 (1.06 vs. 1.36), $t(19) = 3.69, p < .01, d = 0.48$, but then returned to rates that were not significantly different from the novel trial on Trials 4 and 5 (Trial 1 vs. Trial 4 (1.06 vs. 0.85): $t(19) = 1.83, p = .08, d = 0.43$; Trial 1 vs. Trial 5 (1.06 vs. 1.14): $t(19) = 0.99, p = .34$). These comparisons were also reliable when a Bonferroni correction was used. Thus, the pattern of mean whistle rates (on the reflective trials) support **H₁**, namely that mean whistle rates would increase across successive exposures. **H₃** was also supported as mean whistle rates were greater on the reflective trials than the non-reflective trials.

Burst Pulse Rates

Figure 4 reports burst pulse rates across the 10 trials collapsed across reflectivity. A repeated-measures ANCOVA indicated that lagoon was not a reliable covariate, $F(1, 18) = 0.01, p = .93$, however a significant effect of trial number was found, $F(9, 162) = 48.05, p < .001, \eta_p^2 = 0.73$. Bonferroni-corrected comparisons indicated that mean burst pulse rates generally increased across trials (Trial 1 vs. Trial 2 (0.03 vs. 0.13), $p < .001$;

Trial 1 vs. Trial 4 (0.03 vs. 0.26), $p < .001$; Trial 1 vs. Trial 5 (0.03 vs. 0.25), $p < .001$;
 Trial 1 vs. Trial 6 (0.03 vs. 0.13), $p < .001$; Trial 1 vs. Trial 7 (0.03 vs. 0.38), $p < .001$;
 Trial 1 vs. Trial 9 (0.03 vs. 0.22), $p = .001$; Trial 1 vs. Trial 10 (0.03 vs. 0.44), $p < .001$),
 indicative of sensitization, with the exception of Trials 3 (0.01) and 8 (0.03), which were
 equivalent to Trial 1 (0.03), $p = 0.71$ and $p = 1.0$ respectively.

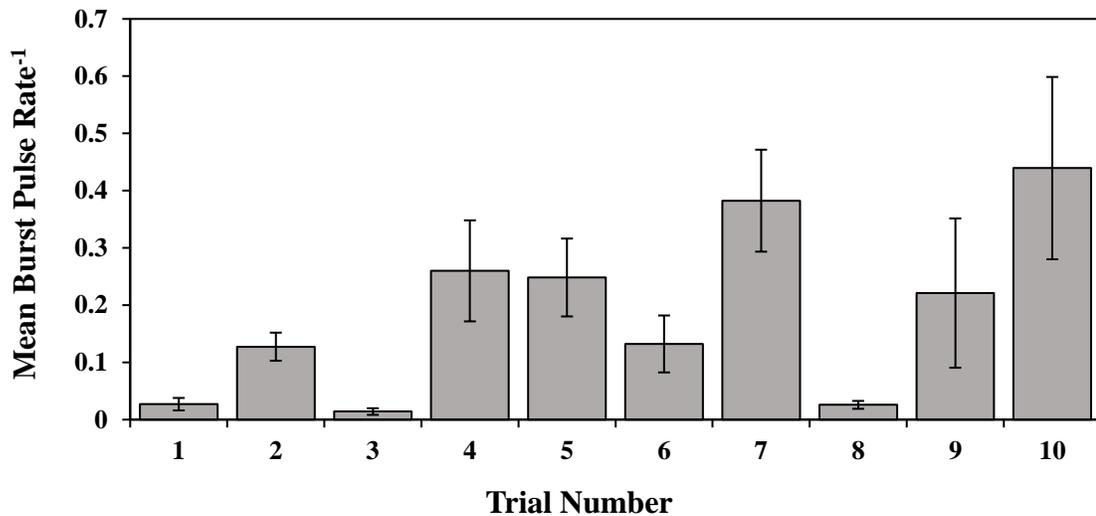


Figure 4. Mean burst pulse rates as a function of trial number collapsed across lagoon habitat. Mean burst pulse rate is computed as number of total burst pulses emitted in the trial divided by the trial's duration (minutes), divided by the number of dolphins in the habitat (burst pulse rate⁻¹). Error bars represent 95% confidence intervals.

The next set of analyses examined burst pulse rates as a function of reflectivity and trial number (see Figure 5). A 2 (reflectivity) \times 5 (trial) repeated-measures ANCOVA with lagoon as a covariate was similarly used to analyze burst pulse rates. The covariate of lagoon was not significant, $F(1, 18) = 0.01$, $p = .93$, however a significant main effect of trial was found, $F(4, 72) = 79.43$, $p < .001$, $\eta_p^2 = 0.82$. Post-hoc t -tests revealed that burst pulse rates significantly increased following the novel exposure: Trial 1 vs. Trial 2 (0.08 vs. 0.16), $t(19) = 6.66$, $p < .001$, $d = 1.53$; Trial 1 vs. Trial 3 (0.08 vs.

0.14): $t(19) = 5.05, p < .001, d = 1.23$; Trial 1 vs. Trial 4 (0.08 vs. 0.31), $t(19) = 8.98, p < .001, d = 2.98$; Trial 1 vs. Trial 5 (0.08 vs. 0.26), $t(19) = 4.95, p < .001, d = 1.73$. All comparisons held with a Bonferroni adjustment. Taken together, this pattern of sensitization is consistent with **H₂**, which predicted an increase in burst pulse rates across trials.

A main effect of reflectivity was also found, $F(1, 18) = 31.54, p < .001, \eta_p^2 = 0.64$, which indicated that overall burst pulse rates were greater when the mirror's reflective surface was exposed versus covered (0.35 vs. 0.03). This pattern supports **H₃** which hypothesized that the reflective condition would produce greater burst pulse rates. As with whistle rates, a trial by reflectivity interaction was also found, $F(4, 72) = 147.42, p < .001, \eta_p^2 = 0.89$. Burst pulse rates were low on Trial 1 but increased differentially depending on whether the mirror's reflective surface was exposed or covered. Specifically, for reflective trials mean burst pulse rates showed significant sensitization, increasing across trials: Trial 1 vs. Trial 2 (0.11 vs. 0.31), $t(19) = 12.48, p < .001, d = 1.78$; Trial 1 vs. Trial 3 (0.11 vs. 0.27), $t(19) = 7.62, p < .001, d = 1.38$; Trial 1 vs. Trial 4 (0.11 vs. 0.53), $t(19) = 11.28, p < .001, d = 3.34$; Trial 1 vs. Trial 5 (0.11 vs. 0.51), $t(19) = 5.06, p < .001, d = 1.87$. In contrast however, burst pulse rates remained relatively low or even decreased across non-reflective trials: Trial 1 vs. Trial 2 (0.04 vs. 0.01), $t(19) = 2.52, p = .02, d = 1.03$; Trial 1 vs. Trial 3 (0.04 vs. 0.01), $t(19) = 4.60, p < .001, d = 1.30$; Trial 1 vs. Trial 4 (0.04 vs. 0.08), $t(19) = 3.03, p < .01, d = 0.72$; Trial 1 vs. Trial 5 (0.04 vs. 0.01), $t(19) = 4.41, p < .001, d = 1.45$.

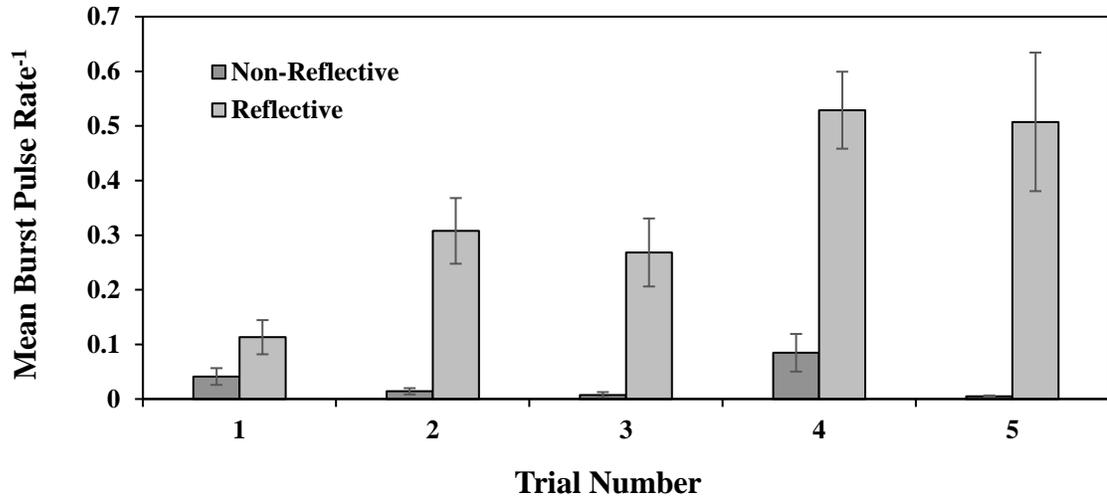


Figure 5. Mean burst pulse rates as a function of trial number collapsed across lagoon habitat. Mean burst pulse rate is computed as number of total burst pulses emitted in the trial divided by the trial's duration (minutes), divided by the number of dolphins in the habitat (burst pulse rate⁻¹). Error bars represent 95% confidence intervals.

Whistles vs. Burst pulses

As a final comparison, a paired samples *t*-test was used to compare overall production rates between whistles and burst pulses. As predicted by **H4**, the mean whistle rate was greater than the mean burst pulse rate (1.34 vs. 0.19), $t(19) = 9.25$, $p < .001$, $d = 2.41$. Additionally, mean whistle rates were negatively correlated to mean burst pulse rates, $r = -0.58$, $p < .01$, indicating that whistles and burst pulses were likely not produced at high rates concurrently. Thus, dolphins were more likely to use one type of vocal response in favor of another, and whistles were utilized more frequently.

CHAPTER IV - DISCUSSION

The bottlenose dolphins in the present study exhibited a neophobic response to a novel mirror, as evidenced by low vocalization rates on novel trials. In addition to neophobia, the results of this study suggested that dolphins sensitized to the apparatus: Both whistle and burst pulse rates were greater on the last exposure to the stimulus when compared to the first when collapsed across reflectivity conditions. Critically, these effects were driven by the rates of both vocal responses when the mirror was presented in the reflective condition. This increase for the reflective condition was particularly robust in burst pulse rates, where rates increased five-fold relative to the initial rate on the first novel trial. In non-reflective condition, burst pulse rates remained low or decreased across the 5 trials. This interaction provides additional evidence that rates of sensitization are influenced by the complexity of the novel object (Mettke-Hofmann et al., 2006).

Taking a closer look at the hypotheses, the first prediction (**H₁**) involved whistle rates increasing across successive exposures, a pattern of sensitization. This hypothesis was moderately supported with two analyses. The first analysis, which used all 10 trials collapsed across reflectivity and habitat, showed that whistle rates were stable for the first three trials, but showed a dramatic increase around Trial 4 and remained high until Trial 8, when rates returned to those recorded for the novel trial. The second analysis, which examined differences between the reflectivity conditions, found that whistle rates on all non-novel reflective trials were greater than the novel reflective trial. The mean whistle rate spiked at the reflective Trial 2 which then decreased to Trial 3, but stabilized across the remaining reflective trials. The pattern was not as clear for the non-reflective trials; There was an initial decrease in whistle rates during the second exposure, but then there

was a sharp rise observed on non-reflective Trial 3. For the last two trials, the rates stabilized around those seen on the novel non-reflective exposure.

A further indicator of sensitization to the apparatus was found when considering the pattern of burst pulse rates. Burst pulse rates increased steadily across all reflective trials, supporting **H₂** which similarly predicted sensitization of burst pulse rates across trials. As with whistle rates, the pattern of sensitization was found when analyzing burst pulse rates in two different ways. The first analysis, which used all 10 trials and collapsed across reflectivity and habitat, showed that burst pulse rates followed a general upward trend as the study progressed, with rates peaking on the final exposure (Trial 10). The trials that did not follow the upward trend (Trials 3 and 8) happened to be the only trials that were non-reflective for all three habitats. The second analysis involved accounting for reflectivity condition and found that mean burst pulse rates were being driven by the robust sensitization found across reflective trials. Burst pulse rates during the non-reflective condition were near zero.

Importantly, across analyses of whistle and burst pulse rates, trial number was found to interact with reflectivity condition, which indicated that sensitization rates were greater for the reflective than non-reflective conditions—a pattern consistent with **H₃**. This increase in sensitization for the reflectivity condition was particularly great for burst pulses, though it was also found for whistle rates. In further support of **H₃**, both whistles and burst pulses were produced more frequently in the reflective condition than the non-reflective.

The final hypothesis (**H₄**) predicted that overall whistle rates would be greater than burst pulse rates—a pattern supported by the data. Additionally, a negative

correlation between whistles and burst pulses was found, indicating that as one vocal type increased, the other decreased. This implies that the dolphins in this study likely favored one type of vocal response over the other when presented with the novel object.

Taking a closer look at the pattern of whistle rates across the course of the study, the first analysis examined how whistle rates change across all 10 trials, regardless of the reflectivity of the mirror. A period of sensitization which peaked at Trial 4 was detected. Interestingly, whistle rates then declined following Trial 4, demonstrating a pattern of habituation. This evidence of habituation was only apparent when the analysis was collapsed across reflectivity conditions in the 10-trial comparison. The other primary variable to consider is the reflectivity (i.e., complexity) of the novel object and how it may be affecting whistle production. In this case, the results indicated that the average of the whistle rates on the 5th exposure to each condition was greater than the novel exposure to each version of the stimulus. From this approach, the results seem to indicate that sensitization in whistle rates occurred across the course of the study. While there was a decrease in mean whistle rates when comparing the 4th exposure to the 3rd exposure regardless of reflectivity conditions, the habituation pattern was limited due to the similarity between exposures 2 and 3 and exposures 4 and 5. A more complete picture begins to emerge when we examine how mean whistle rates change within each reflectivity condition. In the non-reflective condition, whistle rates were relatively low on the first trial and persisted across remaining trials. Only in the reflective condition did sensitization emerge. The most pronounced increase occurred between exposures 1 and 2. Whistle rates remained high during the third reflective exposure, then showed a slight decrease for exposures 4 and 5, culminating with rates that were twice as high as those

recorded on the first exposure to the reflective surface. The degree of sensitization in the reflective trials was sufficient to mask any habituation that may have occurred across the course of all ten trials.

The factors that determine the rate of whistle production in bottlenose dolphins are numerous and varied. In an early demonstration, McBride and Hebb (1948) showed that when bottlenose dolphins were exposed to a novel object, they responded with constant whistling paired with excitement that was expressed by dolphins swimming quickly near the far side of the enclosure in a tightly formed group. The dolphins' heightened response subsided after an hour or two (i.e., habituation), but the object was still avoided well after other behaviors returned to baseline. The present study did not find the same pattern: Whistle rates were low on the novel trial and increased over successive exposures (versus the novel trial). One reason for this discrepancy could be that McBride and Hebb's dolphins may have perceived the novel objects as only a minor disturbance, whereas in the current study, the dolphins could have possibly identified the mirrored surface as aversive or threatening in some way. Bowles and Anderson (2012) reported that dolphins limit vocal production in the presence of a predator and/or threat to avoid detection. This difference in perceived threat might help to explain why the dolphins in the current study were somewhat quiet on the novel exposure to each condition.

A second reason the results of the current study do not align with McBride and Hebb (1948) could reflect methodological differences. Kuczaj et al. (2002) systematically demonstrated that the presentation schedule of a novel object (i.e., an environmental enrichment device designed for the animal to interact with, such as a chew toy or ball)

has a significant impact on the rate of habituation, with 2 hour-long trials showing significantly greater rates of habituation than 15 trials lasting from 1-15 minutes. McBride and Hebb introduced the novel object into the habitat and then left it for an extended period of time (many hours). Thus, the relatively short 20-minute trials used in the present experiment may have been an insufficient amount of time to produce habituation.

As previously mentioned, elevated whistle rates appear to be related to stressful situations including extraction, mother/calf separation, and isolation (e.g., Esch et al., 2009; Janik & Sayigh, 2013). Lopes et al. (2016) found that the dolphins in the present study showed more instances of aggressive non-vocal behavior in later versus earlier trials. A neophobic response including aggressive behaviors could be indicative of a stressful situation (Herman & Tavalga, 1980). Therefore, it is likely that the elevated whistle rates during the non-novel reflective trials were an additional signal indicating the stimulus was perceived as more aversive after the initial exposure, potentially as a result of the uncertainty involved with the reflective surface.

The patterns found in the rate of burst pulses were consistent with whistle rates though sensitization was far more pronounced. Burst pulses were rarely produced on the novel trial in each condition, but when the reflective surface was exposed, the rates of burst pulses increased dramatically. This robust sensitization under the reflective condition was contrasted with rates remaining extremely low across all of the non-reflective trials. These results support the interpretation presented by Mettke-Hofmann et al. (2006) who suggested stimuli that contain more complexities (e.g., size, shape, surface irregularities, etc.) amplify the strength of the behavioral response towards a novel object,

whether these responses be explorative or avoidant. The reflective surface of the mirror in the present study contained more visual information (e.g., moving colors and shapes of various brightness levels) than the non-reflective surface, thus making the mirrored surface the more visually complex of the two conditions.

The large increase in burst pulse rates across trials is consistent with the increase in aggressive behaviors from early to late trials reported by Lopes et al. (2016). Burst pulse signals have frequently been correlated with aggressive or agonistic contexts such as when frightened (Caldwell et al., 1962) or making threat (Blomqvist & Amundin, 2004). The results of this study therefore parallel past literature in that as aggressive behaviors increased, so did the rate of burst pulse production.

Additionally, Lopes et al. (2016) reported that the majority of interactions with the apparatus in the present study involved at least two dolphins. This implies that there may have been a social aspect involved with gaining enough motivation to interact with a potentially dangerous stimulus (Stowe et al., 2006). Whistle rates have been reported to increase in both social (e.g., new dolphins join groups that are whistling; whistle rates increase when feeding, (Quick & Janik, 2012; Acevedo & Guitierrez, 2004)) and stressful situations (Esch et al., 2009), and exposure to a novel object may be perceived as either a social or stressful scenario. However, burst pulse rates have traditionally been correlated with a more limited number of contexts including aggression, male herding behavior, and cooperation on a task (Conner & Smolker, 1996; Eskelinen et al., 2016; Overstrom, 1983). Therefore, the results that whistles were produced more frequently than burst pulses throughout the entirety of the study may be explained by the greater variety of contexts where whistle production occurs compared to burst pulses.

This study did have several limitations which will be discussed in the following section. One concern emerged when analyzing vocal rate patterns: The results indicated that differences existed across the three lagoons in whistle rates (see Appendix). The use of an ANCOVA, statistically accounted for these lagoon level effects, however, it may be worthwhile to consider possible reasons the three populations of dolphins may have responded differently to the apparatus. Some influential factors may be the number of mother-calf pairs present in each location as well as the age of the calf. The North lagoon housed three mothers with dependent calves while the other two habitats only contained one mother-calf pair each. South Lagoon housed Squirt, mother of Tashi, a calf that was a couple of weeks old at time of study. Interestingly, Lopes et al. (2016) found Squirt had the most aggressive interactions of all the dolphins. More generally, Lopes et al (2016) reported females had more aggressive interactions than males and adults and calves were more aggressive than all other age classes. In line with those findings, the present analysis found that habitat-based differences trending in burst pulse rates may be influenced by the proportion of mother-calf pairs in North Lagoon. North Lagoon exhibited the highest rates of burst pulses during the non-reflective trials and showed the most robust increases across the reflective trials. These exaggerated responses exhibited by the dolphins living in the North Lagoon could be the result of the mothers threatening the unknown stimulus to protect their calves (Overstrom, 1983). Concurrently, the burst pulses could be a disciplinary action toward overly curious calves (McCowan & Reiss, 1995). The present study also found that either North or South Lagoon had the highest whistle rates across the three habitats on each trial of the study. For non-reflective trials, the North lagoon exhibited the greatest whistle rates across the three habitats on three of

the five exposures. The same was true for the whistle rates on the novel exposure to the reflective surface. Of the three habitats, the South Lagoon showed the greatest increase from the novel to non-novel trials during the reflective condition, as well as the highest whistle rates during all non-novel reflective trials. These mothers may be using whistles to request their calf's presence (Kuczaj et al., 2015), to express excitement (McBride & Hebb, 1948), or to convey uncertainty (Janik & Sayigh, 2013). Despite these trends, more research is needed to elucidate how the age of calves present and the proportion of mother-calf pairs in a population may affect vocal production in response to a novel stimulus.

When considering interpretations for differences in vocal rates across the three lagoons, it is essential to recall that each habitat experienced a different presentation order of reflective and non-reflective trials, which presents another limitation to this study. Any differences found across the three habitats could either be attributed to that lagoons' specific presentation order or individual/group differences between the habitats. This methodological choice adds variability to the results and makes the interpretation of the habitat-based differences more difficult. Future research should attempt to replicate all aspects of the methodology across populations to enhance the power of population or habitat-based comparisons.

Another limitation to this study involved the inability to localize specific vocalizations to individual dolphins. As such, the analyses required that when computing vocal responses for each dolphin, these data points were the average of all vocal responses for the entire habitat, not for each individual dolphin. This analysis eliminated any individual variability each dolphin may have produced, preventing any analysis of

individual differences such as age, sex, personality, maternal status, or rank in the dominance hierarchy. Future research would greatly benefit from utilizing a hydrophone array which would enable each vocalization to be triangulated to a particular dolphin. It is likely that variation in acoustic output can be predicted by individual differences and improved methodology could help elucidate those relationships.

Conclusion

In summary, dolphins in this study exhibited vocal patterns indicative of neophobia, with low rates of whistles and burst pulses on novel trials suggesting extreme uncertainty. As the experiment progressed, the dolphins' vocalizations increased across the course of the trials, as compared to the novel trials, which could be interpreted as sensitization. The pattern was most clearly seen with burst pulse signals, the vocal type commonly associated with aggression, but only in the reflective condition. Burst pulse rates were near floor during the non-reflective trials. Therefore, both complexity and number of previous exposures to an object appear to affect vocal response patterns.

APPENDIX A – Supplementary Material

Facility	Subject	Sex	Age Class
Dolphins Plus Bayside/ Cove	Alfonz	M	Adult
	Kimbit	M	Adult
	Samantha	F	Adult
	Leo	M	Subadult
	Elvis	M	Subadult
	Nica	F	Subadult
	Julie	F	Juvenile
	Isaac	M	Calf
Dolphins Plus Oceanside/ Island Dolphin Care/ North	Sarah	F	Adult
	Dinghy	F	Adult
	Jessica	F	Adult
	Fiji	M	Subadult
	Zoe	F	Calf
	BB	F	Calf
	Grace	F	Calf
Dolphins Plus Oceanside/ Dolphin Cove South	Bob	M	Adult
	Bella	F	Adult
	Squirt	F	Adult
	Lotus	F	Juvenile
	Tashi	M	Calf

Table A1. Subjects in each of the habitats as a function of age class and gender.

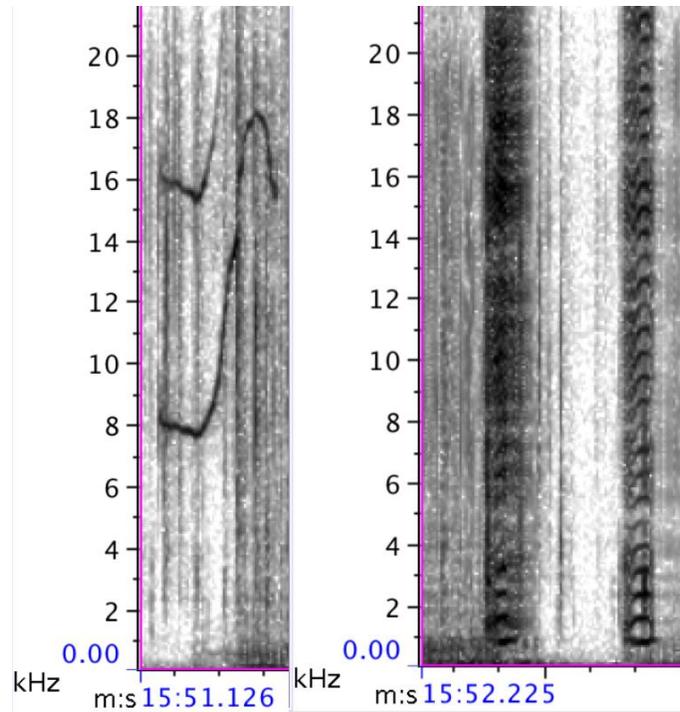


Figure A1. Spectrograms depicting a whistle (left) and a burst pulse (right). Frequency (kHz) is displayed on the y-axis while the x-axis shows time (m:s). A whistle is operationally defined as a narrowband signal with a pure tone that can be modulated by frequency. Whistles were included in the analysis if they were separated from one another by .25 seconds or more. Burst pulses were defined as tight packets of click emissions that appear as one unit on the spectrogram due to a low inter-click interval and clear beginning and end points. Burst pulses typically have an inter-click interval of less than 0.01 seconds (Eskelinen et. al, 2016).

Trial	Bayside (Cove)	IDC (South)	Oceanside (North)
1	NR (T ₁ _NR)	NR (T ₁ _NR)	R (T ₁ _R)
2	R (T ₁ _R)	R (T ₁ _R)	NR (T ₁ _NR)
3	NR (T ₂ _NR)	NR (T ₂ _NR)	NR (T ₂ _NR)
4	R (T ₂ _R)	R (T ₂ _R)	NR (T ₃ _NR)
5	R (T ₃ _R)	R (T ₃ _R)	R (T ₂ _R)
6	NR (T ₃ _NR)	R (T ₄ _R)	R (T ₃ _R)
7	R (T ₄ _R)	R (T ₅ _R)	NR (T ₄ _NR)
8	NR (T ₄ _NR)	NR (T ₃ _NR)	NR (T ₅ _NR)
9	NR (T ₅ _NR)	NR (T ₄ _NR)	R (T ₄ _R)
10	R (T ₅ _R)	NR (T ₅ _NR)	R (T ₅ _R)

Table A2. Presentation order of reflectivity condition across habitats (R = reflective, NR = non-reflective, subscript = trial number relative to condition).

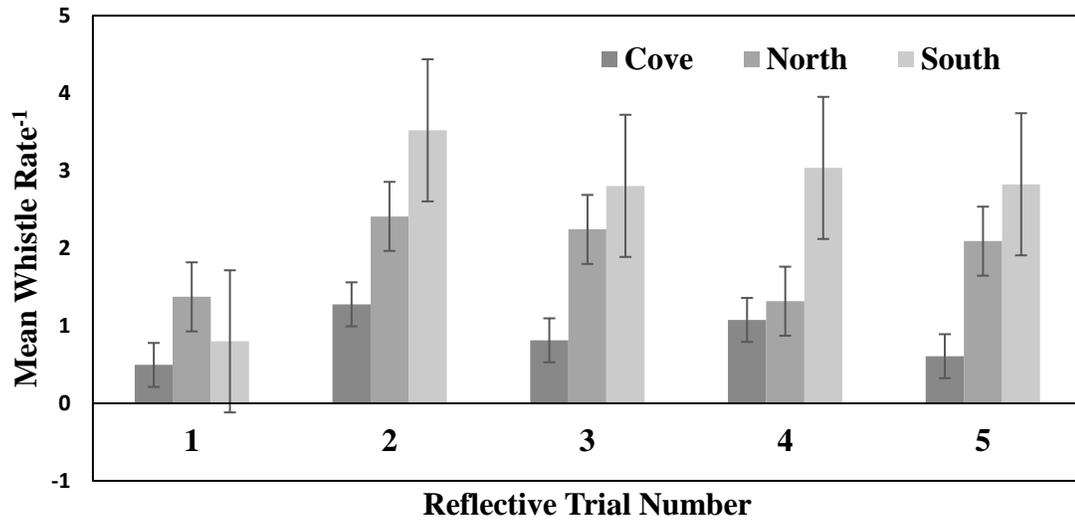


Figure A2. Mean whistle rates as a function of habitat across the 5 reflective trials. Error bars represent 95% confidence intervals.

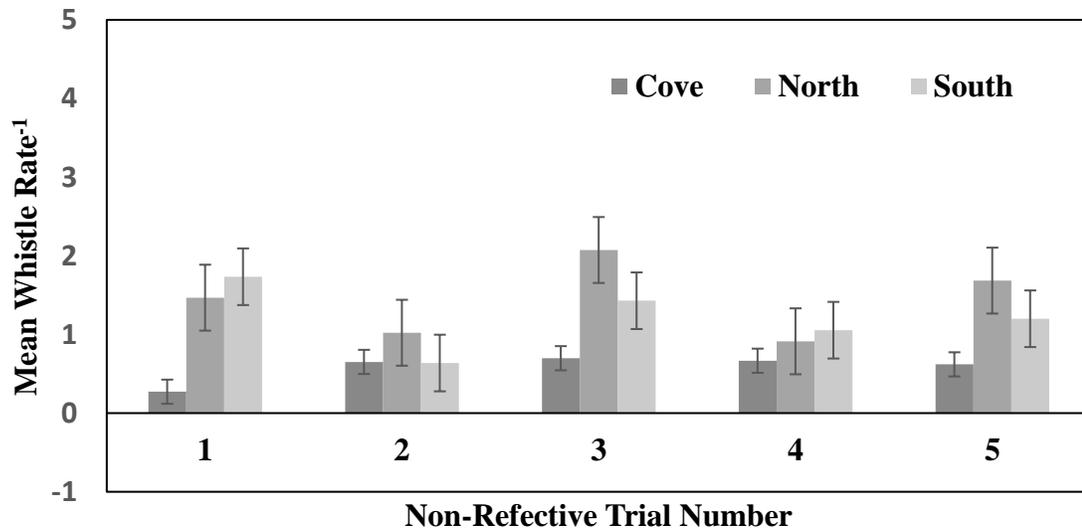


Figure A3. Mean whistle rates as a function of habitat across the 5 non-reflective trials. Error bars represent 95% confidence intervals.

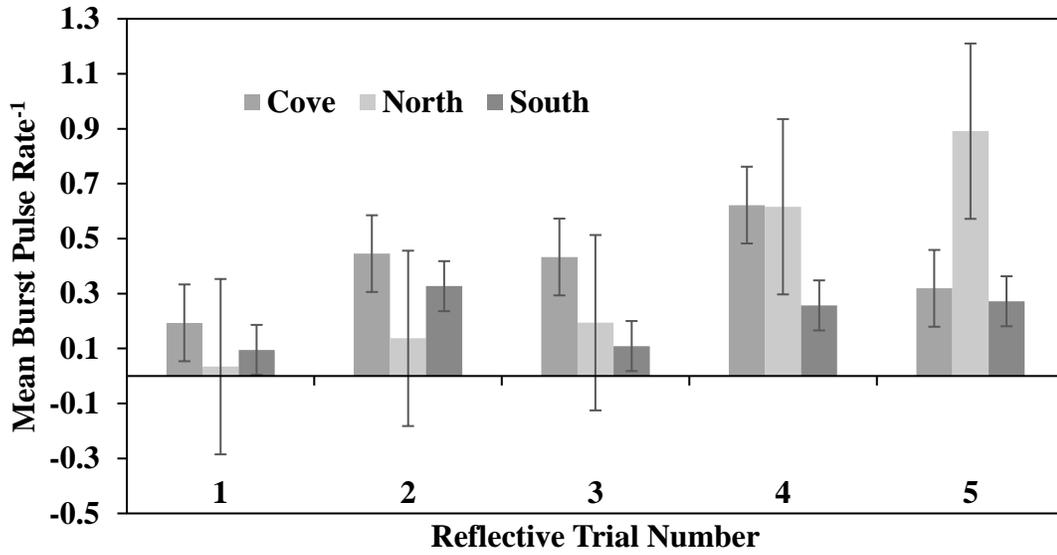


Figure A4. Mean burst pulse rates as a function of habitat across the 5 reflective trials. Error bars represent 95% confidence intervals.

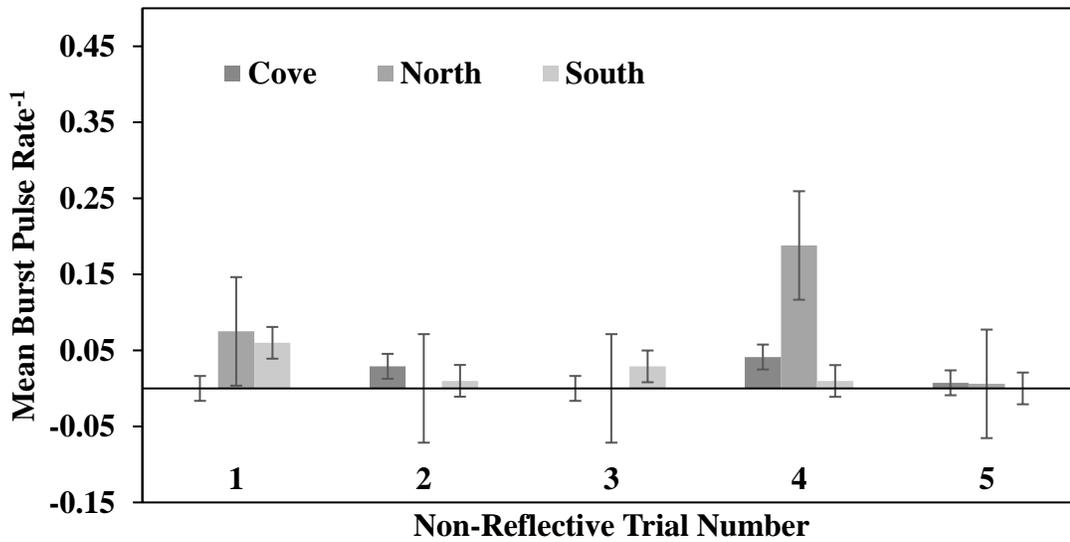


Figure A5. Mean burst pulse rates as a function of habitat across the 5 non-reflective trials. Error bars reflect 95% confidence intervals.

APPENDIX B – IACUC Approval Letter



THE UNIVERSITY OF
SOUTHERN MISSISSIPPI

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

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NOTICE OF COMMITTEE ACTION

The proposal noted below was reviewed and approved by The University of Southern Mississippi Institutional Animal Care and Use Committee (IACUC) in accordance with regulations by the United States Department of Agriculture and the Public Health Service Office of Laboratory Animal Welfare. The project expiration date is noted below. If for some reason the project is not completed by the end of the approval period, your protocol must be reactivated (a new protocol must be submitted and approved) before further work involving the use of animals can be done.

Any significant changes should be brought to the attention of the committee at the earliest possible time. If you should have any questions, please contact me.

PROTOCOL NUMBER: 17110901
PROJECT TITLE: Vocal Response of Bottlenose Dolphins to a Novel Stimulus
PROPOSED PROJECT DATES: 11/2017 – 09/2020
PROJECT TYPE: New
PRINCIPAL INVESTIGATOR(S): David Echevarria
DEPARTMENT: Psychology
FUNDING AGENCY/SPONSOR:
IACUC COMMITTEE ACTION: Full Committee Review Approval
PROTOCOL EXPIRATION DATE: September 30, 2020

Jake Schaeffer, PhD
IACUC Chair

November 9, 2017

Date

FOOTNOTES

¹While methodological constraints prevented their inclusion in the current study (i.e., their appearance on a spectrogram was indiscernible from the local fauna [e.g., popping shrimp] and noise from the apparatus itself), the final broad category of dolphin acoustic signals is echolocation clicks. They were first described by Kellogg, Kohler, and Morris (1953) as rapidly occurring clicks that appeared to be used in ‘echo-investigation’. Wood et al. (1953) described being able to elicit these clicks with the introduction of a novel object. Unfortunately, the technology of that era limited the ability for these early investigators to collect meaningful data by modern standards. More recently, echolocation click trains have been associated with foraging/feeding (Herzing, 1996) and object identification, localization and exploration (Kuczaj et al., 2015). Overall, echolocation clicks do not seem to be used as a primary vocal for social communication, rather click trains tend to serve a more practical function of maintaining spatial awareness.

²The significant covariate reveals that reliable habitat-related differences in dolphin vocal responses were found. These results could reflect individual differences across dolphins in the various lagoons such as the presence of multiple mother-calf pairs in selective habitats. This topic will be addressed in more detail more in the discussion section.

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