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Bubble Stream Production by Belugas (Delphinapterus leucas)

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UNIVERSITY OF SAN DIEGO

San Diego

Bubble Stream Production by Belugas (*Delphinapterus leucas*)

A thesis submitted in partial satisfaction of the requirement for the degree of

Master of Science in Marine Science

by
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DEDICATION

This thesis is dedicated to “Stone Cold” Steve Austin.

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ABSTRACT

Bubble stream production in belugas has been poorly characterized and its function is not well understood. I examined behavioral states when producing bubble streams (“bubbling”), and when bubbling calls, to determine whether bubbling was significantly associated with a particular call category or behavioral state. Using 19 hours of video and audio recordings collected over a two-day period, I quantified bubble streams of a 4-month old calf and an unrelated adult female housed together. Based on the overall activity budgets and pool of vocalizations for both animals, I calculated the expected counts of bubble streams with and without vocalizations, assuming that they occurred randomly ($\chi 2$ Goodness of Fit, $p < 0.001$). I also compared rates of behavior in time blocks (95% CI). The calf produced 419 bubble streams, and the adult female produced 98. Bubble streams were significantly more likely than expected during social-affiliative interactions, but when the whales were not in close contact. The calf vocalized with 20% of bubble streams (86 vocalizations) and the adult female with 18% (18 vocalizations). The animals produced vocalizations with only a small proportion (3.3%) of their bubble streams. Synchronous bubbling was highly correlated to call type in the case of the adult female – she only produced bubbles with one call type. No significant relationship between bubble usage and call type was found for the calf. Overall, the results were most consistent with the hypothesis that bubble streams serve as a visual cue during social-affiliative interactions between belugas.

CHAPTER 1: INTRODUCTION

1.1 Introduction

Basic Biology and Life History

Belugas inhabit Arctic and subarctic waters (Laidre 2000, Huntington 2000, Karlsen 2002). The species ranges from the St. Lawrence River, around the Arctic Ocean to the Bering Sea and into Cook Inlet in Alaska (Huntington, 2000). Where it has been studied, the species has been separated into a number of stocks. In Alaska, there are five distinct stocks, Beaufort Sea, eastern Chukchi Sea, eastern Bering Sea, Bristol Bay, and Cook Inlet (Hill and DeMaster 1998). However, in other parts of the range, belugas are still poorly studied (Laidre et al. 2000), and their biology is still not well understood compared to other cetaceans.

Belugas travel in groups, with mothers and calves usually traveling separately from adolescent and adult males (Paine 1995, Colbeck et al. 2012). These groups are usually relatively small, consisting of 2-10 closely-related individuals. However, larger aggregations of unrelated animals form during migrations and molting periods (Colbeck et al. 2012). After sea ice breaks up during the late spring and summer, most groups migrate into warmer, shallower waters, such as rivers and estuaries, where breeding and molting take place (Boily 1995), before returning to open water wintering grounds (Laidre 2000, Huntington 2000, Colbeck et al. 2012). These shallow waters may also have more food for the belugas, allowing for the buildup or retention of their blubber stores before returning to colder waters during the winter.

Most breeding takes place between March and May, with gestation lasting 12-14 months (Robeck et al. 2005). During the first month of life, a calf spends almost all of its time at its mother’s side, making nursing, swimming, sleeping, and predator avoidance easier (Krasnova et al. 2009, Hill 2009). After about a week, the calf may swim short distances away from the mother for brief periods, and by the second month, swimming abilities are developed enough to allow the calf to venture farther from its mother for longer periods of time (Krasnova et al. 2009). After a few weeks, interactions with other young calves begin, becoming more frequent with time (Hill 2009). These interactions with coeval calves and eventually older calves are thought to be important for the development of hunting skills, social behaviors, and general locomotion (Krasnova et al. 2009).

Behavior

Cetacean behavior is difficult to observe in the wild, so many studies to date have focused on captive animals, particularly bottlenose dolphins (*Tursiops truncatus*), the cetaceans most commonly kept in zoos and aquaria. While each study uses slightly different terminology and focused on somewhat different behaviors, there is general agreement among most studies on the behavioral states of small cetaceans. Understanding of the individual behaviors (events) that compose those behavioral states is weaker, but most studies acknowledge behavioral events falling into the categories solitary, play, social-affiliative, and agonistic, which encompasses both aggressive and submissive behaviors exhibited in conflicts (e.g., Recchia 1994, Hill 2009, and Hill et al. 2015). Most studies with mixed-sex groupings of animals also include a category for sexual behavior.

Categorization of behavior in the wild is often slightly different, considering both different day-to-day activities (e.g., captive dolphins do not need to forage or travel, but wild dolphins do) and different observation abilities of the researcher (e.g., a researcher may not be able to study fine-scale behaviors or infer the contexts in which they were produced). For these reasons, studies of wild cetaceans often group behaviors into larger categories, such as traveling, foraging, and resting (e.g., Henderson et al. 2012, Alekseeva et al. 2013, Howe et al. 2015). These studies often treat the focal unit as a group instead of individuals and classify the current behavior as the behavior that the majority of the animals present are performing (Mann 1999). Thus, individual behaviors and social roles are often unknown except where underwater video observations have been possible (Herzing 1996, Dudzinski 1996).

Play behavior appears in many species of social cetaceans, both wild and captive (Paulos et al. 2010). In species that have been studied, it is observed in all sexes and age groups, although the problem of distinguishing between social play and other social behaviors has not been addressed rigorously. It likely has a particularly important function in young animals during development of their motor and cognitive abilities (Kuczaj et al. 2006, Paulos et al. 2010). Hill et al. (2015) observed more play activity in social groupings with young belugas than in groups with just adults or adults and juveniles. Although all animals in their study were observed playing, the older belugas played less on average, with the exception of two adult females with calves.

Development of play behavior follows a consistent pattern in bottlenose dolphins, with social play first appearing around 2 weeks of age, followed by bubble play (including the production of bubble clouds or bubble rings with which the animals interact) at around 1 month of age and object play at around 2 months of age (Tizzi 2000). The two latter stages are characterized by the animal first observing an example of a play behavior by another animal, then manipulating a bubble or object firsthand.

Little work has been done to directly compare behavior of wild cetaceans to those in captivity, but Dudzinski (2010) collected some preliminary data to address the question. She asked dolphin trainers at institutions in several countries to watch a 25-minute video of wild dolphins exhibiting various behaviors and complete a survey rating the similarities between the behavior of wild dolphins (*Stenella frontalis, Tursiops aduncus,* and *Tursiops truncatus*) and captive dolphins (*Tursiops truncatus*) with which they worked. The trainers reported more behavioral similarities than differences; 4 of the 17 trainers who responded to the survey reported no noticeable differences between the groups, and 2 trainers also reported having seen all of the behaviors from the wild group exhibited by their captive group. Additionally, the trainers categorized behaviors as aggressive, sexual, and affiliative, agreeing in 95% of the samples with the researcher's ratings based on wild dolphins (Dudzinski 2010). While it may not be justified to assume that the same behaviors mean the same things in both groups, the fact that the researchers observed the same behaviors in seemingly similar contexts in both wild and captive animals was certainly suggestive of general behavioral similarity between the two groups. Dudzinski et al. (2010) compared incidence of a single class of behavior (pectoral touching) among dolphins in an oceanarium and wild dolphins from two populations and found that the behavior was exhibited similarly in social contexts regardless of living environment.

Although bubble formations are among the more detectable behaviors and can often be attributed to individuals, they have largely been studied in the context of play even under captive conditions and have received surprisingly little attention in studies of wild cetaceans. One study looking at bubble production by dusky dolphins (Trudelle 2010) only categorized the context of the behavior in terms of movement patterns, as “swimming”, “floating”, or “diving”, and only assessed behavior during one specific activity, foraging. The relationship of bubbling behavior to vocal activity was not measured.

This lack of detail is common to studies of free-ranging behavior, particularly among belugas, which live in a difficult environment for research and are never found in clear water. A study of belugas in Eagle Bay, Cook Inlet, only listed behavioral states as “milling” or “traveling” (Howe et al. 2015). Though the authors did go further to describe individual “behaviors”, most of them were descriptions of the components of behaviors visible from the surface, like tail slapping or body positions, and all social interactions were pooled into one category. In explaining this potential limitation, the authors mentioned that belugas did not exhibit many aerial behaviors and most of their behaviors below the surface were not observable due to turbidity. A more recent study expanded on previous work on White Sea belugas by including behavioral categories such as “relaxation”, “playing”, “race”, and “sex and hierarchical behavior”, describing behavior in these wild belugas in more detail than previously, but without linking the behaviors to descriptions in other species (Alekseeva et al. 2013). Video data along with visual observations were taken from a 40m tower on shore, which likely decreased the accuracy with which the researchers could categorize behavior and link it to individuals.

Vocalizations

Beluga whales, like other cetaceans, use vocalizations extensively in many aspects of their lives. Historically, vocalizations of cetaceans have been separated into three general categories: clicks, burst-pulse sounds and whistles (Au 1993). However, the categories are broad and in some cases not clearly distinguished. For example, echolocation is by definition composed of click trains, but may be distinguished from burst-pulse sounds only by subtle differences in spectral characteristics and inter-pulse intervals that must be defined for each species and functional context (e.g., Au et al. 1974, Branstetter et al. 2012).

The category of whistles is particularly broad, encompassing a range of signals sharing only a generally tonal timbre to the human ear. The best available evidence currently suggests that whistled sounds are produced by mechanisms similar to those that generate clicks and burst-pulse sounds (Madsen et al. 2011). As explained by Watkins (1968), when clicks are produced at a high enough repetition rate, they resolve to a fundamental frequency with sideband harmonics in a spectrogram display based on the Fast Fourier Transform. These calls may sound like whistles due to their high frequency, relatively narrow-band components, but these tonal components are actually created by producing trains of pulses with short inter-pulse intervals. Several studies have recognized this production mechanism for other odontocetes (Marcoux et al. 2012 with narwhals, Herzing 2000 with wild Atlantic spotted and bottlenose dolphins, Madsen et al. 2011 with bottlenose dolphins).

In the literature, these sounds can grade from pure tones without obvious harmonics (at least within the limits of the recording system) to complex, modulated tonals with many harmonics, or tonal sounds mixed with clicks or burst pulses. For most odontocetes, tonal sounds have been characterized by the term ‘whistle’ because they sound like high-pitched whistles to the human ear, but in the killer whale, they are given the more accurate term “pulsed call”. For this species, the tonals are recognized as a product of pulse frequency and inter-pulse interval (Watkins 1967). It is important when reading literature about this subject to remember that the same terminology may not always refer to the same kind of vocalization depending on the species being discussed. For the purposes of this paper, I will refer to vocalizations that have a frequency-modulated tonal quality as “whistles”, following terminology that has been applied to belugas previously.

In addition to whistles, broadband vocalizations with more evident pulses in spectrograms with typical sampling windows (i.e., long inter-pulse intervals) will be referred to as “burst-pulse” vocalizations. Finally, vocalizations that contain overlapping sounds of different types will be referred to as “combined calls”. The overlapping sounds are referred to as components, such as a burst-pulse component and a concurrent or subsequent tonal component, or two overlapping tonal components with diverging frequencies.

For the beluga, vocalizations have been categorized as either “whistles” or “pulsed” calls (Sjare and Smith 1986, Belikov and Bel’kovich 2006, Vergara and Barrett-Lennard 2008), with clicks and burst-pulsed sounds pooled as “pulsed” unless echolocation was studied explicitly (e.g., Au et al. 1985). A few studies also include a third category of “noisy” or “combined” calls (Sjare and Smith 1986, Thomson 1995, Karlsen et al. 2002, Belikov and Bel’kovich 2008), suggesting commonalities with the vocalizations of killer whales, which are often composed of multiple sound types.

The narrowband tonal components of whistles often include apparent harmonics (additional tonal components at integer multiples of the fundamental frequency), although it is likely that these are better interpreted as evidence of a pulsed origin, as demonstrated for the killer whale and bottlenose dolphin (Watkins 1967, Madsen et al. 2011). Beluga whistles are characterized by modulation of the fundamental frequency to varying degrees. They are thought to be used for social communication because they occur most often in social contexts (Karlsen et al. 2002, Chmelnitsky and Ferguson 2012). Whistles make up the majority of the beluga’s repertoire (Karlsen et al. 2002, Belikov and Bel’kovich 2006).

Several studies have suggested that a subset of these whistles may serve as contact calls. Contact calls are known from bottlenose dolphins (Janik and Slater 1998), and elements of the killer whale dialect are used in the context of maintaining contact (Filatova et al. 2011). In bottlenose dolphins, for which the evidence of contact function is strongest (Janik and Slater 1998), whistles promote group cohesion and reunions after separation (Janik and Slater 1998, Shapiro 2006, Sayigh et al. 2007, Harley 2008, Quick et al. 2012).

As inhabitants of the dark waters of the Arctic (Boyd et al. 2010), maintenance of group cohesion through vocalizations may be especially important for belugas. The evidence available suggests that whistles have some function as contact calls. Some whistles are unique to individuals and are used in exchanges with other belugas (Morgan 1979, Van Parijs et al. 2003, Bel'kovich and Kreichi 2004, Vergara et al. 2010). A recent paper by Morisaka et al. (2013), based on data collected in an oceanarium, described a burst-pulse call similar in sound to a door creaking, termed "PS1", that seemed to serve as a contact call. Each beluga in their study produced a version of the call that was significantly different from those of the other animals, and the production of a PS1 call by one animal was almost always met with the return of another animal's PS1 call within 1 sec. When a PS1 call was not returned quickly, the animal producing the first call usually repeated their call until it received a response (Morisaka et al. 2013). The fact that these calls were distinct for each individual suggests they function as name-like identification calls (in the bottlenose dolphin, they are termed "signature whistles"), that identify the individual. The process of exchanging these distinct calls supported the contact call hypothesis. The results of Morisaka et al. (2013) are consistent with an earlier study by Vergara and Barrett-Lennard (2008) showing that a beluga calf at the Vancouver Aquarium appeared to learn calls characteristic of adult social partners in its environment.

A final category of vocal production for cetaceans is echolocation, which was not included in this study. Echolocation consists of trains of broadband clicks with intervals between pulses that are generally longer than in pulse bursts and more variable. In this case, the inter-pulse interval is functional, controlled by the clicking animal as it explores its target. Echolocation is used for navigation, prey detection and capture, and object identification in the beluga, as in other odontocetes (Au et al. 1985, Au et al. 1987, Belikov and Bel’kovich 2008). This category of vocalization was not included in analyses for this study, because it is not known to be communicative.

Visual Cues in Communication

Visual cues are also used in communication between whales. Belugas, like other odontocetes, have two high-resolution areas in their retinas and are therefore capable of good vision (Pryor and Norris 1998, Mass and Supin 2002). Additionally, odontocetes’ eyes are horizontally elliptical, which allows a relatively broader area of the retina to perceive objects in their peripheral field of view compared to most terrestrial animals (Connor and Peterson 1994).

Odontocetes use a number of postural gestures to communicate. Belugas have considerably more flexibility of their head and neck than other cetaceans (O’Corry-Crowe 2002), and they may be especially able to use such cues for communication. For example, dolphins and beluga whales often utilize an s-shaped posture as a low-amplitude threat (Pryor 1990, Horbeck et al. 2010). Another study found that an inverted swimming posture by spinner dolphins (*Stenella longirostris*) usually precedes sexual activity (Norris et al. 1994). Captive bottlenose dolphins in a study by Xitco et al. (2001) pointed with their rostrum and body to specify certain objects in the presence of humans, even looking back and forth from the object to the human until the human approached and retrieved the object to give to the animal. This pointing only occurred when humans were in the water, implying that the dolphins recognized that their pointing could be seen and interpreted when in the same medium. It is possible that they could apply this concept to communicating with fellow dolphins as well. A study focusing on gestures of beluga whales found that individuals often produced a specific head-turning motion when another animal had encroached on their individual space (Suzuki 2007). However, there has been no comprehensive study of such gestures, and it is unclear how individual behaviors combine to form a functional system of communication. There is certainly a need for more research on the use of visual cues in beluga whales.

Bubbling

Bubbling is a highly salient cetacean visual behavior. Bubble formations take several forms, including clouds, streams, and rings. Each type of bubbling has been studied to varying degrees in different cetacean species, and each are thought to have different functions. In the case of bubble rings, most is known about use by bottlenose dolphins. McCowan et al. (2000) studied all bubbling behaviors in 4 captive-born juvenile males aged 3 to 8 years. Of all bubbling events, play with bubble rings accounted for 94%, while bubble streams accounted for 5% and bursts and clouds together made up the remaining 1%. These last three types fell into “Social Bubbling Behavior,” since they were usually made during social surprise, whistle production, or chases between individuals, while bubble rings were considered separately as “Solitary Play Bubble Behavior”. Additionally, the data showed that 89% of the bubble rings were manipulated by the animal that created them, and anecdotal evidence suggested that infants observed juveniles and adults producing bubble rings before attempting them. These findings supported the hypothesis that bubble rings served as “toys” to be used for solitary play. Another study on bottlenose dolphins reported on bubble ring play, but instead of producing the rings with air from their blowholes, the animals had spontaneously started using their flukes to make the bubble rings (Pace 2000). By hitting their flukes against the surface of the water, the animals could produce bubble rings under water that they would subsequently interact with as a form of solitary play.

Very little is known about the use of this behavior in other cetaceans, including belugas. While a few studies reported that belugas have been observed producing bubble rings, they focused on bubble clouds (Delfour and Aulagnier 1997, Hill et al. 2011) or pooled bubble rings into the category of “play behavior” (Hill et al. 2015), and failed to study them further.

Of all the types of bubbling, bubble clouds are seemingly the most well-studied both within and across species. These formations are sometimes referred to differently in the literature; for example, a study of bottlenose dolphins by McCowan et al. distinguished between “bubble bursts” produced in fright or surprise and “bubble clouds” produced in aggressive contexts, though the two were not clearly distinguishable (2000). Another study on belugas refers to the formations as “bubble blows” (Delfour and Aulagnier 1997). These formations are usually produced rapidly in a single burst, resulting in the sudden appearance of a large cloud made up of many-sized bubbles that rises to the surface. They may even ripple the water at the surface or be accompanied by an audible roiling sound from the force of exhalation. In a study by Delfour and Aulagnier (1997), bubble blows were observed from 5 captive belugas using focal animal sampling. They found that the animals produced more bubbles in early morning and early afternoon than in late afternoon, a pattern that corresponded with the times the whales were more active in general. The authors also found inter-individual differences in production but no significant difference based on age. While one male in the study produced no bubble clouds at all, the youngest and the oldest animals did produce them. Clouds were never produced when animals were together, and the producer of the cloud was often seen biting, kicking, following, or looking at the bubble cloud. Based on these findings, the authors concluded that, under the conditions of their study, bubble cloud production was used as a form of solitary play, defining play as a behavior that provides no obvious benefits (Delfour and Aulagnier 1997).

While Delfour and Aulagnier provided information about bubbling behavior in belugas, several additions to the methodology would have been more informative. First, video recordings of the study animals could have provided more in-depth analysis of the contexts of the behavior, including how close other animals were or subtleties of the behaviors before and after bubbling. The authors did not record the animals' behaviors or behavioral states at the time of bubbling other than noting if the animal interacted with the bubbles after producing them. In addition, audio recordings from this time period could have provided information about how often the animals vocalized during or in close conjunction with bubble clouds.

Another study on bubble clouds involved 4 captive mother-calf beluga pairs (Hill et al. 2011). Using about 32 hours of video data, the authors were able to relate the type of bubbling with behavioral context for each bubbling event. Contexts included “Play”, “Reactive”, “Pair Swim”, and “Interaction”. Bubble formations were categorized as “bursts”, “streams”, “rings”, or “small to medium individual bubbles”.

Out of 856 bubbling events, 814 were bursts and the remaining 42 fell into one of the other categories. The mothers produced bursts significantly more than the calves during pair swimming and reactive contexts (where an animal reacts with a startle or flight response), while calves produced them significantly more than the mothers during solitary activity and social interaction (Hill et al. 2011). These results suggested interesting differences in the way that young animals and adults used bubble formations. As the authors explained, the behavior may have multiple functions depending on context, with mothers appearing to use it as a protective display or threat, and calves using it as part of affiliative social interactions.

Additionally, calves produced significantly more bubble streams (characterized by the gradual release of small bubbles from the blowhole) than expected by chance alone, compared their mothers, suggesting two possible conclusions: calves might have to learn the context for using this type of bubbling behavior, or similar to the findings on bubble burst use, bubble streams may serve different purposes for adults and calves. To determine this, future studies would need to look at calves of different ages or in different social groupings to determine if bubble streaming changes over time or with social context. Additionally, audio data would be helpful for considering bubbling in relation to vocalization behavior.

Little is known about bubbling in wild odontocetes of any species. In turbid water, bubbling behaviors can be difficult to see from both above and below the surface, and information about the individual producing the behavior or the context associated with it is usually difficult to obtain. In their review of play in cetaceans, Paulos et al. (2010) mention that bubble rings have been reported in wild belugas and wild Atlantic and Pacific spotted dolphins, but no evidence of a play (or other) purpose exists to this point.

One study looked at bubbling in wild dusky dolphins. Trudelle (2010) collected 800 minutes of underwater video footage during feeding bouts off New Zealand. The author described bubble type and size, behavioral context, and behaviors occurring directly before and after bubble emissions. Of 323 samples, 54% were trains (mostly horizontal to the dolphin's back as a stream of small-sized bubbles), 36% were columns (mostly vertical with medium-sized bubbles), and 9% were clouds. Likely, trains would have appeared instead of columns if the animal had been moving or possibly bubbling with less force. Since bubbles were most often produced at a distance of 2-4m from their prey, the authors concluded that the bubbles were not aiding in corralling or catching fish. This finding, along with the observation that individuals often synchronized their sequences of behaviors immediately after one of them produced bubbles, suggests that the bubbles function as communication of some sort.

Trudelle's study was limited to a very specific behavioral context (foraging) and only categorized behavioral states as "swimming", "diving", or "floating". The work was interesting because foraging is a very different behavior in captivity (e.g., performing human-conditioned behaviors to obtain food), and the use of bubbles has not been studied in this context. Without a comparable study in the captive environment, it is difficult to draw comparisons. Nonetheless, Trudelle's findings hint at a communicative use for bubbling that can be probed in captive studies. It is also worth noting that "trains" are synonymous in this case with other descriptions of bubbles as "streams" or "trails". The term "bubble stream" is used here.

Of all bubbling types, arguably the least is known about bubble streams. This is certainly the case for belugas. Several studies of belugas mention the behavior, but most do so either in passing (Delfour and Aulagnier 1997, Pace 2000), without explanation about possible function (Hill et al. 2015), or as a means to identify which animal in a group is producing a vocalization (Vergara and Barrett-Lennard 2008). The most convincing study to date looking at bubble stream production by belugas was in the report by Hill et al. (2011) on bubble bursts. Though the authors placed bubble streams in context to some degree, their behavioral categories were relatively general; for example, the category "interaction" included both affiliative and agonistic interactions, each of which could have involved one of several behaviors. Without more detail, the authors could only say that the contexts in which calves and mothers produced bubble streams differed. Additionally, without audio recordings, there was no way to know how often vocalizations accompanied the bubble streams and, if so, what kinds of vocalizations were used.

Bubbling is a visual cue, but may be combined with acoustic signaling. First, the bubble formation might produce a sound during production, therefore serving as both a visual and auditory cue. Caldwell and Caldwell (1971) reported that "underwater bubbles from the blowhole produce sound", at least to the human ear. Bubble formations could also be detected by active echolocation, which cetaceans can use interchangeably with vision to obtain information about their environment (Pack and Herman 1995). There is no evidence to date that they detect bubble streams using this modality, but it would be reasonable to expect that they might. Pryor and Shallenberger (1991) speculated that bubble streams could provide information beyond visual cuing by this means. As described above, they can be manipulated during play and might conceivably be used as a defensive behavior in the presence of predators to draw the focus of a nearby animal from the bubbling individual.

Studying Behavior

While studies of wild animals are preferred for producing results that can be generalized to wild populations as a whole, field research on behavior can be prohibitively difficult and expensive to undertake. Captive environments differ from those in the wild, but studies of captive animals make it possible to observe animals closely and for longer periods of time, facilitating the examination of phenomena like social structure, behavior, and communication. This method also allows researchers to work with animals that have known sex, age, health, social associations, etc., and consider these variables when interpreting findings.

Just as studying behavior is an extremely important step in understanding animals, accurately recording and classifying this behavior is also imperative. To achieve this, researchers use ethograms, inventories of behaviors selected for the purposes of a given study. These behaviors are usually defined so that they can be identified reliably and described to others (Altmann 1978). In studies of terrestrial animals, most if not all of the behaviors are associated with known behavioral states and are assigned a recognized or hypothesized function that can be supported with preliminary observations.

Ethograms have been used in many cetacean behavioral studies (e.g., Krasnova et al. 1994, Recchia 1994, Dudzinski 1996, Hill 2009, and Tizzi et al. 2010). While each author categorizes behavior slightly differently, all generally group behaviors into a consistent inventory of states. These are swimming, resting, social interactions, which may be affiliative (mutual and positive) or agonistic (negative, covering both the aggressor's behavior and those of animals receiving the aggression) and those used during play, which may include behaviors from a variety of states. In the case of play, context (e.g., object play in isolation) removes ambiguities about the function of the behavior. Some authors have included detailed descriptions for their categories of behavior, particularly in cases where subjects are clearly visible underwater (e.g., Recchia 1994, Dudzinski 1996). Similarly, many ethograms include sexual behaviors (e.g., Recchia 1994, Hill 2009, Campbell 2011). Ethograms may also include relative movements and positions, such as one animal joining a group. For wild odontocetes, "milling" with others (e.g., Karlsen et al. 2002) encompasses a large range of social behaviors, including play, agonism, and sexual behavior, which makes it difficult to interpret the function of specific gestures, such as bubbling, that may accompany the behavior.

1.2 Justification for Study

An understanding of behavioral signals and vocalizations is important for both the management of wild populations and the care of captive beluga whales and other cetaceans. Bubble formations are highly salient gestures whose functions are poorly understood, and the least studied are bubble streams. In order to better understand this behavior, my research quantified the behavioral states associated with the production of bubble streams by belugas, and the vocalizations (if any) that accompanied them.

1.3 Significance and Implications

This research has implications for both understanding a marine mammal species and contributing to its conservation. As discussed earlier, the communication systems of cetaceans, including vocal and visual aspects, are still surprisingly unknown. In particular, little is known about beluga whale behavior – their arctic habitat makes field studies nearly impossible, and they are less common in captive environments than bottlenose dolphins. This research will expand our understanding of a previously unstudied behavior that is known to occur in at least three odontocete species (McCowan and Reiss 1995, Hill et al. 2011, Bowles et al. 2015). Especially significant for this study are two opportunities. First, video and audio recordings of a well-characterized captive group were available, with information available about which animal was vocalizing at least a proportion of the time, and which animal was producing bubble streams at all times. In addition, the genetic relationships among the animals, the age and sex of the callers, and the behaviors that coincided with bubble stream production were known. Second, there was an opportunity to study bubbling in a developing calf and an adult, allowing us to observe differences in when and how this behavior starts in belugas.

CHAPTER 2: Variation in Beluga Bubble Formations with Behavioral and Social Context

2.1 Introduction

Odontocete cetaceans produce several types of bubble formations (e.g., McCowan et al. 2000, Hill et al. 2011). Previous studies have shown that some or all of these formations can function in the context of play (Delfour and Aulagnier 1997, Hill et al. 2011), but also serve as visual gestures in the context of communication. However, research on the communicative function of bubble formations has been limited, and there is surprisingly little information on bubble streams, despite their association with vocalizations in what are apparently multimodal communicative behaviors. In the case of the beluga (*Delphinapterus leucas*), data on the function of bubble formations outside play are very limited.

Odontocete cetaceans typically produce social vocalizations (“calls”) without any visible behavior, making it difficult to attribute them to specific callers. However, in a small proportion of cases, calls are produced synchronously with a bubble formation, the bubble stream, which indicates the caller and possibly other information. Since bubble streams emitted synchronously with vocalizations are a relatively reliable way of identifying callers, some studies of bottlenose dolphins have focused their analyses on vocalizations accompanied by bubble streams (McCowan and Reiss 1995). In these studies, there was no unbiased way to compare bubbled and non-bubbled calls of individuals, or to estimate individuals’ true vocalization rate.

Bubble streams do not accompany every vocalization. The rate of bubble stream production has been documented in belugas, bottlenose dolphins, and killer whales (Paine 1995, Herzing 1996, Mann et al. 2000, Bowles et al. 2015), showing that bubble streams are, on average, emitted synchronously with only a few percent of vocalizations, and that they may also be emitted without a vocalization, particularly in the case of young animals. This adds an additional layer of complexity to both our understanding of the behavior and the behavior itself. Bubble streams may also be emitted preferentially in a subset of behavioral or social contexts (Fripp 2005).

Although belugas are so highly vocal that they have been called “the canaries of the sea”, their vocal communication system has been studied very little by comparison with those of bottlenose dolphins and killer whales. Therefore, examining the development and use of bubble formations and association of the behavior with vocalizations could provide important insights into the function of a highly salient aspect of their social communication and shed light on the function of bubble formations generally.

Advances in video and audio recording in zoological settings make it possible to attribute both bubbled and non-bubbled calls to an individual with relative certainty. As a result, we can study how bubble streams relate to social context and individual characteristics of the caller. This information could give valuable insight into the behavior and communication of cetaceans. This study used combined video and audio data from belugas living in facilities at SeaWorld San Diego to explore the function of bubble streams in varying behavioral and social contexts. Specifically, we hypothesized that bubble streams would not always be accompanied by a vocalization, and that the adult beluga would show more consistency in her use of bubble streams than the calf.

2.2 Methods

Study site

Data for this study were collected in September of 2010 (Fig. 1). The study animals were F1, an adult female, and F2, a female calf. F1 was about 27 years old and had an alloparental relationship with F2. F2 was about 4 months old at the time of the study. They were observed in a complex of two pools (Fig. 2). Two other belugas, an adult female (the calf's mother) and an adult male, were in an exhibit pool separated from the study animals. Recordings were not available from the main exhibit pool during the study period.

The behavior of the two study animals, F1 and F2, was captured using three video cameras providing nearly complete coverage of the two study pools from the surface. Vocalizations were collected through a hydrophone placed at the far end of the larger back pool, but with line of sight into the smaller husbandry pool (Fig. 2).

The husbandry pool was a rectangle measuring 3.4 m wide by 6.7 m long and 2.4 m deep. The back pool was an octagon measuring about 8.5 m across and 3.7 m deep. The back pool and husbandry pool were connected by a gateway wide enough for the animals to swim through. On two occasions, trainers isolated F2 in the husbandry pool for feeding, at which time the gateway

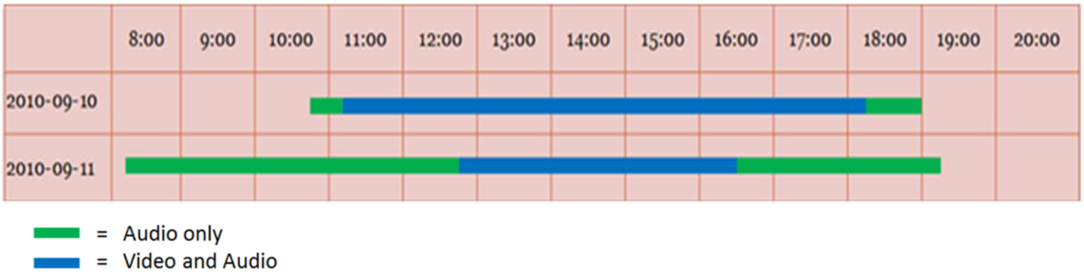


Figure 1: Summary of recording effort for September 10th and September 11th, 2010. The green bars indicate the time periods during which only audio was recorded, while the blue bars indicate the time periods during which video and audio were collected simultaneously.

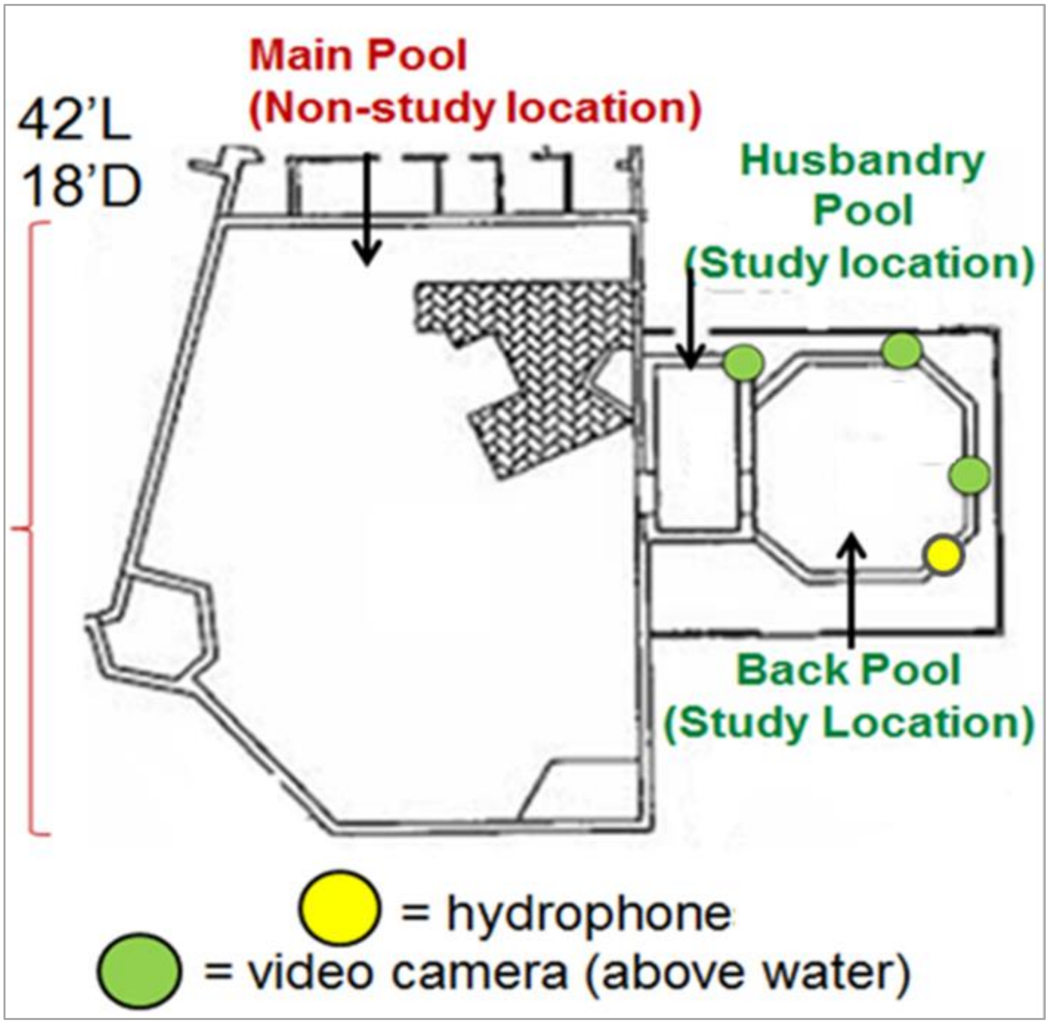


Figure 2: Map of study pools. The study animals, F1 and F2, were housed in either the husbandry pool or the back pool for the entirety of the study and usually had access to both. Two other belugas, an adult male and an adult female, were housed in the main pool for the entirety of the study and were not focal subjects. The yellow circles indicate the location of the hydrophone, while the green circles indicate the locations of the 3 video cameras.

was closed, but at all other times, the animals were free to swim between the two pools.

In addition to the belugas' vocalizations, the audio recordings included observer commentary from two research assistants stationed poolside during recording periods. Observers were instructed to focus on an exhaustive inventory of behavioral states and a small list of events relevant to the study, including vocalizations audible in air, bubble formations, and human activities going on in the pools (e.g., presence of trainers or guests, animals being fed)

Recording Configuration:

Vocalizations were captured using an ITC 6050C hydrophone (Channel Technologies, Santa Barbara, CA) connected to a 4-channel Edirol R44 digital audio recorder (Roland Corporation, Los Angeles, CA). The system frequency range was 50 Hz to 48 kHz (sampling at 96 kHz, 12-bit resolution). The hydrophone was placed with its acoustic center at 1.25 m depth. It was lowered into a section of PVC pipe open to the water at the bottom and perforated by 3 cm holes drilled at intervals in the sides. The pipe was anchored to the side of the pool on an aluminum frame. This configuration protected the hydrophone from the whales while minimizing interference with vocalizations other than high frequency clicks.

Behavior data were collected using three CCD cameras connected to a Panasonic DMR-E85H Digital Video Recorder (Panasonic Corporation US, Chesapeake, VA) through a Robot 4-channel multiplexer (Sensormatics Video Products, San Diego, CA). The three cameras were placed to provide as much coverage of the two study pools as possible (>90%). This arrangement provided a triplexed video stream with a view across the husbandry pool looking south, a view across the back pool toward the gateway into the husbandry pool looking southwest, and a view from above looking down on the rub rope in the back pool (see Fig. 3). The multiplexer provided a time code on the multiplexed image.

Data Management:

Since the audio and video data were collected on separate equipment, they were not synchronized initially. Several measures were used to align the sequences of audio and video events with as little error as possible during processing. First, the data files were collected with time information. There was a time stamp on the video images, and the file names of the audio recordings were automatically generated with the date and time they were recorded, both to the nearest second. Because the two types of time stamps were not always perfectly synchronized, a satellite-linked clock that was visible to observers was used to add verbal “time stamps” to the observer tracks of the recordings at the same time that a hand was swiped across the video recording. This provided an independent check on the synchrony between audio and video time stamps. Time synchronization was good throughout the recordings (within 2 sec). When times disagreed between data sources, the verbal “time stamps” given by the observers were accepted as the correct reference.

Behavioral events could also be used to synchronize events. However, this source of information was used only as a reality check, to identify possible problems with synchronization by other methods. Once video and audio sources

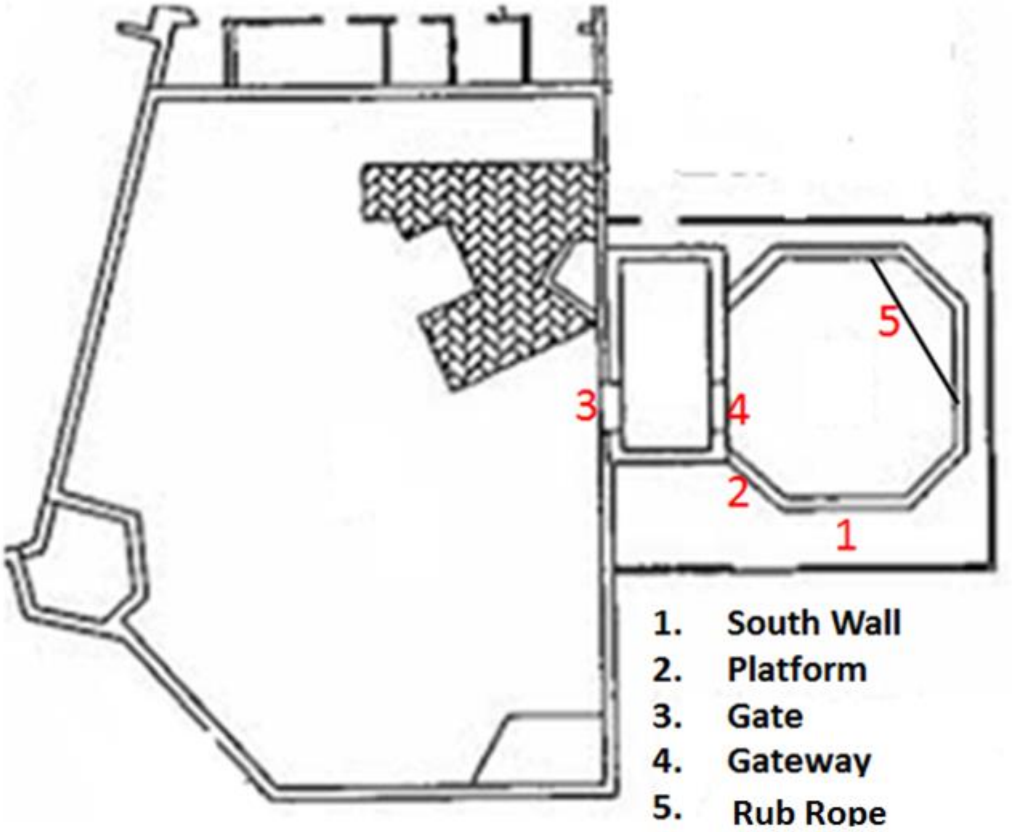


Figure 3: Select locations in the study pools. The study animals frequented these locations often, as discussed later. The gate (location #3) adjacent to the main pool allowed some degree of visual contact between the study animals and the other belugas, but did not allow physical contact between them due to a mesh net in the gate.

were aligned correctly, they were combined in Adobe Premiere Elements (Adobe Systems Incorporated, San Jose, CA) and scored (Fig. 4). Bubble formations were not reliably observed from the overhead camera view, so the reports from onsite observers were used to detect these events (Fig. 5).

Videos synchronized with audio from the September observation periods were analyzed for all bubbling events. These events were recorded individually onto a data sheet for each animal along with the time of the event, type of bubbling, concurrent behaviors, location of the animal, any human activity occurring near the pools, and the proximity of the bubbling animal to the other animal in the study pools (Table 1). More detailed explanations of the factors recorded on the data sheet are shown in Table 2. Behaviors were scored from an ethogram (Table 3), a catalog of behavioral states and events relevant to the study. Finally, locations were scored based on certain landmarks and areas around the pools (Table 4).

All bubbling events and the concurrent behavioral state of the bubbling animal were scored for analysis. This method of “continuous” or “all-occurrence” sampling of focal animal behavior has been supported by previous papers (e.g., Altman 1974, Mann 1999). The time was marked when a focal whale entered a behavioral state, irrespective of bubbling behavior, and a new record entered any time they transitioned to a different behavioral state. Subtracting the transition time from the start time in each behavioral unit gave the minutes spent in that behavioral state. The total time spent in each state was calculated for the entire study period for that animal. From this, the percentage of the day spent in each

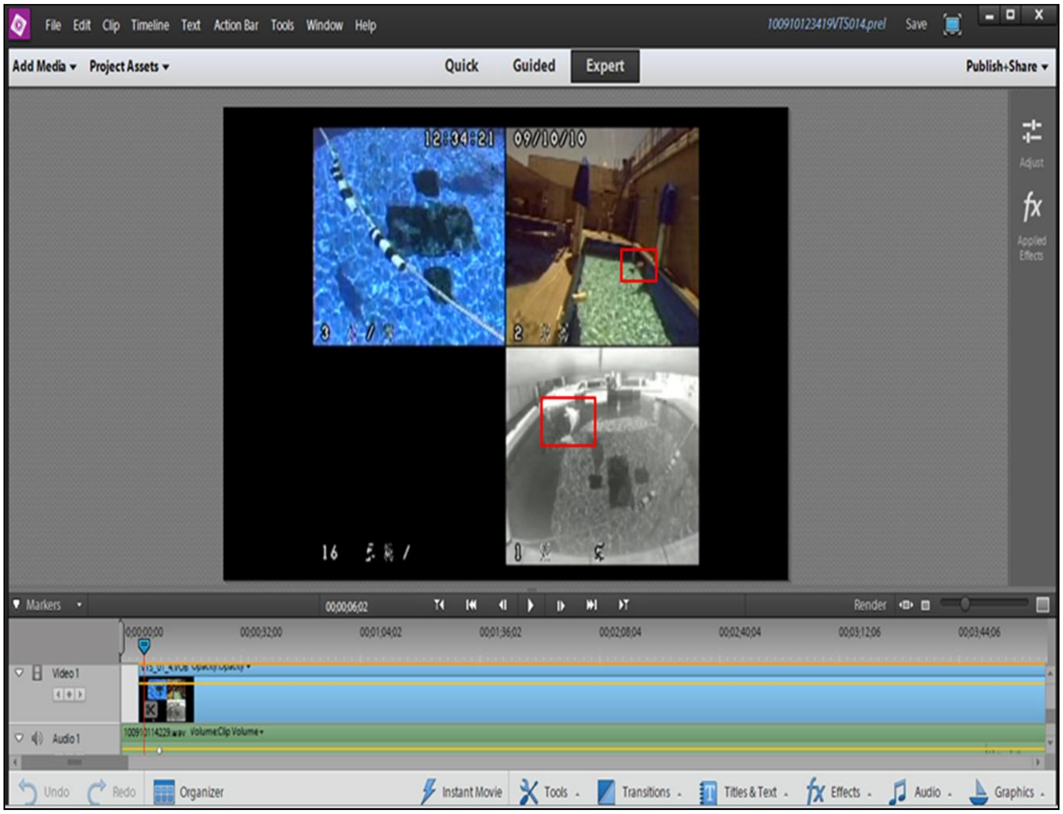


Figure 4: Screenshot of video data viewed in Adobe Premiere Elements. Red boxes show F2 in the top-right panel and F1 in the bottom-right panel. While bubble streams were not visible on the video, the research observers were close enough to observe them happening, and the video allowed for documentation of the remaining factors noted on the data sheets.

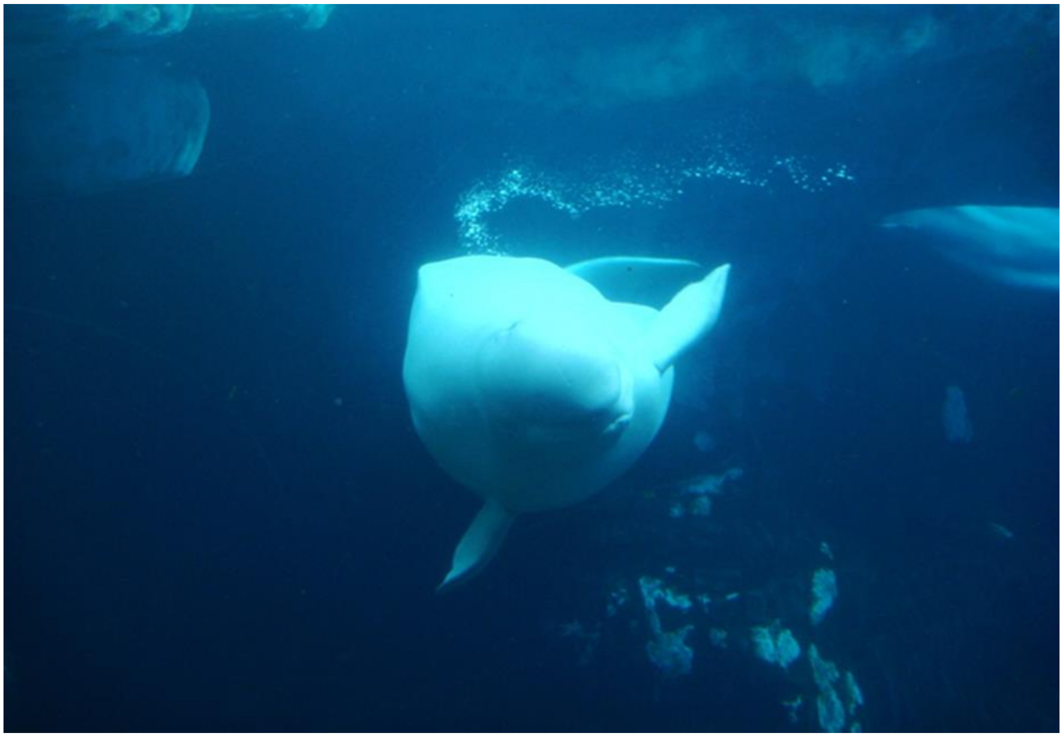


Figure 5: A beluga producing a bubble stream. Bubble streams were not visible on the video recordings, but the research observers could see bubble streams from their position at the edge of the pool. Photo by A. Bowles.

Table 1: Summary of factors studied and the categories within each factor.

Factor:	**Categories Included:**
Behavioral State	
	Swim
	Rest
	Soc-Affil
	Soc-Agon
	Nurse
	Solitary
	Play
	Human
Pool	
	Husbandry
	Back
Location	
	Gate
	GW
	South
	Plat
	Rope
	Other
Human Activity	
	TPres
	TInter
	Guests
	None
Proximity of Other Animal	
	No
	Yes: 1
	Yes: 0.5
	Yes: 0

Table 2: Category codes of the factors recorded on the data sheet when bubbling occurred. These same factors were recorded when producing the daily activity budgets for each animal.

Code:	**Description:**
File	File name (year, month, day, hour, minute, second), refers to Adobe Premier Elements file with video and audio synced
Scan Time	What time the behavior mentioned started (in hr:min:sec format; real time, not file time)
Bubble	Presence of a bubble stream or bubble cloud (left blank if none)
Behav	Behavior occurring during time of scan based on ethogram (see below)
State	Behavioral state into which the behavior falls (see below)
Pool	In which pool the behavior was occurring
Location	Where the behavior was occurring in the pool
Anim Near	Whether or not the other animal in the pool was in proximity to the focal animal
Body Length	Number of adult body lengths between focal animal and other animal (0.0 = touching, 0.5 = less than 1 length but not touching, 1.0 = 1 length)
Activity	Human activity going on near the pools (trainers present, trainers interacting with animal(s), guests present, or none)

Table 3: Category codes of the behaviors recorded on the data sheets, as well as the behavioral state into which each behavior fell and a detailed description of each behavior.

Code:	**Behavior:**	**Behavioral State:**	**Description:**
SWIM	Swim	Swim	Absence of any behavior other than swimming
INACT	Inactive	Rest	Not swimming, just resting or being still
AFC	Affiliative contact	Soc-Affil	Pec rubs, nosing, rubbing along whale, mouthing pecs or flukes
GATE	Gate Interaction	Soc-Affil	Touching gate or stopped at gate
ROLL	Rolling	Soc-Affil	Rolling around with no obvious direction of travel
UNDR	Under whale	Soc-Affil	Calf upright under whale, in contact with body (often bumping)
SACT	Surface Active	Soc-Agon	Slapping body parts, breaching
SUCK	Suckling	Nurse	Calf with head or mouth in contact with vents
HEAD	Head Out	Solitary	Head out of water, may be looking at trainers or observers
RUB	Rub	Solitary	Rubbing on pool surface
OBJ	Object	Play	Interacting with an object placed in the pool
HUM	Human Interaction	Human	Actively interacting with trainers

Table 4: Category codes for each location recorded on the data sheets, as well as a detailed description of each location. Numbers in parentheses refer to the locations mapped in Fig. 3.

Code:	**Location:**
GATE (3)	Gate between exhibit pool (not part of study area) and husbandry pool
GW (4)	Gate between husbandry pool and back pool
SOUTH (1)	South wall of back pool
PLAT (2)	Weighing platform near south wall of back pool
ROPE (5)	Rope for animal enrichment in back pool
OTHER	Any location not mentioned above

behavioral state could be calculated by dividing the total minutes in a given behavioral state by the total minutes of observation for the animal.

Data Analysis:

Analysis of Event Rate by Behavior State:

The events were summed to get the total number of bubble streams produced in each behavioral state by time of day and animal. Further analyses were based on these data. The proportion of bubble streams produced in each behavioral state was defined as the number of bubble streams in a given behavioral state divided by the total number of bubble streams produced during the study by the animal. Once both the bubbling percentages and the activity budget percentages had been calculated, it was possible to compare the proportion of bubbling events in each behavioral state to the proportion of time they spent in the state overall.

The process of summing the bubbling events, summing the minutes spent for the given categories of the variables irrespective of bubbling, calculating the respective percentages, and comparing them to each other, was repeated for each factor being studied. This analysis produced the percentage of bubbling events for comparison with overall activity using the following factors: behavioral state, pool, location, human activity, and inter-individual proximity (see Table 5 in Results section).

Analysis of behavioral events concurrent with behavioral state or other contextual information has been used to study the function of behavioral events previously (e.g., Recchia 1994, Vergara and Barrett-Lennard 2008, Vergara et al.

2010, Musser et al. 2014, Bowles et al. 2015). In order to compare counts of events such as bubbling and vocalizations per unit time (frequency data) with time spent in various states (time-activity budgets), the expected count per unit time had to be calculated and compared with observed counts.

Comparisons were made using the chi-squared goodness-of-fit test (Kramer and Schmidhammer 1992, Sharpe 2015). The test uses percentage data to determine similarity between two distributions by comparing observed data (the counts obtained during the study) to expected data (counts calculated based on assumptions about how events should be distributed in time). For this study, expected values were calculated based on the assumption that there would be no difference in the rates of behavioral events among categories – here, the factors of a variable, such as behavioral state.

The chi-squared statistic made it possible to generate expected values based on the percentage of time spent in each category (behavioral state, location, etc.). The null hypothesis was that the animals bubbled at the same rate regardless of the variable factor representing each context (e.g., behavioral state). This hypothetical rate was used to calculate the expected count of events given the observed time spent in each category. When looking at the “Pool” variable only, the chi-squared test was conducted as a test of independence with Yates’ continuity correction, appropriate for a 2x2 table.

The validity of the chi-squared statistic depends on a number of assumptions. First, factors and categories included in the test must have at least some probability of being associated with bubbling events or vocalizations. If the test table includes more than a small number of cells with none of the events of interest, the test could be biased. Thus, the tests were based on the assumption that data had been collected for long enough to detect bubbling events in any of the contexts represented by factors (e.g., location = back pool), if they were likely to occur. Conformity with this assumption is discussed below.

Second, the test assumed that successive samples of events were independent of each other. Conformity with this assumption is also discussed below.

Finally, the test assumed that the events of interest were so short that their duration did not bias the overall activity budget. The continuous stream of behaviors included both vocalizations and bubble streams, sometimes overlapping, so their influence on the budget as a whole had to be considered. However, each event lasted on the order of a second compared to a total dataset lasting more than 68 x 10^3 sec for each of the whales. The temporal resolution of behavioral state measurements was on the order of seconds, while state durations were substantially longer (see Results). In addition, the durations of the bubble streams could not be measured precisely. In the worst case, the cumulative duration of events represented less than 1% of the total observation time. On this basis, both bubble streams and vocalizations were treated as instantaneous events for the purposes of analysis.

Event Independence:

The assumption of event independence was tested by examining intervals between bubbling events for evidence of bouting. Historically, this has been done by generating a log-frequency plot (natural logarithm) of bout intervals and looking for distinct modes in the distribution (Martin et al. 1993, Dawkins 2007). The cutoff between modes in the graph is taken as the cutoff point separating individual events from bouts. This approach has the disadvantage that there may not be a clear minimum that distinguishes modes, leading to uncertainty about the break point. A more rigorous approach is described by Slater and Lester (1982) and Sibly et al. (1990). Interval data are displayed as a probability density plot (the proportion of samples in each frequency bin across the range of possible intervals). Intervals are broken into the smallest bin widths consistent with a smooth probability plot. Each bar represents the proportion of the total dataset in each bin.

The proportions are then used to calculate log-survivorship plots, which show the logged cumulative proportion of the dataset (Slater and Lester 1982) across the range of intervals. Distinct ‘breaks’ in this representation provide an estimate of the boundary between types of bouts. In most treatments, the break is the crossing point between two distributions that appear linear in the log-survivorship plot but have different slopes. This approach assumes that the distribution is produced by two processes, one with short intervals and one with longer intervals.

A number of authors have recommended methods for finding this break point. However, the precision of these break points depends on large datasets and at least some independent evidence for the 2-process interval model (e.g., Tolkamp and Kyriazakis 1999).

The models for bouts in studies of marine mammals (typically for call or dive intervals) have been developed with 2-process or 3-process models (e.g., Berdoy 1993, Parks and Tyack 2005, Janik et al. 2013, Rekdahl et al. 2015), but generally much smaller datasets.

My datasets (bubble streams and calls with bubble streams) were small, and I was mainly interested in evidence for clusters of events with short intervals that would indicate lack of independence. I based my analysis on an empirical approach used by Janik et al. (2013) to find a conservative criterion interval for short bouts of whistles emitted by bottlenose dolphins. They were interested in identifying bouts of similar whistles from individual bottlenose dolphins as a way to isolate signature whistles. Their approach was as follows:

1) Generate a histogram with the smallest bin interval that produces a smooth plot.
2) Convert it to a probability density plot (the proportion of events in each bin plotted against bin interval).
3) Generate a log-survivorship plot using the proportions (log of the cumulative proportion in successive bins plotted against bin interval)
4) Look for a strong peak in the probability density function. If the peak corresponds to a break in the log-survivorship plot, even if the break is relatively gradual, the peak will be a conservative estimate of the break point between short and longer intervals.

The approach of Janik et al. (2013) likely underestimated the total count of whistles with short intervals, because it defined the break point as the mode in the probability density plot. However, this approach gave them a conservative break point, ensuring that events in their data were reliably within bouts.

I was interested in the effect on my analysis of bouts of events with short intervals, i.e., of the possibility that my samples of calls or bubble streams were not independent, and therefore that my sampling unit should have been bouts as opposed to individual calls or streams. I chose a less conservative break point to include all events that could be part of a bout. This point was the upper limit of the first mode in the probability density plot, confirmed by a break in the log-survivorship plot from one linear segment to another or from an initial linear to non-linear segment.

Based on this break point, I used bouts as the unit of measurement instead of individual events and recalculated the chi-squared analysis.

Behavior Accumulation Curves:

Considering that observations and scoring of behavior can be time-consuming, there is a tradeoff between the duration of observations and the time it takes to process them. It behooves the researcher to sample enough events that there is a high probability of representing the animal's true repertoire of behaviors, but not much more. To address the adequacy of the observation period for the behavioral states used in the analysis, I calculated a behavior accumulation curve (BAC). A BAC shows the cumulative count of behaviors observed over time. In behavioral studies, the frequency of new behaviors decreases as sampling effort increases until a complete inventory of the behaviors has been captured (Dias et al. 2009). With continued sampling, the cumulative count of behavioral events or states reaches an asymptote. Typically, sampling should continue for several multiples of this asymptotic time to ensure that rare events are encountered, preferably over a time period adequate to account for diurnal or other cyclical patterns of importance. If the sampling time is not adequate to detect a behavioral state of interest, then the behavioral state should be pooled with others (categories collapsed) or removed from the analysis.

Related cells were collapsed to reduce the number of cells in the data tables where cell sample sizes were small. For example, the stream of focal whale data was initially categorized as a sequence of individual behavioral events. However, it was not possible to observe some behaviors reliably, and others were so rare that they were never observed synchronously with bubbling or call events. Instead, these behavioral events were pooled into behavioral states, which could be identified reliably and yielded blocks of time adequate to detect bubbling events. Basing the analysis on states also made it easier to spot meaningful patterns in behavior. For example, while it may not be meaningful if an animal bubbles more when doing one social-affiliative behavior compared to another social-affiliative behavior, it could be very meaningful if they bubble more when doing social-affiliative behaviors compared to solitary behaviors. A similar process was used to pare down locations; since the animals used some locations often and others rarely, the locations with low frequencies were pooled into a separate “other” category. Pooling continued until behavior accumulation curves showed that all the behavioral states were exhibited within at most the first 1/3 of the observation period.

Statistical Analysis:

Data analysis was conducted in the R programming environment (Version 3.0.3, R Studio package, www.r-project.org/, R Core Team 2014). The chi-squared test showed overall whether it was likely that there were significant differences in rates of bubbling events among the categories included in the test (i.e., behavioral states). However, the result would not indicate which states contributed most to the difference. There were several techniques for *post-hoc* analysis of chi-squared findings to glean more information about the relative importance of categories.

Sharpe (2015) described several methods for conducting *post-hoc* tests. First, the residuals may be used to make comparisons. Residuals were calculated along with the overall results of the chi-squared test. These residuals were a measure of the differences between the observed and expected values for each cell of the chi-squared comparison, corrected for the total sample size.

The equation for standardized residuals is:

$$sr_i = \frac{(x_i - \bar{x})}{\sqrt{\dfrac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{n}}}$$

The larger the absolute value of the residual, the more the observed data in that cell differ from expectation. The residual comparison must be made in the context of the original count of events, because an absolute difference of 5 means something different if the total count is 100 than if the count is 10.

The chi-squared distribution could be used to calculate criterion values for the residuals in the same manner as for the test overall. Using z-scores (Sharpe 2015) to estimate the proportion of the chi-squared distribution exceeding a criterion for α, standardized residuals (abbreviated as “stdres” from here on) were significant as follows: at $\alpha = 0.05$, a stdres of ± 1.96 was significant; at $\alpha = 0.01$, a stdres of ± 2.58 was significant; at $\alpha = 0.001$, a stdres of ± 3.30 was significant.

A positive standardized residual value indicates observed values higher than expected, and a negative value indicates observed values lower than expected. For example, in the current study, a positive standardized residual of 3.87 for F2 for bubbling in the presence of humans would suggest that F2 bubbled significantly more when interacting with humans than expected, and the magnitude of the residual suggested that the difference was significant at a p-value < 0.001 (see Table 6).

Confidence Intervals:

The chi-squared analysis did not quantify variability in behaviors per unit time. I double-checked the results of my analysis and got an indication of variability in the rates of behaviors by comparing the proportions of observed behavior states or other factors by time block. Taking these blocks as samples, I calculated 95% Confidence Intervals (CI) by factor. If activity did not vary much between time blocks, and events were associated with particular factors, the 95% CI across factors would not overlap or only overlap slightly. If the variance was large between time blocks, the overlap would be large and there would be no evidence for a relationship between events and the factors of interest.

In simple terms, the chi-squared goodness-of-fit test showed the significance of the observed differences between rates of bubbling and time in behavioral states or other factors overall, while the 95% CI showed how confident I could be in any differences I observed in light of the variance throughout the study period. I used 30-minute intervals to calculate the 95% CI values. The intervals were long enough for one or more changes in behavioral state, and almost always contained one or more bubble streams.

2.3 Results

A total of about 19 hours of data were collected over 2 days in September 2010, of which 10 hours had video coverage. F1 (the adult female) produced a total of 98 bubble streams in this time, and F2 (the female calf) produced 419 bubble streams.

For each factor studied, the chi-squared goodness-of-fit test showed whether percentages of bubbling events differed significantly from expected based on overall activity throughout the study period. Percentages for F1 are shown in Table 5 and percentages for F2 are shown in Table 6. Significant results for both animals are summarized in Table 7. The factors studied and the categories within each factor are described in Table 1.

Differences in behavior were great enough between the two animals that their results were analyzed separately. For both animals, there were significant

Table 5: Summary of results for each factor studied when bubbling compared to overall activity for F1. P-values showing the significance of the difference between bubbling and overall activity are given below each table. Asterisks next to bubbling percentages indicate that that individual factor contributed to the significant difference from the chi-squared analysis. Factors that were removed from analysis are shaded in gray. Numbers in parentheses are standardized residuals. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$

F1

Behavioral State

	Bubbles (%)	**All Activity (%)**	**Bubbles (Freq)**	**All Activity (Minutes)**
Swim	18.82*** (-5.07)	43.61	18	278
Rest	30.59* (2.07)	22.16	30	141
Soc-Affil	47.06*** (8.47)	15.99	46	102
Solitary	2.35*** (-3.36)	14.38	2	92
Human	1.18 (-1.44)	3.65	1	23
xSoc-Agon	0.00	0.21	0	1
xNurse	0.00	0.00	0	0
xPlay	0.00	0.00	0	0

$p < 0.001$

Pool

	Bubbles (%)	**All Activity (%)**	**Bubbles (Freq)**	**All Activity (Minutes)**
Back	54.12*** (-8.23)	84.50	53	538
Husbandry	45.88*** (8.23)	15.50	45	99

$p < 0.001$

Location

	Bubbles (%)	**All Activity (%)**	**Bubbles (Freq)**	**All Activity (Minutes)**
GW	14.12*** (-4.74)	37.38	14	238
Gate	42.35*** (11.65)	8.82	42	56
Plat	18.82 (0.21)	17.62	18	112
South	20.00 (-1.37)	26.13	18	166
Other	4.71 (-1.76)	10.05	5	64
xRope	0.00	0.00	1	1

$p < 0.001$

Human Activity

	Bubbles (%)	**All Activity (%)**	**Bubbles (Freq)**	**All Activity (Minutes)**
Tpres	12.94*** (-6.36)	45.10	13	287
Tinter	0.00* (-2.28)	4.55	0	29
Guests	7.06 (-1.49)	11.62	7	74
No	80.00*** (8.50)	38.72	78	247

$p < 0.001$

Proximity

	Bubbles (%)	**All Activity (%)**	**Bubbles (Freq)**	**All Activity (Minutes)**
No	89.80*** (4.73)	67.28	88	429
Yes: 0.0	4.08 (-0.83)	6.48	4	41
Yes: 0.5	3.06*** (-3.56)	15.95	3	102
Yes: 1.0	3.06* (-2.34)	10.30	3	66

$p < 0.001$

Table 6: Summary of results for each factor studied when bubbling compared to overall activity for F2. P-values showing the significance of the difference between bubbling and overall activity are given below each table. Asterisks next to bubbling percentages indicate that that individual factor contributed to the significant difference found in the chi-squared analysis. Factors that were removed from analysis are shaded in gray. Numbers in parentheses are standardized residuals. * = p < 0.05, ** = p < 0.01, *** = p < 0.001

F2

Behavioral State	**Bubbles (%)**	**All Activity (%)**	**Bubbles (Freq)**	**All Activity (Minutes)**
Swim	19.66*** (-8.09)	60.02	82	382
Rest	13.43 (1.59)	8.93	56	57
Soc-Affil	45.80*** (7.74)	16.61	192	106
Nurse	0.00* (-2.03)	4.00	0	25
Solitary	5.28 (-0.65)	6.51	22	41
Play	4.32*** (INF)	0.05	18	0
Human	11.51*** (3.87)	3.84	48	24
xSoc-Agon	0.00	0.05	0.00	0

p < 0.001

Pool	**Bubbles (%)**	**All Activity (%)**	**Bubbles (Freq)**	**All Activity (Minutes)**
Back	8.59*** (-6.77)	42.11	36	268
Husbandry	91.41*** (6.77)	57.89	383	369

p < 0.001

Location	**Bubbles (%)**	**All Activity (%)**	**Bubbles (Freq)**	**All Activity (Minutes)**
GW	15.87 (-0.80)	19.28	66	123
Gate	42.55** (3.24)	27.93	178	178
Plat	1.44 (-1.92)	6.04	6	38
South	3.85* (-2.05)	10.36	16	66
Other	35.82 (1.50)	28.65	150	183
Rope	0.48** (-2.77)	7.74	2	49

p < 0.001

Human Activity	**Bubbles (%)**	**All Activity (%)**	**Bubbles (Freq)**	**All Activity (Minutes)**
Tpres	33.57* (2.24)	24.48	141	156
Tinter	12.23*** (4.20)	4.39	51	28
Guests	0.48 (-1.80)	3.56	2	23
No	53.72** (-3.06)	67.57	225	430

p < 0.001

Proximity	**Bubbles (%)**	**All Activity (%)**	**Bubbles (Freq)**	**All Activity (Minutes)**
No	79.19*** (3.73)	60.88	332	388
Yes: 0.0	3.11** (-2.94)	13.18	13	84
Yes: 0.5	8.13*** (-3.35)	21.55	34	137
Yes: 1.0	9.57** (2.84)	4.39	40	28

p < 0.001

Table 7: Summary of all significant results for F1 (top) and F2 (bottom) when comparing bubbling events to overall activity.

F1

Variable:	Bubbling More Likely:	Bubbling Less Likely:
Behavioral State	Social-Affiliative, Rest	Swim, Solitary
Pool	Husbandry pool	Back pool
Location	Gate	Gateway
Human Activity	No activity	Trainers present, Trainers interacting
Proximity of Other Animal	Not in proximity	1.0 body lengths away, 0.5 body lengths away

F2

Variable:	Bubbling More Likely:	Bubbling Less Likely:
Behavioral State	Social-Affiliative, Play, Human	Swim, Nurse
Pool	Husbandry pool	Back pool
Location	Gate	South wall, Rope
Human Activity	Trainers present, Trainers interacting	No activity
Proximity of Other Animal	Not in proximity, 1 body length away	0.0 body lengths away, 0.5 body lengths away

differences in all of the variables when comparing bubbling events to overall activity throughout the day. These factors were the animal’s behavioral state, which pool they were in, their specific location in the pools, human activity going on near the pools, and their proximity to the other animal (see Table 2 above). However, there were some states atypical of one or both of the animals. For example, F1 did not have any behaviors falling into the “Play” category, so this category was eliminated from her analysis.

When the 10 hours of video data were broken into 20 30-minute time blocks, activity could be examined over time. These samples were used to produce behavioral accumulation curves (Fig. 6). The entire repertoire of behavioral states included in the final sampling protocol was reached after a small proportion of the total sampling period. Both animals demonstrated all known behavioral states within the first 2.5 hours. F2 demonstrated all of the behavioral states within 1 hour of the start of the study. Thus, the total sampling time was at least 4-fold longer than the duration needed to obtain a sample with all behavioral states, as was true with the other factors studied. In addition, data collection took place over two days, ensuring at least a degree of independence among the time blocks.

Activity budgets of both animals were plotted over the 2-day period of the study to visualize the variance in their activity (F1 in Fig. 7, F2 in Fig. 8). Activity was quite variable throughout both days, showing that significant findings were

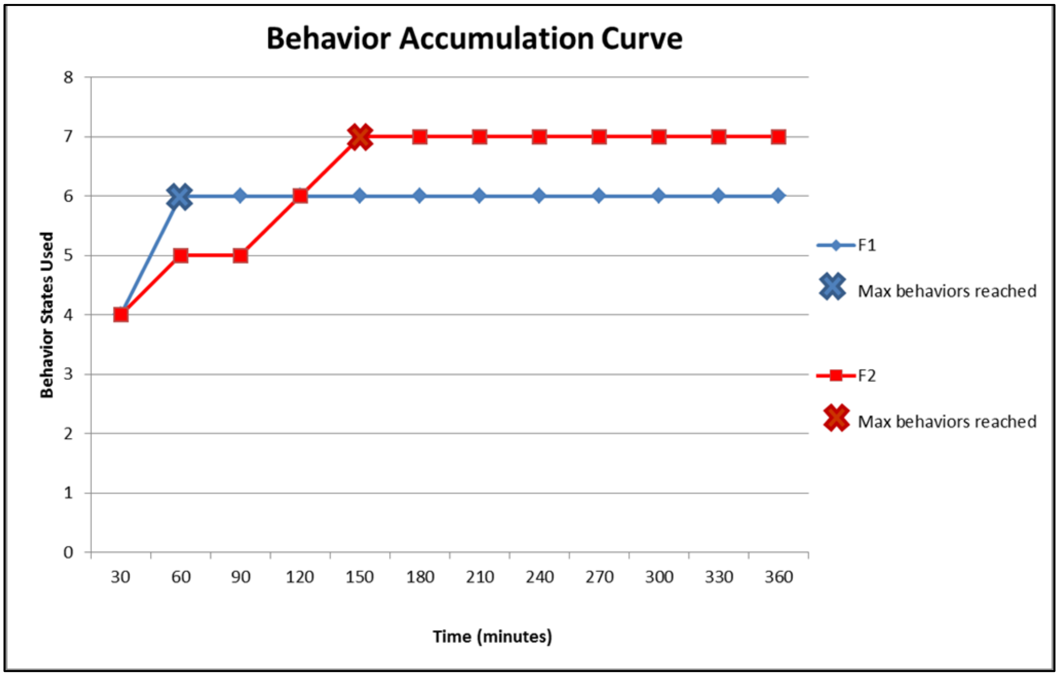


Figure 6: Behavior Accumulation Curves for F1 (blue line with diamond markers) and F2 (red line with square markers) showing how many behavioral states the animal had used by the given time. The x-axis shows the time in minutes that had elapsed since the start of the study. X's denote the time by which each animal had demonstrated all of their behavioral states seen in the study. F1 did not engage in play behaviors at any point in the study, so she had a maximum of 6 behavioral states while F2 had 7.

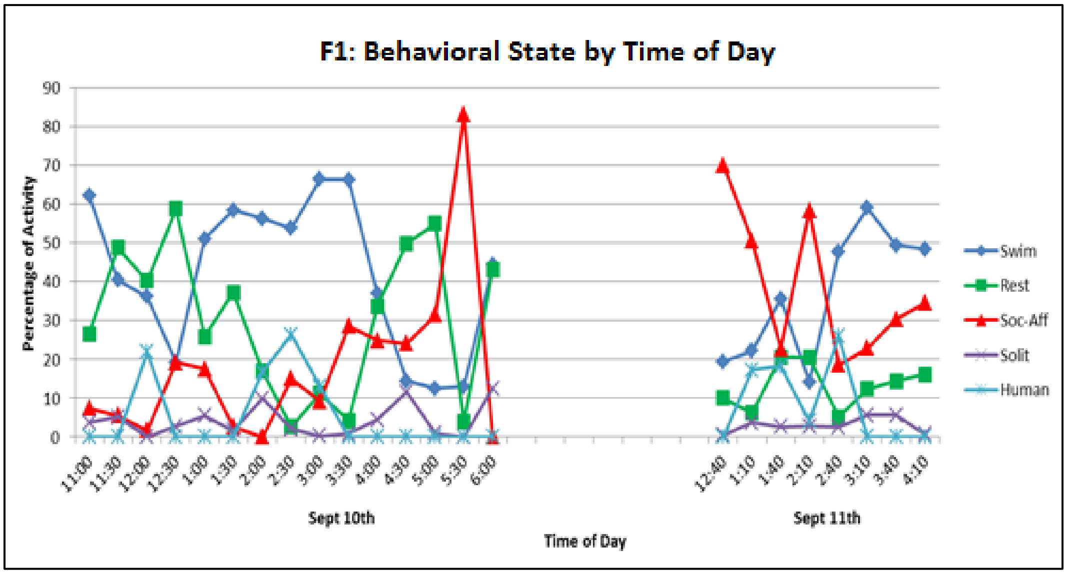


Figure 7: Behavioral state by time of day for F1. Times are from 11:00am-6:00pm on September 10th and 12:40pm-4:10pm on September 11th. Values are the percentage occupied by the given behavioral state for the 30-minute time period shown on the x-axis.

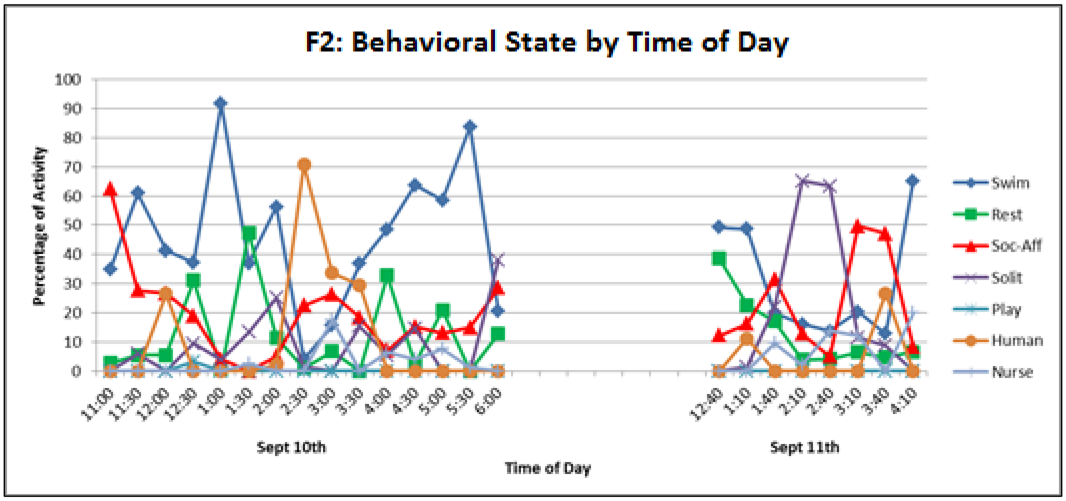


Figure 8: Behavioral state by time of day for F2. Times are from 11:00am-6:00pm on September 10th and 12:40pm-4:10pm on September 11th. Values are the percentage occupied by the given behavioral state for the 30-minute time period shown on the x-axis.

not the result of a few instances of high activity of a certain type outweighing overall inactivity (or vice versa).

Behavioral State

Based on the previous literature, the social-affiliative behavioral state was of special interest in relation to bubble streams (Fripp 2005). I plotted the activity budgets with the social-affiliative behavioral state highlighted and compared them with counts of bubble streams and bubbled calls over the study period (F1 in Fig. 9, F2 in Fig. 10). Red boxes in these figures show relative peaks in social-affiliative behavior in the top plot of each figure, as well as corresponding peaks in bubble stream production overall in the middle plot and bubbled call production in the bottom plot. The high degree to which the peaks in production of both categories of bubble streams corresponded to peaks in social-affiliative behavior suggested that bubble streams were preferentially used in this context, possibly for communicative purposes.

The behavioral state “Play” was eliminated from the analysis for F1 because she did not produce behaviors in that category when either bubbling or overall. “Social-Agonistic” was eliminated for both whales because the proportion for that activity overall was less than 1% and could not be pooled logically with any of the other states. This left 5 behavioral states for F1: “Swim”, “Rest”, “Social-Affiliative”, “Solitary”, and “Human” and 7 behavioral states for F2: “Swim”, “Rest”, “Social-Affiliative”, “Nurse”, “Solitary”, “Play”, and “Human”.

There was a significant difference between predicted and observed bubble stream counts among the behavioral states for both F1 ($\chi^2 = 89.74$, df = 4, p <

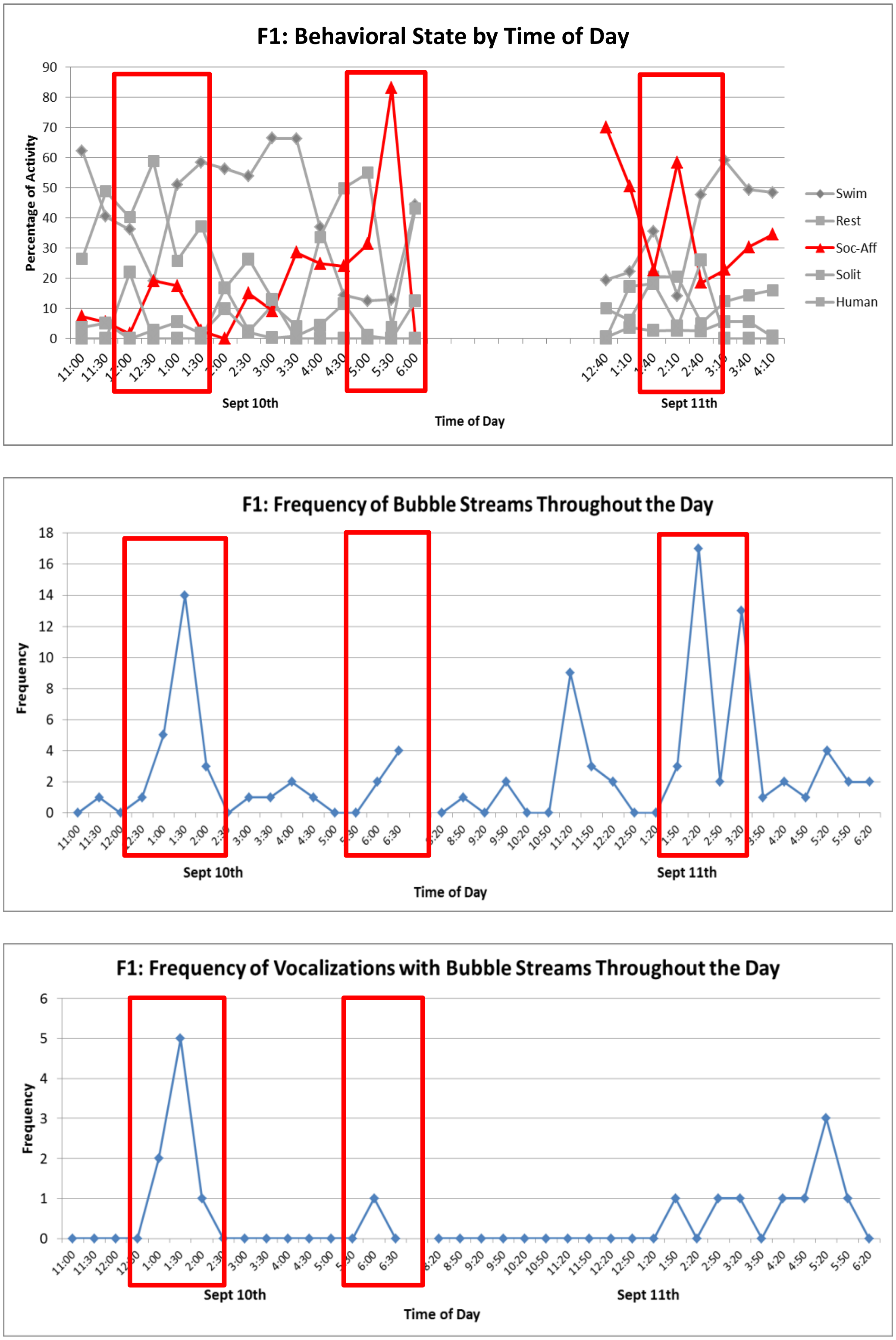


Figure 9: Behavioral state by time of day for F1 (top), followed by frequency of bubble streams overall (middle) and frequency of bubble streams with a vocalization (bottom) for F1. The red line on the top graph highlights the social-affiliative behavioral state, and red boxes highlight where peaks in that behavioral state correspond to peaks in bubble stream production.

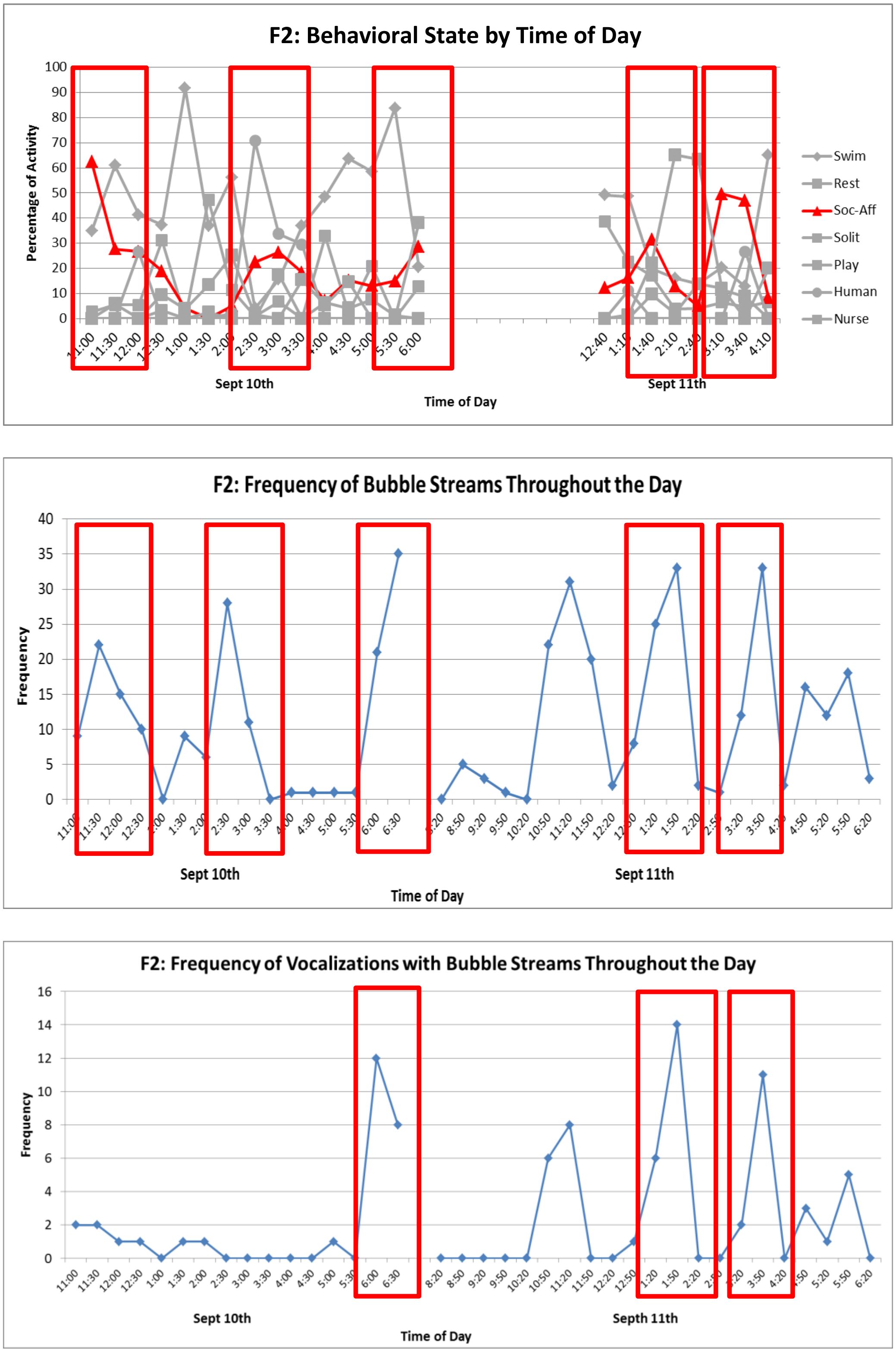


Figure 10: Behavioral state by time of day for F2 (top), followed by frequency of bubble streams overall (middle) and frequency of bubble streams with a vocalization (bottom) for F2. The red line on the top graph highlights the social-affiliative behavioral state, and red boxes highlight where peaks in that behavioral state correspond to peaks in bubble stream production.

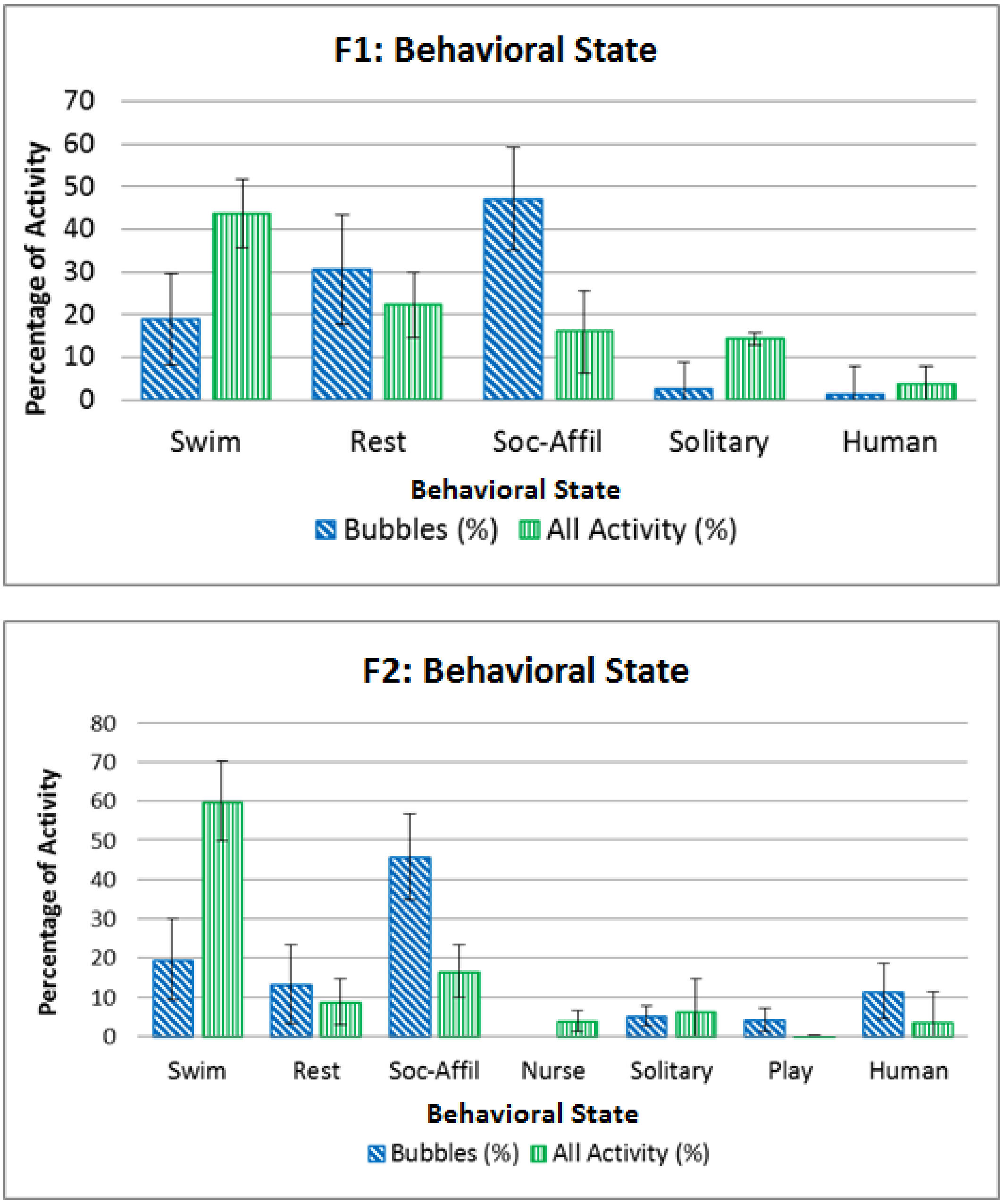


Figure 11: Percentage of time spent in each behavioral state when bubbling (blue cross-hatched bars) compared to overall activity (green lined bars). F1's results are shown in the upper panel and F2's results are shown in the lower panel. Error bars denote 95% confidence intervals.

0.001) and F2 ($\chi^2 = 28$, df = 6, $p < 0.0001$). These results are shown in Figure 11. There was clear evidence that bubbling events were not equally likely in all behavioral states. F1 bubbled significantly less than expected in the categories "Swim" (stdres = -5.07, $p < 0.001$) and "Solitary" (stdres = -3.36, $p < 0.001$), and bubbled significantly more than expected when in "Rest" (stdres = 2.07, $p < 0.05$) and "Social-Affiliative" (stdres = 8.47, $p < 0.001$). While resting, F1 often performed a sequence of behaviors consisting of floating slowly toward the platform wall in the back pool, bumping into the wall slowly with her melon, and pushing back off the wall gently while releasing a bubble stream. This sequence accounted for 27.3% of her bubble streams while resting and 25.3% of her total time resting. This use of bubble streams did not appear in F2's repertoire.

F2 bubbled significantly less than expected in the categories of "Swim" (stdres = -8.09, $p < 0.001$) and "Nurse" (stdres = -2.03, $p <0.05$). Like F2, she bubbled significantly more when in the "Social-Affiliative" state (stdres = 7.74, $p < 0.001$). In addition, she bubbled more often than expected in the "Play" (stdres = INF, $p < 0.001$), and "Human" (stdres = 3.87, $p < 0.001$) behavioral states.

The 95% CI's supported these statistical findings for the most part, showing high confidence for all of the significant factors except "Rest" for F1. This might be explained by her stereotyped resting behavior, which usually involved bubble streams. Additionally, the CI's for "Play" for F2 do not appear to overlap, but since the counts were small to begin with, more data would be needed to interpret this factor conclusively.

Pool

There was a significant difference in observed and expected incidence of bubbling between pools for both F1 ($\chi^2 = 67.68$, df = 1, $p < 0.001$) and F2 ($\chi^2 = 45.82$, df = 1, $p < 0.001$). These results are shown in Figure 12. F1 and F2 both bubbled significantly less than expected in the back pool (F1: stdres = -8.23, $p < 0.001$; F2: stdres = -6.77, $p < 0.001$) and significantly more than expected in the husbandry pool (F1: stdres = 8.23, $p < 0.001$; F2: stdres = 6.77, $p < 0.001$. The 95% CI's for this factor agreed well with the statistical results, with no overlap between them for either animal or either pool.

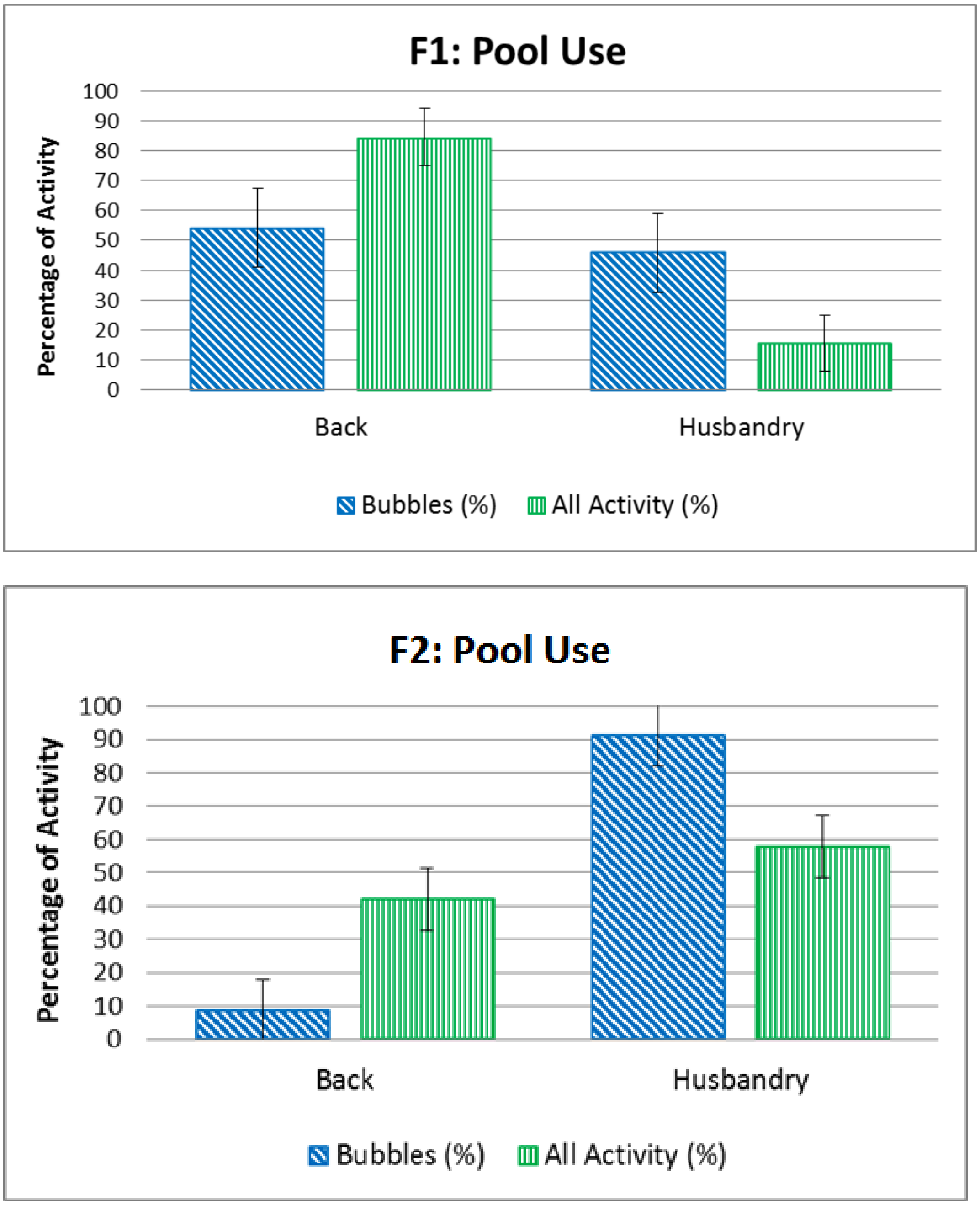
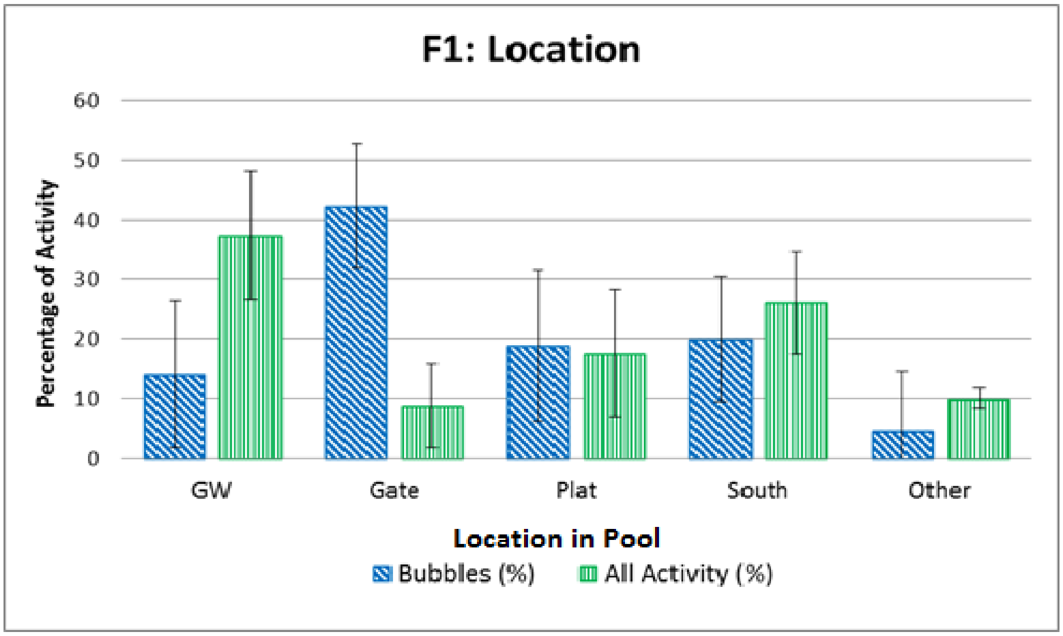


Figure 12: Percentage of time spent in each pool when bubbling (blue cross-hatched bars) compared to overall activity (green lined bars). F1's results are shown in the upper panel and F2's results are shown in the lower panel. Error bars denote 95% confidence intervals.

Location

When looking at the animals' locations in the pools separately from the pools themselves, there was a significant difference in bubbling with location (Fig. 13) for both F1 ($\chi^2 = 141.95$, df = 4, $p < 0.001$) and F2 ($\chi = 23.995$, df = 5, $p < 0.001$). F1 bubbled significantly more than expected at the gate to the main exhibit pool (stdres = 11.65, $p < 0.001$) and significantly less than expected at the gateway between the husbandry and back pools (stdres = -4.74, $p < 0.001$). F1 did not spend any time close to the hydrophone in either period, so that location was eliminated for her. F2 bubbled significantly more than expected at the gate (stdres = 3.24, $p < 0.01$) and significantly less than expected at the south wall (stdres = -2.05, $p < 0.05$) and near the rub rope (stdres = -2.77, $p < 0.01$).

While the 95% CI's for F1 show no overlap between bubbling and overall activity for the significant factors mentioned above, there was considerable overlap for F2 in "Gate" and "South". However, the magnitude of the statistical significance of the differences between the two conditions based on residuals was large. The contradiction can be explained by long periods during which the calf was at the gate and not always bubbling frequently. There were 30-minute intervals during which she spent all of her time at the gate and some when she spent no time at the gate. This likely led to large variation in the values for this factor when bubbling and during overall activity, inflating the CI's.



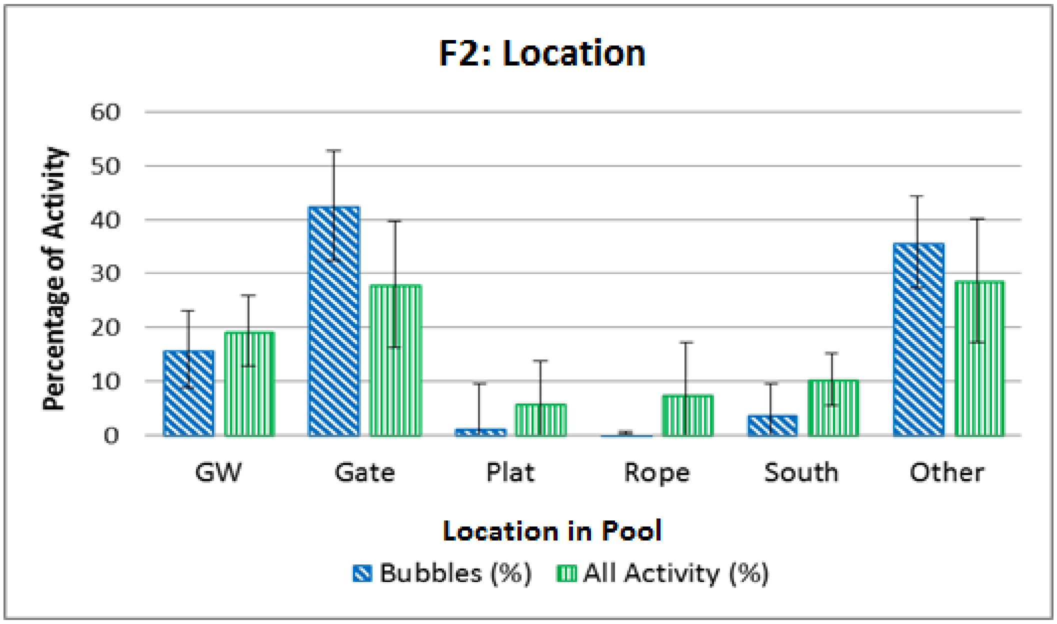


Figure 13: Percentage of time spent in each location in the pools when bubbling (blue crosshatched bars) compared to overall activity (green lined bars). F1's results are shown in the upper panel and F2's results are shown in the lower panel. Error bars denote 95% confidence intervals.

Human Activity

Bubbling differed from expectation in relation to human activity in the vicinity of the test pools (Fig. 14) for both F1 ($\chi^2 = 73.697$, df = 3, p = $p<0.001$) and F2 ($\chi^2 = 26.85$, df = 3, $p < 0.001$). For F1, bubbling exceeded expectation significantly when no human activity was occurring (stdres = 8.50, $p < 0.001$), while bubbling was observed significantly less than expected when trainers were present (stdres = -6.36, $p < 0.001$) or interacting with her (stdres = -2.28, $p < 0.05$). For F2, bubbling occurred significantly more than expected when trainers were present (stdres = 2.24, $p < 0.05$) and when trainers were interacting with her (stdres = 4.20, $p < 0.001$), while bubbling occurred significantly less than expected when no human activity was going on near the pools (stdres = -3.06, $p < 0.01$).

For F1, the 95% CI's showed no overlap between the significant factors, while they overlapped considerably for the factors found to be significant for F2. Again, her behavior seemed to be more variable; in an immature animal this variability can be expected. An older calf, having learned the appropriate context for bubbling and other interactions with humans, might show less variance in these factors.

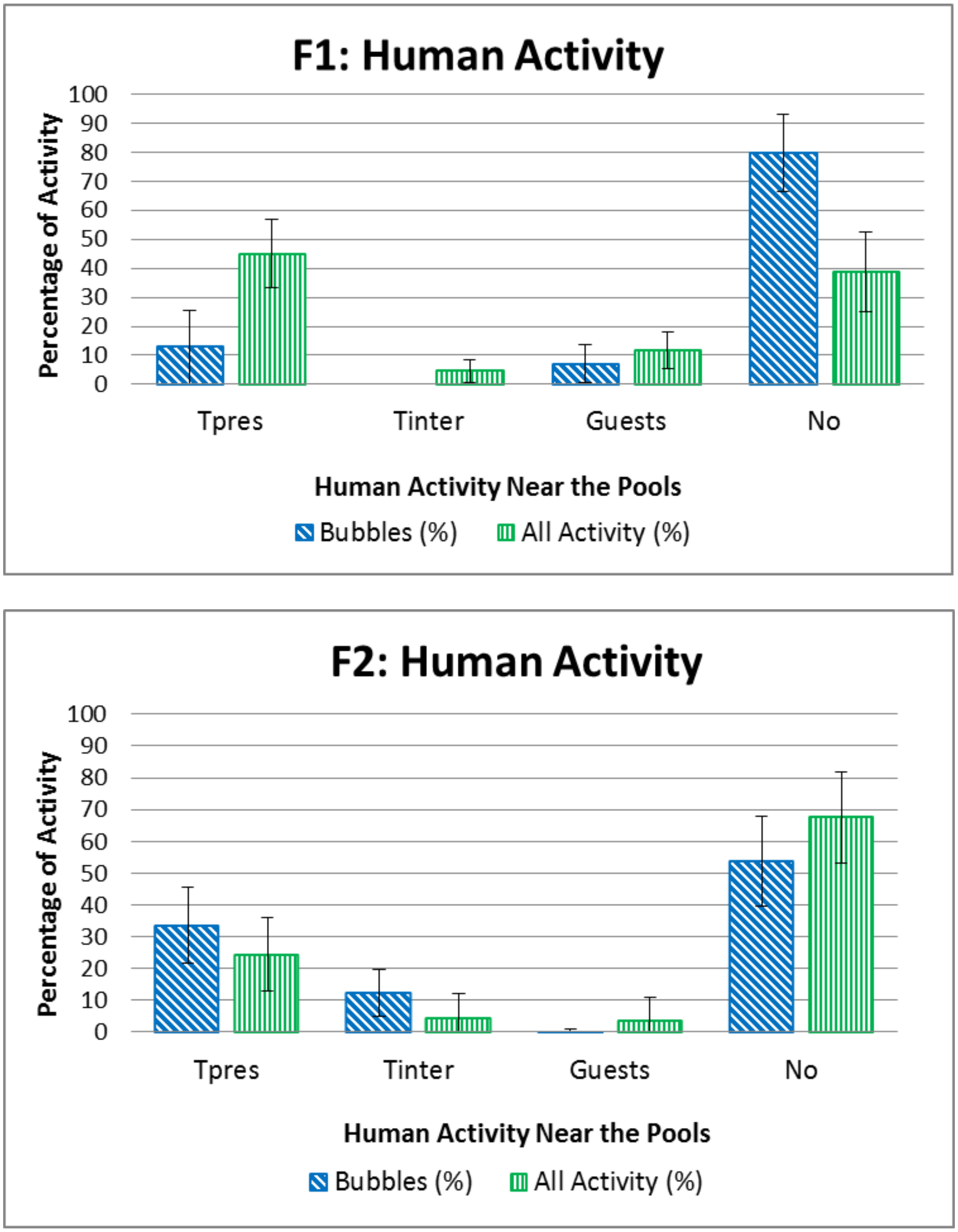


Figure 14: Percentage of time spent bubbling (blue crosshatched bars) compared to overall activity (green lined bars) with various human activities going on near the pools. F1's results are shown in the upper panel and F2's results are shown in the lower panel. Error bars denote 95% confidence intervals.

Proximity of Other Animal

There was a significant difference in the proximity of the other animal to the focal animal when bubbling occurred compared to overall activity for F1 (χ^2 = 23.41, df = 3, $p < 0.001$) and F2 (χ^2 = 29.45, df = 3, $p < 0.001$). These results are shown in Figure 15. F1 bubbled significantly more than expected when F2 was not in close proximity (greater than 1.0 adult body length away; stdres = 4.73, $p < 0.001$) and significantly less than expected when F2 was close, either 0.5 (stdres = -3.56, $p < 0.001$) or 1.0 body length away (stdres = -2.34, $p < 0.05$). F2 also bubbled significantly more than expected when F1 was not in close proximity to her (stdres = 3.73, $p < 0.001$) or was 1.0 adult body length away (stdres = 2.84, $p < 0.01$), and bubbled significantly less than expected when F1 was touching her (stdres = -2.94, $p < 0.01$) or was 0.5 body length away (stdres = -3.35, $p < 0.001$).

The 95% CI's supported these statistical findings, especially when looking at times when the animals were not in close proximity to each other. However, at times when F1 was 1.0 body length away from F2, F2's bubbling activity was variable and therefore the CI's overlapped. Perhaps for this factor, the activity going on at the time explained bubbling behavior better than the proximity of the other animal, at least for the inexperienced calf.

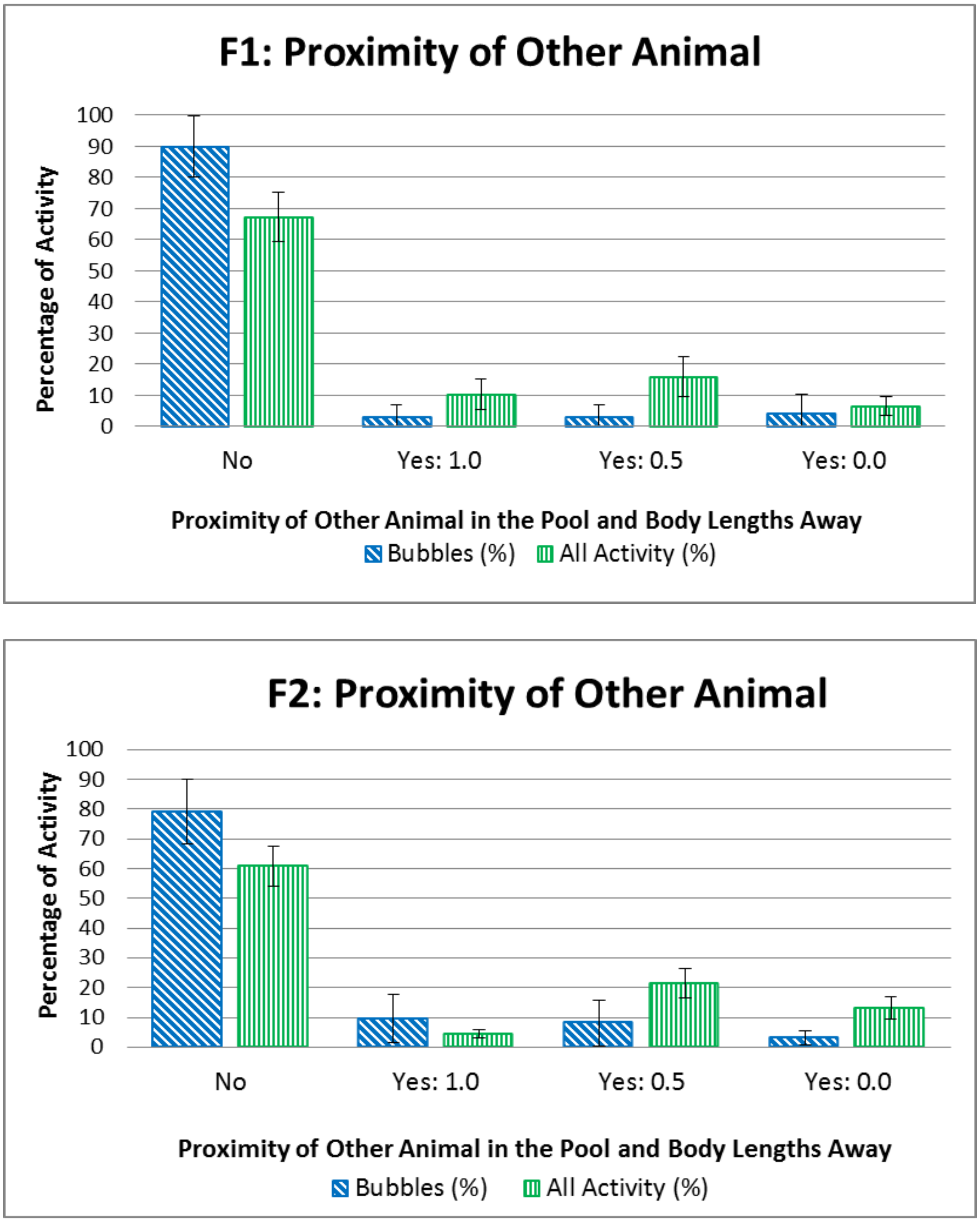


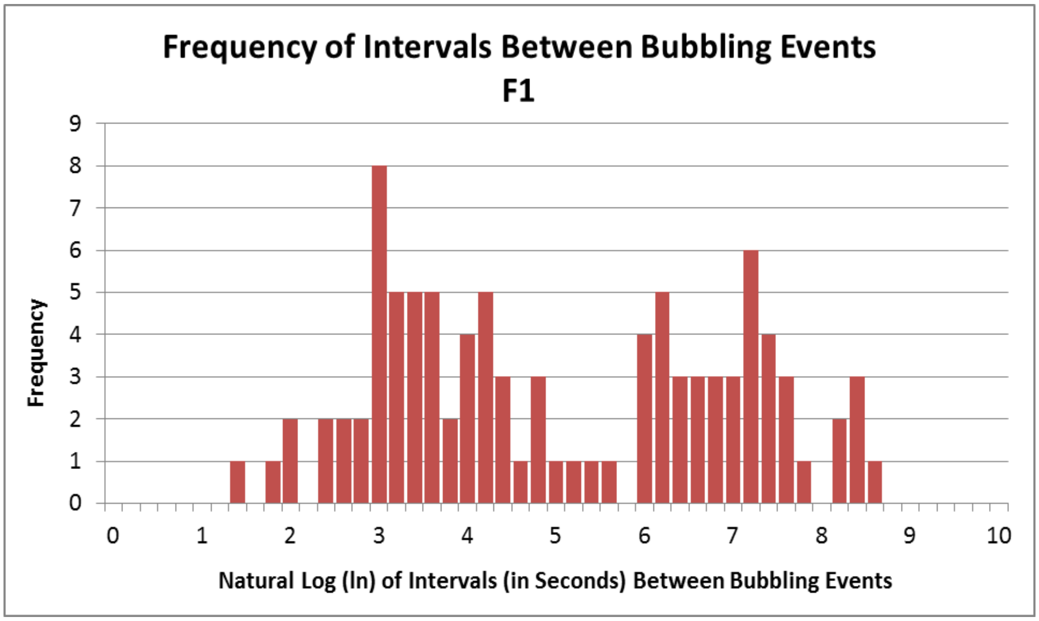
Figure 15: Percentage of time spent bubbling (blue crosshatched bars) compared to overall activity (green lined bars) when in varying proximity to the other animal in the pool. F1's results are shown in the upper panel and F2's results are shown in the lower panel. Error bars denote 95% confidence intervals.

Bout Analysis

Log-normal plots of the distribution of bubbling intervals showed an approximately normal distribution for F2 in the case of bubble streams (bottom of Fig. 16). The distribution of bubble streams was concentrated below ln(interval) = 150 s. Given this single mode in the distribution, successive samples might have been independent of each other. However, for F1 the natural log of the intervals revealed a bimodal distribution (top of Fig. 16), suggesting that two different types of bubble stream intervals were possible, that is, that bubble streams occurred in “bouts”.

To refine the estimate of the bout break point, the data for both whales were examined using the method of Janik et al. (2013). Figure 17 shows the frequency plot for F1 and F2 with the data binned into 10 sec intervals. Both plots show a peak at 20 sec, although it was weak in the data for F1, and the rest of the intervals were widely spread across the range from 10 sec to 2000 sec. The probability density plot (Fig. 18A) shows that the greatest proportion of the sample was found at long intervals (200 sec or more). Thus, in her case, evidence for bouting was weak. However, the concentration of short intervals was at or below 110 sec, and the log-survivorship showed a break between linear and non-linear decay below this interval.

In the case of F2, a high proportion of the intervals were concentrated in the first 110 sec as well (bottom of Fig. 17), with a clear peak at 20 sec and no mode at longer intervals. If I had wanted to guarantee that bubble streams were from single bouts, I could have taken this as the break point, but the concern in



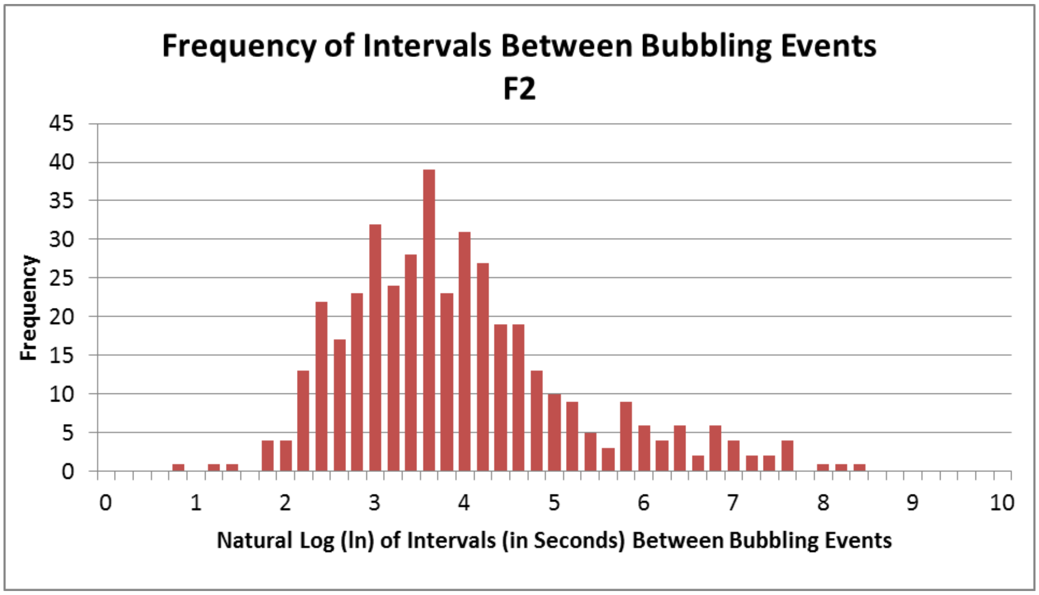
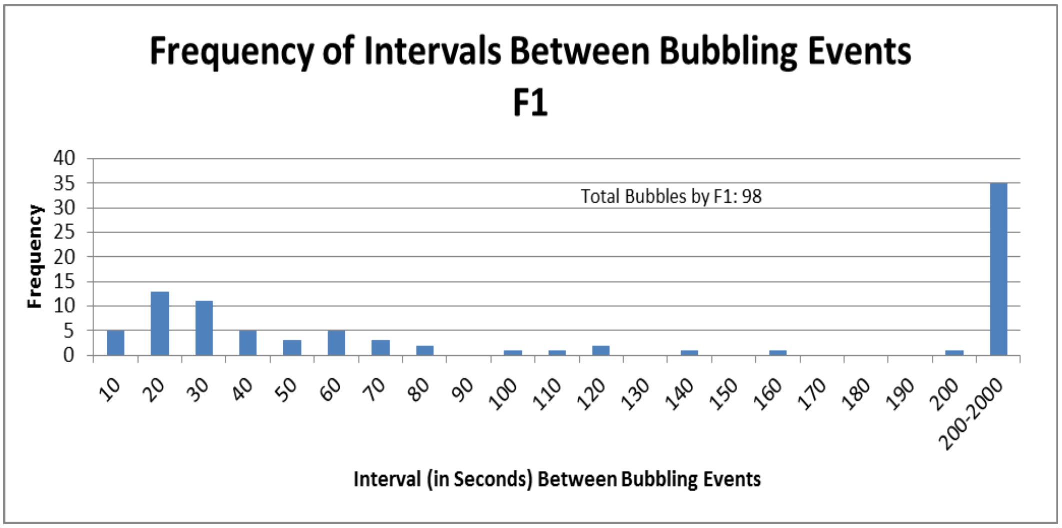


Figure 16: Natural log (ln) of frequency of intervals between bubbling events for F1 (top) and F2 (bottom). The graph for F2 shows a normal distribution, but the bimodal distribution for F1 suggests possible bouting behavior in relation to bubbling.



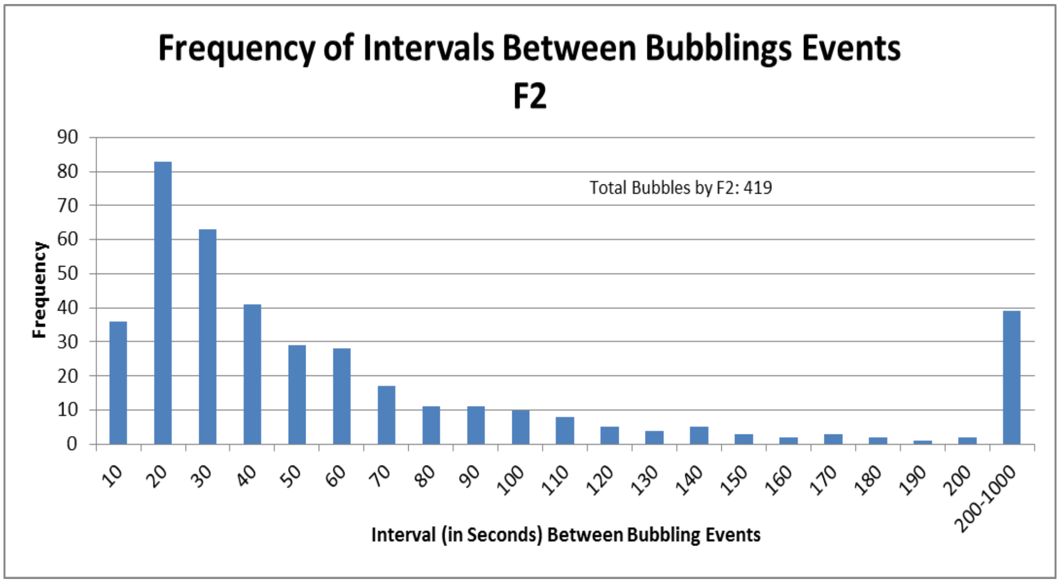
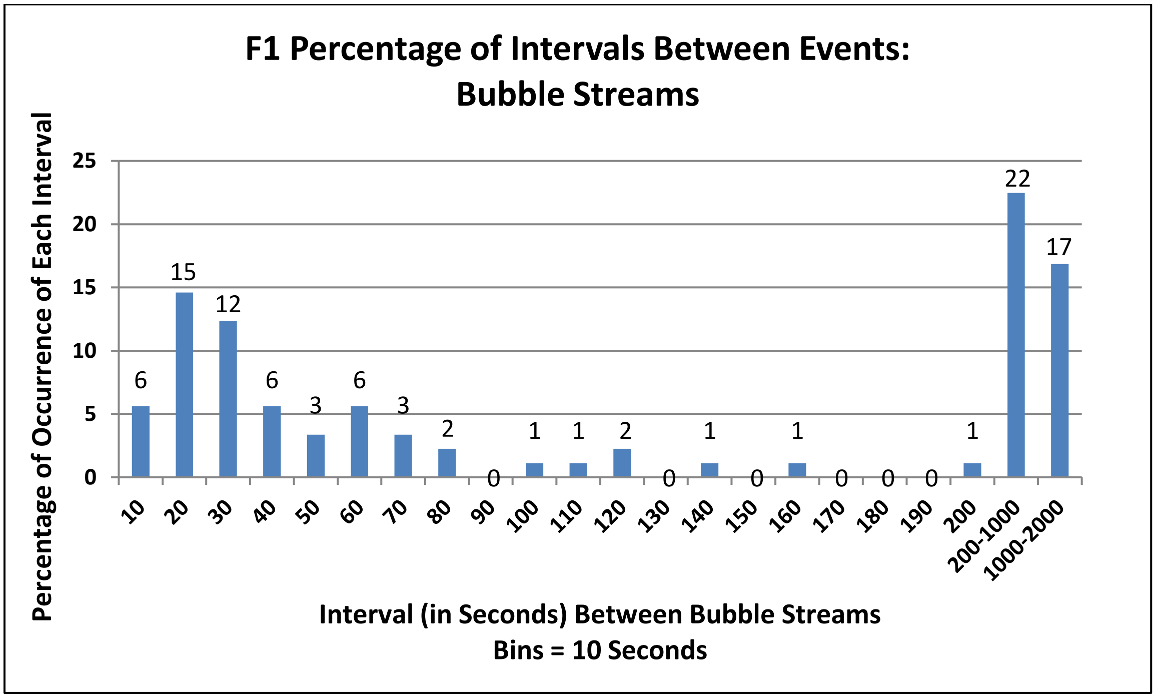
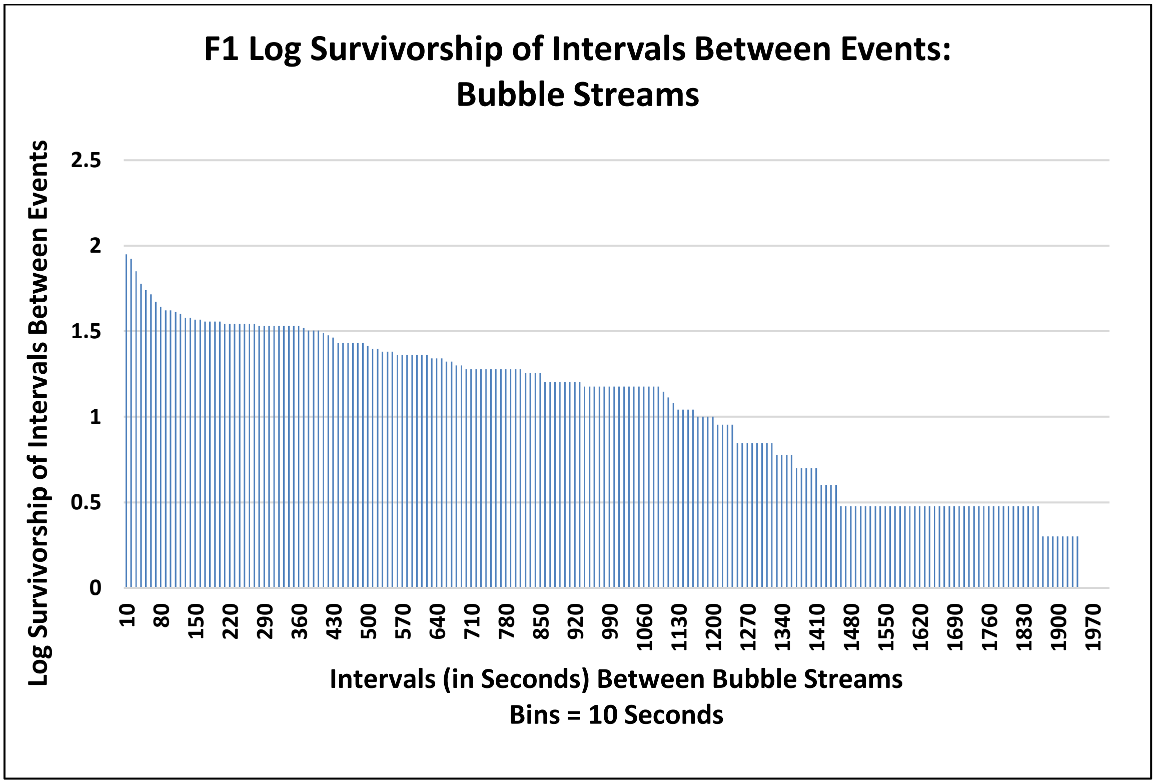


Figure 17: Frequency of intervals between bubbling events for F1 (top) and F2 (bottom). Intervals are shown in bins of 10 seconds; the bar over interval “10” represents intervals of 0-10 seconds in duration, the bar over interval “20” represents intervals of 11-20 seconds in duration, and so on.



A



B

Figure 18: Percentage of intervals between events (A) and log survivorship of intervals between events (B) for F1's bubble streams.

this case was to ensure independence between successive bubbling events. Thus, there was evidence for a process concentrated at short intervals, and I took 110 sec as a conservative estimate of the break point. The log-survivorship plot (Fig. 19B) showed that this was also the point at which decay shifted from linear to non-linear, consistent with the frequency and probability density plots.

The log-survivorship plot for F1 also suggested that the second mode in the initial log-normal plot (compare Fig. 18B to top of Fig. 17) was part of an additional type of interval, very long intervals (perhaps a third-order or higher order process). However, the count of these bouts would have been small, and the long intervals made them difficult to treat as events. They were not analyzed separately, but might be considered in future, larger datasets.

Because the distribution for F1 at short intervals was at least consistent with the break point for F2, I applied the same break point to both whales. In the data for F2, bubbling events separated by more than 110 sec were treated as individual events, while those separated by less than 110 sec belonged to the same bout.

For F1, I found 45 bouts ranging from 1-12 bubble streams per bout. On average, a bout contained 2.17 bubble streams, with only 5 of the 45 bouts containing 5 or more bubble streams. Thus, bouts typically were not long. Analysis for F1 based on bouts as the sampling unit gave results similar to bubble streams analyzed as independent events (Figs. 20-24). Behavioral state ($\chi^2 = 60.518$, df = 4, $p < 0.001$), pool use ($\chi^2 = 30.724$, df = 1, $p < 0.001$), location in the pools ($\chi^2 = 60.63$, df = 4, $p < 0.001$), human activity ($\chi^2 = 63.927$, df = 3, $p <$

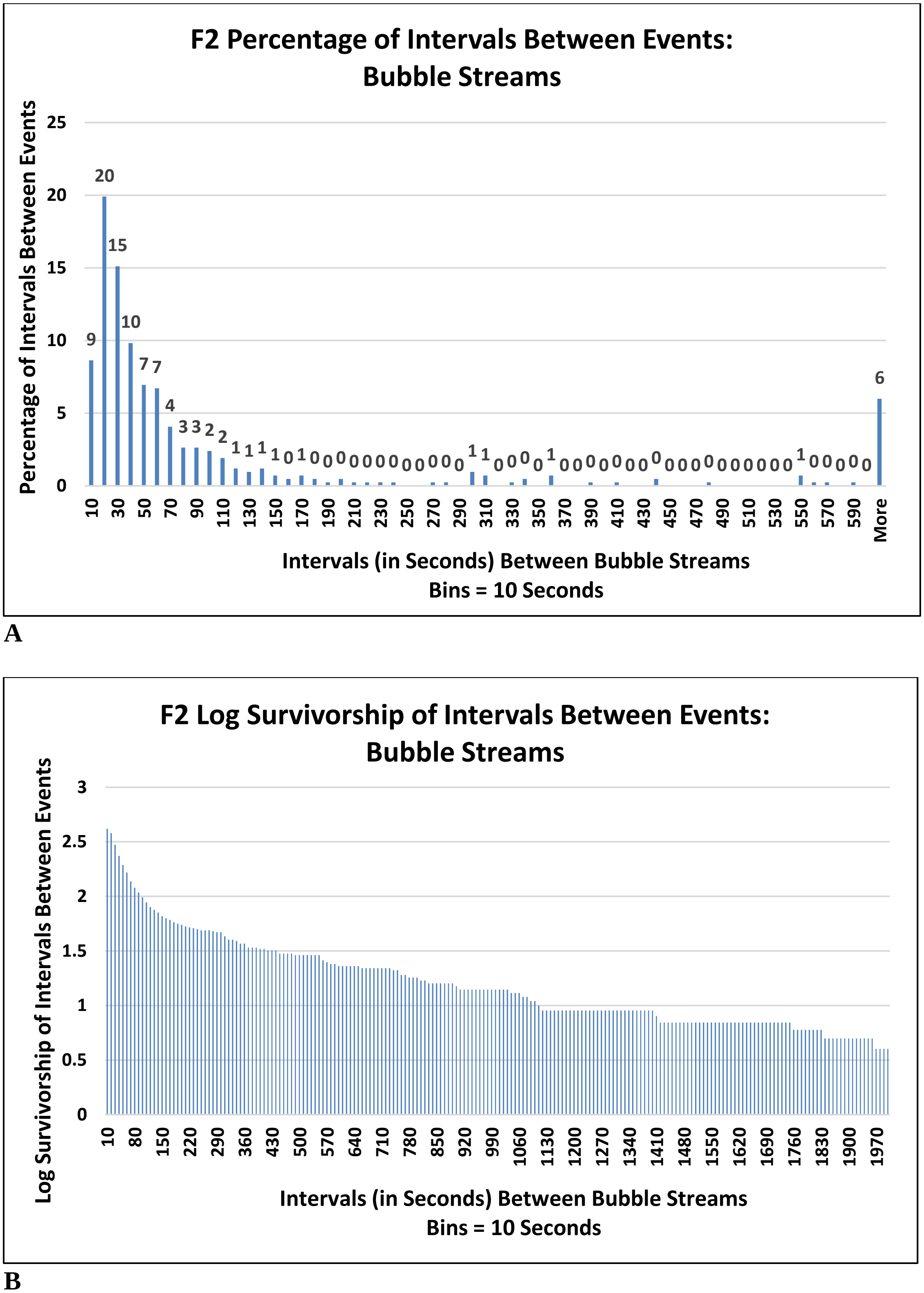


Figure 19: Percentage of intervals between events (A) and log survivorship of intervals between events (B) for F2’s bubble streams.

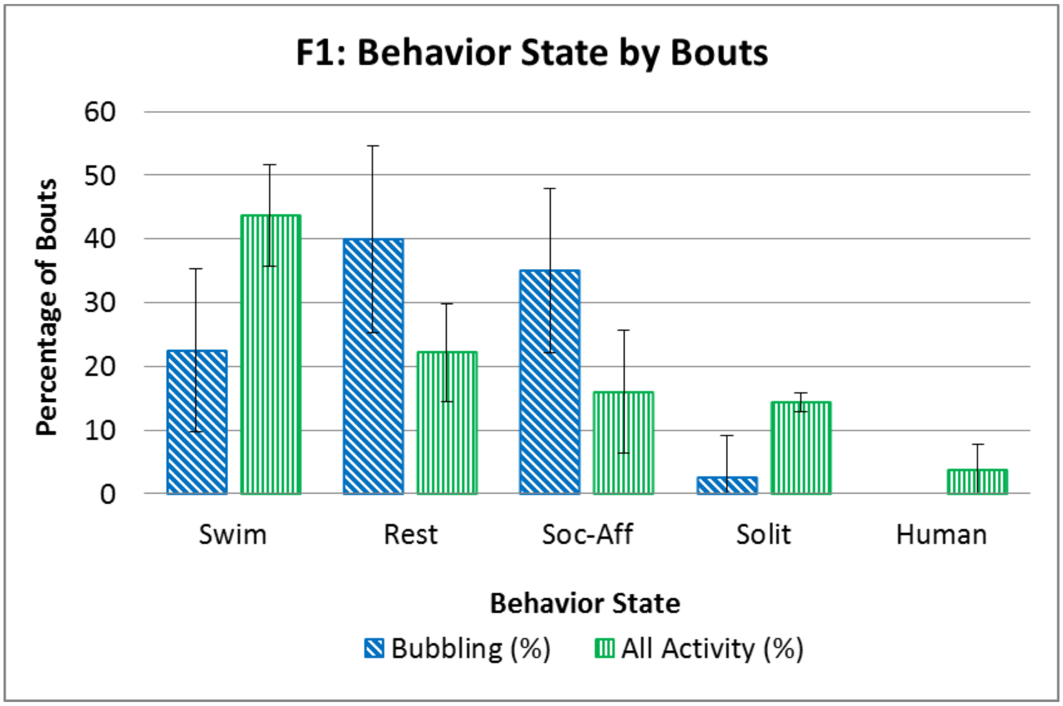


Figure 20: Percentage of bubbling bouts (blue crosshatched bars) compared to overall activity (green lined bars) with varying behavioral state. Error bars denote 95% confidence intervals.

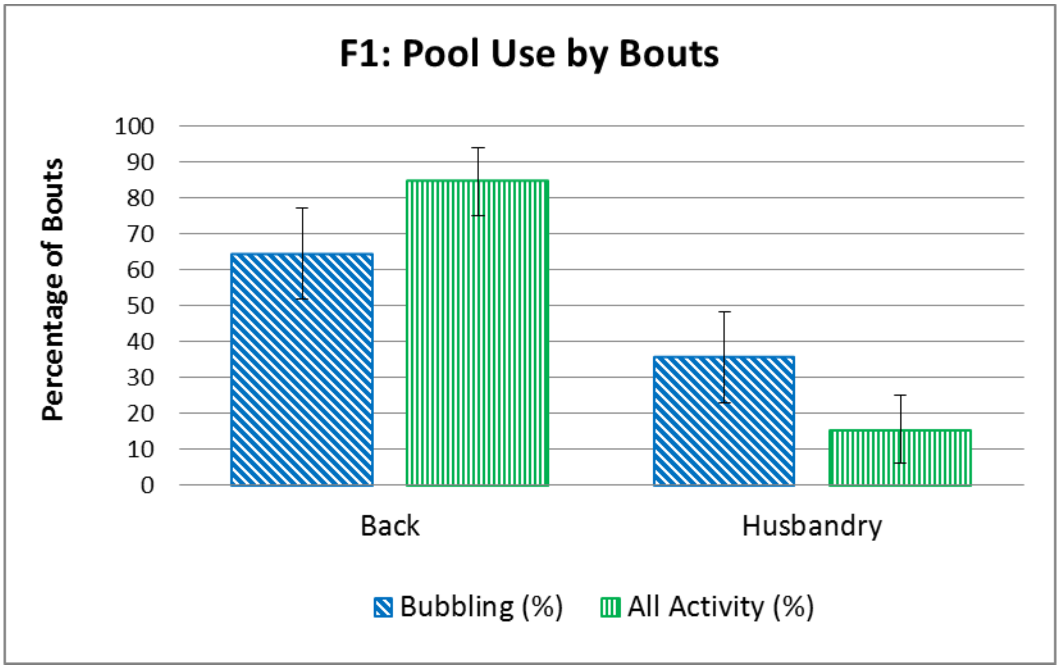


Figure 21: Percentage of bubbling bouts (blue crosshatched bars) compared to overall activity (green lined bars) when in either pool. Error bars denote 95% confidence intervals.

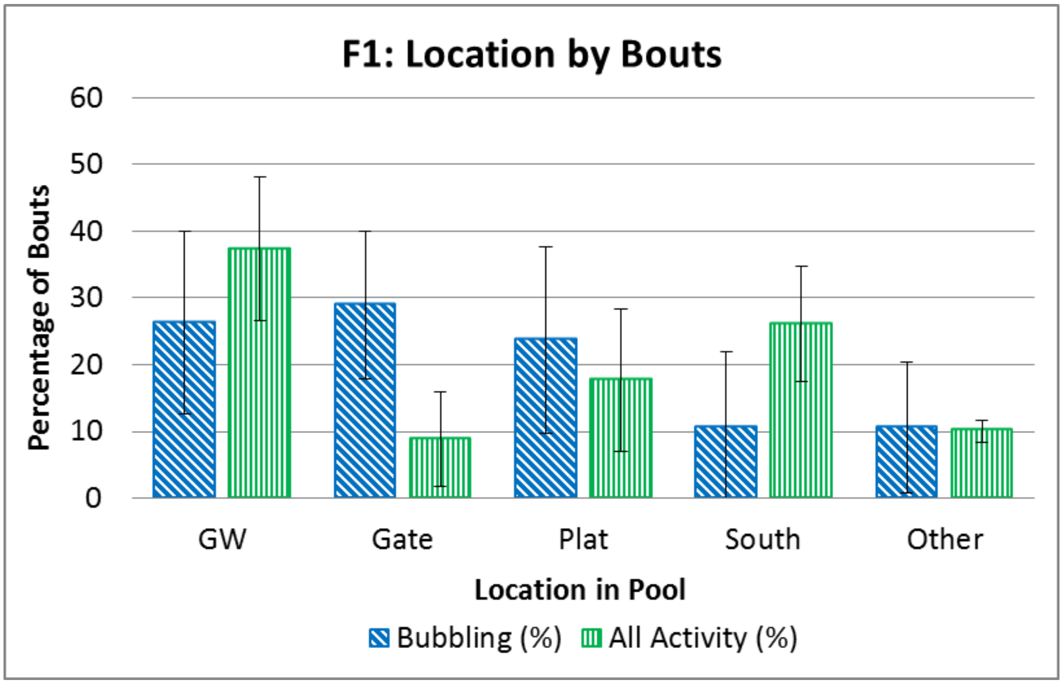


Figure 22: Percentage of bubbling bouts (blue crosshatched bars) compared to overall activity (green lined bars) when in various locations in the pools. Error bars denote 95% confidence intervals.

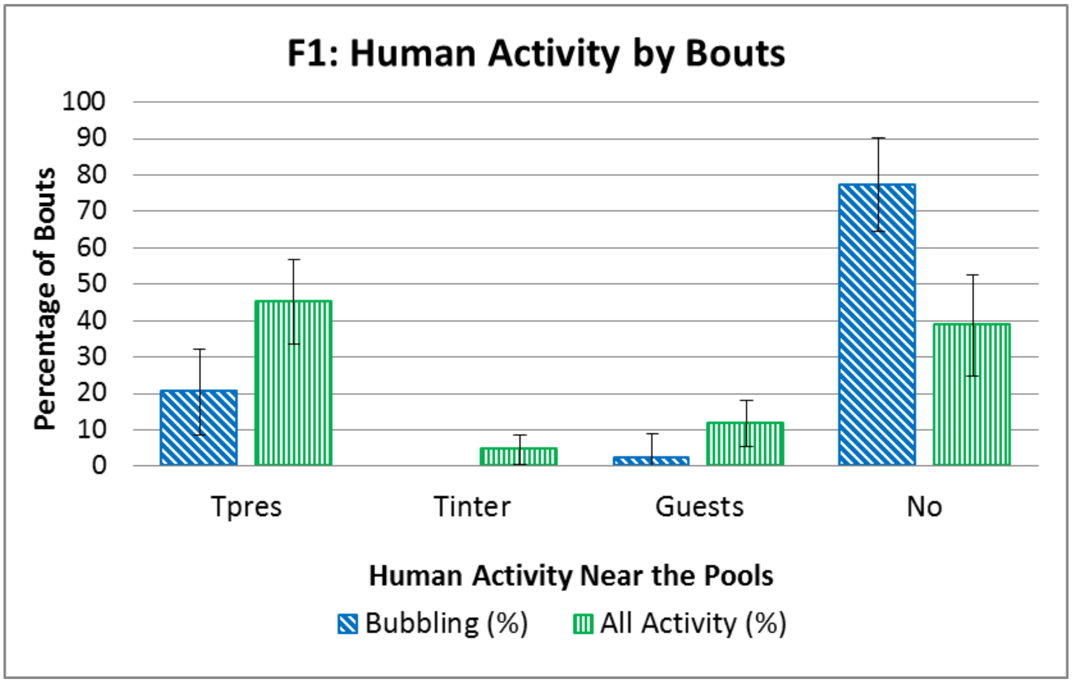


Figure 23: Percentage of bubbling bouts (blue crosshatched bars) compared to overall activity (green lined bars) with varying human activity occurring in the exhibit. Error bars denote 95% confidence intervals.

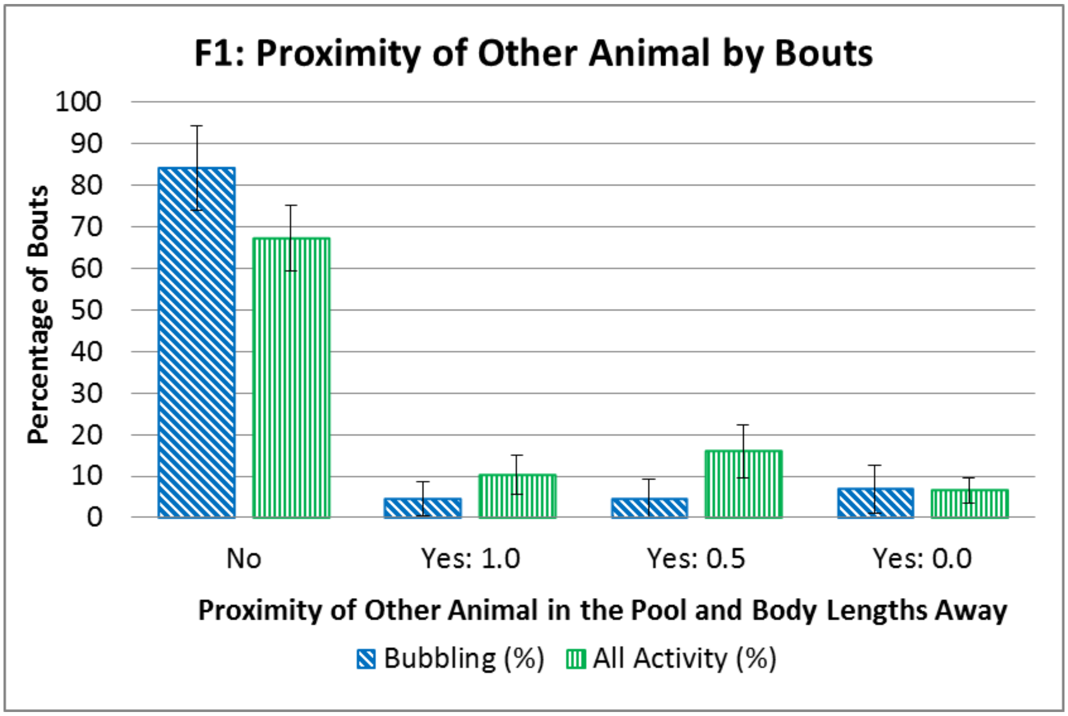


Figure 24: Percentage of bubbling bouts (blue crosshatched bars) compared to overall activity (green lined bars) when in varying proximity to the other animal in the pool. Error bars denote 95% confidence intervals.

0.001), and proximity of the other animal ($\chi^2 = 15.576$, df = 3, p = 0.001) were all significantly different from expectation for F1 when comparing bubbling bouts to overall activity (see Table 8). Though 95% confidence intervals were larger due to lower sample size (bouts instead of events), the same general patterns were found and the CI's indicated similar distinctions. F1 bubbled significantly more than expected while in a resting or social-affiliative behavioral state, when in the husbandry pool, at the gate, when no humans were present, and when not in close proximity to F2. F1 bubbled significantly less than expected while swimming and solitary, when in the back pool, at the gateway and south wall, with trainers or guests present, and when 0.5 body length away from F2.

Table 8: Summary of results for each factor studied comparing bubbling bouts to overall activity for F1. P-values showing the significance of the difference between bubbling bouts and overall activity are given below each table. Asterisks next to bubbling percentages indicate that that individual factor contributed to the significant difference found in the analysis. Numbers in parentheses are standardized residuals. The number of bouts eliminated due to no majority behavioral state are shown below each table.
* = p < 0.05, ** = p < 0.01, *** = p < 0.001

F1

Behavioral State	**Bubbling (%)**	**All Activity (%)**	**Bubbling Bouts (Freq)**	**All Activity (Minutes)**
Swim	22.50*** (-4.27)	43.61	9	278
Rest	40.00*** (4.28)	22.16	16	141
Soc-Affil	35.00*** (5.17)	15.99	14	102
Solit	2.50*** (-3.39)	14.38	1	92
Human	0.00 (-1.95)	3.65	0	23

p < 0.001 **No Majority:** 5

Pool Use	**Bubbling (%)**	**All Activity (%)**	**Bubbling Bouts (Freq)**	**All Activity (Minutes)**
Back	64.44*** (-5.54)	84.50	29	538
Husbandry	35.56*** (5.54)	15.50	16	99

p < 0.001 **No Majority:** 0

Location	**Bubbling (%)**	**All Activity (%)**	**Bubbling Bouts (Freq)**	**All Activity (Minutes)**
GW	26.32* (-2.29)	37.38	10	238
Gate	28.95*** (7.10)	8.82	11	56
Plat	23.68 (1.59)	17.62	9	112
South	10.53*** (-3.55)	26.13	4	166
Other	10.53 (0.16)	10.05	4	64

p < 0.001 **No Majority:** 7

Human Activity	**Bubbling (%)**	**All Activity (%)**	**Bubbling Bouts (Freq)**	**All Activity (Minutes)**
Tpres	20.45*** (-4.95)	45.10	9	287
Tinter	0.00* (-2.18)	4.55	0	29
Guests	2.27** (-2.92)	11.62	1	74
No	77.27*** (7.91)	38.72	34	247

p < 0.001 **No Majority:** 1

Proximity of Other Animal	**Bubbling (%)**	**All Activity (%)**	**Bubbling Bouts (Freq)**	**All Activity (Minutes)**
No	84.09*** (3.58)	67.28	37	429
Yes: 1.0	4.55 (-1.89)	10.30	2	41
Yes: 0.5	4.55** (-3.11)	15.95	2	102
Yes: 0.0	6.82 (0.14)	6.48	3	66

p = 0.001 **No Majority:** 1

2.4 Discussion

Little is known about the context of bubble streams in odontocete cetaceans generally. We know that bubble streams are not an unavoidable byproduct of vocalization (e.g., Pryor 1990, Bowles et al. 2015) and are therefore almost certainly produced to communicate. Pryor (1990) interpreted them as a sign of highly-active social interactions, while Bowles et al. (2015, 2016) associated them with positive (social-affiliative) and highly active states. This is the first study of belugas to look specifically at bubble stream production to probe the possible function(s) and context(s).

I found significant differences in bubbling events for several of the factors studied: the animal's behavioral state, which pool they were in, their specific location in the pools, human activity, and their proximity to the other animal. These results supported the hypothesis that bubble stream production occurs more often in certain contexts and provides added evidence that bubbling may aid in communicating at a distance and getting attention.

Bubbling most often accompanied social-affiliative behaviors for both animals. Social-affiliative behaviors occur when whales are interacting in a positive way, as opposed to social-agonistic behaviors, which are negative, ranging from defensive submission to aggression. The social-agonistic behavioral state was removed from analysis for both animals because it was so rare. The finding is consistent with a much larger dataset collected by Graham and Noonan (2010) for the killer whale under controlled conditions. Overtly agonistic interactions almost never occurred for either whale, and bubble streams were never produced in this state.

Behaviors in the social-affiliative category (Table 3) included affiliative contact with another animal (e.g., touching pectoral fins), interacting at the main gate through which animals in another pool were visible, and rolling with frequent contact with the other whale (the state called ‘milling’ or ‘socializing’ in free-ranging cetaceans; see Section 1.1). In the dataset presented here, the social-affiliative state was made up almost entirely of interactions at the main gate with animals in the adjacent exhibit pool.

Blomqvist et al. (2005) showed the existence of a “play-fighting signal”, a specific vocalization used by bottlenose dolphins to distinguish play-fighting from true aggression. Studies of horses (McDonnell and Poulin 2002), canids (Feddersen-Petersen 1991), primates (Clemente and Lindsley 1965) and rats (Pellis and Pellis 1987) have found similar evidence for meta-signals indicating play-fighting. McDonnell and Poulin (2002) describe it as “similar to serious adult fighting behavior, but with more of a sporting character than serious fighting,” and note that “the cohorts appear to alternate offensive and defensive roles, spar on as if to ‘keep the game going’, and stop short of injury.” The data on rats provide strong evidence that these interactions are positive and reinforcing (Panksepp 2003). It can be difficult to operationalize the subtle differences distinguishing the two types of aggression, but exaggerated movements or vocalizations are often involved (Feddersen-Petersen 1991). While play-fighting has not been studied much in cetaceans, their highly social nature lends support to the idea that they would participate in play-fighting as well. The study by Blomqvist et al. (2005) certainly suggests that bottlenose dolphins perform play-fighting, and that they use a certain vocalization as a meta-signal to differentiate it from true aggression. Similar to that vocalization, it is possible that bubble stream production could be a meta-signal for belugas and other cetaceans to clarify the context in which a behavior occurs or somehow alters its meaning. This seems likely, since they often produce the same behaviors and vocalizations both with and without bubble streams.

F2, the calf, was also more likely than expected to be playing or interacting with humans when bubbling. These results could be one line of evidence that bubble stream production occurs during social interactions (with humans in this case) or during behaviors with positive affect. However, bubble streams could also be interpreted as a behavior associated with high states of positive arousal generally. This has been suggested in multiple previous studies on cetaceans, which found bubbles to be associated with higher vocal activity (Dudzinski 1996), “excitement vocalizations” (Herzing 1996), large groups (Pryor 1990), and close-range high-activity states in general (Bowles et al. 1988, 2015).

It is possible that F2 bubbled in a greater number of contexts than F1 because she was young and had limited experience with social interactions. F1 might have learned to be more selective about the contexts in which she bubbled over time, whereas F2 had not learned the “correct” contexts for bubbling yet. Or, bubbling might be an age-specific behavior. F1 never exhibited play behaviors, which might be relatively rare in older belugas (Paulos et al. 2010, Hill et al.

2015). The study was short enough that uncommon behaviors might not have been detected. Also, since she had a great deal of experience with training sessions and was asked to perform behaviors during training sessions (unlike the calf), she may simply have been focused on performing when interacting with the trainers, as opposed to treating the interactions as a purely social encounter. It is also possible that F2 had transferred the use of this behavior from communicating with conspecifics to communicating with humans, who had been part of her social environment since birth, unlike the case for F1. F2 was born in the study facility and was under 1 year old at the time of the study, while F1 was born in the wild and rescued as a young animal. Based on this hypothesis, F2 might try to communicate with or get the attention of trainers by bubble streaming as she would with other belugas.

A study by Akiyama and Ohta (2006) on three captive bottlenose dolphins lends some support to the last hypothesis. They found that their subjects whistled more when people were present, used a greater variety of whistles when more people were in the water with them, and whistled more frequently and longer when interacting with a new person. These findings are consistent with the suggestion that F2 used more bubble streams when people were around as a form of communication. F1, as a 30-year old wild-born animal, might have seen the trainers as different from the start or learned over time that humans do not respond to bubble streams as conspecifics do, reducing her use of this behavior with humans over time.

If the hypothesis about attention-getting is to be supported, then F1's bubble streams at greater than expected rates when resting, which would appear to be a completely different context, must be explained. For her, bubbling in this context seemed to be part of a habitual behavior performed while resting (see section 2.3 under Behavioral State). This behavior consisted of floating slowly toward the platform wall in the back pool, bumping slowly into the wall with her melon, and pushing back off the wall gently while releasing a bubble stream. Her slow, fixed movement pattern and lack of interaction with F2 during this behavior suggest she was resting or sleeping at the time. It is possible for cetaceans to sleep and perform familiar tasks at the same time for long periods (e.g., Ridgway 2009, Branstetter et al. 2012). I have seen a bottlenose dolphin in another aquarium performing a similar string of behaviors while sleeping: floating vertically underwater, slowly bobbing to the surface, spitting water from its mouth, and sinking back down underwater. The animal repeated this succession of movements several times per minute while resting, and trainers in the exhibit corroborated that this was a "relaxing" habit that she performed often when sleeping. The behavior by F1 in this study appeared similar, and it is plausible that she incorporated bubble streams into this routine. The behavior did not cause physical damage, nor was it associated with negative social interactions. Since cetaceans sleep with one hemisphere of their brain asleep and the other hemisphere still active (e.g., Ridgway 2002, Lyamin et al. 2008, Branstetter et al. 2012), movements with this complexity are certainly possible while the animal is sleeping. Although it is unclear why bubbling became integrated into this habitual behavior sequence, it certainly led to over-representation of bubbling in the resting state. Thus, although they did not form a bout within the bout analysis, the bubbling events in this context were probably not independent and did not have the same function as in other contexts.

A communicative, attention-getting use of bubble streams is also supported by the fact both whales bubbled more often than expected in the husbandry pool and, specifically, most at the gate between the main exhibit pool (outside the study area) and the husbandry pool. This gate consisted of mesh netting across the opening between the pools, allowing for visual and vocal, but not physical, interaction between the study animals and the adult male and female who were not part of the study. If bubble streams are a visual cue for getting another animal’s attention or communicating some kind of meta-information associated with vocalizations, it would make sense to bubble most at the gate providing visual and vocal access to animals in the exhibit pool.

Both animals bubbled more than expected when they were more than 1 body length from each other. This suggests that bubbling is designed to get attention and possibly to indicate a high state of social arousal when whales are not in close proximity, but still within each other’s visual field. This finding may seem counterintuitive as bubbling occurred more during social-affiliative interactions that, in theory, could involve the animals being close to each other. However, at less than 1 body length, the animals might not be able to see each other, and the visual bubble stream might be missed (Mass and Supin 2001). Head-to-head postures of the two animals would allow for close proximity as well as bubble stream perception, but this kind of body orientation is usually thought to be agonistic, at least in bottlenose dolphins (Blomqvist and Amundin 2004). Since the whales were usually in close proximity to each other (closer than 1 body length) when they were swimming or resting, communicating with bubble streams may not have been effective. However, when they were far enough from each other to be out of direct contact (more than 1 body length away) but still close enough to see each other, the use of a bubble stream to get the attention of the other animal would seem fitting. Unlike F1, F2 also bubbled more than expected when 1 body length away, suggesting that she might be bubbling at closer range to ensure she stayed in contact with her allomother F1. Over time, we might expect that F2 would narrow the contexts in which she produced bubble streams to fit that of the adult beluga.

Proximity scores in this study did not take into account the proximity of the animals in the main exhibit pool, so it is also possible that, though F2 and F1 were not in close proximity to each other while bubbling, non-study animals could have been close on the other side of the gate. In this case, one of those animals might have been the object of the bubbling behavior.

The finding that bubbling events were organized in bouts for F1 raises the question of whether bouting is developed over time. This could explain why the calf did not show structure in the pattern of bubbling. This explanation may be consistent with the four-fold greater count of bubbling events emitted by F2 compared to F1. However, both whales vocalized with nearly the same proportion of bubble streams (~20%), and F1 only produced two bubble streams on average in bouts, a small additional level of structure relative to F2. Thus, differences in bubbling behavior by the young whale appeared to be a matter of quantity and context rather than structure. Longitudinal studies could probe this question further with a greater sample of whales, particularly exploring whether most adult belugas bubble in bouts and whether bout length is similar My small sample size made it difficult to tease out the prevalence and importance of bubbling occurring in bouts, and whether there was anything unusual about the few long bouts. However, based on the data, I believe that bouts, if present, were short and did not change my conclusions about associations between bubbled calls and the factors studied (behavioral state, location, etc.).

2.5 Summary

Overall, this study showed that there was a significant difference in the contexts in which two belugas, a calf and an adult, used bubble streams. Bubble streams were emitted more often than expected during affiliative social interactions when animals were separated in some way (distance, across a gate, across the air-water interface). Bubble streams occurred most often at a gate separating the two study animals from two adult belugas, showing that the behavior was predominantly used with conspecifics, although the calf also bubbled during interactions with human caretakers. Bubbling was most likely to be a visual cue to get the attention of another animal separated from the bubbling individual and possibly modifying the signal value of an accompanying behavior or vocalization. The data in this and the limited number of other studies are consistent with bubble streams as a signal recruiting attention and indicating excited and positive social state. Future research on bubble streams should attempt to utilize larger, more diverse populations, long-term data sets, and wild cetaceans to narrow the specific contexts and uses for bubble stream production.

CHAPTER 3: Bubble Formations Synchronized with Vocalizations in the Beluga: Behavioral and Social Context

3.1 Introduction

As described in Chapter 1, belugas are very social and vocal animals. Based on the available literature, they use three main types of vocalizations in social communication: whistles, burst-pulsed calls, and combined calls in social interactions (Karlsen et al. 2002, Vergara and Barrett-Lennard 2008). Whistles are narrow-band calls with frequency modulation and typically obvious harmonics of a "fundamental" or carrier frequency. Burst-pulse calls are broadband signals with little or no tonal quality. Combined calls contain components with properties of both call types, produced simultaneously, with some degree of overlap, or in sequence.

Although it has been used as an indicator of vocal activity in a number of studies, little is known about the production and social function of the bubble formation most often associated with vocalizations, the bubble stream. It is clear that these streams are not an uncontrollable, mechanical aspect of vocalization, as several studies report that odontocetes produce vocalizations with bubble streams in only a small percentage of cases [Paine 1995, belugas (*Delphinapterus leucas*); Herzing 1996, Atlantic spotted dolphins (*Stenella frontalis*) and bottlenose dolphins (*Tursiops truncatus*); Mann et al. 2000, review on cetaceans; Bowles et al. 2015, killer whales (*Orcinus orca*)]. However, many researchers have reported that bubble streams are associated with vocalization (e.g., McCowan and Reiss 1995, Dudzinski 1996, Vergara and Barrett-Lennard 2008). There has been little effort to study synchronous bubbling with vocalizations systematically, and its function is not understood. This study was designed to determine the proportion of vocalizations associated with bubble formations in the beluga, as well as to classify the pattern of use of specific vocalization types and categories with bubble streams.

3.2 Methods

Study site

Data for this study were collected at SeaWorld San Diego in September of 2010. The animals present were F1, an adult female, and F2, a female calf. F1 was 27 years old and F2 was about 4 months old during the time of the study. Two other belugas, an adult female and an adult male, were in the main exhibit pool separate from the study animals. Recording equipment was not present in the main exhibit pool, so the two belugas in that pool were not involved in data collection for this project. The behavior of the two study animals, F1 and F2, was captured through video cameras angled into the pools, and their vocalizations were captured through a hydrophone array placed in the pools.

In addition to capturing the animals' vocalizations, the audio recordings also included observer commentary from research assistants who were stationed poolside during recording periods. One research assistant was assigned to each pool at a given time, meaning that the assistant covering the husbandry pool was responsible for providing commentary for all animals in that pool at the time, whether that meant observing one animal, both animals, or neither animal depending on their locations in the pools. Observer commentary included information about behavior, any audible vocalizations, observed bubble streams, and human activities near the pools (e.g. trainers or guests present, animals being fed, etc.). Research assistants were present poolside, providing observations during all recording periods using an ethogram of behaviors and pool locations to describe their activities. Research assistants had been trained to identify each behavior being studied, including bubble streams, and were instructed to give as much detail as possible about what the animals were doing and where they were located at all times.

Recording Configuration:

Vocalizations were captured using an ITC 6050C hydrophone (Channel Technologies, Santa Barbara, CA) connected to a 4-channel Edirol R44 digital audio recorder (Roland Corporation, Los Angeles, CA). The system frequency range was 50 Hz to 48 kHz (sampling at 96 kHz, 12-bit resolution). The hydrophone was placed with its acoustic center at 1.25 m depth. It was lowered into a section of PVC pipe open to the water at the bottom and perforated by 3 cm holes drilled at intervals in the sides. The pipe was anchored to the side of the pool on an aluminum frame. This configuration protected the hydrophone from the whales while minimizing interference with vocalizations other than high frequency clicks.

Behavior data were collected using three CCD cameras connected to a Panasonic DMR-E85H Digital Video Recorder (Panasonic Corporation US, Chesapeake, VA) through a Robot 4-channel multiplexer (Sensormatics Video

Products, San Diego, CA). The three cameras were placed to provide as much coverage of the two study pools as possible (>90%). This arrangement provided a triplexed video stream with a view across the husbandry pool looking south, a view across the back pool toward the gateway into the husbandry pool looking southwest, and a view from above looking down on the rub rope in the back pool (see Figure 3). The multiplexer provided a time code on the multiplexed image.

Data Management:

Since the audio and video data were collected on separate equipment, they were not automatically synchronized. Several measures were used to align the sequences of audio and video events with as little error as possible during processing. First, the data files were collected with time information. There was a time stamp on the video images, and the file names of the audio recordings were automatically generated with the date and time they were recorded, both to the nearest second. Because the two types of time stamps were not always perfectly synchronized, a satellite-linked clock that was visible to observers was used to add verbal “time stamps” to the observer tracks of the recordings at the same time that a hand was swiped across the video recording. This provided an independent check on the synchrony between audio and video time stamps. Time synchronization was good throughout the recordings (within 2 sec). When times disagreed between data sources the verbal “time stamps” given by the observers were accepted as the correct reference.

Behavioral events could also be used to synchronize events. However, this source of information was used only to identify possible problems with synchronization by other methods. Once video and audio sources were aligned correctly, they were combined in Adobe Premiere Elements (Adobe Systems Incorporated, San Jose, CA) and scored. Bubble formations were not reliably observed from the overhead camera view, so the reports of onsite observers were used to detect these events.

Data Analysis:

Behavior during Bubbling:

Videos with synchronized audio from September were analyzed for all bubbling events. These events were recorded individually for each animal along with the time of the event, type of bubbling, concurrent behaviors, location of the animal, any human activity occurring near the pools, and the proximity of the focal animal to the other animal in the pools. After all bubbling events were identified and the concurrent behavioral state was noted, the events were summed to get the total number of bubble streams produced in each behavioral state during the study period. For more information about analyzing behavior data, see Data Analysis section of Chapter 2.

Comparisons were made using a chi-squared goodness-of-fit test (see Data Analysis section of Chapter 2 for more details). Data analysis was conducted in the R programming environment (Version 3.0.3, R Studio package, www.r-project.org/, R Core Team 2014). Once calculated, the chi-squared test would show whether it was likely that there were significant differences in rates of bubbling events among the categories included in the test (i.e., behavioral states). However, the result would not indicate which states contributed most to the difference. For this, I calculated the standardized residuals for each test to show the degree to which each cell in the test was contributing to significance (see Data Analysis section of Chapter 2 for more details).

Vocalizations during Bubbling:

The focus of this portion of the study was the use of vocalizations in regard to bubble streams. To study this aspect, several steps were taken. The audio files were processed with the detection algorithm in Raven Pro 1.5 to find beluga vocalizations. An iterative process was used to develop an efficient detector. Characteristics included a minimum frequency of 2 kHz, a maximum frequency of 48 kHz, minimum duration of 0.1 s, maximum duration of 5 s, minimum separation of 0.05 s, signal-to-noise ratio (SNR) threshold above 7 dB (meaning the signal was at least 7 dB above the background noise), and a minimum occupancy of 50% (meaning at least 50% of the samples within a selection exceeded the background noise by the SNR threshold). Other parameters were set to Raven's default values, including a block size of 2 sec (the width of the block used to calculate the background noise level), a hop size of 0.5 sec (meaning the successive window overlapped the preceding window by 25%), and a noise power estimation exceeding the 20th percentile. These attributes produced the highest proportion of correct detections (picking out a signal that was truly a vocalization) as opposed to other sounds (such as pool noise) or missed detections.

Once vocalizations were detected, two scorers reviewed them to remove any incorrectly detected sounds and to select any missed vocalizations that could be found while quickly scrolling through the files. All vocalizations were placed into categories I developed based on their aural properties and spectrograms, similarly to previous studies on beluga vocalizations (e.g., Sjare and Smith 1986, Recchia 1994, Karlsen et al. 2002, Belikov and Bel'kovich 2006, Chmelnitsky and Ferguson 2012). I identified 10 categories (Figure 25).

The benefits of categorization of vocalization by "hand" (by human eye and ear) as opposed to automated classification by computer are debated (e.g., Angiel 1997, Janik 1999, Chmelnitsky and Ferguson 2012, Shamir et al. 2014). To ensure that the categories were not arbitrary, an impartial judge who was not familiar with the vocalizations or categories scored a sample of the calls. The judge was presented randomly with 2 examples of vocalizations from each category (20 vocalizations total) and taught by the experimenter how to categorize them. After learning the categories, the judge was presented randomly with 6 new examples of vocalizations from each category (60 vocalizations total) and asked to categorize them without help from the experimenter. The judge correctly categorized 48 of the 60 test vocalizations (80%). Two vocalizations, C and E, were most often categorized incorrectly (only 42% correct between the two), possibly because the call types were graded signals. When those were removed, the judge correctly categorized 43 of 48 vocalizations (90%).

After all vocalizations were categorized, they were cross-referenced with bubble stream events. Any vocalization that occurred within 1 sec of a bubble stream reported by an observer and not accompanied by an overlapping vocalization was considered to be co-occurring with the bubble stream. The number of bubble streams accompanied by vocalizations and the number of bubble streams produced overall were calculated by variable and factor (e.g., behavioral state). These totals were turned into percentages by dividing the number of bubble streams accompanied by vocalizations for the given variable factor by the total number of bubble streams accompanied by vocalizations. To determine the proportion of bubble streams with and without vocalizations, counts by factor were divided by the total number of bubble streams with or without vocalizations for the entire study. These percentages were then compared using a chi-squared goodness-of-fit test to expected values from the animals’ overall activity budgets (see Methods section of Chapter 2 for further description).

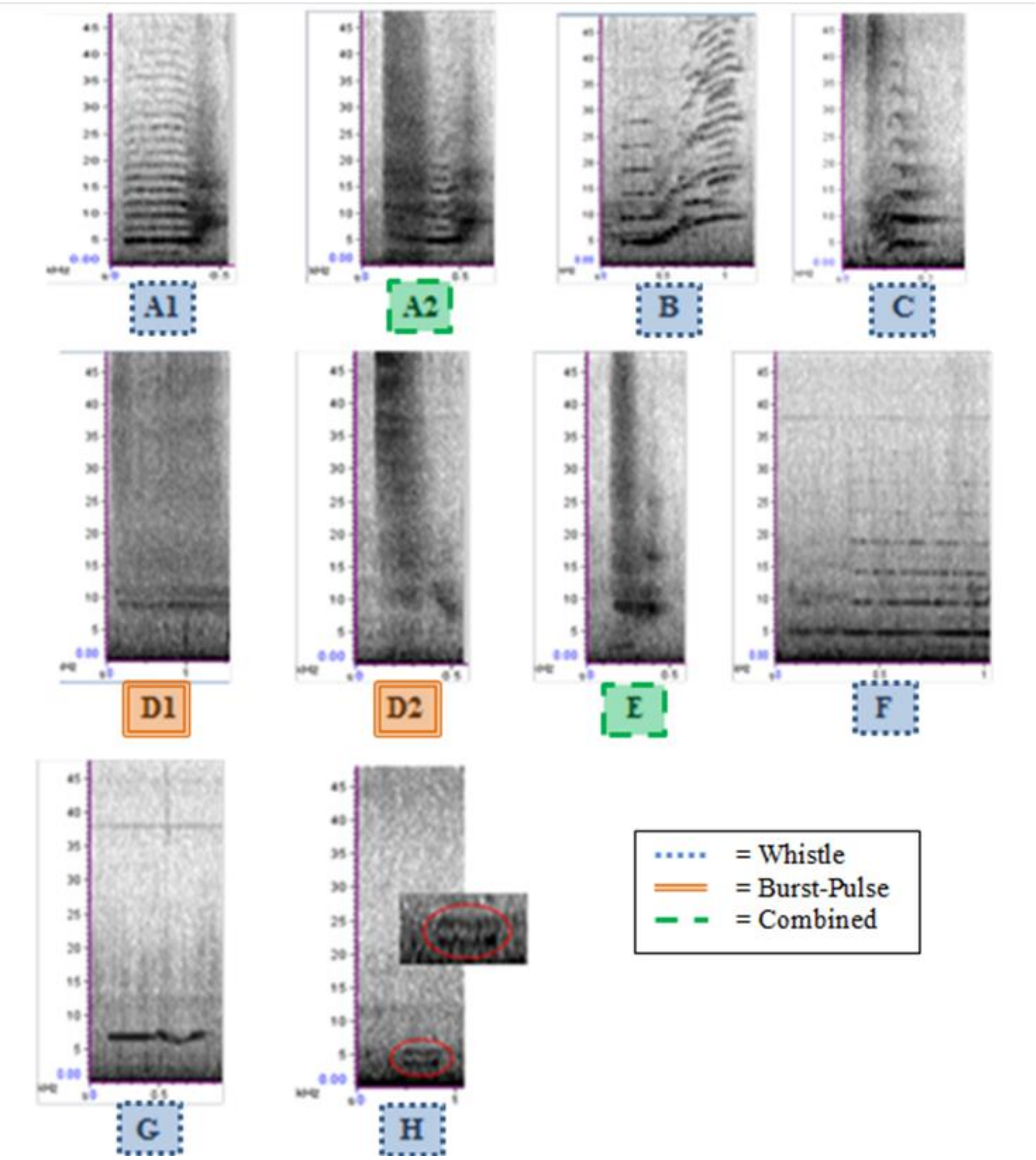


Figure 25: Spectrograms of each of the 10 types of vocalizations produced during the study. The y-axis shows frequency in kilohertz with time in seconds on the x-axis. C and E could not be reliably distinguished from each other, so they are pooled in the results section. The H vocalization only occurred when the calf was actively nursing or swimming under the allomother in the nursing position. This vocalization was low-frequency and quiet, so a magnified version is inlaid at the top-right of the original vocalization, and red ovals indicate the vocalization.

3.3 Results

Bubble Streams With and Without Vocalizations

A total of about 19 hours of recordings were made over 2 days in September 2010. Of the 98 bubble streams produced by F1 (the adult female) in this time, 18 (18.4%) were synchronized with a vocalization. Of the 419 bubble streams produced by F2 (the female calf) during this time, 86 (20.7%) were synchronized with a vocalization. Thus, although the calf was four times as likely to produce bubble streams, the proportion synchronized with a vocalization was similar for both whales.

For each categorical variable, a chi-squared goodness-of-fit test was performed to determine whether the respective percentages of each factor differed significantly when producing a bubbled call compared to producing a bubble stream in general (i.e., all bubble streams regardless, of whether a vocalization accompanied them). Data for the two animals were analyzed separately (Table 9 for F1, Table 10 for F2). The significant results for both animals are shown in Table 11. When an animal did not bubble in a given state, the state was eliminated from analysis. For example, since F1 did not bubble when interacting with trainers, this category was eliminated from her dataset, but not for F2, who did bubble with trainers.

Table 9: Summary of results for each factor studied when producing bubbled calls compared to overall bubbling for F1. P-values showing the significance of the difference between bubbled calls and overall bubbling are given below each table. Asterisks next to bubbled call percentages indicate that that individual factor contributed to the significant difference found in the chi-squared analysis. Factors that were removed from analysis due to low occurrence are shaded in gray and preceded by "x.". Numbers in parentheses are standardized residuals. * = p < 0.05, ** = p < 0.01, *** = p < 0.001

F1:

Behavioral State	**Bubbled Calls (%)**	**All Bubbles (%)**	**Bubbled Calls (Freq)**	**All Bubbles (Freq)**
Swim	38.89*** (4.39)	21.43	7	21
Rest	55.56*** (4.55)	33.67	10	33
Soc-Affil	0.00*** (-8.51)	41.84	0	41
Solitary	5.56* (2.54)	2.04	1	2
Human	0.00 (-1.01)	1.02	0	1
xPlay	0.00	0.00	0	0
xSoc-Agon	0.00	0.00	0	0

p < 0.001

Pool	**Bubbled Calls (%)**	**All Bubbles (%)**	**Bubbled Calls (Freq)**	**All Bubbles (Freq)**
Back	88.89*** (6.44)	57.14	16	56
Husbandry	11.11*** (-6.44)	42.86	2	42

p < 0.001

Location	**Bubbled Calls (%)**	**All Bubbles (%)**	**Bubbled Calls (Freq)**	**All Bubbles (Freq)**
GW	0.00*** (-3.89)	13.27	0	13
Gate	5.56*** (-6.75)	37.76	1	37
Plat	44.44*** (5.34)	22.45	8	22
South	33.33*** (4.28)	17.35	6	17
Other	11.11 (1.58)	7.14	2	7
Rope	5.56* (2.52)	2.04	1	2

p < 0.001

Human Activity	**Bubbled Calls (%)**	**All Bubbles (%)**	**Bubbled Calls (Freq)**	**All Bubbles (Freq)**
Tpres	11.11 (0.04)	11.22	2	11
Guests	11.11 (2.15)	6.12	2	6
No	77.78 (-1.39)	82.65	14	81
xTinter	0.00	0.00	0	0

p = 0.096

Proximity	**Bubbled Calls (%)**	**All Bubbles (%)**	**Bubbled Calls (Freq)**	**All Bubbles (Freq)**
No	94.44 (1.48)	89.80	17	88
Yes: 0.0	5.56 (0.80)	4.08	1	4
Yes: 0.5	0.00 (-1.76)	3.06	0	3
Yes: 1.0	0.00 (-1.76)	3.06	0	3

p = 0.08

Table 10: Summary of results for each factor studied when producing bubbled calls compared to overall bubbling for F2. P-values showing the significance of the difference between bubbled calls and overall bubbling are given below each table. Asterisks next to bubbled call percentages indicate that that individual factor contributed to the significant difference found in the chi-squared analysis. Factors that were removed from analysis are shaded in gray and preceded by "x.". Numbers in parentheses are standardized residuals. * = p <0.05, ** = p <0.01, *** = p < 0.001

F2:

Behavioral State	Bubbled Calls (%)	All Bubbles (%)	Bubbled Calls (Freq)	All Bubbles (Freq)
Swim	25.58 (0.92)	21.75	22	91
Rest	16.28 (0.40)	15.38	14	64
Soc-Affil	43.02 (-1.50)	50.66	37	212
Solitary	3.49 (-1.04)	5.84	3	24
Human	8.14*** (4.42)	1.59	7	7
Play	3.49 (-0.67)	4.77	3	20
xSoc-Agon	0.00	0.00	0	0

p < 0.001

Pool	Bubbled Calls (%)	All Bubbles (%)	Bubbled Calls (Freq)	All Bubbles (Freq)
Back	6.98 (-0.71)	8.59	6	36
Husbandry	93.02 (0.71)	91.41	80	383

p = 0.48

Location	Bubbled Calls (%)	All Bubbles (%)	Bubbled Calls (Freq)	All Bubbles (Freq)
GW	12.79 (-0.88)	15.75	11	66
Gate	40.70 (-0.46)	42.96	35	180
Plat	1.16 (0.16)	1.43	1	6
South	3.49 (-0.69)	4.77	3	20
Other	41.86 (1.44)	34.61	36	145
xRope	0.00	0.48	0	2

p = 0.63

Human Activity	Bubbled Calls (%)	All Bubbles (%)	Bubbled Calls (Freq)	All Bubbles (Freq)
Tpres	38.37 (1.11)	33.41	33	140
Guests	2.33** (2.58)	0.48	2	2
No	51.16 (-0.62)	53.94	44	226
Tinter	8.14 (-1.20)	12.17	7	51

p = 0.03

Proximity	Bubbled Calls (%)	All Bubbles (%)	Bubbled Calls (Freq)	All Bubbles (Freq)
No	86.05 (1.73)	79.24	74	332
Yes: 0.0	3.49 (0.29)	3.10	3	13
Yes: 0.5	4.65 (-1.23)	8.11	4	34
Yes: 1.0	5.81 (-1.40)	9.55	5	40

p=0.28

Table 11: Summary of all significant results for F1 (top) and F2 (bottom) when comparing bubbled calls to bubbling events overall

F1

Variable:	Bubbled Calls More Likely:	Bubbled Calls Less Likely:
Behavioral State	Swim, Rest, Solitary	Social-Affiliative
Pool	Back pool	Husbandry pool
Location	Platform, Rope, South wall	Gate, Gateway
Human Activity	N/A	N/A
Proximity of Other Animal	N/A	N/A

F2

Variable:	Bubbled Calls More Likely:	Bubbled Calls Less Likely:
Behavioral State	Human	N/A
Pool	Guests present	N/A
Location	N/A	N/A
Human Activity	N/A	N/A
Proximity of Other Animal	N/A	N/A

Behavioral State:

F1 showed a significant difference in behavioral state when producing bubbled calls compared to expectations from the overall bubbling rate ($\chi^2 = 78.24$, df = 4, $p < 0.001$). These results are shown in Figure 26. She vocalized with bubble streams more than expected in the categories of “Swim” (stdres = 4.39, $p < 0.001$), “Rest” (stdres = 4.55, $p < 0.001$), and “Solitary” (stdres = 2.54, $p < 0.05$). Despite the greater number of bubble streams than expected in the “Social-Affiliative” state, she did not vocalize with bubble streams in this state (stdres = -8.51, $p < 0.001$). Since she did not bubble with social-agonistic behaviors, this state was removed (this was true for both whales).

Based on the chi-squared analysis, F2 also showed a significant difference in behavioral state when producing bubbled calls compared to producing bubbles in general ($\chi^2 = 22.51$, df = 5, $p < 0.001$). These results are also shown in Figure 26. However, in her case, the difference was explained by significantly more bubbled calls than expected in the category “Human” (stdres = 4.42, $p < 0.001$), i.e., when humans were engaged in an activity with her. The 95% CI analysis supported this result. Although the proportion of time spent interacting with humans was relatively small, 7 of the 48 bubble streams in this state were vocalized, and the 95% CI ranges were disjunct. As with F1, the category “Social-Agonistic” was removed, since F2 did not bubble in this behavioral state.

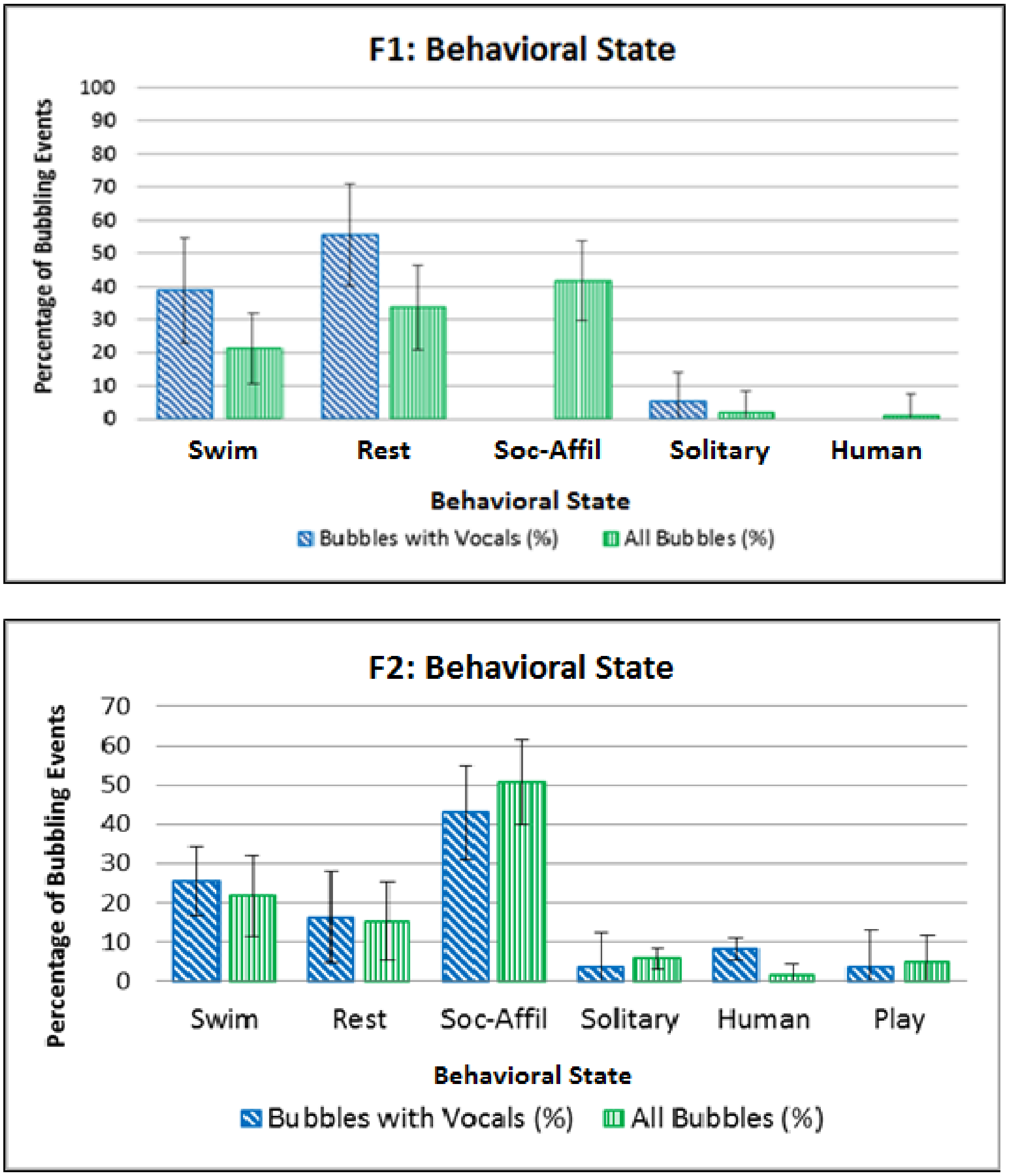


Figure 26: Percentage of time spent in each behavioral state when producing bubbled calls (blue crosshatched bars) compared to overall bubbling (green lined bars). F1's results are shown on the top graph and F2's results are shown on the bottom graph. Error bars denote 95% confidence intervals.

Pool:

For F1, there was a significant difference in pool use when producing bubbled calls compared to overall bubbling ($\chi^2 = 41.49$, df = 1, $p < 0.001$). F1 produced bubbled calls significantly more than expected in the back pool (stdres = 6.44, $p < 0.001$) compared to the husbandry pool (stdres = -6.44, $p < 0.001$). However, there was no significant difference in pool use when producing bubbled calls compared to overall bubbling for F2 ($\chi^2 = 0.498$, df = 1, $p = 0.48$). Results for both animals are shown in Figure 27. It is worth noting that the back pool was larger than the husbandry pool and was also the only pool in which F1 interacted with trainers

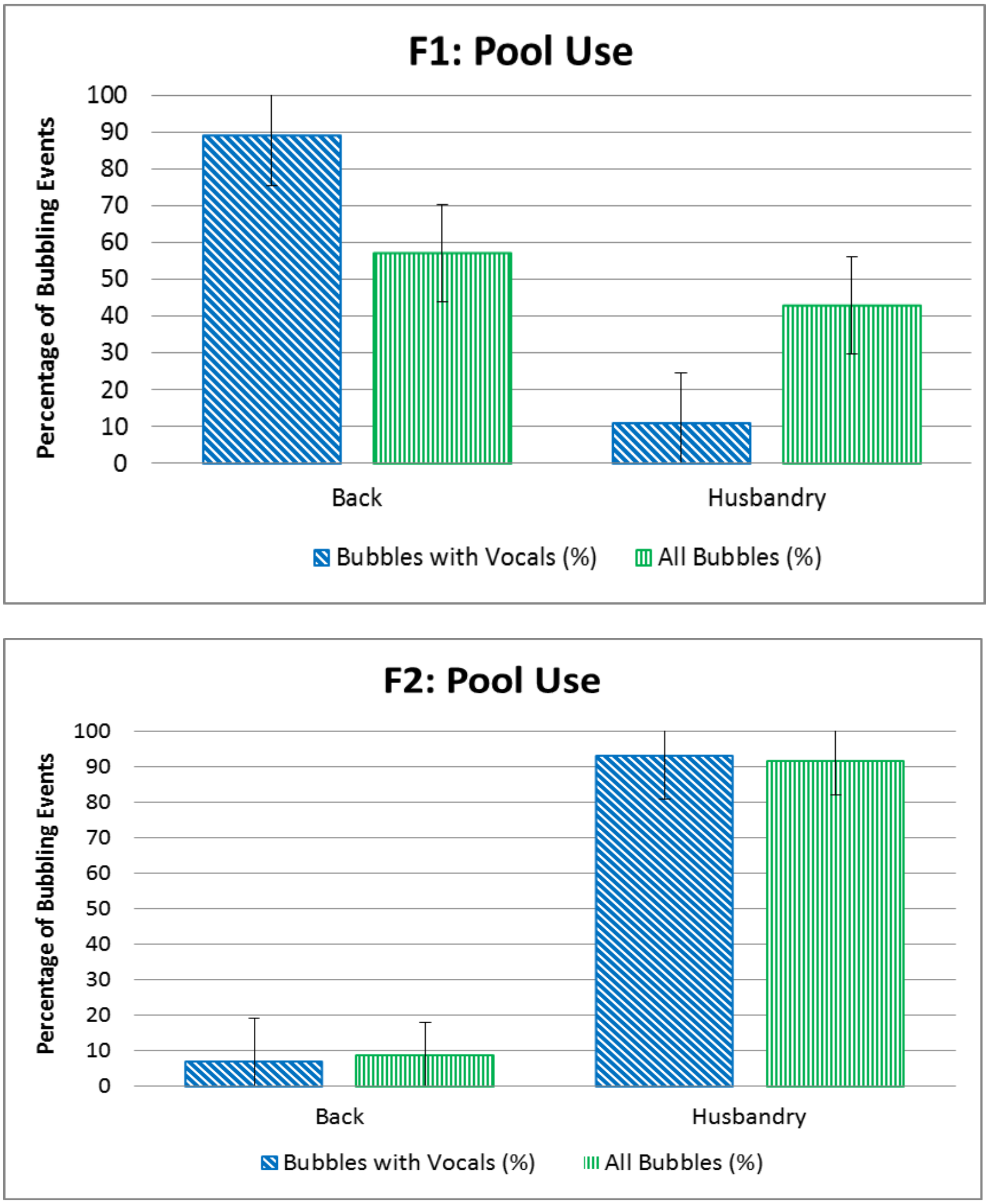
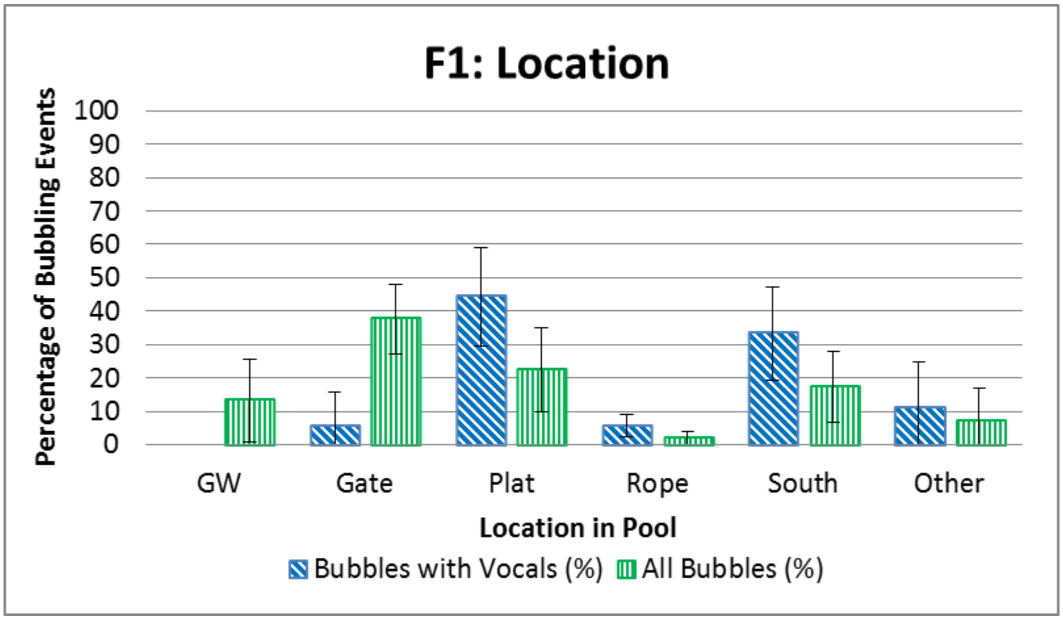


Figure 27: Percentage of time spent in each pool when producing bubbled calls (blue cross-hatched bars) compared to overall bubbling (green lined bars). F1's results are shown on the top graph and F2's results are shown on the bottom graph. Error bars denote 95% confidence intervals.

Location:

When looking at the animals' locations in the pools separately from the pools themselves, there was a significant difference in location between bubbled calls and bubbling events in general for F1 ($\chi^2 = 87.13$, df = 5, $p < 0.001$). F1 produced bubbled calls significantly more than expected at the platform (stdres = 5.34, $p < 0.001$), near the rub rope (stdres = 2.52, $p < 0.05$), and along the south wall (stdres = 4.28, $p < 0.001$). All of these locations were part of the back pool. F1 produced bubbled calls significantly less than expected at the gate (stdres = -6.75, $p < 0.001$) and the gateway (stdres = -3.89, $p < 0.001$). Based on the 95% CI values, the gate was the only location that was significantly different. This suggests that the other locations were more variable between times of bubbled calls and bubbling overall, and more data would be required to show that they were significantly different.

F2's locations when comparing bubbled calls and bubbling events in general were not significantly different ($\chi^2 = 2.59$, df = 4, $p = 0.63$). Results for both animals are shown in Figure 28.



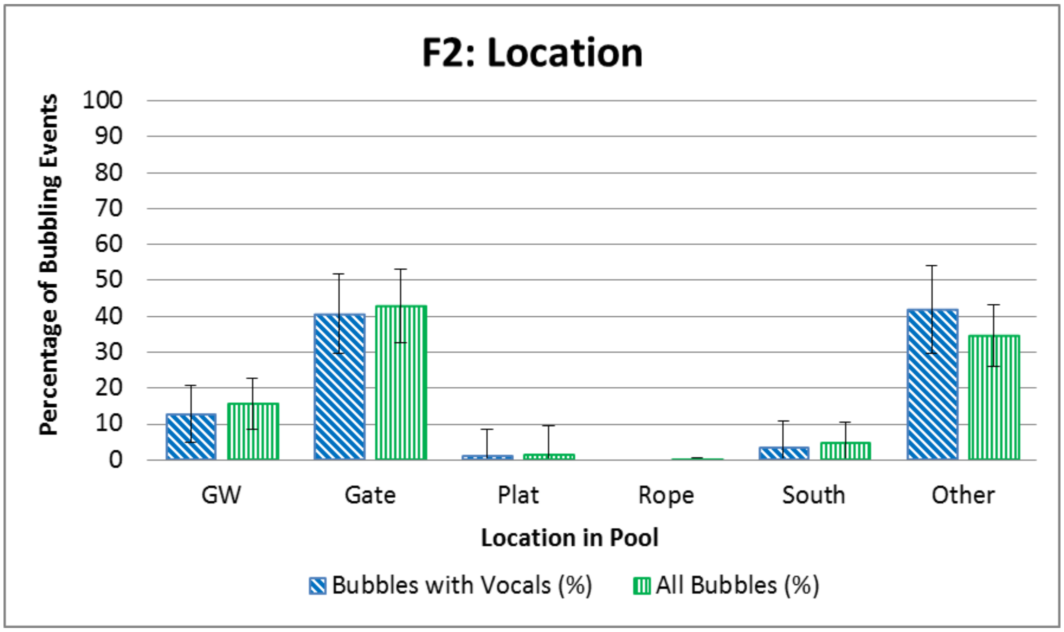
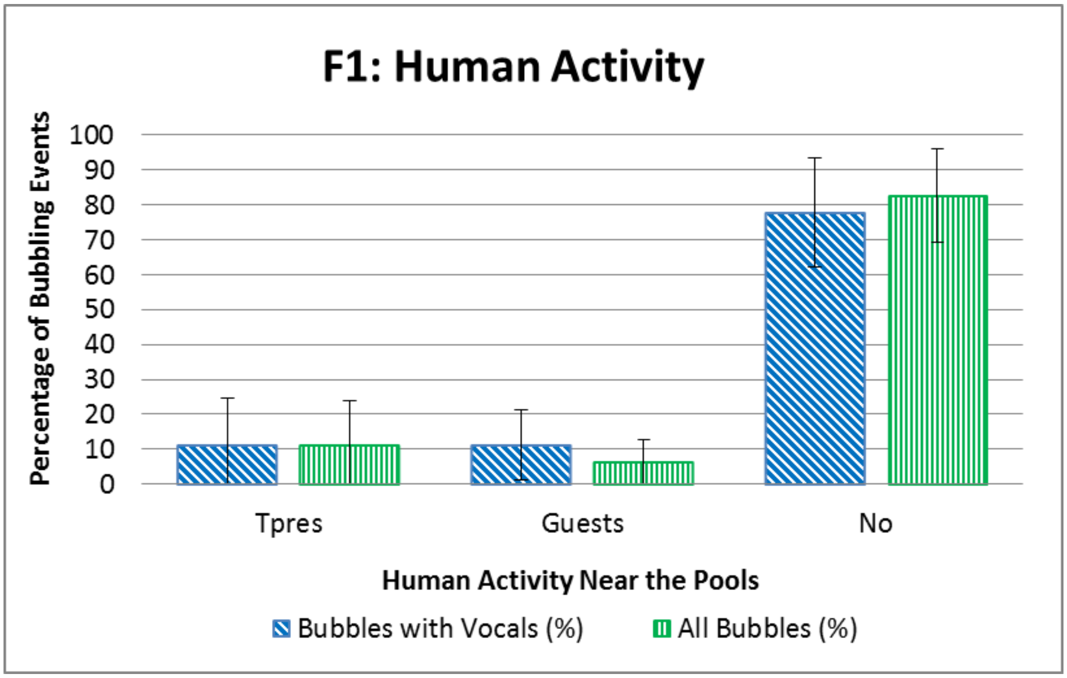


Figure 28: Percentage of time spent in each location when producing bubbled calls (blue cross-hatched bars) compared to overall bubbling (green lined bars). F1’s results are shown on the top graph and F2’s results are shown on the bottom graph. Error bars denote 95% confidence intervals.

Human Activity:

For F1, there was no significant difference in bubbled calls vs. overall bubbling based on human activity ($\chi^2 = 4.68$, df = 2, p = 0.10). For F2, this difference was significant ($\chi^2 = 8.92$, df = 3, p = 0.03), but the 95% CI analysis (Figure 29) did not suggest robust differences (all 95% CI ranges overlapped). Results for both animals are shown in Figure 29. Based on the chi-squared analysis, F2 produced bubbled calls significantly more than expected when guests were present (stdres = 2.58, p = 0.01). Since research assistants were always present, the category “guests present” only referred to guests who accompanied trainers to view the animals. As is clear from Figure 29, the proportion of time spent in this state was small, so the difference could have been a matter of chance. During these visits, trainers interacted with the animals and rewarded them for stationing or other behaviors. They usually entered from the main door into the pool area, about 5 m south of the back pool. The group usually paused by the door for a moment or two, and then approached the husbandry pool where F2 was most often located and stood within 2 m of the south wall of the husbandry pool.



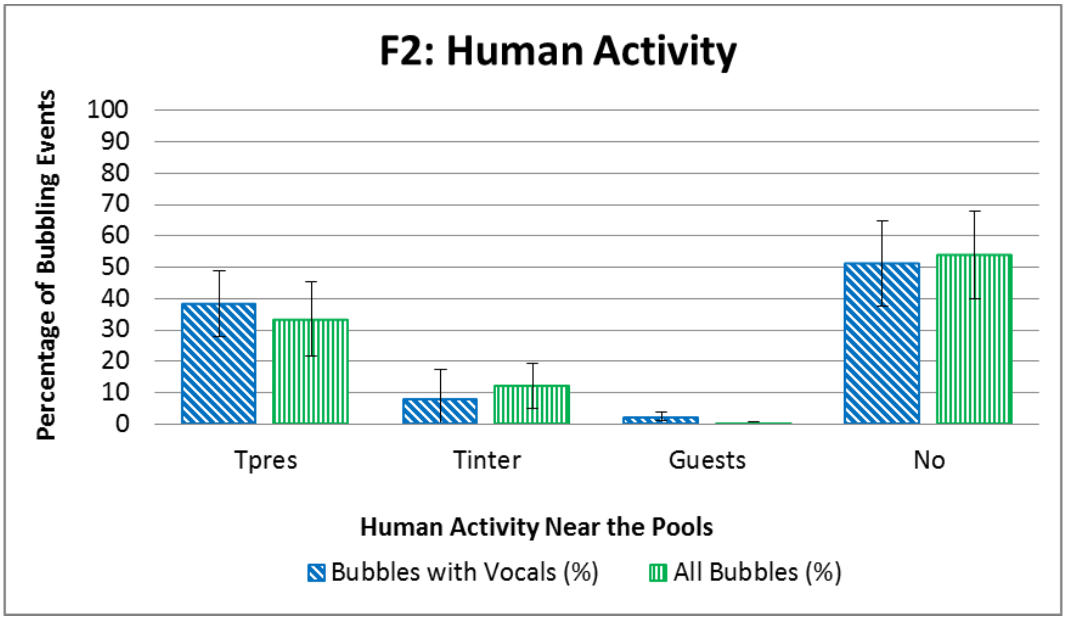
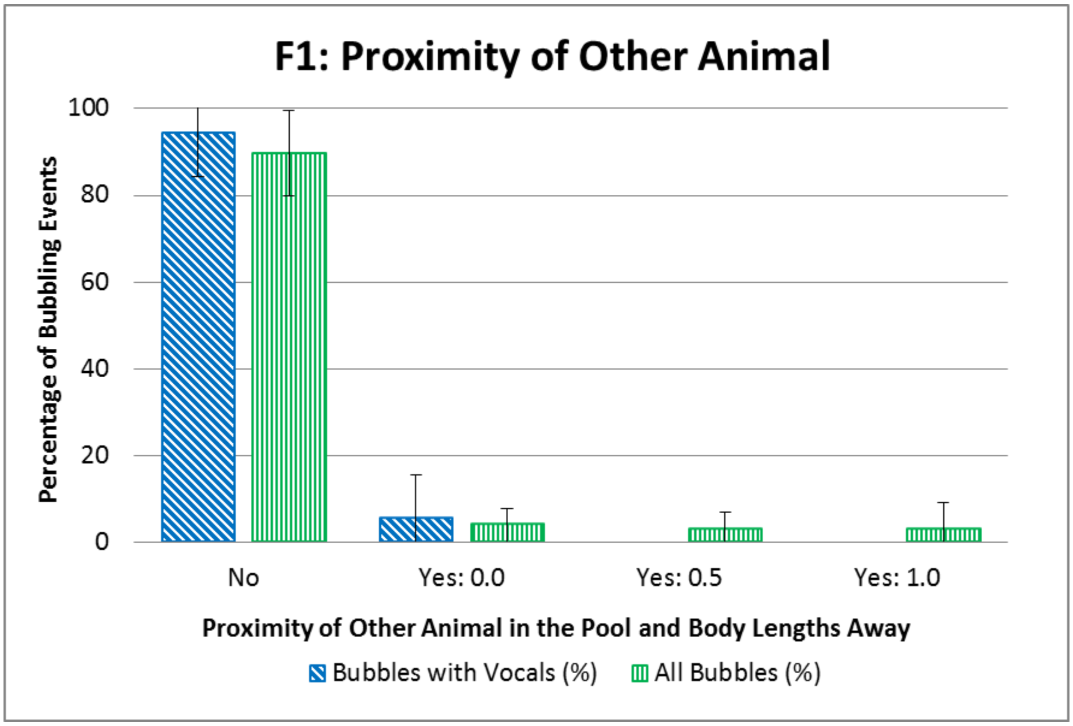


Figure 29: Percentage of time spent producing bubbled calls (blue cross-hatched bars) compared to overall bubbling (green lined bars) with various human activities going on near the pools. F1's results are shown on the top graph and F2's results are shown on the bottom graph. Error bars denote 95% confidence intervals.

Proximity of Other Animal:

There was no significant difference in bubbled calls and overall bubbling based on the proximity between the two animals for F1 ($\chi^2 = 6.83$, df = 3, p = 0.08) or F2 ($\chi^2 = 3.87$, df = 3, p = 0.28). These results are shown in Figure 30. The values for proximity included more than 1.0 adult body length from each other, 1.0 body length away, 0.5 body length away, and 0.0 body length away (touching). The 95% CI values support this finding, as all intervals overlapped almost completely.



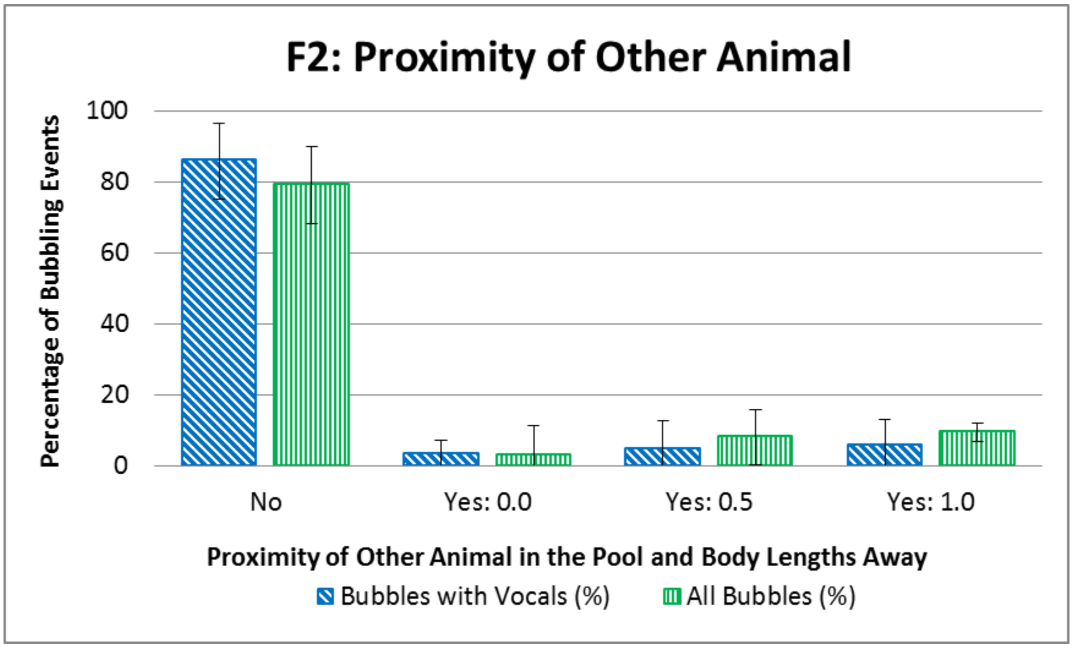


Figure 30: Percentage of time spent producing bubbled calls (blue cross-hatched bars) compared to overall bubbling (green lined bars) when in varying proximity to the other animal in the pool. F1's results are shown on the top graph and F2's results are shown on the bottom graph. Error bars denote 95% confidence intervals.

Vocalizations With and Without Bubble Streams:

In addition to comparing bubble streams with vocalizations ("bubbled calls") to all bubble streams, I compared the vocalizations made synchronously with bubble streams to vocalizations in general (i.e. vocalizations with and without bubble streams). To do this, I detected all vocalizations in the audio files and categorized them based on their aural properties and the visual properties of their spectrograms (see Methods in Chapter 3).

The belugas were highly vocal during the 19 hours of recordings; a total of 3,136 vocalizations were detected. However, synchronous bubbling with vocalizations was not common. Of all vocalizations, 18 were associated with bubble streams produced by F1, and 86 were associated with bubble streams produced by F2. Thus, only 3.3% of all vocalizations were bubbled. This is the first estimate of the percentage of bubbled vocalizations by belugas and is comparable to those of bottlenose dolphins (1% from Fripp 2005) and killer whales (4% from Bowles et al. 2015)

While the 18 and 86 vocalizations for F1 and F2, respectively, could be attributed to the individual animal because of accompanying bubble streams, the remainder could not be attributed. It is possible that a small proportion were produced by whales from the exhibit pool spending time in the gate aperture. Because they could not be attributed to the individual subjects, the remaining vocalizations were pooled and considered as the expected repertoire. Proportions of call types within this pooled dataset were treated as expected values.

The 10 categories of vocalizations fell into the expected broad vocalization categories described for wild belugas (whistles, burst-pulse calls, and combined calls; see Chapter 1). Spectrograms of the vocalizations and categories are shown in Figure 25. Whistles were characterized by a narrow-band fundamental frequency, usually accompanied by some degree of frequency modulation and harmonics of the fundamental frequency. Burst-pulse calls were characterized by a broadband signal with little or no tonal quality. Finally, combined calls contained properties of both whistles and burst-pulse calls, produced either simultaneously or in a continuous sequence.

Two calls, A1 and A2, were classified as subsets of each other, because A2 was almost identical to the A1 call, with the only difference being that the A1 call consisted only of a whistle, while the A2 call had the same whistle with a burst-pulse component at the beginning of the whistle. It is important to note that labeling these calls “A calls” is not meant to suggest that they relate to the “Type A” calls described by Vergara and Barrett Lennard (2008); while my A1 and A2 calls relate to each other, the specific use of the letter “A” is arbitrary. A similar distinction was made for the D1 and D2 calls. D1 sounded like the “raspberry” sound made by rude English speakers, with a duration of approximately 1 sec. The D2 call had a similar aural quality but a much shorter duration (usually less than 0.5 sec long).

F1 associated particular vocalizations with bubble streams more often than expected ($\chi^2 = 890$, df = 9, $p < 0.001$), as did F2 ($\chi^2 = 123.40$, df = 9, $p < 0.001$). F1 produced only D1 vocalizations (a burst-pulsed call) when bubbling (stdres =

29.83, $p < 0.001$). Thus, the other vocalizations were bubbled significantly less often than expected (stdres values for remaining categories were between -6.12 and -2.05, p between 0.05 and < 0.001). The D1 vocalizations associated with F1's bubble streams were noticeably louder and harsher than those recorded when F1 was not bubbling (prospectively produced by F2). F2 produced calls significantly more often than expected of type C (stdres = 2.58, $p = 0.01$), D1 (stdres = 9.00, $p < 0.001$) and G (stdres = 3.00, $p < 0.01$), and less than expected of H calls (stdres = -6.12, $p < 0.001$). It is likely that all G and H calls recorded during the study were produced by F2, as she was either interacting at the main gate or nursing (in the case of the H vocalization, explained further in the discussion section) when these calls were recorded. When calls C and E were pooled into one call type and the analysis was run again, that joint call type was not produced differently than expected when comparing vocalizations with and without bubble streams. For the sake of this study, I kept the C and E calls separate until more analysis can be done to determine if they are similar enough to be considered one call type. Note that the D1 call was most often bubbled by F2 and was the only call bubbled by F1. Calls bubbled significantly more often than expected included two types of whistles (C and G) and one burst-pulsed call (D1). The call produced least often was a whistle (H). Results for both animals are shown in Figure 31 and are summarized in Table 12 for F1 and Table 13 for F2. Table 14 summarizes the significant results for both animals.

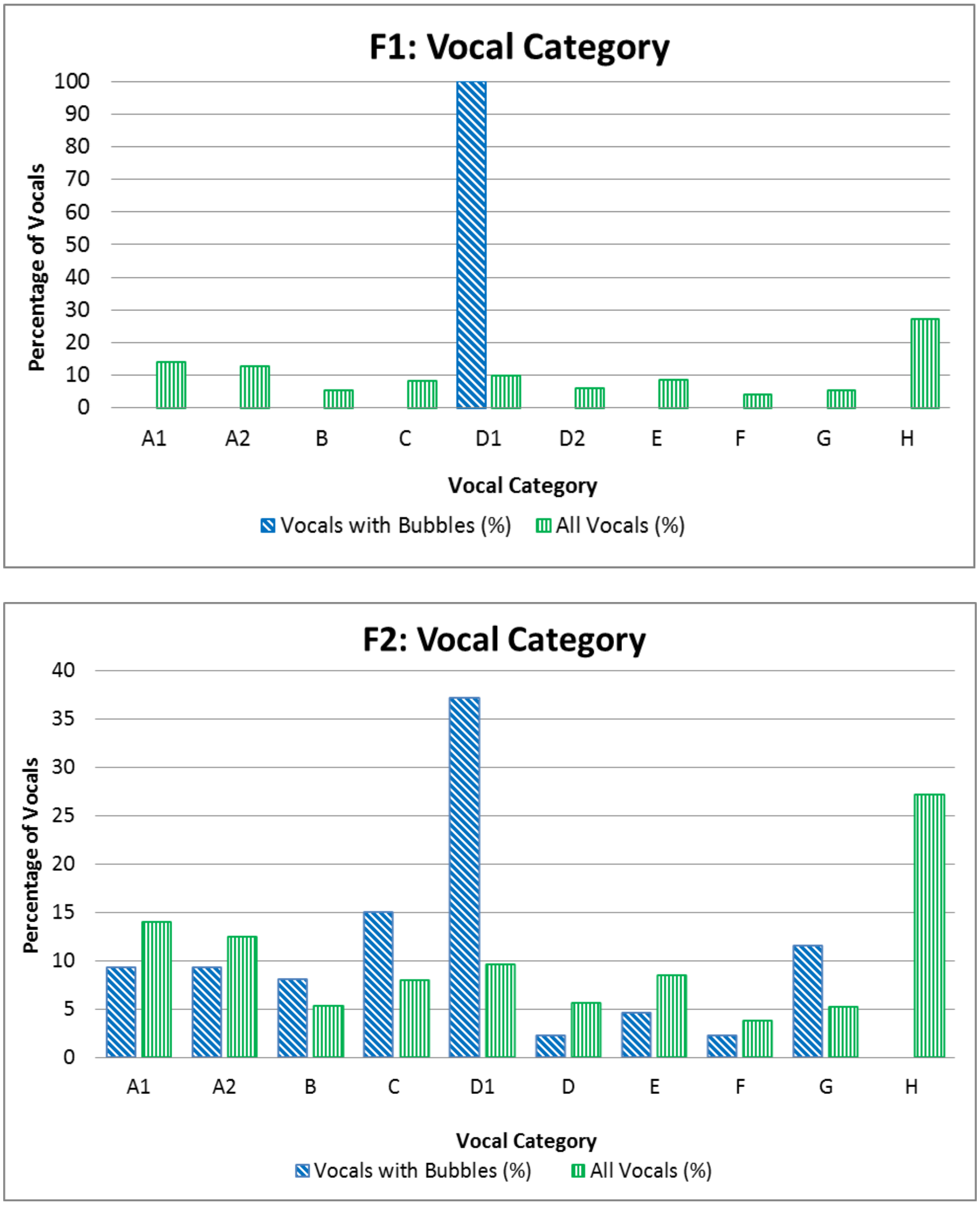


Figure 31: Percentage of each type of vocalization used when bubbling (blue cross-hatched bars) compared to overall vocalizing (green lined bars). F1's results are shown on the top graph and F2's results are shown on the bottom graph.

Table 12: Summary of results for each vocal category when producing bubbled calls compared to overall vocalizing for F1. The columns on the left show the percentages (indicated by "%") while the columns on the right show the raw values from which the percentages were calculated (indicated by "freq"). P-values showing the significance of the difference bubbled calls and overall vocalizing are given below each table. Asterisks next to percentages indicate that that individual category contributed to the significant difference found in the chi-squared analysis. Numbers in parentheses are standardized residuals.
* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$

F1

Vocal Category:	**Bubbled Calls (%)**	**All Vocals (%)**	**Bubbled Calls (Freq)**	**All Vocals (Freq)**
A1	0*** (-4.06)	14.00	0	439
A2	0*** (-3.71)	12.47	0	391
B	0* (-2.31)	5.36	0	168
C	0** (-2.96)	8.04	0	252
D1	100.00*** (29.83)	9.66	18	303
D2	0* (-2.54)	5.71	0	179
E	0** (-2.96)	8.48	0	266
F	0* (-2.05)	3.86	0	121
G	0.00* (-2.31)	5.23	0	164
H	0.00*** (-6.12)	27.20	0	853

$p < 0.001$

Table 13: Summary of results for each vocal category when producing a bubbled call compared to overall vocalizing for F2. The columns on the left show the percentages (indicated by “%”) while the columns on the right show the raw values from which the percentages were calculated (indicated by “freq”). P-values showing the significance of the difference between bubbled calls and overall vocalizing are given below each table. Asterisks next to percentages indicate that that individual category contributed to the significant difference found in the chi-squared analysis. Numbers in parentheses are standardized residuals.

* = p < 0.05, ** = p < 0.01, *** = p < 0.001

F2

Vocal Category:	**Bubbled Calls (%)**	**All Vocals (%)**	**Bubbled Calls (Freq)**	**All Vocals (Freq)**
A1	9.30 (-1.39)	14.00	8	439
A2	9.30 (-0.86)	12.47	8	391
B	8.14 (1.41)	5.36	7	168
C	15.12** (2.58)	8.04	13	252
D1	37.21*** (8.995)	9.66	32	303
D2	2.33 (-1.56)	5.71	2	179
E	4.65 (-1.26)	8.48	4	266
F	2.33 (-0.87)	3.86	2	121
G	11.63** (3.00)	5.23	10	164
H	0.00*** (-6.12)	27.20	0	853

p < 0.001

Table 14: Summary of all significant results for F1 (top) and F2 (bottom) when comparing bubbled calls to vocalizations overall.

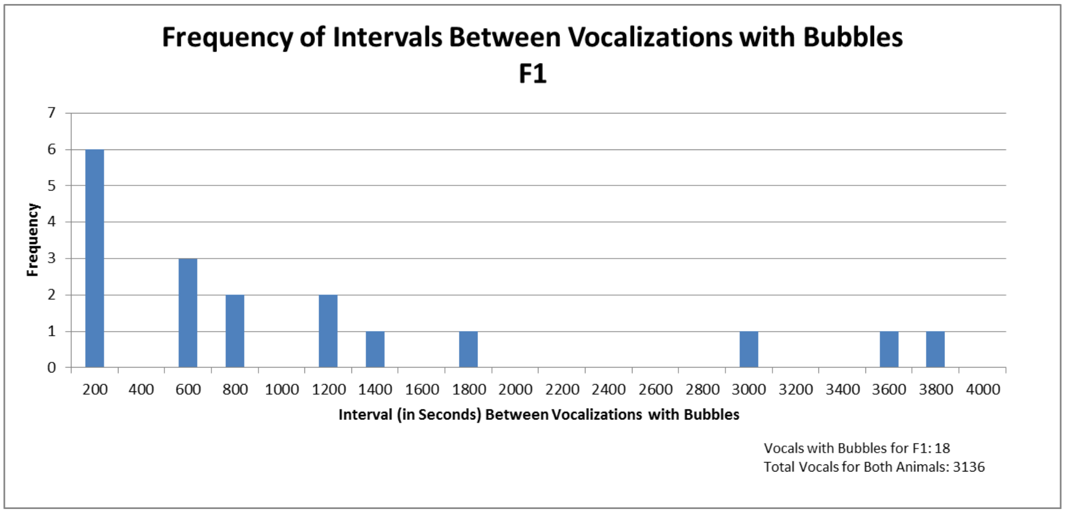
F1

Variable:	**Bubbled Calls More Likely:**	**Bubbled Calls Less Likely:**
Vocal Category	D1	All other categories

F2

Variable:	**Bubbled Calls More Likely:**	**Bubbled Calls Less Likely:**
Vocal Category	C, D1, G	H

For F1, the sample of bubbled calls was clearly biased with respect to behavioral state and very small. Thus, I did not attempt to look for evidence of bouts for F1. Even so, I include the interval frequency plot for F1 along with the plot for F2 (Fig. 32). The probability density plot and log-survivorship curve for F2 are shown in Figure 33. These plots do not support a 2-process model for bubbled calls, at least in this young animal. The modal peak was at 20 sec, as for bubble streams overall, but the percentage of calls at this peak was small (12%), and there was no clear mode in the graph, nor a break between an initial linear decline in log survivorship and another slope or slopes. This meant that a single process model would be as defensible as a 2-process or multiple-process model. Therefore, there was no reason to suspect that bubbled calls were not independent; in other words, there was no reason to suspect bouting behavior in F2's production of bubbled calls.



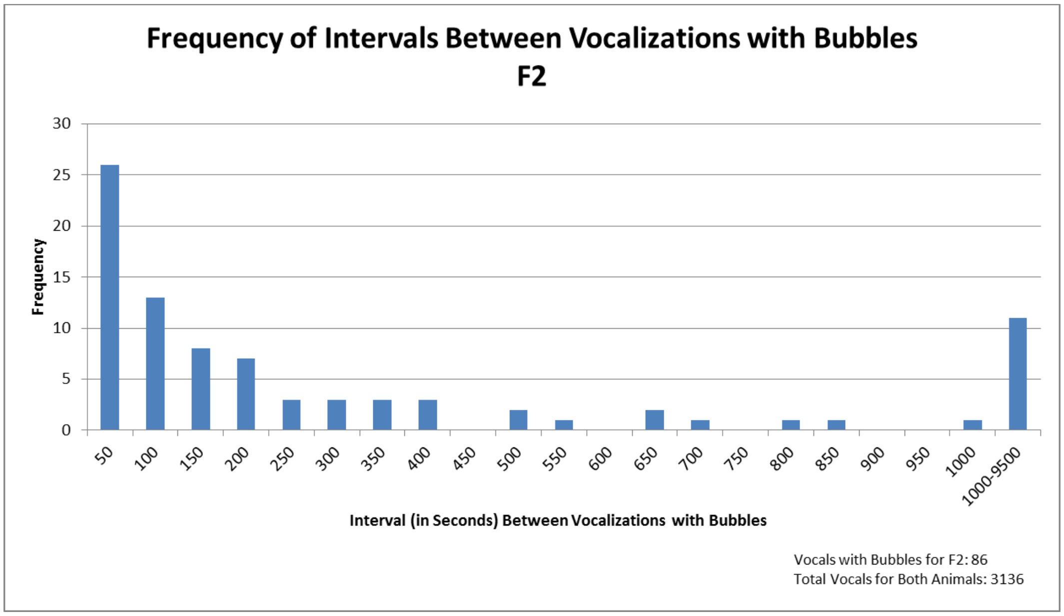


Figure 32: Frequency of intervals between bubbled calls for F1 (top) and F2 (bottom). Intervals are shown in bins of 50 seconds for F2; the bar over interval “50” represents intervals of 0-50 seconds in duration, the bar over interval “100” represents intervals of 51-100 seconds in duration, and so on. Intervals are shown in bins of 200 seconds for F1, as her vocalizations were fewer and farther between.

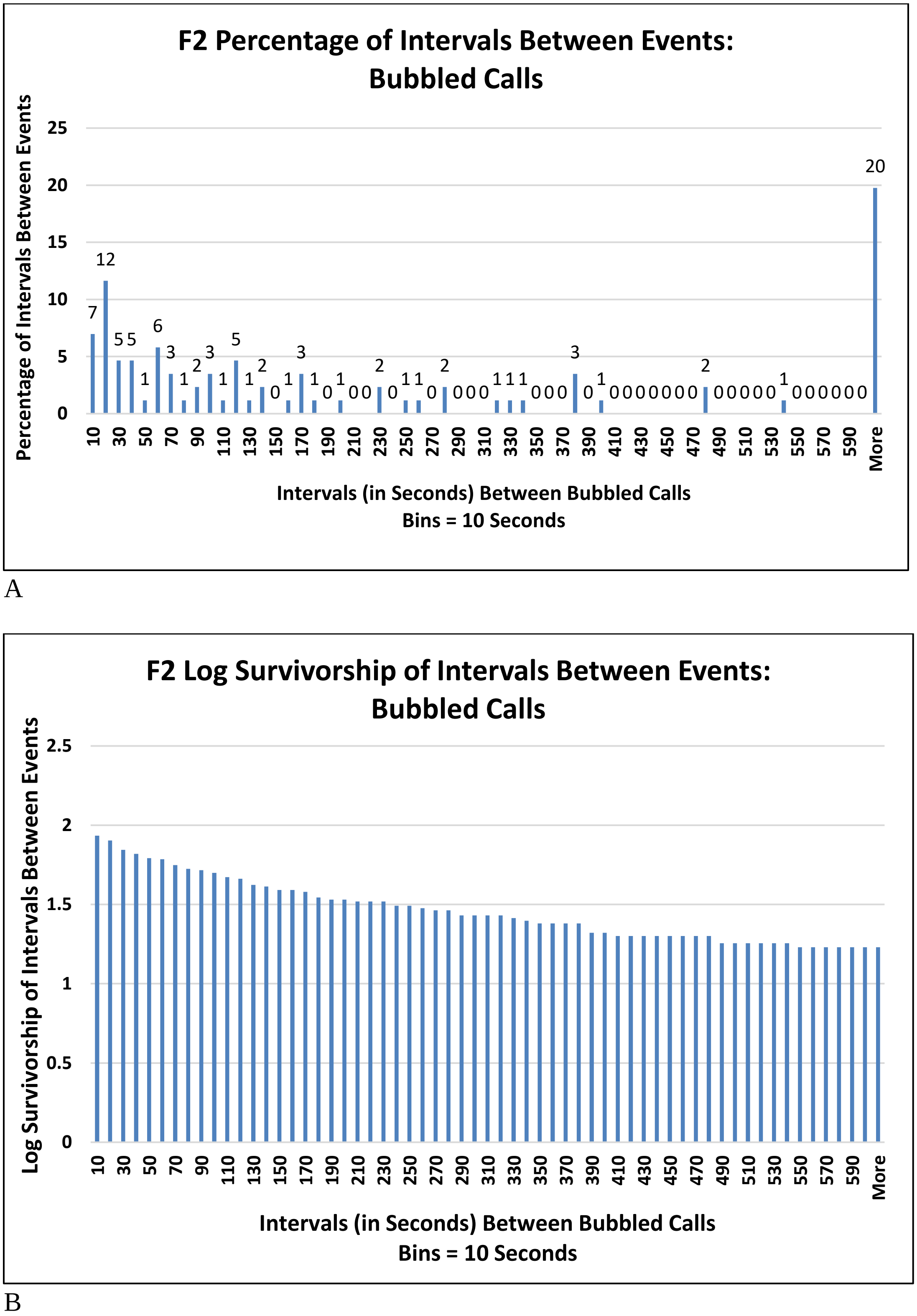


Figure 33: Percentage of intervals between events (A) and log survivorship of intervals between events (B) for F2’s bubbled calls.

3.4 Discussion

The goal of this portion of the study was to evaluate the relationship between bubble streams and vocalizations produced by belugas. Bubble streams were compared with and without vocalizations, as were vocalizations produced with and without bubble streams.

Previous studies of bubble stream production have reported that the behavior accompanies every vocalization (e.g., McCowan and Reiss 1995, Dudzinski 1996, Vergara and Barrett-Lennard 2008), while a few studies have disputed that conclusion (e.g., Pryor 1990, Fripp 2005). Some research on other cetacean species has found that different types of vocalizations are bubbled (e.g., Herzing 1996, *Stenella frontalis* and *Tursiops truncatus*; Bowles et al. 2015, *Orcinus orca*), but no study on belugas has focused on quantifying the use of bubble streams or their association with particular vocalization types.

Odontocete cetaceans accompany vocalizations with a stream of bubbles a small percentage of the time (1%, Fripp 2005; 4%, Bowles et al. 2015). Both this study and Bowles et al. (2015) found that the same vocalization could be produced with or without a bubble stream, showing that bubble streams are not produced uncontrollably as a result of the vocal production mechanism. Instead, they must be produced by the animal deliberately. This usage supports the hypothesis that bubble streams are a visual cue that alters the information in the vocalization somehow. I found that an average of 3.3% of vocalizations were bubbled by belugas, comparable to the percentages reported for other species. Future studies where all vocalizations can be attributed would be desirable to confirm this value, as the count of vocalizations in this study could have been affected by unseen animals.

This was also the first study of belugas to examine production of bubble streams overall and in behavioral context, which has not been examined systematically in other odontocetes. In this study, bubble streams were produced concurrently with a vocalization in only ~18-20% of cases (F1, 18.4%; F2, 20.5%).

Unlike the data for Chapter 2, data for Chapter 3 did not need to be reanalyzed with bouts of behavior as the unit of measurement instead of individual bubbling events. A probability density plot of intervals between bubbling events showed no clear peak, suggesting F2's calls were not being made in bouts. The data for F1 were not reanalyzed either, as the sample size was too small to look for bouting.

Vocalizations during bubble stream production gave support for the same possible functions of bubbling as described in Chapter 2. F1 produced more bubble streams with vocalizations than bubble streams overall in certain contexts, such as swimming, resting, and solitary behavior, as well as when she was located in areas near the south part of the back pool. She produced fewer bubble streams with vocalizations than bubble streams overall during social-affiliative behaviors in the husbandry pool and near the gate and gateway. This could suggest that, when the animals are already within line-of-sight of each other, they do not need to use a vocalization in addition to a bubble stream to get each other's attention.

Interestingly, F2 only showed a significant difference in vocalization behavior when bubbling in two contexts. She produced more bubbled calls than expected when interacting with humans, and possibly more often when guests were present. However, the sample involving guests present was small.

The findings suggest a somewhat different use of bubbled calls between F1, the adult female, and F2, a calf. Even so, the results reinforce the finding in Chapter 2 that bubble stream production has a signaling function in positive social situations. This is consistent with the previous literature that has touched on this subject (e.g., Bowles et al. 1988, Pryor 1990). It may seem counterintuitive that F1 produced bubbled calls more during non-social behavioral states, including swimming, resting, and solitary behavior, but it may be that she vocalized in these states to initiate a social interaction with F2 when they were not already in contact. It may be that vocalizations with bubble streams work to initiate an interaction, while bubble streams thereafter bring special attention to certain actions or intentions. There might not have been a need for a vocalization with a bubble stream when the two animals were already interacting, which would explain why F1 vocalized during bubble stream production less often during social-affiliative behavior, i.e., when in close contact. When located in the back pool and specifically near the platform, rub rope, or south wall, F1 might need to add a vocalization to her bubble stream to get the attention of F2, who was usually in the husbandry pool. Based on this interpretation, it would make sense that bubbled calls would occur less when F1 was in the husbandry pool, near the gateway, or near the gate. In all three cases, F1 was close enough to F2 that she could be sure the calf would know where she was and that she could contact the calf quickly if necessary. Additionally, at the gate, she may not have needed a vocalization along with her bubble stream to communicate with the two other adult belugas in the main pool (not part of the study area) if they were already close to the gate themselves or already attending to her visually.

F2 produced bubbled calls more often than expected when interacting with trainers and when guests were present. Two possibilities fit the data. First, as suggested in Chapter 2, she may be more oriented to social interactions with humans because she was captive-born. In that case, the bubble streams or vocalizations might have been added as emphasis or because of high excitement. Akiyama and Ohta (2006) showed that bottlenose dolphins in a similar situation whistled more and with greater variety when people were present.

However, F2 produced more bubbled calls (n = 86) and bubble streams overall (n = 419) than F1 (n = 18 and n = 98). This means that, although human-oriented contexts were the only ones in which F2 showed significant differences in use, she still bubbled with and without vocalizations more often in other contexts than F1. It is possible that, as a young animal, F2 was less discriminating while she was still learning and practicing both bubble streams and vocalizations. A study by Vergara and Barrett-Lennard (2008) showed that beluga calves displayed evidence of vocal babbling in the first year of life when developing their repertoire. Their calf simultaneously began producing a larger variety of sounds in general while certain individual vocalizations became more stereotyped. F2 was of a similar age (4 months old) to the calf in that study, and likely exhibited babbling as well. This might explain why F2 did not show more distinction between the contexts in which she vocalized with her bubble streams and the contexts in which she did not, as F1 did. Over time, we might find that F2's contextual use of vocalizations during bubble stream production would narrow, either as part of the normal developmental progression of the behavior or through learning from F1.

Bubbled Calls vs. All Vocals

Both animals used vocalizations differently when bubbling compared to overall. However, part of this finding may have been the result of methodological limitations. First, observers could not attribute vocalizations to individual animals except when they were synchronous with bubble streams. They could not hear vocalizations from the surface or distinguish other gestures synchronous with vocalizations. This meant that all vocalizations not synchronous with bubble streams had to be considered representative of the repertoire of both animals. However, this assumption may not have been justified. First, vocalizations may have been somewhat contaminated with sounds from the adjacent exhibit pool. In addition, one or the other of the two whales may have produced the majority of these calls. Finally, there may have been a strong bias in the types of calls bubbled. Certainly, there seemed to be a large bias in F1's choice of vocalizations to bubble (only D1).

The D1 vocalizations attributed to F1's bubble streams as well as many of the un-attributable D1 vocalizations had a different timbre than other vocalizations, being much harsher and louder. If this subset of D1 calls was the repertoire produced by F1, then F2 may have produced the more diverse repertoire revealed by synchronous bubbling as part of her development, consistent with the babbling observation of Vergara and Barrett-Lennard (2008). Previous studies have found that cetacean calves start out producing many types of vocalizations, most of which disappear as they develop an adult repertoire that more closely resembles those of other adults in their social group (Bowles et al. 1988, Snowdon and Hausberger 1997, Fripp et al. 2005, Vergara and Barrett-Lennard 2008, Tizzi et al. 2010). If this hypothesis is correct, F1 produced almost exclusively D1 calls, and there was little if any difference between the category of vocalization she used when producing bubble streams compared to vocalizing in general.

F2 used more C, D1, and G vocalizations when bubbling than when vocalizing overall. However, when the C call type was pooled with the E call, since the two were often confused with each other, only the D1 and G vocalizations were used significantly more than expected. These vocalizations might have been used more often when bubbling because they were vocalizations used more often in the contexts in which bubbling most often occurred. For example, F2 may have used these calls more often during social-affiliative behaviors or when she was 1.0 body length or more away from F1, as were the bubble streams themselves. If this was the case, the use of these vocalizations would have been affected more by behavioral context and the bubble streams would have been a meta-communicative signal with a separate function.

It is interesting that two of the three vocalizations produced more often with bubble streams, C and G, were whistles. Previous research (e.g., Karlsen et al. 2002, Belikov and Bel'kovich 2007) has shown that whistles are the largest part of an odontocete's repertoire, although these observations were made without regard to behavioral context. Some whistle types, such as bottlenose dolphin signature whistles, appear to be contact calls, i.e., they are context sensitive (Janik and Slater 1998, Harley 2008). However, if unstereotyped and stereotyped whistles are pooled, social odontocetes apparently use whistles more often during social interactions (e.g., Dudzinski 1996, Karlsen et al. 2002, Akiyama and Ohta 2006, Belikov and Bel'kovich 2007, Chmelnitsky and Ferguson 2012). The limited published data on the use of bubble streams support my observation that bubble streams are used during social interactions (Pryor 1990) and that they are used with particular calls (Fripp 2005, Bowles et al. 2015) and in association with particular behavioral states (Fripp 2005).

Two vocalizations, G and H, were especially interesting in both their sound quality and context. Both vocalizations were whistles. Based on general cues such as activity and social interaction at the gate, they appeared to be used predominantly by the calf F2. The G vocalization had no visible harmonics, leading me to wonder initially whether the sound was coming from some mechanical apparatus within the pools. However, A. Bowles, who was very familiar with the facility, reported that there had been no transducers in the pools that produced a sound similar to this vocalization since the birth of F2. The G vocalization was not associated with any particular time of day or human activity.

Additionally, it was not produced deliberately by staff. Finally, it was observed synchronously with bubble streams produced by F2. This evidence suggested that it was not simply an artifact or a human-made cueing tone, but a pure tone sound either initiated or imitated by the calf (perhaps learned from whales in the exhibit pool). No other calls were pure tones without harmonics.

The H vocalization was also unique. It was heard continuously over a period of 17 min and 24 sec on September 10th, with a narrow frequency band (around 3-4 kHz), short duration (about 1 sec), and constant interval between calls (about 2 sec). It was not heard in any other part of the dataset. Throughout this short period, F2 was observed either actively suckling or swimming close to F1's mammaries, suggesting that the call may have had some relation to suckling or nursing. F1 was not F2's biological mother but had started to lactate spontaneously shortly after she and the calf were introduced to each other. This behavior has been described in captive belugas previously (Leung et al. 2010). The vocalization could have served as some sort of signal to F1 that stimulated lactation, like crying in human babies. This supposition is supported by Morisaka et al. (2001), who found that two neonate bottlenose dolphins produced significantly more whistles leading up to suckling behavior, suggesting that the whistles could be a "begging call" to stimulate milk production. If true, this study would provide the first evidence of such a call in belugas. For this vocalization, as well as the G vocalization, more data would be helpful in supporting the interpretation of these findings.

Because my results confirmed earlier reports that vocalizations synchronous with bubble streams occur at relatively low percentage, my sample of synchronous behaviors was small. It is possible that some of the differences observed could have been the result of chance. A previous study on bottlenose dolphins by McCowan (1995) with similar methodological constraints found no difference in vocal production rates for bubbled and non-bubbled calls, so similar findings might be expected with belugas, given a more robust data set. However, the McCowan study has been controversial, because other studies have found evidence of bias in both the vocalization type and behavioral context of bubbled vocalizations (see Harley 2008), including a study of another species. Bowles et al. (2015) found that killer whales preferentially bubbled one type of call, stereotyped pulsed calls with divergent biphonation, although individuals could bubble multiple call types with this characteristic. These authors noted that bubble streams were associated with close-range interactions and high activity states, and suggested that they were used to draw attention to a concurrent call or to the individual producing it. Their subjects were not calves, so age-related use of bubble streams could not be explored with their dataset.

The weight of evidence suggests that odontocete behavioral state affects use of certain vocalizations and the use of bubble streams, as opposed to a close link between vocalizations and bubble streams (e.g., McCowan and Reiss 1995, Dudzinski 1996, Vergara and Barrett-Lennard 2008). Since bubble streams only accompanied vocalizations a small proportion of the time in this study – the whales bubbled 80% of the time without vocalizations - it is likely that the visual aspect of the bubble stream, and not vocalization, was the salient feature of the behavior.

The pattern of bubbling certainly suggested a signaling function, but its association with vocal behavior was unclear. Any roiling produced by bubble streams was inaudible at the corner hydrophone. If bubbling served an auditory function – say by soliciting attention with a roiling sound– we would expect the animals to use it at a range of distances, and possibly mostly while in close proximity. Similarly, if bubbling had no significance to the animals and occurred solely as a byproduct of vocalization, we would expect it to show no correlation with the animals' proximity to each other. Instead, this pattern of use when the animals were near each other but not 1 body length away or closer suggests that their ability to see the display may be the aspect of it that is pertinent.

Future studies should further contextualize bubble stream production in belugas and other cetaceans. Larger, more diverse groups of animals would support this end. Also, characterization of this bubbling behavior in wild populations, particularly in conjunction with acoustic recordings designed to localize callers, would clarify the use of these behaviors. If bubble streams are indeed visual cues that provides reliable information about behavioral state, they could be used by researchers in future studies to monitor interactions and assess behavioral state.

3.5 Summary

This study described the contexts in which two belugas, an adult female and a female calf, produced bubbled calls compared to the contexts in which they bubbled overall and vocalized overall. The results suggest that F1 produced more bubbled calls than bubble streams overall when she was not already involved in social behaviors and produced them less when she was involved socially. This could mean that adding a vocalization to a bubble stream helps to initiate social interaction. The female calf, F2, produced more bubbled calls than bubble streams overall at times when she was interacting with humans and when observers were present, pointing toward her use of vocalizations with bubble streams during high excitement. F2's contextual use may change as she ages and learns the "correct" contexts for vocalizing, bubble streaming, and both together. Her pattern of use is also likely influenced by the fact that she was born and raised in captivity, meaning she likely sees the trainers and guests who interact with her as conspecifics with whom she can communicate vocally. F1, as a wild-born animal, likely does not.

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