Observation and analysis of sonar signal generation in the bottlenose dolphin (*Tursiops truncatus*): Evidence for two sonar sources

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ABSTRACT

Indirect evidence for multiple sonar signal generators in odontocetes exists within the published literature. To explore the long-standing controversy over the site of sonar signal generation, direct evidence was collected from three trained bottlenose dolphins (*Tursiops truncatus*) by simultaneously observing nasal tissue motion, internal nasal cavity pressure, and external acoustic pressure. High-speed video endoscopy revealed tissue motion within both sets of phonic lips, while two hydrophones measured acoustic pressure during biosonar target recognition. Small catheters measured air-pressure changes at various locations within the nasal passages and in the basicranial spaces. Video and acoustic records demonstrate that acoustic pulses can be generated along the phonic fissure by vibrating the phonic labia within each set of phonic lips. The left and right phonic lips are capable of operating independently or simultaneously. Air pressure in both bony nasal passages rose and fell synchronously, even if the activity patterns of the two phonic lips were different. Whistle production and increasing sound pressure levels are generally accompanied by increasing intranarial air pressure. One acoustic "click" occurred coincident with one oscillatory cycle of the phonic labia. Changes in the click repetition rate and cycles of the phonic labia were simultaneous, indicating that these events are coupled. Structural similarity in the nasal apparatus across the Odontoceti suggests that all extant toothed whales generate sonar signals using the phonic lips and similar biomechanical processes.

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1. Introduction

Locating the site and identifying the driver of odontocete sonar signal generation has long challenged investigators. Early on, the debate centered on whether the clicks were generated in the larynx or in the nasal complex. This argument was eventually settled in favor of the nose. The past half century of debate and controversy is now covered by several reviews (Cranford, 2000; Cranford and Amundin, 2003; Cranford et al., 1996; Evans, 1973; Norris, 1964, 1968, 1969, 1975; Norris and Harvey, 1974; Norris et al., 1972). Also, Mead (1975) and Popper (1980) have written comprehensive reviews of the pertinent issues.

A broad array of sophisticated animal sonar systems can actively and quickly – within milliseconds – alter their outgoing signals in response to the task they encounter, or the target, or the echoes from previous clicks (Au et al., 1985; Kalko and Schnitzler, 1993). Observations also suggest physiological limits to their adjustments. Within these limits, animals can modify the spectral characteristics, the repetition rate of the sonar signals, the number of generator(s) and their location(s), and the time required to alter or tune the mechanical system used to generate signals (Ridgway, 2011). These limits themselves provide clues to the mechanics of sound generation and the structure of the sound-generation apparatus. In this paper, we focus especially on the number of sonar signal generators in the bottlenose dolphin. Possibly, much of what we report here can be generalized and extrapolated to other non-physeteroid odontocetes.

Norris et al. (1972), Evans (1973), and Lilly (1978) presented indirect evidence that dolphins have more than one sonar signal generator. More recent studies have suggested that multiple sonar signal generators exist (Cranford et al., 1996; Markov and Ostrovskaya, 1990; Sigurdson, 1997) in all odontocetes except the sperm whales (Physeteridae and Kogiidae). But the matter has rarely been directly

investigated; more often it has been left to inference. A recent report does suggest a single (right side) location for sonar click generation in the harbor porpoise (*Phocoena phocoena*) (Madsen et al., 2010), but some doubt has been cast on the interpretation of these observations (Cranford, 2011). Lammers and Castellote have also collected indirect evidence that the white whale (*Delphinapterus leucas*) produces sounds from two click generators (Lammers and Castellote, 2009). In this paper, we describe the first direct evidence for click generation from both the left and right phonic lips in bottlenose dolphins (*Tursiops truncatus* Montagu).

We know that the number of generators, their locations, relative positions, size, shape, material composition, and duty cycles can all influence, limit, or otherwise determine the range of acoustic parameters, such as pulse repetition rate, signal bandwidth, spectral frequency composition, and/or transmission beam geometry. In this wealth of new understanding, our project has focused on two primary goals: (1) to determine with high-speed video endoscopy the sonar signal source location and number in a bottlenose dolphin; and (2) to investigate via recording pressurization events within various cephalic air spaces the physiological mechanism that bottlenose dolphins use to produce their sonar signals.

2. Materials and methods

This project depended crucially on precise (to the millimeter) placement of endoscopes and pressure catheters within the dolphin's airways in order to observe otherwise invisible tissue movements. This capability required the cooperation of dolphins, their human trainers, and veterinarians. The veterinary staff placed the endoscopes and catheters within the dolphin's head, and the trainers requested performance of the requisite bioacoustic tasks. This cooperation is built on years of trust and experience, as the dolphins themselves ultimately controlled our ability to gain and record data.

Three bottlenose dolphins (*Tursiops truncatus*) were trained to produce sounds—pulses or whistles (see Table 1 for physical characteristics of the dolphins). Our methods in large part follow those described by Ridgway and Carder (1988), who measured air pressure in nasal and laryngeal cavities in a live, phonating white whale (*Delphinapterus leucas*). As they did, we trained our dolphins to echolocate underwater and respond to targets by whistling when they recognized a specific target. Elsberry (2003) presents the details of our equipment configurations and procedures.

2.1. High-speed video endoscopy

In addition to recording nasal air pressure, we used a dual-camera, high-speed video system (NAC HSV-400 high speed video camera system) attached to fiber optics endoscopes to investigate the movement of various tissue components within and along the dolphin's airways during click and whistle generation. Goller and Larsen (1997) have used endoscopic probes to investigate sound generation in birds. To our knowledge this is the first time that a dual, high-speed system has been used for such investigations of odontocetes.

Both high-speed video cameras were synchronized so that their shutters opened and closed simultaneously. This dual-camera and video system let us synchronously observe and record two concomitant events.

Table 1
Physical characteristics of dolphin subjects.

Name	Sex	Month/year of birth	Mass (kg)[a]	Length (m)[a]
BRT	Female	06/1961 (est.)	197	2.52
SAY	Female	06/1979	263	2.78
BUS	Male	06/1980	192	2.50

[a] Mass and length values are averages during the study period.

We could select video frame rates of 200 or 400 Hz. One camera recorded tissue movements made visible through an endoscope placed within the complex airways of the dolphin's head (Dormer, 1979; Green et al., 1980; Houser et al., 2004; Lawrence and Schevill, 1956; Mead, 1975; Reidenberg and Laitman, 2008). The other camera sometimes monitored the tissue motion visible through another endoscope, but most often it focused on oscilloscope traces of acoustic pressure from a hydrophone placed in the water near, and in front of, the dolphin's head.

At various times we used different endoscopes, all manufactured by Pentax. The endoscope's outside diameter (OD) was usually 3.8 mm, but on rare occasions we used an endoscope with an OD of 12 mm because the larger fiber optics bundle provided an increased viewable field and light gathering capacity. We determined the breadth of the endoscope's field of view (FOV) by using a long piece of translucent surgical tubing whose inside diameter was just large enough to accept the endoscope. This tubing was attached so that it projected about two millimeters beyond the tip of the endoscope and kept the surrounding tissue from touching the optical element. In so doing, it maintained a constant distance to the target tissue and so set the field of view. The tip of the tubing was marked in 1-millimeter circumferential increments, letting us estimate distances within the FOV.

2.2. Behavioral methods and acoustic recordings

We recorded activities within the nasal and pharyngeal cavities of three bottlenose dolphins (*Tursiops truncatus*) in each of two recording situations. In one situation, the dolphin was stationed with most of its head just under the surface of the water (only the blowhole was exposed to the air) and its rostrum in the trainer's hand. During these trials, the dolphin was not performing an echolocation task but was asked to produce whistles or clicks by a tactile cue given with the trainer's free hand. This allowed us to probe or otherwise move the endoscope to a desired location and test the dolphin's capacity to produce sounds when the endoscope was in selected airways, anywhere between the larynx and the blowhole. It also let us interfere with the dolphin's sound producing ability by inserting the endoscope between vibrating elements. These "interference experiments" were particularly useful for pinpointing the location of the sound source(s).

In the other recording situation, we placed the endoscope in any one of various locations within the air passageways and then directed each dolphin to station itself on a bite-plate 0.5 m below the surface of the water. At this station, the blindfolded dolphin performed an echolocation task (target recognition) (Cranford et al., 1997; Ridgway and Carder, 1988). Endoscopic observations focused primarily upon the region of the phonic lips, about 2.5 to 3 cm below the dolphin's blowhole on the left and right side of the nasal apparatus. In both recording situations, the dolphins allowed us to probe most of the air spaces between the nasal and pharyngeal airways. This included the vestibular and spiracular portions of the nasal air sac system; both bony nasal passages and the openings to the pharyngotympanic tubes (Eustachian tubes); the nasopharyngeal cavities in the basicranial space; the lumen of the larynx; and the pharynx itself. All the while, the dolphins performed bioacoustic trials.

The only major air spaces that we could not probe with the endoscope were the premaxillary sacs and nasofrontal sacs. The nasofrontal sacs are long narrow air spaces that originate from the inferior vestibule and encircle the main (spiracular) nasal passage. The inferior vestibule opens into the posterior wall of the spiracular cavity, ventral to an overhanging tissue mass that includes the phonic lips, as part of the "monkey lips/dorsal bursae" (MLDB) complex (Cranford, 1992). This intricate configuration made the nasofrontal sacs inaccessible to our endoscopes. The premaxillary sacs, also left unexplored, overlie the flattened shelves of the premaxillary bones, just anterior to the openings of the superior bony nares, forming large

pouches off of the spiracular cavity. Complete descriptions of the nasal air sac system can be found in multiple papers (Dormer, 1974; Heyning, 1989; Lawrence and Schevill, 1956; Mead, 1975; Reidenberg and Laitman, 2008; Schenkkan, 1973).

We also measured three physiological parameters while the dolphin used echolocation to accomplish the target recognition task. Small pressure catheters measured pneumatic changes at the same depth in each bony nasal passage while we monitored the tissue motion at both (left and right) sets of phonic lips using high-speed video endoscopy. At the same time, we recorded sound pressure in the water outside the dolphin's head. A Brüel and Kjäer Model 8103 hydrophone and Brüel and Kjäer Model 2635 charge amplifier recorded the high-frequency underwater dolphin sounds. These acoustic signals were filtered through a Krohn-Hite high-pass filter module (8-pole Butterworth, cut-off frequency of 80 Hz). The filtered acoustic output was passed into a National Instruments PCI-MIO-16E-1 multifunction input–output data acquisition card.

2.3. Internal nasal air pressure recordings

Our standard equipment for observation and recording also included catheters containing one or three pressure sensors (Elsberry, 2003). These Millar pressure catheters measured intranarial air pressure. Three different pressure catheter models were used at different points during the experimental period. Two flexible catheters (models Mikro-Tip PC-350 Size 5 French and Mikro-Tip SPR-524 Size 2.5 French), had single element strain-gage transducers at the end of each catheter. The other catheter (Mikro-Tip SPR-673 Size 6 French) had three strain-gage transducers. The trainer inserted the catheter into the bony nasal passages (approximately 10 cm below the blowhole) and monitored the dolphin continuously while it stationed on a bite-plate underwater, performing an echolocation (target recognition) task.

We could place catheters into any of the nasal or pharyngeal airways, except the nasofrontal sacs and premaxillary sacs. Measurements of intranarial pressure were most often recorded with the catheters at a depth of 10 cm from the blowhole, which put the sensors within the bony nasal passages. This technique, like that used by Ridgway et al. (1980) and Ridgway and Carder (1988), allowed us to track pressure changes during our observation periods. The objective here was to determine the magnitude of pneumatic forces and compare them to the timing of events seen through the endoscope during sound generation. This arrangement also provided a means to relate internal nasal pressure to the acoustic pressure and to spectral components of the sonar signals generated. These relationships will be the subject of another report.

2.4. Additional video devices

The high-speed video cameras and recorder (NAC HSV-400) included a built-in screen splitter that allowed the output of two high-speed video cameras to be recorded simultaneously on a single videotape. Each camera wrote simultaneously on one half of each video frame. This split screen captured the view from the endoscope and either the screen of the oscilloscope that displayed the hydrophone record or else the view from another endoscope. In some circumstances we needed to capture two endoscope displays on the same half-frame. A special adapter allowed the second field of view to be mounted off-axis in the principal field of view. The result was a composite video frame that included two endoscopic views on one half-frame, and the oscilloscope screen on the other half-frame (see video clip #1). This device provided all three video feeds synchronously on the same VHS video tape. Video clip #1 shows views of both phonic lips simultaneously from two of the 3.8 mm endoscopes. Video clip #2 shows a high speed video recording of a single set of phonic lips using the 12 mm endoscope.

The composite video frames from the high-speed video recorder were passed to a Videonics MX-1 video mixer, so that we could add the output from a separate video camera that recorded the experimental setup. This additional camcorder (standard VHS operating in air) was used to obtain a view of the experimental working area, including the trainer interacting with the dolphin and the veterinarian operating the endoscope(s). This camera also provided a microphone record of the various airborne interactions between investigators.

3. Results

The right and left sets of phonic lips lie at the dorsal boundary of the spiracular cavity, one on either side of the membranous nasal septum. Each set of phonic lips comprises what we will refer to as an
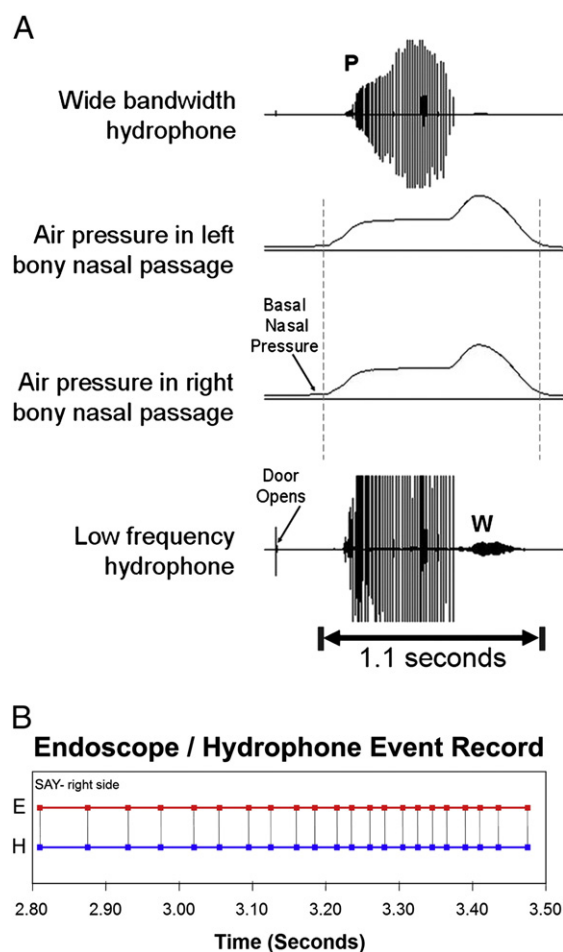
Fig. 1. A. Representative recording of a biosonar trial. All records are aligned across time. Beginning at the left, the first event on the hydrophone channels is the sound-proof door opening, leaving the opaque plastic screen in place. Then pressure in both nasal passages rises simultaneously (at the first vertical dashed line) to a point where the dolphin begins making a series of sonar pulses (P) that she uses to identify the target. These pulses are recorded on both a wide bandwidth (high frequency) hydrophone and a low frequency hydrophone. The dolphin (BRT) was trained to produce a whistle to indicate that she had identified the target. In preparation for producing the whistle (W), she raises the pressure to a level that is almost twice the pressure used for generating the sonar pulses. The whistle registers easily on the low frequency hydrophone. After the whistle, the internal nasal pressure drops to the basal level (second vertical dashed line). The pressurization event (between the two vertical dashed lines) lasts 1.1 s. During the whistle, the maximum intranarial pressure difference reaches 121.8 kPa (915.8 mm Hg) above the basal pressure, which remained at 8.44 kPa (63.5 mm Hg). B. Simultaneous event records for cyclic motions of the phonic labia seen through the endoscope (E), and acoustic pulses recorded by an underwater hydrophone (H). These events are apparently coupled because their rates change together. These observations are of the right phonic lips in the dolphin SAY.

anterior labium and posterior labium; in each set, a phonic fissure runs between these labia where they meet along their length. Right and left, the sites of click generation lie along these phonic fissures. Odontocetes produce sonar clicks by pushing air across the phonic labia and through the phonic fissure, which vibrates the labia and the associated tissues (MLDB complex). Through direct endoscopic observations, we have identified the source and site of bottlenose dolphin sonar signals to be the labia of the phonic lips, at various locations along the length of the phonic fissures. These observations comprise more than two thousand click-trains, over fifteen thousand individual clicks, and about six hundred whistles. Fig. 1A shows a representative recording of a trial.

3.1. Site of click generation

One set of endoscopic observations required the dolphin to station at the side of the enclosure and produce pulses *ad libitum*, instead of performing an echolocation task. An acoustic pulse occurred coincident with each cycle of the phonic labia. A cycle begins with the phonic labia parting at the phonic fissure, followed by an eruption of air and serous fluid spewing from the phonic fissure, and concludes with closure of the phonic labia. Fig. 1B shows that changes in the cyclic rate of these phonic labia are simultaneous with the repetition rate of acoustic pulses recorded on the hydrophone. The rates and periods of the two types of events are synchronous; we conclude that they are coupled. Several of these opening/closing cycles of the phonic labia can be viewed in the supplemental video clips (see video clip #1 and video clip #2). Fig. 2A and B show some details of video clip #1, including the location of the phonic fissure between the labia of the phonic lips.

During the first set of observations we used one endoscope. After some practice, we could reliably move the endoscope to see where tissue movements were synchronous with the production of acoustic pulses. The synchronous vibrations always occurred along the span of either left or right phonic fissure. We never observed simultaneous vibrations in any other location, despite probing every other portion of the contiguous air cavities, except those that were inaccessible (nasofrontal and premaxillary sacs). Contrary to the suggestion of Purves and Pilleri (1983) and Purves (1966), there were no perceptible differences in the sequence of events that accompanies the production of clicks, whether for sonar or ad libitum.

The tip of the endoscope often lay near where some movement could be detected while sounds were being generated, but that movement was not exactly within the field of view. This usually occurred when the tip of the endoscope was within a centimeter or two of the site of sound generation. In these instances, we could often move the endoscope laterally along the phonic fissure to find the generation site where the labia parted cyclically as air and fluids were now visibly expelled. The sound generation site was small, merely 2–4 mm across. The site was not always in exactly the same location along the phonic fissure, which are approximately 1.5 cm (left) and 2.5 cm (right) long in bottlenose dolphins (Cranford, 1992). The dolphins appeared to be able to change or adjust the site of sonar signal generation along the length of each phonic fissure.

3.2. Verification of the sound generation site by interference

We verified that the site of sonar signal generation is the phonic labia by using the endoscope to interfere with the process. To do this, we placed the tip of our endoscope between the phonic labia to interfere with sound generation, and then retracted it to eliminate the interference. In these instances, we used the endoscope in the retroflexed position, with the tip turned 180° into a J-shape so it looked back on itself. We found that if the endoscope was straight the dolphins quickly learned to shunt it aside, to the nearest lateral corner of the spiracular cavity, in order to comply with the trainer's request to produce sounds. We do not know how the
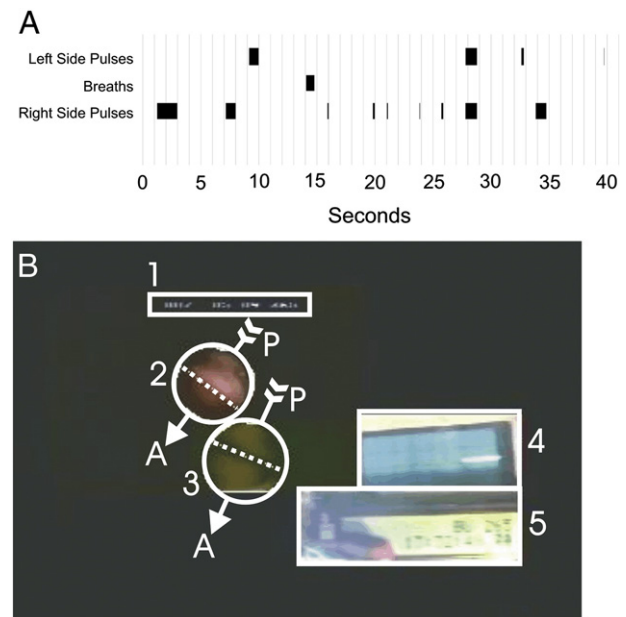
Fig. 2. A. Synopsis of a 40-second segment from a dual endoscope event record (seen in video clip #1). This is from a single trial of ad libitum sounds by the male dolphin (BUS). Event types are: taking a breath, or generating pulses with the right and/or left phonic lips. B. Frame components of video clip #1. Block #1 represents the frame counter. Two circles, labeled #2 and #3, show views through the two (3.8 mm diameter) endoscopes of the right and left phonic lips respectively. The dotted line across each circle indicates the phonic fissure, the boundary between the anterior labium and posterior labium phonic labia of each set of phonic lips. The arrows point from "P" posterior to "A" anterior. Block #4 shows the oscilloscope screen that displays the high frequency hydrophone's trace of acoustic pressure. Block #5 displays the subject's name, the date and time stamp, and the trial number. In block #4, during the video clip a flat line indicates quiet on the oscilloscope screen, but clicks are deflections that briefly occupy part of the display.

dolphins pushed the endoscope aside. However, the muscles in this region are arranged in layers with fan-like origins and can produce facile and complex multi-directional movements (Heyning, 1989; Lawrence and Schevill, 1956; Mead, 1975).

The dolphin could not move the retroflexed endoscope enough to avoid its interference. A few trials were required to pinpoint the sound generation site to a narrow region along the length of each phonic fissure, where the anterior and posterior phonic labia come into contact with one another. During these interference experiments, the dolphins were asked to generate clicks but could not do so, instead producing a raspy series of ineffectual "Bronx cheer" type sounds (Evans and Prescott, 1962).

3.3. Multiple pulse generation sites

Using simultaneous endoscopic observations of both (left and right) phonic lips, we observed directly that bottlenose dolphins possess at least two, separately controllable click generators. Two small endoscopes (3.8 mm OD) were placed at the same depth (2.5 cm) below the blowhole, each aimed at one set of phonic labia. The adult male dolphin (BUS) was cued to produce clicking sounds ad libitum. One particular trial, lasting 2:35 min, contained 29 distinct sound generation events. The entire (2:35 min) trial shows that four sets of pulses were generated at the phonic fissure on the right, after which a whistle was generated at the left phonic fissure. Of the next seven multi-pulse events, two were produced on the left. After taking a breath, the dolphin produced nine more multi-pulse events; one occurred on both sides simultaneously and two were exclusively on the left. After another breath, BUS made a final sequence of six pulsed

events from the right. To augment this paper, we are happy to provide inquirers with the entire video trial for further examination. Here though, we selected a 41-second segment (video clip #1) from this 2:35 min trial, that shows clicks generated independently from the left phonic lips (4 click trains) and the right phonic lips (9 click trains). There is one instance in this video segment where it appears that both phonic lips are operating simultaneously. But the whistling heard in the video clip came from the trainer's whistle, not from the dolphin. The relative timing of the events in this video clip can be found in Fig. 2A and a map of the video frames is in Fig. 2B.

Our video record establishes that our dolphins are capable of producing acoustic pulses from both phonic lips, independently or simultaneously. We have not seen evidence that these animals can produce clicks simultaneously from multiple locations along a single phonic fissure.

That dolphins use multiple sound generators is also borne out by the acoustic "signatures" in our recordings (Figs. 3 and 4). When we tested echolocating animals, the positions of both the recording hydrophones and the dolphins were kept constant. Consequently, the variations seen in the acoustic characteristics are due to changes in the outgoing signal or its propagation pathway, not to changes in recording positions within the sound field.

Plots of peak frequency versus estimated radiated acoustic power are shown in Fig. 3. We applied the directivity index of 25.8 dB, as measured by Au and colleagues for bottlenose dolphins (Au et al., 1986), to estimate radiated acoustic power, rather than measure the directivity index for each subject. In Fig. 3, panel 3A shows the collected results for all three dolphins, while panels 3B, 3C, and 3D separately plot the color-coded results for each individual dolphin. In all three dolphins there was a distinct tendency for the peaks to fall

into two broad frequency bands. These bands or clouds of plotted points are centered above and below 70 kHz and may indicate sound source signatures. Despite this central tendency, one can easily distinguish individual differences in Fig. 3. For example, the female (SAY) plotted in yellow, produced high-frequency/high-power signals almost exclusively. This female had many years of experience performing echolocation tasks. She also used the fewest clicks to successfully complete tasks.

Similarly, the peak frequency for all clicks can be displayed in a histogram for each of our three individual bottlenose dolphins (Fig. 4). All three animals produced clicks with either of two spectral peaks. Although there is substantial overlap in the position (frequency) of the peaks, there are clearly individual differences, as seen by comparing the three dolphins in Fig. 4.

A single sonar click train can contain two spectrally stable peaks. These spectral frequency peaks can occur simultaneously, in isolation, or in succession. Dolphins can switch from one peak to the other, or differentially emphasize both peaks within a single click train. Fig. 5A shows the spectrum for each click in a train produced by a single stationary animal. In this figure the click spectra are displayed according to the click number in the train with a constant offset from one another, rather than separated by their actual timing. The entire 0.78-second click train begins with a single high-frequency click, after which comes a series of 4 clicks with low peak frequency spectra, followed by 2 clicks with bimodal spectra of nearly equal magnitude, then 17 clicks with high-frequency emphasis, where each spectrum contains both high and low frequency components of varying magnitude.

So, while it is clear from Fig. 5A that the spectral frequency emphasis or "tone" of each click in a train can vary within certain limits, the "tempo" or repetition rate of click generation can also be
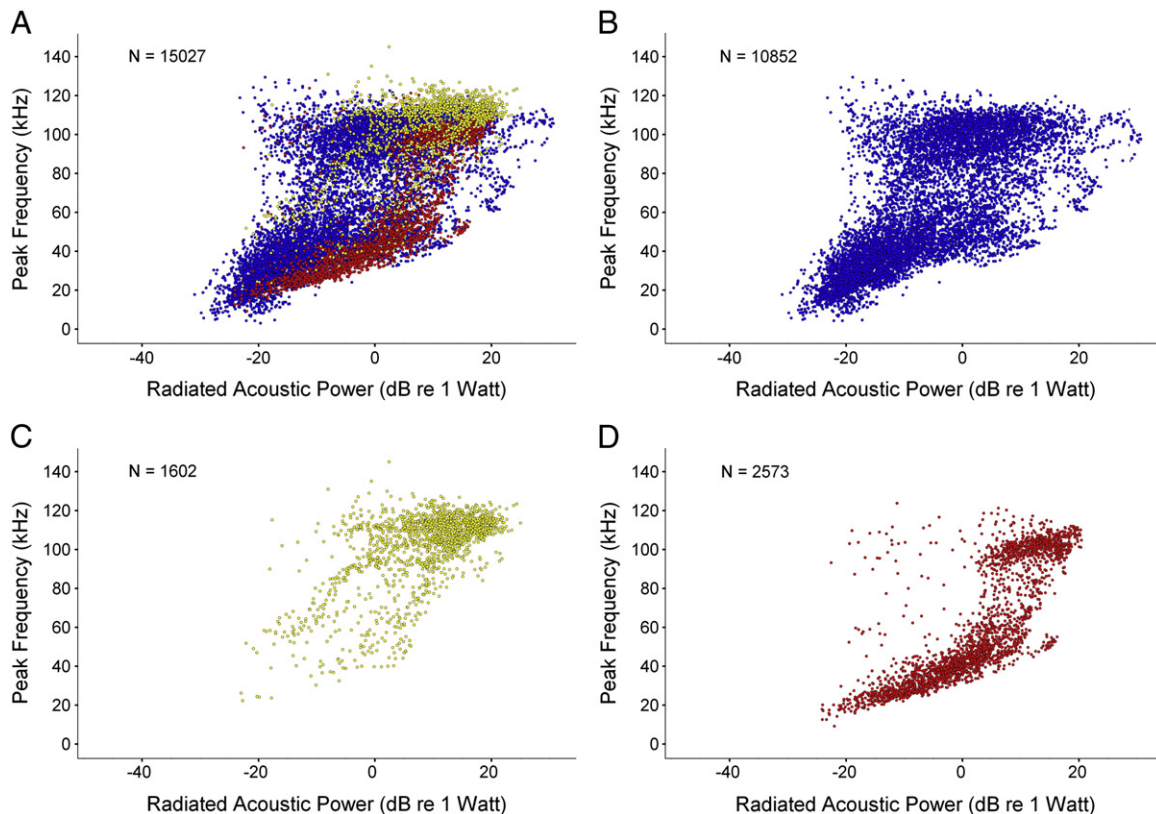
Fig. 3. Peak frequencies of echolocation clicks plotted against radiated acoustic power for 15,027 clicks. Panel 3A shows the collected plots of echolocation clicks for all subjects, color coded for each individual dolphin. Panel 3B shows in blue the plots for the adult female (BRT). Panel 3C shows in yellow those results for the adult female (SAY). Panel 3D plots in red those for the adult male (BUS).
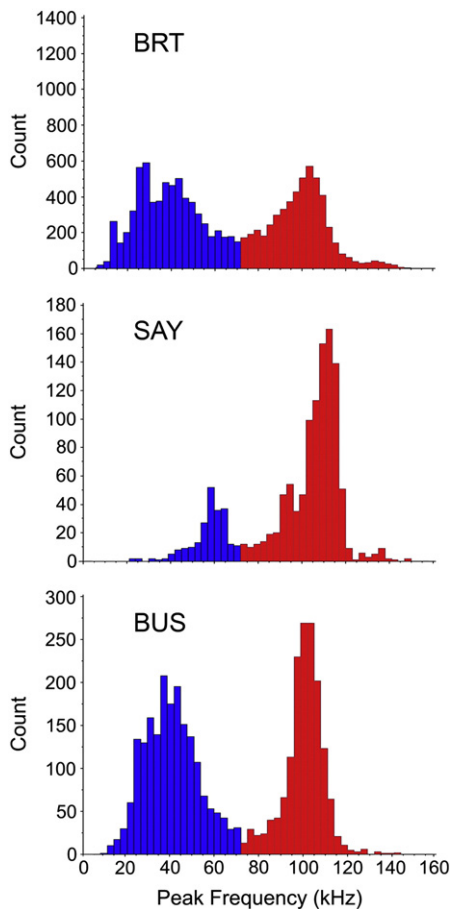
Fig. 4. Histograms of the highest peak frequency for each click from three individual dolphins (BRT at the top, SAY in the middle, BUS at the bottom). The colors distinguish the bimodal nature of the histograms for the three dolphins. Although there are individual differences, each peak fell into one or the other of two frequency bands indicated by different colors.

adjusted by the dolphin. Fig. 5B shows the acoustic power and intranarial pressure being adjusted over the course of clicks in a train. This train of 92 clicks was generated in 1.84 s. The clicks are represented by a series of circles that are laid upon a line showing the elapsing time-course of the train. The circles are black as the clicks increase to peak power and pressure, and change to gray as power and pressure falls off. These changes in the physiologic parameters suggest a sound generation apparatus that must be started, ramped up to some functional performance level, and then shut down. Fig. 5B shows a system that begins (lower left) at low power and pressure, runs up to high power and high pressure (upper right), and then shuts down, all in less than two seconds. This was not the only start-stop pattern we observed, but it was the most prevalent.

3.4. Collateral events

We observed the phonic labia to vibrate only in synchrony with the pulse repetition rate. We did, however, make other endoscopic observations that bear on our understanding of sonar signal generation.

The formation of parallel furrows on the posterior mucosa of the spiracular cavity, immediately ventral to the phonic lips, is foremost among these collateral events (see video clip #3). We saw these furrows on two occasions, once during a simple endoscopic exploration of the nasal apparatus. On this occasion, the furrows were formed by ridges that emerged suddenly and in parallel on the mucosal surface. Three or four of the ridges could be seen across the endoscope's field of view, about one millimeter apart. Since they

occurred during a time when the dolphin was not making sounds, the furrows did not appear to be the result of air flowing over the surface of the mucosa. In the span of a few seconds, one or more sets of ridges and furrows formed in varying directions simultaneously and instantaneously. Although the ridges and their corresponding furrows were parallel, they were not always in straight lines. Sometimes curving parallel ridges and furrows formed. The furrows appeared as if they were made by fibers being drawn taut within underlying layers of the mucosa. The origin or mechanism of this remarkable capability remains unknown.

Another time when we observed the formation of parallel furrows and ridges was during the interference experiments, when the endoscope was retroflexed. During those sessions, the optical tip of the endoscope often lay just below (ventral to) the phonic labia, when the trainer requested a series of pulses from the dolphin. Just prior to the animal's attempt to make pulses, the parallel ridges and furrows formed on the posterior wall of the mucosa, always in straight lines and perpendicular to the long axis of the phonic fissure. In these cases, the furrows and ridges ran to the phonic labia, but the video resolution did not allow us to discern whether the furrows matched the permanent grooves that traverse the labia themselves (Cranford et al., 1996).

Another set of intriguing collateral observations occurred during endoscopic investigation of the nasopharynx during sound generation. The process of making clicks and whistles is apparently powered in part by the nasal plug muscles, the muscles of the face (Mead, 1975) and in particular by contractions of the palatopharyngeal muscle (PP) complex (Dormer, 1979; Green et al., 1980; Reidenberg and Laitman, 1987; Reidenberg and Laitman, 1994). While the endoscope peered into the bony nasal passages as sound generation commenced, the PP muscles contracted, and the bulging muscle bellies rapidly approached the endoscope's lens. As the muscles relaxed and retreated from the lens, sounds ceased, and a dense cloud of vapor formed within the bony nasal passages. As soon as the muscles contracted again and moved toward the endoscope, the vapor cloud disappeared, indicating that vapor formation was mediated by pressure (e.g., adiabatic cooling) (Coulombe et al., 1965). Endoscopic observations also revealed that the pumping action of the PP muscles, and the concomitant rise in pressure in the bony nasal passages, is part of the power source that pneumatically drives sound production.

Finally, we have seen that the nasal apertures of the pharyngo-tympanic tubes (Eustachian tubes) close just prior to the onset of sound generation. This is a necessary precursor, because the Eustachian tube opens into the pressurized volume in the bony nasal passage at the angle of the Eustachian notch of the pterygoid bone (Mead and Fordyce, 2009).

4. Discussion

Early studies, relying on remote or indirect observations, could not determine if dolphins generated sonar signals at one or more exact sites within the head. For a few decades, investigators incorrectly identified the nasal plug nodes as the site of signal generation (Diercks et al., 1971; Dormer, 1979; Evans, 1973; Evans and Prescott, 1962; Evans et al., 1964; Mackay, 1980; Mackay and Liaw, 1981). Our study offers direct multi-modal evidence that bottlenose dolphin sonar signals are generated by pneumatically driven cyclic actions of the phonic labia, components of each set of phonic lips. We have also found strong evidence for at least two sonar signal sources, the left and right phonic lips. Additional information on the anatomy of this region can be found in these references (Cranford, 1999a, 1999b; Cranford et al., 1996, 2008b; Mead, 1975).

4.1. Site of sonar signal generation

In the bottlenose dolphin, each (right and left) set of phonic lips is at the center of a tissue complex (MLDB) described by Cranford
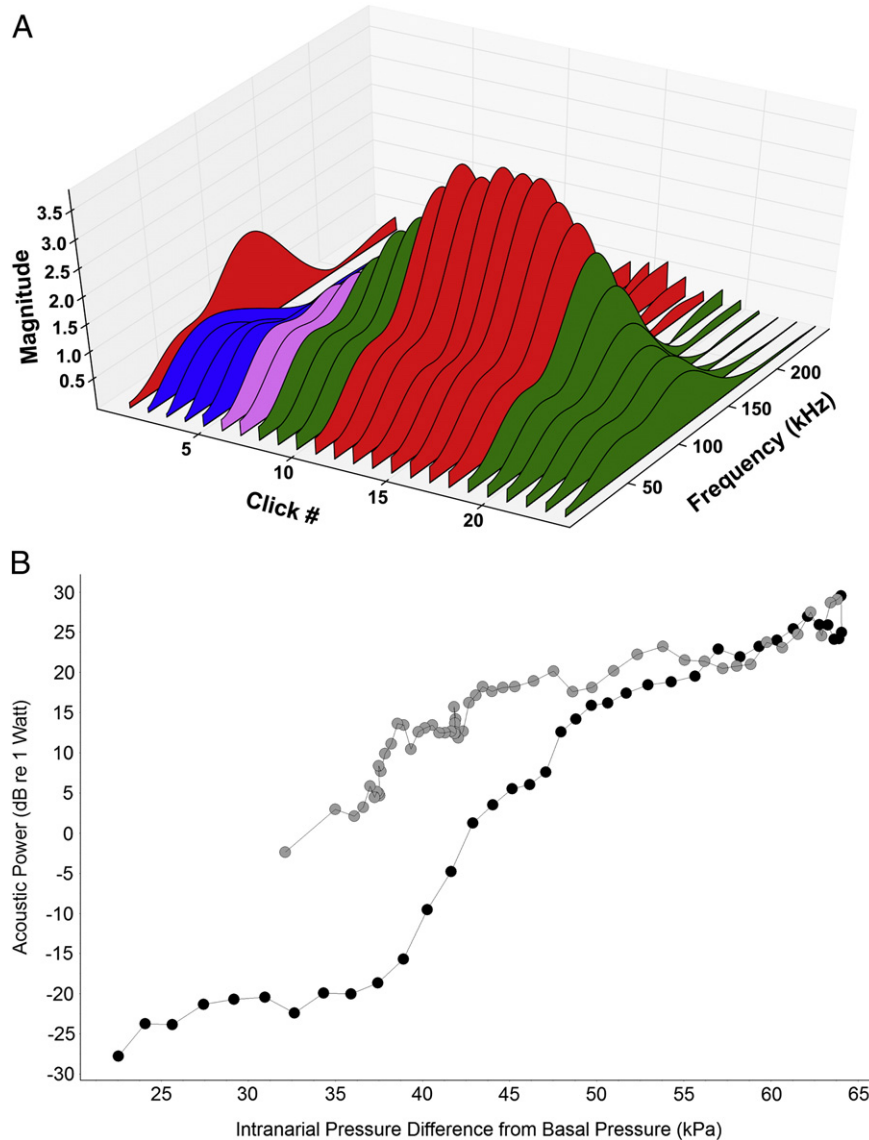
Fig. 5. A. The 24 clicks shown here were generated in one 0.78-second train by SAY during a biosonar trial. Each colored trace shows a click's spectral frequency composition and relative amplitude along that spectrum. The clicks are separated using a constant offset in this display, rather than the actual temporal spacing. Differences in the number of peaks and their frequency let us sort each click into these color-coded classes: Blue = unimodal low-frequency, Red = unimodal high-frequency, Green = dual-peaked with high-frequency dominance, Pink = bimodal peaks. B. The dots in this display represent clicks in a train from BRT for one trial. They show the progression of click generation as the dolphin adjusts the intranarial pressure and the acoustic power output. The pulse train begins at the lower left origin of the graph (black dots) and ramps up to the high-power and high-pressure clicks in the upper right, after which, as the train continues, they fall off (gray dots) to end at higher levels than where they began. The 92 clicks in this trail were generated in 1.84 s.

(1992). We can now report that click sounds are produced as air is pushed across the vibrating labia of the phonic lips. We have examined the nasal anatomy of 32 species of extant Odontoceti, including at least one example apiece from seven of the ten families (Physeteridae, Kogiidae, Ziphiidae, Pontoporiidae, Monodontidae, Delphinidae, and Phocoenidae). All of the specimens except sperm whales (Physeteroidea = Physeteridae + Kogiidae) possess bilaterally arranged phonic lips with a complement of associated structures: the MLDB complexes (Cranford, 1988, 1992; Cranford et al., 1996). Only the physeteroids deviate dramatically from this morphological pattern (Kernan and Schulte, 1918; Klima et al., 1986a, 1986b; Morris, 1973, 1975; Norris and Harvey, 1972; Raven and Gregory, 1933; Schenkkan and Purves, 1973). Physeteroids have only one set of phonic lips associated with the increased complexity of the right side of the nasal apparatus. This complexity itself reflects the extreme enlargement (by hypertrophy or hyperplasia) of the nasal soft tissues on the right side (Cranford, 1999b), along with some diminution or

alternate specialization of the left side. The diminutions of the left side indicate acoustic and respiratory functional specialization rather than atrophy (Cranford et al., 1996). For example, the sperm whale's nasal anatomy has been specialized for breathing (on the left) and sound production (on the right) (Møhl, 2001; Møhl et al., 2003; Møhl, et al., 2000). Consequently, no obvious homologue of the left phonic lips in other whales can be found in the three living sperm whale species. Since all odontocetes share this basic nasal structural unit (the MLDB complex) (Cranford et al., 1996), whether bilateral or unilateral (Norris and Harvey 1972; Møhl 2001), one may surmise that it is the likely source of impulse sounds used by all extant toothed whales and that its precursor existed in a common ancestor.

Many aspects of the sound generation anatomy have been described (Evans and Maderson, 1973; Green et al., 1980; Heyning, 1989; Lawrence and Schevill, 1956, 1965; Mead, 1975; Schenkkan, 1971, 1973; Schenkkan and Purves, 1973; Raven and Gregory, 1933; Norris and Harvey, 1972). More recently, others have described the
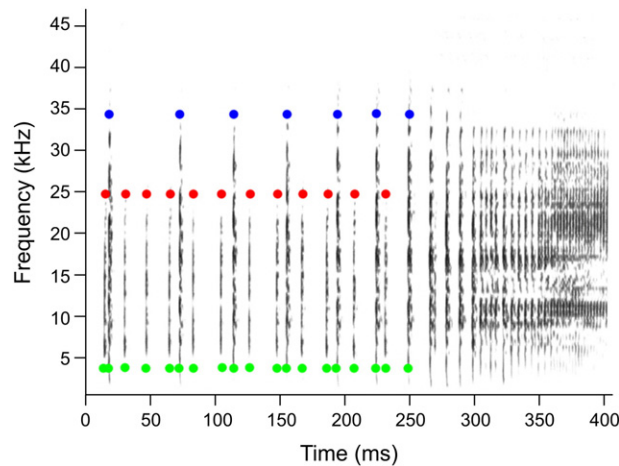
Fig. 6. Periodicity of pulse train components indicates two independent click sources. This pulse train was recorded from a stationary solitary adult female bottlenose dolphin (Scylla). During the first 250 ms, the accumulated pulses appear to have an irregular rhythm (green dots). Alternatively, this irregular train of clicks could be interpreted as being separated into two component trains, each with a different rate. In that case, the rate of the blue-dot click train is increasing, while the rate of the red-dot click train is decreasing. Beyond 250 ms the trains appear to become synchronous and the repetition rate rises rapidly into a pulse burst with harmonic structure. Dr. William E. Evans kindly provided us with this record.

sound generation complex quantitatively, in terms of structural shapes, sizes, and material composition (Cranford et al., 1996, 2008b; Soldevilla et al., 2005). Still others have offered details about the interfaces between structures (Harper et al., 2008; McKenna, 2005, in press). The microanatomy of the phonic lips and the other tissues comprising the sound generation apparatus have been investigated in dolphins by Degollada (1998) and in harbor porpoises by Prahl (2007) and Prahl et al. (2009). They both have reported a wealth of information that bears upon our results here, so we will summarize some of their most pertinent findings.

Degollada (1998) and Prahl (2007) found that the epithelium of the phonic labia along the region of the phonic fissure is keratinized and thickened, with many compact cell layers. This specialization resembles the structure found in the phonic lips of the sperm whale, as reported by Norris and Harvey (1972), who referred to it as the *museau de singe* rather than the phonic lips (Cranford, 1999b).

Degollada and Prahl independently described the permanent parallel ridges and grooves of the dermis and epidermis in the phonic labia (Degollada, 1998; Prahl et al., 2009). These are likely to be the grooves we see in the epithelium of the phonic labia during dissection. But they are not related to the formation of the dynamic furrows we see in video clip #3, that form in the mucosa of the posterior wall of the spiracular cavity (immediately ventral to the phonic lips). These dynamic furrows more likely reveal the action of muscles, acting on what Degollada calls the Laminar Fibrous Complex, which he described in detail (Degollada, 1998). According to Degollada and Prahl, innervation of the MLDB complex is significant. They note that the nerve fibers terminating in this region are much more numerous than in the surrounding tissue (Degollada, 1998; Prahl et al., 2009). Degollada and Prahl have also corroborated earlier findings of Evans and Maderson (1973), identifying glandular tissues just ventral to the phonic lips. These tissues likely produce the copious amounts of fluid that spew forth from between the phonic fissure when clicking sounds are generated (seen clearly in video clip #2), probably lubricating the vibrating phonic labia.

The evidence that sonar pulses in the bottlenose dolphin are generated using a pneumatic mechanism along the phonic fissures that are formed by the phonic labia, is unequivocal. Partly, it is based on the structural similarity of the nasal (MLDB) complexes across the Odontoceti (Amundin and Cranford, 1990; Cranford, 1992; Cranford et al., 1996). And now, using direct endoscopic observations, we have corroborated the functional and physiological findings of Ridgway and colleagues, as well as those of Amundin and Andersen (1983) and

Ridgway et al. (1980). Amundin and Andersen (1983) also palpated vibrations coming from the right side's phonic lips area during pulse generation. Our pressure catheter recordings demonstrate that click generation is powered by the compression of air within the bony nasal passages and basicranial space, primarily by the pumping action of the palatopharyngeal muscle complex, but likely also with the aid of other muscle groups such as the laryngeal and gular musculature (Dormer, 1979; Green et al., 1980; Lawrence and Schevill, 1965; Norris et al., 1971; Reidenberg and Laitman, 1987, 1994; Huggenberger et al., 2008). Previous studies have arrived at similar conclusions using nasal pressure catheters and electromyography (Amundin and Andersen, 1983; Ridgway and Carder, 1988; Ridgway et al., 1980). Endoscopic observations within the bony nasal passages during pressurization of the air, explain the closure of the pharyngotympanic tubes (Eustachian tubes) by muscle action as pressure increases. The implication is that closure at the nasal orifice of each tube is required to help maintain the increased nasal air pressure used for sound generation.

The most compelling evidence for the site of sound generation was gleaned by our high-speed video endoscopy. First, the event synchrony shows that the acoustic signals and the activity at the phonic fissure are coupled (Fig. 1B). Second, the interference experiments pinpoint the phonic labia as the location of pulse generation. And finally, despite extensive exploration of all respiratory passages between the larynx and the blowhole, we did not find any other tissues vibrating synchronously with sound generation events.

4.2. Mechanism of sonar signal generation

The mechanism by which odontocetes generate their sonar signals has attracted considerable investigative attention, primarily in conjunction with the search for the location of the signals' source(s) (Cranford, 2000; Cranford and Amundin, 2003; Cranford et al., 1996; Evans, 1973; Madsen, 2002; Madsen et al., 2010; Norris, 1964, 1968, 1969, 1975; Norris and Harvey, 1974; Norris et al., 1972; Popper, 1980). Three primary nasal mechanisms have been proposed over the first 50 years of research into this question. Cranford (2000) and Cranford et al. (1996) reviewed and discussed these mechanisms and concluded that the "pneumatic mechanism" was more plausible than the friction-based stridulatory mechanism (Evans, 1973) or a cavitation-based mechanism based upon the synchronous collapse of bubbles (Goodson et al., 2003).

Fletcher (1992) calls a mechanism that is driven by air pressure a "pneumatically excited sound generator." In these systems, such as

the vocal folds of the human larynx (Titze, 1976, 2002) or the thin vocal membranes of bat larynges (Suthers, 1973, 1988) or the tympaniform membranes in various configurations of the avian syrinx (Suthers, 1988, 1990; Suthers and Zollinger, 2004), air flow sets membranes into oscillation. In odontocetes the mechanism is somewhat different because the vibrating components of the MLDB complexes are orders of magnitude greater in mass than the vibrating membranes used in air by the larynx/syrinx of other animals (humans, bats, and birds). Coupling sound-generating vibrations to water rather than to air requires a different set of parameters, primarily those producing power in the outgoing signal and coupling it effectively to water.

The sound generation mechanism in odontocetes, when viewed through the endoscope, appears to be similar to the action of the lip-reed of human brass players (Fletcher and Rossing, 1998; Stevenson, 2009), essentially a pressure-controlled valve formed by the two lips. According to Fletcher and Rossing (1998), "the motion of the valve is controlled by the pressure difference across it, and the frequency with which it vibrates is controlled partly by its own natural frequency and partly by the resonances of the instrument air column to which it is connected." In the case of dolphin click production, it is the vibrations within the tissue that are eventually coupled to the water, instead of those in the air column. We cannot be certain what the "resonances of the instrument" are in dolphin click production. But since the clicks originate from, and propagate in soft tissue, it is there that functionally analogous structures will likely be found. One place to begin the search for these analogs is at the boundaries between tissues and organs, like the melon, and the acoustically significant voids of the nasal air sacs.

The phonic lips of the dolphin apparently operate similarly to the lips of human brass players, the human larynx, and the avian syrinx. Theoretically, pressure-controlled valves fall into three simplified model types according to how they react to constant pressure applied to the inlet and outlet ports, only two of these three concern us here. The two applicable models are: (1) the "outward-striking" or "outward swinging door", which will tend to be forced open when the inlet pressure is positive and the outlet pressure is negative; or (2) the "sideways-striking" or "sliding door", which will tend to be forced open when both inlet and outlet pressures are positive, though still with a pressure differential (Fletcher and Rossing, 1998). We believe this second model resembles what we have observed in the dolphin, but we are cautious because the view from the endoscope is essentially two dimensional and real-world valves may not quite match theoretical constructs.[1]

The process in a human brass player begins with a build-up of pressure behind the valves (lips) until the closing force is overcome and the lips swing forward (open). The air rushes out through the opening between the lips, the pressure drops, and the lips close, partially due to the suction created by the pressure drop and the springy nature of the lips under muscular tension. Compared to the human lip reed, in most odontocetes the posterior labium of each set of phonic lips has the potential to be more mobile, and perhaps freer to vibrate than the anterior labium. This reasoning includes these facts: the posterior labium of each set of phonic lips is part of a peninsula of soft tissue that is bounded by nasal diverticula and passageways of air that may facilitate vibrations (Cranford et al., 1996), whereas the anterior labium is integrated into a large inertial mass of forehead tissue containing the melon. In effect, the dolphin's phonic labia may act like a hammer and anvil, more akin to the theoretical "sliding-door" model than to an "outward-swinging" door pressure-controlled valve system (Fletcher, 1993; Fletcher and Rossing, 1998).

Dubrovskiy and Giro (2004) and Dubrovsky et al. (2004) tested a physical model of the click generation process. Their simple model consisted of a valve formed by a compression ring around an underwater tube that was connected to a source of compressed air. They state, "The oscillating ring is the source of acoustic clicks, which are time-locked to the bends of the displacement curve." The "bends" they refer to are the maximum and minimum points or "inflection points" in the graph of displacement for a point on the outer edge of the compression ring. "Because the acoustic pressure gradient is proportional to the second time derivative of the displacement, clicks arise at the bends of the displacement waveform" (Dubrovsky et al., 2004). This bending mechanism is appealing because it can be scaled to account for odontocetes of vastly different sizes, and the basic model is elegant in its simplicity.

Cranford, Amundin, and Norris (1996) offered their "unified hypothesis" for odontocete pulse generation, a pneumatic mechanism where the phonic lips (previously known as the "monkey lips") are driven by a pressurized air stream from the bony nasal passages. They proposed:

> "In our scenario, the generators are driven pneumatically by pressurized air from the nares. Sounds are generated as the airstream is forced between the monkey lips, setting an MLDB complex (or portions of it) into vibration. The expended air is then captured by the vestibular air space, for recycling, as shown by Norris and his colleagues ('71). The periodic opening and closing or smacking of the monkey lips together should break up the airstream and determine the click repetition rate of the train. The analogy of a trumpet player comes to mind here because human lips break up the airstream in a similar fashion (Martin, '42a,b; Mackay, '80b). The difference is that the musician is concerned with the consequent vibrations set up in the air (Martin,'42), whereas for the toothed whales the vibrations set up in the tissue will be most effectively transmitted into the aqueous environment (Amundin, '91b). This is primarily because the soft tissues (and especially acoustic fats) have acoustic impedance values close to water."

Our endoscopic observations provide evidentiary support for such a hypothetical mechanism. We envision a similar mechanism, one that compresses the air, initiates pneumatic sound generation events in tissue vibrations, and partially controls the repetition rate, a mechanism similar to the lip reed of human brass players (Chen and Weinreich, 1996; Fletcher and Rossing, 1998; Fletcher and Tarnopolsky, 1999; Martin, 1942; Newton et al., 2008; Stevenson, 2009).

The palatopharyngeal muscle complex originates within the bony nasal passages (Green et al., 1980; Lawrence and Schevill, 1965) and forms a sphincter that wraps around the extended anterodorsal spout of the larynx, which is composed of the paired corniculate cartilages and the epiglottis (Reidenberg and Laitman, 1987, 1994). The palatopharyngeal muscle complex elevates the spout of the larynx into the internal bony nares or "choanae" of the basicranium. This motion, along with an observable bulging of the contracting muscle belly, appears to compress the air trapped between the rigid walls of the bony nares by the spout of the larynx and the sphincter portion of the palatopharyngeal muscle complex, in a space capped off dorsally by the nasal plugs. This compressed air appears to be the primary source of sound-generating power for the labia of the phonic lips. Additionally, dolphins apparently can 'charge' or 'recharge' the nasopharynx with air from the lower respiratory tract by opening the laryngeal spout and moving air from the lower airways.

4.3. Kinds of odontocete clicks

The clicks produced by odontocetes are of several sorts (Cranford, 2000; Evans, 1973; Kamminga, 1988; Norris, 1969; Popper, 1980; Tyack and Clark, 2000), differing largely by their repetition rate,

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[1] Model type 3, as described by Fletcher and Rossing (1998), is a theoretical valve that works similar to the reed of woodwind instruments. This mechanism is not applicable to odontocete sound production.

acoustic frequency spectrum, and apparent or associated function. Any proposed sound generation mechanism must account for these differences in the kinds of sounds made. The most parsimonious view is that all such clicks, regardless of repetition rate or function, are likely generated at the same locations (along either or both phonic fissures) and largely by the same (pneumatic) mechanism.

Recording opportunistically in the wild without control over the relative position between the animal and the recording transducer probably accounts for some of the reported spectral variability of clicks. Also, some of the earliest reports relied on recording gear with insufficient bandwidth to capture the primary energy in the highest frequency bands (Evans and Prescott, 1962). Consequently, several of these early reports focused on the low frequency component of the signal. These often audible low frequencies probably include the "machine noise" (Kenneth S. Norris, pers. comm.), that may accompany click generation but seems unlikely to be used for echolocation. Reports of this sort of noise have been largely absent from the literature once researchers began using high frequency recording gear (Cranford, 2000). These low frequency elements have been distinguished and separated from the high frequency ones in porpoises by Amundin (1991a), who used helium-charged air, which has also been used to investigate sound generation in bats (Roberts, 1973) and birds (Nowicki et al., 1989). The early, limited bandwidth recordings suffice to capture repetition rates but not the clicks' broadband spectral properties (Cranford, 2000).

The waveforms of odontocete clicks can be classified very usefully by incorporating information theory (Wiersma, 1988). Wiersma suggests that there are two basic types of sonar signals based upon the time-bandwidth product. Short-duration, wide-bandwidth signals (few cycles or "oligocyclic" clicks) (Cranford, 1992) are those that we commonly record from bottlenose dolphins and other delphinids (Au, 1993). Relatively long-duration, narrow-bandwidth signals (many cycles or "polycyclic" clicks) (Cranford, 1992) are like those recorded from porpoises (Møhl and Andersen, 1973), a few cephalorhynchid delphinids (Kamminga, 1988), at least a few species of *Lagenorhynchus* (Kyhn et al., 2010), and the pygmy sperm whale (Caldwell and Caldwell, 1989; Madsen et al., 2005; Marten et al., 1989; Ridgway and Carder, 2001).

According to Wiersma, both signal types approach the asymptotic curve of the theoretical minimum time-bandwidth product, but in different locations. He states that the minimum time-bandwidth signals are highly detectable even when the noise spectrum is unknown (Wiersma, 1988). So, while dynamic characteristics do unite these two signal types, some species-specific detailed anatomic differences distinguish each signal type (Amundin, 1991b; Amundin et al., 1988; Cranford et al., 1996). Regardless of signal type or anatomic type, all evidence points to a common location (the phonic lips) for the generation of clicks. Indeed, all odontocetes have at least one set of (homologous) phonic lips, along with the associated structural (MLDB) complex (Cranford, 1988, 1992). These homologous locations probably play a similar functional role in the sound generation process across the Odontoceti (Cranford and Amundin, 2003; Cranford et al., 1996).

From what we know about the click generation process; what physiological mechanisms might account for the observed spectral differences in the clicks and their associated anatomic configurations? Factors that can determine the output of any sound source include size, shape, material composition, and driving force characteristics. Of these, we may assume pretty safely that the properties of the tissues comprising the sound source(s) are similar across the Odontoceti, except possibly for the chemical composition of the specialized lipids or "acoustic fats." In addition, the fatty structures (bursae or spermaceti organs) of the sound generation system are similarly shaped across all odontocetes, i.e. elongate rods that are rounded on both ends (Cranford, 1992; Cranford et al., 1996). However, the size range of these fatty structures across the Odontoceti is extreme, from just under 1 cm long in porpoises (Amundin and Cranford, 1990) to

upwards of 20 m long in the sperm whale (Cranford, 1999b; Nishiwaki et al., 1963). Of course, the dynamic particularities of the driving force and any structural adjustments to tension should also significantly affect acoustic output, fine-tuning the generators and their signals or their repetition rates. But, generator size is perhaps the most significant parameter that could affect the spectral properties of the acoustic output.

What could account for the differences between the narrow-band porpoise-like (polycyclic) signal and the broad-band dolphin-like (oligocyclic) signal (Cranford, 2000; Kamminga, 1988)? The long duration (~150 μs) polycyclic signals may tell us that both left and right generators are used with a constant phase shift to form a single, porpoise-like polycyclic click (Cranford and Amundin, 2003), or perhaps the interference between a direct path and a reflection from an air space would accomplish the same result. Madsen and colleagues report that a multiple source mechanism is not required for this signal type (Madsen et al., 2010). One of several potential problems with Madsen's explanation is that the configuration of the air spaces in *Phocoena phocoena* would seem to hinder the direct acoustic pathway out of the head, that Madsen and colleagues believe they have recorded (Cranford, 2011). In particular, the enlarged distal portions of the nasofrontal sacs are positioned immediately anterior to each anterior dorsal bursa (Cranford et al., 1996; Huggenberger et al., 2009). A direct acoustic pathway out of the porpoise head from the phonic lips does seem difficult to imagine anatomically, and polycyclic porpoise clicks may result from interference of more circuitous and/or reflected pathways.

In porpoises (Phocoenidae) and at least some cephalorhynchids like Commerson's dolphin (*Cephalorhynchus commersonii*), both generators are roughly similar in size, shape, and material composition; and so we might expect them both to produce spectrally similar signals (Amundin and Cranford, 1990; Amundin et al., 1988). The narrow bandwidth nature of the polycyclic pulse could then be the "combined" signals from both sets of phonic lips (Cranford et al., 1996) that are slightly shifted in phase (delayed actuation on one side). This might also account for the reduced-amplitude "squiggle" that typically occurs over a couple of cycles within the last third of the waveform in the harbor porpoise and Commerson's dolphin signals (Kamminga, 1988): this oddity, again, may indicate interference between two signals. This is clearly not the case in the pygmy sperm whale (*Kogia breviceps*), which produces polycyclic narrow bandwidth signals but without the squiggle in the waveform. Kogiids have only a single set of phonic lips, and they lack nasofrontal sacs. Direct pathways are possible in the Kogiidae, and the lack of a "squiggle" in their polycyclic waveform indicates a lack of multi-source interference. This exception has been discussed (Cranford and Amundin, 2003). Although the site and mechanism of sound generation in bottlenose dolphins may be the same as it is in the other odontocetes, there are most likely differences in what happens to the signal along the sound propagation pathways that affect the biosonar emissions. At the same time, let's acknowledge how very little we know about the bioacoustic behavior of *Kogia* spp. and other aspects of its deep-diving teuthophagus habits (Amundin and Andersen, 1983; Caldwell et al., 1966; Goold and Clarke, 2000; Marten et al., 1989; Ridgway and Carder, 2001).

Clearly, the air stream figures importantly in the pneumatic mechanism (Dormer, 1979; Mackay, 1980; Ridgway and Carder, 1988; Ridgway et al., 1980). Two types of small air channels that we report here may significantly affect air flow to and across the labia of the phonic lips. The first type comprises the small permanent grooves that cross the phonic labia perpendicular to their long axis in a wide variety of odontocetes, from dolphins to sperm whales. The details of these grooves have been described from histological sections (Degollada, 1998; Norris and Harvey, 1972; Prahl et al., 2009). Postmortem examinations of various odontocetes confirm the existence of these small, permanent grooves in all 32 species dissected so far (Cranford, 1992). The second set of channels includes the

intriguing ephemeral, dynamic furrows that we have observed endoscopically (video clip #3). It may be that these dynamic furrows control the flow of air generally to the phonic fissures or to specific parts of them. Conceivably, their almost instantaneous dynamic nature could alter the direction or other traits of air flow, depending on demand or other circumstances. One endoscopic session showed that furrow/ridge formation is dynamic, instantaneous, and malleable (video clip #3). The dynamic ephemeral furrows must be driven by muscle action, since they form even in the absence of sound generation or, presumably, air flow. This opens the possibility that air streams could be directed via furrows to either or both sets of phonic fissures—or even to multiple locations along each fissure, although we have not yet seen evidence of this latter suggestion.

Multiple sonar signal generators can function in the bottlenose dolphin. If these animals can produce clicks from more than one site along a single phonic fissure, the possibility arises that sonar signal generation sites are numerous.

4.4. Multiple sites for sonar signal generation

Indirect evidence exists for multiple dolphin sound sources (Cranford, 1999a; Cranford et al., 1996; Evans, 1973; Evans and Maderson, 1973; Evans and Prescott, 1962; Lilly, 1961, 1962, 1966, 1978; Lilly and Miller, 1961; Mackay and Liaw, 1981; Markov and Ostrovskaya, 1990; Murray et al., 1998; Norris et al., 1972). However, until now direct investigations have not established a minimum or maximum number of dolphin sonar signal generators.

Our bottlenose dolphins tended to produce oligocyclic echolocation signals with durations near 50 μs. Some odontocete species appear to use polycyclic echolocation signals that are three times longer (~150 μs) (Amundin, 1991b; Kamminga, 1988; Kyhn et al., 2010; Møhl and Andersen, 1973). Generating those longer duration clicks might require particular mechanisms, such as symmetrical bilateral anatomy, and/or multiple generators actuated with precise, phase-shifted timing. Phase shifting between two sonar sources could explain the peculiar squiggle in the signal waveform of harbor porpoises and Commerson's dolphins. Fig. 6 shows evidence of precise timing between two sources. Our endoscopic observations suggest that oligocyclic clicks in the bottlenose dolphin do not strictly depend on multiple generative sources. But we did find evidence of bilateral sound generators that can be operated independently or synchronously (Figs. 2 and 6, plus Fig. 5A and video clip #1).

Four lines of evidence, enumerated here, indicate two separately controllable click generators in the nasal apparatus of the bottlenose dolphin.

First, odontocete nasal anatomy contains two (MLDB) complexes (Cranford, 1992) that are similar in all non-physeterid odontocetes (Lawrence and Schevill, 1956; Mead, 1975) and are implicated in sound generation. The phonic lips are anatomic landmarks of these complexes, visible at the dorsal boundary of the spiracular cavity, on either side and a few millimeters above the dorsal boundary of the membranous nasal septum. Cranford, Amundin, and Norris (1996) identified these bilateral nasal complexes as possible sonar click generators. The bilateral arrangement of these potential sound generation structures across the Odontoceti prompted our investigation.

In his classic monograph on delphinid facial anatomy, Mead (1975) recognized the importance of the spiracular cavity, and referred specifically to portions of it as the anterior and posterior folds. "It has been generally ignored as an anatomical entity in this group, but is important from a comparative standpoint, as it becomes highly modified in some other odontocetes" (page 9, Mead, 1975). Mead's work is still the best description of delphinid forehead anatomy and was the first to recognize the posterior bursa, a yellowish elliptical fat body that "lies between the blowhole ligament and the posterior wall of the nasal passage, and produces the faint ridge of the posterior fold" (page 9, Mead, 1975). This "faint ridge of the posterior fold" is the structure we now refer to as the posterior labium of the phonic lips.

Mead's taxonomic comparisons of this region and the analysis of Cranford, Amundin, and Norris (1996) imply that natural selection maintains the structural arrangement and composition within some range of tolerance. Otherwise, we would expect heritable "structural imperfections" to accumulate over time. On the contrary, the phonic lips are present and likely functioning similarly in all extant odontocetes (Cranford et al., 1996).

The casual inquirer might conclude that bilateral directional asymmetry in the odontocete nasal apparatus is an indication of atrophy from disuse. But comparisons across the Odontoceti argue that nasal asymmetry, most dramatic in sperm whales, more likely results from selection for different sized sound generators and the concomitant spread in the toothed whales' acoustic spectrum (Cranford et al., 1996; Mead, 1975).

The second line of evidence for dual sonar signal generators in odontocetes, reported here, is the direct and simultaneous endoscopic observations of both phonic lips (video clip #1). We emphasize again that our dolphins were capable of producing acoustic pulses from both pairs of phonic lips independently or simultaneously (Fig. 2A). Our findings corroborate previous work with hydrophone arrays that reported clicks emanating from either or both sides of the head in bottlenose dolphins (Diercks et al., 1971; Evans, 1973; Lilly, 1962, 1978). Mackay and Liaw (1981), using Doppler ultrasound investigations of bottlenose dolphins also reported multiple sound generation sites.

The third line of evidence is based on distinct acoustic "signatures" (Figs. 3 and 4) in recordings made during our observations. The most plausible inference is that the separate peaks in the power spectrum result from different center frequencies generated by different sound sources. The alternative explanation – that these peaks are produced by the same sound source – is less likely, since the peaks do not appear to be harmonically related (Fig. 4).

Plots of peak frequency versus estimates of radiated acoustic power (Fig. 3) show that all three of our bottlenose dolphins demonstrated a tendency to produce peaks in two frequency bands, one above and one below 70 kHz. Despite this shared tendency, they showed distinct individual differences. For example, one female (named SAY), plotted in yellow (Fig. 3), had considerable echolocation experience and produced clicks characterized by high frequency and high power. SAY also used the fewest number of clicks to complete a task. If these bands indicate sound source signatures, then the difference in SAY's behavior, compared to that of the other dolphins, suggests that using both sound sources was not required to recognize the target. The differences we observed in the number and characteristics of the echolocation clicks used by individual animals may be attributable to "superstitious behavior"—behavior which is not required to complete the task but which may have been inadvertently rewarded and inculcated during the long string of approximations that attended training.

The dolphin's ability to switch between spectral peaks within a single click train, as shown in Fig. 5A, may also result from switching between one sound source and another; even further implying two such generators. Those who choose to deny multiple sonar signal generators in non-physeterid odontocetes need to account for the varied parameters we report in stationary echolocating animals.

At this time, we cannot establish a maximum number of sonar sources in the bottlenose dolphin, but we have offered, above, three lines of evidence for at least two separately controllable click generation sources.

• First, Cranford, Amundin, and Norris (1996) have implicated bilateral anatomic complexes, one associated with each nasal passage, as sonar sources.

- Second, direct simultaneous endoscopic observations of both these putative sonar signal generation apparatuses reveal that pulses can be generated from either set of phonic lips (left or right) or both, and from different locations along them.
- Third, a single sonar pulse sequence can contain two stable spectral peaks (Figs. 3 and 4) that can occur simultaneously, in isolation, or in succession (Fig. 5A) (see also Au et al., 1995; Sigurdson, 1997).

A fourth line of evidence comes from an unusual recording, previously unpublished, made by Dr. William E. Evans (pers. comm.). His data bear so centrally on ours that we report them here, with his kind permission. Dr. Evans placed a contact hydrophone on the head of a bottlenose dolphin (named Scylla) in a tank by itself. The animal was stationed within inches of the tank wall, which was lined with sound-absorbing material. As we read it, the intriguing sonogram (Fig. 6) shows a bottlenose dolphin click train composed of two distinctive but complexly intermingled sets of pulses, with independent repetition rates that change in opposite directions. In this example, the clicks in the train also have distinct spectral and amplitude signatures (Fig. 6).

In Fig. 6, the rate of occurrence or "rhythm" across the first group of pulses in this sonogram (as represented by the green dots) is complex and irregular, without an apparent pattern. It seems very unlikely to us that a single biological system could stop and start to produce such an irregular rhythm in a fraction of a second. A more convincing interpretation is that the clicks within the first 250 ms come in two trains with rates that change gradually but are opposite in repetition frequency (represented by the red dots and blue dots). That is, the repetition rate for the red dots increases over time, while the repetition rate for the blue dots decreases. Thus these click trains seem to reveal two independent pulse generators in this solitary, stationary animal. Now consider what happens to these two click trains after 250 ms, as the repetition rate rises dramatically. The two hypothesized generators appear to become synchronized, and their timing precision becomes astounding, achieving an estimated repetition rate of more than 400 clicks per second during the final 100 ms of the sonogram. There may be alternative interpretations for this sonogram; but since the animal is alone and stationary, dual sources may be the most parsimonious explanation.

These observations, those from the literature as previously mentioned, and our direct observations combine to suggest most strongly that bottlenose dolphins have at least two sonar signal generators. The actual degree of independence in these bilateral generators remains open to conjecture and discussion, since the air pressure that drives them appears to rise and fall in both bony nasal passages in unison, as shown in Fig. 1A.

4.5. What can we learn from other physiologic parameters during sound production?

Many animals produce sounds from multiple sources (Frey et al., 2006; Nowicki and Capranica, 1986; Suthers, 1990). But recognizing their very multiplicity in a dolphin, forces us to reevaluate earlier views of the physiological limits on pulse repetition rate, signal bandwidth, frequency composition, and transmission beam geometry.

Our recordings demonstrate that acoustic pulses can be generated by bottlenose dolphins using the asymmetrically sized phonic lips (larger right and smaller left) and associated MLDB complexes. In our dolphins, we observed that whistles were only generated by the left phonic lips but we assume that whistles could be produced on either side because: (1) other than size, the two sound generation complexes are anatomically similar; (2) we observed equal pressurization patterns in both bony nasal passages (Fig. 1A), even if the activity patterns at the two sets of phonic lips are different; and (3) the results reported in the established literature concurs (Lilly, 1962; Mackay, 1980). In contrast to our observations, Lilly (1962, p. 522)

states that one of his bottlenose dolphins tended, "to click only on the left side and whistle only on the right side and can do so on both sides simultaneously or separately."

Can bottlenose dolphins pressurize each bony nasal passage independently? This seems plausible in light of the dual source evidence, and since the palatopharyngeal muscles play a role in pressurization and attach more than three quarters of the way up into each bony nasal passage. Even though we never observed such independent pressurization, nor, to our knowledge, have previous workers (Amundin and Andersen, 1983; Ridgway and Carder, 1988; Ridgway et al., 1980), we surely have not yet seen all of our dolphins' nasal behavior. But we observed more than 15,000 clicks, and we have noted a relationship, albeit weak, between increasing acoustic power, increasing intranarial pressure, and increasing peak frequency (Fig. 3). Au, Moore and their colleagues also observed this (Au, 2000; Moore and Pawloski, 1990).

Whistle production invariably required close to twice the nasal air pressure that pulse generation did (Fig. 1A). Ridgway et al. (2001) reported a similar pattern. Ridgway and Carder (1988) observed pressures as high as 106.4 kPa (800 mm Hg) in the nasal cavity of a whistling white whale (*Delphinapterus leucas*), while Amundin and Andersen (1983) reported intranarial maxima of 80.86 kPa (608 mm Hg) for *Tursiops truncatus* and 54 kPa (406 mm Hg) in *Phocoena phocoena*. The highest intranarial pressure we recorded was equivalent to 164.92 kPa (1240 mm Hg). A full report on our nasal air pressure observations is forthcoming.

How might dolphins adjust click production from two sources to achieve arrival-time differences in the sound field on the order of tens to hundreds of microseconds?

In the bottlenose dolphin, the phonic lips (and MLDB complexes) are bilaterally asymmetric, 5 to 12 cm apart (depending upon the size of the animal), and set at an angle of about 30° to each other (Cranford et al., 1996). The speed of sound in sea water and tissue is roughly 1500 m/s, which is equivalent to about 1.5 mm/μs. This geometric arrangement of the phonic lips (and associated MLDB complexes) is such that switching between left and right sound generation anatomy exercises control over where sounds are generated, thereby adjusting the relative path length for pulses and so the precise time they take to reach any destination. In this paper, we have also reported that bottlenose dolphins are capable of sliding or slightly altering the exact site of sound generation along individual phonic fissures. This apparent millimeter-scale control over the sound generation site could also contribute small changes to the path length and arrival time differences.

For a pair of pulses produced in unison from the two MLDB complexes, the arrival-time difference at any location within the sound field will be a function of the path length from each source. The greatest time difference between the pulses will occur out to the sides, some 90° from the midsagittal plane of the skull, since this maximizes the distance between the sound generators with respect to any point in the sound field. Similarly, the least difference in arrival-time should occur between pulses that propagate directly ahead of the animal, along the primary beam axis, where there is maximum overlap, interference, and sound pressure level (SPL). These suppositions about sound field characteristics from two pulse generators have been corroborated by Lammers and Castellote (2009) in their study of sonar in *Delphinapterus leucas* and by Starkhammar et al. (2011).

Another mechanism that could produce arrival time differences between pulses from two sources was proposed by Cranford et al. (1996) and Cranford (1992, 2000). It would be realized by small delays in the actuation of one in a pair of sound generating phonic labia. For example, small changes to the tension on the posterior labium and bursa by the intrinsic musculature of the posterior nasofrontal sac could retard the repetition rate for the phonic labia on that side, slightly adjusting the timing in a functioning apparatus.

In a sound generation apparatus that ramps up to cycle hundreds of times per second, it is conceivable that small adjustments in the exact site of click generation (combined with the distance and angles between the MLDB complexes), and/or actuation timing of the sound generators, could produce sub-millisecond timing differences in the interference beams from two sources. It is also plausible that these mechanisms could produce two overlapping transmission beams that interfere in the far field to produce movement in the axis of the sonar beam (i.e., beam steering) (Amundin, 1991b) or else a stable beam containing combined spectral properties from the "signatures" of two sources. Finite element modeling techniques could test these sorts of ideas about the actuation and timing between sonar sources, acoustic beam shape, direction, or frequency composition (Cranford et al., 2008a, 2010; Krysl et al., 2006, 2008). Or one might employ, as we and others have (Amundin, 1991b; Au et al., 2010; Moore et al., 2008; Starkhammar et al., 2011), animals exquisitely trained for additional relevant laboratory experiments.

Backus and Schevill showed that sperm whales could time their pulse generation events better than human drummers and could precisely entrain the pulse sequences to an echo-sounding machine (Backus and Schevill, 1966). This lends credence to the suggestion that odontocetes can adjust the rhythm of the clicks within a train to a precision of tens to hundreds of microseconds.

Multiple click generation sites may also help explain how dolphins can achieve click repetition rates that have been reported to reach at least two thousand clicks per second (Herzing, 1996; Lilly, 1962), or perhaps a few to a handful of thousands of clicks per second (Busnel and Dzeidzic, 1966; Murray et al., 1998). True, some findings give one pause. Thus, Weir et al. (2007) report a 1.6 kHz repetition rate for clicks in sperm whales, an animal with only a single set of phonic lips within their gigantic nose and yet a complex sound generation system (Cranford, 1992, 1999b; Norris and Harvey, 1972; Raven and Gregory, 1933; Schenkkan and Purves, 1973). But sperm whales may be a special case, having as they do the world's largest and most powerful nasal musculature.

These high repetition rates suggest that these animals cannot control each pulse independently, because the rate is faster than the highest nerve firing rates. Instead, it would seem that they must actuate their signal generators and then "tune" them to circumstances or needs (Ridgway, 2011). To human ears, of course, quickly repeated pulses merge together into audible tones related to the time between pulses, tones known as "time separation pitch" or TSP. Sonograms show them as harmonic structure, where the frequency represents the repetition rate interval (Murray et al., 1998; Watkins, 1967). But it is important to remember that these are pneumatic pulses, driving the lips to vibrate—and to emit acoustic pulses in response.

Could these multi-kilohertz repetition rates be achieved by multiple sources working in concert? The individual pulses in these bursts are so close together that they are difficult to distinguish. But one might analyze the spectral properties of alternate clicks in such very fast trains to detect more than one sound source "signature." The precise timing required to produce these high repetition rates may seem improbable, but so are the demonstrated rates themselves. At least we know now that the pressures in both bony nasal passages rise and fall together (Fig. 1A). This much of a glimpse into this puzzling phenomenon may help guide us toward eventual clarity.

We know that these extremely rapid pulse trains cannot be controlled one pulse at a time; Bullock and Ridgway (1972) found that the bottlenose dolphin central nervous system simply cannot follow pulse repetition rates higher than 600/s, as shown with evoked potentials in the inferior colliculus.

Can sounds be generated from two parts of a single source—that is, from more than one location along the same phonic fissure at the same time? We cannot eliminate this possibility. We can imitate such a mechanism crudely by pinching our own lips together in the middle while blowing "raspberry" sounds out on either side of the pinch. The odontocete nasal and gular apparatuses are richly supplied with highly mobile muscle groups, some of which are reminiscent of those in the human lips and tongue (Heyning, 1989; Lawrence and Schevill, 1956, 1965; Mead, 1975; Schenkkan, 1973). So very fine motor control in this region is possible. If multiple simultaneous pulse sources are possible along a single set of phonic lips, then the dynamic furrows we have seen (video clip #3) are likely an integral part of that process.

In our own studies so far, we have not observed the sound generation process with enough temporal resolution, or sufficiently under the "burst pulse" condition, to determine whether many click generation sites (more than two) may contribute to a train. To some degree, sonar systems depend on the consistency and stability of transmitted signals, so that changes in the returning echoes reveal changes in the target (e.g., speed, aspect, or distance). Changes in the outgoing signals would seem to complicate matters unless they are integrated into a larger context, perhaps by their very rapidity in pulse trains.

4.6. Bimodal distribution of peak frequency

In an elegant series of experiments, Moore and Pawloski (1990) demonstrated experimentally that bottlenose dolphins generate bimodal spectra and have some control over spectral content and signal amplitude. Evidence also suggests that odontocetes can adjust the frequency spectrum of their outgoing sonar signals in response to tasks or conditions (Au et al., 1985, 1995; Sigurdson, 1997).

We know that the tissue complexes immediately around the phonic lips are moderately asymmetric in most delphinids and many other odontocete species. This nasal asymmetry is termed "directional", meaning that the right side is always larger than the left (Cranford et al., 1996). This asymmetry may explain the bimodal frequency distribution we observed in the sonar pulses in this study (Figs. 4 and 5A), a pattern that has drawn the attention of others too, Au (1993, 2000, 2004) and Starkhammar et al. (2011). The frequency peaks may simply be related to the different dimensions of the fatty bursae within the left and right phonic lips, as may be inferred from Fig. 6 and has been suggested elsewhere (Cranford, 1992, 2000; Cranford and Amundin, 2003). One difficulty with this interpretation is that if these bimodal signals are the combined result of two sources, they would need to be kept precisely out of phase or adeptly synchronized. This precise timing requirement also confronts our proposal for how the polycyclic signals are formed.

When Au and his colleagues conducted their echolocation experiments under stimulus control in a laboratory setting, they recorded from a consistent position in the sound field, and click spectra tended toward standard forms (Au et al., 1995). Their analysis of echolocation clicks from a false killer whale (*Pseudorca crassidens*) sorted easily into four classes according to spectral composition (see their Fig. 2, page 53). Most of their spectra were bimodal, but they also found two unimodal forms. It is possible to align the spectral peaks across all four of their classes. Doing so suggests that, at most, two major peaks occurred, one between 35–40 kHz and the other between 100–110 kHz. These peaks do not appear to be harmonically related. How could these two peaks be produced from a single sound source? Instead, their results may be explained as the acoustic "signatures" of two sonar sources.

This "bimodal signature" notion could also explain the unimodal frequency peaks in our Figs. 3, 4, and 5A: they could result from just one or the other set of phonic lips operating alone. We have seen that dolphins can switch between spectral peak configurations within the course of a single click train (Fig. 5A). Other studies, too, have provided evidence of switching back and forth between dominant spectral peaks within a single click train (Au et al., 1995; Sigurdson, 1997).

Endoscopes, pressure-recording catheters, sonograms, extraordinary experimental animals, and their trainers have combined with

earlier studies to illuminate much about odontocete sonar signal generation. Nevertheless, puzzles big and small continue to challenge us, and their solutions continue to reward the patient investigator.

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Acknowledgments

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