Analysis of Northern Bottlenose Whale Social Clicks to Improve Our Understanding of Behaviour and Regional Population Structure

Scotian Shelf and Newfoundland Populations

Submitted to:

Katherine Hastings Species at Risk Management Division - Ecosystem Management Branch
Department of Fisheries and Oceans Canada

Report for Contract F5299 – 200035

Date: March 26, 2021

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Project Summary

The northern bottlenose whale (NBW), *Hyperoodon ampullatus,* Scotian Shelf Population (SPP), is classified as Endangered under Canada's Species At Risk Act (SARA). In an effort to protect this population, DFO has proposed an action plan for the SSP (2016) outlining the areas where additional research is needed to meet recovery objectives. Key to those recovery measures is understanding the population structure of the SSP and its linkages with other populations in the North Atlantic Ocean. Currently, it is known that the SSP is genetically distinct from whales found in the Davis Strait, Baffin Bay and off Iceland (Feyrer *et al.*, 2019); however, the connectivity between the SSP and the recently discovered aggregation off Newfoundland is less clear. In this project, we aim to describe and analyze NBW *social click* patterns along the Scotian Shelf and Newfoundland. If NBW *click patterns* are socially learned, the spatial distribution of *click pattern* dialects could reveal socially mediated population structure.

Here, we describe methods used to validate and classify *click patterns* in recordings collected by the Whitehead Lab between 2015 - 2019. By incorporating click bearing analysis, we show that the majority of click patterns suspected by listening to recordings were often artefacts: parts of quieter click trains or overlapping click trains produced by different whales. This underscores the importance of validating aurally determined click patterns with directional information. While robust, this protocol yielded a very small sample of click patterns. With this reduced dataset, we found evidence that variation among manually classified click sequences is continuous rather than discrete. Next steps will include to analyze the entire available dataset to supplement our analysis, and to compare *click pattern* repertoires among the SSP and Newfoundland aggregation. We recommend that future analysis of possible NBW *social clicks* incorporates data generated by tag-based studies being conducted in other less-threatened populations, and that methods to stream-line the validation of apparent click patterns be developed.

Objectives

The objectives of this project were to test the hypotheses that NBW (1) have stereotypical social *click* patterns and that (2) these click patterns vary between groups of socially or geographically separated individuals.

Deliverables

- A description of the methods used to validate NBW click patterns
- A description of social clicks documented in the Newfoundland and Scotian Shelf Populations in 2015 – 2019

Methods

<u>1. Data collection</u>

Analysis was based on recordings collected during the summers of 2015, 2016, 2017, and 2019 in dedicated surveys in the 12 m research vessel *Balaena*. Surveys took place along the continental shelf edge from the Scotian Shelf to southern Labrador. A substantial amount of time was spent searching for and following NBW in the Gully, Haldimand, and Shortland Canyons, as well as the Sackville Spur off Newfoundland. Throughout these years, recording setups changed slightly (Table 1).

2. Acoustic analysis

2. a) Defining click patterns

In order to consistently differentiate *click patterns* from regular clicks, we formulated a working definition of *click patterns* based on previous descriptions of NBW clicks intervals (Moors-Murphy, 2015; Clarke *et al.*, 2019; McAllister, 2019). Thus, we initially defined *click patterns* as series of 2 – 10 consecutive clicks separated from other click trains by > 1s and produced by a single whale as confirmed by methods described below.

Additionally, we explored whether distinct click patterns could be found within longer click sequences. We based this approach on previous descriptions of sequences of irregular clicks by HMM (Hilary Moors-Murphy; unpublished data) and buzzes by Wahlberg *et al.* (2011). HMM described click trains which started with 1 – 3 clicks which were considerably shorter than the following clicks and referred to those as *quick – starts*. *Buzzes* have been documented for NBW, as well as other beaked whales, during echolocation click trains (Johnson *et al.*, 2006; Wahlberg *et al.*, 2011). Additionally, it has been noted that NBW click trains are remarkably more irregular than those described for other echolocating cetaceans (Wahlberg *et al.*, 2011). Here, we studied whether distinctive click patterns could be objectively identified in click trains usually associated with echolocation. For this, we extracted the first 10 clicks of click trains that could be subjectively classified as *regular* when we perceived consistent inter-click intervals (ICIs), *irregular* when ICIs were perceived to be variable, *quick-starts* when the first few ICIs were noticeably shorter than the following clicks, and *buzzes* when the first few clicks were so close together that they produced a buzzing sound. We refer to these sequences as *10-click segments* and note that these clicks do not represent an entire click train but only its first 10 clicks.

2. b) File selection

2015 – 2019 Recordings

All recordings were processed through the beaked whale detector process developed by Beslin and Stanistreet (REF). The beaked whale detector combines automated detection parameters and manual validation to perform streamlined analysis of acoustic recordings for 4 species of beaked whales (Sowerby's, NBW, Cuvier's and Trues/Gervais). All recordings with NBW detections were manually reviewed and confirmed. Files for which the nearest detection included NBW and that were collected within 30 min of the start or end of a visual encounter were selected for further analysis of click patterns. This yielded 1302 files (192.67 hrs). However due to the constant presence of NBW echolocation clicks in recordings collected in the Gully interfering with the localization of click patterns, the initial dataset was reduced to a random subsample of 25% of files per day and excluded those collected in the Gully, (163 files – 23.33 hrs) (Figure 1).

Selected recordings were then filtered using a 10th Order Butterworth 5 – 90 kHz bandpass filter in Matlab (after Baumann-Pickering *et al.*, 2013) to facilitate acoustic detection and provide clearer waveforms. Filtered recordings were visualized in a spectrogram and listened to in PAMlab Lite (Jasco Applied Sciences, Dartmouth, Nova Scotia). FFT settings were set to 94 Hz frequency step, a 0.001s frame length, a 50% overlap, and a Hamming window. Initially, we visually inspected spectrograms in a 30s window searching for characteristic NBW clicks (Clarke *et al.*, 2019). Where potential NBW clicks trains were identified, they were inspected in a 0.003s window (375 Hz frequency step, 0.000266 frame length, 6% overlap, and a Hamming window) to confirm visual characteristics of click spectrograms. Where NBW clicks were visually confirmed, we played back file sections to listen for possible patterns at 0.75 – 1.0 speed. Individual clicks from apparent click patterns were each annotated in a 0.003 sec window.

Initially, we annotated *click patterns* and *10-click segments* using the amplitude of clicks to discern individual trains from clicks produced by other whales. Where click trains appeared to overlap and were suspected to belong to multiple whales, we manually inspected the time of arrival of echo (TOA) produced by individual clicks. As echo TOA is correlated with the depth of an individual, it can be used to help separate clicks produced by individuals at different depths (Zimmer, 2011). However, when whales are found at similar depths, this method cannot separate click trains produced by different whales.

Consequently, we processed recordings containing *click patterns* and *10-click segments* in PamGuard (Gillespie *et al.*, 2009) to display click bearings and confirm whether patterns belonged to a single individual. The click detector analyzed filtered audio files through an additional 20 – 50 kHz 6th order band-pass Butterworth Filter, and detected clicks 10dB above background noise through a 15 kHz 6th order trigger filter. Click bearings were estimated and displayed, which allowed for distinct click trains to be visualized. Clicks for which a consistent bearing was confirmed were then annotated in PAMlab and associated *click patterns* and *10-click segments* were included in further analysis. We refer to these as *validated click patterns.*

3. Analysis of Click Patterns

3. a) Click extraction

Validated click patterns and *10-click segments* were extracted using a custom written Matlab routine adapted by JS from Baumann-Pickering *et al.*, (2013). Acoustic measurements, including peak and center frequency were extracted from clicks to finally confirm species identity by excluding patterns for which the average center frequency exceeded 35 kHz. Inter-click-intervals (ICI's) were also obtained as the time difference in seconds between the peak amplitude of an annotated click and that of the following click.

3. b) Description and classification of click patterns

Initially, *validated click patterns* were classified by the number of clicks present. For each click type, n-1 ICIs were available, n being the total number of clicks. However, in the case of doublets, which are formed by pairs of clicks separated from each other by longer intervals, we analyzed the short ICI within click pairs and the long ICI between click pairs (Moors-Murphy, 2015).

Statistical analysis of clicks was restricted for those click types for which sufficient validated samples were available. This was only possible for *10-click segments.* To explore whether manually classified 10-click segments could be grouped based on the distribution of their ICI's, we conducted a principal components analysis (PCA). Additionally, to test whether clicks could be categorized, we ran a k-means clustering analysis. This method requires that the number of clusters (k) be pre-defined. While there are different methods for finding the optimal number of clusters, we used the optimum gap statistic (Tibshirani *et al.*, 2001). This metric compares the clustering performance that results from different cluster numbers to a null model that assumes no clusters exist in the data. This method provides the advantage that it can identify no clustering (k = 1) as an optimum value.

Results

We screened 163 recordings (23.33 hrs) collected between 2015 – 2019 (Table 2). NBW whale clicks were present in 63.19% of these recordings. We were able to confirm the presence of *click patterns* in 19.19% of files where NBW were detected. Of all initially annotated click patterns, we were able to validate 33.65%. The remainder of the patterns were either a loud portion of a longer click train (48.25% - Supplementary Figure 1), artefacts of multiple overlapping trains (6.67% - Supplementary Figure 2), or other artifacts (Figure 2). This resulted in 2.75 *validated click patterns* for each hour of recordings during which NBW were detected.

The *click patterns* which were most likely to be confirmed were doublets and triplets (Figure 2), as well as *10-click segments*. *Click patterns* with 4 – 9 clicks were not only less common but were also most likely to be artefacts. Overall, ICIs for *click patterns* as well as *10-click segments* had a median of 0.37 seconds (s.d. = 0.15) (Figure 3). While there was some variation among different *click patterns* and *10-click segments*, mean ICIs for all were close to the overall mean (Table 3). We concentrated further analysis of individual *click pattern* types on doublets, triplets and *10-click segments*, as these were the only types for which enough samples were found (Table 4).

1. Doublets

The majority of doublets documented were part of a train of consecutive doublets pairs (Figure 4). This resulted in 5 trains made up of 2 – 5 consecutive doublets (Figure 4). Often, doublet trains were followed by regular click trains (e.g., Supplementary Figure 3). However, 6 single doublets were also found. Doublet-like patterns were also perceived within trains (Figure 2) but were excluded from this analysis as they not fit our definition of *click patterns*. On average, the 2^{nd} (long) ICI for doublets was 3.19 times (s.e. = 0.35) as long as the 1^{st} (short) interval (Figure 5). While the average short ICI in doublets was similar to that of all other documented ICIs, long ICIs were substantially larger (Table 3). Additionally, the variability in long ICIs was nearly five times as large as that documented for the entire dataset.

2. Triplets

The majority of the 12 triplets documented occurred in isolation, but we found two pairs of triplets which followed each other within 2.57 – 2.66 sec, and one which was closely followed by a regular click train (Supplementary Figure 4). ICIs within triplets were highly regular, with an average 2^{nd} to 1^{st} ICI ratio of 1.11 (s.e. = 0.03) (Figure 5). The 2^{nd} to 1^{st} ICI ratio of triplets was also substantially less variable than that of doublets (Figure 5).

3. 10-click segments

Manual classification of *10-click segments* into *regular clicks, irregular clicks, buzzes,* and *quick-starts* resulted in different ICI distributions (Figure 6). Segments classified as regular clicks had highly consistent ICIs throughout the click sequence, and very little variation across individual *regular click* trains (Supplementary Figure 5). *Irregular clicks* had similar average ICIs as those found in *regular clicks* but were much more variable within as well as across segments (Figure 6). *Buzzes* had extremely short ICIs (< 0.1 sec) in the first 4 – 5 clicks, with gradually increasing ICIs. The rate at which the transition from “buzzing” to longer clicks was variable across samples. We also found that buzzes were often preceded by “squeals” which could not be annotated as individual clicks (Supplementary Figure 6). On the other hand, *quick-starts* had initial ICIs in the first 1 – 3 clicks that were longer than those found for *buzzes*, but still nearly half as long as those found in *regular clicks* (Supplementary Figure 7). The transition from short clicks in *quick-start* patterns was also generally more abrupt than that of *buzz* patterns (Figure 6).

Although manual classification of *10-click segments* resulted in differences in the ICI distributions among *regular clicks, irregular clicks, quick-starts* and *buzzes,* PCA analysis revealed a high degree of overlap among all manually designated categories (Figure 7). *Regular clicks* were shown to be a subset of *irregular clicks. Buzzes* had the least overlap with the other types, but stull had a high degree of overlap with *quick starts.* Furthermore, we found no evidence to support the existence of discrete *10-click segment* types as the optimum number of clusters for k-means analysis was 1 (Figure 8).

Discussion

Our validation protocol of NBW click patterns revealed that the majority of patterns initially annotated were artefacts of varying amplitudes within a train and the overlapping of click trains produced by multiple individuals. While this procedure required a significant amount of time and yielded a very small sample size (50 *click patterns* and 43 *10-click segments* – Table 4), it provided a robust means of extracting click patterns. Our ability to validate these patterns relied on the existence of bearing information provided by the use of a directional hydrophone array. For the time being, this means that recordings collected with omnidirectional hydrophones (including those collected between 1988 – 2007 by the Whitehead Lab) may not yet be used for this analysis. Other means of identifying individual click trains that do not rely on directional recordings, including the cross-correlation of acoustic properties of clicks, may be able to overcome this constraint in the future (Starkhammar *et al.*, 2011).

The most common cause for incorrectly annotated click patterns was the increased amplitude of short sections within a longer click train (Supplementary Figure 1). Often, these surrounding quieter clicks were not audible nor visible in a spectrogram. Beaked whales can regulate the amplitude within an echolocation click train as they search for their prey (Madsen *et al.*, 2013). At the same time, because toothed whale clicks are directional, changes in the position of an individual relative to the hydrophone can result in differences in the received amplitude (Zimmer *et al.*, 2005). These changes can result from an individual changing their direction as well as by them moving their head, which is common during foraging in other beaked whales (Zimmer *et al.*, 2005). Whether the changes in amplitude we observed within results from a change in source volume produced by the whale or a change from its position with respect to the hydrophone is uncertain. However, it is possible that changes in amplitude within a click train could be used as a means of transferring information, similar to raising one's voice. In any case, this property of NBW echolocation click trains highlights the importance of complementing purely aural analysis with a sensitive click detector and bearing information.

We found that click patterns which had been previously described were present in the dataset analyzed. The doublets we found had similar temporal characteristics as those described by Moors-Murphy in the Gully in 2006 (2015), with long intervals between doublets being on average nearly 3 times as long as short intervals within click pairs. The high degree of similarity in the temporal patterning of doublets we analyzed – collected between 2015 – 2019 in the Scotian Shelf and Newfoundland – suggests this click pattern may be stable across time and populations. However, we note that our sample size (23 individual doublets; 5 doublet trains) is insufficient, and that more samples will be necessary to determine whether subtler differences are present between previous reports and our own, as well as between the SPP and

Newfoundland aggregation. ICIs we found for triplets were also similar to those reported by McCallister (2019). However, we were not able to confirm the presence of quicker click patterns described before (McAllister, 2019). It is possible that quick patterns, for which ICIs averaged 0.16 sec (s.d. = 0.05), were in reality produced by multiple overlapping individuals (see Supplementary Figure 2). Conversely, trains of very short ICIs may be found within longer click trains, which would have excluded them based on our working definition of *click patterns*.

Our initial classification of *10-click segments* is aligned with previous research that described distinct temporal patterns within the starts of NBW click trains. However, through clustering analysis we found evidence that that the distinction between these based on temporal patterns may be continuous rather than discrete. Except for buzzes, which have been described as part of the final phase in prey detection in other echolocating species, the role of these different temporal patterns within click trains in echolocation remains unknown (Madsen, 2005; Wahlberg *et al.*, 2011). In beaked whales, *regular* click trains produced during foraging dives are remarkably irregular, which may point to a different way of echolocating than that of other odontocetes (Wahlberg *et al.*, 2011). Alternatively, it may be that information is being transmitted through these temporal patterns. In both cases, other means of characterizing the temporal structure of click trains over different temporal scales may produce valuable insight into the vocal behaviour of NBW and other beaked whales (Ravignani *et al.*, 2019; Burchardt and Knörnschild, 2020).

Recommendations for future work

A better understanding of NBW vocalizations will contribute not only to our knowledge of their social and foraging behaviours but could provide crucial information for future threat assessments. Currently, the wide variations in amplitude within click trains and overlapping of multiple click-trains in NBW recordings pose a challenge for accurately extracting click patterns from passive acoustic recordings. Here, we overcame this challenge by incorporating click bearing information made available by a directional hydrophone array. However, this protocol was time consuming and resulted in very small sample size returned, which hindered our ability to compare vocalizations across populations and time. We propose future steps to address this challenge as well as to develop the state of knowledge on NBW vocal behaviour:

1. Streamlining the click-pattern validation process and adapting it to data for which no bearing information is available may yield a substantially large sample in considerably less time. As a benchmark, previous quantitative analysis and comparisons of sperm whale social vocalizations have been based on sample sizes of over 500 codas (Rendell and Whitehead, 2003), which is an order of magnitude larger than what we obtained. We have proposed to adapt a click train identification method based on click spectra cross-correlation analysis to the existing dataset in order to optimize this process (Starkhammar *et al.*, 2011). This will allow us to conduct quantitative clustering analyses and compare repertoires across the SSP and Newfoundland populations.
2. As NBW are highly social species whose experience of their environment is primarily acoustic, it is likely that the communicate through acoustic signals, some of which may remain undiscovered by passive acoustic monitoring. Our analysis of NBW recordings relies entirely on clicks that match the characteristics of those previously described for the species (Clarke *et al.*, 2019). But if communication is achieved through other signals, they are being omitted by our current approach. There are currently plans by Dr. Sascha

Hooker to study NBW acoustic behaviour by tagging individuals in the Eastern North Atlantic Ocean. The information collected by this means would provide ground-proofing for the click-patterns we have documented and may reveal the existence of other types of vocalizations previously unreported for NBW. For instance, acoustic tags have revealed the existence of whistles and rasps in Blainville's beaked whales which likely are used for communication (Aguilar de Soto *et al.*, 2012).

3. Our protocol was based on a pre-defined working definition of *click-patterns* informed by previous reports. While some of the patterns we observed fit our criterion, there may be important information in the temporal structure within longer sequences of click trains. Future work will concentrate in studying temporal structures in these click trains over different scales (for example, within pairs, tens, or hundreds of clicks). By combining quantifications of temporal structure with observation behavioural data, we will be able to better understand the role of NBW vocalizations. This may then be incorporated into temporal and spatial analyses of social and foraging behaviours, which can then inform future vulnerability assessments.

References

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Tables

Table 1. Hydrophone setup and settings for recordings collected by the Whitehead Lab from 2015 - 2019

Year	Hydrophone setup	Sensitivity	Preamplifier	Sampling Rate	Frequency Range (kHz)
2015 & 2017	Two-element hydrophone array (0.5m separation)	-204 dB re: 1 μPa	Magrec HP-02 - 29 dB gain	192 kHz	0 – 96
2016	Two-element hydrophone array (0.5m separation)	-204 dB re: 1 μPa	Magrec HP-02 - 29 dB gain	June – July: 96 kHz	0 – 48
				August: 192 kHz	0 – 96
2019	Two-element hydrophone array (0.43m separation)	-204 dB re: 1 μPa	Magrec HP-02 - 29 dB gain	192 kHz	0 – 96

Table 2. Analyzed by year, and location. Canyons in the Scotian Shelf are denoted as "SS"

Year	Region	Encounters	Recordings	Hours
2015	SS Haldimand	11	35	5.53
	SS Shortland	1	13	0.47
2016	SS Haldimand	13	22	3.22
	SS Shortland	28	41	5.89
	Newfoundland	9	26	3.75
2017	Haldimand	3	5	0.83
	Newfoundland	7	16	2.67
2019	SS Shortland	2	6	0.97
	Total	74	163	23.33

Table 3. Summary statistics of inter-click intervals (ICI) (sec) collected for NBW click patterns. For doublets, the summary of ICIs is shown for the short and long intervals.

Click pattern type	number of ICIs analyzed	Median	Mean	Standard deviation
regular	139	0.375	0.389	0.071
irregular	126	0.402	0.408	0.173
quick	106	0.356	0.349	0.134
buzz	54	0.116	0.150	0.137
doublets*				
single	6	0.441	0.444	0.173
short (within train)	17	0.474	0.434	0.151
long (within train)	12	1.710	1.567	0.980
triplet	24	0.380	0.403	0.109
other patterns	56	0.356	0.374	0.171

***** For doublets, three types of ICIs are summarized. Singles refer to click pairs that occurred in isolation, short intervals refer to the ICI within a click pair in a train, and long intervals refer to the ICI between click pairs in a train.

Table 4. Number of validated *click patterns and 10-click segment* types. Those for which sufficient samples were collected are shown in bold

Click pattern type	Number of samples
Doublets	**23**
Triplets	**12**
4clicks	7
5clicks	6
6clicks	1
7clicks	1
9clicks	0
10 click segments	**43**
buzz	**6**
irregular	**13**
quick start	**10**
regular	**14**

Figures

Figure 1. File selection and *click pattern* validation process for current report.

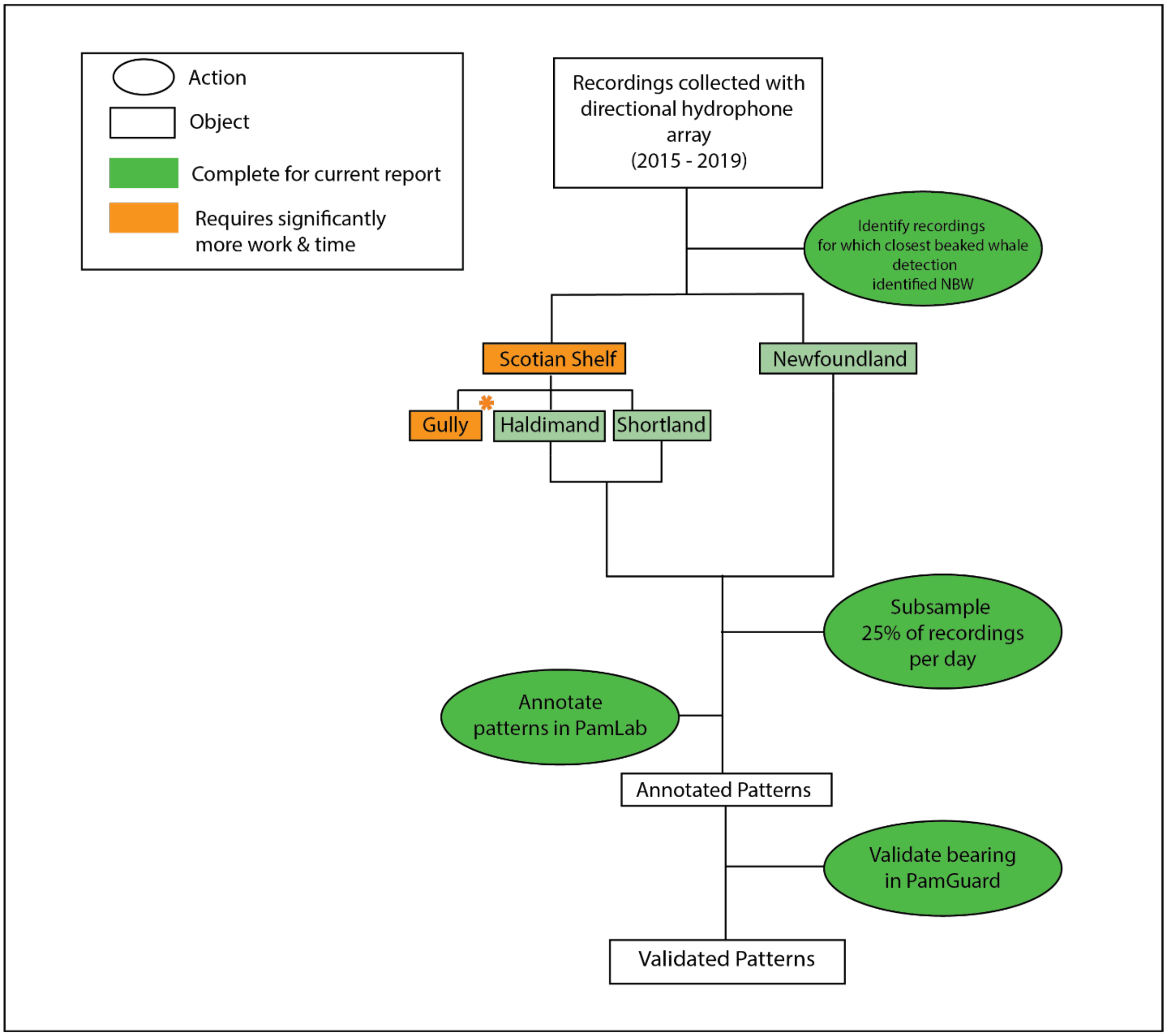


Figure 2. Proportion of validation results for each *click pattern* type after analysis through PamGuard.

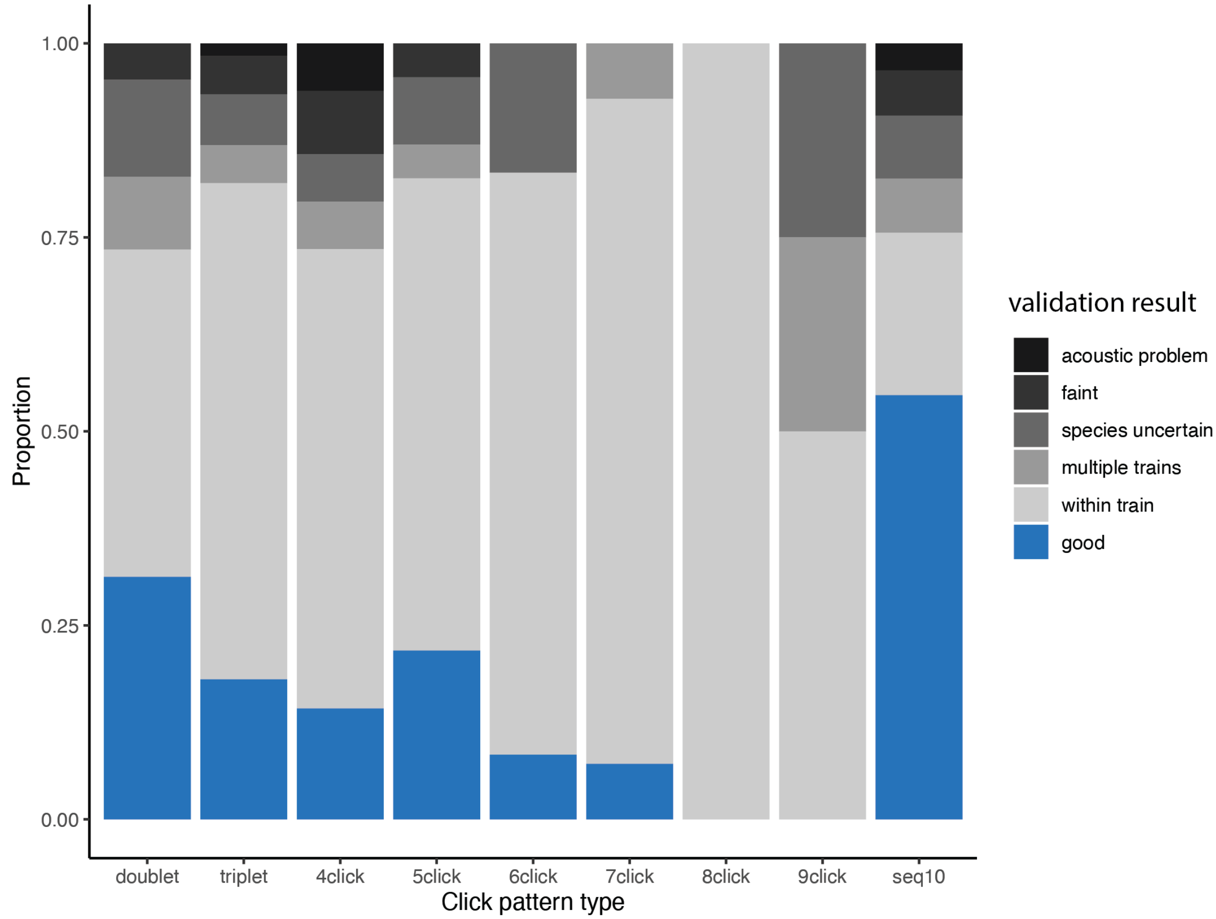


Figure 3. Distribution of inter-click intervals (ICI) in seconds for different *click patterns* and *10-click segments* for Northern Bottlenose Whales. The overall median ICI is shown with a dashed line.

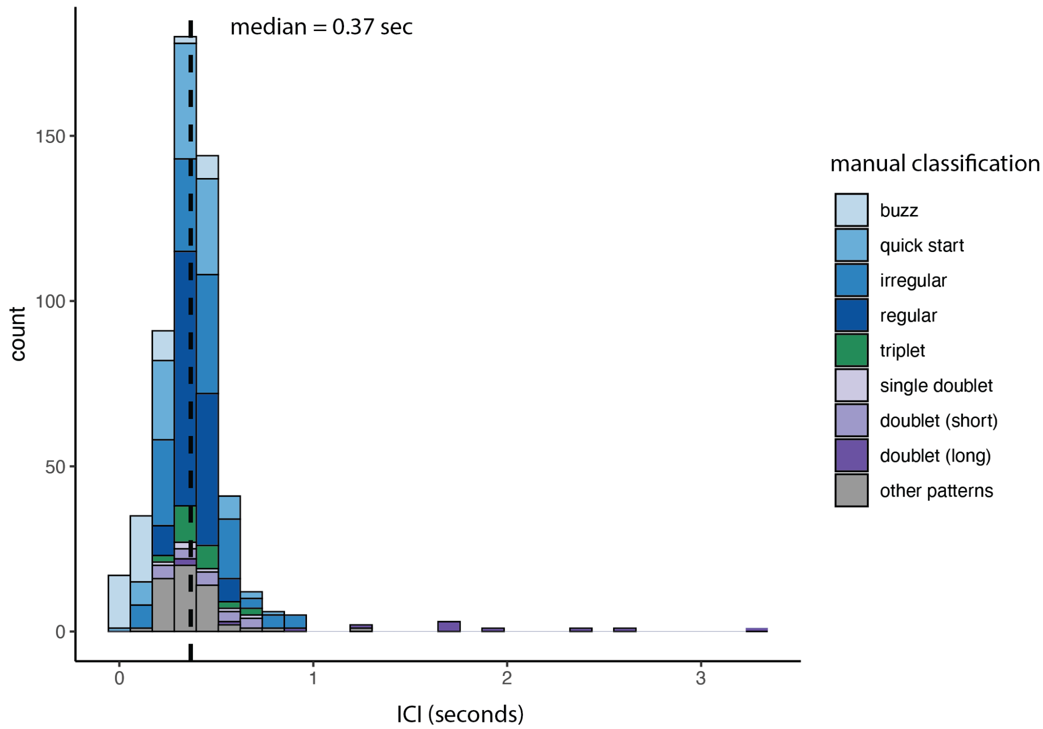


Figure 4. Temporal structure of validated doublet trains for this study. Each blue line represents a click, gray lines indicate "short" inter-click intervals and black lines indicate "long" inter click intervals.

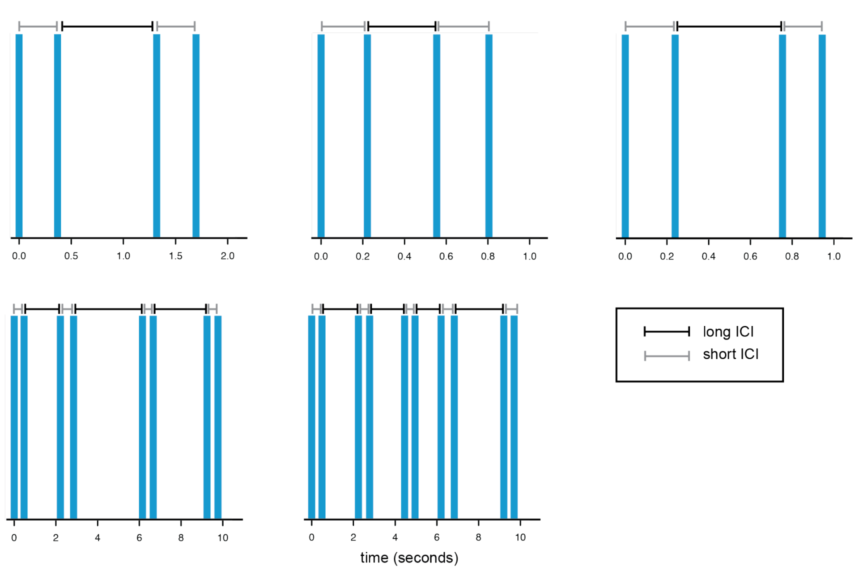


Figure 5. Ratio of 2^{nd} to 1^{st} interval for in doublet trains and triplets. For doublet trains, the ratio of 2^{nd} to 1^{st} interval is equivalent to the long to short interval ratio. A ratio of 1 (where the 2^{nd} and 1^{st} intervals are the same) is marked as a red dashed line.

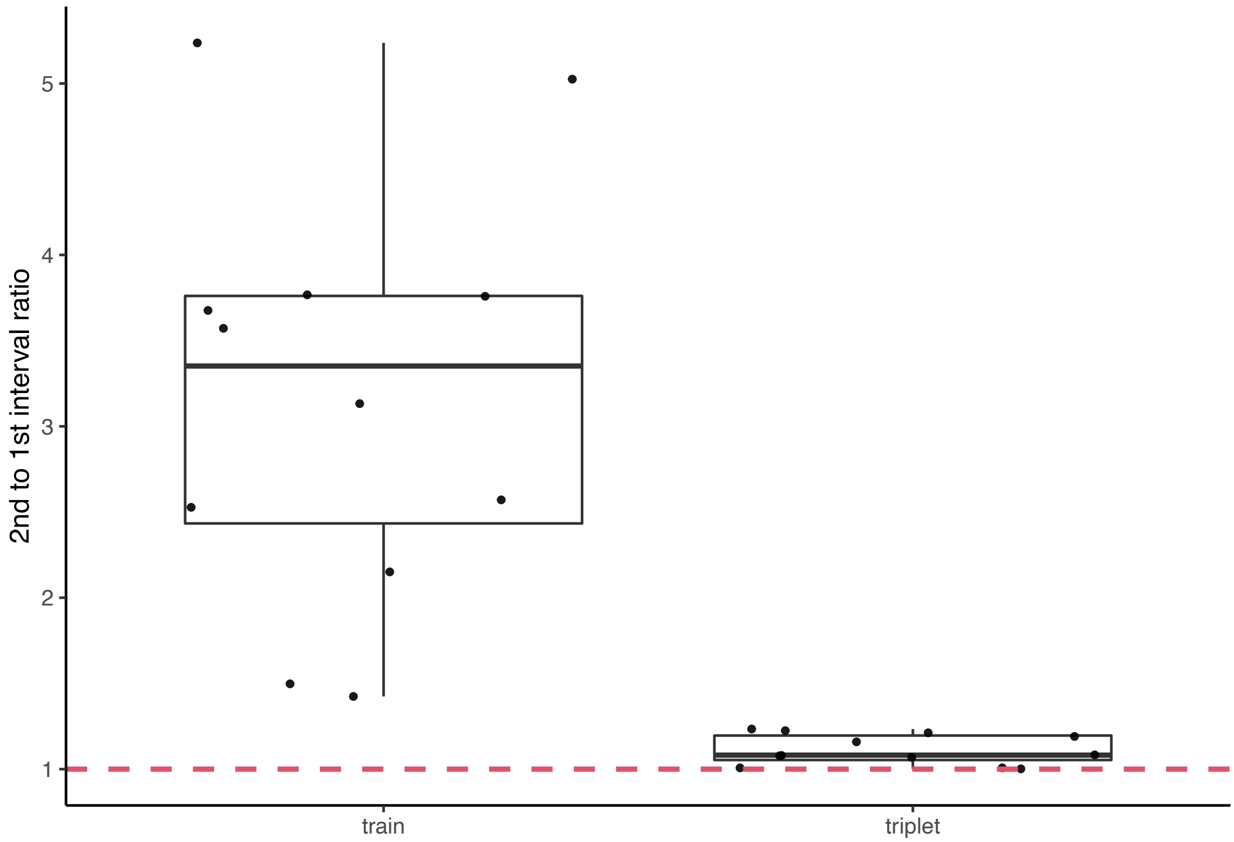


Figure 6. Average inter-click-intervals for the first 10 clicks of *10-click*. Grey ribbons show the corresponding standard error.

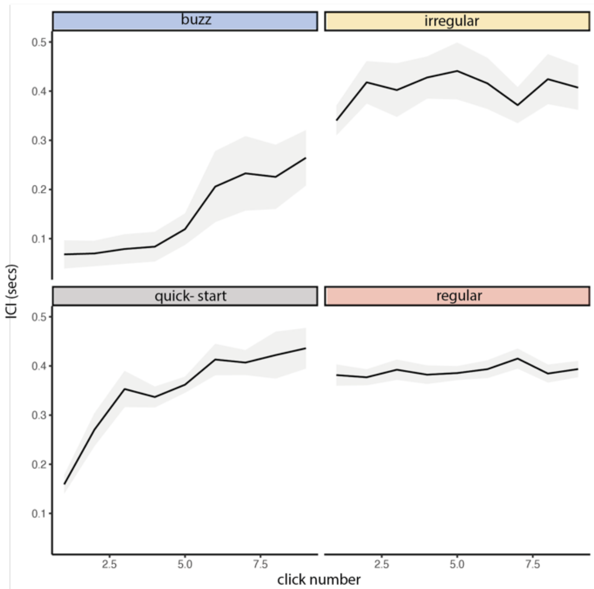


Figure 7. Visual representation of principal component analysis for *10-click segments.* Principal components 1 and 2 are plotted in the x and y axes, respectively, along with the percentage of variance explained by each. Plotted are the 95% confidence ellipses which represent the smallest ellipse that covers 95% of the data points of each manually defined type of *10-click segment.*

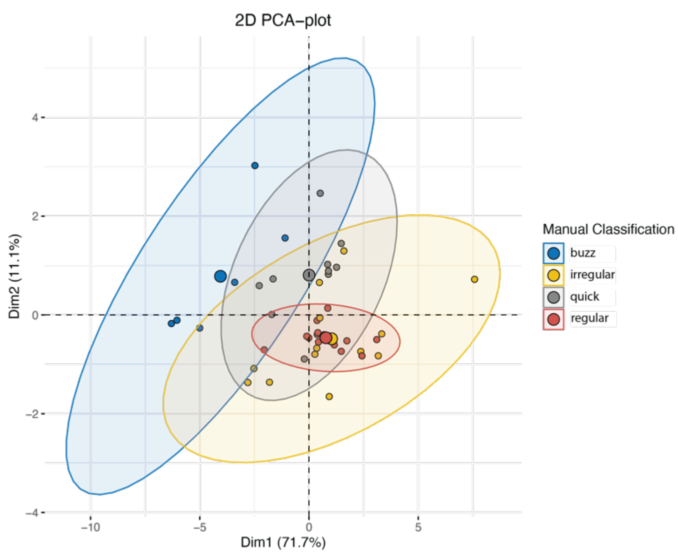
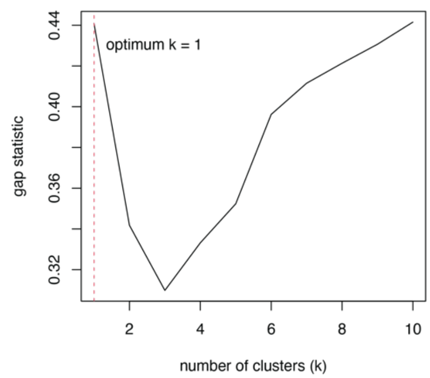


Figure 8. Gap statistics for each number of clusters in k-means analysis. The red dashed line indicates the optimum k value for which the gap statistic is maximized (k = 1).



Supplements

Figure 1. Example of a *click pattern* which was aurally classified as an isolated pattern, but that was found to be part of a quieter preceding click train. The click pattern is shown in a PAMlab spectrogram highlighted in yellow (a). The corresponding amplitude profile (b) and bearing display (c) produced in PamGuard are shown and annotated clicks are highlighted in orange.

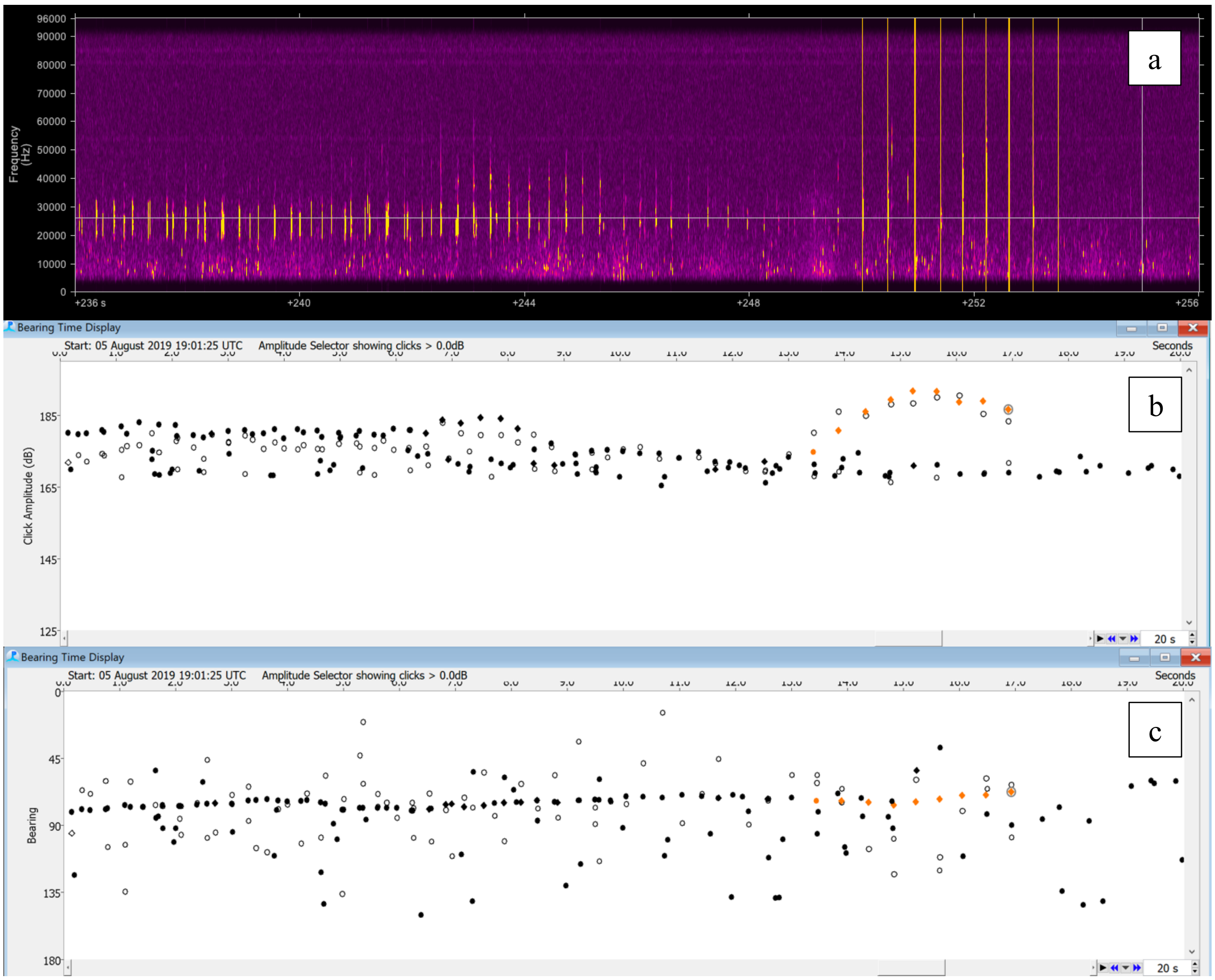


Figure 2. Example of an *apparent click pattern* which was aurally classified as an isolated pattern, but that was found to be produced by multiple overlapping whales. The click pattern is shown in a PAMlab spectrogram highlighted in yellow (a). The corresponding amplitude profile (b) and bearing display (c) produced in PamGuard are shown and annotated clicks are highlighted in orange.

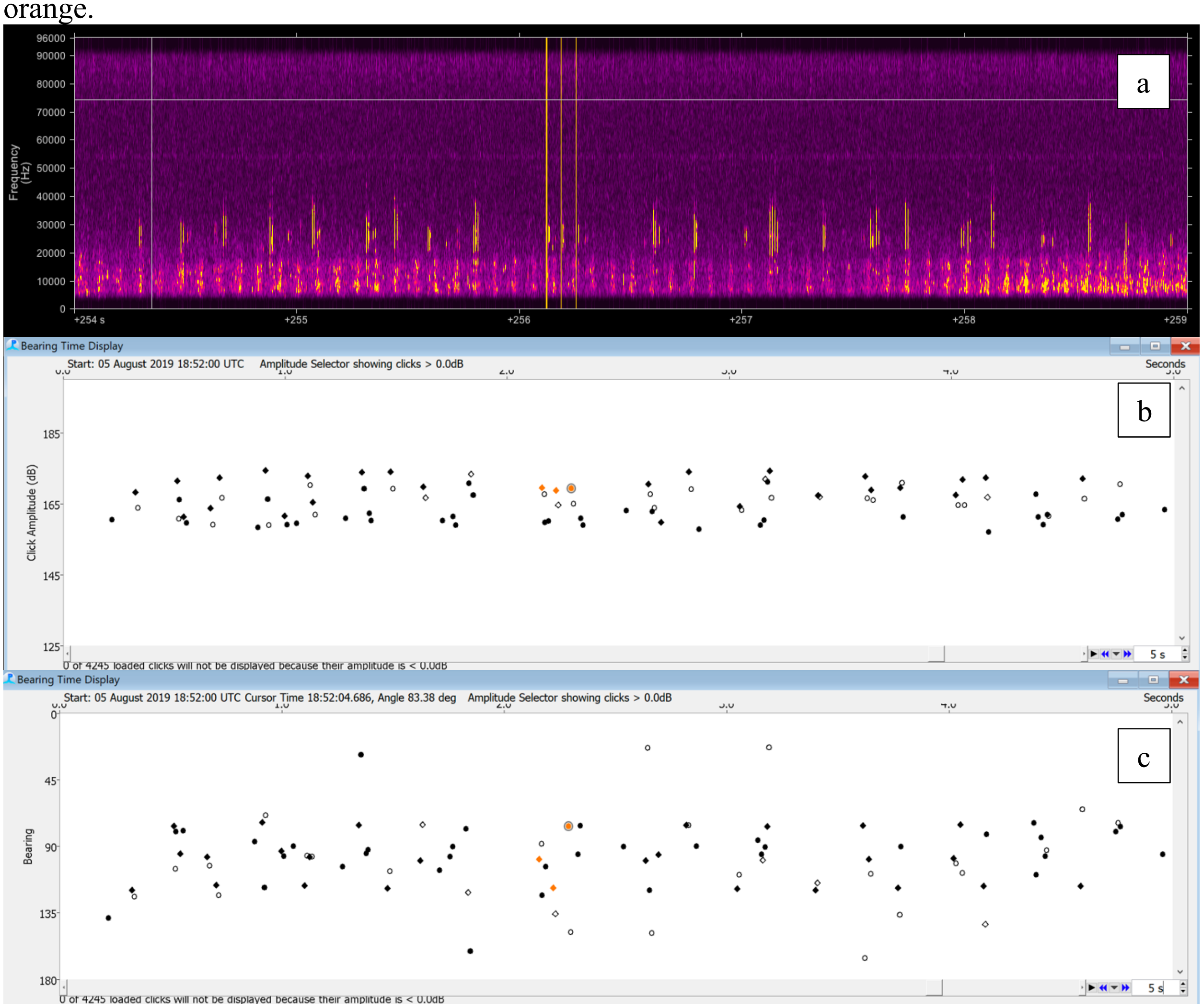


Figure 3. Oscillogram (a) and spectrogram (b) of doublets followed by a regular click train. Doublet pairs are highlighted in yellow and regular clicks are highlighted in green (a). The corresponding bearing display produced in PamGuard is shown below (c).

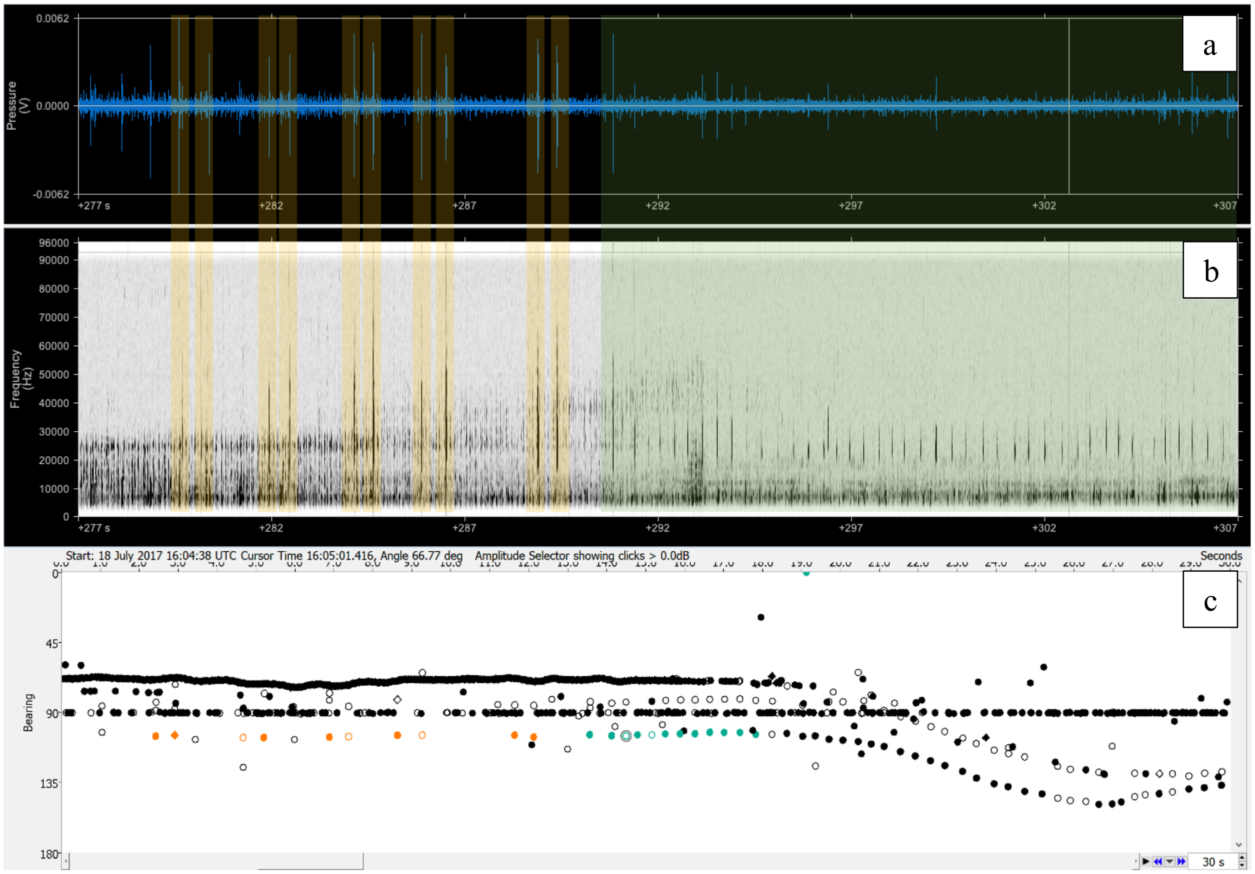


Figure 4. Oscillogram (a) and spectrogram (b) of successive triplets followed by a regular click train. Triplets are highlighted in yellow and regular clicks are highlighted in green (a). The corresponding amplitude (c) and bearing (d) display produced in PamGuard is shown below. Clicks that are not highlighted either belong to a different individual or likely result from noise.

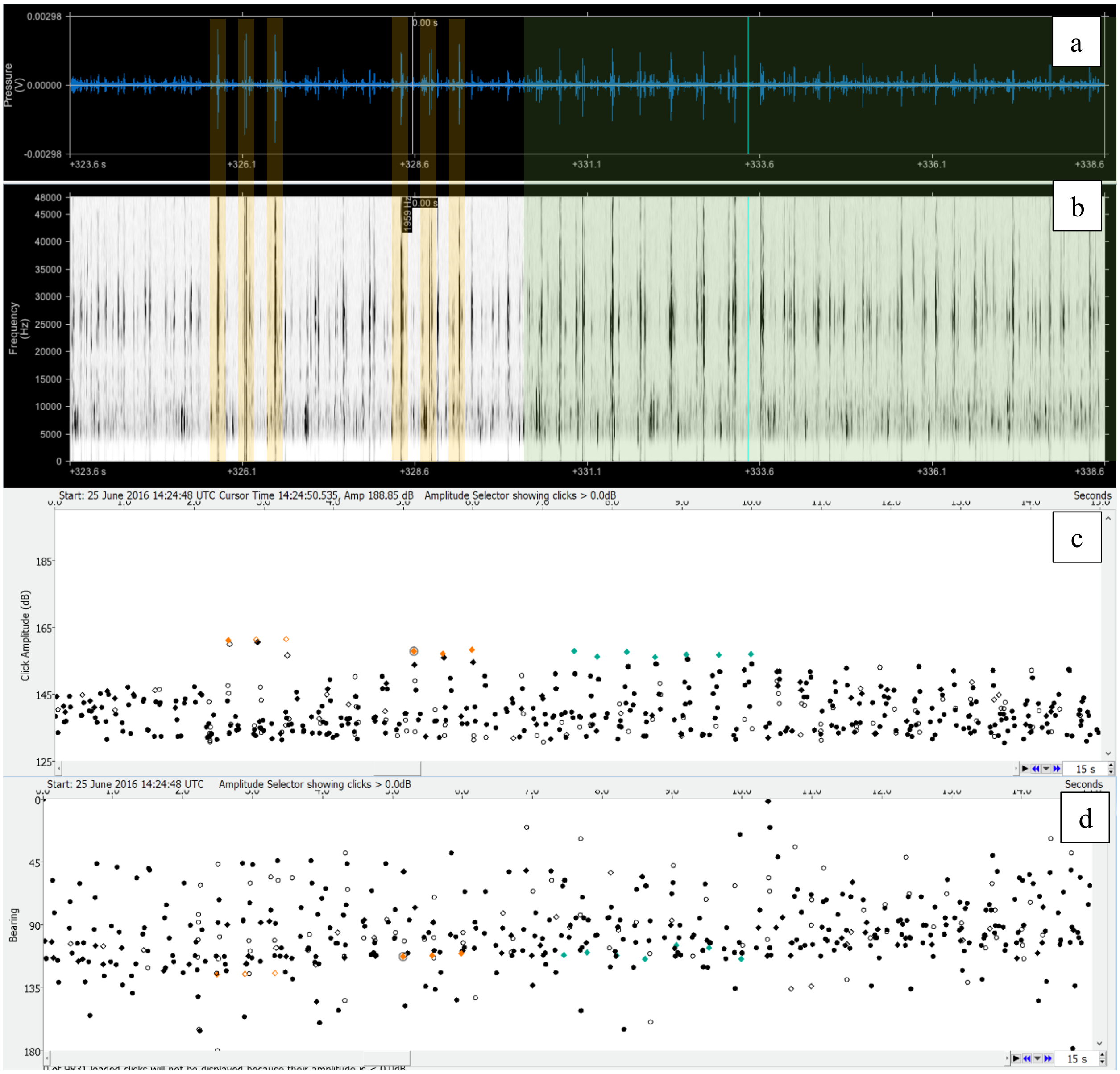


Figure 5. Spectrogram of a *regular click* train (a). Annotated clicks are highlighted in yellow. The corresponding amplitude (c) and bearing (d) display produced in PamGuard is shown below, where corresponding annotated clicks are marked in green.

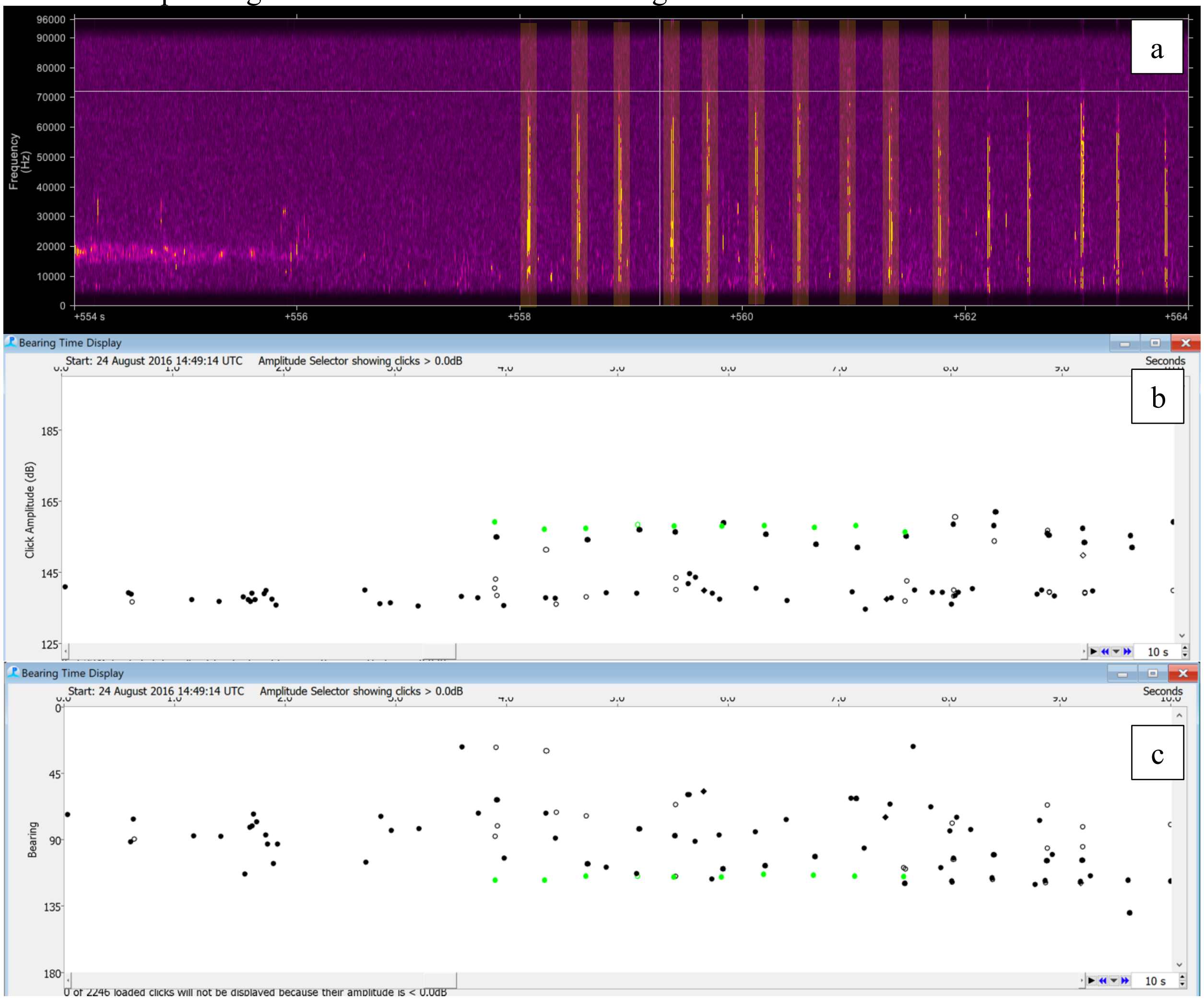


Figure 6. Spectrogram of a *buzz* pattern preceded by a “squeal” (a). Annotated clicks are highlighted in yellow. The corresponding amplitude (c) and bearing (d) display produced in PamGuard is shown below, where corresponding annotated clicks are marked in green.

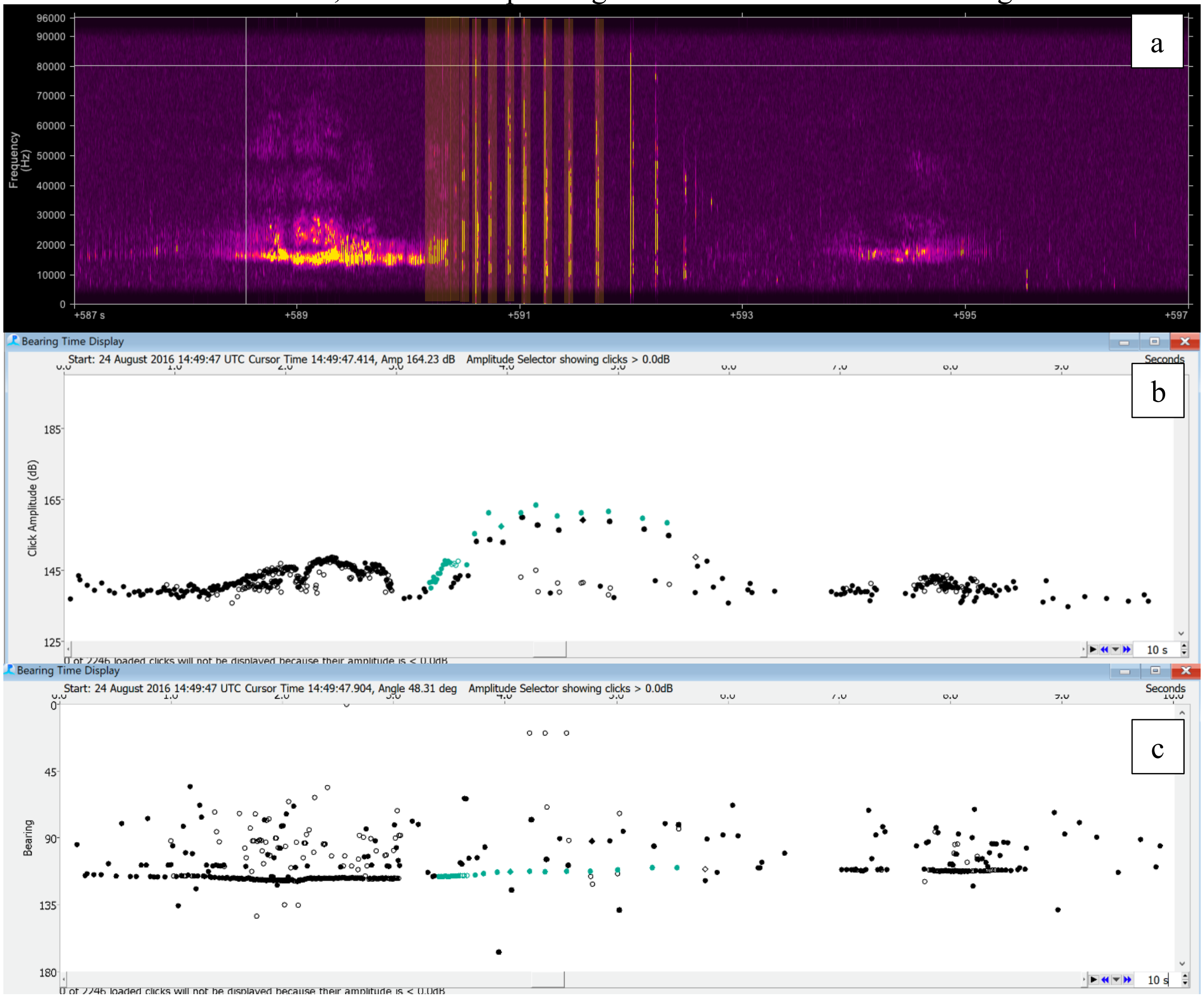


Figure 7. Spectrogram of a *quick-start* pattern (a). Annotated clicks are highlighted in yellow. The corresponding amplitude (c) and bearing (d) display produced in PamGuard is shown below, where corresponding annotated clicks are marked in green.