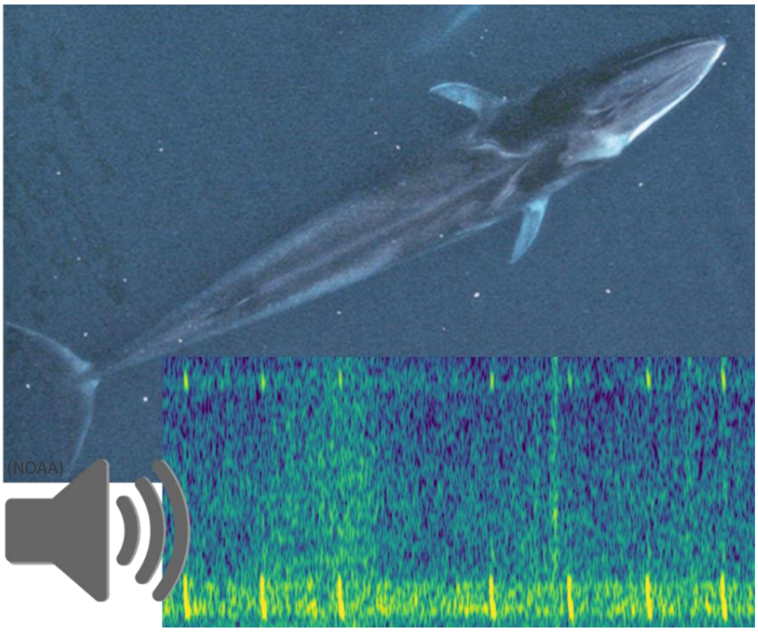
Exploring Geographic Variation in Fin Whale (*Balaenoptera physalus*) Calls From Two Passive Acoustic Monitoring Sites Revealing Population Identities Across the Weddell Sea, Antarctica



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Declaration of honor

I hereby confirm on my honor that I personally prepared the present academic work and carried out myself the activities directly involved with it. I also confirm that I have used no resources other than those declared. All formulations and concepts adopted literally or in their essential content from printed, unprinted or Internet sources have been cited according to the rules for academic work and identified by means of footnotes or other precise indications of source.

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Abstract

Currently, little is known about the population identity of fin whales (*Balaenoptera physalus*) in Antarctic waters but initial analyses of acoustic recordings from the Southern Ocean (SO) have shown that fin whale calls differ between regions, possibly representing different fin whale populations. In the Atlantic Sector of the Southern Ocean, the typical fin whale 20-Hz song is often accompanied by simultaneous higher frequency (HF) component at around 89Hz or 99Hz. However, the distribution of these call types throughout the area and whether there is a clear spatial separation between these call types is so far unknown. In this study fin whale calls were examined between two locations, the Greenwich Meridian and Elephant Island across the Weddell Sea (from 2009, 2011, 2013, and 2015) to gain further insights into the connections between fin whales in this region. The HF call component was found to be significantly (p-value < 2.2e^-16) unique in its frequency at the two locations with 99Hz (97.14Hz ± 3.19) at Greenwich Meridian and 86Hz (86.26Hz ± 1.36) at Elephant Island. The inter-pulse interval (IPI) of both low frequency (LF) (20-Hz) and HF calls were also found to differ between geographic regions, with a median IPI of 14.5 seconds at Elephant Island and median IPI around 10 seconds at Greenwich Meridian. Variation in song IPIs were also investigated between geographic locations, Elephant Island was determined to have a majority singlet song type and Greenwich Meridian was found to have mostly triplet songs. The occurrence of HF and LF calls showed a strong positive correlation, indicating that both call components are produced simultaneously. The characteristic elements for fin whale calls examined in this study all indicate that the fin whale calls recorded at Elephant Island and Greenwich Meridian belong to two distinct acoustic populations. An understanding of how potentially distinct fin whale stocks utilize different geographic regions is fundamental for management and conservation measures aiming to improve the conservation status of this vulnerable species.

Introduction

Current Status of Fin Whales

Due to commercial whaling of fin whales, there was a massive decline in the population. After ~50 years of protection from commercial whaling, very little is known about the current status of fin whales in the Southern Ocean (SO) (Van Opzeeland *et al.* 2014). Commercially exploited to critical population levels, the fin whale is currently listed as a vulnerable species (Santora *et al.* 2014, Cooke, 2018). Studies on the distribution, abundance, and ecological role of cetaceans in the SO have been limited (Baumann-Pickering *et al.* 2015). Little is known about the pelagic distribution of fin whales and other whale species in the SO largely due to visual surveys' operational constraints. Research cruises in the Antarctic are generally restricted to performing fieldwork in the austral summer months where daylight hours, weather, sea ice, and visual detection are optimal for visual surveys (Van Opzeeland *et al.* 2014, Baumann-Pickering *et al.* 2015, Širović *et al.* 2009, Menze *et al.* 2017). Fin whale abundance estimates for the SO have been mostly based on visual sightings (e.g. Santora *et al.* 2014, Branch and Butterworth, 2001). Since visual surveys are restricted to the summer season and ice-free areas, this creates large gaps in population estimations for fin whales in the SO. Due to the lack of data, knowledge gaps for fin whales in the SO include; seasonal distribution patterns (especially during austral winter), whether there are distinct populations throughout the SO, and if so, their distribution/ habitat use*.* The Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR) has planned to establish a Marine Protected Area (MPA) in the Weddell Sea which would also be in favour of further population recovery of Southern Hemisphere fin whales.

Fin Whales in the Southern Ocean

Fin whales have been observed to prefer foraging in areas with complex water circulation (Santora *et al.* 2014), which because of their speed and agility, allows fin whales to exploit high concentrations of prey that might be caught in eddies and fronts (Santora *et al.* 2014). In the SO, fin whale diet is almost exclusively Antarctic krill (*Euphausia superba*). They feed intensively in the summer while mainly fasting during the winter (Aguilar and García-Vernet, 2018). Fin whales are believed to forage throughout the Weddell Sea, exploiting the complex circulation of the Antarctic Circumpolar Current as well as eddies that form hot spots for fin whale feeding (Santora *et al.* 2014, Baumann-Pickering *et al.* 2015).

In the Arctic, fin whales' migration routes have been defined with populations migrating seasonally between high and low latitudes with few individuals remaining at high latitudes year-round (Simon *et al.* 2010). It is unknown if Southern Ocean fin whales in the Antarctic have similar behavioural ecology as the fin whales in the Arctic. Generally, habitat use and relationship to environmental conditions of fin whales in the SO are unknown (Santora *et al.*

2014). Therefore, current knowledge gaps exist for fin whale ecology and the role of fin whales in the Antarctic marine food web. Fin whales in the SO are seldom seen at the ice edge during the summer, and in the winter months, they are thought to migrate to low latitudes to calf and breed (Širović *et al.* 2009). Fin whales are assumed to avoid areas with dense sea ice cover, which could be another reason for fin whales being seen further North in the SO, presumably migrating to lower latitudes when pack ice forms (Širović *et al.* 2004).

Fin whales are known to produce several different vocalizations (e.g. 40Hz feeding call (Romagosa *et al.* 2021)) with the most common one being the 20-Hz call. 20-Hz fin whale calls have been observed to be regular and irregular in frequency. Regular 20-Hz pulses are characteristic and consistent short (1-second duration), repetitive downsweeps (Figure 3) (Širović *et al.* 2004, Simon *et al.* 2010) and have been recorded in every ocean basin. Irregular 20-Hz pulses have been observed to vary in duration and frequency. Fin whale pulses that are defined as a 'song' are a series of 20-Hz pulses occurring in regular inter-pulse intervals (IPIs) which occur for 2 or more minutes up to hours (Helble *et al*. 2020, Morano *et al*. 2012), with breaks in between assumed to be surface breathing. Current knowledge on fin whale calls is that only males create songs that are thought to attract females over long distances since fin whales are solitary animals and are only brought together at feeding aggregations and breeding grounds (Croll *et al.* 2002). Previously all fin whales in the SO are thought to belong to the same population (Širović *et al.* 2009). However, acoustic studies have identified differences in fin whale calls across the SO (Gedamke 2009, Širović *et al.* 2009, Baumann-Pickering *et al.* 2015). These acoustic studies suggest that there are distinct fin whale populations within the SO. The consistent differences in fin whale calls across the SO point to the potential value of using acoustic monitoring to assess the population structure of fin whales. Regional differences in fin whale calls in the North Atlantic and the North Pacific have been used to aid in fin whale stock/ population structure (Gedamke, 2009).

To date, it is unknown whether or not there are distinct populations of fin whales in the Weddell Sea. The spatial separation of fin whale calls from opposite sides of the Weddell Sea could indicate different fin whale populations existing throughout the SO.

Passive Acoustic Monitoring (PAM)

Passive acoustic monitoring (PAM) has become the tool of choice to study the presence, relative abundance, migratory movements, and behaviour of large baleen whales, particularly in polar waters (Simon *et al.* 2010). Baleen whales produce various species-specific calls related to feeding, location, and social behaviours (Baumann-Pickering *et al.* 2015). Fin whales produce vocalizations that are a perfect example of a species that PAM can monitor well because they create powerful, repetitive low frequency (LF) 20-Hz pluses (Simon *et al.* 2010). PAM is a robust method with the ability to monitor the species over long time periods in hard-to-access areas like the SO (Baumann-Pickering *et al.* 2015 and Van Opzeeland *et al.* 2014), which also reduces seasonal bias in data. The basic information that can be attained from acoustic studies is the presence of a species in an area, but over time PAM can provide data on how the presence and properties of whale calls change over time in specific regions (Van

Opzeeland *et al.* 2014). An acoustic study conducted by Gedamke (2009) used regional differences observed in fin whale calls in the North Atlantic and North Pacific. To assist in fin whale population demographic analysis, which improved fin whale stock assessment and conservation management options.

Geographic Variability in Animal Calls

Geographic variability in animal vocalizations can indicate the presence, movements, the seasonality, and describe separate populations by calling types (Stafford *et al.* 2001). Geographical variation within a species call repertoire can be interpreted as initial stages of genetic divergence, adaptations to local environmental conditions, or reflect cultural differences between populations (Samarra *et al.* 2015).

There are several examples of geographic variation in acoustic signals between closely related species or populations within a species, such as frogs, birds, and mammals (mice) where a change in acoustic signals can lead to distinctive populations or subspecies. Jang and colleagues (2011) found that H. japonica had variable geneflow between regions but also geographic variation in calls characteristics between regions. Sexual selection by females for male song was determined to be an underlying factor in geographic variation of male tree frog advertisement calls. A study conducted by Mountjoy and Lemon (1996) on European Starlings hypothesized that female choice for long complex song structure in males was more important in mate choice than the location/ territory the male occupied. Females were reported to find males faster that had complex song even if the location wasn't optimal for nesting compared to a male that had a less complex song but more optimal nesting territory. Females that select males based on long complex songs were also found to be correlated with males in better condition and indicated the age of the male singer to the female as well relaying that the males have the best fitness for future offspring. Male mice have been observed to create high-pitched, ultrasonic, calls relaying information to females. Chabout (2015) found that male mice created long simple vocalizations when singing directly to females but when exposed to female urine, created more complex songs to lure females closer. It was observed that female mice preferred the more complex vocalizations over the simpler longer vocalizations. Generally, this model of sexual selection by females for male song portraying male fitness leads to geographic variation in calling types selected by the females of that population for song structure.

Generally, the divergence of sexual traits between populations can result from several reasons one of which is sexual selection. Sexual selection can often lead to rapid changes of traits that are involved in mate recognition and can create reproductive isolation between lineages (Panhuis *et al.* 2001). Female preferences for the acoustic signals of their own lineage/ population compared to acoustic signals from neighbouring populations/ lineages reduces the likelihood of mating, which can lead to a loss of genetic exchange between the two lineages/populations over time (Panhuis *et al.* 2001). The divergence of mate recognition signals between populations due to behavioural factors such as acoustic signals might not yet be present in the genes of the species but be associated with geographical distance (Hatch and Clark, 2004).

Geographical Variation in Whale Calls

Geographic variability in whale calls can provide information about the acoustic ecology and behaviour of the species. Geographical variation in whale songs can demonstrate changes in communication between conspecifics and describe the caller's location and movements within an area (Stafford *et al.* 2001). Geographic variation in whale calls can also provide information on the seasonal changes of calls within an area; this provides information on the intensity of calls, the migration of calling individuals, or the decrease in calls within an area (Stafford *et al.* 2001). Geographical variation in whale calls interpreted as different populations can also provide information on where whale stocks overlap or do not overlap. This can lead to improved knowledge about shared feeding grounds or overwinter and calving areas between populations (Delarue *et al.* 2009). Investigating geographic variation in intraspecific whale calls can provide insights into the evolution, movements, and cultural traditions of that species (Samarra *et al.* 2015). Geographical variation in whale calls could also provide information on the acoustic environment; factors explaining signal frequency variations between populations could be differences in background noise as well as transmission properties in the surrounding environment (Samarra *et al.* 2015).

Synchronizing of Song

A seasonal synchronizing of song is observed when throughout the rest of the year there is variability in call interval and types but with the onset of mating, season males converge on a certain song type. Humpback whales and blue whales have been observed to have a seasonal synchronizing in song with the onset of their mating season. (McDonald *et al.* 2009; Gavrilov *et al.* 2012). Fin whales from different oceans have been observed to have a seasonal synchronizing in characteristic 20-Hz calls (Morano *et al.* 2012, Oleson *et al.* 2014, Buchan *et al.* 2019). The inter-pulse interval (IPI) is the time between calls and has been examined as a population identifier to distinguish whale populations based on calls (Hatch and Clark, 2004; Gedamke, 2009; Delarue *et al.* 2009). A seasonal lengthening of inter-pulse interval (IPI) of LF fin whale pulses was observed by Oleson and colleagues (2014). At the start of acoustic presence, fin whale calls in the Pacific Ocean were observed to have short IPIs and near the end of the season with long IPIs and they are observed to synchronize before just before the end in acoustic presence for that year. North Atlantic fin whale calls examined by Morano and colleagues (2012) were observed to have a synchronizing pattern within a single month during winter. Seasonal shifts in fin whale pulses vary from what is referred to in their study as " short" IPI a "long" IPI and "transitional" IPIs throughout peak calling time of the year. In the Southern Hemisphere, Buchan and colleagues (2019) observed fin whale 20-Hz calls to have seasonal changes in acoustic presence but the reoccurrence of a song IPI in fin whale calls of a short IPI and long IPI indicating the same group(s) of whales are routinely using the area. These seasonal shifts observed in inter-pulse intervals of LF fin whale calls are thought to be a mechanism for changes in communication between individuals throughout the year with the synchronizing effect just before migration towards lower latitudes interpreted as a possible mating display by male fin whales. Not many studies have investigated inter- and intra-variability of IPIs between and within the same site and this synchronizing effect hasn't been examined in detail between fin whale populations in other ocean basins.

Geographic Variation in Fin Whale Calls

Differences seen in fin whales songs have been observed over all of the world's oceans, new songs that suddenly emerge in an area are hypothesized to be new groups of whales coming into an area or cultural changes in song structure (Helble *et al.* 2020). Male fin whales sing 20- Hz pulse songs with regular repeating inter-pulse intervals and may also alternate the frequency at which they sing (Helble *et al.* 2020). As seen by stocks of fin whales along the Eastern coast of Canada, they are genetically the same but rarely intermingle because of characteristic differences in song structure (Delarue *et al.* 2009). Geographic variation in whale calls has also been used to distinguish between stocks of humpback whales, blue whales, sperm whales, and killer whales (Stafford *et al.* 2001, Delarue *et al.* 2009) and even fin whales in the Northern Hemisphere (Hatch and Clark 2004, Delarue *et al.* 2009).

For fin whales, their calls might also distinguish between populations (Baumann-Pickering *et al.* 2015, Gedamke, 2009). There is some evidence in the Arctic and the SO for geographic variation in fin whale calls. There has been an observed portion of the known 20-Hz call with a simultaneous higher frequency (HF) component of the call. Two different HF pluses have been observed in the SO, one at 89Hz from the Antarctic Peninsula and the other at 99Hz from the East Antarctica region (Van Opzeeland *et al.* 2014). This high-frequency component of fin whale calls is believed to serve as conspecific recognition. The low frequency (LF) component of the fin whale call at 20-Hz is consistent among geographical locations. Still, it is clear that in the Arctic and likely in the SO, the HF component of fin whale call varies between geographical regions and likely distinguishes between fin whale populations (Simon *et al.* 2010, Gedamke, 2009).

In the Weddell Sea, it is unknown where the geographical separation in the different HF components of fin whale calls occurs, which will be the topic of investigation of this study.

Weddell Sea

The Weddell Sea represents one of the largest marginal seas in the Southern Ocean (Diekmann and Kuhn, 1998). It is a pristine example of an area where passive acoustic monitoring would yield useful data on an acoustic species. The Weddell Sea is encompassed by the Antarctic Peninsula on the Western border up until Greenwich Meridian on the Eastern border (Fahrbach *et al.* 2004). The Weddell Sea remains largely ice-covered in both austral summer and austral winter, with permanent ice cover in the western Weddell Sea along the Antarctic Peninsula (Siegel, 2005). This almost constant ice cover is not optimal for visual surveys, but with passive acoustic monitoring, we can observe vocally active species year-round. Fin whales are known to be present in the Weddell Sea, but there are still knowledge gaps in the acoustic ecology of this species (Širović *et al.* 2009). A study by Menze and colleagues (2017) shows that there appears to be a difference in the HF component of fin whale calls around the Greenwich Meridian compared to the fin whale calls observed around Elephant Island in the Scotia Sea, just off the northern tip of the Antarctic Peninsula.

Research Question

The 20-Hz pulses have been observed to vary in frequency from around 15Hz – 42Hz (Helble *et al.* 2020; Van Opzeeland *et al.* 2014). Fin whale 20-Hz songs have also been known to vary geographically in the Inter-pulse intervals (IPIs) which has been hypothesized to identify different acoustic populations in close geographic proximity to one another (Wood and Širović (2020); Hatch and Clark, 2004; Gedamke, 2009; Delarue *et al.* 2009). Preliminary research on acoustic data from the Weddell Sea shows that the typical fin whale call at 20-Hz is often accompanied by a second higher frequency call, ranging from 85Hz to 89Hz around Elephant Island (EI) and even higher near 99Hz along the Greenwich Meridian (GM) (Van Opzeeland *et al.* 2014). The high frequency (HF) component might serve to differentiate between fin whale populations in the Weddell Sea basin. This higher frequency component of fin whale calls has not yet been explored in detail in the Antarctic. Data were analyzed to examine the geographic variation in fin whale 20-Hz pulses throughout the Weddell Sea.

Results from this project could suggest that there are distinct fin whale populations instead of what is currently assumed to be one large population. This research will lead to better our understanding of fin whale call ecology by improving the baseline of acoustic ecology and the occurrence of these marine mammals in the SO. This study will also provide a better baseline for the continuation of fin whale population recovery in the SO by being able to assess the recovery status of separate fin whale populations, further supporting the conservation of this vulnerable species in the Southern Ocean.

Materials and Methods

Acoustic Recorder Deployment

Passive acoustic recorders were deployed at three sites, two in the Weddell Sea and one in the Scotia Sea (Figure 2). Each recording site consisted of a mooring tethered to the seafloor. In general, the mooring consisted of the acoustic recorder as well as other types of oceanographic instrumentation (e.g. CTD) see Figure 1 for an overview of mooring configuration. At Site 1, which was located along the Greenwich Meridian in the Weddell Sea, recordings were made with a MARU (BRP Cornell University) and a SonoVault (SV, Develogic, Hamburg) recorder from 2009 and 2011, respectively. The MARU and SonoVault recorder were deployed at 4,838m and 1,007m, respectively. At Site 4, which was also located along the Greenwich Meridian, south of site 1, data were collected with a SonoVault recorder from 2012-2013, deployed at a depth of 958m. Site 6, which was located at Elephant Island, in the Scotia Sea there was a single mooring with two recorders, the AURAL (Multi-Electronique, Canada) deployed from 2013-2016 at a depth of 210m and a SonoVault recorder at 212m that collected data in 2013. Table 1 shows a summary of recording sites and specific recorder information (e.g. sample rate, recording duration).

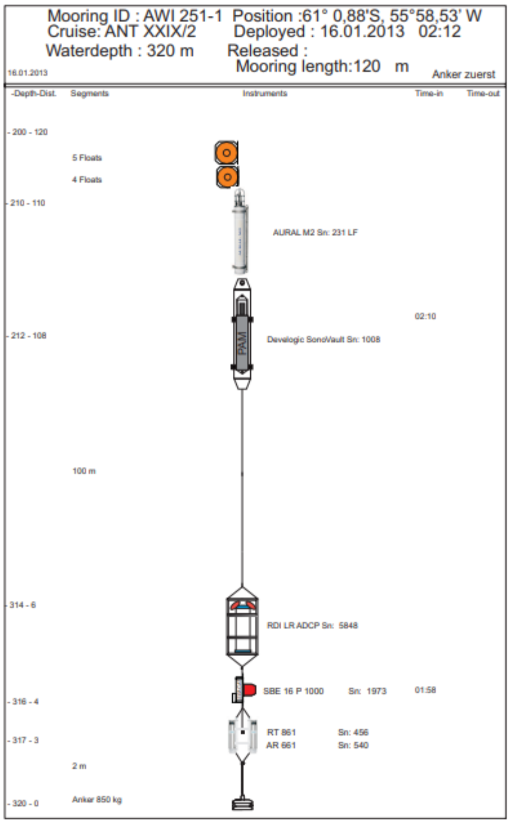

Figure 1. Mooring schematic of passive acoustic monitoring Site 6 located at Elephant Island which had both an AURAL and a SonoVault recorder on the same mooring.

Table 1. Summary of mooring information for the three passive acoustic monitoring sites examined in this study.

Site	Latitude	longitude	Recorder Depth (m)	Recording start yyyymmdd	Recording end yyyymmdd	Recorder type	Sampling Rate
Site 1: Greenwich Meridian	-59.1672	0.0028	4838	2008-12-12	2010-12-03	MARU	2,000Hz
	-59.0503	0.1105	1007	2010-12-11	2011-08-22	SonoVault	5,333Hz
Site 4: Greenwich Meridian	-68.9977	-0.1085	958	2012-12-17	2013-11-13	SonoVault	5,333Hz
Site 6: Elephant Island	-61.0147	-55.9755	210	2013-01-12	2016-05-19	AURAL	32,768Hz
	-61.0147	-55.9755	212	2013-01-12	2013-11-09	SonoVault	5,333Hz

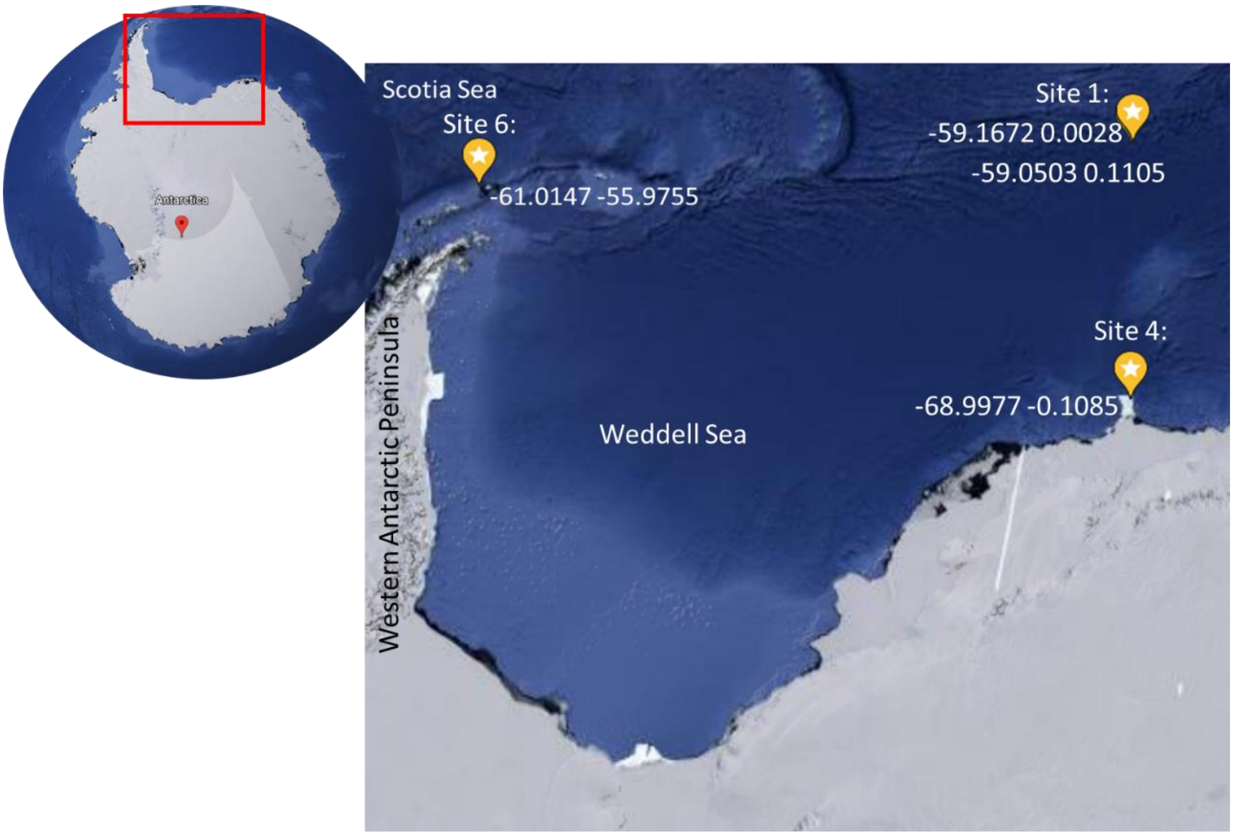

Figure 2. Map of the Weddell Sea with yellow markers indicating the location of passive acoustic monitoring sites in this study. Site 1: -59.1672 0.0028 and -59.0503 0.1105 located along the Northern point of Greenwich Meridian, Site 4: -68.9977 -0.1085 located along the Southern part of the Greenwich Meridian, and Site 6: -61.0147 -55.9755 located at Elephant Island.

Passive Acoustic Data Analysis

Data were analyzed to examine the geographic variation in fin whale 20-Hz pulses throughout the Weddell Sea. The 20-Hz pulses have been observed to vary in frequency from around 15Hz – 42Hz (Helble *et al.* 2020; Van Opzeeland *et al.* 2014). To identify fin whale pulses, which were the main target of the acoustic analyzes, fin whale songs were defined as periods of patterned 20-Hz pulse calls that persisted for two or more minutes, following the protocol described by Wood and Širović (2020). An example of distinct fin whale 20-Hz pulses in a song is shown in Figure 3.

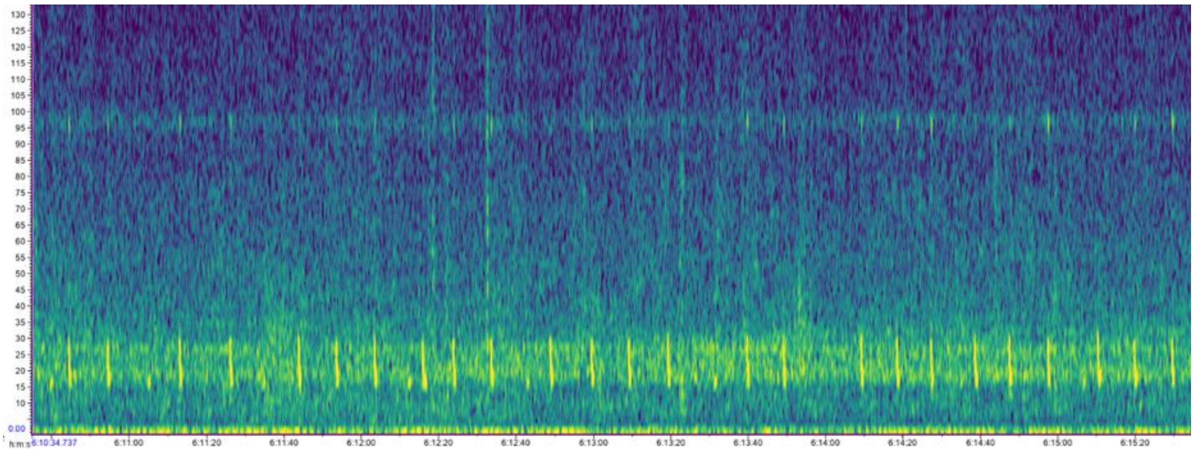

Figure 3. Spectrogram of typical patterned 20-Hz fin whale pulses in song formation longer than 2 min. Spectrogram image is from Site 1: Greenwich Meridian with the visible 99Hz HF call component.

From the acoustic recordings, two days per month were randomly selected for analyzes: one day from the first half of the month (1-15) and the second day from the second half of the month (16-30). If no clear calling bouts longer than 2 min or any fin whale pulses were identified on the randomly selected days, the immediate adjacent days were checked for fin whale song presence (making sure not to switch from one half of the month to the other). In case the immediate adjacent days still had no fin whale calls present, an additional 2 days (or two-day acoustic recording files) were checked adjacent to the initial days chosen for analysis (

Table 2 for visual explanation). If no fin whale calls were found in these additional recordings it was concluded that after checking a total of 4-6 additional days (i.e., a complete day of acoustic recording files), there were no fin whales present during that half of the month.

Table 2. Schematic describing the method of manually checking a full day acoustic recording file for fin whale song presence. If the randomly selected days from the first and the second half of the month did not have fin whale calls, the immediate adjacent days were then checked for fin whale calls.

First half of the month (1-15)					Second half of the month(16-30)				
Additional day 3	Additional day 1	**Initial Day**	Additional day 2	Additional day 4	Additional day 3	Additional day 1	**Initial day**	Additional day 2	Additional day 4

Spectrogram Measurements

Initially, acoustic data were downsampled to a maximum frequency of 500Hz, which made it more feasible to manually identify fin whale songs in the spectrograms. Downsampling of the raw data was done to increase the frequency resolution of the low frequency acoustic signatures. Spectrograms were visualized in Raven Pro 1.6 (K. Lisa Yang, Cornell Lab of Ornithology, 2019). Spectrogram analysis was performed by audio-visually inspecting 120-second paged windows with a frequency range from 5-105Hz and 0.1 s time increments. Each paged Hanning-window had an FFT of 334 points, 90% overlap, frequency resolution 1.5Hz, time resolution 0.67 seconds.

Each 2-min or longer 20-Hz calling bout was logged and the following measurements were extracted from both the low frequency 20-Hz pulse (LF) and the high frequency (HF) pulse (if present): the beginning time and end time of each pulse. The Min frequency and Max frequency of each pulse, the peak frequency, and delta time of each pulse. From this information, the inter-pulse interval (IPI) could be extracted (Figure 4). The IPI is defined as the calculated difference in start time between the first pulse and the next pulse in a calling bout. The labels of LF and HF were later used in IPI comparisons as well as the occurrence of pulses. Peak frequency was used for comparison of the HF calls in frequency within sites and between sites. Delta time was used to check pulses compared to echoes. At this point, Site 4 was excluded from any further analysis because no fin whale calls were detected at this site.

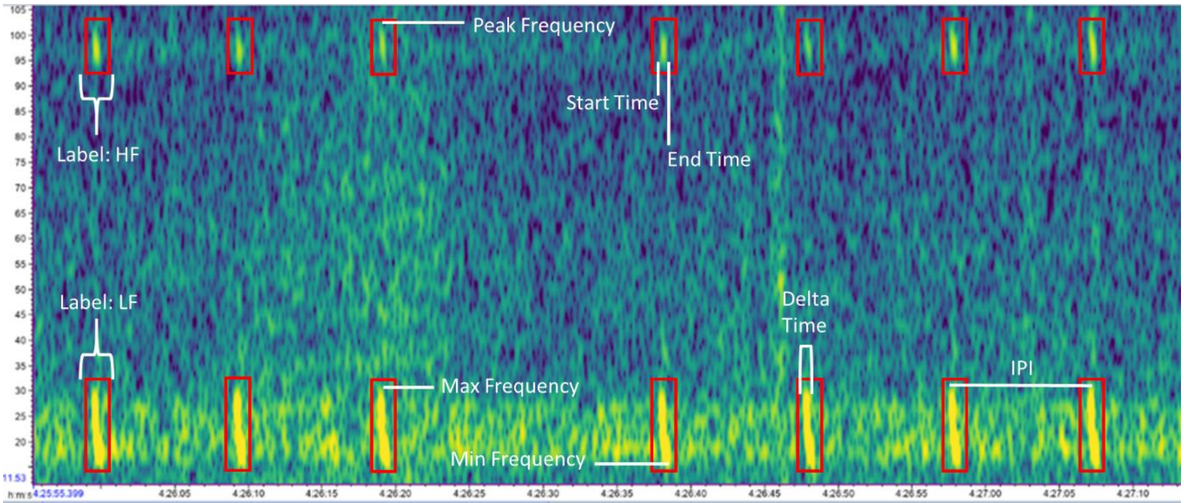

Figure 4. Spectrogram schematic indicating the measurements extracted for analysis from each fin whale song that was 2-min or longer. Spectrogram from Greenwich Meridian with an HF call component of 99Hz.

Song Quality

Before statistical analyses were performed, all recorded fin whale pulses (calls) were classified by the quality of the pulses on a scale of 1-3. With 1 being excellent clear quality, 2 being good, clear calls, and 3 being obscure but still visible. In Figure 5A is an example of a clear, bold fin whale calls that is classified as a level 1 calling bout is shown. Fin whale pulse sequences that are classified as level 2 calling bouts are not as bold as level 1 calls an example in Figure 5B. Figure 5C gives an example of fin whale calls that are classified as a level 3 calling bout. Fin whale calls that were classified as Level 1 or Level 2 in quality were later used in the annual and interannual analysis of IPIs and analysis of IPIs between sites. Level 1 and Level 2 calls were also used for HF call frequency comparisons between sites and used to investigate the relationship between HF and LF calls. Level 1 and Level 2 quality calls were also used to determine if there was a dominant IPI variant per year or site. Fin whale calls classified as level 1, level 2, and level 3 songs were used to examine the acoustic presence of fin whales per month. All levels were used in the fin whale call detection method comparison between analysts.

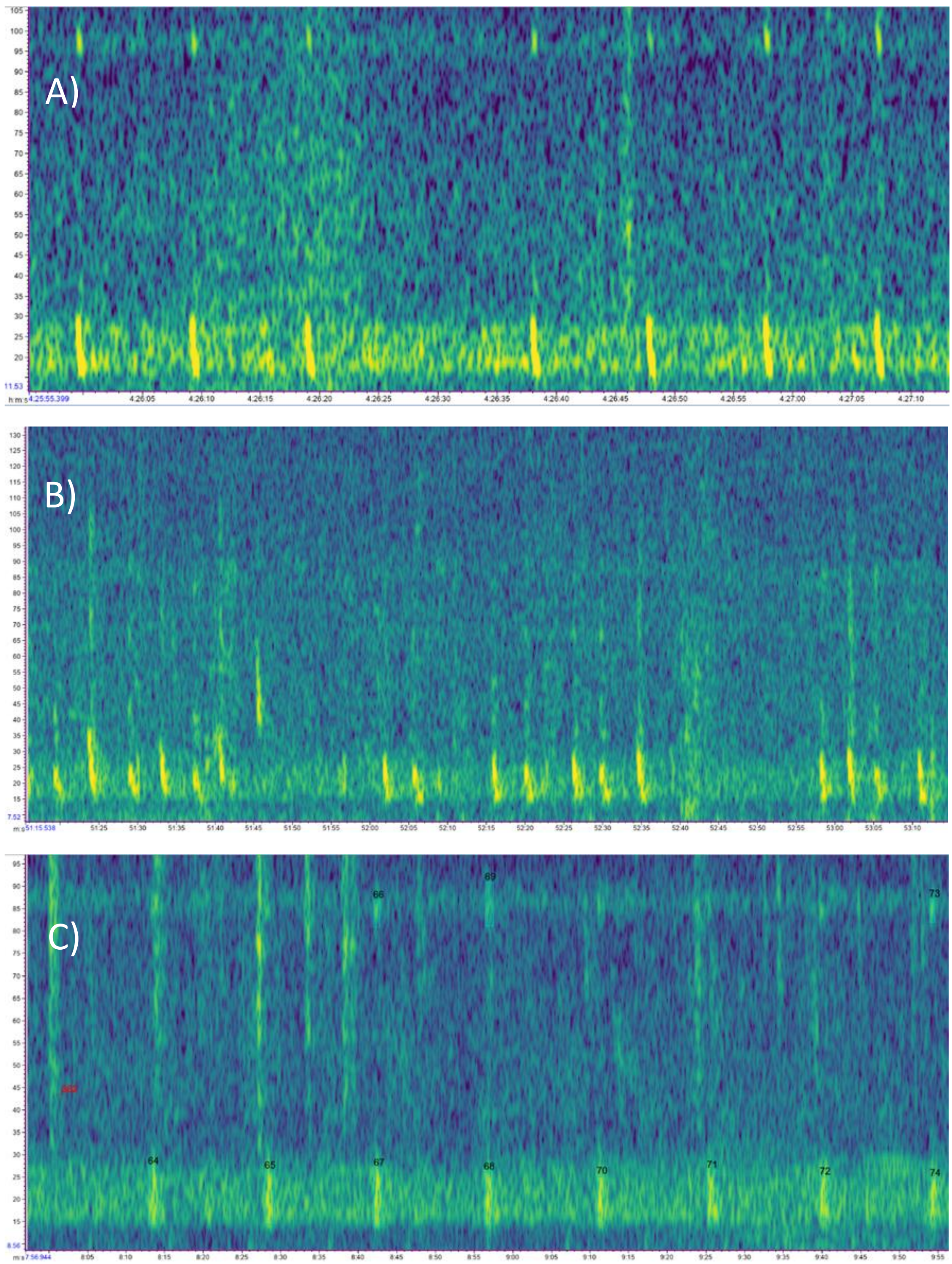

Figure 5. Three spectrogram images indicating the different classification of song quality Spectrogram A represents what is classified as a level 1 quality song. Spectrogram B represents what is classified as a level 2 quality song and spectrogram C indicates an example of a level 3 classified song.

Method Comparison

To explore the reproducibility and robustness of the manual analysis method, three separate analysts blindly analyzed the same two full day recording files to compare between the manual analyses of calling data (Figure 6). Each analyst independently boxed fin whale calls that were visually detected by manually searching through spectrograms using the method mentioned above (Figure 4).

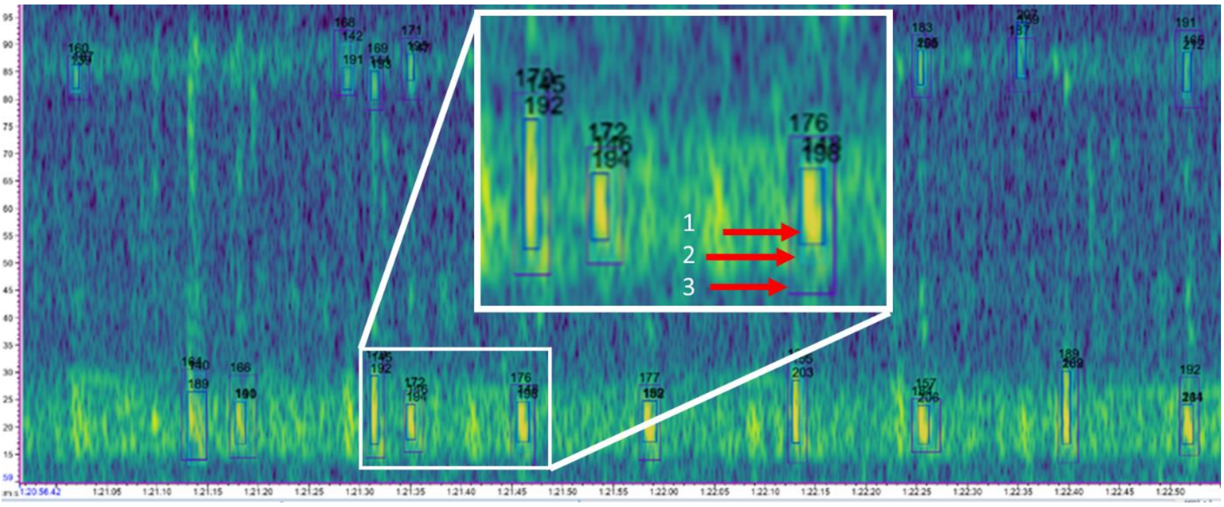

Figure 6. Spectrogram indicating an example file independently analyzed by three different analysts (different colours indicated by the three red arrows a pale blue box, a dark blue box, and a purple box) of fin whale calls for methodology comparison.

Data Analysis

Before data analysis was carried out, any fin whale calls that were questionable or unclear were excluded from any further analysis to ensure more robust results and rule out any outliers. Therefore, only quality levels 1 and 2 calls were used in the following data analyses. R (R Core Team, 2020) and RStudio (RStudio Team, 2020) were used to statistically test for differences in annual and interannual data as well as between sites. The following R packages were used in data analysis: ggplot2, Viridis, dplyr, tidyverse, hrbrthemes (Wickham, 2016 (ggplot2), Garnier *et al*. 2021 (Viridis), Wickham *et al*. 2021 (dplyr), Wickham *et al*. 2019 (tidyverse), Rudis *et al*. 2020 (hrbrthemes)) see Appendix for R scripts used in this study.

Fin Whale Acoustic Presence

To examine the trends in fin whale presence between years and between sites, the number of detected fin whale calls were plotted (Figure 7). Fin whale acoustic presence was expressed as the number of calls counted per month. However, since the search effort for each month was different, the number of fin whale calls counted was normalized by the search effort for each month (Figure 9). Fin whale presence was first investigated over the course of a year, then between years at the same site. Then fin whale presence was explored in detail between sites.

Inter-pulse Interval Within and Between Sites

To explore patterns in the inter-pulse interval (IPI) across the year, within and between sites, the IPIs for LF and HF fin whale calls were plotted. The boxplots were used to summarize the data distribution of IPIs for each month across the year and highlight the median IPI for each month where fin whale calls were present (refer to Figure 11 and Figure 13). Any months that had less than 5 IPI data points were removed from the analysis.

First, to examine differences in IPI values within sites, IPI values were compared between March 2009 and March 2011 for Site 1 (data only available for March between the two years). A Wilcox Rank Sum test was applied to determine if the median IPI values were statistically different from one another between years. Then, to investigate if there was a significant difference in median IPI values at Site 6, a Wilcox Rank Sum test was used to compare months of interest in more detail (e.g., June 2013 and June 2015, and July 2013 and July 2015). IPI values were then examined between sites to determine if there are significant differences between sites in median IPI values. The months at the end of the year where it is assumed that song convergence occurs with the onset of breeding season were investigated with special consideration. June 2009, June 2013, and June 2015 were compared using a Kruskal-Wallis test and if there was a significant difference, the months were cross compared with one another to determine which months were significantly different from one another. Additionally, a Kruskal-Wallis test was run for March between 2009, 2011, 2013, and 2015 with data from both recording sites. Then if a significant value was returned individual months were cross-referenced to determine which months were significantly different.

HF and LF Call Occurrence

To test if HF and LF calls occur simultaneously, LF pulses (20-Hz) were graphed as occurring 100% of the time and the occurrence in % of HF calls associated with the LF calls was graphed on top. Four graphs were made, one for each year, to examine any trends among months in the percent occurrence of HF calls (Figure 15). Unclear HF calls were removed from this analysis for clarity (associated LF calls were also removed if present). The presence of a large amount of noise made the measurements indeterminate if LF calls were present or not. The number of clear HF and LF calls were then graphed together to explore if there was a significant difference between the number of calls detected each month which could be influencing the percentage occurrence of the HF and LF calls.

To statistically test the temporal correlation between the percent occurrence of HF and LF calls, the number of associated calls were plotted in a scatterplot to visualize the trend in the data. Then a Spearman’s correlation coefficient test was run on the data.

HF Call Frequency

To explore the peak frequency of the HF call in detail the HF calls from each year were plotted. Comparisons could then be made within sites between years as well as between sites. Box plots displaying all data points of the peak frequency in the HF fin whale calls were plotted for individual years (Figure 18). It was compared to see if this HF call component for fin whale calls was stable across years within the same site. The HF frequency was examined between sites to investigate if there was a significant difference in the HF call component or if there was any overlap in frequency.
To test if there were significant differences in the peak frequency of the HF call, the peak frequency was compared within sites across years with a Wilcox Rank Sum test to test if there was any significant difference between median peak frequency values for the year. Subsequently, HF calls were examined to determine if there was any significant difference in median frequency values between sites.

Dominant IPI Within and Between Site

Each year was broken down into songs (sequence of 20-Hz pulses). Each song was then classified into singlet, doublet, or triplet. Singlet songs were classified as a song with one reoccurring dominant IPI with a secondary non-dominant high IPI value. A doublet song was classified as a song with two reoccurring dominant IPI's again with a secondary non-dominant IPI. A triplet song variant was classified as a song with three or more dominant and reoccurring IPIs with a secondary non-dominant IPI. Song IPIs between Site 1 and Site 6, for each year were compared by the number of repetitive IPIs within a song into the above described song variant categories. Pie charts were made to display the percentage of each song type within a year between sites. From this information trends in IPI variants for singlet, doublet or triplet were extracted and if there was a dominant song type per year within sites or between sites.

Results

The hypothesis explored in the following section is that there are two distinct acoustic fin whale populations in the Weddell Sea, the Greenwich Meridian (Site 1) population and the Elephant Island (Site 6) fin whale population.

Search Effort for Fin Whale Calls for Each Month

To examine if the search effort was sufficient per month the number of full day files manually searched in this study were plotted. For peak calling time (March to June), a search effort of two days per month was sufficient. However, greater search effort was required for non-peak months (i.e., January, February, July). Only pre-screened files (from previous projects for fin whale calls) were manually searched, resulting in months which had low search effort and no fin whale calls present in the acoustic files. Months from 2009, 2011, 2013, and 2015 that have no search effort reflect that none of the previously pre-screened files contained fin whale calls. The months which only have a search effort of 2 day files represent months for which two initially randomly selected call days both had fin whale calls present and therefore no additional days needed to be searched for that month. For months with only one acoustic day file searched for fin whale presence in 2009, this was the only day with fin whale calls based on the pre-screening (Figure 7).
At Site 6, as expected there was a greater search effort needed to find fin whale calls in non-peak calling months e.g. January and February, and July (Figure 7). In months with maximum search effort such as August to December, no fin whale calls were found (Figure 7). For 2011, there were only data for March, so interannual comparisons could only be made at Site 1 between 2009 and 2011. At Site 6, data were available from January to July so annual comparisons, as well as interannual comparisons between 2013 and 2015, could be made. At site 4, even with maximum search effort for all pre-screened files, no fin whale calls were found (Figure 7). Indicating that there is no fin whale acoustic presence at the southern recording site of the Greenwich meridian.

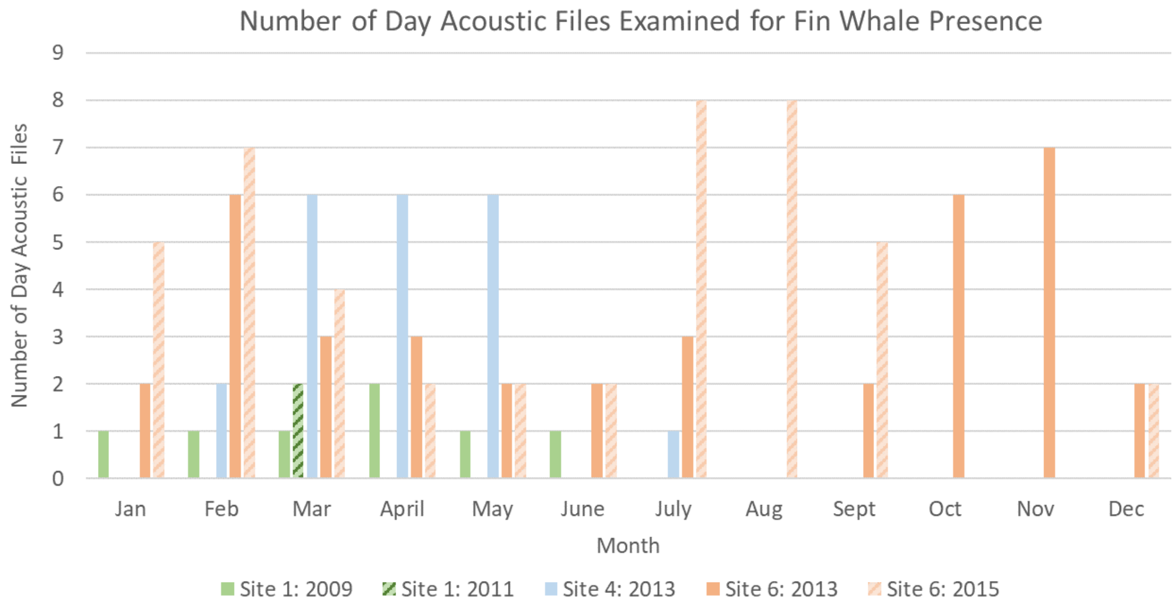

Figure 7. The total search effort between months and years for fin whale calls in full day acoustic recordings. A total of 6 day files were searched before determining that there was no fin whale presence for that month. The blank months for the years indicate there were no data available.

Acoustic Presence of Fin Whale Calls Between Sites and Years

An additional question in this study was if there are different peaks in the acoustic presence of fin whales between the two passive acoustic monitoring sites (Site 1 and Site 6). Between Site 1 indicated in green and Site 6 indicated in orange in Figure 8, there does appear to be a difference in acoustic presence between the two sites. Site 1 (green) has two peaks in fin whale acoustic presence in the course of the year, an initial peak in March/April and then a later peak in June in 2009 (Figure 8). In 2011, there were only data for March available, yet the acoustic presence for fin whales is quite high compared to all the other counts, of acoustic presence of other years and other months. For Site 6 (orange) there is a clear peak in acoustic presence in May for both 2013 and 2015 with some interannual variability between the years (Figure 8). It also appears that there is a smaller peak in the acoustic presence of fin whales in April for both 2013 and 2015. Unlike Site 1, Site 6 has an initial low presence of fin whale calls in early January with a gradual increase until the peak in May for both 2013 and 2015 (Figure 8). After the peak in May, there is a gradual decrease in fin whale calls until August when no whale calls were detected.

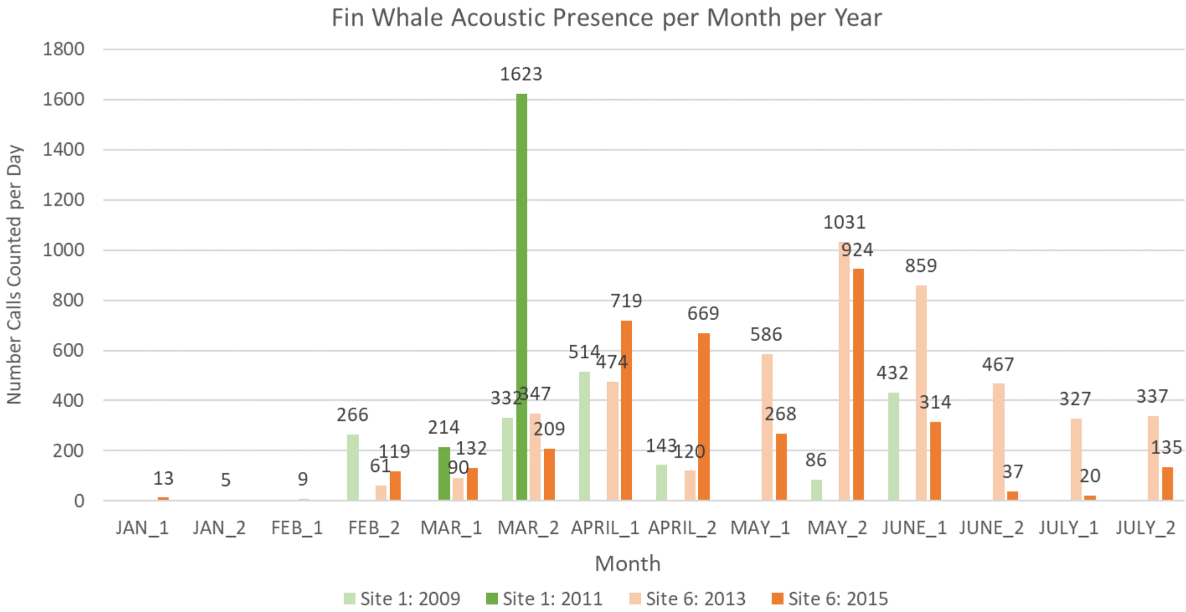

Figure 8. Fin whale acoustic presence determined by the number of fin whale pulses counted in daily acoustic files from the first and second half of the month. The variation in green represents Site 1 data from 2009 and 2011. The orange represents the fin whale presence at Site 6 from 2013 and 2015. The X-axis is the fin whale calls counted per day from the first half of the month (1-15) e.g. Mar_1 and the second half of the month (16-30) e.g. Mar_2.

By normalizing the call counts for the first half of the month and second half of the month it was determined whether the number of day files searched for fin whale presence per month (i.e. the search effort) had any effect on the acoustic presence. Fin whale acoustic presence when normalized by the search effort for each half of the month (Figure 9) did not change the acoustic presence of fin whale calls. At Site 1 the March 2011 result is still a large outlier compared to other months and years of acoustic presence. There are still two visible peaks in fin whale acoustic presence in 2009, initially in March/April then a later peak in June (Figure 9) At Site 6, there is still a gradual increase in acoustic presence before peak presence observed in May for both 2013 and 2015 and then a gradual decrease before no fin whale calls were detected in August.

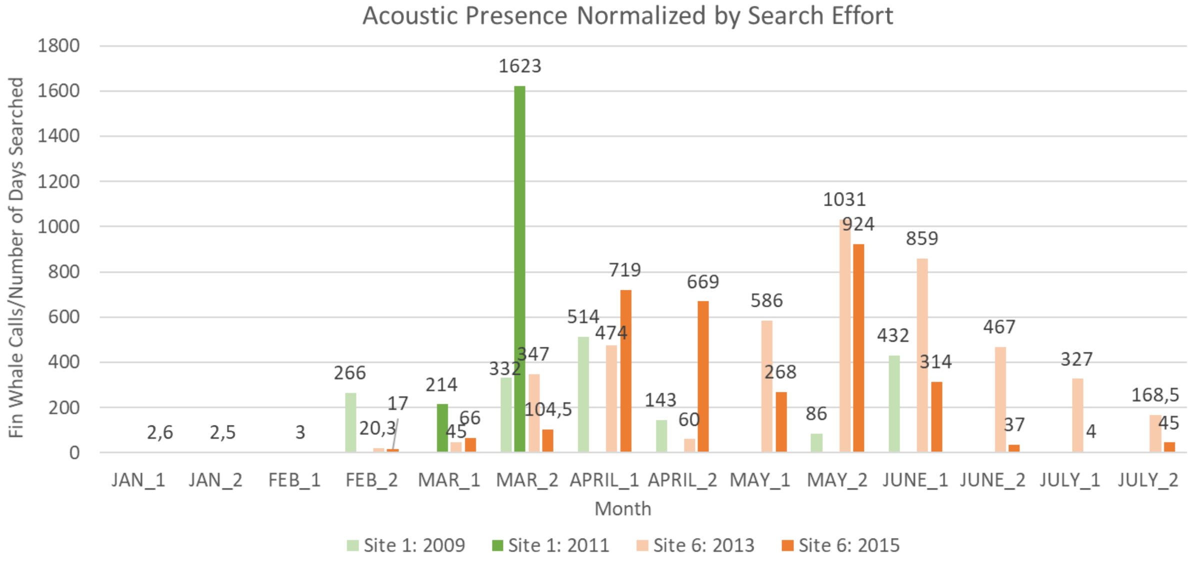

Figure 9. The acoustic presence of fin whale calls normalized by the search effort per half of the month. Green indicates fin whale presence from Site 1 and orange indicates fin whale presence from Site 6.

Are There Characteristic Differences in Fin Whale Songs Between Greenwich Meridian and Elephant Island?

The main question investigated in this study is if there are two distinct acoustic fin whale populations; the Greenwich Meridian (Site 1) population and the Elephant Island (Site 6) population. To explore this question in more detail, characteristic aspects of fin whale calls were examined such as the inter-pulse interval, the low frequency, and high-frequency calls. In addition, data were tested to determine if the LF and HF calls occur simultaneously or also separately from each other. The HF call peak frequency was measured and then compared between recording years and recording sites to investigate if there was any overlap in characteristics at either site. Finally, all IPIs were classified into song types as described in the methods to determine if there was a dominant song type across years within the same site and between sites.

How Does the Inter-Pulse Interval (IPI) of LF Fin Whale Calls Vary Among Geographic Regions Over Months?

The IPI values for both LF and HF calls were plotted for every month to visualize the spread of the IPIs within each month through the years examined in this study. After the IPIs were plotted then data could be compared across years within the same site and between sites. When visually looking at the spread of IPIs across years, it appeared that the IPIs had a large spread during the peak calling months from March to June (Figure 10). Then there was a shift in IPI values to a tighter distribution towards the onset of the breeding season at the end of June beginning of July (Figure 10).

The median IPI values between Site 1 and Site 6 were found to differ by almost 5 seconds. At Site 1 (Greenwich Meridian) there is a median IPI of around 10 seconds (9.87 sec. ±5.7 sec.) across all months. Compared to Site 6 (Elephant Island), which had a median IPI of 14.5 seconds (12.55 sec. ± 5.23 sec.).

To explore if the IPI for LF calls was the same across years within the same site, IPIs were initially compared at Site 1. First, IPIs from this site were examined between March 2009 and March 2011. Testing IPIs for LF calls in a Wilcox Rank Sum Test resulted in a p-value < 2.2e^-16 which indicated to reject the hypothesis that IPIs are independent of one another, suggesting that the LF calls from Site 1 are from the same population. For Site 6 both halves of the month July for 2013 and 2015 were tested to investigate trends in IPIs for LF calls within Site 6. In July there was a low spread in IPIs and it was expected for there to be a convergence in song type. If this song convergence is consistent among years at Site 6, no significant difference in the median IPI values between years is expected. The Wilcox Rank Sum Test for independence for the first half of July (2013 and 2015) resulted in a p-value of 5.632e^-10 which indicated to reject the hypothesis for independence and that the IPIs for LF calls likely belong to the same population. The second half of July (2013 and 2015) IPI data were tested as well using the Wilcox Rank Sum test for independence which resulted in a p-value of 5.743e^-09 which indicated to reject independence and that the IPIs from the second half of July from 2013 and 2015 likely come from the same population.

To examine IPI trends in LF calls between sites, data from March were investigated across all years and sites. It was expected that months with peak acoustic presence, such as March, would have a large range in IPI values. A Kruskal-Wallis test was run to determine if there were significant differences in the IPI between years between sites for the second half of March. A p-value < 2.2e-16, was calculated indicating to reject the hypothesis that all years are the same. Next, a Wilcox Rank Sum Test was run on the individual month comparisons to determine which years had significant differences in median IPIs. Table 3 below summarizes the p-values resulting from the comparative Wilcox Rank Sum Tests between the years. Since March has a large spread in IPIs, it was expected that there would be overlap between years and sites. As expected, comparing IPI's from 2009 and 2011 had a p-value = 0.0001614 which indicated to reject the hypothesis of independence and that the IPIs had no significant difference. The LF call IPIs from 2009 compared to 2013 indicated that the hypothesis of independence is supported (Table 3). The IPI of LF calls compared between 2009 (Site 1) and 2015 (Site 6) had just a slightly significant p-value which indicated that independence was rejected and that the median values are not significantly different. The IPIs from 2011 (Site 1) compared to 2013 (Site 6) and 2015 (Site 6) indicate that independence is supported and that there were differences in IPI values between years. Unexpectedly, LF call IPIs from 2013 compared to 2015 had a non-significant p-value of 0.1199 indicating that independence between years is supported (Table 3).

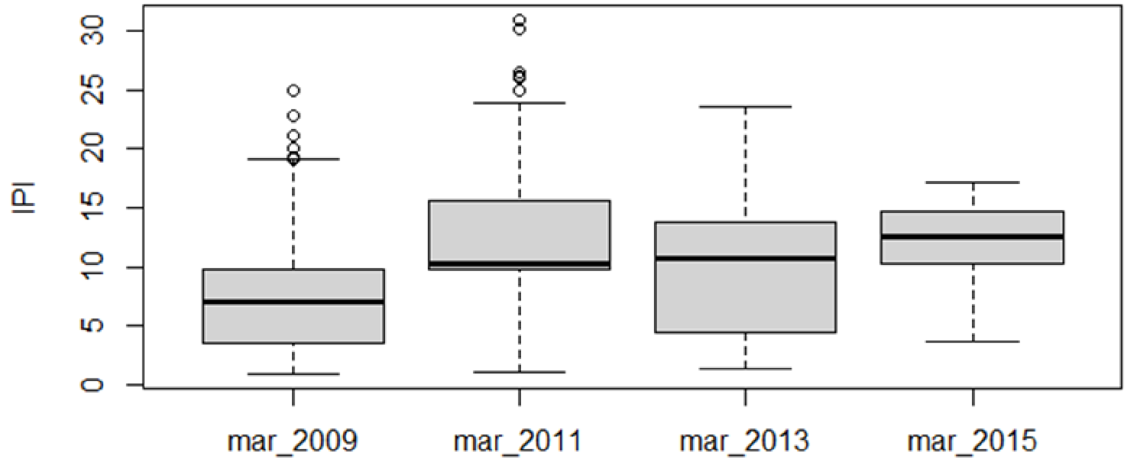

Figure 10. The distribution of IPI (seconds) data points for LF calls during the second half of March for each year 2009, 2011 from Site 1 and 2013, 2015 from Site 6.

Table 3. List of calculated p-values after running the Wilcox Rank Sum Test assuming independence of median IPIs of LF calls for the second half of March for 2009, 2011, 2013, and 2015. The resulting p-values were extracted from R (RStudio Team, 2020) with the red numbers indicating significant p-values and the green numbers indicating non-significant p-values.

Comparison years of March IPI values	**P-Value**	**Site conclusions**
2009 and 2011	p-value = 0.0001614	No diff. within Site 1
2009 and 2013	p-value = 0.1301	Diff. Site 1 and Site 6
2009 and 2015	p-value = 0.04773	No diff. Site 1 and Site 6
2011 and 2013	p-value = 0.4452	Diff. Site 1 and Site 6
2011 and 2015	p-value = 0.1278	Diff. in Site 1 and Site 6
2013 and 2015	p-value = 0.1199	Diff. within site 6

Continuing to examine if IPIs between sites were different, the month of June between 2009, 2013, and 2015 was compared. June was expected to be the early onset of male fin whales converging on a specific song type with the onset of the breeding season (Oleson *et al.* 2014). It was expected that June 2009 (from Site 1) would be significantly different from the LF IPI values of June 2013 and June 2015 (from Site 6). A Kruskal-Wallis test was run on the LF calls IPI values of June 2009, June 2013, and June 2015 to test if the median IPIs from the years are the same. The test resulted in a p-value = 0.004557which indicated that at least one of the years was different from the others. Then IPIs from 2009, 2013, and 2015 were tested individually using a comparative Wilcox Rank Sum Test to determine which year was driving the significant difference. Testing if there was a significant difference in median values between June 2009 and June 2013 returned a p-value = 0.1389, indicating that the median IPI values are independent of one another and likely from different populations. IPI values tested between June 2009 and June 2015 LF calls were found to be significantly different from one another a Wilcox Rank Sum test returned a p-value = 0.5929 indicating that the IPIs were indeed independent of one another and likely from two different populations. The independence of IPIs is also clear from the distribution of LF call IPIs in Figure 11. June 2009 IPIs have a greater spread compared to June 2015 IPIs, which have already conformed to what we expect with the onset of the breeding season. Finally, testing if there was a significant difference between June 2013 and June 2015 a Wilcox Rank Sum Test was run and resulted in a p-value of 4.648e^-5 which indicated that the IPIs were significantly different from independence and likely are from the same population.

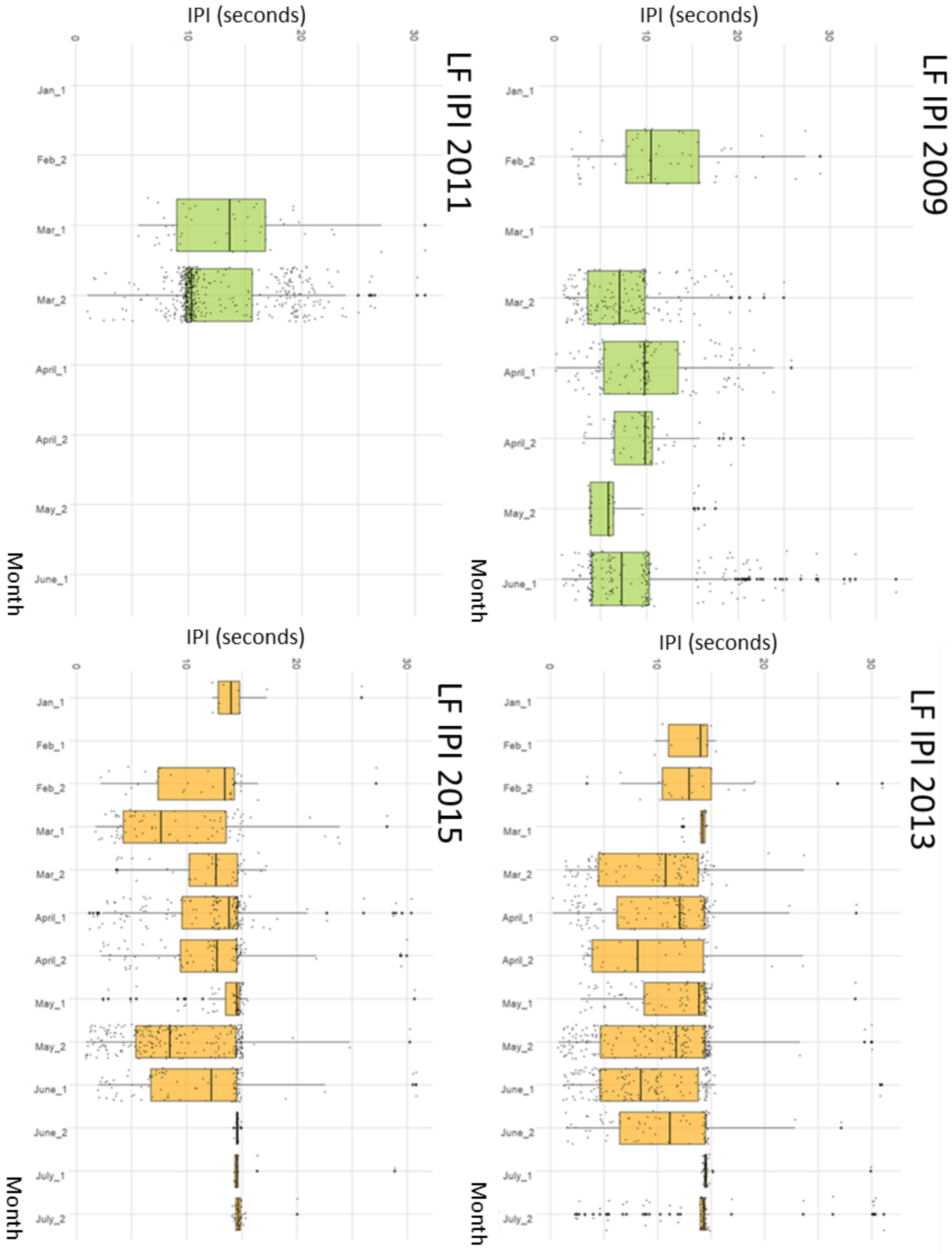

Figure 11. Inter-pulse interval of LF fin whale calls between Site 1 and Site 6. Site 1 (Greenwich Meridian) fin whale IPI in seconds indicated in green and Site 6 (Elephant Island) fin whale IPI indicated in orange. Breaks in data indicate that there was no fin whale call IPIs for that half of the month in the timeline.

How Does the Inter-Pulse Interval (IPI) of HF Fin Whale Calls Vary Among Geographic Regions Over Months?

The HF call IPIs are expected to give a clearer picture of the spread of the IPIs over the months, years within sites, and between sites. HF calls can only be detected when the calling animal is in close proximity to the recorder. In contrast, LF calls can be detected when the calling animal is further away from the recorder. Therefore, when HF calls are visible on the spectrograms this indicates that the calling animals are in close proximity to the recorder allowing for clearer measurements. Visual inspection of the spread of IPIs in June 2009 at the Greenwich Meridian (Site 1) shows the tight conformity which is expected of the IPI towards the start of Southern Hemisphere fin whale breeding season, with an IPI of ~10 seconds (10.24 sec.± 5.80) (Figure 13). Which also then shows the same general pattern for Elephant Island (Site 6) with the conformity of IPI at 14.5 seconds (14.22 sec. ± 5.67 sec.) with the onset of the breeding season. The same tests which were run on the LF call IPIs were then run on the HF call IPIs. First, the median IPI values were tested to determine if there was a significant difference within sites across multiple years. Initially, Site 1 was examined for IPIs from the second half of March between 2009 and 2011. It was expected that there should be no significant difference in the median IPIs between years within the same site. After running a Wilcox Rank Sum Test a p-value = 0.0001614 was returned which indicated to reject the hypothesis of independence and that the IPIs likely come from the same population, which was also supported by the visual impression that there appears to be no difference in the distribution of IPIs (Figure 13). When examining if there was a significant difference in median IPI values for Site 6, the second half of July (2013 and 2015) was examined since HF calls were only present in that half of the month. After running a Wilcox Rank Sum Test on these data the resulting p-value of 4.795e-09 indicated that the IPIs from HF calls were not independent and likely come from the same population.

To investigate trends in HF call IPIs between sites the IPIs were examined, first the LF calls were compared for March and tested across all years (Figure 12). A Kruskal-Wallis test was initially run to determine if IPIs between years for HF calls were the same. The p-value of 0.002514 indicated that at least one year of IPIs was different from the others. Next, a Wilcox Rank Sum Test was run on the individual year comparisons for March to determine which years had a significant difference in median IPIs. Table 4 below summarizes the p-values resulting from the comparative Wilcox Rank Sum Tests between the years. As expected the HF calls IPIs from March 2009 and 2011 resulted in a p-value of $<2.2e^{-16}$ indicating that the IPIs for HF calls are not independent and likely come from the same population. The comparative Wilcox Rank Sum Tests resulted in significantly different outcomes compared to the LF calls. Unexpectedly, IPIs for HF calls from 2009 compared to 2013 and 2015 indicated that the IPIs for HF calls were not independent and there are no significant differences between groups. Which is a different observation than what was determined for the IPI values across years for LF calls. Additionally, when HF call IPIs from 2011 were compared to 2013 and 2015 p-values indicate that years are not independent and there is no significant difference between the years (Table 4). Unexpectedly, the HF call IPIs from 2013 compared to 2015 return a p-value of 0.7634 indicating that independence is true.

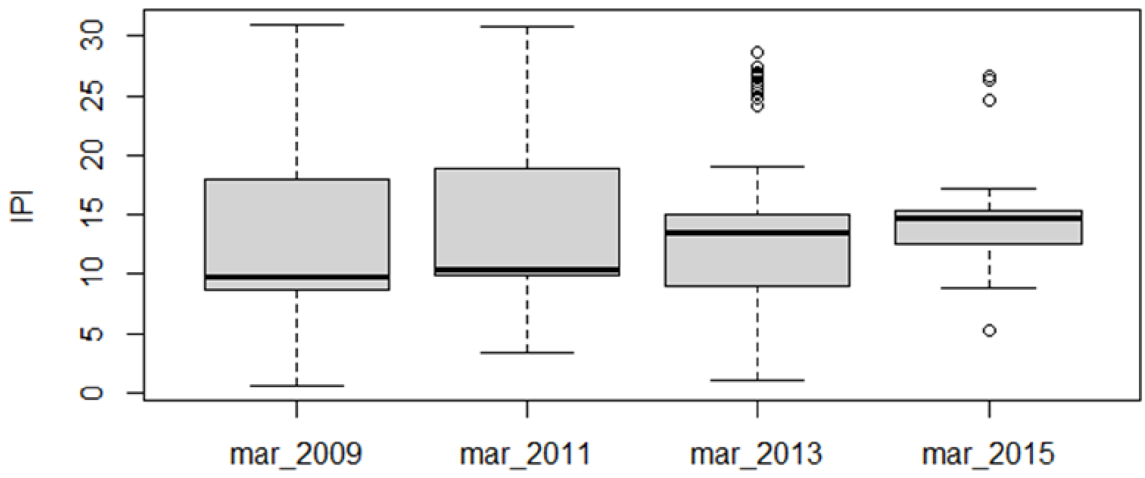

Figure 12. The distribution of IPI (seconds) data points for HF calls during the second half of March for each year 2009, 2011 (Site 1), 2013, and 2015 (Site 6).

Table 4. List of calculated p-values after running the Wilcox Rank Sum Test assuming independence of median IPIs of HF calls for the second half of March for 2009, 2011, 2013, and 2015. The resulting p-values were extracted from R (RStudio Team, 2020) with the red numbers indicating significant p-values and green numbers indicating non-significant p-values.

Comparison years of March IPI values	**P-Value**	**Site conclusions**
2009 and 2011	p-value < 2.2e^-16	No diff. within Site 1
2009 and 2013	p-value = 3.46e^-6	No diff. Site 1 and Site 6
2009 and 2015	p-value = 2.018e^-5	No diff. Site 1 and Site 6
2011 and 2013	p-value = 0.0494	No diff. Site 1 and Site 6
2011 and 2015	p-value = 0.001218	No diff. Site 1 and Site 6
2013 and 2015	p-value = 0.7634	Diff. within site 6

Further testing whether or not there is a significant difference in median IPI values for HF calls between Site 1 and Site 6 the month of June was compared across 2009, 2013, and 2015. To test if there was a significant difference between June of 2009, 2013, 2015 a Kruskal-Wallis test was run and resulted in a p-value = 8.67e^-5, indicating that there was a significant difference in the median of IPI values for June between at least one of the years. Next, individual years were tested to determine which years were driving the significant difference in median IPIs for HF calls. As expected, median IPI values of June 2009 (Site 1) and June 2013 (Site 6) were tested resulting in a p-value = 0.1389 indicating that HF IPIs from 2009 and 2013 are independent of one another. The HF calls IPIs tested between 2009 (Site 1) and 2015 (Site 6) resulted in a p-value of 0.5929 indicating that the IPIs from 2009 and 2015 are independent of one another and are unlikely to come from the same population. While comparing June HF IPIs between 2013 and June 2015 a Wilcox Rank Sum Test resulted in a p-value of 4.648e-05 which indicated independence is rejected and the HF call IPIs are likely from the same populations.

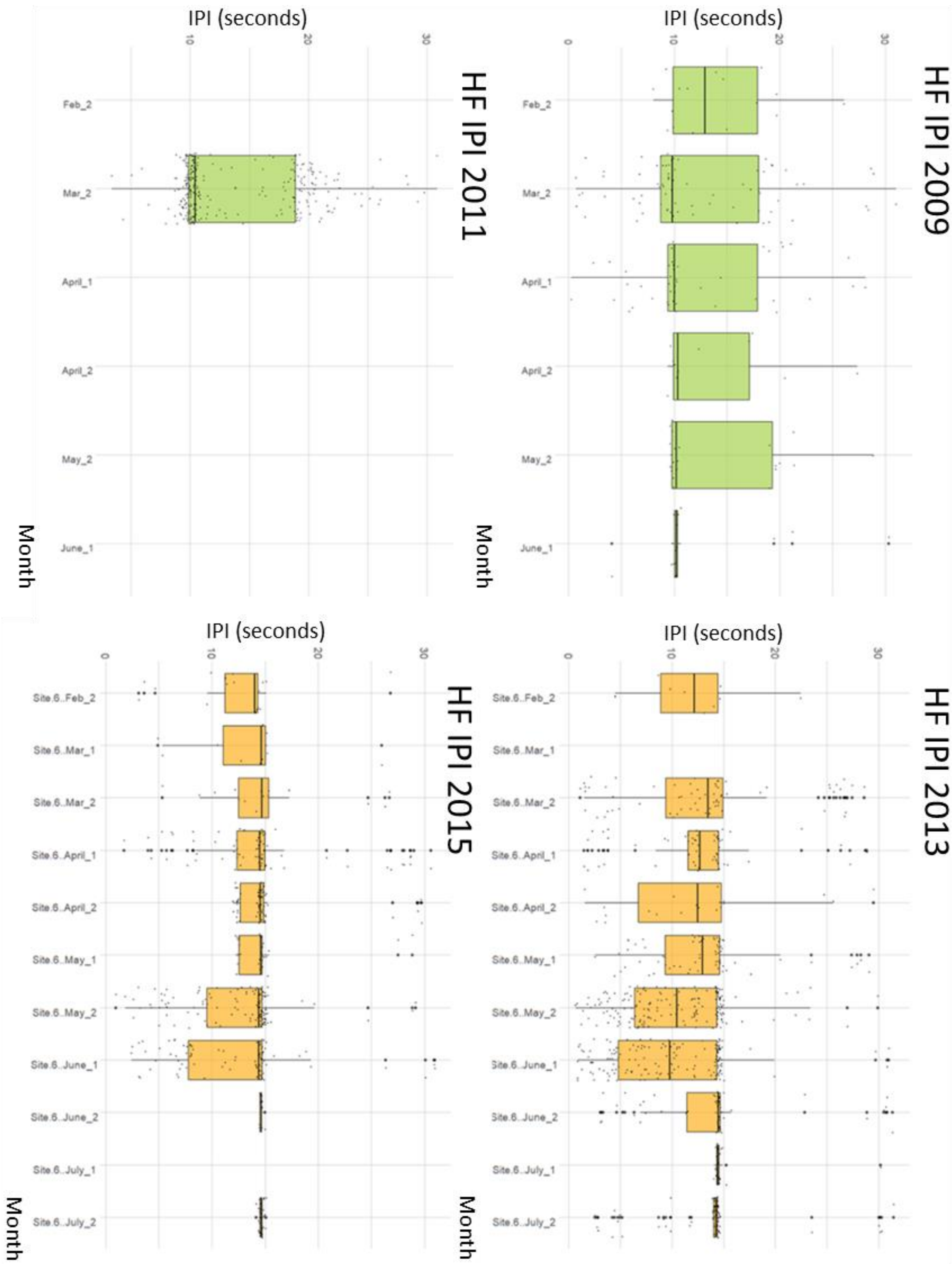

Figure 13. Inter-pulse interval (seconds) of HF call data between the months. Green indicates Site 1, Greenwich Meridian, fin whale calls and orange indicates Site 6, Elephant Island, fin whale call data. Breaks or blanks in the months indicate that no fin whale IPI data points for that half of the month in the timeline.

Is the HF Fin Whale Call Simultaneous to the LF Call or a Separate Call?

To examine whether the HF and LF call occurred simultaneously or not, the percent occurrence was plotted with the LF calls occurring 100% of the time. The months with only a few fin whale calls detected, or no HF calls detected, are the months at the early onset of acoustic occurrence during austral summer(January, February, and early March) for Site 1 and Site 6 (Figure 15). During months of high occurrence, often there were energy bands for the HF call frequency but no individual HF calls were detected (Figure 14– spectrogram image showing the energy band of HF call frequency).

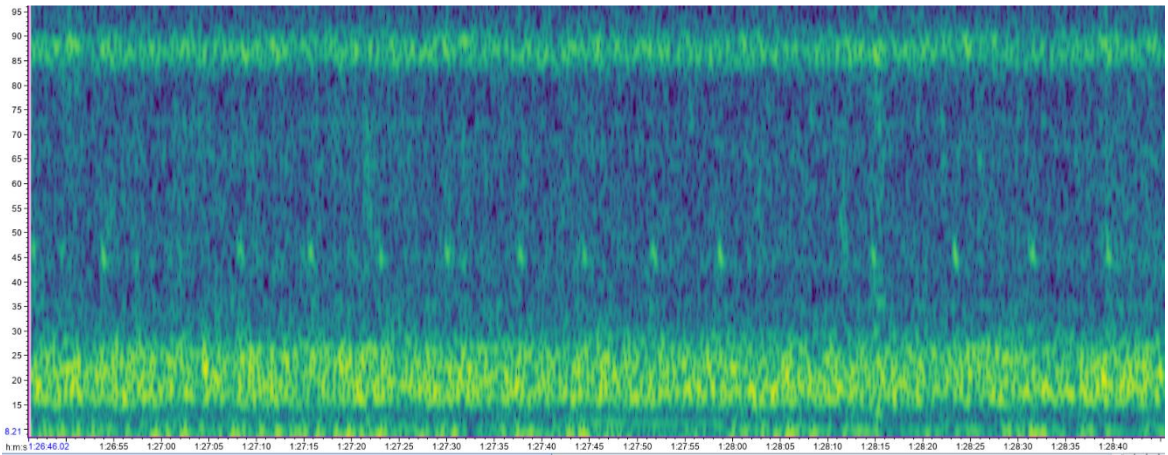

Figure 14. Spectrogram from Elephant Island indicating clear energy bands for both the LF and HF (89Hz) frequency but no clear individual calls can be identified (Site 6, file:20130424).

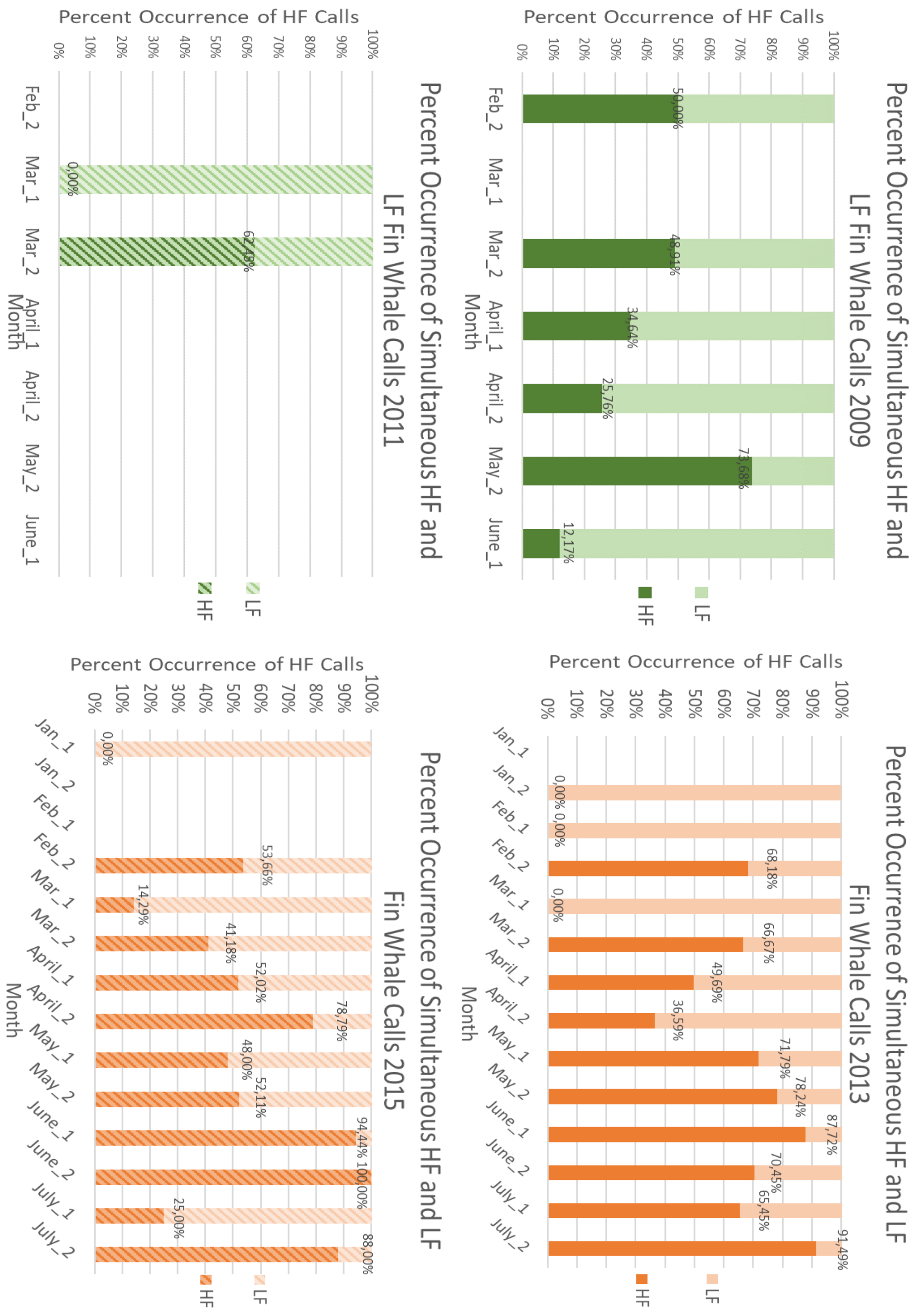

Figure 15. The percent occurrence of HF calls associated with LF calls fin whale calls. Orange indicates Site 6, Elephant Island, occurrence data and the green indicates Site 1, Greenwich Meridian, occurrence data. Blank months without data in the timeline indicate that there were no fin whale calls detected in that month.

To investigate if the HF and LF calls were strongly correlated or not, HF and LF calls were tested using a spearman's correlation coefficient. A spearman's correlation on the occurrence of HF and LF calls resulted in a p-value of 1.038e-10 and a spearman's correlation coefficient of 0.86 (Figure 16). Indicating a strong positive relationship between HF and LF calls.

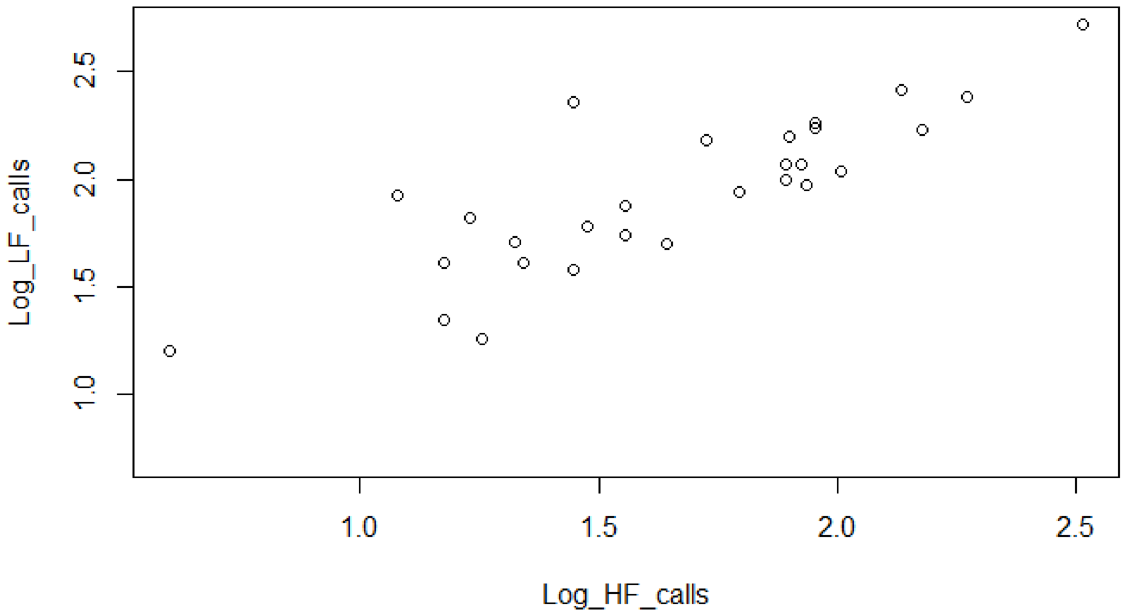

Figure 16. A strong positive linear correlation of HF and LF calls from both recording sites across all years examined in this study after being normalized by a logarithmic scale.

To examine if there was a trend in occurrence the number of HF calls associated with LF calls was also plotted (Figure 17). For months with little to no percent occurrence of HF calls there were only very few LF calls (Figure 17). There was a clear difference in the number of HF and LF calls, the majority of months having just slightly fewer HF calls compared to LF calls as a general trend in the data. Except for June 2009 from Site 1 (Greenwich Meridian) which has a much larger number of LF calls compared to the number of HF calls detected (Figure 17). June 2009 also has some LF calls that differ largely in the IPI as well (Figure 11), suggesting that these calls might not be fin whales at all.

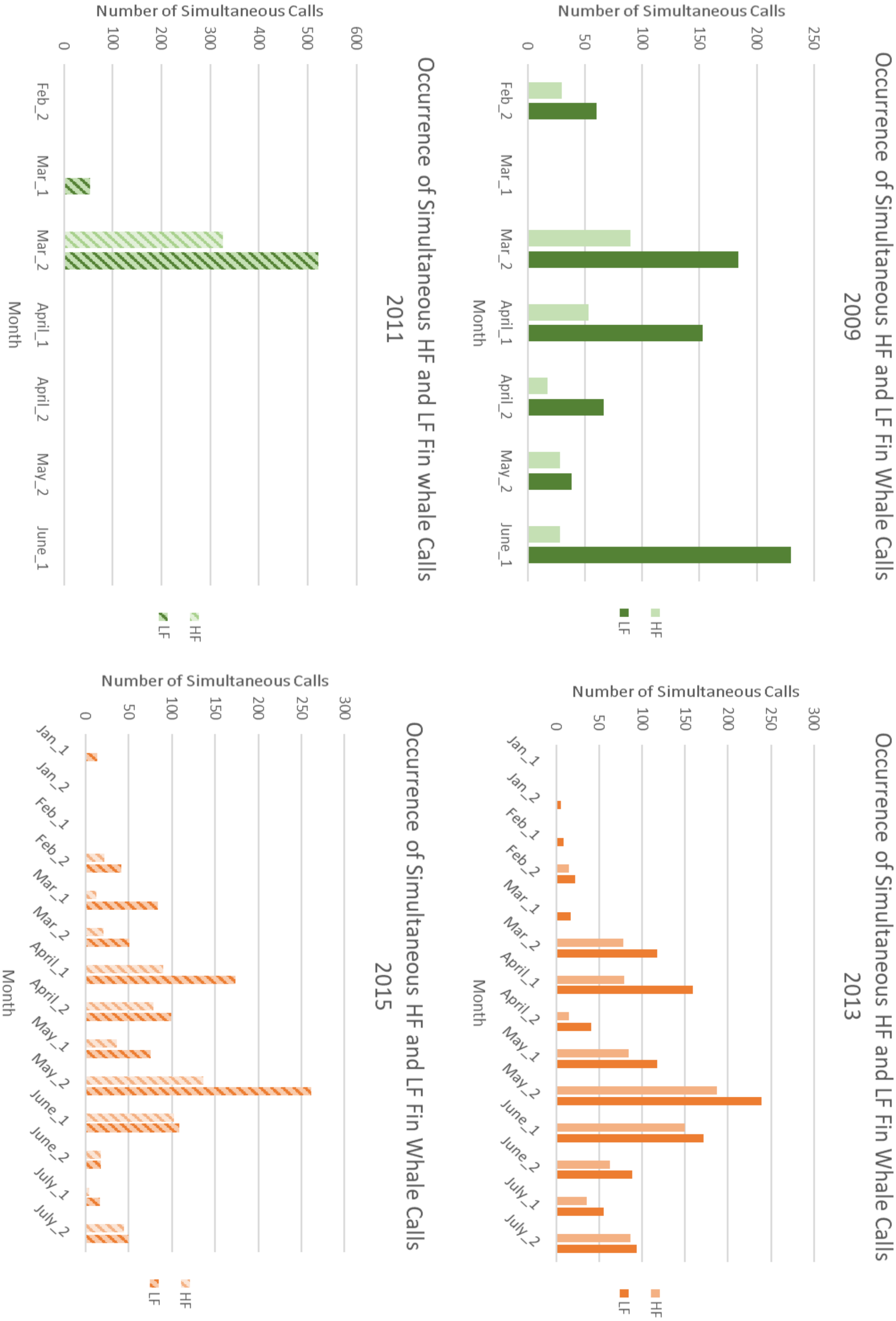

Figure 17. The number of HF and LF fin whale calls that occur in calling bouts for each half of the month. Green indicating Site 1, Greenwich Meridian, HF and LF fin whale calls, and orange indicating the number of HF and LF fin whale calls at Site 6, Elephant Island. Blank month data indicates that no fin whale calls were detected for that month in the timeline.

Is There Any Overlap in the Frequency of the HF Fin Whale Calls Between Sites?

After plotting the distribution of the peak frequency of HF calls for each year between sites, the frequency differences appear to be very distinct between the two sites (Figure 18). Site 1 (Greenwich Meridian) fin whales call at an HF call of 99Hz (97.14Hz ± 3.19) while Site 6 (Elephant Island) fin whales have an HF call of around 86Hz (86.26Hz ± 1.36). At Site 1, Greenwich Meridian, the frequency average for 2009 is 97.59Hz and the average frequency for 2011 is 97.03Hz implying an estimated drop in frequency of 0.28Hz per year. Site 6, Elephant Island, the average frequency for 2013 is 86.42Hz and for 2015 was an average frequency of 85.86Hz leading to an estimated drop in frequency per year of 0.27Hz.

After running a Kruskal Wallis Test to examine if the peak frequency in HF calls between Site 1 and Site 6 belong to the same group the resulting p-value of < 2.2e-16 indicating to reject the hypothesis of equal groups. There is a statistically significant difference in the HF call peak frequency between the two sites. Indicating that the HF call is significantly unique in its frequency to the site location where the fin whales have been recorded.

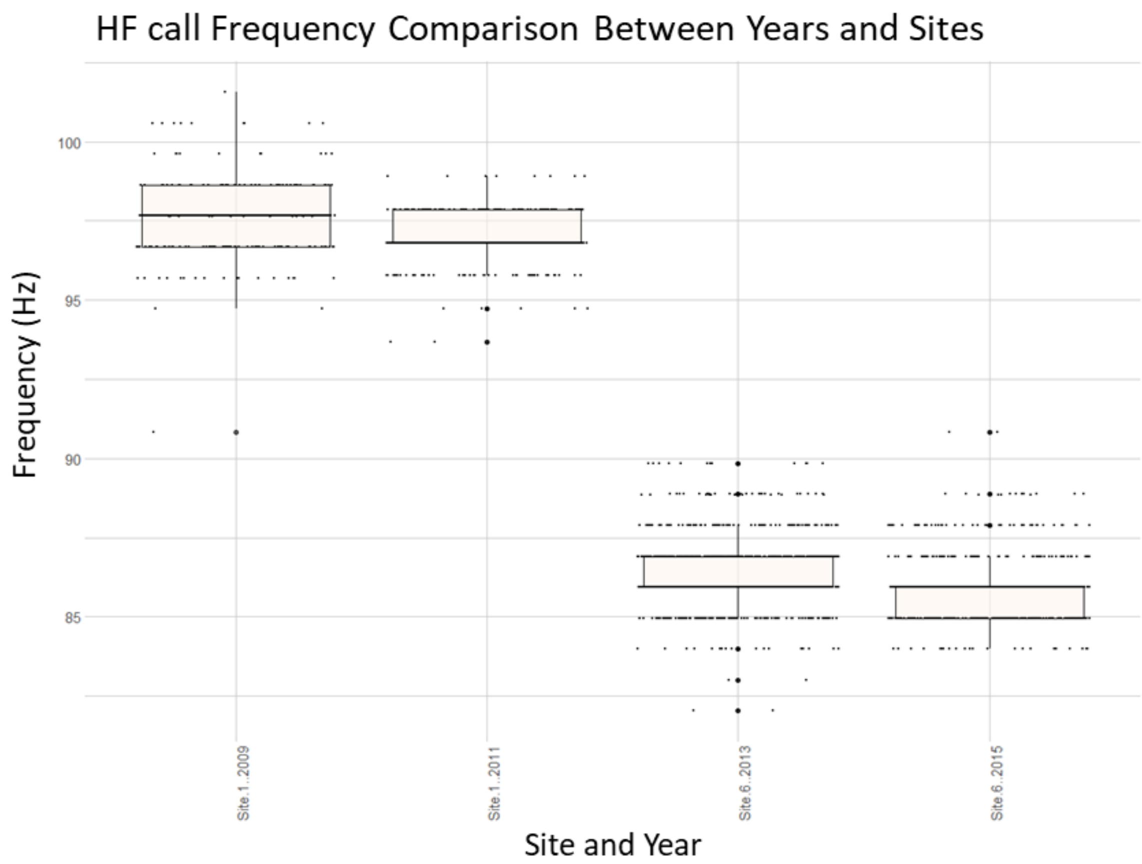

Figure 18. The HF fin whale call in peak frequency between Site 1 (Greenwich Meridian) 2009 and 2011, and Site 6 (Elephant Island) 2013 and 2015.

How does the IPI Song Variant Differ Between Sites and Years?

A dominant singlet song with an IPI of ~ 14.5 seconds for LF pulses was observed around Site 6, Elephant Island. A dominant song IPI of ~10 seconds was observed at Site 1, Greenwich Meridian, with a secondary IPI of ~18 seconds. All songs were observed to have a larger IPI of 27-30 second breaks from the dominant IPIs in the song. Site 6 (Elephant Island) was found to have the majority of singlet songs across both 2013 (45.45%) and 2015 (56.00%) (Figure 19). Site 1 (Greenwich Meridian) was found to have the majority of triplet calls across both 2009 (61.29%) and 2011 (72.00%) (Figure 19). At Site 1, fin whale songs appear to shift from 2009 to 2011 away from singlet calls and to more doublet with the majority still being triplet call variants. At Site 6, fin whale calls appear to shift from a large portion of triplet calls in 2013 to more doublet calls in 2015 with the majority of calls being singlet calls in both 2013 and 2015.

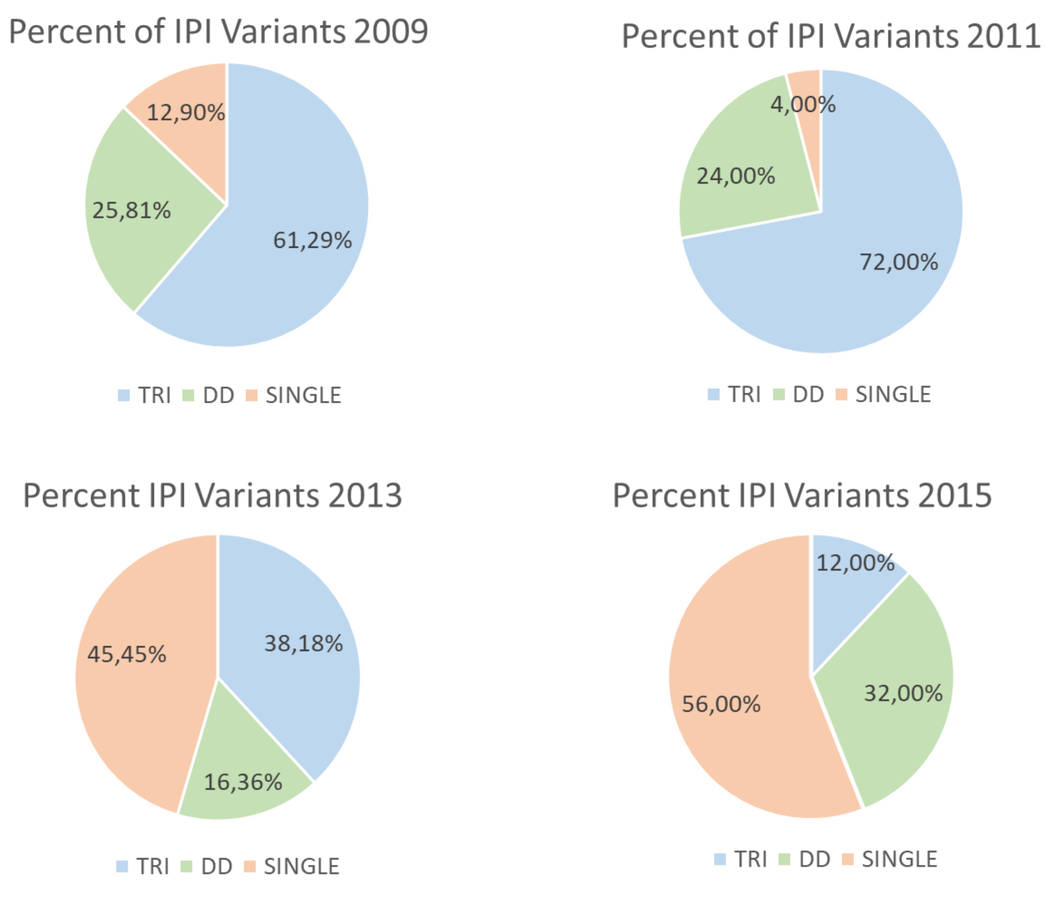

Figure 19. The percent IPI variant of each song type, singlet, doublet, and triplet of LF fin whale calls from both Site 1 and Site 6 across all years. Singlet calls are indicated in orange, doublet calls are indicated in green, and triplet calls are indicated in blue. The upper row shows IPIs are Site 1, Greenwich Meridian, and the bottom row are Site 6, Elephant Island.

Discussion

Does the Acoustic Presence in Fin Whale Calls Differ Between Years and Sites?

Acoustic presence can be used as an indicator for physical presence thus indicating the seasonal movements of fin whales. Many baleen whales spend summer months feeding in cold waters at high latitudes and migrate to overwinter in warm tropical water at low latitudes (e.g. Aguilar and Garcia-Vernet, 2018 and Baumann-Pickering *et al.* 2015). Fin whales from the Southern Hemisphere are assumed to migrate to higher latitudes during the austral summer months, spending the majority of the time feeding in the cold, high productivity, nutrient-rich polar waters and then migrate northward to lower latitudes during austral winter to the breeding and calving grounds. Studies on Arctic fin whale populations have identified no clear migration routes yet but, most acoustic monitoring studies suggest acoustic occurrence and seasonal changes in acoustic activity as evidence of migration. Fin whale acoustic studies from the Arctic suggest that with decreasing acoustic activity in April towards the summer season, fin whales are likely to migrate to higher latitudes (Simon *et al.* 2010). The same pattern in the seasonal acoustic occurrence of fin whale calls has been observed in Southern Hemisphere fin whale studies, indicating that acoustic occurrence is linked to migration throughout the year (Wood and Širović, (2020)). Fin whales off the coast of Chile are thought to migrate to the Southern Ocean, however, fin whale migration routes in the southern Pacific or the Southern Ocean to date have not been explored in detail. Weather conditions in the southern environment make it difficult to perform research as well as the travel time to get to the Southern Hemisphere is often long and expensive (Buchan *et al.* 2019). More work needs to be done identifying fin whale migration routes to and from the Southern Ocean, as this would provide valuable information for improving conservation measures for this vulnerable species.

Differences in peak acoustic presence between the two sites explored in this study suggest that there could be two different populations of fin whales migrating to different preferred feeding grounds in the Southern Ocean. The two peaks in the acoustic presence of fin whale calls seen in 2009 (Figure 8) could be interpreted as an initial migration into the area in March/ April followed by a later northbound migration in June past the recorder out of the Southern Ocean back towards lower latitude breeding grounds. The migration pattern interpreted by the acoustic presence seen in both 2013 and 2015 at Elephant Island is a gradual increase in fin whale presence starting the migration in January and having peak occurrence in May then a slow northward migration back towards lower latitudes. Research on fin whale acoustic patterns off Elephant Island over a longer time scale conducted by Burkhardt and colleagues (2021) suggests that fin whales migrate annually from Elephant Island to coastal waters off Central Chile. This finding has also been supported by Buchan and colleagues (2019), who observed fin whale calls off the coast of Central Chile, which had a corresponding acoustic occurrence in fin whale calls suggesting migration to the Southern Ocean.

Sea Ice Concentration and Fin whale Calls

The huge peak observed in fin whale acoustic presence for March of 2011 could be linked to the low ice concentrations reported during 2011. Fin whales are not known to have a close association with the sea ice edge in the same way that blue whales have been reported close to the ice edge (Širović *et al.* 2006 and Širović, 2004). Between 2010 and 2012 a La Niña event was reported, this can have a large influence on the current circulation in the Southern Ocean (Loeb and Santora, 2014). Since La Niña events can cause warm water to be pushed further southward with the upwelling of cold bottom water off South America, it is believed that the warm water caused lower than normal sea ice conditions for March 2011. Lower sea ice concentrations can explain a large amount of fin whale acoustic presence compared to previous years. Average daily sea ice concentrations from satellite data were plotted over the year 2011 at the location of where the SonoVault recorder was deployed (Figure 20). From the plot, it is clear that there was no sea ice present around the recorder until after July 11th, 2011. Additionally, sea ice concentration also can have a biological impact on Antarctic Krill (Siegel, 2005). Antarctic Krill are the primary food source of fin whales in the Southern Ocean (Agular and Garcia-Vernet, 2018). Krill overwinter under the sea ice and feed on algae that grow in the low light conditions under the ice. If there was a year where lower than normal ice concentrations caused a drop in the krill population, it is possible a larger number of fin whales would be moving around and through the area to look for large shoals of krill caught in eddies.

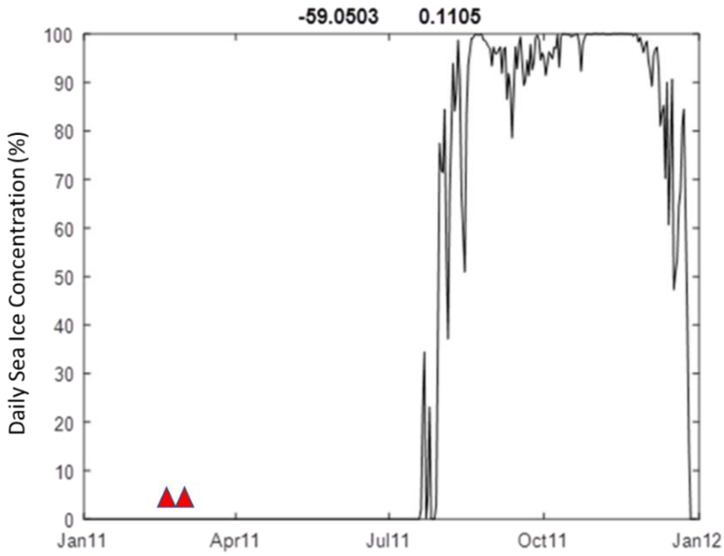

Figure 20. Average daily sea ice concentration displayed in percent for 2011. From the SonoVault deployment at the Greenwich Meridian at Site 1. In a radius of 30km around the recorder. The Red triangles indicate months with fin whale acoustic presence.

Fin whales have previously been observed to have a negative correlation with sea ice and do not associate with the sea ice edge(Širović *et al.* 2006 and Širović *et al.* 2004). Since fin whales are known to have a negative association with sea ice this is a possible explanation for why there were no fin whale calls recorded a Site 4 in the southern Greenwich Meridian. Fin whales have been observed to migrate northward with the increase in daily average sea ice concentrations at the end of July (Širović *et al.* 2004). This is also observed in the acoustic occurrence pattern seen in 2009, 2013, and 2015 at both Site 1 and Site 6. Site 4, located further south than Site 1, is much closer to the ice shelf than the other two sites examined in this study (Figure 2). The average daily ice concentration for Site 4 was plotted for the year 2011 (Figure 21). It is clear from the plot there are only a few days at the end of austral winter with no sea ice at the location of the recorder. Site 4 has a higher average daily sea ice concentration compared to the seasonal ice concentrations observed at the other two sites (Figure 20 and Figure 22). Historically, during visual surveys, fin whales have not been observed as far south as Site 4 (Branch and Butterworth, 2001), indicating that fin whales are not present that far south and likely stay in the northern regions of the Weddell Sea, away from the sea ice.

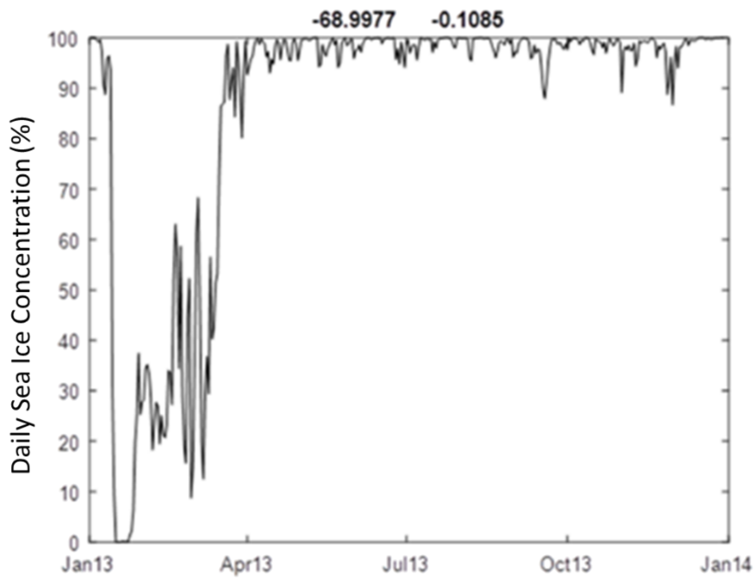

Figure 21. Average daily sea ice concentration in a radius of 30km around the recording site during 2013 at Site 4. Located on the Southern Greenwich Meridian.

The seasonal trends in acoustic activity at Elephant Island from 2013 and 2015 point towards the same group or populations of whales migrating through the area every year. Fin whales off the coast of Elephant Island consistently spend the austral summer season foraging in the cold coastal waters before migrating out of the area. These findings are supported by Burkhardt and colleagues (2021), who observed the same peak in acoustic presence over a longer time series (January 2013 to February 2016). The steady decrease in the acoustic presence of fin whales at Elephant Island can also be explained by a change in sea ice concentrations (Figure 22). It is known that with an increase in sea ice concentration at the end of austral summer, fin whales then begin their migration to warmer waters in lower latitudes. The timing of average daily sea ice concentration and acoustic occurrence of fin whales off the coast of Elephant Island is coordinated perfectly between both 2013 and 2015.

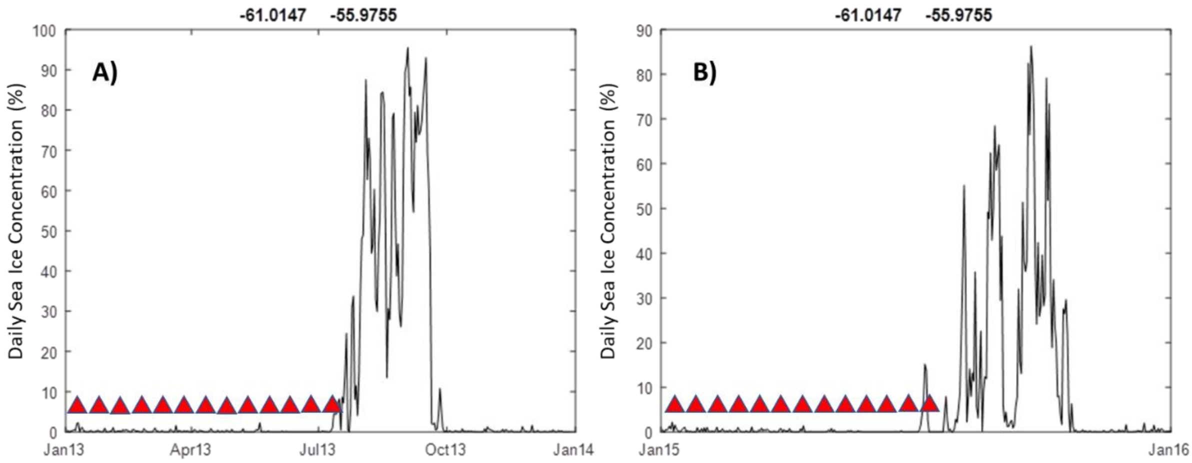

Figure 22. Average daily sea ice concentration in a radius of 30km around the recording site during 2013 (A) and 2015 (B) off the coast of Elephant Island, Site 6. Red triangles indicate months with fin whale acoustic presence.

There are differences in peak timing between the sites, Greenwich Meridian having two peaks. An initial peak in March/April and then a second later peak in June. Elephant Island fin whales have one large peak in May (Figure 8). These differences between sites suggest different migration patterns in fin whales from Greenwich Meridian and Elephant Island migrating from northern (low latitude) breeding grounds back towards the Southern Ocean feeding grounds. The structure and geography of the Southern Ocean between these two sites also suggest that fin whales recorded from Greenwich Meridian likely migrate to and from Southern Africa, the Indian Ocean, and towards Australia. Fin whales that are recorded off Elephant Island likely migrate to and from the Central and Southern Pacific. Fin whales from Greenwich Meridian have a shorter distance to migrate to warmer water when migrating towards African waters and the Indian Ocean. Fin whales migrating from Elephant Island have a shorter travel distance to the South American waters. These global features could also play a role in separating two stocks of fin whales from Site 1 and Site 6, but more work needs to be done on fin whale migration and seasonal occurrence to clarify where fin whales migrate to after leaving the Southern Ocean.

Differences Observed in Inter-Pulse Interval

The inter-pulse interval or IPI has been described as a possible population identification tool when characterizing baleen whale songs from different ocean basins. Several studies from the North Atlantic, and the North Pacific have suggested that the IPI could be used to delineate between acoustic populations. Acoustic populations are described as a group or population of animals that highly rely on audible communication for several behaviours (such as feeding and socialization) but especially mate recognition. Acoustic populations are defined as having different acoustic signatures in call behaviour between groups making populations distinct from one another even if there might not be genetic differences observed. Blue whale and humpback whale songs have been observed to have large variations in IPI throughout the year but just before the onset of the breeding season, they converge on a certain song type which has been identified as a characteristic difference between breeding populations (Oleson *et al.* 2014). After a widespread study on the call behaviour of blue whales across all ocean basins, McDonald and colleagues (2006) found that there are at least nine distinctive blue whale populations based on the characteristic differences in the IPI.

Also in fin whales, the duration of IPIs in songs has been observed to vary geographically between regions, which could indicate possible populations between ocean basins (Hatch and Clark, 2004; Delarue *et al.* 2009; Castellote *et al.* 2012; Oleson *et al.* 2014). In fin whale songs from the North Pacific, patterns in IPIs are short during the summer months and are then observed to shift and become longer towards the winter months (observed by Buchan *et al.* 2019; Oleson *et al.* 2014; Širović *et al.* 2017). Different IPIs have been shown to represent possible populations (acoustic populations) in the Atlantic, Mediterranean, and Pacific (Hatch and Clark, 2004; Constaratas *et al.* 2021; Geijer, 2016). Fin whale calls have previously been observed to have a similar synchronizing seasonal pattern in the IPI like what has been observed in blue whale and humpback whale songs. The synchronizing in IPI of male fin whale songs have been observed to occur just before the onset of migration towards breeding grounds in lower latitudes. The IPIs calculated from LF calls analyzed in this study, exhibited a clear seasonal pattern in the spread of IPIs (Figure 11). During the early migration of fin whales to the Southern Ocean at the end of the austral winter months there is a large spread in the IPIs. The IPI then shifts at the end of austral summer and there is a synchronizing of IPIs seen in both 2013 and 2015 at Elephant Island. This synchronizing of call behaviour supports the hypothesis that since it is male fin whales that sing, the song is a mating display, and males which conform to the dominant song type have a higher chance of breeding success. At Elephant Island, the median IPI throughout the year across all months is 14.5 seconds. At the end, of June 2015 and July 2013 and 2015, the fin whale pulses synchronize to the dominant IPI of 14.5 seconds. Oleson and colleagues (2014); observed a similar pattern in fin whale songs from the North Pacific with a lengthening of IPIs seasonally. At the beginning of the season fin whale song was observed to have short IPIs and at the end of the season longer IPIs. The IPI was then observed to level off just before the end of the calling period. In this study, in June of 2009 at Greenwich Meridian, the LF calls do not show as clear a pattern as they do at Elephant Island. The HF IPI gives a clearer picture of the spread of the IPIs over the months between sites. Since the HF calls can only be picked up close to the recorder, while the LF calls can be detected up to 100km away from the recorder (Burkhardt *et al.* 2021). The

HF call IPI at Greenwich Meridian shows the tight conformity of the IPI towards the onset of migration out of the area with an IPI of ~10 seconds, which is the dominant IPI recorded for Greenwich Meridian. This is evidence for the hypothesis that male song conforms at the onset of the breeding season to convey male fitness and increase the chances of breeding success.

When examining the IPI values for LF calls within Site 6 between 2013 and 2015, the statistical test suggests independence between groups which was unexpected (Figure 10). The month of March is during peak acoustic presence of fin whale call detections and in 2013 there were a lot more IPI data points than in 2015. After closer examination of the LF IPIs from 2013, there is a much larger spread of low IPIs, which could indicate multiple whales calling at the same time compared to the LF IPIs from 2013. This could explain why the LF calls between 2013 and 2015 within Site 6 are suggested to be independent. Additionally, in the examination of LF call IPIs from 2009 (Site 1) and 2015 (Site 6) were tested to have just under the significant factor to have no difference in IPIs between sites. The number of data points between 2009 and 2015 are very few which could influence the test between years and visually there is little overlap in the IPI distributions (Figure 11). Very likely with additional data points between 2009 (Site 1) and 2015 (Site 6) the p-value would be over 0.05 indicating that IPI values for LF calls between sites are different from one another.

While examining the IPI values for HF calls within and between sites during the month of March, the same pattern of independence as seen in LF calls within Site 6 is also seen here in the HF calls (Table 6). Again, this could be a result of the number of data points influencing the statistical test. If the number of data points between the two groups being compared is largely skewed this can return a significant result even if data were not significantly different between the two groups being tested. Other unexpected results from statistically testing HF call IPIs include: 2011 (Site 1) compared to 2013 and 2015 (Site 6) from the distribution of the IPIs (Figure 12), there appears to be a clear distinction between Site 1 and Site 6 HF call IPI values. Therefore, it appears that the difference in the number of data points is influencing the statistical results of the test applied to data between sites but the distribution in IPIs still shows a clear difference between sites and similarity within sites.

Characteristic differences in IPI values observed between Elephant Island and Greenwich Meridian suggest that these fin whale recordings belong to two different populations. These characteristic differences in IPI values such as seasonal patterns recorded across multiple years as well as the synchronizing of the dominant IPI type just before the drop in acoustic presence are evidence that these two sites represent different fin whale populations. The seasonal changes are observed in both the LF and the HF call IPIs adding even more evidence that the Elephant Island and Greenwich Meridian fin whale calls belong to distinct populations.

Differences Observed in Song Variants

The three-song variants differed in percent occurrence over the three years but all song variants were always present across all years. Variation in IPIs have been classified into songs types which include, singlet calls, doublet calls, and triplet calls. There is still a lot of new work being done on the pulse classification of fin whale songs. A study by Wood and Širović (2020), which is one of the first known studies to examine song variants in fin whale calls, found three different song variants present across a three-year study of fin whale songs off the Western Antarctic Peninsula. The most common song type was a singlet call with an IPI of 14.5 seconds. This finding is comparable to what was observed in fin whale song variants from Elephant Island in this study. Elephant Island fin whale calls had a common song type of a singlet across both years and the singlet IPI was 14.5 seconds. The percentage of song variant in 2015 reported in Wood and Širović (2020) also closely match the calculated percentage of song variants from 2015 fin whale call data in this study.

Song variation between fin whale populations being a new topic of interest does not have a lot of background information to back up the findings and there has been no recorded song variation exploration done for Greenwich meridian fin whale calls at this point in time. However, findings from Wood and Širović (2020) agree with the findings from the Elephant Island fin whale song variants observed in this study, suggesting that the characteristic differences seen in the percentage of song variants across the years point to the Elephant Island and Greenwich Meridian indicate that there are two distinct fin whale populations.

Is the HF Call and LF Call Simultaneous or Separate?

Findings from this study seem to support the hypothesis that the HF call component is assumed to be produced simultaneously with the LF call. To date, there hasn't been much research done on comparing the cooccurrence of HF and LF fin whale calls. A previous study conducted by Constaratas and colleagues (2021) examined fin whale song differences based on the HF call alone because the HF call is often thought to be a clear indication of calling behaviour. HF calls can only be detected in close proximity of the recorder because high frequency sounds dissipate in water faster than low frequency sounds do. It has been long hypothesized that the HF and LF fin whale calls occur simultaneously and when they have not been recorded together there are three possible explanations. The first situation is LF calls are recorded on their own and this is thought to be because the calling animal is too far away from the recorder for the HF pulse to be recorded but there is still the LF pulse present because LF calls can travel longer distances. The way sound dissipates in the water column is that low frequency sounds travel further through the water column and it is assumed this is the reason why many baleen whale species use low frequency sounds for long distance communication. The second situation is that there are LF calls present but no identifiable HF calls. This occurs when there is too much energy in the HF call frequency band of the spectrogram. Visually there is a clear energy band where the HF call should be, but no individual HF calls can be identified. The situation with no HF calls but clear LF calls can be especially true during the peak occurrence of acoustic activity since so many whales are calling at the same time that individual pulses cannot be counted. For this reason, other studies suggest not using call counts alone but in combinations with spectral density measurements and acoustic power analysis (Buchan *et al.* 2019). The third situation is HF calls being detected without simultaneous LF calls. There were only 5 occasions during analysis where this situation occurred and it can be attributed to there being too much ambient noise present in the low frequency energy to determine if the LF calls are there but are just hidden by noise (see Figure 23). All the calling bouts with HF calls and no clear LF calls were from Elephant Island, a coastal area, having a lot more ambient noise and tidal influence than the Greenwich Meridian recording site being located in an open ocean environment. The IPIs of the HF calls alone were measured and fall within the range observed for other HF calls with clear LF calls associated with them. This result further supports that the LF and HF calls occur together but under circumstances of large ambient noise where LF calls are not visible, HF calls might still be visible. Then under the circumstance where the whale is far away from the recorder the LF call might be visible but not the HF call.

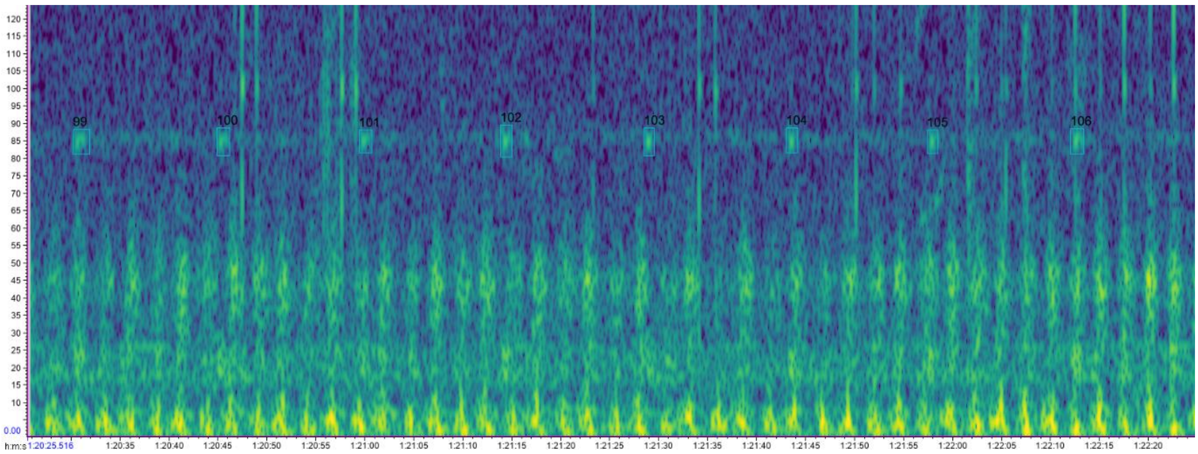

Figure 23. Spectrogram from Elephant Island fin whale recording shows an example of the situation where HF calls were recorded without LF calls. From the spectrogram, it is clear there was a lot of noise in the LF frequency range making the LF calls unclear.

At both Site 1 and Site 6, the seasonal onset of acoustic activity included months that had very few fin whale calls detected and often also had very few or no HF calls. One explanation is that the LF and HF are not simultaneous, but another explanation is that the whales were too far away from the recorder for the HF pulses to be recorded. At the early onset of acoustic occurrence, fin whales are still far away from the recorder migrating towards it suggesting that the HF call might not be recorded during the times where fin whales are migrating into the area of the recorder or migrating away from the recorder. Months during peak acoustic activity with LF calls recorded without HF calls often showed bold energy bands at the HF call frequency. The bold energy bands indicate that the HF call is being produced but no individual

HF calls were visible to be counted (Figure 24). During the peak times in acoustic activity, it is possible that there were too many whales calling at the same time for individual HF calls to be visible in the energy band.

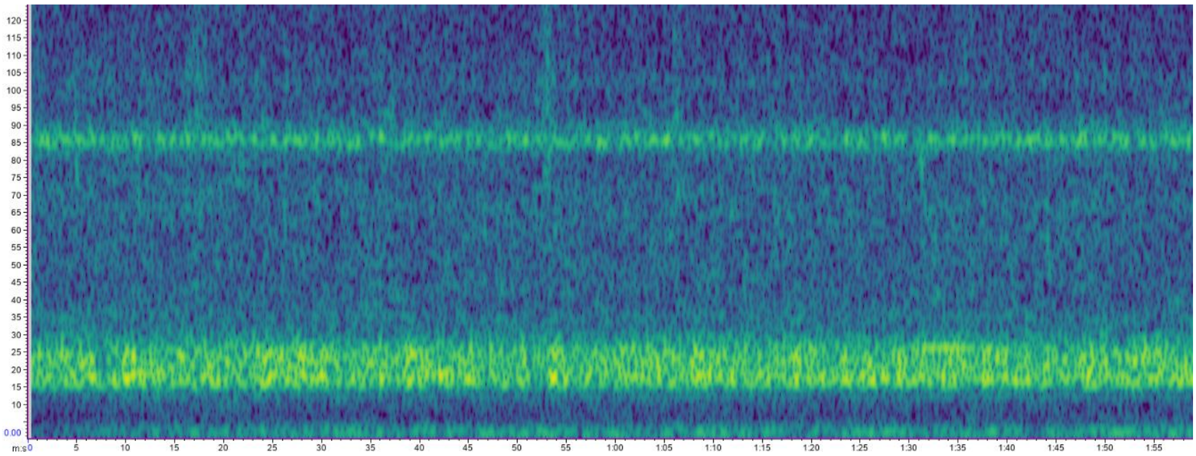

Figure 24. Spectrogram from Elephant Island fin whale call data indicating the situation where there is fin whale acoustic presence but there is too much energy in the LF and HF frequency bands for individual pulses to be recognizable.

Another challenge when examining the occurrence of HF and LF calls was that there are some ambiguous calls. In June 2009 at Site 1 there was a much higher recorded number of LF calls than HF calls and one reason for this could be because this is the time when a drop in acoustic occurrence was noticed, indicating that fin whales are starting to migrate northwards to lower latitudes. So, if fin whales are moving away from the recorder, not as many HF calls will be detected as LF calls. Another reason for this large difference in LF calls counted compared to HF calls could be that some of the more ambiguous calls as seen in Figure 25 below are possibly blue whale calls. When looking at the IPI there are also some LF IPIs that are large outliers compared to the other fin whale calls recorded in this file (Figure 11). Buchan and colleagues (2014) reported several different types of blue whale calls some being pulses referred to as ‘D calls’ which can sometimes look similar to fin whale calls and might be mistaken for fin whale calls. This suggests some calls counted as fin whale calls in this study may actually be blue whale calls.

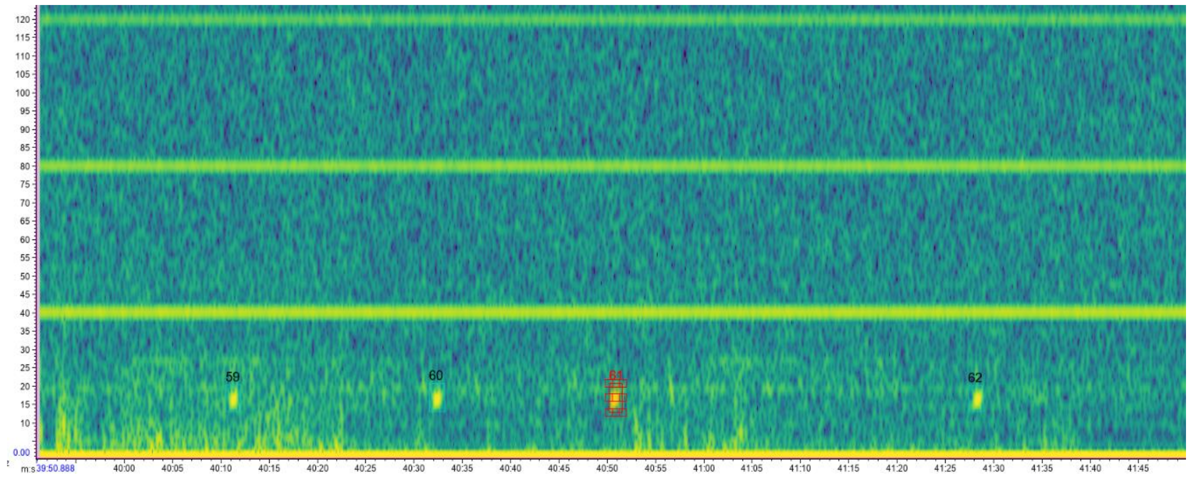

Figure 25. Spectrogram from Greenwich Meridian indicating a calling bout of LF calls which could be fin whale calls but they have a much longer IPI than other calls recorded at this site.

There is still a lot unknown about the high frequency call component of fin whale calls, therefore a lot more work needs to go into exploring the use of this call. There needs to be further investigation into long-term studies for the presence of high frequency calls and also if the HF call is used by fin whales at the breeding location as well as feeding grounds. It is assumed that the HF call is used by fin whales to identify male singers as belonging to a certain population and the LF call is for long distance communication. If it is assumed that the HF and the LF calls are simultaneous, then the hypothesis of LF calls being used for long distance communication and HF calls for short distance mate recognition is probable.
Do the HF and LF calls occur simultaneously? Given the high percentage of occurrence of HF and LF calls occurring simultaneously and what is known about sound dissipation in water, the results confidently indicate that the HF and LF calls occur simultaneously even if it doesn't always appear that way in spectrogram recordings.

Is There any Overlap in the Frequency of the HF Fin Whale Call Between Passive Acoustic Monitoring Sites?

The HF call component observed in this study from Elephant Island was found to be between 86Hz and 85Hz with no overlap to the HF calls found at Greenwich Meridian with an HF call component of 99Hz-97Hz. The high frequency call component of fin whale song has been discussed as an additional population identifier (Gedamke *et al.* 2009). In the Northern Hemisphere, fin whale songs have been observed to have an HF call component of 135Hz to 140Hz (Buchan *et al.* 2019; Hatch and Clark, 2004). Constaratas and colleagues (2021) suggest that both IPI and the presence of HF calls might be two methods of describing acoustic populations. Fin whales in regions across the Southern Ocean also have been found to produce HF calls that vary in frequency. Gedamke and colleagues (2009) studied fin whale calls off the western coast of Australia, across the Southern Ocean (east of the Greenwich Meridian), and off the coast of Tasmania. Fin whale calls recorded off the western coast of

Australia were found to have an HF call component at a peak frequency of 99Hz. Fin whale calls recorded off the coast of Tasmania have two distinctive HF call components, one at a peak frequency of 82Hz and a second HF call component at a peak frequency of 94Hz. Suggesting that there are three populations of fin whales present in the Southern Ocean off of the western coast of Australia and off Tasmania.

Fin whale HF calls were observed to have a drop in frequency between years. Fin whale HF calls from Greenwich Meridian (Site 1) were estimated to drop 0.28Hz per year and the Elephant Island (Site 6) fin whale HF calls were estimated to drop 0.27Hz per year. There is a known phenomenon for whale species of a drop in song frequency as years progress which has been observed in fin whales and is also known for other species like blue whales. Buchan and colleagues (2019) reported that fin whale calls off the coast of Chile have an HF call component of 85Hz. From what has been previously reported by Širović and colleagues (2004) fin whales off the Western Antarctic Peninsula have a reported HF call component of 89Hz. Buchan and colleagues (2019) reported that with the known phenomenon of a drop in frequency over time and with an average drop in the frequency of 0.22Hz per year, fin whales studied in Širović and colleagues (2004) and Buchan and colleagues (2019) could belong to the same population. Another study by Leroy and colleagues (2018) found that fin whales that produce an HF call component of 99Hz saw an average drop in the frequency of 0.22Hz per year, which agrees with estimates of a drop in frequency of HF calls which was also observed in this study. At site 1, Greenwich Meridian, the average frequency for 2009 is 97.59Hz and 97.03Hz for 2011, which accounts for an estimated drop in frequency 0.28Hz per year. Site 6, Elephant Island, the average frequency for 2013 is 86.42Hz and for 2015, 85.86Hz with an estimated drop in frequency per year of 0.27Hz.

In conclusion, with such a clear difference in the HF calls between Greenwich Meridian and Elephant Island and no overlap in the HF call frequency between sites. The differences in HF calls likely indicate two distinct populations. Other researchers have reported that the HF call off the Western coast of Australia was 99Hz and the HF call component off the Western coast of Chile was 85Hz further suggesting that these two HF call components belong to two distinct fin whale populations. The Elephant Island fin whale population likely migrating northward towards Chile/Central Pacific and the Greenwich Meridian population likely migrating towards Australia/ Indian Ocean during austral summer.

Singing Behaviour of Male Fin Whales

Synchronizing in song IPI observed just before the end in acoustic presence could suggest a mating display and female fin whales from Greenwich Meridian (Site 1) and Elephant Island (Site 6) are influencing song by sexual selection. Males that produce songs in any species while the female of that species has not been recorded to produce songs infer that song plays an important role in mate recognition and mate selection (Croll *et al.* 2002). In general song characteristics that are linked to breeding success appears to be observed only in male singers, usually, because females invest large amounts of energy by rearing the offspring so female mate choice for male fitness is an important investment. The production of low frequency, high amplitude calls are known to be energetically expensive for males to produce, suggesting that male song is a proxy for fitness and increases breeding success (Oleson *et al.* 2014). The sexual selection pressures from females' preference for male song, has been seen in birds and frogs as well as baleen whales and can play an important role in breeding population structure since females will not mate with a male who is performing a different song than the preferred one for each population. Evidence from the synchronizing effect of the IPIs seen at the Greenwich Meridian and Elephant Island, and both sites having a different IPI that male fin whales synchronize too. Suggesting that the females from the Greenwich Meridian and Elephant Island are defining the dominant song time and males conform to this song time in order to have the highest breeding success. Additionally, separation of male song structure between stocks is visible from the different HF calls between Elephant Island and Greenwich Meridian there is no overlap in frequency between the two sites. Which suggests that female fin whales from the Greenwich Meridian breeding population select for an HF call of ~99Hz, compared to the Elephant Island females selecting for an HF call of ~86Hz, since it is likely that, females will only breed with males from the same population they belong to. The HF call in combination with the IPI can be used by female fin whales to discriminate between males from different breeding populations.

General Findings

Spectrogram Noise

The locations, environmental conditions, and internal noise of each recorder can influence the recordings and measurements from each site. The recorder, data are unique with each deployment and the environmental conditions that are present at each mooring site. Each recorder also had differences in the level of internal noise within the recorder which can affect the signal-to-noise-ration of the spectrogram. The Site 6 (Elephant Island) recorder (AWI251-01_AU0231) is located in a coastal environment, which can have a lot more coastal background noise recorded. Site 6 also has a larger tidal influence which can lead to tidal noise influencing the mooring which is picked up in the recorder. Site 1 (Greenwich Meridian) (MARU-1 recorder and the AWI227-11_SV0002), located in the northern part of the Greenwich Meridian is an open ocean location. Being located in the open ocean there is not as great of a tidal influence on ambient noise observed at Site 6. However, the MARU-1 recorder has some internal noise from the recorder itself which can be seen as loud horizontal bands in the spectrogram images (Figure 26, spectrogram C).

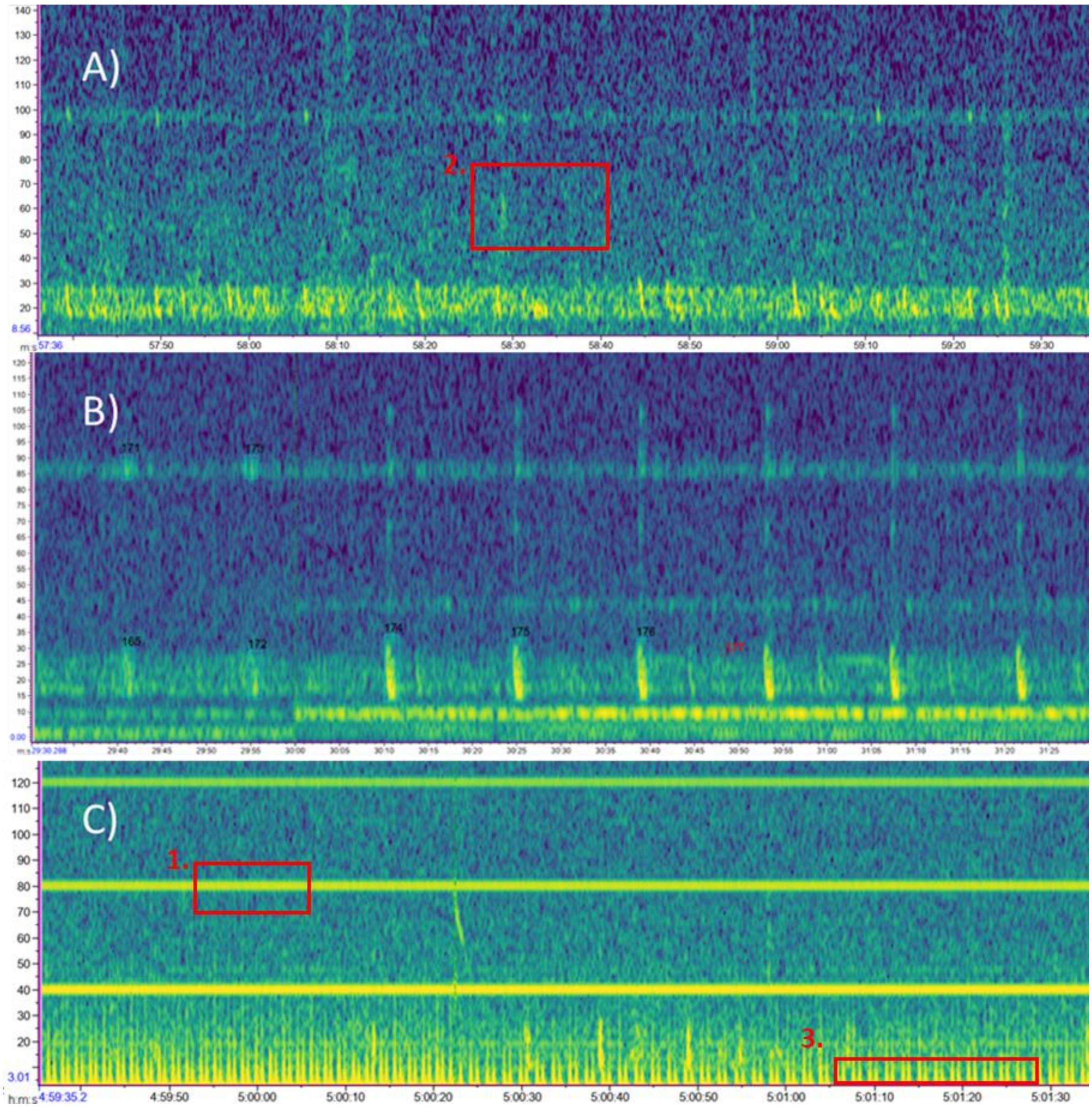

Figure 26. Spectrogram images from the three recorders examined in this study. Indicating different levels of noise from each recorder. Spectrogram A) is a spectrogram from a SonoVault recorder located at Site 1 (Greenwich Meridian). Spectrogram B) is from an AURAL recorder located at Site 6 (Elephant Island). Spectrogram C) is from the MARU recorder located at Site 1 (Greenwich Meridian). Red box 1. indicates one of the noise lines seen in the MARU recordings. Red box 2. Indicates ambient noise in the SonoVault recorder from Site 1. Red box 3. Indicate some bold ambient noise in the low frequency band.

Does Analyst Variability Substantially Impact Call Counts?

To determine if the measurements extracted in this study are reliable, call counts were examined within one analyst over time and also between three independent analysts from different experience backgrounds of fin hale call manual analysis. Boxing of fin whale calls can pose a challenge when calls are close together. In RAVEN, the thickness of the lines for boxing calls is fixed. The thickness of lines used to create boxes around fin whale calls can cause variability even within one analyst's boxing of the same calls. To investigate the variability in measurements within one analyst, calls were boxed at the start of acoustic analysis and again at a later stage, the same calls were boxed. This served two purposes in this thesis the first was to eliminate the learning bias in the analyses and the second was to explore differences in boxing of calls. The initial call counts compared to the later call counts within one analyst were slightly different but within reasonable bounds with initially learning the method and later call counts after being more confident in counting calls. Files that were recounted were the initial eight day files from the Maru recorder and the SonoVault recorder, both from Site 1. These initial eight day files were chosen to be redone while the method of acoustic analysis was learned during these call file counts. The redone call count files were the files later used in the acoustic analysis in call comparisons between sites.

Table 5. Fin whale call counts of one analyst's initial counts and secondary counts for MARU recordings from May 29th, 2009 at Site 1. Indicating the total number of calls counted, the total high frequency calls counted. The low frequency calls and the calculated (%) of calls detected between initial and secondary call counts.

File Name:	**MARU-01 20090529**		
	Initial Counts	Secondary Counts	% Difference
Total Calls	114	86	32.56%
LF calls	90	58	55.17%
HF calls	24	28	14.29%

The acoustic recordings from the AURAL recorder look different from the acoustic recordings from the MARU recorder (and SonoVault recorder). These differences in the acoustic recordings from the different recorders can lead to slight differences in counted calls between analysts. Differences that were observed between analysts were expected because each analyst had a different level of experience in the acoustic analysis of fin whale calls and had their method to determine which is a call and which is not a call. The differences due to identification were expected to be small because analysts were using the same set of rules for measuring and boxing of calls (refer to Table 6 Table 7). Since each analyst agreed on using the same set of rules/ spectrogram settings while calls were boxed, suggests that the method is very robust with a very small amount of disagreement between analysts. To test if these differences would meaningfully influence call counts, three different analysts blindly counted fin whale calls from two different recorders, these call counts are summarized in Table 6 and Table 7. The largest differences between analysts were in the HF call counts in both the AURAL recorder and the MARU recorder (Table 6 and Table 7). The differences between analysts are not different enough to influence the acoustic presence of fin whale calls in terms of call counts.

Table 6. Fin whale call counts of the three analysts for AURAL recordings from March 20th, 2013 at Site 6. Indicating the total number of calls counted, the total high frequency calls counted. The low-frequency calls and the calculated difference (%) of calls were detected between the three analysts.

File name:	**20130320_000000_AWI251-01_AU0231_500Hz**					
Analyst	Analyst 1		Analyst 2		Analyst 3	
Total # of calls	354		273		286	
# HF calls	144		81		110	
# LF calls	210		192		176	
% Diff.	Analyst 1	Analyst 2	Analyst 1	Analyst 3	Analyst 2	Analyst 3
% difference total	29.67%		23.78%		4.55%	
% difference HF	78%		31%		26%	
% difference LF	9%		19%		9%	

Table 7. Fin whale call counts of three analysts for MARU recordings from June 8th, 2009. Indicating the total number of calls counted, the total high frequency calls counted. The low-frequency calls and the calculated difference (%) of calls were detected between the three analysts.

File name:	**20090608_000916_MARU01_MA0001_500Hz**					
Analyst	Analyst 1		Analyst 2		Analyst 3	
Total # of calls	494		537		431	
# HF calls	29		22		21	
# LF calls	465		515		410	
% Diff.	Analyst 1	Analyst 2	Analyst 1	Analyst 3	Analyst 2	Analyst 3
% difference total	8.01%		14.62%		24.59%	
% difference HF	32%		38%		5%	
% difference LF	10%		13%		26%	

At the Northern Greenwich Meridian, the calls are faint and often much closer together than the calls that are observed at the Elephant Island site. An example of the faint calls typical of the Greenwich Meridian can be seen in spectrogram A in Figure 26. Typical-looking fin whale calls which are observed at Elephant Island can be seen in Figure 26, spectrogram B. Other factors that can influence how analysts count calls are the amount of ambient noise, the colour theme used in RAVEN, and the screen/ external monitor each analyst uses.

From what was observed on the variability of inter-analyst boxing the AURAL recording from Elephant Island fin whale calls appear to be largely equal between analysts (Figure 27). In contrast, comparisons from the MARU recorder have more variability between analysts. Inter analyst's variability for boxing of fin whale calls for the MARU file has a much larger variability (Figure 27). These differences between boxing could be caused by differences in the spectrogram settings each analyst used in RAVEN. Or just reflect each analyst's personality, it appears that analyst 1 is a very tight boxer while analyst 3 is a broader boxer of fin whale calls.

Originally it was discussed that each analyst would use the same settings and colour scheme in RAVEN. Yet the external monitor used to view spectrograms and the screen setting made it impossible for each analyst to use the same settings to view spectrograms. The differences in screen setting between analysts could be an explanation for some of the differences observed in analyst call counts. Additionally, an interesting observation was that the colour scheme used can influence the visibility of calls. The colour scheme used during analysis was the one recommended by Širović *et al.* 2015 (personal communication) called "cool" (i.e. a dark blue background and bright yellow for loud sounds (as seen in Figure 26) The other independent analysts used a black and white colour scheme (black being bright/loud sounds, white being background noise). The black and white colour scheme resulted in some additional pulses that analyst 1 initially did not discover. Especially in circumstances with large amounts of background noise, analysts were still able to identify some faint unclear pulses, using the black and white colour scheme, while in the blue and yellow colour scheme those pulses were not identifiable.

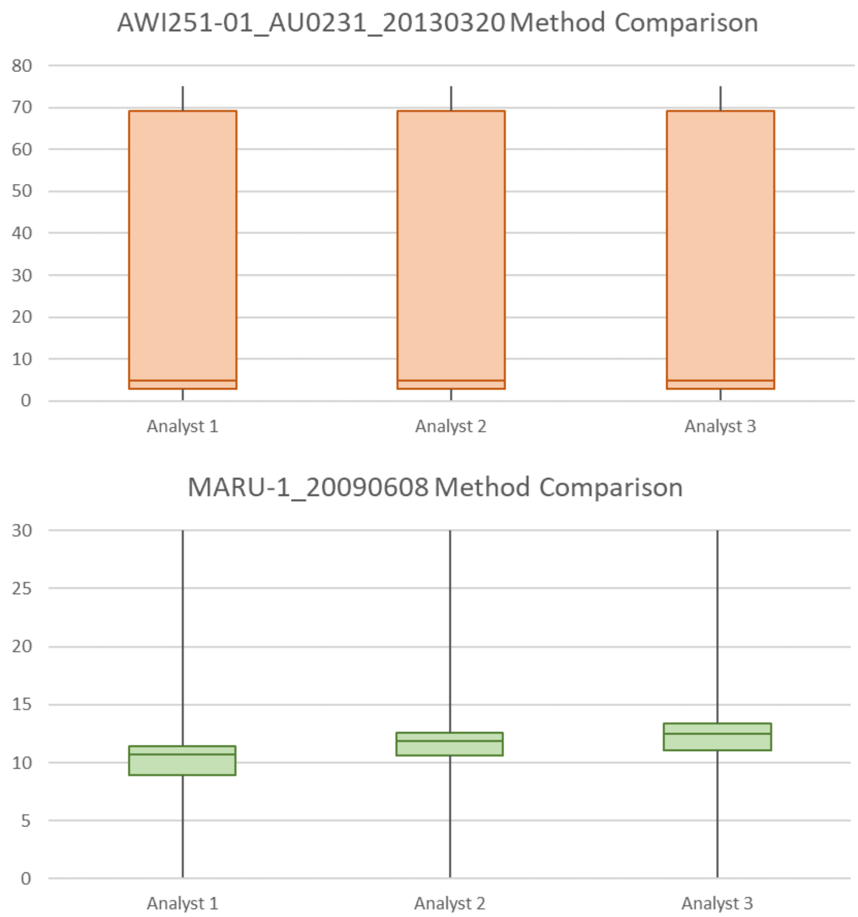

Figure 27. Method comparison of boxing of fin whale calls between three analysts from two different recorders. Orange represents call counts from Elephant Island during the 2013 deployment and green represents the MARU recorder from the Greenwich Meridian location during the 2009 deployment.

Other studies working on acoustic data of baleen whale calls have also examined inter-analyst variability when manually identifying baleen whale calls. The different personality types of the analysts can influence how precise measurements were recorded in each study. A study conducted by Leroy and colleagues (2018) found that analyst personality has a slight effect on how precise or more broad measurements are made. Additionally, a study that found some biases in the call counts due to analysts' personality differences was a study by Širović and colleagues (2015) which examined biases in data and found that there were differences between a developed call index for fin whale pulses compared to individually counted calls by different analysts. In both above mentioned studies, acoustic measurements were still determined to be reliable with little variability observed between analysts.

In conclusion, similarly to what other researchers have found inter-analyst variability does not influence the measurements to a large degree. As seen in the inter-analyst comparisons in this thesis, the counts performed by three analysts with different personalities, backgrounds, and experience in manual call counts, were relativity similar supporting the accuracy of these measurements. Allowing any conclusions made from the measurements taken in this study to be accurate and can be replicated with little variability. Trusting the conclusions that fin whale calls recorded from Site 1 (Greenwich Meridian) and Site 6 (Elephant Island) reflect different acoustic populations.

Conclusion and Outlook

Conclusion

Passive acoustic data from 2009 and 2011 at Greenwich Meridian, and 2013, 2015 at Elephant Island show characteristic differences in fin whale songs between the two sites. There is a clear seasonality in the acoustic occurrence of whales in the Weddell Sea basin. There are different peaks in the acoustic presence between Greenwich Meridian and Elephant Island, suggesting that the two proposed populations have different migration times and likely arrive at the austral summer feeding grounds from different austral winter breeding grounds. The IPIs observed at Greenwich Meridian and Elephant Island show characteristic differences, suggesting that the songs recorded at Greenwich Meridian and Elephant Island are from two different populations. These characteristic differences between the sites are observed across multiple years suggesting that the IPIs are stable across multiple years, further suggesting that Greenwich Meridian has one population of fin whales and Elephant Island has another. The IPI song variants were determined between the two sites to have a larger difference in the percentage of song variants. Greenwich Meridian was observed to have a dominant song type of triplet calls and Elephant Island was found to have a dominant song type of singlet calls. These differences observed across years within sites further support that Greenwich Meridian and Elephant Island have two proposed fin whale populations. The HF call component has previously been suggested along with IPIs of fin whale calls to delineate possible fin whale populations. The HF calls observed between Greenwich Meridian and Elephant Island further suggest that the HF call component is a probable population identifier with no overlap in peak frequency of the HF call observed between the two sites. After breaking down and examining individual components of fin whale song recorded between two geographic regions in close proximity (3037.87km from Site 1 to Site 6), and there being observed characteristic differences between songs further suggests separate populations. The observed differences in the acoustic occurrence, IPI, song variant, and HF call component between the Greenwich Meridian and Elephant Island all suggest that these locations are visited by/ used by different populations of fin whales.

Next Steps for Future Research

Future research should include creating long-term studies examining more years in the time series in order to get a larger picture of how song characteristics between the Greenwich Meridian and Elephant Island sites change over more time, which would be integral to further population recovery of fin whales. There has been more research on fin whale song from the Western Antarctic Peninsula and Elephant Island than there has been at the Greenwich Meridian so to explore longer time series and how the acoustic occurrence, IPI, and HF call change over longer time periods can lead to further insights into fin whale call behaviour in the Weddell Sea. Additional years of data are needed especially since for 2011 there was only fin whale acoustic occurrence in March but it was also observed that 2011 had environmental conditions different from the normal situation in terms of sea ice concentration. In addition to looking at already existing passive acoustic recording from Greenwich Meridian, an additional recorder should be placed further east of Greenwich Meridian. Additional recorders east of Site 1 could help to fill some knowledge gaps on the migration of fin whales from that area. If the acoustic presence is picked up at sites further east before the acoustic presence at Site 1, this could indicate the fin whales are migrating from Western Australia and the Indian Ocean.

It is known that at the Greenwich Meridian and Elephant Island there has been no recorded overlap in fin whale calls, but it is unknown currently if there could be overlap somewhere between these two recording sites. In the future, it would be key to explore if these two proposed populations or acoustic calls are observed to overlap anywhere in the middle of the Weddell Sea because this can then have implications for the conservation management of the species. If analysis of PAM data from a current passive acoustic monitoring site in the middle of the Weddell Sea would further suggest that these two proposed populations are not interacting. The next step would be to create catered conservation efforts for each population to maximize the recovery status of fin whales in the Southern Ocean.

Song variants are a relatively new exploration into fin whale song structure and need to be investigated further. At this point, there has only been one study looking at song variation in fin whales from the Western Antarctic Peninsula (Wood and Širović, (2020)) and no studies have examined song variants from fin whales at the Greenwich Meridian. Additionally, there have yet to be studies that explore song variation from other proposed fin whale populations from other ocean basins, which might be an additional tool to delineate across acoustic populations. An initial step towards investigating song variants could be to analyze existing acoustic data from different proposed fin whale populations and determine if there are additional song variants that exist in other ocean basins and if there are any overlaps between recording sites in song variants. Additionally, doublet songs often have alternating long and short pulses which is what Helble and colleagues (2020) refer to in their study as 'A' and 'B' pulses. Differences in song structure observed by Helble and colleagues (2020) could also be examined in more detail for fin whale calls between regions in this study to see if the same pattern arises in doublet songs having alternating pulse types.

In cooperation with other countries and research institutes, it would be key to look into the migration of the whales between these two locations. It is theorized that the Elephant Island fin whales migrate to breeding grounds off Chile and move to the Central Pacific. While the Greenwich Meridian fin whales are hypothesized to migrate towards South Africa, the Indian Ocean, and Australia to breeding grounds, however, this remains largely unknown. It would be imperative for conservation efforts to identify the migration route to breeding grounds to determine if there would be any genetic cross-over between the two populations. If there is not which is what is expected then they are in the early stages of divergence and need to be treated as two independent populations. Initially, to examine if fin whales from Greenwich Meridian are migrating east towards western Australia, South African waters, and the Indian Ocean, comparing fin whale recording from Greenwich Meridian to recording from Western Australia and South African waters would be a crucial step. Examining recordings from these different locations for overlap in HF call components along with IPIs and song variants can allow for a better picture of where fin whales are migrating to once they leave the Southern Ocean. Additionally, in the future, as technologies improve, to have satellite-monitored radio tags on whales from several sites, monitoring migration movements for several years would be a game-changer into the understanding of where whales migrate to after spending austral summer in the Southern Ocean. The implementation of satellite-monitored radio tags has already been done in a study by Lydersen and colleagues (2020) monitoring Northern Hemisphere fin whale migration routes from summer feeding grounds off the coast or Norway and winter feeding grounds off Portugal. Satellite-monitored radio tagged fin whales from Lydersen and colleagues (2020) revealed migratory movements of fin whales in detail, with some whales remaining at higher latitudes all year long. To date, there are no studies examining/ satellite-monitored radio tagged fin whales from the Southern Ocean and where fin whales migrate too after leaving summer feeding grounds.

Currently, the IUCN red list lists fin whales as vulnerable with recovering population numbers, but there is still so much unknown about fin whales in the Southern Ocean. Proposed populations have different environmental stressors, etc., and may need independent conservation measures. Therefore, to continue the positive trend of fin whale population recovery, different aspects of fin whale behaviour must be examined further.

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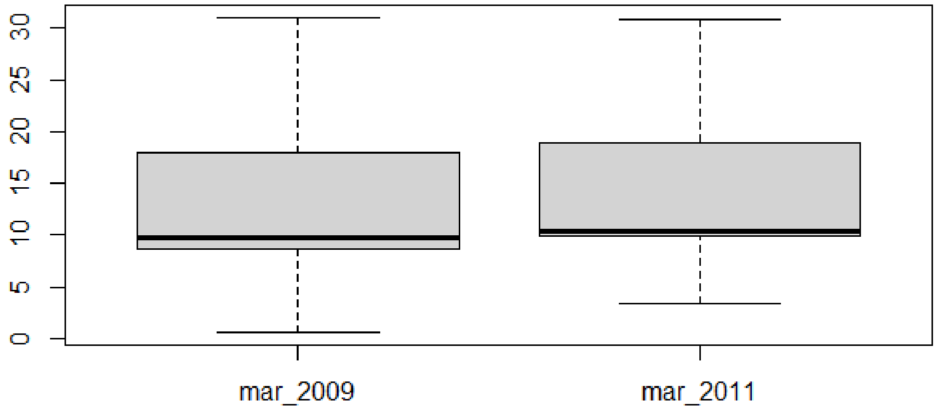
Appendix

Overview of Appendix

Statistical Test Results

March HF calls 2009 and 2011



> wilcox.test(IPI~ï..month_year, data = IPI_mar_2, paired = FALSE)

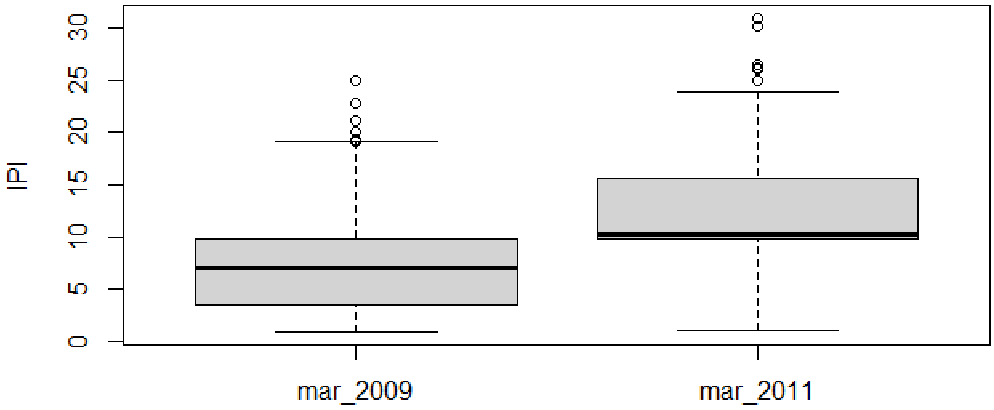
Wilcoxon rank sum test with continuity correction

data: IPI by ï..month_year

W = 8187, p-value = 0.0001614

Since the number of calls is different, had to used paired false and assume to test that they are independent. From the low p-value we reject that the groups are independent and that they belong to the populations

March_2 2009 and 2011 of LF calls



wilcox.test(IPI~ï..month_year, data = IPI_mar_2, paired = FALSE)

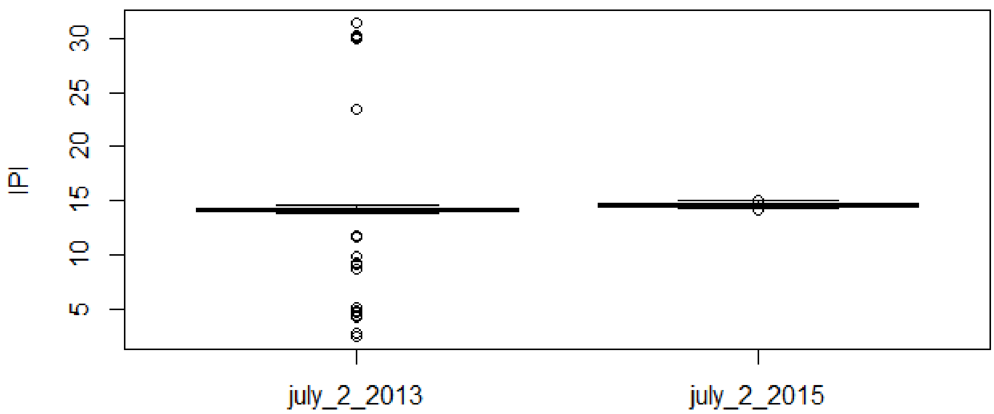
Wilcoxon rank sum test with continuity correction

data: IPI by ï..month_year

W = 18958, p-value < 2.2e-16

The different number in calls had assume paired is false, which meant testing for independence. The low p value suggests to reject the hypothesis. The LF calls between 2009 and 2011 are not different.

July_1_LF calls 2013 and 2015



wilcox.test(IPI~ï..month_year, data = IPI_july_1, paired = FALSE)

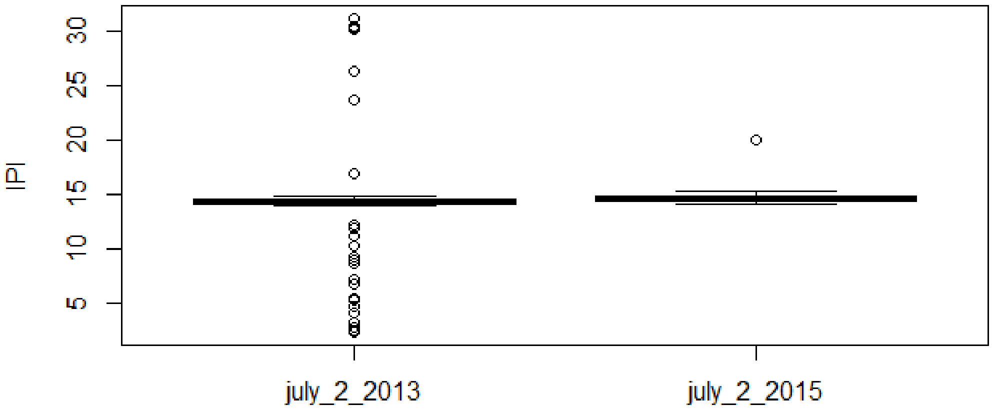
Wilcoxon rank sum test with continuity correction

data: IPI by ï..month_year

W = 436, p-value = 5.632e-10

Had to test paired = false, because different number in observations. The Ho: is testing for independence. The low p=value below 0.05 suggests to reject Ho and that the LF calls from site 6, belong to the same population.

July_2 LF calls between 2013 and 2015



wilcox.test(IPI~ï..month_year, data = IPI_july_2, paired = FALSE

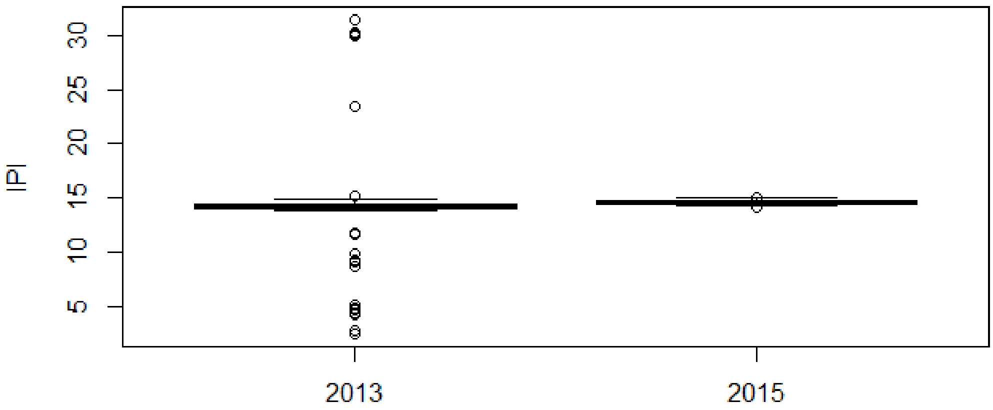
Wilcoxon rank sum test with continuity correction

data: IPI by ï..month_year

W = 778, p-value = 5.743e-09

Had to test paired = false, because different number in observations. The Ho: is testing for independence. The low p=value below 0.05 suggests to reject Ho and that the LF calls from site 6, belong to the same population.

July HF calls 2015 and 2015 only for second half of July



wilcox.test(IPI~ï..month_year, data = IPI_HF, paired = FALSE

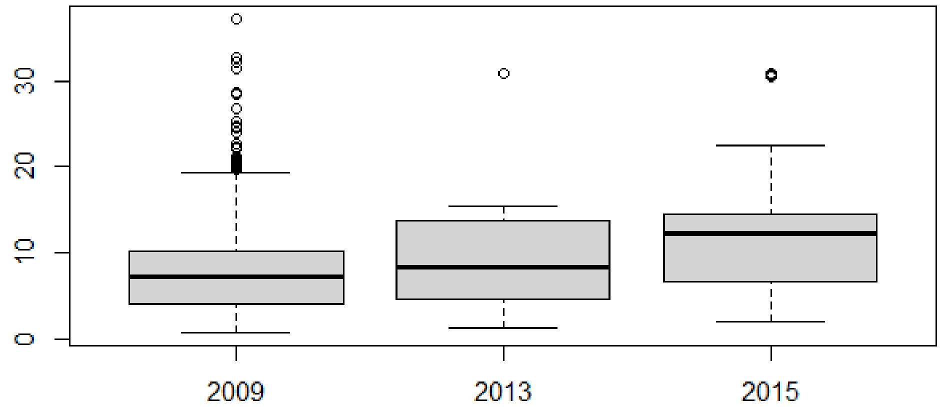
Wilcoxon rank sum test with continuity correction

data: IPI by ï..month_year

W = 731, p-value = 4.795e-09

Had to test paired = false, because different number in observations. The Ho: is testing for independence. The low p=value below 0.05 suggests to reject Ho and that the HF calls from 2013 and 2015 at site 6, belong to the same population.

June_1_2009, 2013 and 2015 LF calls



kruskal.test(IPI~ï..month_year, data = IPI_LF)

Kruskal-Wallis rank sum test

data: IPI by ï..month_year

Kruskal-Wallis chi-squared = 10.782, df = 2, p-value = 0.004557

Kruskall wallis test, tests if the groups are the same and if at least one group is different the test will return a significant p-value indicating one of the groups is different. Then need to test between the years to see which is different.

Testing between 2009 and 2013

IPI_2009_2013 <- read.csv("HF_june_2009_2013_IPI.csv")

wilcox.test(IPI~ï..month_year, data = IPI_2009_2013, paired = FALSE)

data: IPI by ï..month_year

W = 822, p-value = 0.1389

The non significant p-value agrees that the two groups being tested are independent and not paired together.

Testing IPI for LF calls between 2009 and 2015

Wilcoxon rank sum test with continuity correction

data: IPI by ï..month_year

W = 2184, p-value = 0.5929

The non significant p-value agrees that the two groups being tested are independent and not paired together.

Testing for independence between 2013 and 2015 of LF calls

wilcox.test(IPI~ï..month_year, data = IPI_2013_2015, paired = FALSE)

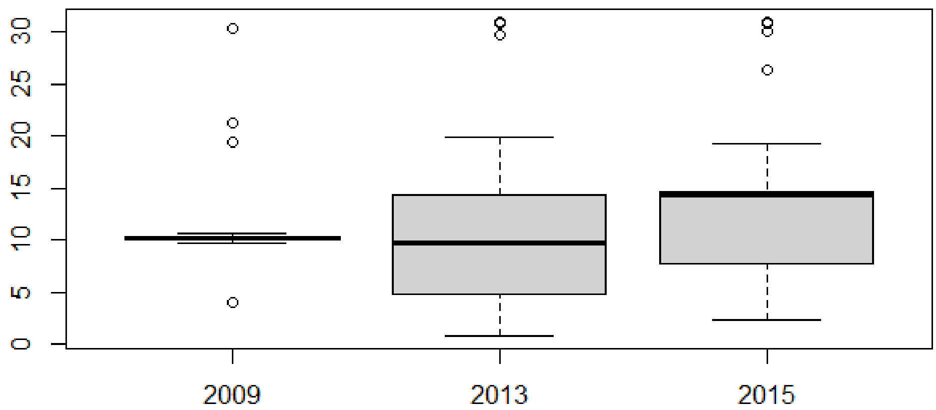
Wilcoxon rank sum test with continuity correction

data: IPI by ï..month_year

W = 8518, p-value = 4.648e-05

The significant p value, suggests to reject the independence and that LF calls data from 2013 and 2015 belongs to the same population.

Testing the HF calls from 2009, 2013, and 2015



Kruskal-Wallis rank sum test

data: IPI by ï..month_year

Kruskal-Wallis chi-squared = 18.705, df = 2, p-value = 8.674e-05

Kruskall wallis test, tests if the groups are the same and if at least one group is different the test will return a significant p-value indicating one of the groups is different. Then need to test between the years to see which is different.

Testing the HF calls between June 2009 and 2013

Wilcoxon rank sum test with continuity correction

data: IPI by ï..month_year

W = 822, p-value = 0.1389

Testing for independence because of different sample size and the non-significant p-value agree with the Ho that these are independent populations.

Testing the HF calls between June 2009 and 2015

wilcox.test(IPI~ï..month_year, data = IPI_2009_2015, paired = FALSE)

Wilcoxon rank sum test with continuity correction

data: IPI by ï..month_year

W = 2184, p-value = 0.5929

The non significant b values suggests that indeed these are independent, and the HF calls from 2009 site 1 and 2015 site 6 are from different populations.

Testing the HF calls between June 2013 and 2015

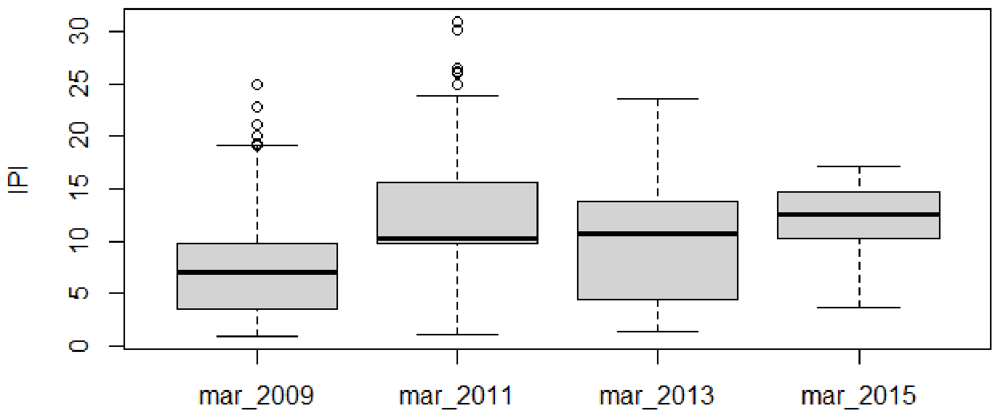
Wilcoxon rank sum test with continuity correction

data: IPI by ï..month_year

W = 8518, p-value = 4.648e-05

The p value is significant different, rejecting the Ho: that these populations are independent, that the HF calls between 2013 and 2015 belong to the same population.

Testing the second half of march across all years for LF calls



Kruskal-Wallis rank sum test

kruskal.test(IPI~ï..month_year, data = IPI_LF)

data: IPI by ï..month_year

Kruskal-Wallis chi-squared = 129.22, df = 3, p-value < 2.2e-16

Significant p-value rejecting the Ho: that all groups are the same, so now to test independently what years are significantly different from one another.

Testing march LF calls between 2009 and 2011

Wilcoxon rank sum test with continuity correction data: IPI by ï..month_year

W = 8187, p-value = 0.0001614

Reject the Ho; that the two groups are independent, and that the groups are likely part of the same population.

Testing march LF calls between 2009 and 2013

Wilcoxon rank sum test with continuity correction

data: IPI by ï..month_year

W = 2235.5, p-value = 0.1301

alternative hypothesis: true location shift is not equal to 0

Reject the Ho; that the two groups are independent, and that the groups have no significant differences between them.

Testing march LF calls between 2009 and 2015

Wilcoxon rank sum test with continuity correction

data: IPI by ï..month_year

W = 772, p-value = 0.04773

alternative hypothesis: true location shift is not equal to 0

Reject the Ho; that the two groups are independent, and that the groups have no significant differences between them.

Testing march LF calls from 2011 and 2013

Wilcoxon rank sum test with continuity correction

data: IPI by ï..month_year

W = 9434, p-value = 0.4452

alternative hypothesis: true location shift is not equal to 0

Reject the HO: that the two groups are independent.

Testing the march LF calls from 2011 and 2015

Wilcoxon rank sum test with continuity correction

data: IPI by ï..month_year

W = 3277, p-value = 0.1278

alternative hypothesis: true location shift is not equal to 0

reject the Ho: that 2011 and 2015 LF calls are independent

Testing the LF calls from March 2013 and 2015

Wilcoxon rank sum test with continuity correction

data: IPI by ï..month_year

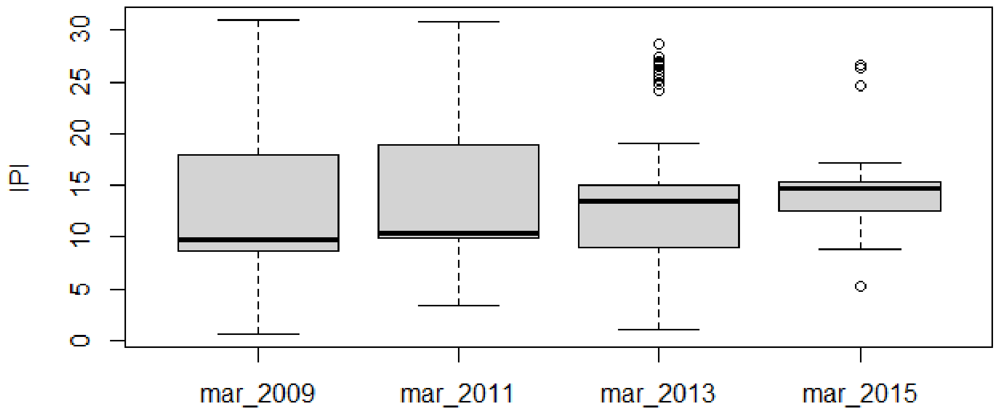
W = 729, p-value = 0.1199

alternative hypothesis: true location shift is not equal to 0

do not reject the HO: that the LF calls form 2013 and 2015 suggest two independent groups

---

Testing the HF calls from march_2 across all years for HF calls



Kruskal-Wallis rank sum test

kruskal.test(IPI~ï..month_year, data = IPI_HF)

data: IPI by ï..month_year

Kruskal-Wallis chi-squared = 14.807, df = 3, p-value = 0.001989

So reject the Ho: that the groups are all the same there are differences among groups and now need to independently test to determine what those differences are.

Test mar_2 Hf calls from 2009 and 2011

Wilcoxon rank sum test with continuity correction

data: IPI by ï..month_year

W = 18958, p-value < 2.2e-16

Reject Ho that the HF calls from 2009 and 2011 are not independent and likely are form the same population.

Testing between mar_2 HF calls from 2009 and 2013

Wilcoxon rank sum test with continuity correction

data: IPI by ï..month_year

W = 7593.5, p-value = 3.46e-06

Significant p-value, reject Ho that these are independent and there is no significant difference between groups.

Testing between mar_2 HF calls 2009 and 2015

Wilcoxon rank sum test with continuity correction

data: IPI by ï..month_year

W = 7877, p-value = 2.018e-05

Significant p-value, reject the HO: that the 2 groups are independent from one another.

Testing between mar_2 HF calls from 2011 and 2013

Wilcoxon rank sum test with continuity correction

data: IPI by ï..month_year

W = 37995, p-value = 0.0494

Reject the Ho: that these two groups are independent suggests that there is no significant difference between the two groups.

Testing the HF calls from mar_2, 2011 and 2015

Wilcoxon rank sum test with continuity correction

data: IPI by ï..month_year

W = 40490, p-value = 0.001218

Low p-value, reject the Ho: the two groups are not impendent and there is no significant diff between groups.

Testing between HF calls mar_2 from 2013 and 2015

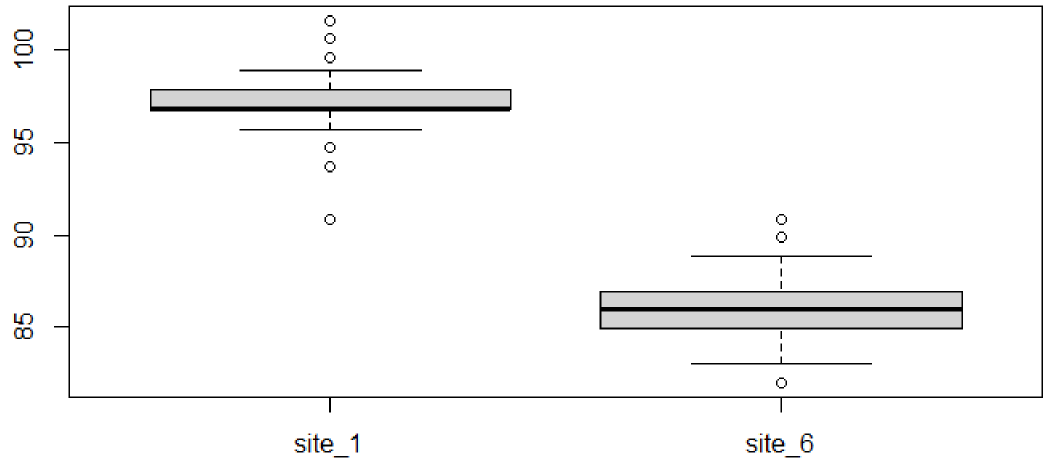
Wilcoxon rank sum test with continuity correction

data: IPI by ï..month_year

W = 7396, p-value = 0.7634

Not significant p-value, so do not reject the Ho: the groups are independent.

Testing HF peak frequency between site 1 and site 6



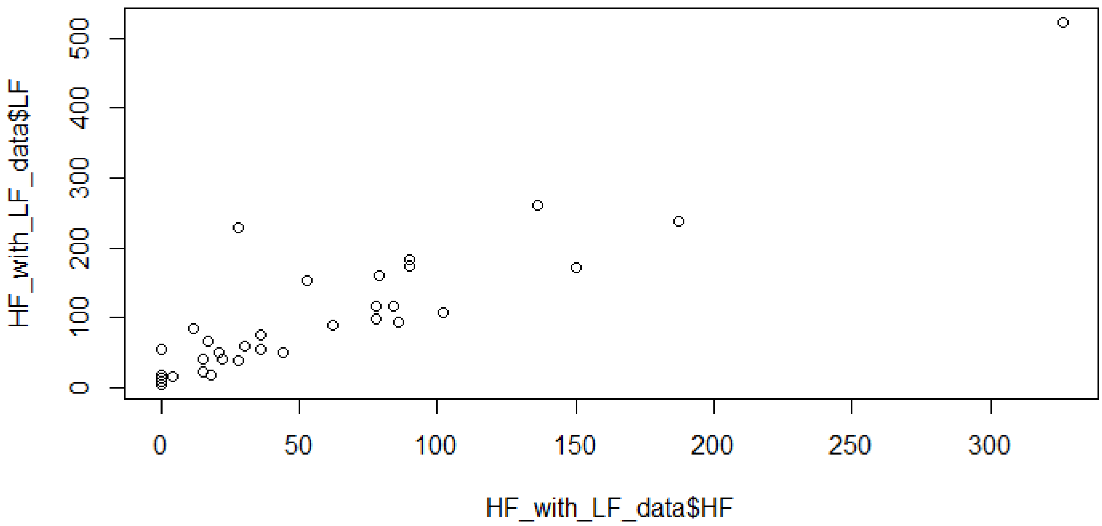
Kruskal-Wallis rank sum test

data: ï..frequency by site

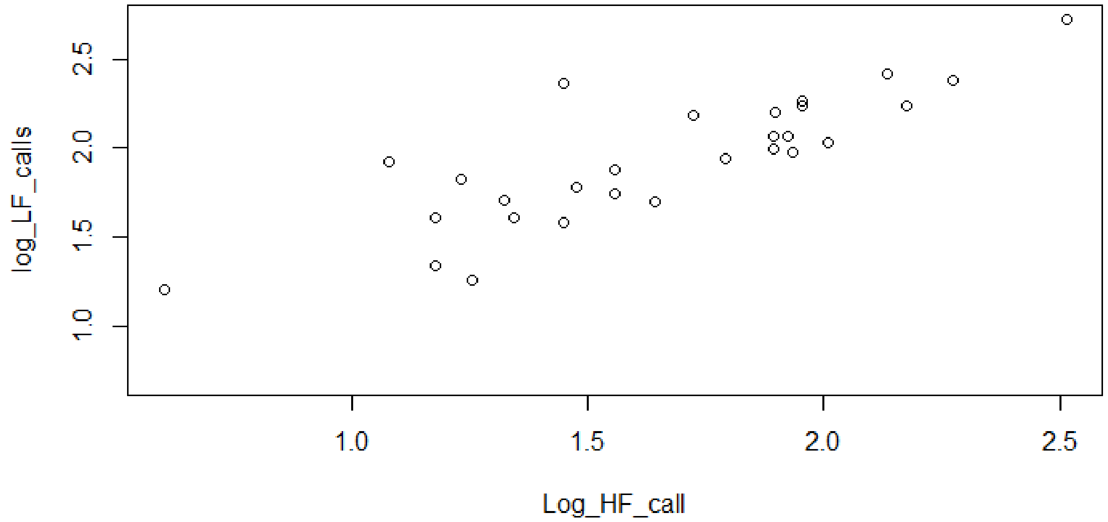
Kruskal-Wallis chi-squared = 1244, df = 1, p-value < 2.2e-16

Indicating there is a difference between groups.

Correlation of HF and LF calls



After taking the log when trying to normalize the data and use parametric test:



Spearman's rank correlation rho

data: HF_with_LF_data$log_HF and HF_with_LF_data$log_LF

S = 819.34, p-value = 1.038e-10

alternative hypothesis: true rho is not equal to 0

sample estimates:

rho = 0.8630784

Call Quality Assessment

Table 8. Indicating all the acoustic day files from the different recorders examined in this study from both sites across all years wit the respective percentage quality in Level 1, 2 and 3 calls.

Site	File Name	% Quality 1 Calls	% Quality 2 Calls	% Quality 3 Calls
Site 1:	20090221_MARU-01	0.00%	34.96%	65.04%
	20090330_MARU-01	0.00%	83.43%	16.57%
	20090402_MARU-01	14.40%	26.65%	58.95%
	20090421_MARU-01	0.00%	56.64%	43.36%
	20090529_MARU-01	0.00%	72.15%	36.71%
	20090608_MARU-01	29.63%	38.19%	32.18%
	20110304_AWI227-11_SV0002	0.00%	25.35%	74.65%
	20110322_AWI227-11_SV0002	34.75%	19.16%	46.09%
	20130126_AWI251-01_AU0231	0.00%	100.00%	0.00%
	20130202_AWI251-01_AU0231	0.00%	100.00%	0.00%
	20130226_AWI251-01_AU0231	0.00%	59.68%	40.32%
	20130312_AWI251-01_AU0231	0.00%	51.61%	48.39%
	20130320_AWI251-01_AU0231	13.87%	58.96%	27.17%

Site 6:	20130414_AWI251-01_AU0231	1.90%	48.73%	49.37%
	20130424_AWI251-01_AU0231	0.00%	60.00%	40.00%
	20130504_AWI251-01_AU0231	17.41%	15.87%	66.72%
	20130524_AWI251-01_AU0231	7.85%	52.18%	39.98%
	20130610_AWI251-01_AU0231	30.81%	29.60%	39.59%
	20130622_AWI251-01_AU0231	0.00%	56.34%	43.66%
	20130708_AWI251-01_AU0231	16.40%	13.88%	69.72%
	20130718_AWI251-01_AU0231	24.03%	34.42%	41.23%
	20150114_AWI251-01_AU231	0.00%	100.00%	0.00%
	20150226_AWI251-01_AU0231	0.00%	58.82%	41.18%
	20150306_AWI251-01_AU0231	0.00%	43.94%	56.06%
	20150318_AWI251-01_AU0231	4.31%	30.14%	65.55%
	20150406_AWI251-01_AU0231	7.21%	43.45%	48.96%
	20150420_AWI251-01_AU0231	17.31%	16.73%	65.38%
	20150504_AWI251-01_AU0231	6.02%	49.62%	44.74%
	20150528_AWI251-01_AU0231	11.04%	34.63%	54.33%
	20150610_AWI251-01_AU0231	5.48%	68.06%	26.45%
	20150626_AWI251-01_AU0231	0.00%	100.00%	0.00%
	20150708_AWI251-01_AU0231	0.00%	100.00%	0.00%
	20150720_AWI251-01_AU0231	0.00%	69.63%	30.37%

R Scripts for Plots

Initial Plot was to plot the LF and HF calls IPI values from all years across all months with data to visualize the spread of IPIs between months.

```
### first to read to file where your data is set your working directory ###
setwd("C:/Users/tfield/Documents/Field_MSc_Thesis_2021/bioacoustic data analysis/inital
results/edited IPI files/2009/csv files for R")
HFdataIPI    <- read.csv("Boxplot_HF_all_data_points.csv", fileEncoding="UTF-8-BOM")
#
### check if it reads in correctly ###
str(HFdataIPI
View(HFdataIPI)
###delete the unneeded columns###
     datasetHF    <- HFdataIPI[,-c(1)]
    View(LFdataIPI)
###########################################
### need to have mean and the SD to put into the graph of each column whish is the rnorm(#,#,#)
for the values###
###also in the name of column beside the name the # is the total data point for each boxplot###
  mean("site 1: Feb_1, 2009")
#then the SD
  Sd("site 1: Feb_1, 2009")
  str(HFIPIsitecomparison)
```

```
 View(HFIPIsitecomparison)
##############################################
# Plot
HFIPIsitecomparison
 ggplot(HFIPIsitecomparison, aes(x=name, y=value, fill=name)) +
 geom_boxplot() +
 scale_fill_viridis(discrete = TRUE, alpha=0.6) +
 geom_jitter(color="black", size=0.4, alpha=0.9) +
 theme_ipsum() +
 theme(
   legend.position="none",
   plot.title = element_text(size=11)
 ) +
 ggtitle("HF IPI From Site 1 and SIte 6 between Years") +
 xlab("site per year")
 ylab("IPI")
##############################################
###the above plot is for a boxplot with the individual data points laid over top to see the distribution
###
```

The R script below as used to plot the peak frequency of all HF calls across the years 2009, 2011, 2013 and 2015 between Site 1 and Site 6.

```
### first to read to file where your data is set your working directory ###
#
setwd("C:/Users/tfield/Documents/Field_MSc_Thesis_2021/bioacoustic data analysis/inital results/csv files for R")
#
##### when only a single csv file in folder use read.csv (specify file to prevent reading in data copies!!!)####
HFdataIPI    <- read.csv("HF_comparsion_edited .csv", fileEncoding="UTF-8-BOM")
#
### check if it reads in correctly ###
str(HFdataIPI)
#########################################
# Libraries
library(tidyverse)
library(hrbrthemes)
library(viridis)
#
install.packages("ggplot2")
library("ggplot2")
#
install.packages("viridis")
library(viridis)
#
library(ggplot2)
library(dplyr)
#
```

```
library(hrbrthemes)
#                                    #seashell -for HF comparisons #orange3=2015
                                    #yellowgreen= 2011 #orange=2013 #palegreen3 = 2009 data
#
### the plot below plots the whole graph in a single colour
# Plot
HFdataIPI
 ggplot(stack(HFdataIPI), aes(x = ind, y = values, fill= ind )) +
 geom_boxplot(color="black", fill="seashell", alpha=0.6) +
 geom_jitter(color="black", size=0.4, alpha=0.9) +
 theme_ipsum() +
 theme(
  legend.position="none",
  plot.title = element_text(size=12),
  axis.text.x = element_text(angle = 90)
 ) +
 ggtitle("HF Call Frequency Comparison Between Sites") +
 xlab("Site and Year") + ylab("Frequency")
#
 #scale_fill_manual(values=c("#69b3a2", "grey")) + use this line to highlight specific column colours
##############################################
```

R scripts for Statistical Analysis

The following r-script was used to compare IPI values within sites, starting with Site 1 comparing March 2009 and 2011. Then is same basic test and outline was then used to compared the month of July between 2013 and 2015 at Site 6 for both HF and LF calls.

```
setwd("C:/Users/tfield/Documents/Field_MSc_Thesis_2021/bioacoustic data analysis/inital results/csv files for R")
#
IPI_mar_2 <- read.csv("IPI_HF_mar_2_2009V2011.csv")
#
names(IPI_mar_2)
#
View(IPI_mar_2)
#
boxplot(IPI~ï..month_year, data = IPI_mar_2)
#
#need to make subsets for each year.
#
mar_2009 <- subset(IPI_mar_2, ï..month_year=="mar_2_2009")
#
mar_2011 <- subset(IPI_mar_2, ï..month_year=="mar_2_2011")
#
```

```
boxplot(mar_2009$IPI, mar_2011$IPI, names =c("mar_2009", "mar_2011"))
#
#now after visualizing the data we test for normality
#
shapiro.test(mar_2009$IPI)  #p-value = 0.0001636
#
shapiro.test(mar_2011$IPI)  #p-value < 2.2e-16
#
#
#so we know that only 2013 is significant diff from normality so we need to first try to normalize it
#since normality tests are thought to be stronger than non-parametric tests, but applications of
normality have to be applied ot both
#
mar_2009$exp_IPI_2009=exp(mar_2009$IPI)
#
mar_2011$exp_IPI_2011=exp(mar_2011$IPI)
#
boxplot(mar_2009$exp_IPI_2009, mar_2011$exp_IPI_2011, names =c("mar_2009", "mar_2011"))
#
#now after visualizing the data we test for normality
#
shapiro.test(mar_2009$exp_IPI_2009)  #p-value < 2.2e-16
#
shapiro.test(mar_2011$exp_IPI_2011)  #p-value < 2.2e-16
#
#just trying the log of the info to normalize to see how it looks different:
#
mar_2009$log_IPI_2009=log10(mar_2009$IPI)
#
mar_2011$log_IPI_2011=log10(mar_2011$IPI)
#
boxplot(mar_2009$log_IPI_2009, mar_2011$log_IPI_2011, names =c("mar_2009", "mar_2011"))
#
#now after visualizing the data we test for normality
#
shapiro.test(mar_2009$log_IPI_2009)  #p-value = 1.178e-07
#
shapiro.test(mar_2011$log_IPI_2011)  #p-value = 5.98e-16
#
#OKAY so still no matter if its the log10 to try and normalize the data or the exp to try and normalize
the data it is nor normally distributed
#now visualize it again to see if we could normalize it, also test for normality again using shapiro.test
#
#
#so still significantly different from normality, so that means we need to use non-parametric tests
# so we use the Mann-Whitney test between the median values of the sites
#
march_2009 = mar_2009
#
march_2011 = mar_2011
#
```

```
wilcox.test(IPI~ï..month_year, data = IPI_mar_2, paired = TRUE)
####################################################################################
####################################################################################
#Wilcoxen signed ranks test
#Wilcoxen signed ranks test for independent groups (paired = FALSE) also called Mann-Whitney U
test
#wilcox.test(IPI~month_year, data = IPI_mar_2, paired = FALSE)
# Group A and Group B are independent,
#H0 => there is a difference between Group A and Group B = Group A and B are different
#p<0.05 reject H0 and accept H1 = there is no difference between Group A and Group B = Group A
and Group B are the same
#Wilcoxen signed ranks test for dependent groups (paired = TRUE)
#wilcox.test(round(IPI~month_year, data = IPI_mar_2, paired = TRUE)
  # Group A and Group B are dependent, H0 => there is no difference between Group A and Group B
#= Group A and Group B are the same --> p<0.05 reject H0 and accept H1 = there is a difference
between Group A and Group B
#= Group A and Group B are different
```

Next the following r-script was used to compare IPIs across sites, with specific interest on the months before onset in mating season. Starting with comparing the month of June between 2009, 2013 and 2015 between both HF and LF call IPIs.

```
#####################################################################################
#Ho: The IPI per month median is different by site?#############################
#####################################################################################
setwd("C:/Users/tfield/Documents/Field_MSc_Thesis_2021/bioacoustic data analysis/inital
results/csv files for R")
#
IPI_HF <- read.csv("june_HF_1_2009_2013_2015.csv")
#
names(IPI_HF)
#
View(IPI_HF)
#
#need to make subsets for each year.
#
june_2009 <- subset(IPI_HF, ï..month_year=="june_2009")
#
june_2013 <- subset(IPI_HF, ï..month_year=="june_2013")
#
june_2015 <- subset(IPI_HF, ï..month_year=="june_2015")
#
boxplot(june_2009$IPI, june_2013$IPI, june_2015$IPI, names =c("2009", "2013", "2015"))
#
#now after visualizing the data we test for normality
#
shapiro.test(june_2009$IPI)  #p-value = 6.101e-06
#
shapiro.test(june_2013$IPI)  #p-value = 4.83e-12
```

```
#
shapiro.test(june_2015$IPI)  #p-value = 4.914e-10
#
#so we know that they are all significant diff from normality so we need to first try to normalize it
#since normality tests are thought to be stronger than non-parametric tests
#
june_2009$exp_IPI_2009=exp(june_2009$IPI)
#
june_2013$exp_IPI_2013=exp(june_2013$IPI)
#
june_2015$exp_IPI_2015=exp(june_2015$IPI)
#
boxplot(june_2009$exp_IPI_2009, june_2013$exp_IPI_2013, june_2013$exp_IPI_2015, names
=c("2009", "2013", "2015"))
#
#now after visualizing the data we test for normality
#
shapiro.test(june_2009$exp_IPI_2009)  #p-value = 5.284e-09
#
shapiro.test(june_2013$exp_IPI_2013)  #p-value  < 2.2e-16
#
shapiro.test(june_2015$exp_IPI_2015)  #p-value  < 2.2e-16
#
#
#just trying the log of the info to normalize to see how it looks different:
june_2009$log_IPI_2009=log10(june_2009$IPI)
#
june_2013$log_IPI_2013=log10(june_2013$IPI)
#
june_2015$log_IPI_2015=log10(june_2015$IPI)
#
boxplot(june_2009$log_IPI_2009, june_2013$log_IPI_2013, june_2015$log_IPI_2015, names
=c("2009", "2013", "2015"))
#now after visualizing the data we test for normality
#
shapiro.test(june_2009$log_IPI_2009)  #p-value = 5.251e-05
#
shapiro.test(june_2013$log_IPI_2013)  #p-value = 7.153e-12
#
shapiro.test(june_2015$log_IPI_2015)  #p-value = 2.702e-10
#
#OKAY so still no matter if its the log10 to try and normalize the data or the exp to try and normalize
the data it is nor nomrally distributed
#now visualize it again to see if we could normalize it, also test for normality again using shapiro.test
#
#
#so still significantly different from normality, so that means we need to use non-parametric tests
# so we use the Mann-Whitney test between the median values of the sites
#
#merge the months and IPI so we can compare by each
#
```

```
kruskal.test(IPI~ï..month_year, data = IPI_HF)
#
#	Kruskal-Wallis rank sum test

#data:  IPI by ï..month_year
#Kruskal-Wallis chi-squared = 16.699, df = 2, p-value = 0.0002365
#Kruskal-Wallis rank sum test
###so the three months of June are significantly different from one another now to test individually
which one is driving the sign. diff.
#I am just going to sort the months out into
#
IPI_2009_2013 <- read.csv("HF_june_2009_2013_IPI.csv")
#
wilcox.test(IPI~ï..month_year, data = IPI_2009_2013, paired = FALSE)
#
#Wilcoxon rank sum test with continuity correction

#data:  IPI by ï..month_year
#W = 822, p-value = 0.1389
#alternative hypothesis: true location shift is not equal to 0
#
#2009  and 2013 are not sign diff from independence.
#########################################################################################
IPI_2009_2015 <- read.csv("HF_june_2009_2015_IPI.csv")
#
wilcox.test(IPI~ï..month_year, data = IPI_2009_2015, paired = FALSE)
#
#Wilcoxon rank sum test with continuity correction

#data:  IPI by ï..month_year
#W = 2184, p-value = 0.5929
#alternative hypothesis: true location shift is not equal to 0
#
#2009 and 2015 are not sign. Diff from independence.
#########################################################################################
IPI_2013_2015 <- read.csv("HF_june_2015_2013_IPI.csv")
#
wilcox.test(IPI~ï..month_year, data = IPI_2013_2015, paired = FALSE)
#
#Wilcoxon rank sum test with continuity correction
#data:  IPI by ï..month_year
#W = 8518, p-value = 4.648e-05
#alternative hypothesis: true location shift is not equal to 0
#
#2013 and 2015 are significant so reject the Ho: reject the independence and the groups are likely
paired and not different from one another.
###########################################################################################
######################################################################################
```

The following r-script was used to examine IPIs for both HF and LF calls for March between 2009, 2011, 2013 and 2015.

```
#####################################################################################

#Ho: The IPI per month median is different by site?##############################
#####################################################################################
setwd("C:/Users/tfield/Documents/Field_MSc_Thesis_2021/bioacoustic data analysis/inital results/csv files for R")
#
IPI_HF <- read.csv("IPI_HF_mar_all_years.csv")
#
names(IPI_HF)
#
View(IPI_HF)
#
boxplot(IPI~ï..month_year, data = IPI_HF)
#
#need to make subsets for each year.
#
Mar_2009 <- subset(IPI_LF, ï..month_year=="mar_2009")
#
Mar_2011 <- subset(IPI_LF, ï..month_year=="mar_2011")
#
Mar_2013 <- subset(IPI_LF, ï..month_year=="mar_2013")
#
Mar_2015 <- subset(IPI_LF, ï..month_year=="mar_2015")
#

boxplot(Mar_2009$IPI, Mar_2011$IPI, Mar_2013$IPI, Mar_2015$IPI, names =c("2009", "2011", "2013", "2015" ))
#
#now after visualizing the data we test for normality
#
shapiro.test(Mar_2009$IPI)  #p-value = 0.0001636
#
shapiro.test(Mar_2011$IPI)  #p-value < 2.2e-16
#
shapiro.test(Mar_2013$IPI)  #p-value = 0.0002805
#
shapiro.test(Mar_2015$IPI)  #p-value = 0.003762
#
#so we know that only 2013 is significant diff from normality so we need to first try to normalize it
#since normality tests are thought to be stronger than non-parametric tests, but applications of normality have to be applied ot both
#
Mar_2009$exp_IPI_2009=exp(Mar_2009$IPI)
#
Mar_2011$exp_IPI_2011=exp(Mar_2011$IPI)
```

```
#
Mar_2013$exp_IPI_2013=exp(Mar_2013$IPI)
#
Mar_2015$exp_IPI_2015=exp(Mar_2015$IPI)
#
boxplot(Mar_2009$exp_IPI_2009, Mar_2011$exp_IPI_2011, Mar_2013$exp_IPI_2013,
Mar_2015$exp_IPI_2015, names =c("2009", "2011", "2013", "2015"))
#
#now after visualizing the data we test for normality
#
shapiro.test(Mar_2009$exp_IPI_2009)  #p-value  < 2.2e-16
#
shapiro.test(Mar_2011$exp_IPI_2011)  # p-value < 2.2e-16
#
shapiro.test(Mar_2013$exp_IPI_2013)  #p-value  < 2.2e-16
#
shapiro.test(Mar_2015$exp_IPI_2015)  #p-value = 2.362e-09
#
#just trying the log of the info to normalize to see how it looks different:
#
Mar_2009$log_IPI_2009=log10(Mar_2009$IPI)
#
Mar_2011$log_IPI_2011=log10(Mar_2011$IPI)
#
Mar_2013$log_IPI_2013=log10(Mar_2013$IPI)
#
Mar_2015$log_IPI_2015=log10(Mar_2015$IPI)
#
boxplot(Mar_2009$log_IPI_2009, Mar_2011$log_IPI_2011, Mar_2013$log_IPI_2013,
Mar_2015$log_IPI_2015, names =c("2009", "2011", "2013", "2015"))
#
#now after visualizing the data we test for normality
##OKAY so still no matter if its the log10 to try and normalize the data or the exp to try and
normalize the data it is nor normally distributed
#now visualize it again to see if we could normalize it, also test for normality again using shapiro.test
#
shapiro.test(Mar_2009$log_IPI_2009)  #p-value = 1.178e-07
#
shapiro.test(Mar_2011$log_IPI_2011)  #p-value = 5.98e-16
#
shapiro.test(Mar_2013$log_IPI_2013)  #p-value = 1.094e-06
#
shapiro.test(Mar_2015$log_IPI_2015)  #p-value = 0.003123
#
#
#so still significantly different from normality, so that means we need to use non-parametric tests
# so we use the kruskal-Wallis test for all values between the median values of the sites
#
kruskal.test(IPI~ï..month_year, data = IPI_LF)
#
#           Kruskal-Wallis rank sum test
```

```
#data:  IPI by ï..month_year
#Kruskal-Wallis chi-squared = 14.308, df = 3, p-value = 0.002514
#
# ############################So since we car See there is a sign difference in variances but
we need to test which are driving the differences.
#
mar_2009_2011 <- read.csv("IPI_HF_mar_09_11.csv")
#
wilcox.test(IPI~ï..month_year, data = mar_2009_2011)
#
#          Wilcoxon rank sum test with continuity correction

#data:  IPI by ï..month_year
#W = 8187, p-value = 0.0001614
#alternative hypothesis: true location shift is not equal to 0

###########################so they are significantly diff. from one another, unexpected yet
mar 2011 is a weird year for presence.
#
#
mar_2009_2013 <- read.csv("IPI_HF_mar_09_13.csv")
#
wilcox.test(IPI~ï..month_year, data = mar_2009_2013)
#
#          Wilcoxon rank sum test with continuity correction

#data:  IPI by ï..month_year
#W = 2235.5, p-value = 0.1301
#alternative hypothesis: true location shift is not equal to 0
#
# ######################## 2009 and 2013 march are not significantly different from one
another, unexpected.
#
#
mar_2009_2015 <- read.csv("IPI_HF_mar_09_15.csv")
#
wilcox.test(IPI~ï..month_year, data = mar_2009_2015)
#
#
#          Wilcoxon rank sum test with continuity correction

2#data:  IPI by ï..month_year
#W = 772, p-value = 0.04773
#alternative hypothesis: true location shift is not equal to 0
#
#
####################2009 and 2015 are NOT sign. diff. from one another
#
mar_2011_2013 <- read.csv("IPI_HF_mar_11_13.csv")
#
```

```
wilcox.test(IPI~ï..month_year, data = mar_2011_2013)
#
#
#          Wilcoxon rank sum test with continuity correction

#data:  IPI by ï..month_year
#W = 9434, p-value = 0.4452
#alternative hypothesis: true location shift is not equal to 0
#
#
# ##################are not significantly different from one another, unexpected.
#
#
mar_2011_2015 <- read.csv("IPI_HF_mar_11_15.csv")
#
wilcox.test(IPI~ï..month_year, data = mar_2011_2015)
#
#
#          Wilcoxon rank sum test with continuity correction

#data:  IPI by ï..month_year
#W = 3277, p-value = 0.1278
#alternative hypothesis: true location shift is not equal to 0
#
#
# ####################are significantly different from one another. as expected
#
#
mar_2013_2015 <- read.csv("IPI_HF_mar_15_13.csv")
#
wilcox.test(IPI~ï..month_year, data = mar_2013_2015)
#
#
#          Wilcoxon rank sum test with continuity correction

#data:  IPI by ï..month_year
#W = 729, p-value = 0.1199
#alternative hypothesis: true location shift is not equal to 0
######################################################################################
######################################################################################
```

The following r-script was used to test the peak frequency of the HF calls from 2009, 2011, 2013, 2015 between Site 1 and Site 6.

```
#######################################################################################
#Ho: The IPI per month median is different by site?###############################
#######################################################################################
setwd("C:/Users/tfield/Documents/Field_MSc_Thesis_2021/bioacoustic data analysis/inital results/csv files for R")
```

```
#
HF_frequency <- read.csv("HF_frequency_by_site_info.csv")
#
names(HF_frequency)
#
View(HF_frequency)
#
#need to make subsets for each year.
#
GM_HF <- subset(HF_frequency, site=="GM")
#
EI_HF <- subset(HF_frequency, site=="EI")
#
boxplot(GM_HF$ï..frequency, EI_HF$ï..frequency, names =c("site_1", "site_6"))
#
#now after visualizing the data we test for normality
#
shapiro.test(GM_HF$ï..frequency)  #p-value < 2.2e-16
#
shapiro.test(EI_HF$ï..frequency)  #p-value  < 2.2e-16
#
#so we know that they are all significant diff from normality so we need to first try to normalize it
#since normality tests are thought to be stronger than non-parametric tests
#so I want to see on a logarithmic scale if that normalizes the data
##################################################################
GM_HF$log_HF_GM=log10(GM_HF$ï..frequency)
#
EI_HF$log_HF_EI=log10(EI_HF$ï..frequency)
#
#now visualize it again to see if we could normalize it, also test for normality again using shapiro.test
#
boxplot(GM_HF$log_HF_GM, EI_HF$log_HF_EI, names =c("site_1", "site_6"))
#
#now after visualizing the data we test for normality
#
shapiro.test(GM_HF$log_HF_GM)  #p-value < 2.2e-16
#
shapiro.test(EI_HF$log_HF_EI)  #p-value < 2.2e-16
#
#so still significantly different from normality, so that means we need to use non-parametric tests
# so we use the Mann-Whitney test between the median values of the sites
#
#merge the months and IPI so we can compare by each
#
wilcox.test(ï..frequency~site, data = HF_frequency, paired = FALSE)
#
#           Wilcoxon rank sum test with continuity correction
#data:  ï..frequency by site
# = 1, p-value < 2.2e-16
#alternative hypothesis: true location shift is not equal to 0
################################################################################
```

The following r-script was used to examine the relationship between HF and LF calls. The HF and LF call occurrence as correlated using a spearman correlation.

```
#####################################################################################
###correlations of HF and LF calls####################################################
#####################################################################################
setwd("C:/Users/tfield/Documents/Field_MSc_Thesis_2021/bioacoustic data analysis/inital results/csv files for R")
#
HF_with_LF_data <- read.csv("HF_matched_LF.csv")
#
names(HF_with_LF_data)
#
View(HF_with_LF_data)
#
#now plot to see how the data looks
#
plot(HF_with_LF_data$HF, HF_with_LF_data$LF)
#
#next test whether each variable significantly is different from normal distribution
#
shapiro.test(HF_with_LF_data$HF)   #p-value = 9.161e-06
#
#
shapiro.test(HF_with_LF_data$LF) # p-value = 1.497e-05
#
#since there is significant diff. from normal we have to try and normalize it first
#
HF_with_LF_data$log_HF=log10(HF_with_LF_data$HF)
#
HF_with_LF_data$log_LF=log10(HF_with_LF_data$LF)
#
#now visualize it again to see if we could normalize it, also test for normality again using shapiro.test
#
plot(HF_with_LF_data$log_HF, HF_with_LF_data$log_LF, xlab= "Log_HF_call", ylab= "log_LF_calls")
#
#now after visualizing the data we test for normality
#
shapiro.test(HF_with_LF_data$log_HF)  #p-value = NA
#
shapiro.test(HF_with_LF_data$log_LF)  #p-value = 0.5879
#
#we want to look at the correlation between the two use cor.test
cor.test(HF_with_LF_data$log_HF, HF_with_LF_data$log_LF, method = "pearson")
#
plot(HF_with_LF_data$log_HF, HF_with_LF_data$log_LF, xlab = "HF_calls", ylab = "LF_calls")
#
```

```
#SO, since this data does not have equal sample sizes becuase we are missing some HF calls we need to use spearman correlation
#
#so i want to see what the correlation looks like from the non-log values:
cor.test(HF_with_LF_data$log_HF, HF_with_LF_data$log_LF, method = "spearman")
#
#Spearman's rank correlation rho
#data:  HF_with_LF_data$log_HF and HF_with_LF_data$log_LF
#S = 819.34, p-value = 1.038e-10
#alternative hypothesis: true rho is not equal to 0
#sample estimates:
#     rho 0.8630784
#
# so from this we can see that it is a positive strong correlation
#we can then create a final scatter plot to visualize the abline in the data:
plot(HF_with_LF_data$log_HF, HF_with_LF_data$log_LF, xlab = "Log_HF_calls", ylab =
"Log_LF_calls")
#
#add in the abline
#
abline(lm(HF_with_LF_data$log_HF~HF_with_LF_data$log_LF), lyt="dashed", col="red")
#
#
```