Geographic variation in the time-frequency characteristics of high-frequency whistles produced by killer whales (*Orcinus orca*)

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Abstract

Investigating intra-specific variation in acoustic signals can indicate the extent of isolation and divergence between populations and adaptations to local environments. Here we analyze the variation in killer whale high-frequency (>17 kHz) whistles recorded off Norway, Iceland, and the North Pacific. We used a combination of methods including multivariate comparisons of spectral and temporal parameters and categorization of contours to types. Our results show that spectral and temporal characteristics of high-frequency whistles recorded in the North Pacific show significant differences from whistles recorded in the Northeast Atlantic, being generally stereotyped, lower in frequency, and slightly longer in duration. Most high-frequency whistles from the North Pacific were downsweeps, while this was one of the least common types recorded in the Northeast Atlantic. The repertoire of whistles recorded in Norway was similar to Iceland, but whistles produced in Norway had significantly lower maximum frequency and frequency range. Most methods were able to discriminate between whistles of the North Pacific and the Northeast Atlantic, but were unable to consistently distinguish whistles from Iceland and Norway. This suggests that macro- and microgeographic differences in high-frequency whistles of killer whales may reflect historical geographic isolation between ocean basins and more recent divergence between adjacent populations.

Keywords: ultrasonic whistles, orcas, geographic variation

Introduction

When describing geographic variation in acoustic signals it is useful to divide differences into macrogeographic or microgeographic variation, depending on whether populations being compared are neighboring, making interbreeding and social interaction a possibility, or geographically separated, and thus socially and reproductively isolated (Mundinger 1982). Comparative studies of odontocete whistles have shown more pronounced inter- than intra-specific variation in whistle spectral parameters, which could arise if intra-specific variability is constrained to occur within a species-specific framework (Steiner 1981, Ding *et al*. 1995*a*, Matthews *et al*. 1999, Rendell *et al.* 1999, Podos *et al*. 2002). Variation in whistle structural parameters has previously been described for populations of the same dolphin species both at microgeographic (*e.g*., Azevedo and Van Sluys 2005, Morisaka *et al*. 2005) and macrogeographic levels (*e.g*., Camargo *et al*. 2006, Baron *et al*. 2008). Intra-specific geographic variations may occur due to genetic divergence, adaptations to local environments or cultural differences (Janik 2009). A general correlation of whistle variation with distance has been proposed whereby neighboring populations appear to have more similar whistle structure than geographically distant populations (Ding *et al*. 1995*b*, Azevedo and Van Sluys 2005, Rossi-Santos and Podos 2006, May-Collado and Wartzok 2008), although this is not always the case (Camargo *et al.* 2006). Within populations, there is often variation in duration and complexity of signals (*i.e*., number of inflection points) (Steiner 1981, Azevedo and Van Sluys 2005, Morisaka *et al*. 2005), while the time-frequency contour of whistles often varies among individuals, providing information on individual identity (*e.g*., bottlenose dolphin, *Tursiops truncatus*; Janik *et al*. 2006; common dolphin, *Delphinus delphis*: Caldwell and Caldwell 1968; Pacific humpback dolphin, *Sousa chinensis*: Van Parijs and Corkeron 2001). Understanding the variation in the acoustic repertoire within and between different populations may shed light on the evolution, movements, and cultural traditions of a species.

There are known differences in the structure and use of pulsed calls and whistles between different killer whale populations. In the North Pacific, pods of resident (fish-eating) killer whales produce unique and stable repertoires of stereotyped pulsed calls (Ford 1989, 1991). These calls differ between matrilines within pods (Ford 1991, Miller and Bain 2000, Deecke *et al.* 2010), and to a lesser degree between individuals within the same matriline (Nousek *et al.* 2006). The stability of resident pods may explain why the variation in pulsed calls primarily encodes group, rather than individual, identity (Tyack 1986). Killer whale whistles are generally more complex and longer than other delphinid whistles (Thomsen *et al.* 2001) and some have stereotyped frequency contours that are often produced in complex sequences (Riesch *et al.* 2006, 2008). Resident killer whale groups in British Columbia that do not share pulsed calls share stereotyped whistles, which may provide a community-level means of recognition, facilitating social interactions (Riesch *et al.* 2006). Transient (mammal-eating) killer whales in the North Pacific also produce stereotyped whistles and the repertoire seems to be shared by all members of the population (Riesch and Deecke 2011). Despite being sympatric with resident killer whales, transients have distinct, population-specific repertoires of both pulsed calls and whistles (Ford and Fisher 1982, Riesch and Deecke 2011).

In Iceland and Norway, early studies suggested that killer whales also produce group-specific call repertoires (Moore *et al.* 1988, Strager 1995). A common ecotype, known as the "herring-eating Scandinavian killer whale" (Simon *et al.* 2007) has been suggested for killer whales in this region due to close genetic relatedness (Foote *et al.*

2009*b*, Morin *et al.* 2010) and similar acoustic and foraging behavior. Killer whales are thought to have regularly migrated between these two locations (Jonsgård and Lyshoel 1970) due to the distribution of their main prey, Atlantic herring *Clupea harengus* (Sigurjónsson *et al.* 1988, Similä *et al.* 1996, Simon *et al.* 2007). The migration route of the Atlanto-Scandian herring stock spanned from western Norway to eastern Iceland prior to the stock collapse in the 1960's (Jakobsson and Østvedt 1999). The migration patterns of the Icelandic and Norwegian herring stocks changed following the collapse and remained closer to each country's coast (Jakobsson and Stefánsson 1999, Kvamme *et al.* 2003). Photo-identification studies dating to the 1980's show no matches between Iceland and Norway (Sigurjónsson *et al.* 1988, Foote *et al.* 2009*a*), suggesting that if killer whales migrated between those two locations in the past, there is currently little to no interaction between these populations. Nevertheless, some Icelandic killer whales have been re-identified as far east as Shetland (Foote *et al.* 2009*a*), showing that the movements of some individuals do extend beyond coastal waters. The suggested historic contact of killer whales between Iceland and Norway may have influenced their acoustic repertoire. However, comparisons of their pulsed call type repertoire have found apparently little repertoire sharing between the two locations (Moore *et al.* 1988, Strager 1995, Stenersen and Similä 2004, Shamir *et al*. 2014). If killer whales in Iceland and Norway were in contact in the past and their acoustic repertoires were similar, it is possible that sufficient time has passed for call repertoires to have diverged - explaining the pattern observed today. We might then expect that other signals produced by these whales may also show divergence. A divergent call repertoire may serve as a population-identifier, however, the extent of stereotypy and geographic variation in other signals is little understood.

High-frequency whistles ranging up to 75 kHz were first reported from herring-eating Northeast Atlantic killer whales (Samarra *et al*. 2010). High-frequency whistles have since been described from killer whale recordings made in the North Pacific (referred to as ‘high-frequency modulated signals’ and ‘ultrasonic whistles’; Simonis *et al.* 2012, Filatova *et al.* 2012) and in the Antarctic (Trickey *et al*. 2014), indicating that this is a widespread sound type produced by several populations although they do not appear to be ubiquitous (Samarra *et al.* 2010, Filatova *et al*. 2012). In the North Pacific, whistles were consistently downsweeps and, in some cases, highly stereotyped (Simonis *et al*. 2012, Filatova *et al.* 2012). Here, we analyze qualitatively and quantitatively the variation in time-frequency contours of high-frequency whistles recorded from herring-eating killer whales in Norway and Iceland, and compare them to stereotyped high-frequency whistles recorded in the North Pacific. Our objectives were: 1) to investigate the degree of micro and macro-geographic variation of time-frequency parameters and; 2) to investigate whether high-frequency whistles produced by killer whales in Iceland and Norway were also stereotyped and, if so, evaluate the spatial distribution of different whistle types. This study of geographic variation is a starting point to considering the potential function(s) of these poorly understood signals.

Methods

In the Northeast Atlantic, acoustic recordings were collected in Tysfjord, Vestfjord and Ofotfjord (Northern Norway) between 2005 and 2009 and off the Vestmannaeyjar archipelago and the Reykjanes peninsula in 2004, 2008 and 2009 (Iceland, Fig. 1). In both locations, recordings were collected during the day and most sounds were recorded from feeding or socializing whales. No other cetaceans were concurrently sighted. Recording systems used varied with year and location and included towed and vertical hydrophone arrays, as well as sound recording tags attached to whales using suction cups ("Dtags"; Johnson and Tyack 2003). All systems sampled at 96 or 192 kHz (Table 1). Further details of all systems used and data collected are given in Samarra *et al*. (2010). High-frequency whistles analyzed here are the same as those reported in that study, including whistles with frequency contours at least partially below 48 kHz (up to 48 kHz whistles) and whistles with frequency contours entirely above 48 kHz (>48 kHz whistles), when available. Only 2.6% of whistles at least partially below 48 kHz recorded at 192 kHz sampling rate crossed 48 kHz, i.e. had minimum frequency below 48 kHz and maximum frequency above 48 kHz. This suggests that the lower sampling rate of 96 kHz likely resulted in a negligible loss of whistles with frequency contours at least partially below 48 kHz that could be sampled. High-frequency whistles were defined as signals with contours entirely above 17 kHz (Samarra *et al*., 2010), as this was the maximum frequency previously reported for killer whale whistles (Thomsen *et al*. 2001).

To compare whistles produced in the Northeast Atlantic with those produced by killer whales in the North Pacific, we analyzed high-frequency whistles described by Simonis *et al*. (2012). The recordings were collected at sampling rates of 192 or 200 kHz from either a ship-based hydrophone array or a high-frequency acoustic recording package (HARP) and were scanned for high-frequency whistles through the use of Long-Term Spectral Averages (LTSAs) (Wiggins and Hildebrand 2007). To date, recordings from 19.9 cumulative years at 18 different sites across the North Pacific have been examined by analysts trained to recognize the presence of these signals (Fig. 1). There have been 19 acoustic encounters of killer whales at 9 different locations that included HFM signals, of which the temporal and spectral characteristics of a subset from 6 locations are described here. The recording locations of signals described here include: Aleutian Islands, Washington Coast, Southern California Bight, Hoke Seamount, Kauai, and Pearl and Hermes Atoll (Table 1, Fig. 1). High-frequency whistles were attributed to killer whales by their clear association with other sounds known to be produced by killer whales or by visual observations of killer whales present in the area (Simonis *et al*. 2012). To ensure the data sets from the two ocean basins were comparable we only used signals detected in the North Pacific where contours were also entirely above 17 kHz. Table 1 shows the total number of whistles analyzed from each location. We pooled all data from the North Pacific for subsequent analyses, as sample sizes were too small to compare each location separately. Following inspection of recordings using Adobe Audition 2.0© (Blackmann-Harris window; FFT=2048 and 4096, for 96 kHz and 192 or 200 kHz sampling rates, respectively; 100% window width) whistle contours were traced from visual inspection of the spectrogram using a peaks contour extraction algorithm as developed by Buck and Tyack (1993; Hann window; frequency resolution=46.875 Hz; time resolution=0.667 ms). The following descriptive parameters were measured from the extracted fundamental frequency contour: start, half-way duration point (mid) and end frequency, minimum and maximum frequency, frequency range (maximum-minimum frequency) and duration.

All high-frequency whistles were assigned a quality score based on visual assessment of signal to noise ratio and overlap with other sounds, between 1 (poor, when the signal was barely detectable in the spectrogram) and 3 (high, when the full contour was clearly visible). Only clearly visible contours (of quality 3) with sufficient signal-to-noise ratio to extract the measurements required were extracted in our data sets from both ocean basins. It is likely that some whistles in our sample were produced by the same group or the same individuals (mean ± stdv [min; max] of number of whistles per recording day: 22.4 ± 22 [1; 95]). Photographs of whales were opportunistically collected while recordings were made in Iceland and Norway. Estimates of group size were not consistently collected across recording sessions in these two locations, but in general varied between 4-15 animals to large aggregations containing 50-100 individuals. Photo-identification was particularly challenging in Norway due to low-light conditions in winter and analysis has not been completed. In Iceland, on average 17 ± 14 [2; 41] individuals were identified per recording session and for those individuals that were resighted at least once (52 of 86) the number of resightings was 4 ± 2 [1; 9]. Recordings in the North Pacific were mostly from a single day in each location, and group sizes were generally not available (Table 1). It was not possible to identify which individuals produced high-frequency whistles recorded in this study, however, it is unlikely that the sampling procedure across different days or across wide geographic areas (such as for the North Pacific data) biased the repeated sampling of a small number of individuals within each location.

Stereotypy of high-frequency whistles

To investigate whether, like pulsed calls, high-frequency whistles fell into stereotyped categories, the data set was categorized using two different methods: 1) visual categorization by human observers as commonly used to identify whistle types and; 2) automated categorization using ARTwarp (Deecke and Janik 2006).

1) Categorization by a human observer

We first conducted visual classification by a human observer using the entire sample of high-frequency whistles to identify differences in the repertoires of different whistle types across locations. Spectrograms of all whistles were generated in MATLAB (version 7.0.4; spectrogram parameters: FFT=2048 or 4096, for 96 kHz and 192 or 200 kHz sampling rates, respectively; overlap=87.5%; window function=Hann). The observer was blind to the origin of high-frequency whistles being classified. High-frequency whistles seemed to show continuous rather than discrete variation, and as a result were grouped into broad categories (e.g., Azevedo and Van Sluys 2005): upsweeps (rise in frequency with no inflection points), downsweeps (decrease in frequency with no inflection points), ascending-descending (rise in frequency followed by one inflection point and then a decrease in frequency), descending-ascending (decrease in frequency followed by one inflection point and then an increase in frequency), constant (small or no change in frequency and no inflection point), and other (if a contour did not fit any of the previous categories, Fig. 2).

2) Categorization using an automated method

To inspect variation at a finer scale, we conducted an independent automated categorization in MATLAB (version 7.11). A subset of the extracted fundamental frequency contours were analyzed by an adaptive resonance theory neural network that uses dynamic time-warping to calculate contour similarity (ARTwarp; Deecke and Janik 2006). Computation time constraints meant that it was impossible to input all extracted contours unless whistles were downsampled, reducing their time resolution. However, downsampling by a factor of 3 resulted in severe misclassification of the data (18 out of 19 categories included misclassified whistles) based upon visual inspection of generated categories. Therefore, the original time resolution was used (0.667 ms), but only a subset of randomly chosen whistle contours was categorized.

ARTwarp compares the similarity between an input contour and a contour that defines each category (reference contour) to a user-defined similarity value, called the vigilance parameter, to decide how each input contour should be categorized (Deecke and Janik 2006). To find the categorization that would explain most variation in high-frequency whistles with the least number of categories, the vigilance parameter was set to values between 0% and 100% in 50 logarithmic steps, and the subset of contours was categorized for each vigilance parameter value. The optimal categorization should have the highest variance ratio, calculated as the ratio of average within-category similarity over average between-category similarity as in Deecke and Janik (2006). This method was adopted because it has successfully classified the signature whistles of bottlenose dolphins and pulsed calls of killer whales (Deecke and Janik 2006).

Multivariate analysis of variation in time-frequency parameters

We conducted a multivariate discriminant function analysis (DFA) to investigate differences between whistles from different locations. All frequency and time parameters were included, except frequency range, as it is already represented by minimum and maximum frequency. We used location as the grouping variable (‘Iceland’, ‘Norway’ or ‘North Pacific’) and used a jackknife cross-validation technique implemented in the lda function of package MASS version 7.3-16 (Venables and Ripley 2002) in R 2.11.1 for Mac OS (R Development Core Team 2011). The overall proportion of correct classifications and the proportion of correct classifications by location were calculated. These were compared to the proportion of by-chance accuracy, calculated as the sum of the squares of all prior probabilities, assumed to be equal for all locations.

Results

We measured the parameters of 985 high-frequency whistles ($n_{Iceland}$ = 570; n_{Norway} = 256; $n_{Pacific}$ = 159). One of the whistles in our sample (from Norway) was a clear outlier, with duration of 4.2 s, much longer than the majority of remaining whistles and was removed from the analyses. Table 2 presents the descriptive statistics of all contour parameters measured. Duration and frequency range showed the highest coefficients of variation across all locations. All other frequency parameters had much lower coefficients of variation, however variation for Iceland and Norway was consistently larger than variation in the North Pacific. This could be due to the smaller sample size of whistles from the Pacific. However, in the North Atlantic very high-frequency whistles were recorded that likely explain the larger coefficients of variation observed here. There was a clear gap in the distribution of frequency parameters between whistles with fundamental contours up to 48 kHz and whistles with fundamental frequency contours entirely above 48 kHz (Fig. 3). Whistles with contours entirely above 48 kHz were recorded in a small number of encounters ($n_{Iceland}$ = 4; n_{Norway} = 2). They appeared as clear outliers in the distributions and were thus removed from the comparisons of parameter distributions (Fig. 3).

There was considerable overlap between the distributions of some of the contour parameters from Iceland and Norway (Fig. 3). To compare these parameters across locations, we employed a Mann-Whitney U test, due to the non-normality of most distributions (Shapiro-Wilk normality tests: *P*<0.0001, except for frequency range in the North Pacific with p=0.09). We used Bonferroni correction to adjust the significance level to account for multiple comparisons (0.05/21=0.002). Despite the apparent overlap, comparisons between the parameter distributions from Iceland and Norway revealed significant differences in end frequency (Mann-Whitney U test: W=74148; *P*=0.0004), frequency range (Mann-Whitney U test: W=81867; *P*<0.0001) and maximum frequency (Mann-Whitney U test: W=74203; *P*=0.0003). End frequency and maximum frequency were significantly correlated within the two locations (Pearson correlation; Iceland: end *vs*. maximum frequency: r=0.96, *P*<0.001; Norway: end *vs*. maximum frequency: r=0.98, *P*<0.001), which likely reflects a whistle upsweep shape. Whistles recorded in the North Pacific tended to have lower start, mid, end, minimum, and maximum frequencies, a slightly longer duration and a greater frequency range. The start frequency was not significantly different between Iceland and the Pacific (Mann-Whitney U test: W=49578; *P*=0.008), while all other parameters were (*P*-values<0.0001). All parameters were also significantly different between the North Pacific and Norway, with the exception of duration (Mann-Whitney U test: W=16777; *P*=0.11).

Categorization by a human observer

The visual classification of whistles showed that in Iceland the majority of whistles were upsweeps, followed by descending-ascending whistle types, while in Norway, upsweeps and descending-ascending whistles were equally common (Table 3). Overall, the different whistle types recorded from Norway and Iceland largely resembled each other in spectral and temporal characteristics (Fig. 2), in agreement with the similarities suggested by the overlapping parameter distributions. In contrast, whistles from the North Pacific were all downsweeps, with only one whistle being classified as ‘Other’. These clear differences in repertoire correspond to the differences in extracted frequency parameters (above). Nevertheless, the simple structure of downsweeps recorded in the North Pacific was similar to that of the few downsweeps recorded in the Northeast Atlantic (Fig. 2).

Categorization using an automated method

An assessment of the geographic variation of whistles was also completed using 50 randomly chosen whistles from each location. The results of the ARTwarp categorization showed that an increase in the vigilance parameter resulted in an increase in the number of categories generated. The chosen categorization divided the 150 high-frequency whistles into 27 categories, containing between 1 and 18 contours (mean ± standard deviation of contours in each category: 6 ± 4.6). This categorization corresponded to the local maximum in variance ratio achieved when the vigilance parameter was 95.6%. All but five categories included more than one whistle. Of the five categories containing only one whistle one category contained one whistle from the Pacific, two categories contained single whistles from Norway, and two categories contained single whistles from Iceland. Of the 22 categories with more than one whistle, five included only high-frequency whistles from the Pacific. The remaining high-frequency whistles from the Pacific were grouped into three more categories that also included whistles from Norway: two categories containing only two whistles (one from the Pacific and one from Norway) and; one category including 17 whistles from the Pacific and one whistle from Norway. All remaining categories included whistles from both Norway and Iceland.

Multivariate analysis of variation in time-frequency parameters

The multivariate DFA showed high macrogeographic variation between the Northeast Atlantic and North Pacific groups, with the first discriminant function accounting for 98.9% of between group variability. The loadings of the first discriminant function revealed that duration, maximum frequency, and end frequency were the main discriminating predictors (Loadings: duration = -2.81; maximum frequency = 0.33; end frequency = -0.46). The cross-validated classification showed an overall correct classification of 62.4% of whistles to the correct location, compared to a by-chance proportion of 33.3%, calculated as the sum of the squares of all prior probabilities. Correct classification scores per location revealed that the North Pacific was the location with the highest score (95.0%), with only 8 of its 159 whistles misclassified as being from Norway. Lower correct classification scores (63.9% and 52.4%) were achieved for whistles from Norway and Iceland, respectively. Most misclassifications for Norway (77 of 84) were assigned to Iceland and conversely, most misclassifications for Iceland (254 of 261) were assigned to Norway. The remaining 7 misclassifications from each location were assigned to the North Pacific. Figure 4 shows the first two discriminant functions and illustrates how the first discriminant function achieved the most discrimination between locations, with whistles from the North Pacific being clearly different from those recorded in the Northeast Atlantic, while whistles from Norway and Iceland overlapped to a much greater extent. However, at high values of the second discriminant function there was little overlap between Iceland and Norway (Fig. 4). Inspection of these whistles revealed that these have high frequency range and possibly are driving observed statistically significant differences in this parameter between locations.

To investigate whether discrimination between Iceland and Norway was more apparent within whistle types, discriminant function analyses were carried out for the two most common whistle types (upsweeps and descending-ascending) at these locations. Descending-ascending whistles were correctly classified at rates of 60% and 66.7% for Iceland and Norway, respectively. Overall correct classification was of 62.7%, compared to a by-chance proportion of 50%, suggesting a slight improvement in classification when using this whistle type, but still considerable similarity between locations. The overlap in the distribution of the discriminant scores resulting from the first discriminant function is presented in Figure 5, illustrating how the discrimination between these two locations was poor. Correct classification of upsweeps was 58.7% and 58.5% for Iceland and Norway, respectively. For upsweeps, overall correct classification was 58.6%, compared to a by-chance proportion of 50%, which also suggests an overlap in the discriminant scores within this type (Fig. 5).

Discussion

We found clear macrogeographic variation between high-frequency whistles recorded in the North Pacific and the Northeast Atlantic, while within the Northeast Atlantic only subtle microgeographic variation was observed. In all locations whistles could be manually classified into broad stereotyped categories, but the diversity of whistle types identified varied between ocean basins. While the majority of whistles recorded in the North Pacific consisted of only one whistle type, the Northeast Atlantic repertoires included more types and the repertoires were similar between locations. In all locations the within-location variation in most frequency parameters was small in comparison to variation in frequency range and duration. It is possible that some degree of this variation is due to varied signal-to-noise ratio conditions under which recordings were collected. Nevertheless, in other delphinids intra-specific variation in frequency parameters was generally low, while variation in whistle duration tended to be comparatively higher (*e.g.*, Ding *et al*. 1995*b*, Morisaka *et al*. 2005). It is possible that frequency parameters are constrained by morphology, while the variability of whistle duration may be related to social and non-social behavior or environmental factors, or random individual variation (*e.g.*, May-Collado and Wartzok 2008).

Macrogeographic variation in high-frequency whistles

Within the North Pacific, acoustic encounters of killer whales were not common at any of the recording sites, and high-frequency whistles were infrequently observed. There was broad similarity in the recorded whistles despite the considerable geographic range covered by the recordings. The consistency in whistle type (downsweeps) and similarities in frequency characteristics to those reported by Filatova *et al*. (2012) suggests that within the Pacific Ocean there may be little variability in high-frequency whistles. However, not all populations appear to produce these signals; both Northeast Pacific resident and transient killer whales apparently do not produce high-frequency whistles (Samarra *et al*. 2010, Filatova *et al*. 2012) but the ecotypes of those whales that do produce them in the North Pacific remain largely unknown, with the exception of North Pacific offshores (Simonis *et al*. 2012, Filatova *et al*. 2012). While Pacific offshore, resident and Northeast Atlantic killer whales are all closely related genetically (Morin *et al*. 2010), it is curious that Pacific resident killer whales have not been recorded using the signals shared by their nearest relatives. In contrast to the apparent similarity within the Pacific Ocean, there were clear differences between whistles recorded in the Pacific and Atlantic Ocean basins.

Whistles recorded in the North Pacific had consistently lower frequency parameters, while frequency range was significantly higher in the North Pacific than in either of the Northeast Atlantic populations. Differences in duration were less pronounced and significant differences were only detected in comparison to Iceland. The discriminant function analysis was able to correctly classify the vast majority of whistles from the North Pacific, assigning duration and maximum and end frequency as main discriminating predictors. Whistle types identified were also considerably different between ocean basins; while downsweeps were the most common whistle type in the North Pacific, this whistle type was uncommon in the Northeast Atlantic. The automated categorization also grouped most whistles from the Pacific into distinct categories. This divergence in whistle types will likely influence some of the observed differences in frequency parameters particularly the start, mid and end frequency but does not explain differences in minimum and maximum frequencies, which should not be affected by whistle shape. Thus, we believe that the observed differences in frequency parameters between ocean basins are not exclusively due to differences in whistle type usage but reflect a real divergence in the whistle frequency produced.

Divergence in frequency at macrogeographic scales could reflect divergence in geographically isolated populations that could originate from a wide range of genetic and social mechanisms. Indeed genetic data suggests considerable variation between populations in the Atlantic and the Pacific in comparison to variation between Iceland and Norway (Morin *et al*. 2010, Foote *et al*., 2011). Differences in the acoustic environment, such as background noise or transmission properties (*e.g*., Morisaka *et al*. 2005, May-Collado and Wartzok 2008), have also been proposed as factors explaining variations in signal frequency characteristics between populations. In both ocean basins recordings were collected over wide geographic areas, and we therefore cannot identify large and consistent habitat differences that could clearly explain the patterns of variation observed in this study. Body size, however, is known to vary between the two ocean basins; killer whales in the Northeast Atlantic in general have smaller body sizes than killer whales in the Northeast Pacific (Christensen 1984, Stenersen and Similä 2006), but Northeast Pacific offshores have smaller body size than resident and transient killer whales (Ford *et al.* 2000, Dahlheim *et al.* 2008). Nevertheless, we cannot exclude the possibility that divergence in frequency characteristics between these ocean basins may be related to morphological constraints. The reasons behind the consistent production of downsweeps in the North Pacific in contrast to the more variable repertoires recorded in the Northeast Atlantic are unknown. Although intriguing, to fully understand the reasons behind variations in frequency and usage of whistle types across ocean basins more effort is required to record killer whales with adequate sampling rates in other locations. This will reveal the extent of the consistent downsweep repertoire across the North Pacific or the existence of variable repertoires in other ocean basins as well as how frequency characteristics may vary in other habitats.

Microgeographic variation in high-frequency whistles

Within the Northeast Atlantic, we found similarities in the repertoire and characteristics of high-frequency whistles produced by killer whales in Norway and Iceland. None of the quantitative methods employed was able to distinguish between whistles from Iceland and Norway as clearly as between whistles from Northeast Atlantic and North Pacific, suggesting different levels of divergence between locations. Despite the apparent overlap in parameter distributions in the Northeast

Atlantic, whistles from Norway showed significantly lower end frequency, maximum frequency and frequency range when compared to Iceland. The discriminant function analysis showed some degree of correct classifications, although many whistles from both locations were misclassified, while the automated categorization included whistles from both locations in most of the categories generated. Although the overall repertoire was similar between the two locations, there were some differences in the usage of whistle types. Upsweeps were the most common whistle type in Iceland, while in Norway both upsweeps and descending-ascending whistles were equally common. Even when trying to discriminate between locations within whistles of the same type, discriminant function analyses still misclassified a large proportion of whistles, suggesting similarity between whistles produced. The similarity in both time-frequency parameters and overall repertoire of high-frequency whistles between Norway and Iceland is in striking contrast to the divergence in pulsed call repertoires (Moore *et al*. 1988, Strager 1995, Stenersen and Similä 2004, Shamir *et al.* 2014). At present we have little evidence of contextual production of high-frequency whistles to help us identify their function. Nevertheless, this divergence between pulsed calls and high-frequency whistles possibly reflects different functions of these different signal types.

Microgeographic variation in acoustic signals may be shaped by genealogy, the timing of separation, ranging behavior of individuals, or cultural divergence in the case of learned acoustic signals (*e.g*., Ding *et al*. 1995*b*, Azevedo and Van Sluys 2005, Papale *et al*. 2013). For killer whales in Norway and Iceland evidence for (a recent) common ancestor comes from historic ranging patterns of prey, genetics (Jonsgård and Lyshoel 1970, Foote *et al*. 2009*a*), and high-frequency whistle repertoires, while the unique pulsed call repertoires of each region may reflect more recent divergence (Moore *et al*. 1988, Strager 1995, Stenersen and Similä 2004, Shamir *et al.* 2014). A small number of high-frequency whistles have also been recorded from killer whales in Shetland (Samarra *et al*. 2010), an archipelago situated between Iceland and Norway where some Icelandic killer whales are known to travel (Foote *et al*. 2009*a*). Further recordings from this location and others should provide an interesting comparison to evaluate the degree of divergence between adjacent populations. Although the function(s) of these high-frequency whistles remain unclear, the signals analyzed in this study offer a window to understanding how acoustic behavior may relate to ancestry and dispersal patterns of killer whale populations on multiple scales.

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Table 1. Specifications of recordings containing high-frequency whistles analyzed in this study (further details are given in Samarra *et al*. 2010 and Simonis *et al*. 2012).

Ocean basin	Location	Recording days	Recording time (h)	Sampling rate (kHz)	Recording method	Whistles analyzed
Northeast Atlantic	Iceland	19	64	96 and 192	Vertical hydrophone array Towed hydrophone array Dtag	570
	Norway	18	104	96 and 192	Towed hydrophone array Dtag	257
North Pacific	Aleutian islands	2	3.1	200	HARP (depth 783 m)	44
	Hoke Seamount	1	0.6	200	HARP (depth 770 m)	22
	Kauai	1	0.6	200	HARP (depth 706 m)	36
	Pearl and Hermes Atoll	1	0.6	200	HARP (depth 753 m)	10
	Southern California	1	1.9	200	HARP (depth 1295 m)	22

	Bight					
	Southern California Bight	1	0.5	200	Ship-based hydrophone array	11
	Washington Coast	1	0.7	192	Ship-based hydrophone array	14

Table 2. Descriptive statistics of high-frequency whistles analyzed in this study. Sample sizes for each location are included in brackets. For each parameter, values given are mean ± standard deviation [minimum – maximum], with coefficient of variation (calculated as the ratio of the standard deviation to the mean) given as a percentage within brackets. All frequency parameters are presented in kHz and duration is presented in milliseconds (ms).

Location	Iceland (*n*=570)	Norway (*n*=256)	North Pacific (*n* = 159)
Start frequency	32.6 ± 9.1 (27.9%) [16.9 – 71.2]	34.7 ± 11.0 (31.7%) [18.3 – 71.0]	29.6 ± 5.1 (17.2%) [19.3 – 44.0]
End frequency	38.2 ± 8.7 (22.8%) [19.4 – 74.7]	37.4 ± 9.0 (24.1%) [21.5 – 68.3]	20.8 ± 3.2 (15.4%) [17.1 – 33.4]
Mid frequency	33.8 ± 8.6 (25.4%) [17.6 – 68.8]	34.6 ± 9.6 (27.7%) [19.0 - 64.3]	25.3 ± 4.3 (16.9%) [18.4 – 39.4]
Minimum frequency	31.7 ± 8.6 (27.1%) [16.9 - 68.3]	33.0 ± 9.2 (27.9%) [18.1 – 64.3]	20.8 ± 3.2 (15.3%) [17.1 – 33.4]
Maximum frequency	38.4 ± 8.7 (22.7%) [19.4 – 74.7]	38.4 ± 10.2 (26.6%) [22.3 – 71.0]	29.7 ± 5.1 (17.1%) [19.3 – 44.0]
Frequency range	6.8 ± 3.7 (54.4%)	5.4 ± 2.9 (53.7%)	8.9 ± 3.8 (42.6%)

	[0.7 – 21.2]	[1.0 – 19.9]	[1.6 – 20.2]
Duration	138.1 ± 135.9	143.6 ± 143.1	142.6 ± 74.2
	(98.4%)	(99.7%)	(52.1%)
	[6 - 814]	[10 - 1300]	[37.8 – 371.2]

Table 3. Proportion of high-frequency whistle types produced in different locations. Desc-Asc stands for descending-ascending and Asc-Desc stands for ascending-descending whistles.

	Whistle categories						
Location	Upsweep	Downsweep	Desc-Asc	Asc-Desc	Constant	Other	Total
Iceland	68.4%	1.4%	28.6%	0%	0.2%	1.4%	570
Norway	41.2%	9.3%	42.8%	0.8%	0%	5.8%	257
North Pacific	0%	99.4%	0%	0%	0%	0.6%	159
Total	50.3%	19.3%	27.7%	0.2%	0.1%	2.4%	986

Figure captions

Figure 1. Map illustrating the approximate locations (stars) where recordings containing high-frequency whistles were collected in: top) the Northeast Atlantic and; bottom) the North Pacific. SCB stands for Southern California Bight. Locations where recordings were conducted but no high-frequency whistles were detected are also shown (circles).

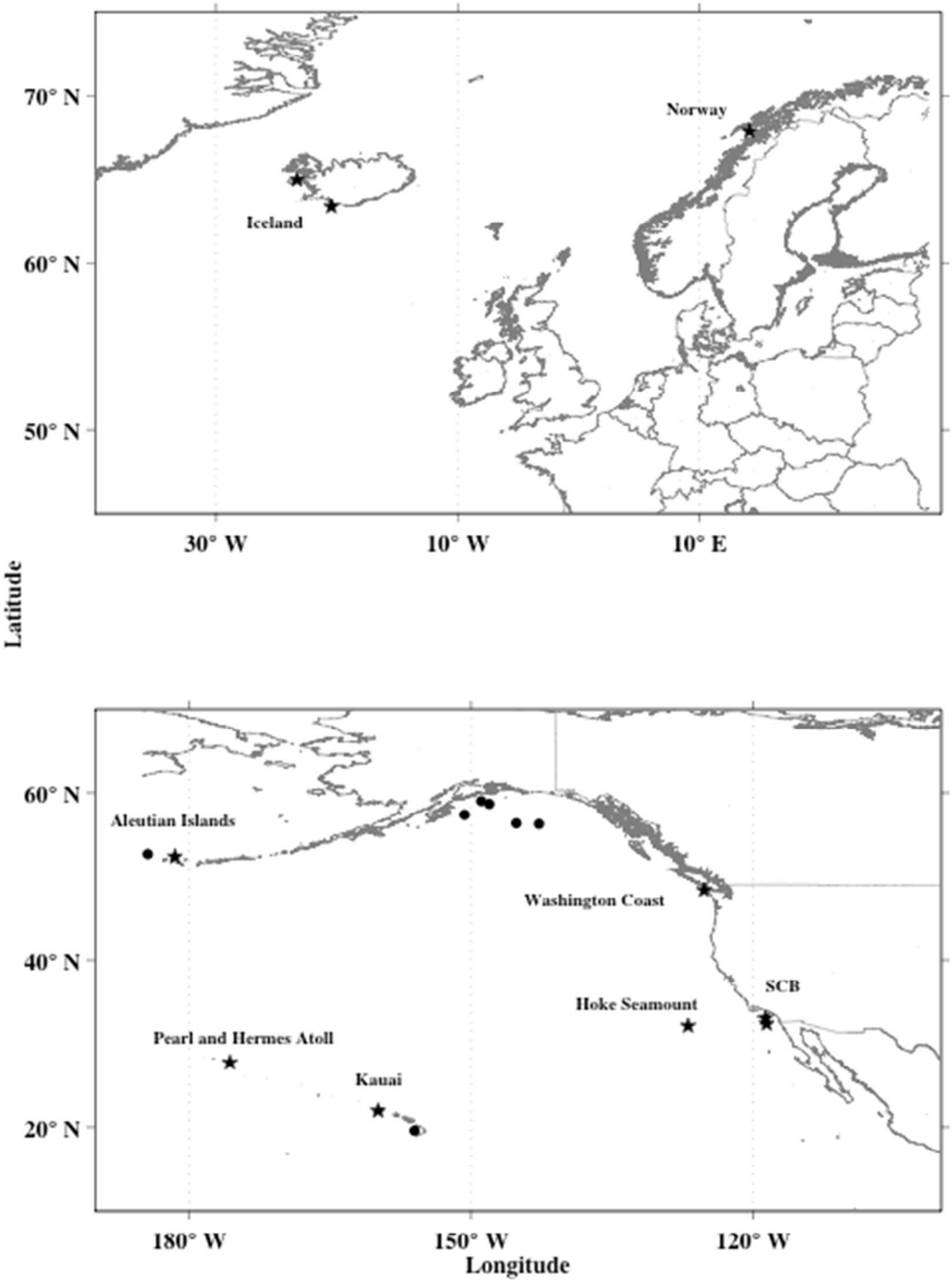
Figure 2. Spectrograms showing examples of different high-frequency whistle types from Iceland, Norway and the North Pacific. If a specific whistle type was only produced in one location only one example was shown. Note the different y-axis scaling for the Constant whistle type.

Figure 3. Distribution of all frequency parameters extracted from whistle contours. Horizontal lines represent medians, boxes represent inter-quartiles, and whiskers represent values within 1.5 times the inter-quartile range from the boxes. Outliers are plotted as single points. Frequency range is plotted separately due to its different y-axis scale, as is duration.

Figure 4. Plot of the first two discriminant functions for the comparison between whistles recorded in Iceland (I), Norway (N) and the North Pacific (P). Colors follow the same legend as in Figure 2. Note the overlap between whistles from Norway (N) and Iceland (I) in contrast to discrimination between whistles from the NE Atlantic and whistles from the Pacific (P).

Figure 5. Boxplots illustrating the distribution of discriminant scores (DF1) for descending-ascending whistles (Desc-Asc) for the two groups (Iceland and Norway) and discriminant scores for upsweep whistles for the two groups. Colors follow the same legend as in Figure 2.

737 **Figure 1**



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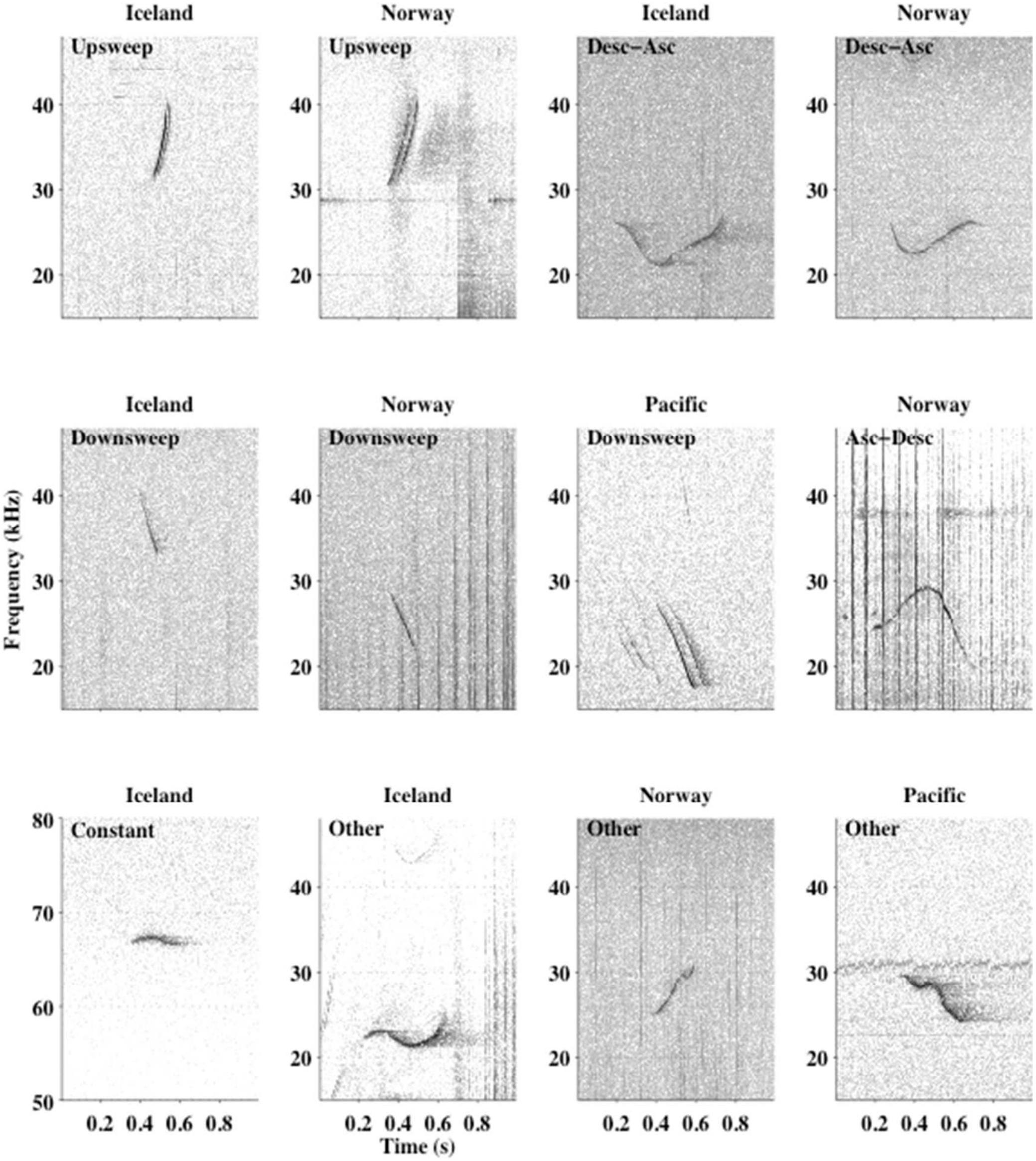
740 **Figure 2**



741

Figure 3

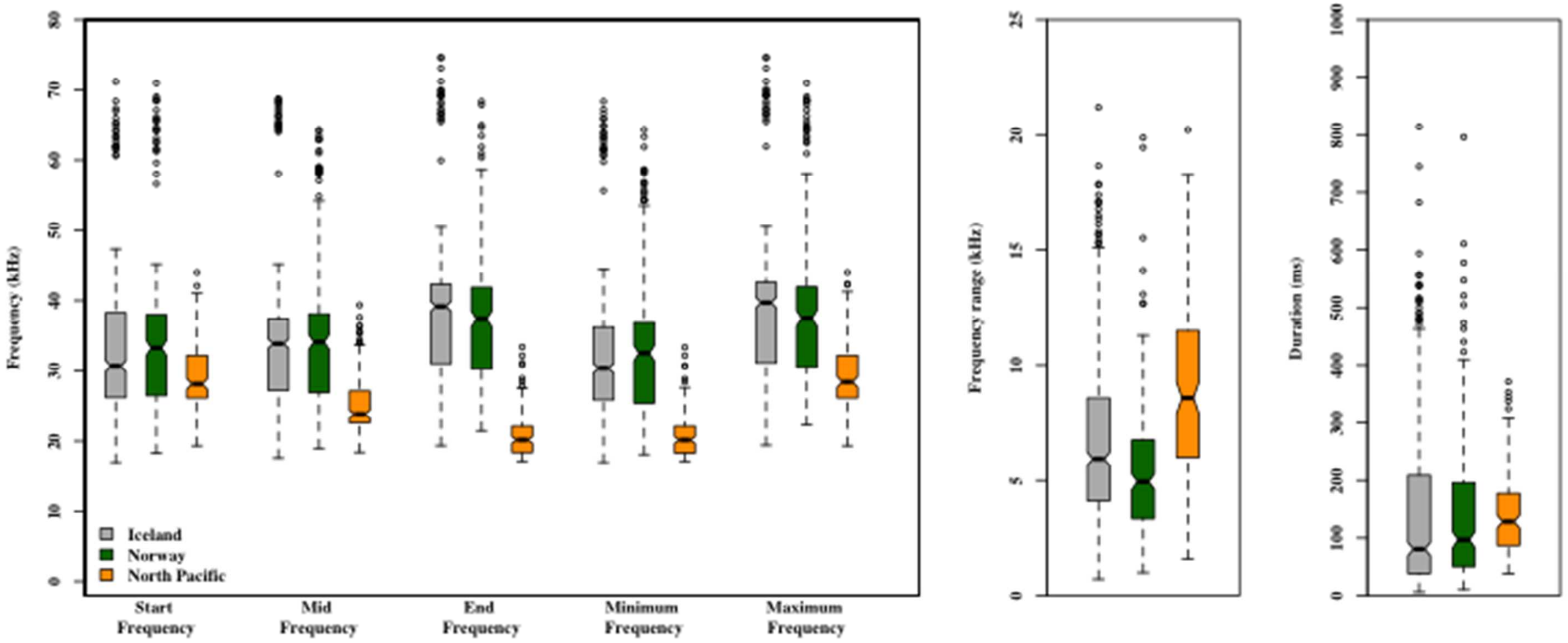
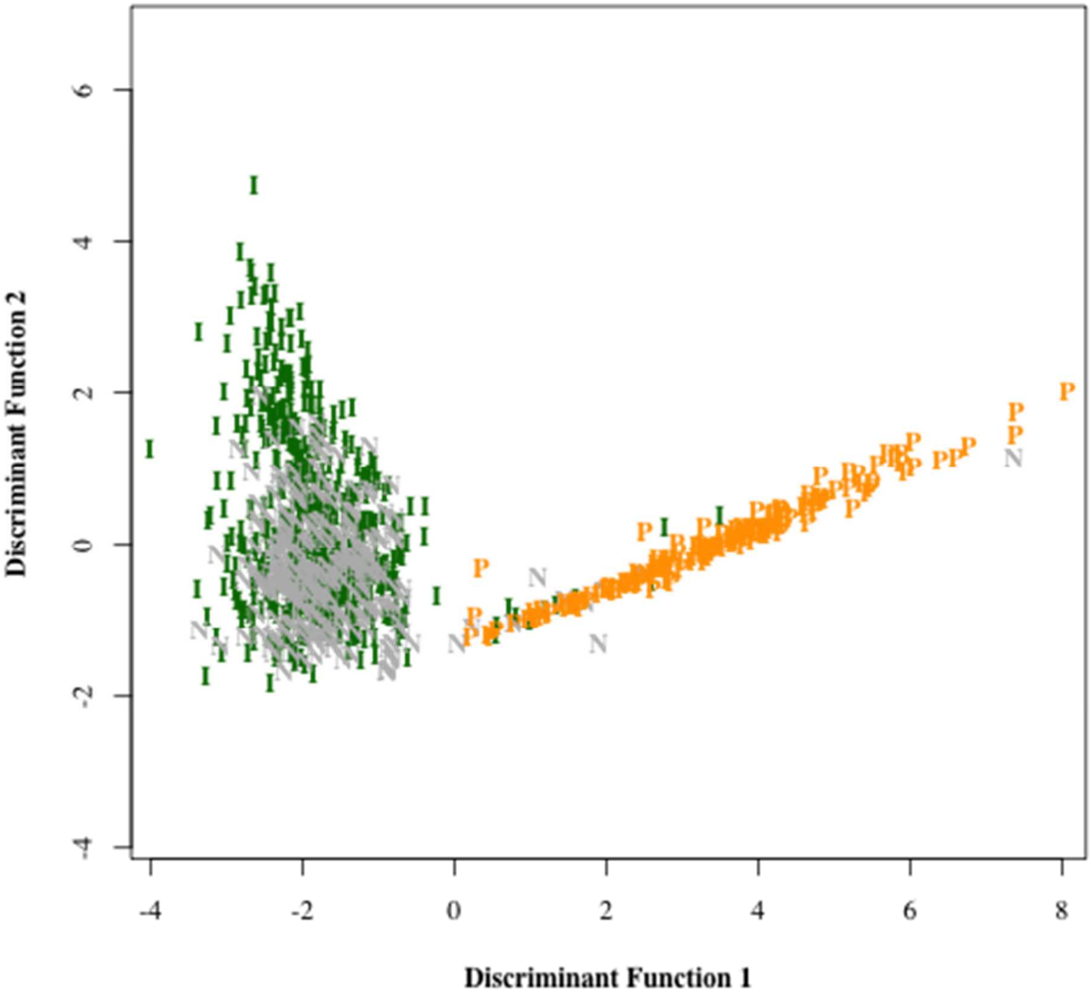

Frequency (kHz)
0
10
20
30
40
50
60
70
80
Iceland
Norway
North Pacific
Start Frequency
Mid Frequency
End Frequency
Minimum Frequency
Maximum Frequency
Frequency range (kHz)
0
5
10
15
20
25
Duration (ms)
0
100
200
300
400
500
600
700
800
900
1000

Figure 4


Discriminant Function 2
Discriminant Function 1
6
4
2
0
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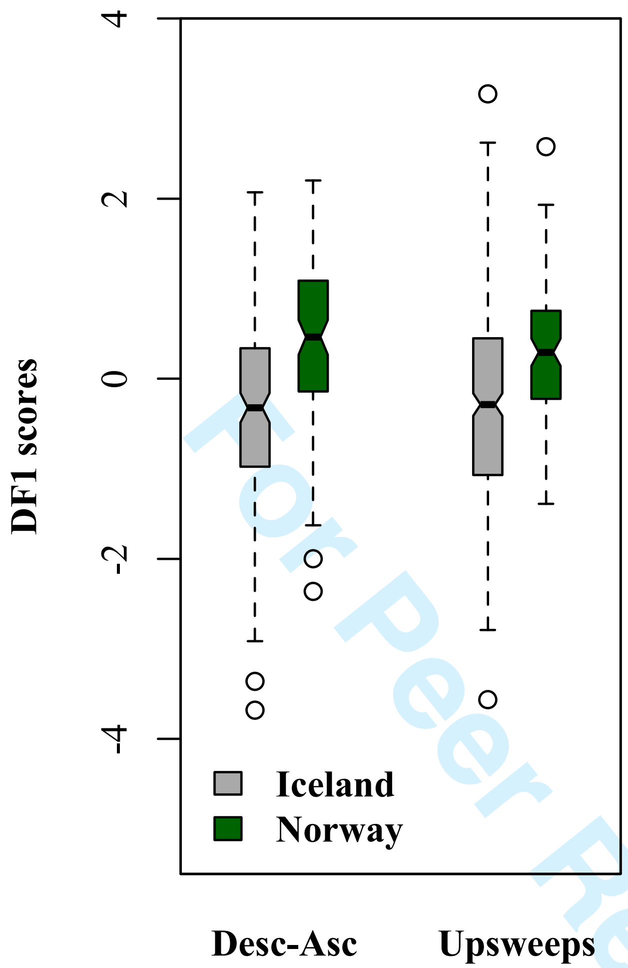
746 **Figure 5**



747