Geographic Variation in the Whistle Characteristics of Bottlenose Dolphins (*Tursiops truncatus*) between Cardigan Bay, Wales, the Shannon Estuary, Ireland, the Molène Archipelago, France and the Sado Estuary, Portugal

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A thesis submitted for the degree of
Marine Biology M.Sc.

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Declaration

This dissertation has not previously been accepted in substance for any degree and is not being concurrently submitted for any degree.

This dissertation is being submitted in partial fulfilment of the requirements for the degree of M.Sc. Marine Biology.

This dissertation is the result of my own independent work / investigation, except where otherwise stated.

Other sources are acknowledged with references. A bibliography is appended.

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The thesis work was conducted from May to September 2012 in collaboration with the Sea Watch Foundation, under the supervision of Dr. Peter Evans at Bangor University.

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Date: 21/09/2012

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Abstract

The whistles of bottlenose dolphins can differ between geographic locations, but the reasons behind this variation remain unclear. It is important to study geographic variation in whistle characteristics of dolphins as it could be reflective of culture, genetic differences, and the importance of learning within different populations. In this study, the whistle characteristics of bottlenose dolphins (*Tursiops truncatus*) were compared between four different geographic locations (Cardigan Bay, Wales, the Shannon Estuary, Ireland, the Molène Archipelago, France and the Sado Estuary, Portugal). It was predicted that variation between populations would be greater than the variation within populations. Recordings from the four locations were collected using either hydrophones or bottom-moored autonomous recorders between 2001 and 2012. Whistles were extracted from the recordings, and nine whistle characteristics were measured from each whistle. One-way ANOVAs and Kruskal-Wallis tests were undertaken on each of the nine whistle characteristics to determine the ways in which whistles varied between location. The frequency and intensity variables of whistles from the Sado Estuary were significantly higher than in other areas. This variation could be due to differences in background noise levels, genetic differences, the openness of populations, or differences in body size. It seems most likely that differences in background noise levels between populations would explain the variation, due to the high levels of boat traffic in the Sado Estuary. Future studies should focus on more conclusively determining the reasons behind the existing variation between these four populations of bottlenose dolphins.

Acknowledgements

I am very grateful to Peter Evans for his guidance and supervision throughout the project. Further, I am thankful to members of the Sea Watch Foundation, in particular Daphna Feingold, Rachel Lambert, Danielle Gibas, Gemma Veneruso and all the volunteers for all their help during the data collection, as well as to Katy Thompson, with whom I carried out the data collection in Cardigan Bay. I am also thankful to the skippers of the *Dunbar Castle II* and the *Ma Chipe Seabrin*. Finally, I am very grateful to Anneli Englund of County College Cork, Lucia Di Iorio of ENSTA and Manuel Dos Santos of ISPA, for kindly sharing their data from their respective regions for use in this investigation.

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1 | Introduction

Bottlenose dolphins (*Tursiops truncatus*), along with many other marine mammals, rely heavily on sound in various aspects of their lives, such as communication (Morisaka *et al.*, 2005b; Ansmann *et al.*, 2007), foraging (Herzing, 1996; Herzing and dos Santos, 2004; dos Santos *et al.*, 2005), and group cohesion (Janik and Slater, 1998; Janik, 2000; Morisaka *et al.*, 2005b; Rossi-Santos and Podos, 2006; May-Collado and Wartzok, 2008). Dolphins typically produce three different categories of sound: echolocation click trains (Herzing and dos Santos, 2004), high energy burst- pulsed sounds (Perrin *et al.*, 2008), and un-pulsed, tonal acoustic signals, or whistles, all of which have different functions. The clicks and buzzes produced by dolphins during echolocation are mostly used whilst foraging (Au *et al.*, 1978; Herzing, 1996), but have also been observed during courtship and discipline behaviours in Atlantic spotted dolphins (*Stellena frontalis*) and bottlenose dolphins (*Tursiops truncatus*). Burst-pulsed sounds have been observed during echolocation and during social events (Blomqvist and Amundin, 2004), as well as during aggressive and sexual behaviour in bottlenose dolphins (Herzing, 1996; Blomqvist and Amundin, 2004). This study focuses on whistles, the third type of vocalisation produced by dolphins.

In bottlenose dolphins, whistles appear to be used mostly for communication (Janik, 2000). It has previously been suggested that the characteristics of dolphin whistles may vary depending on geographic area (Wang *et al.*, 1995; Morisaka *et al.*, 2005b), but it remains unclear as to why this is. This study therefore aims to compare the whistle characteristics of bottlenose dolphins from different populations in the eastern North Atlantic Ocean (Cardigan Bay, Wales; Shannon Estuary, Ireland; Molène Archipelago, Iroise Sea, France; Sado Estuary, Portugal). Variation in the whistle characteristics of bottlenose dolphins and other sound dependent marine mammals may be reflective of social structure within populations and environmental differences between populations (Rendell *et al.*, 1999). As such, by determining whether variation exists between the whistle characteristics of different populations of bottlenose dolphins, it may be possible to better understand reasons behind any existing dissimilarities, and therefore gain a more insightful understanding into how they may be affected by environmental and anthropogenic factors.

Bottlenose dolphins are toothed whales (sub-order Odontoceti) belonging to the Delphinidae family (Perrin *et al.*, 2008). They are widely distributed, existing in inshore and offshore populations in temperate and tropical areas (Wells and Scott, 2002; Perrin *et al.*, 2008). Inshore populations tend to be of smaller group size than offshore populations, comprising of between two and fifteen dolphins in contrast to groups of up to one hundred dolphins in offshore populations (Perrin *et al.*, 2008). It is important to study and monitor bottlenose dolphin populations acoustically, as it is likely that increasing amounts of anthropogenic disturbance in the oceans will have an effect on the way in which they use sound, and it is necessary to understand what these effects will be.

1.1 Dolphin Whistles

Dolphin whistles are un-pulsed and tonal acoustic signals (May-Collado and Wartzok, 2008), which have been found to be used during different types of social interactions such as mother-calf interactions (Smolker *et al.*, 1993; Herzing, 1996; Jones and Sayigh, 2002) and courtship (Smolker and Pepper, 1999). However, dolphins have also been observed to use whistles whilst foraging (Herzing, 1996). The whistles of dolphins have been found to consist of different acoustic characteristics, such as duration, inflection points and a number of frequency variables. These characteristics have previously been used to look at the variation in dolphin whistles that might exist between or within populations (Wang *et al.*, 1995; Rendell *et al.*, 1999; Azevedo *et al.*, 2007).

Bottlenose dolphins have a repertoire of whistles, which grows with age as a result of their ability to mimic sounds (Tyack, 1997; Lima *et al.*, 2012). This mimicry appears to be a form of learning, and it suggests that dolphins have the ability to continue learning throughout their lives. It has been found that individual bottlenose dolphins develop a 'signature whistle' at an early age, and it is believed that vocal imitation is fundamental to this development (Tyack, 1997; Janik, 2000; Rossi-Santos and Podos, 2006). Signature whistles have been described as whistles that are specific to individual dolphins, and which are used as a form of identification. These whistles tend not to change over time (Caldwell and Caldwell, 1965; Smolker *et al.*, 1993; Tyack, 1997; Cook *et al.*, 2004; Janik *et al.*, 2006; May-Collado and Wartzok, 2008; Nakahara and Miyazaki, 2011), and appear to be important in group cohesion (Sayigh, 1992; Janik *et al.*, 2006) due to their apparent ability to be used as signals of recognition. For instance, mothers and calves who have been separated have been observed to use signature whistles in order to find each other and re-group (Smolker *et al.*, 1993; Herzing, 1996). Janik *et al.* (2006) discovered that bottlenose dolphins recognised particular signature whistles, and that this recognition was not due to the voice associated with the whistle, but due to the recognition of the whistle itself. Caldwell *et al.* (1969) suggested that bottlenose dolphins have the capacity to recognise up to eight different dolphins based on their signature whistles, whilst Sayigh (1992) found that dolphins respond more strongly to signature whistles emitted by individuals with whom they have stronger social affiliations. Since dolphins exist in fission-fusion societies (Connor *et al.*, 2000; Reynolds, 2000; López, 2011), an individual dolphin will have different affiliations with different dolphins over time, suggesting that they would not need to recognise over eight individual signature whistles at any one time (Tyack, 1997).

Bottlenose dolphins also produce whistles that are not signature whistles, and these whistles occur more frequently during social interactions than during any other type of behaviour (Cook *et al.*, 2004). Whilst signature whistles appear to have a specific function, it is still unclear as to what function non-signature whistles play in communication between bottlenose dolphins, though it is likely that they transmit information, perhaps about food or danger (Tyack, 1986).

1.2 Variability of Dolphin Whistles

1.2.1 Inter-specific Variation in Whistle Characteristics

Considering the variation in the whistle characteristics both between and within different species seems essential in terms of understanding the reasons behind whistle variation. Rendell *et al.* (1999) studied the intra- specific and inter- specific variation in the whistles of five different species of odontocetes: the false killer whale (*Pseudorca crassidens*), short-finned pilot whale (*Globicephala macrorhynchus*), white-beaked dolphin (*Lagenorhynchus albirostris*), long-finned pilot whale (*Globicephala melas*) and the Rissos dolphin (*Grampus griseus*). It was discovered that the whistles of the five species differed significantly from each other, but that when compared within species, the whistles were more similar. This indicates that whistles are species- specific, and it was further suggested by Rendell *et al.* (1999) that the variation within a population may indicate the state of social relationships and the importance of culture in the populations and societies of many marine mammals. Similar results were found by Steiner (1981), who suggested that the reason for these results was that whistles contained information that was specific to a particular species. This would be important for the reproductive isolation of different species inhabiting the same geographic area (Steiner, 1981). It is therefore apparent that whistles produced intra-specifically are similar, whilst whistles produced inter-specifically are different.

1.2.2 Geographical Variation in Whistle Characteristics

Geographical variation in the whistle characteristics of dolphins and other toothed whales has been widely studied (Wang *et al.*, 1995; Rendell *et al.*, 1999; Jones and Sayigh, 2002; Parsons *et al.*, 2002; Bazua-Duran, 2004; Bazua-Duran and Au, 2004; Morisaka *et al.*, 2005b; Rossi-Santos and Podos, 2006; Ansmann *et al.*, 2007; Baron *et al.*, 2008; May-Collado and Wartzok, 2008), and whilst some studies have found apparent dissimilarities between the whistles of different populations, others have found no such differences. However, the reasons behind any existing variances remain unclear.

Variation in whistle characteristics between populations has been attributed to different factors, such as the nature of the relationships between dolphin populations that are inhabiting different areas of the same region (Steiner, 1981; Wang *et al.*, 1995), genetic differences between populations (Pichler *et al.*, 1998; Parsons *et al.*, 2002; Rossi-Santos and Podos, 2006; Baron *et al.*, 2008; Frere *et al.*, 2010; Tyson *et al.*, 2011), group size (Jones and Sayigh, 2002; Cook *et al.*, 2004; Ansmann *et al.*, 2007; May-Collado and Wartzok, 2008; Quick and Janik, 2008), the body size of individuals (Ansmann *et al.*, 2007; May-Collado and Wartzok, 2008) and different levels of ambient noise in different locations (Wang *et al.*, 1995; Buckstaff, 2004; Morisaka *et al.*, 2005a; May-Collado and Wartzok, 2008).

It has been proposed that geographic variation may be higher between populations that exist further apart than between sympatric populations. For example, Wang *et al.* (1995) compared the structure of bottlenose dolphin whistles between five different regions, including the Gulf coast of Texas (within which three regions were observed), Argentina, the Gulf of California, Japan and Australia. Differences in whistle structure were greater between dolphin populations that were located further apart, whilst whistle structures of dolphin populations that were located closer to one another were quite similar in strucure (Wang *et al.*, 1995). The whistle structures from the dolphins along the Gulf coast of Texas were found to be significantly different from each other, though they shared some similarities. It was suggested that any similarities were due to movements between different populations of dolphins, such that dolphins belonging to one population would hear whistles from dolphins belonging to another population, and due to their ability to imitate sounds (Janik, 2000), they might retain some aspect of the foreign whistle and introduce it into their own population (Wang *et al.*, 1995). It was further suggested that the significant differences in the whistle structure of the five different populations of bottlenose dolphin was due to their geographic isolation from one another (Wang *et al.*, 1995).

By contrast, Steiner (1981) studied the whistle characteristics of five different species of dolphin, and discovered that even when the species were sympatric, the whistle characteristics were significantly different from each other. It was suggested that in the case of sympatric species, the whistles of each species would need to be distinguishable from one another to avoid hybridisation and to promote reproductive isolation (Steiner, 1981). This implies that whistles do in fact contain species- specific information, as explained previously, clarifying why in the case of Wang *et al.* (1995) there could have been some whistle mixing between populations of the same species along the coast of Texas, whereas in the case of Steiner (1981), whistles of sympatric species did not exhibit any similarities.

It has been proposed that genetic information from different populations or different species of dolphins could also explain any variations in whistle characteristics. Pichler *et al.* (1998) investigated the genetic structure of different populations of Hectors dolphins (*Cephalorhynchus hectori*) around the coast of New Zealand, and it was found that the genetic structure of the populations reflected strong geographic isolation. Although the whistle characteristics of these populations were not studied, it would be interesting to determine whether there would be significant differences in whistle characteristics between populations, and whether the genetic isolation of the populations would be able to account for these differences. Other studies have also examined the possibility of genetics as an explanation for whistle variation. Parsons *et al.* (2002) looked at the genetic diversity of the different populations of bottlenose dolphins around the United Kingdom. It was found that the dolphins inhabiting the waters of the Moray Firth in Scotland were genetically similar to the dolphins inhabiting the waters along the Welsh coast to a greater extent than to other bottlenose dolphin populations on the west coast of Scotland (Parsons *et al.*, 2002). However, the whistle characteristics of the different populations of dolphins were not analysed, and comparing the vocalisations of the dolphins in the different areas may reveal whether genetic differences could also account for whistle variations.

Geographic variation may also be explained by variation in group sizes of dolphins within populations. Several studies have analysed the effects of group size on the vocalisations of dolphin species. For example, Jones and Sayigh (2002) compared the vocal behaviour of bottlenose dolphins between different geographic locations, whilst taking group size and activity of the dolphins into account. It was found that with increasing group size, vocal production also increased. Furthermore, they found that vocal production was highest during social activities. This suggests that animals that are part of larger groups vocalise more, perhaps to keep in contact with fellow group members. However, not all studies examining the effects of group size on vocal production have found these results. Quick and Janik (2008) found that, in contrast to Jones and Sayigh (2002), the rate of vocalisation decreased with increasing group size. However, it was also noted that the rate of whistle production increased as groups spread out, regardless of group size. Perhaps the dolphins studied by Jones and Sayigh (2002) were more spread out, such that it seemed as though they were vocalising more frequently.

Individual body size may also contribute to geographical variation in whistle characteristics. In looking at the ways in which body size might influence whistle variation in cetaceans, May-Collado *et al.* (2007) suggested that the minimum frequency produced by the animals was physiologically limited through body size, but that maximum frequency was not. It is therefore likely that other factors influence the variability of other frequency variables in cetaceans.

Because of the importance of sound to marine mammals, it is important to consider the potential impacts that anthropogenic sources of sound could have upon them. Several studies have considered that geographical variation of whistles between populations of dolphins could be due to differing levels of background noise. In particular, various studies have looked at the effects of vessel noise on the communication between dolphins (Buckstaff, 2004; Morisaka *et al.*, 2005a; May-Collado and Wartzok, 2008). For example, in comparing the whistles of bottlenose dolphins in different areas of the western Atlantic Ocean, May-Collado and Wartzok (2008) found that the dolphins inhabiting the area with the highest amount of boat traffic whistled at higher frequencies than dolphins inhabiting other areas. The authors further found that dolphins inhabiting areas with high noise levels at low frequencies whistled at higher frequencies than other dolphin populations, suggesting that the dolphins inhabiting areas of high boat noise altered their whistles in order to avoid masking effects. Buckstaff (2004) looked at the effects of boat noise on the acoustic behaviour of bottlenose dolphins in Florida, and did not find any variation in whistle frequency as a result of boat noise. However, it was found instead that dolphins whistled more frequently in the presence of a moving vessel, and less frequently as the vessel moved away. Although it is unclear as to why this reaction occurred, it suggests that the presence and approach of vessels did have an impact on the acoustic behaviour of bottlenose dolphins, though not in terms of their whistle frequency. Buckstaff (2004) suggested that the reason for the increased frequency of whistle production was to ensure that their acoustic communication was not masked by the noise of the boat, or to increase group cohesion and proximity to one another. However, it has previously been found that bottlenose dolphins tend to whistle more frequently when excited or agitated (dos Santos *et al.*, 2005), and it would seem likely that the increase in the frequency of whistles produced would serve to re-group the dolphins. It is therefore likely that whistles are in fact often used for group cohesion.

Other studies have found no effects of boat noise on bottlenose dolphin acoustic behaviour (Lemon *et al.*, 2006). Whilst studying the potential effects of powerboats on acoustic as well as surfacing and travelling behaviour in bottlenose dolphins from Australia, Lemon *et al.* (2006) found that there were no effects on acoustic behaviour induced by approaching boats, although it was observed that they altered surfacing behaviour and moved away from vessels. Thus, although there are differences in findings regarding acoustic behaviour of dolphin species, it is apparent that vessels may have behavioural effects on the animals. Nevertheless, it also seems likely that variation in noise levels within different marine mammal habitats could contribute to the observed geographical variations in whistle characteristics among populations of the same species of marine mammal, as has been suggested by Morisaka *et al.* (2005b).

Morisaka *et al.* (2005a) also proposed that the vocalisations of dolphin populations change naturally over time, due to their ability for learning and imitation, such that members of the same species living in different populations would have different whistle characteristics. Furthermore, McCowan *et al.* (1998) compared whistle characteristics of captive dolphins from the same and from different populations, and found an indication that the characteristics of the whistles produced by bottlenose dolphins are influenced by social familiarity, such that particular members of a population, with whom an individual may have a strong social bond, might influence the way in which whistles are structured. However, this study was undertaken using captive dolphins, and it is unclear as to whether the same results would be found in the wild, though it seems highly plausible due to the high learning capabilities that are exhibited by dolphins.

Although it remains unclear to what extent each of these variables contributes to geographic variation, it seems likely that it is a combination of some or all of these factors. Understanding the reasons for these geographical variations between the whistles of bottlenose dolphins is necessary, as they could be reflective of the importance of learning and culture. Furthermore, geographic variation could indicate genetic differences between different populations.

1.3 Rationale and Hypotheses

Whistle characteristics of different species of dolphins can differ between regional populations (Wang *et al.*, 1995; Morisaka *et al.*, 2005b). However, the whistle characteristics from bottlenose dolphin populations around the eastern North Atlantic Ocean have as of yet not been compared in great depth. The four bottlenose dolphin populations in this study are located in four different areas of the eastern North Atlantic Ocean (Cardigan Bay, Wales; the Shannon Estuary, Ireland; Molène Archipelago, France; the Sado Estuary, Portugal) (Figure 1.1). All four areas experience differing levels of boat traffic (Gregory and Rowden, 2001; Foley and McGrath, 2010; Veneruso and Evans, 2012), but also act as areas of conservation (Harzen, 1998; Le Duff *et al.*, 1999; Englund *et al.*, 2007; Veneruso and Evans, 2012). By comparing the whistle structure between different populations of bottlenose dolphins, it may be possible to better understand the ways in which dolphins use sound to communicate.

As discussed previously, there are various potential reasons for geographic and local variation in the whistle characteristics of dolphin species. However, by further investigating the vocal behaviour of dolphins it may be possible to better understand the ways in which whistles are used. Ultimately, by attempting to understand the ways in which sound is used by bottlenose dolphins, it will allow for a better understanding how populations might be affected by sound pollution and increasing levels of anthropogenic noise.

This study therefore aims to compare the whistle characteristics of bottlenose dolphins between Cardigan Bay (Wales), the Shannon Estuary (Ireland), the Molène Archipelago (France) and the Sado Estuary (Portugal), through recording whistles of bottlenose dolphins in Cardigan Bay, and obtaining access to existing whistle recordings from the other three areas. This study further aims to determine whether there is greater variation between populations than within populations. It was predicted that there would be significant differences in the whistle characteristics of bottlenose dolphins between the different geographic locations. This is due to differences in environmental variables, such as depth and water temperature, as well as the variation that is likely to exist between areas in background noise levels occurring mainly from boat traffic. Furthermore, it was predicted that the variation between populations would be greater than the variation within populations. This is due to the fact that bottlenose dolphins are highly social, such that culture and learning are important. It is for this reason that it seems likely that if different groups of bottlenose dolphin existed in the same region, they would interact and therefore be exposed to whistles from different groups. As such, it is possible that features of the whistles from one group would be used in another group.

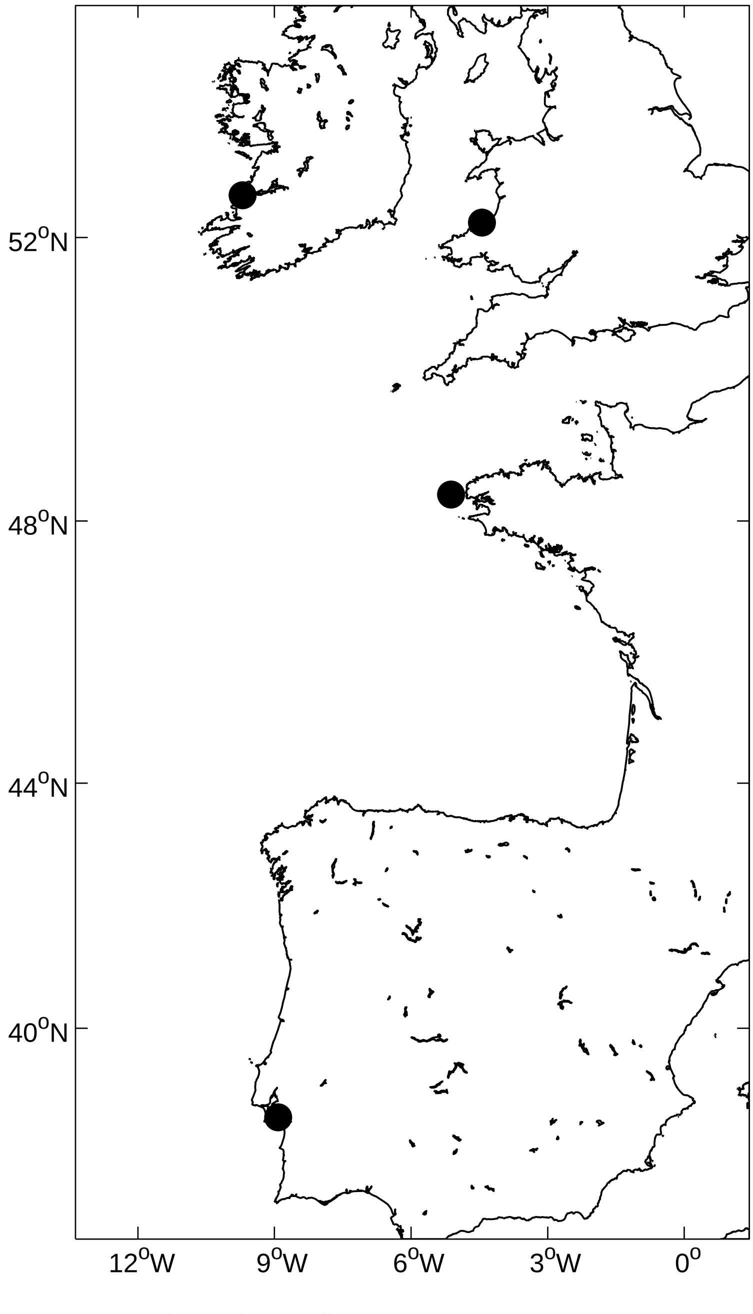


Figure 1.1: Locations of the four different bottlenose dolphin populations (Shannon Estuary (52° N, 009° W), Cardigan Bay (52° N, 004° W), Molène Archipelago (48° N, 005° W), and Sado Estuary (38° N, 008° W)) from which recordings were taken and analysed. The different locations are marked in black.

2 | Methods

2.1 Study Organisms

Recordings from four different populations of bottlenose dolphin were analysed for this study. The French population, residing in the Molène Archipelago region, currently consists of approximately 40 individuals, and is neighboured by a smaller population around the Ile de Seine (Le Duff *et al.*, 1999). The Cardigan Bay population is a semi-resident population, which currently consists of between 200-300 individuals (Veneruso and Evans, 2012). The population residing in the Shannon Estuary consisted of approximately 120-140 individuals in 2008 (Englund *et al.*, 2007, 2008; Mirimin *et al.*, 2011). This population is a closed and genetically isolated one (Mirimin *et al.*, 2011). The smallest population from which data were used is that from the Sado Estuary, consisting of approximately 25-30 individuals (dos Santos *et al.*, 2005). This population is thought to be isolated and in decline (dos Santos *et al.*, 2005). All populations of bottlenose dolphins in this study were inshore, although two (the Shannon Estuary and the Sado Estuary) were estuarine habitats, one was a shallow open bay (Cardigan Bay), and one was an island Archipelago facing the Atlantic Ocean (Molène Archipelago). Data collection from the Cardigan Bay population was undertaken under licence from the Countryside Council for Wales (CCW). This licence allowed for an active yet careful approach of the dolphins in order to collect the relevant data.

2.2 Study Areas

Recordings from four different populations of bottlenose dolphins from the North Atlantic Ocean were analysed (Figure 1.1) in order to compare the whistle characteristics of the dolphins within and between each population.

2.2.1 Cardigan Bay, Wales

Cardigan Bay is a large expanse of water, covering approximately 5500 km^2 (Ceredigion County Council *et al.*, 2001; Gregory and Rowden, 2001), situated in the Irish Sea, off the western coast of Wales (5219’ N, 445’ W) (Figure 2.1).

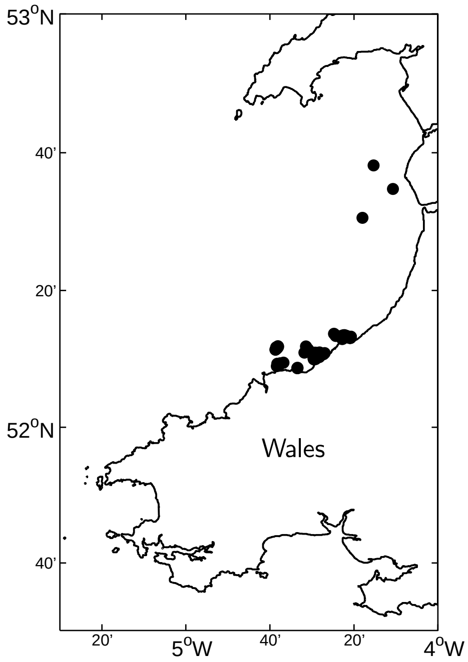


Figure 2.1: Cardigan Bay, Wales (52° N, 004° W), where some of the bottlenose dolphin whistles used for analysis in this study were recorded. The GPS coordinates of the areas in which dolphins were recorded are depicted by the black marks.

Within this bay resides the largest semi-resident population of bottlenose dolphins in the United Kingdom (Veneruso and Evans, 2012). It contains two Special Areas of Conservation (SACs), one at the northern end (Pen Llyn a’r Sarnau SAC), and one at the southern end (Cardigan Bay SAC), which is where the majority of the data in this study were collected. The Cardigan Bay SAC reaches up to 60 metres in depth, and has an area of 958.65 km^2 (Veneruso and Evans, 2012). Boat traffic is evident throughout Cardigan Bay (Gregory and Rowden, 2001; Lohrengel *et al.*, 2012).

2.2.2 The Shannon Estuary, Ireland

The Shannon Estuary, located off the west coast of Ireland (5230' N, 0922' W), is a 75 km long stretch of water through which the River Shannon into the Atlantic Ocean (Foley and McGrath, 2010) (Figure 2.2). It is also a Special Area of Conservation, hosting the only truly resident population of bottlenose dolphins in Ireland (Foley and McGrath, 2010). Boat traffic is high in the Shannon Estuary, as it is an important and well used shipping route, such that anthropogenic noise in this area is likely to be high (Rogan *et al.*, 2000; Foley and McGrath, 2010).

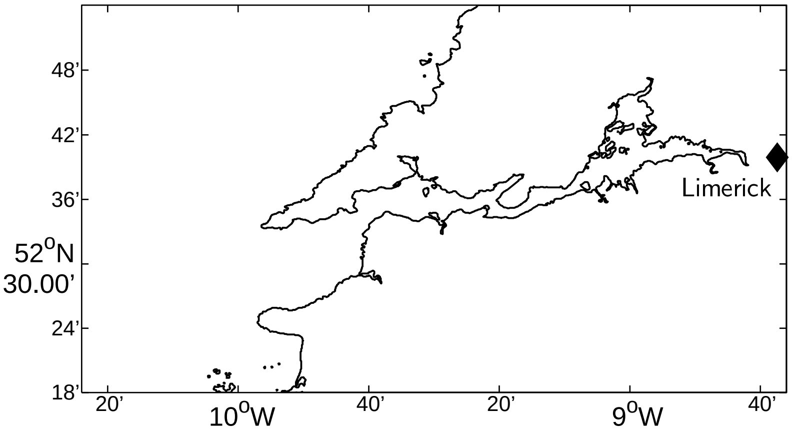


Figure 2.2: The Shannon Estuary, Ireland (52° N, 009° W), where some of the whistles of bottlenose dolphins analysed in this study were recorded.

2.2.3 Molène Archipelago, Iroise Sea, France

The Molène Archipelago is located off the North West coast of France (4813' N, 0502' W), near Brest, and forms a part of the Atlantic Ocean (Figure 2.3). It is situated in the Iroise Sea within a marine park, which covers 3550 km^2, and has a depth of up to 50 metres (Le Duff *et al.*, 1999). Within this marine park exist two different groups of bottlenose dolphins, a larger group around the Molène Archipelago, and a smaller group near the Ile de Seine (Le Duff *et al.*, 1999). The data in this study were collected from the larger group of dolphins existing around the Molène Archipelago region, which is approximately 70 km^2 in area and consists of 11 islands (Genov *et al.*, 2009; Jaud *et al.*, 2011).

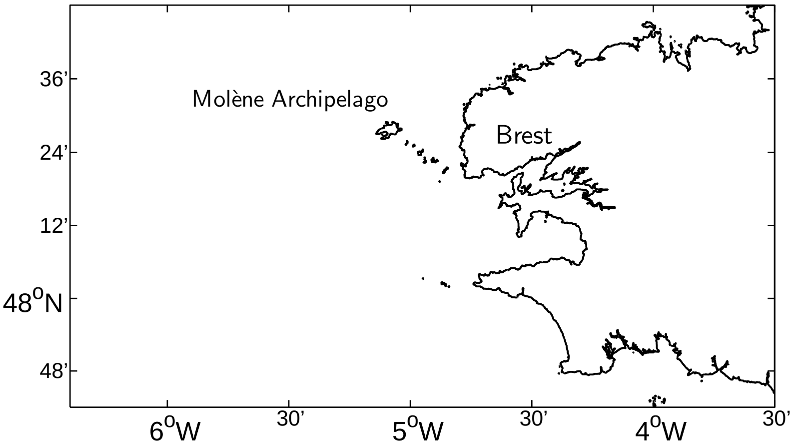


Figure 2.3: The Molène Archipelago in the Iroise Sea (France) (48° N, 005° W), where some of the whistles of bottlenose dolphins analysed in this study were recorded.

2.2.4 The Sado Estuary, Portugal

The Sado Estuary, located off the west coast of Portugal (3829' N, 0855' W), connects to the Atlantic Ocean by the Sado River, which runs through it (Figure 2.4). The area of the Sado Estuary is approximately 150 km^2 in area, with depths of between 5 and 40 metres (Harzen, 1998; dos Santos *et al.*, 2007). It is also an important shipping route, such that anthropogenic noise and boat traffic in this area are likely to be high.

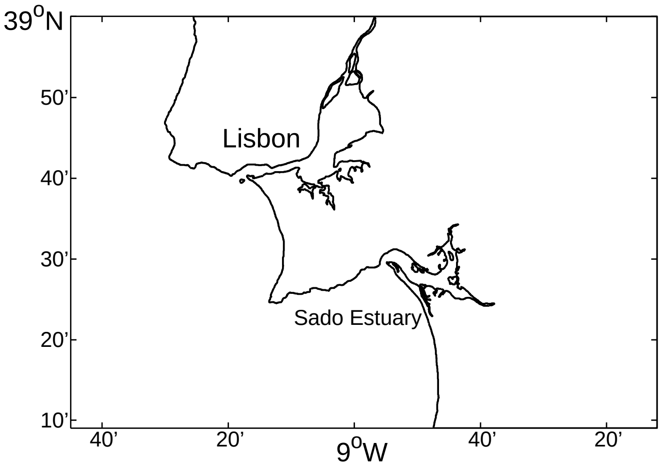


Figure 2.4: The Sado Estuary, Portugal (38° N, 008° W), where the some of the whistles of bottlenose dolphins analysed in this study were recorded.

2.3 Experimental Procedure

2.3.1 Data Collection

2.3.1.1 Cardigan Bay

Recordings of bottlenose dolphins were collected between the months of May and July 2012, using a hydrophone (model C55RS; linear frequency range (+/- 3 dB) (kHz): 0.020-44; useable frequency range (+ 3/- 12 dB) (kHz): 0.009-100; transducer sensitivity (dB, re 1 V/uPa): -200), which was omnidirectional below a frequency of 10 kHz. The hydrophone was connected to a NEMA-4X water resistant battery box (Signal output: female BNC cable) powered by one 9V battery. The battery box was connected to the Olympus L5-11 EU recorder, which was set to record in uncompressed wave format at a sampling rate of 96 kHz, with a MIC-BNC cable. Headphones were plugged into the Olympus recorder in order to hear the sounds that were being recorded in real time (Figure 2.5).

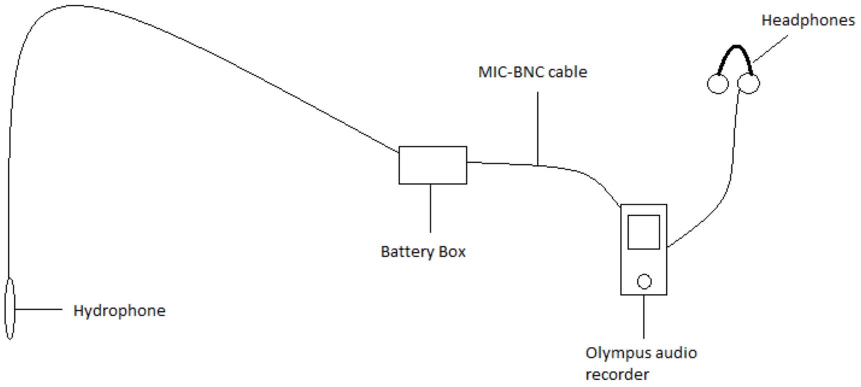


Figure 2.5: Arrangement of the equipment used to record bottlenose dolphin vocalisations in Cardigan Bay between May and July 2012.

2.3.1.2 The Shannon Estuary, Ireland

Recordings from the Shannon Estuary were obtained by contacting Anneli Englund of University College Cork. These data consisted of recordings from five different encounters in 2008 and 2010, and were collected using an omnidirectional hydrophone (HTI 96-MIN, High Tech, Inc.) connected to a solid state recorder (Edirol 09HR) which sampled at a rate of 96 kHz.

2.3.1.3 Molène Archipelago, The Iroise Sea, France

Recordings from the Molène Archipelago were provided by Lucia Di Iorio of École Nationale Supérieure de Techniques Avancées (ENSTA), Brittany. These data consisted of acoustic data that had been recorded continuously during a period of ten days at the beginning of September, 2010. The data were collected using a bottom-moored autonomous recorder (AURAL, Multi-Electronique, Quebecec) at a sampling rate of 32 kHz.

2.3.1.4 The Sado Estuary, Portugal

Recordings from the Sado Estuary were obtained by contacting Manuel dos Santos of Instituto Universitário (ISPA), Portugal. These consisted of acoustic data that had been recorded from a variety of encounters in 2001 and 2002. Some recordings were collected using a Bruel and Kjaer 8103 hydrophone, connected to a Bruel and Kjaer preamplifier 2646 (27 V battery), whilst others were collected using Cetacean Research Technology C303 hydrophone. In all cases, a DAT recorder (Sony TCD-D10 Pro) was used, which sampled at a rate of 44 kHz.

2.3.2 Boat Protocol in Cardigan Bay

A combination of line-transect and *Ad libitum* surveys were undertaken on various vessels within Cardigan Bay (Table 2.1). Surveys were undertaken during the day if the visibility was good ($\geq$1.5 km), if the sea conditions were appropriate (Beaufort Sea State ≤ 2, low swell), and if there was no precipitation.

Once on the boat, the equipment was set up as shown in Figure 2.5, and when dolphins were spotted, they were approached slowly according to the Sea Watch Foundation licence protocol. The hydrophone was then held over the side of the boat such that it was submerged to a depth of approximately 3 metres. Recordings were made for as long as the dolphins were in range. Whilst the hydrophone was submerged, other data (time of day, GPS coordinates, group size, group composition and dolphin behaviour) were collected on standardised behaviour forms (Appendix Figure A.1) at three minute time intervals, until the hydrophone was retrieved from the water. Recordings were made at group level or individual level. At the group level, the hydrophone was held in the water as described above. At the individual level, the hydrophone was mounted onto a 3 metre pole, and a bowl was placed around the hydrophone with the purpose of acting as a parabolic reflector, making the hydrophone more directional. As it was rarely possible to point the hydrophone at a single individual dolphin, it was pointed in the general direction of a group of dolphins, such that the identity of the individuals could seldom be recorded.

Table 2.1: Details of the four different boats (boat length, maximum speed, type of engine) that were used to collect recordings of the vocalisations of bottlenose dolphins in Cardigan Bay between May and July 2012.

Vessel	**Length (m)**	**Speed (Kn)**	**Engine Type**
Dunbar Castle II	9.7	7	120 hp diesel
Ma Chipe Seabrin	10	10	Twin 220 hp diesel
Boat Gallois	5	8	60 hp petrol

When on dedicated line-transect surveys, the hydrophone was most often deployed whilst the engine of the boat was running, and was frequently towed through the water as the boat was moving. However, on the *Boat Gallois Ad libitum* surveys, the engine was always switched off for the purpose of recording the dolphin whistles. If the dolphins were in travel mode during the *Ad libitum* surveys, the boat would go ahead of the group in their predicted path, such that recordings could be taken while the dolphins were travelling past the boat.

2.4 Acoustic Analysis

The recordings were analysed using *Ishmael* software (version 2.3 David Mellinger, NOAA/PMEL, 7600 Sand Point Way NE, Seattle, WA). This software produced spectrograms of the recordings, such that whistles could be visually located within the recordings (Figure 2.6).

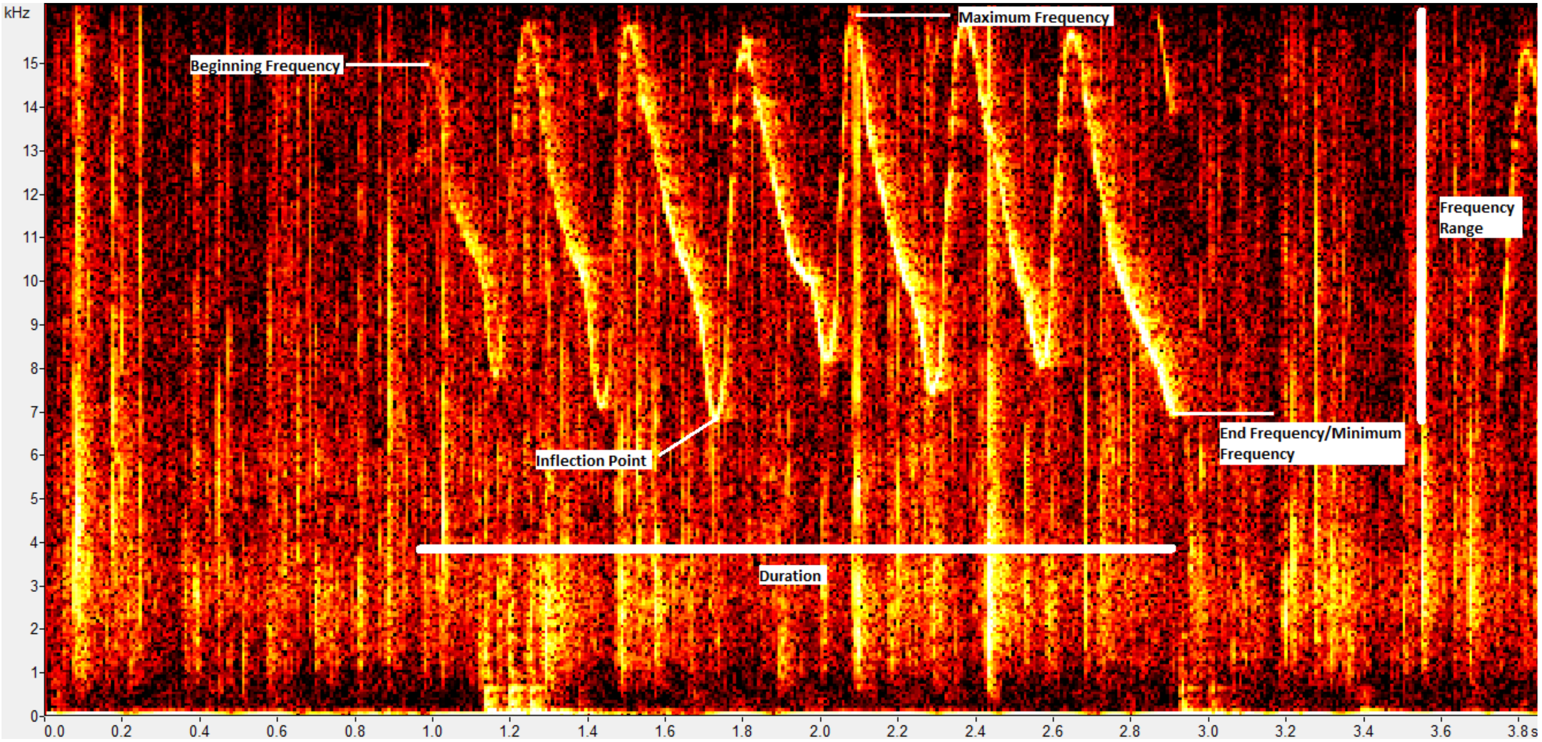


Figure 2.6: An example of a spectrogram of a sinusoidal bottlenose dolphin whistle, showing 7 of the 9 whistle characteristics (beginning frequency, end frequency, minimum frequency, maximum frequency, frequency range, duration, and number of inflection points) that were calculated from each whistle.

Each whistle was selected manually, and *Ishmael* then calculated the whistle characteristics (beginning frequency, end frequency, minimum frequency, maximum frequency, peak frequency, maximum intensity, start time, and end time) of the selected whistle (Table 2.2).

Table 2.2: Description of the 10 different whistle characteristics that were collected and analysed from recordings taken from the four different populations of bottlenose dolphins.

Whistle Characteristic	**Description**
Beginning Frequency (Hz)	The frequency at the start of the whistle
End Frequency (Hz)	The frequency at the end of the whistle
Minimum Frequency (Hz)	The lowest frequency that occurs during the whistle
Maximum Frequency (Hz)	The highest frequency that occurs during the whistle
Peak Frequency (Hz)	The frequency that occurs at the point of highest intensity in the whistle
Frequency Range (Hz)	The difference between the minimum frequency and end frequency
Maximum Intensity (dB)	The highest intensity of the whistle
Inflection Points	The number of points at which the inclination of the whistle changes direction
Duration (s)	Calculated by subtracting the start time of the whistle from the end time

Such whistle characteristics have previously been used to compare the whistles of bottlenose dolphins between populations (Wang *et al.*, 1995; Rendell *et al.*, 1999; Bazua-Duran and Au, 2004; Azevedo *et al.*, 2007; López, 2011), and it is thus considered an appropriate way to make such comparisons. The number of inflection points in each whistle were counted by eye. The whistle duration was calculated by subtracting the start time from the end time, and the frequency range of each whistle was calculated by subtracting the start frequency from the end frequency. Whistle characteristics for every good quality whistle were obtained from every recording. Whistles were organised into six different categories (upsweep, downsweep, sinusoidal, convex, concave and constant frequency) (Figure 2.7), using the same definitions as Ansmann *et al.* (2007) and Petrella *et al.* (2012).

2.5 Statistical Analysis

Statistical analyses were carried out using IBM SPSS Statistics Version 20. Descriptive statistics including the mean, standard deviation and coefficient of variation were calculated for each of the nine whistle characteristics within each of the four populations of bottlenose dolphin.

2.5.1 Variation Within Populations

To look at the variation in whistle characteristics within each of the four populations of bottlenose dolphins, the coefficient of variation was calculated for each whistle characteristic and then plotted graphically. This allowed for a visual comparison of the amount of variance that occurred in each whistle characteristic within populations. In order to compare the amount of variation that occurred within each population with the amount of variation occurring within other populations, a One-way Analysis of Variance (ANOVA) was carried out on the coefficients of variation between each population.

2.5.2 Variation Between Populations

The number of whistles obtained from each area was different, such that two separate analyses were undertaken on the data. In the first analysis, the whistle characteristics from all four areas were compared. In the second analysis, the whistles from the Sado Estuary were excluded, as the number of whistles obtained from this region was much smaller than the rest (Table 3.1). A column of random numbers was generated in Microsoft Office Excel, and the data were then sorted in order of the random number column. In this way, a number of whistles corresponding to the country with the least amount of whistles was randomly selected from the data set from Cardigan Bay and the Molène Archipelago. In the first analysis, 162 whistles were randomly selected from each area, and in the second analysis, 30 whistles were randomly selected from each area for comparison. The data were tested for normality and homogeneity of variance using a Kolmogorov-Smirnov test and Levene's test (Levene, 1960), respectively. If the data were not normaly distributed, Kruskal-Wallis tests (Kruskal and Wallis, 1952) were undertaken. If the results of the Kruskal-Wallis test were significant, Mann-Whitney U tests were undertaken to determine where the significance lay. If the data were normal, One-way ANOVA tests were undertaken to compare each whistle characteristic between the different geographical areas. *Post Hoc* Fisher's Least Significant Difference (LSD) (Hayter, 1986) tests were conducted on the data if the One-way ANOVA held significant results. The significance level used for all tests was $P = 0.05$.

2.6 Within Cardigan Bay Comparisons

The whistles from Cardigan Bay were analysed in more depth. Chi Square tests were undertaken on behaviour and whistle type, behaviour and group size, and whistle type and group size, to determine whether any correlation existed. Behaviour was split into two categories: 'Travelling' and 'Feeding.' Other behaviour types such as socialising and bow-riding were discarded, due to low sample sizes. Group size was split into three categories as described in Table 2.3. Whistle type was split into six categories as described in Table 2.4.

Table 2.3: The way in which the group size of bottlenose dolphins was categorised.

Category of Group Size	Group Size Cluster (number of dolphins)
1	1 - 4
2	5 - 8
3	9 - 12

Table 2.4: The way in which the types of bottlenose dolphin whistles were categorised (Category 1 = upsweep, 2 = downsweep, 3 = sinusoidal, 4 = convex, 5 = concave, and 6 = constant frequency).

Category	Whistle Type
1	Upsweep
2	Downsweep
3	Sinusoidal
4	Convex
5	Concave
6	Constant Frequency

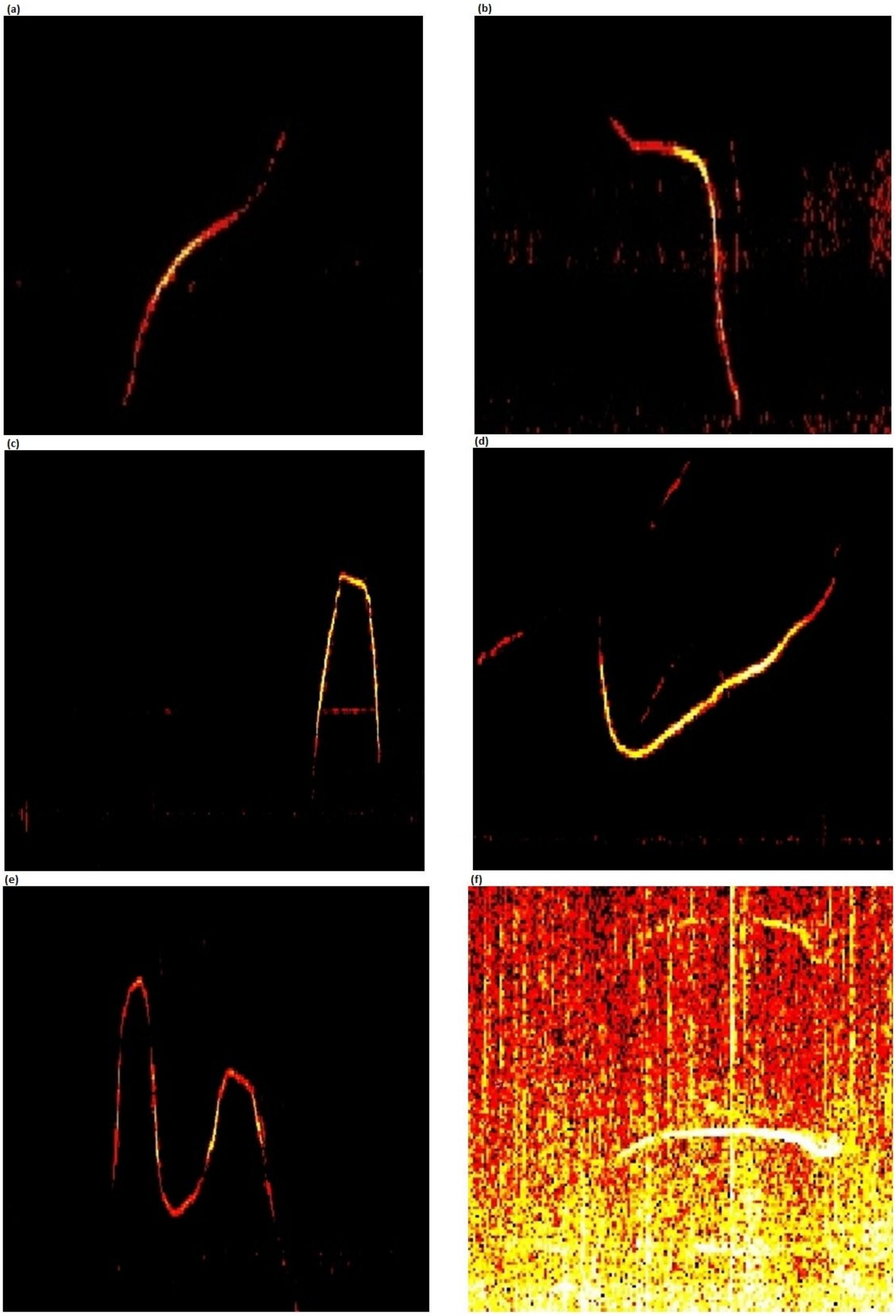


Figure 2.7: The six different categories of bottlenose dolphin whistles that were analysed in this study ((a) upsweep, (b) downsweep, (c) convex, (d) concave, (e) sinusoidal, and (f) constant frequency).

3 | Results

A total of 1198 whistles and 11 hours, 13 minutes of recordings were analysed from the four populations combined (Table 3.1).

Table 3.1: Total number of whistles and total number of minutes of recording that were analysed from each of the four populations of bottlenose dolphin.

Population	**# Whistles Analysed**	**Amount of Recording Analysed (min)**
Cardigan Bay, Wales	307	418.38
Shannon Estuary, Ireland	162	87.54
Molene Archipelago, France	699	127.05
Sado Estuary, Portugal	30	75.54

Descriptive data (mean, standard deviation, minimum value, maximum value and coefficient of variation) are shown in Table 3.2 for all four areas. In Cardigan Bay and the Sado Estuary, upsweeps were the predominant whistle type (62% in Cardigan Bay and 46% in the Sado Estuary). By contrast, downsweeps were the most predominant whistle type in the Shannon Estuary and the Molène Archipelago (38% in the Shannon Estuary and 67% in the Molène Archipelago). Comparisons between the four areas of the mean values for each whistle characteristic can be seen in Figure 3.1.

Table 3.2: The descriptive statistics (mean, standard deviation, minimum, maximum and coefficient of variation) for each of the 9 bottlenose dolphin whistle characteristics ("Min." = Minimum frequency, "Max." = maximum frequency, "Beg." = beginning frequency, "End" = end frequency, "Peak" = peak frequency, "Range" = frequency range, "Intensity" = maximum intensity, "Duration" = duration) from Cardigan Bay, the Shannon Estuary, the Molène Archipelago and the Sado Estuary.

		Frequency (Hz)								
Study Site	**Descriptive Statistics**	**Min.**	**Max.**	**Beg.**	**End**	**Peak**	**Range**	**Intensity (dB)**	**Duration (s)**	**Inflection**
Cardigan Bay	Mean	6566.500	13069.941	8135.146	11116.211	9145.681	6504.692	9.840	0.732	0.526
	S.D	1535.922	3105.343	3342.585	3713.313	5493.593	2965.037	2.183	0.655	1.031
	Minimum	3063.490	6839.420	3205.980	4025.280	3617.580	659.130	5.000	0.130	0.000
	Maximum	11864.300	22190.200	20931.150	21245.900	101218.800	16131.130	13.600	10.280	6.000
	CoV	0.234	0.238	0.411	0.334	0.601	0.456	0.222	0.895	1.964
Shannon Estuary	Mean	7680.967	14854.546	10259.795	11466.680	11164.002	7173.579	9.887	0.756	1.870
	S.D	2740.929	3613.942	4144.719	9204.070	4124.856	2852.550	1.065	0.764	3.793
	Minimum	3644.590	8917.610	3954.770	4109.850	4312.500	1550.920	6.880	0.050	0.000
	Maximum	17990.300	23961.200	23883.680	113085.620	22500.000	15276.250	12.140	7.660	36.000
	CoV	0.357	0.243	0.404	0.803	0.369	0.398	0.108	1.011	2.028
Molene Archipelago	Mean	7964.765	12125.455	10991.818	8891.497	9519.702	4160.690	10.196	0.590	1.478
	S.D	1680.722	2433.702	2380.674	2577.855	2009.738	2122.486	0.886	0.308	2.267
	Minimum	688.180	1646.850	767.590	1958.670	896.000	317.620	7.850	0.090	0.000
	Maximum	12851.800	16384.000	16225.190	16304.600	15808.000	10269.810	12.780	3.100	25.000
	CoV	0.211	0.201	0.217	0.290	0.211	0.510	0.087	0.522	1.534
Sado Estuary	Mean	14279.049	26699.072	16160.455	20923.393	21151.045	12420.022	14.364	0.429	0.867
	S.D	5167.039	7594.743	7495.524	8641.468	7286.307	5720.695	1.027	0.804	1.106
	Minimum	164.560	325.950	202.530	199.370	218.750	161.390	9.010	0.070	0.000
	Maximum	25935.400	39237.500	36859.450	37305.330	36562.500	22071.100	14.740	4.610	3.000
	CoV	0.362	0.284	0.464	0.413	0.344	0.461	0.071	1.874	1.276

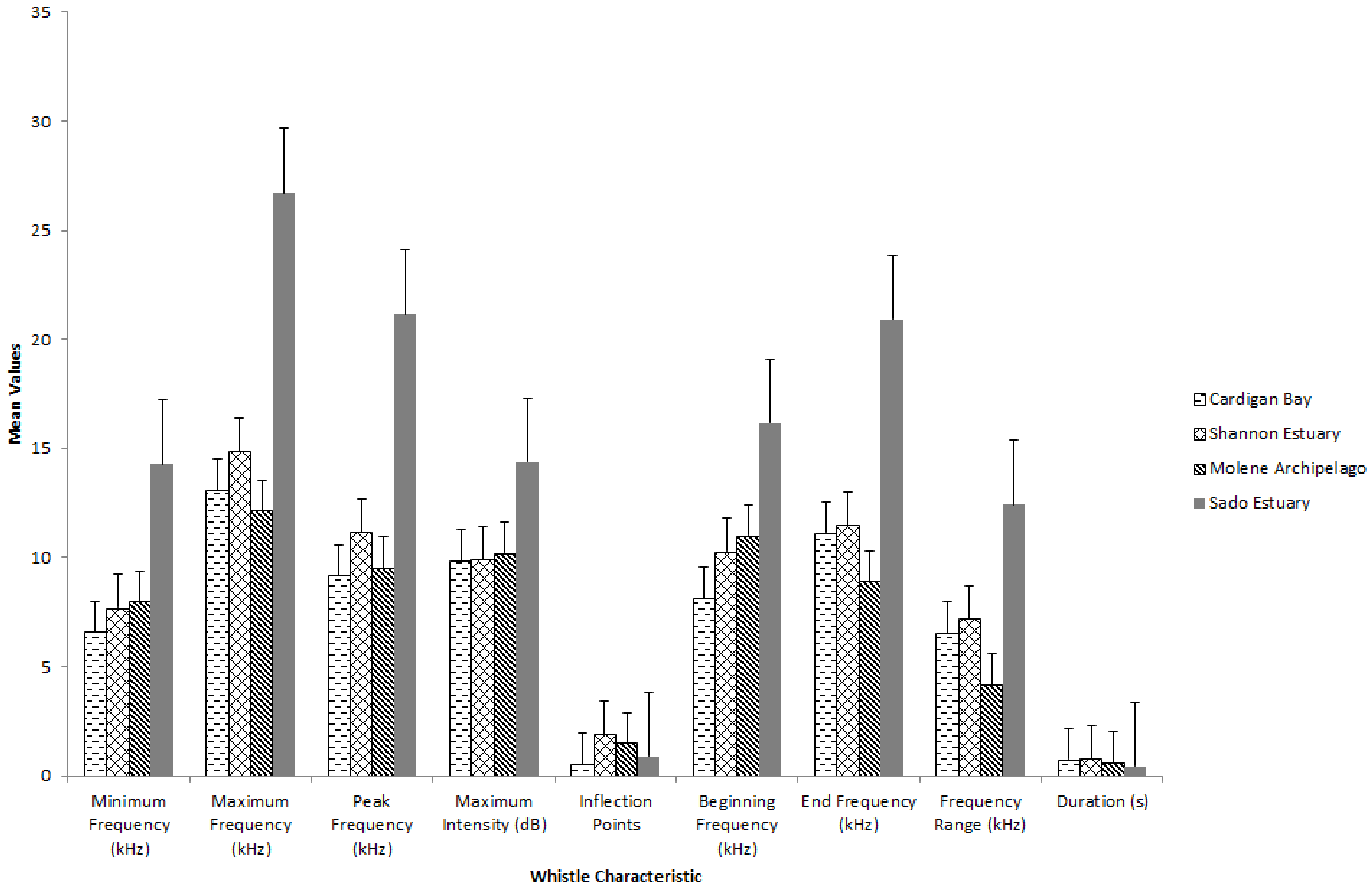


Figure 3.1: Mean values +/- Standard Error for each of the bottlenose dolphin whistle characteristics from Cardigan Bay, the Shannon Estuary, the Molène Archipelago and the Sado Estuary.

3.1 Variation Within Populations

The whistles from the Sado Estuary exhibited the highest mean frequencies for all frequency variables (Figure 3.1), whilst the whistles from Cardigan Bay, the Shannon Estuary and the Molène Archipelago exhibited similar mean frequency values for all frequency characteristics (Figure 3.1). The whistles from all four populations exhibited similar mean values for the number of inflection points and the duration (Figure 3.1). For the most part, frequency variables (beginning frequency, end frequency, peak frequency, maximum frequency, minimum frequency and frequency range) showed the lowest coefficients of variation in all four areas, whilst duration and number of inflection points showed the highest coefficients of variation in each area (Figure 3.2). The whistles from Cardigan Bay, the Shannon Estuary and the Molène Archipelago showed the highest variation within their populations in the number of inflection points, whilst the whistles from the Sado Estuary showed the highest variation in the duration. The maximum intensity variable showed the least variation in all four populations. The whistles from the Sado Estuary population displayed the highest mean frequency variables out of the four different areas, whilst the other three areas exhibited similar mean frequency variables (Figure 3.1). The whistles from the Cardigan Bay population had the lowest mean number of inflection points, whilst the whistles from the Shannon Estuary had the highest mean number of inflection points (Figure 3.1). The whistles from all four places had similar mean durations (Figure 3.1). Between the four different populations, there was no difference in the amount of variation occurring within populations (One-way ANOVA: $F = 0.298$, d.f. $= 3$, $P > 0.05$). Thus, the bottlenose dolphin whistle characteristics from each area had similar amounts of within-population variation.

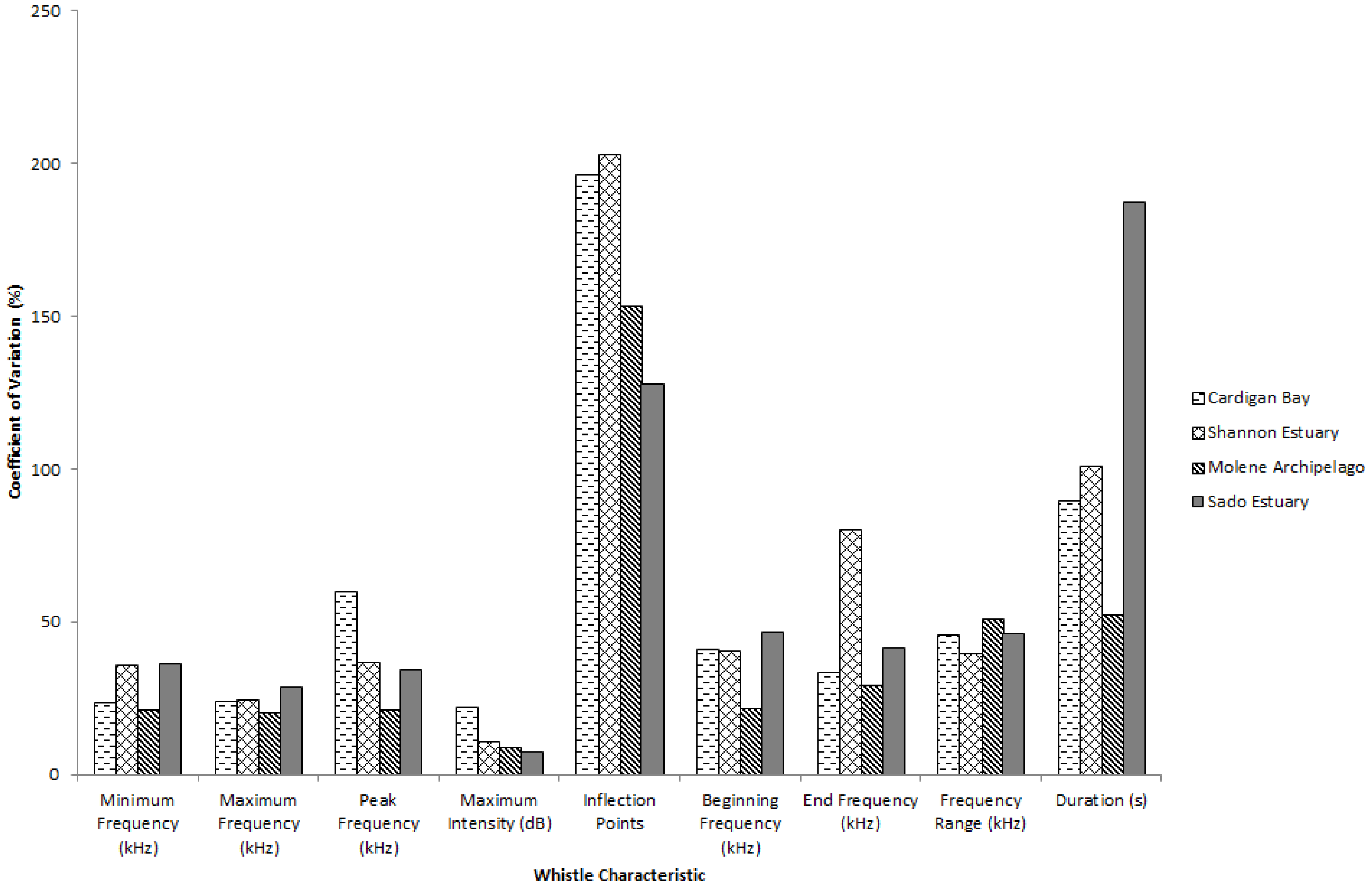


Figure 3.2: Coefficient of variation for each of the 9 whistle characteristics from the whistles of bottlenose dolphins from Cardigan Bay, the Shannon Estuary, the Molène Archipelago and the Sado Estuary.

3.2 Geographical Variation in Whistle Characteristics

3.2.1 Comparison between Cardigan Bay, Shannon Estuary, Molène Archipelago and Sado Estuary

Neither the duration of whistles nor the number of inflection points in whistles differed significantly between the four populations (duration: One-way ANOVA: F = 1.235, d.f. = 3, $P > 0.05$; Number of inflection points: Kruskal-Wallis: $X^2 = 1.640$, d.f. = 3, $P > 0.05$). The whistles from the Sado Estuary had significantly higher peak frequencies than the whistles from the other three populations, none of which differed significantly (One-way ANOVA: F = 8.227, d.f. = 3, $P < 0.05$; *Post-Hoc* LSD test: Sado Estuary > Molène Archipelago = Shannon Estuary = Cardigan Bay). The whistles from the Sado Estuary exhibited significantly higher frequency variables than the whistles from any other area (Appendix Table B.1). The whistles from the Sado Estuary also exhibited significantly higher maximum intensities than the whistles from any other area (Appendix Table B.1). The whistles from Cardigan Bay, the Shannon Estuary and the Molène Archipelago did not differ from one another in maximum intensity or in peak frequency (Appendix Table B.1).

3.2.2 Comparison between Cardigan Bay, Shannon Estuary and Molène Archipelago

Maximum intensity did not differ significantly between the whistles of bottlenose dolphins from Cardigan Bay, the Shannon Estuary and the Molène Archipelago (Kruskal-Wallis: $X^2 = 2.759$, d.f. = 2, $P > 0.05$). However, the three populations did differ significantly from each other in beginning frequency, peak frequency, maximum frequency, minimum frequency and frequency range (Appendix Table B.2). The whistles from Cardigan Bay and the Shannon Estuary were similar in end frequency, but those from the Molène Archipelago had significantly lower end frequencies than the whistles from Cardigan Bay and the Shannon Estuary (Appendix Table B.2). The whistles from Cardigan Bay and the Shannon Estuary had similar durations, but were of significantly longer duration than those from the Molène Archipelago (Appendix Table B.2). The whistles from the Shannon Estuary and the Molène Archipelago contained similar numbers of inflection points, but those from Cardigan Bay had significantly less inflection points than the whistles from the other two populations (Mann-Whitney U: Shannon Estuary > Molène Archipelago > Cardigan Bay) (Appendix Table B.2).

3.3 Bottlenose Dolphin Whistles from Cardigan Bay

There was a significant correlation between behaviour and the type of whistle produced by bottlenose dolphins in Cardigan Bay (Chi-Squared test: $\mathrm{X}^2(4) = 9.918$, $P < 0.05$). During travel, upsweeps were the most common type of whistle and concave whistles were the least commonly used whistle, whereas during feeding, whilst upsweeps were the most common type of whistle, convex whistles were the least common (Figure 3.3).

There was also a significant correlation between group size and dolphin behaviour (Chi-Squared test: $\mathrm{X}^2(2) = 33.981$, $P < 0.05$), with feeding being most common in smaller groups (group size 1; Table 2.3), and least common in larger groups (group size 3; Table 2.3), and travelling being most common in larger groups and least common in smaller groups (Figure 3.4).

There was a correlation between group size and the type of whistles produced (Chi-Squared test: $\mathrm{X}^2(8) = 48.785$, $P < 0.05$). Upsweeps were the most common type of whistle that occurred when dolphins were in groups of between 1 and 4, and sinusoidal whistles were least common in small groups (Figure 3.5). In group sizes of between 5 and 8 dolphins, downsweeps were the most common types of whistle, and concave whistles the least common (Figure 3.5). In groups of between 9 and 12 individuals, upsweeps were the most common types of whistle used whereas neither downsweeps nor concave whistles were used at all. Sinusoidal shaped whistles occurred most frequently when dolphins were in larger groups.

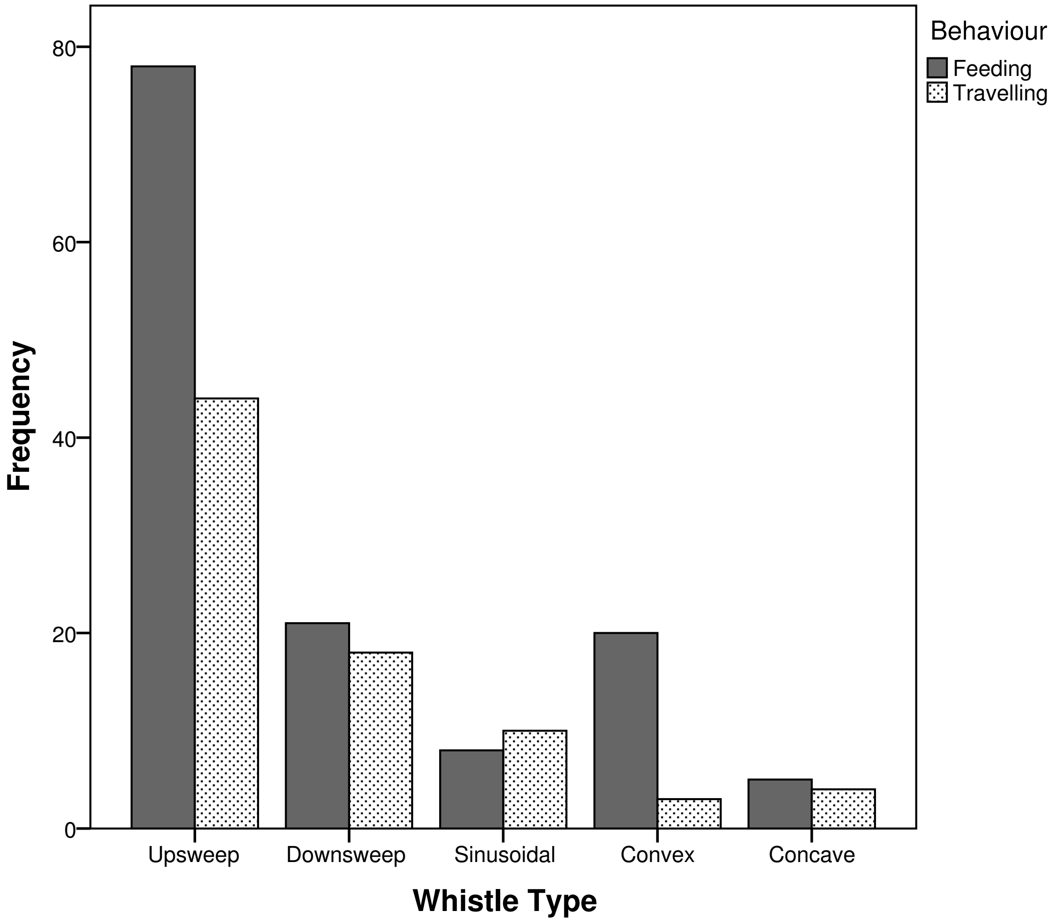


Figure 3.3: The frequency of different whistle types (upsweep, downsweep, sinusoidal, convex, or concave) during different types of behaviour (feeding or travelling) in the bottlenose dolphin population of Cardigan Bay.

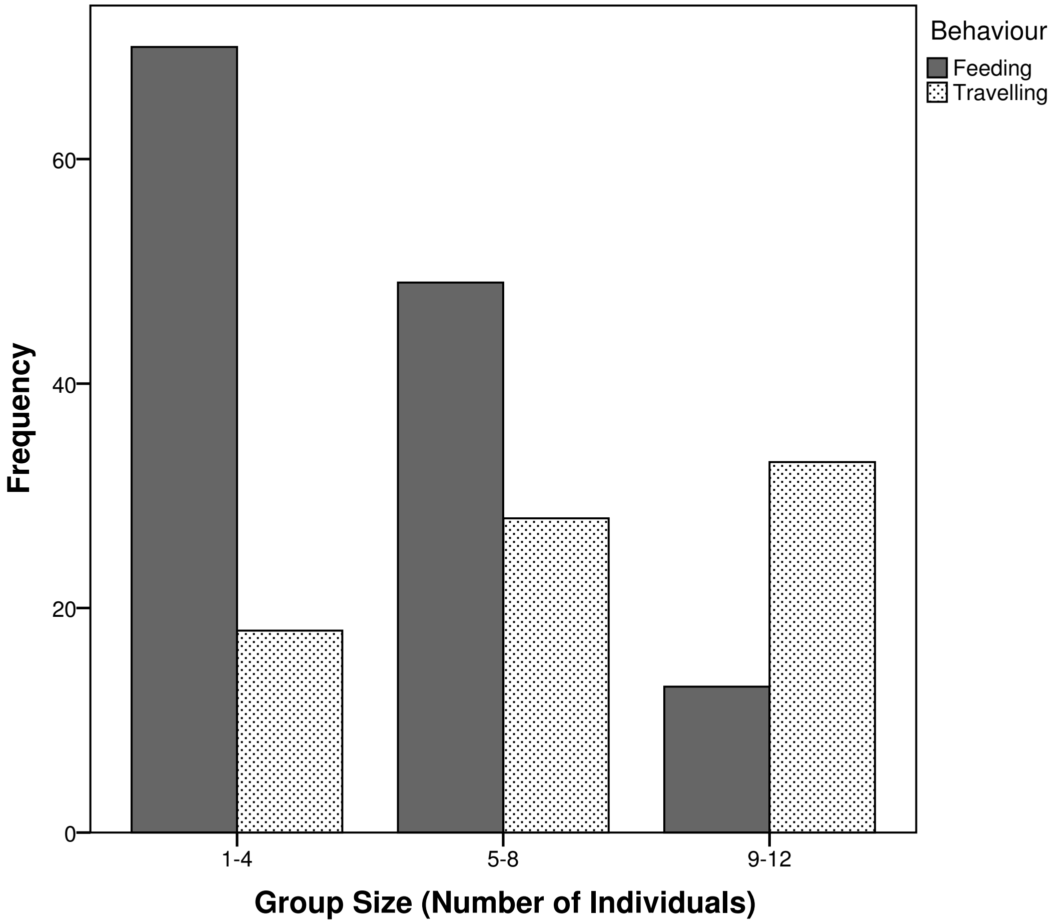


Figure 3.4: The frequency of different behaviours (feeding or travelling) according to three different group sizes of bottlenose dolphins in Cardigan Bay.

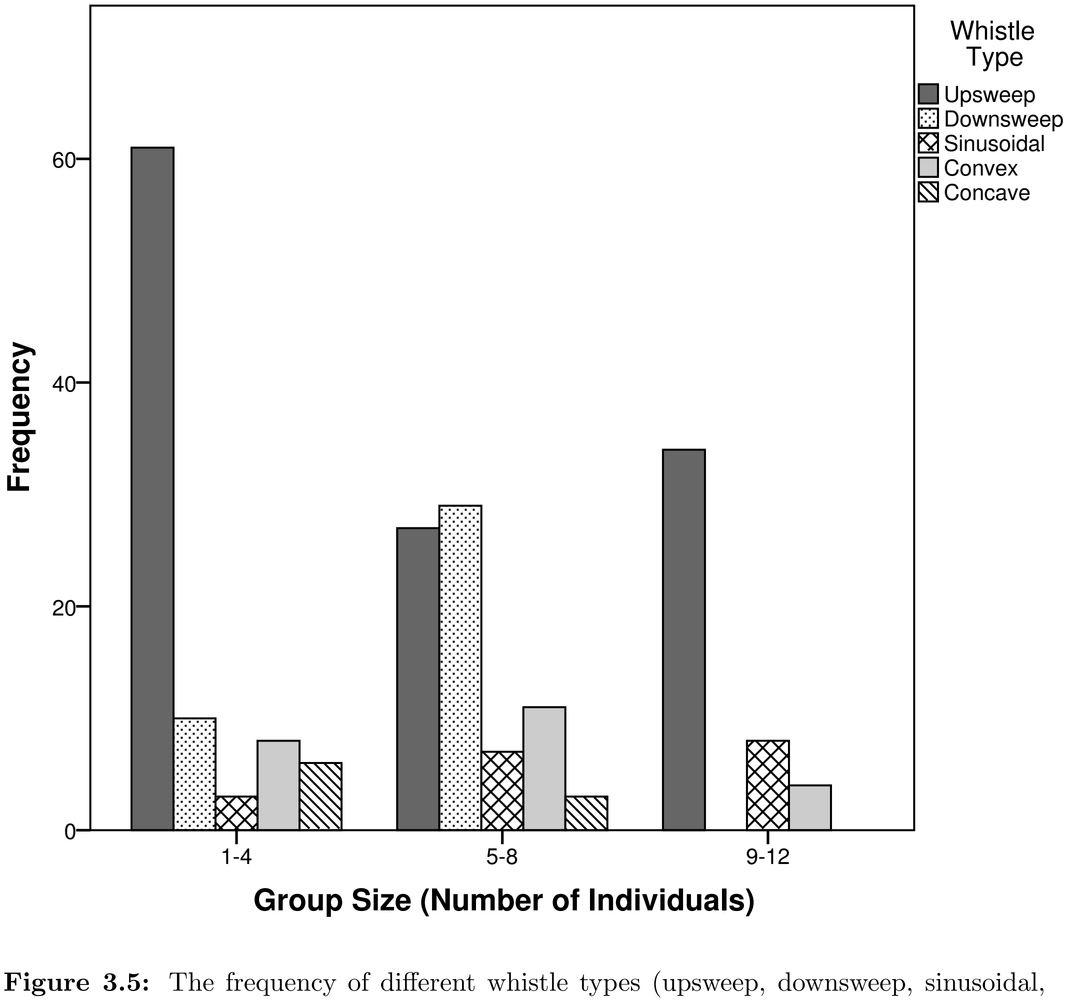


Figure 3.5: The frequency of different whistle types (upsweep, downsweep, sinusoidal, convex, or concave) according to different group sizes in the bottlenose dolphin population of Cardigan Bay.

4 | Discussion

This study has shown that many of the whistle characteristics of bottlenose dolphins (*Tursiops truncatus*) differ depending on geographic location in the eastern North Atlantic Ocean. The findings that upsweeps were most common in Cardigan Bay and the Sado Estuary, whilst downsweeps were the most predominant whistle types in the Shannon Estuary and the Molène Archipelago are both in agreement and disagreement with previous findings. It seems that upsweeps contribute a significant amount to the vocal repertoire of bottlenose dolphins (Cook *et al.*, 2004; López, 2011), as well as other species of dolphin (Lima *et al.*, 2012). Cook *et al.* (2004) found that in bottlenose dolphins, 19% of whistles were upsweeps, which is in contrast to 46%, 29%, 62% and 15% for the Sado Estuary, the Shannon Estuary, Cardigan Bay and the Molène archipelago respectively. Perhaps the reason behind the differences in the composition of the whistle repertoires of the four populations is due to differences in behaviour at the time of recording in each of the four places. Although the behaviour and group size of the bottlenose dolphins at the time of recording in Cardigan Bay were known, these factors were not recorded in any of the other three areas, such that it is possible that differences in behaviour or group size contributed to the differences found here between sites. However, it is also possible that the samples retrieved from each site were representative of a variety of behaviours, such that the difference in whistle composition could indicate a difference in culture or population structure. Perhaps the bottlenose dolphins in the different areas use different whistle types more frequently during different behaviours.

4.1 Comparison of Whistle Characteristics Within Populations

Within all four populations, it was the number of inflection points and the duration of whistles that exhibited the highest amount of variation (Figure 3.2), with little variation occurring in the frequency and intensity of whistles. These findings are similar to those of several other studies (Wang *et al.*, 1995; Azevedo *et al.*, 2007; May-Collado and Wartzok, 2008; Petrella *et al.*, 2012).

It is thought that it is the number of inflection points and the duration of whistles that carry information about particular behaviours as well as emotional information such as the presence of food or danger. (Wang *et al.*, 1995; Janik and Slater, 1998; Morisaka *et al.*, 2005b; López, 2011; Petrella *et al.*, 2012). It is also thought that these acoustic features provide the mechanism whereby individuals are able to create their own signature and be recognised by other individuals (Sayigh, 1992). The high intra-regional variability in these two whistle characteristics observed in this investigation could therefore be indicative of the variation that exists at an individual level, such that the variation in the number of inflection points and duration of whistles could contribute to the manner in which individuals recognise each other through signature whistles. May-Collado *et al.* (2007) suggest that the variation of inflection points and duration of whistles within a population could be influenced by social structure, whilst the frequency and intensity variables of whistles appear to be limited by the body size of the dolphins emitting the sounds. This could explain why within populations, whistles did not differ in either frequency or intensity variables. Perhaps the dolphins within each population were physiologically limited to produce whistles of certain frequencies and frequency ranges. However, the low variation in frequency and intensity variables observed in this study could also be a result of dolphins in each location adjusting the frequency and intensity of their whistles to account for the level of background noise in their environment, in order to avoid the masking of their communication. It thus seems likely that the high variation in the duration and number of inflection points of whistles within populations is reflective of the social nature of dolphins, such that dolphins use these characteristics to modify the information that different whistles may carry, as has been suggested by others (Sayigh, 1992; Wang *et al.*, 1995; Janik and Slater, 1998; Morisaka *et al.*, 2005b; López, 2011; Petrella *et al.*, 2012), whilst low variation in frequency and intensity could be due to levels of background noise.

4.2 Geographical Variation in Whistle Characteristics

The whistles from the Sado Estuary had much higher frequencies than the whistles from any other area (Figure 3.1). Whistle frequencies also differed between Cardigan Bay, the Shannon Estuary and the Molène Archipelago. Other studies have observed similar findings (Wang *et al.*, 1995; Morisaka *et al.*, 2005b; Rossi-Santos and Podos, 2006; Baron *et al.*, 2008; May-Collado and Wartzok, 2008).

The variation in whistle characteristics between the bottlenose dolphins observed here could be a result of the varying levels of background noise between the different populations. It seems likely that elevated noise levels caused by vessels could have masking effects on the communication between dolphins, as the frequency of boat noise (0.1-10 kHz) often overlaps with the frequency range of bottlenose dolphins (0.2-24 kHz) (Buckstaff, 2004). Therefore, to overcome this masking, animals may adjust the frequencies of their whistles, as well as perhaps the duration of their whistles. Previous studies have observed differences in whistle characteristics of different populations of dolphins, and have attributed the variability to the levels of background noise present in each population. For instance, Morisaka *et al.* (2005b) examined the geographical variation of whistles in Indo-Pacific bottlenose dolphins (*Tursiops aduncus*), and discovered that the frequency range of the whistles emitted by the dolphins at three sites in Japan were different between sites. Ambient noise levels at these three sites also differed, suggesting that the geographical variation in the whistle characteristics of these dolphins was due to the varying levels of anthropogenically produced sound between the three locations. Further evidence that bottlenose dolphins may be able to adapt to their acoustic environment has been proposed by May-Collado and Wartzok (2008), who found significant differences in the whistle characteristics of bottlenose dolphins between different geographic populations. They found that in areas of high boat traffic, dolphin whistles had higher maximum frequencies, contained more inflection points, and were longer in duration, suggesting that dolphins were adapting their whistle structures in order to avoid masking effects. These studies indicate that animals are able to adapt to their surrounding environment in order to avoid masking effects. It therefore seems likely that the whistles emitted by bottlenose dolphins living in each of the four localities studied in the present investigation could have been representative of the ways in which the dolphins had adapted acoustically to their respective environments.

The lack of difference between the within-population variances of whistle characteristics and the significant difference in whistle characteristics between populations (Figure 3.2) further suggests that the animals belonging to their respective populations have acoustically adapted to the environment in which they live. If frequency variables were solely limited by body size, it would be less likely for dolphin whistles to exhibit such strong differences between populations, as although they may differ slightly in body size, it seems unlikely that they would differ enough to cause such significant differences between populations. Therefore, it is probable that environmental factors and levels of background noise are also important variables in explaining the differences in whistle characteristics. Thus, the difference in the frequency characteristics of the whistles between populations could have been due to significant differences in exposure to boat traffic and vessel noises.

Harzen (1998) studied the habitat use of bottlenose dolphins in the Sado Estuary and found that the dolphins predominantly used the shipping channel and part of the canal zone, through which passes a large amount of boat traffic. In the present study, the bottlenose dolphin whistles from the Sado Estuary had significantly higher frequency variables than the whistles from the other areas (Figure 3.1). The reason for the notable difference in the whistle characteristics between the whistles from the different populations may thus be that the dolphins in the Sado Estuary are more exposed to boat noise, which has caused them to adapt acoustically to an environment of higher noise levels in order to avoid masking effects. Therefore, the difference in the frequency characteristics of the whistles between populations could be due to significant differences in exposure to boat traffic and vessel noises.

High noise levels from boats or other anthropogenic sources such as military sonar, seismic surveys, and pile driving could not only cause masking of acoustic behaviour of marine mammals, but could also cause temporary threshold shifts in their hearing. Temporary threshold shifts have been observed experimentally in bottlenose dolphins in several studies (Nachtigall *et al.*, 2004; Finneran *et al.*, 2005). Finneran *et al.* (2005) looked at the effects of mid- frequency naval sonar on the hearing thresholds of bottlenose dolphins and found that they caused temporary threshold shifts in the study animals. This suggests that dolphins inhabiting waters with high noise levels could, over time, even exhibit permanent threshold shifts in their hearing. It seems likely that with permanent threshold shifts, dolphins would alter whistle frequencies to reflect their altered hearing ranges. As the dolphins from this study exist in locations that have varying levels of background noise, the whistle variation observed here could be due to temporary or permanent threshold shifts in the hearing of dolphins, causing alterations in their whistle frequency and intensity variables to avoid masking effects.

It has previously been suggested that environmental factors such as depth and salinity may have an effect on the way sound is able to travel through water (Quick *et al.*, 2008). It is also possible that differences in depth and heterogeneity of the marine environments between populations could influence the way in which sound travels through the water, and therefore the ways in which the dolphins in each area could be affected by the levels of background noise. For example, the Shannon Estuary and the Sado Estuary are relatively narrow and shallow (Englund *et al.*, 2007; dos Santos *et al.*, 2007), with steep sides occurring in the Shannon Estuary (Englund *et al.*, 2007). These features would perhaps amplify the levels of exposure that the animals in these areas experience. Conversely, although shallow, the Molène Archipelago and Cardigan Bay are relatively large and open areas (Le Duff *et al.*, 1999; Gregory and Rowden, 2001), such that any sound emitted under water would be less likely to reflect. As such, it is less probable that the bottlenose dolphins existing in Cardigan Bay or the Molène Archipelago would be heavily impacted by boat traffic. Similarly to the Sado Estuary, the Shannon Estuary is host to a heavy shipping route, with ten million tonnes of boat traffic passing through it each year (Englund *et al.*, 2007). If background noise levels were the only factor contributing to geographical differences in whistle characteristics, it is probable that the whistles from the Shannon Estuary and the Sado Estuary would show similar differences from the whistles from Cardigan Bay and the Molène Archipelago. Although the whistles from the Shannon Estuary had some significantly higher frequency variables than the whistles from Cardigan Bay and the Molène Archipelago, they were not as great as the observed differences between the frequency variables from the Sado Estuary and the Welsh and French populations. However, it is possible that the amount of boat traffic and therefore background noise is much higher in the Sado Estuary than in the Shannon Estuary. Nevertheless, it remains possible that additional factors may contribute to the geographical variation observed in this investigation.

For instance, it has been found that the bottlenose dolphin population residing in the Shannon Estuary is genetically isolated (Mirimin *et al.*, 2011). Mirimin *et al.* (2011) observed that bottlenose dolphins that were resident to the Shannon Estuary were very infrequently observed to venture away from the estuary. It is therefore unlikely that the dolphins from the Shannon Estuary would mix with other groups of dolphins, and improbable that they would learn and acquire whistle characteristics from other groups of dolphins. Perhaps it is for this reason that their whistles were different from the whistles of the dolphins in Cardigan Bay, the Molène Archipelago and the Sado Estuary. The population from the Sado Estuary has also been suggested to be genetically isolated, and it could be this factor that creates the distinct geographical differences in the whistle structure of the bottlenose dolphin populations studied here. Furthermore, it has been suggested that genetic distinction and isolation may sometimes be related to variation in vocal behaviour between dolphins (Bazua-Duran and Au, 2004; Morisaka *et al.*, 2005b; Baron *et al.*, 2008), such that this could be a contributing factor to any existing variation between the four populations. The genetic isolation of the Shannon Estuary could therefore be a contributing factor the geographic differences in the frequency variables between the dolphins from the Shannon Estuary and Cardigan Bay and the Molène Archipelago.

Furthermore, the level of residency or openness of the populations could contribute to the observed variations. Other studies have suggested that variation between whistles may be greater between populations that exist further apart. For example, Wang *et al.* (1995) compared the structure of bottlenose dolphin whistles between five different regions, including the Gulf coast of Texas (within which three regions were observed), Argentina, the Gulf of California, Japan and Australia. It was found that differences in whistle structure were greater between dolphin populations that were located further apart, whilst whistle structures of dolphin populations that were located closer to one another were quite similar in strucure. It was proposed that any similarities were due to movements between different populations of dolphins, such that dolphins belonging to one population would hear whistles from dolphins belonging to another population, and due to their ability to imitate sounds (Janik, 2000), they might retain some aspect of the foreign whistle and introduce it into their own population. It was further suggested that the significant differences in the whistle structure of the five different populations of bottlenose dolphin was due to their geographic isolation from one another.

The bottlenose dolphin population at Cardigan Bay has been observed to be semi-resident, such that they reside in the bay mostly during the summer months, between April and October, and migrate further north during the winter (Pesante *et al.*, 2008; Feingold *et al.*, 2011; Veneruso and Evans, 2012). The type of residency as well as the size of the populations could be a contributing factor to the geographical variation in whistle characteristics. It is possible that during their travels, this population might encounter groups of dolphins that are not part of their population. Similarly, the bottlenose dolphins residing in the Molène Archipelago exist in close proximity with another group of bottlenose dolphins, which reside further south at the Ile de Seine, as well as a large bottlenose dolphin population in Normandy and the Channel Islands. It is therefore highly likely that they would also mix with these other groups of dolphins, thus perhaps acquiring a greater range of whistle characteristics. In contrast, the bottlenose dolphins inhabiting the Shannon and Sado Estuaries are isolated populations, inhabiting these areas year round (Englund *et al.*, 2007; dos Santos *et al.*, 2007). Furthermore, the Sado Estuary population of bottlenose dolphins is one of the smallest resident populations of bottlenose dolphins (dos Santos *et al.*, 2007). The population currently consists of approximately 25 individuals, and has been observed to be in decline (dos Santos and Almada, 2004). It would thus be expected that the whistles within the Sado Estuary in particular, but also the Shannon Estuary would be less variable than in other areas. Although the findings from this study suggest that they exhibit similar amounts of within-population variation as the other two populations, it is apparent from Figure 3.2 that the variation in whistle characteristics from the populations inhabiting the Shannon Estuary and the Sado Estuary is generally greater than the variation from the whistles of dolphins from Cardigan Bay and the Molène

Archipelago. Perhaps with larger sample sizes, a difference in the within-population variation would become apparent between the four different populations of bottlenose dolphins studied here.

Although the differences between Cardigan Bay, the Shannon Estuary and the Molène Archipelago were less obvious than the differences between the Sado Estuary and the other three areas, they did exist nonetheless. Most notable was that the whistles from the Shannon Estuary had the highest maximum frequency, peak frequency, and frequency range (Figure 3.1). However, the whistles from the three areas also shared some similarities in whistle characteristics. The Shannon Estuary and Cardigan Bay had similar end frequencies and durations, whilst the Shannon Estuary and the Molène Archipelago had a similar number of inflection points. The Molène Archipelago had the lowest end frequencies, maximum frequencies, duration and frequency range out of the three populations. An explanation for the differences between the three populations could be differences in body size. Perhaps the dolphins in the Molène Archipelago are slightly smaller in body size, thus being more limited in their frequency range, though it is also possible that they are not so affected by high levels of background noise, such that they do not need to whistle below or above certain frequencies.

Previous studies have also considered body size to be a contributing factor in whistle variation between populations. For instance, May-Collado *et al.* (2007) looked at the ways in which body size might be related to the frequency of whistles that cetaceans are able to emit, and found that variations in the minimum frequency of whistles were negatively correlated with variations in body size of the animal emitting the whistle. This suggests that the animals had evolved to emit lower frequency sounds, but that lower frequency sounds were limited by the body size of the animals. The differences in the minimum frequencies between the bottlenose dolphins from the four populations may therefore possibly be partially attributed to differences in the body sizes between populations. Perhaps the reason for the significantly higher mean minimum and maximum frequency variables in the Sado Estuary (Figure 3.1) is that dolphins are significantly different in size, thus limiting their minimum frequency, as suggested by May-Collado *et al.* (2007), whilst maximum frequency may be determined by the significantly higher level of boat traffic in the area.

Other factors may also have contributed to the observed geographical variation. For example, the group sizes of the dolphins in each area could have differed, causing different vocal behaviour at the time of recording. It has previously been found that vocal production increases with group size in dolphins (Jones and Sayigh, 2002), and that as groups become more spread out, the rate of whistle production increases (Quick and Janik, 2008). It seems likely that dolphins would exhibit different vocal behaviour during different activities. For example, if groups were spread out, it is likely that whistle production would be dominated by signature whistles for the purpose of group cohesion. Therefore, at the time of recording, dolphins in each of the four areas may have been behaving differently, thus perhaps also causing variation in their vocal behaviour. Ansmann *et al.* (2007) suggest that a combination of group size and ambient noise levels in a particular area are the most likely causes of variations in dolphin whistles. They studied and compared the vocalisations of different populations of short beaked common dolphins (*Delphinus delphis*) in two different geographical areas, and discovered that both group size and different behaviours contributed to geographical variations in whistle characteristics. However, it was also suggested that other factors would most likely contribute to variations in addition to those mentioned, and they suggested that the different areas studied could have been used for different purposes, such as breeding or feeding. Nevertheless, it seems that group size and composition of dolphins from all areas may be an important factor to take into consideration when comparing whistle characteristics geographically in future studies, as these factors may influence the vocal behaviour and therefore whistle variation of dolphins.

4.3 Variation Within Cardigan Bay

Within Cardigan Bay, there were correlations between whistle type and behaviour of the dolphins (Figure 3.3), such that upsweeps were most frequently used during feeding. Certain whistle types have previously been associated with different behaviour types. For example, Janik and Slater (1998) suggested that upsweeps occurred most often when bottlenose dolphins were travelling, which disagrees with the findings from this investigation, where it was found that upsweeps were more predominant during feeding than during travelling (Figure 3.3). However, this could be due to more vocalisations occurring during feeding than during travelling in the Cardigan Bay recordings. In addition, it was possible to record more whistles whilst dolphins were feeding as they did not tend to move as much as when they were travelling. Therefore, this correlation could be skewed due to the fact that more recordings were made of feeding dolphins than of travelling dolphins.

However, it is also likely that group size has an effect on whistle type, as Janik and Slater (1998) found that captive bottlenose dolphins emitted signature whistles more frequently when the group was separated, and produced more non-signature whistles when the group was re-united. In this study, there was a correlation between group size and whistle type, which indicated that upsweeps and downsweeps were most common in smaller group sizes, and that whistles containing inflections (sinusoidal, convex, concave) were more common in larger groups of dolphins (Figure 3.5). Thus upsweeps and downsweeps may be used more by dolphins when travelling or feeding in smaller groups, whilst more complex whistles have other purposes such as group cohesion and socialising. More investigation into the uses of different whistles for different activities in bottlenose dolphins should be undertaken, as this would provide a better understanding of the ways in which a variety of whistles are used.

This investigation further demonstrated that there was a correlation between behaviour and group size, with feeding occurring most frequently in smaller sized groups, and travelling occurring most frequently in larger groups (Figure 3.4). This finding is counter-intuitive, as it has previously been observed that group sizes, as well as whistle production, tend to increase during feeding events (Acevedo-Gutiérrez and Stienessen, 2004).

4.4 Limitations

Although significant differences were found between the four populations of bottlenose dolphins studied, it is necessary to consider some of the limitations that could have influenced the results. Firstly, whereas the method of recording was similar in Cardigan Bay, the Shannon Estuary and the Sado Estuary, with hydrophones being deployed from boats, in the Molène Archipelago, recordings were made continuously by using a bottom-moored recorder. If dolphins vocalise differently in the presence of vessels, then the recordings from Cardigan Bay, the Shannon Estuary and the Sado Estuary could consist of a different repertoire of whistles than those from the Molène Archipelago, where the effects of vessel presence were partially controlled for. However, as the most notable difference occurred between the whistles from the Sado Estuary and the other three locations, it seems unlikely that this difference in recording would have greatly influenced the results.

A second potential confounding factor is that recordings were used from different years at different times. The whistles from Cardigan Bay were most recent, being collected between May and July of 2012, whilst the whistles from the Sado Estuary were oldest, having been collected in 2001 and 2002. Perhaps the whistles of bottlenose dolphins from the Sado Estuary were different in 2001 and 2002 than to the present time. It is possible that the whistles of dolphins change naturally over time due to their abilities for mimicry and learning in fission fusion societies, as has been proposed by Morisaka *et al.* (2005a). In order to truly compare whistle characteristics between different populations of bottlenose dolphin, it seems that it would be useful to use recordings that have been made within a few years of each other, or ideally within the same year. Nevertheless, it is possible that the whistles of bottlenose dolphins do not change much over time, such that this limitation may be negligible.

The small sample size of thirty whistles per population used for the analysis in this investigation should be taken into consideration. However, it seems that a small sample size would be more likely to cause results to be non-significant. In this case, there was a significant difference in the whistle characteristics between the four populations, such that the results from this study could actually underestimate apparent existing geographic variation. The difference between the dolphin population in the Sado Estuary and the dolphin populations in the other three areas should nevertheless be further investigated with greater sample sizes in order to discover more about the differences between the populations. In future studies it would be wise to take behaviour, group size and group composition into account in all study areas, in order to be able to determine more conclusively the reasons for geographic variation between whistle characteristics.

4.5 Conclusion

This study has shown that geographical variation exists between the whistle characteristics of bottlenose dolphins from Cardigan Bay, the Shannon Estuary, the Molène Archipelago and the Sado Estuary, supporting the predictions. Most notably, the Sado Estuary population of bottlenose dolphins is seemingly very different acoustically from the populations of Cardigan Bay, the Shannon Estuary and the Molène Archipelago. Whilst it could be a combination of the variables discussed above that affect the variation in the whistle characteristics between the four populations, it seems most likely that it is the significantly higher level of background noise in the Sado Estuary that has caused such marked differences in whistle characteristics. If this is the case, it would confirm that dolphins do in fact adapt their whistle characteristics in order to avoid the masking effects of high levels of background noise. Future studies looking at geographic whistle variation between these four locations should therefore attempt to determine the reasons for the variation more conclusively. In particular, finding out more about the differences in levels of anthropogenic background noise could provide important information about the ways in which bottlenose dolphins adapt to their surrounding environment. Although more research is needed in order to be able to determine the reasons behind this acoustic diversity between populations, the results from this study give indication that bottlenose dolphins have evolved to have different whistle characteristics depending on geographic location, most likely through adapting acoustically to their environment. The variations depicted in this study could also reflect different cultures of bottlenose dolphins.

This study has therefore made it clear that there are still many factors to be taken into consideration when comparing whistle characteristics of bottlenose dolphins geographically, and it is apparent that much more research should be done in this subject to better understand the way in which dolphins use sound. It seems that further research should also be undertaken to understand the reasons for variation within populations, as this would further our understanding of the vocal behaviour of dolphins. Through this understanding, it would be possible to better comprehend the effects that anthropogenic sound sources may have on bottlenose dolphin populations, which is important with the increasing amounts of anthropogenic disturbances in the oceans.

References

Acevedo-Gutiérrez, A. and Stienessen, S. (2004). Bottlenose dolphins (*Tursiops truncatus*) increase number of whistles when feeding. *Aquatic Mammals*, **30**(3):357–362. 44

Ansmann, I., Goold, J., Evans, P., Simmonds, M., and Keith, S. (2007). Variation in the whistle characteristics of short-beaked common dolphins, *Delphinus delphis*, at two locations around the British Isles. *Journal of the Marine Biological Association of the UK*, **87**:19–26. 1, 4, 5, 21, 43

Au, W., Floyd, R., and Haun, J. (1978). Propagation of Atlantic bottlenose dolphin echolocation signals. *The Journal of the Acoustical Society of America*, **64**:411–422. 1

Azevedo, A., Oliveira, A., Rosa, L., and Lailson-Brito, J. (2007). Characteristics of whistles from resident bottlenose dolphins (*Tursiops truncatus*) in southern Brazil. *The Journal of the Acoustical Society of America*, **121**(5):2978–2983. 2, 21, 36

Baron, S., Martinez, A., Garrison, L., and Keith, E. (2008). Differences in acoustic signals from Delphinids in the western North Atlantic and northern Gulf of Mexico. *Marine Mammal Science*, **24**(1):42–56. 4, 5, 37, 40

Bazua-Duran, C. and Au, W. (2004). Geographic variations in the whistles of spinner dolphins (*Stenella longirostris*) of the Main Hawai ian Islands. *The Journal of the Acoustical Society of America*, **116**(6):3757–3769. 4, 21, 40

Bazua-Duran, C. (2004). Differences in the whistle characteristics and repertoire of Bottlenose and Spinner Dolphins. *Annuals of the Brazilian Academy of Sciences*, **76**(2):386–392. 4

Blomqvist, C. and Amundin, M. (2004). High-frequency burst-pulse sounds in agonistic/aggressive interactions in bottlenose dolphins, *Tursiops truncatus*. In *Echolocation in bats and dolphins*, pages 425–431. (Thomas, J., Moss, C. and Vater, M., Eds.), Chicago, Ill.: University of Chicago Press. 1

Buckstaff, K. (2004). Effects of watercraft noise on the acoustic behavior of bottlenose dolphins, *Tursiops truncatus*, in Sarasota Bay, Florida. *Marine Mammal Science*, **20**(4):709–725. 5, 7, 37

Caldwell, M. and Caldwell, D. (1965). Individualized whistle contours in bottle-nosed dolphins (*Tursiops truncatus*). *Nature*, **207**:529–531. 3

Caldwell, M., Caldwell, D., and Hall, N. (1969). An experimental demonstration of the ability of an Atlantic bottlenosed dolphin to discriminate between whistles of other individuals of the same species. *Los Angeles Museum of Natural History Foundation*, **6**:67–74. 3

Ceredigion County Council, Countryside Council for Wales, Environment Agency Wales, North Western and North Wales Sea Fisheries Committee, Pembrokeshire Coast National Park Authority, Pembrokeshire County Council, and D'r Cymru Welsh Water (2001). *Cardigan Bay Special Area of Conservation Management Plan* 190pp. 13

Connor, R., Wells, R., Mann, J., and Read, A. (2000). The Bottlenose Dolphin: Social Relationships in a Fission-Fusion Society. In *Cetacean Societies: Field Studies of Dolphins and Whales*, pages 91-126. (Mann, J., Connor, R.C., Tyack, P.L., Whitehead, H., Eds.). University of Chicago Press, Chicago and London. 3

Cook, M., Sayigh, L., Blum, J., and Wells, R. (2004). Signature-whistle production in undisturbed free-ranging bottlenose dolphins (*Tursiops truncatus*). *Proceedings of the Royal Society: Biological Sciences*, **271**:1043–1049. 3, 5, 35

dos Santos, M. and Almada, V. (2004). A case for passive sonar: Analysis of click train production patterns by bottlenose dolphins in a turbid estuary. In *Echolocation in Bats and Dolphins*, pages 400–403. (Thomas, J., Moss, C. and Vater, M., Eds.), Chicago, Ill.:University of Chicago Press. 41

dos Santos, M., Louro, S., Couchinho, M., and Brito, C. (2005). Whistles of bottlenose dolphins (*Tursiops truncatus*) in the Sado Estuary, Portugal: characteristics, production rates, and long-term contour stability. *Aquatic Mammals*, **31**(4):453–462. 1, 8, 12

dos Santos, M., Coniglione, C., and Louro, S. (2007). Feeding behaviour of the bottlenose dolphin, *Tursiops truncatus* (Montagu, 1821) in the Sado estuary, Portugal, and a review of its prey species. *Zoociências*, **9**(1):31–39. 15, 39, 41

Englund, A., Ingram, S., and Rogan, E. (2007). Population status report for bottlenose dolphins using the Lower River Shannon SAC, 2006-2007. Technical report. 9, 12, 39, 41

Englund, A., Ingram, S., and Rogan, E. (2008). An updated population status report for bottlenose dolphins using the lower River Shannon SAC in 2008. Technical report. 12

Feingold, D., Baines, M., and Evans, P. (2011). Cardigan Bay bottlenose dolphin social and population structure: findings from a ten-year photo ID dataset. In *25th European Cetacean Society Conference.* 41

Finneran, J., Carder, D., Schlundt, C., and Ridgway, S. (2005). Temporary threshold shift in bottlenose dolphins (*Tursiops truncatus*) exposed to mid-frequency tones. *The Journal of the Acoustical Society of America*, **118**(4):2696–2705. 39

Foley, A. and McGrath, D. (2010). Social Structure Within the Bottlenose Dolphin (*Tursiops truncatus*) Population in the Shannon Estuary, Ireland. *Aquatic Mammals*, **36**:372–381. 9, 14

Frere, C., Krutzen, M., Mann, J., Connor, R., Bejder, L., and Sherwin, W. (2010). Social and genetic interactions drive fitness variation in a free-living dolphin population. *Proceedings of the National Academy of Sciences*, **107**(46):19949–19954. 5

Genov, T., Wiemann, A., and Fortunam, C. (2009). Towards identification of the bottlenose dolphin (*Tursiops truncatus*) population structure in the North-eastern Adriatic Sea: preliminary results. *Varstvo narave*, **22**:73–80. 14

Gregory, P. and Rowden, A. (2001). Behaviour patterns of bottlenose dolphins (*Tursiops truncatus*) relative to tidal state, time-of-day, and boat traffic in Cardigan Bay, West Wales. *Aquatic Mammals*, **27**(2):105–113. 9, 13, 39

Harzen, S. (1998). Habitat use by the bottlenosed dolphin (*Tursiops truncatus*) in the Sado Estuary, Portugal. *Aquatic Mammals*, **24**(3):117–128. 9, 15, 38

Hayter, A. (1986). The Maximum Familywise Error Rate of Fisher's Least Significant Difference Test. *Journal of the American Statistical Association*, **81**(396):1000–1004. 23

Herzing, D. and dos Santos, M. (2004). Functional aspects of echolocation in dolphins. *Echolocation in bats and dolphins. Chicago:*, **53**(386-393). 1

Herzing, D. (1996). Vocalisations and associated underwater behavior of free-ranging Atlantic spotted dolphins, Stenella frontalis and bottlenose dolphins, *Tursiops truncatus*. *Aquatic Mammals*, **22**(2):61–79. 1, 2, 3

Janik, V. and Slater, P. (1998). Context-specific use suggests that bottlenose dolphin signature whistles are cohesion calls. *Animal Behaviour*, **56**(4):829–838. 1, 36, 37, 43, 44

Janik, V., Sayigh, L., and Wells, R. (2006). Signature whistle shape conveys identity information to bottlenose dolphins. *Proceedings of the National Academy of Sciences*, **103**(21):8293–8297. 3

Janik, V. (2000). Whistle matching in wild bottlenose dolphins (*Tursiops truncatus*). *Science*, **289**:1355–1357. 1, 3, 5, 41

Jaud, V., Iorio, L. D., Gervaise, C., and Chauvaud, L. (2011). Analysis of long term measurements of very shallow water sounds, a perspective for coastal monitoring. *UAM 2011.* 14

Jones, G. and Sayigh, L. (2002). Geographic variation in rates of vocal production of free-ranging bottlenose dolphins. *Marine Mammal Science*, **18**(2):374–393. 2, 4, 5, 6, 7, 43

Kruskal, W. and Wallis, W. (1952). Use of ranks in one-criterion variance analysis. *Journal of the American Statistical Association*, **47**(260):583–621. 23

Le Duff, M., Hily, C., and Glemarec, M. (1999). Environement Naturel de l'Iroise, Bilan des Connaissances et Intérêt Patrimonial. **2**. 9, 12, 14, 39

Lemon, M., Lynch, T., Cato, D., and Harcourt, R. (2006). Response of travelling bottlenose dolphins (*Tursiops aduncus*) to experimental approaches by a powerboat in Jervis Bay, New South Wales, Australia. *Biological Conservation*, **127**:363–372. 8

Levene, H. (1960). *Contributions to Probability and Statistics: Essays in Honor of Harold Hotelling.* (Olkin, I., Ed.), Stanford University Press. 23

Lima, I., Andrade, L., Carvalho, R., Lailson-Brito, J., and Azevedo, A. (2012). Characteristics of whistles from rough-toothed dolphins (*Steno bredanensis*) in Rio de Janeiro coast, southeastern Brazil. *The Journal of the Acoustical Society of America*, **131**(5):4173–4181. 2, 35

Lohrengel, K., Veneruso, G., Feingold, D., Pesante, G., Ugarte, F., and Evans, P. G. H. (2012). Boat traffic trends and effects on bottlenose dolphin sighting rates in Cardigan Bay, Wales. In *26th European Cetacean Society Annual Conference, Galway, Ireland. (proceedings published).* 13

López, B. D. (2011). Whistle characteristics in free-ranging bottlenose dolphins (*Tursiops truncatus*) in the Mediterranean Sea: Influence of behaviour. *Mammalian Biology-Zeitschrift für Säugetierkunde*, **76**:180–189. 3, 21, 35, 36, 37

May-Collado, L. and Wartzok, D. (2008). A comparison of bottlenose dolphin whistles in the Atlantic Ocean: factors promoting whistle variation. *Journal of Mammalogy*, **89**(5):1229–1240. 1, 2, 3, 4, 5, 7, 36, 37

May-Collado, L., Agnarsson, I., and Wartzok, D. (2007). Reexamining the relationship between body size and tonal signals frequency in whales: a comparative phylogenetic approach. *Marine Mammal Science*, **23**:524–552. 7, 36, 42

McCowan, D., Reiss, D., and Gubbins, C. (1998). Social familiarity influences whistle acoustic structure in adult female bottlenosed dolphins (*Tursiops truncatus*). *Aquatic Mammals*, **24**(1):27–40. 8

Mirimin, L., Miller, R., Dillane, E., Berrow, S., Ingram, S., Cross, T., and Rogan, E. (2011). Fine-scale population genetic structuring of bottlenose dolphins in Irish coastal waters. *Animal Conservation*, **14**(4):342–353. 12, 40

Morisaka, T., Shinohara, M., Nakahara, F., and Akamatsu, T. (2005a). Effects of ambient noise on the whistles of Indo-Pacific bottlenose dolphin populations. *Journal of Mammology*, **86**(3):541–546. 5, 7, 8, 45

Morisaka, T., Shinohara, M., Nakahara, F., and Akamatsu, T. (2005b). Geographic variations in the whistles among three Indo-Pacific bottlenose dolphin (*Tursiops aduncus*) populations in Japan. *Fisheries Science*, **71**:568–576. 1, 4, 8, 9, 36, 37, 40

Nachtigall, P., Supin, A., Pawloski, J., and Whitlow, W. (2004). Temporary threshold shifts after noise exposure in the bottlenose dolphin (*Tursiops truncatus*) measured using evoked auditory potentials. *Marine Mammal Science*, **20**(4):673–687. 39

Nakahara, F. and Miyazaki, N. (2011). Vocal exchanges of signature whistles in bottlenose dolphins (*Tursiops truncatus*). *Journal of Ethology*, **29**:309–320. 3

Parsons, K., Noble, L., Reid, R., and Thompson, P. (2002). Diversity and population structuring of UK bottlenose dolphins (*Tursiops truncatus*): Is the NE Scotland population demographically and geographically isolated? *Biological Conservation*, **108**:175–182. 4, 5, 6

Perrin, W., Würsig, B., and Thewissen, J. (2008). *Encyclopedia of marine mammals*. London: Elsevier Inc. 1, 2

Pesante, G., Evans, P., Baines, M., and McMath, M. (2008). *Abundance and Life History Parameters of Bottlenose Dolphin in Cardigan Bay: Monitoring 2005-2007*. CCW Marine Monitoring Report No. 61, 1-75. 41

Petrella, V., Martinez, E., Anderson, M., and Stockin, K. (2012). Whistle characteristics of common dolphins (*Delphinus* sp.) in the Hauraki Gulf, New Zealand. *Marine Mammal Science*, **28**(3):479–496. 21, 36, 37

Pichler, F., Dawson, S., Slooten, E., and Baker, C. (1998). Geographic isolation of Hector's dolphin populations described by mitochondrial DNA sequences. *Conservation Biology*, **12**(3):676–682. 5, 6

Quick, N. and Janik, V. (2008). Whistle rates of wild bottlenose dolphins (*Tursiops truncatus*): Influences of group size and behavior. *Journal of Comparative Psychology*, **122**(3):305–311. 5, 7, 43

Quick, N., Rendell, L., and Janik, V. (2008). A mobile acoustic localization system for the study of free-ranging dolphins during focal follows. *Marine Mammal Science*, **24**(4):979–989. 39

Rendell, L., Matthews, J., Gill, A., Gordon, J., and Macdonald, D. (1999). Quantitative analysis of tonal calls from five odontocete species, examining interspecific and intraspecific variation. *Journal of Zoology, London*, **249**:403–410. 2, 4, 21

Reynolds, J.E., W. R. E. S. (2000). *The Bottlenose Dolphin. Biology and Conservation.* University Press of Florida, Gainesville. 3

Rogan, E., Ingram, S., Holmes, B., and O'Flanagan, C. (2000). A survey of bottlenose dolphins (*Tursiops truncatus*) in the Shannon estuary. *Marine Institute Resource Series*, **9**:46. 14

Rossi-Santos, M. and Podos, J. (2006). Latitudinal variation in whistle structure of the estuarine dolphin, *Sotalia guianensis*. *Behaviour*, **143**:347–364. 1, 3, 4, 5, 37

Sayigh, L. (1992). Development and functions of signature whistles of free-ranging bottlenose dolphins, *Tursiops truncatus*. *PhD Thesis at Woods Hole Oceanographic Institution.* 3, 36, 37

Smolker, R. and Pepper, J. (1999). Whistle convergence among allied male bottlenose dolphins (Delphinidae, *Tursiops* sp.). *Ethology*, **105**:595–617. 2

Smolker, R., Mann, J., and Smuts, B. (1993). Use of signature whistles during separations and reunions by wild bottlenose dolphin mothers and infants. *Behavioral Ecology and Sociobiology*, **33**:393–402. 2, 3

Steiner, W. (1981). Species-specific differences in pure tonal whistle vocalizations of five western North Atlantic dolphin species. *Behavioral Ecology and Sociobiology*, **9**:241–246. 4, 5, 6

Tyack, P. (1986). Population biology, social behaviour and communication in whales and dolphins. *Trends in Ecology and Evolution*, **1**:144–150. 3

Tyack, P. (1997). Development and social functions of signature mistles in bottlenose dolphins, *Tursiops truncatus. Bioacoustics: The International Journal of Animal Sound and its Recording*, **8**:21–46. 2, 3

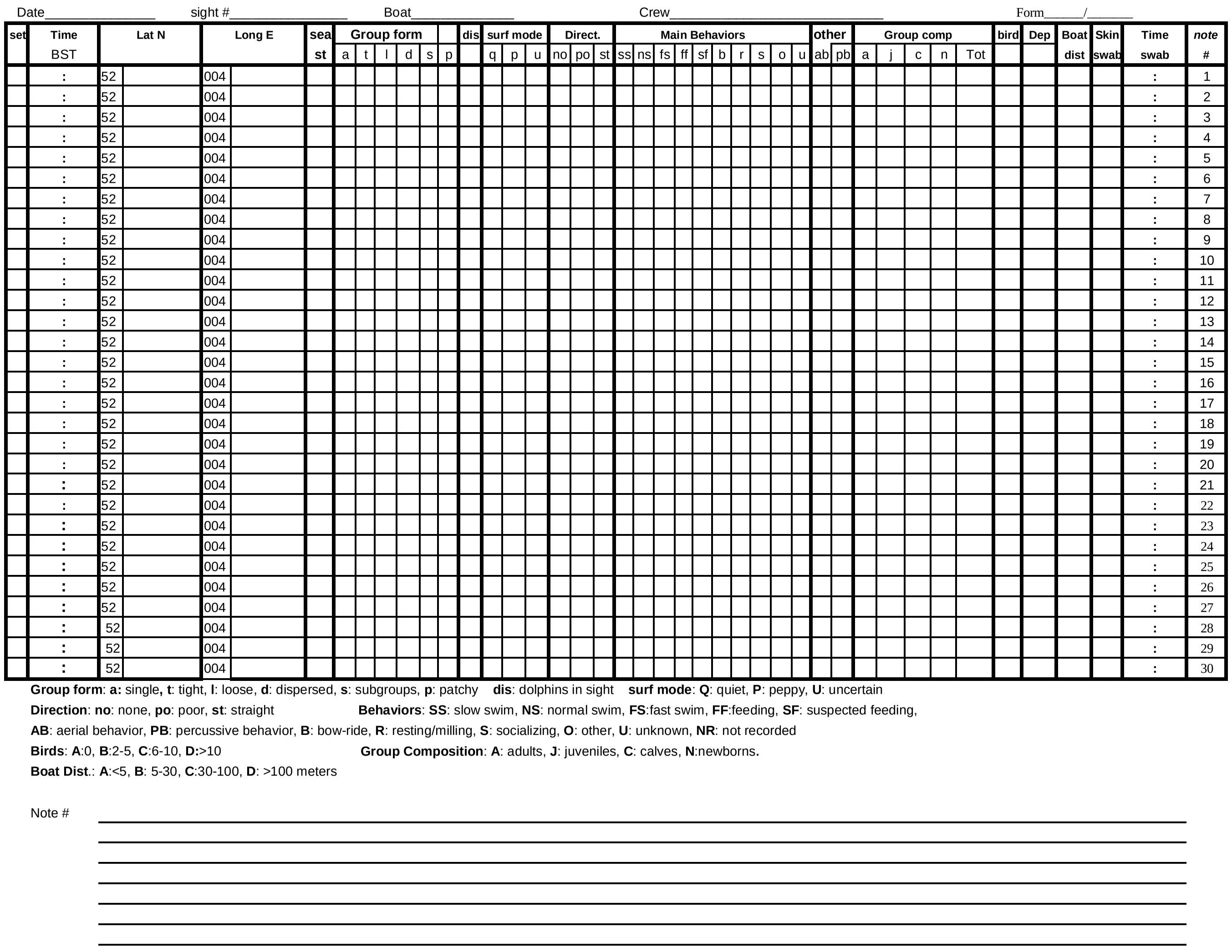
Tyson, R., Nowacek, S., and Nowacek, D. (2011). Community structure and abundance of bottlenose dolphins, *Tursiops truncatus*, in coastal waters of the northeast Gulf of Mexico. *Marine Ecology Progress Series*, **438**:253–265. 5

Veneruso, G. and Evans, P. (2012). *Bottlenose dolphin and harbour porpoise monitoring in Cardigan Bay and Pen Llyn a'r Sarnau Special Areas of Conservation.* CCW Monitoring Report No. 95, 65pp. 9, 12, 13, 41

Wang, D., Wursig, B., and Evans, W. (1995). Whistles of bottlenose dolphins: comparisons among populations. *Aquatic Mammals*, **21**(1):65–77. 1, 2, 4, 5, 6, 9, 21, 36, 37, 40

Wells, R. and Scott, M. (2002). Bottlenose dolphins (*Tursiops truncatus* and *T. aduncus*). In *Encyclopedia of Marine Mammals*, pages 122–128. 2

A | Methods



Date_______________ sight #________________ Boat______________ Crew_____________________________ Form______/_______

set	Time	Lat N	Long E	sea	Group form						dis	surf mode			Direct.			Main Behaviors										other		Group comp					bird	Dep	Boat	Skin	Time	*note*
	BST			st	a	t	l	d	s	p		q	p	u	no	po	st	ss	ns	fs	ff	sf	b	r	s	o	u	ab	pb	a	j	c	n	Tot			dist	swab	swab	#
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Group form: **a:** single**, t**: tight, **l**: loose, **d**: dispersed, **s**: subgroups, **p**: patchy **dis**: dolphins in sight **surf mode**: **Q**: quiet, **P**: peppy, **U**: uncertain

Direction: **no**: none, **po**: poor, **st**: straight **Behaviors**: **SS**: slow swim, **NS**: normal swim, **FS**:fast swim, **FF**:feeding, **SF**: suspected feeding,

AB: aerial behavior, **PB**: percussive behavior, **B**: bow-ride, **R**: resting/milling, **S**: socializing, **O**: other, **U**: unknown, **NR**: not recorded

Birds: **A**:0, **B**:2-5, **C**:6-10, **D:**>10 **Group Composition**: **A**: adults, **J**: juveniles, **C**: calves, **N**:newborns**.**

Boat Dist.: **A**:<5, **B**: 5-30, **C**:30-100, **D**: >100 meters

Note #

Figure A.1: Standardised behaviour form, used for recording dolphin behaviour at three minute intervals during hydrophone deployment

B | Results

Table B.1: The *Post-Hoc* Mann-Whitney U test results to show the ways in which the whistle characteristics from each of the four populations (Cardigan Bay, the Shannon Estuary, the Molène Archipelago, the Sado Estuary) of bottlenose dolphins (*Tursiops truncatus*) differ from each other

Whistle Characteristic			**Mann Whitney U**	**Z**	***P***
Beginning Frequency	Cardigan Bay	Shannon Estuary	114.000	-4.968	0.000
		Molene Archipelago	69.000	-5.633	0.000
		Sado Estuary	53.000	-5.870	0.000
	Shannon Estuary	Molene Archipelago	395.000	-0.813	0.416
		Sado Estuary	253.000	-2.913	0.004
		Cardigan Bay	114.000	-4.968	0.000
	Molene Archipelago	Sado Estuary	198.000	-3.726	0.000
		Cardigan Bay	69.000	-5.633	0.000
		Shannon Estuary	395.000	-0.813	0.416
	Sado Estuary	Cardigan Bay	53.000	-5.870	0.000
		Shannon Estuary	253.000	-2.913	0.004
		Molene Archipelago	198.000	-3.726	0.000
End Frequency	Cardigan Bay	Shannon Estuary	446.500	-0.052	0.959
		Molene Archipelago	261.000	-2.794	0.005
		Sado Estuary	152.000	-4.406	0.000
	Shannon Estuary	Molene Archipelago	259.000	-2.824	0.005
		Sado Estuary	161.500	-4.265	0.000
		Cardigan Bay	446.500	-0.052	0.959
	Molene Archipelago	Sado Estuary	44.000	-6.002	0.000
		Cardigan Bay	261.000	-2.794	0.005
		Shannon Estuary	259.000	-2.824	0.005
	Sado Estuary	Cardigan Bay	152.000	-4.406	0.000
		Shannon Estuary	161.500	-4.265	0.000
		Molene Archipelago	44.000	-6.002	0.000
Maximum Frequency	Cardigan Bay	Shannon Estuary	283.000	-2.469	0.014
		Molene Archipelago	112.000	-0.562	0.574
		Sado Estuary	31.000	-6.195	0.000
	Shannon Estuary	Molene Archipelago	225.000	-3.327	0.001
		Sado Estuary	63.000	-5.722	0.000

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Table B.1 – Continued

Whistle Characteristic			**Mann Whitney U**	**Z**	***P***
		Cardigan Bay	283.000	-2.469	0.014
	Molene Archipelago	Sado Estuary	30.000	-6.210	0.000
		Cardigan Bay	112.000	-0.562	0.574
		Shannon Estuary	225.000	-3.327	0.001
	Sado Estuary	Cardigan Bay	31.000	-6.195	0.000
		Shannon Estuary	63.000	-5.722	0.000
		Molene Archipelago	30.000	-6.210	0.000
Minimum Frequency	Cardigan Bay	Shannon Estuary	225.500	690.500	0.001
		Molene Archipelago	102.000	-5.145	0.000
		Sado Estuary	30.000	-6.210	0.000
	Shannon Estuary	Molene Archipelago	415.000	-0.517	0.605
		Sado Estuary	133.000	-4.687	0.000
		Cardigan Bay	225.500	690.500	0.001
	Molene Archipelago	Sado Estuary	48.000	-5.944	0.000
		Cardigan Bay	102.000	-5.145	0.000
		Shannon Estuary	415.000	-0.517	0.605
	Sado Estuary	Cardigan Bay	30.000	-6.210	0.000
		Shannon Estuary	133.000	-4.687	0.000
		Molene Archipelago	48.000	-5.944	0.000
Maximum Intensity	Cardigan Bay	Shannon Estuary	430.000	-0.296	0.767
		Molene Archipelago	414.000	-0.532	0.595
		Sado Estuary	18.000	-6.387	0.000
	Shannon Estuary	Molene Archipelago	416.000	-0.503	0.615
		Sado Estuary	25.000	-6.284	0.000
		Cardigan Bay	430.000	-0.296	0.767
	Molene Archipelago	Sado Estuary	28.000	-6.239	0.000
		Cardigan Bay	414.000	-0.532	0.595
		Shannon Estuary	416.000	-0.503	0.615
	Sado Estuary	Cardigan Bay	18.000	-6.387	0.000
		Shannon Estuary	25.000	-6.284	0.000
		Molene Archipelago	28.000	-6.239	0.000
Frequency	Cardigan Bay	Shannon Estuary	420.000	-0.444	0.657

Continued on Next Page…

Table B.1 – Continued

Whistle Characteristic			**Mann Whitney U**	**Z**	***P***
Range		Molene Archipelago	186.000	-3.903	0.000
		Sado Estuary	182.000	-3.962	0.000
	Shannon Estuary	Molene Archipelago	153.000	-4.391	0.000
		Sado Estuary	174.000	-4.081	0.000
		Cardigan Bay	420.000	-0.444	0.657
	Molene Archipelago	Sado Estuary	79.000	-5.485	0.000
		Cardigan Bay	186.000	-3.903	0.000
		Shannon Estuary	153.000	-4.391	0.000
	Sado Estuary	Cardigan Bay	182.000	-3.962	0.000
		Shannon Estuary	174.000	-4.081	0.000
		Molene Archipelago	79.000	-5.485	0.000

Table B.2: The *Post-Hoc* Mann-Whitney U test results to show the ways in which the whistle characteristics from each of the three populations (Cardigan Bay, the Shannon Estuary, the Molène Archipelago) of bottlenose dolphins (*Tursiops truncatus*) differ from each other

Whistle Characteristic			**Mann Whitney U**	**Z**	***P***
Beginning Frequency	Cardigan Bay	Shannon Estuary	8829.500	-5.092	0.000
		Molene Archipelago	6080.000	-8.353	0.000
	Shannon Estuary	Molene Archipelago	9634.500	-4.137	0.000
		Cardigan Bay	8829.500	-5.092	0.000
	Molene Archipelago	Cardigan Bay	6080.000	-8.353	0.000
		Shannon Estuary	9634.500	-4.137	0.000
End Frequency	Cardigan Bay	Shannon Estuary	12484.000	-0.757	0.449
		Molene Archipelago	7791.500	-6.323	0.000
	Shannon Estuary	Molene Archipelago	10047.000	-3.647	0.000
		Cardigan Bay	12484.000	-0.757	0.449
	Molene Archipelago	Cardigan Bay	7791.500	-6.323	0.000
		Shannon Estuary	10047.000	-3.647	0.000
Peak Frequency	Cardigan Bay	Shannon Estuary	9272.000	-4.567	0.000
		Molene Archipelago	11089.000	-2.412	0.016
	Shannon Estuary	Molene Archipelago	10834.000	-2.714	0.007
		Cardigan Bay	9272.000	-4.567	0.000
	Molene Archipelago	Cardigan Bay	11089.000	-2.412	0.016
		Shannon Estuary	10834.000	-2.714	0.007
Maximum Frequency	Cardigan Bay	Shannon Estuary	9156.500	-4.704	0.000
		Molene Archipelago	11167.000	-2.319	0.020
	Shannon Estuary	Molene Archipelago	7168.500	-7.062	0.000
		Cardigan Bay	9156.500	-4.704	0.000
	Molene Archipelago	Cardigan Bay	11167.000	-2.319	0.020
		Shannon Estuary	7168.500	-7.062	0.000
Minimum Frequency	Cardigan Bay	Shannon Estuary	10629.500	-2.957	0.000
		Molene Archipelago	7294.500	-6.912	0.000
	Shannon Estuary	Molene Archipelago	11194.000	-2.287	0.022
		Cardigan Bay	10629.500	-2.957	0.000
	Molene Archipelago	Cardigan Bay	7294.500	-6.912	0.000

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Table B.2 – Continued

Whistle Characteristic			**Mann Whitney U**	**Z**	***P***
		Shannon Estuary	11194.000	-2.287	0.022
Maximum Frequency	Cardigan Bay	Shannon Estuary	12291.000	-0.986	0.324
		Molene Archipelago	13008.500	-0.135	0.893
	Shannon Estuary	Molene Archipelago	11547.000	-1.868	0.062
		Cardigan Bay	12291.000	-0.986	0.324
	Molene Archipelago	Cardigan Bay	13008.500	-0.135	0.893
		Shannon Estuary	11547.000	-1.868	0.062
Frequency Range	Cardigan Bay	Shannon Estuary	11220.500	-2.255	0.024
		Molene Archipelago	7233.000	-6.985	0.000
	Shannon Estuary	Molene Archipelago	5386.000	-9.176	0.000
		Cardigan Bay	11220.500	-2.255	0.024
	Molene Archipelago	Cardigan Bay	7233.000	-6.985	0.000
		Shannon Estuary	5386.000	-9.176	0.000
Inflection Points	Cardigan Bay	Shannon Estuary	8332.500	-6.317	0.000
		Molene Archipelago	7666.500	-7.110	0.000
	Shannon Estuary	Molene Archipelago	13062.000	-0.075	0.940
		Cardigan Bay	8332.500	-6.317	0.000
	Molene Archipelago	Cardigan Bay	7666.500	-7.110	0.000
		Shannon Estuary	13062.000	-0.075	0.940
Duration	Cardigan Bay	Shannon Estuary	12516.500	-0.718	0.473
		Molene Archipelago	9356.000	-4.467	0.000
	Shannon Estuary	Molene Archipelago	10993.000	-2.525	0.012
		Cardigan Bay	12516.500	-0.718	0.473
	Molene Archipelago	Cardigan Bay	9356.000	-4.467	0.000
		Shannon Estuary	10993.000	-2.525	0.012