BOTTLENOSE DOLPHIN SIGNATURE WHISTLE VARIATION: A PERSPECTIVE ON PHYSICAL CHARACTERISTICS, LONG-TERM STABILITY, RELATEDNESS, AND DISTURBANCE EFFECTS

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Bottlenose dolphin signature whistle variation: A perspective on physical characteristics, long-term stability, relatedness, and disturbance effects

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ABSTRACT

Bottlenose dolphin signature whistles are individually specific, modulated tonal sounds that have been the focus of interest for studying the social acoustic repertoire in this species. However, there is still limited understanding about the factors that might drive variation in these signals. The dolphin community in Sarasota Bay is ideal for investigating the influence of interspecific and intraspecific characteristics as well as the social structure on signature whistles as it has been a long-term study for over 30 years. In this thesis, I investigated whether sex, age and size affected the acoustic parameters and modulation patterns of signature whistles. I also determined the variation in signature whistle acoustic characteristics over a long period of time to test the stability of contour patterns. To look at the possible influence of genetic relationships on whistle structure, I studied the influence of maternal kinship (including mothers, grandmothers, siblings and aunts or uncles) and associations during the first year of life on signature whistle acoustic parameters and contour modulation patterns. Since short-term responses to environmental changes can also be encoded in whistles and can lead to changes in whistle parameters, I investigated the effect of carrying digital acoustic devices (D-TAGs) after brief capture-release session on the acoustic properties of signature whistles and dolphin behaviour. The overall results of this thesis highlighted that signature whistles are arbitrary signals that have long-term stability allowing them to be reliable signature calls. Mother-calf relations and social interactions during very early development were found to have the highest influence on the crystallisation of a signature whistle modulation pattern. Carrying suction cup D-TAGs, on the other hand, had no clear effect on the dolphins' signature whistles and surface behaviour, confirming that these research methods are appropriate for studying vocal behaviour in bottlenose dolphins.

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I was admitted as a research student in February 2012 and as a candidate for the degree of Doctor of Philosophy in February 2013; the higher study for which this is a record was carried out in the University of St Andrews between 2012 and 2016.

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CHAPTER 1

GENERAL INTRODUCTION

1.1 INTRODUCTION

Acoustic signals are used by a diverse number of taxa as sound can be highly useful for communication when individuals inhabit environments where other senses are limited to short distance communication (Hauser, 1996; Bradbury & Vehrencamp, 1998; Konishi, 1999). For instance, aquatic animals are exposed to different environmental pressures than their terrestrial counterparts. Acoustic signals are transmitted further underwater than light. Therefore, they tend to be an important path of communication for many marine organisms like crustaceans, fish and marine mammals (Tyack, 1998; Zelick *et al*., 1999; Fay *et al*., 2008).

Social structure might also influence acoustic communication. Solitary species usually only interact during the reproductive season and use acoustic signals to advertise fitness to possible mates (Ryan, 1988). On the other hand, social species use sounds in a wider range of activities, such as maintaining group cohesion, kin recognition, finding potential mates, advertising food sources and possible danger to conspecifics (McComb & Semple, 2005; Hollen & Radford, 2009; Clay *et al.*, 2012; Chaverri *et al*., 2012). The types, composition and structure of calls that organisms produce have evolved by natural selection in view of the environment pressures, social structure, neurological and learning capacities, and also due to sexual selection (Ryan, 1986; Bass *et al*., 1999; Rendall *et al*., 2009). In the following sections, I discuss how acoustic signals can vary in different species, the production of acoustic signals in dolphins, and the importance of vocal learning and social structure in bottlenose dolphins for the study of signature whistles.

1.1.1 VARIATION IN ANIMAL ACOUSTIC SIGNALS

From a physics perspective, animal acoustic signals can be divided into two main categories: pulsed signals that are broadband sounds with relatively short duration, and tonal signals that are narrowband sounds with relatively long duration (Au & Hastings, 2008). Environmental constraints, physiological changes and social learning might lead to long term adaptations of acoustic signals represented in variation in frequency structure as well as production rate (Janik, 2000; Brumm & Slabbekoorn, 2005). For variation to alter acoustic communication, it has to be perceived by the receiver and has to carry information (Taylor & Reby, 2010). Within a species, differences can be represented as variation of calls within populations of the same species, variation within related groups in a population, and variation at individual level.

Acoustic variation can be found in all species that use sounds, but its extent can be limited if learning has little or no influence on call development. Vocal learning, also called production learning, refers to the ability to modify vocalisations as the result of the influence of sounds in the environment from conspecifics, and not only the capacity to alter the comprehension and usage of sounds, which would be defined as contextual learning (Janik & Slater, 2000; Boughman & Moss, 2003). Vocal learning is not commonly found in animals other than humans. However, it has been shown for species of birds and mammals. For example, passerine birds, parrots, hummingbirds, bats, elephants, seals and cetaceans (Marler, 1970; Caldwell & Caldwell, 1972; Nottebohm, 1972; Pepperberg, 1994; Boughman, 1998; Reichmuth & Casey, 2014; Poole *et al*., 2015).

For species that lack vocal learning ability, variation in calls might be primarily driven by environmental pressure, physiological characteristics and sexual selection. In many insects, for example, songs are used mostly to attract a mate. In the case of oceanic field crickets (*Teleogryllus* *oceanicus*), the mating songs are linked to a genetic imprint, which means that specific genes have been found to be related to song production that passes from one generation to the next (Bentley & Hoy, 1972) without learning being involved. This call show high species specific stability. However, acoustic variation can occur in hybridisation zones or due to a male-male interaction while singing (Ritchie, 1992; Mousseau & Howard, 1998). Within other arthropods, spiders use stridulatory organs to produce sounds or use pedipalps to transmit vibrations through the ground in mating courtship. The variation in call rate and frequency of tremulation signals are used by females to assess mate quality, as an increase on these parameters could indicate a larger male (Uetz & Stratton, 1982; Gibson and Uetz, 2008; Uhl & Elias, 2011). Similarly, variation in fish calls happens mostly in temporal patterns and can be associated with dominance and size, territorial behaviour, competition for mates, and geographic location (Colleye *et al*., 2009; Radford *et al*., 2014; Wilson *et al*., 2014; Parmentier *et al*., 2005). For mammals that are not vocal learners, variation in their acoustic repertoire can be linked to physiological differences, for example by age in baboons (*Papio ursinus*) (Ey *et al*., 2007) or sexual dimorphism in red deer (*Dama dama*) and colobus monkey (Koffi Bene & Zuberbueler, 2009; Charlton & Reby, 2011). Moreover, variation can be linked to specific contexts, for example in rhesus macaque variation in ‘coo’ calls during foraging (*Macaca mulatta*) (Hauser, 1991). However, these type of variations are not acquired by production learning.

On the other hand, species with vocal learning abilities have vocalisations that might vary not only under environmental and biological pressure, but also influenced by sounds from social interactions within individuals the community. For example, bird songs might vary geographically leading to a process of speciation, and can also show differences within the same individual by age and season (Borror, 1961; Irwin, 2000; Podos & Warren, 2007; Benedict & Bowie, 2009). However, songs are learnt from other adults and variation can also occur when there is a disturbance in the learning process. For example indigo buntings (*Passerina cyanea*) that grow isolated develop abnormal songs, while individuals interacting socially with adults develop the same songs those adults (Payne, 1981). Similarly, in bats, differences in social vocalisations have been found by geographic location, sex-related characteristics, age, and animal size (Moss, 1988; Davidson & Wilkinson, 2002; Kazial & Masters, 2004), but also by context-dependent behaviour and variation driven by mother-calf association (Moss *et al*., 1997; Gadziola *et al*., 2012). Moreover, African elephants (*Loxodonta africana*) vocal activity varies with group size and reproductive status (Payne et al., 2003). Vocalisation rates and call types differ by sex, and the fundamental frequency decreases with age (Poole, 1994; Stoeger-Howarth *et al*., 2007). Also, calls vary when individuals are in presence of dominant animals and depending on group behaviour (Soltis *et al*., 2005; Wood *et al*., 2005).

Within marine mammals, cetaceans are particularly of interest for their vocal learning abilities. For example, humpback whales (*Megaptera novaeangliae*) show a complex structured song during the breeding season that is learnt and varies with every breeding season (Cerchio *et al*., 2001; Mercado *et al*., 2005; Green *et al*., 2010). In addition, geographic variation associated to population boundaries has been reported in blue whales (*Balaenoptera musculus*) and fin whales (*Balaenoptera physalus*) (McDonald *et al*., 2006; Oleson *et al*., 2014). Many baleen whale species tend to produce simple communication calls with stereotyped acoustic structure for different species (Richardson *et al*., 1995; Au & Hastings, 2008). In contrast, toothed whales produce a wide repertoire of social sounds and also echolocation pulses (Au & Hastings, 2008; Morisaka, 2012). For example, belugas are well known for their varied modulated and pulsed calls that are complex to categorise, but individuals possibly produce population specific vocalizations (Karlsen *et al*., 2002; Garland *et al*., 2015). Killer whales (*Orcinus orca*) and sperm whales (*Physeter macrocephalus*) are the only known species of odontocetes with local 'dialects' by specific matrilineal group or geographic clans (Deecke *et al*., 2000; Rendell & Whitehead, 2005). Furthermore, in killer whales, variation in peak frequency amongst ecotypes is related to feeding habits (Foote & Nystuen, 2008), and harmonic energy differences have also been found between the sexes (Miller *et al*., 2007). In addition, for oceanic dolphins, variation in whistle and pulse production can be linked to geographic location, ambient noise, and social context (Rendell *et al*., 1999; Rossi-Santos & Podos, 2005; Rankin *et al*., 2007; May-Collado & Wartzok, 2008; Oswald *et al*., 2008; Quick & Janik, 2012; King *et al*., 2013). Short term changes in vocalizations are more difficult to study since context effects have to be considered. In a later chapter, I will therefore investigate the influence of one of our research methods on vocal variation.

1.1.2 SOUND PRODUCTION AND ACOUSTIC REPERTOIRE IN DOLPHINS

In order to investigate acoustic variation in animal calls, it is relevant to understand the sounds production mechanisms in the species. Cetaceans have been of great interest in the field of bioacoustics due to their underwater physiological adaptations in order to use sounds for navigation, foraging and communication (Richardson *et al*. 1995, Au & Hastings, 2008). The aquatic lifestyle has modified the way cetaceans communicate in comparison to their terrestrial relatives. This is reflected in highly specialised hearing systems and sound production structures in cetaceans, as the physical properties of water allow sound to travel further and faster than in air, while light is restricted to the top layer of the water column (Pryor, 1990; Richardson *et al*., 1995; Berta *et al*., 2006).

Sound production in most mammals is performed with the structures of the larynx. Vocal folds vibrate with the flow of air and these vibrations are transmitted into the air through the mouth and nose cavities (Fitch, 2006; Elemans *et al*., 2015). However, cetaceans have a very different cranial anatomy where the nostrils are situated at the top of the head. The nasal cavity is also modified, and the larynx is positioned low inside the body. The oesophagus and the trachea are completely separated by a modification in the epiglottis. Therefore, cetaceans are able to swallow and breathe at the same time (Berta *et al*., 2006).

Sound production differs between the two groups of cetaceans. Mysticetes vocalisations are most likely produced using their larynx, vocal folds and laryngeal sac (Mercado *et al.*, 2010; Adam *et al*., 2013). In odontocetes, the supracranial airways consist of a complex system of nasal passages, air sacs, nasal plugs and the phonic lips (Cranford *et al*., 1996). Unlike terrestrial mammals and mysticetes, odontocetes produce sounds using their nasal structures, where the larynx is not involved in sound production. Their sounds are produced at a source in the nasal tract, called the phonic lips. These structures are used for the production of tonal and pulsed sounds in odontocetes (Berta *et al*., 2006; Madsen *et al*., 2011) and their function is analogous to that of the vocal folds in a terrestrial mammal's larynx. Both types of sound can be produced at the same time since each individual has two pairs of phonic lips that can be used independently (Cranford *et al*., 1996).

Most dolphin species produce tonal and pulsed sounds classified into three main groups that compose their acoustic repertoire: (1) whistles, (2) echolocation clicks and (3) broad-band pulsed calls including buzzes, chirps, screams and other categories commonly classified as 'burst pulses' (Richardson *et al*., 1995; Au & Hastings, 2008). Furthermore, they produce other types of sounds, like jaw clapping or noise with their blowhole (Nachtigall *et al*., 2000). Echolocation clicks are used for navigating, finding prey and communication (Au, 1980; Dawson, 1991; Tyack & Clark, 2000), while burst pulses (Blomqvist & Amundin, 2004b; Lammers *et al*., 2006) and whistles (Janik & Sayigh, 2013; Janik, 2014) are primarily used in social contexts.

1.1.3 SIGNATURE WHISTLES AS INDIVIDUAL SIGNALS

Whistles are found commonly in the family Delphinidae with a few exceptions, the Lissodelphinae (right whale dolphins) and the genus *Cephalorhynchus* (May-Collado *et al*., 2007). A special whistle category in the repertoire of the bottlenose dolphin is the signature whistle, an individually distinctive, modulated, tonal signal (Sayigh & Janik, 2009). It is not known if all dolphin species produce signature whistles, but positive evidence has been shown in captive spotted dolphins (*Stenella attenuata*) and pacific white-sided dolphins (*Lagenorhynchus obliquidens*) (Caldwell & Caldwell, 1968; Caldwell & Caldwell, 1971; Caldwell *et al*., 1973). Additionally, the presence of signature whistles has been suggested for other species of delphinids, like the Pacific humpback dolphin (*Sousa chinensis*) (Van Parijs & Corkeron, 2001) and the marine tucuxi (*Sotalia guianensis*) (De Figuereido & Simão, 2009). However, bottlenose dolphins (*Tursiops truncatus*) are probably the best known producers of signature whistles in captivity and in the wild (Caldwell & Caldwell, 1965; Sayigh *et al*., 1990), with around half of the whistles produced by free ranging bottlenose dolphins in Sarasota Bay being signature whistles (Cook *et al*., 2004). The signature whistles in this species have been of significant interest when studying the social behaviour and individual interactions amongst animals, and regardless of some scepticism towards their existence (McCowan & Reiss, 2001), they have been demonstrated to be real individual signature signals corroborated by the correct classification of the whistles with the identity of the vocaliser

(Sayigh *et al*., 2007; Janik & Sayigh, 2013). Furthermore, it has been shown dolphins are able to recognise signature whistles of familiar individuals based solely on the modulation pattern of the fundamental frequency, even if the exact acoustic parameters vary and vocal cues are absent, indicating that the modulation contour conveys individual information (Janik *et al*., 2006; Harley, 2008). Nevertheless, variation in acoustic parameters in whistles is suggested to also transmit additional context-specific information (Janik *et al*., 1994; May-Collado & Wartzok, 2008).

It is important to note that signature whistles develop in the first months of life (Caldwell & Caldwell, 1979; Killebrew *et al*., 2001; Fripp & Tyack, 2008), and their crystallisation appears to be influenced by vocal learning (Janik & Sayigh, 2013). The relevance of vocal learning in bottlenose dolphin acoustic communication is discussed in the following section.

1.1.4 VOCAL LEARNING AND REFERENCING IN BOTLLENOSE DOLPHINS

Vocal learning and referencing are features of communication systems that can radically increase the quality and quantity of the transmitted information. As stated in a previous section, vocal learning or production learning refers to the ability to modify vocalisations as the result of the influence of sounds in the environment from conspecifics (Janik & Slater, 2000). On the other hand, referential signals can be defined as those signals that have potential of conveying enough specific information about a situation or object for the receivers to select an appropriate response for that event (Macedonia & Evans, 1993; Evans, 1997; Townsend & Manser, 2013). This ability can be linked to contextual learning, where individuals are capable of learning to react or respond in specific ways depending on the sound uttered by conspecifics (Janik & Slater, 1997). Vocal learning and referencing are not necessarily mutually inclusive. For instance, grey parrots

(*Psittacus erithacus*) are vocal learners and capable of producing new labels for food and other object from labels given by humans showing a good capacity for referencing calls (Giret *et al.*, 2009). Meanwhile, vervet monkey (*Chlorocebus pygerythrus*), which are not vocal learners, seem to have specific calls to refer to three different types of predators (Seyfarth *et al.*, 1980).

Bottlenose dolphins are well known vocal learners and are able of referencing using acoustic signals (Herman & Forestell, 1986; Janik, 2013). This makes them an interesting species to study when exploring communication complexity. The abilities of this species for imitation, new signals production and labelling acoustically have been documented in captivity and in the wild, where individuals copy whistles of conspecifics and use novel signals to refer to objects (Richards *et al.*, 1984; Tyack, 1986; King *et al.*, 2013). Therefore, their vocal plasticity allows dolphins to modify and generate new signals or base new ones on other, pre-existing sounds from their surroundings. Furthermore, it has been claimed that dolphins in captivity can transmit information acoustically about specific tasks to other dolphins without any other cue, indicating that the acoustic signals exchanged contained enough information for the other dolphin to perform the task without previous knowledge (Bastian, 1967).

Signature whistle development seems to be influenced by vocal learning during early age, and these whistles can be shaped by the interactions between calves and their mothers or other conspecifics in the community (Sayigh *et al.*, 1995; Fripp *et al.*, 2005). Similarly, individuals in captivity growing with only one adult in the pool are likely to develop a signature whistle similar to that adult (Tyack, 1997). Moreover, vocal learning abilities in bottlenose dolphins are not exclusive to early age, but remain throughout their life. Adult dolphins are capable to spontaneously imitate, modify whistles and include new signals in their repertoires (Richards *et al.*, 1984; Reiss & McCowan, 1993; Sigurdson, 1993). Also, adult male dolphins can modify parts of their signature whistle contour to match the one of other males (Watwood *et al*., 2004). These variations can be meaningful for the individuals, as this species is known to be able to discriminate relatively small variations between whistle contours (Harley, 2008).

Recognising the capacity of modifying vocalisation and the use of referential signals in this species is relevant to understand whether specific acoustic parameters and modulations in signature whistles can be linked to characteristics such as, sex, age and size. These variations could be working as referential sections of the signature whistle to convey such information. However, dolphins could be inventing freely their own signature whistle as an arbitrary signal that can be potentially influenced by sounds in their social environment or by kin relations instead of being limited by physical characteristics. Moreover, vocal learning can also influence the stability of signature whistles over time (Tyack, 1997), allowing dolphins to maintain the individuality of the signal even if some sections of the contour are modified (Watwood *et al*., 2004). Therefore, investigating how stable signature whistles are over long periods of time is important to contribute to a better understanding of the function of these calls.

Signature calls could work as reference signals in a social community. In bottlenose dolphins, signature whistles are used for addressing others in the group (King & Janik, 2013) and when they join new groups in the wild (Quick & Janik, 2012). While an animal producing its own signature whistle does not provide evidence for reference, using a copy of another animal's signature might. The ability of innovation and imitation of acoustic signals in bottlenose dolphins throughout their lives is likely to be related to the constantly changing social structure in the species and the need to maintain individual relations with different dolphins in the community (Tyack, 1997). Therefore, understanding the social structure and social dynamics in bottlenose dolphins is necessary in order to investigate their acoustic communication and use of signature whistles as a main signal for individual recognition.

1.1.5 SOCIAL STRUCTURE AND SIGNATURE WHSITLES IN BOTTLENOSE DOLPHINS

The bottlenose dolphin is a long-lived species that lives in fission-fusion societies where individually distinctive signature calls are well suited for recognition. Animals living in highly stable associations such as stable groups or families (Tyack, 1986) have fewer requirements for individual recognition and tend to develop group-specific calls instead. There is an expectation for signature calls to be stable over time to convey information about individuality in groups with regularly changing composition (Shapiro, 2010). Sayigh *et al*., (1990) have shown that signature whistles seem to remain stable for at least a decade, but it is still unknown if this is the case for longer periods of time.

In these dynamic societies, bottlenose dolphins have relationships with other individuals in the community that vary over time, and where communication is important to maintain group cohesion. Moreover, they also show some long lasting relationships between specific pairs of individuals (Connor *et al*., 2000; Connor, 2007; Wells, 2009). For example, mother-calf bonds are strong and may last for the first 3-8 years of the new-born's life (Gibson & Mann, 2008). Mother and calf whistle similarity appears to be sex-biased since it has been claimed that female calves produce contours more different to those of their mothers than their male counterparts do (Sayigh *et al*., 1995). Calves remain highly associated with their mothers during the first year of life which could influence the crystallisation of the signature whistle modulation (Mann, 2000; Killebrew *et al*., 2001; Fripp & Tyack, 2008). Furthermore, calves seem to model their signature whistles based on signature whistles from individuals in their community (Fripp *et al*., 2005). However, there is no clear understanding whether high levels of association amongst calves during their first year of life might lead to whistle similarity. Moreover, considering that to optimize individual distinctiveness individuals should aim for signature whistles that are distinctive within a community (Janik & Sayigh, 2007), it could be expected that individuals would show signature whistles less similar to their kin. Furthermore, investigating whether and how different kin relations influence signature whistles modulation and acoustic parameters is important to recognise any potential genetic influences on the development of signature whistles. However, the influence of kin relations other than mothers in the crystallisation of signature whistles has not been studied.

Between the time after weaning and becoming sexually mature, juveniles form mixed-sex groups. However, as adults, there is general sex segregation in the species. Adult females form separate groups with their offspring, whilst male-female associations depend on the reproductive state of the female (Smolker *et al*., 1992). Adult males remain in long-term associations, forming alliances consisting of two or three individuals depending on the geographic area, with triads being rare in Florida (Connor *et al*., 2000; Owen *et al*., 2002). Moreover, in Australia, second-level alliances have been observed (Connor *et al*., 1992). Interestingly, from the acoustic communication perspective, members of male alliances tend to converge part of their signature whistle modulation patterns and general whistle repertoire (Smolker & Pepper, 1999; Watwood *et al*, 2004).

The formation of these alliances is likely to help when competing for access to females with other males, since one of the mating strategies of the species consists in coercion of females by males in order to achieve impregnation (Scott *et al*., 2005). The time that males escort females might last from a few hours to several days (Connor *et al*., 2000). Considering this mating strategy, acoustic sex recognition would involve a great benefit for females trying to avoid potentially aggressive males or to select, to a certain level, male alliances for mating. For males, acoustic sex recognition could help to locate potential females, and conveying sex information could advertise dominance to other male alliances. In addition, morphological sexual dimorphism in this species (Tolley *et al*., 1995) could lead to constraints in acoustic parameters. Signature whistles could convey sex information in acoustic parameters that differ significantly between both sexes and/or sex-specific modulation patterns. Moreover, this acoustic information could be linked to individual size and possible age, considering that smaller and younger males might not represent the same potential threat to a group of females as big, older and experienced males. In this case, some acoustic parameters might show a relationship with size or age, and certain modulation patterns or sections of the contours could be limited to specific age groups.

Additionally, from the perspective of bioacoustics research, the potential of sex, age and size identification from individual bottlenose dolphins, could lead to a more efficient automatic categorisation for studying population dynamics using passive acoustics.

Most populations of this species live in a type of fluid and dynamic society previously explained. The three main areas where bottlenose dolphin social communities have been studied on a long-term basis are the Moray Firth in Scotland, Shark Bay in Western Australia, and Sarasota Bay in Florida. The work for this thesis was conducted on the animals in Sarasota Bay, the longest standing study out of the three. Detailed information about the study area can be found in Chapter 2 (section 2.2).

1.1.6 BIOLOGGING BOTTLENOSE DOLPHINS: COLLECTING ACOUSTIC DATA

Collecting acoustic data has become easier with the development of attachable devices that allow monitoring and recording individuals with the minimum human intervention (Bogard *et al*., 2010; Walker *et al*, 2012). This is particularly important for marine mammals, as access for studying communities in marine environments is limited by the difficulty of obtaining information from multiple individuals that are out of sight for most of the time (Johnson *et al*., 2009). However, the use of biologging devices can present some issues for some species, especially if these devices alter their natural behaviour (McMahon *et al*., 2012). Anthropogenic influences might induce variation in the acoustic structure of whistles in dolphins (Buckstaff, 2004; May-Collado & Wartzok, 2008; May-Collado & Quiñones-Lebron, 2014). Therefore, it is important to investigate whether biologging might also cause a variation effect on signature whistle acoustic characteristics. In bottlenose dolphins, signature whistles have been studied in free-ranging individuals using passive acoustic localization arrays and single hydrophone recordings (Quick & Janik, 2012; Janik *et al*., 2013; Gridley *et al*., 2014; Kriesell *et al*., 2014). Attaching digital recording devices to individuals has become more popular in recent years in the attempt to unravel more information about the use of cetacean calls in the wild. One risk when using such devices is that acoustic variations in signals and changes in behaviour may be caused by a disturbance from carrying the acoustic device. Thus the influence of tagging on dolphin behaviour needs to be investigated in order to validate, support and promote the use of new technologies for the study of acoustic communication in marine mammals.

1.2 THESIS OVERVIEW

Signature whistles are distinctive, modulated, tonal sounds that convey individual information (Sayigh *et al*., 2007). Many studies of signature whistles in bottlenose dolphins have focused mostly on the general acoustic characteristics of the fundamental frequency (Buck & Tyack, 1993; Janik & Slater, 1998; Sayigh *et al*., 2007), on the use of these whistles as contact calls for group cohesion and addressing conspecifics by copying (Watwood *et al*, 2005; Quick & Janik, 2012; King *et al*., 2013), and on the variation in acoustic parameters according to the context and geographic location (Janik *et al*., 1994; Ding *et al.*, 1995; Quick & Janik, 2008; Gridley, 2010). The use of a long-term acoustic database, such as the one that has been collected to date from the dolphin community in Sarasota Bay, Florida, allows us to further explore specific variations that can occur in these signals by using large sample sizes of individuals, reliable acoustic comparison methods and robust statistical analyses to establish predictions, relationships and differences in order to comprehend what additional information might be encoded in signature whistles. In this thesis, I investigate (a) whether differences related to selected anatomical and physiological characteristics might affect acoustic properties and influence modulation patterns, (b) how stable these patterns are over time and (c) how similarities in signature whistles can be a consequence of relatedness or early associations. In Chapter 3, I investigate the effect of sex, age and size on signature whistle acoustic characteristics and whether specific modulation patterns are associated with sex and age groups. In Chapter 4, I evaluate the stability of those acoustic characteristics and modulation patterns within the same individual in a period of over 20 years. In addition, in Chapter 5, I examine the influence of relatedness, and associations amongst calves during the first year of life in the crystallisation of the signature whistle. Finally, in Chapter 6, I investigate the effects of carrying biologging devices (D-Tags) on the acoustic parameters of signature whistles and surface behaviour of bottlenose dolphins followed after capture-release sessions, as a way to investigate whistle parameter variation depending on disturbance context.

In summary, the aim of this thesis is to investigate signature whistle variation, in relation to sex, age, relatedness, calf associations, and disturbance context, in order to understand what additional information could be potentially transmitted and which factors could influence the acoustic structure of these signals in bottlenose dolphins.

CHAPTER 2

METHODS:
STUDYING WHISTLES, STUDY AREA, AND ACOUSTIC DATA COLLECTION

2.1 INTRODUCTION

Dolphins are known for producing a wide repertoire of sounds (Au & Hastings, 2008). As stated in Chapter 1, dolphins produce pulsed and tonal sounds. Whistles are the tonal sounds that are associated with social activities (Richardson *et al*., 1995; Connor *et al*., 2000), and they have been the centre of many studies trying to understand social acoustic communication in dolphins (Lang & Smith, 1965; Tyack, 1986; Smolker *et al*., 1993; Janik & Slater, 1998). However, in order to be able to understand the use of these signals in dolphin species, it is necessary to establish methods to measure whistle characteristics. In the following sections, I discuss the different approaches that have been used for studying dolphin whistles and the general approaches used throughout the chapters in this thesis.

2.1.1 APPROACHES FOR MEASURING ACOUSTIC PROPERTIES OF WHISTLES

One of the most widely used tools in bioacoustics studies is representing sound graphically with spectrograms using the fast Fourier transform (Koenig *et al*., 1946; Openheim, 1970; Tyack *et al*., 1992). Whistles are represented as narrowband signals in spectrograms. They consist of a fundamental frequency and generally several harmonics (Watkins & Wartzok, 1985). These tonal sounds are usually frequency modulated, which is represented as a curved line on a spectrogram.

These can be analysed by pattern-recognition tools (computer-based categorization tools) or by the human eye.

Due to the use of graphic representation of these sounds, one classic approach to study these signals is a qualitative categorisation of modulation patterns (Tyack, 1986, Caldwell *et al*., 1990; Sayigh *et al*., 1990). In addition, quantitative acoustic measurements in frequency and time can be used to represent characteristics of the signals (Steiner, 1981; Morisaka *et al*., 2005; May-Collado, 2010) or comparing fundamental frequency modulation (Buck & Tyack, 1993; McCowan, 1995; Esfahanian *et al*., 2014). Whistle categories and acoustic parameters can then be studied for associations with specific situations (behaviours, context, disturbances, etc.), for relationships with physical characteristics (sex, age, social groups, kin, etc.), and to determine similarity of repertoires (Tyack & Buck, 1993; Sayigh *et al*., 1995; May-Collado, 2010).

2.1.2 CALCULATING WHISTLE PAIR SIMILARITY

Scientists have used different methods to compare similarity between dolphin whistles. Qualitative methods based on visual comparisons have been used by judging similarity to classify modulations (Sayigh *et al*. 1990; Luis *et al*., 2015). Moreover, the similarity of two contours can be ranked by human volunteers using an arbitrary scale commonly scaled from 1 (not similar) to 5 (very similar) (Sayigh *et al*., 1995; Janik, 1999; King *et al*., 2013; Bebus & Herzing, 2015). There is the benefit that human classification does not require quantitative computing effort. However, the resulting similarity values are subjective and several observers have to be used in order to create an objective average score. Furthermore, the amount of possible comparisons is limited by the time available from human volunteers, which can be problematic for very large sample size.

As for quantitative methods, cross-correlation of entire spectrograms has been used to compare similarity amongst acoustic signals in birds (Nowicki & Nelson, 1990; Clark *et al*., 1987). For dolphin whistles, this method has been tested to calculate pairwise similarity amongst extracted contours from their fundamental frequency instead of the complete spectrograms, which can help to avoid ambient noise interference. However, cross-correlation techniques does not work well for calculating frequency modulation similarity values for whistles of different length, as the method cannot be implemented to ignore differences in duration (Janik, 1999). McCowan (1995) proposed a method consisting of sampling a number of points at regular intervals relative to the length of the signal in order to create contours of equal length for comparisons, and then using point measures to calculate pairwise Pearson product-moment correlation coefficients to obtain indices of similarity, as this coefficient measures the association between two sets of variables (Derrick *et al*., 1994). The number of equidistant points sampled varies amongst studies that have followed this method. McCowan (1995) measured 20 points and preserved the negative values from the Pearson product-moment correlation coefficients. Smolker & Pepper (1999) measured 100 points to increase the representation of the modulation and converted negative values to zero, because they considered negative correlation to indicate a level of dissimilarity equal to that at zero. Deecke *et al*. (2010) working on killer whale calls also used the 100 equally spaced points and then measured similarity between two calls using pairwise comparisons. There are benefits to this point sampling method as it preserves the modulation of the signal when a large amount of points are sampled. However, the accuracy of the similarity values can be misleading as the 'standardisation' of the signal contorts the modulation pattern to one of standard duration, but does not take account of time dilation and enough frequency points that could lead to errors (Figure 2.1).

Dynamic time warping (DTW) is a technique that can solve these problems. It compares the distance similarity of two time-dependent sequences (whistle frequency contours in this case) by allowing modification in time of the whistle, eliminating the time-axis fluctuations in order to find the best alignment of the contours that are being compared (Buck & Tyack, 1993; Wang & Gasser, 1997; Deecke & Janik, 2006; Muller, 2007).

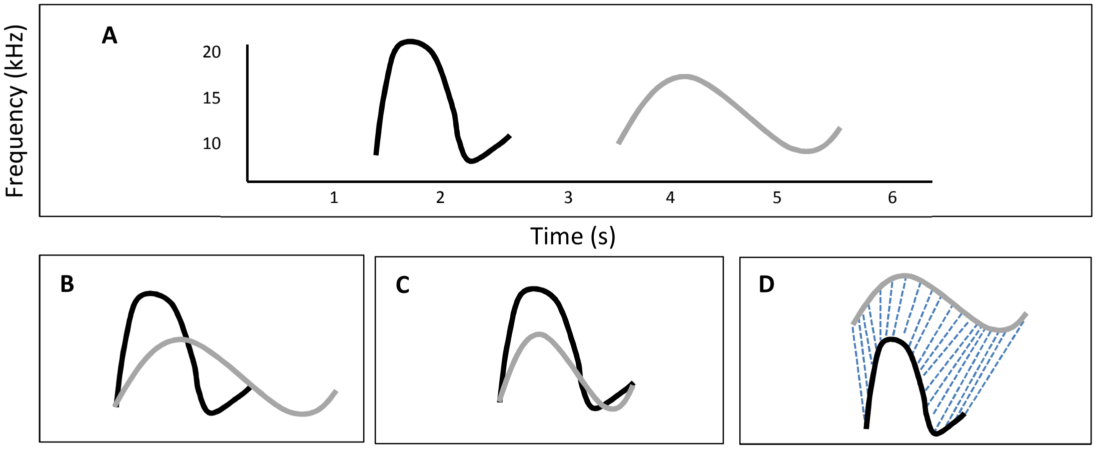


Figure 2.1. Diagram showing an example of two whistle fundamental frequency contours (A), and how they are matched with cross-correlation of both contours (B), a method point-sample and 'standardised' duration (C), and dynamic time warping (D).

2.1.3 WHISTLE CLASSIFICATION AND CATEGORIES

In the previous section, I discussed the methods used for comparing similarity between two whistles. The establishment of similarity values can be used to create general categories to classify whistles. Categorising these acoustic signals can help to understand dolphin acoustic repertoires and differences amongst species (Janik, 1999; Oswald *et al*., 2003).

Since the existence of spectrograms, one of the first ways to classify dolphin whistles was visually (Dreher & Evans, 1964; Caldwell & Caldwell, 1965; Tyack, 1986). This first approach consisted of establishing specific categories (e.g. upsweep, downsweep, constants, concave, etc.) that could be used to analyse repertoires in the same species (Bazua-Duran & Au, 2002; Rasmussen & Miller, 2002; Azevedo & Van Sluys, 2005; Ansmann *et al*., 2007). However, this method was quite limiting considering the complex repertoire of tonal signals found in dolphin species (Connor *et al*., 2000; Au & Hastings, 2008). Therefore, there has been an active search for quantitative methods to complement the visual categorisation method.

For instance, cross-correlation techniques can be used to calculate similarity of whistle contours and then classify whistles using those similarity values with cluster analysis (Janik, 1999). On the other hand, the method proposed by McCowan (1995) consists in classifying whistles using the coefficients of a pairwise product-moment Pearson correlation in k-means cluster analysis to create categories with similar whistles. However, Janik (1999) argued that human categorisation seems to perform more accurately when classifying signature whistles than modulation point sampling or cross-correlation techniques, likely because humans use the overall shape of whistles and the tested computing methods have difficulties separating small differences. In search of quantitative methods that could be as reliable as human classification, Deecke & Janik (2006) proposed a method based on using contour extraction, dynamic time warping comparisons and neural network analysis to classify odontocetes tonal calls. The characteristics of the software and method are explained in the following section.

2.1.4 ARTWARP SOFTWARE FOR CONTOUR CATEGORISATION AND COMPARISON

For this thesis, ARTWARP software (Deecke & Janik, 2006) was used for categorising whistle contours (Chapter 3) and to obtain similarity values for whistle pairs (Chapter 4 and 5). ARTWARP is software that combines dynamic time warping (DTW) and adaptive resonance theory (ART2 neural network) that allows comparing and categorising modulated sounds. The programme compares the input contours and puts them either in the same or in different categories depending on the similarity of their frequency modulation pattern. It also establishes a reference contour for each category. For each new contour addition to a category, the frequency content, relative length, and duration of the reference is modified (Figure 2.2). ARTWARP creates the categories based on the parameter of vigilance that is set by the user. This value refers to the minimum percentage match required between a new input contour and the reference for this new contour to be added to that specific category (Deecke & Janik, 2006).

ARTWARP is a method of automatic whistle categorisation that performs better than other automatic classifiers (Deecke & Janik, 2006). It has been used to analyse geographic variation in whistles (Gridley, 2010), to confirm human categorisation of whistles when detecting signature whistles from free-ranging bottlenose dolphins (Quick & Janik, 2012), and to identify possible signature whistles from Indo-Pacific bottlenose dolphins (Gridley et al., 2014). The proven capacity of ARTWARP to categorise signature whistles in a reliable way led to its use in the investigation of other frequency modulated sounds. For example, Deecke *et al*. (2011) used ARTWARP to classify call types from killer whales (*Orcinus orca*) in order to find if they correlated with two different feeding habits and Bazua-Duran *et al*. (2013) used it to investigate the complexity of repertoires of two species of dolphins.

In addition, it is possible to use the DTW algorithm in the software to obtain a percentage of similarity between two whistle contours. This algorithm in ARTWARP allows horizontal and vertical jumps in three frequency points in the signal, which means that the algorithm can modify the whistle contour by a factor of three in the frequency and time domain to achieve the best match when comparing two whistle contours. The algorithm in ARTWARP also calculates the relative percentage of similarity in frequency between both by dividing the smaller frequency value by the larger value at each point of the comparison and then multiplying it by 100 (Deecke & Janik, 2006).

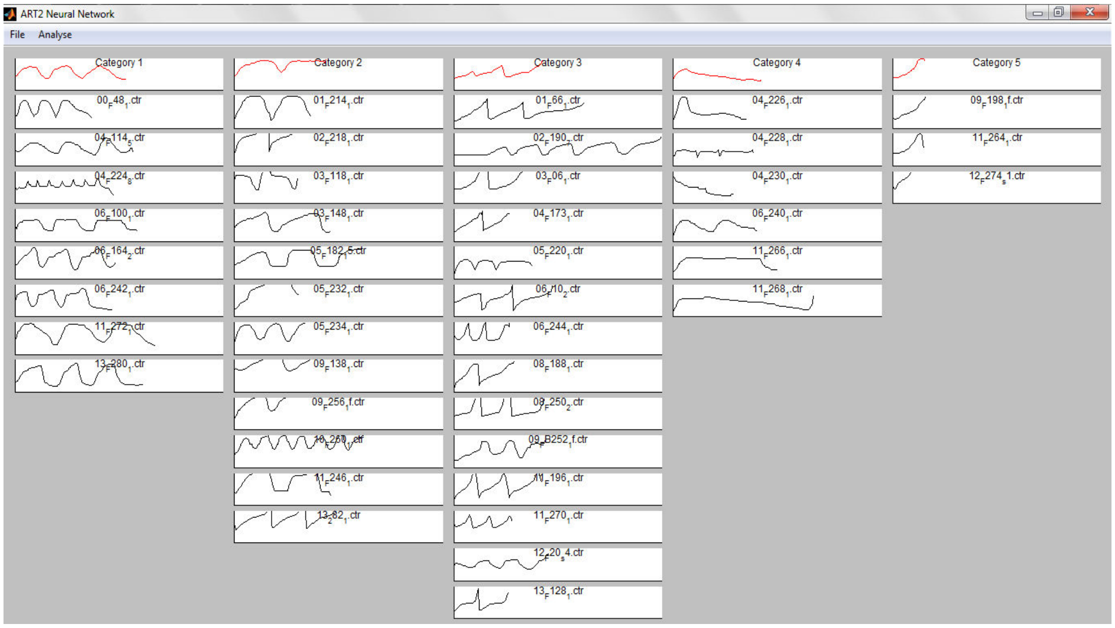


Figure 2.2. Example of ARTWARP software window showing how the categorisation works. The red contours indicate the neuron (contour base) of each category (five categories in this example). The black contours indicate the whistles classified in each category.

2.1.5 MEASURING REPERTOIRE COMPLEXITY FOR COMPARING GROUPS OF INDIVIDUALS

Complexity in whistle repertoire is defined in this thesis as the calculated index based on the amount of signature whistles of different contour modulation found in a given group of dolphins. Repertoires with whistles showing patterns with little modulation were considered less complex than those repertoires with whistles showing highly modulated patterns. In Chapter 3, I calculated values of *proportional variability* and *complexity index* in order to compare the repertoire complexity between sexes and age groups based on the formulas presented by Bazua-Duran *et al*. (2013). Detailed information about the individuals, groups and procedure can be found in the methods section of that chapter. In this section I describe the concepts of those values and the possible outcomes.

First, the proportional variability (PV) indicates the percentage of categories established by ARTWARP compared with the total number of whistle contours in a group of dolphins. Therefore, the higher the PV value (the closer to 100%) the higher the number of categories that the whistles were classified in the group, indicating a more variable repertoire in the group. The formula is computed as follow:

(1) $$PV = \left(\frac{\mathrm{CAT}}{\mathrm{TW}}\right) * 100$$

Where, ***PV*** indicates the Proportional variability, ***CAT*** indicates the number of categories given by ARTWARP, and ***TW*** indicates the total number of signature whistles in the group of dolphins.

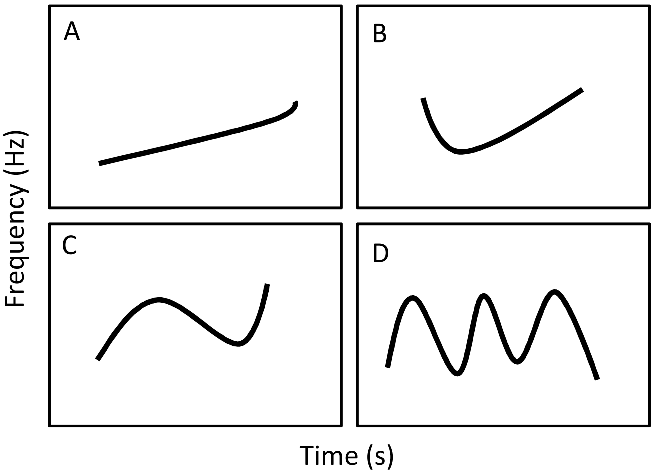


Figure 2.3. Example of whistle modulation patterns representing each category depending on the degree of increasing modulation in the fundamental frequency contours: (A) non-modulated, (B) slightly modulated, (C) major modulation, and (D) very modulated.

The complexity index (CI) is calculated classifying the whistles in a group into four categories according to the degree of modulation (Figure 2.3), and calculating the index value with a weighted formula (2). Weighting is useful because it gives more influence to elements representing highly modulated whistles, which presence is considered to indicate a more complex repertoire in this study. The resulting value indicates an index going from 0.25 when all the contours are non-modulated whistles to 1 when all the contours are highly modulated. Values between 0.26 and 0.59 indicate a repertoire closer to non-modulated contours. Moreover, values between 0.60 and 0.70 indicate evenly distributed contours in all the categories. And, values between 0.71 and 0.99 indicate that the group repertoire includes more whistles towards the highly modulated category (Figure 2.4). It is recommendable to plot the number of whistle contour per category joined with the complexity index value for a better representation and understanding of the value. The formula is computed as follows:

$$CI = (4 * \mathrm{D} + 3 * \mathrm{C} + 2 * \mathrm{B} + 1 * \mathrm{A})/(4 * \mathrm{TW}) \qquad (2)$$

Where, ***CI*** indicates the Complexity index, ***A*** indicates the number of signature whistles in the category "non-modulated", ***B*** indicates the number of signature whistles in the category "slightly modulated", ***C*** indicates the number of signature whistles in the category "major modulation", ***D*** indicates the number of signature whistles in the category "multi-modulated", and ***TW*** indicates the total number of signature whistles in the group of dolphins.

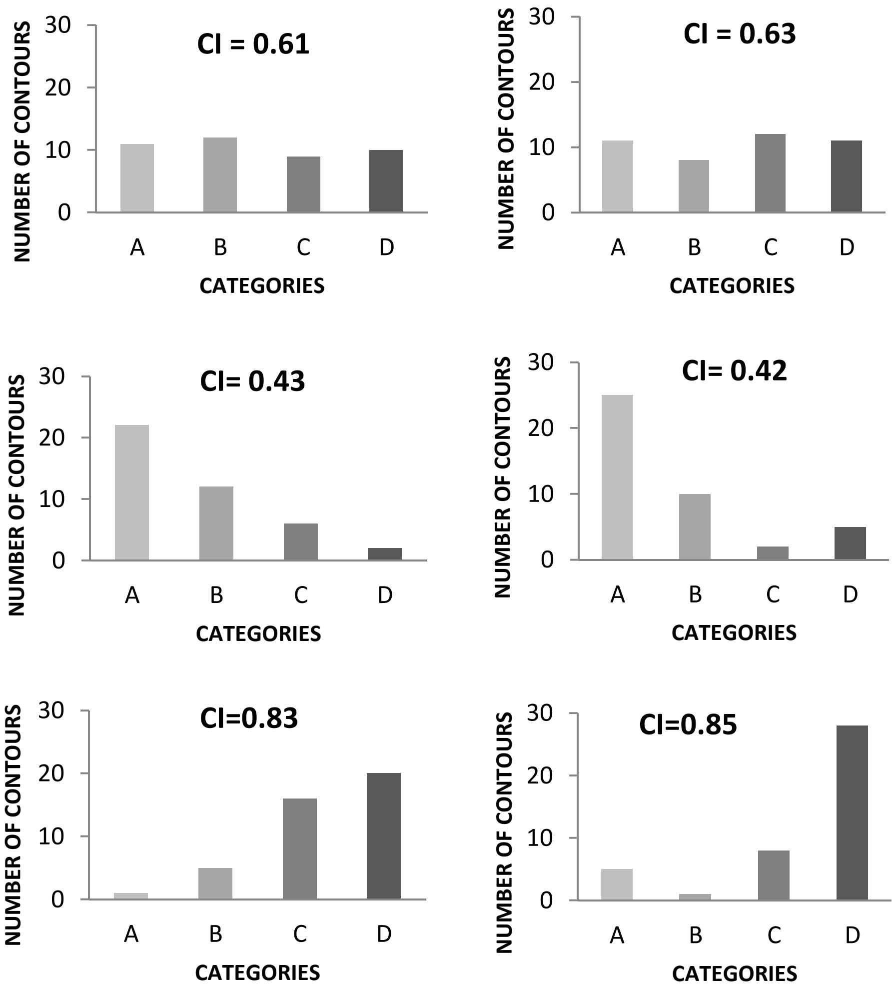


Figure 2.4. Six examples of how the complexity index can be represented graphically with the number of contours in different categories. The categories (Figure 2.3) are shown in the x-axis with each bar in different grey shade, and the complexity index (CI) value is indicated in the centre of each graph. Note the highest CI value when most of the contours correspond to category D.

2.1.6 WHISTLE PARAMETERS AND CONTOUR MODULATION APPROACH IN THIS THESIS

Throughout this thesis, I chose to analyse signature whistles based on (a) the properties of acoustic parameters that place the fundamental frequency in a frequency-time space, and (b) the contour shape of the fundamental frequency even if acoustic parameters do not necessarily differ.

Signature whistles acoustic parameters show a certain degree of variation (Janik *et al*., 1994), but it has been shown that the contour modulation patterns are sufficient to transmit individual identity information (Janik *et al.*, 2006; Harley, 2008). This does not mean that acoustic parameters are not transmitting relevant information for the dolphins. In fact, it has been shown that signature whistles transmit information on context and internal state (Janik *et al*., 1994, Esch *et al.,* 2009a). Moreover, measuring specific acoustic parameters is important to analyse individual differences in whistle frequency-time structure (McCowan, 1995). Thus, both approaches should be used to fully explore whistle variation.

In Chapter 3 and Chapter 4, I used acoustic parameters and contour modulation patterns to investigate relationships with physical characteristics (sex, age, size in different individuals), and stability over time (age within individuals). In addition, for Chapter 5, I used two different methods to establish similarity between signature whistle pairs in order to investigate influence of kin relationships and calf associations. The contour modulation similarity is important as identity information is contained in the pattern (Janik *et al*., 2006), and it has been used to compare stability, mother-calf relations and vocal mimicry accuracy (Sayigh *et al*., 1990; Sayigh *et al*., 1995; King *et al*., 2013). Besides, the situations where acoustic parameters are similar can bring a different set of answers from just the contour comparisons, even if the modulations are not similar. Acoustic parameters can reflect the general position of the whistle in a multivariate space (frequency and time) and bring information about ranges and limits for individual whistle production (Figure 2.5). Using solely acoustic measurements in order to investigate similarity and classify calls has been implemented in other species (Wood *et al*., 2005; Knörnschild *et al*., 2007; Risch *et al*., 2007), and has the potential to give relevant information for whistle comparisons in bottlenose dolphins. For Chapter 6 I followed this approach and focused only on acoustic parameter variation in whistles, as it has been shown that some acoustic parameters vary with context, possible stress, and anthropogenic noise (Janik *et al*., 1994; Esch *et al*., 2009b; May-Collado & Wartzok, 2008).

Detailed information about the specific analyses and procedures can be found in the methods section of each chapter.

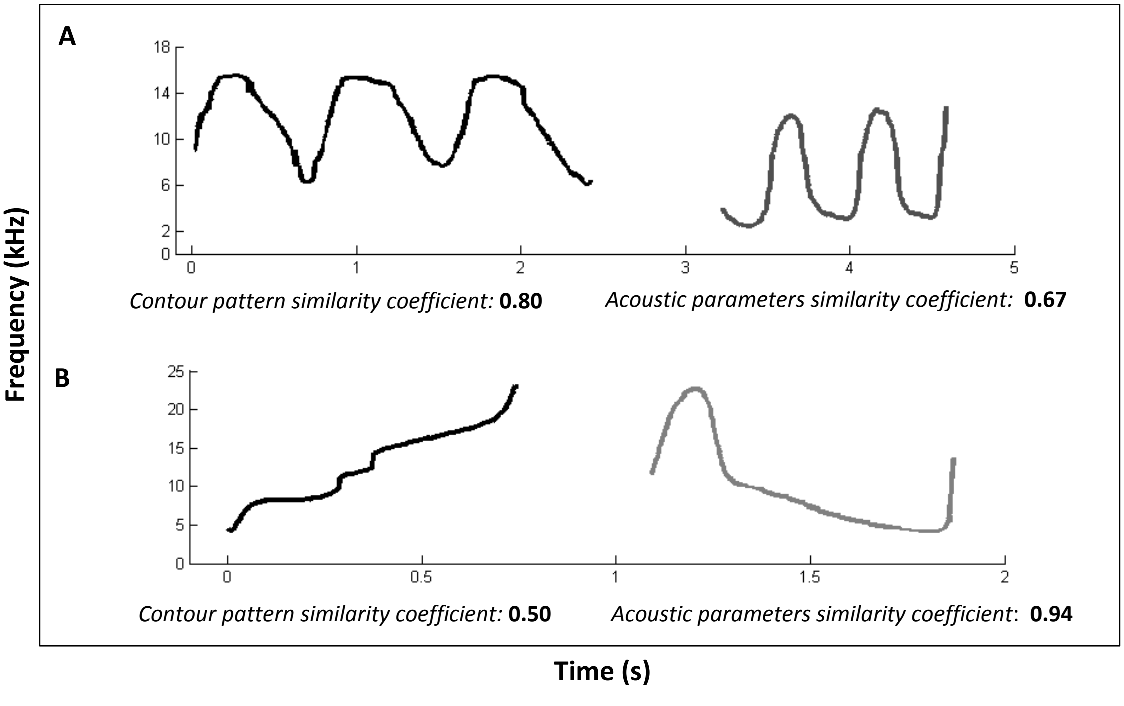


Figure 2.5. Examples of how two whistles with higher similarity in contour patters can have lower similarity in acoustic parameters (A), and two whistles with higher similarity acoustic parameters than similarity in contour pattern (B). The values of coefficient of similarity by contour match and parameter measurements are shown below each pair. Values closer to 1 indicate more similarity.

2.2 SARASOTA BAY: LONG-TERM DOLPHIN COMMUNITY STUDY

2.2.1 STUDY AREA

Sarasota Bay lies on the west coast of Florida in the Gulf of Mexico (Figure 2.6). It is a semi-closed body of shallow water, 10 metres at its deepest and an average of 5 metres, consisting of several embayments and passes for water exchange in the bay. The area is surrounded by sandy, muddy coast, and mangroves near to a highly developed tourist area with constant boat traffic. The area is a habitat for many species of fish, birds and mammals (Kish *et al.*, 2007; NEPCCR, 2007).

Sarasota Bay is the home of a resident community of bottlenose dolphins. A continuous long-term study has been implemented for over 30 years and continues to date. There is a current register of around 160 individuals that visit the area regularly (Allen, 2014).

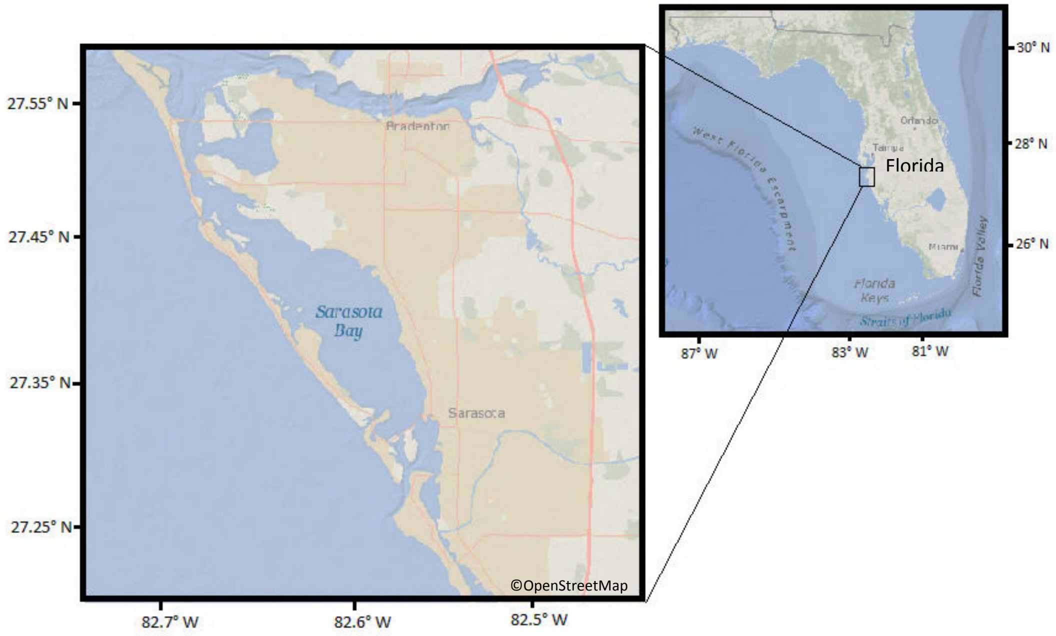


Figure 2.6. Location of Sarasota Bay in the Gulf of Mexico, on the west coast of Florida.

2.2.2 RESEARCH IN SARASOTA

The dolphin research in Sarasota, Florida, started in the 1970s and it has been the longest continuous research program of bottlenose dolphins. Studying this dolphin community has helped to understand the biology, health, social structure, anthropogenic effects and acoustic communication in the species (Wells, 1991; Wells, 2014).

Long-term studies in long living animals are considered the optimum method of understanding intricate social networks (Wells, 2014). Studies in Sarasota Bay have helped to unveil the abundance, distribution, feeding habits and social networks of bottlenose dolphins in the area, (Irvine & Wells, 1972; Hohn *et al*., 1989; Barros & Wells, 1998; McCabe *et al*., 2010; McHugh *et al*., 2011; Dunshea *et al*., 2013; Rossman *et al*., 2015), and even though there are differences amongst populations, this can provide a baseline to study other populations and even other dolphin species in coastal habitats.

Furthermore, a yearly health assessment has provided an opportunity to evaluate health parameters in individuals (Hart *et al*., 2015) and helped to discover facts about the physiology of bottlenose dolphins involving thermoregulation, hormones and pathology (Barbieri *et al*., 2009; Bryan *et al*., 2007; Hart *et al*., 2015; Miller *et al*., 2011). It also helped to understand the impacts of new stressors. For example, Sarasota dolphins served as control when the oil spill in the Gulf of Mexico occurred since they were not exposed to the oil (Balmer *et al*., 2015). In addition, a large number of acoustic studies in Sarasota Bay addressed topics from communication (e.g. Sayigh *et al*., 1990; Cook *et al*., 2004; Quintana-Rizo *et al*., 2006; Janik & King, 2013; King *et al*., 2013) to the effects of anthropogenic noise (e.g. Nowacek *et al*., 2001; Buckstaff, 2004; Buckstaff *et al*., 2013).

The characteristics of the Sarasota Bay bottlenose dolphin community and the long term controlled research effort that has been applied in this area, makes it an ideal area for conducting the study presented in this thesis.

2.3 ACOUSTIC DATA COLLECTION FROM CAPTURE-RELEASE SESSIONS

Recordings used for Chapters 3, 4 and 5 were collected from brief capture-release events for health assessments in Sarasota Bay from 1975 to 2013. After initial capture, each animal was held in place by a group of trained people. During capture periods, the dolphins were maintained in water and/or on a veterinary examination vessel. Recordings in 1975 and 1976 were collected in air using a microphone near the dolphin's head and an Uher Report L or Report IC reel-to-reel tape recorder (frequency response: 40-20000 Hz). For the years after, vocalisations were recorded using custom-built hydrophones (Woods Hole Oceanographic Institution, frequency response 2 Hz to 30 kHz ± 1 dB) or High Tech Inc. SSQ94 hydrophones (frequency response 2 Hz to 50 kHz ± 1 dB) attached to suction cups and placed directly on the melon of each dolphin. Before 2004, the acoustic recordings were obtained at 96 kHz and 16 bits using either Panasonic AG-6400 or AG-7400 video-cassette recorders (frequency response 20-32000 Hz ± 3 dB) or Marantz PMD-430 or Sony TC-D5M stereo-cassette recorders (frequency response of 20-18000 Hz ± 5 dB). The recordings were digitised at 96 kHz and 16 bits. In the following years Sound Devices 744T digital recorder (frequency response: 10-48000 Hz ± 1 dB) was used. Up to four hydrophones were connected to separate channels for simultaneous recordings. In addition, length, weight and sex were registered on every occasion. The age of each dolphin was determined by registering the date of possible birth based on observations of known mothers with new-born calves in the field, and also by counting the growth layers in teeth (Hohn *et al*., 1989; Irvine, 2011).

Acoustic and behavioural data collection for Chapter 6 was conducted during focal follows in 2013 and 2014. More detailed information can be found in the methods section of that chapter.

2.4 ACOUSTIC DATA PROCESSING FOR WHISTLE CONTOUR AND PARAMETER EXTRACTION

Sayigh *et al*., (2007) showed that each dolphin uses almost only one whistle type in the recording contexts during capture-release sessions in Sarasota. This whistle type is a signature whistle, the most common whistle when an animal is isolated. These whistles can account for 80% to 100% of all the whistles produced in isolation (Janik & Sayigh, 2013). Acoustically, signature whistles were defined as tonal sounds of over 100 ms that can consist of a continuous modulated signal or several units (defined as LOOPS in this thesis) which are separated by silence intervals of less than 250 ms (Esch *et al*., 2009a).

For this thesis, I processed recordings from 1975 to 2013 from capture-release sessions. A different subset of years and individuals was used for Chapter 3, 4, and 5. The description of years and number of individuals and whistles used for each analysis is specified in each chapter. In addition, for Chapter 6, I processed whistles from individuals sampled during focal follows in 2013 and 2014. Detailed information about individuals and sample size can be found in the methods section of that chapter.

For each individual, whistles were inspected visually from the audio files using spectrograms (512 FFT, Hamming window, overlap 90% and resolution of 48 kHz) created with Adobe Audition 2.0 (Adobe Systems). Then, the fundamental frequency contour of each whistle was extracted using the software 'Beluga' in MATLAB (R2014b) (http://biology.st-andrews.ac.uk/soundanalysis/) which tracks contours based on a supervised peak-finding algorithm (Deecke *et al*., 1999). Whistle contours were used to investigate the repertoire complexity and classification by sex and age (Chapter 3), the contour stability (Chapter 4), and the whistle similarity amongst individuals (Chapter 5).

Once the contours were extracted, 11 parameters were measured (Table 2.1) using a MATLAB (R2014b) custom routine. Inflexion points (IP) and number of units that constitute a whistle (LOOPS) were counted directly from the spectrogram. For multi-unit whistles, the total duration was measured including all the units and the silent spaces between them. Duration of silence (SIL D) was calculated by adding the duration of the spaces between all units (Figure 2.7). Lastly, the frequency variability index (FVI) is a value that estimates the magnitude of fundamental frequency change in the whistle contour calculated dividing the variance in fundamental frequency by the square of the mean frequency, and multiplied by 100 (McCowan *et al*., 1998). Then, these acoustic parameters were used in statistical analyses to investigate relationships with physical characteristics (Chapter 3), variation of acoustic parameters over time (Chapter 4), similarity of acoustic parameters amongst individuals (Chapter 5) and variation with disturbance context (Chapter 6).

Table 2.1. Descriptions of each of the parameters measured from every whistle contour.

CODE	ACOUSTIC PARAMETER	DEFINITION
BEG F	*Beginning Frequency*	First frequency point in the contour (Hz).
END F	*End Frequency*	Last frequency point in the contour (Hz).
MAX F	*Maximum Frequency*	Highest frequency point in the contour (Hz).
MIN F	*Minimum Frequency*	Lowest frequency point in the contours (Hz).
DUR T	*Total Duration*	Time of the whistle including all its units (ms).
MEAN F	*Mean Frequency*	Average of all frequency points in the contour (Hz).
MID.F	*Median Frequency*	Frequency point at half duration (Hz).
F RANGE	*Frequency Range*	Span from the highest frequency point to the lowest (Hz).
F.GRAD	*Frequency Gradient*	Overall gradient/steepness of the whistle.
IP	*Inflection Points*	Number of points when the frequency changes direction.
SIL.D	*Duration of Silence*	Time of silence between the units in a whistle (ms).
LOOPS	*Units on the Whistle*	Number of units that compose the whistle.
FVI	*Frequency Variability Index*	Calculated as the variation divided by the square mean multiplied by 100

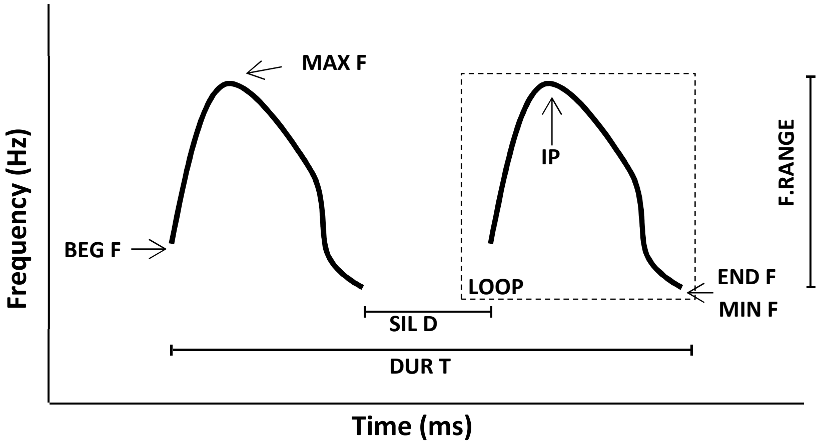


Figure 2.7. Diagram showing some of the parameters measured on the whistle contours (Table 2.1).

CHAPTER 3

BOTTLENOSE DOLPHIN SIGNATURE WHISTLES: DO SEX, AGE AND SIZE MATTER?

3.1 INTRODUCTION

Age, growth and sexual maturity influence the morphology and function of structures during the ontogeny of individuals. Particularly, hormonal factors are highly related not only to the differentiation between the sexes, but also to ageing and growth (Bustad, 1982; Everitt & Meites, 1989; Çitfici, 2013). In many animals, growth and body mass also have an influence on features of their vocalizations. Similarly, sex can be encoded in specific parameters of animal sounds. Such general features that affect all vocalizations of an animal are commonly referred to as voice features. Differences in voice features can be a by-product of morphological differences, transmitting sex, size and quality of the caller (Reby & Charlton, 2011).

Sex recognition is fundamental for the reproductive success of gonochoric species. Sexual dimorphism refers to specific morphological and/or behavioural characteristics that differ between males and females (Isaac, 2005; Berta *et al*., 2006). These differences might be driven by sexual selection and influenced by the mating system in the species. Therefore, polygamous species tend to be more sexually dimorphic than monogamous species (Frayer & Wolpoff, 1985; Owens & Hartley, 1998). Differences in vocal repertoires of males and females can occur in modulation and structure of the signal or specific types of calls, and might be caused by sexual selection, morphological features or sex-dependant social experiences (Green, 1981). Acoustic sexual dimorphism has been demonstrated to some degree in several terrestrial species. Some examples are baboons (*Papio ursinus*) (Ey *et al*., 2007), zebra finches (*Taeniopygia guttata*) (Riede *et al*., 2010), red deer (*Cervus elaphus*) (Reby & Charlton, 2012), bullfrogs (*Rana catesbeiana*) (Boyd *et al.*, 1999) and big brown bats (*Eptesicus fuscus*) (Grilliot *et al*., 2009). Similar examples can be found for differences according to age or size of animals. In some species of bats, the number of harmonics, the duration and the average frequency of the calls vary with age and might be used for recognition (Habersetzer & Marimuthu, 1986; Russo *et al*., 2001; Liu *et al.*, 2007; Hiryu & Riquimaroux, 2011). In baboons, it has been found that as animals get older, their fundamental frequencies are lower and calls get longer probably due to increase in size (Ey *et al.*, 2007). These variations might be associated with the changes in the tissues related with sound production, general growth in size and/or changes in behaviour. Body size is correlated to the size of the air cavities inside the animals in allometric conditions. This situation affects the resonance properties when these animals produce their vocalisations, so a bigger animal will produce a lower frequency (Fletcher, 2004). Acoustic differences that indicate size, age or sex differences can often be detected by animals as is known from birds (Yamaguchi, 1998) and marmosets (Smith *et al*., 2009). Similarly, big brown bats females seem to be able to identify echolocation patterns of males (Kazial & Masters, 2004), and differences in frequency components of the echolocation pulses between males and females depend on being uttered in a social context (Grilliot *et al*., 2009).

In odontocetes, it has been found that new-born finless porpoise (*Neophocaena phocaenoides*) produce low frequency pulsed sounds with long duration during the first hours after birth, but after the first month they produce low and high frequency sounds simultaneously where low frequency pulses are weaker and shorter in duration than the new-borns (Li *et al*., 2008). Also, it has been found that sperm whale (*Physeter macrocephalus*) neonates produce clicks with low directionality, lower frequency and long duration than those produced by adults. Those sounds do not resemble the coda type sounds used by juveniles and adults, but older calves may produce repetitive trains that can be understood as the coda pattern precursor (Madsen *et al*., 2003). In addition, for the same species, Schulz *et al*. (2011) found that a calf and its mother had distinctive coda patterns suggesting the use of these signals to identify individuality or age class.

For bottlenose dolphins (*Tursiops truncatus*), individual recognition is an important factor for their social structure. On the coast of Florida, studies have shown that bottlenose dolphins are slightly sexually dimorphic in size, where males are longer and heavier than females (Tolley *et al*., 1995). Also, males have several body measurements (*e*.g., wider flippers or more teeth) that are larger than in females (Hersh *et al*., 1990; Tolley *et al*., 1995). Similarly, cranial measurements show some degree of sexual dimorphism (Turner & Worthy, 2003). These physical differences may lead to acoustic differences between sexes, although it is known that some species are acoustically dimorphic even without a strong size dimorphism as a result of difference in their sound production structures or behaviour (Boyd *et al*., 1999; Fitch, 2000). New-born dolphins’ sound production begins early in life (Killebrew *et al*., 2001) and the complex repertoire of each individual changes as they grow, and it is affected by the social context during vocal learning (McCowan & Reiss, 1997; Janik, 2000; Quick & Janik, 2012). Mother and calf recognition is also achieved acoustically using signature whistles (Smolker *et al*., 1993). Animal size and sound production structures affect the frequency and the resonance frequencies that are emitted (Fletcher, 2004). Therefore, the proportions of structures related to sound production in younger dolphins are immature and this changes as they grow. New-borns show rapid growth whilst suckling during the first year of life, continuing at a slower rate until a plateau is reached more or less around the period when sexual maturity is reached (Reynolds *et al*., 2000).

Bottlenose dolphins have a wide repertoire of sounds including pulsed (echolocation clicks and burst pulses) and tonal signals (whistles) (Richardson *et al*., 1995; Au *et al.*, 2000). Within the latter group, signature whistles are specific modulated tonal signals (Sayigh *et al.*, 2007) known to convey individual information that allows dolphins to identify each other (Janik & Slater, 1998; Janik *et al*., 2006). They are used to address conspecifics and when joining a new group (Quick & Janik, 2012; King *et al*., 2013). Therefore, information related to morphology and physiology of an individual might be transmitted through this type of signals by specific acoustic parameter values or modulation patterns associated to sex and age group.

Vocal learning ability in bottlenose dolphins includes copying whistles and using novel sounds as labels for objects (Richards *et al*., 1984; King *et al*., 2013). This learning capacity is involved in the development of unique signature whistles early in life (Janik, 2013). It has been shown that signature whistles are signals used for individual recognition, as mothers respond significantly more to signature whistles of their independent calves than other similar age individuals in the community (Sayigh *et al*., 1999). Moreover, dolphins respond to significantly more to playbacks of whistles produced by familiar individuals even without voice cues present (Janik *et al*., 2006). Nevertheless, these whistles not only convey individuality, but might also contain context-related information, as frequency and time parameters showed significant difference between isolation and training contexts in a captive bottlenose dolphin (Janik *et al*., 1994). Thus, specific individual information about the animal such as sex, age and size might be encoded in areas of the modulation or acoustic parameters, considering that those could be constrained by physical characteristics.

The objective of this study was to investigate whether acoustic parameters and modulation characteristics in signature whistles show relationship with sex, age and size of the individual transmitting, in the sense of being constrained to a certain limit by these physical characteristics or whether signature whistles acoustic parameters and modulations are selected arbitrarily by each individual. The analyses focused primarily on the acoustic properties and modulation in the signature whistle fundamental frequency. However, as it has been shown that dolphins are capable of discriminating differences in harmonics (Branstetter *et al.*, 2013) and in other delphinids there has been suggested a difference in harmonic energy content between sexes (Miller *et al.*, 2007), I also considered the harmonic energy content in comparison to the fundamental frequency as this could indicate another path for transmitting information.

3.2 METHODS

3.2.1 DATA PROCESSING

Recordings were collected from brief capture-release events for health assessments in Sarasota Bay. The information about the study area and data collection is explained in Chapter 2 (section 2.2 and 2.3).

For this chapter, I processed recordings from 2000 to 2013, and obtained recordings of 85 individuals. The signature whistle of each individual was identified by comparing with the established whistle catalogue. A total of 20 non-consecutive signature whistles from each of 85 individuals were selected in order to obtain a sample size across the whole recording session. Signature whistles of individuals in Sarasota are known and catalogued. Signature whistles from the capture-release sessions were compared to the catalogue for positive confirmation. Then, the acoustic parameter extraction was carried out as stated in Chapter 2 (section 2.4).

In addition to the acoustic parameters already described in this section, I used the fundamental frequency contours in a neural network analysis with ARTWARP software (Deecke & Janik, 2006), and obtained spectral measurements of signature whistles from a subsample of the individuals. These analyses are explained in the following sections of methods.

3.2.2 ANALYSIS ON FUNDAMENTAL FREQUENCY PARAMETERS

From the 85 individuals sampled, only individuals with complete information about sex, age, length and weight were used for the statistical analysis, because not all individuals during the capture-release sessions can be weighed. Therefore, 78 individuals were included in the analysis, 39 females and 39 males. The average values of the whistles' parameters for each individual were calculated and organised in a database with the information about sex, age, length and weight. All the statistical analyses were performed using R software (R 3.2.0, GNU project).

HIERARCHICAL CLUSTER ANALYSIS

The 13 whistle parameters were first standardised as z-scores in order to avoid giving different weight in the analysis to different magnitudes of the measurements. Then a hierarchical cluster analysis was run to investigate if the parameters would classify the whistles into clusters with a pattern based on sex, age or size. For this analysis the R package *pvclust* (Suzuki & Shimodaira, 2006) was used. The cluster was run with using Euclidean distances and a bootstrap of 10000 iterations. This routine assesses uncertainty in hierarchical cluster analysis by performing a multiscale bootstrap to calculate the probability defined by Suzuki & Simodaira (2006) as AU

(Approximately Unbiased) of the clusters classified by real similarities. It also calculates a Bootstrap Probability value.

RANDOM FOREST

Random Forest analyses (Breiman, 2001) were run with the 13 parameters for each response variable (SEX, AGE, LENGTH and WEIGHT). This analysis provides the order of importance of each parameter to categorise individuals in relation to the independent variable (see Appendix 1). The three parameters with the least importance for all four response variables (IP, SIL.D and LOOPS) were not included the rest of the analysis. Random forest was used because it is a robust analysis against overfitting and outliers, and performs well compared to other classifiers like discriminant function analysis because of the use of randomly selected predictors per each dividing node that minimise the correlation between predictors in the forest, increasing accuracy as a classifier (Breiman, 2001; Liaw & Wiener, 2002).

MULTIVARIATE MULTIPLE REGRESSION

The rest of the variables were investigated for univariate normality visually using histograms and quantile-quantile plots. Then, the data was standardised rescaling it with z-scores for the accurate comparison amongst coefficients of parameters measured in different scales of magnitude and the predicted variables in the analysis. Standardised scores units are measure as standard deviations (σ) of the predictor, in a way that coefficient estimates in a regression can be interpreted as the amount of negative or positive change in the predicted variable for each standard deviation change in the predictor variable.

A multivariate multiple regression was fitted using sex, age, length and weight as response variables and BEG.F, END.F, MAX.F, MIN.F, MEAN.F, MID.F, RANGE.F, GRAD.F, FVI and DUR.T as predictor variables. The binary variable sex was use for the regression using the value 0 for females and 1 for males. Binary variables in multivariate analyses allow the use of multiple predictors and its use does not affect the robustness of the analysis (Hellevic, 2009). Moreover, when at least a moderate correlation structure exists among the predicted variables, as the case of increasing size with the increase of age and sexual dimorphism in size towards males, a multivariate regression is more effective than regressions analyses for each dependent variable separately (Hartung & Knapp, 2005). Moreover, the multivariate model fit was estimated by Pillai's test statistic.

In addition, diagnosis of the model fit was revised by using the Mahalanobits quantile plot and the studentised residuals histogram (Appendix 1). It is worth consider that with a large sample size of over 20 per variable the Multivariate Central Limit Theorem holds and we can assume the multivariate normality assumption holds and that test results are reliable even with a slight skewness in the normal distribution of the residuals (Dinov *et al*., 2008)

3.2.3 ANALYSIS ON CONTOUR MODULATIONS

As mentioned in Chapter 2, the acoustic parameters measured do not necessarily capture the modulation complexity of the fundamental frequency in the signature whistle,. Hence, a neural network analysis using ARTWARP software was run in order to investigate if the number of contour modulations categories and level of modulation differ by sex or age group. Information about ARTWARP software is explained in detail in Chapter 2 (section 2.1.4).

In this study, I am applying the benefits of automatic categorization in order to identify if signature whistle patterns can be used to indicate sex or age of a dolphin. In order to establish the parameters for the classification in ARTWARP, I took in account that Deecke & Janik (2006) tested the neural network with signature whistles of the same individual and found that the value of 0.96 was the highest vigilance value that still classified the signature whistles in a single category. Therefore, in the analysis for this study the vigilance was selected at 0.90 to allow some flexibility in the formation of categories in the analysis as a value of 0.96 would be too strict setting each individual whistle in a different category. This is important as the analysis consist in comparing signature whistles of different individuals in order to identify whether those signals of different sex or age group consist of more categories (are more variable). In this case a lower vigilance value than 0.96 would allow to group whistles that are similar without requiring to be identical, which was the aim for the categorisation performed by Deecke & Janik (2006).

One whistle contour was selected randomly for each individual in order to optimise the speed of the analysis in ARTWARP, and considering that according to the example of Deecke & Janik (2006), any signature whistle from the same individual would cluster into the same category with any vigilance value lower than 0.96.

A total of 85 dolphins were used in this analysis. For the analysis, the signature whistles were separated into two sets of groups: by sex (43 males and 42 females, including all ages) and by age group (42 sub-adults and 43 adults, including both sexes). The division between sub-adult and adult animals was selected at the age of 10 as this is the approximate age of sexual maturity (Reynolds *et al.*, 2000). An additional control group including all the whistles together was also used in the analysis. Then, the neural network analysis was run for each group. A resulting classification including the number of categories and the contours classified in each category was shown in the software.

In addition, each of the 85 signature whistles contours were categorised by three inexperienced volunteers into 4 categories established on the amount of increasing modulation in the contour, from non-modulated to highly modulated (Figure 3.1). Each whistle was allocated in a category if at least two of the three volunteers agreed.

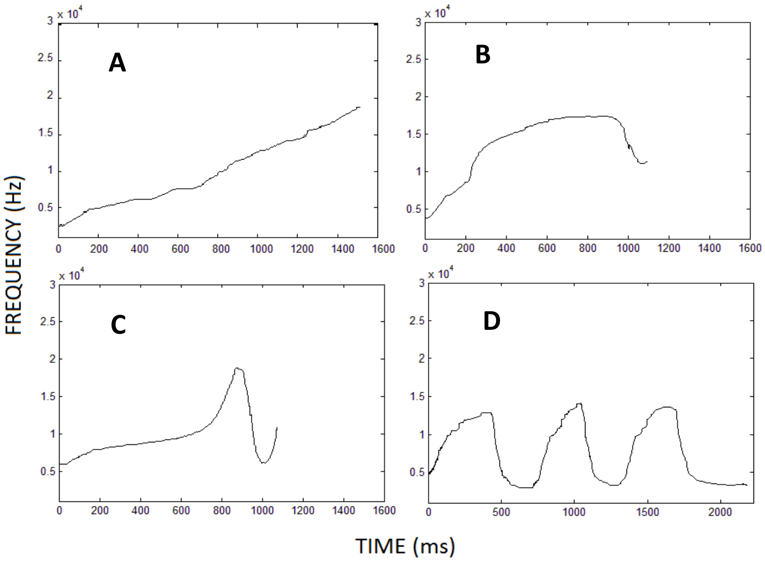


Figure 3.1. Examples of categories for level of modulation in the sample. **A**: non-modulated, **B**: Slightly modulated, **C**: Major modulation, and **D**: Highly modulated.

The proportional variability and the complexity index were calculated based on a modification of the formulas used by Bazua-Duran *et al.* (2013) to calculate the complexity in whistle repertoires of dolphins. The description of the calculations and interpretations are stated in Chapter 2 (section 2.1.5). The values were calculated for each of the groups (MALES, FEMALES, SUB-ADULTS, and ADULTS).

3.2.4 DIFFERENCES IN RELATIVE ENERGY BETWEEN FUNDAMENTAL FREQUENCY AND SECOND HARMONIC

Harmonic energy can show variation related to physical characteristics (Fletcher, 2004). Odontocetes have shown some degree of variation in harmonic energy and bottlenose dolphins are capable of discriminate directionality of whistles based on harmonics (Miller *et al*., 2007; Branstetter *et al*., 2013). Therefore, after analysing parameters in the fundamental frequency and modulation patterns, this analysis aim is to tests if the difference in energy between fundamental frequency and second harmonic could indicate a relationship with sex, age and length.

For this analysis only those individuals with at least five signature whistles with a good quality recording were used. Good quality signals were defined as those at least 10 dB above ambient noise and without any overlapping signals. A total of 54 individuals with information about age, sex and length were used for statistical analysis. Weight data was missing in several of these individuals, and it was not included. However length was used as a parameter for size.

The measurements were made in MATLAB directly from the 3D spectrogram (Figure 3.2) of the normalised signals (1024 FFT, Hamming Window, 50% overlap) by clicking on the output figure. For each whistle, I selected the point of highest relative energy from the fundamental frequency (F_0) and the highest energy point in the second harmonic (H_2). The values are given in dB/Hz by the 'spectrogram' function in MATLAB. Then calculated the difference between both values subtracting value of the second harmonic to the fundamental frequency, as the fundamental frequency always had more energy. These values were averaged for each individual and organised in a database including sex, age and length. Only values for the second harmonic were measured to avoid a bias in the comparisons, as higher harmonics show higher directional properties (Branstetter *et al.*, 2012). The difference in energy parameter was tested for normal distribution with a Shapiro-Wilks test ($p > 0.05$), and inspected graphically with a quantile-quantile plot for confirmation. Then, a multivariate linear regression was performed and fitted similarly to the MMR explained previously, using the values of sex, age, and length as predicted variables of the model, and the difference between fundamental frequency (F_0) and the second harmonic (H_2) as the predictor variable. The multivariate model fit was estimated by Pillai's test statistic.

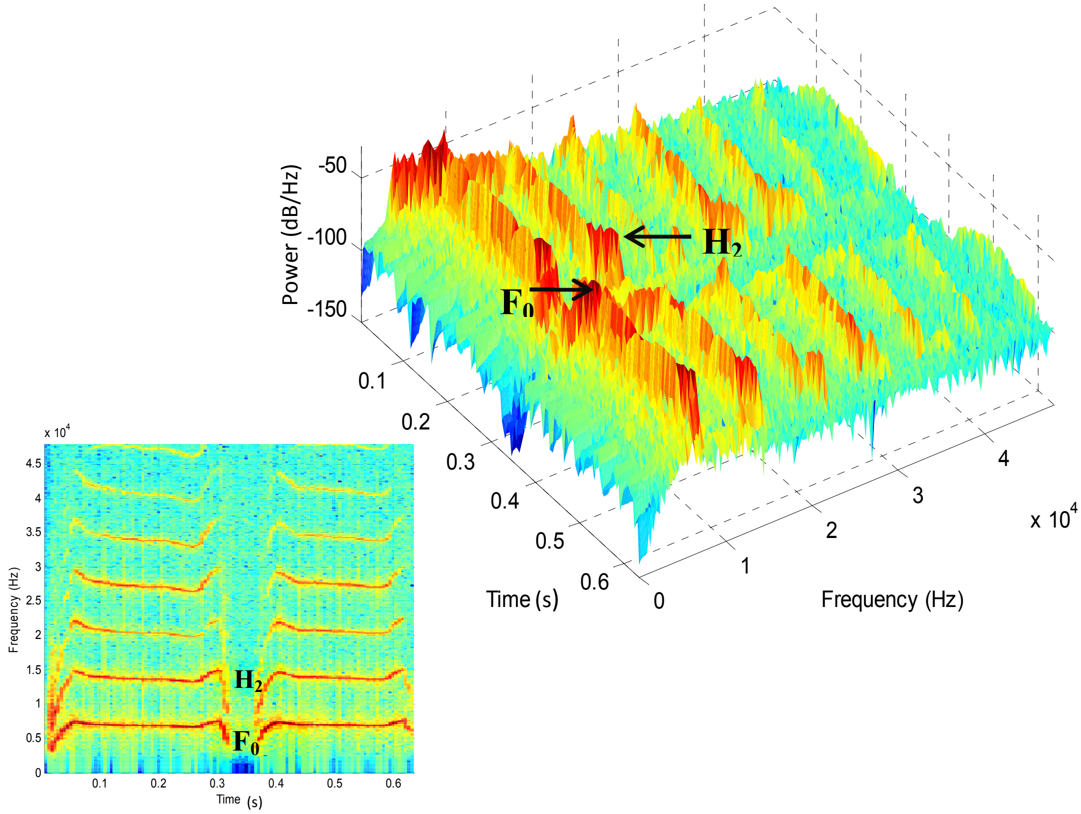


Figure 3.2. Example of 2D and 3D spectrograms of a signature whistle showing the peak energy values in the fundamental frequency (F_0) and the second harmonic (H_2) shown with arrows. Spectrogram parameters: 1024 FFT, 50% overlap, Hamming Window.

3.3 RESULTS

3.3.1 FUNDAMENTAL FREQUENCY

HIERARCHICAL CLUSTER ANALYSIS

The parameters grouped individuals of different sizes, ages and both sexes together. The groups in the dendrogram contain signature whistles with similar acoustic parameter values. This was the case for significant groupings as well as for the rest of the clusters. Thus, the measured parameters do not seem to indicate established clusters based on sex, age or size (Figure 3.3).

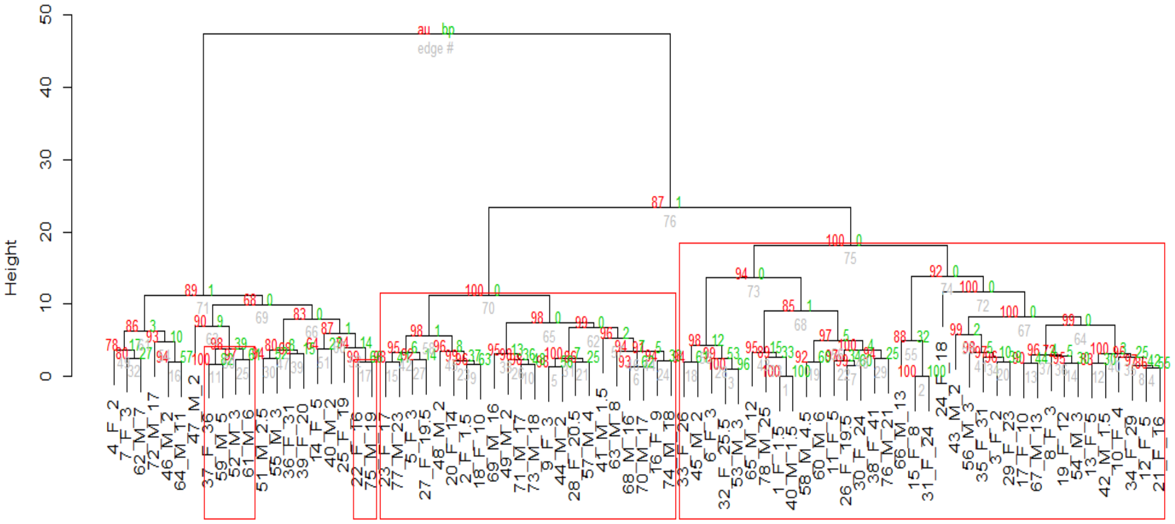


Figure 3.3. Hierarchical Cluster highlight in red rectangles the groups that were formed significantly more often than by chance (AU= 95%, p <0.05). Note that at the end of each branch, each individual label consists of identification number, followed by sex (M or F) and age. The red numbers are the AU (the Approximately Unbiased p-value calculated by multiscale bootstrap resampling) and the numbers in green are the BP (the Bootstrap Probability calculated by normal bootstrap resampling).

ACOUSTIC PARAMETERS: MULTIVARIATE MULTIPLE REGRESSION

In the multiple regression analysis, there were no significant relationships for any parameter when tested against sex, age, length and weight. The summary for the univariate outputs for each predicted variable of the analysis are shown in Tables 3.2a and 3.2b.

The Pillai's multivariate test statistic was calculated in order to test the overall fit of the model. This test calculates the linear hypothesis that the different responses for the dependent variables (SEX, AGE, LENGTH and WEIGHT) are the same across the parameters. The null hypothesis (that there is no significant relationship between predictors and predicted variables) is accepted if this test statistic is small. The results of the multivariate model test did not find any parameter with a significant effect on prediction for the four dependent variables (Table 3.3).

Table 3.2a. Estimate coefficients, t-value and *p* values of the 10 predictor variables for SEX and AGE. Values of F statistics and *p* values for the univariate regression output of each predicted variable in the MMR model are also included.

SEX

	Estimate	t value	Pr(>|t|)
BEG.F	-0.137	-0.751	0.455
END.F	0.363	1.36	0.178
MAX.F	-0.209	-0.5	0.619
MIN.F	0.021	0.115	0.908
F.RANGE	-0.023	-0.063	0.950
DUR.T	-0.154	-1.222	0.226
MEAN.F	-0.191	-0.784	0.436
MID.F	0.338	1.755	0.083
F.GRAD	-0.339	-1.143	0.257
FVI	0.074	0.71	0.479
		F-statistic	*p*-value
		0.9175	0.5227

AGE

	Estimate	t value	Pr(>|t|)
BEG.F	-1.766	-0.488	0.627
END.F	-0.695	-0.131	0.896
MAX.F	1.667	0.201	0.842
MIN.F	2.856	0.77	0.444
F.RANGE	2.156	0.287	0.775
DUR.T	1.368	0.546	0.587
MEAN.F	-4.770	-0.986	0.328
MID.F	0.494	0.129	0.898
F.GRAD	0.701	0.119	0.906
FVI	-0.053	-0.026	0.980
		F-statistic	*p*-value
		0.6529	0.7631

Table 3.2b. Estimate coefficients, t-value and *p* values of the 10 predictor variables for LENGHT and WEIGHT. Values of F statistics and *p* values for the univariate regression output of each predicted variable in the MMR model are also included.

LENGTH

	Estimate	t-value	Pr(>|t|)
BEG.F	-8.56	-0.861	0.392
END.F	4.89	0.336	0.738
MAX.F	3.07	0.135	0.893
MIN.F	7.91	0.777	0.440
F.RANGE	2.82	0.137	0.891
DUR.T	-0.71	-0.103	0.918
MEAN.F	-24.34	-1.832	0.071
MID.F	13.43	1.277	0.206
F.GRAD	-4.62	-0.286	0.776
FVI	2.33	0.409	0.684
		F-statistic	p-value
		0.686	0.7337

WEIGHT

	Estimate	t value	Pr(>|t|)
BEG.F	-14.93	-0.751	0.455
END.F	12.59	0.433	0.667
MAX.F	26.77	0.587	0.559
MIN.F	8.87	0.436	0.664
F.RANGE	-16.80	-0.408	0.684
DUR.T	-0.68	-0.05	0.960
MEAN.F	-47.95	-1.806	0.076
MID.F	28.60	1.361	0.178
F.GRAD	-9.65	-0.299	0.766
FVI	4.93	0.433	0.667
		F-statistic	p-value
		0.5778	0.8264

Table 3.3. Values of the multivariate test statistic model fit of the MMR using Pillai's test statistic showing the approximate F and *p* values for each of the 10 predictor variables. Note that none of the parameters showed statistically significant relationship with the predicted variables.

	Test statistic	Approx. F	Pr(>F)
BEG.F	0.0258	0.4235	0.791
END.F	0.0307	0.5062	0.731
MAX.F	0.0527	0.8914	0.474
MIN.F	0.0333	0.5528	0.697
F.RANGE	0.0578	0.9816	0.424
DUR.T	0.0327	0.5409	0.706
MEAN.F	0.0635	1.0863	0.370
MID.F	0.0823	1.4360	0.232
F.GRAD	0.0234	0.3844	0.819
FVI	0.0127	0.2064	0.934

These results from the multivariate multiple regression can be represented graphically using visual hypothesis plots with the package "heplots" in R (Friendly *et al.*, 2007). The relationships between the different parameters and the predicted variables showed different degrees of influence, but none of them were significant. None of the lines in the visual hypothesis test plots from the multivariate multiple regression protruded beyond the error ellipse indicating that there was no significant influence of any of the parameters (Figure 3.4).

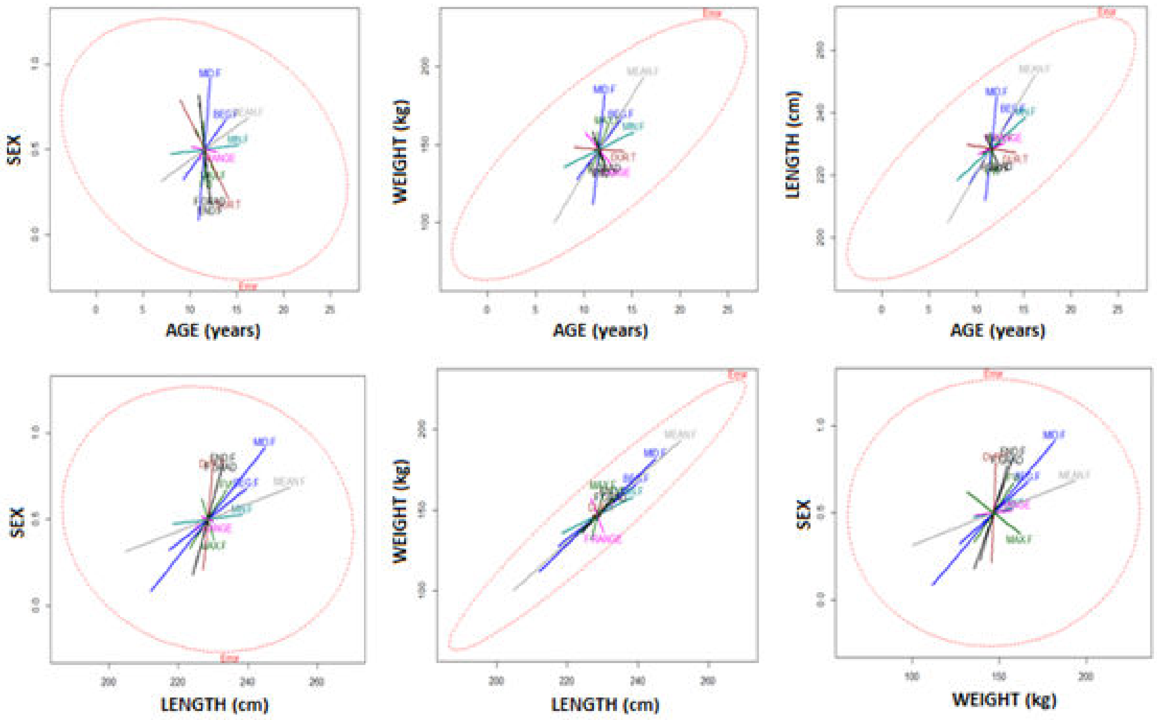


Figure 3.4. Visualisation plots of hypothesis tests in multivariate multiple regression analysis showing the interactions of the 10 parameters and the independent variables: SEX (females=0, males=1), AGE, LENGTH and WEIGHT using the package "heplots" (Friendly, 2007) in R software. The different colour of each line represents a dependant variable. The red circle indicates the error limits. The length of the line indicates the influence of the variable. MEAN F (grey line) and MID F (dark blue) are not significant but show the longest lines.

CONTOUR MODULATION: NEURAL NETWORK ANALYSIS ARTWARP

The results from the ARTWAP analysis showed that the number of categories in which the signature whistles were categorised by group (males, females, sub-adults and adults) did not differ from each other (Table 3.4). A high proportion of variability (PV) indicates that the number of categories is closer to the number of whistles in a group, meanwhile a low PV indicates that several whistles in the group fall into just few categories. The values of proportional variability were very similar for the four groups, indicating that the number of categories set by ARTWARP was almost the same for both sexes and age groups. At the same time, it is important to notice that the value of proportional variability (PV) was lower for the control group that includes all the individuals. This indicates that there was almost the same amount of categories with the double number of contours than with each individual group, suggesting that the majority of categories are shared amongst all four groups and there are not specific categories for sex or age group.

The complexity index was highly similar for the four groups and the control, showing that the different degrees of modulation can be found in any sex or age group. The CI values between 0.60 and 0.65 indicate that the number of contour types is evenly found in the group. This can be corroborated assessing with a barplots that indicate similar number of contours in the different categories between males and females, and between subadults and adults (Figure 3.5). This suggests that specific categories of whistle contour modulation are not specific to one sex or age group.

Table 3.4. Neural network analysis values in ARTWARP showing the total number of whistles, categories, the proportional variability (PV). Note the similar values for all the groups.

	MALES	**FEMALES**	**SUB-ADULTS**	**ADULTS**	**ALL**
Number of Signature Whistles	43	42	42	43	85
Number of Categories	21	22	21	23	29
Proportional Variability (%)	*50*	*51.2*	*50*	*53.4*	*34.1*

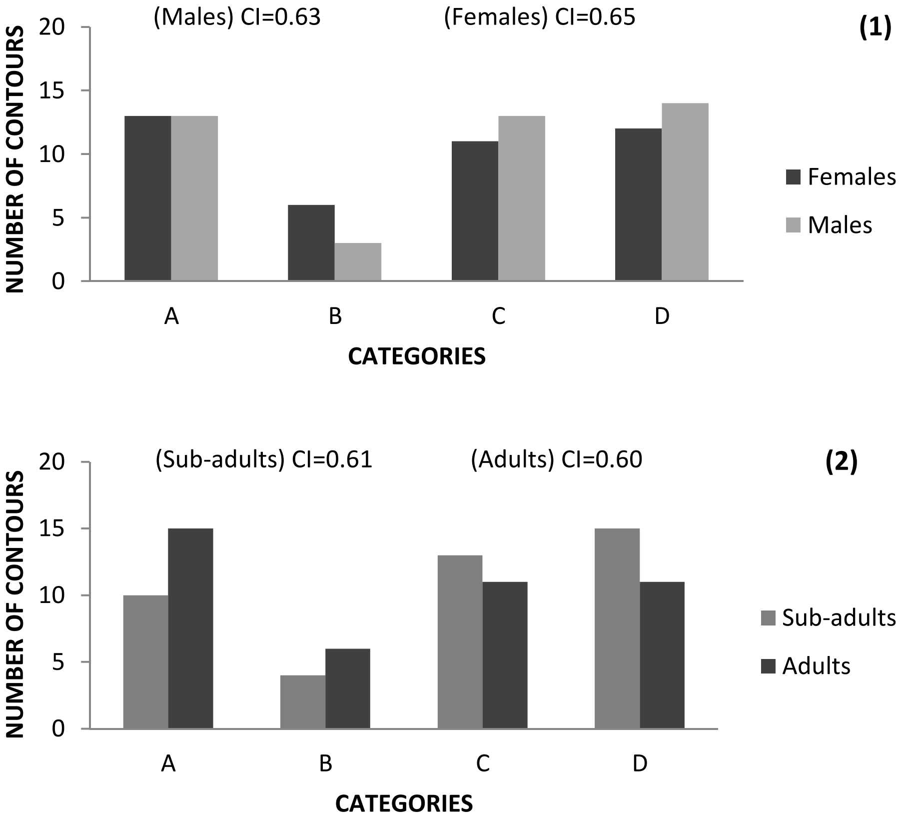


Figure 3.5. Comparison of the number of signature whistles for each modulation category (A, B, C, D) by sex (**1**) and age group (**2**). Complexity Index (CI) values for each group are shown in the top of each graph.

2.3.2 HARMONIC ENERGY CONTENT

The results for the multivariate regression showed that the difference between the peak energy in the fundamental frequency and the peak energy in the second harmonic showed a positive relationship with age (t= 3.125, *p*= 0.003) and length (t= 2.123, *p*= 0.038). This relation was not found for sex (Table 3.5). The graphic output of the relation between the response variables and the predictor variable also showed the significance for AGE and LENGTH (Figure 3.6). In general terms, older individuals tended to have higher peak energy values in the fundamental frequency in comparison to the second harmonic, therefore, a higher difference value. Younger individuals tended to have similar peak energy values for both, the fundamental frequency and the second harmonic, resulting in a lower peak energy difference.

Table 3.5. Estimate coefficients and *p* values for the univariate outcome for the relationships between each variable (sex, age and length) and the differences in peak energy. Moreover, bellow is including Pillai's test for the multivariate model.

		Estimate	*t value*	*F-statistic*	*Pr(>|t|)*
SEX	**F_0-H_2**	0.002089	0.208	0.04319	0.836
AGE	**F_0-H_2**	0.5695	3.125	9.764	**0.003**
LENGTH	**F_0-H_2**	0.9695	2.123	4.507	**0.038**

	Pillai's test statistic	*Approx. F*	*Pr(>F)*
F_0-H_2	0.1829	3.7312	**0.0169**

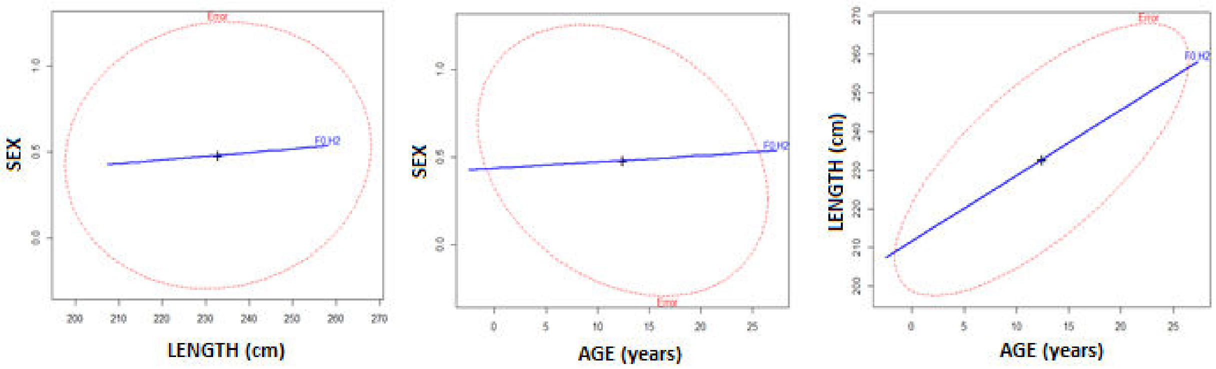


Figure 3.6. Visualisation plots of the interactions of the calculated difference between fundamental frequency and second harmonic (F_0-H_2) and the independent variables: SEX (female=0, male=1), AGE and LENGTH using the package "heplots" (Friendly, 2007) in R. The length of the line indicates the influence of the variable. The variable lines protrude the error ellipse for the relations of AGE /SEX, but only in direction of AGE, and for AGE/LENGTH which indicates significance for the AGE and LENGTH.

3.4 DISCUSSION

Understanding the information that acoustic signals convey can help to underline how they transmit honest information about morphological, physiological and individual information. Many signals are constrained by specific characteristics of individuals conveying information about age, sex, group and size. This information is shared through the acoustic structure of the calls, patterns and sequences. This situation can be found in a wide number of taxa such as primates (Ey *et al*., 2007; Lameira *et al*., 2013), pinnipeds (Sanvito *et al*., 2007), birds (Riede *et al*., 2010), red deer (Reby & Charlton, 2012), and frogs (Boyd *et al.*, 1999; Smith & Roberts, 2003). These are driven to some degree by sexual and natural selection. For all the aforementioned cases, the acoustic signals are not signature calls, but consist of a shared repertoire amongst conspecifics.

For bottlenose dolphins, I focussed on fundamental frequency parameters. Their signature whistle seems to be arbitrary modulation patterns that individuals invent themselves (Janik, 2013). This species has shown that they are able to use arbitrary sound, for example, they copy signature whistles to address conspecifics (King *et al*. 2013; King & Janik, 2013). Therefore, a motivation for this study was to determine to what extent there are other constraints on the development of these signals. The results in this chapter showed that the main parameters measured in signature whistles do not group individuals by sex, age or size with an unsupervised method as simple clustering (Figure 3.3). Likewise, the results from the multivariate regression of this study (Table 3.2) indicated that none of the parameters measured from signature whistles correlated directly with sex, age or size (Figure 3.4). There are examples of species where acoustic signal parameters do not convey morphological information either. Some species of horseshoe bats (*Rhinolophus spp.)* do not show acoustic sexual differences in their calls (Siemers *et al*., 2005). Likewise, male and female brown skuas (*Stercorarius antarcticus*) do not show significant differences in acoustic parameters measured in their three main calls (Janicke *et al*., 2007).

Mean frequency showed a trend for an inverse correlation with age and size. This means that large animals tend to have lower mean frequencies than small individuals. Even though this parameter was not statistically significant in the relationship with size, it had the strongest influence in the model. Such pattern with an inverse relationship between mean frequency and body size has been found for several species of odontocetes using all the whistle repertoire and not specifically signature whistles (Matthews *et al*., 1999). There is also a general correlation between size and sound production, where larger individuals produce lower fundamental frequencies (Fitch, 2000; Fletcher, 2004). Following this idea, acoustic signal's properties include not only the fundamental frequency but also their harmonic content. The energy difference between the fundamental frequency and the second harmonic in signature whistles varied significantly with age and length but not with sex. Unlike the relationship with mean frequency, this relationship was significant. This inverse relationship between having higher energy in the fundamental frequency than in the second harmonic and age could be caused by differences in sound production organs or tissues rather than a total length, as it has been shown in brown bats and baboons that ageing has an effect on their sound production organs (Moss, 1988; Ey *et al*, 2007). The lack of a relationship with sex could indicate a lack of differentiation in sound production structures between males and females. The general tendency was that the relative energy difference in fundamental and harmonics was higher in older and in longer animals than in smaller and younger ones, with a larger peak energy in the fundamental frequency then in the second harmonic. Miller *et al*., (2007) found that killer whales' energy ratios were higher in males than in females. They suggested that the large difference in size could be an important factor. The energy distribution across harmonics also varies with the density of the medium and the depth (Ridgway *et al.*, 2001; Madsen *et al*, 2012). However, the recordings compared in this work were obtained near the surface and in semi-controlled conditions so that this was not a factor here. Spectral characteristics might vary depending on morphological characteristics. Therefore, specific internal structures in bottlenose dolphins such as air nasal sacs and nasal cavities size could account for the difference found in my work.

It is possible that dolphins use harmonic information. Branstetter *et al*., (2013) have shown that dolphins can detect variation in harmonic content to a certain degree to identify the direction of whistle. This harmonic information could be used near the surface by dolphins to extract recognition information. Nonetheless, more experiments are necessary to understand if harmonic energy content plays an important role in age or size recognition, considering that in water, the speed of sound and the pressure could complicate the detection of relative energy differences in high harmonics during deep dives (>100m). Hence, even though dolphins can use harmonic information, other parameters or characteristics that could be more easily perceived at higher depths may play a more relevant role in recognition.

It has been shown that the fundamental frequency contour pattern without vocal cues is enough for a dolphin to recognise a familiar signature whistle (Janik *et al*, 2006) and Janik (1999) argued that this robustness to noise and pressure may have contributed to the evolution of vocal learning in bottlenose dolphins. Thus, the signature whistle modulation pattern represents a very specific adaptation to individual recognition in the marine environment. Interestingly, the parameter found to vary with age and size here (relative energy distribution across harmonics) do not affect the modulation pattern of the signature whistle and therefore do not compromise the encoding of identity. The contour modulation analysis showed that the different groups (MALES, FEMALES, SUBADULTS and ADULTS) had similar degrees of whistle modulation complexity in their signature whistles (Figure 3.5). Most of the contour categories in the ARTWARP analysis were shared (Table 3.4) indicating that similar types of modulation patterns are used for signature whistles across age and sex classes. Thus, physiological and morphological characteristics do not seem to determine the parameters of the signature whistle fundamental frequency, nor its modulation pattern. There is a lack of evidence for specific male or female signature whistles or size defined parameters in whistle contours. This supports the idea that these whistles are truly arbitrary signals that are independently defined by vocal learning as suggested by Janik (2013).

The function of many vocal signals in the acoustic repertoire of bottlenose dolphins remains unknown. Especially the role of non-signature whistles and other pulsed sounds is still poorly understood. These calls may carry information on sex, age, and size of the animal. Furthermore, studies on general voice features in dolphins are rare. My study here was the first to address the role of voice in sex, age and size communication and found little evidence for such information encoded in signature whistles. Instead, I have found that signature whistle modulation patterns seem to be truly arbitrary signals. Sex, age and size do not constrain the acoustic parameters or modulation patterns that individuals use in signature whistles. Dolphins might use visual and acoustic cues combined with their long-term memory (Bruck, 2013) for sex and age recognition, and even to determine individual sizes. Therefore, further research on different signals in the bottlenose dolphin repertoire can help to unravel the transmission mechanisms of that information in this species.

CHAPTER 4

BOTTLENOSE DOLPHINS THROUGH TIME: HOW STABLE ARE SIGNATURE WHISTLES?

4.1 INTRODUCTION

Growth and ageing are fundamental parts in the development of animals. Senescence rates vary across species and can be dependent on environmental factors and genetic traits (De Magalhães, 2006; Nussey *et al.,* 2013). In addition, ageing might also account for changes in behaviour and the way individuals interact with each other as well as the cognitive abilities involved (Willis *et al*., 2009; Burke *et al.*, 2012), and might also affect the signals that are used for social interactions.

Acoustic signals are widely used for communication, ranging from simple sounds, alarm signals, nonlinear signals and complex songs in a variety of taxa (Wiley & Richards, 1982; Edds-Watson, 1997; Zelick *et al*., 1999; Ladich, 2000; Gillooly & Ophir, 2010). Social acoustic communication is essential for the cohesion and interactions of a group in species that rely highly in sounds. Variation of acoustic signals related to age has been found in species of bats and primates (Moss, 1988; Moss *et* al., 1997; Ey *et al*, 2007). Ageing of tissues involved in sound production is an important part of the change in some properties of acoustic signals. However, behavioural changes due to specific contexts (e.g. mating season, perceived danger) and interactions with conspecifics also intervene in the shaping of social vocalisations. For example, the variation of alarm calls in male meerkats (*Suricata suricatta*) is not only influenced by social context, but also by the motivation of the caller (Townsend *et al.*, 2012). Egyptian fruit bats

(*Rousettus aegypticus*) develop similar repertoires to those individuals in the colony as long as they have social interactions (Prat *et al*., 2015). Likewise, it has been shown that budgerigars (*Melopsittacus undulatus*) can learn songs as adults through vocal imitation during exposure to social interactions (Farabaugh *et al*., 1994). Moreover, the stability of social acoustic signals and group dialects might vary depending on the context over time. Bird song might stay stable depending on the environmental conditions. For example, zebra finches (*Taeniopygia guttata*) produce learnt songs that will remain stable all their lives, as long as their hearing abilities remain intact (Lombardino & Nottebohm, 2000). In addition, O'Loghlen *et al* (2013) studied the whistle song of brown-headed cowbirds (*Molothrus ater*) of the Convict dialect in California, finding that the song is remarkably stable for as long as 30 years, regardless of the differences from neighbour dialects. On the other hand, changes can occur at group level gradually over time as a consequence of individuals modifying acoustic signals without being directly the result of physical ageing but rather innovation. For example, humpback whale (*Megaptera novoangliae*) songs have shown a continuous change in time in some populations (e.g. shifts, additions or deletions in components of song structure). In various geographic locations, songs vary with a longitudinal direction (East or West) and gradually generate new songs (Payne & Payne, 1985; Payne, 2000; Eriksen *et al*., 2005). Here, variation seems to be related to social interactions and exposure to acoustic elements of other songs (Mercado *et al*., 2005). On the contrary, social calls in this species in the South Pacific seem to remain stable over time with minor variations (Rekdahl *et al.*, 2013).

Within odontocetes, killer whales (*Orcinus orca*) dialects from the North Eastern Pacific remain stable within clans. However, variation in specific calls can be caused by oblique transmission which means that they can be learnt from other clans and not only pass from mothers to offspring (Deecke *et al*., 2000). Furthermore, killer whale clans in the Northern region share stereotyped whistles amongst them, but those repertoires are different from those in the Southern clans (Riesch *et al.*, 2006). Similarly, sperm whales (*Physeter macrocephalus*) produce clan distinct coda dialects that have been shown remain stable for at least 6 years (Rendell & Whitehead, 2005). In birds, the yellow-naped amazon (*Amazona auropalliata*) has different dialects corresponding to geographic areas, and the acoustic properties of those calls remain stable for as long as 11 years with a stable geographic distribution (Wright *et al.,* 2008).

The effects that ageing might have on acoustic repertoires and the stability of vocalizations differ depending on the stage of physical development of an organism, varying across species. For example, new-born finless porpoises (*Neophocaena phocaenoides*) produce long duration, low frequency pulsed sounds during the first hours after birth, and only after the first month do they start producing high and low frequency sounds simultaneously (Li *et al*, 2008). Sperm whale neonates produce clicks with lower directionality, lower frequency and longer duration than those produced by adults. Older calves may produce repetitive trains of clicks that are sometimes considered to be precursors to the coda pattern of adults (Madsen *et al*., 2003).

Signature vocalisations are specific signals that present acoustic characteristics sufficiently distinctive for a receiver to identify the caller (Shapiro, 2010). A high degree of stability would be expected in these types of signals since the individual identity of a caller does not change over time. Slow changes may not affect individual recognition if those changes are not detected by the receiver or if receivers learn to recognise the new variant of the individual call. Greater horseshoe bats (*Rhinolophus ferrumequinum*), for example, show a decrease in the peak frequency of their echolocation clicks over time (Jones & Ransome, 1993). These signals are also used for mother-pup recognition, but each infant only depends on the mother for a very short time period, over which acoustic parameter changes in the mother's clicks would be minor. Signature vocalizations have been found in several species. Common marmosets (*Callithrix jacchus)* produce a ‘phee call’ that is a tonal sound that differs from their alarm calls and with properties specific to an individual (Jones *et al*., 1993). African grey parrots (*Psittacus erithacus*) produce a specific call that is learnt by their offspring (Giret *et al.*, 2012). In squirrel monkeys (*Saimiri sciureus)*, mothers can recognise their offspring by the ‘isolation peep’ call produced by their own infant (Symmes & Biben, 1985). Likewise, the existence of signature vocalizations has been also claimed for narwhals (*Monodon monoceros*) (Shapiro, 2006), belugas (*Delphinaptarus leucas*) (Morisaka *et al.*, 2013), fallow deer (*Dama dama*) (Reby *et al*., 1998) and meerkats (Townsend *et al*., 2011), although there have not being studies about their long-term stability.

Bottlenose dolphins (*Tursiops truncatus*) are very social animals that live in fission-fusion societies and acoustic recognition plays an important role for their social interactions in order to maintain cohesion. Bottlenose dolphins are also capable of vocal learning (Janik, 2014). They modify whistles, innovate signals, and copy their conspecifics (Janik, 2013). Signature whistles start developing in early in life, during the first months since birth when calves start producing whistle-like sounds until a specific modulation pattern becomes stereotypical (Caldwell & Caldwell, 1979; Killebrew *et al*., 2001; Fripp & Tyack, 2008). The crystallisation of the signature whistle seems to be influenced by sounds in their environment or whistles from others individuals in the community (Miksis *et al*., 2002; Fripp *et al*., 2005). In addition, signature whistles are believed to be highly stable, as Sayigh *et al*. (1990) suggested that signature whistles contours from a sample of mothers and calves remain stable for at least a decade. However, it has been shown that some acoustic parameters in whistles might vary with time. For example, Smolker & Pepper (1999) showed that adult males are able to modify their whistle repertoire to match their alliance partner, and Cadwell & Cadwell (1979) found a degree of variation in whistles of calves during the first months of life.

In Chapter 3, I showed that signature whistle parameters are not constrained by sex, age and size for different individuals in the Sarasota Bay community. Therefore, the aim of this study was to address whether there are parameters that vary and remain stable within signature whistles of the same individuals and how stable the contour modulation of these signals is across a period over 20 years.

4.2 METHODS

4.2.1 DATA PROCESSING

Information about data collection during capture-release session and the study area of Sarasota Bay are explained in Chapter 2 (sections 2.2 - 2.4). For this chapter, I reviewed the general database of recorded animals from 1975 to 2013 in order to identify potentially useful individuals for whistle stability analysis. As most individuals were recorded more than once, the aim was to select individuals with a long record of capture-release sessions. I then selected recordings of at least three different years for each individual from the youngest year possible to adulthood. From the database, 26 individuals with long records of up to 29 years were available.

For analysing older recordings taken with video-cassettes or stereo-cassettes it was necessary to digitise them first using a Panasonic AG-7350 VHS connected to a Sound Devices 744T digital recorder. A total of 94 tapes were digitised at 96 kHz and 16 bits. For data after 2005, digital recordings were available to be used directly. Then, each recording was reviewed visually with spectrograms using Adobe Audition 2.0 (Adobe Systems) with parameters of 512 FFT size, 85% overlap, Hamming window and 48 kHz resolution window. Several of the recordings for the analysis were discarded, either because the signal-to-noise ratio in the recording was too low or because there were no signature whistles during the recording. The final sample size was 17 individuals with three to six sampled years including recordings of animals of 1 to 50 years of age (Table 4.1).

For the analysis in this chapter, I extracted 20 signature whistles for each given sampled year for each individual, and extracted contours and acoustic parameters as stated in Chapter 2 (section 2.5).

Table 4.1. Individuals selected for the analysis of the stability of signature whistles, including information about age at the time of recording, sex and years covered for each individual.

INDIVIDUAL	SEX	AGES						YEARS	YEAR SPAN
FB06	Male	1	4	10	19			1985-2003	18
FB09	Female	1	6	20				1985-2004	19
FB11	Female	1	5	14	20	24		1985-2008	23
FB07	Female	1	19	27				1985-2012	26
FB92	Male	2	7	13				1990-2001	11
F157	Female	2	11	18				1990-2006	16
FB20	Male	2	6	17	23			1991-2012	21
FB33	Female	2	8	12	18	24	31	1984-2013	29
FB62	Male	3	16	22				1976-1995	19
FB55	Female	3	8	16	23			1989-2009	20
FB10	Male	4	12	25				1985-2006	21
FB05	Female	12	29	38				1975-2001	26
FB54	Female	13	27	35				1984-2006	22
FB90	Female	20	28	36	43			1990-2013	23
FB63	Female	22	31	40	47			1976-2001	25
FB48	Male	25	32	41				1984-2000	16
FB15	Female	34	41	50				1984-2000	16

These parameters were used in a linear mixed model (LMM) in order to investigate what parameters might vary and stay stable over time in the same signature whistle. In addition, a comparison of the fundamental frequency contour between the sampled years in order to determine stability of modulation patterns was also performed. The detailed information about these analyses is described in the following methods sections.

4.2.2 LINEAR MIXED MODEL USING ACOUSTIC PARAMETERS

A linear mixed model (LMM) was used to assess whether acoustic parameters measured on the signature whistles of an individual changed across years. The mixed model was chosen to give more power including the whole set of individuals in a single model and to be able to account for the effect of individual variation in the dataset in contrast to a regular linear model.

The analysis was run using the package “lme4” (Bates *et al*., 2015) in R software (R 3.2.0, GNU project). First, a revision of the collinearity of the acoustic parameters was conducted, creating a table of correlation for all the parameters (Appendix 2). For every compared pair of parameters, one of the parameters was dropped if showed a high correlation (> 0.55) with the other. This threshold value was selected to be restrictive enough (Dormann *et al*., 2013). This was done to avoid issues in the mixed model, as even if collinear variables are not necessarily redundant, a large number of fixed effects can complicate the calculation of random factors and models fail to converge values (Dormann *et al*., 2013; D. Russell, pers. comm. 2016.). Then, the remaining parameters were used in a forward selection using the AIC (maximum likelihood) value to select the best model (MAX.F, MIN.F, MEAN.F, DUR.T, LOOPS, F.RANGE and IP). A forward selection is a suitable option when it is unclear if all the parameters are well suited for the model, and it is a valid model selection because it does not compromise the significance of the final model, as the AIC only represents a comparison value of the fit of each model (Bozdogan, 1987;

Hosmer & Lemeshow, 2004; D. Russell, pers. comm. 2016). The forward selection consists of adding a new parameter to the model at a time until the AIC value is minimised. If adding a new parameter increases the AIC value, the best model is the one without that added parameter. For the model I set a random intercept for individual and a random slope for each predictor variable. Random slopes are often discarded, but they are necessary when there is a valid assumption that there could be variation within individuals for each predictor in the model, as is the case for different signature whistles. Avoiding including slopes in mixed models can lead to non-conservative *p*-values (Schielzeth & Forstmeier, 2009; Barr et al. 2013). Therefore, it is recommended to add all the slopes for the fixed effects that might be important for the interpretation. The best model had AGE as a predicted variable and the acoustic parameters MAX.F and MEAN.F as predictor variables. Moreover, individual (ID) was included as a random effect, including the random slopes for MEAN.F and MAX.F. The criterion of convergence was checked to be <0.001. Finally, p values for the fixed effects were calculated from F statistics via Kenward-Roger approximation using the “sjPlot” package in R (Lüdecke, 2016) as “lme4” does not calculate this value (Bates *et al*., 2015).

4.2.3 MODULATION PATTERN SIMILARITY COMPARISON OVER TIME

To analyse the variation in modulation of the fundamental frequency, the whistle contours from the individuals were used for comparing the similarity of the patterns over time. For those whistles composed of more than one unit, only the first unit was used since all units of each multi-looped whistles in this sample had the same modulation pattern. This decision helped to avoid bias introduced by silent intervals between loops.

For each of the 17 individuals, the whistle contours were compared using the dynamic time warping (DTW) algorithm in the ARTWARP code, which was described in Chapter 2 (section 2.1.5).

For each individual, five random signature whistle contours were selected for each sampled year. Each of the five contours was used in a pairwise comparison with the five contours of the consecutive sampled year using ARTWARP to obtain a percentage of match between contours (e.g. the individual when 1 year old vs. same individual when 6 years old, then when 6 years old vs. 14 years old, then 14 years old vs. 23 years old, and so on) (Figure 4.1). From these, an average similarity was calculated for the comparison between subsequent sampled years. Using the same procedure, I also compared the whistle contours between the first sampled year and the last sampled year (Y1/Yn) for each individual to examine the total variation in the span of sampled years. The number of consecutive comparisons varied as the number of years sampled was not the same for all individuals (e.g. 4 sampled years equal to 3 possible consecutive comparisons) (Table 4.1).

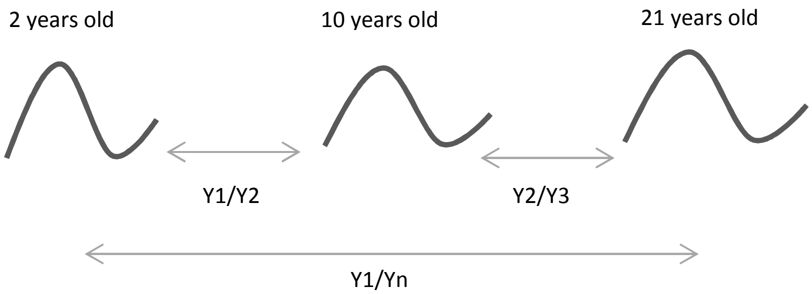


Figure 4.1. Example of the procedure used to compare the contours for each individual across the years. The lines indicate the contours compared. The comparisons were performed between consecutive sampled years (Y1/Y2; Y2/Y3), and between the first year sampled and the last year sampled (Y1/Yn).

To test the stability of whistle modulation patterns, a Wilcoxon paired signed-rank test was used to compare the mean values of similarities between the first year and the second sampled year (Y1/Y2) and the similarity between the first and last sampled year (Y1/Yn) of all the individuals. To test whether males and females differed in their stability across years, a Mann-Whitney U test was performed comparing the similarities between the first and last sampled year (Y1/Yn), the whole time span of the sample, between both sexes.

4.3 RESULTS

4.3.1 PARAMETER VARIATION AND STABILITY

The results from the linear mixed model showed that only one parameter (MEAN.F) showed a significant relationship with age. For the fixed effects, maximum frequency showed a fitted increase of 1 kHz by every 0.9 years increase in age, but this was not statistically significant (p =0.209). Mean frequency showed a significant fitted decrease of one kHz by every 5.9 years (p= 0.030) for the overall modelled fixed effect (Table 4.2 and Figure 4.2). The intercept value is not of particular interest when predictors are continuous as it represents the mean of the outcome when the predictors are zero (Berry & Feldman, 1985; Jaccard & Turrisi, 2003). Moreover, there was a significant effect of the random intercepts and slopes compared with a model with only random intercepts (χ^2= 449.4, p <0.0001) or without any random effect (χ^2=1641.5, p <0.0001). In the model, the random effects of Individual (ID) and slopes accounted for most of the variance (99%) (Table 4.2). Therefore, it is important to have accounted for intercept and slopes. The linear trend lines for both predictors are shown in Figure 4.2.

It is necessary to take in account that these relationships are modelled for all the individuals with variable intercepts and slopes, allowing considering the possibility of differences amongst the individuals and giving a general trend. It has to be noticed that there is variation within the of signature whistle in the dolphins trends showed in the fitted random intercepts and slopes for both predictors (Figure 4.3).

Visual inspection of residual plots did not reveal any obvious deviations from the assumptions and confirmed a good model fit (Appendix 2).

Table 4.2. Results from the linear mixed model (LMM), indicating the coefficient estimates, standard error and t-values. The p-values were calculated via Kenward-Roger approximation. Random effects variance values are also shown.

Fixed effects:	*Estimate*	*S.E.*	*t-value*	*p-value*
Intercept	57.72	26.10	2.211	0.027
MEAN.F	-5.92	2.67	-2.187	0.030
MAX.F	-0.93	0.74	1.255	0.209
Random effects:				
Intercept and slope	*ID (MEAN.F)*	*ID (MAX.F)*	*Residuals*	
Variance	8842.1	2418.3	33.94	
Groups, ID	17			
Observations	1218			

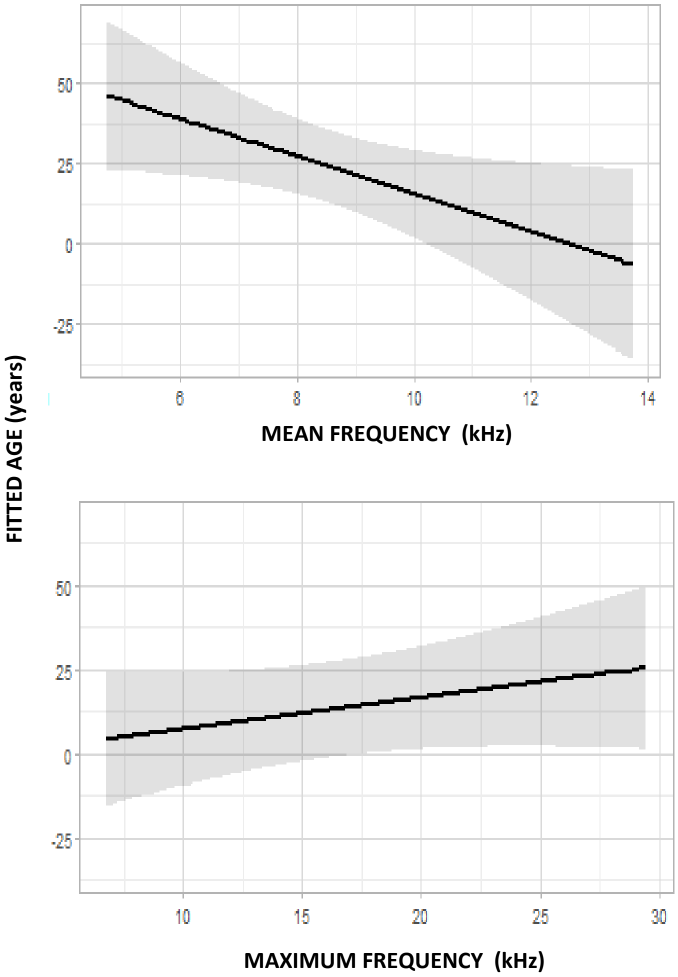


Figure 4.2. Plots showing the relationship of fixed effects from the linear mixed model (LMM). The black solid lines indicate the best fit linear trend lines for the combined overall effect of each dolphin (ID= 17) with 95% confidence intervals (grey shade) for each predictor: mean frequency and maximum frequency. Mean frequency tends to decrease significantly over time (*p*= 0.030).

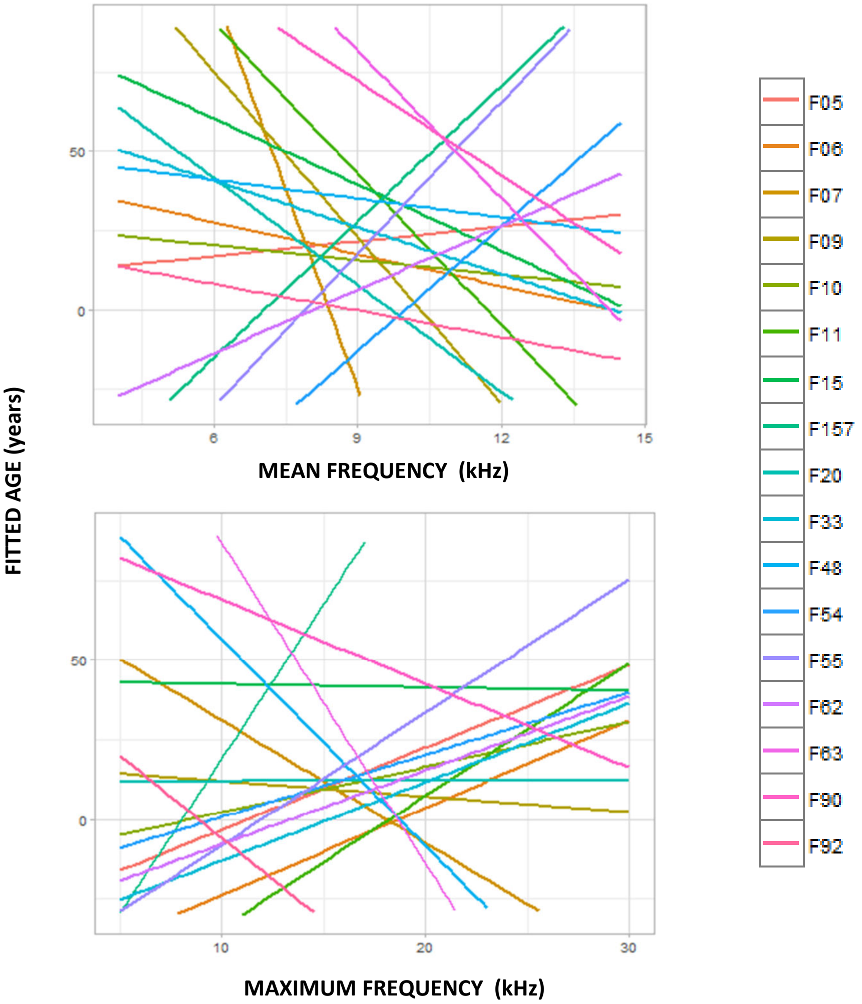


Figure 4.3. Plots showing the relationship of random effects (intercept and slopes). The solid lines indicate the linear trend lines of each dolphin for both predictor variables: mean frequency and maximum frequency. Each colour represents one individual in the sample. Note that x-axes are in different scale between both acoustic parameters.

4.3.2 MODULATION PATTERN STABILITY

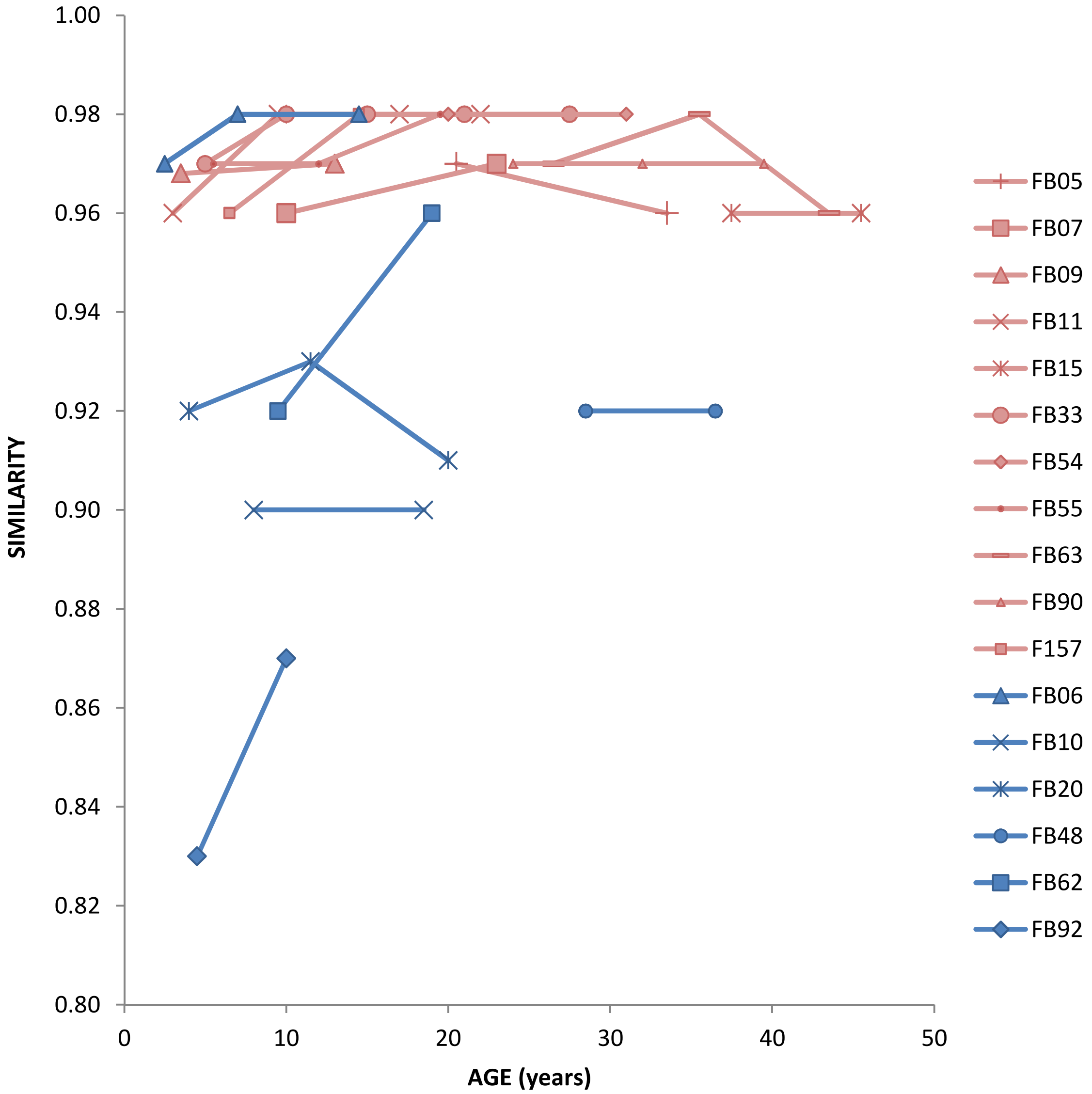


Figure 4.4. Similarity values for each pair of continuous sampled years for all the individuals. Data points are placed along the *x*-axis half-way between the two compared ages for each whistle comparison. Points from the same individual are connected by a straight line. Blue colours correspond to males and pink colours to females.

The contour similarity analysis showed that the modulation patterns of signature whistles remain over 90% similar across the years for up to 29 year (Figure 4.4). The only exception was a male dolphin, FB92, which showed the lowest similarity between the first and the second sampled year, 83%. However, none of the whistle contours showed lower matches, indicating a high stability over time.

The results of the Wilcoxon paired rank test (V=51, *p* = 0.72) showed that the similarities between the first sample year and the second sampled year (Y1/Y2) of all the individuals were not statistically different than the similarities between the first sampled year and the last sampled year (Y1/Yn) (Figure 4.5). This indicates that each signature whistle contour has remained relatively stable throughout the year span between 11 and 29 years (Table 4.1).

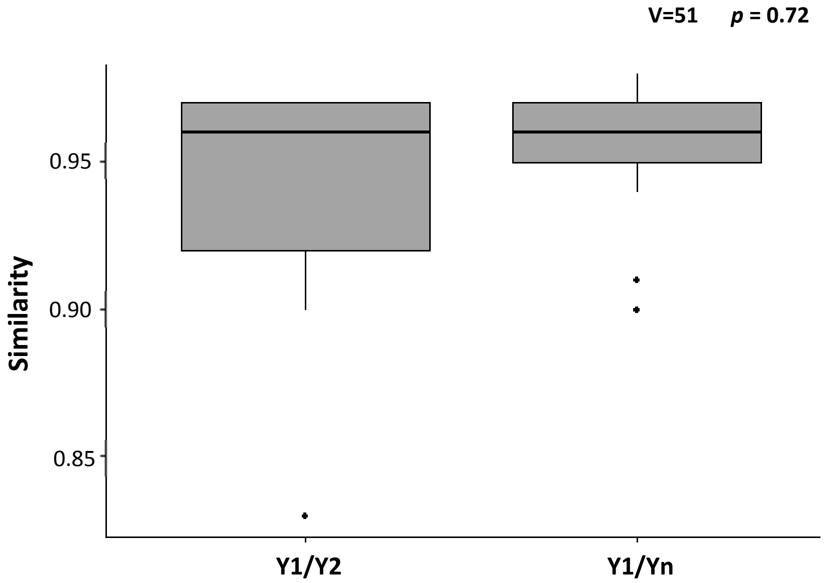


Figure 4.5. Boxplots showing the mean value and standard deviation for the comparisons between the similarity of whistles from the youngest sampled year and the consecutive year (Y1/Y2) versus the similarity of whistles from the youngest and oldest sampled years (Y1/Yn) for all individuals. Note that no significant difference was found. Values of the Wilcoxon paired test are shown in the upper-right corner on the graph.

The results of the Mann-Whitney U test (W=58, $p = 0.01$) showed that the similarities between the first year sampled and the last year sampled were significantly different between males and females. This means that males modify their signature whistles over time to a higher degree than females (Figure 4.6).

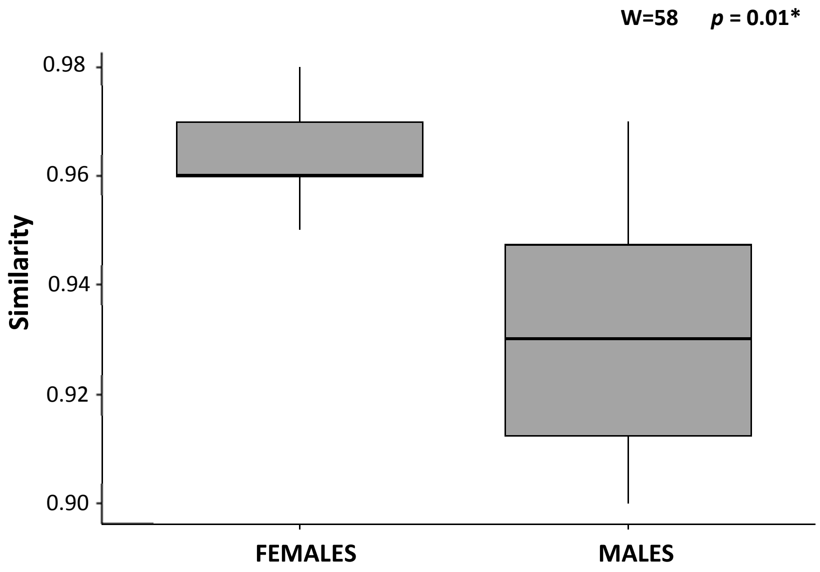


Figure 4.6. Boxplots showing the mean value and standard deviation of the comparisons between the first sampled year and the last sampled year (Y1/Yn). There was a significant difference in the similarity of whistles from the youngest and oldest sampled years between males and females. Values of the Mann-Whitney U test are shown in the upper-right corner on the graph.

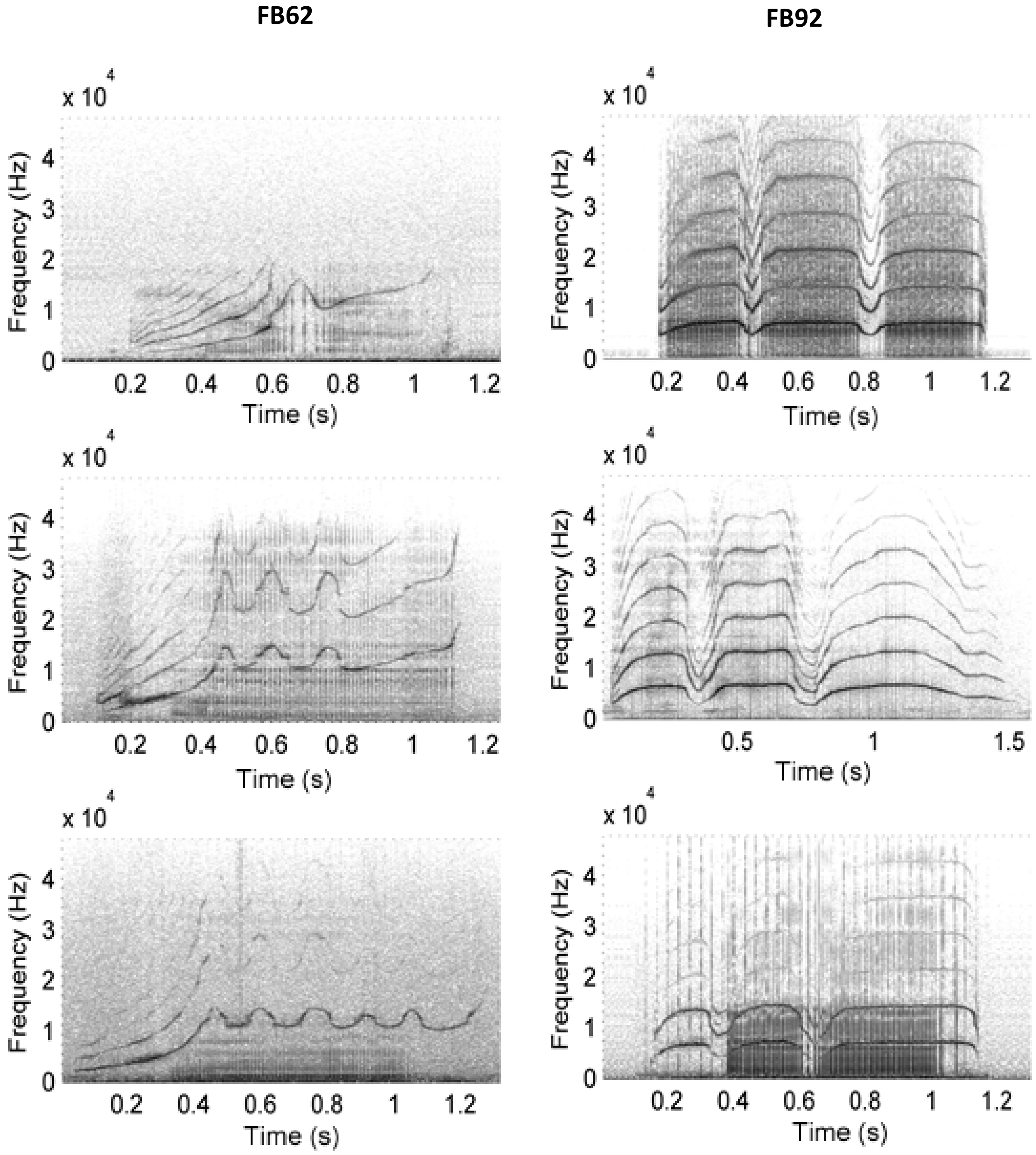


Figure 4.7. Spectrograms showing the differences through time in the signature whistles of two males, FB62 (right) and FB92 (left) at the age of **3**, **16** and **22** for FB62 and at the age of **2**, **7** and **13** for FB92. Spectrograms parameters: 1024 FFT, Hamming Window, 50% overlap, 48 KHz resolution. These two individuals showed highest variation across the years. However the similarity between years was never lower than 83% for FB92 and 92% for FB62.

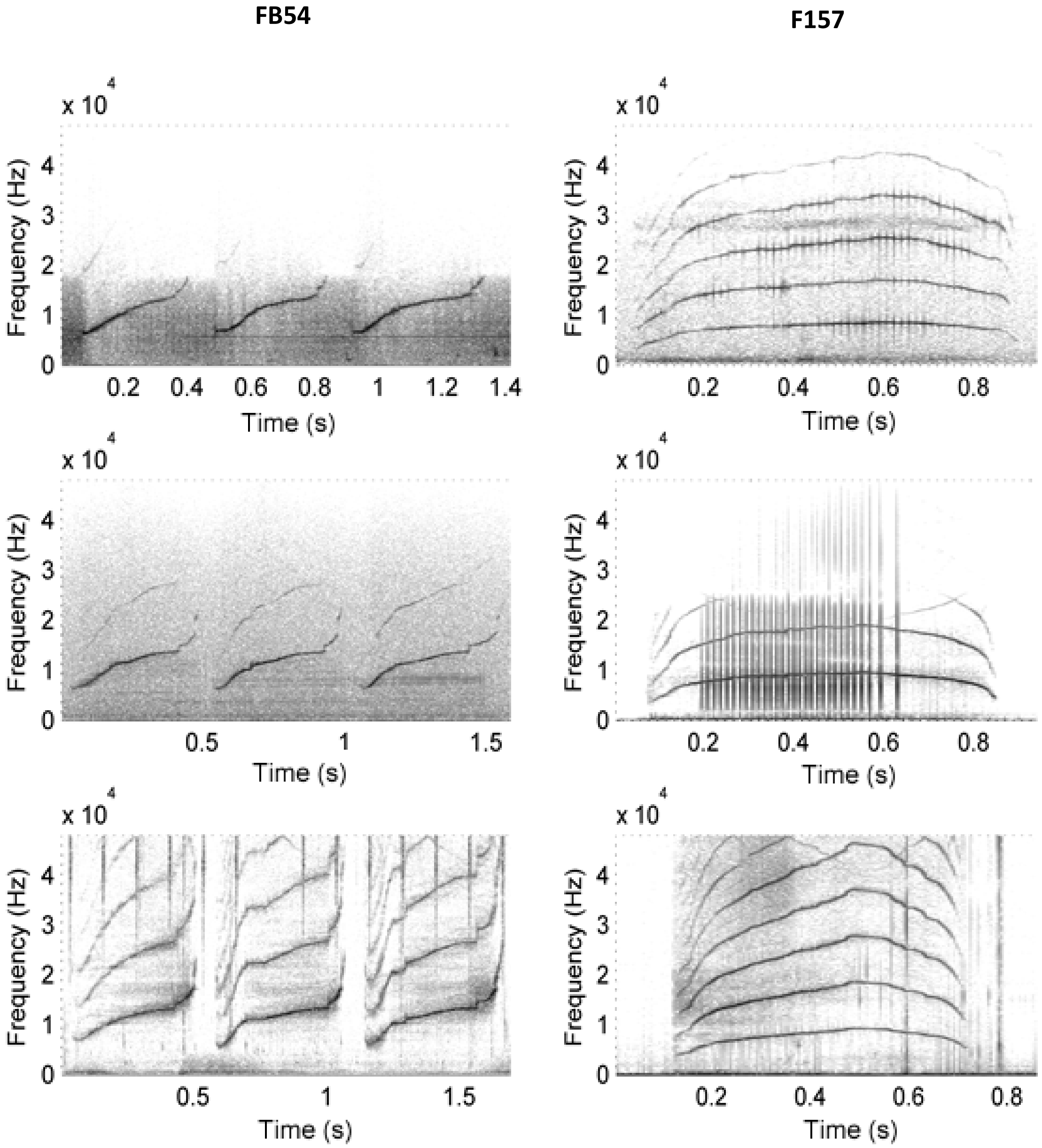


Figure 4.8. Spectrograms showing the differences through time in the signature whistles of two females, F157 (right) and FB54 (left) at the age of **2**, **11** and **18** for F157 and at the age of **13**, **27** and **35** for FB54. Spectrograms parameters: 1024 FFT, Hamming Window, 50% overlap, 48 KHz resolution. These two individuals showed low degree of variation across the years, showing a similarity between years was never lower than 96%.

4.4 DISCUSSION

The stability of signals through time is highly important when specific information needs to be transmitted repeatedly and in a reliable way. For species that use songs, acoustic displays are usually for reproductive and mating purposes. Here, changes over time may be desirable if they help to establish fitness and increase competition and selection. However, a high degree of stability of those characteristics in signature calls that are important to convey identity information is expected as long as identification is required over that period of time (Shapiro, 2010).

Signature whistles convey information through the fundamental frequency modulation pattern (Janik *et al*., 2006). However, little is known if there are specific parts of that modulation that convey more information and how much this signature call change over time. Moreover, it is know that some whistle parameters might vary with context (Janik *et al*., 1994; May-Collado, 2010), but long term individual signature whistle stability has been only studied using contour modulation similarity (Sayigh *et al*., 1990).

Acoustic parameters measured in the fundamental frequency of other species vocalisations like yellow-naped amazon calls, eagle owls individual calls (*Bubo bubo*), and male blue monkeys (*Cercopithecus mitis stuhlmanni*) individual ‘pyow’ calls have shown stability for 2 to 10 years (Butynski *et al*., 1992; Lengagne, 2001; Wright *et al.,* 2008). For the signature whistles used in this study, most of the acoustic parameters could be considered to be stable over time, as only maximum frequency and mean frequency contributed to the best model fit in the linear mixed model (LMM), and only the latter showed a significant relationship with age. Mean frequency in many mammals generally can be used to indicate or predict age in a population, as with the increase of age the mean frequency commonly decreases, considering that older individuals are larger (Moss, 1988; Fletcher, 2004). Moreover, when comparing tonal calls from different odontocetes, there seems to be a correlation between mean frequency and body size (Matthews *et al*., 1999). However, this relation refers to the whole repertoire of tonal sounds from different species.

Unlike the results for the multivariate multiple regression (MMR) in Chapter 3 from this thesis, an inversely proportional relation between mean frequency and age was found for individual signature whistles over time. However, this apparent discrepancy can be explained by the fact that for the MMR analysis the data consisted in different signature whistles of a large number of individuals collected at just one point in time (each individual sampled just at one year). The aim of that analysis was to detect if any of the variables measured could predict age, sex or size in any signature whistle. Those results showed that mean frequency does not predict age for different signature whistles. On the other hand, in this chapter, I used several sampled years and the LMM accounted for different intercepts and slopes by individuals in order to fit the best model with parameters that could predict age within the same signature whistle over time and investigate stability in parameters. In this case, even though the mixed model showed a decreasing linear trend in the relationship between the mean frequency and age, each dolphin's signature whistle shows a different mean frequency/age relationship intercept and slope (Figure 4.3). These results do not suggest that all signature whistles with relatively low mean frequency will always belong to any older individuals, but that within the same individual signature whistle, there is a high probability that lower mean frequency is predicting older ages. This type of relationship of average fundamental frequency and age in calls has been found for some terrestrial species (Fitch, 2006; Ey et al., 2007). However, even if a significant relationship between mean frequency and age was found in individual signature whistles, this acoustic parameter is independent from the modulation pattern of the signal.

I found evidence that signature whistles general modulation shape remains similar for a longer period than the decade stated by Sayigh *et al*. (1990). Moreover, Bruck (2013) found that dolphins can remember signature whistle contours for as long as 20 years, confirming that relatively high stability in the contour of these signals are important long-term. In addition, unlike Sayigh *et al*. (1990), this study also investigated variation in males from calves to adulthood. The results of the modulation pattern comparison analysis showed that for most of the individuals, contour remained around 90% similar over time of up to three decades (Figure 4.4). Moreover, there was no significant difference in the Wilcoxon paired test between modulation similarities values of the first and second sampled years versus the first and last sampled years within the same individuals (Figure 4.5). This situation indicates that the general modulation pattern of the signature whistle remains stable over time, with only a degree of variation that still ensures signature whistle recognition. However, the degree of similarity is sex-biased, with female signature whistles' modulation remaining highly stable (>96%), and male signature whistles' modulation showing lower values of similarity (Figure 4.4). There is a significant difference when comparing those similarity values between males and females (Figure 4.6), with males showing on average a lower similarity value between the first and last sampled year than females. This sex-segregated degree of similarity can be explained by the social structure in bottlenose dolphins. Adult females stay in close proximity with their calves and form groups with loose bonds amongst each other. In contrast, adult males form long-term alliance pair bonds (Wells & Scott 1999; Connor *et al*., 2000). Once an alliance is formed, it might last for a lifetime (Wells *et al*., 1987; Wells, 2003). It has been shown that male alliances Woodare more likely to have more noticeable changes in their signature whistle (Figure 4.7). Male alliances change their whistle repertoire to be more similar with their alliance partner (Smolker & Pepper, 1999) and signature whistles tend to become more similar for allied pairs (Watwood *et al.*, 2004). Thus, it can be argued that these close interactions combined with their vocal learning capacities lead to the convergence of whistle patterns to reinforce the alliance once it is formed. On the other hand, female whistle patterns are not exposed to that pressure of converging to others as they do not form close alliances with selected individuals in the same way as males do (Figure 4.8).

Although signature whistle modulation patterns seem to be crystallised in the first months of life (Tyack, 1997; Sayigh *et al*., 2007), the control of the sound production organs might involve a period of adaptation and learning (Killebrew e*t al.*, 2001; Fripp & Tyack, 2008). Therefore, it is worth noticing that the values of similarity for comparisons in consecutive sampled years showed a tendency of increasing similarity values towards older sampled years for several individuals (e.g. FB11 = 0.96, 0.98, 0.98 ; FB55 = 0.97, 0.98, 0.98 ; FB06 = 0.97, 0.98, 0.98; FB92 = 0.83, 0.87) (Figure 4.4). This means that the lowest values of similarity in most individuals were shown in the comparisons of the first sampled year and the second sampled year. This suggests that the degree of variation might be reduced as the animal gets older, meaning that the signature whistle modulation tend to vary less with increasing age.

It is important to mention that relatively small changes in the signature whistle do not necessarily have to compromise the transfer of individual information. Bottlenose dolphins are well known vocal learners, with the capacity to innovate and modify sounds, as well as copy and use signals to address conspecifics in their communities (Miksis *et al*., 2002; Quick & Janik, 2012; King e*t al*., 2013; King & Janik, 2013; Janik, 2014). Even though physiological changes with age and maturity might limit the acoustic capacities of an individual to a certain extent, this does not necessarily influence the modulation pattern of the fundamental frequency, since only general voice cues are reported to be affected by physiological age variation (Fitch, 2006; Fletcher, 2004). Social experiences and behavioural changes seem to have the greatest effect on modulation patterns (Janik *et al*., 1994; Smolker & Pepper, 1999). Hence, the contour variations found in this study are unlikely to be caused by the physical growth of the animal, but are mostly due to vocal learning through social interactions. Clearly, the modulation patterns play a more important role in recognition than the acoustic frequency parameters as it is known that the contour shape conveys individual information (Janik *et al*., 2006).

In conclusion, the general modulation pattern of signature whistles is highly stable over time, with individual variation in acoustic parameters and modulation linked to vocal learning through social interactions. The sex segregated system of social structure drives the differences found in male and female stability at least in some part of their life. Thus, signature whistles can be reliably used by researches in long-term studies involving individual identification.

CHAPTER 5

THE INFLUENCE OF RELATEDNESS AND CALF ASSOCIATION ON SIGNATURE WHISTLE SIMILARITY

5.1 INTRODUCTION

Kin relationships can be defined in terms of shared somatic gene information, and it usually refers to first and second degree family bonds such as parents, siblings, grandparents, uncles and aunts, (Reis & Sprecher, 2009; Penn & Frommen, 2010). The association of kin related individuals varies and depends on the social structure of the species (Swarts & Rosenblum, 1981). Kin relations might manifest themselves as a form of preserving genetic material to pass to future generations. Kin recognition is vital to maintain kin associations and can be achieved by different mechanisms (Green *et al*., 2015). For many species, acoustic recognition is used as an important part of communication with conspecifics and for mating purposes (Penn & Frommen, 2010). Similarity or variation in acoustic signals is caused by different mechanisms amongst species. For some insects like crickets, their mating song is predisposed genetically (Bentley & Hoy, 1974; Mousseau & Howard, 1998) and genetic similarity translates into acoustic similarity. In contrast, song birds learn songs' structure from their parents and other conspecifics (Williams, 2004; Greig *et al*., 2012). Here, similarity is achieved by learning from a model, and in order to maintain kin recognition, animals need to learn from relatives. Thus, the complexity of the social networks in a community might affect the way vocalisations evolve, as those vocal patterns are likely influenced by social interactions (Tyack, 1997; Deecke *et al*., 2010).

Studying acoustic kin recognition in odontocetes is particularly interesting due to the diverse social structures that can be found and their vocal learning abilities (Connor *et al*., 1998; Janik, 2014). For instance, killer whales (*Orcinus orca*) form stable matrilineal groups that exhibit philopatry in both sexes. This species show distinctive repertoires for different kin related groups which are transmitted vertically to the offspring. Killer whales from the North East Pacific produce specific discrete calls shared by individuals within a pod and seem to remain stable for several decades. These specific repertoires seem to serve as vocal indicators of relatedness and social affiliation. In addition, call structure seems to play a role in kin recognition as the shape of the calls are correlated with matriarch relatedness, shaping associations and helping to make vocal communication within a pod more efficient (Ford, 1991; Deecke *et al.*, 2000; Deecke *et al*., 2010).

In contrast, small delphinids generally exhibit female-biased philopatry, where male bonding occurs in species with small sexual size dimorphism and male-biased operational sex ratio, and it is independent of dispersal tendencies (Möller, 2012). For instance, bottlenose dolphins (*Tursiops truncatus*) live in complex fission-fusion societies with some long lasting relations between their members that vary over time (Wells, 2009). They may form parties with individuals of both sexes. However, same sex groups tend to associate more closely than those of mixed sex. Mother-calf bonds are strong and may last for the first 3-8 years of the new-born (Gibson & Mann, 2008). Adult females are usually found to form groups with their offspring separated from adult males. Therefore, male-female associations depend more on the reproductive state of the female (Smolker *et al*., 1992). On the other hand, adult males form long lasting alliances (Owen *et al*., 2002). Living in a fission-fusion society results in the creation of different degrees of kinship association. It has been shown that adult males form alliances with genetically related males in the Bahamas (Parsons *et al*., 2003), but male alliances are formed by unrelated individuals in South East Australia (Möller *et* al., 2001),

Vocal learning in dolphins might have evolved due to the need of developing individual recognition under voluntary control in an aquatic environment where other sensory cues are limited (e.g. involuntary voice cues) (Tyack, 1997). Therefore, vocal learning plays an important role for the development of signature whistles in early life, as the crystallisation of the stereotyped modulation pattern occurs in the first month after birth (Tyack, 1997; Killebrew *et al*., 2001; Fripp &Tyack, 2008). However, bottlenose dolphins maintain the ability to copy and modify whistle repertoires throughout their lives, considering that adult individuals in captivity are capable of integrating new signals influenced by sounds that occur in their surroundings (Richards *et al*., 1984; Tyack, 1997). In addition, considering that signature whistles appear to be developed to be different from those of other animals (Janik & Sayigh, 2007), there might be an active pressure to sound different from kin. It has been shown that signature whistles can be influenced by sounds in their immediate environment in captivity (Miksis *et al*., 2002), and it has been suggested that signature whistles from male calves tend to be more similar to those of their mothers, whilst female calves generally showed a dissimilar modulation, with some of their signature whistles being very unlike of those of their mother pattern (Sayigh *et al*., 1995). Consequently, associations with kin and other dolphins in the population may play an important role during the developing of the acoustic repertoire of both sexes. Mother and calf pairs are the most common strong bond that occurs for genetically related individuals, as paternal care is absent in cetacean species.

It is known that males forming alliances develop similar characteristics in their whistle repertoires as the alliance becomes stronger (Smolker & Pepper, 1999; Watwood *et al*., 2004). Furthermore, it has been found that bottlenose dolphin calves produce similar signature whistles to those of individuals in the same community (Fripp *et al*., 2005). Given these multiple influences on vocal development in bottlenose dolphins, it is unclear whether animals can recognise relatedness from whistles of conspecifics. In fact, Janik *et al*. (2006) did not find similarities between signature whistles of related animals when using a cross-correlation of the modulation pattern of signature whistles. However, that study looked only at a sample size of 14 dolphins.

Considering that there might be a pressure in bottlenose dolphins to develop a signature whistle different from their kin, and that social interactions seem to play an important role for shaping acoustic vocalisations in cetaceans (Tyack, 1997), the aim of this study was to investigate whether kinship and calf associations during the first year of life amongst bottlenose dolphins in the Sarasota Bay community influence signature whistles fundamental frequency contour modulation and acoustic parameters similarity by comparing pairs of individuals with different coefficient of relatedness, specific kin relations, and calves with different coefficients of association.

5.2 METHODS

5.2.1 DATA PROCESSING

The detailed information about the study area, capture-release and equipment is described in Chapter 2 (sections 2.2 and 2.3).

For studying kin relation influence on signature whistles, I used 74 individuals of different ages and both sexes (46 females and 28 males) from recordings between 1984 and 2013, with at least one kin relationship with another individual in the sample. The kin relationships used were established as: Mother and calf, siblings, grandmother and calf, uncle or aunt and calf, and cousins. This was possible because the mother-calf relations are known in Sarasota Bay through field surveys that register new-born dolphins every year. The mother is tightly associated with the new-born and can be identified with the dorsal fin. The rest of the kin relations where derived by knowing all the calves for each mother in the sample in order to establish which individuals were grandmothers, sibling, uncles, aunts and cousins.

On the other hand, considering that bottlenose dolphins live in a fission-fusion society and their social dynamics are very active, coefficients of association for two individuals cannot be calculated across different years as they are time-dependent. Also, it has been shown that bottlenose dolphins develop their signature whistle during the first months of life (Killebrew *et al*., 2001) and this can be considered a critical time for influencing the modulation contours of the individual. Thus, for association analysis, I focused on individuals that were born in the same year. A minimum of 4 individuals were used for each year. The seven years of birth sampled, including in brackets the number of individuals per year, were as follows: 1984 (4), 1996 (5), 1999 (5), 2000 (4), 2002 (7), 2008 (4) and 2010 (4), a total of 33 dolphins. The individuals used for this analysis were not related to each other on the maternal line.

For all the individuals, recordings were visually reviewed using Adobe Audition 2.0 (Adobe Systems) with spectrograms at 512 FFT, Hamming window and 48 kHz resolution. Then, 20 signature whistles for each individual with a good comparable quality were selected randomly across the audio file. Then, the fundamental frequency contours were extracted using 'Beluga' software in MATLAB (R2014b) as explained in Chapter 2 (section 2.4) and saved for further analyses.

CALCULATING SIMILARITY FOR WHISTLE PAIRS

For this study, similarity values were calculated with two methods: (1) Parameters similarity using pairwise Pearson product-moment correlation coefficients of the measurements, and (2) contour similarity match obtained through ARTWARP software (Deecke & Janik, 2006) calculated using dynamic time warping on signal pairs. These two approaches were used because measuring acoustic parameters can give information about the general position of a signal in a multivariable space regardless of the contour shape, and the contour match can indicate a similar modulation pattern regardless of the actual position of the contour in frequency-time space, as explained in Chapter 2 (section 2.1.6).

The first method consisted of calculating whistle similarity from the average value of 13 parameters of the 20 whistles for every individual. Whistle contour extraction and parameter measurement procedure is explained in Chapter 2 (Section 2.3). After that, pairwise Pearson product-moment correlation coefficients were calculate in order to establish the similarity between the vectors of parameter values for every pair in the sample, and to build a matrix of similarity.

For the second method, I used dynamic time warping (DTW) in order to compare modulation patterns of fundamental frequencies. I used the algorithm in ARTWARP software was in order to calculate similarities. The specifications about the software and how it calculates the match between contours are stated in Chapter 2 (section 2.1.4). This algorithm allows compression and expansion of the time and frequency axis of a signal in order to fit with a reference signal (Deecke & Janik, 2006), which is not accounted for with the parameter correlation method. Warping methods have also being used successfully for comparing bird vocalisations

(Kogan & Margoliash, 1998; Meliza *et al*., 2013) and for killer whales calls (Brown & Miller, 2007). Furthermore, the rate of correct classification for the same call type into the same category is high when using this method combined with a neural network analysis to categorize calls into classes (Deecke & Janik, 2006). In this case, I selected three random contour whistles from the 20 of each individual in the sample. This was decided in order to reduce computing time in ARTWARP as every pairwise contour comparison was calculated one at a time for every pair of individuals. Then, the values were ordered in a matrix of similarity. This procedure was repeated three times, one for each whistle selected. Then I calculated the average of the three matrices to obtain one single matrix with the average contour similarity of each pair of individuals. This average was considered valid as the match results for the 3 comparisons were the same or with only 0.01 difference (e.g. 0.91, 0.91, 0.90), and ARTWARP categorised all the contours of the same individual in the same category at a vigilance value of 0.99 (Explained in Chapter 2). In addition, the contour comparisons involved the matching of 2 contours with the DTW ignoring the parameter fluctuations. Moreover, for individuals with signature whistles of multiple units, only the first unit was used for comparison of the contour pattern in order to reduce the effect of silence spaces between units in the whistle. This was justified because all of these types of whistles consisted of repetitions of identical units and were sometimes produced as single units by the individual. The pairs that ARTWARP output contour similarity of zero were not included in the analyses in order to avoid a possible bias in the correlations tests as they were likely outliers from the rest of the data points, considering the lack of similarity pair values between 0.45 and 0.0.

Unequal average comparisons are problematic only in nested or factorial situations within the same statistical test. For this chapter, there was no use statistical test to compare between the similarities obtained from different method, but only within the same method. I considered that the similarities by parameters and by contour are exposing different characteristics of the signature whistle as explained in Chapter 2 (section 2.1.2).

KIN RELATIONSHIPS

Matrilineal kin relationships amongst the individuals were used based on the information held in the Sarasota Dolphin Research Program. The coefficient of relationship was established based on the generic percentage of DNA autosomal consanguinity in mammals (Wright, 1922; Hill & Weir, 2011) in order to create a continuum of values. For mothers and calves, the coefficient of relationship was set to 0.5. For siblings, considering they are likely to be half-siblings, uncles or aunts, and grandmothers, the values were set as 0.25. Finally, for cousins, coefficients of relationship were set to 0.125, and for unrelated animals it was 0.0. As individuals within a population will have a degree of relatedness, for this study, unrelated individuals were defined as those pairs that do not share a maternal link in first or second degree based on DNA autosomal consanguinity (<0.125). With this information one matrix with the coefficient of relationship values was created with 74 individuals.

Furthermore, in order to separate the effect of each of the relatedness categories from each other, four different binary matrices were built for the kin relations. For each matrix, the other kin relations were not included, giving value 1 to the pairs with the specific relationship for that matrix and value 0 for the unrelated pairs. The number of individuals in each matrix was set as follows: 1) Mother and Calf (69 individuals), 2) Siblings (42 individuals), 3) Grandmother and Calf (17 individuals), and 4) Aunt or Uncle and Calf (22 individuals). A binary matrix for cousins was not used because only 3 individuals had this relation.

CALCULATING ASSOCIATION OF ONE YEAR OLD CALVES

The Coefficients of Association (CoAs) were calculated between the mothers of the individuals compared in each year using the Half Weight Index when the calves were in their first year of life (Cairns & Schwager, 1987). Calves have the highest association with their mothers during the first year and mothers are easier to sight in the field. As a calf will be always associated with their mothers, the CoAs of the mothers are valid to use for comparing whether the calves of two mothers were associated during that particular period.

The Half Weight Index (HWI) is a common measure to describe the level of association of two individuals in a period of time (Cairns & Schwager, 1987; Wells *et al.*, 1987; Bräger *et al.*, 1994). Every method for calculating coefficients of association presents some bias. The HWI works best for populations where there is a high probability to underestimate the association between pairs of individuals. This might inflate the association indices for some pairs. To counteract this possible bias, I only used HWI values for pairs of individuals that were observed together at least 10 times during the same year.

The index is given by the equation:

(1)

$$\mathbf{HWI} = \frac{x}{x + \frac{1}{2}(Ya + Yb)}$$

Where ***x*** is the times that both individuals were sighted together, and ***Ya*** and ***Yb*** are the number of sightings for each individual respectively. The coefficient of association ranges from 0.0 when a pair of individuals was never seen together to 1.0 when the pair was seen always together.

A separate matrix of association was created for each year.

5.2.2 DATA ANALYSIS

All tests were carried out in R software (R 3.2.0, GNU project). A series of Mantel matrix permutation tests were calculated using package '*vegan*' (Oksanen *et al*., 2015). Matrices were compared using the function *mantel* setting the number of permutations to 1000, giving a 95% confidence interval level, and using method "pearson". The test calculates the probability of similarity between two distance matrices using permutation tests (Mantel, 1967; Sokal, 1979). This type of test has been used successfully before in comparison of matrix correlation of acoustic parameters with genetic relatedness and association in killer whales (Deecke *et al*., 2010) and Campbell's monkeys (*Cercopithecus campbelli*) (Lemasson *et al*., 2011).

A correction for multiple comparisons for using parameter similarity and contour similarity was not applied considering that the aim in the study of comparing the two methods of measuring similarity was planned (Rothman, 1990). Corrections are usually recommended when the number of tests is high in order to avoid increasing the error Type I (false positives). This is common in gene base studies, where a large number of comparisons are made. However, this situation changes as the number of comparisons drops (Johnson *et al*., 2010). When the analyses consist in just a few comparisons (in this case two methods for calculating similarity), using corrections that could result to be too conservative would lead to an increase of error Type II (false negative), becoming a concern the possibility of accepting a null hypothesis that is not true (Rothman, 1990; Johnson *et al*. 2010; Gelman *et al*., 2012)

RELATEDNESS

The coefficient of relationship matrix was compared to two whistle similarity matrices in two separate Mantel tests: one with the contour similarity matrix and the other with the parameters similarity matrix in order to test if percentage of genetic closeness correlates with whistle similarity. For this test, the kin relations of siblings, grandmothers, uncles or aunts (0.25 autosomal genetic share) are not considered separately, but as part of the spectrum in the coefficient of relationship.

On the other hand, in order to separate and compare whether specific kin relations are more likely to be more similar than unrelated animals independently, two Mantel tests using the contour match similarity and the parameter correlation similarity matrix, were run for each of the four family relation types. Each matrix for one kin relation excludes the other kin relations. This is a valid approach as the Mantel test can also be used to compare a dissimilarity matrix with a numerical binomial model matrix to test the correspondence strength between matrices (Legendre & Fortin, 1989). In addition, Mann-Whitney U tests were performed to investigate the differences in whistle similarity between the male and female calves for the mother-calf pairs.

ASSOCIATIONS

Mantel tests were performed using CoA matrices to compare with whistle similarity matrices. One test per year was run in order to examine correlation of calves by specific year of birth: 1984, 1992, 1999, 2000, 2002, 2008 and 2010.

A single Mantel test comparing a single matrix with the possible pairs of calves across the years could not be performed because coefficients of association cannot be calculated from different years in order to build a matrix (e.g. CoA from one calf from 1999 with one calf from 2008). In addition, those comparisons would not be from two one-year-old animals anymore. Therefore, in order to be able to compare across the years, I used the calf pair values of every year (47 pairs) in a Spearman´s correlation test in order to assess whether there was a correlation between the similarity index (contour match and parameters) and the index of association. Spearman’s correlation was used because it is not sensitive to violation of normality or presence of outliers (Abdullah, 1990). In addition, Kruskal-Wallis tests were performed to investigate if there is a significant difference in the median of the signature whistle similarity amongst calf pairs by sex (i.e. male-male, female-female and female-male).

5.3 RESULTS

5.3.1 INFLUENCE OF RELATEDNESS

The Mantel test results showed a low positive correlation between coefficient of relationship and contour similarity which was statistically significant (Mantel R= 0.058, *p*=0.01). The Mantel test for parameter measures of similarity showed no correlation or significance (Figure 5.1).

The results of the analyses with the kin binary matrices showed no significant correlations with exception of the mother-calf comparison calculated with contour similarity, which also showed the highest Mantel R value of correlation (Mantel R= 0.05, *p*= 0.009) (Figure 5.2). For both comparisons, correlations were positive but low, but the significant matrix correlation was only for the modulation comparison. Therefore, dolphins tend to have a similar contour pattern to their mothers, but are not necessarily using the same frequency-time parameters as the results from the Mantel test calculated with similarity of parameters was not statistically significant (R=0.01, *p*=0.27).

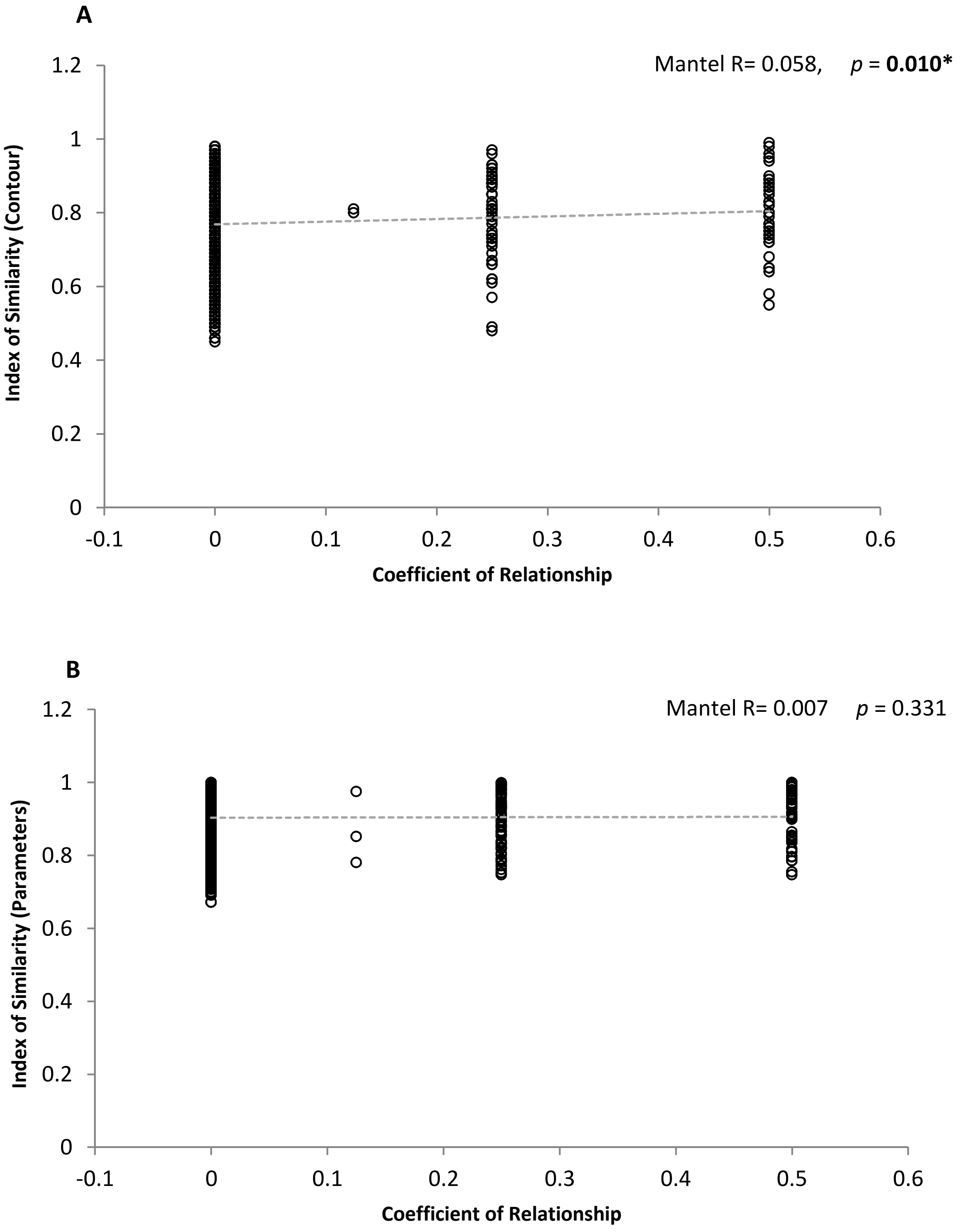


Figure 5.1. Scatterplots show the correlation between coefficient of relationship (matrilineal consanguinity) and index of similarity. The dotted line represents a tendency line. The results for the Mantel test are shown in the right corner of each graph. [**A**] Similarities calculated with contour matches and [**B**] Similarities calculated using parameter values.

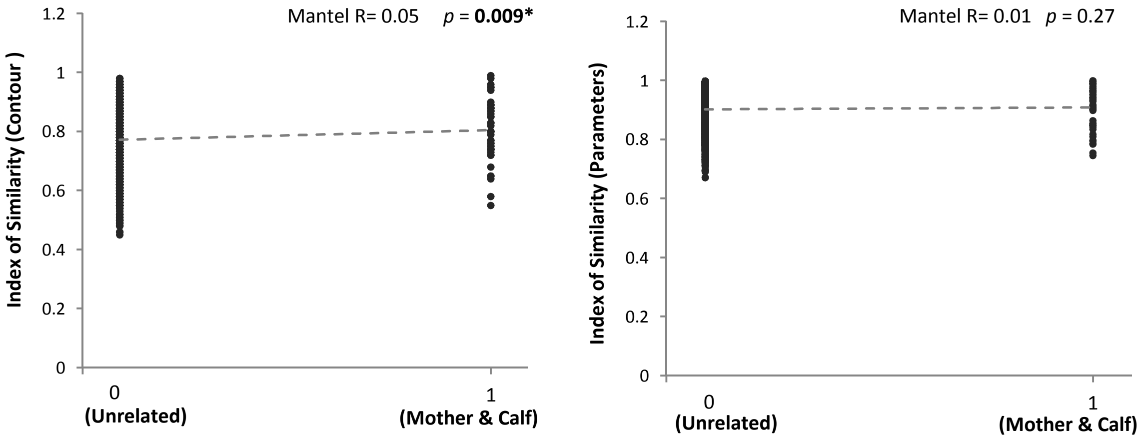


Figure 5.2. Scatterplots show the relationship between the mother/calf binary model and index of similarity. The results for the Mantel test are shown in the right corner of each graph. On the left graph the similarities calculated with contour match, and on the left with parameter values. The grey dotted line represent the linear tendency.

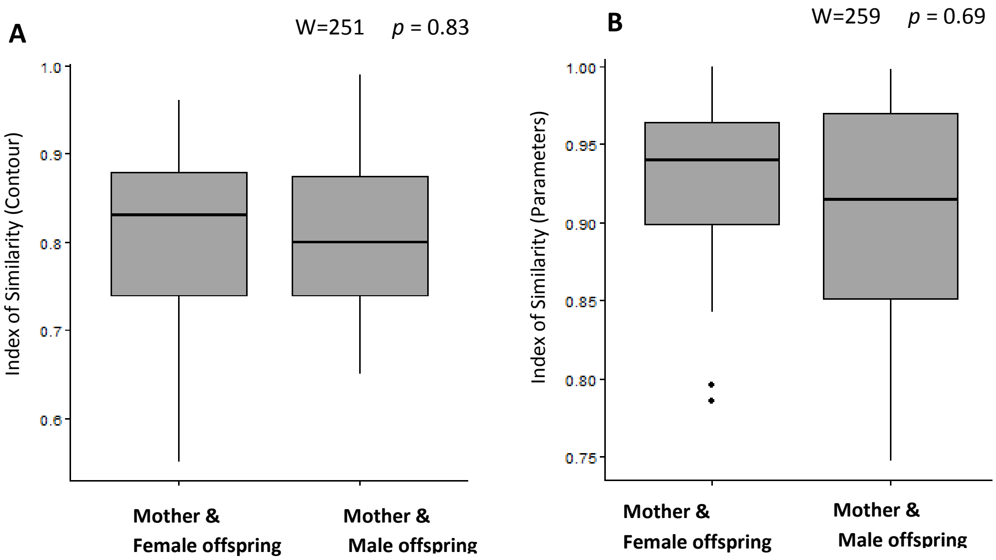


Figure 5.3. Box plots show the median value for the index of similarity for female and male calves with their mothers. The results for the Mann-Whitney U test are shown in the right corner of each graph. [**A**] Similarities calculated with contour matches and [**B**] Similarities calculated using parameter values.

In addition, a Mann-Whitney U test showed that there was no significant difference in the index of similarity for male and female calves with their respective mothers (pairs mother-male calf, n=23; pairs mother-female calf, n=21) for either contour similarity or parameters similarity (Figure 5.3). This suggests that the probability of whistle similarity with their mother is not affected by sex.

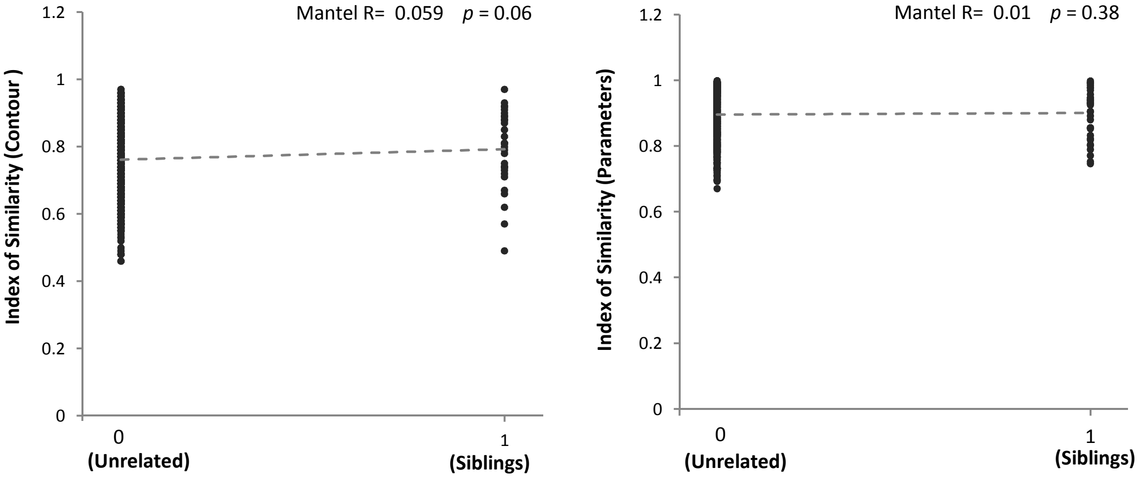


Figure 5.4. Scatterplots showing the relationship between the siblings binary model and the index of similarity. The results for the Mantel test are shown in the right corner of each graph. [**A**] Similarities calculated with contour matches and [**B**] Similarities calculated using parameter values. The grey dotted line represent the linear tendency.

The comparisons for pairs of siblings showed no significant correlation for contour similarity or parameters similarity (Figure 5.4). However, the correlation was positive for the contour similarity analysis. The Mantel tests values for grandmother-calf pairs and aunt or uncle-calf pairs were also low and not statistically significant (Figures 5.5 and 5.6).

These results suggest that only the relation of mothers and calves have a significant influence in contour similarity within the kin relations in the sample.

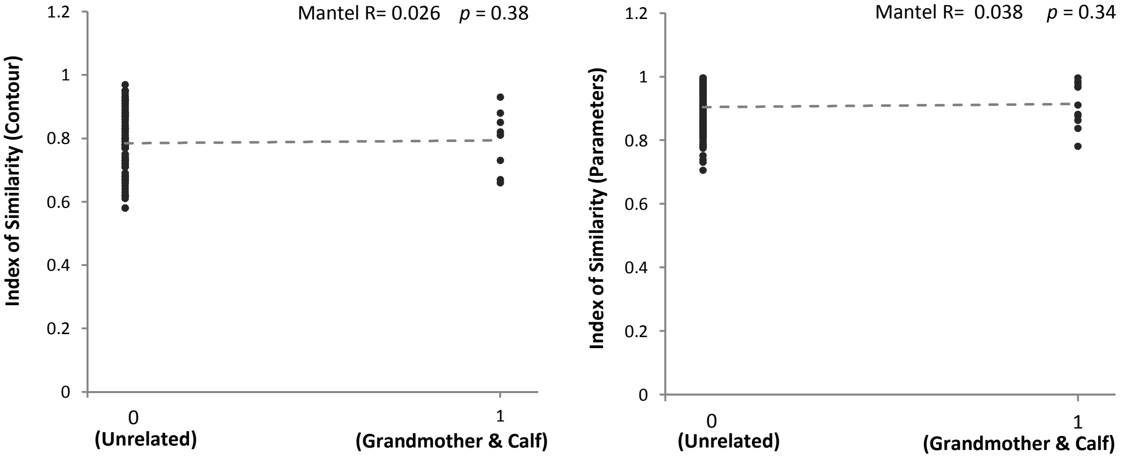


Figure 5.5. Scatterplots showing the relationship between the grandmother/calf binary model and the index of similarity. The results for the Mantel test are shown in the right corner of each graph. [**A**] Similarities calculated with contour matches and [**B**] Similarities calculated using parameter values. The grey dotted line represent the linear tendency.

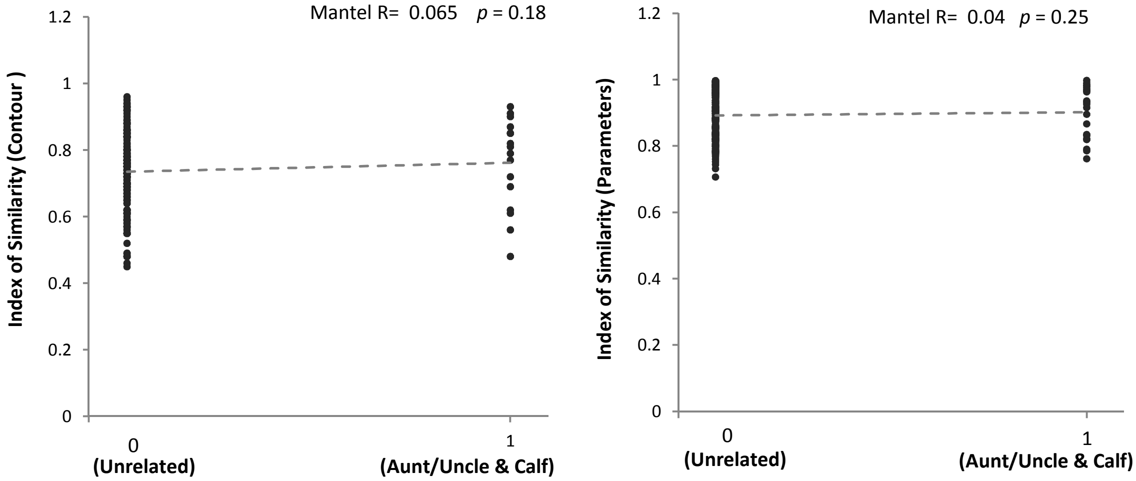
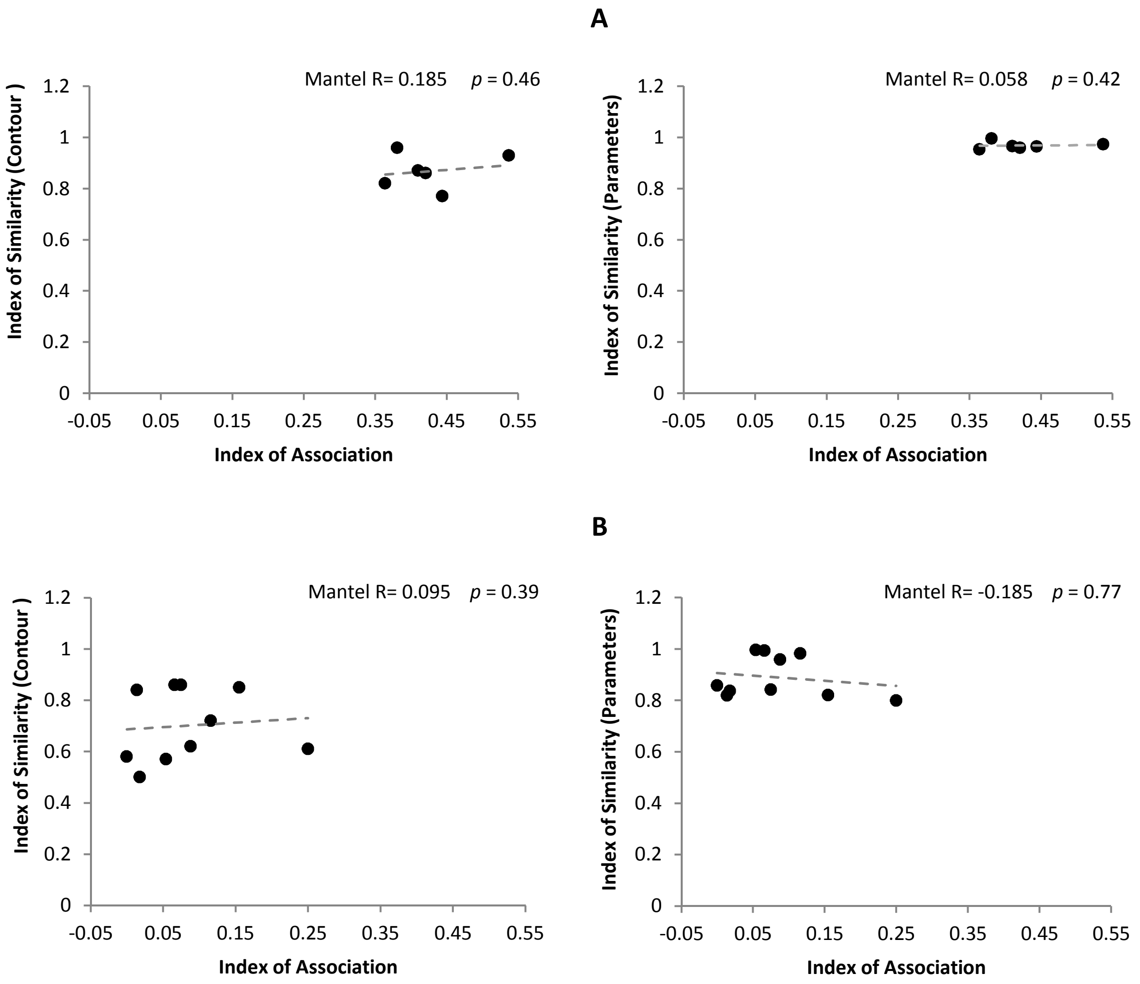
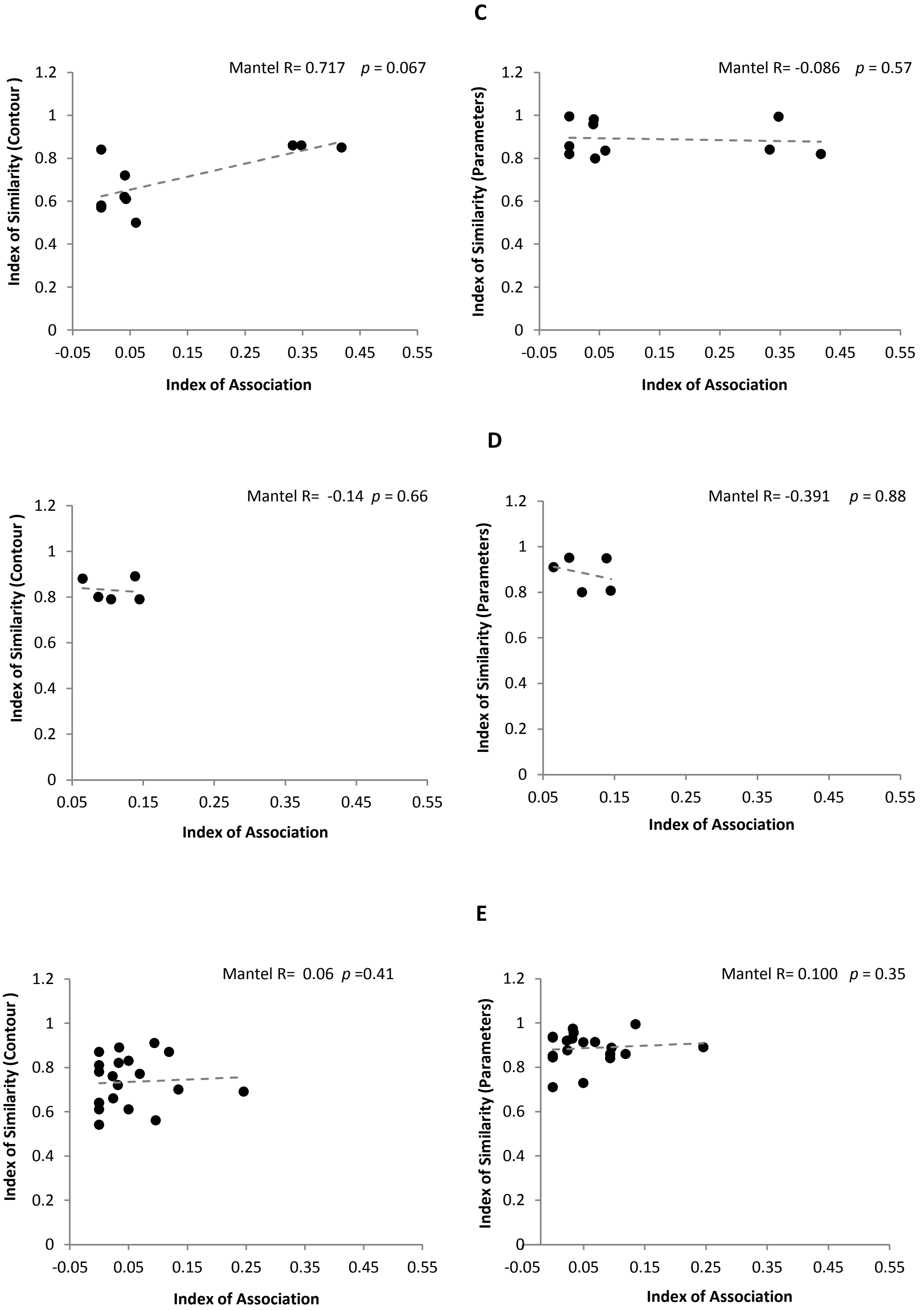


Figure 5.6. Scatterplots showing the relationship between the uncle/aunt binary model and the index of similarity. The results for the Mantel test are shown in the right corner of each graph. [**A**] Similarities calculated with contour matches and [**B**] Similarities calculated using parameter values. The grey dotted line represent the linear tendency.

5.3.2 INFLUENCE OF ASSOCIATION FOR ONE YEAR OLD CALVES

The results of individual Mantel test by year showed no significant correlation between the index of association and the index of similarity for both measurements (Figures 5.7).




C
Mantel R= 0.717 *p* = 0.067
Index of Similarity (Contour)
Index of Association
Mantel R= -0.086 *p* = 0.57
Index of Similarity (Parameters)
Index of Association
D
Mantel R= -0.14 *p* = 0.66
Index of Similarity (Contour)
Index of Association
Mantel R= -0.391 *p* = 0.88
Index of Similarity (Parameters)
Index of Association
E
Mantel R= 0.06 *p* =0.41
Index of Similarity (Contour)
Index of Association
Mantel R= 0.100 *p* = 0.35
Index of Similarity (Parameters)
Index of Association
0
0.2
0.4
0.6
0.8
1
1.2
-0.05
0.05
0.15
0.25
0.35
0.45
0.55

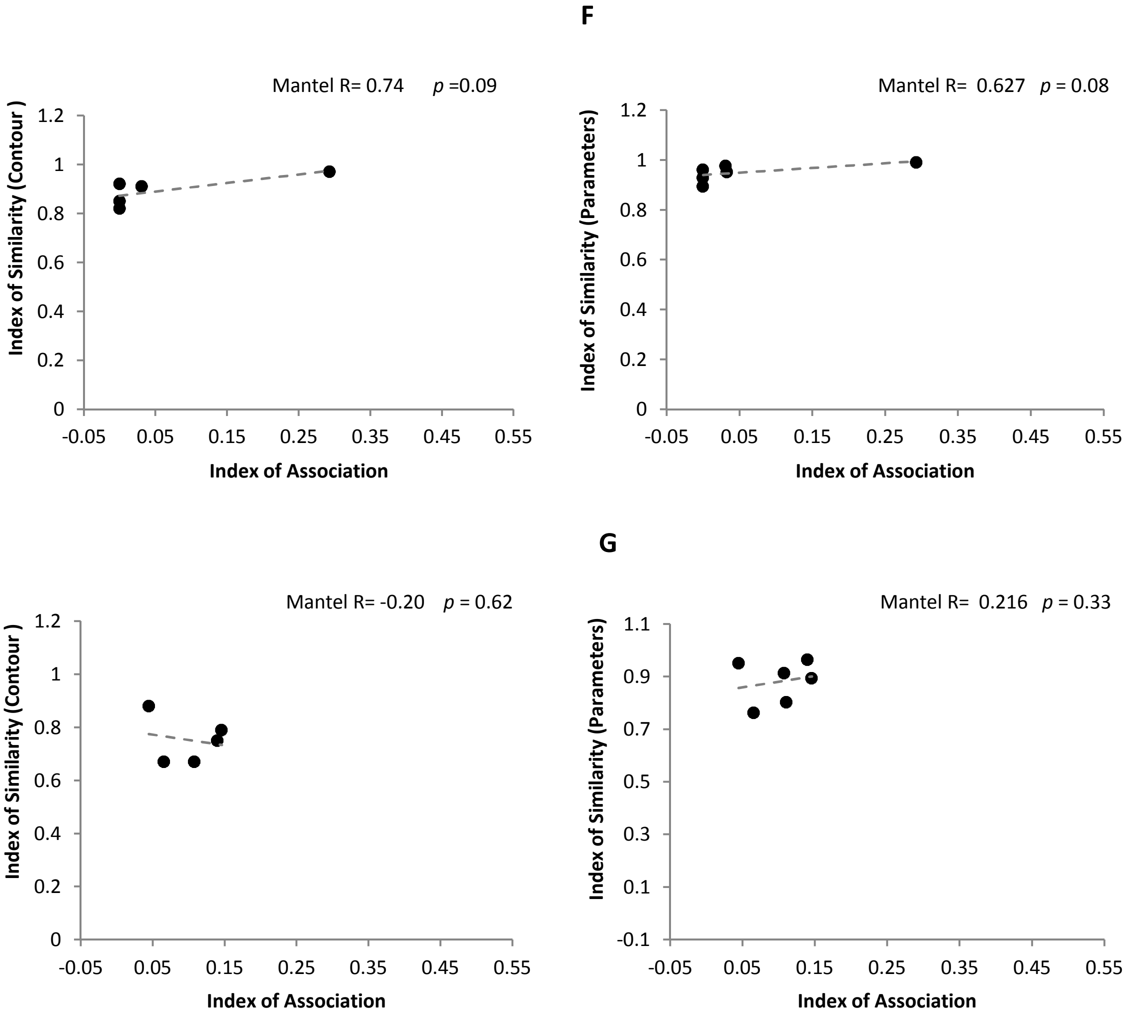


Figure 5.7. Scatterplots show the relationship between the index of association (CoA's) and index of similarity for pairs of calves in: **A) 1984, B) 1996, C) 1999, D) 2000, E) 2002, F) 2008, and G) 2010**. The results for the Mantel test are shown in the right corner of each graph. The grey dotted lines represent regression tendency lines. On the left graph are the similarities calculated with contour matches, and on the right with parameter values. The axes are in the same scale in all the graphs for comparison.

Even though Mantel tests are a powerful permutation test, one of the main issues encountered in matrix correlation analysis by year is that for most of the years, ranges of associations were very limited. Only the values for the year 1999 showed a relatively wide range of CoAs values (0 to 0.45). It is worth noticing that the correlation for the analysis of that year was the highest correlation of all the individual years (Mantel R= 0.717, p value= 0.067) (Figure 5.7 C).

The results from the Spearman's correlation test showed a significant positive correlation between signature whistle similarity calculated with contour matches and index of association of the individuals during their first year of life (r= 0.32, p = 0.028) (Figure 5.8). This analysis showed that the correlation value was relatively low, but positive and statistically significant. This might suggest that associations with other calves during the first year of life have an influence on the signature whistle modulation pattern to some degree. Following this idea, it is interesting to notice by visual examination of the correlation plot, that those pairs with coefficients of association higher than 0.4 had always relatively high contour similarity values, and those with contour similarities of zero were always lower, showing a coefficient of association of around 0.1. In addition, the correlation using parameter similarities was not statistically significant but still positive (n= 65, r= 0.16, p=0.191). The same tendency of the lowest values of whistle similarity coinciding with the lowest value for Coefficients of association can be observed (Figure 5.8).

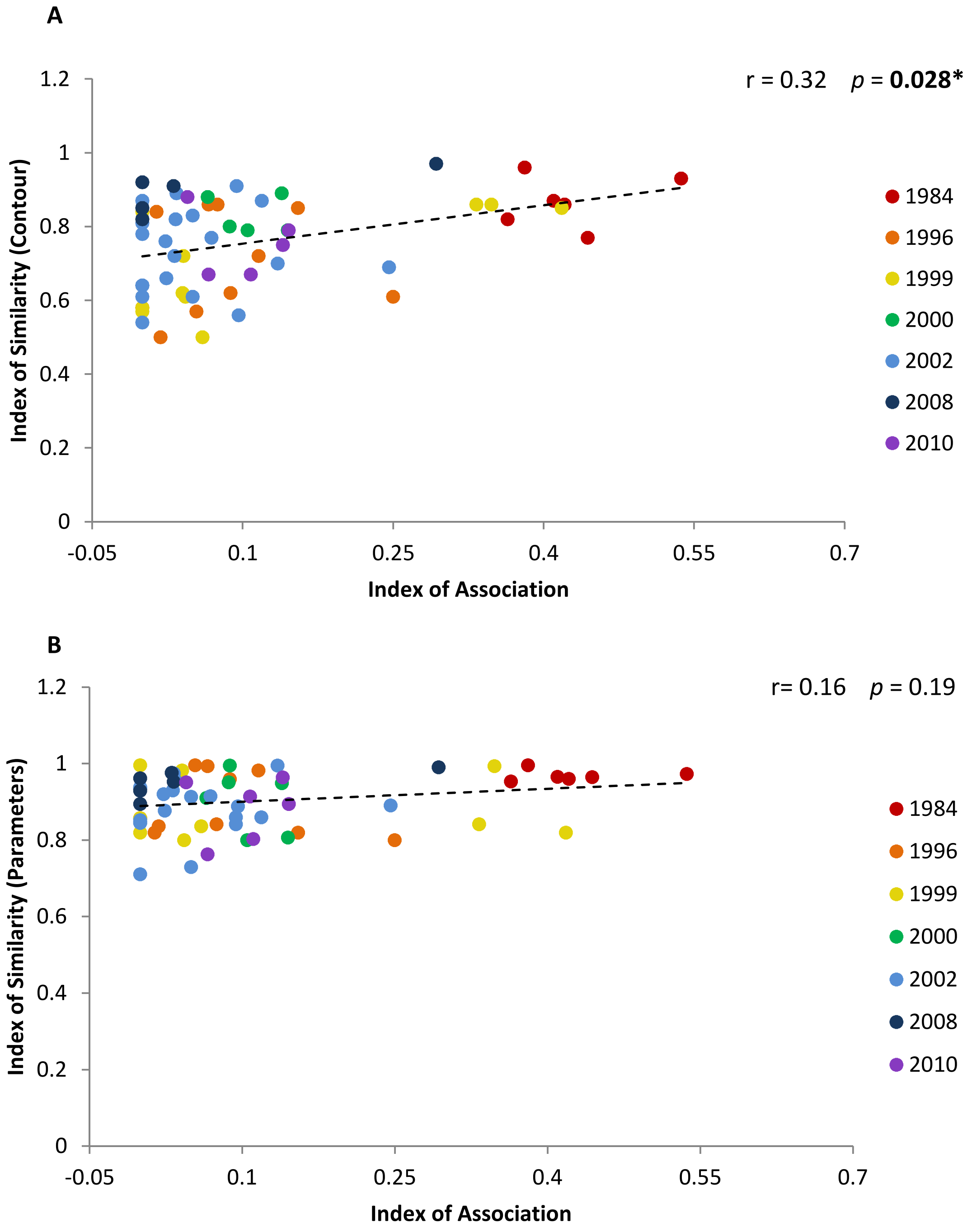


Figure 5.8. Scatterplots show the relationship between index of association and index of similarity. The results for Spearman’s Correlation test are shown in the right corner of each graph. The colour code identifies the first year of life for each pair. For [**A**], the index of similarity was based on contour matches, and on [**B**], it was based on parameter similarities. The tendency lines are shown as black dotted lines.

The sex composition of the calf pairs (female-female= 13; male-male= 18 or female-male=30) did not show a specific tendency for whistle similarity (Figure 5.9). There was no difference in the median of the signature whistle contour similarities (Kruskal-Wallis Test, χ^2= 5.21, *p*= 0.08) or parameters similarities (Kruskal-Wallis Test, χ^2= 4.64, *p*= 0.10) amongst pairs of males, females or mixed sex pairs, suggesting that the sex of the individual a calf associates do not increases or decreases the probability of having a similar contour pattern (Figure 5.10).

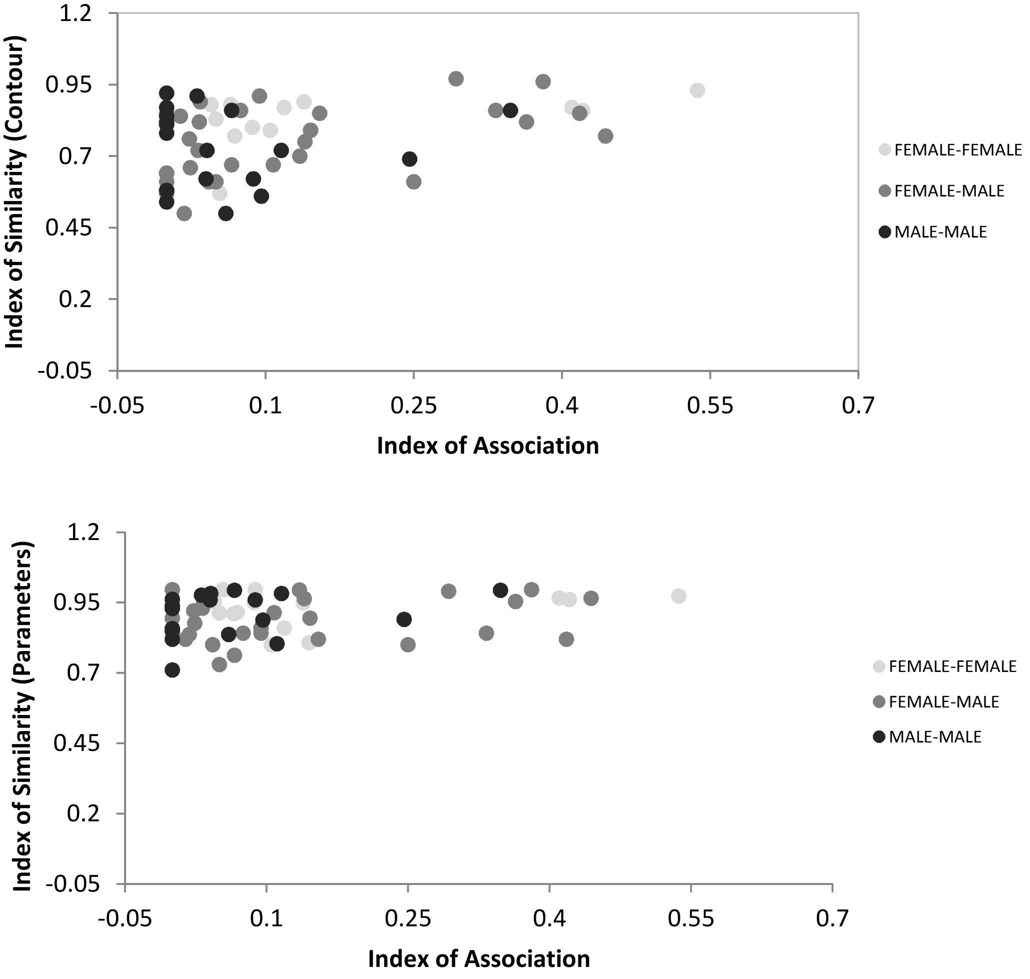


Figure 5.9. Scatterplots show the relationship between index of association and index of similarity. The colour code identifies the sex composition of each pair. For [**A**], the index of similarity was based on contour matches, and on [**B**], it was based on parameter similarities.

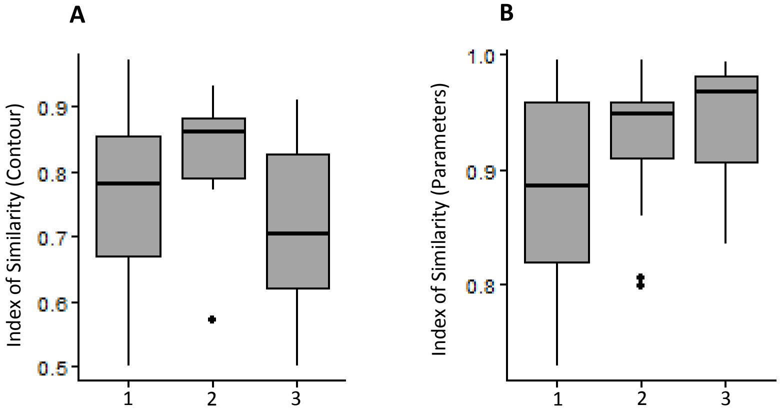


Figure 5.10. Box plots comparing the median value for mixed sex calf pairs (**1**), female calf pairs (**2**) and male calf pairs (**3**). Each graph corresponds to: **A**) Contour similarity and **B**) Parameter similarity. There is no statistically significant difference amongst calf pairs.

5.4 DISCUSSION

Measuring acoustic parameters is a wide spread method to compare dolphin whistles, and it can give information about the general position of a signal in a multivariable space. However, it is important to note that parameter correlations might be high for whistles that have dissimilar contour modulation patterns. Likewise, the contour match can indicate a similar modulation pattern regardless of the actual position of the contour in frequency-time space. This is important considering that signature whistles can convey identity information in their modulation patterns independent of vocal cues (Janik *et al.*, 2006). Similarity values calculated using parameters showed no significant values in any analysis, and showed low correlation. Acoustic parameters in signature whistles seem to vary with context (Janik *et al.*, 1994) whilst modulation patterns are relatively stable as shown in Chapter 4 in this thesis. King *et al*. (2013) showed that dolphins copy signature whistle contour shapes of close associates, but without matching all acoustic parameters of the copier's whistle. Likewise, Ralston & Herman, (1995) showed that dolphins seem to be capable of processing the whistle contour without using the absolute frequency. Taking this in account, acoustic parameters do not necessarily reflect the modulation pattern of the whistle, and dissimilar contours might show absolute frequency similarity. Therefore, contour similarity using dynamic time warping algorithm stated in (Deecke & Janik, 2006) seems to be better method to represent for signature whistle similarity than using parameters correlation.

Studying signature whistle contour similarity amongst bottlenose dolphins can help to unravel how individuals learn their signature whistle and how the community influences the vocal learning process, especially taking in account that the modulation pattern is sufficient to convey individual information (Janik *et al*., 2006). In this study, the significant results of the Mantel test for coefficient of relationship and contour similarity (Figure 5.1) indicates that generic genetic closeness increases the probability of two individuals of having similar whistle contours. However, this significant result of a relationship of the index of relationship and whistle contour similarity could be confounded with the significant relationship of similarity found for mothers and calves. The mother and calf degree of contour similarity does not necessarily has to be based on the genetic closeness, but on a high association in early life (Wells & Scott, 1999; Mann, 2000) as I will discuss below, especially taking in account that the results of the binomial Mantel tests for siblings, grandmothers, and aunts or uncles showed that those kin relations are not more likely to have similar whistles than unrelated individuals (Figure 5.2, Figure 5.4-Figure 5.6).

Mother and calf pairs showed a significant positive correlation with contour similarity and the fact that they are the kin with the highest coefficient of relatedness could explain the result of the analysis for genetic similarity. In this case, the results showing that individuals have more probability to have similar whistles to their mothers can be explained by their degree of association in their youngest years. Dolphin calves of both sexes spend up to 5 years of their lives highly associated with their mothers until weaning, while slowly the calves become more independent and start associating with other individuals (Connor *et al*., 2000; Wells, 2009). Sayigh *et al.* (1995) found that male calves tended to have more similar whistles to their mothers than female calves, although not all the male calves of the same female necessarily developed a signature whistle similar to their mothers. However, in this study, there was no significant difference in the median similarity values between calves of both sexes neither when using contour or parameter similarity (Figure 5.3), suggesting that males and females have similar probability of developing a signature whistle similar to their mothers. Both sexes are exposed to their mother's signature whistle during the first year of life, and the identity of the mother could be influencing more whether a calf develops a similar or dissimilar whistle than just sex (Sayigh *et al*., 1995). The discrepancy of results with Sayigh *et al.* (1995) could be related most likely to the difference in the method of calculating similarity between both studies and the smaller span of years sampled (1976-1990) in comparison with this thesis. Sayigh et al. (1995) calculated as a categorical rank of similarity going from 1 (not similar) to 5 (very similar) using human observers. I compared 44 calves (1984-2013) in this study using a quantitative method of similarity match. The limited qualitative scale (categorical variable) could drive observers to rank whistles with a relative low similarity for female calves, that quantitatively (using DTW), would have a higher percentage similarity match (continuous variable).

In addition, the lack of high similarity values between calves and grandmothers compared with unrelated individuals, but high similarity between mothers and calves could suggest that dolphins select sections of their mother's signature whistle to model their own, but these sections are not necessarily the same as the ones used by the next generation (grandchildren), avoiding of a matrilineal tendency. Ultimately, a signature call is meant to be sufficiently distinctive to determine an individual (Shapiro, 2010). In the fission-fusion social structure of bottlenose dolphin populations, kin relationships other than mothers with their calves are not necessarily bonded strongly. For example, in Sarasota, male alliances are usually between unrelated males (Connor *et al*., 2000). In contrast, other dolphins like killer whales tend to maintain pod-specific repertoires (Yurk *et al*., 2002; Miller *et al*., 2004), but this is related to matriarchal defined stable groups, a situation that does not occur in bottlenose dolphins. This lack of kin bonds might suggest that the degree of association would become more relevant for influencing acoustic similarities than the genetic relatedness itself. This premise is supported by the results in this study showing no significant correlation between signature whistle similarity and any kin other than mothers. Thus, the similarity in signature whistles in bottlenose dolphins might be influenced more by the social interaction rather than maternal kinship.

Associations within bottlenose dolphin communities can vary greatly across the years (Irvine *et al*., 1981; Wells *et al*., 1987; Bräger *et al*., 1994). Sometimes, the limitation of comparative data across years when studying associations might lead to non-representative results. For instance, the lack of significance with strong correlation values for the results of Mantel tests when comparing index of association and whistle similarity by year (Figures 5.7), could be explained by the short range in the index of association values of specific individuals sampled. For example, the Mantel test results for the individuals in the year 2000 (Figure 5.7 A) and 2010 (5.7 B) showed not relationship between index of association and whistle similarity. However, the values for index of association ranged only from 0.0 to 0.18, a very low value in a narrow range. In contrast, the model that fit with the highest correlation and the lowest p value is the one for year 1999 (Figure 5.7 C). It showed the broadest range for index of association values of all years (0.0 to 0.45). In addition, for analysis of independent years the sample size might also have an effect in calculating the yearly relationship accurately. Therefore, the relationship of signature whistle similarity and association becomes clearer with the when all the pairs from each year were combined in a Spearman's Correlation test (Figure 4.8) and the range of coefficients of association ranged from 0.0 to 0.55.

The values used for the association analyses were only representative for the first year of life, which is considered the moment when dolphins are developing their signature whistle modulation pattern (Killebrew *et al*., 2001; Fripp & Tyack, 2008). An influence of those associations on signature whistle modulation patterns might be seen during the youngest period of a dolphin's life. Bottlenose dolphins are vocal learners with the capacity of innovate sounds to add to their repertoires (Janik, 2014). Therefore, associations with individuals during the first year of life might have an important role in shaping signature whistles, especially when the individuals associated are also one year old. Calves associate with all the individuals that their mother associates with and the sounds those individuals produce appears to influence the repertoire of the calves (Fripp *et al*., 2005). Many juveniles in Sarasota show a high philopatry and once they are independent from their mothers, they tend to associate with individuals that they used to interact with when they were calves (McHugh *et al*., 2011). My results suggest that these continuous associations seem to play some role in the development of signature whistle modulation patterns. It has been shown that individuals in captivity spontaneously use acoustic signals from their surroundings to add new signals to their repertoires (Tyack & Sayigh, 1997; Miksis *et al*., 2002). Also, social companions have a strong influence on whistle similarity in whistle repertoires in captive adult females (McCowan *et al*., 1998). In the wild, for the area around West Florida, Fripp *et al*. (2005)

found that calves in Sarasota show a higher whistle contour similarity with individuals which associate regularly but not the most in the community. Their results suggest that individuals that are encountered a lot might not be the main ones influencing signature whistle modulation in calves. However, Fripp *et al.* (2005) investigated all associates of the calves for one single year, and the measurement of association was established as percentage of time. For this study, I used coefficients of association (CoA) based in several encounters, and compared a larger number of calves. In addition, I only compared newborn calves with one another. There seems to be an influence of calves on calves, that is not apparent amongst adults and calves.

I also found that the sex of calves is not relevant for the degree of whistle similarity displayed by a pair, as pairs consisting of different or same sex calves showed no significant difference in their contour or parameter similarity (Kruskal-Wallis Test, $p>0.05$) (Figure 5.9). Therefore, the association between calves itself, regardless of sex of the other individual, is what might have influence on the similarity of signature whistle contours amongst one year old calves. However, this might change when males become independent and reach sexual maturity, as male alliances seem to modify certain characteristics in their whistle contour to converge whistle repertoires (Smolker & Pepper, 1999; Watwood *et al.*, 2004).

In conclusion, using the contour comparison with dynamic time warping seems to be a better method to calculate similarity between signature whistles than using parameter pairwise correlations, especially when the interest is about modulation patterns. In addition, this study showed that the high coefficients of associations amongst unrelated calves correlate significantly with high signature whistle contour similarity. On the other hand, mother and calf pairs are more likely to show high whistle similarity, but the same is not true for other kin relationships (sibling, grandmothers and calves, and uncles or aunts and calves) which did not show significant correlation between coefficient of relatedness and contour similarity. Therefore, considering that mothers are the highest associated individual of their offspring during the first year of life (Mann, 2000), it could be suggested that social interaction between individuals in highly dynamic societies, especially at very young age, seems to have more influence on signature whistle crystallisation and modulation changes than relatedness itself.

CHAPTER 6

EFFECTS OF CARRYING A TAG ON BOTTLENOSE DOLPHIN SIGNATURE WHISTLES AND SURFACE BEHAVIOUR

6.1 INTRODUCTION

Advancing technology has allowed for more efficient data collection to study animal populations. Biologging technology helps to obtain information from free-ranging animals and provides effective data when access to individuals is limited (Bograd *et al*., 2010; Walker *et al*., 2012). Many attachable devices allow researchers to collect diverse parameters simultaneously, from physiological data to environmental information in order to understand the dynamics of the animals carrying these tags (Payne *et al*., 2014). Tags for obtaining telemetry data have been used especially for studying spatial movements, behaviour and ecology (Seegar *et al*., 1996). For instance, in land animals, radio and satellite trackers have been used in bats (Castle *et al*., 2015), frogs (Gourret *et al*., 2001), birds (Walls & Kenward, 1998; Barbraud & Weimerskirch, 2012), carnivores (Delibes & Beltran, 1986), amongst others.

For marine animals, tagging devices have been used for obtaining continuous biological information, access to which can be otherwise limited by the elusiveness of free-ranging marine organisms. Some examples include acoustic tags on roundfish (Claireaux & Lefrancois, 1998), tracking tags on sea turtles (Mansfield *et al.*, 2014; Hart *et al*., 2015), radio tracking tags on dolphinfish (*Coryphaena hippurus*) (Taquet *et al*., 2007; Merten *et al*., 2014), and whale shark (*Rhincodon typus*) telemetry tags (Eckert & Stewart, 2001).

Similarly, marine mammal studies have extensively used tags to obtain biological information. Some examples include tagging seals to investigate movements and oceanographic parameters (Robinson *et al*., 2012; Roquet *et al*., 2014), using acoustic and accelerometer tags for tracking blue whales (*Balaenoptera musculus*) to study their surface behaviour around boats and possible boat collisions (McKenna *et al*., 2015), tagging pantropical spotted dolphins (*Stenella attenuata*) to obtain data on underwater behaviour and during night time when visual methods cannot be used (Baird *et al*., 2001), and using radio transmitters to track dolphin movements and habitat use (Lynn & Würsig, 2002). Using acoustic recording tags is important as marine mammals use sound for many of their activities and for sensing their environment. These tags can be very useful to study vocal behaviour, spatial movements and other ecological factors from the perspective of the individual (Johnson & Tyack, 2003; Johnson *et al*., 2009).

Tagging devices can provide continuous data over long periods of time. However, there is concern over possible effects from tagging and carrying the tag. The aim of researchers is to reduce any effects while increasing the quality of data that can be obtained (Wilson & McMahon, 2006). Negative effects are most likely when using invasive devices inserted into the tissue, which can remain attached for long periods of time (Walker *et al*., 2012; Robbins *et al*., 2013), since they might result in inflammation and lesions. But even with less invasive designs, it is always necessary to take the size, shape and form of the attachment into account. Fast-setting glue, tape and suction cups provide an alternative to invasive attachment methods and a hydrodynamic design can help to reduce the drag in fast swimming animals (Wilson *et al*., 1997; McMahon *et al*., 2008; Pavlov & Rashad, 2012; Shorter *et al*., 2014). For instance, antennae from transmitters attached with adhesive tape on Magellanic penguins (*Spheniscus magellanicus*) have shown an effect on the efficiency of catching prey depending on the hardness of the antenna. Soft antennae reduce this effect considerably. The antenna material and size have a large influence on drag in diving animals (Wilson *et al*., 2004). Moreover, Ropert-Courdet *et al*. (2007) found that a large size device affects diving behaviour in little penguins (*Eudyptula minor*) more than a small one. For any biologging research, it is important to reduce the possible negative effects that a device could cause to an animal, in order to guarantee obtaining quality data. McMahon *et al*. (2008) found that tags attached with glue to elephant seal (*Mirounga leonina*) brought important information for conservation of the species and did not affect the survival of the individuals.

Quantifying impacts of tags on marine animals becomes difficult considering the complications of obtaining the same resolution of performance tracking the individuals when they are not carrying the tag (Shorter *et al*., 2014). However, it is known that species react differently to tag attachment. For example, sperm whales (*Physeter macrocephalus*) showed a brief reaction by decreasing the time they spent at the surface and the time spent foraging compared with periods around 20 hours later after the tag had been removed (Isojunno & Miller, 2015). On the other hand, Sakai *et al*. (2011) found that Heaviside's dolphins (*Cephalorhynchus heavisidii*) showed negligible impact to tagging attempts with suction cup tags, by returning to the behaviour they were performing shortly after a dive. Antarctic killer whales (*Orcinus orca*) did not show a long-term (around 26 days) effect to carrying a satellite tag, with little or no reaction to the attempts of tagging (Reisinger *et al*., 2014). However, Van der Hoop *et al*. (2014) found that even though carrying a tag does not necessarily affect the metabolic rate in bottlenose dolphins in captivity, tagged individuals showed lower swimming speeds, suggesting that dolphins modulate this behaviour in order to avoid increasing energy expenditure that the extra drag of a tag could cause.

On the other hand, changes in acoustic signals also can be induced by anthropogenic influence. For instance, Buckstaff (2004) showed that bottlenose dolphins (*Tursiops truncatus*) in

Sarasota increase whistle rates when there is presence of noise emitted by boat activities. Furthermore, general whistle frequency parameters and duration vary with watercraft activities close to bottlenose dolphins in Boca del Toro, Panama (May-Collado & Quiñonez-Lebron, 2014). However, the possible effect of carrying a device on the acoustic behaviour and signature whistle production in bottlenose dolphins is not yet understood. This is relevant to ensure obtaining accurate acoustic data that is not biased by the collecting method.

In Sarasota Bay, Florida, radio tags have been used to investigate movements and habitat use of bottlenose dolphins in the area (Irvine *et al*., 1981). Additionally, a long-term health assessment programme has been conducted in the area using brief capture-release session to collect data (Wells *et al*., 2004). Similar techniques are used in Indian River Lagoon, Florida for health studies (Fair *et al*., 2006). Possible stress effects related to these captures in Sarasota have been investigated using acoustic data from restrained and free-ranging dolphins in Florida where Esch *et al.*, (2009b) found that the whistle rate and number of loops (repetitive modulations sequences) were higher at the beginning of the capture-release session, but decrease throughout the end of the session. However, it is not known if the deployment of a digital acoustic recording tag (D-Tag) (Johnson & Tyack, 2003) causes a change in acoustic parameters in the bottlenose dolphins signature whistles in in Sarasota or if the surface behaviour might change whilst carrying the tag. Context-specific variation in acoustic parameters can occur in this species (Janik *et al*., 1994; May-Collado, 2010). Therefore, attached devices could alter the whistles acoustic parameters and call rates as a response of disturbance context. In addition, alterations of their vital activities such avoiding feeding or limiting socialising could indicate a negative impact of carrying these devices.

Obtaining accurate and unaltered information about the vocal activity of cetaceans is one of the main goals of using acoustic D-Tags. Therefore, the aim of this study was to investigate the possible effect that carrying a D-Tag has on bottlenose dolphin signature whistle acoustic parameters and surface behaviour after capture-release sessions in Sarasota Bay.

6.2 METHODS

6.2.1 DATA COLLECTION

Johnson & Tyack (2003) developed a digital acoustic recording tag (D-Tag) that attaches with suction cups to the surface of cetaceans. The original version contained a Li-Ion battery, a solid memory up to 3 GB and an in-built hydrophone. The third version of the D-Tag was developed in Woods Hole Oceanographic Institute, and contains 2 hydrophones, a solid memory of up to 64 GB and 160 kHz recording bandwidth (http://soundtags.st-andrews.ac.uk/dtags/dtag-3). This version was the one attached to the dolphins during this study.

To test the possible effects of carrying a D-Tag on a dolphin's surface and acoustic behaviour, a series of focal follows were carried out. In the first phase we obtained surface behavioural data and simultaneous acoustic recordings from focal individuals when carrying a D-Tag. As a control, we located the same individuals one week later and followed them for a similar period of time without the D-Tag. For bottlenose dolphins, the dorsal fin can be used to identify individuals in the long term using its shape and natural marks (Kareczmarski & Cockcroft, 1998). This method of recognition allows focal follows with specific dolphins.

A total of 21 D-Tags were place on 9 pairs and 3 solitary dolphins during brief capture-release sessions in 2013 and 2014 for health assessments in Sarasota Bay, Florida, USA. The D-Tags were placed on the animals towards the end of the health assessment shortly before release. Immediately after release, a focal follow was conducted on board a small vessel. Recordings were obtained using two HTI 94 SSQ hydrophones (frequency response 2 Hz to 50 kHz ± 1 dB) towed on chains on either side of the boat and approximately 1.5 m below the water surface. The hydrophones were connected to a 4 channel 96/24-bit Tascam DR-40 Digital Recorder (frequency response 20 Hz to 20 kHz ± 3 dB) with a 32GB SD card for storage. All the recordings were collected at a frequency sample of 96 kHz.

In order to maintain a comparable method, recordings obtained from the D-Tag were not used because the measurement of acoustic parameters and whistle rates would not be comparable with those collected with the towed hydrophones during the second week when the dolphins are not carrying the D-Tag. Therefore, the same method of obtaining the acoustic recordings was use throughout both weeks. Whistles can be recognised by comparing them with the signature whistle catalogue in Woods Hole Oceanographic Institution and the recordings of past capture-releases sessions, as the group composition is known for every follow.

Behavioural data were collected on spread sheets on an Apple iPad G4 using point sampling with a 3 minute interval between points (Altman, 1974). The focal behaviours were divided into 5 categories that are explained in Table 6.1. Additionally, information about the group size, group composition, bearing, and the GPS position of the boat (with a Garmin GPSMAP 76CS) was also collected along with the behaviour.

Each animal was followed until the D-Tag was detached either by the dolphin itself or by the release programming in the suction cups. For cases where the D-Tag was programmed to come off after dusk, the follow was ended at dusk. In these cases, the D-Tag was recovered the next day. If a focal follow ended early in the afternoon, the vessel returned to where the next health assessment session of the day took place to start a new follow.

Focal follows in the second week required locating the same individuals that were followed in the first week. We then performed focal follows over a similar time period as in the first week registering the same behavioural and acoustic data as in the first week. Unfortunately, it was not possible to locate every individual that was tagged in the first week for a follow during the second week.

Table 6.1. Behaviour categories sampled during focal follows.

Behaviour	Description
Resting	Slow movements, floating at surface, frequent change of direction.
Socialising	Movements without specific direction, breaching, body contact.
Feeding	Variable movements, repetitive diving, prey jumping and/or in mouth.
Travelling	Straight directional swimming with constant breathing.
Milling	Movements without consistency and frequent changes in direction.

6.2.2 DATA ANALYSIS

ACOUSTIC DATA

Digital recordings were visually reviewed using Adobe Audition 2.0 (Adobe Systems) with the following spectrogram settings: FFT size of 512 points, Hamming window, and frequency resolution of 48 kHz. This first review was used to identify the quality of each recording and to locate signature whistles. The signature whistles of each individual were identified visually comparing with the signature whistle catalogue from Woods Hole Oceanographic Institute and the recordings of signature whistles from the capture-release sessions of each individual for confirmation. Signature whistles were counted and saved in separate files for processing. The number of whistles obtained varied with each individual. For statistical pairwise tests, the maximum number of signature whistles collected in either situation determined the total number of pairs possible to be used for each individual in parameter measurement analysis.

Individuals usually produce signature whistles during social interactions. Therefore focal follows of solitary dolphins or of animals with few interactions reduced the available total sample size of signature whistles. For some individuals, it was not possible to find a sufficient number of signature whistles in one or both of the conditions (tagged or non-tagged). These individuals were not used in the acoustic analysis. In order to use an individual for acoustic analysis, it had to have at least 5 signature whistles in each condition. Only the recordings of 6 individuals (4 males and 2 females) met these criteria.

Once the signature whistles were saved in individual files, contour extraction was performed using the software 'Beluga' in MATLAB (R2014b) (http://biology.st-andrews.ac.uk/soundanalysis/) which extracts contours based on a peak-finding algorithm (Deecke *et al*., 1999). This was performed in a similar way as the extraction of contours for the acoustic data collected during capture-release sessions stated in Chapter 2 (section 2.4). However, a set of only six parameters was extracted using a custom routine in MATLAB (R2014) (Table 6.2). The number of loops was counted visually directly from the spectrogram. A loop was defined as in Esch *et al*., (2009a) as a repetition of the similar modulation pattern within a continuous whistle or a unit composing the signature whistle with separation less than 250 ms for non-continuous signature whistles. The chosen frequency and time parameters have been used in previous studies investigating variation in whistles due to stress and anthropogenic effects on free-ranging dolphins (May-Collado & Wartzok, 2008; Esch *et al*., 2009b; May-Collado & Quiñones-Lebron, 2014). Contour modulation comparisons were not performed as it has been shown in Chapter 4 that the modulation remains highly similar over long periods of time, and that acoustic parameters seem to show changes in disturbances and context (Janik *et al*., 1994; Buckstaff, 2004; Esch *et al*., 2009b).

Table 6.2. Parameters measured from the fundamental frequency contour of each signature whistle.

CODE	PARAMETER
BEG F	*Begin frequency*
END F	*End frequency*
MAX F	*Maximum frequency*
MIN F	*Minimum frequency*
MEAN F	*Mean frequency*
DUR T	*Total duration*
LOOP	*Number of continuous repeated modulation patterns*

All statistical analyses were performed using R software (R 3.2.0, GNU project). First, the parameters were tested for normality using Shapiro-Wilks tests and observing the quantile-quantile distribution plots. Since most of them were non-normal, a series of paired Wilcoxon Tests were performed in order to investigate the variation in each individual in both conditions (with D-Tag and without D-Tag) considering that each signature whistle is distinctive. In this case, a Bonferroni correction was applied due to the large number of comparisons to avoid false positives.

Signature whistle rates were calculated for every individual counting the number of signature whistles in the audio file and dividing that number by the total time of the follow in minutes as the recording time was the same as the time of each focal follow in both conditions, with the D-Tag attached and without the D-Tag. This was performed in order to establish the parameter for comparison with all the individuals. Signature whistle rate data was tested for normality using Shapiro-Wilks tests for when animals were tagged (p=0.19) and non-tagged (p=0.15). Then, paired T-tests were used to investigate if there was a statistically significant difference between the signature whistle rates in both conditions.

BEHAVIOURAL DATA

Only those focal individuals that were followed for at least 63 minutes in order to obtain a sample size of at least 21 points of 3 minute behavioural samples in both conditions (tagged and non-tagged) to be used for surface behaviour analysis. Using larger sample size (>20) helps to increase power for fitting the model.

In order to test if there was a significant difference between the behaviours during the follows when the animals were carrying a tag and the follows a week after the tagging, I used

Generalised Estimating Equation based-models (GEEs). The statistical analyses were performed using R software (R 3.2.0, GNU project) using the package *geepack* (Højsjsgaard *et al*., 2006).

GEE models are similar to General Linear Models (GLMs), but they allow taking into account the dependency of one behavioural data point with the consecutive point, and are better suited for longitudinal data (Ballinger, 2004).

The behavioural information for each three min sample during the focal follows was organised in columns in a spread sheet for the analysis. Then, the model was constructed with the response variable set as a binary presence absence of the D-Tag (with D-Tag=1, without D-Tag= 0), and behaviour was set as the independent categorical variable. The behavioural data was coded in other column as categories giving numbers from 1 to 5: 1) Milling, 2) Feeding, 3) Travelling, 4) Resting and 5) Socialising. Each individual was organised consecutively in a column where the variable individual (ID) was included as the grouping variable in order to take into account individual variation in behavioural response. Finally, the family for the model was chosen as 'binomial'. The individuals were included together to give more power to the model.

Then, the GEE model was run to calculate the probability of each behaviour proportions to be significantly different when the dolphin is carrying a tag and not carrying a tag, taking into account the variation within each individual in the sample. The probability and confidence intervals of the behaviours to be significantly different between both conditions were calculated using a bootstrapping routine (written by D. Russell, 2015) and plotted. Therefore, if there is no statistical difference between proportions of one behaviour in both conditions ($p > 0.05$), the probability plot would show the confidence intervals of that behaviour in the 0.5 line. On the other hand, if any behaviour is significantly different ($p < 0.05$), the probability plot would show the confidence intervals for that behaviour above or below the 0.5 probability line to the direction of the condition were happened more, either with the D-Tag (1.0) or without the D-Tag (0.0).

6.3 RESULTS

A total of 66.8 hours of focal follows were conducted (Table 6.3). During the week of capture-release sessions with the D-Tags in place, were 12 focal follows with a total duration of 43.9 hours took place. The follows the week after capture-release sessions without the D-TAGs consisted of 9 focal follows with a total duration of 22.9 hours.

Table 6.3. Total number of individuals tagged for focal follows in each condition during 2013-2014.

Tagged animals ID	Focal Individual	Year of Follows	Duration with D-Tag (mins)	Duration without D-Tag (mins)	Pair Relation
F221	F221	2013	136	272	Alone
F138	F138	2013	30	—	Alone
F128 & F187	F128	2013	183	301	Male/Female
F280	F280	2013	237	235	Alone
FB33 & F268	FB33	2013	324	33	Mother/Calf
FB90 & F270	FB90	2013	485	—	Mother/Calf
F196 & F268	F196	2014	155	183	Male Alliance
F276 & F142	F276/F142	2014	281	138	Male Alliance
F197 & F243	F197	2014	265	72	Mother/Calf
F133 & F245	F133	2014	341	63	Mother/Calf
F185 & F249	F185	2014	110	—	Mother/Calf
F164 & F242	F164	2014	85	—	Male Alliance

A total of 9 focal individuals for which we had behavioural data and acoustic recordings in both weeks were used for analyses. During the follow of F276 and F142, the D-Tag on F276 got detached after a couple of hours, and it was necessary to switch to F142 as focal individual for the rest of the follow. The individuals used for the analyses are found in Table 6.4.

Table 6.4. List of Individuals used to compare both conditions (with D-Tag and without D-Tag) showing their age, sex and year of capture. The analyses column gives a code indicating which of the analysis each individual was part, **a** (acoustic), **b** (behavioural).

Individual	Sex	Age at capture	Year of follows	Analyses
F221	Female	4	2013	b
F128	Male	21	2013	a & b
FB33	Female	31	2013	a
F280	Male	3	2013	a & b
F133	Female	15	2014	b
F196	Male	16	2014	a & b
F197	Female	11	2014	a & b
F142	Male	22	2014	a
F276	Male	22	2014	b

6.3.1 SIGNATURE WHISTLES PARAMETERS AND RATES.

The results from the Wilcoxon tests indicated that none of the parameters measured showed significant differences between the signature whistles recorded when the individuals were tagged and when they were non-tagged (Table 6.5). In addition, the lack of strong variation between both conditions can be visualised in Figure 6.1 and 6.2 by individual. Most individuals showed marginal differences in the mean values, with exception of F196. This individual showed the lowest *p* values of all the individuals in the analysis for BEG F and MEAN F, with higher mean values for both parameters during the follows without the D-Tag attached. However, none of its parameters result was significantly different.

The average number of signature whistles was relatively higher during the follows when the individuals were non-tagged than when they were carrying the D-Tag. Even though the average follow duration was lower in the second week, the number of signature whistles and average signature whistle rates showed an apparent increase in the second week of follow when the D-Tag was not attached (Table 6.6). This could be caused by individual variation in whistle rates, as individual FB33 showed a high whistle rate differently to the rest of the individual during the second follow, increasing the general average. However, there was no significant difference in signature whistle rate between both conditions (t=-2.27; p=0.073) (Figure 6.2).

Table 6.5. Mean values and standard deviation for each parameter by individual. Results of paired Wilcoxon tests are also shown (Bonferroni correction *p* <0.001).

Parameters	*Individuals*	**No. Whistles in each condition**	***mean ± SD***		Wilcoxon paired test	
			WITH TAG	**WITHOUT TAG**	***V***	***p- value***
BEG F (kHz)	FB33	10	6.16 ± 0.24	6.08 ± 0.17	28.5	0.509
	F128	5	4.21 ± 0.32	4.13 ± 0.47	8	1.000
	F142	10	6.23 ± 1.63	7.65 ± 0.84	13.5	0.169
	F196	8	5.04 ± 0.45	7.71 ± 1.21	0	0.010
	F197	6	7.00 ± 0.72	7.02 ± 1.21	7	0.528
	F280	8	4.96 ± 0.84	5.18 ± 0.67	17	0.944
END F (kHz)	FB33	10	18.23 ± 0.39	17.22 ± 3.71	20	0.832
	F128	5	14.27 ± 1.49	13.16 ± 1.14	14	0.125
	F142	10	9.08 ± 3.02	7.02 ± 0.75	36.5	0.209
	F196	8	12.11 ± 1.54	13.96 ± 1.58	6	0.109
	F197	6	6.22 ± 0.68	5.92 ± 0.84	13	0.688
	F280	8	3.25 ± 0.58	3.67 ± 0.70	12	0.439
MAX F (kHz)	FB33	10	18.61 ± 0.5	18.50 ± 0.62	29	0.473
	F128	5	14.44 ± 1.52	13.39 ± 1.00	13	0.188
	F142	10	16.42 ± 2.27	15.98 ± 1.09	33	0.625
	F196	8	18.32 ± 1.26	19.45 ± 1.05	5.5	0.092
	F197	6	15.3 ± 0.41	14.42 ± 1.40	18	0.141
	F280	8	12.57 ± 0.47	13.02 ± 0.78	7	0.271
MIN F (kHz)	FB33	10	5.96 ± 0.21	6.00 ± 0.23	20	0.811
	F128	5	4.22 ± 0.32	3.49 ± 0.51	15	0.063
	F142	10	5.98 ± 1.37	6.53 ± 0.48	29.5	0.444
	F196	8	4.08 ± 0.413	6.36 ± 1.11	0	0.014
	F197	6	5.53 ± 0.66	5.63 ± 0.67	9	0.844
	F280	8	2.95 ± 0.46	2.86 ± 0.29	10.5	1.000
MEAN F (kHz)	FB33	10	11.38 ± 0.47	11.68 ± 0.62	13	0.160
	F128	5	8.29 ± 0.83	7.54 ± 0.29	14	0.125
	F142	10	10.50 ± 1.94	11.16 ± 0.38	28	1.000
	F196	8	9.96 ± 0.52	13.42 ± 1.11	0	0.010
	F197	6	8.53 ± 0.27	9.05 ± 0.59	1	0.063
	F280	8	7.69 ± 0.33	7.68 ± 0.48	17	0.945
DUR T (s)	FB33	10	0.90 ± 0.32	0.85 ± 0.25	34	0.557
	F128	5	0.84 ± 0.36	0.77 ± 0.23	8	1.000
	F142	10	0.91 ± 0.55	1.42 ± 0.70	8	0.050
	F196	8	1.87 ± 0.44	1.10 ± 0.63	28	0.183
	F197	6	2.07 ± 0.57	1.43 ± 0.84	15	0.438
	F280	8	1.60 ± 0.39	1.53 ± 0.32	20	0.844
LOOPS	FB33	10	1.8 ± 0.42	1.6 ± 0.51	7.5	0.424
	F128	5	1.4 ± 0.54	1.6 ± 0.54	2	0.772
	F142	10	2.6 ± 1.07	2.4 ± 1.07	26	0.713
	F196	8	4.8 ± 0.71	6.6 ± 1.59	0	0.050
	F197	6	2.7 ± 0.51	1.8 ± 0.75	6	0.174
	F280	8	2.6 ± 0.52	2.3 ± 0.52	14	0.484

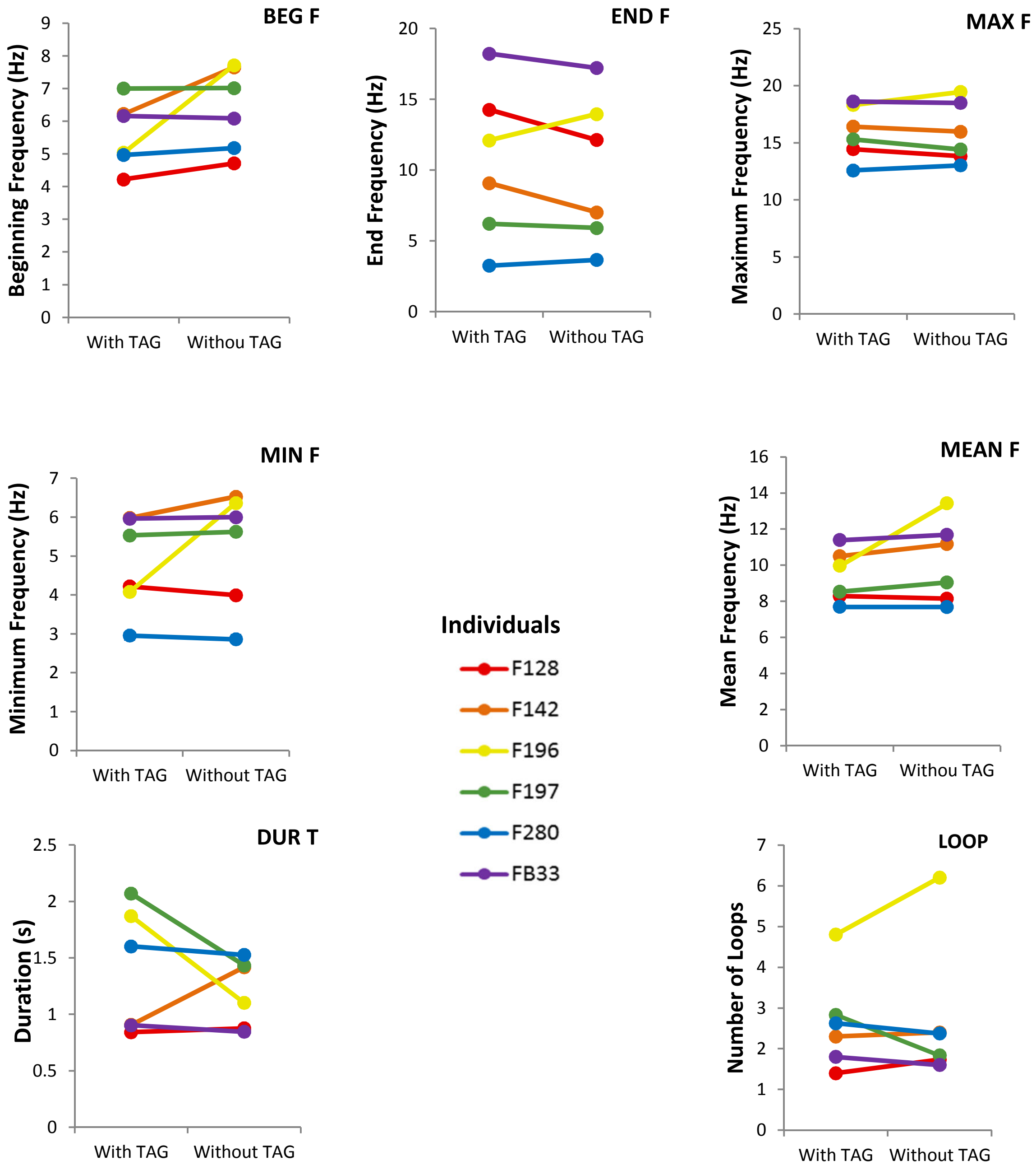


Figure 6.1. Comparisons of the mean for the parameters values for each individual during the week with the D-Tag and the week after without the D-Tag. Lines connect the same individual. Note that individual F196 had the relatively most variable minimum and mean frequency parameters, but there was no significant difference for any parameter of any individual.

Table 6.6. Focal individuals, number and rates of signature whistles in both conditions.

WITH D-TAG

Focal Individual	Average Groups Size	Follow Duration (mins.)	Number of signature whistles	whistles/minute
F280	1.00	240	8	0.033
F197	2.48	265	22	0.083
F196	1.90	155	10	0.065
F142	2.36	281	7	0.025
F128	2.66	183	5	0.027
FB33	2.02	324	24	0.074
Average values	2.2	241.3	12.7	0.051

WITHOUT D-TAG

Focal Individual	Average Groups Size	Follow Duration (mins.)	Number of signature whistles	whistles/minute
F280	1.97	235	29	0.123
F197	3.44	72	6	0.083
F196	3.60	183	8	0.044
F142	5.80	138	17	0.123
F128	6.26	301	26	0.086
FB33	2.36	35	10	0.257
Average values	3.7	160.7	15.8	0.120

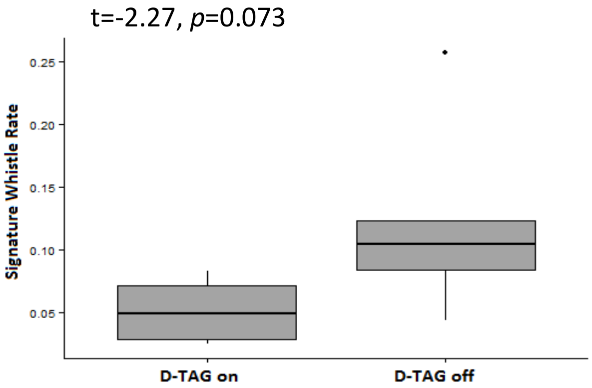


Figure 6.2. Boxplots comparing the signature whistle rate between dolphins carrying a D-tag and not carrying a D-tag. The t-test results for comparing the whistle rates between the follows in both conditions showed that the differences were not statistically significant (t and *p* values are shown in the upper left corner of the graph).

6.3.2 SURFACE BEHAVIOUR: WITH TAG VS WITHOUT TAG

Travelling was the most common behaviour for both conditions (with and without a D-Tag), occurring around 80% of the time (Figure 6.3).

The results from the GEEs showed that there was a no significant difference in the time the dolphins spent travelling, milling or socialising during the follows carrying a D-Tag and the follows the week after without the D-Tag in both conditions. However, there were significant differences between the time spent in feeding and resting during the follows when the animals were tagged and the follows the week after the tagging (Table 6.7).

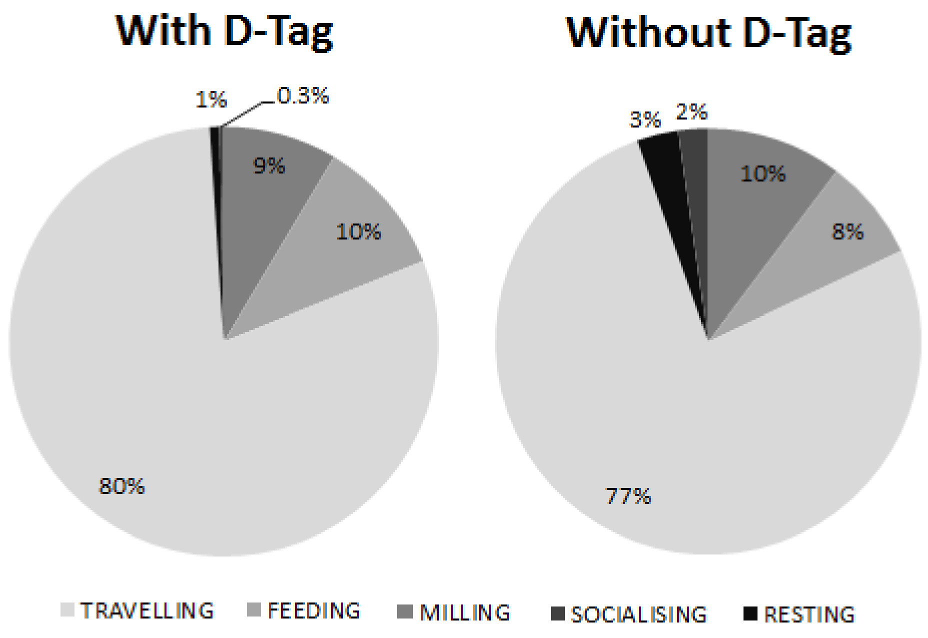


Figure 6.3. Average behaviour proportions when individuals where carrying a tag and without the tag. Note that travelling behaviour represented the highest percentage in both conditions. In addition, the proportion of resting behaviour is higher during the follows with individuals without the D-Tag, and the proportion of feeding behaviour is higher when the dolphins are carrying the D-Tag.

The results from the GEE model indicate the probability of an individual to be tagged or not tagged based on the behaviour proportions in a period of time (longitudinal dependent data) (Figure 6.4). This inference means that dolphins seem to have spent more time feeding during the follow when the individuals were carrying the D-Tag than without the device. In addition, the time dolphins spent resting was higher during the follows without the tag attached. Meanwhile, the rest of the behaviours were not significantly different in both conditions.

Table 6.7. Estimates coefficients, standard errors and *p* values from the GEE model comparing the behaviours between the follows with the dolphins with a D-Tag and without the D-Tag. Note that feeding behaviour happened significantly more when individuals were carrying a tag, and resting behaviour happened significantly more when the individuals were not carrying a tag.

BEHAVIOUR	**Estimate**	**Std. Err**	**Wald**	**P value**
MILLING	2.47e-16	0.381	0.00	1.0000
FEEDING	9.73e-01	0.427	5.20	**0.0227**
TRAVELLING	-9.26e-02	0.506	0.03	0.8547
RESTING	-2.64e+00	0.785	11.29	**0.0008**
SOCIALISING	1.61e+00	1.31	1.51	0.21893

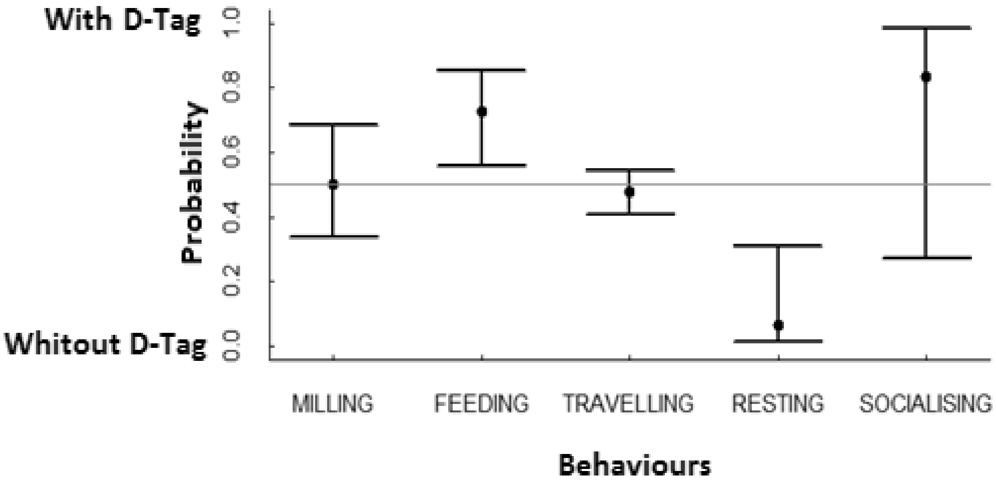


Figure 6.4. Probability of each behaviour to be associated with individuals with a tag or without a tag. The grey line at 0.5 indicates the area where behaviours are the same in both conditions. The bars indicate 95% confidence interval. Note that feeding behaviour occurs significantly more when the animals are carrying a tag, and resting behaviour occurs significantly more when the individuals are without a D-Tag.

6.4 DISCUSSION

Collecting biological information from animal populations may result in disturbance to individual and group behaviour. Therefore, it is necessary to show that research efforts have no effect on general animal fitness or that the possible effects are known (McMahon *et al*., 2012). The attachment of tracking devices to individuals may or may not result in a variety of disturbances. Most importantly, effects that result in a long-term deterioration of vital rates and performance of the organism are to be avoided. Furthermore, it is in the researcher’s interest to minimise disturbance caused by the method when collecting data to ensure a representative sample.

Cetaceans are exposed to a variety of possible effect during a tagging process, such as the approaching of the boats, the attachment of the tag, the drag when carrying the tag and in specific occasions, the process of capture-release of the animals. These species might show aversive reaction to tag attachments, especially during boat surveys. For instance, bottlenose dolphins in Doubtful Sound increased evasive behaviours during attempts to attach suction-cup tags from a boat (Schneider *et al*., 1998). In this study, D-Tag devices were placed on dolphins at the end of capture-release sessions before the animal was released. With the animals restrained during these sessions, I could not assess the effect of attaching a tag.

Stress responses during health assessments in Sarasota have been investigated using acoustic information. Esch *et al.* (2009b) found that the number of loops in signature whistles and the whistle rate were significantly higher at the beginning of a capture compared to the end before the animal is released, and that this increase could be attributed to stress related to being the capture. Physiological responses to capture-release health assessments in bottlenose dolphins from South Carolina and East Florida showed a pattern of decreasing short-term disturbances, where the concentrations of epinephrine and dopamine increased during the pre-examination and then decreased with time closer to the post-examination time (Fair *et al*., 2014). The recordings during follows for this study started just after the release of tagged animals, and the results showed that there was no significant difference in the number of loops (Table 6.5) or average whistle rate (Figure 6.2) between the week when the animals were carrying a D-Tag and the second week without the D-Tag. Thus, considering that the whistle rate and number of loops decrease by the end of the restrain session before the release compared with the beginning of the session and when comparing individuals during the restrain sessions versus undisturbed conditions (Esch *et al*., 2009b), the lack of statistically significant difference in these parameters after the release with a D-Tag and the week after without the D-Tag suggests that the reduction in stress towards the end of the capture remained after the release, and that carrying the acoustic device did not represent a negative impact on the acoustic behaviour.

Other studies have shown that whistle parameters might change in the presence of anthropogenic disturbances. For example, bottlenose dolphins seem to produce whistles with lower frequencies when dolphin-watching boats were around the animals in Panama (May-Collado & Quiñones-Lebron, 2014). Moreover, Indo-Pacific bottlenose dolphins also produce whistles with lower frequencies and less modulation in a noisy environment (Morisaka *et al*., 2005b). However, I found that none of the other acoustic parameters in signature whistles differed significantly between the dolphins when tagged and untagged (Table 6.5 & Figure 6.1). The results were highly similar for most individuals. Even though none of the parameters measured for dolphin F196 were significantly different, this individual was the one that showed the relatively highest change for minimum frequency and mean frequency. It is unclear why this dolphin showed relatively high variation in those parameters, as this individual is an adult male that has been part of the health assessments before. It has been shown that dolphins can modify acoustic parameters in their signature whistles depending on the context (Janik *et al*., 1994) but that variation does not necessarily reflect stress, as Esch *et al*. (2009b) found that number of loops seems to vary significantly as possible stress response, but for F196 that parameter remained very similar. This lack of effect on signature whistle rates and acoustic parameters supports the use of acoustic recording tags for studying calling rates, body position and diving behaviour in cetaceans as it has been used in fin whales (*Balaenoptera physalus*) (Stimpert *et al*., 2015), sperm whales (Madsen *et al*., 2002), and pilot whales (*Globicephala melas*) (Jensen *et al*., 2011).

On the other hand, changes in surface behaviour of odontocetes have been used to identify potential disturbances cause by anthropogenic activities (Nowacek *et al.*, 2001; Constantine *et al.*, 2004). Behavioural changes can also be used also to identify potential effects after attaching a tag or similar device. For example, attaching D-Tags to sperm whales caused only short-term effects with reduction of non-foraging time that ceased in a few hours after tagging, with no evidence for long-term changes affecting locomotion cost or foraging success (Isojunno & Miller, 2015). Moreover, Antarctic killer whales showed no negative effects of carrying a satellite tag for a period of 26 days (Reisinger *et al*., 2014). For this study, it is important to mention that none of these devices were set to remain on the dolphins for more than a 24 hour period. Therefore, long-term attachment effects were not investigated.

Changes in behaviour are not necessarily a negative response. It has been a concern that tag attachments can affect the foraging behaviour of animals. For example, mallard ducks (*Anas platyrhynchos*) reduced their time feeding and increased their time resting when they were carrying harness radio transmitters (Pietz *et al*., 1993). However, the results of the GEE analysis showed that the proportion of time that dolphins spent feeding was longer during the time that the dolphins were carrying the D-Tag than during the follows without it. Therefore, the decrease in time spent foraging during the follows when the animals were not tagged can be attributed to other factors such as their daily feeding cycles (Wells *et al*, 2013). Moreover, in Sarasota, dolphins tagged with satellite transmitters were observed feeding in their normal cycle during the periods carrying the transmitter (Wells *et al*., 2013).

On the other hand, Van der Hoop *et al*. (2014) found that dolphins in captivity seem to swim slower in order to avoid increasing energy expenditure that could result by the tag-induced drag, as the flow disturbance and turbulence around the tag would tend to increase with high speed (Munson *et al*., 2006). Moreover, Blomqvist & Amundin (2004a) found that two tested dolphins in captivity swam slower and spent more 'logging' (resting) during the periods carrying an acoustic tag on their dorsal fins. However, the results from the GEE model in this study showed the dolphins rested significantly more during the time they were not carrying the D-Tag (Figure 5.4), suggesting that the suction cup tag did not have an impact on their swimming behaviour. Designs of the D-Tag have been improved to reduce drag in fast swimming animals (Shorter *et al*., 2014).

In addition, there were no significant differences in the proportions of time spent travelling, milling and socialising in both conditions, suggesting that dolphins continued interacting with conspecifics whilst carrying a D-Tag. This can be indicative of negligible effect on their socio-sexual behaviours. Similarly, Amazon river dolphins (*Inia geoffrensis*) tagged with radio transmitters showed no effect on their reproductive success for at least three years compared with non-tagged individuals (Martin *et al*., 2006).

In respect of anthropogenic effects, it is important to note that mathematical models have predicted that bottlenose dolphins seem to have a good tolerance to disturbances and that some changes in behaviour do not necessarily decrease vital rates and general success of the individuals as long as they can compensate for the effects (New *et al*., 2013).

In conclusion, this study suggests that dolphins carrying a D-Tag do not change significantly their signature whistle parameters or signature whistle rates. In addition, differences in feeding and resting behaviour in both conditions do not show an impact on foraging or swimming while carrying a D-Tag, suggesting that there is no immediate negative effect on bottlenose dolphin surface behaviour when carrying these devices.

The use of D-Tags has been of great benefit to provide information about cetacean movements, acoustic repertoires and anthropogenic effects (Johnson *et al.*, 2009; Schmidt *et al.*, 2010; Tyack e*t al.*, 2011; Goldbogen *et al*., 2014; Stimpter *et al*., 2014), and contribute to obtaining valuable data about the environment (Payne *et al*., 2014). It is recommended for any project using attachable devices to investigate possible long-term effects of these devices on animal populations to ensure that research tools are not interfering with the survival of the animals.

CHAPTER 7

GENERAL DISCUSSION

7.1 BOTTLENOSE DOLPHINS AND SIGNATURE WHISTLES

Bottlenose dolphins (*Tursiops truncatus*) are social animals that communicate using a complex vocal repertoire (Wells & Scott, 1999; Connor *et al*., 2000; Au & Hastings, 2008). Within this repertoire, signature whistles are tonal signals conveying individual information through their modulation pattern (Caldwell & Caldwell, 1965; Janik *et al*., 2006; Sayigh *et al*., 2007) that are used during group joins at sea (Quick & Janik, 2012), and which can be copied to address conspecifics (King *et al*., 2013). In addition, signature whistles develop in early stages of life (Killebrew *et al*., 2001) influenced by vocal learning and are relatively stable once crystallised (Sayigh *et al*., 1990; Fripp *et al*., 2005). Although male bottlenose dolphins seem to still modify to some degree the modulation when they form alliances (Watwood *et al*., 2004). Yet, the use of these signals for recognition of physical characteristics and the variation of signature whistles over long periods of time (>10 years) remained unstudied. For this thesis, I investigated whether signature whistles are arbitrary and long-term stable, individual signals, the importance of the social structure for the crystallisation of the modulation patterns, and effects of the use of biologging for acoustic data collection.

7.2 SIGNATURE WHISTLES AS ARBITRARY SIGNALS IN BOTTLENOSE DOLPHIN REPERTOIRES

An arbitrary signal in communication can be defined as a symbol or code that is created to be associated to a specific reference, and are typical for human language (e.g. word <-> meaning, name <-> person). Humans use a vast number of vocal arbitrary symbols that are learnt, but their use is rare in other animal communication systems (Nowak & Komarova, 2001; Hurford, 2004).

Vocal learning requires interactions with conspecifics in a social environment. As mention in Chapter1, vocal learning can be defined as the ability of modifying acoustic signals and integrating them to the own repertoire after exposure to external sound sources (Janik & Slater, 2000). On the other hand, functional referencing is defined as the use of specific signals to convey an object of action (Macedonia & Evans, 1993; Evans, 1997). Animals displaying vocal learning and referencing generally live in social groups (Blumstein, 1999; Wilbrecht & Nottebohm, 2003; Nowicki & Searcy, 2014). For example, adult spectacled parrotlets (*Forpus conspicillatus*) produce signature calls (Wanker *et al*., 1998) which are modified versions of signatures from family members (Wanker *et al.*, 2005). Similarly, green parrots (*Forpus passerinus*) learn their signature calls from their parents (Berg *et al*., 2012). On the other hand, vervet monkey (*Chlorocebus pygerythrus*) alarm calls seem to be mostly innate, with a limited range of acoustic plasticity determined by the sound apparatus (Hufford, 2004).

As stated in Chapter 1, dolphins possess a sound production system that allows them to produce a wide variety of vocalisations and even produce multiple sounds simultaneously (Berta *et al*., 2006; Au & Hastings, 2008). The arbitrary modulation of tonal sounds requires good control of the vocal apparatus and neurological processing (Marino *et al*., 2007). Even if vocal organs impose a limit on what frequencies a modulation can reach, the innovative sequences, loops and inflections can result in a wide variety of patterns.

Signature whistle modulations and acoustic parameters seem not to be influenced or constrained tightly by physical characteristics such as sex or size of the individuals as stated in the results obtained in Chapter 3, meaning that this type of information is not necessarily encoded in specific modulation pattern type or specific acoustic parameters found across individuals. On the contrary, that indicates a high level of arbitrariness of this individual signal. Neither sex, nor age, nor size showed significant effects on the modulation and fundamental frequency parameters. This independence in acoustic parameters also was observed in Chapter 6, where the use of tags did not alter the acoustic parameters of signature whistles within individuals (Table 6.5). Variation may be constrained to certain limits if crystallised patterns and fundamental frequency ranges function as an individual referential signal themselves, and need to be maintained relatively stable. Considering that, any possible effect of stress reflected in signature whistles seems to be expressed primarily in vocalisation rates and loop increase (Esch *et al*., 2009b).

Contextual variation in acoustic signals has been studied in other species. For example, juvenile hyenas (*Crocuta crocuta*) produce calls with higher maximum frequencies and shorter inter-call intervals during a context of social excitement (Theis *et al*., 2007). Moreover, big brown bats (*Eptesicus fuscus*) show variation in emission patterns and specific syllable structures in their social vocalisations during different behaviours of aggressiveness and appeasement (Gadziola *et al*., 2012). Furthermore, male fallow deer (*Dama dama*) produce higher mean fundamental frequency in presence of females when other males are around than when other males were not close (Charlton & Reby, 2011). In addition, male zebra finch (*Taeniopygia guttata*) songs vary in tempo depending whether they are directed to a female or sung in isolation (Cooper & Goller, 2006). Similarly, bottlenose dolphins have shown context-specific acoustic variation in whistle production that could be transmitting additional information about situations (Janik *et al*., 1994). However, this variation of acoustic parameters in signature whistles is not necessarily perceived by the receiver animal as an obstacle for individual recognition, considering that the contour modulation itself contains enough information for the dolphins to recognise individuality (Janik *et al*., 2006).

The development of signature whistles as referential signals is influenced by vocal learning, and it seems that early exposure to surrounding sounds, including conspecifics' signature whistles help to shape the crystallisation of signature whistles of new-borns (Tyack & Sayigh, 1997; Miksis *et al*., 2002; Fripp *et al*., 2005). As calves spend most time with their mothers, the fact that mothers seem to have a higher influence than other genetically related dolphins (e.g. siblings, grandmothers) can be understood. Even though in the past it has been claimed that male and female calves produce signature whistles influenced by their mothers in different ways (Sayigh *et al*., 1995), it seems that using a different approach with measurement of similarity using a dynamic time warping algorithm, the influence of the mother's whistle on the calf's whistle development is not different between the sexes. The association degree of mothers and calves is similar regardless of the calf's sex, as the weaning period is similar for males and females (Mann, 2000). However, as the age of weaning approaches the association with the mother reduces as the newly independent calves associate more with other individuals (Gibson & Mann, 2008). Once they reach sexual maturity, the interactions are ruled by the sex-segregated social structure (Wells & Scott, 1999; Connor *et al*., 2000).

The importance of having referential labels lies in the structure of a dynamic society. Bottlenose dolphins tend to show fluid associations with other individuals within a population.

Thus, having individual labels helps to carry information about those dolphins that are familiar and remembered. The fact that physical characteristics are not transmitted by signature whistle modulation might be countered by the capacity to remember past encounters. For instance, when dolphins meet, they would exchange signature whistles (Quick & Janik, 2012) and they would be able to identify the age group and sex of the individual with which they are interacting regardless of the quality of the interaction. This information would stay in memory of a dolphin associating that particular whistle modulation with all the information about the individual. The sex and age recognition might be done by using other signals of their acoustic repertoire, using vision or a combination of both. Dolphins in captivity have shown that they are capable of using visual cues to identify the main trainers by patterns in their clothing (Tomonaga *et al*., 2015). They have good vision capacities similar to their terrestrial relatives, artiodactyls (Pryor, 1999; Tomonaga *et al*., 2014) and they could use a combination of visual cues and signature whistles to build a memory of the individuals in their community. For instance, it is known that bottlenose dolphins can remember signature whistles from old conspecifics in captivity for up to 20 years (Bruck, 2013). In addition, the fact that dolphins can recognise signature whistles from familiar individuals without vocal cues (Janik *et al*., 2006) is likely to be linked to their capacity to associate memories of those modulation patterns to specific individuals.

Signature whistles' modulation patterns are relatively stable over time, with at least 90% of contour similarity for at least 29 years, as shown in Chapter 4. However, there is a sex difference in the degree of modification, where males modify their signature whistles to a higher degree than females. Female whistles stay up to 96% similar for the sample in this study (Figure 4.4). This situation is related to the social behaviour of the sexes in the fission-fusion society with sex-segregated adult groups and male alliances (Wells & Scott, 1999; Wells, 2009). The loose bonds amongst females offer little pressure to converge modulation patterns, meanwhile the tight relationship of male pair alliances may favour convergence in modulation patterns and repertoire possibly to strengthen the bond (Smolker & Pepper, 1999; Watwood *et al*., 2004). In this regard, the variation in stability might not be innately sex specific if the abilities for vocal learning are considered to be similar in both sexes (Richards *et al*., 1984; Reiss & McCowan, 1993; Janik & Slater, 1997).

At the same time, vocal production structures do not appear to be sexually dimorphic as energy distribution differences were not significant for sex (Figure 3.6). However, it is important to mention that voice cues do not affect the modulation pattern of the signature whistle fundamental frequency. Therefore, a difference in energy distribution correlated with age is likely to affect any vocalisation that the individual produces and could be understood as an effect of the growth of the cranial bones and organs (Fletcher, 2004; Fitch *et al*., 2006).

The cues individuals use for sex and age recognition from unknown individuals might lie in other acoustic or visual cues as bottlenose dolphins possess good underwater sight (Berta *et al*., 2006). However, the fact that signature whistles specifically seem not to carry information about physical characteristics in the modulation does not mean that acoustic cues are not used for such recognition at all. Certain acoustic patterns or sequences not investigated in this thesis might help to identify sex and age for approaching dolphins. For example, sex and age recognition would be advantageous for a receptive female with the potential of being coerced by males.

Fundamental frequency modulation is produced by sound production organs considering the source of vibration (e.g. vocal cords, syrinx and phonic lips.), whilst voice cues represent the filtering caused by resonance in air cavities in the path of the sound waves. This filtering can alter the harmonic energy content in vocalisations (Fitch, 2000; Riede *et al*., 2006). Besides, the vocalisation characteristics from the source and the filtering can vary independently (Fitch & Hauser, 2003).

Humans use voice cues to recognise individuals regardless of what the speaker is saying. However, humans also use individual, referential labels (e.g. names) when meeting specific people, and those encounters help to learn about physical characteristics (e.g. sex, age group, race) of the person using the sight and memory. Therefore, an exposure to a specific 'name' would recall the learnt information even with the lack of voice cues, although this would not apply to other reference signals in speech for other objects (Hurford, 2004; Formisano *et al*., 2008). Similarly, bottlenose dolphins in a semi-resident community would interact with each other exchanging signature whistles and learning those referencing labels of their associates as they age. Therefore, it appears to be more important to recognise those specific modulation patterns than voice cues that could vary with depth (Ridgway *et al*., 2001). However, if sex or age recognition comes from other vocalisations, the harmonic content could be of use near the surface considering that coastal bottlenose dolphins mostly inhabit very shallow areas and that most of the dives in offshore bottlenose dolphins are in shallow depths (Wells & Scott, 1999; Klatsky *et al*., 2007).

Even though bottlenose dolphins are a phenotypically plastic species, social structure seems to be relatively similar in the populations distributed worldwide (Connor *et al*., 2000; Reynolds *et al*., 2000), with the exception of the Doubtful Sound population where individuals form stable groups that are atypical for the species (Lusseau *et al*., 2003). Despite this situation, the results presented in this work might be a starting point for comparisons with other populations. For instance, individuals in the North Atlantic are bigger in size than their tropical counterparts. The results in this thesis suggest that size does not affect modulation patterns in fundamental frequencies. However the difference in length for the North Atlantic populations could show a stronger tendency in respect of the energy distribution in the second harmonic than what was found in Chapter 3 for the community in Sarasota. Tropical populations show similarity in maximum sizes and sexual dimorphism which has to be considered when extrapolating results. Also, as signature whistles seem to be arbitrary, referential signals that are not influenced by physical characteristics and seem to have the same function in all populations, the results of this thesis may be applicable to populations outside of Sarasota Bay.

In addition, it is important to highlight the use of appropriate tools for measuring acoustic parameters in order to obtain meaningful information to answer research questions. The ARTWARP method (Deecke & Janik, 2006) has been successfully used for bottlenose dolphin whistle repertoire classification and comparison (Bazua-Duran *et al*., 2013; Gridley *et al*., 2014). Furthermore, the use of dynamic time warping to compare modulation patterns of signature whistles can be as objective as the approach using multiple volunteers for human classification with the advantage of not having to relay in volunteer availability. However, this does not mean that classical measurements are not useful, but that simple frequency parameters might not reflect the modulation. To date, we know that the modulation pattern of signature whistles carries the individual information (Janik *et al*., 2006) and therefore, modulation comparisons could be valuable for similarity measurements as used for Chapter 4 and Chapter 5 in this thesis. Meanwhile, classical measurements can tell about the limits on frequency or time ranges, and spectral measurements can give information about voice cues in relation to other parameters (Chapter 3 & Chapter 6).

7.3 USING BIOLOGGING FOR ACOUSTIC COMMUNICATION RESEARCH IN DOLPHINS.

Dolphins live in dynamic groups and interactions with conspecifics rule their behaviour. Therefore, obtaining reliable undisturbed acoustic behavioural data can be a challenge due to the difficulty in accessing those groups in the wild. Advances in technology have helped greatly to obtain information about the life of wild dolphin populations, where disturbances caused by research vessels are kept to a minimum by maintaining distance from focal groups. For instance, the use of towed hydrophones has facilitated the acquisition of recordings of wild odontocete populations, but large ranges to the animals and the directionality of some of the sounds might lead to situations in which the whole repertoire of vocalisations is not always obtained.

It is important to consider any effects that the use of specific research tools might have on the studied species. Hence, as the use of attached devices for measuring a diverse number of parameters is expanding, an increasing need of understanding possible effects on animal behaviour has emerged to improve devices and guarantee unbiased data collection (Hooker *et al*., 2007; Bograd *et al*., 2010)

In Chapter 6, I investigated the effect of capture and the attachment of suction-cup acoustic tags on bottlenose dolphin acoustic and surface behaviour, showing that carrying the attached device itself had no measurable effect on the surface behaviour. Due to the data collection logistics in Sarasota Bay, it was not possible to completely separate the effect of capture-release sessions from the tagging itself, as every tagging process is preceded by a capture-release session. Nevertheless, the results obtained during the follows after the captures could support the idea that the effects of the capture of dolphins on their acoustic behaviour in the same population are reduced at the end of the capture when the number of loops get reduced

(Esch *et al*., 2009b), and the lack of differences in the acoustic parameters during the follows in both conditions (with a tag and without a tag) suggests a negligible effect of carrying the tag on the signature whistles. Even though vocalisation rates might increase as a result of stress at the beginning of a capture (Esch *et al*., 2009b) or variation in whistle parameters might occur depending of specific contexts (Janik *et al*., 1994), variation in acoustic parameters of signature whistle might not affect their function as individual reference signals as long as the modulation pattern remains recognisable to the receiver. It would be interesting to continue a long-term investigation of the tagging effects on wild bottlenose dolphin populations, especially isolating the tagging effect from other disturbances.

7.4 CONCLUSIONS

Signature whistles are arbitrary, learnt individual signals that are not influenced in parameters of the fundamental frequency by physical characteristics such as sex, age or size. Therefore, sex and age recognition might be achieved by different paths or a combination of paths. Signature whistles are highly stable through time, but with variation by sex, where males show a higher degree of variation over time than females, likely related to alliance formation. The crystallisation pattern seems to be primarily influenced by social interactions through vocal learning rather than a genetic imprint of related individuals. Fundamental frequency characteristics in signature whistles do not seem to be altered by the use of biologging devices, neither does the whistle rate. Differences in surface behaviour do not seem to represent a negative impact caused by carrying a tag.

Future work should explore other sounds of the bottlenose dolphin repertoire and the use of voice cues in those vocalisations for sex and age recognition. Moreover, it would be interesting to investigate the long-term relation of association patterns and the evolution of similarity in signature whistles within specific pairs of individuals in several years (e.g. male alliances) in order to understand the rules of stability of signature whistles in bottlenose dolphins.

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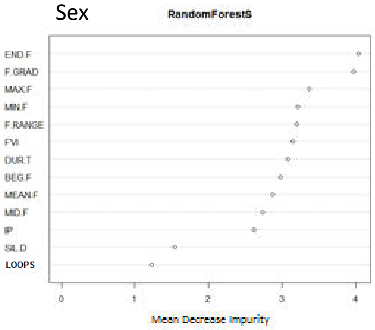
APPENDICES

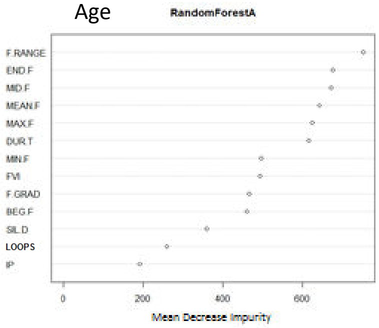
APPENDIX 1

Plot results of the Random Forest Analysis and Multivariate Multiple regression fit diagnostics from Chapter 3.

Sex



Age



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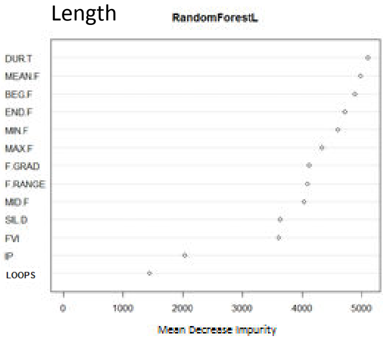
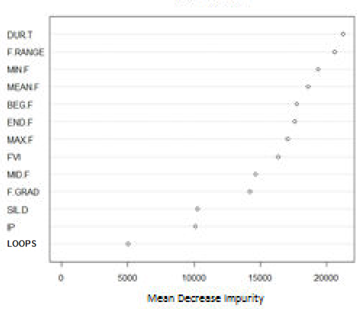
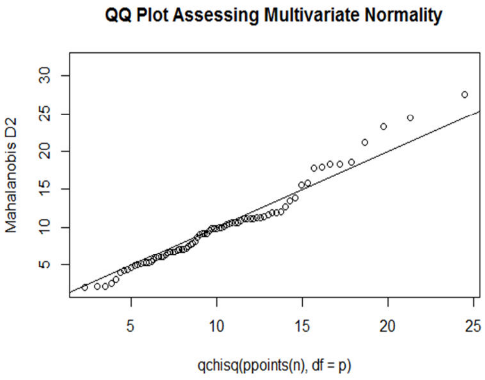
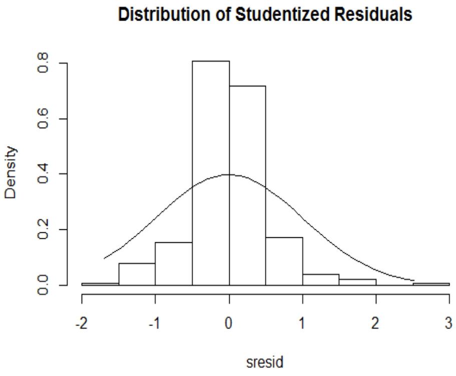


Weight



For relative large sample sizes (> 20) the multivariate normality of residuals can be considered true even with slightly skewed plots.





APPENDIX 2

Pearson's correlation *r* values for the parameters measured in whistle contours for Chapter 4. Light grey cells indicate the values r > 0.6 for the parameters that were not included for the forward selection of the linear mixed model to avoid collinearity.

	BEG.F	END.F	MAX.F	MIN.F	MEAN.F	MID.F	F.RANGE	DUR.T	FVI	SIL.D	STEPS	IP
BEG.F	1.00											
END.F	0.22	1.00										
MAX.F	0.08	0.76	1.00									
MIN.F	0.86	0.35	0.22	1.00								
MEAN.F	0.19	0.49	0.49	0.37	1.00							
MID.F	-0.02	0.21	0.49	0.13	0.85	1.00						
F.RANGE	-0.16	0.51	0.59	-0.10	0.38	0.19	1.00					
DUR.T	0.22	0.07	0.11	0.12	0.11	0.05	0.04	1.00				
FVI	-0.18	0.67	0.95	-0.08	0.69	0.46	0.62	0.07	1.00			
SIL.D	0.24	0.23	0.25	0.24	0.13	-0.13	0.02	0.55	0.18	1.00		
STEPS	0.24	0.18	0.24	0.19	0.17	-0.05	0.00	0.53	0.19	0.84	1.00	
IP	-0.13	-0.51	-0.45	-0.32	-0.29	-0.03	-0.15	0.31	-0.37	-0.26	-0.12	1.00

Quantile-Quantile plot for normality distribution (A) and Fitted Residual plot (B) for the linear mixed model. Both plots indicate a relative good fit of the model. Big sample size (>1000 samples as in this case) account for good normal fit even when there is slightly skewed plots.