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THE USE OF ACCELEROMETRY, VIDEO-TRACKING AND ACOUSTIC RECORDINGS TO STUDY THE BEHAVIOR OF CAPTIVE BOTTLENOSE DOLPHINS

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Courage does not always roar. Sometimes courage is a little voice at the end of the day that says:

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- Mary Anne Radmacher -

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PALAVRAS-CHAVE

Acelerometria; Video-tracking; Bioacústica; Golfinhos-roazes

KEYWORDS

Accelerometry; Video-tracking; Bioacoustics; Bottlenose dolphins

Categorias de Classificação da tese

PsycINFO Classification Categories and Codes:

2200 Psychometrics & Statistics & Methodology

2221 Sensory & Motor Testing

2400 Animal Experimental & Comparative Psychology

2440 Social & Instinctive Behavior

RESUMO

Ao longo dos anos, vários métodos de análise automática e semiautomática têm sido desenvolvidos para superar as limitações e a subjectividade da análise comportamental manual. A acelerometria, por exemplo, tem mostrado um enorme potencial em fornecer informações sobre o comportamento animal. Para este projecto, o comportamento de dois machos adultos de golfinho-roazes (*Tursiops truncatus*) foi estudado em cativeiro, através de diferentes metodologias: acelerometria, *video-tracking* e a análise acústica. Para os dados de acelerometria foi desenvolvido um algoritmo que classificou correctamente 86 comportamentos, agrupados em 8 categorias. O comportamento mais observado foi a Natação Dorsal e a análise de entropia revelou que o comportamento dos golfinhos possui uma estrutura altamente organizada e que poderá permitir a previsão da sequência de movimentos que sucedem a determinada acção. Adicionalmente, foi desenvolvido um sistema de *video-tracking* semi-automático*,* o D-Track. Este sistema quantifica, de forma não-invasiva, as trajectórias em 3D dos golfinhos na água com uma reconstrução tridimensional da piscina, usando câmaras de vídeo comuns. Os resultados mostraram que ambos os animais despenderam 85% do tempo à superfície na Área Profunda da piscina (5 metros de profundidade) e apresentaram velocidade média constante, com predominância das velocidades baixas (máximo de 1,7 ms^{-1}). Este sistema é uma ferramenta inovadora que pode ser usada por laboratórios e parques temáticos para monitorizar as preferências e rotinas dos seus animais.

As emissões acústicas dos golfinhos-roazes são divididas em sons pulsados (cliques de ecolocalização e *burst-pulses*) e não pulsados (assobios, podendo estes ser estereotipados ou variantes), sendo frequentemente associados com outros comportamentos. Numa primeira fase foram analisadas as taxas de emissão de assobios de seis golfinhos em cativeiro, em dois períodos distintos e em três contextos diferentes. Apenas uma categoria de assobios foi observada em ambos os períodos e considerada assobio assinatura, tendo sido associada ao mesmo animal em ambos os períodos. As taxas de emissão dos assobios foram 7,8 vezes mais elevadas em isolamento, comparando com o contexto social. Os assobios recolhidos entre 2012 e 2014 foram analisados através de dois softwares comerciais para extrair assobios (BELUGA) e categorizá-los automaticamente (ARTwarp). No entanto, os resultados mostraram que o ARTwarp não se aplica adequadamente a este tipo de dados, uma vez que muitos assobios são apenas abreviaturas de vocalizações mais longas, e mostrando que a classificação visual ainda é o melhor método de classificação destes sons.

A combinação destas metodologias possui um enorme potencial para fornecer informações sobre movimentos, rotinas e vocalizações associadas aos sujeitos. O D-Track foi usado na tentativa de identificar o emissor mais provável das vocalizações, enquanto a acelerometria foi usada para explorar uma possível relação entre o comportamento e emissões acústicas específicas até agora sem sucesso.

Em suma, o principal objectivo deste abordagem multi-disciplinar foi desenvolver métodos, num ambiente controlado, que possam ajudar a melhorar o bem-estar dos golfinhos em cativeiro e criar ferramentas que possam ser aplicadas em estudos no meio natural. Espera-se que os esforços deste projecto venha a contribuir para o avanço do conhecimento sobre estes animais.

ABSTRACT

Over the years, automated and semi-automated methods have been developed to overcome the limitations and subjectivity of manual behavioral analyses. Accelerometry, for instance, has shown an enormous potential in providing information about animal behavior. In this project, two male common bottlenose dolphins (*Tursiops truncatus)* kept at a zoological facility, were used to study behavior, using accelerometry, video-tracking and acoustical techniques. An algorithm was developed that, using acceleration data, correctly classified 86 behaviors, grouped into 8 categories. The most common behavior observed was Dorsal Swim, and the entropy analysis indicated that the dolphins' behavior contains a high order structure that may allow the prediction of which behavior will follow a specific action. A semi-automatic tracking system, D- Track, was also developed. It is able to quantify, non-invasively, the 3D trajectories of dolphins in the water, through a three-dimensional reconstruction of the pool using standard cameras. The data showed that both animals spent around 85% of the time at the surface of the Deep Area of the pool (5 meters deep) and showed a stable average speed, with slow speeds predominant (maximum 1.7 ms^{-1}). This system is a novel tool that offers possibilities for laboratories and marine parks to monitor the preferences and routines of their animals.

The underwater acoustical emissions of bottlenose dolphins are divided in pulsed (echolocation clicks and burst- pulses) and non-pulsed sounds (whistles, stereotyped or variant patterns), and it is known that various vocal emissions are frequently associated with other behavioral displays. For a primary analysis, the whistle emissions of six captive dolphins, in two separate time sets and three different contexts, were analyzed. Only one whistle contour category was found in both time sets and considered a signature whistle. This contour was associated with the same animal in both time sets. Whistle emission rates were 7.8 times higher in isolation as compared with social context. The data collected from 2012 to 2014 was also analyzed using two commercial software to extract (BELUGA) and automatically categorize (ARTwarp) the whistles; however, the results showed that ARTwarp does not work with this data set, since most of the whistles are just abbreviations of longer version emissions, and human-based visual classification proved to be a better classification method.

The use of all these techniques combined has great potential in providing information on the movements, routines and associated individual vocalizations of the focal animals. The D-Track was used in an attempt to identify the "most-likely" emitter of the sounds while the accelerometer data was explored in an effort, so far unsuccessful, to find a relationship between behavioral patterns and specific vocal emissions.

The overall purposes of this multi-approach research were to use the conditions of a controlled environment to develop methods that may help to improve the welfare of captive dolphins, and to create tools that are applicable to the investigations of these animals in the wild. It is hoped that some novel contributions and advances may come out of various developments and examples here presented.

TABLE OF CONTENTS

INDEX OF FIGURES

INDEX OF TABLES

CHAPTER 1 - GENERAL INTRODUCTION

Bottlenose dolphins, *Tursiops truncatus* (Montagu, 1821), are cosmopolitan marine mammals that have been the focus of numerous studies both in the wild and in captivity. Most of the research done involves social structure dynamics, behavior, communication, ecology, distribution and anatomy.

Three main sections integrate this thesis: accelerometry analysis, allowing the analysis of fine detailed behavior; the development of a tracking system, which enables the quantification of the position of the animal in an artificial environment; and acoustical analysis, to have a clear understanding of the communication of dolphins in different contexts. All sections had the purpose of collecting extensive detailed behavior of captive bottlenose dolphins for: 1) development of methods that could applied in data from wild dolphins, where recordings are difficult to obtain; and 2) understanding of movements and routines of these animals to increase their quality of life in artificial environments. These two approaches will contribute to the understanding of the movements and actions of bottlenose dolphins in their natural behavior.

In the next section, some historical notes about behavioral research, as well as a small introduction regarding acoustics, movements and routines in marine mammals are presented, focusing on the bottlenose dolphin.

1. Behavioral Research

1.1. Some historical notes.

During the seventeenth and eighteenth centuries, naturalists often disputed the interpretation of animal's behaviors, debating whether the activities were congenitally fixed or rather a consequence of the animal's choice (Richards, 1989). The dawn of contemporary behavior science was in the nineteenth century and Charles Darwin's publication "*On the Origin of Species by Means of Natural Selection or the Preservation of Favoured Races in the Struggle for Life*", in 1859, was one of the most important events (Boakes, 2010). Charles Darwin was the first scientist to reconstruct the evolutionary history of behavior by using modern comparative methods (Ghiselin, 1973; Boakes, 2010), postulating how natural selection operates in various kinds of traits (e.g. both anatomical and behavioral) (Boakes, 2010). Two of his major books, "*The Descent of Man and Selection in Relation to Sex*" and "*The Expression of the Emotions in Man and the Animals*" (1871 and 1872, respectively) also contributed to a new growing field, ethology.

Later, when trying to understand animal behavior in the wild, Oskar Heinroth also attempted to apply Darwin's theories (Heinroth, 1909, 1922, 1931, 1941; for more information see Workman, 2013). In the light of Darwin's Theory of Evolution, Heinroth studied innate behavior in birds, arguing that it is possible to track instinctive behavior patterns back to common ancestors (Workman, 2013). The notion of ritualization courtship displays was one of the first and original Heinroth's ideas. Around the same time, Julian Huxley also studied courtship and sexual habits in waterfowls, creating the definition of ritualization and converging it to the study of instinctive behavior as the foundation of ethology (Heinroth, 1909, 1922, 1931, 1941; Schulze-Hagen & Birkhead, 2015). Huxley also highlighted three biological aspects: i) mechanistic-physiological, ii) adaptive-functional and iii) evolutionary or historical, which could overlap but were different in nature (Huxley, 1914; Thorpe, 1979).

Nikolaas Tinbergen, in the 1960s, contributed to the definition of the above mentioned Huxley's aspects by adding ontogeny as a fourth dimension (Tinbergen, 1963; Hladký & Havlíček, 2013; Calisi, 2014): i) mechanism, which deals with immediate causes of the behavior; ii) function, related to the evolutionary processes that give rise to a specific behavior; iii) phylogeny, referring to the history of the trait; and iv) ontogeny, related to the development processes that have shaped a specific behavior (Tinbergen, 1963; Hladký & Havlíček, 2013). These four questions became the roadmap of ethology's development (Burkhardt, 2005), where behavior was not just assumed to have a specific function, but was empirically studied in the context in which each behavior occurs (Röell, 2000).

In 1973, Nikolaas Tinbergen, along with Konrad Lorenz and Karl von Frisch, won the Nobel Prize in Physiology or Medicine for their discoveries in organization and elicitation of individual and social behavior patterns (Burkhardt, 2005).

The methodology developed in this thesis is the first step into dealing with Tinbergen's first question, the behavior mechanism.

2. Different Types of Behavioral Analysis: Automatic vs. Manual

The study of behavior can be done through direct observation, by manually recording the events during sampling periods, or semi-automatically, by observing and entering data into a pre-defined software (Lind et al*.,* 2005; Noldus et al*.,* 2001; Noldus et al*.,* 2002). These techniques have various advantages (Noldus et al*.,* 2001; Noldus et al*.,* 2002). However, there is the drawback of being a very time-consuming process and susceptible to errors due to fatigue, drift and subjectivity of the observers (Lind et al*.,* 2005; Martin & Bateson, 2007).

Although manual recordings offer vast and interesting possibilities to researchers (Ballesta et al*.,* 2014), automated observations may become more reliable, since a computer algorithm can be more stable and consistent than a human eye (Noldus et al*.,* 2001; Noldus et al*.,* 2002). Consequently, this automation has also improved the accuracy of scientific conclusions, allowing for a better understanding of some aspects of behavior otherwise not quantifiable (Lind et al*.,* 2005; Ravi et al*.,* 2005; Wilson et al*.,* 2008). Pattern recognition algorithms that extract quantitative measurements of the animals' behavior enable the detection of both brief and long events, periods of inactivity and daily variations (Noldus et al*.,* 2001; Noldus et al*.,* 2002). This methodology became suitable to measure locomotor behavior, expressed in spatial measurements such as distance or speed, which the human observer is unable to accurately assess (Spink et al*.,* 2001).

The quantitative movement analysis remains as one key interests of the field, since knowing where a subject is, in space and time, can provide relevant information regarding its behavior, ecology and social interactions (Rutz & Hays, 2009). The use of automatized behavior recordings gives the opportunity to study not only the animal itself, but also how it interacts with the environment, facilitating new strategies for its conservation (Cooke et al., 2004; Ropert-Coudert & Wilson, 2005).

2.1. Behavior patterns.

The systematic description of the patterns and behaviors, as classic catalogue prototypes, started with Heinroth and the comparative ethologic study of the duck courtship (Heinroth, 1910), followed by Huxley's studies with the grebe (Huxley, 1914) and Lorenz's with corvids (Lorenz, 1931). After the 1940s, in an attempt to study and better understand the behavior of animals, numerous behavior pattern catalogues, named ethograms, were compiled (Schleidt et al*.,* 1984).

2.2. Ethograms.

An ethogram is a detailed catalog of the behaviors displayed by animals of a certain species. To build an ethogram it is necessary to collect behavioral observations from a sample of subjects, detailed description of the various behaviors and, when possible, images or drawings of said behaviors (Tillberg et al., 2007).

Müller, Boutière, Weaver and Candelon compiled, in 1998, information about bottlenose dolphin movements in the wild, from different regions, with emphasis in solitary and social behaviors (Figure 1.1) (Müller et al., 1998).

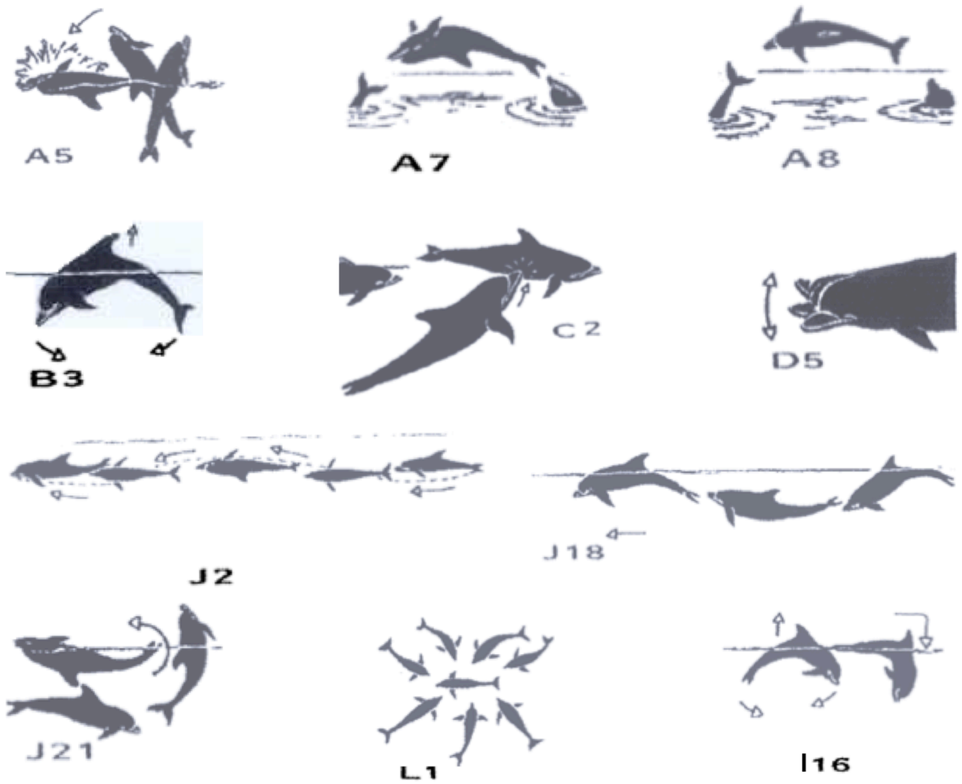

Figure 1.1. Examples of dolphins' behavior drawings in the wild. A5 represents a back breach; A7 is an Inverted Leap; A8 a Lateral Leap; B3 is a Dorsal Arch; C2 is Rostral Nudge; D5 is a Head Nod; J2 is a Corkscrew Swimming; J18 is an Undulating Swim; J21 is a Back Head Swim; L1 is a Spoke Formation and I16 is a Back Up (From Müller et al., 1998).

3. Study of Cetacean Behavior

3.1. Captive dolphins.

Because of their coastal nature and widespread use in zoos and aquaria, bottlenose dolphins are one of the best-known cetacean species (Shane et al*.,* 1986). Due to the difficulties of collecting data in the wild, research done in zoos or marine parks provides a unique opportunity to study specific aspects of this species.

To ensure all their needs, health requirements and safety, wild animals under human care are often managed under strict regimes (Hosey et al., 2009) which are highly based on positive reinforcement training, meaning a desired reward is given after an increase in frequency of a selected behavior (Skinner, 1938; Brando, 2010). This type of reinforcement training achieves total cooperation of the animal, thus decreasing stress and enhancing reliability of the collected data (Laule et al., 2003).

3.1.1. Behavioral studies.

Obtaining detailed information about marine mammals' behavior in the wild can be a challenge due to methodological difficulties (Mann, 1999) and to the fact that most of these animals often move rapidly, quickly covering many kilometers. Hence, behavioral research of these animals is mainly done from boats and through aerial observations, providing limited information about their behaviors which occur underwater, where they spend most of their lives (Costa, 1993; Mann, 1999; Yasuhiko, 2004). Despite these difficulties, over the past 40 years we have witnessed great progress in the knowledge of the behavior, social organization and ecology of these marine species (Shane et al., 1986).

Behavioral parallels between the captivity and wild environments suggest that captive animals may serve as models to better understand cetacean behavior (Samuels & Gifford, 1997).

Numerous behavioral studies with captive dolphins were carried out over the years. Since 1940, small cetaceans at Marine Studios have been the subjects of published studies. In McBride (1940) and McBride and Hebb (1948), the authors showed a detailed description of dolphins' behavior and their vocalizations without using any listening instrumentation, associating sounds with actions (Wood, 1953; Defran and Pryor, 1980). McBride and Kritzler, in 1951, were the first to describe pregnancy and birth in the bottlenose dolphin. In 1953, F. G. Wood Jr. collected information by linking distinct types of acoustic signals to movements, e.g. jaw clap as a sign of intimidation by a dominant animal; specific whistles associated with different types of behavior such as play, sexual behavior or other activities (McBride & Kritzler, 1951; Wood, 1953).

David and Melba Caldwell were among the first scientists to accept that individuality and attachment played a key role in a dolphin's life. In 1964, they carried out numerous studies concerning the behavior of dolphins in captive environments (Samuels & Tyack, 2000).

Pepper and Defran, in 1975, highlighted learning as an important aspect in a dolphin's training (Pepper & Defran, 1975; Shane, 1990). They recommended the use of visual or acoustic cues, which the animals are trained to follow and, thus, performing an action defined by the trainer (Pepper & Defran, 1975) through positive reinforcement and operant conditioning (Defran & Pryor, 1980).

Samuels and Gifford, in 1997, studied the agonistic behavior of the bottlenose dolphin among individuals in the same facility. They described the male's dominance and the female's stable dominance relationships based on age (Samuels & Gifford, 1997).

Despite the number of studies in captivity, some authors consider that captive environment may alter the behavior of the animals (Akiyama & Ohta, 2007; McPhee, 2003), leading to a decrease in the ability to respond to a variable and unpredictable environment (McPhee, 2003).

3.1.2. Behavioral stereotypies.

For welfare purposes, stereotypies are defined as repetitive, unvarying, abnormal and apparently functionless behavior patterns (Ödberg, 1978; Broom, 1983; Mason & Latham, 2004); and have been given increasing importance over the years. Therefore, understanding its causes is important not only to comprehend the organization of behavior, but also to evaluate its consequences on the subjects' quality of life (Rushen & Mason, 2006). Animals in unchanging environments, as captive habitats, are typically the ones performing stereotyped behaviors (Broom, 1983).

Some authors suggest that any level of stereotypy is an indicator of poor welfare (Ödberg, 1978; Mason 1991; Lawrence & Rushen, 1993; Garner & Mason, 2002), others claim that stereotypy should only be a concern if it affects more than a percentage of animals within a group (see Mason & Latham, 2004).

Stereotyped behavior can be observed as: i) behavior patterns repeated in a rigid way; ii) rigid spatial movement; and iii) increase of stereotype behaviors in expectation for food (Gygax, 1993).

There are several studies of stereotypies with various animal species (see Rushen & Mason, 2006). Carnivores living in zoos are very prone to stereotypies, mainly locomotor ones. The characteristics of this behavior, in terrestrial mammals, are repetitive pacing or circling, with consequences such as abrasions, sores, as well as abscesses (Clubb & Vickery, 2006). In marine carnivores, as Lorenz observed in 1969 in sea lions, stereotypies consist of swimming in rigid routines, with turns in predictable locations (Gygax, 1993).

The first reports of stereotypic behavior in captive dolphins date from 1977 (Greenwood, 1977). The lack of social interactions and the size of the artificial environment are often associated with this type of behavior, which means that both factors are highly relevant to the welfare of dolphins (Ridley and Baker, 1982; Mason, 1991; Gygax, 1993).

3.1.3. Welfare concerns.

A careful observation of an animal’s behavior may provide information about its needs, preferences and internal state (Mench, 1998). How the animal behaves is the key to answer most of the welfare concerns (Dawkins, 2004), and much progress has been made over the years to develop new techniques to increase animal welfare quantitative indices. Nevertheless, a measure of stereotypy that truly correlates with welfare is not straightforward and there is still no widely accepted system to measure it (Gygax, 1993; Dawkins, 2004; Mason & Latham, 2004).

4. Acoustic Behavior in Cetaceans

Common bottlenose dolphins produce a wide variety of underwater sounds (Lammers & Oswald, 2015), allowing the detection and discrimination of the position of a sound in three-dimensional space (Mooney et al*.*, 2008). These animals are also known for their vocal learning capabilities, with high ability to imitate other animals' whistles, including man-made whistles or artificial sounds (Lilly, 1965; Janik & Slater, 1997; Tyack, 1997; Janik, 2000; Miksis et al., 2002). This ability comes from the dolphins' need to modify and adjust their own vocalizations to be able to communicate successfully with other animals of the same group (Janik & Slater, 2000), maintaining the social cohesion (Tyack, 2008).

McBride and Hebb (1948) and Schevill and Lawrence (1949) published the first qualitative reports on whistles, marking the beginning of dolphins' acoustic behavior studies. In the 1960s, Melba and David Caldwell, in Marineland Aquarium, made important discoveries on acoustic behavior. The Caldwells put forward the notion of a unique and individual sound, later known as signature whistle (Caldwell & Caldwell, 1965; Caldwell & Caldwell, 1968; Caldwell et al., 1970). In the 1980s, signaling pattern full descriptions began to emerge, as well as attempts to identify the whistle emitter (Lammers & Oswald, 2015).

Some examples of acoustic studies in the wild include: changes in the acoustic behavior near operating vessels (Nowacek et al., 2001; Lusseau, 2003; Luís et al., 2014); signature whistle and long-term stability (Sayigh et al., 1990; Janik & Slater, 1998; Janik, 2000; dos Santos et al., 2005; Luís et al., 2016a); analysis of pulsed sounds, the burst-pulses (Herzing, 1996; Luís et al., 2016b) and sonar emissions (Capus et al., 2007; Au, 1993).

4.1. Different types of vocalizations in bottlenose dolphins.

Bottlenose dolphins produce an extensive variety of sounds, which are divided into two categories: pulsed emissions, i.e. echolocation clicks or burst-pulsed sounds; and non-pulsed signals, i.e. whistles (Caldwell et al., 1990; Janik, 2013) (Figure 1.2).

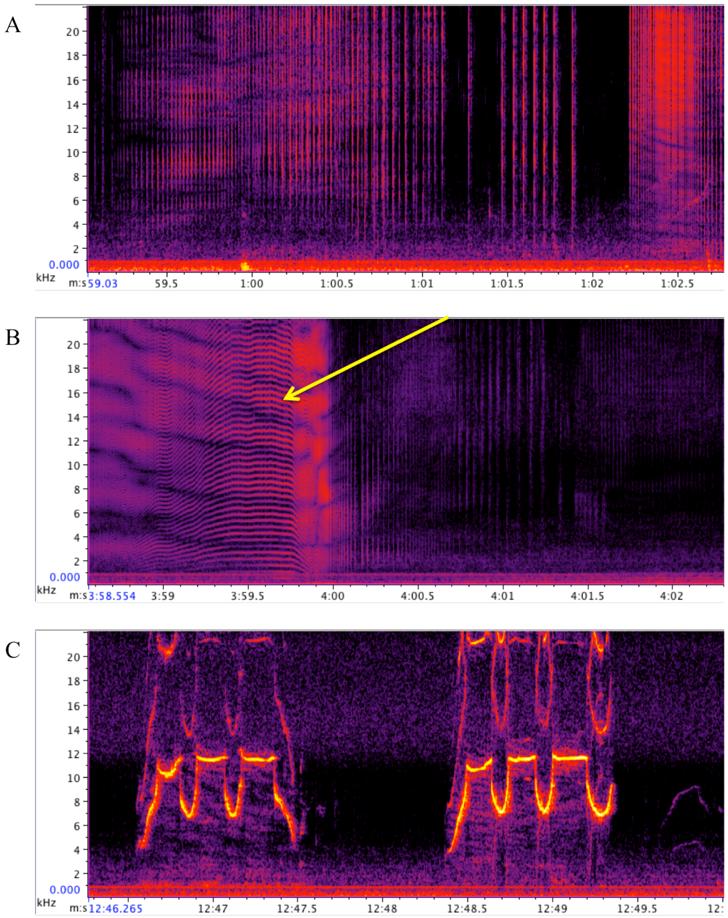

Figure 1.2. Different types of dolphin communication sounds. A) Echolocation clicks, B) Burst pulse sound with a yellow arrow showing the sound itself, followed by echolocation clicks and C) Frequency-modulated whistle.

Pulsed signals are directional and high-frequency broadband clicks, with dominant frequencies commonly higher than 50-60kHz, used to detect, discriminate and recognize objects in the environment, including potential preys, based on the returning echo (Caldwell et al., 1990; Au, 1993; Janik, 2013). Burst-pulses are clicks emitted at a high repetition rate, sometimes as high as 800-1450 clicks per second (Dolphin et al., 1995; Mooney et al., 2009) that seem to function not only as tools to prey catching (Miller et al., 2004), but also to carry information about the emitter’s motivational/emotional state (Foote et al., 2008). This system is known as echolocation or biosonar (Griffin, 1958).

4.1.1. Whistles.

Whistles are narrowband frequency modulated sounds, consisting of a fundamental frequency contour and often with several harmonic bands (occurring at multiples of the fundamental) (Herzing, 2000). These sounds usually vary from 4 to 15 kHz and may have a social function, such as individual identification (Caldwell et al., 1990; Janik, 2013). They often last less than one second, and they can go from one to multiple loop repetition (Figure 1.3), having highly variable frequency modulation contour shapes, from concave to convex, upsweep to downsweep, and with intermediary forms between categories (Bazúa-Durán & Au 2002; Dudzinski et al., 2009). The features can vary depending on the context. For example, reports in isolation described that dolphins tend to modify whistle time-frequency parameters, number of loops and emission rate compared with normal situations (Caldwell et al., 1990; Weary & Fraser, 1995; Watts & Stookey, 2001; Barton, 2006; Esch et al., 2009a; Esch et al., 2009b).

Whistles may be generated by one of two functionally separated phonic lip pairs, also known as “museau de singe”, one for each nasal passage (Cranford et al., 2008). Several studies reported that these two sound generators are capable of simultaneous clicks and whistle production (Lammers et al., 2003; Lilly, 1962; Markov & Ostrovskaya, 1990).

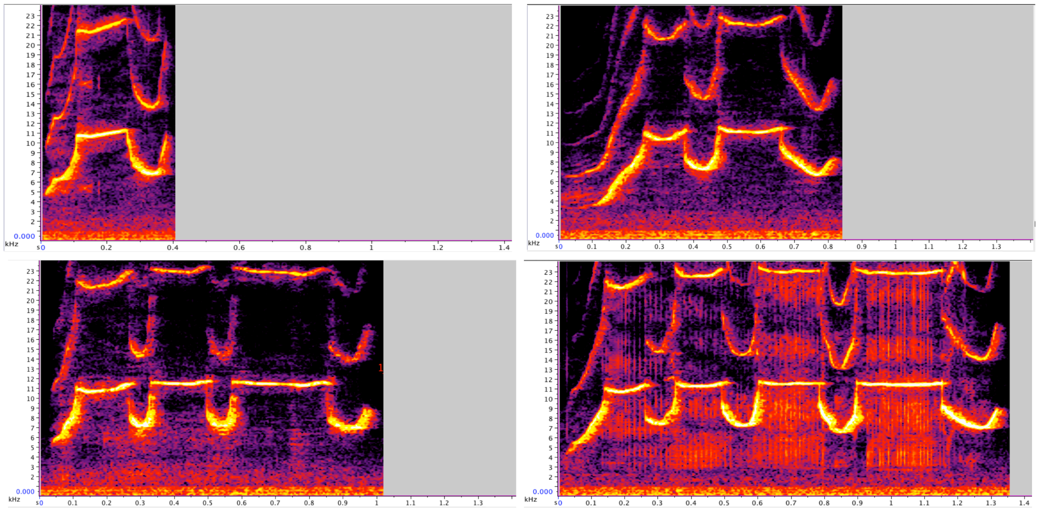

Figure 1.3. Example of a whistle contour with different loop number, from one loop to four loops.

4.1.2. Signature whistles.

Based on the definition of Caldwell et al., (1990), signature whistles are stereotyped and individually distinctive whistles, recognizable by the frequency modulation pattern of the contour (Janik et al., 2006). These vocalizations are documented as the most commonly whistle type produced by an animal in isolation (Caldwell et al., 1990), ranging from 80% to 100% of whistles emissions (Caldwell & Caldwell, 1965; Caldwell et al., 1990; Janik & Slater, 1998; Sayigh et al., 2007; Janik et al., 2013).

David and Melba Caldwell were the first investigators postulating that signature whistles may represent a unique vocalization, different for each dolphin. These stereotyped whistles seem to be stable for a long time (Sayigh et al., 1990, 2007; Bruck, 2013; Luís et al., 2016a), suggesting that these signals may be involved in individual identification (Caldwell & Caldwell, 1965; Tyack, 1986; Caldwell et al., 1990; Sayigh et al., 1990; dos Santos et al., 2005).

Acoustical reports suggest that signature whistles are developed through vocal learning during the first year of a dolphin's life, and may remain stable throughout an individual's lifetime (Caldwell & Caldwell, 1979; Caldwell et al., 1990; Sayigh et al., 1990; Janik & Sayigh, 2013). Dolphin's calves are known to model their signature whistles on sounds in their acoustic environments (Fripp et al., 2005; Tyack & Sayigh, 1997), including man-made whistles (Miksis et al., 2002). Male dolphin's calves are more likely to produce whistles similar to those of their mothers, while females tend to produce more distinct signature whistles. This scenario is not surprising since females tend to remain associated with their mothers and hence the convenience of a more distinctive whistle contour (Sayigh et al., 1990, 1995).

5. Automatized Behavior Recording in Marine Mammals

Currently, the behavior of individuals in the wild can be studied in greater detail, using radio, acoustic and satellite tags for movements near the surface. Hydrophones and video cameras may also be useful in the study of underwater behavior with continuous recordings. However, the use of video equipment is frequently limited (Mann, 1999).

There are several types of techniques, equipment and algorithms that can measure the different components of behavior. The most widely studied behaviors are: locomotor behavior, using equipment such as accelerometers; routines, using video cameras to record the spatial occupancy of the animals; and acoustic behavior, using hydrophones to record the dolphins' underwater emissions.

5.1. Sensors used in behavioral recordings.

Numerous studies use a variety of sensors for behavior recordings on marine mammals. The most frequent used one is the acoustic recording sensor (e.g. dos Santos et al., 2005; Luís et al., 2016a,b); however, nowadays there are data loggers that measure a vast number of parameters, such as: acceleration, water depth, water temperature, orientation, vocalizations, GPS position and underwater light level, among others.

5.1.1. Locomotor behavior.

Locomotor behavior is characterized by movement, or absence of it (Wilson et al*.,* 2008), which may vary as a response to environmental changes (Shepard et al., 2003, 2008). Movement can be defined as a change in the spatial location of an individual in time (Nathana et al., 2008).

Research in the ethology field has been actively seeking techniques that provide a better understanding of the movements of an animal. Thus, equipment like accelerometers may play a fundamental part in the increase of knowledge about action and movement.

5.1.2. Accelerometers as motion sensors.

Accelerometers are motion sensors that measure proper acceleration (g-force), which is the velocity variation in time experienced by an object. This instrument gives an estimate of the acceleration along the x-y axis if biaxial, or x-y-z axis if triaxial, from which velocity and displacement can also be estimated, as well as body position and posture (Ravi et al., 2005).

Since accelerometers react to the earth's gravitational field, as well as to acceleration brought by the subject (Wilson et al., 2008), they provide information about movements not detectable by the human eye, through the measurement of static and dynamic acceleration (Ravi et al., 2005; Shepard et al., 2003, 2008; Watanabe et al., 2005; Wilson et al., 2008).

An accelerometer resting on the Earth's surface will indicate 9.8 m/s^2 upwards, since any point on the Earth's surface is accelerating upwards regarding to the local inertial frame. An object in free fall will have a maximum acceleration of 9.8 m/s^2. When or if the object reaches the terminal velocity (constant velocity), its variation will be zero, meaning zero acceleration (Fishbane et al., 1996). Newton's second Law explains this phenomenon:

Force (F) = mass (m) x acceleration (a)

Weight (W) = mass (m) x gravitational acceleration (g)

For a free-falling object, the net external force is just the weight of the object:

Force (F) = Weight (W)

mass (m) x acceleration (a) = mass (m) x gravitational acceleration (g)

acceleration (a) = gravitational acceleration (g)

There are several types of accelerometers. A capacitance-sensing accelerometer, used in this thesis, consists of two types of plates: independent fixed plates; and plates attached to the moving mass. Acceleration deflects the proof mass and unbalances the differential capacitor, resulting in a sensor reading whose amplitude is proportional to acceleration (Figure 1.4) (Rutz & Hays, 2009).

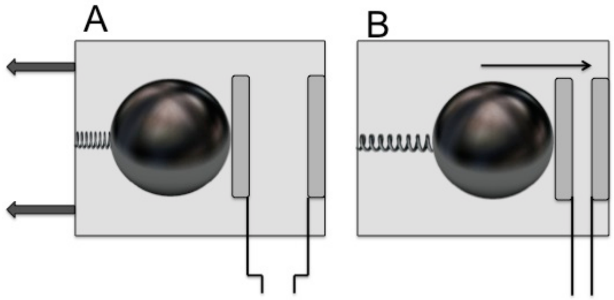

Figure 1.4. How a capacitive acceleration sensor works. The broad concept of a capacitive accelerometer is that A - as the accelerometer box moves to the left, B - the black mass is forced to move and pushes the metal plates close together, changing their capacitance in a measurable manner.

Even though the intention of the scientist is to avoid interfering with the natural behavior of the animals, methods involving equipment directly attached on bodies may still have some effect on their lives and activities (Ravi et al., 2005).

5.1.3. Static and dynamic acceleration.

Static acceleration is a measure of the inclination of the accelerometer with respect to earth's gravitational field, it allows the characterization of an animal's posture or body angle (Shepard et al., 2003, 2008; Wilson et al., 2008). Low frequency signals typically represent changes in posture (Shepard et al., 2003, 2008).

Dynamic acceleration, obtained from the movement of the body, is representative of locomotion and represented by changes in velocity (Shepard et al., 2003, 2008; Wilson et al., 2008). This component can be obtained by subtracting the overall acceleration from the static component (Wilson et al., 2008) and it is usually represented by high frequency signals (Shepard et al., 2003, 2008).

Static and dynamic acceleration components, which are generally associated, give a more precise estimate of the movement, even at fine scales (Shepard et al., 2003, 2008).

5.1.4. Accelerometry and marine mammals.

In marine carnivore species, such as sea lions and seals, accelerometers are usually glued to their heads or jaws to study feeding attempts and dives, quantifying the acceleration features of head and jaw movements during these activities (Davis et al., 1999; Naito et al., 2010; Viviant et al., 2010; Iwata et al., 2012).

In cetacean species, accelerometers are typically used in tags or data-loggers that are able to collect more information about the environment. The use of the accelerometers is extensive, ranging from the determination of the stroke energy during different types of dives in beaked whales (López et al., 2015) to the prediction of energetic needs in Pacific white-sided dolphin (Rechsteiner at al., 2013) and to the study of speed, swimming effort and stroke energy in bottlenose dolphins (Akiyama et al., 2015).

Despite the studies carried out in the wild using accelerometers, there is a lack of knowledge concerning fine scale behavior. Captive cetaceans may help to fill this gap in knowledge, since they are in a controlled and stable environment, and it is much easier to observe and record.

5.2. Video tracking.

Over the years, various techniques have been developed, and several attempts have been made to automatically monitor the position and/or movements of animals by using sensors attached to their bodies (Ravi et al., 2005; Wilson et al., 2008). Video-based tracking methods, on the contrary, do not require any equipment to be attached to the subjects. This method was developed in controlled environments, like captivity, to explore and extract the detailed information that is contained in the videos of moving animals (Kabra et al., 2013). These systems were introduced in the early 1990s, offering advantages of flexibility, spatial resolution and temporal precision for many applications. The principles of these analyses are often based on image-subtraction algorithms, grayscale threshold and/or statistical models (Lind et al., 2005). Due to the high temporal and spatial resolution of these techniques, it is now possible to perform video tracking of different animals and transform the video data into trajectories of positions over time (Kabra et al., 2013, Pérez-Escudero et al., 2014).

5.2.1. Video tracking in different animal models.

These video tracking techniques have been widely used for commercial video analysis systems in a variety of experiments (Hashimoto et al., 1999; Spink et al., 2001; Khan et al., 2005; Dell et al., 2014) with different animal models such as *Caenorhabditis elegans* (Geng et al., 2004; Simonetta & Golombek, 2007; Machino et al., 2014), fish (Kato et al., 2004; Delcourt et al., 2009; Mirat et al., 2013; Pérez-Escudero et al., 2014), insects (Martin, 2004; Branson et al., 2009; Pérez-Escudero et al., 2014), rodents (De Chaumont et al., 2012; Ohayon et al., 2013; Giancardo et al., 2013; Matsumoto et al., 2013; Pérez-Escudero et al., 2014; Wiltschko et al., 2015), primates (Ballesta et al., 2014) and pigs (Lind et al., 2005; Ahrendt et al., 2011). However, the studies mentioned above have been mostly limited to small cages and restricted environments, as noted by Ballesta et al., (2014). The techniques used were unsuitable to study the movements of large animals such as bottlenose dolphins in their sizable pools.

One of the video tracking field key interests is the quantitative movement analysis, since knowing where an animal is, in space and time, can provide important indirect information about its behavior, ecology and social interactions (Rutz & Hays, 2009).

6. Aim of the Project

The aim of this research was to develop and integrate a suite of methods that might allow a less subjective, as well as a faster approach to analyze detailed behavior. This study comprises three major sections: the analysis of behavior using accelerometers; the analysis of movements and detection of routines using a tracking system; and acoustic recordings of vocal emissions using a hydrophone.

The behavioral study with accelerometers in dolphins allows the quantification of patterns, providing information about the type of behaviors they execute, how often they do it and its sequence and transitions. The goal of this section is to start building a behavioral map of captive dolphins that has a potential application to the study of wild marine mammals. Nowadays, almost all of the equipment used to study marine mammal movements, like tags and GPS emitters, has a two or a three-dimensional accelerometer; however, most of the time the information is not fully understood. For that reason, it is important to have a validation in an environment where more variables can be controlled and where the movements are visible on video recordings. This approach will allow, in the future, a more complete understanding of the behavior flow in dolphins, both in wild and captive environments.

The analyses of dolphin movements and routines using a tracking system provide an opportunity for the study of occupancy and habitat use, as well as semi-automatic detection of stereotypies in captive environments, using only two video cameras. To overcome the obstacles of tracking robust animals in a large pool with various depths, we have developed D-Track, a software that tracks dolphins in their habitat without disturbance. This is an innovating methodology, since the D-Track system works with cameras from different angles and heights, and not only from above or laterally, as usually happens in existing systems. It represents an important improvement that may allow marine parks and laboratories to easily be aware of the needs of their animals whilst diminishing the involved effort and time.

The acoustic behavior recordings using hydrophones bring the possibility of application to both captive and wild marine mammals, and there are many proven and potential advances in the understanding of dolphin acoustic behavior resulting from captive studies.

With the combination of these three methodologies, a renewed attempt will be made to incorporate various aspects of the dolphins’ behavior: the detailed movements and the 3-D use of space with the acoustic emissions. We are able to study if there is any association between behavior and acoustic signals. The combined use of these techniques is an opportunity to link the behaviors in an integrated approach, with facilitated analysis, that will allow new understanding of how bottlenose dolphins move according to their preferences and how they vary their acoustical emissions according to specific activities or locations in the pool.

CHAPTER 2 - METHODS TO ANALYZE BEHAVIOR AND MOVEMENTS OF CAPTIVE BOTTLENOSE DOLPHINS

1. Marine Park

The subjects used for this study were kept in an artificial habitat at Zoomarine, Guia (Algarve, Portugal; 37º 7.6654 N, -8º 18.8333 W).

Zoomarine opened in 1991 and receives, in average, 500 thousand visitors per year. Zoomarine also participates in scientific projects, from Masters to Doctoral projects, and international conferences to increase the knowledge of the species that live in the park, from welfare to physiology, behavior, and others.

At the moment, Zoomarine has around 20 dolphins and a big number of different species, from sea lions, seals, paradise birds, birds of prey and countless number of fish species. Dolphins are distributed by three different habitats, Enseada, Blue Lagoon and Delphinarium. All dolphins spend time in hall habitats, especially between Enseada and Blue Lagoon. Delphinarium, generally, is where they are born and spend the first years with their mother.

This thesis data collection was mainly done in the Blue Lagoon habitat; however, some recordings were also obtained in the Delphinarium pool.

This study followed careful procedures to minimize disturbance to the animals, and was approved by Zoomarine's Board and all technical Directors.

2. Subjects

The subjects of this study were two male common bottlenose dolphins (*Tursiops truncatus*) here designated as HM5 and AM4. HM5 was born in 1995 and AM4 in 2004. Their weights and total lengths are, respectively, 185 kg, 2.46 m and 172 kg, 2.55 m. Both subjects were trained with operant conditioning techniques and positive reinforcement to tolerate a small waterproof box held by two suction cups near the right pectoral fin. Both animals learnt how to remove the equipment and for this reason they received fish rewards during all accelerometer sessions as an incentive to keep the apparatus.

Information about specific routines and social preferences were taken into account for the accelerometer sessions to ensure minimal interference with the behavior of the focal animal. All accelerometer sessions were planned according to the trainers' availability and carried out depending on the animals' cooperation.

3. Arena

The subjects were housed in the Blue Lagoon pool, which is about 47 m long, with 2382.3 m^3 of artificial salt water, divided into 4 smaller sections separated by fiberglass doors and panels with circular holes (Figure 2.1). The data collection was carried out in a pool section measuring, approximately, 21 m diameter, with a maximum depth of 5 m (average depth of 1,9 m). This pool section, containing only the subjects, was physically isolated from other dolphins, but not acoustically, during measurements. During daily routines, the subjects were used to spend time in each section.

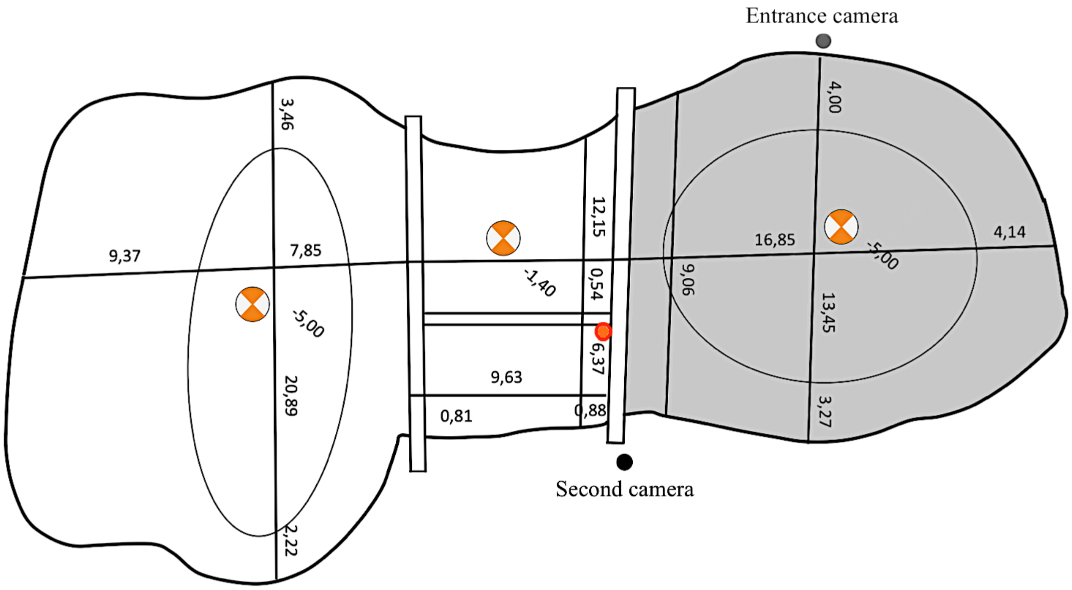

Figure 2.1. Shape and dimensions of experimental pool. Image showing dolphins' complete habitat dimensions and depth. The grey part of the figure represents the section where the experiments occurred. The orange-and-white circles represent the maximum depth point in each of the pool sections. The colored circles represent the approximate positions of the cameras; 1 - red circle indicates the position of the hydrophone and the underwater camera; 2 - grey circle shows the position of Entrance Camera; 3 - black circle illustrates the position of Second Camera. Both cameras enable capturing the entire pool for the tracking analysis.

4. Equipment Set-Up

The experimental set-up consisted of three GoPro Hero2 video cameras (GoPro, Inc.); one Cetacean Research C55 hydrophone with a MicroTrack II (M-Audio) recording system; and one Kinetamap accelerometer (SparkFun Electronics, Inc.) inside a waterproof box (Snap Sights SC25 from Intova) (see details below).

The video cameras were positioned in fixed locations for a complete recording of the animal's movements. Two cameras were placed 4 m high (opposite to each other) on a pole, and one camera was attached to one of the underwater fiberglass doors, inside a hole in one of the doors. The hydrophone was positioned near the underwater camera in a small adjacent pool with no animals (Figure 2.1). The hydrophone was kept, approximately, one meter deep.

Each trial required the complete preparation, placement and removal of the set-up. In the beginning of each session, both trainer and researcher sat on a bench near the pool to avoid direct interaction with the subject. Training of other dolphins, passage of trainers through various parts of the pool area and feeding sessions in other sections of the pool occurred normally, with minimal interference concerning the schedule and routines of the other animals.

4.1. Equipment.

4.1.1. Video cameras.

The video cameras were used with f/2.8 170° lens with free focus adjustment and an effective sensor resolution of 11.0 megapixels. Wide-angle lenses were preferred because they allow the monitoring of the entire experimental pool. These lenses have the disadvantage of adding fish-eye distortions to the images. Videos were recorded into 32 GB SD cards in MP4 format, with a resolution of 720p and 30 frames per second. The data from the video was used both to study the routines of the animals, as well as a validation method for the accelerometer data.

4.1.2. Hydrophone.

For this project, a C55 hydrophone connected to a digital recording device, the M-Audio MicroTrack II, was used. A 9 V battery polarized the C55 hydrophone. It presents a linear frequency range of ± 3 dB in the 0.016 – 44 kHz, a usable frequency range of +3/-20 dB in the 0.006-203 kHz band; a transducer sensitivity of -185 dB re 1V/μPa; and an effective sensitivity of -165 dB re 1V/1 μPa.

The MicroTrack II was set to record WAV files with 24-bit resolution at 48 kHz sampling rate into CompactFlash cards of 2 GB capacity.

4.1.3. Accelerometer.

The Kinetamap accelerometer consists of a digital 3 axis sensor (ADXL345) (Figure 2.2) that records data at 65 Hz into an internal 1 GB microSD memory card and has a USB rechargeable internal 1100 mAh LiPo battery.

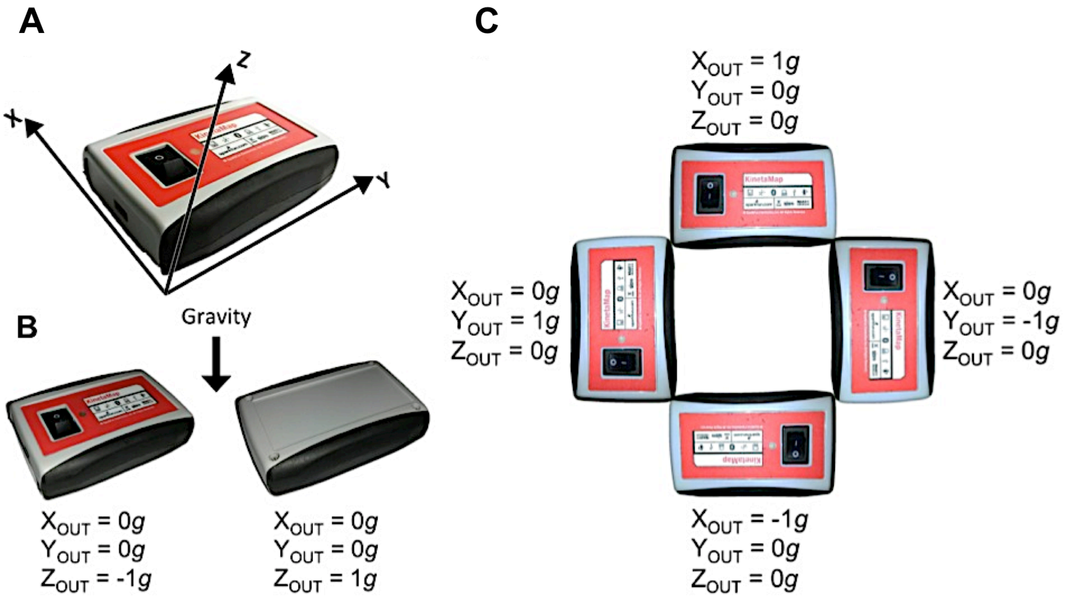

Figure 2.2. Accelerometer orientation. Image showing the orientation of the axis of the accelerometer used. A – Axis of acceleration; B – Representation of the orientation in relation to gravity and; C – Representation of the output responses of the accelerometer in each position.

The ADXL345 is a capacitance-sensing accelerometer consisting of two types of plates: independent fixed plates and plates attached to the moving mass. Acceleration deflects the mass and unbalances the differential capacitor, resulting in a sensor output whose amplitude is proportional to acceleration. This is a high-resolution accelerometer, with 8 G range, 16 bits of digital output data per sample and a sensitivity of 3.9 m*g*/LSB, which enables the detection of inclination changes of less than 1°.

The Kinetamap accelerometer, with the dimensions of 2.8 x 6.3 x 9.4 cm and 103 g, was contained in a 4.2 x 7.3 x 11.1 cm waterproof box with a total weigh of, approximately 250 g, representing around 0,14% of the dolphins' weighs.

The values recorded by this equipment were in voltage (mV) and were later transformed in *g* ($1g = 9.81\ ms^{-2}$).

4.1.4. Accelerometer parameters.

According to this project focus, it was possible to configure some parameters of the accelerometer. When parameters were set to 0 it means that they were disabled. On the contrary, 1 means enabled. The configurations used in each session are described in Table 2.1. The parameters for Bluetooth and GPS functions were turned off in all sessions, since the animals were underwater and their movements had small coordinate variation.

Table 2.1. Set of parameters that were used in the accelerometer data of the ADXL345.

Parameters	Default Value	Value Used
config_menu	0	0
log_gps	1	**0**
log_accel	1	1
send_gps_to_bt	0	0
log_batt	1	1
log_nmea	1	**0**
enable_waas	1	**0**
full_scale_accel	0	**1**
enable_rmc	0	0
enable_gga	1	1
accel_frequency (Hz)	10	**100**

The log_accel and log_batt parameters allowed the storage of the acceleration data and the monitoring of the battery voltage value in the SD Card, respectively.

The full_scale_accel parameter sets the range of the accelerometer. With the parameter set to 0 the range was 2G mode, and with 1, it was set to 8G. Although the 2G mode has higher resolution in the intermediate values, it saturates faster, with the consequent risk of information loss. In the 8G mode, saturation is less likely. Since 8G also means a bigger dynamic range, this parameter was set to 1.

The last parameter, the accel_frequency (Hz), was set to the maximum value to increase acceleration data collection of the animals. The accelerometer data were recorded with an average sampling frequency of 65 Hz.

4.1.5. Accelerometer positioning.

The waterproof box containing the 3D accelerometer was secured to the subject by two suction cups, which were specially designed for cetaceans by Cetacean Research ™ (the GelRelease ™ suction cup) (

Figure 2.3), and it was positioned near the dolphin's pectoral fin (Figure 2.4). The 3 axis correspond to dorso-ventral (x), anterior-posterior (y) and lateral (z).

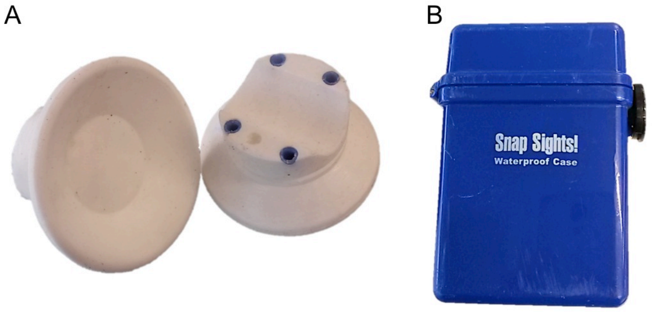

Figure 2.3. Equipment used to attach the accelerometer to the dolphin. A - Cetacean Research™ GelRelease™ suction cups that were used to attach the B – waterproof box with the accelerometer to the dolphin.

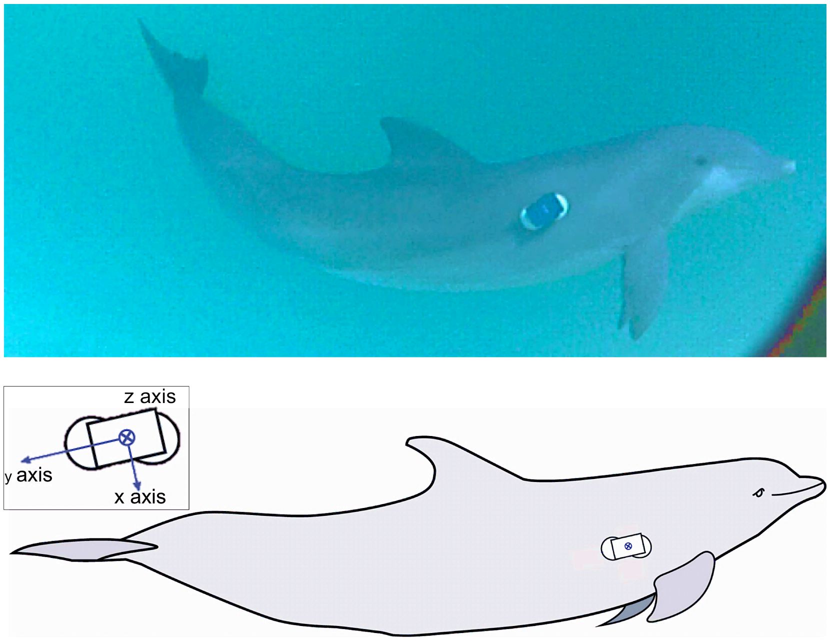

Figure 2.4. Placement of the accelerometer in the dolphin’s body. Top image, a frame from an underwater recording of the dolphin with the accelerometer. Bottom image, drawing showing the position of the accelerometer in the dolphin and the direction of three axis (x, y and z).

5. Data Collection

Data collection was conducted from November 2012 to April 2014 during the winter season (from November to April), at times in which the park was closed to the public or with lower attendance of visitors. During the closing period, animals kept their training schedule and the same daily routines they usually have when the park is open.

All equipment was synchronized in the beginning of each session with a chronometer. To synchronize the cameras, a Wi-Fi remote control from GoPro was used. The videos and chronometer were turned on at the same time and the rest of the equipment was turned on with the chronometer in front of the camera to record the exact millisecond.

Every session started, whenever possible, with some habituation period to avoid any behavior change due discomfort related to the experimental set-up.

5.1. Trajectory data collection (video and acoustics recordings).

Video and acoustics were recorded in two different contexts: subject "alone" and "accompanied with another animal" in the pool. Videos and acoustics were recorded in non-consecutive sessions of 60 minutes each, from 07:00 AM to 07:00 PM, for animal and context, totaling 2880 minutes of behavioral and acoustical recordings throughout 48 sessions. The purpose of these recordings was the subsequent comparison of the subject's behavior with and without accelerometer to evaluate the impact of the equipment in the subjects.

These videos had an additional goal of studying the pool location preference for both animals, using trajectories across sessions.

For the acoustical recordings, the intent was to study the sound produced by the focal animal and to compare it in both contexts, by isolating the frames in which the focal dolphin was closer to the hydrophone.

5.2. Acceleration data collection (video, acoustics and accelerometer recordings).

Accelerometer data were also collected in two different contexts: subject "alone" and "accompanied with another animal". In contrast to video recordings, acceleration sessions had different duration depending on the animal's motivation to participate and on trainers' availability. Since the data collection required voluntary participation of the subjects with positive reinforcement, every time the animal showed any signs of decreased motivation or distress, the session was aborted by the trainers. Sessions shorter than 20 minutes were discarded, since there was no certainty that the behaviors recorded followed habituation of the animal to the accelerometer or not. Sessions were recorded non-consecutively from 09:00 AM to 6:00 PM for each animal and context, and are showed in Table 2.2.

Table 2.2. Acceleration and acoustic sessions for HM5 and AM4 in different contexts, “alone” and “with company”.

	HM5	**AM4**
Acceleration “Alone”	625 minutes (17 sessions, from 22 to 60 minutes)	620 minutes (12 sessions, from 32 to 68 minutes)
Acceleration “With Company”	691 minutes (16 sessions, from 25 to 64 minutes)	647 minutes (14 sessions, from 27 to 64 minutes)
Acoustics “Alone”	542 minutes (15 out of 17 sessions)	468 minutes (9 out of 12 sessions)
Acoustics “With Company”	529 minutes (13 out of 17 sessions)	605 minutes (13 out of 14 sessions)

Some sessions were also discarded because of equipment malfunctions or recording errors.

6. Features

6.1. Video camera calibration.

Since the wide-angle lenses distorted the recorded images, it was necessary to calibrate the cameras to match the pixel coordinates and to correct the position and dimensions of the animal.

To perform the calibration, images of a black and white chessboard were used. This method enables us to know the positions of pattern corners with respect to the coordinate system of the target (see Carstensen, 2001). Thus, the distortion matrix used was [-0.335050702095, 0.120226070285, 0.0, 0.0, -0.0201695654541].

The following formula is a camera matrix, or a matrix of intrinsic parameters:

A = [[654.55, 0, 1279.0/2]

[0, 654.55, 719.0/2]

[0, 0, 1]]

These values were correction factors to compensate camera distortion and gave the precise position and orientation of the camera with respect to the target.

6.2. Tracking system.

To perform the tracking of the animals in the pool, the foreground detection technique, or background subtraction, was used to detect and recognize the dolphin. For this project, a Python 2.7.3 script (Python Software Foundation) was developed and called D-Track. The script was used with an OpenCV 3.0 library to define the threshold for each frame of the video (Figure 2.5). The threshold is the only parameter that had to be manually defined because it depends on the weather and light conditions.

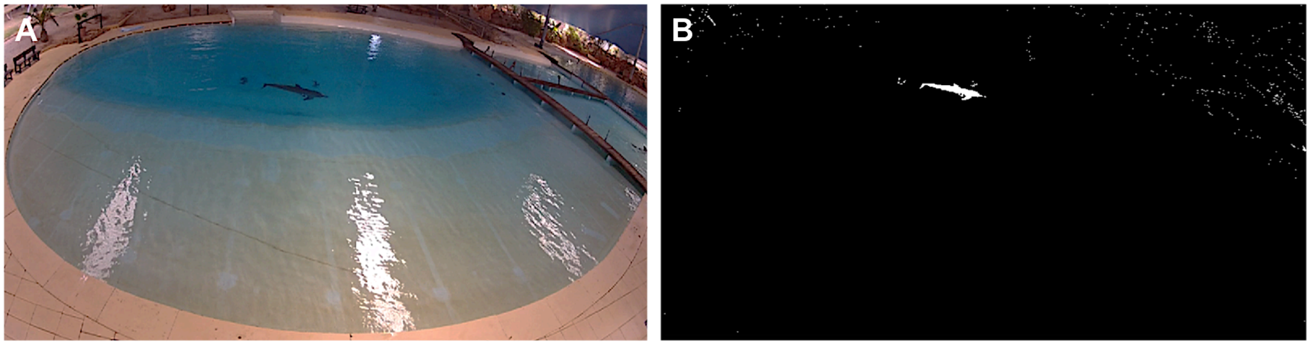

Figure 2.5. Visual example of how the tracking algorithm works. A - An example of a video frame recorded with the focal animal; and B - the same frame with the background subtracted for analysis, showing the focal animal in white.

Each camera frame was used to find the 2D centroid of the moving subject. The centroid, in pixels, corresponds to a vector originated from the focal point of the camera to the subject in the pool. The intersection of the two vectors, one from each camera, was used to calculate the 3D position of the animal in the pool.

The following formulas were used to find the pixel vectors for both cameras:

$$\begin{bmatrix} x \\ y \\ z \end{bmatrix} = R \begin{bmatrix} X \\ Y \\ Z \end{bmatrix} + t$$

$$x' = x/z$$

$$y' = y/z$$

$$\mu = f_x * x' + cx$$

$$\nu = f_y * y' + cy$$

Where X, Y and Z were the coordinates of the 3D point in the coordinate space; R and t are the rotation and translation matrix; μ and ν were the coordinates of the projection points in pixels; cx and cy were the main points that are usually at the image center; and f_x and f_y were the focal lengths expressed in pixel units.

6.3. 3D tracking system.

Two opposite cameras were used to record the entire habitat from two distinct perspectives to obtain the 3D data. Detailed measurements and distances of the pool were included in the D-Track software where five points visible from both cameras were chosen as input calibration points to be used in the RANSAC Perspective-*n*-Point algorithm (Fischler & Bolles, 1981). The tiles on the floor were also measured and the dimensions were included in the software for image calibration in each session (Figure 2.6).

The images of both cameras were then merged to reconstruct the 3D scenario.

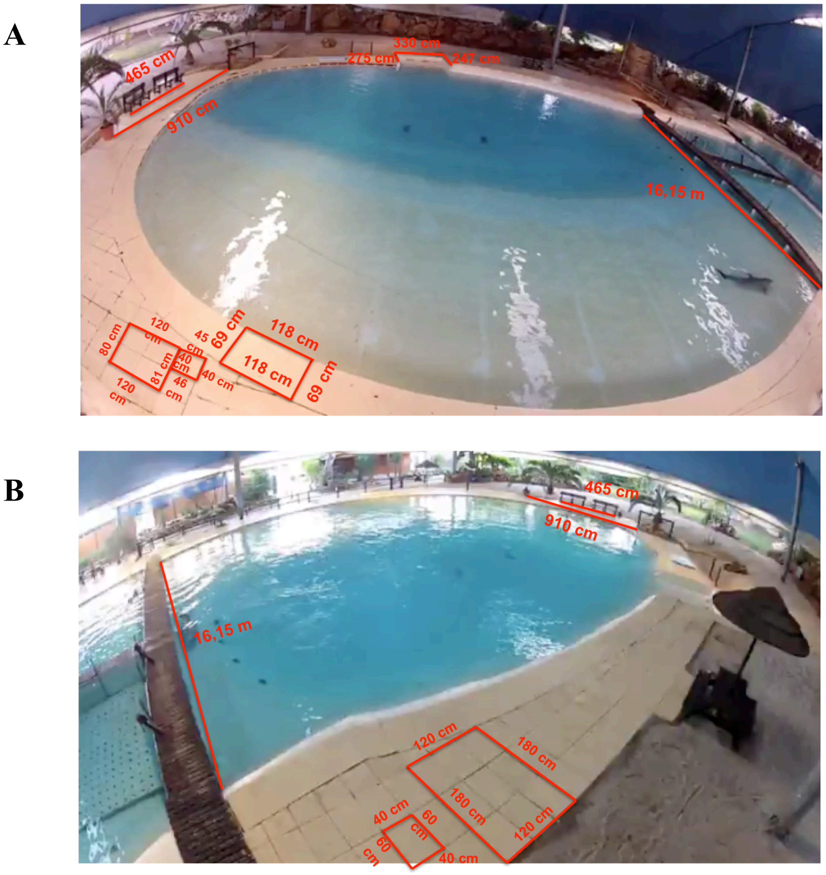

Figure 2.6. Measures in the experimental pool from both cameras point of view. A – View from the entrance camera with the measures of the floor tiles and bridge; and B - View from the second camera with the measures of the tiles and the same bridge.

The tracking algorithm applied a mask filter to the images to ignore the regions outside the pool. Several imaging filters, such as an adaptive threshold, color ranges filtering, erosion and dilation, were used to extract the subject's blob and to remove the remaining noise.

After applying the filters, the pixel coordinates corresponding to the centroid of the dolphin in each frame image were extracted. Subsequently, a Gaussian filter (sigma=32) was applied to all the resulting coordinates to smooth and eliminate some centroid moving or jittering (momentary tracking errors). A refraction index of 1.4 was estimated based on the depth of the pool and specific measurements with known distances of the pool. Afterwards, the intersection of the vectors that correspond to the subject centroids was calculated, giving the exact 3D point of the position of the subject (Figure 2.7).

Figure 2.7. Three-dimensional reconstruction of the pool. A – Image from the entrance camera showing the dolphin and the red dot from the tracking recognition. B - Image from the second camera showing the dolphin in the same frame as image A, and the red dot representing the centroid of the animal. C – Three-dimensional reconstruction of the pool using the algorithm. The two vectors are going from the two cameras to the dolphin (intersection of both vectors) giving the 3D position of the animal in the pool (red dot).

To avoid fictitious fluctuations in the data, an outlier analysis was made calculating the median, lower quartile, upper quartile, interquartile range, inner and outer fences of the data. The major outliers, which resulted from the calculations with the outer fences, were discarded.

6.3.1. Defining animal spatial preferences in the pool.

To analyze the occupancy/preferences of the animals in the pool, it was necessary to isolate the portion of the scenario to quantify. This isolation will give information about the frames to be analyzed. The frames associated with the maximum depth area were labeled as the Deep Area of the pool. The difference between the total frames and those corresponding to the Deep Area were named the Shallow Area of the pool.

To study the tridimensional preferences of the animals in relation to the deepest part of the pool (close to the 5 meters depth), the water below the maximum depth of the Shallow Area (approximately 1.4 meters depth) was named the Bottom (Figure 2.8).

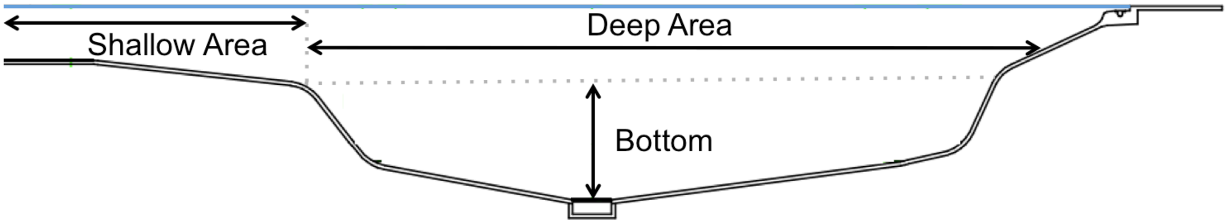

Figure 2.8. Side view of the experimental pool with the representation of the different parts of the scenario to the analyses of the preference/occupancy of the dolphins.

6.3.2. Dolphins' speed in the pool.

The speed of the animals was calculated from the centroid position of the dolphin in each frame, subtracting the previous frame, thus obtaining the distance moved per unit of time. The range of speeds was divided into three categories to create a scale from low to high speed (tertiles).

6.3.3. Quantifying dolphins' routines in the pool.

To study the dolphins' routines, the total position data from all sessions was plotted for both animals to analyze its variation throughout sessions. Important occurrences like swimming circle sizes (complete circles around the pool); the influence of the trainers' presence; and interaction of the focal animal with animals in adjacent pools were explored. To study the variation in the swimming circle sizes, the distance between the opposite points in the circle was measured, as well as the speed. To study the influence of the trainers' presence in the routines of the focal animals, as well as the interaction of the subject with other dolphins in the adjacent pool, the corresponding section of the scenario was isolated.

The Kruskal-Wallis statistical test was used to examine differences in the size and duration of the swimming circles across HM5 and AM4 and among sessions of each subject.

All statistical analyses were performed using *IBM SPSS Statistics 21* (IBM Inc.).

6.4. Accelerometer.

The accelerometer operated at a variable sampling frequency during the sessions, so it was necessary to group the values into one second samples. Because the video is at 30 frames per second, the data was transformed into 30 Hz using an interpolation. The reason to use this interpolation was the necessity to have a function that could transform the data, no matter how long or short it was, in specific number of samples without losing important information. The method used was the piecewise cubic spline interpolation (Boor et al., 1978), or cubic spline, because the error's probability is smaller, since the function adjusts itself to go through all the data points.

After the interpolation, a transformation to the gravitational G had to be applied since the original file comes expressed as voltage (V).

6.4.1. Gravitational & body components separation.

The separation between the two components is important since it helps to discriminate specific movements or behaviors performed by an animal. To perform this separation, it is necessary to have a gyroscope. However, for this project, it was not possible to have an accelerometer and a gyroscope integrated. For that reason, an iPhone 4S with Sensor Data App iPhone (Wavefront Labs) application was used in one small session of 10 minutes.

The Sensor Data App used gave direct access to the data, which was transferred directly from the electronic equipment as a CSV (comma separated values) data file.

The 3D acceleration of the application is given in units of g and the data from the 3D gyroscope enabled the necessary calculations to separate the two acceleration components, gravitational and body. A median filter was applied to decrease the noise in the raw data. A correlation was tested for different cutting values until the separation was similar to that performed by the gyroscope (

Figure 2.9 and Figure 2.10). The cutting value, or high pass filter, that permits the better separation between body acceleration and gravitational acceleration in dolphins is 0.45 Hz. The Gravitation Acceleration component is obtained by simply subtracting the Body Acceleration from the original acceleration data. All these data were analyzed in Matlab-based software.

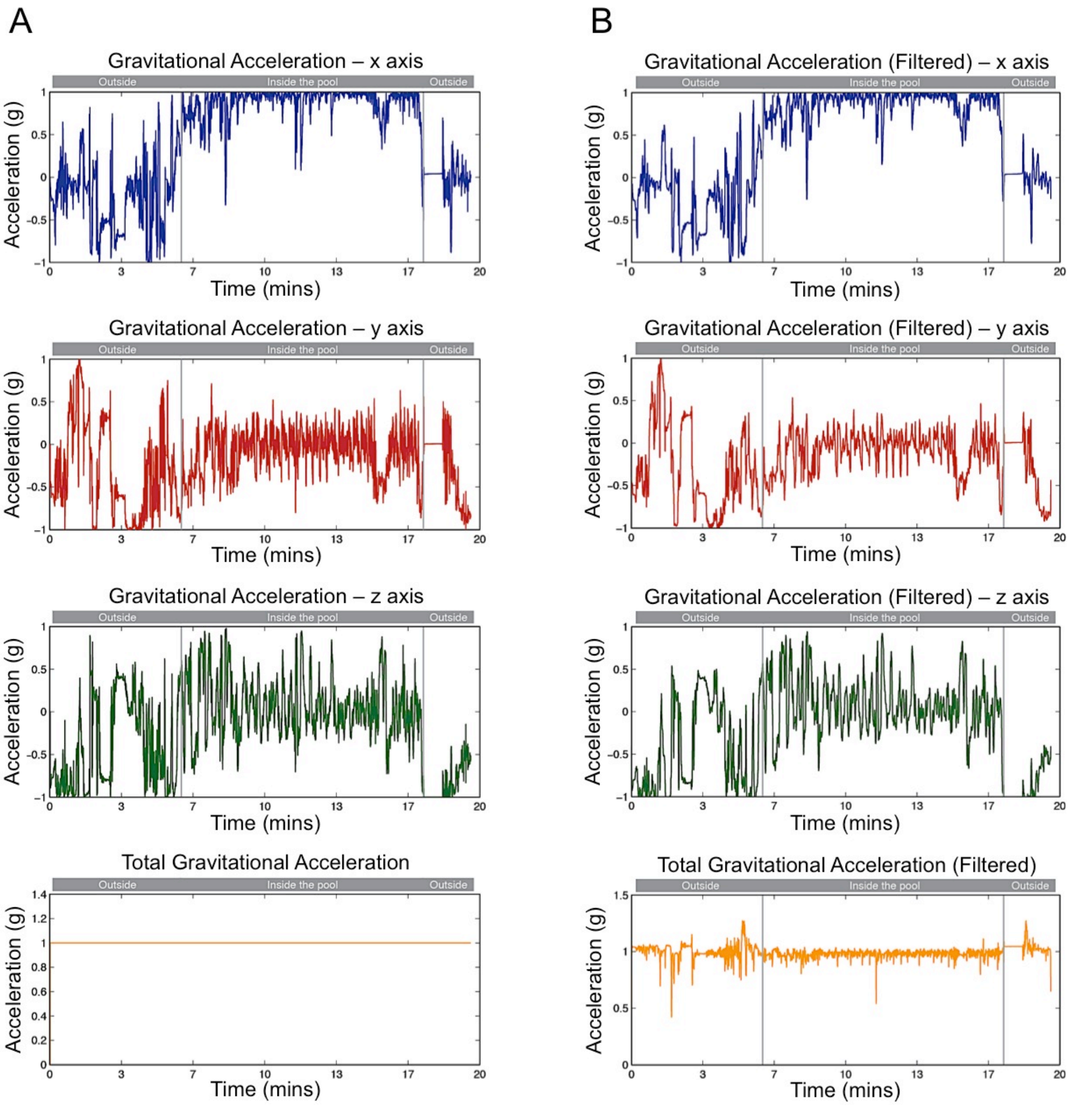

Figure 2.9. Definition of the high pass cutoff filter for the separation of the acceleration components. Column A represents the values of the gravitational acceleration obtained by the subtraction of the gyroscope data, and column B represents the gravitational acceleration separated from the body acceleration with a high-pass 1st order Butterworth filter with 0.45 Hz cutoff frequency. The grey part on the top of the graphs indicates when the accelerometer is outside and inside of the pool. Inside the pool means that it is attached to the dolphin. The two columns are very similar for the three axis. The last graph of both columns represents the total gravitational acceleration, which means that the sum of the three axis should have acceleration of 1, as shown in the last graph of column A. The last graph of column B doesn't show a sum of 1 but it is very close (mean value ~1 g).

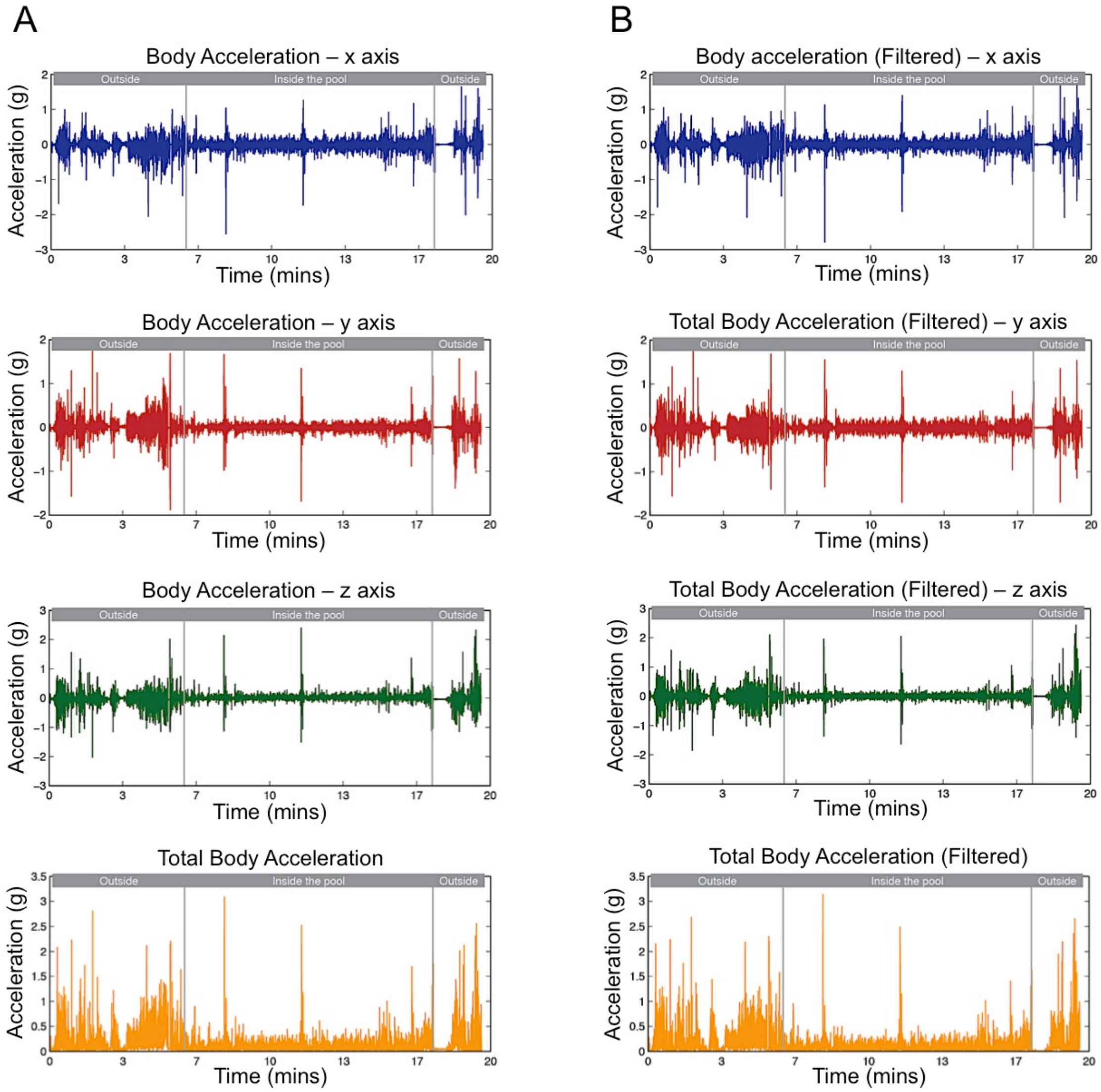

Figure 2.10. Definition of the high pass cutoff filter for the separation of the body acceleration. Column A represents the values of the body acceleration obtained by the subtraction of the gyroscope data, and column B represents the body acceleration separated from the gravitational acceleration with a high pass 1^{st} order Butterworth filter with 0.45 Hz cutoff frequency. The grey part on the top of the graphs indicates when the accelerometer is outside and inside of the pool. Inside the pool means that it is attached to the dolphin. The two columns are very similar for the three axis, showing that the cutting value correctly separates both components.

6.4.2. Stop & movement analyses.

In pattern recognition analysis, it is usual to start with the identification of the two types of movements: stop and motion. For this purpose, two videos were categorized "by hand" considering the stop and movement behaviors to compare the automatized code for the identification.

The analysis "by hand" considered the three following behaviors for this discrimination: Stop (physically and spatially), Glide (physically stop – no muscle movement - but moving in space) and Motion (physically and spatially). The challenge was to define when the dolphin is really stopped, since it seems to be always in movement.

For this analysis, a Matlab-based algorithm was used to calculate and determine the points of the acceleration data that are considered stop or motion. To determine these values, a histogram of the frequencies was made and the code was written to choose the value in which the first 5% of the bins have, at least, 80% of the frequency.

6.4.3. Cluster analyses.

Cluster analysis has the purpose of grouping similar values in one specific group. With the separation of the body and gravitational acceleration components, it was necessary to define a time window and the cutoffs to identify and separate the behaviors.

The window size was selected as one second, like the accelerometer data. The chosen resolution was the 30 Hz just to facilitate the identification of the clusters in the video, since it is also 30 frames per second.

To determine the cutoffs for the cluster analysis, three sessions of each animal were randomly selected, analyzed together and treated as a single session. The overall cutoff values for the combined sessions were used as the best cutoff value for each animal. These cutoffs were then applied in the analysis of each session to determine the number of clusters in the session.

To be able to separate the behaviors successfully, more than one component was chosen. Four variables were selected to be analyzed together: the gravitational acceleration component of the 3 axis (x, y and z) and the logarithm of the total body acceleration component.

After the selection of the variables, the distance between the points of the analysis was measured; the affinity between clusters was calculated, as well as the number of clusters in each session.

To be able to validate the cluster algorithm, it was necessary to perform manual behavior analysis, as the behavior labeling serves as a ground truth for the algorithm analysis.

6.4.4. Manual labeling of the behaviors.

Random sessions of the animals "alone" were analyzed and a manual identification of the less dubious behaviors was produced. The main behaviors chosen for the manual labeling were: Lateral behavior positions, Inverted behavior positions, Head Up, Eating and Spinning. Some variations of these behaviors were also included in the analysis, like inverted playing.

Using behavioral software developed by Champalimaud Center for the Unknown's Scientific Software Development team, the Python Video Annotator (https://github.com/UmSenhorQualquer/pythonVideoAnnotator), it was possible to analyze the videos and register the behaviors at the same time, extracting, in the end, the corresponding frames of the annotations.

6.4.5. Cluster analysis and change points.

We applied affinity propagation (Frey & Dueck, 2007) to group data points into meaningful behavior blocks. Affinity propagation is an unsupervised clustering algorithm that identifies exemplars, data points that represent a group of similar data points, and forms clusters of data around the exemplars. It considers all points as potential exemplars and exchange data points until a good set of exemplars is defined and clusters are formed. This cluster analysis was performed separately for each dolphin and each session.

One-second sliding windows were then used for change-point detection, i.e. to discover time points at which properties of time-series data change. Change-point detection is done by thresholding the average distance between neighboring sliding windows, therefore obtaining stationary segments of putative behavior blocks ranging from one to several seconds.

6.5. Acoustics.

This part of the project was divided into two major components: the visual acoustic analysis of the whistles of specific animals with data collected in 2008 and subsequently in 2012; and an automatic acoustic analysis of data collected in the 2012-2014 period with accelerometer data. For this second part, since it was impossible to physically isolate the focal dolphins from the other dolphins in the pool, the sound detected by the hydrophone includes the focal dolphin's emissions as well as other sounds from its environment (Figure 2.11).

Figure 2.11. Experimental pool. Picture of the experimental pool with a red circle indicating the hydrophone position.

6.5.1. Visual acoustic analysis.

As explained in Chapter 5 and Chapter 6 in more detail, the identification of the whistle emissions, as well as the counting and classification, were obtained visually and aurally, using Raven Pro 1.4 (Cornell Lab of Ornithology, Ithaca, NY). Whistles were selected with a minimum of 300 ms duration; sharp and defined contours and no overlapping with other vocalizations. Whistles with less than 300 ms, as defined by Richards et al. (1984), were considered just chirps, or short whistles with unknown function, and for that reason they were excluded from the analysis.

For the visual categorization, stereotyped whistle contours were cross-compared for frequency modulation similarity and assigned to mutually exclusive groups. Each whistle category had to include at least five similar contours in the total recordings. Whistle contours with low representation were labeled as “variants”.

A subset of 50 randomly selected whistles was used with 2 naïve voluntaries to calculate the interobserver reliability.

6.5.2. Acoustic data analysis.

Given the relevance of the signature whistles in dolphin communication, group cohesion and social interaction, it was important to study this type of sounds throughout the years and in different contexts. To identify signature whistles, the SIGnature IDentification (SIGID) criteria (Janik et al., 2013) were used. This method proposes that a vocalization can be identified as signature whistle if 75% of all whistles in a bout consists of that such stereotyped whistle. Each bout must be composed of sequences of the same contour in intervals of one to ten seconds. These criteria were applied bidirectionally, thus a whistle to be counted as part of a bout had to be followed or preceded by another whistle of the same type inside the time interval.

To validate this approach, the whistles of specific animals were extracted from the isolation recordings. Following Caldwell & Caldwell (1965) the dominant whistle type emitted by each individual (i.e. the most frequently emitted whistle contour) could be considered to be its signature whistle. The Kruskal-Wallis test was used to examine differences in whistle emission rates according to the context (isolated, segregated and social) for specific dolphins.

To study the effect of isolation, segregation and social contexts on whistle characteristics, a full-factorial multivariate analysis of variance (MANOVA) with Games-Howell post-hoc test was performed for the acoustic features of the whistles assigned to the two dolphins that were present in all contexts. All statistical analyses were performed using *IBM SPSS Statistics 21* (IBM Inc.).

6.5.3. Automatic acoustic analysis.

Because dolphins are very inquisitive animals, the hydrophone had to be placed in an adjacent, connected part of the pool to avoid changes in the behavior of the focal animal.

The identification of the whistle emission, as well as the counting and classification, were done using spectrograms produced with Raven Pro 1.4. As in the previous section, whistles were selected with a minimum of 300 ms duration; sharp and defined contours and no overlapping with other vocalizations.

After selecting the whistles, the analysis proceeded with the Matlab-based algorithm, BELUGA, developed by Volker Deecke and Vincent Janik (St Andrews University) for whistle frequency contour extraction.

After the extraction analysis, the contours were processed in a Matlab-based algorithm, ARTwarp, also developed by the authors of BELUGA, to automate the categorization of the sounds. This algorithm combines dynamic time warping, to measure the similarities of the contours, with the adaptive resonance theory to group the sounds into different categories (Deecke & Janik, 2006).

For the previously selected whistles, the start, end, minimum, maximum, mean, range frequencies (all in kHz) and duration (in ms) were measured.

With all the equipment synchronized, it was possible to determine when the focal animal was closer to the hydrophone, and which whistles were produced in that instant. It was assumed that whistles recorded in the frames in which the focal animal was closer to the hydrophone were from this emitter.

To define the section of the pool that will be identified as the hydrophone section, a selection of the frames was made. Since the hydrophone is omnidirectional and knowing the dimensions of the pool section, a virtual circle, with the approximate hydrophone position in the center, was defined.

Every time the dolphin entered the predefined circle, the corresponding frames were considered for the acoustical analysis. Since the equipment was synchronized, a corresponding match between the frames and the acoustical data was made and the vocalizations were extracted.

Whistles that occur one second before and/or after the specific frames were included in the analysis to avoid losing data with the transitions of the delimited circle.

CHAPTER 3 – ACCELEROMETRY DATA – TOWARDS AN IN-DEPTH ANALYSIS OF CAPTIVE DOLPHIN BEHAVIOR

(Pre-final version of the manuscript to be submitted)

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1. Abstract

Obtaining detailed information about the behavior of wild marine mammals has been a challenge over the years and new techniques have been developed to automatically monitor behavior. Accelerometry has shown an enormous potential in providing information about an animal's behavior; however, a validation of the behavior patterns is necessary. The goal of unsupervised classification is to be able to automatically identify the behavior performed by a dolphin without resorting to video cameras. This validation is often impossible in the wild so it is essential to do it in a controlled environment. For a data collection involving two male adult dolphins (contexts: "alone" and "accompanied by other animal" in the pool), a total of 86 behaviors, grouped into 8 categories, were correctly classified by the algorithm: the Lateral Ascent, Lateral Swim, Lateral Dive, Inverted Dive, Glance Up, Dorsal Swim, Dorsal Dive and the Head Up. The most common behavior observed for both dolphins and contexts was Dorsal Swim. A sequence of behaviors was also analyzed and the results showed a similarity of the behavior sequences between both animals and both contexts, emphasizing that these animals did not change their behavior when they were alone or accompanied. An entropy analysis indicated that the dolphins' behavior contains a high order structure that may allow the prediction, by a

statistical model, of which behavior will follow a specific action. As far as we know, this is the first study building a behavioral map with the combination of the possible postures for a more complete behavior analysis. The unsupervised classification algorithm will make it possible to extract rare behavior patterns or those that researchers do not normally observe directly. Even thought the algorithm is specific for bottlenose dolphins, it has the potential to be applied to other cetacean species.

2. Introduction

Obtaining detailed information about the behavior of wild marine mammals has been a challenge over the years. Scientific equipment has become more efficient, though the data remains hard to collect. Most of these animals often move or migrate for many kilometers a day, and research is mainly done from boats and through aerial observations, providing limited information about their underwater lives, where they spend most of their time (Costa, 1993; Yasuhiko, 2004). Over the years, techniques have been developed to automatically monitor behavior. Examples are data transmitters directly attached to the animals (Ravi et al., 2005; Wilson et al., 2008). Accelerometry has shown an enormous potential in providing information about an animal's behavior. When attached to an animal's body, this type of equipment can record orientation and movement through the acceleration. This way, with the accelerometer data alone, some aspects of animal behavior can be inferred (Soltis et al., 2012). The use of pattern recognition algorithms may allow the extraction of quantitative measurements and enable the detection and the monitoring of actions of freely moving subjects. Both brief and long events, periods of inactivity and diurnal variations, can provide a window to the behavior of the animals through their movement profiles (Noldus et al., 2001; Noldus et al., 2002; Machado, et al., 2015). Since accelerometers react to the earth´s gravitational field as well as to acceleration produced by the animal (Wilson et al., 2008), they provide information about movements not detectable by human eye. Acceleration can be divided into static and dynamic components (Ravi et al., 2005; Shepard et al., 2003, 2008; Watanabe et al., 2005; Wilson et al., 2008). Static acceleration, represented by low frequency signals, is a measure of the inclination of the accelerometer with respect to the earth's gravitational field and it allows the characterization of the animal's posture or body angle. Dynamic acceleration is represented by high frequency signals, obtained from the movement of the body (Shepard et al., 2003, 2008; Wilson et al., 2008), generally associated with locomotion. These components of acceleration can provide a measure of the animal's motion and allow a more accurate estimate to the identification of the behavior pattern (Albareda, et al., 2003; Shepard et al., 2003, 2008).

The aim of this research was the development of a method that allows not only a less subjective approach to analyze behavior but also a faster one. This study was motivated by the possibility of using the development of techniques in captive animals to apply them to the study in wild marine mammals with existing equipment. Nowadays, almost all the equipment used to study marine mammals, such as tags and GPS emitters, have a 2- or a 3-axis accelerometer; however, most of the times the full potential of these data is not obtained for lack of validation. For that reason, the validation between accelerometer and video is important to enable, in the future, a more complete mapping of cetacean behavior.

3. Materials and Methods

3.1. Subjects and facility.

The subjects of this study were two captive common bottlenose dolphins (*Tursiops truncatus*) at Zoomarine, Algarve (Portugal). Both animals were adult males, HM5 (born in 1995 and with 185 kg, 2.46 m) and AM4 (born in 2004 and with 172 kg, 2.55 m) which at the time of this study were held in a covered pool and with natural light, having artificial lighting when necessary.

The complete pool, 47 m long, was divided into 4 smaller sections that were separated by perforated fiberglass doors and panels (Figure 3.1). The animals were filmed in a pool section with approximately 21 m diameter and with varying depth, maximum depth, 5 m. Sessions duration, subjects motivational condition and annoyance signals were carefully monitored by dolphin trainers so that no distress was imposed on the animals by the experiments.

This study followed careful procedures to minimize disturbance to the animals, and was approved by Zoomarine's Board and all technical Directors.

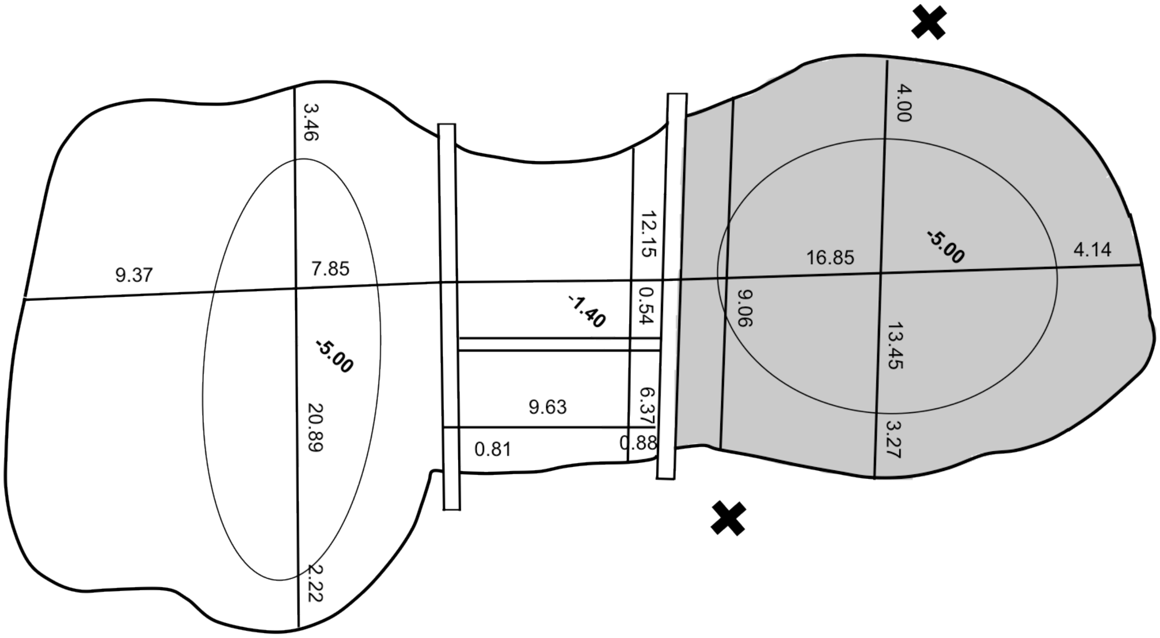


Figure 3.1. Pool's scheme and dimensions. Image showing the complete habitat dimensions and depth of the dolphins' pool. The grey part of the scheme represents the portion of the pool where the experiment occurred. The black cross represents the position of the cameras.

3.2. Set-Up of the equipment.

The set-up consisted of two 170º wide-angle GoPro Cameras (GoPro Hero 2, ©GoPro, Inc.) and one three-axis accelerometer Kinetamap (SparkFun Electronics, Inc.) inside a waterproof box (Snap Sights SC25 from Intova) measuring 10.80 x 8.89 x 3.81 cm, with a total weight of 250 gr, representing only 0.14% and 0.15% of HM5 and AM4's weighs, respectively.

The videos were recorded in a 32 GB SD card in MP4 format with a resolution of 1280x720 and at 30 frames per second. The video cameras were positioned in the locations indicated on Figure 3.1 to enable the complete recording of each animal's behaviors. Due to the pool size constraints, the two cameras were placed 4 m high (one opposite to the other) on the top of a pole (Figure 3.1).

The Kinetamap device recorded acceleration data at an average of 65 Hz (65 points per second) into an internal microSD memory card and had a USB rechargeable internal 1100 mAh LiPo battery. This was a high-resolution accelerometer, with 16 bits of digital output data (capturing 65536 possible values per data point) and a sensitivity of 3.9 m*g*/LSB, which enables the detection of inclination changes less than 1°. The values given by this equipment were proportional to *g* due to gravity (1 $g = 9.81\ ms^{-2}$) (Figure 3.2).

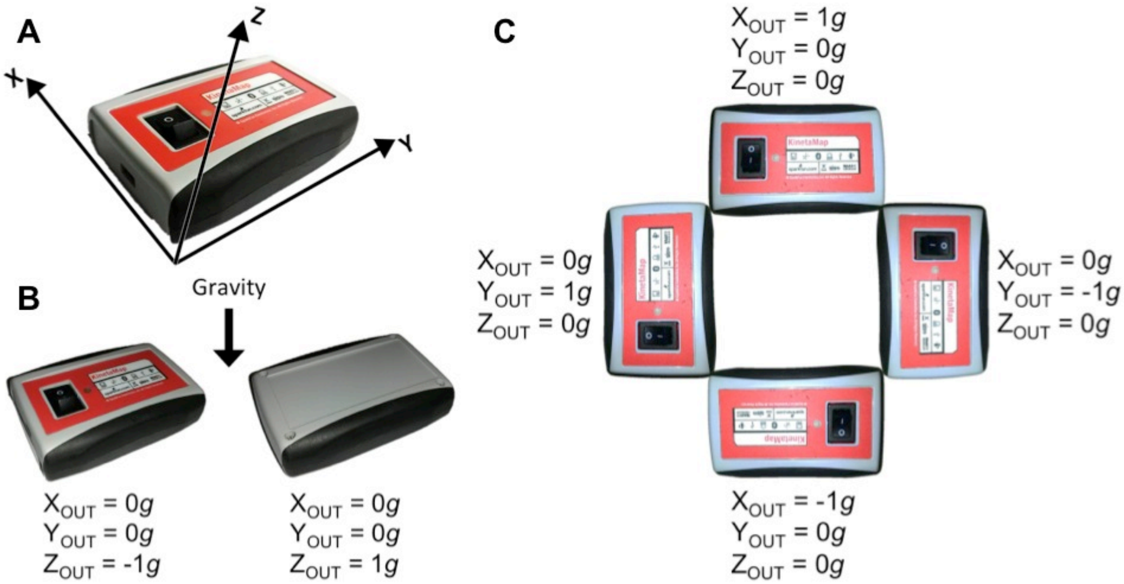


Figure 3.2. Kinetamap device orientation. Image showing the orientation of the axes of the accelerometer used. A – Axes of acceleration sensitivity; B – Orientation to gravity and; C – Output responses of the accelerometer in each position.

Each trial needed the complete preparation, placement and removal of the set-up. All experimental activities occurred with minimal interference regarding the schedule and routine of the animals.

3.2.1. Accelerometer placement.

The 3D accelerometer, inside a waterproof box, was attached near the pectoral fin of each subject (Figure 3.3) by two suction cups specially designed for cetaceans (Saddle Suction Cups from Cetacean Research Technology). The animals were trained, through positive reinforcement, to tolerate the waterproof box near the right pectoral fin. To reward the dolphins for tolerating and keeping the equipment in position, they were regularly reinforced with fish throughout the sessions. The three-axis corresponded to dorso-ventral (x), anterior-posterior (y) and lateral (z).

The location of the equipment on the dolphin's bodies was as exact as possible for each session, since dynamic components of acceleration depend on the position in which the accelerometer is placed.

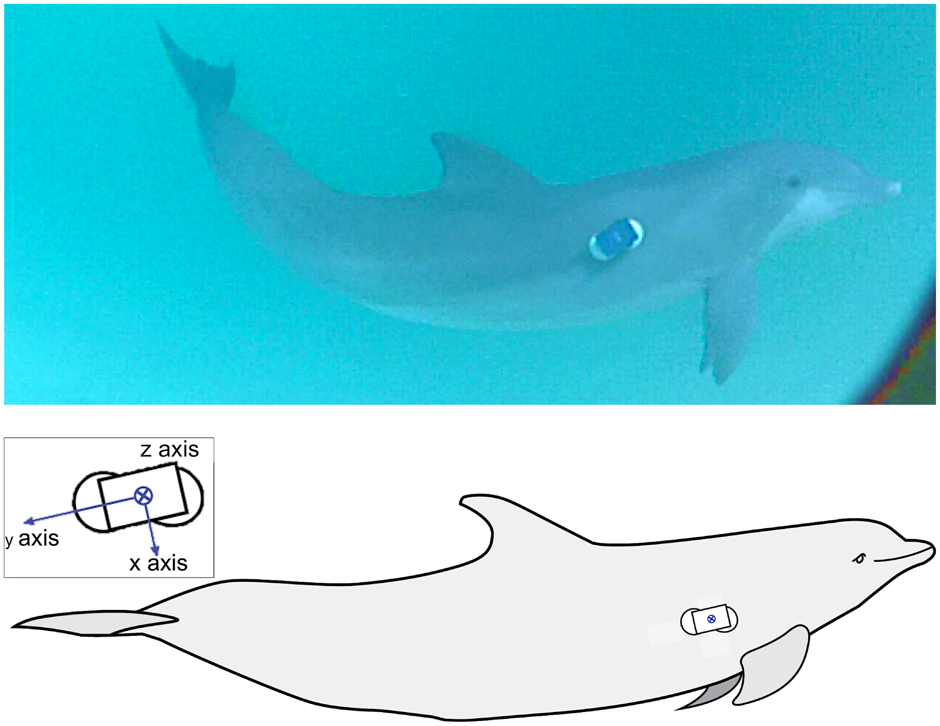


Figure 3.3. Placement of the accelerometer in the dolphin's body. Top image, a frame from an underwater recording of a dolphin with the accelerometer. Bottom image, drawing of the position of the accelerometer in the dolphin and the direction of three axis (x, y and z).

3.2.2. Experimental protocol.

Data collection was conducted from November 2012 to April 2014 during the winter seasons (from November to April), which corresponded to the time the park was closed for the public or with low numbers of visitors. During the closing period, the animals kept their training schedules and routines.

All equipment was synchronized in the beginning of each session with a chronometer. The cameras and chronometer were turned on at the same time and the accelerometer was turned on next to the chronometer and in front of the camera to record the starting time. To synchronize the cameras, we used a Wi-Fi remote control.

3.2.3. Data collection with video & accelerometer recordings.

Accelerometer data was collected in two different contexts: when the animal was alone in the pool and when it was accompanied by another animal. Whenever the animal was not cooperative data collection was aborted to avoid unnatural behaviors. It was stipulated that sessions with less than 20 minutes would be discarded, since that was the time deemed necessary to be sure of habituation of the animal to the accelerometer. Sessions were recorded from 09:00 AM to 6:00 PM for each animal and context, as shown in Table 3.1.

Table 3.1. Time of sessions, in minutes, recorded for each experimental animal and corresponding number of sessions, for both contexts.

	HM5	AM4
"Alone"	625 mins (17 sessions from 22 to 60 mins)	620 mins (12 sessions from 32 to 68 mins)
Accompanied	691 mins (16 sessions from 30 to 64 mins)	647 mins (14 sessions from 27 to 64 mins)

Since the accelerometer performed at an average of 65 Hz and because of the video sampling frequency at 30 frames per second, the data were converted to 30 Hz, or 30 points per second using an interpolation. The reason to use this method was the necessity to have a function that could transform the data, no matter how long or short the session was, into a specific number of samples without losing information. The method used was the piecewise cubic spline interpolation (Boor et al., 1978), or cubic spline. Since the function adjusts itself to go through all the data points, the probability of error is smaller.

A calibration of the accelerometer data around zero axes was performed to eliminate the associate error for the fact that the accelerometer is not exactly located in the same body position during all sessions. Another way to guarantee that all sessions had the accelerometer in similar location in the dolphin was by selecting the ones that had the peaks around the same values (for example, for x_GA component, the first peak should be around -1 to guarantee that all sessions are measuring the same movements).

3.3. Behavior analysis.

We quantified animal behaviors using continuous acceleration measurements based on the following features: (i) gravitational acceleration along the dorsal-ventral axis (x_GA), which measures one component of the body rotation, (ii) gravitational acceleration along the anterior-posterior axis (y_GA), which captures body inclination, (iii) gravitational acceleration along the mediolateral axis (z_GA) to measure another component of the body rotation, and (iv) total body acceleration (totAccelBA), which captures periods of movement versus rest (Figure 3.3).

Total body acceleration was defined as:

$$Total\ Acceleration\ BA = \sqrt{x\_BA^2 + y\_BA^2 + z\_BA^2}$$

where x_BA, y_BA and z_BA denote the body acceleration in the dorsoventral, anterio-posterior and mediolateral axis, respectively, with respect to the animal's head (Figure 3.3). The individual BA components were calculated by median-filtering the raw acceleration time series and by subsequent high-pass (0.45 Hz) filtering with a fourth-order Butterworth filter. Gravitation acceleration, GA, was obtained for each axis by median and subsequent low-pass filtering (0.45 Hz cutoff, fourth-order Butterworth filter). This high pass filter was calculated by using a combination of a gyroscope and an accelerometer in one session and the best-fit value for the measurements was chosen (for more details see Chapter 2).

The x_BA, y_BA, z_BA and totAccelBA time series were binned (1000 ms length with 80% overlapping) and discretized to obtain a low-dimensional histogram representation of each time bin. These sliding windows were then used for change-point detection, i.e. to discover time points at which properties of time-series data change. Change-point detection is done by thresholding the average distance between neighboring sliding windows, therefore obtaining stationary segments of putative behavior blocks ranging from one to several seconds (Figure 3.4).

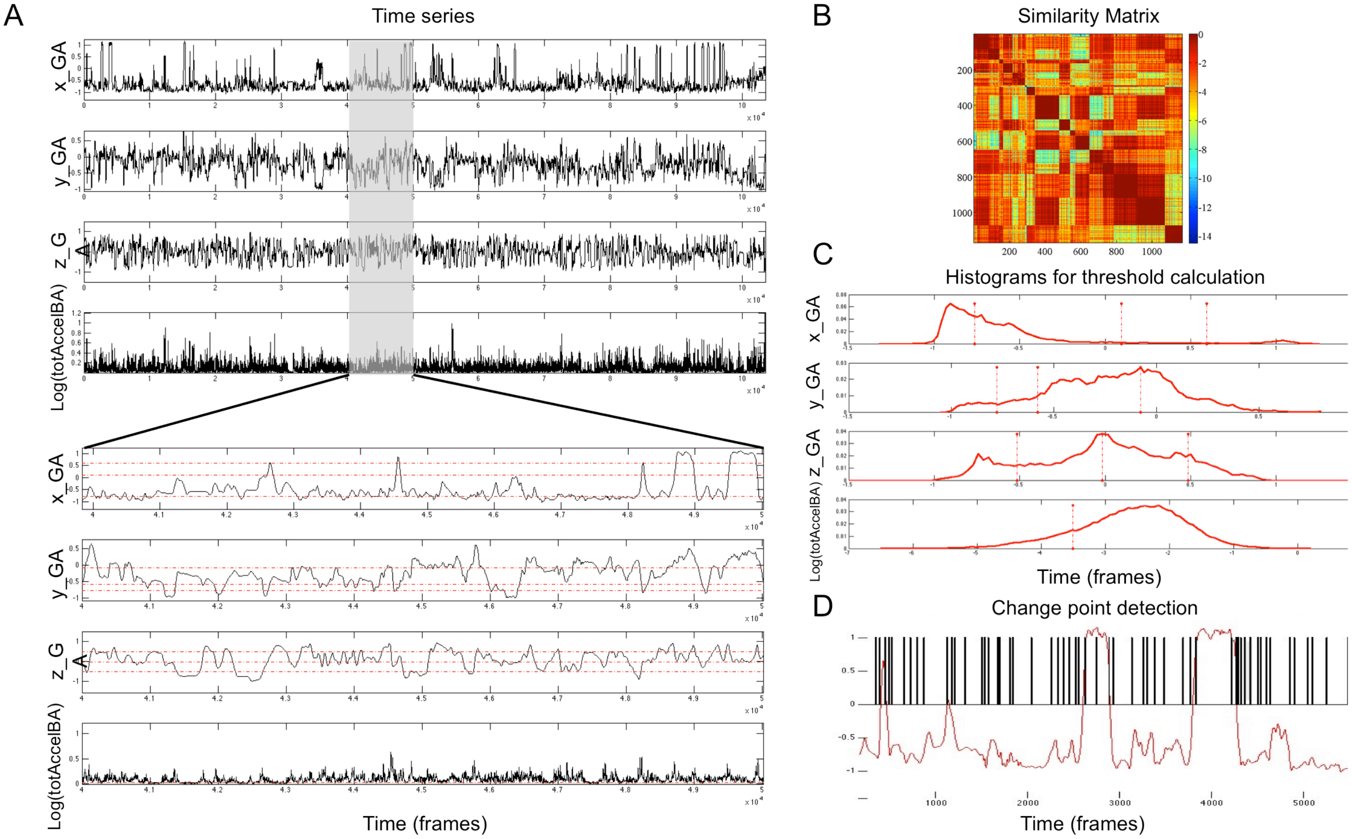


Figure 3.4. Behavioral clustering algorithm method. A) Example of a time series for an accelerometer session (top). A detailed view of 1000 frames is shown (below) with the thresholds lines in red. B) An example of a matrix of pairwise similarities. C) A histogram for the threshold calculation showing one session and the corresponding thresholds for the different variables. D) An example of the change points detection for the x_BA signal. Bigger spaces between the black lines represent bigger behavior blocks.

For each "behavior" segment and feature, values were discretized using one or three thresholds: For totAccelBA, a single threshold was used to separate moving from the resting. The threshold was the same for both subjects and it was set to the value separating the bimodal distribution of BA (visualized in logarithmic scale). For x_GA, y_GA and z_GA, three thresholds were used separating “dives” (y_GA), “turns” (x_GA and z_GA) and “movement” (logarithm of the total acceleration of the Body acceleration component). The resulting histograms, i.e. 4 per behavior segment, were individually normalized to obtain probability distributions, and then used to calculate pairwise similarities between segments. The measure of similarity, S, was based on histogram intersection similarity (Zhang & Lu, 2003).

These similarity values were clustered using affinity propagation (Frey & Dueck, 2007), generating a continuous unbiased classification of the behavioral states. Affinity propagation is an unsupervised clustering algorithm that identifies exemplars, data points that represent a group of similar data points, and forms clusters of data around the exemplars. It considers all points as potential exemplars and exchanges data points until a good set of exemplars is defined and the clusters are formed.

To calculate the transition probabilities from one behavior to the next, a first-degree Markov Chain analysis was performed. This analysis indicates how dependent a specific behavior is from the previous or the next events. Behaviors were also grouped in bigrams and trigrams to study transitions.

3.3.1. Markov chain, Shannon’s entropy and statistics.

Markov Chain is a group of possible states of a system in which the possible transition paths between two states depends on the first one (Stewart, 1994). This first order stochastic process can be defined as $p(X_t|X_{t-1})$, where p is the probability of X_t following X_{t-1}.

Shannon’s Entropy measures the internal dynamics of a system, in this case, the sequences between the behavior states. Zero-order entropy measures the behavior repertoire diversity and it is defined by $H_0 = log_2 N$, where N is the number of states. First-order entropy expresses the internal organization structure and it is defined by $H_1 = \sum_{i=1}^{m} p_i \, log_2 p_i$. Higher entropy orders, second and/or third, indicates the complexity of a system and how states interact within a repertoire. Second-order entropy, equivalent to the first order Markov chain, is defined by $H_2 = \sum_{i=1}^{m} p_i \sum_{j=1}^{m} P_{j|i} \, log_2 P_{j|i}$ and third-order entropy is described as

$H_3 = \sum_{i=1}^{m} p_i \sum_{j=1}^{m} P_{j|i} \sum_{k=1}^{m} P_{k|j,i} \, log_2 \, P_{k|j,i}$. The value of each entropy order will drop from one order to the next if the behavioral sequence complexity increases.

To study the effect of the difference between the observed frequency of behaviors and the expected ones, a chi-square test was applied to the bigrams and trigram analysis. All coding and statistical analyses were performed using *MATLAB R2011a* (The MathWorks, Inc., USA).

3.3.2. Manual behavior classification.

For the validation of the unsupervised approach, a manual behavior classification from the video, “serial recording” method, was performed. The animals’ activity was coded using a published ethogram with descriptions for wild dolphins (see Müller et al., 1998). The behaviors most often observed are described in Table 3.2.

Table 3.2. Behavior classification through observation analysis of the video recordings. Brief descriptions adapted from Müller et al., 1998, except the behaviors marked with *.

Behavior	Code	Brief description
Head Up Swim	1	Repetitive head exposure during dorsal swimming with rostrum oriented upward.
Lateral Swim	2	90-degree rotation from the dorsal position swim, one pectoral swim upward and the other downward.
Head Up	3	Exposure of the foresection of the head at the surface in vertical or near vertical position, briefly stationary with pronounced arch.
Inverted Swim	4	Forward swimming with belly up.
Eating *	5	Head exposure oriented upward, near the edge of the pool, stationary
Glide	6	Dorsal forward displacement without tailstock movements, and with body fully extended.
Corkscrew Swim	7	Forward swimming with repetitive longitudinal rotations, with body parallel to the water surface, at the surface or underwater.
Underwater Swim *	8	Underwater forward swimming in dorsal position.
Stop *	9	Stationary position, at surface or underwater, without body movement.
Surface Swim (“Sharking”)	10	Dorsal forward swimming with exposed dorsal fin.
Dive	11	Movement of the head from the surface to underwater with body arching, exposing the tail

4. Results

With more than 40 hours of data recordings, it was possible to compare the acceleration values between two dolphins, alone and with company in the pool. More importantly, it was also possible to validate an algorithm that enables the automatic detection of different blocks of behavior.

4.1. Behavior classification.

The aim of this unsupervised classification code is to consistently identify specific actions or behaviors. Through more than 12 hours of non-consecutive sessions ("alone"), the algorithm classified 41 and 39 behavioral clusters for HM5 and AM4, respectively. Figure 3.5 shows the histograms of the selected parameters: the x_GA data graph gives dorso-ventral information, such as dorso-lateral-inverted posture; the y_GA graph gives anterior-posterior information, as vertical-horizontal posture; the z_GA graph gives right-left information, and all three have 3 thresholds each; and the log(totAccelBA) graph gives stop-moving information and has 1 threshold. Dolphin schematics in the graphs represent the animal postures. The letters/numbers inside the graphs represent different segments in which the threshold is applied. Each component has only one possible segment that should be combined with the other segments from the different components. Each segment of each graph will give information about the dolphin's posture. Figure 3.5 can be read as a combination between components and segmentations.

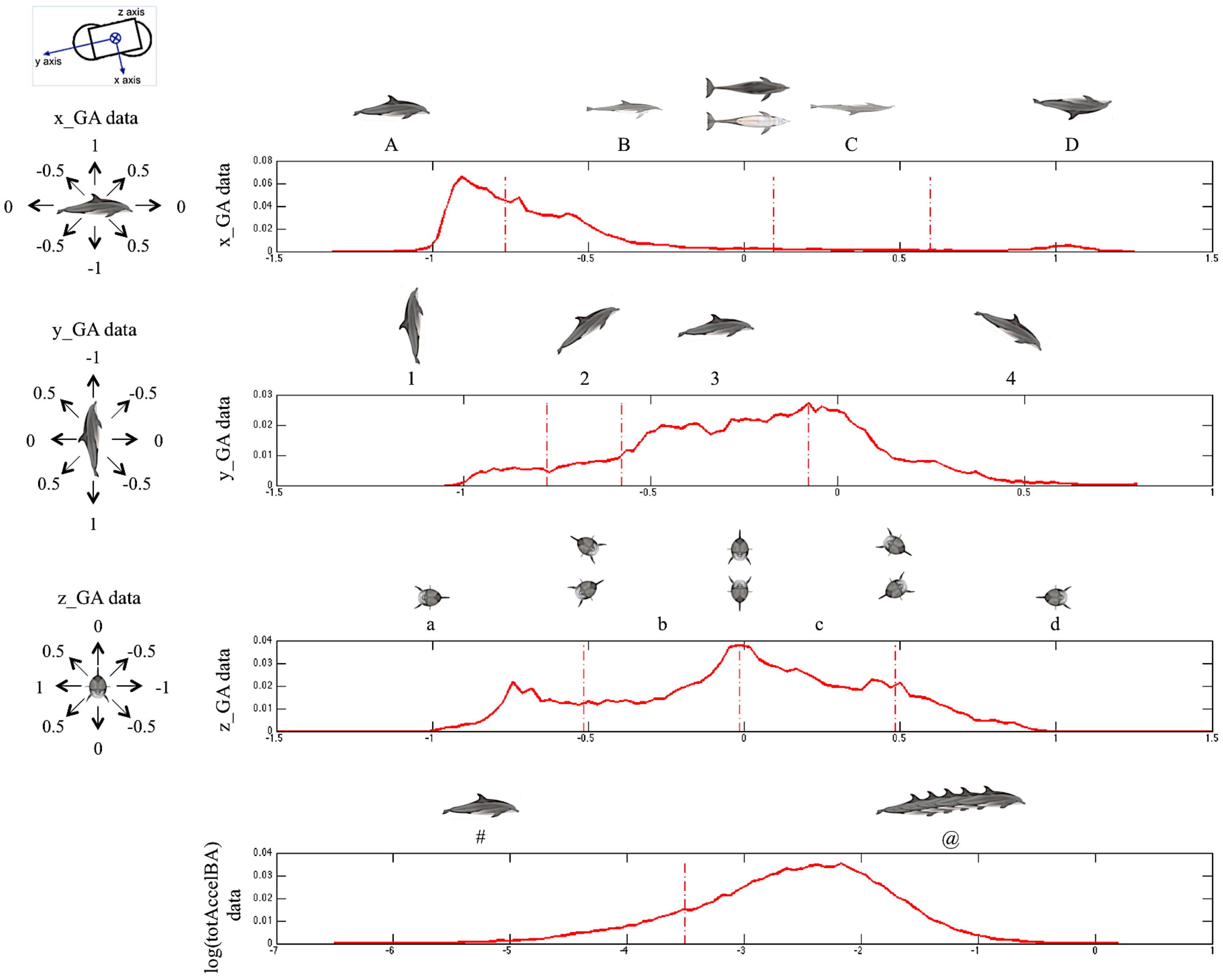


Figure 3.5. Histograms for the four parameters analyzed and their respective thresholds (vertical dotted line). The small dolphin's images represent the position of the animal that corresponds to each threshold. The letters/numbers inside the graphs represent different segments in which the same threshold is applied.

Thresholds from "A" to "D" represent a body rotation from dorsal position (A) to inverted position (D) throughout the lateral transition postures (B and C). The histogram of this component shows that most of time dolphins are in dorsal position.

Thresholds from “1” to “4” represent a body inclination from vertical position, “Head Up” (1) to dive movement (4) throughout the transition postures (2 and 3). The histogram of this component shows that mostly dolphins are in horizontal or diving position.

Thresholds from “a” to “d” represent another component of the body rotation, with discrimination of left and right axial movement, from “Lateral Left” (a) to “Lateral Right” (d) through the transition lateral postures (b and c). The histogram of this component shows that, frequently, dolphins are in dorsal or inverted position.

Thresholds “#” and “@” represent, respectively, stillness and movement. The histogram of this component shows that dolphins are rarely motionless.

4.2. Behavior profiles and classification of the behavior.

To explore the behavior profiles detected by the algorithm, an affinity propagation cluster was performed. Figure 3.6 shows an example of the output of this cluster for one session.

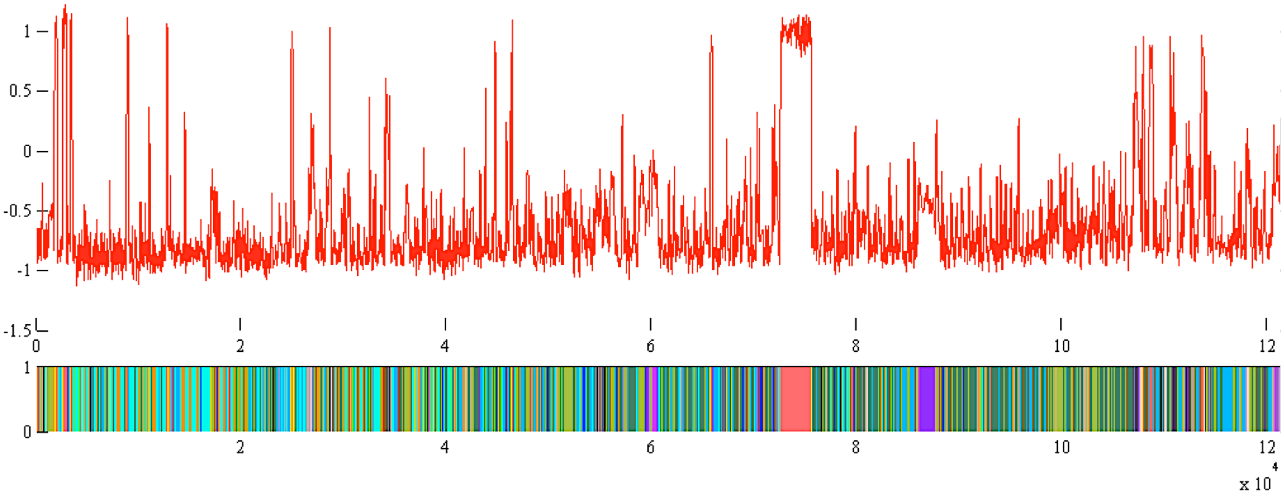


Figure 3.6. Graph with acceleration data. Top image represents the acceleration of the animal for the chosen parameter x_GA that will give information about acceleration during different postures, in this case dorso-ventral movements. Bottom image shows the behavior discrimination with each specific cluster (in this case a behavior pattern) represented by a color.

4.3. Identification of the behavior clusters.

The analysis of the unsupervised algorithmic identification of the behaviors produced 86 different clusters that were labeled with the correspondent behavior using the information contained in Figure 3.5. Table 3.3 show an example of how the automatic decision was made to identify each of the clusters and behaviors.

To decide which behavior was represented in each cluster, only values above 0.60 were considered (see example in Table 3.3). Values below 0.60, which have an equal probability of being another behavior pattern, were labeled as undetermined.

Table 3.3. Examples of the cluster list with the values for the different thresholds that give information about the behavior. On the left of the table, there is the identification of the behavior with its variants. Values in grey represent the sections of the thresholds that identify the behavior. Only values above 0.60 were used to identify the behaviors. Columns "D" and "d" from the x_GA data and z_GA data were excluded from the table for being irrelevant for the examples.

		x_GA data			**y_GA data**				**z_GA data**			**log(totAccelBA) data**	
	Cluster	***A***	***B***	***C***	***1***	***2***	***3***	***4***	***a***	***b***	***c***	**#**	**@**
Lateral Right Swim	**6**	0.08	0.92	0.00	0.00	0.00	1.00	0.00	0.14	0.86	0.00	0.00	1.00
	40	0.00	0.00	0.78	0.00	0.11	0.89	0.00	0.94	0.06	0.00	0.07	0.93
Head Up	**9**	0.00	1.00	0.00	1.00	0.00	0.00	0.00	0.00	0.83	0.17	0.10	0.90
	10	0.00	1.00	0.00	0.89	0.11	0.00	0.00	0.00	0.00	1.00	0.03	0.97
	37	0.00	0.05	0.95	1.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00
Dorsal Stop	**35**	1.00	0.00	0.00	0.00	0.00	0.91	0.09	0.00	0.00	1.00	0.62	0.38
Dorsal Dive Stop	**62**	1.00	0.00	0.00	0.00	0.00	0.10	0.90	0.00	0.98	0.02	0.68	0.32

4.4. Unsupervised behavior analysis across sessions and across animals.

After grouping all sessions for both animals and contexts, the cluster analysis was performed. As Figure 3.7 shows, both dolphins present similar behavior clusters compared with each other and between contexts.

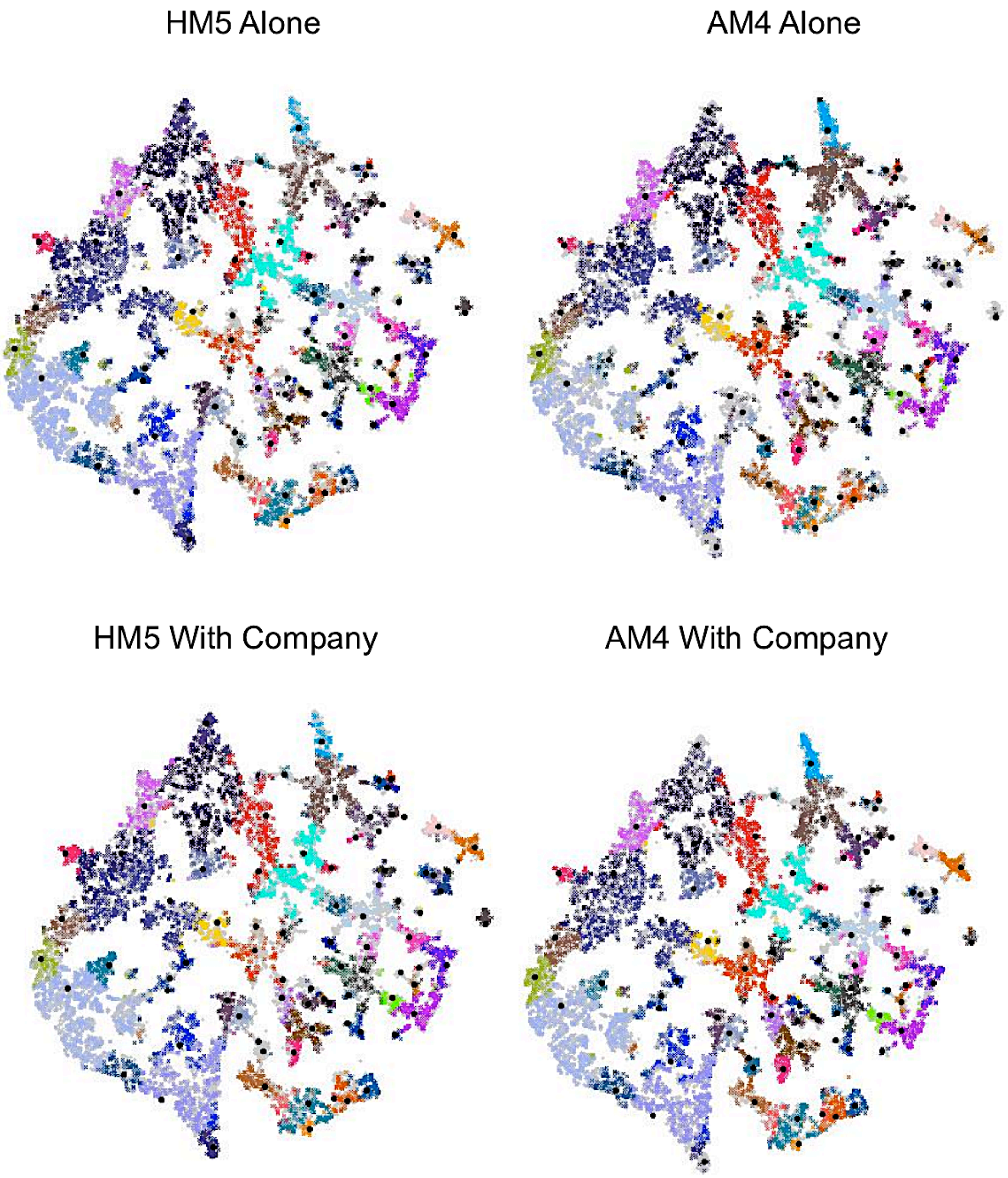


Figure 3.7. Behavior “regions”. Cluster analysis from all sessions with a total of 86 identified clusters. Different colors mean different actions/behaviors; however, same color represents same action/behavior throughout the graphs. Bi-dimensional spatial plots represent distances from each data point to its exemplar.

Since the behavior identification for both animals is similar, a grouped cluster analysis was performed and showed in Figure 3.8.

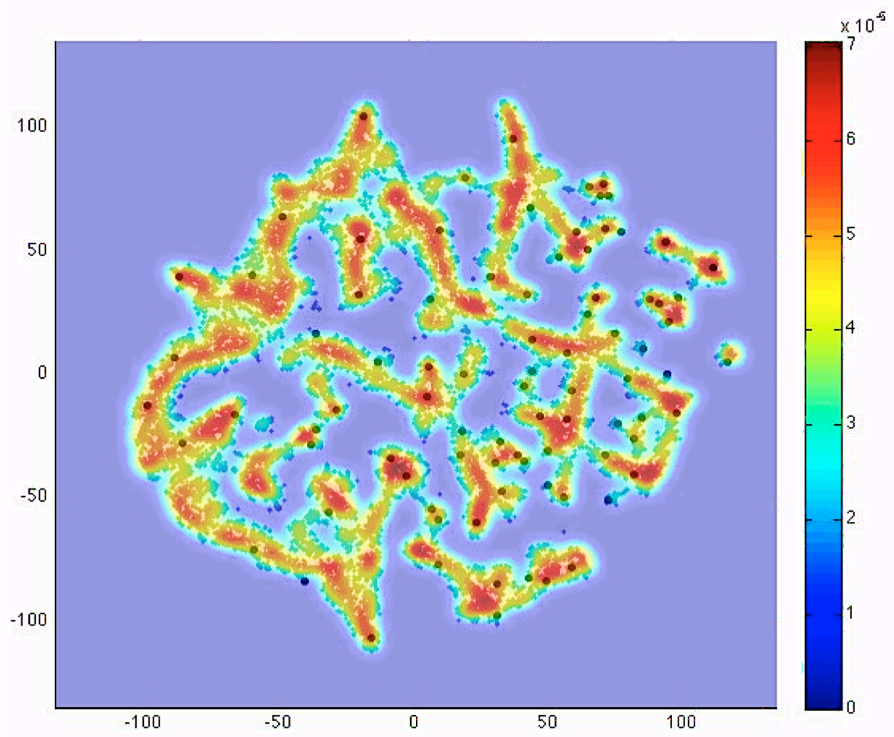
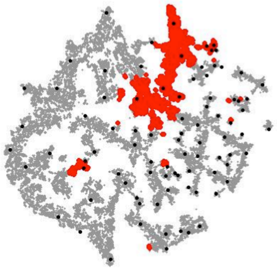


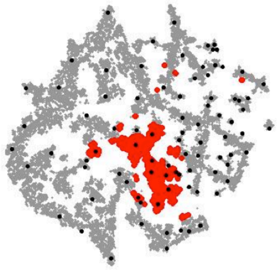
Figure 3.8. The combination of HM5 and AM4 alone and accompanied clusters into one graph with red showing the higher density of points per cluster and the black dot representing the exemplar value of each cluster. The color bar represents the concentration of behaviors/actions that are found closer (red) or more distant (blue) from the exemplar. Note that the exemplars, represented as a black dot, are found where the red color is stronger, showing that the values of each cluster tend to be localized around the exemplar.

A t-SNE Mapping was performed for behavior dimension reduction that enabled the identification of the behavior clusters for all sessions together (HM5 “alone” + HM5 with another animal + AM4 “alone” + AM4 with another animal) (Figure 3.9).

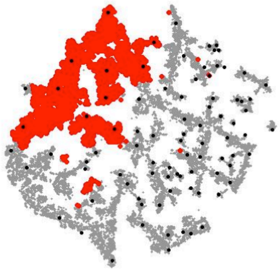
Lateral Right Swim



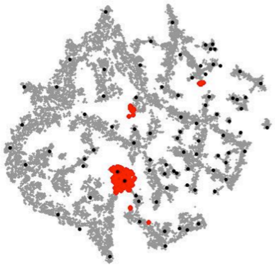
Lateral Left Swim



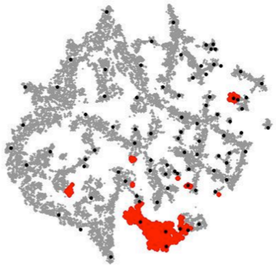
Dorsal Swim



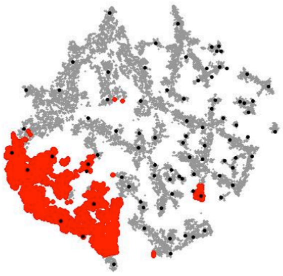
Lateral Right Dive



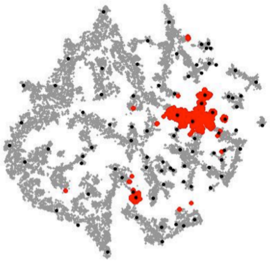
Lateral Left Dive



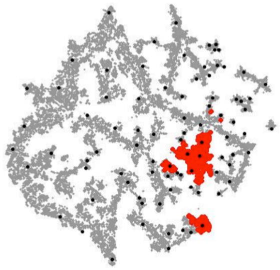
Dorsal Dive



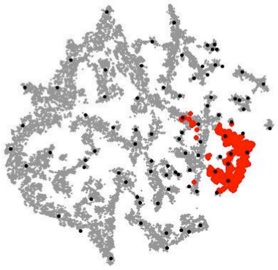
Lateral Ascent Right Swim



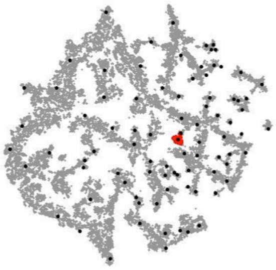
Lateral Ascent Left Swin



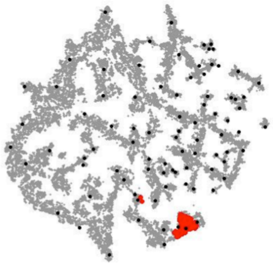
Head Up



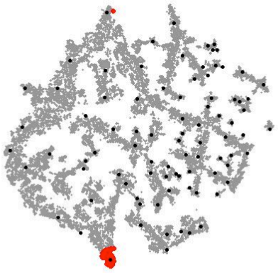
Lateral Right Dive/Stop



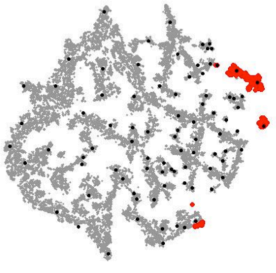
Lateral Left Dive/Stop



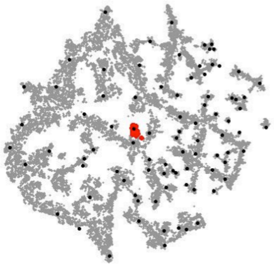
Dorsal Dive Stop



Inverted Dive



Lateral Left Stop



Dorsal Stop

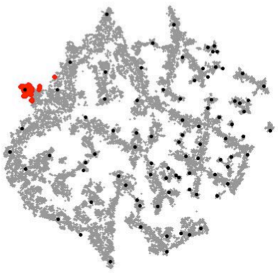


Figure 3.9. Segmentation of the behavior "regions". Graphs representing the different behavior categories and their specific locations in the behavioral map. Organization of behavioral space into "regions" with similar movement types (red). The black dots represent the exemplars of each behavior cluster.

4.5. Behavioral sequences.

Behavioral sequences are important to understand how behavior is organized, and can provide information about the animal's internal/emotional state, stereotypies or simply to characterize the activity itself.

For the behavioral sequence analysis, the monograms, bigrams and trigrams were calculated. The most frequent behavior for HM5 and AM4, for both contexts, was Dorsal Swim.

The behavioral sequence as bigrams and trigrams for both animals alone are shown in Table 3.4 and Table 3.8. Same sequences for both animals, when they are accompanied with another dolphin in the pool, are shown in Table 3.6 and Table 3.7.

Table 3.4. Table of bigrams for both dolphins "alone" with behaviors.

AM4 "Alone" Behaviors	HM5 "Alone" Behaviors
Dorsal Swim - Dorsal Swim	Dorsal Dive - Dorsal Swim
Dorsal Dive - Dorsal Swim	Dorsal Swim - Dorsal Swim
Dorsal Swim - Dorsal Dive	Dorsal Swim - Dorsal Dive
Dorsal Swim - Lateral Right Swim	Dorsal Dive - Dorsal Dive
Lateral Right Swim - Dorsal Swim	Dorsal Swim - Lateral Right Swim

Table 3.5. Table of trigrams for both dolphins “alone” with behaviors.

AM4 “Alone” Behaviors	HM5 “Alone” Behaviors
Dorsal Swim - Dorsal Dive - Dorsal Swim	Dorsal Swim - Dorsal Dive - Dorsal Swim
Dorsal Swim - Dorsal Swim - Dorsal Swim	Dorsal Dive - Dorsal Swim - Dorsal Swim
Dorsal Swim - Lateral Right Swim - Dorsal Swim	Dorsal Dive - Dorsal Swim - Dorsal Dive
Dorsal Dive - Dorsal Swim - Dorsal Swim	Dorsal Dive - Dorsal Dive - Dorsal Swim
Dorsal Dive - Dorsal Dive - Dorsal Swim	Dorsal Swim - Dorsal Swim - Dorsal Dive

Table 3.6. Table of bigrams for both dolphins “accompanied by other animal” in the pool with behaviors.

AM4 Accompanied Behaviors	HM5 Accompanied Behaviors
Dorsal Dive - Dorsal Swim	Dorsal Dive - Dorsal Swim
Dorsal Swim - Dorsal Swim	Dorsal Swim - Dorsal Swim
Dorsal Swim - Dorsal Dive	Dorsal Swim - Dorsal Dive
Dorsal Dive - Dorsal Dive	Dorsal Dive - Dorsal Dive
Dorsal Swim - Lateral Right Swim	Lateral Right Swim - Dorsal Swim

Table 3.7. Table of trigrams for both dolphins “accompanied by other animal” in the pool with behaviors and their quantities.

AM4 Accompanied Behaviors	HM5 Accompanied Behaviors
Dorsal Swim - Dorsal Dive - Dorsal Swim	Dorsal Swim - Dorsal Dive - Dorsal Swim
Dorsal Dive - Dorsal Swim - Dorsal Dive	Dorsal Dive - Dorsal Swim - Dorsal Swim
Dorsal Dive - Dorsal Swim - Dorsal Swim	Dorsal Swim - Dorsal Swim - Dorsal Dive
Dorsal Dive - Dorsal Dive - Dorsal Swim	Dorsal Dive - Dorsal Swim - Dorsal Dive
Dorsal Swim - Dorsal Swim - Dorsal Swim	Dorsal Swim - Dorsal Swim - Dorsal Swim

4.6. Entropy.

Table 3.8 presents the values of the Shannon’s entropy for the behavior of each dolphin for both contexts, “alone” and accompanied with another animal in the pool.

Table 3.8. Behavior entropy values, from zero-order to higher orders, for HM5 and AM4 for both contexts, “alone” and accompanied.

	HM5 “Alone”	**HM5 Accompanied**	**AM4 “Alone”**	**AM4 Accompanied**
$\mathbf{H_0}$	6.43	6.43	6.36	6.43
$\mathbf{H_1}$	2.92	2.96	2.97	3.02
$\mathbf{H_2}$	1.85	2.00	2.06	2.06
$\mathbf{H_3}$	0.02	0.01	0.03	0.01

As showed in Table 3.8, the decreasing entropy values, from H_0 to H_3, indicate an increase in the information content, as well as complexity, of the behavior sequences.

5. Discussion

Accelerometers have been used over the years to study behavior and movements in different species of marine mammals, such as pinnipeds (Davis et al., 1999; Naito et al., 2010; Viviant et al., 2010; Iwata et al., 2012) and cetaceans (Rechsteiner et al., 2013; Akiyama et al., 2015; López et al., 2015). Bottlenose dolphins, due to their coastal nature and widespread use in aquariums, are one of the best-known cetaceans (Shane et al., 1986). Despite the work done with this species, few are the studies using accelerometry data to examine their behavior. A validation of the manually vs. automatically identified behavior is not always possible in wild environments and, for that reason, it is important to quantify the most common behaviors of captive animals of a species, and to use that information when carrying out studies in the wild.

The available literature suggests a high potential for the use of accelerometry equipment to provide accurate and reliable information about postures, transitions and behavior patterns. Detailed behavioral analysis is difficult and very time consuming and the use of such equipment may enable more precision.

The present study was able to successfully identify bottlenose dolphin's behaviors using 3-axis accelerometer.

5.1. Classification of the behavior.

Various behaviors were correctly classified by the algorithm, a total of 86 for both dolphins and contexts. The total behaviors for each animal and contexts were grouped into 8 categories, Lateral Ascent, Lateral Swim, Lateral Dive, Inverted Dive, Glance Up, Dorsal Swim, Dorsal Dive and Head Up. Each category is represented by a variety of points that characterize different postures of the same behavior.

As far as we know, this is the first study building a behavioral map with the combination of the possible postures for a more complete behavior analysis. Work done in more controlled conditions allows the recording of the animals and enables the comparison between the acceleration profile and the movement recorded by the video cameras.

5.2. Identification of the behavior clusters.

The goal of the unsupervised classification is to be able to automatically categorize the behavior performed by a dolphin without resorting to video cameras, which in wild are, sometimes, impossible to use. Also, it is important since with correct calibration it decreases subjectivity in the behavior classification process because there is no manual labeling involved.

Postures with lateral, inverted or vertical movements were easier to be recognized by the algorithm, compared with dorsal and static positions. Static positions were particularly difficult to identify, not because of the algorithm accuracy, but because these dolphins are rarely motionless. Buoyancy and breathing imply that dolphins are almost always moving.

In our study, we identify four specific parameters with concomitant threshold segments that were able to separate 15 different behaviors/movements, giving information about the animal posture. As far as we know, this is the first study identifying these behaviors without recurring to manual labeling or video visual inspection. The latter was used only as a validation of the method.

5.3. Unsupervised behavior analysis across sessions and across animals.

Both animals presented similar behavioral acceleration signatures and, for that reason, the analyses were combined. It was important to establish that, even though both animals are different in terms of acceleration, the behaviors seem to have the same pattern, at least at this data resolution, for both contexts, “alone” and accompanied with another animal in the pool.

The algorithm separated the data in specific blocks and we could even identify differences between left lateral and right lateral. These results show that the accelerometer is differentiating successfully the posture of the animal, even though the behavior analysis is slightly different from the conventional ethogram done by observation.

Despite being able to define a group of points representing a Lateral Left Swim behavior in our data, for example, this means that each one of the points in Figure 3.9 represents slightly different lateral left postures that will represent the Lateral Left Swim. The same applies to the Dorsal Swim behavior, due to the variation in body posture, it is represented in a very large spectrum of the graph and has very distant points from each other, depending if the body is pending more to the right or left.

Head up, Inverted Swim and Lateral Ascent Swim, due to the specificity of the movement, have their location much closer to each other and its distribution is very restricted in the graph.

The Stop movement is dispersed through the behavioral map, since each posture has its own stopped version. Despite the dolphin's ethogram, a stop behavior means no movement, to the accelerometer readings represents not only the absence (or low components of acceleration) of motion, but it also measures the different postures that the animal is assuming. For that reason, in Figure 3.9 the stop movement is represented in the edge of the graph.

Due to the training time and equipment tolerance constraints, it was not possible to collect data from a third animal. It would be important to increase the power of this analysis by adding other animals. In the future, this algorithm should be tested in data collected in the wild to test if captive and wild dolphins can, or not, be compared.

5.4. Behavioral sequences.

The behavioral sequences for both animals were analyzed using mono, bi and tri-grams. The most common behavior observed for both dolphins and contexts was Dorsal Swim.

As for the behavioral sequence, both AM4 and HM5 alone performed more transitions from Dorsal Swim to Dorsal Swim and from Dorsal Dive to Dorsal Swim. The analysis of the trigrams showed that Dorsal Swim - Dorsal Dive - Dorsal Swim was the most common combination for both animals followed by Dorsal Swim - Dorsal Swim - Dorsal Swim for AM4 and Dorsal Dive - Dorsal Swim - Dorsal Swim for HM5.

When accompanied, both AM4 and HM5 alone performed more transitions from Dorsal Dive to Dorsal Swim and from Dorsal Swim to Dorsal Swim. The analysis of the trigrams showed that Dorsal Swim - Dorsal Dive - Dorsal Swim was, again, the most common combination for both animals followed by Dorsal Dive - Dorsal Swim – Dorsal Dive for AM4 and Dorsal Dive - Dorsal Swim – Dorsal Swim for HM5.

The similarity of the behavior sequences between both animals and both contexts emphasizes that these animals did not change their behavior when they were alone or accompanied. These results are not unexpected since the animals are used to spend time with other dolphins and an additional known dolphin in the pool does not constitute a surprise for the focal dolphin.

Since behavior is an expression and a result of how the animal interacts with its environment, knowledge about behavior sequences can provide information about animal needs, preferences and internal states (Mench & Mason, 1997). Accelerometry methods provide a fast and non-subjective approach to study how behavior is organized and how this organization may differ from subject to subject and, ultimately, from species to species.

Quantifying the behavior in wild animals that are hard to track and observe in a 24-hour basis, as cetacean species, has been challenging. Behaviors that are hidden from the observer most of the times may contain important information that will be important for an effective conservation strategy. Our approach has the potential to show unknown aspects of dolphin's underwater life, and may shed light on the unseen behaviors of these animals in the wild.

5.5. Entropy.

Entropy analysis is widely used to study sequence complexity in different types of data. In cetaceans is normally used to investigate the acoustical communication complexity (see McCowen et al., 1999; Suzuki et al., 2006; Doyle et al., 2008) but it also can be applied to measure the complexity of the dolphin's behavior repertoire. Our data indicates that the dolphin's behavior contains a high order structure that will allow the prediction, by a statistical model, of which behavior will follow a specific action. This type of analysis could be particularly interesting in data from the wild, since it could enable the prediction of the behavior where it is impossible to use a camera or direct observations.

The fact that there is a pattern in the entropy orders for both animals and in both contexts of signal, $H_0>H_1>H_2>H_3$, suggests that there is an increase of behavior complexity through the behavioral sequences.

HM5 H_0 entropy values, alone and accompanied, are the same, which seems to indicate no behavioral modification at this level. H_1 and H_2 values have a ratio of 0.99 and 0.93, respectively, implying that the information contained in the behavior is not changed when the animal is alone or accompanied. H_3 values decrease from context "alone" to "accompanied" by a ratio of 2, which may indicate an adjustment in the behavior at this level in the presence of other animal in the pool.

AM4 H_0 and H_1 entropy values have a ratio of 0.99 and 0.98, respectively, suggesting no modification in the behavior information between both contexts. H_2 entropy values are the same for both contexts, which seems to indicate no behavioral modification at this level. H_3 values have a ratio of 0.99 and 0.93, respectively, implying that the information contained in the behavior is not changed when the animal is alone or accompanied. H_3 values, similarly to HM4's entropy, decrease from context "alone" to "accompanied" but by a ratio of 3, which may indicate some adjustment in the behavior at this level, in the presence of another animal in the pool.

6. Conclusion

An unsupervised machine-learning method was used to automatically identify the most common behaviors of bottlenose dolphins in a captive environment, using accelerometer data. The results are potentially very promising, since the algorithm was able to identify 7 of the 11 behaviors previously identified manually. There are still some technical problems that must be corrected; an example is that the accelerometer should have a gyroscope and a stable frequency sample. Nevertheless, the use of this algorithm may answer questions about the behavior of dolphins in the wild, where a video camera or an observer are not an option. The next step is to try it with data from other dolphins and see if the identification of the clusters is animal-based or if it is independent. The main goal is to use this algorithm in wild dolphins in an attempt to start analyzing in detail their most common behaviors in their natural environment.

The unsupervised classification algorithm will make it possible to extract rare behavior patterns or those that researchers do not normally observe directly. Even thought the algorithm is specific for bottlenose dolphins, it has the potential to be applied to other cetacean species.

CHAPTER 4 - D-TRACK – A SEMI-AUTOMATIC 3D VIDEO TRACKING TECHNIQUE TO ANALYZE MOVEMENTS AND ROUTINES OF AQUATIC ANIMALS WITH APPLICATION TO CAPTIVE DOLPHINS

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1. Abstract

Scoring and tracking animal movements manually is time consuming, subjective, and susceptible to errors due to fatigue. Automated and semi-automated video-based tracking methods have been developed to overcome the errors and biases of manual analyses. We present D-Track, an open-source semi-automatic tracking system able to quantify the 3D trajectories of dolphins, non-invasively, in the water. This software produces a three-dimensional reconstruction of the pool and tracks the animal in different depths, using standard cameras. D-Track allows the determination of spatial preferences of the animals, their speed and the identification of behavioral routines. We tested the system with two captive dolphins during different periods of the day. Both animals spent around 85% of the time at the surface of the Deep Area of their pool (5 meters depth). Both dolphins showed a stable average speed throughout 31 sessions, with slow speeds predominant (maximum 1.7 ms^{-1}). Circular swimming was highly variable, with significant differences in the size and duration of the "circles", between animals and within-animals across sessions. The D-Track system is a novel tool to study the behavior of aquatic

animals, and it offers possibilities for laboratories and marine parks to monitor the preferences and routines of their animals.

2. Introduction

Ethologists and other behavioral scientists have traditionally recorded and scored animal movements in experimental settings manually. This technique has the advantage of low investment (Noldus et al., 2001; Noldus et al., 2002; Lind et al., 2005) but it has the drawback of being a very time consuming process and susceptible to errors due to fatigue, drift and subjectivity (Lind et al., 2005; Martin & Bateson, 2007). To overcome these problems, automated video-based tracking methods have been developed over the years in controlled environments to explore and extract the detailed information that is contained in videos of moving animals (e.g., Kabra et al., 2013). Due to the high temporal and spatial resolution of these techniques, it is now possible to perform the tracking of different animals and transform the video data into trajectories of positions over time (Kabra et al., 2013; Pérez-Escudero et al., 2014).

These techniques are now widely used with commercial video analysis systems in a variety of experiments (Hashimoto et al., 1999; Spink et al., 2001; Khan et al., 2005; Dell et al., 2014), with different animal models such as *Caenorhabditis elegans* (Geng et al, 2004; Simonetta & Golombek 2007; Machino et al., 2014), fish (Kato et al., 2004; Delcourt et al., 2009; Mirat et al., 2013; Pérez-Escudero et al., 2014), insects (Martin 2004; Branson et al., 2009; Pérez-Escudero et al., 2014), rodents (de Chaumont et al., 2012; Giancardo et al., 2013; Matsumoto et al., 2013; Ohayon et al., 2013; Pérez-Escudero et al., 2014; Wiltschko et al., 2015), primates (Ballesta et al., 2014) and pigs (Lind et al., 2005; Ahrendt et al., 2011). However, the studies mentioned above have been mostly limited to small cages and restricted environments, as noted by Ballesta et al., 2014. The approaches used in previous studies seemed less suited to track the movements of animals such as bottlenose dolphins in their sizable pools.

To overcome the obstacles to animal tracking in a large pool with varying depths, we developed D-Track, a software that tracks aquatic animals in their habitat, without disturbance only from top or lateral views (as it usually happens in existing systems) because it combines the information of the measurements of the pool with the images of both cameras to reconstruct a 3D environment. It is also prepared to perform the analysis with different light and weather conditions, as well as with the animals at different depths. The tracking is semi-automatic and does not suffer from propagation of errors, requiring only some adjustments according to the sunlight and wind levels at the site. D-track was designed to be adaptable to other captive settings and it needs no special modifications for different species. It represents an improvement that may allow marine parks and laboratories to easily be aware of behavioral or movement anomalies with their animals without spending too much effort or time.

3. Methods

3.1. Subjects and facility.

The subjects of this study were two captive common bottlenose dolphins (*Tursiops truncatus*) held at the marine theme park Zoomarine, located in the Algarve region (south of Portugal). Both animals were adult males: HM5 (born in 1995 and with 185 kg, 2.46 m) and AM4 (born in 2004 and with 172 kg, 2.55 m). At the time of this study, these dolphins were housed in a covered pool (Lagoa Azul). Artificial illumination supplemented natural light when necessary.

The complete pool, 47 m long, is divided into 4 smaller sections that are separated by perforated fiberglass doors and panels (Figure 4.1).

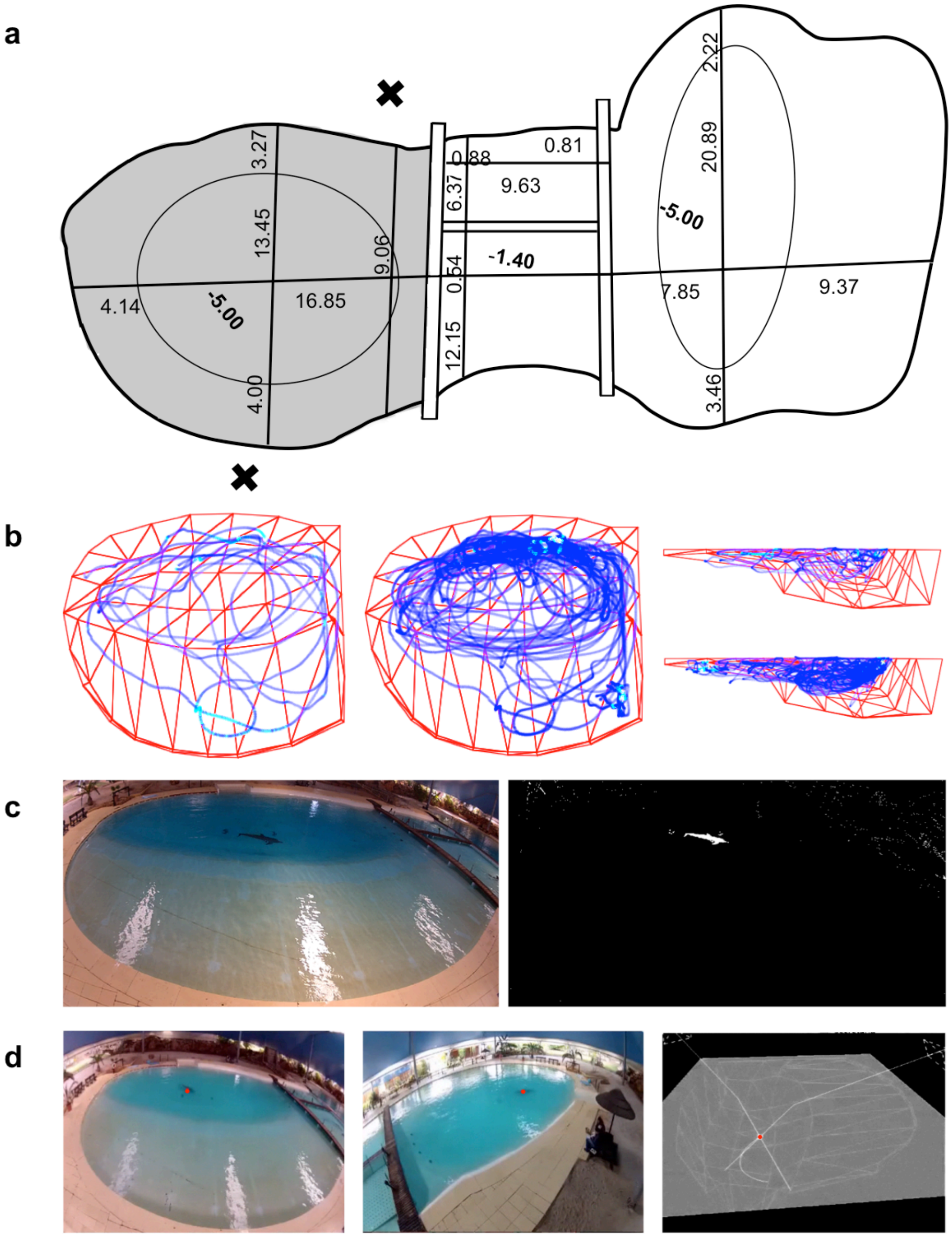

Figure 4.1. Entire pool with dimensions and sample trajectories. (a) Diagram of the experimental pool. Black X marks show the position of the two video cameras, with 170º lenses at approximately 4-m high. (b) Example of the trajectory of the focal dolphin in the pool, after 5 and 30 minutes of a session, from above and laterally. (c) An example of a video frame recorded with the focal animal (left), and with the background subtracted for analysis (right). The threshold had to be defined manually depending on the weather conditions. (d) Images showing the dolphin,

with the red dot from the tracking recognition, and with the three-dimensional reconstruction of the pool. The intersection of the two vectors from the cameras to the dolphin determine the 3D position of the animal in the pool.

The animals were video recorded in a pool section (the experimental pool), approximately circular, with 21 m diameter and a maximum depth of 5 m. The subjects routinely spend time in all the pool section areas. The animals in the experimental pool were separated from the remaining animals by fiberglass doors, with visual and acoustic contact among them.

Two cameras (GoPro Hero 2, ©GoPro, Inc.), with 170º lenses, 1280x720 pixel resolution and 30 frames per second video, were mounted in approximately opposite locations around the pool.

This study was reviewed and approved by Zoomarine's Ethical and Animal Welfare Committee and followed careful procedures to minimize disturbance to the animals.

3.2. Data collection.

Behavioral data collection was conducted between November 2012 and April 2014. Video recordings of single animals (either HM5 or AM4) were made in 31 sessions, from 20 to 60 minutes duration, between 9 AM and 6 PM, always in the presence of trainers, totaling 1080 minutes. The set up of the equipment was prepared 30 to 10 minutes before the data collection, to minimize the animal's reactivity.

The movements of trainers through the different areas of the pool, as well as feeding and training sessions with other animals, were carried out normally.

3.3. Calibration of the cameras.

The cameras covered the entire experimental pool with their wide-angle lenses, adding a fish-eye effect that distorted the image, as well as the distances and relative sizes. Therefore, it was necessary to calibrate the cameras to be able to match the pixels coordinates to the correct position of the animal, as well as the size. To perform the calibration, images of a chessboard were used (see Carstensen, 2001). The following camera parameters were used:

Distortion matrix = [-0.335050702095, 0.120226070285, 0.0, 0.0, -0.0201695654541]

The following formula is a camera matrix, or a matrix of intrinsic parameters:

A = [[654.55, 0, 1279.0/2]

[0, 654.55, 719.0/2]

[0, 0, 1]]

3.4. Overview of the system.

To obtain the 3D data, two synchronized video cameras, recording in 32 GB SD card and .mp4 format, were fixed in approximately opposite locations to enable the recording of the entire habitat from two different perspectives. The exact information about the position and orientation of the cameras was necessary for the calculation of the animal's 3D position and movement data. Therefore, detailed measurements of the pool were included in the D-Track software, where five points visible from both cameras were chosen as input calibration points to be used in the RANSAC Perspective-*n*-Point algorithm (Fischler & Bolles, 1981).

The images of both cameras were then merged to reconstruct the 3D scenario.

Details of 3D video analysis system are explained in the following sections in three steps: 3D data acquisition, dolphin tracking, and quantification of the dolphin's position in the pool.

3.5. 3D data acquisition.

For the present study, a Python 2.7.3 script (Python Software Foundation) with OpenCV 3.0 library was used to apply the color filters to the video frames and to solve the RANSAC Perspective-*n*-Point (Figure 4.1c).

For the animal tracking, the frames of each camera were used to find the 2D centroid of the moving subject. The centroid, in pixels, corresponds to a vector originated from the focal point of the camera to the subject in the pool. The intersection of the two vectors, one of each camera, is used to calculate the 3D position of the animal in the pool.

To find the pixel vectors for both cameras, the following equations, from the pinhole camera model, were used:

$$x' = x/z,\ y' = y/z,\ u = f_x * x' + cx,\ v = f_y * y' + cy,$$

$$\Leftrightarrow$$

$$x = (u-cx)*z/ f_x,\ y = (v-cy)*z/ f_y,$$

where u and v are the coordinates of the projection points in pixels; cx and cy are the principal point that is usually at the image center; and f_x and f_y are the focal lengths expressed in pixel units.

To obtain the vector we calculated the points for $z = 0$ and $z = 1$.

3.6. Dolphin tracking.

The tracking algorithm applies a mask to the images to ignore the regions of the pool that are not important. Several imaging filters, such as an adaptive threshold, color ranges filtering, erosion and dilation, were used to extract the subject's blob and to remove the remaining noise. The parameters for these filters had to be defined manually depending on the weather conditions.

After applying the filters, the pixel coordinates corresponding to the centroid of the dolphin in each frame image is extracted. Subsequently, a Gaussian filter (sigma=32) is applied to all the resulting coordinates to smooth the data and eliminate some centroid jittering, and correct momentary tracking errors. A refraction index of 1.4 was estimated based on the depth and specific measurements of known distances, and applied. Then, the intersection of the vectors that correspond to the subject centroids is calculated, giving the exact 3D point of the position of the dolphin (Figure 4.1d).

To avoid fluctuations that would be artifacts in the data, an outlier analysis was made calculating the median, lower quartile, upper quartile, interquartile range, inner and outer fences of the data. The major outliers, obtained from the calculations with the outer fences, were discarded.

3.7. Quantification of the position of the dolphin in the pool.

The D-Track permits different types of analyses depending on the parameters defined, producing information about the trajectories, as well as the pool section occupancy/preferences, speed and routines of the animals in the pool.

Trajectories - To study the dolphin's 3D trajectories and routines in the pool throughout sessions, the animal's position in each frame is extracted. Plotting the positions of the animals through time makes it possible to observe the entire trajectory and routines of the animals.

Pool section occupancy/preferences - To analyze the occupancy/preferences of the animals in the pool, it was necessary to isolate the portion of the arena to quantify. This isolation will determine the frames to be analyzed. The frames associated with the maximum depth area were labeled as Deep Area at the Surface of the pool. The difference between the total frames and those corresponding to the Deep Area were designated the Shallow Area of the pool. To study the preferences of the both animals in relation to the deepest part of the pool (close to the 5 meters depth), the area below the maximum depth of the Shallow Area (approximately 1.4 meters depth) was selected and named Bottom.

Speed – The speed of the animals was calculated through the difference between 3D positions of the dolphin in two consecutive frames ($\text{position}_{\text{frame\_index}} - \text{position}_{\text{frame\_index-1}}$), which will give the distance moved between each two frames (33.33 milliseconds). The maximum speed of the animals was the highest speed value registered. This value was then divided in three (tertiles) to create a scale from low to high speed. This velocity, in meters per frame, was then transformed in meters per second and divided into three categories based on the tertiles: low (0 to 1.7 ms^{-1}), intermediate (1.7 to 3.4 ms^{-1}) and high speed (3.4 to 5.1 ms^{-1}).

Dolphin's Routines – To study the routines of the animals, the total position data from all sessions was plotted for both animals, to study variation in the positions throughout the sessions. Important occurrences were analyzed in this section: swimming ring sizes (complete circles around the pool); trainers' presence influence; and interaction of the focal animal with animals in adjacent pools. To study the variation in the swimming ring sizes, the position of the beginning and the end of each ring was extracted, and the distance between both points was measured, as well as the speed from one point to another. To study the influence of the trainers' presence in the routines of the focal animals, as well as the interaction of the subject with other dolphins in the adjacent pool, the corresponding section of the scenario was isolated, as for the occupancy/preferences part of the study.

3.8. Statistical analysis.

The Kruskal-Wallis test was used to examine differences in the size and duration of the swimming rings across HM5 and AM4 and between sessions of each subject.

All statistical analyses were performed using *IBM SPSS Statistics 21* (IBM Inc.).

4. Results

4.1. System overview.

The behavioral arena used to develop and test the D-Track system consists of a 21 m diameter pool with variable depth, increasing from 1.4 m to a maximum of 5 m (Figure 4.1a). Two 170º lenses GoPro Cameras (GoPro Hero 2, ©GoPro, Inc.), with 1280x720 pixel resolution and 30 frames per second video, were mounted in opposite sides of the pool to enable the recording of the entire habitat from two different perspectives. The D-Track software component consisted of a tracker for dolphin trajectories from captured digital video (Figure 4.1b). Both cameras had wide-angle lenses that distort the image as well as relative distances, and had to be calibrated (following Carstensen, 2001). Image-subtraction techniques were used to detect the moving subject through the contrast that separates the animal from the background (Figure 4.1c).

Detailed measurements of the habitat were introduced to the D-Track software, with five visible points outside of the pool, from both cameras, as input calibration points to the RANSAC Perspective-*n*-Point algorithm (Fischler & Bolles, 1981). Images of both cameras were then merged to reconstruct 3D scenarios, and the thresholds for the dolphin tracking were defined manually since they are highly influenced by the light and weather conditions. After applying the filters, the pixel coordinates corresponding to the centroid of the dolphin in each frame image were then extracted.

To test the potential for semi-automated tracking analysis, we quantified the occupancy/preferences of two adult animals in the pool (labeled HM5 and AM4), their instantaneous speeds and circular swimming paths. We then compared the two dolphin tracks. The analysis resulted from 31 sessions (from November 2012 and April 2014 during the winter seasons) that ranged from 20 to 60 minutes, totalizing 18 hours of video recordings.

The D-track software, written in Python (version 2.7, Python Software Foundation) detects the moving objects, frame by frame, and so the focal dolphins in the pool are semi-automatically tracked.

For the animal tracking, each camera image pinpoints the moving subject at its 2D centroid, and the position is determined as a vector from the cameras to the dolphin. The intersection of the vectors of both cameras was used, together with the arena spatial data, to calculate the 3D position of the animal in the pool (Figure 4.1d).

4.2. Quantification of the positions of the dolphin in the pool.

The D-Track system permits different types of analyses according to the parameters defined, providing information about the actual trajectories, as well as the occupancy/preferences for the pool sections, and the speeds and routines of the animals. D-track is mostly automatic, although some features need to be assigned manually, namely the image thresholds, and some artifact corrections to the tracking may be necessary.

3D trajectories in the pool throughout the sessions - To study the dolphin's 3D trajectories and routines in the pool throughout the sessions, the animal's position in each frame is extracted. By plotting the positions of the animals, it is possible to observe their entire trajectories and routines. Figure 4.2 shows the plots of the tracking sessions for the two focal dolphins.

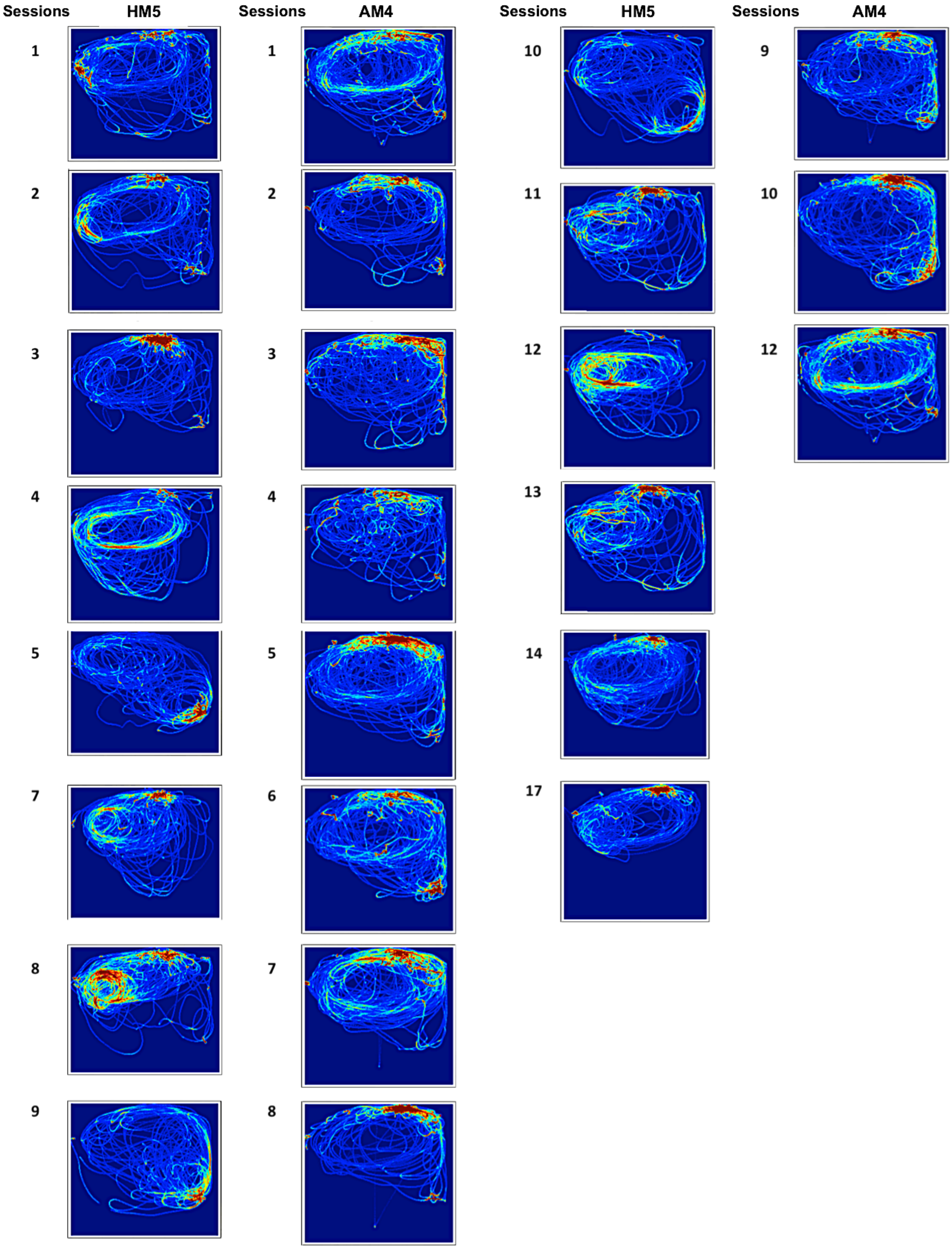

Figure 4.2. Trajectories of both dolphins throughout the tracking sessions. Red indicates higher occupancy.

Occupancy / preferences of the animals in the pool - To analyze the occupancy patterns of the animals, it is necessary to define the subsection of the pool to be analyzed (Figure 4.3a). The frames associated with the area of maximum depth area were labeled Deep Area of the pool. The remaining area was named Shallow Area of the pool. The deep area below 1.4 meters depth (corresponding to the maximum depth of the Shallow Area) was named the Bottom.

HM5 and AM4 seem to present the same occupancy/preferences in the pool. The animals spent more time in the Deep Area (Figure 4.3a, approximately 85% of the total time (406.85 mins (SD = 9.99 mins) and 444.39 mins (SD = 10.15 mins) for HM5 and AM4, respectively). In relation to the Bottom of the pool, both dolphins spent only around 7% of the non-consecutive 18 hours, about 36.84 minutes (SD = 4.34 mins) and 39.61 minutes (SD = 4.89 mins) for HM5 and AM4 respectively (Figure 4.3b) (mainly in the 12:00 and 15:00 sessions for HM5 and 09:00, 12:00 and 17:00 sessions for AM4 (Figure 4.3c).

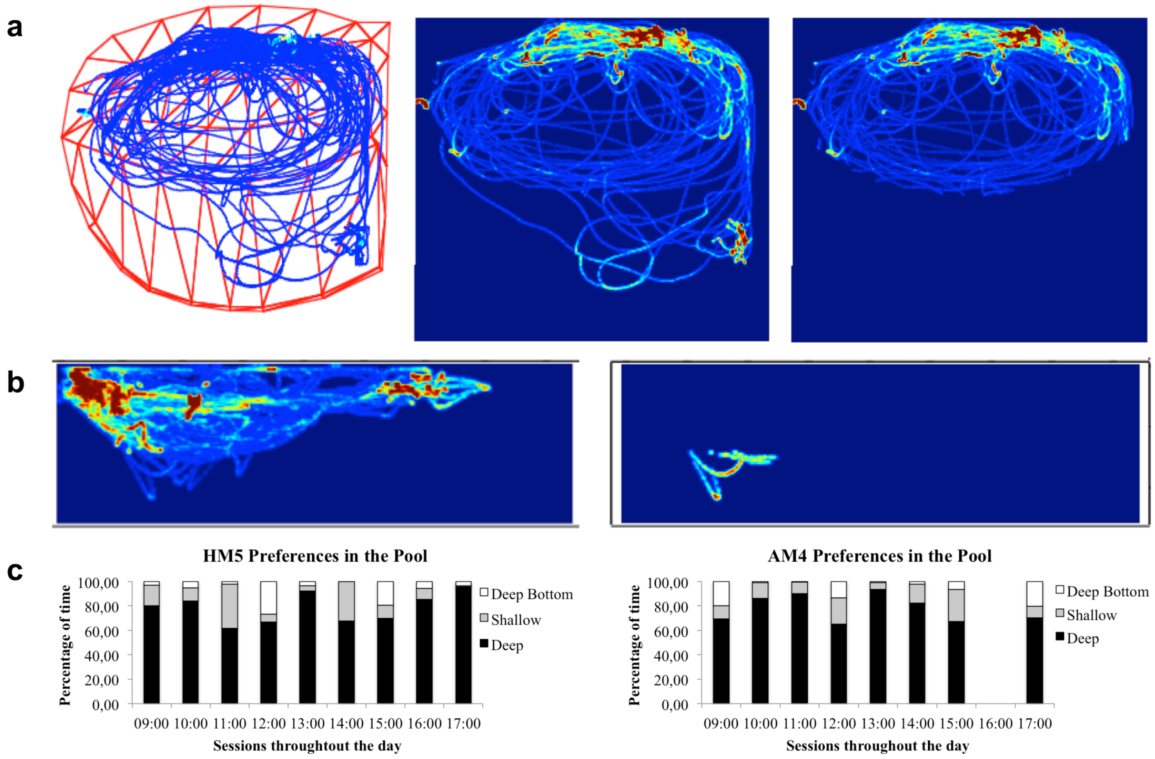

Figure 4.3. D-Track outputs for dolphin trajectories in the 3D-reconstructed pool. (a) Trajectory of AM4 in one of the sessions with red points representing more time spent in a particular section of the pool and light blue lines representing less occupancy. At the far right, an isolation of

AM4's movements just in the Deep Area of the pool. (b) On the left, the complete path travelled by the animal in one of the sessions, represented laterally, showing a wide 3D occupation of the pool, and on the right a selection of AM4's path just when the animal was in the Bottom of the pool. (c) Percentage of the time spent by HM5 and AM4 in the Deep, Shallow and Bottom of the pool throughout the day.

4.3. Dolphin speeds in the pool.

The speed of the animals was calculated using the centroid positions of the dolphins between consecutive frames, which gave distances moved per time unit. The highest speed calculation in the tracking sessions was used to create speed tertiles: low (0 to 1.7 ms^{-1}), intermediate (1.7 to 3.4 ms^{-1}) and high speed (3.4 to 5.1 ms^{-1}).

The animals show very consistent average speeds throughout the day (Figure 4.4a). Both animals spent the same amount of time in the three categories of speed (Figure 4.4b), the low speed tertile being the most common. A total of 202.93 minutes (SD = 6.65 mins) correspond to 58.78% of the total time for HM5, and 244.31 minutes (SD = 3.01 mins) correspond to 57.73% for AM4. The high speed tertile was the less frequent, with HM5 exhibiting 9.53 minutes (SD = 44.34 secs), corresponding to 2.76% of the total time and AM4 presenting 13.89 minutes (SD = 1.01 mins), corresponding to 3.28%.

Using the trajectory information, we were able to study where the animal shows lower, intermediate and higher speeds (Figure 4.4c for HM5 and Figure 4.4d for AM4).

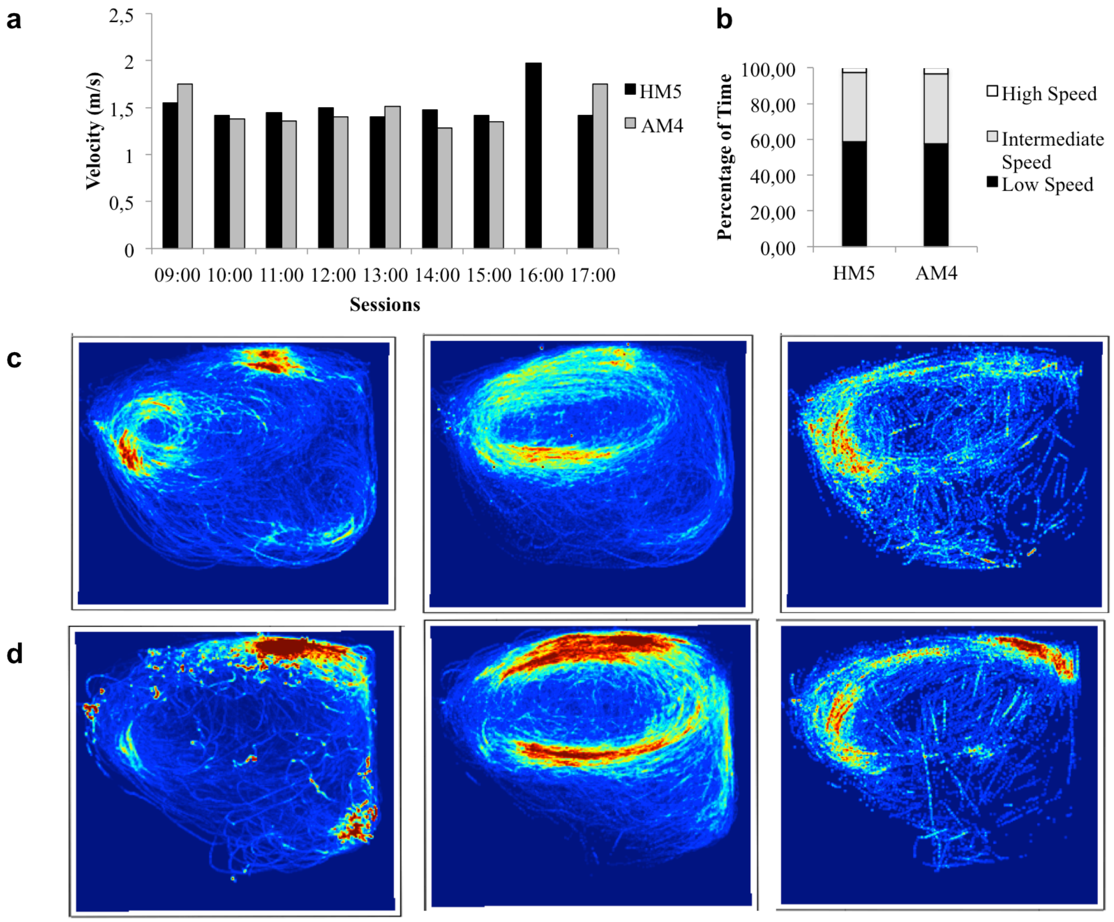

Figure 4.4. Dolphin speeds for all sessions. (a) Mean speed for HM5 and AM4 for all sessions throughout the day. (b) Percentage of time each dolphin spent in the three speed tertiles throughout all sessions. (c) Speed analysis of the entire data collection for HM5 in the speed tertiles: Low, Intermediated and High speed. The colors represent the occupancy of the animal in the pool, with red points showing more time spent in that particular location at each speed tertile. (d) The same as c) for AM4.

4.4. Routines of the animals in the pool.

To study the movement routines of the dolphins, the total position data from all sessions was plotted for both animals. Significant aspects were analyzed: “swimming circle” sizes (complete circles around the pool); the possible influence of the trainers’ presence at the pool’s edge; and possible interaction of the focal animal with dolphins in adjacent partition of the pool.

Even though both animals present a preference for the Deep Area of the pool, in general the animals used the entire partition throughout the sessions, as shown in Figure 4.5.

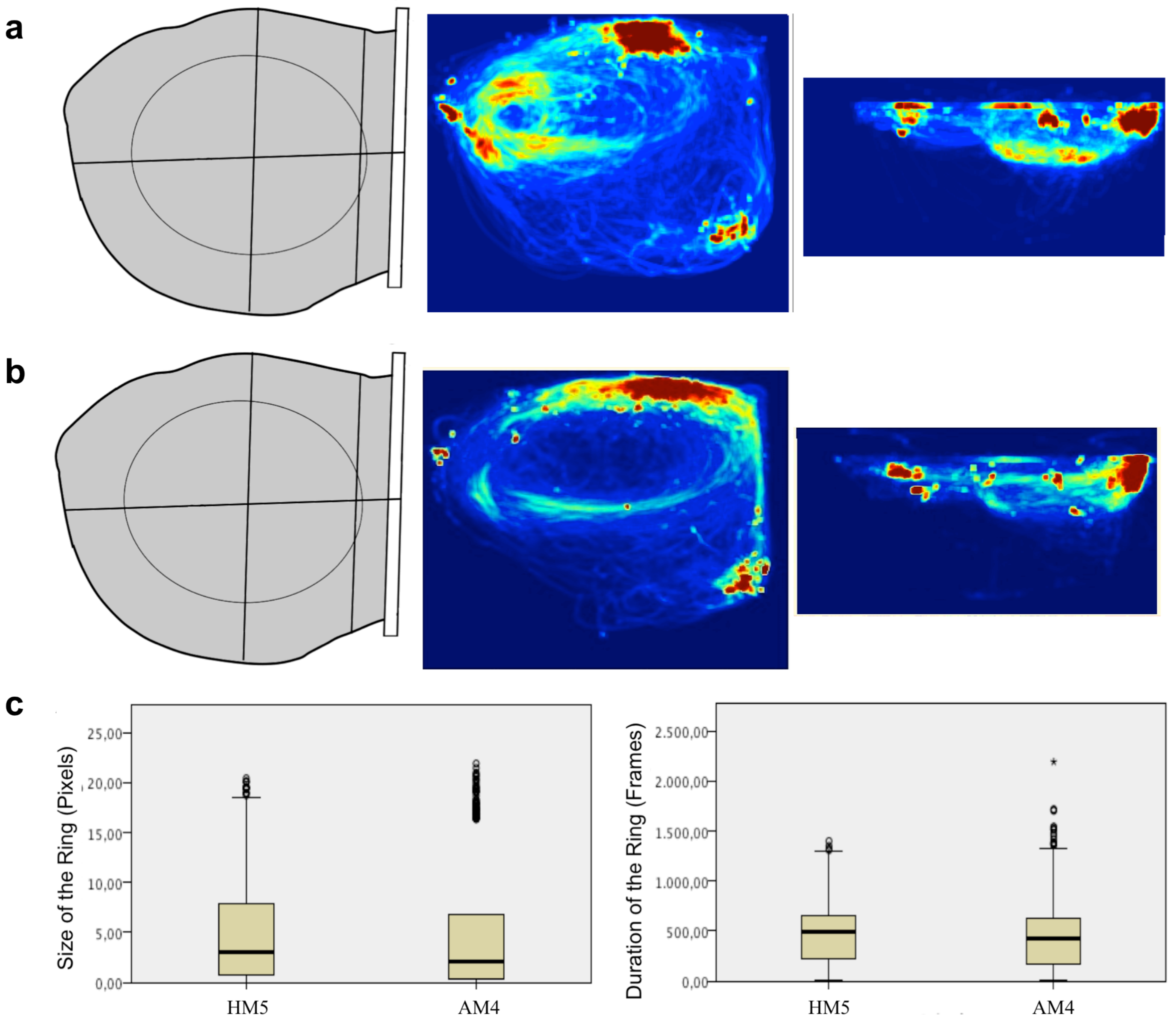

Figure 4.5. Movement routines of the Dolphins in the pool throughout the sessions. a) Routines of HM5 and b) Routines of AM4 in all sessions, combining the information from the top and lateral views. c) Size and Duration of the swimming rings with significant differences between dolphins (both $p < 0.001$).

To study the variation in the swimming ring sizes, the position of the beginning and the end of each ring was extracted from the trajectory, and the distance between both points was measured, as well as the frame-by-frame instantaneous speeds from one point to the other.

The analysis of ring duration resulted in a mean number of 469 frames per ring (SD = 303.56 frames), corresponding to 15.63 seconds for HM5, and a mean number of 433 frames per ring (SD = 278.25 frames), corresponding to 14.43 seconds for AM4. This apparent homogeneity hides relevant variation. In fact, the Kruskal-Wallis test revealed significant differences in the size and duration of the rings between animals, (H (1) = 38.570, $p < 0.001$ (Figure 4.5c), suggesting individual patterns of circling. It also revealed changes in the size and duration of the rings during individual sessions of HM5 (H (13) = 48.757, $p < 0.001$ and H (13) = 224.675, $p < 0.001$, respectively) and AM4 (H (10) = 129.701, $p < 0.001$ and H (13) = 84.736, $p < 0.001$, respectively), and between sessions H (1) = 26.162, $p < 0.001$), supporting the notion of variability in the 3D movement trajectories of the dolphins.

To study the possible influence of the trainers' proximity in the movement routines of the subjects, the section of the 3D scenario nearer the trainers' position was extracted and analyzed separately. The data show that the animals do not spend much time near the trainers' position, with HM5 spending just 6% of the total time in that area (31.29 mins with SD = 2.29 mins) and AM4 spending around 9% of the time (52.88 mins with SD = 4.42 mins).

As to the possible influences on the subject's movement patterns of other animals in the adjacent partitions of the pool, the trajectory data show that both animals spent little time near the gates that connect the partitions, where the other dolphins could be seen by the subjects. The percentages of total time were 5% and 6% for HM5 and AM4, respectively.

5. Discussion

Bottlenose dolphins in captivity are held in habitats that are obviously much smaller and less complex than in natural environment, which inevitably leads to behavioral alterations (as assessed by, e.g. Defran & Pryor, 1980). New methods are being developed to improve the quality of life of marine mammals living under human care. It is important to understand all factors that can interfere with the health of animals in captivity, and also to devise ways to monitor and improve their welfare, reducing boredom, stress and stereotyped behaviors. For that, any automatized or semi-automatized system to record and analyze movements over extended periods is highly valuable.

We have optimized a system, labeled the D-Track, which allows the semi-automatic video tracking of aquatic animals in a pool, and also performs multiple analyses flexibly. The specific novelty in this development is the ability of tri-dimensional tracking of a large aquatic animal in a wide arena, without the need of a camera above the pool or at a particular angle. The D-Track is adaptable to different artificial settings, and it is easy to implement, requiring only some specific measurements for the 3D reconstruction of the arena. D-Track simplifies movement analyses that had to be done manually (e.g. Shyan et al., 2002) in a way prone to error. This video tracking system is mostly automatic, although some features need to be assigned manually, namely some thresholds and some tracking corrections. Since captive settings for this type of animals are, usually, outdoors, the tracking system must be able to locate the animal with varying light conditions and wind effects, sometimes requiring these manual corrections. However, it may add small quick jumps in the data (*jittering*), so an outlier's analysis was carried out. The results confirmed that it is possible to analyze with high detail movement data even with variable light conditions, such as during sunrise and sunset.

Knowledge of enclosure preferences by the animals, easy to detect with D-Track, allows a better assessment and improvement in their quality of life. It is also useful in the planning of future modifications in the pools or space management.

Results show that both animals spent about 85% of the time in the Deep Area of the pool, but mostly at the surface, and only 7% in the Bottom at near the 5 meters maximum depth. This is not surprising since these animals have more stimuli at the surface, e.g. the trainers, food and toys. In the Bottom, on the contrary, the animals find only a canvas surface. While in natural environments dolphins may feel motivated to explore as deeply as possible, in captivity the animals may not prefer to spend much time near the bottom, which could be relevant when planning new habitats.

Both dolphins showed a very consistent average speed throughout the sessions, with the slow speed category (up to 1.7 ms^{-1}) as the most dominant (58.78% and 57.73%, for HM5 and AM4 respectively). The routine swimming speed of a bottlenose dolphin in the wild is between 1.77 and 3.19 ms^{-1} and the maximum speed registered is 9.7 ms^{-1} (see Goforth, 1990 and the review of Fish and Hui, 1991). Our data shows lower values for the predominant speed (up to 1.7

ms^{-1}) and maximum speed (5.1 ms^{-}1), which reflects the environmental constrains. Since the pool is approximately 21 meters of diameter, the dolphins may not be able to reach higher speeds.

The dolphins showed specific movement routines that involve circular swimming over the Deep Area of the pool and use most of their habitat, performing rings with different durations and sizes. These rings showed differences between individuals and were variable across sessions, indicating that these individual patterns of movement are not highly stereotyped. In any case, having a baseline of the normal movements of captive dolphins facilitates the detection of bizarre behaviors that may emerge. Since stereotyped movements and bizarre behaviors are one of the major welfare concerns of trainers and facilities holding captive animals, this semi-automatic tracking system may be valuable.

Results show that the dolphins spent modest percentages of time near the trainers or watching other dolphins in adjacent partitions, displaying some degree of autonomy.

Analysis of the routines of captive animals is a time consuming process that may lead to subjectivity and error. Focusing on the needs for behavioral analysis of captive dolphins, we successfully developed a methodology capable of a three-dimensional reconstruction of the artificial habitat and an algorithm that semi-automatically tracks the position of the subject. The flexibility in the camera types and their placements represent a relevant improvement to the existing methods. The analysis of trajectories allows the measurement and quantification of the animal's preferences for pool sections, as well as the detection of stereotypies. This novel application to dolphin management is a user-friendly tool that may be used by institutions that wish to measure or monitor the behavior of their animals. D-Track is potentially useful to any investigation of motor variability in large aquatic animals, both in intraspecific contexts and in the analysis of interspecific differences.

CHAPTER 5 - WHISTLE STABILITY AND VARIATION IN CAPTIVE BOTTLENOSE DOLPHINS (*TURSIOPS TRUNCATUS*) RECORDED IN ISOLATION AND SOCIAL CONTEXTS

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1. Abstract

Common bottlenose dolphins (*Tursiops truncatus*) produce a range of underwater vocalizations, both pulsed (echolocation clicks and burst-pulses) and non-pulsed (whistles). Whistles may be emitted in stereotyped (signature) or variant patterns, and their production might be affected by sex, age, environmental and social contexts. This study examined, non-intrusively, the whistle emissions of six captive bottlenose dolphins at Zoomarine (Algarve, Portugal) in two separate time sets and three different contexts: two of the animals in isolation (2008), and all six in 2012, both segregated from their group and in social context. From a total of 1,681 whistles, 1,249 were analyzed from 32 samples in different contexts: seven samples in isolation in 2008; 18 in segregation in 2012; and seven in social context. Through visual inspection of spectrograms, whistles were classified into 12 different contour categories. Only one category was found in both time sets and could be considered a signature whistle by SIGID criteria (Janik et al., 2013). This contour was associated with the same animal in 2008 and 2012. Whistle emission rates were 7.8 times higher in isolation as compared with social context, and significant differences were also found in the end and maximum frequencies, as well as number of inflections and loops. Multiloop whistles were more common in isolation than in social contexts. The variant (non-stereotyped) contours dominated the whistle production in segregated contexts (but not by isolated animals) and in social contexts. This study highlights the importance of examining the non-stereotyped portion of the bottlenose dolphin's whistle repertoire in different contexts, as signature whistle production may not be a constant or universal phenomenon.

Key Words: Common bottlenose dolphins, *Tursiops truncatus*, Whistles, Emission rates, Isolation, Social contexts, Captivity

2. Introduction

Common bottlenose dolphins (*Tursiops truncatus*) produce a wide variety of underwater sounds (Lammers & Oswald, 2015) including pulsed emissions, i.e. echolocation clicks and burst-pulsed sounds, and non-pulsed signals, i.e. whistles. Whistles usually occur between 4 and 15 kHz and may have a social function, such as individual identification (Caldwell et al., 1990; Janik, 2013). This type of vocalization often lasts less than one second but shows a range of frequency modulation contour shapes varying from concave to convex, upsweep to downsweep and with intermediary forms between categories (Bazúa-Durán & Au, 2002; Dudzinski et al., 2009).

Bottlenose dolphin whistles may consist of one unit of sound (uniloop) or several units (multiloop), which may be repeated as a single multicontoured whistle (connected) or with short periods of silence (disconnected) (Caldwell et al., 1990; Sayigh et al., 1990, 1995; Janik & Slater, 1998; Esch et al., 2009a; King et al., 2013). Previous research has demonstrated that the number and duration of loops are affected by the behavioral context. Isolation or capture-release contexts revealed higher number of loops of the same repetitive whistle (stereotyped) compared with undisturbed conditions (Esch et al., 2009b). These results suggest that alterations to whistle production may contain information about the emitter's motivational/ emotional state, and that the increase in number of loops might allow dolphins to convey additional information while also communicating identity (Weary & Fraser 1995; Watts & Stookey 2001; Barton, 2006; Esch et al., 2009b).

The fact that dolphins produce stereotyped whistles that are stable over long periods of time (Bruck, 2013; Luís et al., 2016a; Sayigh et al., 1990, 2007) suggests that these acoustical signals may be involved in individual identification and position, thus the notion of signature whistle (Caldwell & Caldwell, 1965; Tyack 1986; Caldwell et al., 1990; Sayigh et al., 1990; dos Santos et al., 2005). Studies on ontogeny of signature whistles revealed that these whistle types are developed through vocal learning during the first year of a dolphin's life, and may remain stable throughout an individual's lifetime (Caldwell & Caldwell, 1979; Caldwell et al., 1990; Sayigh et al., 1990; Janik & Sayigh, 2013). Furthermore, dolphin calves are known to model their signature whistles on sounds in their acoustic environments (Fripp et al., 2005; Tyack & Sayigh, 1997), including man-made whistles (Miksis et al., 2002). Male dolphin calves are more likely to produce whistles similar to those of their mothers, while females tend to produce more distinct signature whistles (Sayigh et al., 1990, 1995). While females seem to produce stable signature whistles throughout their life (Sayigh et al., 1990, 2007), males are more likely to change the modulation pattern of their signature whistles in the process of establishing alliances with other males (Connor et al., 2000, Smolker & Pepper 1999; Watwood et al., 2004).

Signature whistles are individually distinctive, stereotyped and the most commonly produced whistle type in isolation (Caldwell et al., 1990). While in isolation, signature whistles may represent 80% to 100% of whistles' emissions (Caldwell & Caldwell, 1965; Caldwell et al., 1990; Janik & Slater, 1998; Sayigh et al., 2007; Janik et al., 2013). Considering different contexts, however, signature whistles may not be the most abundant type (Cook et al., 2004; McCowan & Reiss, 1995, 2001; Luís et al., 2016a; Watwood et al., 2005). For wild bottlenose dolphins, signature whistle production may be lower, accounting only for 32% to 52% of all whistles (Cook et al., 2004; Luís et al., 2016a; Watwood et al., 2005). Moreover, in captivity contexts, the emission of signature whistles may not even occur (McCowan & Reiss 1995; 2001), hence additional investigations of whistle production remain relevant.

Recently, Janik et al., (2013) proposed a useful approach to identify signature whistles based on their pattern of emission (the SIGnature IDentification (SIGID) criteria): if 75% or more of whistles of the same whistle type are emitted within one to ten secs of another whistle of the same type, this contour may be considered a signature whistle. In this study, the acoustic behavior of six captive bottlenose dolphins of various ages and both sexes was sampled to examine whistle stability and variation, as well as the variability of emission rates and time-frequency characteristics of the whistles. This study focused on not only recording whistles in different social conditions, allowing a contextual comparison, but on recording the same animals across four years, allowing an examination of the stability of the whistles.

3. Methods

3.1. Facility and subjects.

Recordings were conducted at Zoomarine, Algarve (Portugal) in two different periods, between February and April 2008 and between April and May 2012. The subjects of this study were two sub-adult males (coded AM4 and UM3) in 2008; a male calf (PM9), two sub-adult females (AF6 and IF7) and three adult males in 2012 (HM5, UM3 and AM4) (Table 5.1). AM4 and UM3 were the focal animals of this study recorded in different life stages, in 2008 and 2012.

Table 5.1. Bottlenose dolphins held at Zoomarine, Algarve (Portugal) involved in this study, along with their sex, year of birth, identity of mother (when known), and year of recording.

Codename	Sex	Year of Birth	Mother	Recorded in
HM5	M	1995	-	2012
UM3	M	2003	CF8	2008, 2012
AM4	M	2004	MF8	2008, 2012
AF6	F	2006	CF8	2012
IF7	F	2007	MF8	2012
PM9	M	2009	XF9	2012

AM4 and UM3 were together with other dolphins in Sam’s Stadium in 2008. All subjects, with the exception of calf PM9, were together in the same pool, Blue Lagoon, for five months prior to the 2012 recordings. These dolphins had already been part of a larger group in another pool, Enseada, with other adult male and female animals. PM9 was with his mother and other dolphins in a different pool, Sam’s Stadium, prior to joining this social group in Blue Lagoon only two months before the data collection.

This study was reviewed and approved by Zoomarine’s Ethical and Animal Welfare Committee and followed careful procedures to minimize disturbance to the animals.

3.2. Data collection.

2008 – Recordings were carried out at Sam’s Stadium, in a covered support pool (pool E), which is connected to other pools by channels that can be blocked by solid metal panels (Figure 5.1). During isolation sampling, the focal animal (either AM4 or UM3) was placed alone, with all passage panels closed and the other dolphins kept away in control by trainers in pool A.

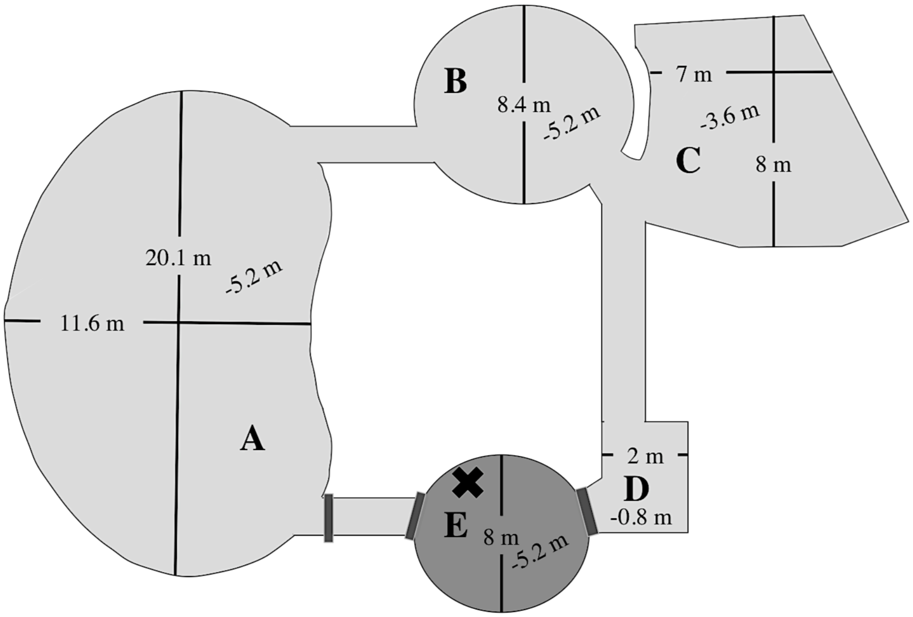

Figure 5.1. Pool dimensions of the Sam's Stadium sections with the experimental pool for the isolation context in dark gray (E) and the closed metal panels in darker grey. The X mark represents the hydrophone positions.

2012 – Recordings were carried out in the covered Blue Lagoon pool, which is divided by perforated fiberglass gates (Figure 5.2). Data collection was carried out in two contexts: 1) segregated (animal alone in one section of the pool, but not acoustically isolated from the others) and 2) social group (one or two more animals in the pool with the focal animal) (Table 5.2).

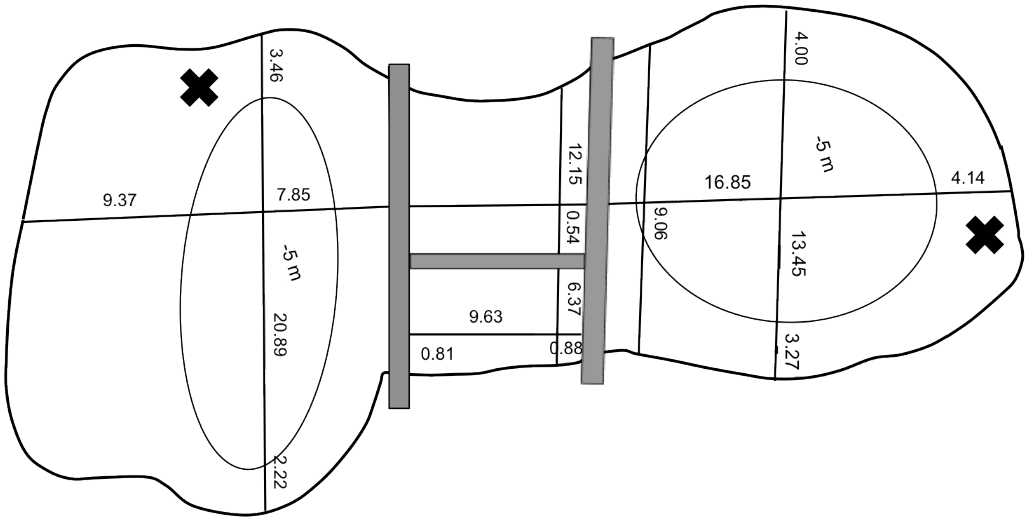

Figure 5.2. Pool dimensions of the Blue Lagoon sections with X marks representing the hydrophone positions and the grey bars representing bridges and partitions connected with perforated fiberglass doors.

Table 5.2. Number of 15-min samples per focal dolphin and context of the acoustic recordings.

	Condition	AM4	UM3	AF6	HM5	IF7	PM9
Feb – April 2008	Isolation	4	3	-	-	-	-
April 2012	Segregated in acoustic contact	3	3	3	3	3	3
April – May 2012	In social group (non-exclusive)	4	2	2	4	4	2

3.3. Acoustic recordings.

In 2008, emissions were recorded using a Cetacean Research Technology SQ26-MT hydrophone, which has a frequency range of 0.020 – 50 kHz; a transducer sensitivity of -194 dB re 1V/µPa; and an effective sensitivity of -169 dB re 1V/µPa. For the 2012 recordings, the hydrophone used was a Cetacean Research Technology C55 which has a linear frequency range of ± 3 dB in the 0.016 – 44 kHz; a usable frequency range of +3/-20 dB in the 0.006-203 kHz band; a transducer sensitivity of -185 dB re 1V/μPa; and an effective sensitivity of -165 dB re 1V/1 μPa. Both hydrophones were connected to a Micro Track II recorder set to record .wav files with a 48 kHz sampling rate and 24-bit resolution in a 2 GB CompactFlash card.

During the data collection, human presence near the pools was kept to a minimum (one trainer and one data recorder).

For the data collected in 2008 at Sam's Stadium pool (Figure 5.1), a minimum habituation period of 10 minutes was established to prepare the set up and to allow the dolphin to adapt to the new context (isolation).

For the data collected in 2012 at Blue Lagoon pool (Figure 5.2), a habituation period of 15 minutes was established to allow the animal to adapt to the session context (segregated from the other animals or in a social group) and to desensitize to the presence of the hydrophone. In 2012 acoustic isolation was not logistically possible, thus an alternative method was used to attribute whistles to emitters. Data collection included notation of intense acoustic emissions recorded when only one dolphin was underwater near the hydrophone, and at least partially oriented towards it, making this animal in those well-defined instances, with reasonable confidence, the "most-likely emitter".

In both years, hydrophones were placed as far away from the other animals as possible (Figure 5.1 and Figure 5.2), at a depth of 1 meter. The duration of the recordings was 15 minutes, and all dolphins were familiar with every section of the pool, since changing sections is part of their routines.

3.4. Acoustic analysis.

In order to identify, count and classify whistle emissions, acoustic recordings were inspected and validated by two independent observers, visually and aurally, using *Raven Pro 1.4* (Cornell Lab of Ornithology, Ithaca, NY) with a Hann window of 512 points (2008 data) and a Hamming window of 512 points (2012 data), both with 50% overlap. Whistles were selected according to Bazúa-Dúran & Au (2002) and Baron et al. (2008), taking into account the following features: minimum of 300 ms duration; sharp and defined contours; no overlapping with other vocalizations; and good signal-to-noise ratio. Since absolute levels were not critical, no attempt was made to obtain calibrated pressure measurements. For the previously selected whistles the following acoustic features were measured using *Raven Pro 1.4*: start, end, minimum, maximum and peak frequencies, duration, number of inflection points and number of loops.

Using visual categorization (Janik & Slater, 1998), stereotyped whistle contours were cross-compared for frequency modulation similarity and assigned to mutually exclusive groups, regardless of the emitter. Each whistle category had to include at least five similar contours in the total recordings. Whistle contours with low representation were labeled “variant”.

Two experienced observers performed the initial contour classification task. Subsequently, a subset of 50 randomly selected whistles was used to test for interobserver reliability, using Kendall’s W coefficient of concordance (Siegel & Castellan, 1988), to confirm the initial classification. Printed sheets (7 x 15 cm) with spectrograms of stereotyped whistles (repeatedly recognizable, stable contours) were presented, in random order, to 2 naïve observers. Each observer was asked to group the whistles into categories according to their graphical appearance.

3.5. Data analysis.

In order to identify signature whistles, the SIGID criteria (Janik et al., 2013) were used. This method proposes that a vocalization can be identified as signature whistle if 75% of all whistles in a bout consist of the stereotyped whistle. Each bout has to be composed of sequences of the same contour in intervals of one to ten seconds. These criteria were applied bidirectionally, thus a whistle to be counted as part of a bout had to be followed or proceed by another whistle of the same type inside the time interval.

To validate this approach, the whistles (*sensu* Caldwell & Caldwell, 1968) of AM4 and UM3 were extracted from the isolation recordings. Following Janik et al., (1994), the dominant whistle type emitted by each individual (i. e. the most frequently emitted whistle contour) could be considered its signature whistle. To assess the differences between contexts of emission in whistle rates and characteristics, all the whistles that could be unambiguously assigned to AM4 and UM3 were selected. The Kruskal-Wallis test was used to examine differences in whistle emission rates according to the context (isolated, segregated and social) for dolphins AM4 and UM3.

In order to study the effect of isolation, segregation and social contexts on whistle characteristics, a full-factorial multivariate analysis of variance (MANOVA) with Games-Howell post-hoc test was performed for the acoustic featured of the whistles assigned to the two dolphins that were present in all contexts (UM3 and AM4). All statistical analyses were performed using *IBM SPSS Statistics 21* (IBM Inc.).

4. Results

A selection of 1,249 good-quality whistles (651 from 2008 and 598 from 2012) was extracted for analysis, representing 77.53% of the total whistle samples. Based on visual classification, a total of 466 stereotyped whistles from three categories (labeled with arbitrary codes LS, HOa and HOb; Table 5.3; Figure 5.3) and 185 non-stereotyped whistles (variant) were identified in the isolation context.

Table 5.3. Values represent percentages of stereotyped whistles occurring with intervals between one to 10 seconds (SIGID criteria). Those in bold refer to the contour qualifying as a signature whistle (> 75% occurrence). Hyphens represent the categories of contours absent in the datasets.

Contour	SIGID percentage in isolation context	SIGID percentage in segregated / social context
AR	-	0%
BA	-	0%
BG	-	0%
ES	-	0%
HOa	56.94%	-
HOb	56.25%	-
LC	-	57.14%
LS	**93.65%**	**88.89%**
MN	-	0%
PL	-	14.29%
VL	-	57.14%

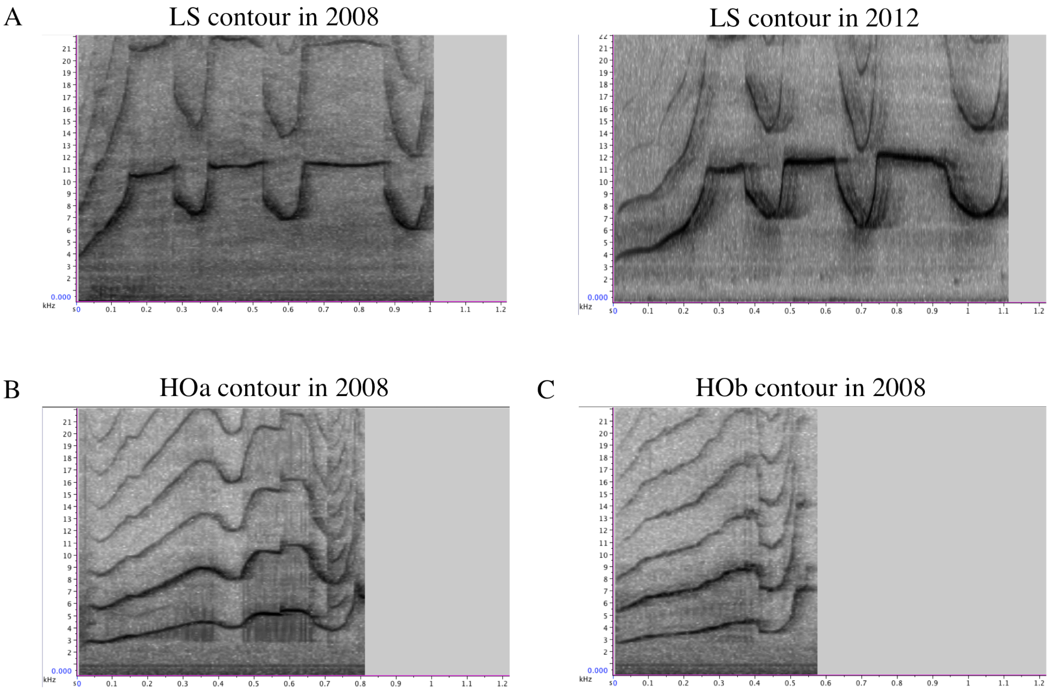

Figure 5.3. A. LS contour observed in all contexts (2008 and 2012). B. HOa contour observed only in isolation (2008). C. HOb contour observed only in isolation (2008).

From the 2012 dataset, a total of 464 whistles with variable contours were observed, and 134 whistles of nine stereotyped contours were identified (codes AR, BA, BG, ES, LC, LS, MN, PL and VL; Figure 5.4). These categories were obtained with high concordance strength ($W = 0.846$, $p < 0.001$) when compared across observers.

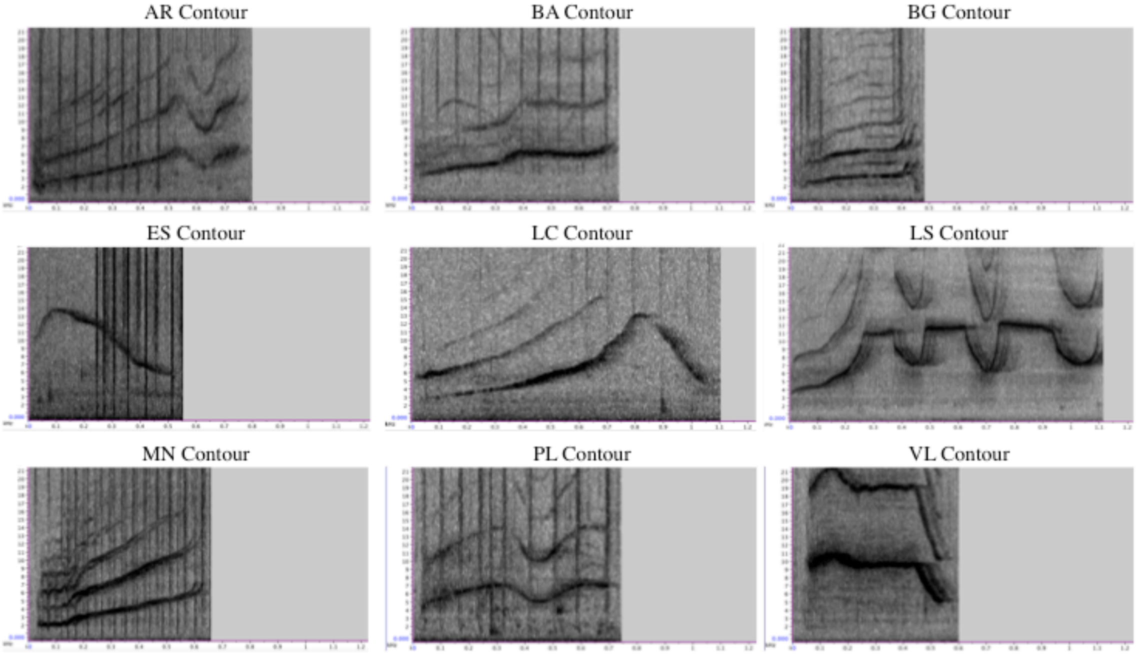

Figure 5.4. Whistle contours observed in 2012 for segregated and social contexts.

When young males AM4 and UM3 were isolated (2008 dataset), they mostly emitted three stereotyped whistles (LS, HOa and HOb). For AM4, the dominant contour was LS, representing 79.04% of his total whistle emissions. For UM3, the dominant contour was HOa (37.31% of his total emission), followed by HOb (16.58% of his total emission).

In the 2012 sample of good quality whistles (N=598), a total of 404 whistles could be attributed to their most-likely emitter based on the focal observations during segregated and social contexts. Of these attributed whistles, only 33.17% were stereotyped whistles (N=134). Analyzing these stereotyped whistles and their most-likely emitters, the majority was the LS contour (68.25%) and was once again attributed to the dolphin AM4. Animal HM5 also produced the LS contour, but at a lower percentage (12.70%). It should be noted that the contours BA, ES and MN, despite their low number, all were attributed to a defined likely emitter. For example, the ES contour was only shared by females, and BA and MN are shared between dolphins that were together in the same pool when they were calves (Table 5.4).

Table 5.4. Number of whistles attributed to their most-likely emitters (from the oldest to the youngest animal from left to right) in contexts segregated and social (2012). "Variants" refers to non-stereotyped whistles.

Contour	**Most-likely Emitters**							
	HM5	UM3	AM4	AF6	IF7	PM9	Occurrences not attributed	**Total of whistles by category**
AR	0	3	1	1	1	1	3	**10**
BA	0	2	1	3	0	0	0	**6**
BG	0	0	0	1	3	2	3	**9**
ES	0	0	0	2	4	0	0	**6**
LC	0	0	1	0	0	0	13	**14**
LS	8	0	43	0	0	0	12	**63**
MN	0	1	0	0	4	0	0	**5**
PL	1	0	3	2	4	0	4	**14**
VL	0	1	0	0	0	0	6	**7**
Variants	24	40	58	65	69	54	154	**464**
Total of whistles by emitter	**33**	**47**	**107**	**74**	**85**	**57**	**195**	
Total								**598**

The LS contour was the only whistle type observed in all contexts: in isolation (2008 dataset), segregation and social contexts (2012 dataset) (Figure 5.3).

Using SIGID criteria, only LS qualified as a signature whistle (Figure 5.3), as 93.65% of the LS emissions occurred in isolation (by AM4) and 88.89% occurred in segregated and social contexts (Table 5.3). For HOa and HOb whistles (Figure 5.3) only 56.94% and 56.25% of the emissions (by UM3, see Table 5.3) occurred within one to ten seconds of another whistle of the same type and therefore were not in agreement with SIGID conditions. For the segregated and social contexts, no other contour was identified as a signature whistle, with contours LC and VL emitted most frequently (both 57.14%; Table 5.3).

4.1. Emission rates and time-frequency parameters.

For the sessions involving dolphins AM4 and UM3 (in both years), the production rates and acoustic measurements of whistles were analyzed.

The Kruskal-Wallis test revealed significant differences in the whistle emission rates between isolation and social context ($H(2) = 10.104$, $p = 0.006$) (Figure 5.5). During isolation, whistle emission rates were 7.8 times higher than in the social context. Although whistle rates were lower in the segregated context than in isolation and higher than the social context, these differences were not significant.

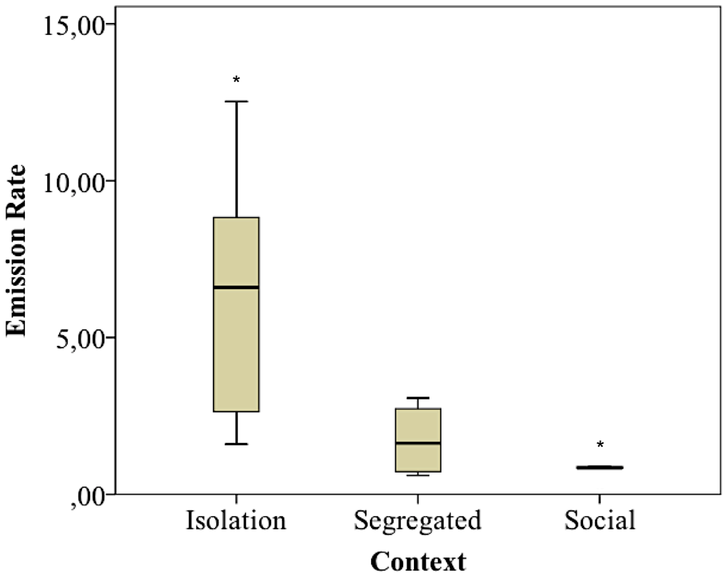

Figure 5.5. Whistle rate (number of whistles per minute per dolphin) in the different contexts. "*" represents significant differences in the whistle's emission rate between conditions isolation and social for sessions involving bottlenose dolphins AM4 and UM3 ($p = 0.006$).

Significant differences were found in the acoustic characteristics of whistles between contexts ($F (16, 1594) = 14.830$, $p < 0.001$). In particular, significant differences were obtained for end and maximum frequencies ($F = 38.125$, $p < 0.001$; $F = 7.193$, $p = 0.001$), the number of inflection points ($F = 35.847$, $p < 0.001$) and the number of loops ($F = 7.337$, $p = 0.001$) (Table 5.5). End and maximum frequencies and number of inflection points were higher in isolation. The percentage of uniloop whistles was 38.86% in isolation, 50.71% in the segregated condition and 80% in social contexts.

Table 5.5. Mean values of whistle acoustic parameters of AM4 and UM3 for each context. Only parameters that revealed significant differences in the MANOVA test were included in the table.

	Whistles Parameters			
Context	End Freq. (kHz)	Max. Freq. (kHz)	Inflection Points	Number of Loops
Isolation	9.06 **ab**	11.39 **ab**	5.59 **ab**	2.08 **ab**
Segregated	7.20 **a**	10.57 **a**	3.25 **a**	1.89 **ac**
Social	7.37 **b**	9.19 **b**	2.67 **b**	1.20 **bc**

Note: Bold letters "a", "b" and "c" indicate pairs with significant differences in the pairwise comparisons. Significance level of 0.05.

5. Discussion

The production of whistles by bottlenose dolphins in captive groups has been the focus of several studies, with divergent results. While some authors consider signature whistles the most common whistle type produced by an individual, regardless of the context of emission (Caldwell et al., 1990; Tyack, 1986; Janik et al., 1994), others emphasize the shared nature of some whistle types (McCowan & Reiss, 1995, 2001). In this study, relevant patterns of whistle production varied according to context, and some common stereotyped contours were only produced in isolation.

The studies of Caldwell et al., (1990) and Sayigh et al., (2007) found the whistle repertoire of isolated dolphins was represented mainly by signature whistles, more than 90% of the total whistle emissions. According to the results presented here, that may not always be the case for all individuals. In the same isolation conditions, two sub-adult males demonstrated distinct acoustic behaviors: AM4 did emit one dominant whistle type (LS represented 79.04% of his whistle production), whereas almost half of UM3's whistles production was made of variant contours and the most frequently occurring whistle represented just 37.31% of the emissions.

Janik and Slater (1998) highlight the importance of habituation when it comes to signature whistle production - dolphins accustomed to captivity often need longer isolation periods before they emit signature whistles. In fact, the habituation/novelty factor seems to be a general issue in whistle production, as initially observed by Tyack (1986), presenting higher whistle rates during novel events. Another relevant factor refers to dolphins' identities and individually specific behavior. Personality traits in nonhuman animals may stay consistent over time (e.g. Capitanio, 1999; Carere et al., 2005; Highfill & Kuczaj, 2007, 2010; Hill et al., 2007), and it is likely that whistling behavior in dolphins depends on personality.

Using SIGID criteria, the LS contour was identified as AM4's signature whistle (93.65% of the vocalizations in isolation and 88.89% in other contexts were produced in bouts with inter-whistles intervals between one and ten seconds, thereby fulfilling the criteria). On the contrary, no signature whistle could be identified in UM3's emissions in isolation. Interestingly, UM3 emitted HOa and HOb in intercalated bouts, thus hindering the identification of both contours as signature whistles. Janik et al., (2013) have suggested that whistle categories with low emission counts might introduce errors in the conservative SIGID method, especially when using it with a small group of animals.

Another possible explanation for the absence of a signature whistle in the recordings of UM3 in isolation could be his fearful behavior profile, recently reported by Neto et al., (2016). The emission of his most common whistles in 2008 (HOa and HOb) was recorded only during his first encounter with the hydrophone, in a repetitive way. Considering that this animal seemed to be more afraid of new things compared with other dolphins in the park, this pattern of emission may indicate that these stereotyped whistles could be not necessarily identity calls, but rather signals related to fear of novel stimuli. The 2012 recordings show that this dolphin does not seem to prefer any stereotyped whistle, at least not in social contexts.

Comparing the two datasets, only one of the contours, LS, was observed in both years, showing a stability already reported by other authors (Sayigh et al., 1990; dos Santos et al., 2005; Luís et al., 2016a) that have observed the same contours lasting for more than a decade.

The overall low occurrence of stereotyped contours and the small group size may have hindered further identification of signature whistles. As to the scarce emission of these contours, it may be a consequence of the artificial environment: the animals are together in the same pool, at close range, and with good visibility conditions, so the emission of identity signals may not be important. As noted by Sayigh et al., (2007), when bottlenose dolphins are in visual contact, signature whistle emission might be suppressed.

Additionally, a clear link between distinct whistle contour category and their most-likely emitter could only be established for the LS contour in segregated and social contexts, with 68.25% of the emissions attributed to AM4. The LS whistle was also emitted by HM5 in a high percentage of its repertoire. This dolphin had a low number of attributed whistles (only 7.98%). It could be that HM5 was copying the LS contour produced by AM4. As suggested by several authors, some signature whistles can be incorporated in other animal's repertoires as a result of copying, even though rarely used, and may be emitted as a label for that particular individual when copied by others (Tyack, 1986; Janik, 2000; King & Janik, 2013; King et al., 2013). Acoustic and play behavior copying is mainly observed between animals with high associations, especially peers with similar age, and is likely to be a type of affiliate display (Kuczaj & Yeater, 2006; King et al, 2013). Dolphins AM4 and HM5 have shared the habitat since the end of 2008, thus the emission of LS contour by HM5 could be a case of copying in a close association.

In this study, different individuals likely produced several stereotyped whistles, but they could not be confidentially attributed to any individual animal. AF6 and IF7, the only female subjects in the study, seemed to share one whistle contour, ES, which is not present in the repertoire of any other dolphin. These emissions may indicate another close affiliation and should be further investigated.

Another interesting case is the BA contour that was emitted by UM3, AF6 and AM4. These individuals were pool companions during their first years of life, and UM3 and AF6 are siblings. As previous studies suggested (Caldwell & Caldwell, 1972; Richards et al., 1984; Janik & Slater, 1998), a dolphin's acoustic repertoire is built through vocal learning and through imitation of sounds that the animals may hear early in life. This could be the case for the BA contour, a whistle type that might have been present in the pool they shared when young, perhaps produced by another individual such as their mother.

PM9's whistle emissions also highlight the importance of an environmental model in whistle production. This calf produced mainly variant contours, and 40.74% of its whistles were upsweeps. As previously reported (Miksis et al., 2002), synthetic stimulus presented during whistle development, such as trainers' whistles, may be used as models, and that could be the case for this young dolphin.

The low number of stereotyped whistles drastically contrasted with the high number of variant contours. These results are in line with what has been described for wild populations, where variant contours represent around 70% of the emissions (dos Santos et al., 2005; Hawkins & Gartside, 2009; Luís et al., 2016a). Throughout the analysis, a high number of incomplete vocalizations were observed. The presence of these short emissions, reminiscent of abbreviations (e.g. resembled start or end of known stereotyped whistle), may be a result of captivity and the stable social context, as these animals are together during most of the year, changing to other pools only when strictly necessary. Tyack (1998) also reported a case of abbreviated whistles in an isolated bottlenose dolphin, noting that the animal devoted less effort to communicate and vocalized less frequently.

5.1. Emission rates and time-frequency parameters.

The whistle emission rate was 7.82 times higher in isolation compared with social contexts. This higher emission rate in isolation is a common response when individuals are separated from their conspecifics (Caldwell et al., 1990; Janik & Slater 1998; Sayigh et al., 2007). The lower emission rates observed in the segregated and social contexts suggest that once at close range and in visual and acoustic contact with other dolphins from the same group, whistle emissions might have secondary relevance in the transmission of information. Considering the acoustic and visual environment in a shallow, limited space such as these pools, it is possible that communication might occur preferentially through visual displays. Future studies should compare the emission rates throughout the day and night to assess variation with light conditions.

Whistles emitted in isolation had significantly higher values for maximum and end frequencies. Similar results have been reported for other mammals in stressful situations and in response to separation, handling and pain (Weary et al., 1997). Although these individuals are used to being separated and handled, the isolation context may trigger stress responses such as the emission of higher frequency whistles.

The higher number of inflection points and loops in isolation also appears to be a common phenomenon: the number of loops was also found to be greater during capture–release events versus undisturbed conditions (Esch et al., 2009b). These findings suggest that multilooped whistles may contain information about the signaler's motivational state, as is the case with social signals of other species (Caldwell et al., 1990; Weary & Fraser 1995; Watts & Stookey 2001; Esch et al., 2009a; Esch et al., 2009b). Despite some studies that relate low frequency modulation whistles to periods of stress in captivity (Lilly & Miller, 1961; Caldwell et al., 1970), this increase may actually represent a higher motivation to communicate rather than an increase in the stress level (Esch et al., 2009b).

In our study, 38.86% of the whistles produced in isolation were multilooped, strengthening the idea of continuous information for the other animals. On the contrary, in social context, it was observed that only 20% of whistles were multilooped, agreeing with the idea that, in social contexts and in captivity, dolphins may not need to emit redundant signals.

In conclusion, this study shows that in a group of captive dolphins, only a single stable signature whistle type was recorded (both in 2008 and 2012) and was emitted mostly by the same dolphin, suggesting that the occurrence of signature whistles may not be a constant or universal phenomenon. Variant contours dominated the whistle production in segregated (but not isolated) and social contexts, possibly because signature calls are less relevant in such situations. Whistle production was much more frequent in isolation, with a higher proportion of multiloop contours, supporting the social cohesion function of these signals. However, this study also highlights the importance of examining the non-stereotyped portion of the bottlenose dolphins whistle repertoire in different contexts, as signature whistle production may not be a constant or universal phenomenon.

CHAPTER 6 – ACOUSTIC BEHAVIOR OF CAPTIVE BOTTLENOSE DOLPHINS DURING MOVEMENT ANALYSIS SESSIONS

(Manuscript in development for later submission)

1. Abstract

In the common bottlenose dolphins (*Tursiops truncatus*) it has been shown, over the years, that vocal emissions are frequently associated with other behavioral displays. This is an acoustical specialized species that uses pulsed and non-pulsed emissions mostly for echolocation and social/foraging events. However, in most cases, it is hard to identify the emitter of the sound. Accelerometry and automated video-based tracking methods have shown great potential in providing information on the movements in animals and to collect detailed information about individual behavior. The subjects of this study were two adult captive bottlenose dolphins trained to tolerate an accelerometer attached near to their pectoral fins. Data collection included acoustical, video tracking and acceleration data. We used two commercial software to extract (BELUGA) and automatically categorize (ARTwarp) the whistles emitted by the dolphins; however, our results showed that ARTwarp does not work with this data set, since most of the whistles are just abbreviations of longer version emissions. The software developed, designated D-Track, was used in an attempt to identify the most-likely emitter of the vocalizations. Although it was possible to proceed with the analysis, more data is needed since captive dolphins have a low emission rate, especially in sessions with the accelerometer, with an average of less than one whistle per minute. Our data showed no correlation between the group size and the emission rate. Each dolphin showed significantly differences between sessions with and without accelerometer in the end, minimum and maximum frequency (for HM5) and in start frequency, minimum and maximum frequencies and duration (for AM4). A contingency analysis between the whistle categories and the movements produced by the focal animals was implemented, revealing no significant relationships. However, this may be a result of a very small sample size, as this work is still in progress. This type of approach might still produce useful results.

2. Introduction

Studies trying to link animal communication signals and social behavior preferably use methods where acoustical signals and movements can be associated (Thomas et al., 2002). In the common bottlenose dolphins (*Tursiops truncatus*) it has been shown, over the years, that vocal emissions are frequently associated with other behavioral displays (Herzing, 1996; Hawkins & Gartside, 2010; Luís et al., 2016b). This species is acoustically specialized and produces a variety of underwater sounds (see review by Lammers & Oswald, 2015). Pulsed emissions, clicks and burst-pulses, are produced in contexts where sonar use is adaptive and during social/foraging events (Au, 2004; Herzing & dos Santos, 2004; Luís et al., 2016a). Non-pulsed emissions, the whistles, may provide information about individual's identity (Caldwell et al., 1990; Janik, 2013). Whistle production rate variation may also contain information about the emitter's motivational/emotional state (Weary & Fraser 1995; Watts & Stookey 2001; Barton, 2006; Esch et al., 2009b). However, for the majority of the cases, it is hard to identify the emitter of the sound to associate it to the behavior display (Thomas et al., 2002). Several methods have been used to overcome the difficulty of the identification of the vocalizing animals in a social environment, in particular attaching sensors to the focal animals (Evans & Sutherland, 1963; Tyack 1991; Nowacek et al., 1998; Laplanche et al., 2015; López et al., 2015).

Accelerometry and automated video-based tracking methods have shown great potential in providing information on the movements in animals and to collect detailed information about individual behavior (Kabra et al., 2013). Accelerometers react to the earth's gravitational field, as well as to body motion (Wilson et al., 2008), providing information about movements normally not detected by the human eye (Ravi et al., 2005; Watanabe et al., 2005; Wilson et al., 2008). Acceleration is divided into static and dynamic acceleration components (Ravi et al., 2005; Shepard et al., 2003, 2008; Watanabe et al., 2005; Wilson et al., 2008). Static acceleration, represented by low frequency signals, is a measure of the inclination of the accelerometer with respect to earth´s gravitational field and it allows the characterization of the animal's posture or body angle (Shepard et al., 2003, 2008; Wilson et al., 2008). Dynamic acceleration, represented by high frequency signals, is obtained from the movement of the body and normally represented by changes in velocity (Shepard et al., 2003, 2008; Wilson et al., 2008).

Automated video-based tracking methods with high temporal and spatial resolution permit the transformation of the video data into trajectories of position over time (Kabra et al., 2013, Pérez-Escudero et al., 2014). This method has been evolving over the years and Rachinas-Lopes et al., (see Chapter 4) developed a system, named D-Track, that enables the tracking of large animals in wide environments permitting the quantification of the animal's routines and the isolation of frames of interest to analyze specific behaviors.

Some specific software, such as BELUGA and ARTwarp (Deecke & Janik, 2006), have been found useful to reduce subjectivity in the acoustic analysis of dolphin vocalization. BELUGA is a whistle-extracting program and ARTwarp is a categorization method that uses an adaptive resonance theory, 'ART', which is an unsupervised neural network that is used to categorize patterns without prior algorithm training. This method allows a sound categorization that is done with flexibility in the time domain and through the perception of the sound frequency (Deecke & Janik, 2006). However, the use of automatic classification for whistles still raises doubts about its performance. Janik (1999) postulated that humans look at the overall shape (contour modulation) of the whistle and its frequency range, while the algorithm strictly analyzes limited temporal/frequency characteristics.

In this study, the acoustic behavior of two young adult male captive bottlenose dolphins was recorded to examine emission rate variability between both animals, as well as the time-frequency characteristics of the whistles. Acceleration was also collected to study the details of the behavior patterns concomitant with the production of specific whistle categories. The automated video-tracking process, D-Track, was used to isolate the frames in which the focal animal was closer to the hydrophone as another method to tentatively identify the likely-emitter of specific whistles.

3. Methods

3.1. Facility and subjects.

The subjects of this study were two captive common bottlenose dolphins (*Tursiops truncatus*) at Zoomarine, Algarve (Portugal). Both animals were adult males, HM5 (born in 1995 and with 185 kg, 2.46 m) and AM4 (born in 2004 and with 172 kg, 2.55 m), which at the time of this study were held in a covered pool and with natural light, having artificial lighting when necessary.

Since the experimental pool is the house for a group of dolphins, isolation of subjects during data collection was not possible. The number of animals changed from 4 to 7 throughout the 2012 to 2014 period. Despite the alterations in the group composition, social structure was already stable and the experimental subjects were familiar with all the animals in the pool during data collection.

3.2. Data collection.

Recordings were carried out in the covered pool, “Blue Lagoon” (Figure 2.1 on Chapter 2), which is perforated fiberglass doors and panels. Data collection was carried out in a segregated context, which means that the focal animal was physically isolated but not visually or acoustically isolated from the remaining group.

Data collection was conducted from November 2012 to April 2014 during the winter seasons (from November to April), which corresponded to the time the park was closed for the public or with low numbers of visitors. During the closing period, the animals kept their training schedules and routines.

All equipment was synchronized in the beginning of each session, with a chronometer. The cameras and chronometer were turned on at the same time and the accelerometer was turned on next to the chronometer and in front of the camera to record the starting time. To synchronize the cameras, we used a Wi-Fi remote control.

Simultaneously with acoustical recordings, accelerometer and video data were collected. The animals were trained, through positive reinforcement, to tolerate a waterproof box held by two suction cups near the right pectoral fin (Figure 2.2 on Chapter 2). To avoid the equipment removal, the experimental dolphins received fish reward during all sessions.

Sessions duration, subjects’ motivational condition and annoyance signals were carefully monitored by dolphin trainers so that no distress was imposed on the animals by the experiments.

This study followed careful procedures to minimize disturbance to the animals, and was approved by Zoomarine’s Board and all technical Directors.

3.3. Accelerometer recordings.

The Kinetamap (SparkFun Electronics, Inc.) sensor consists of a digital 3-axis accelerometer (ADXL345) corresponding to dorso-ventral (x), anterior-posterior (y) and lateral (z) axis. The data was recorded at an average of 65 Hz into an internal microSD memory card. The sensor has a USB rechargeable internal 1100 mAh LiPo battery.

The accelerometer, with the dimensions of 2.8 x 6.3 x 9.4 cm and 103 g, was contained in a 4.2 x 7.3 x 11.1 cm waterproof box with a total weight of, approximately 250 g, representing 0.14% and 0.15% of HM5 and AM4's weights, respectively.

Accelerometer sessions were recorded from 09:00 AM to 6:00 PM for each animal and context, as shown in Table 3.1 on Chapter 3.

3.4. Accelerometer analysis.

The acceleration data captured with the sensor can be divided into gravitation (static) and body (dynamic) components. This separation it is important to discriminate specific movements or behavior performed by the animals (for more information about this separation, please see Chapter 2,

Figure 2.9 and Figure 2.10). In acceleration data, the cutting value, or high pass filter, that permits the better separation between body acceleration and gravitational acceleration in dolphins is 0.45 Hz. After applying the high pass filter to the original data, the body component is obtained.

To perform the whistle trigger behavior analysis, the body acceleration component was transformed into total body acceleration with the following formula:

$$Total\ Body\ Acceleration = \sqrt{(x\_BA^2 + y\_BA^2 + z\_BA^2)}$$

where x_BA, y_BA and z_BA denote the body acceleration in the dorso-ventral, anterio-posterior and medio-lateral axis, respectively, with respect to the animal's head (see Figure 3.3 on

Chapter 3). A selection of the acceleration, 2 second before and after a whistle, was extracted and compared across whistles of the same category.

3.5. Acoustical recordings.

Sound emissions were recorded using a Cetacean Research Technology C55 hydrophone which has a linear frequency response of ± 3 dB in the 0.016 – 44 kHz range; a usable frequency range of 0.006-203 kHz band (+3/-20 dB); a transducer sensitivity of -185 dB re 1V/µPa; and an effective sensitivity of -165 dB re 1V/1 µPa.

The hydrophone was connected to a M-Audio Micro Track II recorder set to a 48 kHz sampling rate and 24-bit resolution recording .wav files in a 2 GB CompactFlash card.

During the data collection, the human interference was kept to a minimum, with the presence of a trainer and the observer. The routines of the animals and feeding time were maintained, to avoid disturbances of the animal behavior and negative implications to the study. The habituation period varied from 5 to 20 minutes after the set up of the cameras.

The acoustic recordings with accelerometer on the subject had a varying duration, from 20 mins to 60 mins, with a total of 980 minutes acquired. The recordings without accelerometer had a fixed duration of 60 mins and a total of 1440 mins were acquired.

As acoustic isolation was not feasible, an alternative method was used to attribute emitters to whistles, involving the D-Track. Two synchronized video cameras were recording the entire sessions allowing the analysis of the position of the animal, especially the frames where the animal was near the hydrophone. The “likely-emitter” identification was attempted using an isolation of the frames in which the dolphin was closer to the hydrophone, given the time scale necessary to extract the whistles. This approach makes it more likely that the emitter of the whistles produced on that specific period of time near the hydrophone is the experimental dolphin.

3.6. Acoustical analysis.

To perform the identification, counting and classification of the whistle emissions, acoustic recordings were analyzed with two different methods. An automatized analysis with a MATLAB-based code program: BELUGA (Deecke & Janik, 2006) for contour extracting, and ARTwarp for the classification of the whistles. BELUGA uses a spectrogram interface to enable the manual extraction of the whistles (Figure 6.1).

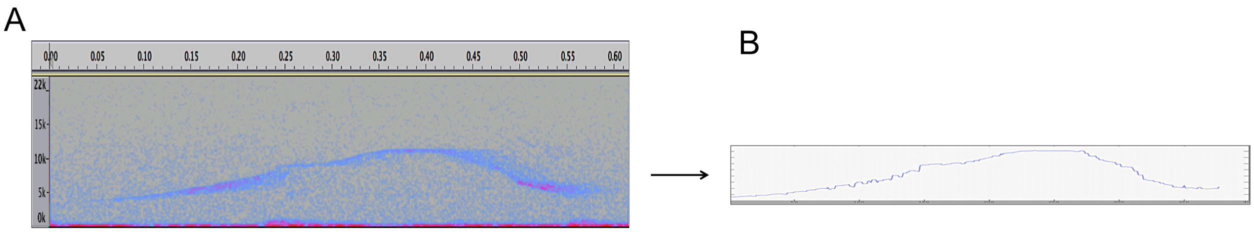

Figure 6.1. Example of a whistle extracted by BELUGA. A) Whistle visualized by BELUGA software as a sonogram B) Extraction of the whistle presented in A.

After extracting the whistles with BELUGA, the output of each whistle (Figure 6.1B) is used for the categorization analysis in ARTwarp. This software categorizes the whistles with a percentage of similarity of 91% (Deecke & Janik, 2006) (see Figure 6.2 as an example). Higher percentage of similarity than the chosen one would create as many categories as the number of whistles. For that reason the 96% considered ideal by Deecke and Janik (2006) could not be used.

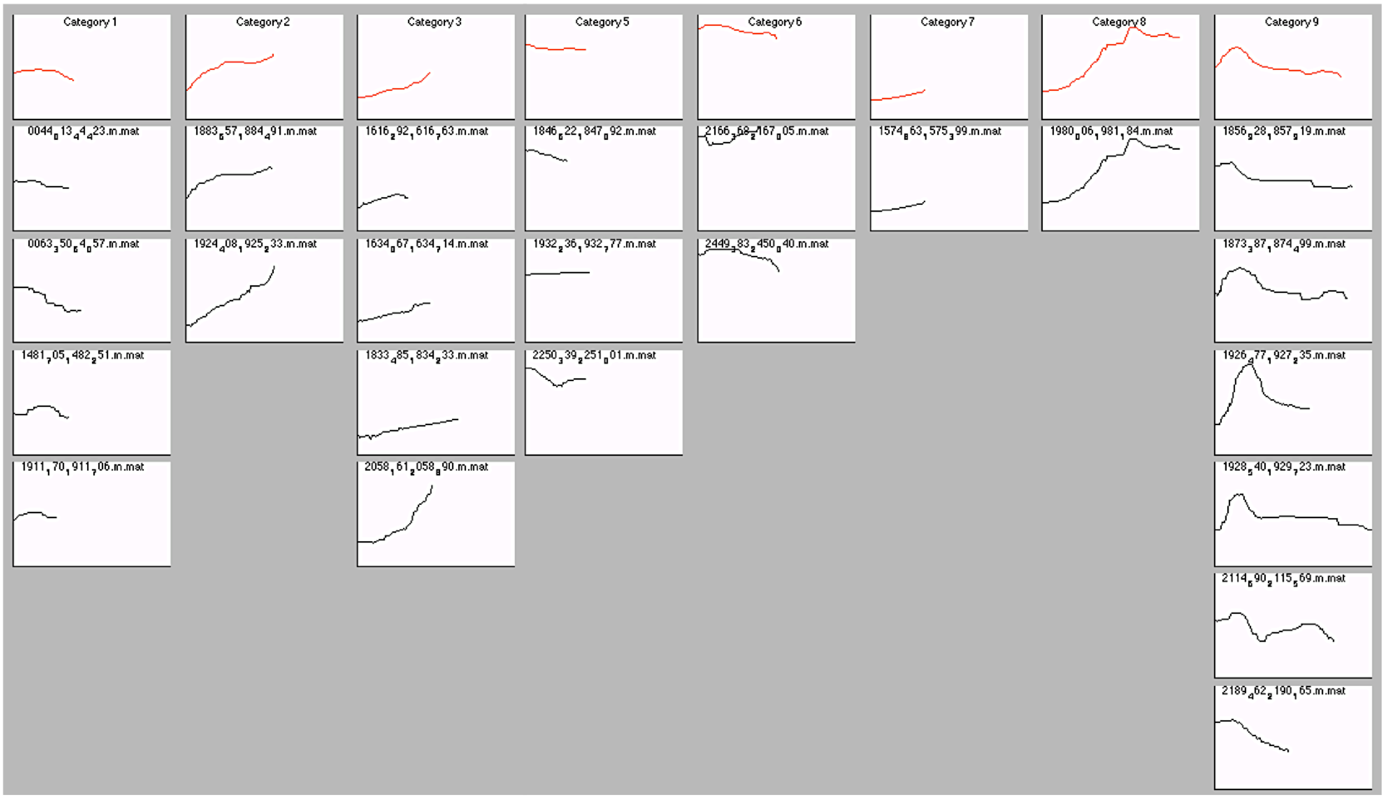

Figure 6.2. Example of an ARTwarp output. Categorization of a set of whistles by the ARTwarp software with 91% similarity.

The non-automatized method, the whistles were visual inspected and validated by one experienced observer, visually and aurally, with spectrogram generated by *Raven Pro 1.4* (Cornell Lab of Ornithology, Ithaca, NY). The selection of the whistles was performed following Bazúa-Dúran and Au (2002) and Baron et al., (2008) procedures. Only whistles with a minimum of 300 ms, sharp and well defined, with no overlapping with other vocalizations, and good signal-to-noise ratio, were included in the analysis. The visual classification (Janik & Slater, 1998) was performed using cross-comparison for frequency modulation and similarity of the stereotyped whistles to assign them to exclusive groups. A category was defined with a minimum of 5 similar contours. The whistles without a category were termed "variant". Subsequently to the classification task, 50 whistle contours were randomly selected and used to test for interobserver reliability, using Kendall's W coefficient of concordance (Siegel & Castellan, 1988). Each spectrogram was printed in sheets with dimensions of 6.5x12.5 cm (FFT, Hamming window; 512

points; 50% overlap) and presented randomly to 2 naïve observers and who were asked to classify the stereotyped whistle according to the patterns in the graphical appearance.

All whistles classified by the MATLAB routines were compared with the visual classification by using Kendall's W coefficient of inter-rater reliability (Siegel & Castellan, 1988), to quantify the similarity between both analyses.

A commercial software package (MATLAB 7.8, The MathWorks Inc., 2009) was used to extract whistle features directly from the contours from BELUGA. The output of the contours consisted in values that corresponded to the fundamental frequency of the whistles, from which were extracted the whistle parameters: start frequency, end frequency, minimum frequency, maximum frequency and duration.

3.7. Video recordings.

To perform the video recordings, two cameras (GoPro Hero 2, ©GoPro, Inc.) were placed in opposite directions, at approximately 4 meters high, to be able to record the 3D movements of the animals (see Chapter 4). The 170º wide-angle lens adds a fish-eye effect distortion to the images, that was compensated, and the cameras were calibrated. The video files were recorded in a 32-GB card in MP4 format, with 1280x720 resolution and 30 frames per second.

Both cameras were digitally synchronized with Wi-Fi remote and the hydrophone was synchronized using a chronometer.

3.8. Video analysis.

To analyze the vocalizations produced by the dolphin near the hydrophone, specific frames were isolated (Figure 6.3) and the frame number was extracted to compare with the vocalizations correspondent to that frame. To increase the likelihood of the experimental dolphin be the emitter, only vocalizations emitted while the animals were near the hydrophone were considered. One second of video, before and after the whistle emission, was also included in the analysis to capture possible adjacent whistles.

This technology was used as an attempt to develop a more accurate approach to identify the likely emitter of a vocalization in an environment that has the presence of other animals. The D-Track enables the isolation of specific points of the trajectory of the animal, namely the frames closer to the hydrophone that will permit the comparison with the times in which the whistles occurred. This way it is possible to identify the whistles that were emitted when a dolphin was in the proximity of the hydrophone without the need of complex data collection by observing the animal and recording manually the times the animal was closer to the hydrophone.

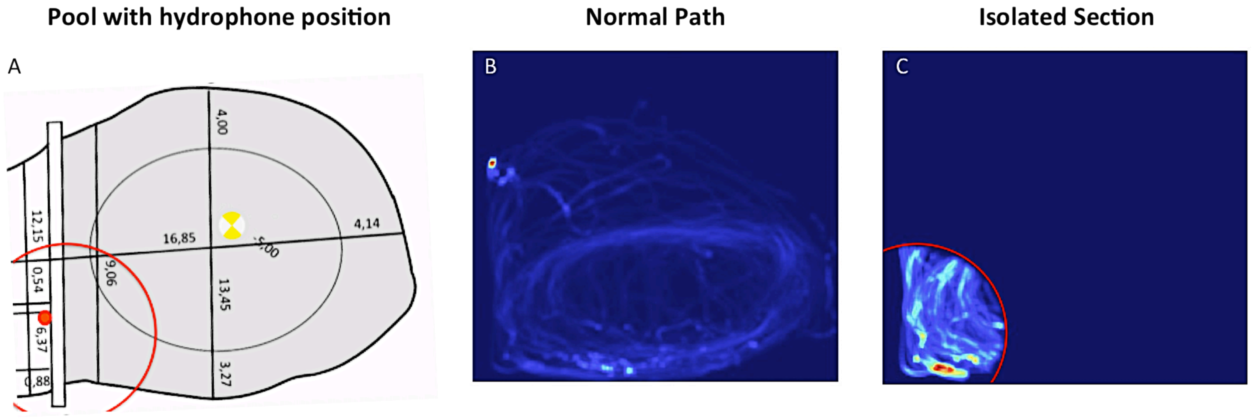

Figure 6.3. Isolation of the section representing the hydrophone. A) Drawing of the experimental pool with the red point representing the hydrophone position and the red circle representing the area assumed for the isolation. B) Trajectory for one of the HM5 sessions showing the occupancy of the pool with light blue representing more time spent in that specific location. C) Isolation of the area in the proximity of the hydrophone and the trajectory that correspond to the frames selected with light colors representing more time spent in that specific location.

3.9. Statistical analysis.

To study the differences between the whistle's parameters, emission rate and the relationship between behavior and whistle during HM5 and AM4 data collections, a series of Kruskal-Wallis analysis of variance non-parametric tests were performed.

To explore the possibility that some behaviors might be associated with any whistle type or, conversely, that some whistles might trigger some behavioral response, a random selection of

30 whistles was made with the corresponding behaviors for a preliminary analysis (at a later opportunity a complete analysis will be performed). These data were then tested with a Monte Carlo simulation.

To compare the number of dolphins in the pool and the whistle production per minute, the Spearman rank correlation coefficient (Zar, 2005) was calculated.

All statistical analyses were done using *IBM SPSS Statistics 22* (IBM Inc.).

4. Results

A selection of 861 good-quality whistles from sessions with HM5 and AM4 with acceleration was extracted for analysis representing 92.58% of the total whistle samples. A selection of 1456 good-quality whistles from sessions with both animals without acceleration was extracted for analysis, representing 89.00% of the total whistle samples.

For sessions without accelerometer, the whistle emission was not significantly different between dolphins (H (23) = 53.05, p = 0.291), with an average of 0.93 and 1.09 whistles per minute for HM5 and AM4, respectively.

For sessions with accelerometer, the whistle emission rate was 1.89 times higher when AM4 was the focal animal compared with HM5, with an average of 1.16 and 0.62 whistles per minute, respectively (H (23) = 4.07, p = 0.044).

4.1. ARTwarp and visual analysis.

For the context "with accelerometer", the acoustical data was analyzed using the ARTwarp software, totalizing 861 stereotyped whistles distributed into 52 categories and 52 non-stereotyped whistles (variant) identified.

For the context "without accelerometer", 476 stereotyped whistles were identified using the visual categorization and distributed into 38 categories. As to variant whistles, 385 emissions were so classified.

The results of automatic and visual categorization were compared using the inter-rater reliability, which showed a non-significant agreement (K = 0.474, p = 0.082) when compared across categorizations.

To validate the visual categorization, two naïve individuals categorized a random selection of whistles, obtaining high concordance strength (W = 0.762, p < 0.001) when compared across all observers.

4.2. Whistle categories in the two contexts.

Using the visual categorization, there was a total of 21 whistle contour categories identified in the study in both contexts (AA, B, BB, CA, CC, DD, GE, H, I, IN, J, K, KL, L, LA, LM, LS, PL, ST, ZA, ZE and the variant whistles, VAR (for more information about the variant whistles and how to categorize it, see Chapter 5). In the accelerometer sessions, 11 whistle categories were identified while in the sessions without accelerometer, were categorized 13. Only 3 contours categories were present in both contexts (AA, BB and LS – see Figure 5.3A to see LS contour). LS contour, as mentioned in Chapter 5, is AM4 most frequent whistle, while no frequent contour was identified for HM5.

Using the video-tracking method to help define the most-likely emitter, in the sessions with accelerometer dolphin AM4 emitted contour LS in higher abundance (16.2% of the total vocalizations) and HM5 emitted contour ST in greater quantity (10.0% of the total vocalizations).

4.3. Emission rate, correlation, and time-frequency parameters.

Comparing whistle emission rates between contexts, the Kruskal-Wallis test revealed non-significant differences (H (47) = 0.17, p = 0.733).

However, during sessions with the accelerometer, the emission rate registered when AM4 was the focal subject was 1.89 times higher compared with the accelerometer sessions of HM5 (H (23) = 4.07, p = 0.044).

The number of animals present in the pool changed from 4 to 7 animals; however no significant differences were found between the number of whistles per minute and group size (r = 0.051, N = 47, p = 0.735).

Comparing the whistle parameters of each focal dolphin in each context, HM5 showed significant differences between contexts in end (F = 12.184, p < 0.001), min (F = 5.848, p = 0.016) and maximum frequencies (F = 21.071, p < 0.001) and all these features were higher for the context "with accelerometer". AM4 showed significant differences between contexts in start (F = 1.223, p < 0.001), duration (F = 189.511, p < 0.001), minimum (F = 25.150, p < 0.001) and maximum frequencies (F = 21.473, p < 0.001). Contrary to HM5, for AM4 all these parameters were lower for the context "with accelerometer" (Table 6.1, Figure 6.4).

Table 6.1. Mean values of whistle acoustic parameters for HM5 and AM4 in the different contexts. Bold values correspond the parameters that revealed significant differences in the non-parametric ANOVA test.

Whistle parameters	HM5		AM4	
	Without Accel.	With Accel.	Without Accel.	With Accel.
Start Freq. (kHz)	4.42	4.77	**6.47**	**5.63**
End Freq. (kHz)	**4.33**	**4.97**	6.12	6.12
Duration (ms)	462	431	**730**	**448**
Min. Freq.	**4.38**	**4.85**	**6.55**	**5.52**
Max. Freq.	**4.26**	**5.12**	**6.52**	**5.57**

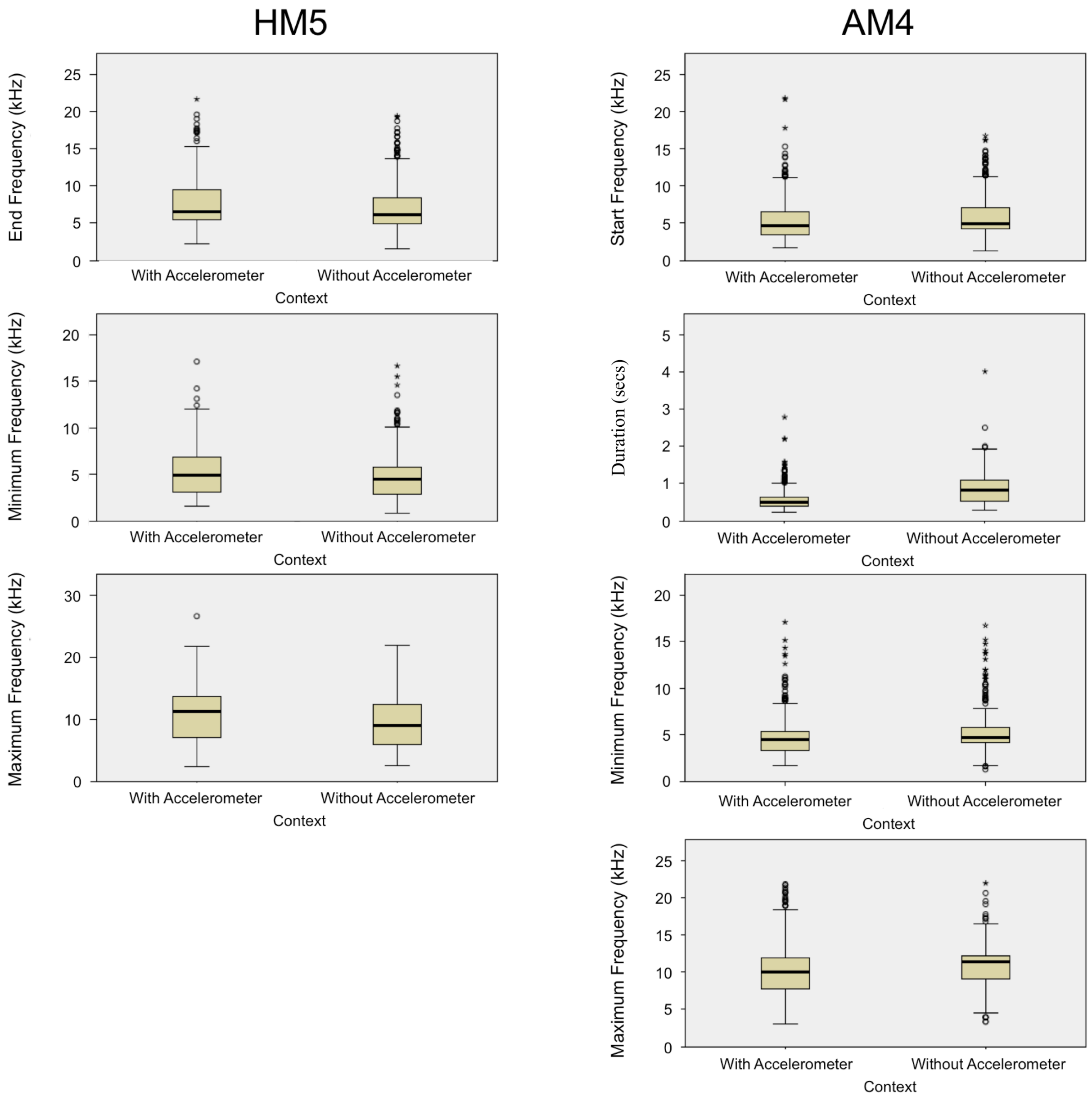

Figure 6.4. Graphs for the parameters that show significant differences between contexts.

4.4. Behavior and whistles.

Using the acceleration data, a non-parametric ANOVA was performed to explore if there is any behavior that is triggered by a specific category of whistles.

The Kruskal-Wallis test showed that there was no specific behavior associated with the acoustic emissions of different categories of whistles, meaning that the behaviors that occurred at the same time as the vocalizations were significantly different from each others (for example for contour CL, F = 6262.01, $p < 0.001$).

The possibility that some behaviors might be associated with any whistle type or, conversely, that some whistles might trigger some behavior pattern was explored. For that analysis a selection of 30 random whistles was made and compared with the behaviors that occurred at the same time. However, no association was found in our data between behavior patterns and acoustic emissions ($\chi^2 = 27.727$, $p = 0.804$) (Table 6.2).

Table 6.2. Z-scores from Monte Carlo analysis for the association between behavior patterns and whistle categories. Values in bold represent the pairs that presented higher association than expected (not relevant given the non-significant result of the χ^2 statistic).

	Whistle Categories					
Behavior	OP	CL	VAR	ZA	BC	MA
Dorsal Dive	**2**	1.3	-1.1	-0.5	-0.7	-0.7
Lateral Left Swim	-0.4	-0.8	1.7	-0.4	-0.6	-0.6
Head Up	-0.5	-0.6	-0.2	-0.5	1.1	1.1
Lateral Right Swim	-0.3	1.7	-1.1	-0.3	-0.4	-0.4
Inverted Dive	-0.5	-0.6	-0.2	**2**	1.1	-0.7
Lateral Ascent Left Swim	-0.2	-0.9	1.3	-0.2	-0.3	-0.3
Lateral Left Dive	-0.3	-1.3	0.4	-0.3	-0.4	**2.5**
Lateral Left Stop	-0.3	0.9	-0.1	-0.3	-0.5	-0.5

5. Discussion

The production of whistles by bottlenose dolphins in captive groups has been the focus of several studies (Tyack, 1986; Caldwell et al., 1990; McCowan & Reiss, 1995, 2001; Janik et al., 1994). In this study, we tested different methods to identify the most-likely emitter of whistle categories and relate the acoustical behavior to the movements of the animals.

Using the MATLAB based software ARTwarp, 809 stereotyped whistles were distributed into 52 categories, and 52 variant whistles were identified from a total of 861 whistles; however, with visual categorization 23 categories and 385 variant whistles were identified. This automatic method has been used for bottlenose dolphins, and other species, by different authors successfully (e.g. Deecke & Janik, 2006; Deecke et al., 2011; Quick & Janik, 2012; Bazúa-Durán et al., 2013; Kershenbaum et al., 2013; Gridley et al., 2014; Samarra et al., 2015), although it did not work as expected for our data. Janik (1999) had already reported that some automatic classification methods revealed a poorer performance in comparison with the human visual classification.

In this study, the differences between automatic and visual categorization may be related to the fact that these animals produce abbreviations of the complete known categories (Rachinas-Lopes et al., 2017 – Chapter 5). This type of whistles may interfere with the automatic software, since the software tries to create a different category for the abbreviated whistles. Automatic categorization also does not take into consideration that the same whistle may be constituted by a different number of loops or units. For this reason, the adopted method to analyze our data was the visual classification.

Identification of the whistle emitter is not always easy (Thomas et al., 2002), even in a controlled environment. Using the D-Track for the isolation of the frames correspondent to the area near the hydrophone, in sessions with HM5, the contour that was vocalized the most was the ST; however, the amount of whistles in these frames for this animal was very low, only 20 whistles in total. For AM4 the most vocalized whistle category was the LS (22 whistles out of 136).

Despite the fact that the whistles were the ones that were more vocalized near the hydrophone, it does not mean that they are the dolphin’s signature whistles. For AM4, we know that it is true, that LS is its signature whistle (Rachinas-Lopes et al., 2017 – Chapter 5); however, for HM5, our data could not support the identification of ST as his signature whistle. Currently the SIGID method (Janik et al., 2013) has been used extensively to test the signature whistle hypothesis (see Chapter 5 for an example); however, the method could not be applied to these data due to the low number of whistles produced by these dolphins.

In a future study, it would be important to increase the duration of the data collection sessions and to isolate the frames of the videos without accelerometry and compare it with the results found for the accelerometer sessions. It will also be a valuable analysis to understand which kind of influence the accelerometer is having in the acoustic behavior of the animals.

Number of emissions per minute revealed non-significant differences between contexts (H (47) = 0.17, p = 0.733) with an average of 0.83 and 1.01 whistles per minute for sessions with accelerometer and without the sensor, respectively. For sessions with accelerometer, the rate per minute was 1.89 times higher when AM4 was the focal animal compared with HM5, with an average of 1.16 and 0.62 whistles per minute, for AM4 and HM5 respectively (H (23) = 4.07, p = 0.044). This difference in the whistle emission rate between dolphins could be related to the dolphin’s age and identity. HM5 was born in 1995 and AM4 in 2004, being 17 and 8 years old when the data collection started. Being a young adult, AM4 may be more curious in relation to the accelerometer sessions (as pointed in a study by Kuczaj et al., 2012). HM5, on the contrary, seemed more vigilant when collecting the data. It is also known that, in bottlenose dolphins, the whistle rate decreases with age (Caldwell et al., 1990; Esch et al., 2009a) and whistling behavior may depend on personality (Highfill & Kuczaj, 2007, 2010; Hill et al., 2007). It will be important to assess personality traits in these animals to better understand differences in reactions to new stimuli, potentially relevant in the training for new research projects.

Even though, the number of animals present in the pool changed from 4 to 7 animals, there was no correlation between the emission rate and the group size by context ($r = 0.051$, N = 47, p = 0.735). This result shows that more dolphins in the pool do not mean that the emission rate should be higher. This is also observed in wild populations of dolphins (dos Santos & Almada 2004; dos Santos et al., 2005; Quick & Janik 2008; Luís et al., 2016a) and may indicate a limit of whistles in a group as a strategy to maximize the information transmission and avoid confusion among emitters (dos Santos et al., 2005).

These emission rates in captivity are much lower compared with the ones in wild environments. Numerous studies with wild bottlenose dolphins reported higher emission rates (e.g. Jones & Sayigh 2002; Díaz-López & Shirai, 2009; Luís et al., 2014). As suggested by several authors, an increase in the emission rates may be related with environment disturbances (Buckstaff 2004; Esch et al., 2009b). In a captive environment, disturbances are reduced to the minimum so the general low emission rate is not surprising. Also, even though segregated, the animals were not visually and acoustically isolated from the other dolphins. In both contexts, the proximity appears to make acoustical communication unnecessary (Rachinas-Lopes et al., 2017 – Chapter 5). In wild bottlenose dolphins, it was suggested that low emission rate may be associated to the familiarity between the members of a group and habitat characteristics (e.g. Jones & Sayigh, 2002).

Whistles emitted by HM5 between sessions with and without accelerometer showed significantly differences in the following parameters: end ($F = 12.184$, $p < 0.001$), min ($F = 5.848$, $p = 0.016$) and maximum frequencies ($F = 21.071$, $p < 0.001$), with all features presenting higher values for the context "with accelerometer". AM4 vocalizations showed significant differences between contexts in start ($F = 1.223$, $p < 0.001$), duration ($F = 189.511$, $p < 0.001$), minimum ($F = 25.150$, $p < 0.001$) and maximum frequencies ($F = 21.473$, $p < 0.001$). Contrary to HM5, AM4's features were lower for the context "with accelerometer". This fact shows that the animals are adapting the acoustical communication in the presence of the accelerometer, which may be related to some "anxiety" in this context, as Weary et al. (1997) mentioned regarding other species. However, further investigation is needed to understand what implications the use of the accelerometer may have in the acoustical behavior of these animals, also considering the potential roles of sex, age, personality and experience. Other fact that may explain these differences between animals are the presence of LS, the AM5 "signature whistle", which might be influencing the values since it was produced in high number, specially, during sessions without the accelerometer.

A contingency analysis between the whistle categories and the movements produced by the focal animals was implemented, revealing no significant relationships. However, this may be a result of a very small sample size, as this work is still in progress and this type of approach might still produce useful results.

CHAPTER 7 - GENERAL CONCLUSION

The overall aim of this thesis was to investigate the behavior of captive bottlenose dolphins to: i) study the behavior patterns of captive dolphins in detail using accelerometry equipment; ii) study the movement patterns of these animals while in a captive environment using video recordings and a custom-made tracking algorithm; iii) study the stability of the acoustic behavior in captivity, available from recordings in two distinct time periods;

Of these three different techniques, the accelerometry shows higher potential to be applied to analysis in the identification of the behavior of wild cetaceans without the need of using video cameras.

1. Accelerometry and Behavior Analysis

With the constant evolution of technology and electronic equipment, it is important to know how to take advantage of it and to understand the type of data that we can extract from such tools. Accelerometry has been showing an enormous potential to help decode the behavior of various species (See Chapter 3 for recent examples). In this project, the accelerometer data allowed the identification of Inverted, Lateral (right and left) and Dorsal Swimming, as well as Head Up, Ascent/Dive and Stop movements. To our knowledge, this was the first study that enabled a completely automatic identification of some behaviors in captive dolphins. Detailed behavior analysis is a very time-consuming process, and it will benefit from automatic algorithms that may help to decrease not only the subjectivity in some analyses, but also the video processing time as well.

One of the key factors when using the accelerometer to analyze behavior is to understand the results that the accelerometers produce. Accelerometers are sensors that can provide a great deal of information about postures and motion; however, the interpretation of the data is not always easy. For example, the Stop behavior in dolphins is one of the behaviors that are hard to observe and, when plotting the behavioral maps, it is spread in various posture/behavior categories. What for a human eye seems to be a stop behavior, for the accelerometer is a sequence of different postures that will vary according to the inclination degrees and, for that reason, it may appear to be spread throughout behavioral maps.

1.1. Future directions.

The goal for the unsupervised classification methodology is to be able to automatically identify the behavior performed by an animal without the need of using video cameras, which in the wild are often impossible to use. It is now important to test the algorithm with a higher number of captive dolphins and, most importantly, with data collected from dolphins in the wild. Currently several tags contain accelerometers (e. g. Rechsteiner at al., 2013; Akiyama et al., 2015; López et al., 2015) but sometimes their data are not used to the fullest extent.

2. Tracking System and Behavior Analysis

Bottlenose dolphins in captive environments are held in much smaller and less complex habitats than the wild. With an increasing concern about the quality of life of these animals that are under human care, it is very important to develop methodologies and strategies to better understand their activities. Being able to understand the animal's preferences can highly contribute to their quality of life. Our system, the D-Track, allowed not only semi-automatic video tracking of aquatic animals in a pool, but also the identification of the preferred areas of the focal dolphins and the quantification of the time spent in each zone.

The D-Track was developed to adapt easily to different artificial settings, and also to be practical to implement, requiring only some specific measurements for the 3D reconstruction of the arena. The setup assembly can be easily learnt, and implementation should be quick. Knowledge about the enclosure preferences of the animals, easy to detect with D-Track, allows for a better assessment and improvement in their quality of life. It is also useful in the planning of future modifications in the pools or space management. With the ever-present concerns about what would be the best dimensions and potential shapes to use in an artificial habitat, the D-Track can be a great solution to study the occupancy of the animals inside the water, including in the depth dimension.

The fact that it was possible to measure the velocity of the animals in the pool was relevant, since it allows a comparison among velocities in the wild and in captivity. Understanding the velocity patterns of dolphins in captive environments is another tool to study the best shape and depth of a pool.

2.1. Future directions.

As future directions, we are working on the tracking algorithm to analyze and quantify the dolphins' preferences in the pool when accompanied by another dolphin. This information can be important to understand, for example, the hierarchy system of these animals in captive environments. Even though the trainers are used to observe the animals' interactions, most of the times they are not present to observe hierarchy dispute behaviors and agonistic asymmetries. With a development of the D-Track system, it will be possible to evaluate, in a single session, how two animals are behaving without the presence of the trainer in the area. It will be possible to quantify how long they stay in specific locations and how they explore the habitat with another animal, dominant or not, in the pool. This type of analysis will help to better manage the dolphins' welfare, reducing recurrent aggressions, potential boredom, stress and stereotyped behaviors in marine parks where the animals exhibit this kind of problems.

3. Acoustic Behavior

The acoustic data was used to clarify the relation between specific whistle categories with their emitters, or in this case, the "likely-emitters". The results suggested that not only whistles carry important information about the emitter, but also that whistle production is dependent on context. Our results, obtained under the same isolation conditions, showed that the two sub-adults had distinct acoustic behaviors, which led us to suggest that, in captivity, controlled environment and visibility conditions may represent different motivations for each animal. In other words, other variables, perhaps psychological or contextual might be more relevant in generating the dolphin's acoustic behavior.

The fact that dolphins are capable of copying acoustic emissions of other dolphins makes it difficult to identify, while in group, the emitter of the vocalizations in our 2012–2015 results.

Because of this, it was important to understand if the focal animals of the accelerometer experiment, HM5 and AM4, had a specific/individual contour that would enable an easier detection of the whistle. For AM4 the LS whistle was identified, was persistent over time and was observed throughout the experiment's time span.

The results also showed that, in captive environments, whistle emission is much lower compared with wild populations (Jones & Sayigh 2002; Díaz-López & Shirai, 2009; Luís et al., 2014). This points to the conclusion that, in these conditions, whistle emissions might not be as relevant to convey information as in wild, and that communication might occur preferentially through visual displays, perhaps easier and more convenient in this environment.

3.1. Future directions.

Acoustic analysis is a complement to study dolphins' behavior. It can provide information about the emitter, environment and other animals. Acoustic analyses in isolation are even more important, since they enable the study of individual sounds simplifying the link between acoustics and behavioral changes, and this of course is much easier to accomplish in a controlled environment.

4. Combination of the Methodologies

The combination of different methodologies can increase the power of a research strategy. In this project, we tried to use available software or develop automatic analyses for the various types of behavioral data. For accelerometry data, we developed a code to automatically identify captive dolphin behaviors. For data using video recordings, we developed a tracking system that enables the semi-automatic study of the movements and occupancy of dolphins in a pool. For acoustical data, two commercially available software were used to extract acoustical contours and to automatically categorize the signals. The acoustic software to automatically categorize the different types of whistles, ARTwarp worked different from the expected with our data.

Automatic classification of whistles with software as ARTwarp seems to work in the wild (Deecke & Janik, 2006; Deecke et al., 2011; Quick & Janik, 2012; Bazúa-Durán et al., 2013; Kershenbaum et al., 2013; Gridley et al., 2014; Samarra et al., 2015); however, at least in this study carried out in captivity, the contour recognition algorithm was not as efficient as expected. As pointed out in Chapter 5 of this thesis, these dolphins seem to use a large percentage of their repertoire as abbreviation of complete whistles. The ARTwarp assumes that all abbreviation is a new whistle, making the analysis very difficult. In these conditions, the best strategy for correct or meaningful pattern recognition apparently still is the “visual” classification operated by human eyes (as already suggested by Janik, 1999). Human eyes work better to detect small variations in the same whistle compared to automatically algorithms. Also, different number of loops does not always mean that they are of a different whistle type, which is another parameter that must be taken into account when developing an automatic classification of sounds. Despite the importance of an automatized method that will improve data analysis and should decrease its processing time, also reducing subjectivity, for this study it was not useful. The impossibility of using the ARTwarp in our data shows how hard it can be to develop a software to perform an automatic categorization of the whistles that will take into account the variation within whistles of the same category. Despite the importance of such software, the visual whistle categorization is still the best way to execute this task.

In a preliminary analysis to explore the possible relationship between whistle categories and movements, the data revealed no significant association between these behaviors. These results may indicate that the focal dolphins are used to the environmental whistle communication that they seem to react normally to the sounds in the pool. The vocalizations of conspecifics in these samples do not seem to produce perceptible changes in the focal animal’s behavior; it is not clear this is a general situation or a result of limited sampling, or a consequence of the artificial context in which these animals live. This work is in progress and this type of approach might still produce useful results in the future.

Regarding the two methodologies developed, the D-Track and the accelerometry algorithm, it is envisaged that they may increase knowledge about captive and wild dolphin’s behavior when associated with acoustic recordings, even though the relationship between movements and emissions, in the present very limited analysis, was not significant.

This project had two different intentions, the study in captive environments, with the main goal the welfare of the animals; and the study with wild dolphins, with the aim of better studying the behavior of these animals in their natural environment with minimal interference and without the need of video cameras.

4.1. Future directions.

The use of a combination of the methodologies should contribute with more precise information about the behavior of a group of captive dolphins. Dolphins do not use sound as the only method to communicate, they also use visual and tactile behaviors, and so, as a next step, it is important to further study the comparison between emissions and movements. Given the fact that visual and acoustics displays are two communication channels in animals, especially in bottlenose dolphins that are acoustically specialized, the relation between these two communication modes could provide important clues about the way these animals integrate sensory information, and interact with their environment. Future analysis should include the tracking of two animals in the pool. This could be an easier and interesting way to study social interactions in a group, even hierarchies, since it is possible to quantify the occupancy of the pool of a focal dolphin and study if the animal has the tendency for dominance or submission. This analysis is especially important when the trainers are not present in the pool area and the animals are by themselves. The addition of the accelerometer data can give valuable information about the behavioral changes in situations such as the previous example regarding agonistic situations. Information about social interactions patterns in captivity can be a major advantage to the study of social interactions in the wild, where usually it is so much more difficult to obtain complete observation data.

In conclusion, these have been the overall purposes of a multi-approach research: to use the conditions of a controlled environment to develop methods that may help to improve the welfare of captive dolphins, and to gain advantages applicable to the investigations of these animals in the wild. It is hoped that some contributions and advances may have come out of these various efforts.

CHAPTER 8 – REFERENCES

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