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Underwater vocalization of bottlenose dolphins in the region of the Sado estuary: acoustic features and occurence patterns

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Abstract

Common bottlenose dolphins, *Tursiops truncatus*, are social marine mammals with complex fission – fusion societies. The acoustic signals propagate efficiently in water, being an ideal form of communication in low visibility waters such as in estuarine habitat. Underwater acustic signals have an important role not only for communication, but also for navigation and prey detection.

The Sado estuary bottlenose dolphins have a wide vocal repertoire that can be divided in three major categories of signals: unpulsed sounds (tonal), such as whistles; echolocation clicks; and burst-pulsed signals, such as creaks, squawks or bangs. This work focus in the brays series, a pulsed vocalization type that combine gulps, grunts and squeaks in bouts.

Bray series have been reported in several, but not in all populations of bottlenose dolphins, however their functional role has yet to be fully understood. This work presents a quantitative analysis of the emission of this signal.

This study was conducted in the Sado estuary region focusing on the resident bottlenose dolphin population. To understand which factors influence this conspicuous vocalization, a set of ecological and behavioural variables were analysed, such as group size, depth, location, pattern of activity, presence of vessels and tidal phase.

A Generalized Linear Model with a Binomial Negative Regression Model was used to test the influence of the variables selected. The results show that group size, depth, location, vessels and tide had no effect on the emission rate of bray series. Only the activity pattern affected the number of bray sequences. Foraging ($\hat{\beta}$ = 1.63±0.51), feeding ($\hat{\beta}$ = 2.13±0.69) and socialization ($\hat{\beta}$ = 2.06±0.81) showed significant higher counts than travelling, supporting notion of social (agonistic or affiliative) function for this signalling emissions.

Key words: *Tursiops truncatus*; Sado estuary; Bioacoustics; Brays Series; Behavioural Analysis

Resumo

Os golfinhos-roazes, *Tursiops truncatus* (Montagu 1821), são mamíferos aquáticos, pertencentes à ordem Cetartiodactyla, infraordem Cetacea, parvordem Odontoceti e à família Delphinidae. Esta espécie apresenta uma ampla distribuição, habitando desde águas tropicais até às águas temperadas, e algumas populações podem ser residentes, habitando baias, lagoas ou estuários, como o do Sado.

A troca de informação é um processo vital na vida animal, e pensa-se que a complexidade das sociedades está relacionada com a complexidade de comunicação entre os seus elementos. Os golfinhos Roazes apresentam uma elevada capacidade cognitiva e são acusticamente especializados, vivem em sociedade de fissão – fusão, formando complexas alianças cuja composição varia ao logo do tempo, dependendo do contexto social.

O vasto repertório vocal de *Tursiops truncatus* pode ser dividido em três categorias de sinais: sons não-pulsados (tonais), cliques de ecolocalização e outros sons pulsados.

Os cliques de ecolocalização pertencem à categoria de sons pulsados, são altamente direcionais e são usados como sonar para orientação e deteção de alvos. O animal emite trens de cliques e obtém informação do ambiente envolvente através dos ecos recebidos, apresentam uma taxa de repetição abaixo de 40 pulsos por segundo.

A categoria de outros sons pulsados é separada dos cliques de ecolocalização por apresentarem uma taxa de repetição muito alta (acima dos 300 pulsos por segundo) e tem um intervalo entre cliques muito curto (menos de 3 ms), que poderá interferir com a função de sonar. O papel funcional destes sinais ainda não é claro, certos estudos, como Herzing (1996) associaram alguns sons pulsados com comportamentos agonísticos ou de cortejo.

No extenso repertório vocal dos golfinhos-roazes existe um grupo de sons pulsados que são combinados em distintas unidades vocais, para produzir sequências rítmicas, chamadas de zurros. Os zurros foram descritos pela primeira vez por dos Santos *et al.* (1990) no estuário do Sado, Portugal, e desde então tem sido descritos e estudados em diversas populações de *Tursiops truncatus*.

Os padrões temporais e estruturais dos zurros ainda se encontram pouco claros. Estes sinais de multiunidades poderão representar um papel específico no repertório vocal, que poderá diferir consoante os componentes dos zurros, podem ser compostos por grunhidos, goles e guinchos. Esta vocalização poderá estar associada a comportamentos de socialização, ou a comportamentos de alimentação.

Para o presente trabalho foi analisado um total de 205 gravações recolhidas na região do estuário do Sado, de março de 2014 a abril de 2017.

O contexto comportamental foi avaliado tendo em conta os padrões de atividade observados à superfície durante as gravações, tendo sido definidos cinco categorias: alimentação (movimentos rápidos à superfície e em várias direções, estando os animais próximos uns dos outros), busca de presa (movimentos erráticos dos animais à superfície, geralmente acompanhados de curtos mergulhos), socialização (estabelecimento de contacto físico à superfície, com curtos períodos de submersão), deslocação (o grupo de golfinhos movimenta-se numa determinada direção, com mergulhos sincronizados) e repouso (animais muito próximos entre si, em movimentação lenta e direção constante ou praticamente imóveis à superfície). O comportamento de repouso não foi observado durante os períodos de amostragem.

Os sons captados durante as gravações foram analisados em laboratório e atribuídos a categorias pré-estabelecidas, de acordo com as suas caraterísticas: Assobios – som tonal modulado e de banda curta; Cliques de ecolocalização - Sinais pulsados direcionais, de banda larga e muito curta duração;

Rangido – som de banda larga com alta repetição (> 40 estalidos por segundo), semelhante ao ranger de uma porta; Chorinco – alta taxa de repetição (maior do que o rangido) e a frequência dominante variável ao longo da emissão, aspeto aural oscilante, assemelha-se a um choro de bebé; Trem de Taxa Variável (TTV) – pode conter cliques discerníveis, rangidos e chorincos, emitidos seguidos e ordem variável, consoante a taxa de emissão dos cliques aumenta ou diminui; Buzz (S-BP) – trem com alta taxa de repetição e curta duração (inferior 0,05 s), semelhante ao de uma abelha; Bang – som pulsado isolado de elevada energia; Grunhido – trem de sons pulsados intensos, com sonoridade estridente e áspera que se parece ao grunhir dos porcos; Gole – som pulsado curto e de baixa frequência, sonoridade de um gole ou soluço; Guincho – curto som pulsado que apresenta uma estrutura harmónica, semelhante a um grito ou ganido agudo.

Através deste estudo verificou-se que os sons mais comuns no repertório dos golfinhos-roazes da população residente do estuário do Sado são os assobios e os trens de cliques, uma vez que, desempenham as funções de comunicação e bio-sonar, respetivamente, e podem ser emitidos em simultâneo, possivelmente transmitindo informação a conespecíficos sobre presença em atividades de alimentação. Estabeleceu-se, também, que os elementos vocais que constituem os zurros são sons comuns no repertório vocal e são mais emitidos em sequência, do que emitidos isoladamente.

Cada sequência de zurro foi definida usando um critério de 0,6 segundos de intervalo silencioso entre elementos vocais, permitindo separar o fim da primeira sequência e o início da segunda, o que permitiu uma análise quantitativa das emissões de zurros.

Tendo em conta as variáveis: local (dentro ou fora do estuário); profundidade; tamanho do grupo; presença ou ausência de embarcações; estado da maré (enchente ou vazante); e atividade dominante usou-se um Modelo Linear Generalizado, com uma distribuição Binomial Negativa para perceber que fatores influenciam a taxa de emissão de zurros, usando 179 gravações, onde os elementos vocais que constituem as sequências de zurros apresentam uma relação de qualidade – ruído média e boa.

Só o padrão de atividade demonstrou diferenças significativas (valores p <0,01), sendo que as atividades de alimentação ($\hat{\beta}$ = 2,13±0,69), busca de presa ($\hat{\beta}$ = 1,63±0,51) e socialização ($\hat{\beta}$ = 2,06±0,81), apresentam taxas de emissão de zurros significativamente maiores que a atividade de deslocação.

Como as sequências de zurros apresentam diversidade de estrutura e composição, as diferentes combinações poderão indicar que este tipo de vocalização é emitida em múltiplos contextos. O contexto social e ecológico onde se verificou maiores taxas de emissão (alimentação, busca de presa e socialização) são complexos, dinâmicos e apresentam diversos estados motivacionais, enquanto, que a atividade de deslocação envolve pouca interação entre indivíduos.

Os fatores local, profundidade, tamanho do grupo, presença de embarcações e estado da maré não revelaram diferenças significativas na taxa de emissão de zurros (valores p> 0,05).

Este estudo conclui que, os zurros são emitidos com mais frequência em padrões de atividades que envolvem mais interação entre os indivíduos e estados motivacionais mais excitados, como a alimentação, a busca de presa e a socialização. Os fatores ambientais não relevaram influências significativas na taxa de emissão destas vocalizações.

Embora o estatuto de conservação da espécie *Tursiops truncatus* seja "pouco preocupante", as populações de golfinhos, como a do estuário do Sado, estão bastante expostas às atividades antropogénicas, como o tráfego de embarcações, poluição e perseguição por parte de embarcações de recreio, entre outras. Estes problemas associados a baixas taxas de reprodução, de recrutamento e continuo envelhecimento da população, apresentam importantes ameaças à sobrevivência destas populações.

Ao estudar as emissões acústicas dos golfinhos no seu meio natural, podemos compreender o papel funcional destas vocalizações e a associação com os contextos comportamentais, e assim, perceber e avaliar os impactos antropogénicos na população, para tomar medidas de preservação adequadas.

Palavras-chave: *Tursiops truncatus*; Estuário do Sado; Bioacústica; Zurros; Análise Comportamental

Table of Contents

List of Figures

List of Tables

1. Introduction

The common bottlenose dolphin, *Tursiops truncatus* (Montagu 1821), is an aquatic mammal, classified in the order Cetartiodactyla, infraorder Cetacea, parvorder Odontoceti and family Delphinidae (Wells & Scott, 2008).

This species has a worldwide pelagic distribution, inhabiting from tropical to temperate waters, both in oceanic and coastal environments, more or less adjacent to continental masses or oceanic islands. In coastal waters, some populations become resident in bays, lagoons or in estuaries and other shallow coastal regions (Bearzi, Fortuna, & Reeves, 2009; Mann, Connor, Tyack, & Whitehead, 2000; Scott & Chivers, 1989; Wells & Scott, 1999).

Bottlenose dolphins (Figure 1.1) are medium size odontocetes that can live more than 40 years. They present a greyish coloration dorsally and laterally, and a light coloration ventrally (Reynolds III, Wells, & Eide, 2000; Wells & Scott, 2008).The adult’s total length ranges from 2 meters to 4 meters, but the genus *Tursiops* presents geographical variation in morphology (Deharveng & Bedos, 2012; Reynolds III *et al.*, 2000; Wells & Scott, 1999).

Figure 1.1 - *Tursiops truncatus* in Sado estuary, photo: Patrícia Rachinas-Lopes.

Dolphins evolved to adapt to the constraints of the marine environment. The fusiform body shape provides an efficient and fast way to swim, with modified appendages, and the respiratory system is modified compared to the terrestrial ancestors, which allowed the ability to dive and communicate (Reynolds III *et al.*, 2000). Although there is no evident sexual dimorphism in adults, in some studies an individual is considered as female when observed regularly with a specific calf (Mann *et al.*, 2000).

The gestation period in *Tursiops* sp. lasts for 12 months, females give birth usually during spring-summer months to a single calf with 84 cm to 140 cm (Deharveng & Bedos, 2012).

Bottlenose dolphins are generalist predators, preying on fishes (such as mullets and European eel), cephalopods (squid and cuttlefish) and even crustaceans (Deharveng & Bedos, 2012; dos Santos, Coniglione, & Louro, 2007; Luís & dos Santos, 2012).

In general, bottlenose dolphins form groups of 2-15 individuals. Nonetheless, there have been reports of groups with more than 1000 individuals. Group size varies according to biogeographic region, prey availability, activity and other factors. For example, in bays and estuaries these dolphins tend to form smaller groups in comparison with the offshore populations (Bearzi *et al.*, 2009; Deharveng & Bedos, 2012).

Bottlenose dolphin societies are characterized by a fission–fusion dynamic, forming complex alliances. Individuals can associate in small groups that change in composition over time, and may depend on the social context, especially in relation to reproductive strategies, (to improve the individuals' fitness) (Augusto, Rachinas-Lopes, & dos Santos, 2012; Connor & Krützen, 2015; Mann *et al.*, 2000; Quintana-Rizzo, Mann, & Wells, 2006). Communication is a vital process to maintain long-term social ties, like the mother-calf bond, one of the strongest associations, or some long-term male-male alliances (Connor, Heithaus, & Barre, 2001; Harley, 2008; Quintana-Rizzo *et al.*, 2006).

Acoustic signals are the ideal form of communication in an aquatic environment, given that the acoustic energy propagates efficiently in water and offers a crucial advantage in turbid waters, where the visibility may be low, like shallow inshore waters, bays or estuary (Au & Hastings, 2008; Wells & Scott, 2008).

The cetacean ear is much different from the other terrestrial mammals. Apparently does not exist a direct connection between the external canal and the tympanic membrane, so the sound is not conducted in the external auditory meatus to the inner ear as in other mammals. Norris (1967) proposed that sounds enter the dolphin's head through the lower mandible and are transmitted to the middle and inner ears through the pan-bone window and the fat channel. (Au & Hastings, 2008).

Tursiops truncatus has one of the widest auditory bandwidths of the cetacean species, hearing up to 150 kHz. This species has a great capability to discriminate frequencies, which is important to detect and process acoustic signals, especially sonar echoes. The sound production is related with movements in the nasal muscles instead of being related with vocal cords in the larynx, like in humans and other mammals (Au & Hastings, 2008).

With a highly developed acoustic system, cetaceans produce a wide variety of underwater signals, reflecting the major role of sound in the ecology and social life of these marine mammals. Communication can occur through vocal and nonvocal sounds, the latter being produced by percussive activity, like "tail slaps" (Au, Popper, & Fay, 2000; dos Santos, Louro, Couchinho, & Brito, 2005; van der Woude, 2009).

Tursiops truncatus has a wide vocal repertoire that can be divided in three major categories of signals: unpulsed sounds (tonal), echolocation clicks and burst-pulsed signals. The burst-pulsed signals can be categorized as social sounds, functioning primarily for communication and have been linked to the social interactions of some species, while echolocation clicks serve for detection of the surrounding environment (Au & Hastings, 2008; Herzing & dos Santos, 2004; Luís, Couchinho, & dos Santos, 2016; van der Woude, 2009; Wells & Scott, 2008).

The tonal sounds which are omnidirectional, narrow-band and frequency-modulated, are known as whistles and their primary function is communication. The fundamental formant is between 3 kHz and 15 kHz and harmonic components may extend above 40 kHz. Whistles can be highly stereotyped and individually specific, being used to communicate identity, location, and possibly emotional states.

Such stereotyped whistles are known as the “signature whistles” (Caldwell & Caldwell, 1965; Janik, 2000b; Luís, Couchinho, & Dos Santos, 2015; Wells & Scott, 2008).

Echolocation click trains, also pulsed signals are highly directional and are used in bio-sonar orientation and target detection (Figure 1.2). The animal emits clicks and obtains a sense of its surroundings from the echoes received. Dolphins and bats have specialized in this capability, which provides an advantage in surviving in habitats with low visibility. It can be used in navigation, foraging and other functions yet to be discovered (Au & Hastings, 2008; Wells & Scott, 2008).

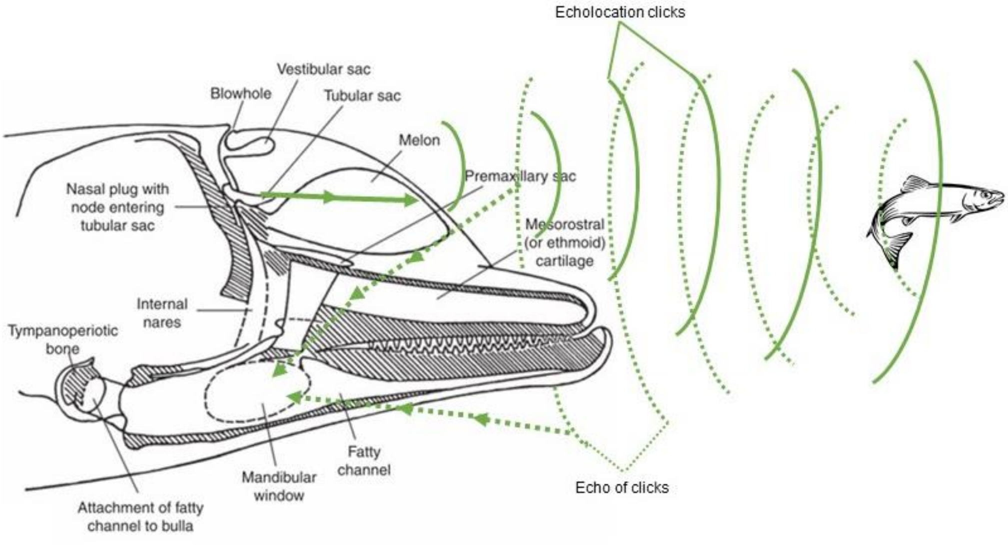


Figure 1.2 – Internal morphology of a bottlenose dolphin head and echolocation mechanism (adapted from: Au, 2008).

Burst-pulses are also formed by broadband pulses, but these may be separated from the echolocation clicks due to the very short inter-click-intervals (ICI), less than 3 ms, and high repetition rate (above 40 pulses per second). In sounds like creaks, squawks and grunts the clicks can’t be perceived individually and the packed clicks may prevent a functional sonar (Au & Hastings, 2008; Luís *et al.*, 2016; van der Woude, 2009). Although Herzing (1996) associated some burst-pulses with courtship or agonistic behaviour, the functional significance of these signals are yet to be fully understood (Au & Hastings, 2008; Herzing & dos Santos, 2004; Luís *et al.*, 2016).

In the extensive *Tursiops truncatus* repertoire there is a group of burst-pulses that can be combined to produce distinct vocal units, called bray series. These rhythmic vocalization sequences were firstly described by dos Santos *et al.* (1990). Since then it has been reported and studied in other populations around the globe (dos Santos, Ferreira, & Harzen, 1995; Gridley, Nastasi, Kriesell, & Elwen, 2015; Herzing, 2015; Janik, 2000a; López & Shirai, 2009).

The temporal and structural patterns of the brays are yet to be clarified. These multi-unit signals may play a specific role within the vocal repertoire, which can differ according to the individual components in the bray series. Brays can be composed by grunts, squeaks and/or gulps (Gridley *et al.*, 2015; Luís, Alves, Couchinho, & dos Santos, 2017). In the resident population of the Sado estuary, bray production was recorded during social behaviour (dos Santos, Caporin, Moreira, Ferreira, & Coelho, 1990; Luís & dos Santos, 2012; Luís *et al.*, 2017), and in Scotland, bray production was reported as food-related call, during the feeding on salmonids (Janik, 2000a; King & Janik, 2015).

The Sado estuary is located on the west coast of continental Portugal, between the city of Setúbal and the Tróia peninsula, and it is the main habitat of a population of bottlenose dolphins, one of the fewest resident populations in Europe (Coelho, 2016; dos Santos & Lacerda, 1987). Since the 80's, several studies have been carried out in the region, about demography, ecology and ethology (Coelho, 2016; dos Santos *et al.*, 2007, 1995; dos Santos & Lacerda, 1987; Gaspar, dos Santos, & Lacerda, 2003; Martinho, Pereira, Brito, Gaspar, & Carvalho, 2014), as well as acoustic communication of this resident population. Acoustic studies explore specific sounds in the wide repertoire of the species, such as whistles (dos Santos *et al.*, 2005; Luís, Couchinho, & Dos Santos, 2015), pulsed sounds (Luís *et al.*, 2016) or bray emissions (dos Santos *et al.*, 1995; Luís & dos Santos, 2012; Luís *et al.*, 2017).

Concerning the conservation status, *Tursiops truncatus* is considered "least concern" by IUCN Red List (Hammond *et al.*, 2012), although the proximity to human activities exposes bottlenose dolphins to numerous threats, such as pollution, habitat alteration, boat traffic, interactions with commercial and recreational fisheries, including bycatch mortality, and dolphin watch harassment (Mann *et al.*, 2000). These factors, associated with apparent low recruitment, low reproductive rates and the ageing of the adults, present a great threat to the survival of resident populations, such as the one in Sado estuary (Gaspar, 2003; Mann *et al.*, 2000). This community used to be composed by, at least 40 dolphins, but is currently reduced to 27 dolphins (Augusto *et al.*, 2012; Coelho, 2016; dos Santos & Lacerda, 1987).

By describing the vocal repertoire of wild dolphins we are contributing to a better understanding of the function of dolphin vocalizations and their relation with the different activities. We can also be in a better position to evaluate the impacts of anthropogenic activities in the population and point out which ones are critical to the survival of a species or a population (Bearzi *et al.*, 2009; Gridley *et al.*, 2015).

Although the study of the acoustic emissions of bottlenose dolphins has been enriching to the understanding of the behaviour, it is still necessary to obtain quantitative correlations between sounds, behaviour activities and ecological circumstances (dos Santos *et al.*, 1990), especially for the pulsed sounds.

1.1 Objectives

This work intends to explore the acoustic repertoire of a small resident bottlenose dolphin population in Sado estuary, Portugal. Specifically, it aims to expand the knowledge concerning burst-pulsed signals, focusing in the bray series, by identifying the factors, or set of factors, that may influence the emission of this type of vocalization, as well as the behaviour contexts associated.

2. Materials and Methods

2.1. Study Area

The Sado estuary is located on the Western continental coast of Portugal, between the city of Setúbal, on the north shore, and the Peninsula of Tróia.

The river mouth is approximately 1.6 km wide. The estuary has two channels divided by sand and mud banks: the South Channel is wider and deeper (maximum depth 25 meters) allowing larger ships to enter the estuary, while the North Channel has shallower waters (depths between 10-15 meters).

The estuary presents a great ability to retain nutrients, with intense insolation which allows higher primary production rates and development of abundant and diversified food chains (Rocha, 2012). As a result, this estuary contains a variety of habitats and high biological richness. To protect the existing biodiversity and sources of biological productivity, in 1980, the upper estuary was established as protected area – The Sado Estuary Nature Reserve, figure 2.1 (Neves, Chozas, Costa, & Rufino, 2004).

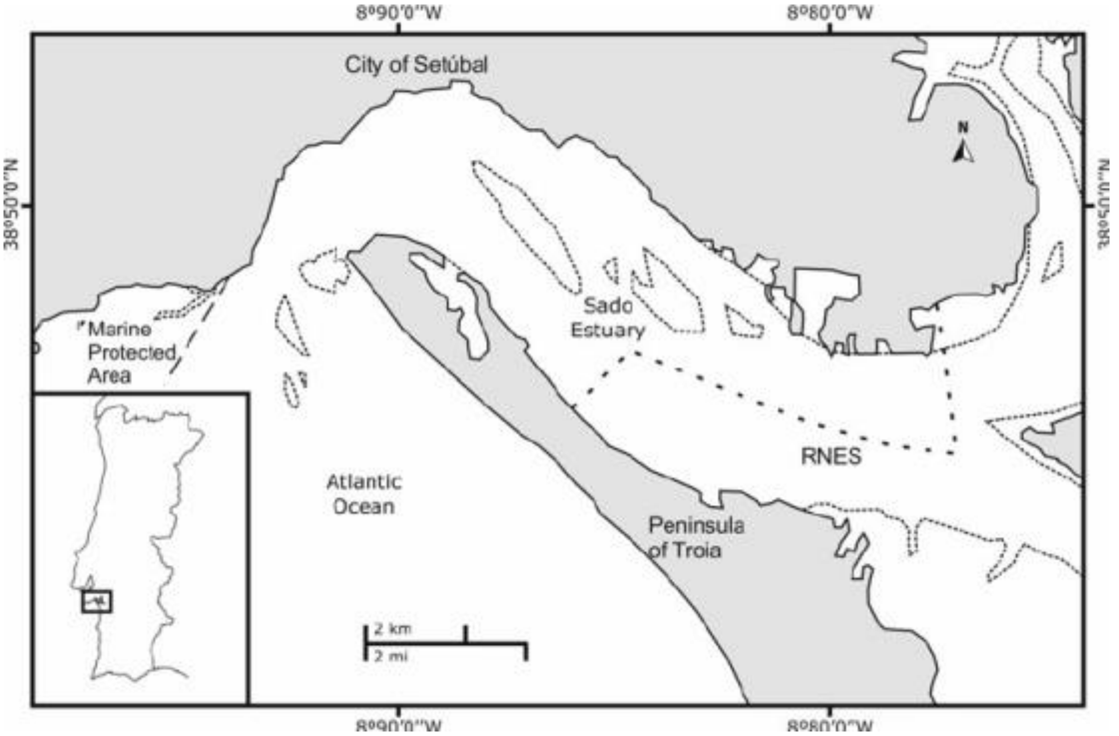


Figure 2.1 - Map of the study area, on the central western coast of Portugal. The small dotted lines represent mud banks and the broken lines represent Marine Protected Areas and RNES (in Augusto *et al.* 2012).

2.2. Data Collection

Field recordings were made in the Sado estuary and adjacent coastal waters from an 8.40 m inboard motor vessel during light hours and with sea state ranging from 0 to 3 on the Beaufort scale.

Data collection went from March 2014 to April 2017, with 6 hours and 7 min of recordings, totalizing 205 different samples, see figure 2.2. The boat-surveys were opportunistic. Once a group of dolphins was observed, a 15 minute habituation period was given.

Figure 2.2 - Sado Estuary map and sample points (2014 - 2017), © 2017 Google.

The recordings were carried out with the research boat positioned about 500 m ahead of the group's location, with the engines and batteries off. A Cetacean Research™ C55 hydrophone was placed at a depth of 5 m or, in case of shallower waters, 3 m. The hydrophone was connected to a Fostex FR-2 digital recorder (Figure 2.3) (effective sensitivity of −185 dB re 1V/1 µPa, frequency response of ±3 dB in the 0.020-44 kHz band and +3/−13 dB in the 0.009-100 kHz band, polarized by a 9 V battery), using a high-pass filter of 100 Hz to decrease the effect of noise generated by the recording platform and low-frequency vibrations. All recordings were made at a 192 kHz sampling rate and with a 24-bit resolution, with the recording level fixed at the calibrated settings and the trim level at −26 dB. The data was stored on Compact Flash memory cards as time-stamped wave files.

Figure 2.3 - Equipment and instrumentation.

While recording, information about GPS position and depth was collected from the research vessel. Additionally, two experienced observers, retrieved data about the dolphin's group composition, behavioural activity, surrounding vessels and other observations considered relevant.

Based on dos Santos, 1998 and dos Santos *et al.*, 2005, the behavioural activity was defined as follows:

Travelling – Constant movement of a group in one direction, with synchronized diving;

Foraging – Diversity of behaviours, characterised by sequences of short dives and surfacing in different directions;

Feeding – Dive pattern similar to foraging, but more localized, with fish tossing, feeding circles or feeding splashes on the water surface;

Socialization – Display of surface behaviour, animals engaging in physical contact with one another; absence of forward movement or prey;

Resting – Individuals close to each other at the surface, with very slow movements or drifting in one direction.

The following variables were selected:

Location – Inside or outside the estuary, using range marker number five (38°29'12.00"N; 8°55'21.60"W);

Vessels – Presence and absence of vessels, in a 1000 m radius;

Tide – Ebb tide and flood tide;

Activity – Feeding, foraging, travel or socialization;

Depth – Estimated using digital charts and the survey boat sounder;

Group Size – number of individuals in the focal group.u

2.3. *Acoustic Analysis*

Recordings were inspected by two trained independent observers, aurally and visually, using *Raven Pro 1.4* (Cornell Lab of Ornithology, Ithaca, NY) with Hann windows of 512 points Fast Fourier Transform (FFT), frequency resolution of 375 Hz and 50% overlap, in order to identify and classify the all vocalizations present in each recording.

Signals were assigned to one of the following pre-established categories, according to their graphical and aural characteristics (see appendix A):

I) Tonal sounds: whistles – narrow-band and modulated signals.

II) Pulsed sounds:

Click trains – discernible click trains, broad band and short duration;

Creaks – long burst-pulse (>0.2 sec.), aurally similar to a creaking door;

Squawks – long burst-pulse (>0.2 sec.), with higher repetition rate than “Creaks”, identical to a crying baby;

TTV – Variable Rate Click Trains, may contain discernible clicks, creaks and squawks with high repetition rate;

Short Burst-Pulses (S-BP) – short burst-pulse (<0.2 sec.), aurally similar to a buzzing bee but brief;

Bangs – isolated high energy pulsed sound;

Grunts – trains of intense burst-pulses, acoustically similar to pig grunts;

Gulps – low-frequency, short-pulse, identical to a sob;

Squeaks – short burst-pulse with a harmonic structure, shrill sound like a scream;

Bray series – rhythmic vocalization composed by different pulsed sounds, such as, gulps, grunts and squeaks, with a variable composition and structure.

All the identified sounds were rated based on signal-to-noise ratio (SNR): 1 – poor-signal and hardly visible on the spectrogram, 2 – fair-signal, visible and with a clear start/end on the spectrogram, and 3 – good-signal, well-marked and with a clear start/end on the spectrogram.

Sounds that compose the brays series, grunts, gulps and squeaks with a SNR of 2 and 3 were selected for further analysis.

Each bray sequence was defined using a 0.6 s bout criterion interval (BCI), to separate the end of the first sequence and the beginning of a second one (Luís, *in prep.*), see appendix B for more information.

2.4. Statistical Analyses

For the analyses of vocalization occurrences, emission rates were computed dividing the number of vocalizations by the number of minutes of each sample.

A Generalized Linear Model (GLM) with a Binomial Negative Regression Model was used to predict the expected value of bray emission rate (the response variable) as a linear combination of a set of observed variables (the predictors), i.e. to determine which predictors had a significant effect on the bray emissions.

The statistics analyses were made in RStudio, Inc., using the nortest, fBasics, MASS and pscl packages.

For more information see appendix C.

3. Results

During the boat surveys, 205 recording samples were collected, totalling 367.15 minutes of sound recordings. Fourty four recordings contained brays series rated as fair or good, in a total of 88.25 minutes (see Table 3.1).

Table 3.1 - Number of recordings, number of brays recordings and total of recording minutes for each year.

Year	Recordings	Recordings with Brays	Total Minutes	Total Bray Minutes
2014	69	22	121.98	42.21
2015	59	11	136.19	31
2016	38	4	56.58	6.45
2017	39	7	52.41	8.59
Total	**205**	**44**	**367.16**	**88.25**

3.1. Vocal Elements

Brays series' elements were common elements in 2014-2017 recordings: Gulps (12%), Grunts (11%) and Squeaks (5%).

Click Trains (38%) and Whistles (21%) were the most frequent vocalizations, and short burst-pulses (<1%) were the least frequent, see Figure 3.1.

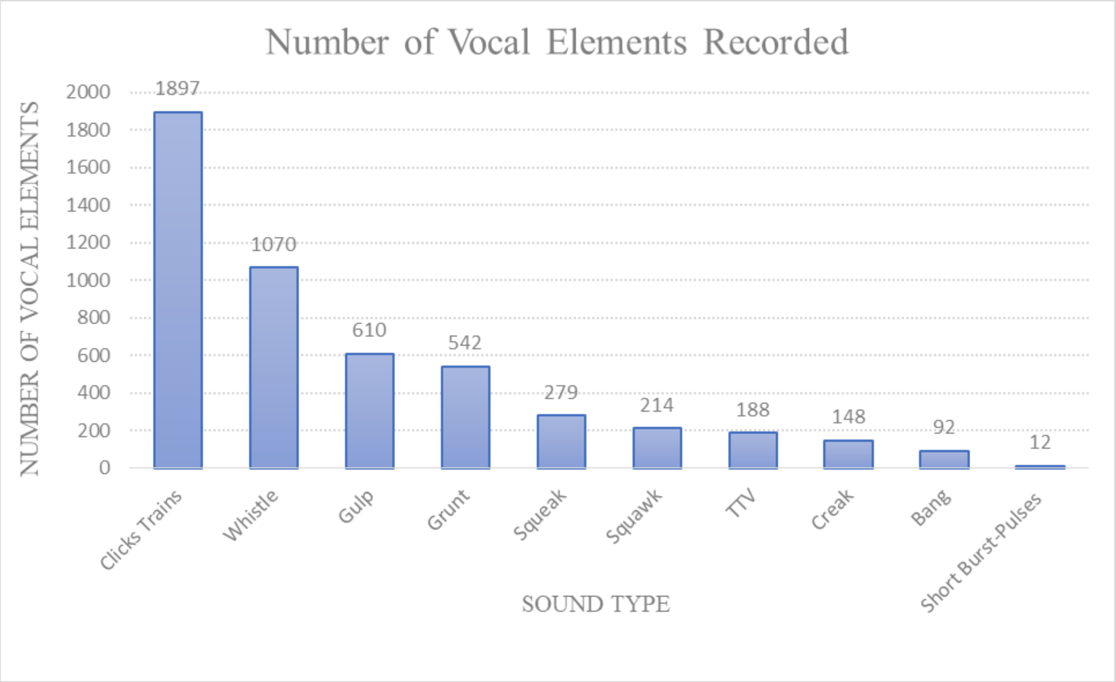


Figure 3.1 – Total number of vocal elements emitted in 2014 – 2017.

Gulps, grunts and squeaks were recorded isolated and as part os bray sequences (Figure 3.2).

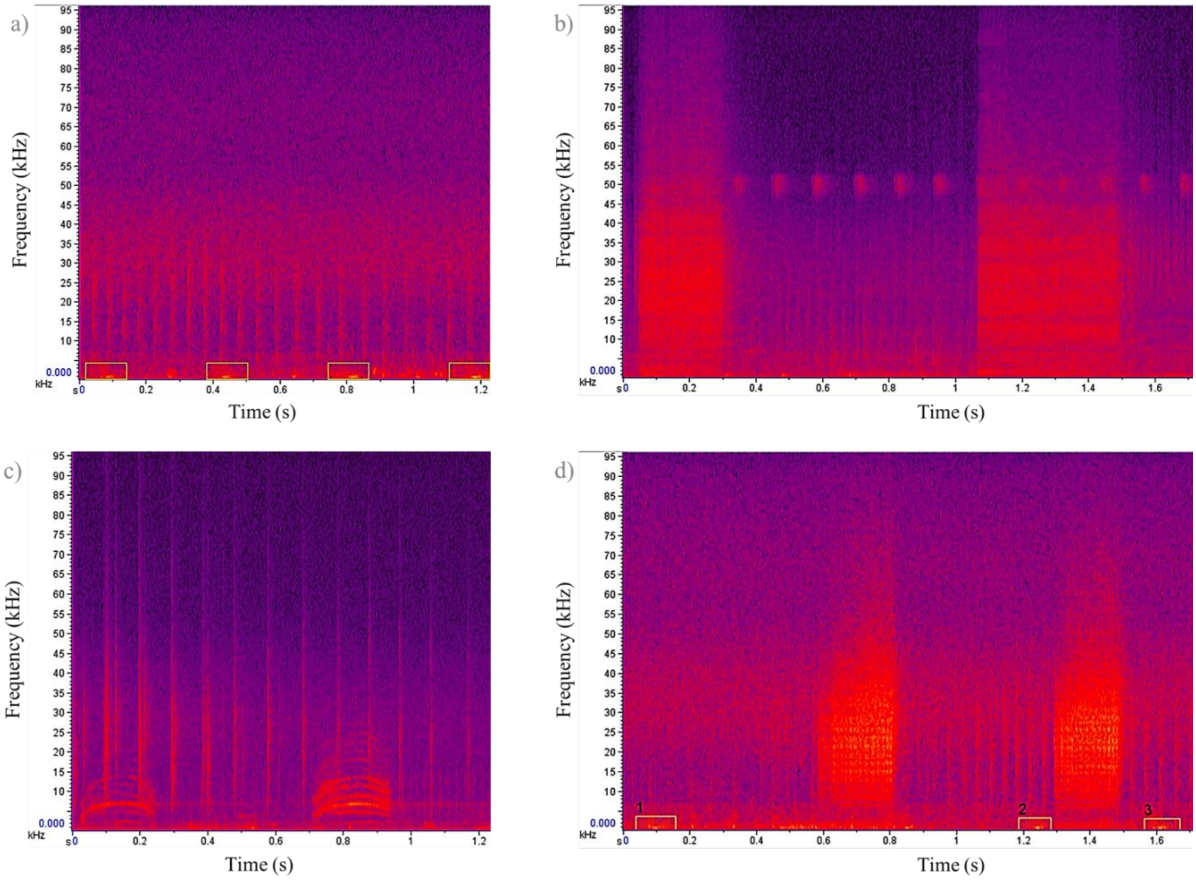


Figure 3.2 - Examples of bray series' elements produced by bottlenose dolphins: a) Gulps (inside the yellow squares), b) Grunts and c) Squeaks. d) An example of a bray sequence. Panels show spectrograms for each signal type, with frequency (kHz) on the y-axis (0 – 96 kHz). Time (s) is on the x-axis. Spectrogram settings: FFT 512, Hann window, overlap 50%.

3.2. Emission Rates

The vocal elements that compose bray sequences were not frequently emitted isolated (Figure 3.3).

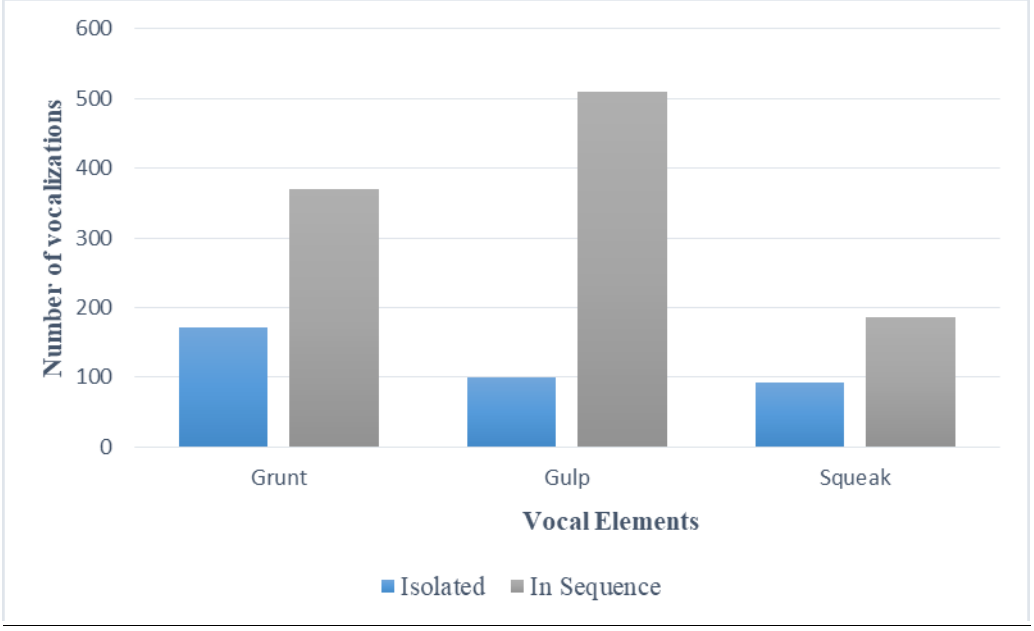


Figure 3.3 - Bray Series Elements, emitted singly and in sequence.

The emission rate of bray sequences did not follow a normal distribution (The Lilliefors (Kolmogorov-Smirnov) normality test, p-value < 0.001) and included an excess of zeros, with 0.75±1.97 mean and 3.9 variance. Therefore, the model that best fit the data series was Negative Binomial Distribution as shown in figure 9, according to the Akaike Criteria (AIC) – 351.7. (For more details see the Appendix C)

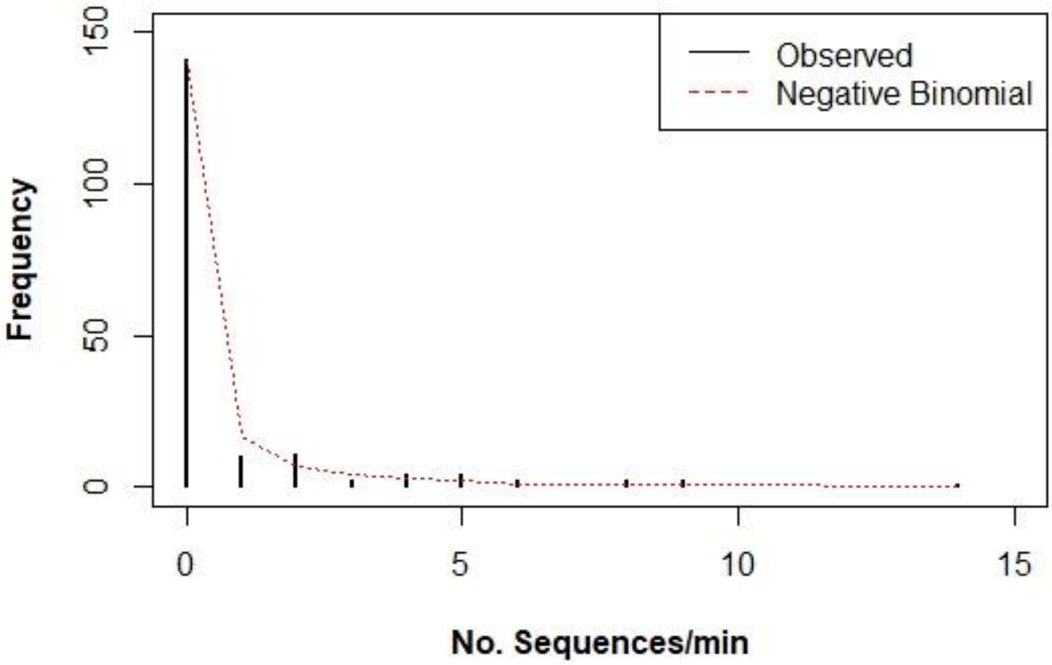


Figure 3.4 - Frequency distribution Number of brays sequences emitted.

The independent variables explored in this study are described in table 3.2. From the 205 recordings, 179 were used that had all the defined variables.

Table 3.2 - Independent variables Summary.

Variables	Description
Group Size	min = 1; max = 20; mean = 8.34; SD = 4.29
Depth	min = 2.7; max = 28.8; mean = 11.65; SD = 5.06
Location	0 – inside the estuary (85.5%) 1 – outside the estuary (14.5%)
Activity	1 – Travelling (55.9%) 2 – Foraging (26.2%) 3 – Feeding (11.2%) 4 – Socialization (6.7%) 5 – Resting (0%)
Vessels	0 – No vessels present in a 1000 m radius (51.4%) 1 – At least one vessel present in a 1000 m radius (48.6%)
Tide	0 – Low Tide (39.1%) 1 – High Tide (60.9%)

According to the GLM, group size, depth, location, vessels and tide were not associated with the emission rate of bray series (Table 3.3). The only variable that was a significant predictor of bray occurences was activity pattern.

Foraging, feeding and socialization showed significant higher counts than travelling. The expected count for foraging was 1.63 higher than the expected count for travelling (the reference group), feeding activity is 2.13 higher and socialization activity is 2.06 higher (see Table 3.3 below).

Table 3.3 – Generalized Linear Model Results.

Variables	***Coefficients Estimate ($\widehat{\beta}$) mean ± SD***		***P-values***
Group Size	0.0280 ± 0.0497		0.573
Depth	-0.00024 ± 0.0479		0.996
Location	0.163 ± 0.644		0.80007
Activity	Foraging	1.633 ± 0.514	0.00150 **
	Feeding	2.131 ± 0.693	0.00211 **
	Socialization	2.055 ± 0.812	0.0114 *
Vessels	0.188 ± 0.436		0.666
Tide	-0.135 ± 0.443		0.762

Note: ‘ ** ’ p – value < 0.001; ‘ * ’ p – value < 0.01

The feeding and socialization activities had the highest bray emission rate and the activity travelling had the lowest (Figure 3.5).

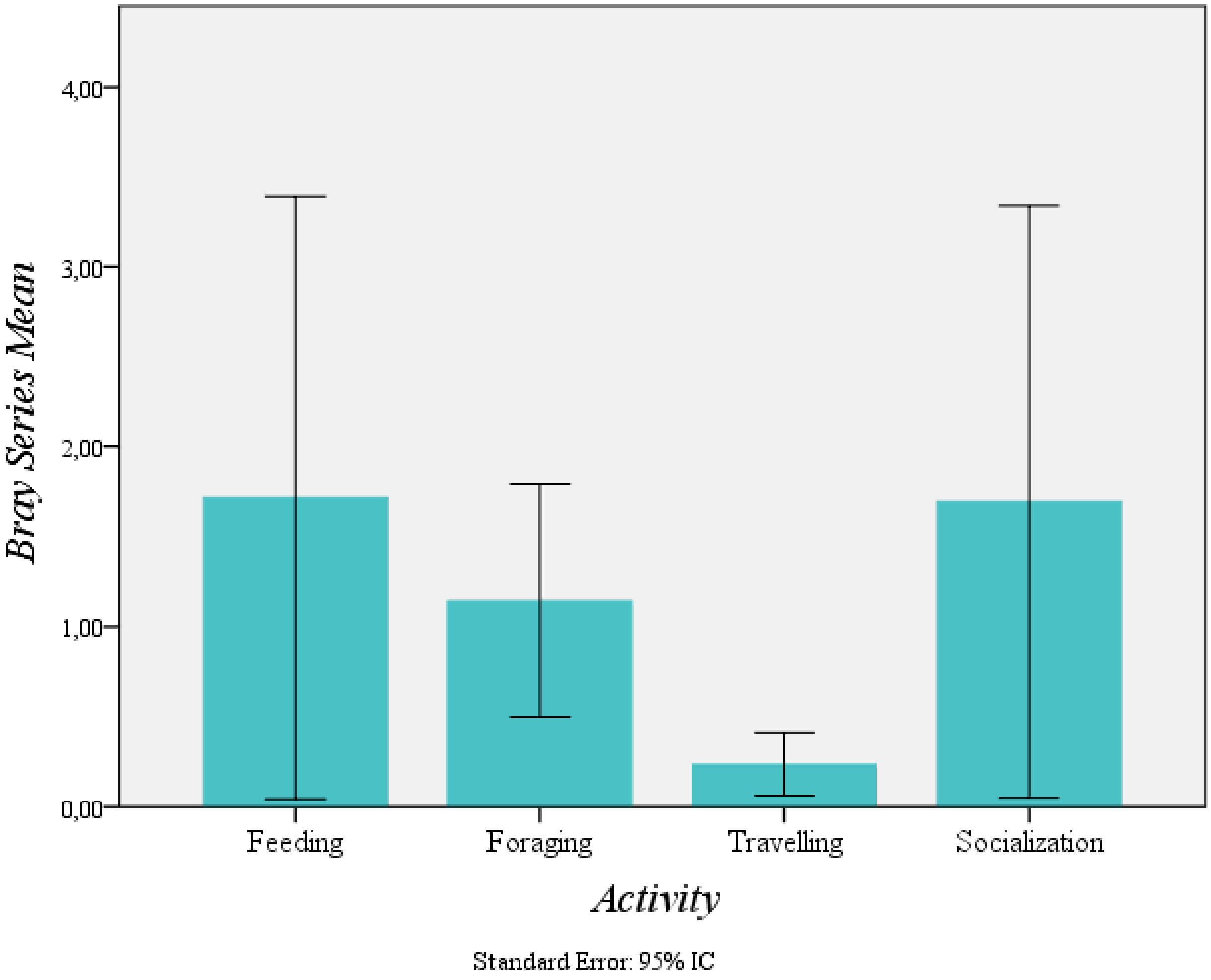


Figure 3.5 - Bray Series emission in each activity with standard error bars of means.

4. Discussion

Bottlenose dolphins are social animals, characterized by a fission–fusion dynamic social structure in which communication is a critical key to the formation of alliances. These animals present an extensive vocal repertoire that can be divided in tonal sounds, echolocation clicks and burst-pulsed signals. The vocalizations can have multiple context-specific functions and studies on cetacean sound production suggest a signal and functional flexibility (Deharveng & Bedos, 2012; Gridley *et al.*, 2015; Simard *et al.*, 2011).

In this study we have focused on the environmental influences and behavioural context of the emission of bray series. The results showed that group size, depth, location, vessels and tide had no effect on the emission rate of bray series, only the activity pattern is significantly related to the number of bray sequences.

Echolocation clicks and whistles are the most frequent vocalizations produced by dolphins. This can be related to the fact that these animals, while in foraging activities, are able to emit whistles and sonar clicks simultaneously, possibly communicating the presence of food to other dolphins (King & Janik, 2015; Ridgway, Samuelson Dibble, Van Alstyne, & Price, 2015).

Previous studies show that whistles often occur in all activity patterns, especially when involving a high level of arousal. The primary function of this signal is communication and it plays an important role in maintaining contact between dispersed individuals of a population (dos Santos *et al.*, 1990; Janik, 2000a; López & Shirai, 2009; Luís *et al.*, 2015). Click Trains are used as bio-sonar orientation and target detection signals. These sounds are also present in all activities, having a major role in the perception of the environment and prey location. The Sado estuary usually has low underwater visibility, and dolphin would be expected to rely more on active echoloction for prey location and capture (dos Santos *et al.*, 1990; Herzing & dos Santos, 2004).

The burst-pulses signals grunt, gulp and squeak are common vocal elements and frequently occur in bouts, forming the brays series. Temporal patterning of vocal elements in brays sequences is poorly defined in the literature (Gridley *et al.*, 2015). This study used a bout criterion interval in order to define a bray sequence, allowing a quantitative approach.

The functional role of this conspicuous vocalization has yet to be fully understood, though in this study it was possible to observe that dolphins emit bray series more frequently in several behavioural patterns, such as feeding, foraging, and socialization, and less in travelling. Brays are present in several populations of bottlenose dolphins. Janik (2000a), suggested that the low – frequency structure of the brays in the dolphin's population in Moray Firth, NE Scotland, occurs within a feeding context, primarily on the prey manipulation of a large fish such as Atlantic salmon (*Salmo salar*). Hastie *et al.* (2006) study also came to the same conclusion, when the dolphins surface with large fish during the bray emissions.

King & Janik (2015), associated the bray emissions, considered food-related calls, with whistles, suggesting a social component and, in the absence of signs of aggression, that these calls are affiliative and helps in transmitting information to conspecifics.

Another study, in the Bahamas, showed that brays occurred during social behaviours, specifically during male fighting and female persecution, suggesting a competitive/agonistic context (Herzing, 2015).

Bray series are a recurrent vocalization type of the Sado estuary bottlenose dolphin population and have been reported in several studies since described by dos Santos *et al.* (1990) (dos Santos *et al.*, 1995; Luís & dos Santos, 2012; Luís *et al.*, 2017; Rocha, 2012).

Based on previous studies of this population (Luís & dos Santos, 2012), higher emission rates were expected during feeding and foraging activities, however it was not possible to determine if it is a cue to conspecifics as suggested by King & Janik (2015) or as manipulating prey behaviour (Janik, 2000a).

Bray sequences are frequently composed by grunts that have been described as aggressive calls (Herzing, 2015; Luís & dos Santos, 2012). The data did not allow to establish bray emissions as an agonistic display during socialization or as a competitive behaviour during feeding when the target prey is not abundant. However, it certainly remains possible that these emissions are mostly agonistic.

Bray series emissions are lowest during travelling activities. This result was expected since this is low interaction activity and dolphins often move in silence (dos Santos *et al.*, 1990).

Each bray sequence presents a variable structure and composition, not all element are present and the order is mutable (Deharveng & Bedos, 2012; dos Santos *et al.*, 1995; Santos, Luís, & Couchinho, 2013). The different combinations could indicate that this type of sound is used in multiple contexts in the wild (Herzing, 2015). The ecological and social contexts where the bray emissions were higher are more complex and have a more diversified motivational states dynamic, than the context of travelling behaviour (dos Santos *et al.*, 2005).

Previous studies found that in the presence of vessels, dolphins may change their emission rates, (Buckstaff, 2004; Luís, Couchinho, & dos Santos, 2014; Rocha, 2012). Vessel noise propagates well underwater, within hearing range of the dolphins, and dolphins in the estuary are constantly exposed to boat traffic, which could make it possible for dolphins to predict the vessels course (Lusseau, 2006). Lemon *et al.* (2006), revealed that dolphins in Jervis Bay, Australia, change their surface behaviour in nearby powerboats, however it did not appear to have an impact in their acoustic behaviour. It might be that coastal dolphins were able to detect incoming vessels at varying distances and adapted their behaviour accordingly. In this study, significant differences in bray series emission were not found in the presence of nearby vessels, a possible explanation could be due to fact that dolphins were able to detect incoming vessels or that episodes with bray series emission are motivationally intense, and dolphins ignore passing vessels, even though they were aware of any vessel in their immediate environment.

Philpott *et al.* (2007) found that acoustic emissions of a bottlenose dolphin’s population in Shannon estuary, Ireland, were associated to tidal state, with more acoustic emissions in the 4 h after high water, possibly reflecting prey-related changes in habitat use. These results differ from observations in the Sado estuary population, which may represent a geographic variation in the diet (Deharveng & Bedos, 2012; Hanson & Defran, 1993). Another explanation could be the fact that the study in Shannin estuary only considers one type of vocalization and not the whole repertoire.

Hanson & Defran (1993) study found that the dolphins they studied in the San Diego area spent more time feeding in the estuary and in shallower waters, where it might be easier to find and capture prey, like the Sado estuary population (dos Santos *et al.*, 2007). Whereas feeding is an arousal activity, which could explain higher emissions of the characteristics vocal elements, such as bray series. It was expected that the number of bray sequences would change depending on whether the dolphins were inside or outside of the estuary. However there was no evidence of this pattern, maybe beacause most of recordings (85%) were made inside the estuary, so it is possible that the variable location was not a significant predictor of bray emissions.

Some studies revealed that dolphins produce more vocalizations when group size increases and, may also depend on the behavioural activity (Henderson, Hildebrand, & Smith, 2011; Nowacek, 2005). In our study, there was no evidence that this happens with bray vocalizations, as was reported for the same population by similar studies, such as Luís & dos Santos (2000). The high levels of background noise may be related to this result. In a noisy habitat, like Sado estuary, each individual may limit its vocal emissions to avoid overlapping and to facilitate the transmittion of the emitted signals, improving the improve communication efficiency (Luís et al., 2015).

Analysing the influence of depth in the emission rate of brays, Hastie *et al.* (2006) observed that dolphins were braying during feeding events, as diving from the surface to depths close to the seabed (mostly between 20 and 30 meters), and were subsequently returning to the surface after the brays. In the present study the described behaviour was not observed, maybe due to the fact that depth was estimated using the boat sounder. In order to retrieve a more accurate conclusion about the depths at which brays were emitted, the method described in Hastie *et al.* (2006) consisting in a vertical array of hydrophones in the water column, to reveal more detailed depth distribution of dolphin vocalisations, could be applied.

4.1. Final Remarks

Bottlenose dolphins exhibit large degrees of behavioural plasticity, both within and between populations, including differences in the vocal repertoires. Not all population vocal emissions are affected by the same variables, such as the ones studied in the present project.

Bray series are a conspicuous component of bottlenose dolphins' repertoire. This quantitative analysis shows significant differences in bray series emission between arousal events, such as socialization, feeding and foraging, and calm activities, like travelling.

Future studies should acquire more data on feeding, foraging and socialization activities, to understand if bray emissions are more related to affiliative or agonistic functions, as well as to study the association of these calls with other vocalizations. A more detailed analysis of behavioural context using simultaneous underwater video and acoustic recording equipment could be a valuable advantage to shed light on the functionality of the bray series, although the application of this method in the Sado estuary is difficult due to the low visibility. Other variables that were not possible to add to this study, such as prey abundance, could be a key to a better understanding about the influence of environmental factors and should be explored in future studies.

5. References

Au, W. W. L., & Hastings, M. C. (2008). *Principles of Marine Bioacoustics.* Noise Control Engineering Journal (Vol. 58). New York, NY: Springer US. http://doi.org/10.1007/978-0-387-78365-9

Augusto, J. F., Rachinas-Lopes, P., & dos Santos, M. E. (2012). Social structure of the declining resident community of common bottlenose dolphins in the Sado Estuary, Portugal. *Journal of the Marine Biological Association of the United Kingdom,* 92, 1–10. http://doi.org/10.1017/S0025315411000889

Bearzi, G., Fortuna, C. M., & Reeves, R. R. (2009). Ecology and conservation of common bottlenose dolphins *Tursiops truncatus* in the Mediterranean Sea. *Mammal Review, 39*(2), 92–123. http://doi.org/10.1111/j.1365-2907.2008.00133.

Bispo, R., & Pinto, F. (2008). *Modelação de contagens com excesso de zeros : aplicação à análise do absentismo no trabalho 1 Introdução 2 Descrição dos dados*. Paper presented at Actas Do XVI Congresso Anual Da SPE, Portugal.

Buckstaff, K. C. (2004). Effects of watercraft noise on the acoustic behaviour of bottlenose dolphins, *Tursiops truncatus,* in Sarasota Bay, Florida. Marine Mammal Science, 20(4), 709–725. http://doi.org/10.1121/1.2799904

Caldwell, M.C. & Caldwell, D.K. (1965) Individualized whistle contours in bottlenose dolphins (*Tursiops truncatus*). *Nature, 207,* 434-435.

Coelho, A. M. (2016). *Analysis of group size, composition and activities, of bottlenose dolphin (*Tursiops truncatus*) in the region of Sado estuary* (Master's thesis, ISPA – Instituto Universitário de Ciências Psicológicas, Sociais e da Vida).

Connor, & Krützen. (2015). Male dolphin alliances in Shark Bay: changing perspectives in a 30-year study. *Animal Behaviour, 103,* 223–235. http://doi.org/10.1016/j.anbehav.2015.02.019

Connor, R. C., Heithaus, M. R., & Barre, L. M. (2001). Complex social structure, alliance stability and mating access in a bottlenose dolphin "super-alliance." *Proceedings of the Royal Society B: Biological Sciences, 268,* 263–267. http://doi.org/10.1098/rspb.2000.1357

Deharveng, L., & Bedos, A. (2012). Diversity Patterns in the Tropics. In White, W.B. & Culver, D.C. (Eds.) *Encyclopedia of Caves* (pp. 238–250). Elsevier. http://doi.org/10.101-6/B978-0-12-383832-2.00032-3

dos Santos, & Lacerda. (1987). Preliminary observations of the bottlenose dolphin (*Tursiops truncatus*) in the Sado estuary (Portugal). *Aquatic Mammals, 13*.2, 65–80.

dos Santos, M.E., Caporin, G., Moreira, H.O., Ferreira, A., Coelho, J.L.B. (1990) Acoustic behavior in a local population of bottlenose dolphins. In Thomas, J.A. & Kastelein, R.A. (Eds.), *Sensory Abilities of Cetaceans: Laboratory and Field Evidence, 196*. Boston: Springer (pp 585–598).

dos Santos, M.E., Ferreira, A.J. & Harzen, S. (1995) Rhythmic sound sequences emitted by aroused bottlenose dolphins in the Sado estuary, Portugal. In Kastelein, R.A., Thomas, J.A. & Nachtigall, P.E. (Eds.), *Sensory Systems of Aquatic Mammals.* Woerden: De Spil Publishers.

dos Santos, M. E., Louro, S., Couchinho, M., & Brito, C. (2005). Whistles of Bottlenose Dolphins (*Tursiops truncatus*) in the Sado Estuary, Portugal: Characteristics, Production Rates, and Long-Term Contour Stability. *Aquatic Mammals, 31*(4), 453–462. http://doi.org/10.1578/AM.31.4.2005.453

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

dos Santos, M. E., Luís, A. R., & Couchinho, M. N. (2013). *Beyond Describing Complex Repertoires : The immediate functions of underwater acoustic emissions of bottlenose dolphins*. Poster presented at 10th National Congress of Ethology, at Lisbon, Portugal.

Gaspar, R. (2003) *Status of the Resident Bottlenose Dolphin Population in the Sado Estuary: Past, Present and Future.* (Doctoral dissertation, University of St. Andrews).

Gridley, T., Nastasi, A., Kriesell, H. J., & Elwen, S. H. (2015). The acoustic repertoire of wild common bottlenose dolphins (*Tursiops truncatus*) in Walvis Bay, Namibia. *Bioacoustics, 24*(2), 153–174. http://doi.org/10.1080/09524622.2015.1014851

Hammond, P. S., Bearzi, G., Bjørge, A., Forney, K. A., Karkzmarski, L., Kasuya, T., Wilson, B. (2012). *Tursiops truncatu*s. In The IUCN Red List of Threatened Species. http://doi.org/http://dx.doi.org/10.2305/IUCN.UK.2012.RLTS.T22563A17347397.en.

Hanson, M., & Defran, R. (1993). The behavior and feeding ecology of the Pacific coast bottlenose dolphin, *Tursiops truncatus*. *Aquatic Mammals*, *19*(3), 127-142.

Harley, H. E. (2008). Whistle discrimination and categorization by the Atlantic bottlenose dolphin (*Tursiops truncatus*): A review of the signature whistle framework and a perceptual test. *Behavioural Processes, 77*(2), 243–268. http://doi.org/10.1016/j.beproc.2007.11.002

Hastie, G. D., Wilson, B., & Thompson, P. M. (2006). Diving deep in a foraging hotspot: acoustic insights into bottlenose dolphin dive depths and feeding behaviour. *Marine Biology, 148*(5), 1181–1188. http://doi.org/10.1007/s00227-005-0143-x

Henderson, E. E., Hildebrand, J. A., & Smith, M. H. (2011). Classification of behavior using vocalizations of Pacific white-sided dolphins (*Lagenorhynchus obliquidens*). *The Journal of the Acoustical Society of America, 130*(1), 557–567. http://doi.org/10.1121/1.3592213

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

Herzing, D.L. (2000) Acoustics and Social Behavior of Wild Dolphins: Implications for a Sound Society In Au, W. W. L., Popper, A. N., & Fay, R. R. (Eds.) *In Hearing By Whales and Dolphins* (pp. 225–272). New York.

Herzing, D. L., & dos Santos, M. E. (2004). Functional Aspects of Echolocation in Dolphins. In In Thomas, J.A., Moss, C.F. & Vater, M. (Eds.), *Echolocation in Bats and Dolphins.* (pp. 386–393).

Herzing, D. L. (2015). Synchronous and Rhythmic Vocalizations and Correlated Underwater Behavior of Free-ranging Atlantic Spotted Dolphins (*Stenella frontalis*) and Bottlenose Dolphins (*Tursiops truncatus*) in the Bahamas. *Animal Behavior and Cognition, 2*(1), 14–29. http://doi.org/10.12966/abc.02.02.2015

Janik, V. M. (2000 a). Food-related bray calls in wild bottlenose dolphins (*Tursiops truncatus*). *Proceedings of the Royal Society B: Biological Sciences, 267*(1446), 923–927. http://doi.org/10.1098/rspb.2000.1091

Janik, V. M. (2000 b). Whistle Matching in Wild Bottlenose Dolphins (*Tursiops truncatus*). *Science, 289*(5483), 1355–1357. http://doi.org/10.1126/science.289.5483.1355

King, S. L., & Janik, V. M. (2015). Come dine with me: food-associated social signalling in wild bottlenose dolphins (*Tursiops truncatus*). *Animal Cognition, 18*(4), 969–74. http://doi.org/10.1007/s10071-015-0851-7

Lemon, M., Lynch, T.P., Cato, D.H. & Harcourt, R.G. (2006). Response of travelling bottlenose dolphins (*Tursiops aduncus*) to experimental approaches by a powerboat in Jervis Bay, New South Wales, Australia. *Biological Conservation, 127*(4), 363-372.

López, B. D., & Shirai, J. A. B. (2009). Mediterranean Common Bottlenose Dolphin's Repertoire and Communication Use. In A. G. Pearce & L. M. Correa (Eds.), *Dolphins: Anatomy, Behavior and Threats* (pp. 1–20). © 2009 Nova Science Publishers, Inc.

Luís, A. R., & dos Santos, M. E. (2012). *Why are bottlenose dolphins banging and braying when toadfish are singing?*. Poster presented at 26th European Cetacean Society Conference, at Galway, Ireland

Luís, A. R., Couchinho, M. N., & dos Santos, M. E. (2014). Changes in the acoustic behavior of resident bottlenose dolphins near operating vessels. *Marine Mammal Science, 30*(4), 1417–1426. http://doi.org/10.1111/mms.12125

Luís, A. R., Couchinho, M. N., & Dos Santos, M. E. (2015). Signature whistles in wild bottlenose dolphins: long-term stability and emission rates. *Acta Ethologica*, 1–10. http://doi.org/10.1007/s10211-015-0230-z

Luís, A. R., Couchinho, M. N., & dos Santos, M. E. (2016). A Quantitative Analysis of Pulsed Signals Emitted by Wild Bottlenose Dolphins. *PLOS ONE, 11*(7). http://doi.org/10.1371/journal.pone.0157781

Luís A. R., Alves, I. S., Couchinho, M. N., & dos Santos, M. E. (2017). *Brays and Bits: Information Theory Applied to Vocal Sequences of Bottlenose Dolphins*. Poster presented at 35th International Ethological Conference (IEC).

Lusseau, D. (2006). The short-term behavioral reactions of bottlenose dolphins to interactions with boats in Doubtful Sound, New Zealand. *Marine Mammal Science, 22*(4), 802–818. http://doi.org/10.1111/j.1748-7692.2006.00052.

Mann, J., Connor, R. C., Tyack, P. L., & Whitehead, H. (2000). *Cetacean Societies: field studies of dolphins and whales.* The University of Chicago Press.

Martinho, F., Pereira, A., Brito, C., Gaspar, R., & Carvalho, I. (2014). Structure and abundance of bottlenose dolphins (*Tursiops truncatus*) in coastal Setúbal Bay, Portugal. *Marine Biology Research,* (July), 1–13. http://doi.org/10.1080/17451000.2014.894244

Neves, R., Chozas, S., Costa, L. T., & Rufino, R. (2004). *Reserva Natural do Estuário do Sado, uma contribuição para o plano de gestão. Instituto da Conservação da Natureza/ Centro de Zonas Húmidas.*

Norris, K. S. (1967). The echolocation of marine mammals. In H. T. Andersen (Ed.), *The Biology of Marine Mammals* (pp. 391–423). Academic Press, New York.

Nowacek, D. P. (2005). Acoustic ecology of acoustic ecology of foraging Bottlenose Dolphins (*Tursiops truncatus*), habitat - specific use of three sound types. *Marine Mammal Science, 21*(4), 587–602. http://doi.org/10.1111/j.1748-7692.2005.tb01253.x

Philpott, E., Englund, A., Ingram, S., & Rogan, E. (2007). Using T-PODs to investigate the echolocation of coastal bottlenose dolphins. Journal of the Marine Biological Association of the UK, 87(1), 11. http://doi.org/10.1017/S002531540705494X

Quintana-Rizzo, E., Mann, D. A., & Wells, R. S. (2006). Estimated communication range of social sounds used by bottlenose dolphins (*Tursiops truncatus*). *Journal of the Acoustical Society of America, (120*), 1671 – 1683.

Reynolds III, J. E., Wells, R. S., & Eide, S. D. (2000). T*he Bottlenose Dolphin. Biology and Conservation*. University Press of Florida.

Ridgway, Samuelson Dibble, D., Van Alstyne, K., & Price, D. (2015). On doing two things at once: dolphin brain and nose coordinate sonar clicks, buzzes and emotional squeals with social sounds during fish capture. *Journal of Experimental Biology, 218*(24), 3987–3995. http://doi.org/10.1242/jeb.130559

Rocha, A.I.L.F.B. (2012) *Vocalizações dos golfinhos-roazes (*Tursiops truncatus*) residentes na região do estuário do Sado e estudo da influência do tráfego marítimo nos padrões de emissão dos assobios*. (Master's thesis, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto).

Rose, C. E., Martin, S. W., Wannemuehler, K. A., & Plikaytis, B. D. (2006). On the use of zero-inflated and hurdle models for modeling vaccine adverse event count data. *Journal of Biopharmaceutical Statistics*, *16*(4), 463–481. http://doi.org/10.1080/10543400600719384

Scott, M. D., & Chivers, S. J. (1989). Distribution and Herd Structure of Bottlenose Dolphins in the Eastern Tropical Pacific Ocean. In Leatherwood, S. & Reeves, R.R (Eds.) *The Bottlenose Dolphin* (pp. 387–402). Elsevier. http://doi.org/10.1016/B978-0-12-440280-5.50026-3

Sibly, R. M., Nott, H. M. R., & Fletcher, D. J. (1990). Splitting behaviour into bouts. *Animal Behaviour*, *39*(1), 63–69. http://doi.org/10.1016/S0003-3472(05)80726-2

Simard, P., Gowans, S., Quintana-rizzo, E., Ii, S. A. K., Wells, R. S., & Mann, D. A. (2011). Low frequency narrow-band calls in bottlenose dolphins (*Tursiops truncatus*): Signal properties, function, and conservation implications. http://doi.org/10.1121/1.3641442

van der Woude, S. E. (2009). Bottlenose dolphins (*Tursiops truncatus*) moan as low in frequency as baleen whales. *Journal of the Acoustical Society of America, 126*(3), 1552. http://doi.org/10.1121/1.3177272

Wells, R. S., & Scott, M. D. (1999). Bottlenose dolphin *Tursiops truncatus* (Montagu, 1821). In S. H. Ridgway & R. Harrison (Eds.), *Handbook of Marine Mammals* (pp. 137–182). San Diego: Academic Press.

Wells, R.S. & Scott, M.D. (2008) Common Bottlenose Dolphin *Tursiops truncatus*. In Perrin, W.F., Würsig, B. & Thewissen J.G.M. (Eds.), *Encyclopedia of Marine Mammals.* London: Academic Press. (pp. 249-255).

Zeileis, A., Kleiber, C., & Jackman, S. (2008). Regression models for count data in R. *J Stat Softw*, *27*(8), 1–25. http://doi.org/10.18637/jss.v027.i08

6. Appendices

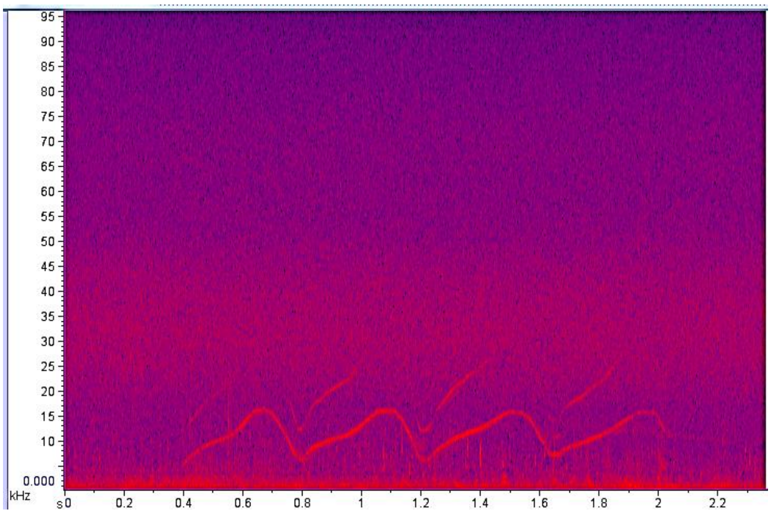
Appendix A

Sado estuary Bottlenose Dolphins Vocal Repertoire

Panels show spectrograms for each signal type, with frequency (kHz) on the y-axis and time (s) on the x-axis. Spectrogram settings: Hann window and overlap 50%.

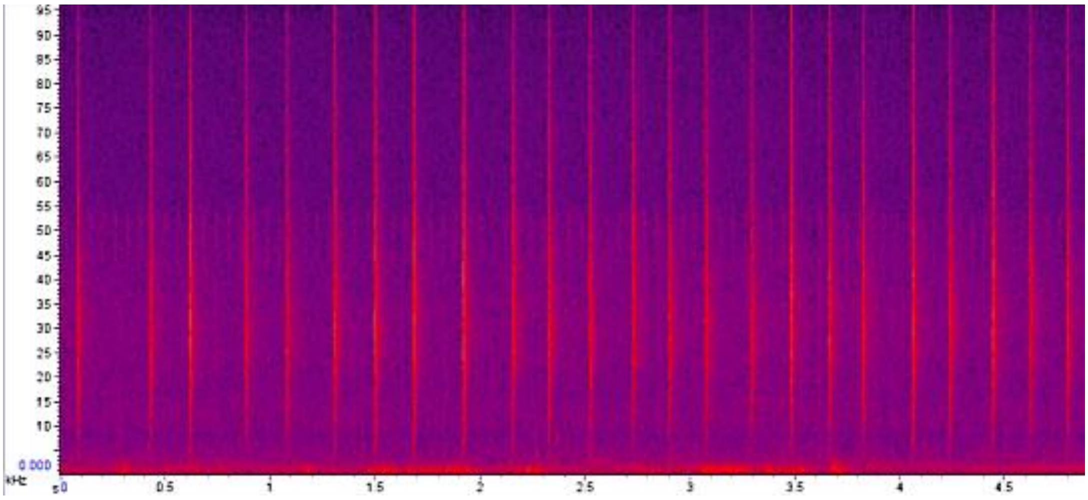
Tonal sounds

Whistles – narrow-band and modulated signals (512 points);

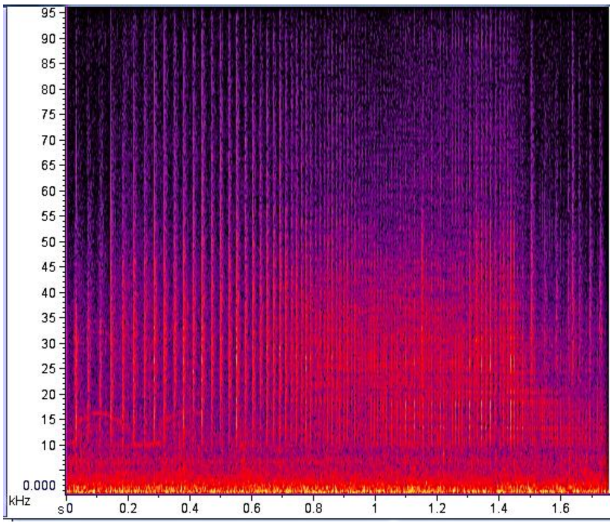


Pulsed sounds

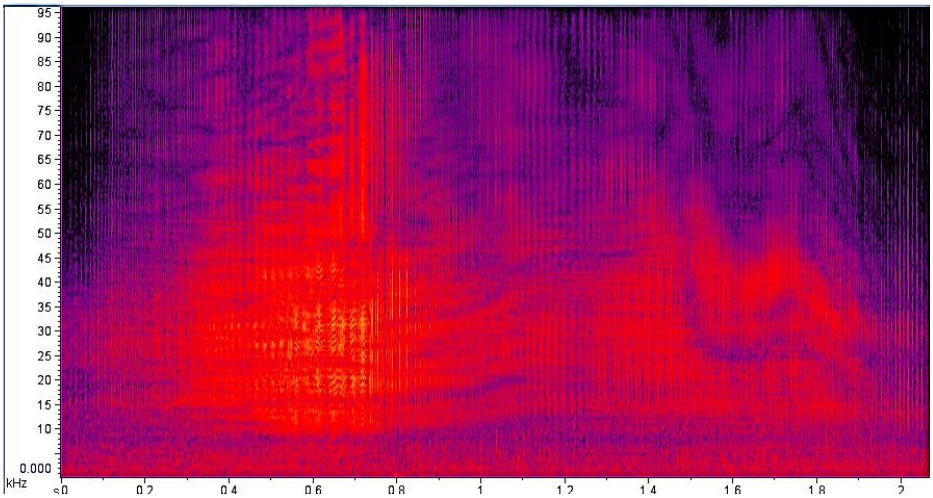
Click trains – discernible click trains, broad band and short duration (512 points);



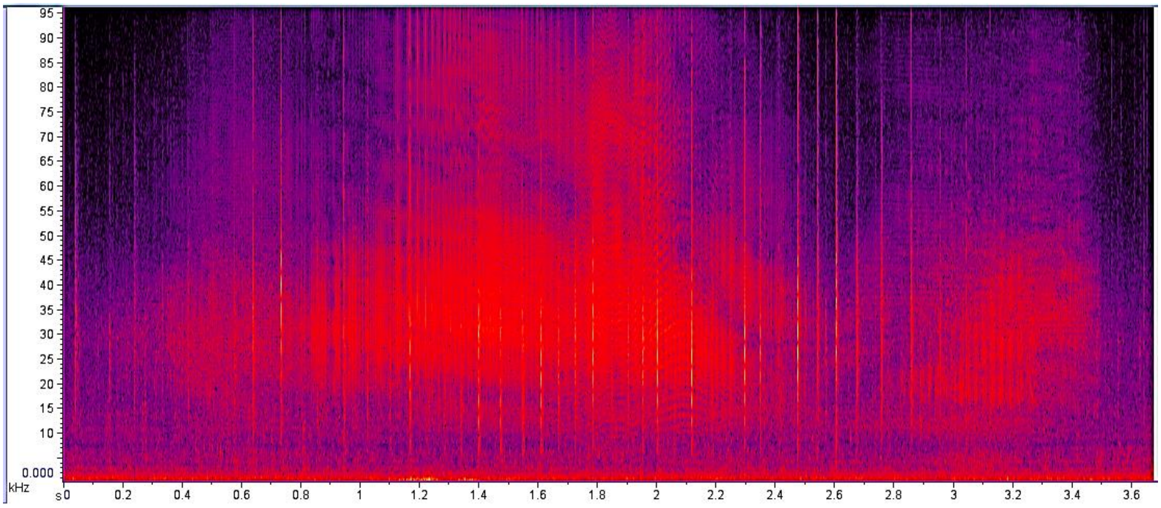
Creaks – long burst-pulse (>0.2 sec.), aurally similar to a creaking door (512 points);



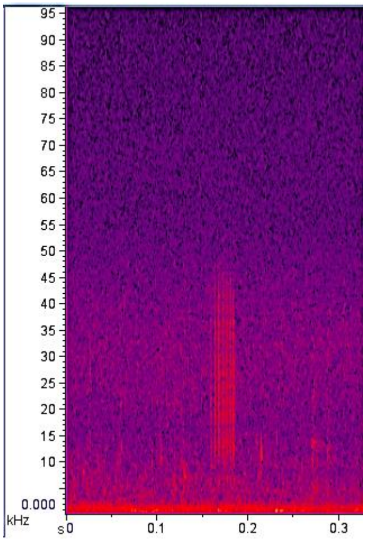
Squawks – long burst-pulse (>0.2 sec.), with higher repetition rate than “Creaks”, identical to a crying baby (512 points);



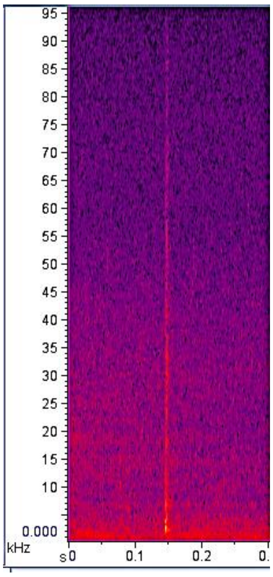
TTV – Variable rate click trains, may contain discernible clicks, creaks and/or squawks with high repetition rate (creak followed by a squawk) (512 points);



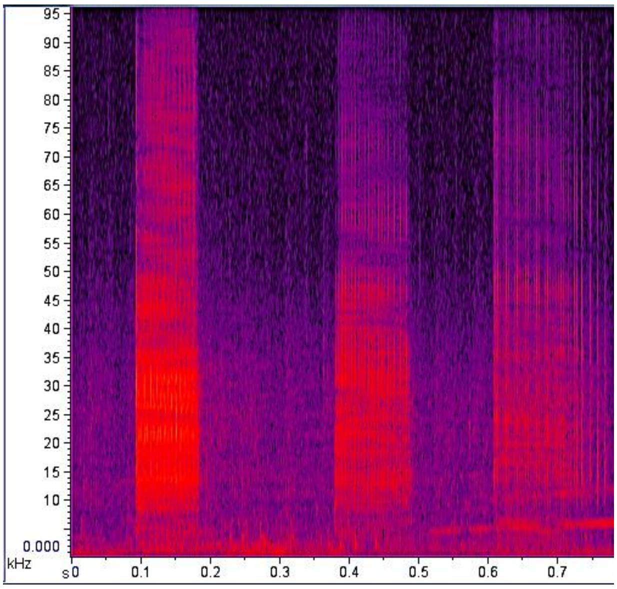
Short Burst-Pulses – < 0.2 sec. duration, aurally similar to a buzzing bee but brief (512 points);



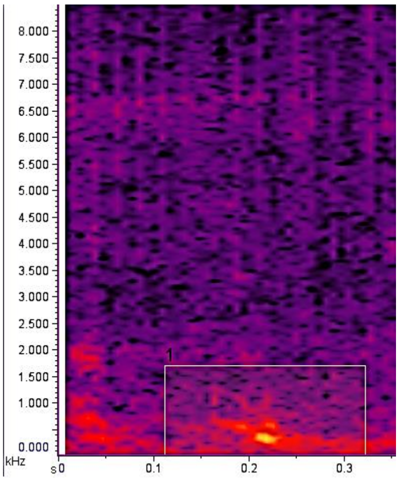
Bangs – Isolated high energy pulsed sound (512 points);



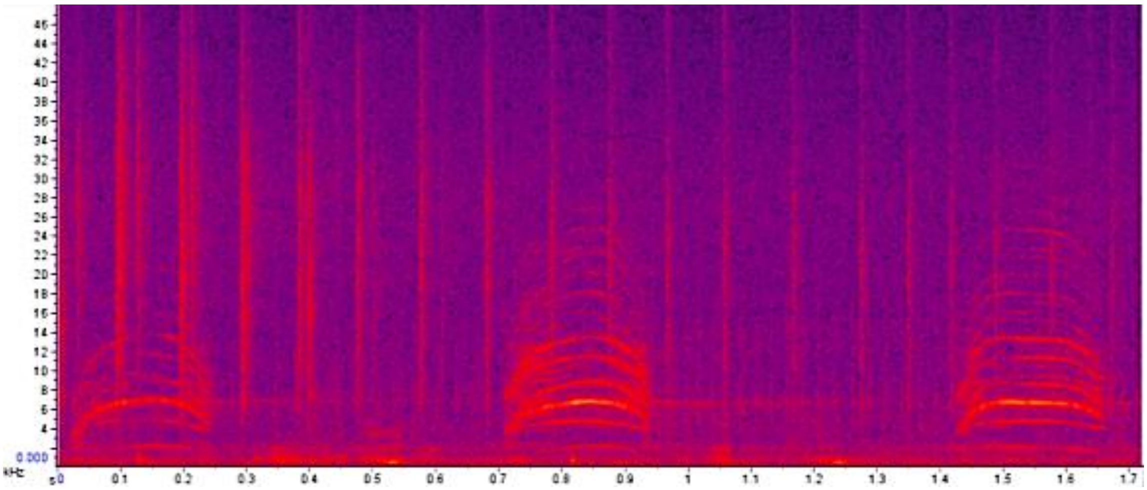
Grunts – Trains of intense burst-pulses, acoustically similar to pig grunts (300 points);



Gulps – Low-frequency, short-pulse, identical to a sip or sob (3000 points);

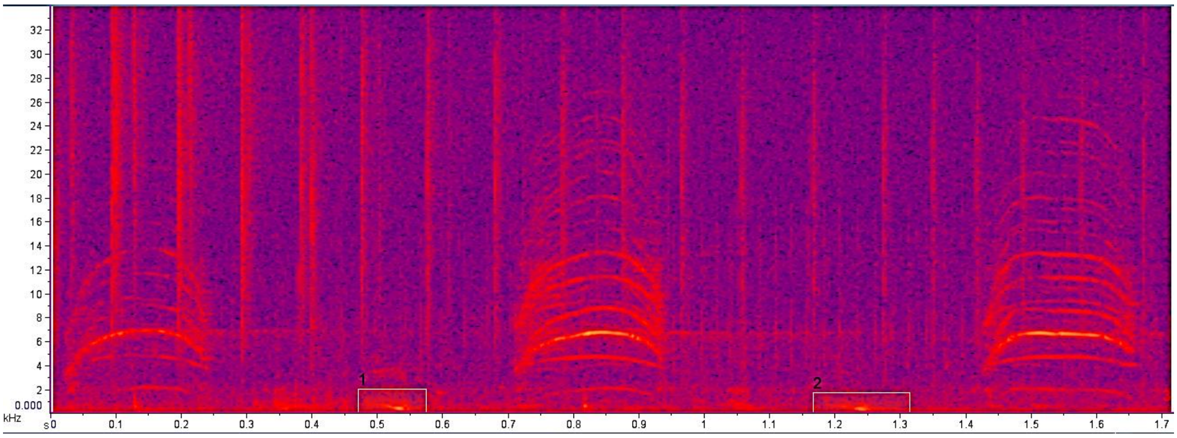


Squeaks – Short burst-pulse with a harmonic structure, shrill sound like a scream (1000 points);

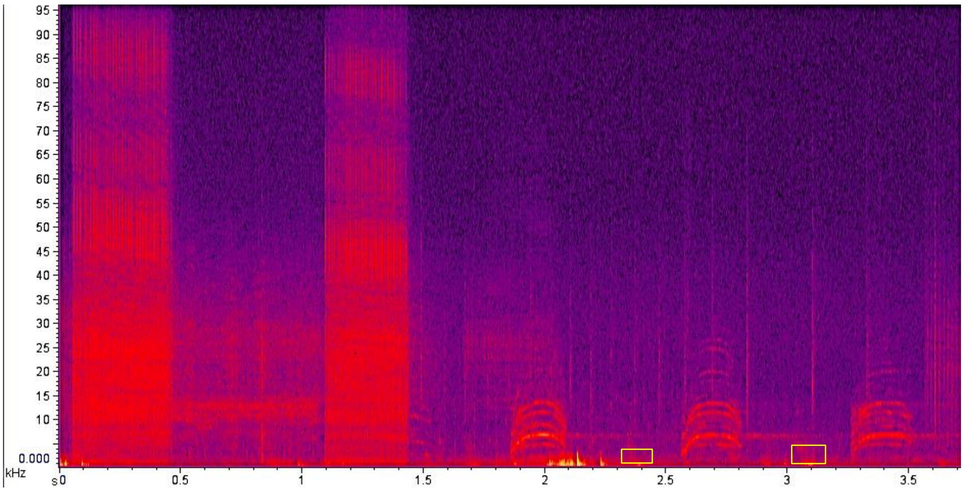


Brays Sequences: rhythmic vocalization composed by different pulsed sounds, such as, gulps, grunts and squeaks, with a variable composition and structure (1500 points)

a) Sequence of squeaks and gulps (yellow square)



b) Sequence of grunts, squeaks and gulps (yellow squares).



Appendix B

Bray Sequence Definition

Bray series' are vocalizations composed by different vocal elements, such as gulps, grunts, and squeaks.

In order to define each sequence the silent gaps were plotted using a log-frequency analysis, and bout criterion interval (BCI) was applied (Sibly, Nott, & Fletcher, 1990) to define each sequence, using only recordings without the noise of vessels. The silent gaps were measured by subtracting the end time of the first element from the start time of the second element.

Bray series' elements were identified in 26.2 % of the recordings.

The majority of bray series' elements were recorded in sequences with very short silent gaps: 58% of all elements were recorded with intervals less than 300 ms in between. Signal overlapping was observed on 6.7% of the emissions. Log-frequency analysis confirmed the bout structure of these emissions (Figure 6.1). A total of 130 sequences were identified.

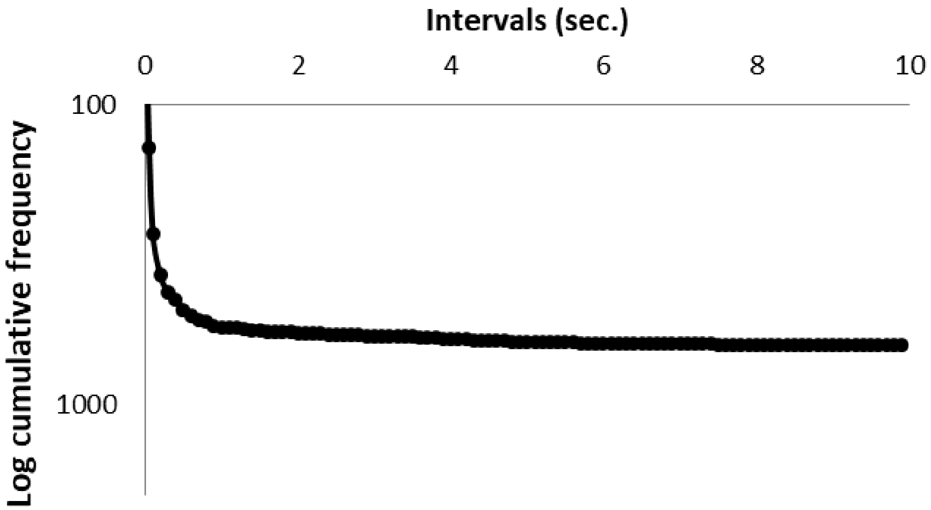


Figure 6.1 - Log survivorship plot of the intervals between vocal units. The clear-cut in slope indicates the bout structure of bray sequences. Bout criterion interval (BCI) estimated at 0.6 seconds.

Appendix C

Statistical Models Analysis

When it comes to modelling counts (numbers greater than or equal to 0), the Poisson regression is often used. This is a generalized linear model where a response is assumed to have a Poisson distribution conditional on a weighted sum of predictors (Bispo & Pinto, 2008; Rose, Martin, Wannemuehler, & Plikaytis, 2006; Zeileis, Kleiber, & Jackman, 2008).

A Poisson distribution is parameterized, the mean and variance are equal, but usually the distribution of the counts does not present this characteristic. There is under- or over dispersion, depending if the variance is smaller or larger than the mean, consequently the performance of a Poisson regression on count data that exhibits this behaviour results and the excess of zeros in a model that won’t fit well (Bispo & Pinto, 2008; Rose *et al.*, 2006; Zeileis *et al.*, 2008).

There are several models that approach hese problems. The Negative Binomial Regression (NB), that belong to the family of generalized linear models (GLM’s) and zero-augmented models, Hurdle models that combine a left-truncated count component with a right-censored hurdle component and the Zero-inflation models mixture models that combine a count component and a point mass at zero (Bispo & Pinto, 2008; Rose *et al.*, 2006; Zeileis *et al.*, 2008).

Descriptive analysis

The Kolmogorov-Smirnov test, used for a number of samples superior to 50 (n>50), shown that the bray emission rate does not follow a normal distribution, once that the p-value < 0.001. Indicating that the null hypothesis (H_0 = the variable presents a normal distribution) should be rejected.

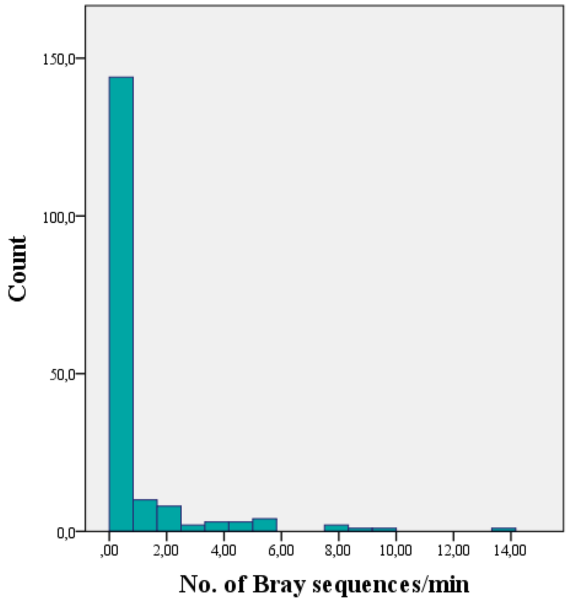


Figure 6.2 - Frequency distribution for No. bray sequences/min

The exploratory analysis presents a variance (3.9) that is higher than the mean (0.75) of the dependent variable, indicating an over dispersion problem, as shown in Figure 6.3.

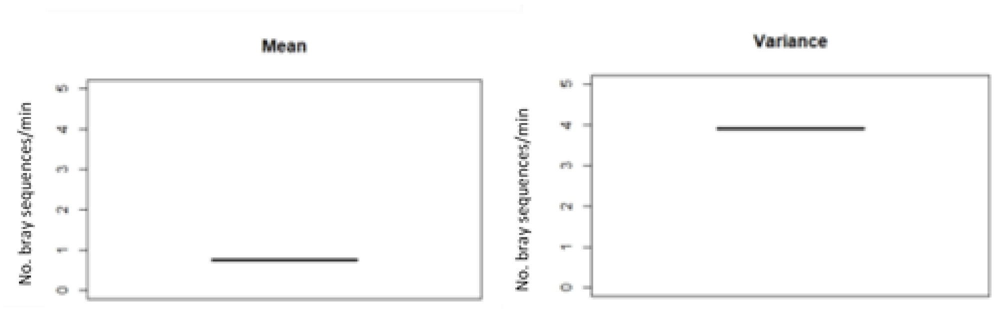


Figure 6.3 - Mean and Variance of the dependent variable - No. bray sequences/min.

Model analysis

When observing the number of bray sequences/ minute distribution graphic (Figure 6.2) it is evident the high number of zero counts. Also, there is a problem of over-dispersion (Figure 6.3), the variance is larger than the mean. Considering these problems, the Poisson Regression Model is inadequate.

By comparing the estimated frequencies of various models (dashed lines in the Figure 6.4) with the frequencies observed, it is possible to perceive the model global fitting to the data. This was made to Poisson regression Model, Negative Binomial Regression Model (NB), Zero Inflated Poisson Model (ZIP) and Hurdle model – Negative Binomial (HNB). Using the using the MASS and pscl packages, in RStudio, Inc. (Figure 6.4).

In the figure 6.4, as in table 6.1, it is possible to observe that the Poisson regression Model underestimates the zero counts and overestimates the values of other counts, not being well adjusted. The ZIP, the NB and the HNB estimated zero count appear to adjust to the observed zeros. Although the ZIP model underestimate the other count values, so this is not the best adjusted model.

The NB and the NHB present similar estimate counts to the observed counts, presenting a good adjustment to the data.

Table 6.1 - Relation between the estimation counts and the observed counts.

Count	observed	Poisson	NB	ZIP	HNB
0	141	99	140	141	141
1	10	45	17	6	10
2	11	21	7	8	8
3	2	9	4	8	6
4	4	3	3	6	4
5	4	1	2	4	3
6	2	0	1	2	1
8	2	0	1	1	1
9	2	0	1	0	0
14	1	0	0	0	0

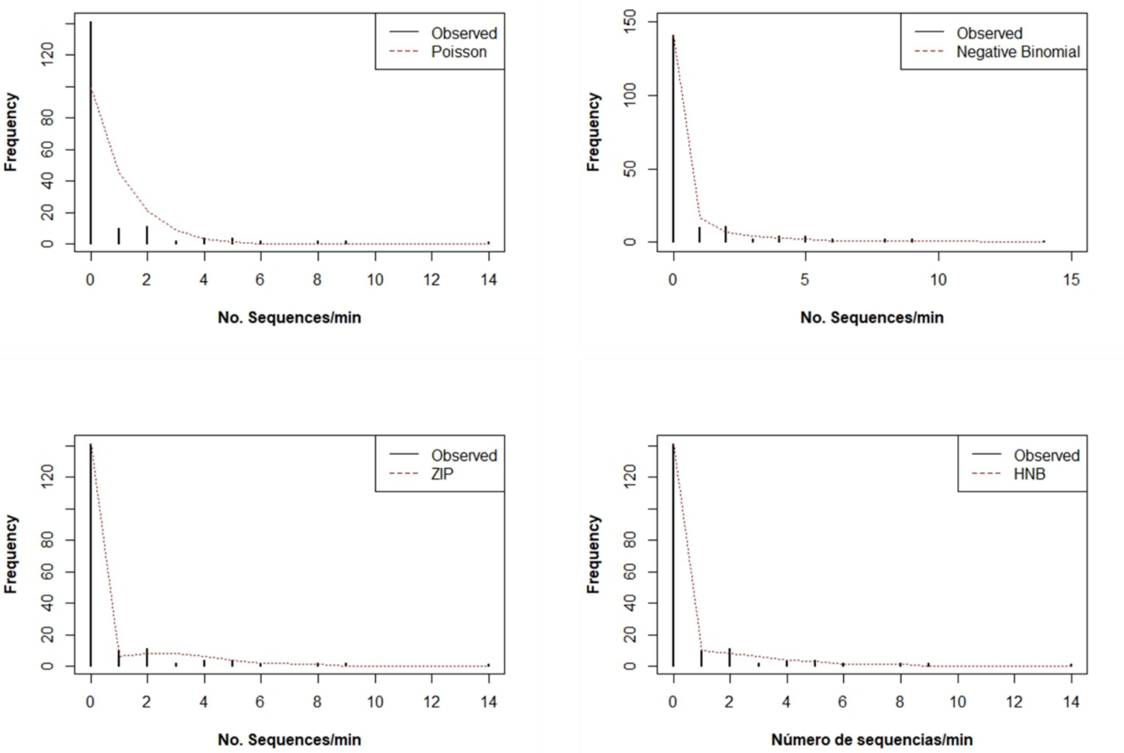


Figure 6.4 - Fitted count regression models for the dependent variable.

Table 6.2 - Adjustment Statistics: logLik – log-likelihood; *df* – degrees of freedom; AIC - Akaike Criteria.

	Poisson	NB	ZIP	HNB
logLik	-282	-171	-177	-168
df	7	8	14	15
AIC	578	358	382	366

When analysing the fit statistics (Table 6.2) the models based on a Negative Binomial Distribution presents better adjustment. NB and HNB present the lowest AIC values (358 and 366 respectively) and higher log-likelihood values (NB = -171; HNB = -168).

The Negative Binomial Model was considered adequate because it addresses the overdispersion as a temporal dependency and/or unobserved heterogeneity (Rose *et al.*, 2006; Zeileis *et al.*, 2008)