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# Variability of signature whistles of bottlenose dolphins (*Tursiops truncatus*) of Losinj archipelago

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**SUMMARY:**

I tursiopi, *Tursiops truncatus*, sono una specie con un'organizzazione sociale molto caratteristica e si affidano al loro complesso repertorio acustico per mantenere il contatto e la comunicazione tra i membri di un gruppo. Esistono tre principali categorie di segnale: click, suoni pulsati e fischi. In questo studio sono messi sotto esame i fischi firma dei tursiopi che vivono nell'arcipelago di Lussino, posto nella parte Nord-Orientale del Mar Adriatico, in Croazia. I fischi firma sono caratterizzati da un preciso andamento della frequenza, diverso per ogni individuo, e da intervalli tra fischi successivi compresi tra 1 e 10 secondi. A partire dal 1987 sono stati effettuati molti studi ecologici, comportamentali e bioacustici sulla popolazione di tursiopi residente nell'area del Quarnaro. I precedenti studi di carattere bioacustico si sono focalizzati prevalentemente sull'impatto delle attività antropiche (presenza di imbarcazioni ricreative o pescherecci) sui fischi emessi dai delfini, mentre questo studio è incentrato sulla variabilità dei fischi firma. In particolare sono stati analizzati gli effetti della diversa composizione dei gruppi (presenza o assenza di cuccioli), del comportamento e della presenza di barche su 7 diversi parametri acustici. Per le analisi sono stati utilizzati campioni registrati con un idrofono RESON TC 4032 tra Maggio e Settembre 2016 e tra Giugno e Settembre 2017, per un totale di 43 avvistamenti e più di 12 ore di registrazioni, contenenti 947 fischi firma. I parametri analizzati sono: durata, range di frequenza, frequenza iniziale, frequenza finale, frequenza minima, frequenza massima e numero di inflessioni. Si è dimostrato che ognuno di questi parametri varia per effetto di almeno uno dei fattori considerati, ma nessun parametro è influenzato da tutti i fattori.

I risultati di questo studio, insieme a quelli delle ricerche precedenti, possono essere utili per valutare lo stato di conservazione dei tursiopi nell'arcipelago di Lussino , per monitorarne i cambiamenti a livello di popolazione e per riconoscere possibili minacce. Tutto ciò è utile per attuare una gestione mirata dei tursiopi e dell'intero ecosistema in cui vivono.

**ABSTRACT:**

Bottlenose dolphin (*Tursiops truncatus*) is a highly social species: individuals rely on vocal communication to keep in contact, to identify themselves and to hunt preys. For this reason bioacustic is an important field of research regarding cetaceans: it can assist monitoring population changes and allow to assess potential threats in a specific area, such as the Cres-Lošinj archipelago (north-east Adriatic Sea, Croatia). In 2014, this area has been declared a Site of Community Importance (SCI) of the NATURA 2000 network.

Data considered in the current study has been collected with a RESON TC 4032 hydrophone between May and September 2016 and June and September 2017: 12 hours and 16 minutes of recordings were collected containing 947 signature whistles. For each whistle seven parameters where analyzed: duration, start frequency, end frequency, minimum frequency, maximum frequencies, frequency range and number of inflection points. Following the whistle characterization phase, the variation in signature whistles parameters was tested according to three factors: group composition, behavioral states and boat presence. Multiple comparisons have been conducted to verify how the combination of different factors would affect whistles parameters. Each whistle parameter has been proved to change according to at least one variable, but neither one parameter is influenced by all the different factors considered in this study.

The results from this study can be used to evaluate the conservational state of bottlenose dolphins population in the Cres-Lošinj archipelago, to monitor its changes and to identify potential threats. Future acoustic studies will help investigating geographical and temporal variations in bottlenose dolphins vocalizations, and will aid understanding the acoustic ecology of the species in this area.

# 1    INTRODUCTION

## 1.1    Framework of the thesis

Bottlenose dolphin (*Tursiops truncatus*) is probably the best-known species among marine mammals and it is regarded as perhaps the most adaptable cetacean, since it occurs all over the world mainly in coastal water at tropical and temperate latitudes. In Cres-Lošinj archipelago (north-east Adriatic Sea, Croatia) a resident bottlenose dolphin population has been consistently monitored since 1990 (Bearzi *et al.*, 1997; Fortuna, 2006).

Dolphins live in complex societies with a wide variety of association patterns and grouping composition (Parra *et al.*, 2011). In this context it is really important to maintain communication with other members of the group i.e. dolphins use signature whistles as contact calls for group cohesion (Janik and Slater, 1998) and for individual recognition (Sayigh *et al.*, 1999).

Boat traffic is the main example of anthropogenic pressure in coastal environments inhabited by bottlenose dolphins (Rako *et al.*, 2013; Pirotta *et al.*, 2015). The presence of boats has been found to cause negative consequences on dolphins that include vertical and horizontal avoidance (Constantine *et al.*, 2004). Furthermore, anthropogenic noise from boat engines has been found to affect vocalization by shifting frequency of dolphin calls (Rako and Picciulin, 2016), increasing emission rate and inducing changes in call duration (May-Collado and Wartzok, 2008; Luís *et al.*, 2014).

The aim of this study is to investigate emission signature whistles of the population of bottlenose dolphins in the Kvarnerić area, with particular attention to the relationships among possible changes in characteristics of signature whistles and group composition, behavioral state and boat presence.



**Fig. 1.1 – Bottlenose dolphin jumping in the waters of Lošinj island (© Photo by Blue World Institute)**

## 1.2    Bottlenose dolphin (*Tursiops truncatus*)

Bottlenose dolphin is one of the most studied cetaceans and it gained popularity thanks to its frequent appearances on television and its worldwide presence in captivity and in research facilities (Jefferson *et al.*, 1993).

*Tursiops truncatus* is a member of the family *Delphinidae*, which is a part of the superfamily *Delphinoidea*, together with *Phocenidae*, *Monodonyidae* and two extinct families: *Kentriodontidae* and *Albireonidae*. *Delphinoidea* belongs to the suborder *Odontocete*, or toothed whales, in the order *Cetacea*. Delphinids quickly radiated into many different morphological and ecological types, from Kentriodontid-like ancestors in mid- to late Miocene. Many of the early delphinid fossils can be assigned to extant genera, particularly *Tursiops* (LeDuc, 2009).

| Kingdom | Animalia |
|---|---|
| Phylum | Cordata |
| Subphylum | Vertebrata |
| Class | Mammalia |
| Order | Catacea |
| Suborder | Odontocete |
| Superfamily | Delphinoidea |
| Family | Delphinidae |
| Genus | Tursiops |
| Species | Truncatus (Montagu, 1821) |

**Tab.1.1 - Taxonomy of Bottlenose dolphin (*Tursiops truncatus*)**

Bottlenose dolphin is a cosmopolitan species, found in tropical and temperate latitudes all over the world. Free-ranging populations can be found in all oceans, as well as in the Black, Red and Mediterrenean Sea (Wells and Scott, 2009). Limits to their range seem to be, directly or indirectly, related to temperature and prey distribution (Wells and Scott, 2009). *Tursiops truncatus* lives both in open waters and in coastal areas, like bays, lagoons, harbors, estuaries and river mouths. There appear to be two ecotypes: coastal and offshore. Population density seems to be higher in coastal ecotype (Wells and Scott, 2009).

### 1.2.1    *Physical characteristics*

Bottlenose dolphins are medium-sized cetaceans with robust body, a moderately falcate dorsal fin and long pectoral fins (Wells and Scott, 2009). Their pigmentation can vary between different shades of gray, with strong countershading: they are dark gray dorsally, while their belly is white or pinkish and there is not a sharp demarcation between the two elements, but a lighter gray covers the sides of these mammals. The belly and lower sides are sometimes spotted (Jefferson *et al.*,

1993). A dark stripe goes from eyes to flippers and a dorsal cape on the back is visible when the pattern is very dark; it is often possible to see white or greyish scars on the body (Jefferson *et al.*, 1993; Wells and Scott, 2009). The species *Tursiops truncatus* can be distinguished from other dolphin species thanks to a marked crease between the melon and the short rostrum (Wells and Scott, 2009). It is also possible to identify singular individual using natural markings, like nicks, scars, scratches and pigment spots, on their dorsal fins (Würsig and Jefferson, 1990): the confirmation of the validity of photo-identification by natural marking has come from studies which combined this technique with various type of tagging (Irvine *et al.*, 1982; Scott *et al.*, 1990). Other features which may help to identify individuals include: shape of dorsal fin, shading of the fin and dorsal body and pigment pattern (Würsig and Jefferson, 1990). The identification of individuals plays a major role in the understanding of biology, ecology, behavior and population dynamics in cetaceans (Hammond, 2009). The natural markings on dorsal fin need to be constantly monitored through time because they can change (Würsig and Jefferson, 1990). These information can be supplemented with the use of facial features and facial symmetry (Genov *et al.*, 2017). Genov and colleagues (2017) found that bottlenose dolphins can be distinguished and identified by facial features and these features are consistent across the left and right sides and therefore symmetrical. Since these are long-lasting markings, the researchers also suggested they may allow calves to be identified after weaning (Genov *et al.*, 2017).



**Fig. 1.2 – Dorsal fin of an adult bottlenose dolphin: many scars and scratches are visible on the fin and very important to identify the dolphin.**
**(© Photo by Blue World Intitute)**

At birth, Bottlenose dolphins are 84-140 cm long and weight 14-20 kg. Adults can measure up to 2,45-3,8 m and 500-650 kg, with males bigger than females, varying by different geographic locations and within different populations (Jefferson *et al.*,

1993; Wells and Scott, 2009). Offshore populations were shown to usually be larger and darker in color than coastal populations, with smaller pectoral fins and different features in hematologic and mitochondrial DNA (Hersh and Duffield, 1990; LeDuc *et al.*, 1999).

Bottlenose dolphins have from 18 to 26 teeth in each jaw. Analysis of dentinal and cement growth layer groups in teeth (Hohn *et al.*, 1989) have shown that females can live to more than 57 years and males up to 48 years in the wild (Wells and Scott, 1999; Jenkins, 2009). Generally, females reach sexual maturity at age from 5 to 13 years old and males later at about 9 to 14 years (Wells and Scott, 2009). Calves can be born in all seasons, but usually with peaks during spring and summer (Urian *et al.*,1996; Thayer *et al.*, 2003). The gestation period last 12 months (Perrin and Reilly, 1984). Lactation last at least one year, but can continue for several more years, since calves stay with their mothers for 3-6 years (Wells and Scott, 1999). In this period calves learn how to catch fish and other important tasks and the separation often coincides with the birth of a new calf (Wells and Scott, 1999; Wells and Scott, 2009).

### 1.2.2  Sociality

Bottlenose dolphins are highly social mammals (Jenkins, 2009) typically found in groups of 2-15 individuals (Culik, 2010), although groups of more than 1000 individuals have been reported (Wells and Scott, 2009). Generally larger groups occur in open waters: this trend could be linked to cooperative foraging strategies and a lower protection degree from predation related to habitat characteristics (Shane *et al.*, 1986). Group composition is dynamic and all populations appear to have a fission-fusion grouping pattern: individuals associate in small groups that change both in size and composition on a daily or even hourly basis (Connor *et al.*, 2000; Mann, 2000). The fission-fusion pattern reflects an adaptation to patchy and irregular prey distribution, with animals spreading into smaller groups to reduce intraspecific competition for food when resources are limited and aggregating in larger groups when food is abundant and predation risk is high (Parra *et al.*, 2011). Associations between individuals of the same sex and also mother and calf bonds are generally strong (Jefferson *et al.*, 1993). Group composition is variable and mixed sex groups are common (Lusseau *et al.*, 2003).

Within this network of changing associates, longer-term relationships coexist. Females tend to associate most often with other females with similar home range and reproductive status (Duffield and Wells, 2002). Female groups composed of preferred associates are known as bands (Wells *et al.*, 1987), and a band membership appears to be stable for periods from years to decades (Connor *et al.*, 2000; Wells, 2003). Mothers that belong to larger band seem to have a greater

reproductive success than females in smaller bands or rearing calves alone. Young females are often recruited back into their natal band (Wells, 2003).

The strongest long-term association pattern in bottlenose dolphins is between adult males (Wells *et al.*, 1987; Connor *et al.*, 1992). Juvenile males begin to develop close relationships with one or two other males of similar age (Wells, 2003). Upon sexual maturity, pairs of males leave the juvenile mixed-sex groups as a closely bonded male alliance. The alliance bond tends to last the lifetime of the males, and in some alliances, a surviving male will form a new partnership with another single male after the death of his original alliance partner (Wells, 2003). One of the primary function of the male alliance bond seems to be improving mating opportunities: receptive females are a patchy resource both temporally and spatially for adult males (Connor *et al.*, 2000).

As in the majority of mammal species, dominance hierarchies have been observed in captivity and aggressive behaviors are used to established and maintain hierarchies (Wells and Scott, 2009). Serious agonistic interactions have been noted also between free-ranging male conspecifics (Parson *et al.*, 2003) as well as between species of dolphins (Herzing *et al.*, 2003).

Furthermore, *Tursiops truncatus* is an extremely vocal species: vocal communication plays an important role in mediating social interactions (Díaz López, 2011).

### *1.2.3 Vocalization*

Bottlenose dolphins present a larynx, but they do not have vocal cords. Sound production is localized in the nasal region. Here there is the so called "*monkey lips/dorsal bursae* (MLDB) complex" called: the *monkey lips,* or phonic lips, are dense connective tissue valves that project into the nasal passage, resembling the region around the mouth of an ape, while the *dorsal bursae* are small ellipsoid fat bodies (Cranford *et al.*, 1996). A pair of *bursae* is associated to a couple of opposite phonic lips to form a MLDB complex (Fig. 1.1). Delphinids present two MLDB complexes that can function independently, so that they can potentially produce different sound simultaneously (Frankel, 2009). Sound production begins when the palatopharyngeal muscles force pressurized air to pass through the phonic lips, causing vibrations in the adjacent *dorsal bursae* (McKenna *et al.*, 2012). Sound vibrations propagate along multiple pathways through the melon and emerge into the environment (Aroyan *et al.*, 1992; Cranford *et al.*, 2008). The melon is an organ placed in delphinids' forehead and composed mainly by fat and connective tissue fibers (Harper *et al.*, 2008), which is thought to focus sound energy generated in the MLDB complex (Cranford *et al.*, 2008).

Bottlenose dolphins have been described to produce three broad categories of vocalizations: echolocation clicks, burst pulsed calls and whistles (Caldwell *et al.*, 1990).

**Clicks:** short broadband high-intensity pulses ranging from tens to 100 kHz and more (Au and Simmons, 2007). Clicks are used for echolocation exploiting returning echoes to explore the surrounding environment (DeLong *et al.*, 2007): dolphins are able to gain information about size, shape, speed, distance and direction of objects and organisms around them, within at least 100 m of distance (Au, 1980). Returning echoes are received through the lower jaw and passed to the inner ear (Arribart *et al.*, 2018). It was found that dolphins presented with an object and rewarded to select an identical object, performed better when objects were presented only for echolocation than when they were presented only for vision (Harley *et al.*, 1996).
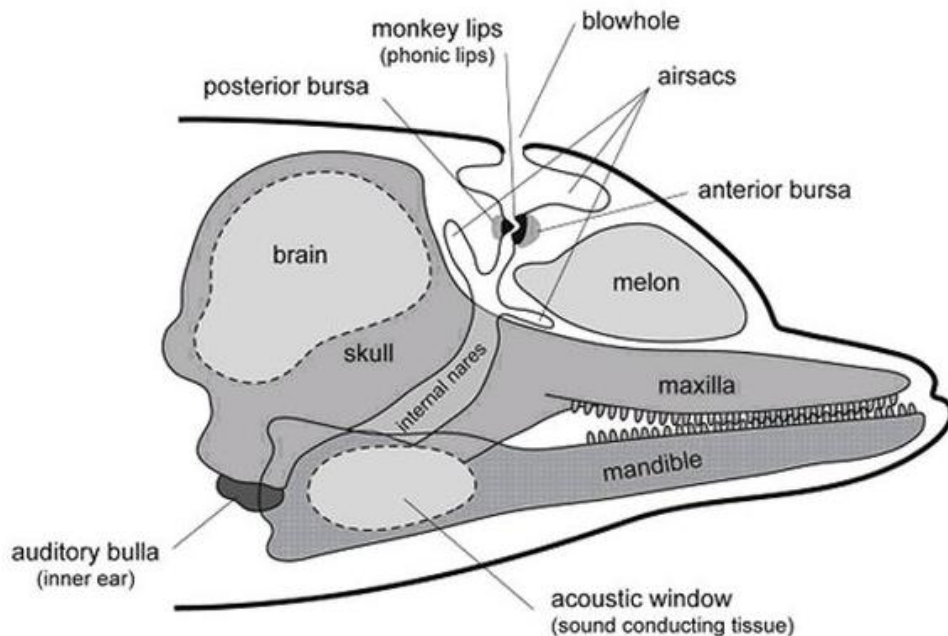


**Fig. 1.3 – Schematic illustration of a dolphin's head anatomy.**
**Sound generator: the Monkey Lips/Dorsal Bursae Complex (MLDB)**
**(modified and adapted from Cranford *et al.*, 1996)**

**Burst pulsed sound:** they are characterized by a repetition rate higher than 300 pulses per second and by interpulse intervals lower than 3 milliseconds (Au, 2000), which cause burst pulsed sounds to be perceived as a continuous sound. Burst pulsed sounds frequency extends beyond 100 kHz (Au *et al.*, 1999) and their structures can vary in amplitude and rate resulting in variations in sound which are perceived by humans as squawks, squeals, cracks, snaps, bleats, barks, groans or moans (Popper, 1980). These vocalizations are used both for navigation and hunting as well as for communication and in social contexts, in particular during play and antagonistic behaviors (Blomqvist and Amudin, 2004). Overstorm (1983)

found burst pulse sound production and duration to be correlated to the aggression level, since they have the potential to provoke auditory discomfort. Burst pulsed sounds can be a safer alternative to physical hitting or fight (Blomqvist and Amudin, 2004). It is common to find whistles overlapping burst pulsed sound at the beginning or at the end (Au, 2000).

**Whistles:** continuous, narrow band, frequency-modulated signals thought to function primarily in social communication. Frequency ranges from 2 to 35 kHz (Oswald *et al.*, 2008), and whistles usually last from several tenths of a second to several seconds (Tyack and Clark, 2000). Dolphins can produce many whistles that differ in frequency, duration and amplitude; whistles can serve a variety of social purposes including communicating social information, coordinating movements during hunting (Norris and Dohl, 1980), identifying individuals through a type of whistle called a "*signature whistle*" (Caldwell *et al.*, 1990), and to some extent also conveying a possible emotional state (Blomqvist and Amundin, 2004). Janik and Sayigh (2013) found that the 38-70% of all whistles emitted are signature whistles: this particular kind of whistles are individually specific signals, each characterized by a unique frequency modulation pattern (Janik and Sayigh, 2013; Janik *et al.*, 2013; King *et al.*, 2013). The inter-whistle interval for signature whistles is within 1-10 seconds, while for non-signature whistles it is below a second (Janik and Sayigh, 2013; Janik *et al.*, 2013). These distinctive vocalizations are particularly used in individual recognition and group cohesion (Janik and Sayigh, 2013): dolphins develop their own signature whistles around the age of four to six months and calves and juveniles are the age classes presenting the higher rates of whistling (Sayigh *et al.*, 1990). Adult females' signature whistles are stable for up to at least 12 years (Sayigh *et al.*, 1990), while males have been shown to modify their whistles to be similar to other members of their alliances, with whom they share a strong social bond (Smolker and Pepper, 1999).
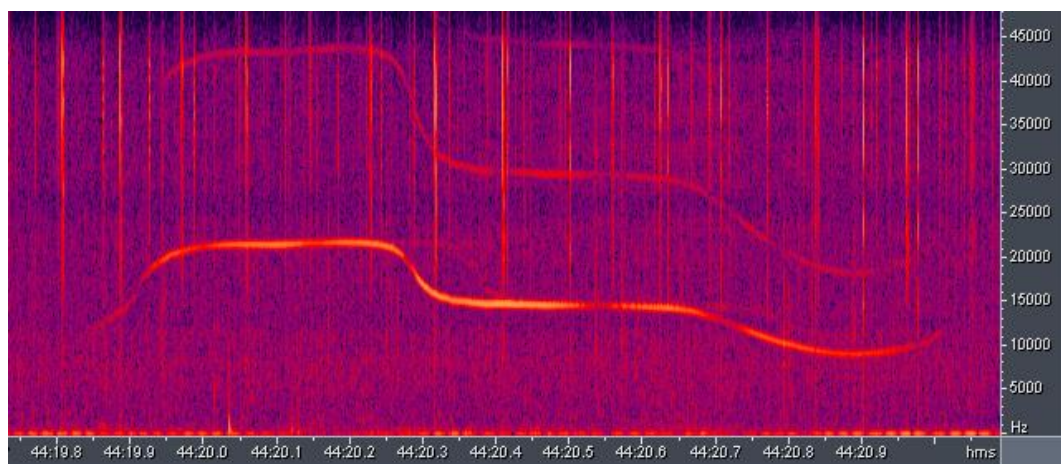


**Fig. 1.4 – Bottlenose dolphins whistle**

### 1.2.4 Bottlenose dolphins of the Kvarnerić

*Tursiops truncatus* is the only cetacean species known to regularly inhabit the northern Adriatic Sea (Bearzi *et al.*, 1997; Bearzi *et al.*, 2004; Zanella and Holcer, 2006; Genov *et al.*, 2008), as confirmed by aerial surveys carried out in 2010 and 2013 to provide a snapshot of the summer distribution and abundance of this species in the entire Adriatic. In the Kvarnerić area there is a resident common bottlenose dolphin population, which has been consistently monitored since 1990 (Bearzi *et al.*, 1997; Fortuna, 2007; Pleslić *et al.*, 2013). The population belongs to the coastal ecotype and it is subject to human pressures that can influence its distribution and abundance (Pleslić *et al.*, 2013). This population went through a period of strong decline (over 30%) between 1995 and 2003, which was probably related to human impact (Fortuna, 2007; Rako *et al.*, 2013). An extensive photo-identification study has been conducted since 2004 in the Kvarnerić and adjacent areas: the data collected until 2011 show that the resident bottlenose dolphin population consists in around 200 individuals, which means that the populations has recovered in size or has shifted the habitat use (Pleslić *et al.*, 2013).

Cres-Lošinj archipelago is characterized by intense boat traffic duringthe summer, intense prey availability and fishing effort: these can be the main causes of the shift in habitat use but further research is required (Pleslić *et al.*, 2013). This dolphin population  is typically spread into small groups, even if they occasionally form larger units. Evidence indicates that there are no major differences between the grouping pattern of males and females, and they are often found together in mixed groups (Bearzi *et al.*, 1997). Furthermore, according to Bearzi and colleagues (1997), the occurrence of calves is higher in large groups, as advantages for calves protection, and the peak in birth is reported for the summer months in the Kvarnerić region, as observed for several other bottlenose dolphin populations (Wells and Scott, 2009).

## 1.3    Adriatic Dolphin Project and the Natura 2000 SCI

The Adriatic Dolphin Project (ADP) started in 1987 and it is currently the longest study of a single resident bottlenose dolphin community in the whole Mediterrenean Sea. The focus of this project is the population ecology and conservation biology of bottlenose dolphins in the Adriatic Sea investigating genetics, bioacoustics, photo-identification, behavioral data, surfacing data, disturbance factors and population and habitat modelling. ADP provides scientific information to the public and the authorities, and promotes the protection of dolphins and their habitat. Between 1987 and 2000, ADP has been run by the Tethys Research Institute (Milan, Italy), after which period the project has been led by the Blue World Institute of Marine Research and Conservation (Veli Lošinj, Croatia), an NGO specifically founded for the purpose of continuing with the

activities of ADP (Fortuna, 2006). Since its foundation, the ADP has shown a successful integration of scientific research and practical conservation, resulting in the declaration of Cres-Lošinj archipelago as a Site of Community Importance (SCI) of the NATURA 2000 network. Now, the Blue World Institute research aids in developing appropriate conservation strategies in the Adriatic Sea and aim to helps in the implementation of the NATURA 2000 priority actions for marine biodiversity, using also new technologies like underwater cameras and drones.



**Fig. 1.5 – Bottlenose dolphin swimming in front of Veli Lošinj**
**(© Photo by Blue World Institute)**

Croatia started protecting bottlenose dolphins in 1994, thanks to the Law on Nature Protection (Rule Book on protection of Certain Mammalian Species, Mammalia). In addition, the *Red Data Book of the Republic of Croatia* lists bottlenose dolphins as *Endangered*. Since bottlenose dolphins research and conservation are a priority under the Croatian Nature Protection Act (Official Gazette 80/13), Croatia has signed a several international conventions that are focused on nature and biodiversity conservation. These include: *Convention on Migratory Species* (Bonn 1979), *Convention on the Conservation of Wild Life and Habitat in Europe* (Bern 1979), ACCOMBAS (*Agreement on the Conservation of Cetaceans in the Black Sea, Mediterranean Sea and contiguous Atlantic Area*, Monaco 1996), *Conservation for the Protection of Marine Environment and Coastal region of the Mediterranean* and *Protocol concerning Specially Protected Areas and Biological Diversity in the Mediterranean*.

Recently the Mediterranean bottlenose dolphin subpopulation has been listed as "Vulnerable" (VU A2code) by the IUCN (Bearzi *et al.*, 2012). But according to the World Conservation Unit (IUCN) criteria, isolated populations with less than 250 individuals are considered as "Critically Endangered" (Fortuna and Mackelworth, 2001): this is the case of the Kvarnerić population, since its abundance is now

estimates around 200 members. Research on population viability in this region estimated that the risk of a local geographic extinction within three generations is very high, 35% (Fortuna, 2006): this means that local population is at risk and needs further monitoring and protection, as it recognized as an important habitat also by UNEP (United Nations Environment Programme, 2011).

## 1.4    Vocal Learning

Vocal learning is the ability of an animal to modify its vocalization as a result of the experience with other vocalizing animals (Janik and Slater, 1997; Janik and Slater, 2000). On the other hand, contextual learning refers to the modification of contextual use or comprehension of a particular vocalization (but not the vocalization itself) due to experience with the vocalization of other individuals (Seyfarth and Cheney, 1997; Janik and Slater, 2000). Many animals that spend at least part of their life in social groups produce calls that indicate group membership. Vocal learning in the context of group living has been reported in songbirds (Brown, 1985; Brown *et al.*, 1988), parrots (Farabaugh *et al.*, 1994), bats (Boughman, 1998), and tentatively in primates (Elowson and Snowdon, 1994; Mitani and Brandt, 1994; Mitani and Gros-Louis, 1998).

Species that produce group-specific vocalization have several common characteristics: comparisons among three different species reveal similarities among songbirds (Australian magpies), parrots (budgerigars) and mammals (greater spear-nosed bats) (Brown *et al.*, 1988; Farabaugh *et al.*, 1994; Boughman, 1998). They all tend to be relatively long-lived species, in which individuals form complex, stable relationships with known individuals. Affiliative interactions usually facilitate the development of group-specific vocalizations, rather than competitive interactions with rivals (Krebs *et al.*, 1981; Nelson, 1997). Vocal learning is a long process and for this reason groups need to be stable long enough for group-specific vocalizations to develop (Boughman, 1998). Another advantage of group-specific calls is to make the requests for assistance of group mates easier, so that they can cooperate to defend scarce high quality territories or to locate patchy resources in unpredictable environments (Brown and Farabaugh, 1997). Also it is important for individuals in a group to recognize one another, to prevent intruders from benefiting, while group stability ensures that individuals are not constantly adjusting their vocalizations for short-term relationships (Boughman, 1998).

Bottlenose dolphins have long been reported to imitate sounds (Evans, 1967; Caldwell and Caldwell, 1972), including human speech (Lilly, 1962) and computer generated tonal, whistle-like sounds (Richards *et al.*, 1984). *Tursiops truncatus* also has characteristics similar to those found in species with group-specific vocalizations: they are long-lived mammals and they form complex long-term

social bonds, even if they have a fission-fusion society, with frequent group changes (Wells, 2003). But also within this network of changing groups, long-term relationships exist: females' bands and males' alliances. An example of group-distinctive vocalization in bottlenose dolphins comes from a study on allied males in Shark Bay, Australia (Smolker and Pepper, 1999). Researchers recorded the whistles produced by three males over a four years period, and they discovered that, as the alliance became stronger, all three males started to produce a very similar whistle type, that had rarely been heard before the alliance formation. This new whistle, apparently develop through convergence, seemed to serve as an "alliance signature", useful to maintain contact between the members or to signal their presence to females or rival males (Smolker and Pepper, 1999). Whistles convergence among allied males suggests that affiliative social relationships may affect vocal learning in bottlenose dolphins.

For females, there may be some differences from males, linked to different priorities in their social interactions. Sayigh and colleagues (1995) compared mother-calf signature whistles from Sarasota Bay, and they found out that female calves were more likely to produce signature whistles that were distinct from those of their mothers, while male calves usually produced whistles similar to those of their mothers. In this region, females often associate with other females of similar reproductive state, also including their mothers (Wells, 1991). Since signature whistles are used for individual identification, it may be more important for daughter to develop a unique signature whistle, because they can end up in the same associative group as their mothers. On the other hand, sons are not going to associate with their mothers post-weaning, so they do not need to develop a really different signature whistle (Sayigh *et al.*, 1995). Furthermore signature whistles are important in mother-calf relationship to keep in contact and to reunite after separations (Smolker *et al.*, 1993), and more generally they are used as contact calls to maintain group cohesion (Janik and Slater, 1998).



**Fig. 1.6 – A female bottlenose dolphin swimming with her calf**
**(© Photo by Blue World Institute)**

## 2    MATERIALS AND METHODS

### 2.1    Study area

The study area of the "Adriatic Dolphin Project" is the Cres-Lošinj archipelago, which is situated in the Kvarnerić area (North-eastern Adriatic Sea), and it covers approximately 2000 km$^2$. This region is punctuated by many islands, islets and channels, including a wide range of habitats, like rocky shores and bottoms, submerged reefs, sea grass flats (*Posidonia oceanica*) and muddy seabeds. These waters are relatively closed and protected, the average depth is around 70 m and it does not exceed 120 m (Arko-Pijevac et al. 2003; Fortuna, 2006), the average salinity is 37,4 psu (Kourafalou, 2001) and the sea temperature ranges between 7 and 15°C in the winter and 22-25°C during the summer months (Favro and Saganić, 2007). The prevalent current in this area is the East Adriatic Current (EAC) that usually peaks in the winter (Orlić *et al.*, 2007). There are two prevalent winds, "bora" and "sirocco", which represent strong mechanical forces generating phenomena like cooling and evaporation of sea surface or upwelling (Cushman-Roisin *et al.*, 2013). This area has relatively low levels of pollution (Gašpić *et al.*, 2002), even if there is a strong human impact influencing both marine and terrestrial ecosystems: industrial marine transport, shipbuilding, oil refineries, oil terminals, power stations, cement industry, tourism and fishery are common human activities of this region (Mackelworth *et al.*, 2003).
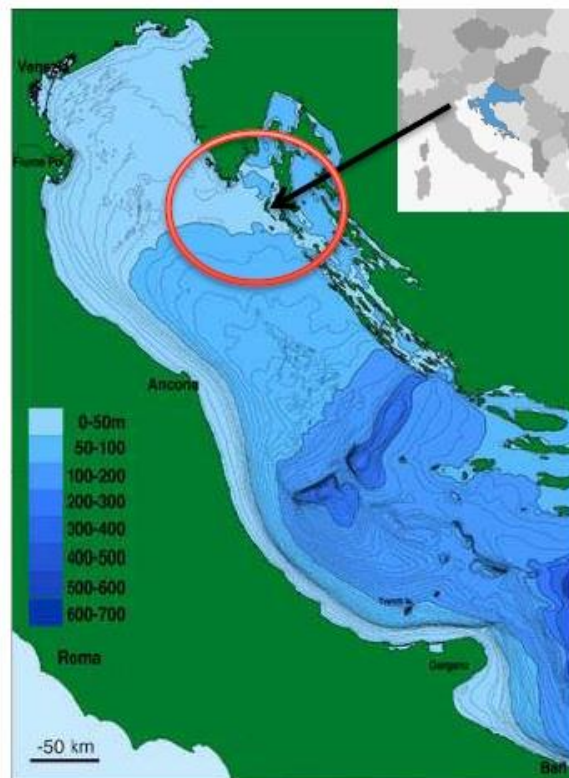


**Fig. 2.1 – Study area**

The Cres-Lošinj archipelago is an important feeding and nursing ground for the resident bottlenose dolphin population (Rako and Picciulin, 2016), it has rich submarine life, historical artefacts, bird nesting sites and is recognized as an important wintering ground for marine turtles (Mackelworth *et al.*, 2003). In 2014 it has been designated as Site of Conservation Interest (SCI) of the NATURA 2000 network.
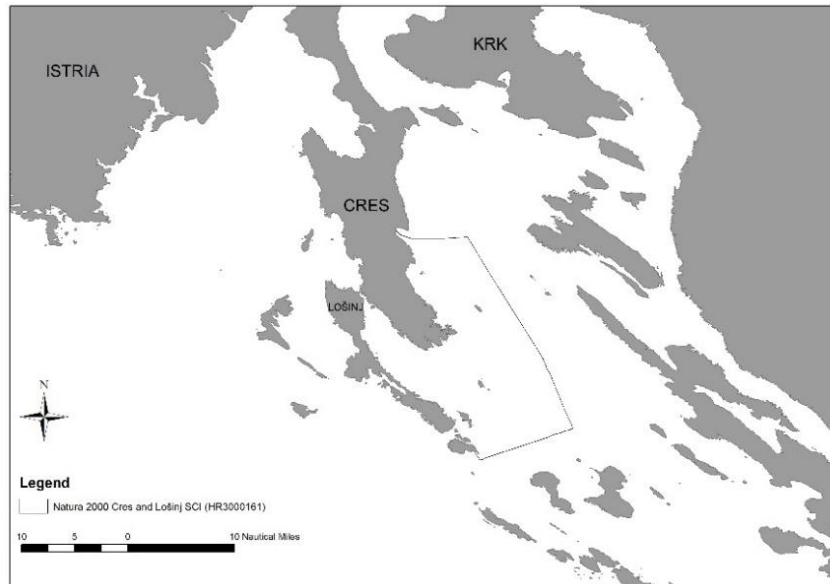


**Fig. 2.2 – The area of Natura 2000 SCI**

For all these reasons, Cres and Lošinj islands represent a very attractive site for tourism: the region records a strong increase of nautical traffic in summer months, which cause significant changes in marine soundscape, which has negative impact on dolphin distribution and habitat use (Rako *et al.*, 2013).

## 2.2 Data collection

The overall data collection took place between 2016 and 2017. I personally collected all the data from June 20th to September 21st in 2017, carrying out 29 surveys for a total of 42 sightings and 11,5 hours of recording. I identified 526 signature whistles in 20 of the 42 total sightings, where I recorded 5,5 hours of recordings. In my analysis I used also 421 signature whistles identified in almost 7 hours of recordings from 23 sightings, from May 22nd to September 12th in 2016. The starting point for the surveys was Veli Lošinj. The research was conducted from a 5,85 m long NOVAMARINE rigid inflatable boat powered by a 4 stroke HONDA 100CV outboard engine. All data were collected in about 6 hours during daylight (time frame between 5,30 AM to 8,30 PM), in good weather conditions and at sea state of Beaufort scale < 3. During active search for dolphins (*positive research effort*), the average speed was kept around 14 knots (25km/h), following *ad libitum* routes (*opportunistic survey*). The crew, from 4 to 9 people, always

including at least two experienced researchers from Blue World Institute, was continuously scanning the area coverin1g 360°, occasionally using FUJINON 7x50 power marine binoculars. During each survey, a tablet, was used to record information on navigation, recording data on time, position, sea state (Beaufort scale), weather conditions and changes in survey conditions, thanks to a specific app, called NaviLog and specifically developed for the purposes of Blue World Institute research. On board there was also a Garmin GPS device (0-15 m accurate), useful to monitor spatial distribution of dolphins and to record the coordinates of dolphin encounters (Fig. 2.3).
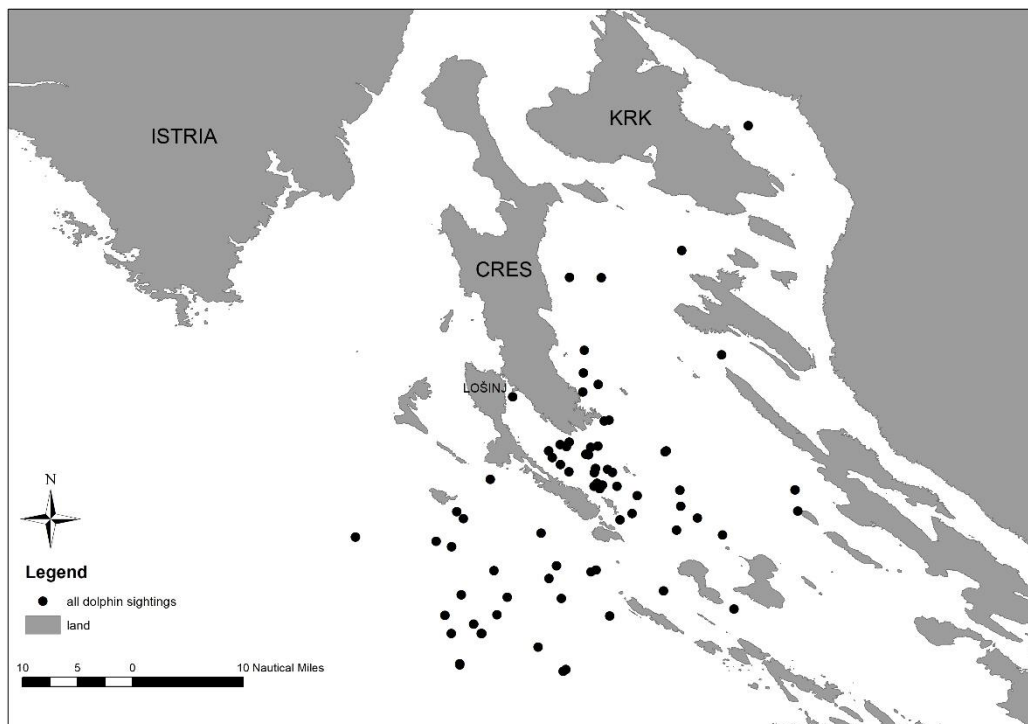


**Fig. 2.3 – Maps of all dolphin sightings in 2016 and 2017**

When dolphins were spotted, the research boat would approach the group travelling parallel to them at the minimum speed, usually 2 knots, in order to minimize disturbance to the group. Many studies found that careful maneuvers can efficiently minimize the research boat influence (Shane *et al.*, 1986; Nowacek *et al.*, 2001; Lusseau, 2003b; Constantine *et al.*, 2004; Bejder *et al.*, 2006a): this involves avoidance of sudden and erratic changes of speed or direction, proceeding parallel to the route of the focal group (Fortuna, 2006) and turning the engine to neutral speed while dolphins are diving.

When dolphins were approached, within safety distance, the sighting started: first of all photo-identification data were collected, following the group for at least 30 minutes, which is considered a sufficient time to record all the required information (Fortuna *et al.*, 1999). A Canon 31 EOS series digital SLR camera with a Canon EF 70-200 mm F/2.8L USM zoom lens was used to take photographs of

the dorsal fin of each dolphin: the pictures allowed the researchers of Blue World Institute to correctly identify all the dolphins present in every encounter. Also photo-identification data were used to confirm the best estimation of dolphin group size, age class composition and behavioral state recorded *in situ*. During dolphin encounters the "group follow" protocol for data collection and the "focal group sampling" method were applied every three minutes based on Altmann (1974) and Mann (1999).

After the photo-identification sampling session ended, the engine would be turned off and the acoustic data collection would begin.

### 2.2.1 Acoustic recordings

Whistles have been recorded using a RESON TC 4032 hydrophone, lowered at approximately 5 m depth, connected to a SOUNDDEVICES 702 high resolution digital audio recorder, set with a sample rate of 192k and a bit depth of 24b. For each sighting, the recording lasted around 20 minutes: if dolphins were not whistling the recording would have ended earlier, and if they were whistling and socializing even for a longer time than 20 minutes , the lasting of the recording would be prolonged. It happened that dolphins kept travelling during the recording: in this situation, the engine was kept at minimum speed to maintain a distance of 200-500 m on average from the group, to allow a good acoustic and behavior sampling. The engine noise have been reduced in the analysis to permit to obtain better distinction of dolphin vocalization within the sample.

### 2.2.2 Group structure and composition

According to Shane (1990) the focal group is "any group of dolphins observed in apparent association, moving in the same direction and often, but not always, engaged in the same activity". All individuals clearly visible within 500 m radius were considered as one focal group. The total number of dolphins in the area, indicated as group size, was constantly monitored, to be sure that it was stable during the sighting. Every time one or more dolphins left or joined the group there was a change of "set" (Bearzi *et al.*, 1997).

Furthermore, two different group associations (group composition) were identified, based on dolphins sex and age:
  – *Females + Calves (FC)*: in the group more than 50% of individuals are females with their newborn or calves;
  – *Adult (A)*: more than 50% of individuals are adults.

Individuals present in the focal group were identified and assigned to their age categories using data from the photo-ID catalogue of Blue World Institute

The four main age categories are defined considering also characteristics such: body size, coloration and the behavior of individual in the group (Bearzi *et al.*, 1997; Fortuna, 2006). According to Bearzi and colleagues (1997) and Fortuna (2006) the four age classes are:

- *Newborn (N)*: it is only 1/3 the length of an adult long, colored in dark grey or brown with visible fetal stripes, constantly in close association with an adult, presumably its mother; its swimming is uncoordinated and has stereotyped surfacing pattern when breathing.
- *Calf (C)*: 1/2 the length of an adult, light grey often still with visible fetal stripes, swimming in association with its mother behind her dorsal fin.
- *Juvenile (J)*: a light grey, usually poorly scarred and rarely nicked individual, 2/3 the length of an adult, often in the same group of its mother, but also swimming independently.
- *Adult (A)*: it is dark grey, usually 2,8-3,0 m long, with scars on its body and distinctive nicks, tooth marks, holes, missing portions and lesions on its dorsal fin.

The gender of bottlenose dolphins was determined based on the overall photo identification data (collected in this area since 1995), observation data and photographs/videos of their genital slits (Whitehead, 2008). Dolphins identified as 'females' were characterized by their gender-specific behavior, such as nursing, which was associated with accompanying a calf for multiple years. Resident animals known as adults for a minimum of 12 years (excluding the period of pre-independence that on average lasts for 4 years) and were never seen in multi annual association with a calf (average age of sexual maturity reported for females in the Adriatic Sea is 12 years; see Pribanić et al., 2000) are considered as 'probable males'.

### 2.2.3 Behavior

During the acoustic sampling behavioral data were collected continuously (*continuous focal group sampling*) in an "*ad libitum*" manner (Altmann, 1974; Martin and Bateson, 1990). The activity in which more than 50% of individuals in the focal group were engaged, was considered as the behavior state of the group. Following Bearzi *et al.* (1999) and Lusseau (2006), group behavior was divided into ten categories: Socialize, Social Travel, Dive, Dive-Travel, Travel, "Active" Trawler Follow, "Passive" Trawler Follow, Surface Feeding, Mill and Mixed Behavior (Table 2.1).

### 2.2.4 Boat presence

Together with behavioral and acoustic data, also information about boat presence within 500 m from dolphin's focal group were recorded. Boat presence data were sampled by eco-volunteers joining the "Adriatic Dolphin Project". Volunteers were previously instructed by Blue World Institute researchers and could always rely on their help during data collection. In this study the focus was only on boat presence and not on boat type and distance from the group.

**Table 2.1 – Definition of behavioral states**

**(Adriatic Dolphin Project behavioral sampling procedure)**

| STATE | DEFINITION |
|---|---|
| SOCIALIZE (S) | Almost constant physical contact with one another; oriented towards one another; no forward movement; display of surface behavior (jumps, leaps, rolling, tail slaps…). |
| SOCIAL TRAVEL (ST) | Moving steadily in one direction while socializing intermittently; tight groups often in physical contact (leaps, rolling…). |
| DIVE (S) | Pattern characterized by cycles of single long dives, lasting up to several minutes; dives are spaced by a cluster of a relatively regular number of ventilations; last in the series of ventilations is often a fluke up or a tail stock submergence, suggesting a vertical dive; submergence and surfacing are usually within the same area; dolphins often dive synchronously. |
| DIVE-TRAVEL (DT) | A pattern that is consistent of both dive and travel, this means that dolphins keep the same direction both underwater and in surface; usually single long dives followed by clustered ventilations; respiration patterns can be highly variable and poorly consistent in comparison with DIVE behavior; groups or sub-groups often synchronous. |
| TRAVEL (T) | Constant directional movement of dolphins, with regular surfacing usually every 10-60 seconds; it can be slow or fast, where slow is associated with resting behavior. |
| "ACTIVE" TRAWLER FOLLOW (ATF) | Following trail of operating trawler, about 150-300 m after the fishing boat; regular single long dives for several minutes accompanied by pattern of regular ventilations. |
| "PASSIVE" TRAWLER FOLLOW (PTF) | Consistent directional movement of dolphins, , with regular surfacing typically every 10-60 seconds, following a trawler at about 150-300 m. |
| SURFACE FEEDING (SF) | Feeding activity performed near water surface (chasing prey, belly up, leaps, jumps…); preys visible near the surface; sometimes also birds congregate in the area. |
| MILL (M) | Dolphins move in different direction in the same location, pretending to dive, but showing no surface behavior and no apparent physical contact between individuals; they usually stay close to the surface, floating and resting. |
| MIXED BEHAVIOR (MB) | No clear prevalence of a single listed behavior in the group. |

## 2.3 Data analysis

Recordings were analyzed processing the sound using Cool Edit Pro 2.1 in spectral view, set on Hamming window (resolution 512 FFT). According to Papale *et al.* (2013 and 2013c), extracted whistles were classified by assigning a signal quality index from zero to three: the assigned score was *zero (0)* when the complete detection of time-frequency contour of the whistle was impossible because of overlapping with other sounds and low intensity; *one (1)* when low intensity and/or low signal to noise ratio prevented the operator from recognizing the complete contour; *two (2)* if the complete contour could be recognized but intensity was low; and *three (3)* when intensity was high and time-frequency contour well defined.
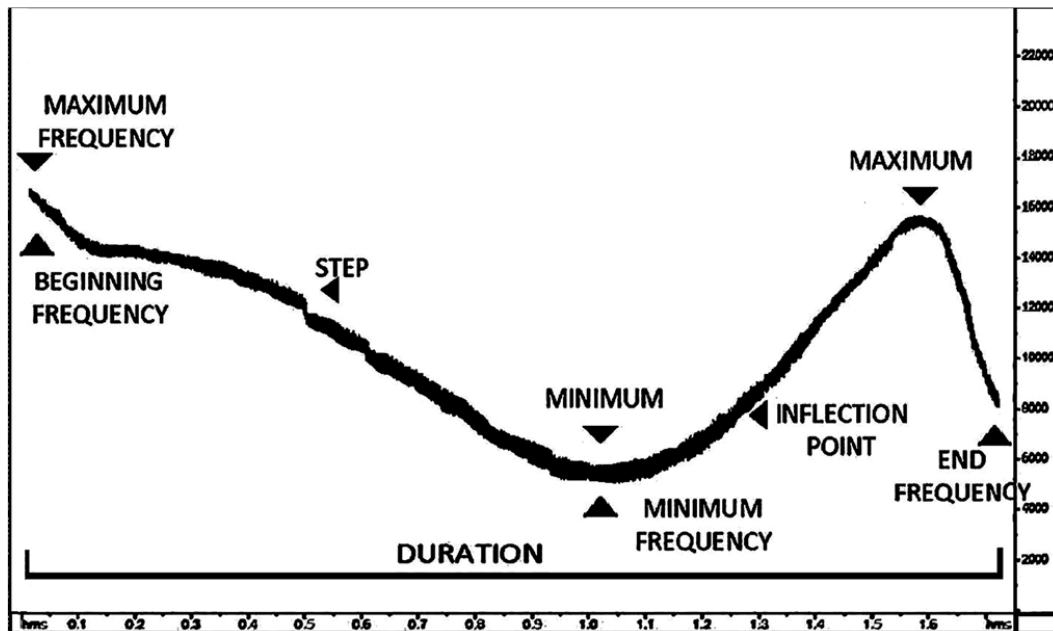


**Fig. 2.2 – Sample spectrogram representing a bottlenose dolphin whistle. Parameters manually measured are shown (Papale *et al.*, 2013)**

According to Oswald *et al.* (2003), Azzolin (2008) and Papale *et al.* (2013c), for each whistle contour, some parameters were considered and measured manually: type of the whistle, duration, beginning frequency, end frequency, minimum frequency, maximum frequency, number of inflection points, number of steps, beginning slope, end slope, number of minima in the contour, number of maxima in the contour, presence/absence of harmonics and presence/absence of interruptions and number of interruptions (Table 2.2). Then frequency range have been calculated (Papale *et al.*, 2013c):

$$Fq\ Range = Max\ Fq - Min\ Fq$$

**Table 2.2 – Whistle parameters and their definitions**

| Parameter | Definition |
|---|---|
| TYPE | Whistles were divided into three different categories depending on the shape of their contour: *rise* (r) for ascending whistles, *down* (d) for descending whistles and *modulated* (m) if the contour was not regular. |
| DURATION | Total signal duration, measured in ms. |
| BEGINNING AND END FREQUENCY | Using the pointer in Cool Edit Pro 2.1, frequencies at the starting and ending point were recorded in Hz. |
| MINIMUM AND MAXIMUM FREQUENCY | Using the pointer in Cool Edit Pro 2.1, frequencies at the minimum and maximum point were recorded in Hz; sometime these parameter can coincide with beginning or end frequencies. |
| BEGINNING AND END SLOPE | Three different categories: *+1* when the curve was rising, *0* when it was flat and *-1* when it was descending (Oswald *et al.*, 2003). |
| NUMBER OF INFLECTIONS | Number of changes from positive to negative or from negative to positive slope (Papale *et al.*, 2013c). |
| NUMBER OF STEPS | It refers to the number of discontinuous changes in frequency (Papale *et al.*, 2013c). |
| HARMONICS | *1* if the whistle has at least one harmonic; *0* if the whistle has no harmonics. |
| NUMBER OF MAXIMA AND MINIMA | Number of relative maximal and minimal points in the whistle contour (Papale *et al.*, 2013c). |
| INTERRUPTIONS | With "interruption" we refer to a time <250 ms that divides two parts of the same whistle, since the typical silent interval between loops of the same whistle is shorter than 250 ms (Esch *et al.*, 2009a). In this study it was considered, first, the presence (*1*) or absence (*0*) of interruptions, and then their number. |

The acoustic structure of signal can have different shape according to different variables, such as environmental conditions (including ambient noise), socio-behavioral characteristics and morpho-physiological features (Bonato *et al.*, 2015). Geographic variation in the structure of acoustic signal may be linked to a combination of these factors and genetic (Papale *et al.*, 2013). Often changes in dolphin whistle features, such as shift in frequencies and call duration, may

represent an adaptation to increased ambient noise (La Manna *et al.*, 2017). Moreover changes in whistle structure may be more pronounced while dolphins are keeping some particular behaviors, involving intense communication among individuals such as foraging and socializing, while variation in modulation parameters (number of inflections, steps, maxima and minima) is probably related to differences in social composition and group size (Azevedo *et al.*, 2007; May Collado and Wartzok, 2008; Papale *et al.*, 2013c). In addition to this, also morphological characteristics may be important in signal modulation: with larger bodies, for instance, lower maximum frequency range can be produced (Berta *et al.*, 2015; Papale *et al.*, 2013). Therefore, whistle parameters can be divided in two categories: the first one includes all those parameters that are modified by the surrounding environment, such as duration, start frequency, end frequency, minimum frequency and maximum frequency (May Collado and Wartzok, 2008). The second group is composed by modulation parameters: they describe the shape and the contour of a whistle and depend on social context (social organization, group composition or behavioral aspects) in which the whistle is emitted. Number of inflections, number of steps, number of minima, number of maxima and number of interruptions belong to the second category (Papale *et al.*, 2013). Some studies highlight the influence of both environmental and socio-behavioral factors for parameters such as signal duration, number of steps and number of minima (Bonato *et al.*, 2015).

## 2.4 Statistical analysis

All data have been recorded with Microsoft Excell and statistically analyzed with R (King *et al.,* 2014). First of all, normality of data distribution was assessed using Shapiro-Wilk normality test and Levene test on homogeneity of variance, in order to apply appropriate tests for statistical analysis. Data were log transformed when necessary. When the assumptions on the normality and homogeneity of variance were met, one-way ANOVA was run to test if each single whistle parameter varied depending on two Factors. The first factor, called Factor_1, represents a behavioral context which combines group behavior with the presence of boats. Here we considered: Travel_MB, Travel_NB, Travel_TW, Feeding_MB, Feeding_NB, Feeding_TW, Social_MB, Social_NB and Social_TW (where NB means "no boats present", MB means "motor boats present" and TW means "trawler boat present"). The second factor, called Factor_2, represents the combination of group composition (Adult (A)/Female-calf group (FC)) with boat presence or absence (FC_MB, FC_NB, A_MB and A_NB). Tukey's post hoc contrast of means was performed on significant factors. Furthermore, a linear Discriminant Function Analysis (L-DFA) was applied to the mean values of each group to verify whether whistles could be attributed to the correct behavioral context.

## 3    RESULTS

### 3.1   Study effort

During 2016 and 2017 summer field work activities, 12 hours and 16 minutes of recordings were collected containing signature whistles, divided into 23 sightings in 2016 and 20 sightings in 2017. The recordings provided 947 signature whistles in total. Table 3.1 shows the distribution of sightings, hours of recordings and number of signature whistles.

**Table 3.1 – Distribution of signature whistles collected during 2016 and 2017**

| Month | Sightings | Hours recorded (hr:min:sec) | N° signature whistles |
|---|---|---|---|
| May 2016 | 2 | 0:20:45 | 18 |
| June 2016 | 7 | 2:12:43 | 239 |
| July 2016 | 6 | 2:16:41 | 82 |
| August 2016 | 6 | 1:28:05 | 69 |
| September 2016 | 2 | 0:32:03 | 13 |
| June 2017 | 2 | 0:10:11 | 27 |
| July 2017 | 9 | 2:24:02 | 189 |
| August 2017 | 5 | 1:11:12 | 201 |
| September 2017 | 4 | 1:28:24 | 109 |
| Tot 2016 | 23 | 6:50:17 | 421 |
| Tot 2017 | 20 | 5:26:20 | 526 |
| Total | 43 | 12:16:37 | 947 |

During dolphins encounter acoustic data were collected for an average of 20 minutes and also data on group composition, dolphin behavior and boat presence were recorded. The majority of the samples were recorded during encounters with groups of females with calves (867 whistles), and I recorded only 80 whistles from groups with adults dolphins. Regarding boat presence, 585 signature whistles were recorded during sightings when motor boats were present and 362 without any other boat. In the end, 333 whistles were sampled during feeding activities, 140 during feeding activities involving trawler boat presence, 419 during travel behavior and 55 while dolphins were socializing. It was decided to use a filtered dataset for the analysis to avoid errors due to pseudo-replication: in each recording, repeated signature whistles have been eliminated in order to reduce the risk of collecting whistles from the same individual and only one signature whistle of each type has been included into the reduced dataset. For statistical analysis, 171 signature whistles were considered: 18 from groups with only adult dolphins (A), 153 from groups with mothers and calves (FC). In total, 71 whistles were recorded without other boats around and 100 with other boats present in

the 500m radius of dolphins. Regarding behavior, 63 whistles were sampled during feeding activities, 19 during feeding involving trawler boat follow, 13 while they were socializing and 76 while they were travelling.

## 3.2    Characterization of signature whistles

All whistles analysis were performed with Cool Edit Pro 2.1 in spectral view, set on Hamming window (resolution 512 FFT). Seven parameters were measured: duration, minimum and maximum frequency, start and end frequency, frequency range and numbers of inflections. Whistles from the entire dataset (INT) have mean duration of 931.9 msec (range: 208.0-2529.0 msec), mean minimum frequency of 6527 Hz (range: 1333-11661 Hz), mean maximum frequency of 15302 Hz (range: 4457-24483 Hz), mean frequency range of 8774 Hz (range: 2400-18003 Hz), mean start frequency of 8558 Hz (range: 1333-23317 Hz), mean end frequency of 11743 Hz (range: 3203-23885 Hz), and mean numbers of inflections of 1.43 (range: 0.00-21.00).

**Table 3.2 – Means and Standard Deviation of each whistle parameter**

| PARAMETERS | INT MEAN (±ST.DEV.) | FC MEAN (±ST.DEV.) | A MEAN (±ST.DEV.) |
|---|---|---|---|
| Duration (msec) | 931.9±408.5 | 884.0±417.3 | 965.5±331.8 |
| Minimum frequency (Hz) | 6527±1973 | 6621±1963 | 5734±1926 |
| Maximum frequency (Hz) | 15302±3861 | 15504±3842 | 13584±3690 |
| Frequency range (Hz) | 8774±3289 | 8883±3262 | 7850±3457 |
| Start frequency (Hz) | 8558±4120 | 8772±4220 | 6738±2557 |
| End frequency (Hz) | 11743±4912 | 11810±4924 | 11176±4906 |
| Number of inflections | 1.43±2.15 | 1.48±2.23 | 1.00±1.28 |

For further analysis two subset have been created: the first one contains all signature whistles from groups of females with calves (FC), while the second one includes whistles from groups of adults (A). In FC dataset there are 153 signature whistles, while in the other one only 18: in A dataset there are not whistles recorded during ATF nor PTF activities and for this reason it was not possible to include some comparisons in the analysis.

Whistles from FC subset have mean duration of 884 msec (range: 208.0-2529.0 msec), mean minimum frequency of 6621 Hz (range: 1333-11661 Hz), mean maximum frequency of 15504 Hz (range: 4457-24483 Hz), mean frequency range of 8883 Hz (range: 2400-18003 Hz), mean start frequency of 8772 Hz (range: 1333-23317 Hz), mean end frequency of 11810 Hz (range: 4457-23885 Hz), and mean numbers of inflections of 1.48 (range: 0.00-21.00).

Whistles from A subset have mean duration of 965.5 msec (range: 478.0-1646.0 msec), mean minimum frequency of 5734 Hz (range: 2540-9930 Hz), mean maximum frequency of 13584 Hz (range: 8813-21246 Hz), mean frequency range

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of 7850 Hz (range: 3247-16628 Hz), mean start frequency of 6738 Hz (range: 2540-11968 Hz), mean end frequency of 11176 Hz (range: 3203-19168 Hz), and mean numbers of inflections of 1.00 (range: 0.00-4.00).

## 3.3    Statistical analysis

A logarithmic transformation was performed on all parameters and then normality of distribution was tested both for the original parameters and for the log-transformed ones. A Shapiro-Wilk test was used to test normality: minimum and maximum frequency and frequency range are normally distributed, one the other hand start and end frequency, number of inflections and duration are not normally distributed, but log-transformed duration (LogDur) is the only transformed variable which is normally distributed. Levene test was used to verify the homogeneity of variance: even if the distribution is not perfectly normal, one-way ANOVA can be used when the assumption of the homogeneity of variance is met. Levene test showed that the assumption on the homogeneity of variance was met for those variables that did not have a normal distribution: number of inflections, start and end frequency.

### *3.3.1    One – way ANOVA test results*
It was possible to apply one-way ANOVA test on the variables: LogDuration, minimum frequency, maximum frequency, frequency range, start frequency, end frequency and number of inflections. Firstly, one-way ANOVA was used to test if group composition influenced different whistle parameters: maximum and start frequency showed p-value to be lower than 0.05 (0.0457 and 0.0473 respectively), this means that they are significantly influenced by the group composition. Then, the influence of the behavior was tested: LogDuration and inflections turned out to be influenced by dolphins behavior (p value: 0.0024 and 0.0395 respectively). Also boat presence was tested, but neither one parameter was found to be influenced by this factor (p values always major than 0.05). Furthermore, one-way ANOVA was applied also to test whether behavior and boat presence would influence whistle parameters differently in groups with different composition. In groups with females and calves behavior was shown to influence LogDuration (p value: 0.0082) and boat presence did not cause whistles to vary, while in groups with only adult dolphins behavior still influenced LogDuration (p value: 0.0195), but also end frequency (p value: 0.0022). Furhermore, boat presence was found to significantly influenced the frequency range (p value: 0.0396) and maximum frequency showed a p value close to significance (0.0538) in groups with adults.

**Fig. 3.1 – Box plot showing parameters significantly influenced by group composition, behavior and boat presence**
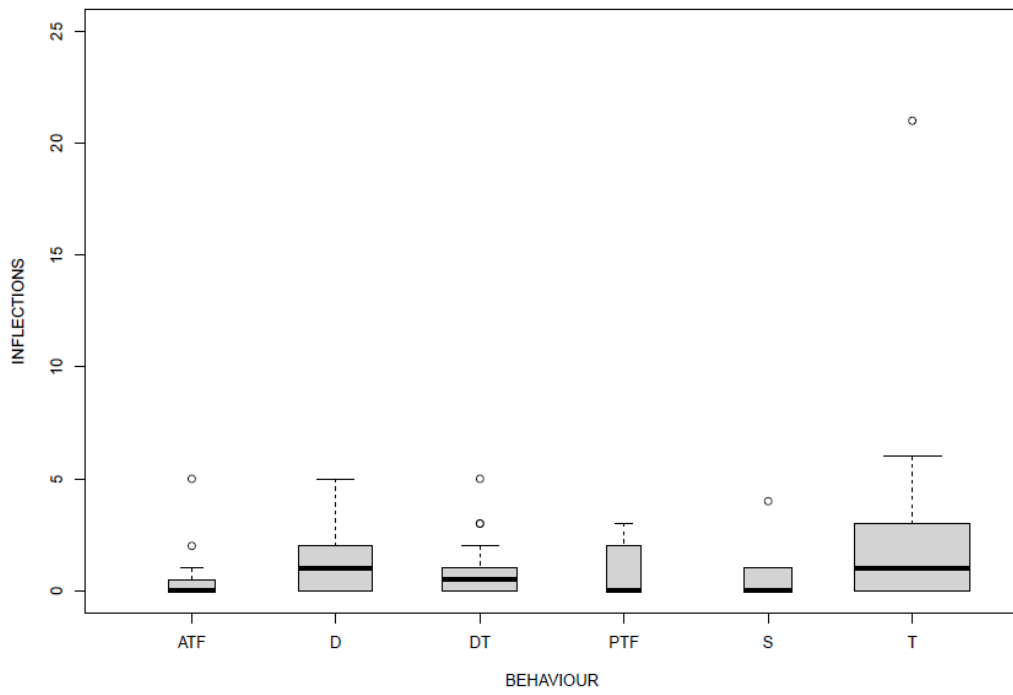
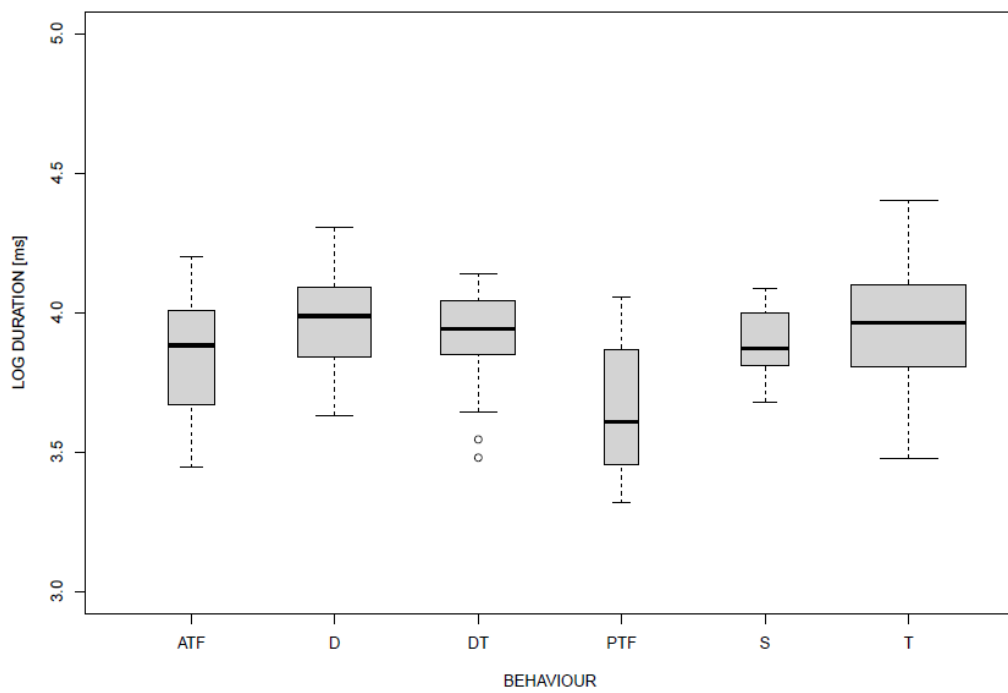3.1 A - Max Frequency is significantly influenced by Group Composition: it's higher in FC groups
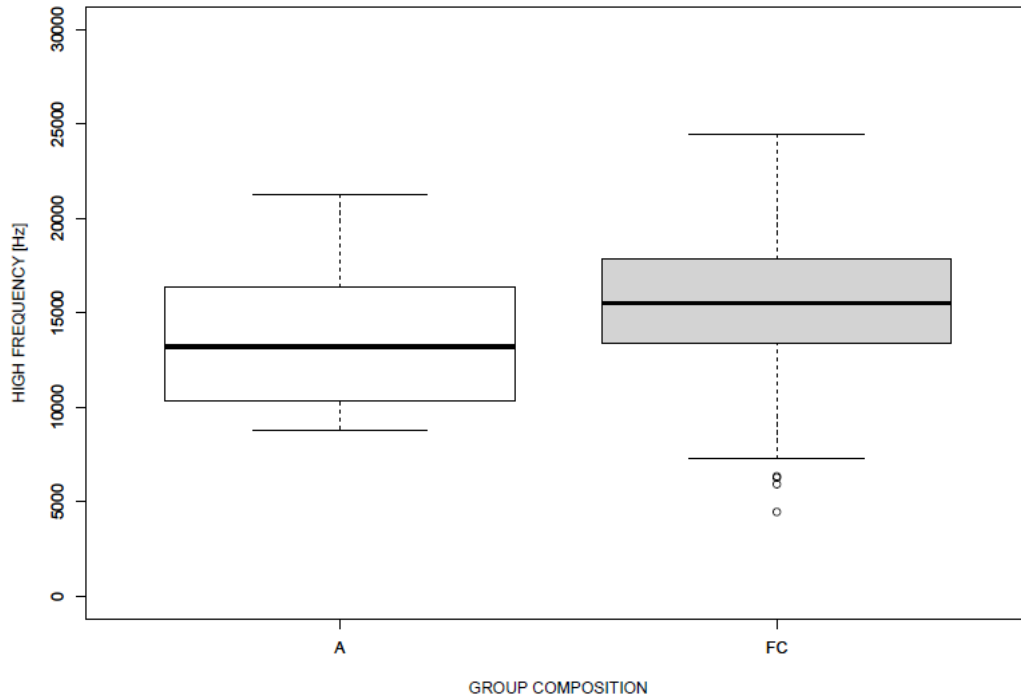


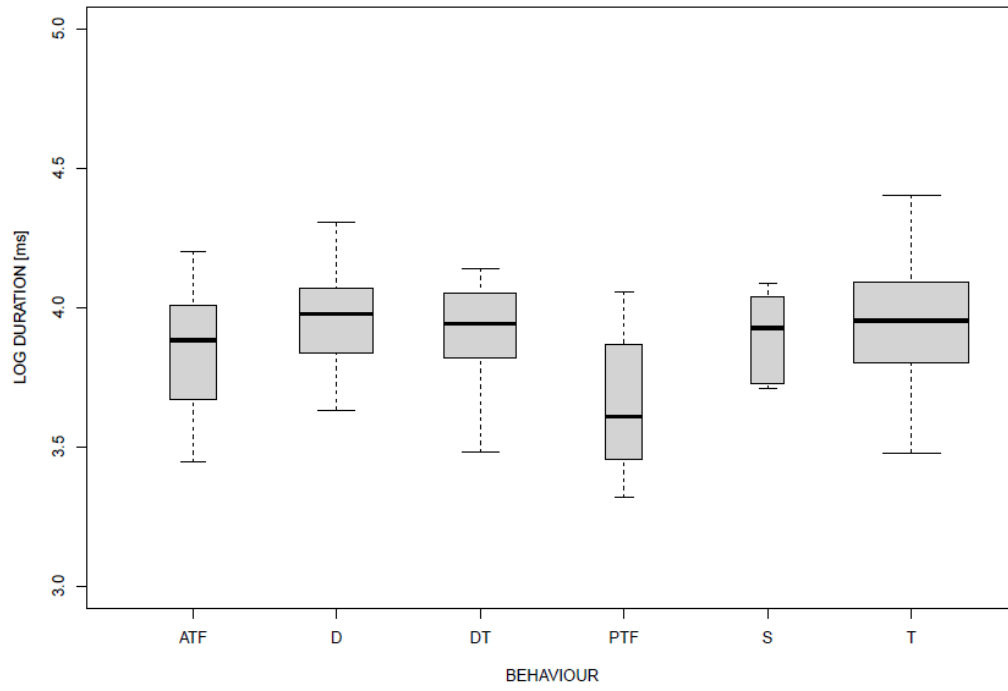3.1 B - Start Frequency is significantly influenced by Group Composition: it's higher in FC groups
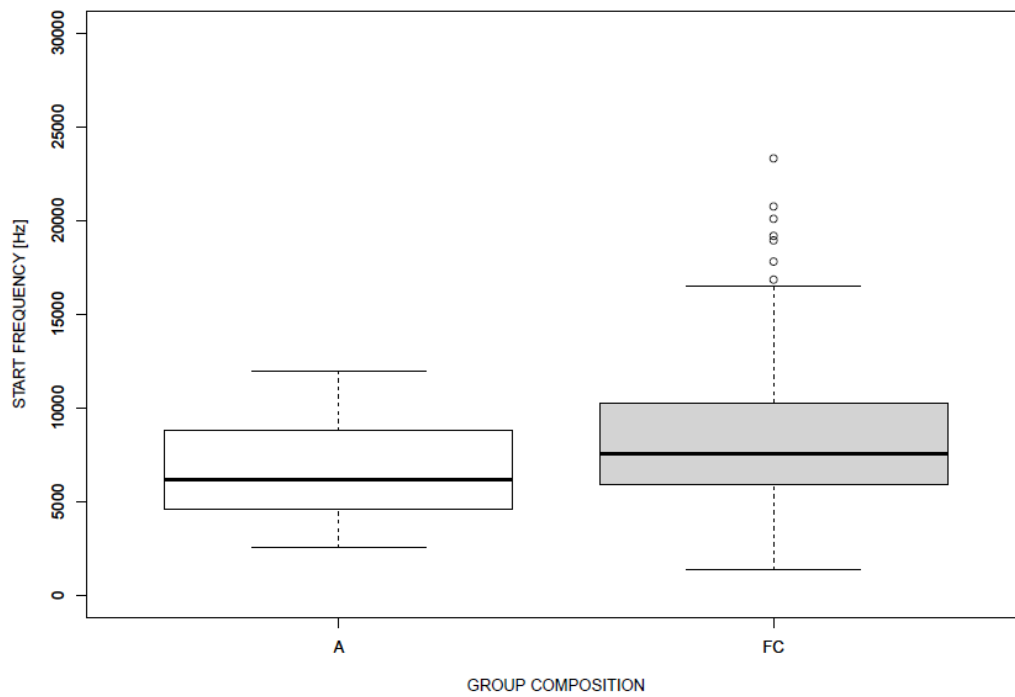
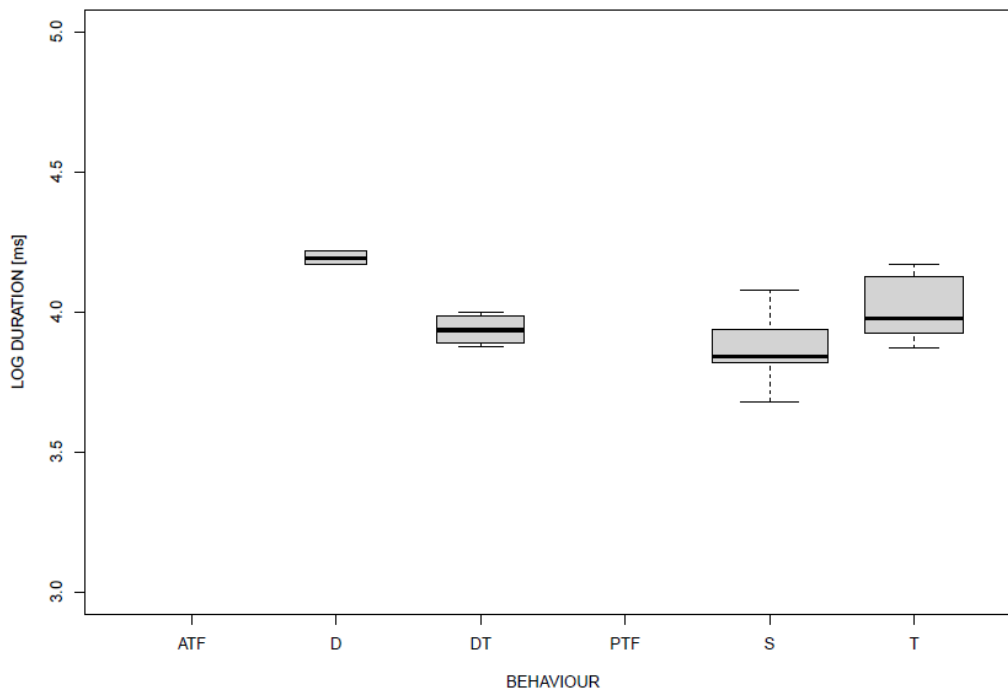## 3.1 C - Number of inflections is significantly influenced by Behavior



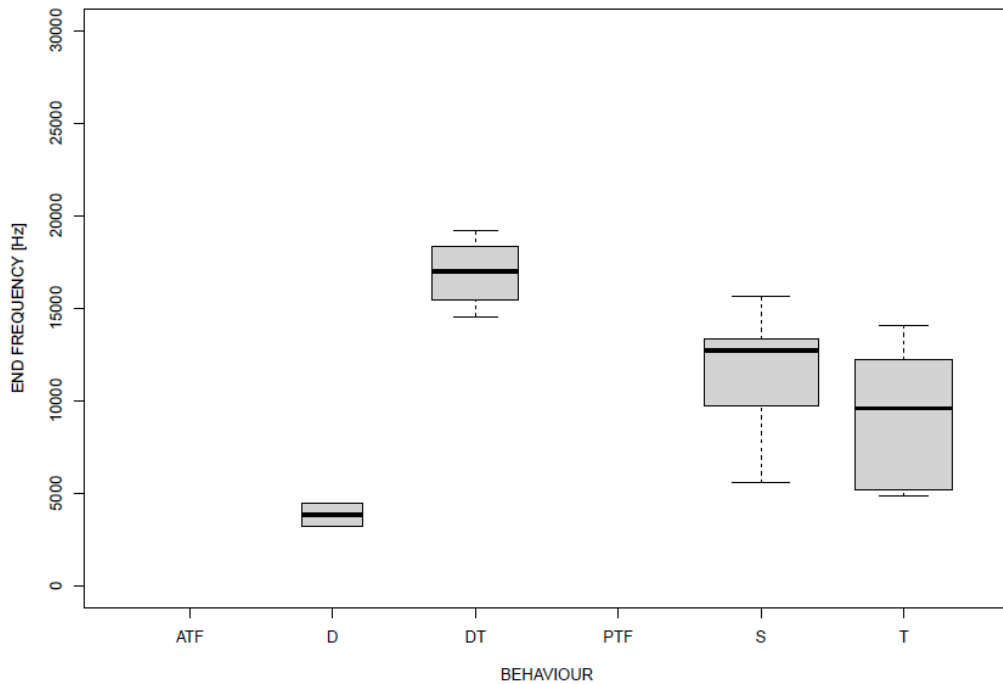## 3.1 D - LogDuration is significantly influenced by Behavior

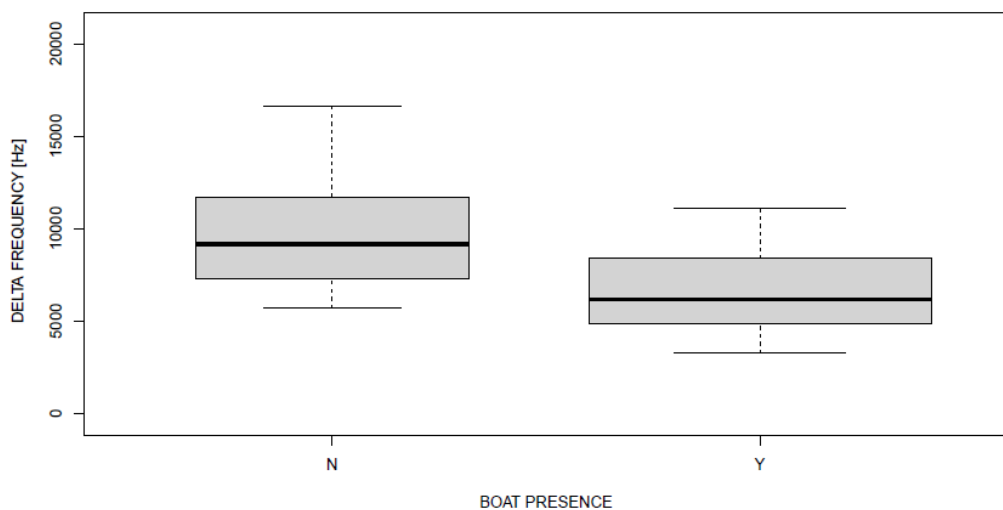## 3.1 E – LogDuration in FC groups is significantly influenced by Behavior



## 3.1 F - LogDuration in A groups is significantly influenced by Behavior

3.1 G - End Frequency in A groups is significantly influenced by Behavior



3.1 H - Frequency Range in A groups is significantly influenced by Boat Presence



### 3.3.2  Multiple comparisons

After testing the effect of each single factor on whistle parameters, further comparison were performed in order to observe possible correlations between factors.

### 3.3.2.1  Factor_1: Behavior and Boat Presence

The combination of behavior and boat presence (Factor_1) was tested with one-way ANOVA test and LogDuration was found to be significantly influenced (p value: 0.0336). Then Tukey Post Hock test was applied to verify between which categories of Factor_1 there were significant differences in LogDuration:

differences were found between Feeding_TW and Feeding_NB, Travel_MB and Feeding_TW, and Travel_NB and Feeding_TW.

One-way ANOVA for Factor_1 was applied also to find effects on group FC and A: in whistles from groups FC there was no significant difference, while in whistles from groups A minimum frequency and inflections were affected (p values: 0.0138 and 0.0096 respectively). Tukey Post Hock test was used: minimum frequency showed differences between Travel_NB and Feeding_NB and between Travel_NB and Travel_MB, while numbers of inflections differed only between Travel_NB and Social_MB. Figure 3.2 shows for each significant results a box plot resulting from ANOVA analysis and additional graphic where it is possible to observe between which categories of Factor_1 the differences were found by Tukey post-hock test.

**Fig. 3.2 – Graphics showing parameters significantly influenced by Factor_1**

3.2 A - Factor_1 has significant effects on Number of Inflections, when only A groups are considered
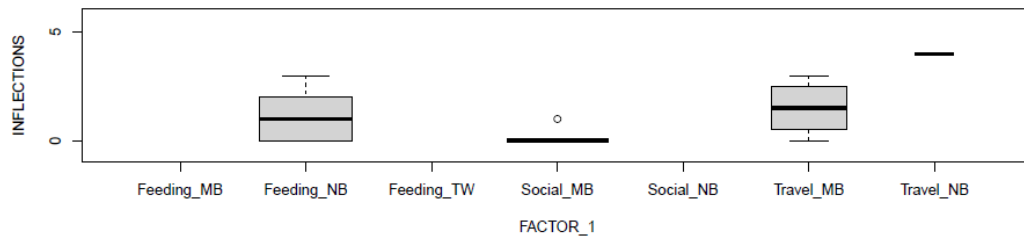


3.2 B – Tukey Post Hock graphic shows the effects of Factor_1 on Number of Inflections, when only A groups are considered
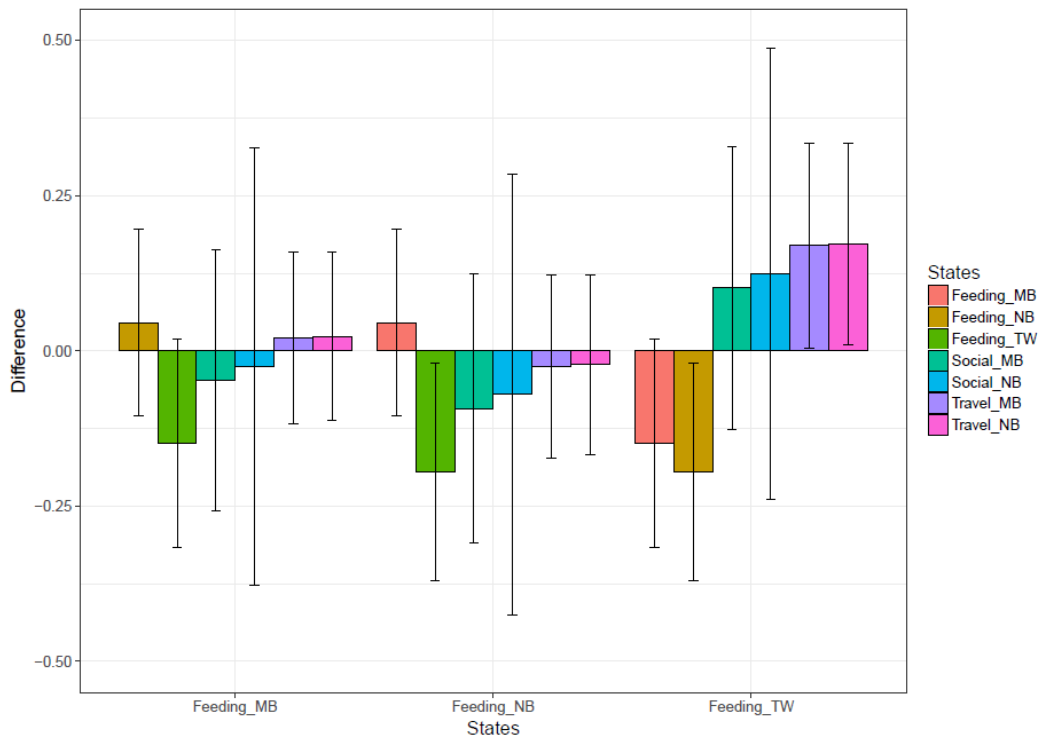
## 3.2 C - Factor_1 has significant effects on LogDuration, when the entire dataset is considered



## 3.2 D – Tukey Post Hock graphic shows the effects of Factor_1 on LogDuration, when the entire dataset is considered
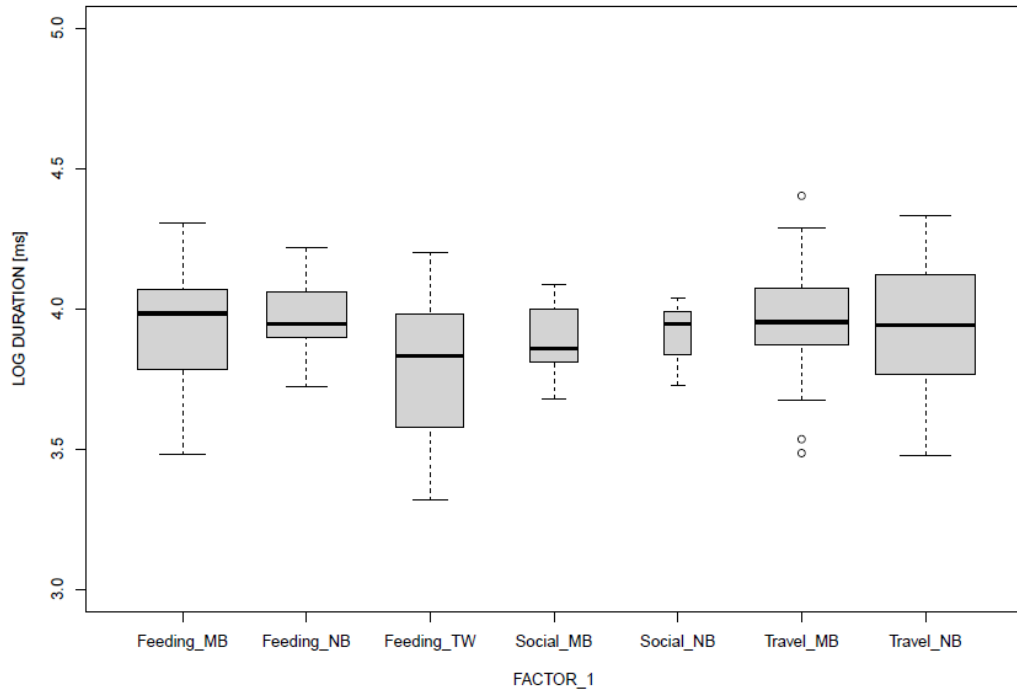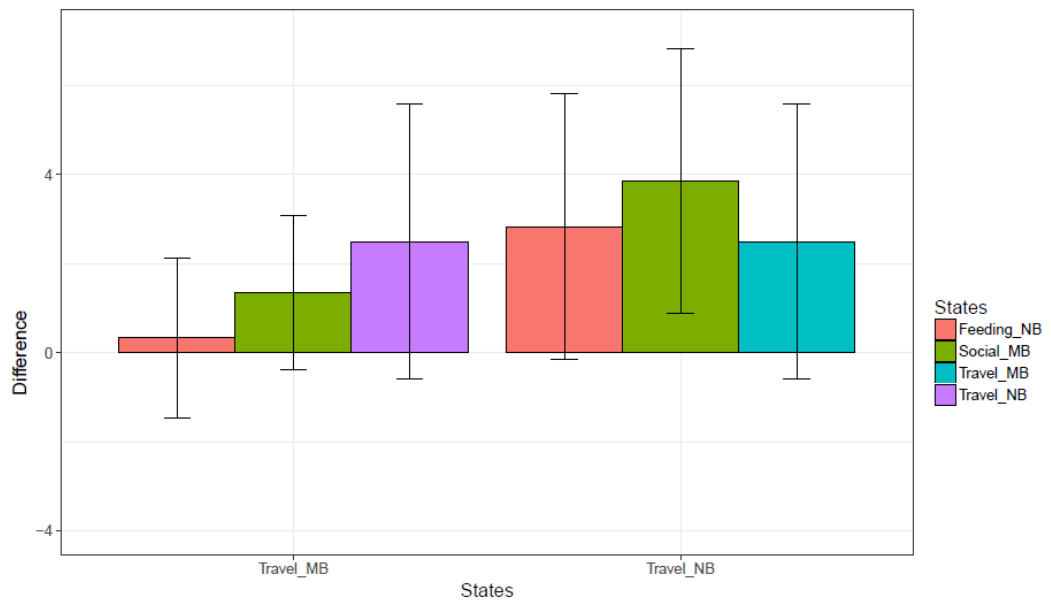
## 3.2 E - Factor_1 has significant effects on Minimum Frequency, when only A groups are considered
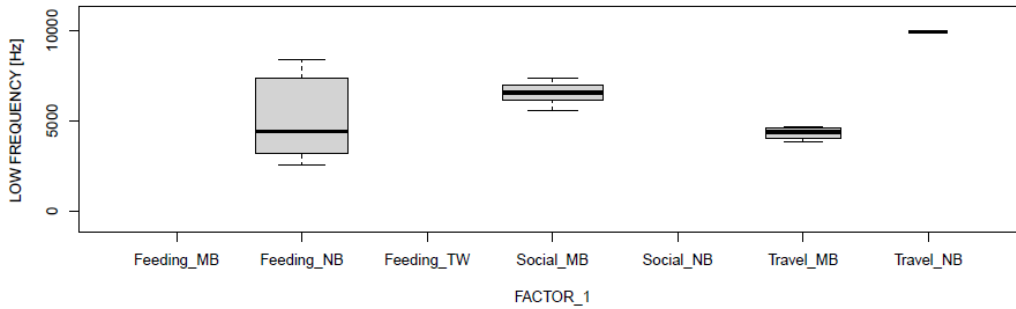


## 3.2 F – Tukey Post Hock graphic shows the effects of Factor_1 on Minimum Frequency, when only A groups are considered
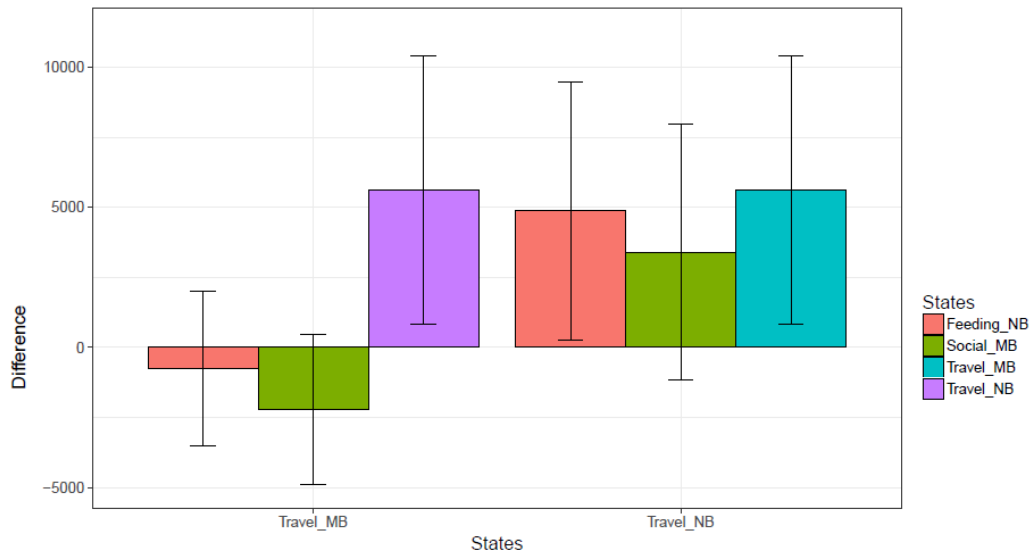


### 3.3.2.2  Factor_2: Group Composition and Boat Presence

In order to test the combination of group composition and boat presence, three subset were created according to behavior: feeding, travelling and socializing. No significant result was found in subsets feeding and travelling, while in the last one number of inflections had significant p value (0.0185). Tukey Post Hock test was applied and differences were observed between FC_MB and A_MB and between FC_NB and FC_MB.

**Fig. 3.3 – Graphics showing how number of inflections is influenced by Factor_2**

3.3 A - Factor_2 has significant effects on Number of Inflections,
when only Socializing behavior is considered
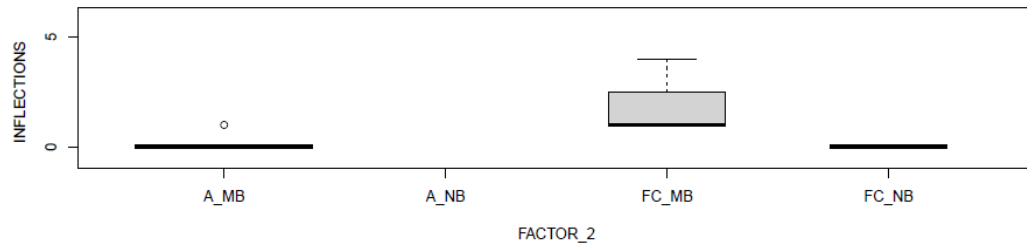


3.3 B – Tukey Post Hock graphic shows the effects of Factor_2 Number of
Inflections, when only Socializing behavior is considered
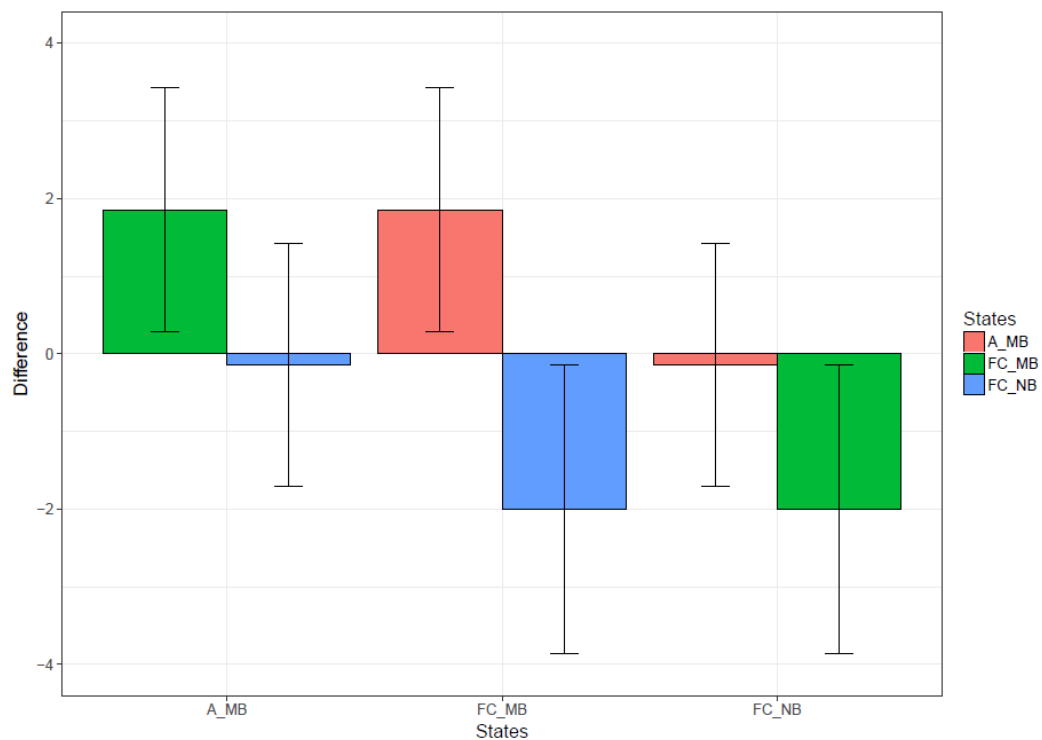


## 3.4    Discriminant Function Analysis (DFA)

Finally a Discriminant Function Analysis (DFA) was conducted in order to verify how many whistles could be correctly assigned to a group using Factor_1, Factor_2, group composition, behavior and boat presence. Thanks to this statistical analysis it was observed that 35% (discrete part) of the entire dataset was correctly grouped into the different Factor_1 categories, between 12 and 13% of the whistles were grouped into Factor_2 categories, 15% into the different group compositions, 20% were grouped according to behavior and 12-13% according to boat presence.

# 4    <u>DISCUSSION</u>

This study describes the variability of signature whistles of bottlenose dolphins in Lošinj archipelago in relation to group composition, behavior and boat presence, for the first time.

Previous studies investigated the effect of anthropogenic noise on the structure of both signature and non-signature whistles considered together (Rako *et al.*, 2012; Rako and Picciulin, 2016): dolphins may shift their whistle frequencies in order to increase transmission efficiency and detectability of their acoustic signals. Other studies offered an insight into factors which can shape the acoustic structure of signals produced by dolphins and on the way in which whistles parameters change according to environmental conditions, socio-behavioral characteristics and morpho-physiological features (Bonato *et al.*, 2015).

Acoustic data from 2016 and 2017 were analyzed in order to investigate the effects of different factors on signature whistle parameters: duration, minimum, maximum, start and end frequencies, frequency range and number of inflections. Each whistle parameter has been proved to change according to at least one variable, but neither one parameter is influenced by all the different factors considered in this study.

It is important to highlight that also other factors may take part in the variation of whistle structure: group size (Oswald *et al.*, 2008), body size (Papale *et al.*, 2013), stress condition (Esch *et al.*, 2009b), characteristics and speed of boats (Buscaino *et al.*, 2016), bathymetric differences and other habitat-dependent variables (May-Collado and Wartzok, 2008; Papale *et al.*, 2013b; Bonato *et al.*, 2015).
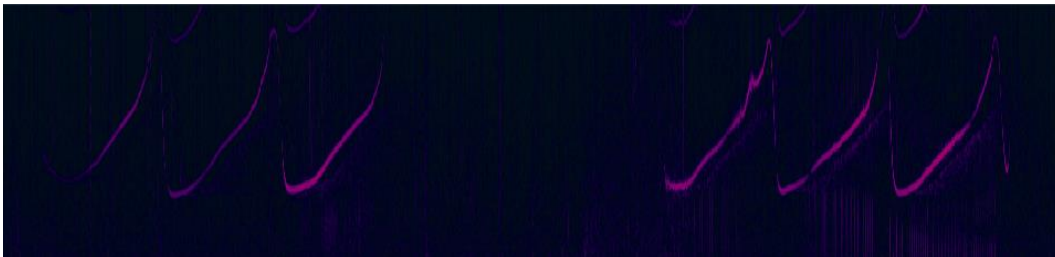


**Fig. 4.1 – Two signature whistles from a bottlenose dolphin**

## 4.1    Group composition

In this study maximum frequency and start frequency are significantly influenced by group composition: looking at Fig. 3.1, it is possible to affirm that both these parameters assume higher values in signature whistles recorded in groups composed by females and calves than in groups with only adult dolphins.

Many studies demonstrate the influence of body size on frequency parameters (Papale *et al.*, 2013b; Berta *et al.*, 2015): the presence of whistles from calves,

which are smaller than adults, may be the reason of the differences found in the results of the current study.

Other studies found that signature whistles duration and number of inflections increase with age (Caldwell and Caldwell, 1979; Caldwell *et al.*, 1990): the results of this study did not highlight any increase in the duration nor in the number of inflection in adult (A) groups compared to females-calves (FC) groups. Another study, from Fripp and Tyack (2008), affirms that calves whistles are longer and have lower frequencies than adults: also these results are not in accordance with the results of the current study, where no difference was found regarding whistle duration in groups with different age composition, and maximum and start frequencies have been shown to be higher in FC groups. Even if higher frequencies attenuate more quickly in marine environment, these frequencies may serve to broadcast location more efficiently over short distances (Esch *et al.*, 2009b). The close relationship between mother and calf can explain the use of higher frequencies: they do not need to communicate over long distances, because the calf uses to swim close to its mother, so higher frequencies may facilitate to keep them in contact (Esch *et al.*, 2009b).

According to the results found by Heiler and colleagues (2016), this study's results from multiple comparison analysis show that in presence of calves there is no variation in whistle's parameters in relation to boat presence. It is possible that the presence of calves may induce a specific pattern of frequencies which reduces the variability in whistle's emission, since calves may have less control over their whistle outputs than adults (Caldwell and Caldwell, 1979) and they may not be able to readily shift to higher frequencies in the presence of boats (Heiler *et al.*, 2016).

For these reasons, it is likely that the influence of boats may be dependent on group composition (Heiler *et al.*, 2016).

## 4.2 Behavior

Behavior has significant effects on duration of signature whistles either when the entire dataset has been analyzed and when only groups FC or A have been considered. In FC group, whistle's duration is the only parameter influenced by behavior, while in the complete dataset both duration and number of inflections have significant differences, and in A groups duration and end frequency show to be affected by dolphins behavior.

It is important to remember that modulation parameters largely depend on the social framework in which the whistle is emitted and for this reason their variation may be linked to behavioral context (Rendell *et al.*, 1999; Gannier *et al.*, 2010; Papale *et al.*, 2013b). Moreover, the variation in call duration and in number of inflections may convey information on behavioral states (Esch *et al.*, 2009b; Gannier *et al.*, 2010; Bonato *et al.*, 2015; Heiler *et al.*, 2016). Both frequency and

modulation parameters have been shown to change during travelling, feeding and socializing behaviors compared to resting behavior, with start, end and minimum frequency lower during resting behavior and maximum frequency, frequency range, duration and number of inflections lower during travelling, socializing and feeding behaviors (Heiler *et al.*, 2016): authors attributed these changes to the difference in the degree of emotional arousal among resting and the other behaviors.

In A groups also end frequency resulted significantly affected by behavior. Changes in frequency parameters have been found in many studies related to boat presence and stressful situations (Esch *et al.*, 2009b; Rako and Picciulin, 2016; Heiler *et al.*, 2016). The current difference in end frequency can be associated to different degrees of activity highlighted in different behaviors: surface foraging, social interaction, aerial and percussive behavior imply a higher level of activity, physical effort and vocal effort than resting or stationary behavior (Díaz López, 2011). It is also possible that the low number of samples in the subset A may be responsible of this result, and more in-depth studies may be required.



**Fig. 4.2 – Bottlenose dolphins jumping: the picture was taken during a sighting when the dolphins were socializing (© Photo by Blue World Institute)**

## 4.3    Boat presence

Results from the entire dataset do not show any significant change of signature whistle parameters in relation to boat presence. Only considering A groups there is a significant difference in frequency range, which seems to be smaller in boat presence (Fig. 3.1), and also maximum frequency value is really close to significance. Still, the difference was found in the subset A, the smaller one, and the result may be linked to the limited number of samples.

From an acoustic point of view, the main repercussion of boat presence is the so called "masking effect": the reduction or limitation of both signaling range and

signal's quality in terms of information (Weilgart, 2007; Oswald et al., 2008; May-Collado and Quinones-Lebron, 2014). The masking effect may have long-term negative impacts on dolphins' life, since they heavily rely on vocalizations to communicate and to transmit information among individuals within a group, to mediate social interaction, to maintain group cohesion (Díaz López, 2011). For this reason, dolphins may alter their whistle structure in order to avoid the decrease in communication efficiency (Rako and Picciulin, 2016). Generally, dolphins may shift their whistle frequencies to the range with lower noise interference, in order to enhance transmission efficiency and signal detectability: in conditions of elevated noise in the low- to mid-frequency range related to small motorized boats (Lesage et al., 1999; Lemon et al., 2006; Jensen et al., 2009), cetaceans shift their whistle frequencies to an higher frequency range (May-Collado and Wartzok, 2008; Rako and Picciulin, 2016). Shifting frequency upward while maintaining other parameters may be advantageous if it allows an accurate transmission of identity and other information in areas with elevated background noise (Heiler et al., 2016). Other studies also found an increased amplitude in presence of high ambient noise and masking effect (Tyack, 1985; Au and Benoit-Bird, 2003; Heiler et al., 2016).

Another way to enhance signal detectability is to prolong call duration when boats are present (Lesage *et al.*, 1999; May-Collado and Wartzok, 2008). However, in the current study no significant results have been found in the comparisons between whistle duration related to boat presence or absence, such as in the studies from Buckstaff (2004) and Luís and colleagues (2014). Generally, the duration of signature whistles depends on behavioral context (Janik *et al.*, 1994; Esch *et al.*, 2009b; Heiler *et al.*, 2016). In the current study only signature whistles have been analyzed, and this can be the explanation for the absence of significant changes in whistle's duration related to boat presence: the influence of behavior dominates the effect of boat noise on whistle parameters (Heiler *et al.*, 2016).

A synergistic negative effect has been found among boat presence, the number of boats present and boat engine noise (Rako and Picciulin, 2016; Heiler *et al.*, 2016) and this can lead to an overall increase in energy expenditure (Williams *et al.*, 2006; Holt *et al.*, 2015) and stress levels (Romano *et al.*, 2004; Rolland *et al.*, 2012). Boats represent also a source of behavioral disturbance (Rako *et al.*, 2013; Pirotta *et al.*, 2015): generally the increasing presence of boats is related to horizontal and vertical avoidance, in relation to the decrease of communication range and of the signal-to-noise ratio in presence of boat traffic (Rako and Picciulin, 2016).

It would be interesting to investigate if the parameters of signature whistles would change in relation both to boats presence and to season, since in Lošinj archipelago there is a peak in nautical traffic during summer months.

**Fig. 4.3 – Bottlenose dolphins swimming around the dolphin-watching boat from Blue World Institute (© Photo by Blue World Institute)**

## 4.4   Multiple comparisons

Factor_1 combines the effects of behavior and boat presence on dolphin whistles. Significant results were found in relation to duration considering the entire dataset, and to number of inflections and minimum frequency when considering only the subset A. Regarding duration in the overall dataset (INT), effects have been observed mostly between feeding and travelling behavior and in presence of trawler boats. The major impact seems to be induced by the presence of trawler boats: signature whistles duration changes between recordings taken during feeding activities in presence of trawlers and without trawlers, but also between different behaviors (feedeing and travelling). Romeu and colleagues (2017) studied the differences present between dolphins which use to feed in cooperation and dolphins which do not cooperate to forage: whistle's duration has been found to significantly decrease in cooperative dolphins, while frequency and number of inflections were lower in non-cooperative dolphins. Authors suggest that this founding can highlight how dolphins are able to use slightly different sounds according to behavioral context. Moreover they found significant differences in whistles emitted during foraging activities in presence or in absence of fisherman. This could suggest the use of specific social sounds according to the feeding context: these whistles may be important both for the coordination of individuals during different foraging activities and for the association between dolphins that use the same foraging tactic (Romeu *et al.*, 2017).

Foraging and socializing generally involve a more intense level of communication among individuals within a group and may suffer a greater impact from boat disturbance (Rako and Picciulin, 2016). During foraging activities, dolphins rely on acoustic communication to coordinate their catch in order to enhance their hunting success (Janik, 2000; Naguib *et al.*, 2009). On the other hand, bottlenose dolphins may increase their whistle rate while they are socializing, so as they are able to maintain contact and to develop social relationship among group members

(Cook *et al.*, 2004; Quick and Janik, 2008). In noisy conditions, the energy spent for vocalization significantly increases and it is possible that dolphin's chances of food intake may suffer a reduction (Williams *et al.*, 2006; Lusseau and Bejder, 2007). Heiler and colleagues (2016) found an increase in the number of inflections from higher to lower state of arousal. In the current study, the number of inflections shows to be influenced by Factor_1 in the subset A, with whistles from the category Travel_NB significantly higher than the ones from Social_MB (Fig. 3.1). Regarding Factor_2, the combination of boat presence and group composition significantly influences the number of inflections registered in signature whistles during socializing behavior, with whistles from the category FC_MB being significantly higher in frequency than the ones from FC_NB and A_MB. Furthermore, for Factor_2, number of inflections changes between the categories FC_MB and FC_NB, which expresses the effect of boat presence on whistle parameters, and between A_MB and FC_MB, which underlines the effect of group composition.

## 4.5   Discriminant function analysis

A linear discriminant function analysis (DFA) is a statistical analysis used to distinguish among predefined groups of samples. In this study, it was verified how many whistles could be correctly assigned to a group using Factor_1, Factor_2, group composition, behavior and boat presence. Factor_1 has been found to be the most powerful factor of discrimination for signature whistles in this study, while Factor_2, group composition and boat presence have a very low data discrimination power, and behavior gives discrete results.

In other studies concerning whistle parameters variation between different areas, DFA has been used to test whether whistles could have been assigned to the right area, with satisfactory results (La Manna *et al.*, 2017). However this is the first study where DFA is used to investigate which factors are the most important to discriminate between signature whistles emitted in different contexts: in this study, the combination of behavior and boat presence has been found to represent to greater discriminant factor, but further analysis are required.

# 5 <u>CONCLUSION</u>

The aim of this study was to identify variations in signature whistles parameters according to different variables: behavior, group composition, boat presence and the combination of behavior and boat presence, and group composition and boat presence. This is important to reach a more in-depth comprehension of the complex communication system of bottlenose dolphins, which have a considerable value for the management and conservation of this cetacean species in Cres-Lošinj archipelago. The study of the acoustic features of bottlenose dolphins can be useful to evaluate their conservation status and to monitor population changes, possibly helping to recognize the dominant threats (Papale *et al.*, 2013). Furthermore, signature whistles carry identity information in their frequency modulation pattern (Janik and Sayigh, 2013) and represent the majority of the whistles emitted both in captivity and by free-ranging dolphins (Buckstaff, 2004; Cook *et al.*, 2004; Watwood *et al.*, 2005; Sayigh *et al.*, 2007). Finding out possible changes in the overall acoustic repertoire of bottlenose dolphins, and in particular in signature whistles, may allow to assess potential threats for the Kvarnerić population.

One limit of the current study may be the omnidirectional recording system, which does not allow for the identification of the "vocalizing" dolphin individual (May-Collado, 2010): identifying the signature whistles of specific individuals within a population may be helpful to recognize them during encounters on the sea, parallel to photo-identification, and may give information on life history traits, underwater behavior and group composition useful for conservation models and management decisions. Some studies have been conducted using both real-time behavioral sampling and video recordings, so that it is possible to link sounds to the real underwater behavioral context, possibly helping with the complete understanding of the function of communicative sounds (Thomas *et al.*, 2002). Other researchers used a suction cup hydrophone, which allowed for the identification of the caller, but also needed more invasive methods, such as at least one capture-release session (King *et al.*, 2013).

A possible bias of this study can be the fact that all the acoustic recordings were collected during daylight: for this reason it is possible that some behaviors that may be more frequent during the night could have been excluded or underestimated, and the noise level in the area could have been overestimated. To avoid this problem, some hydrophones may be placed in fixed points in order to collect data throughout the day.

Future studies in Cres-Lošinj archipelago could investigate the influence of other variables on signature whistle parameters, such as group size or stress condition; moreover the presence and the development of male alliances would be interesting to assess which role can have signature whistles in this context.

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