

UNIVERZA NA PRIMORSKEM  
FAKULTETA ZA MATEMATIKO, NARAVOSLOVJE IN  
INFORMACIJSKE TEHNOLOGIJE

MASTER'S THESIS  
(MAGISTRSKO DELO)

THE EFFECTS OF VESSEL PRESENCE AS A TRIGGER  
FOR BEHAVIOR CHANGES OF BOTTLENOSE  
DOLPHINS (*TURSIOPS TRUNCATUS*) IN CRES-LOŠINJ  
ARCHIPELAGO

(VPLIV PRISOTNOSTI PLOVIL NA SPREMEMBE  
VEDENJA VELIKE PLISKAVKE (*TURSIOPS  
TRUNCATUS*) NA OBMOČJU LOŠINJSKO-CREŠKEGA  
ARHIPELAGA)

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**The effects of vessel presence as a trigger for behavior changes of  
bottlenose dolphins (*Tursiops truncatus*) in Cres-Lošinj archipelago**

(Vpliv prisotnosti plovil na spremembe vedenja velike pliskavke (*Tursiops truncatus*) na  
območju Lošinjsko-Creškega arhipelaga)

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Koper, november 2019

## Ključna dokumentacijska informacija

Ime in PRIIMEK: Brigita ŠIMUNAC

Naslov magistrskega dela: Vpliv prisotnosti plovil na spremembe vedenja velike pliskavke (*Tursiops truncatus*) na območju Lošinjsko-Creškega arhipelaga

Kraj: Koper

Leto: 2019

Število listov: 69                      Število slik: 17                      Število preglednic: 8

Število prilog: 2                      Število stran prilog: 2

Število referenc: 60

Mentor: doc. dr. Peter Mackelworth

Delovna somentorja: dr. Nikolina Rako Gospić, Marko Radulović

UDK: 599.537.6(497.572) (043.2)

Ključne besede: Lošinjsko-Creški arhipelag, *Tursiops truncatus*, vpliv morskih plovil na kite, turistično opazovanje delfinov, analiza markovskih verig

Povzetek: Lošinjsko-Creški arhipelag je pomembno prehranjevalno in razvojno območje za populacijo navadnih pliskavk in zaradi tega tudi NATURA 2000 območje za to vrsto. Majhna, stalno prisotna populacija pliskavk je izpostavljena velikim sezonskim nihanjem v količini pomorskega prometa, vključno s plovili za turistične ogledе kitov in delfinov. Pričujoča magistrska naloga je ovrednotila učinek prisotnosti plovil z uporabo analize markovskih verig. Spremembe vedenjskih vzorcev živali lahko smatramo kot kratkoročne odzive na motnje v okolju in nam lahko služijo kot indikatorji vpliva plovil na morske sesalce. Rezultati te naloge podajo kvantitativne in kvalitativne smernice za monitoring dejavnosti turističnih ogledov kitov in delfinov ter potencialne varstvene ukrepe in nadaljnje raziskave.

### Key words documentation

Name and SURNAME: Brigita ŠIMUNAC

Title of the thesis: The effects of vessel presence as a trigger for behavior changes of bottlenose dolphins of Cres-Lošinj archipelago

Place: Koper

Year: 2019

Number of pages: 69

Number of figures: 17

Number of tables: 8

Number of appendices: 2 Number of appendices pages: 2

Number of references: 60

Mentor: Assist. Prof. Peter Mackelworth, PhD

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UDK: 599.537.6(497.572)(043.2)

Keywords: Cres-Lošinj archipelago, *Tursiops truncatus*, marine vessels impact on cetacean, dolphin-watching tourism, Markov chains analysis

Abstract: Cres-Lošinj archipelago is an important feeding and nursing ground for the resident bottlenose dolphin population and consequently a Natura2000 site for this species. This small population is exposed to extreme seasonal variations in the intensity of the marine traffic, including dolphin-watching activities. This thesis estimates the effects of vessel presence and dolphin-watching tourism on the behavior of focal dolphin groups by applying Markov chain analysis. Changes in behavioral patterns are considered as short-term effects and can be used as an early indicator of the impact of vessels on cetaceans, before they become unmanageable. Results of this thesis provides quantitative and qualitative information for wildlife tourism monitoring with the aim of adopting appropriate conservation measures and provides directions for further research.

## ACKNOWLEDGEMENTS

I would like to thank my mentor Peter Mackelworth for taking me to the Blue World Institute team and giving me the opportunity to spend some time on the sea in the best possible company – with the dolphins. Special thanks go to my co-mentor Nikolina Rako Gospić for all the help, questions answered, suggestions given and all the friendly conversations we had. Thank you, Nike, you're the best mentor that student can wish for! Thanks to my second co-mentor Marko Radulović for introducing me with his namesake in R program and for being so cool when I freaked out about it. Thanks to (Mrs.) Tihana Vučur for helping me with ArcGis program. Thanks to my favorite professor Bojan Lazar for his wholehearted help with shaping this paper. Thanks to Iva Stoiljković for being my best friend (short and simple, just as she likes it 😊). Thanks to my Slovenian translator and friend Matic. Thanks to Mali for being my life companion. Last thanks go to our dear Adriatic dolphins which honored me with their presence and beauty. I am grateful to have them around and hope that generations after me will still have this chance.

## TABLE OF CONTENTS

1 INTRODUCTION .....	1
1.1 BOTTLENOSE DOLPHINS ( <i>TURSIOPS TRUNCATUS</i> ) .....	1
1.2 CONSERVATION STATUS OF THE BOTTLENOSE DOLPHINS.....	3
1.3 BOTTLENOSE DOLPHINS OF THE CRES-LOŠINJ ARCHIPELAGO.....	4
1.4 DOLPHINS – VESSELS INTERACTIONS .....	6
1.5 WHALE-WATCHING INDUSTRY AND OVERVIEW OF ITS EFFECTS.....	7
ON CETACEANS.....	7
1.6 THE AIM AND HYPOTHESIS OF THIS STUDY .....	11
2 MATERIALS AND METHODS .....	12
2.1 STUDY AREA .....	12
2.2 DATA COLLECTION.....	15
2.3 DATA ANALYSIS .....	18
2.3.1 Behavioral transitions and impact conditions.....	18
2.3.2 Variation in behavior in dependence of vessel presence.....	20
3 RESULTS.....	23
3.1 DATA DISTRIBUTION .....	23
3.2 THE DEPENDENCE OF CHANGES IN DOLPHIN’S BEHAVIOR TO IMPACT CONDITIONS .....	28
3.3 BEHAVIORAL TRANSITION PROBABILITIES AND BEHAVIORAL BUDGETS IN DEPENDENCE TO IMPACT CONDITIONS .....	31
4 DISCUSSION.....	39
5 CONCLUSIONS .....	47
6 POVZETEK .....	49
7 REFERENCES.....	53

## LIST OF FIGURES

Figure 1: Distribution of bottlenose dolphin ( <i>Tursiops truncatus</i> ) worldwide .....	2
Figure 2: Bottlenose dolphin ( <i>Tursiops truncatus</i> ).....	3
Figure 3: Dolphin-watching tour organized by the Blue World Limited.....	6
Figure 4: The depths profile of the Adriatic Sea. ....	12
Figure 5: Study area.....	13
Figure 6: Natura 2000 sites in Croatia related to preservation of bottlenose dolphins habitat..	15
Figure 7: Categories of sampled behavioral states on study area.....	24
Figure 8: Proportions of each behavioral state in behavioral budget .....	24
Figure 9: Spatial layout of impact conditions.....	26
Figure 10: Proportions of behavior transition for every vessel type in the initial 3-minute sequence of every sighting .....	27
Figure 11: Proportion of behavior transitions in sightings with only ‘control’, ‘impact DW’ and ‘impact OB’ samples during the whole sightings .....	28
Figure 12: Transition plot for control chain .....	32
Figure 13: Transition plot for ‘impact DW’ chain. ....	33
Figure 14: Transition plot for ‘impact OB’ chain. ....	33
Figure 15: Difference between the transition probability of ‘control’ and ‘impact DW’ condition (a), and control and ‘impact OB’ condition (b).....	35
Figure 16: Behavioral budget for dolphin activity: the proportion of time spent in each behavioral state during ‘control’ (grey), ‘impact DW’ (blue) and ‘impact OB’ (green) conditions .....	36
Figure 17: Cumulative budget estimation for ‘impact DW’ condition (a) and for ‘impact OB’ condition (b) .....	37

## LIST OF TABLES

Table 1: Taxonomy of bottlenose dolphin <i>Tursiops truncatus</i> .....	1
Table 2: Categories of dolphin behavioral states.....	17
Table 3: Dolphins size classes .....	17
Table 4: Vessel categories. ....	18
Table 5: Impact conditions. ....	18
Table 6: Three-minute scans and proportions of behavior transition scans within ‘control’ and both impact conditions. ....	25
Table 7: The dependence of the transition in dolphins’ behavior to impact condition in overall data .....	29
Table 8: Comparison of ‘control’ condition to impact conditions for behavioral transition samples .....	30



## **LIST OF APPENDICES**

APPENDIX A: Code of conduct

APPENDIX B: Behavioral data sheet

## 1 INTRODUCTION

### 1.1 BOTTLENOSE DOLPHINS (*TURSIOPS TRUNCATUS*)

The common bottlenose dolphin, *Tursiops truncatus*, (Montagu 1821) (hereafter ‘bottlenose dolphin’) has been studied intensively throughout the world, and today is presumably the most familiar of the small cetaceans (Bearzi et al., 2008; Franzosini et.al., 2013). It is a member of the family Delphinidae (Table 1) that belongs to suborder Odontocete or toothed whales (Capitanio, 2016).

Table 1: Taxonomy of bottlenose dolphin *Tursiops truncatus* (Marley et al., 2017).

<b>Kingdom</b>	Animalia
<b>Phylum</b>	Chordata
<b>Subphylum</b>	Vertebrata
<b>Class</b>	Mammalia
<b>Order</b>	Cetacea
<b>Suborder</b>	Odontocete
<b>Family</b>	Delphinidae
<b>Genus</b>	<i>Tursiops</i>
<b>Species</b>	<i>truncatus</i> (Montagu, 1821)

Bottlenose dolphins are distributed worldwide through tropical and temperate inshore, coastal, shelf and oceanic waters (Figure 1). They tend to be primarily coastal, but can be found also in pelagic waters (Scheidat et al., 2004).

They have generalized features by which they are recognizable: medium-size, robust body, a moderately falcate dorsal fin, convex flippers with pointed tips, short rostrum and demarcation between the melon (Wells and Scott, 1999). Their color pattern is typically a dark grey back with light grey sides and a near white belly (Figure 2). Adult animals often have scars inflicted by other animals, including rake marks caused by other dolphins’ teeth. Their size reaches up to 3-4 meters with weight around 300 kg when fully grown (Franzosini et.al., 2013). Mean age of sexual maturity of bottlenose dolphin is between 8 and 12 years and the duration of the mother-calf bond last 3-4 years, or longer (Bearzi et al., 1997).

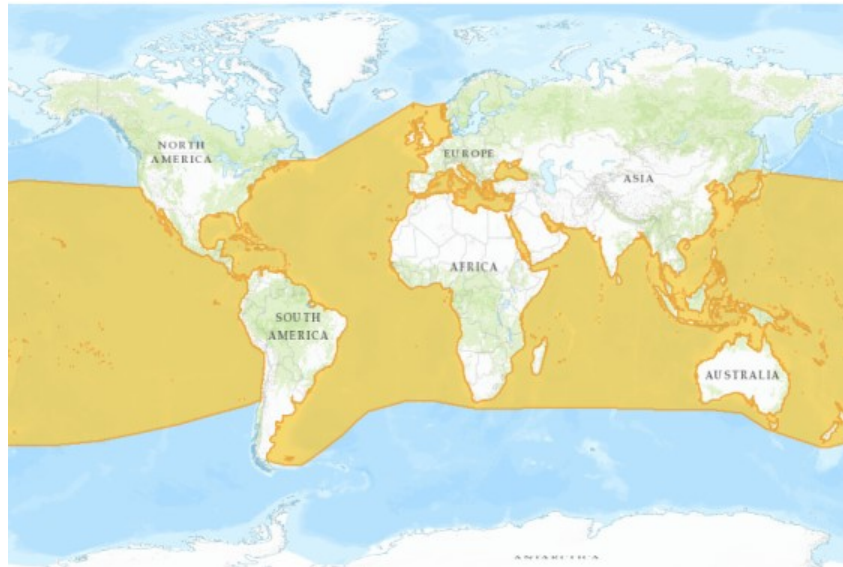


Figure 1: Distribution of bottlenose dolphin (*Tursiops truncatus*) worldwide (Scott et al., 2012).

Being social animals, they usually form groups of 2-15 animals, although groups of more than 1000 have been reported worldwide. In general, bottlenose dolphins in bays and estuaries tend to form smaller groups than those in offshore waters. Group composition is dynamic, but sub groupings may be stable or repeated over periods of years. Sex, age, reproductive condition, familial relationships and affiliation histories appear to be important factors determining group composition (Wells and Scott, 1999). In the wild, bottlenose dolphins seem to be active both during the day and at night, feeding, travelling, socializing or resting. The duration and frequency of activities are influenced by environmental factors, as season, habitat, time of day, and tidal state; and by physiological factors, such as reproductive seasonality (Wells and Scott, 1999).

Like all marine mammals, bottlenose dolphins create sounds and use it to communicate about the danger, food, position of a conspecific, other animal, their own position; identity, territorial and reproductive status. Since the sea water transmits sound more readily than light, most of marine mammals rely on hearing. Odontocete cetaceans have developed a highly sophisticated system of sound production and reception similar to an active biosonar, echolocation (Rako, 2006).



Figure 2: Bottlenose dolphin (*Tursiops truncatus*) (Blue World Institute).

## 1.2 CONSERVATION STATUS OF THE BOTTLENOSE DOLPHINS

The global conservation status of the bottlenose dolphins (IUCN) is classified as least concern. Within the Mediterranean, they are listed as vulnerable (Franzolini et al., 2013). Bottlenose dolphins are protected in Croatia, as well as all cetacean species which happen to occur in the Adriatic Sea, since 1994 by the Law on Nature Protection (NN/31/1995). According to the IUCN, an isolated population numbering less than 250 individuals, which is the case with Cres-Lošinj population, is classified as “critically endangered” (Fortuna and Mackelworth, 2001). According to the Red Book of Mammals of Croatia (2018), they have a status of endangered species. Further, Croatia is a member of several international conventions and agreements relevant to cetacean protection including: “The Convention on the Conservation of Wild Life and Habitat in Europe” (Bern, 1979), “ACCOBAMS” (Agreement on the Conservation of Cetaceans in the Black Sea, Mediterranean Sea and contiguous Atlantic Area; Monaco, 1996), “The Convention for the Protection of Marine environment and Coastal region of the Mediterranean” and “The Protocol concerning Specially Protected Areas and Biological Diversity in the Mediterranean” (Barcelona, 1995; Mackelworth et al., 2013).

Worldwide, bottlenose dolphins face many threats including disease caused by pollution, loss of habitat, fishery, resources degradation, interaction with commercial fisheries and physical and acoustic disturbance caused by human activities, specially increased boat traffic in areas

with high levels of marine tourism (Papale et al., 2011). In Croatia, tourism is the primary economic driver and therefore is the greatest potential threat to the biodiversity of the islands, coastline and sea (Papale et al., 2011). In the Cres-Lošinj archipelago, extreme seasonal variation occurs with intensive and rapid increase of the marine traffic (Rako et al., 2012). For the coastal species, such as bottlenose dolphins, this humanly induced pressure can adversely affect their communication (Rako and Picciulin, 2016), distribution and abundance (Pleslić et al., 2015). Tourism related threats are accompanied by the threats caused by urbanization and unregulated development, fishing, and pollution (Mackelworth et al., 2013).

### 1.3 BOTTLENOSE DOLPHINS OF THE CRES-LOŠINJ ARCHIPELAGO

The Northern Adriatic is considered to be the only Mediterranean area with quantitative historical information about bottlenose dolphin population (Scott et al., 2012). Data indicates decline by at least 50% of bottlenose dolphins over the past 50 years, largely as a consequence of historical killing in extermination campaigns to reduce competition for fish, along with habitat degradation and overfishing. The extermination campaigns were conducted until the early 1960s. For the north-western Mediterranean, the available information suggests similar decline trends (Scott et al., 2012). According to Arcangeli and Crosti (2008) in the Mediterranean Sea bottlenose dolphin populations are scattered and occurrence is not well monitored.

In the Cres–Lošinj archipelago a resident bottlenose dolphin population has been consistently studied within the Adriatic Dolphin Project (ADP). The Adriatic Dolphin Project is the longest consistent study of bottlenose dolphins in the Mediterranean Sea as it has been ongoing since 1987. Since 1999, the project has been implemented by the Blue World Institute for Marine Research and Conservation (BWI), an independent non-profit organization headquartered in Veli Lošinj, on the Lošinj Island. The aim of the Adriatic Dolphin Project is to conduct a research on the bottlenose dolphin population that inhabits Cres-Lošinj area and also to raise public awareness through lectures, media presentations and interaction with other environmentally orientated individuals and organizations. The standard research procedure includes extensive photo-identification, behavioral sampling and acoustic sampling but other information, like dolphin habitat use, association patterns, and reproductive rate are also gained (Rako, 2006).

Since 2001, within the ADP, over 600 individual bottlenose dolphins have been identified in the Cres-Lošinj Archipelago via photo-identification techniques. Population estimation for the period from 1995 to 2003 varies between 80 and 180 dolphins (Radulović, 2016). Last estimations indicate that there are 184 resident individuals living around the islands of Cres and Lošinj (Radulović, 2016; Pleslić et al., 2015).

According to Bearzi et. al. (1997), dolphins of the Cres-Lošinj archipelago showed scattered spatial pattern since the food resources are reportedly scarce, according to local fishermen and unpublished information. Bottlenose dolphins, although occasionally forming large groups, were typically spread into small units, engaged in feeding-related activities, compared to the larger size of travelling and socializing groups. Such a strategy could allow an optimization of foraging efficiency and may indicate that small groups may allow individual foraging in which each animal have a better chance at catching limited prey (Bearzi et al., 1999). The average group size for the period from 2004 to 2011 ranged from 5.9 to 9.3 indicating a decreasing trend (Pleslić et al., 2015). More than 80% of their behavioral budget (proportion of time spent in one behavioral activity) is related to activities of searching for prey and feeding (Bearzi et al., 1999). In Nimak's research from 2006, the most frequent behavioral state was diving (49.4%) followed by dive-travelling (39.4%) while all other behavioral states were recorded less frequently. These dolphins have developed an alternative strategy for finding food described as trawler following making up to 4.6 % of their behavioral budget (Bearzi et al., 1999). They feed mainly on demersal fish which lead them in conflict with commercial fisheries over a food source that is dramatically declining in the Adriatic Sea. However, Cres-Lošinj bottlenose dolphin population can be considered as highly adaptable, feeding on other types of fish as well (Nimak, 2006).

As a response to visitor's desire to see the dolphins in their natural habitat and in a dolphin-friendly and environmentally conscious manner, in 2013, the Blue World Institute registered its own limited company – Blue World Limited, which major activity is organizing dolphin watching trips (Figure 3). Skippers of Blue World Limited operate dolphin-watching boat respecting the “Code of conduct” (Appendix A) which contains strict rules of behavior and time spent in vicinity of dolphin group. In order to evaluate activities of the dolphin watching boat, the Blue World Institute requires the skipper to log boat tracks, locations of dolphin encounters and information on their behavior. In that way, dolphin watching boat can also provide additional information for the research of the local population (Blue World Institute,

2018). This study is one part of the measures by which BWI assesses potential impact of dolphin-watching tour on resident dolphins.



Figure 3: Dolphin-watching tour organized by the Blue World Limited (Blue World Institute).

#### 1.4 DOLPHINS – VESSELS INTERACTIONS

The use of coastal habitats by bottlenose dolphins exposes them to higher levels of human activities than many other cetaceans (Nowacek, 2001). In coastal marine areas, vessel traffic is the most ubiquitous anthropogenic activity with the potential to adversely affect marine wildlife (Marley, 2017). Dolphin reactions to vessel presence are often classified in positive, negative or neutral responses. Positive reactions are considered as vessel approaching and bow riding; negative reactions when the animals move away changing direction/speed or dive longer; and neutral when animals continue their activity without notable change (Capitania, 2016). The studies on cetaceans have shown various horizontal and vertical strategies of avoidance of marine vessels, as a typical negative response. These tactics are very similar to predator avoidance strategies (Lusseau, 2003b). Cetaceans can also be habituated to vessel presence in the areas with no intrusive exposure. Habituation is considered to be a “measure of tolerance”, however, it needs to be interpreted carefully since reduced responsiveness of animals does not directly imply that they are tolerant or habituated to disturbance (Nimak, 2006).

Besides through their physical presence vessels may disturb cetaceans also through the noise that they produce. Noise from anthropogenic sources is now an inescapable life fact for most

cetacean populations worldwide. Such noise, beside vessel presence includes seismic surveying, dredging activities, drilling, explosions, sonar noise and acoustic anti-predator devices (Fortuna et al., 2018). Noise produced by marine vessels can have an adverse effect on animals by causing stress, increasing risk of mortality by distracting the predator/prey detection and avoidance, and by interfering usage of sounds in their communication especially in relation to reproduction and navigation (Rako, 2006).

The literature reports diverse range of vessels-related disturbance to dolphins, from relatively subtle changes in vocal behavior to broad-scale animal movements away from the affected area (Marley et al., 2017). Studies on small cetaceans in the presence of tour vessels have documented short-term changes in animal activity including: breathing rates in *Tursiops truncatus*; swimming directions in *Stenella spp.*, *Orcinus orca* and *Tursiops aduncus* and speed in *Orcinus orca* and *Tursiops truncatus*; diving times in *Tursiops truncatus* and *Sousa chinensis*; phonation rates in *Tursiops truncatus*; changes in specific behavioral states such as travelling and feeding, changes in behavioral state patterns and synchrony in *Tursiops truncatus* (Arcangeli and Crosti, 2008). Studies that quantify the nature and extent of short-term responses to human disturbance, such as changes in animals' behavior, can be useful for alerting researchers to potential population-level effects while they are still reversible (Scheidat et al., 2004).

## 1.5 WHALE-WATCHING INDUSTRY AND OVERVIEW OF ITS EFFECTS ON CETACEANS

In addition to the increased level of boat traffic for transport and recreation, cetaceans are faced with the new challenge of a booming whale-watching industry, focused on various species mostly inhabiting coastal areas (Hoyt, 2001). Global tourism targeting marine mammals has grown dramatically over the past 20 years raising concerns among scientists and managers regarding impacts of these activities on animal populations and individuals. For example, in 1998, the whale watching industry included 9 million whale watchers across 87 countries, and generated over \$1 billion USD in total expenditure.

Ten years later, by 2008, the market grew to 13 million whale watchers across 119 countries and generated a total expenditure of \$2.1 billion USD (Machernis et al., 2018). These numbers are specific only to whale-watching and do not represent the variety of other tourism activities targeting a broader range of marine mammal species (Machernis et al., 2018).



Since cetacean-watching involves an encounter with wild animals, who are not removed or permanently affected by the human impact it belongs to the category of ‘non-consumptive wildlife tourism’ (La Manna et al., unpublished article). According to La Manna et al. (unpublished article) the literature has defined five criteria to identify ecotourism activities: a) nature conservation; b) low environmental impact; c) sustainability; d) the involvement of local communities and e) environmental education. Cetacean-watching tourism should meet all five criteria, but in practice it looks like it is not the case. Many reports describing dolphins’ responses on dolphin-watching tourism have been published: Lusseau and Highman (2004) reported changes in movement pattern, increase in dive intervals and increase in swimming speed of the bottlenose dolphins, as signs of active avoidance (Lusseau and Highman, 2004). Research on minke whale (*Balaenoptera acutorostrata*) at the western coast of Iceland by Christiansen et.al. (2013b) found out that whales responded to whale-watching boats by decreasing their inter-breath interval (IBI) and increasing sinuous movements (decreasing directness index). Similar responses have been observed in other studies of cetaceans with whale-watching activities causing either an increase or decrease in IBI. Lusseau (2003a) found out that boat interactions reduced the overall amount of time bottlenose dolphins spent socializing and resting. Similar response was reported by Arcangeli & Crosti (2008) from Western Australia where duration of travelling, milling and diving increased, while resting, socializing and feeding of bottlenose dolphins decreased, in the presence of the marine vessels. Humpback whales in Ecuador reacted to the approach of whale-watching boats by increasing swim speed significantly and adopted a much more direct path after boats left (Scheidat et al., 2004). Christiansen et al. (2010) reported significant behavioral states changes during tourist boat interaction, with dolphins being more likely to start travelling after a tourist boat interaction instead of staying in a resting or socializing state. The changes in behavioral states also affected dolphins’ behavioral budget. The dolphins spent a smaller proportion of their time foraging, resting and socializing as an effect of tourist boats being present, and instead spent more time travelling (Christiansen et al., 2010).

Recent studies suggested that what we consider as short-term responses to vessel disturbances can lead to biologically significant effects and have unpredicted long-term consequences for the life history of individuals and their population (Lusseau and Bejder, 2007). A decrease in the time spent foraging is likely to result in a reduction in energy procurement due to a decrease in food intake, while a reduction in time spent resting has been shown to induce

physiological stress, cause an increase in heart rate and energetic costs, and reduce energy reserves of an animal (Christiansen et al., 2010). Lusseau and Higham (2004) showed that the disturbance of bottlenose dolphins socializing behavior also has significant consequences for the energetic budget. Socializing is likely to be directly related to the reproduction of a population, hence less time spent socializing might result in lower fecundity rates (Garrafo et al., 2007). Besides that, “play” behavior has been shown to be important for the development of dolphin calves as a method of learning physical maneuvers, object manipulation, problem-solving skills, social behaviors, and foraging methods. Under stressful situation “play” behavior is less likely to occur and could also be disrupted if socializing events become less frequent (Marley et al. 2017).

To what extent behavioral changes might affect individual vital rates depends on the animals’ life history strategy, as well as ecological constraints. The way in which one population responds to vessel presence does not necessarily mean that all populations will respond in the same manner (Marley et al. 2017). For example, if the population’s ecological landscape does not provide opportunity for recovery (time or habitat for resting) or compensation (e.g. feeding at night), behavioral changes are more likely to have an impact on vital rates. Similarly, some species have evolved behavioral strategies with which have better ability to cope with human disturbance (Christiansen et al., 2013b).

Short-term effects have the advantage of being easily detected as avoidance and aggressive behaviors of animal, although if occur, long-term effects are more significant at the population level (Sheidat et al., 2004). Changes in behavioral pattern can be used as an early indicator of the vessel impact on the cetaceans and hence help wildlife management to adopt appropriate conservation measures to reduce the consequences of disturbance (Arcangeli and Crosti, 2008).

On the other hand, wildlife tourism, such as dolphin-watching tours, is often promoted as an activity that contributes to conservation by enhancing environmental knowledge, awareness and appreciation amongst participants for the target animal. While researchers acknowledge the potential for tourism operators to engender a conservation ethic amongst participants, others suggest tourists’ main motivations are consumption and entertainment, and that assumed increased support for conservation is unwarranted. Indeed, tourists often consume wildlife experiences in the form of a short superficial visit with a collection of photographs, souvenirs, and memories (Apps et al., 2017). Unlike any random encounter of recreational (or

any other type) boat and dolphins, dolphin-watching boat is a part of touristic service that offers and charges some time spent with dolphins. Taking good and close pictures of dolphins is a big part of guests' satisfaction, therefore it's very challenging for dolphin-watching boat skippers to operate responsibly, to abide "Code of conduct" - not getting too close nor staying too long with the dolphin group and respecting wildlife above all. That is why it is crucial that wildlife tourism should be managed carefully. Tour operators must have a clear strategy which includes implementing interpretation programmes which reinforce the participants' sense of wonder, awe and excitement. Participants are likely to benefit from examples of practical actions they can take to contribute to the conservation of wildlife (Apps et al., 2017). App et.al. (2018) demonstrates that even a brief, first hand wildlife encounter can have a profound effect on participants' awareness, understanding, attitudes, concern, and behavior towards wildlife conservation (Apps et al., 2017). According to La Manna et.al. (unpublished article) whale-watching has the potential to benefit conservation in long-term, improving participants' awareness and pro-environmental attitude. Considering the economic side, proper wildlife touristic service can generate jobs and become an important tourist attraction for the areas where it is practiced (Notarbartolo di Sciara, 2001).

## 1.6 THE AIM AND HYPOTHESIS OF THIS STUDY

The aim of this study was to investigate the effects that marine vessel presence might have on behavior of bottlenose dolphins of Cres-Lošinj archipelago, and to evaluate the difference in effects between different categories of vessels. The effect was assessed through analysis of the difference between control (only research boat in the vicinity of the focal dolphin group) and impact conditions (impact of dolphin-watching boat and impact of other categories of vessels; Table 5) through the following null hypothesis:

1. There are no significant differences in changes of dolphin's behavior between control and impact conditions.
2. There are no significant differences in dolphin's behavior transition probabilities between control and impact conditions.
3. There are no significant differences in dolphin's behavioral budget between control and impact conditions.

## 2 MATERIALS AND METHODS

### 2.1 STUDY AREA

The Adriatic Sea is a semi-enclosed basin linked to the Mediterranean Sea at the south through the Strait of Otranto. It includes over 1,200 islands, islets and reefs and has almost 8,500 km of shoreline. It is divided into three sub-basins: 1) northern Adriatic - a shallow basin with the bottom reaching a maximum of about 100 m (average depth = 35 m); 2) central Adriatic - with a maximum depth of about 280 m; and 3) southern Adriatic – the deepest part of Adriatic basin (with approximately 1240 meters deep South Adriatic Pit) (Figure 4). Each of these three parts has its own ecological characteristics (NETCET, 2015).

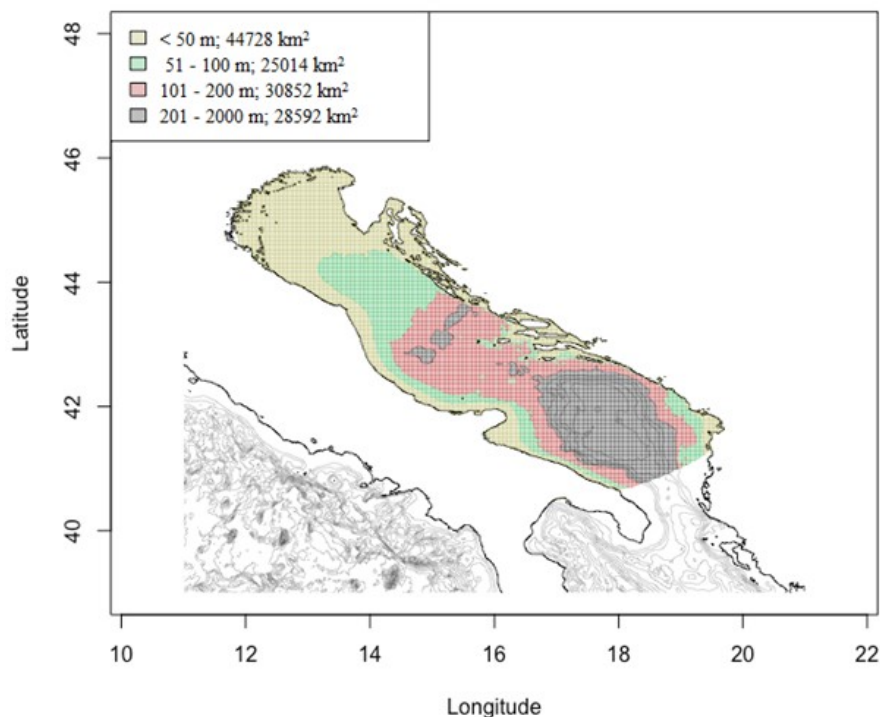


Figure 4: The depths profile of the Adriatic Sea.

The study area, with a surface area of approximately 1600 km<sup>2</sup>, is positioned on the eastern side of the northern Adriatic and includes part of the Velebit Channel and the entire Cres-Lošinj archipelago. This area is called Kvarnerić - the marine area between the first islands to the Croatian mainland: Krk, Rab, Pag and adjacent islands Cres and Lošinj and towards the southeast, the waters around the islands of Silba and Olib (Figure 5). The average sea

depth in this area is 70 m with mostly muddy and sandy sea bed and parts with rocky substrate and seagrass meadows of *Posidonia oceanica* (Pleslić et al., 2015).

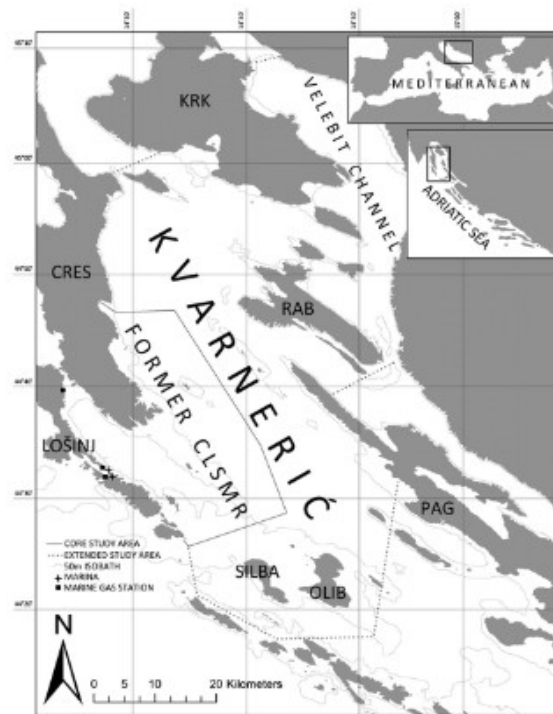


Figure 5: Study area including Kvarnerić, Velebit Channel and islands of Silba and Olib, with indicated location of the former Cres-Lošinj Special Marine Reserve (CLSMR) area (the core study area) (Pleslić et al., 2015).

This shallow part of the Adriatic is sensitive to pollution due to contamination from the rivers Po and Adige and slow water exchange. Besides, Kvarnerić region is affected by many anthropogenic activities including industrial maritime transport, shipbuilding, oil refineries, oil terminals, power stations, cement industry, tourism and fisheries. Therefore, the Northern Adriatic is considered to be one of the most threatened ecosystems in the world (Fortuna et al., 2018; Radulović, 2016).

Waters of this area are an important feeding and nursing ground for the resident bottlenose dolphin population (Rako et al., 2006). Due to its importance, in December 2014, the Cres-Lošinj marine area was designated as a Site of Community Importance (SCI), part of the European Union NATURA 2000 ecological network (Site code: HR3000161; Site name: Cres-Lošinj) (Rako et al., 2017). This designation has a long and difficult history of conservation effort which has led to it, initially enabled thanks to comprehensive data from

Adriatic Dolphin Project. In 1993 a small portion of the Cres-Lošinj archipelago was proposed for protection as a Special Dolphin Reserve. This proposal was later incorporated into the Cres-Lošinj Management Plan, however the Plan was never implemented due to the political upheavals in the region. A new proposal was subsequently prepared by the Blue World Institute, based on the previous draft and new data, which resulted in designation of the Cres-Lošinj Special Marine Reserve (CLSMR) Directive 2009/147/EC in 2006 (Figure 5). Unfortunately, after three years of preventive protection, in 2009, this area was downgraded from the strictest type of protection regime “Special reserve” to less strictly “Regional park” category of protection (Mackelworth et al., 2013; Law on Nature Protection NN 80/13, 15/18).

The Natura2000 network is the EU’s primary framework for nature protection. Europe's most valuable and threatened species and habitats, are listed under annexes within the Directive 2009/147/EC of the European Parliament and of the Council on the conservation of wild birds (Birds Directive) and Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora the Habitats Directive (Natura2000). Within the European Union there are 316 Natura 2000 sites designated for the bottlenose dolphins. There are six Natura2000 sites in Croatian Adriatic Sea related to preservation of bottlenose dolphin’s habitat (Site codes: HR5000032, HR1000035, HR3000419, HR3000469 and HR3000426) including Cres-Lošinj site that overlaps with the study area (Figure 6; The European Environment Information and Observation Network). Total area covered by these six sites: 3,717 km<sup>2</sup> which makes 11.8% of the overall Croatian territorial waters (31,479 km<sup>2</sup>). Appropriate management of these sites would fulfill the Aichi target 11 for Croatia; 10% of coastal and marine areas are conserved and equitably managed by 2020 (Fortuna et. al., 2018).

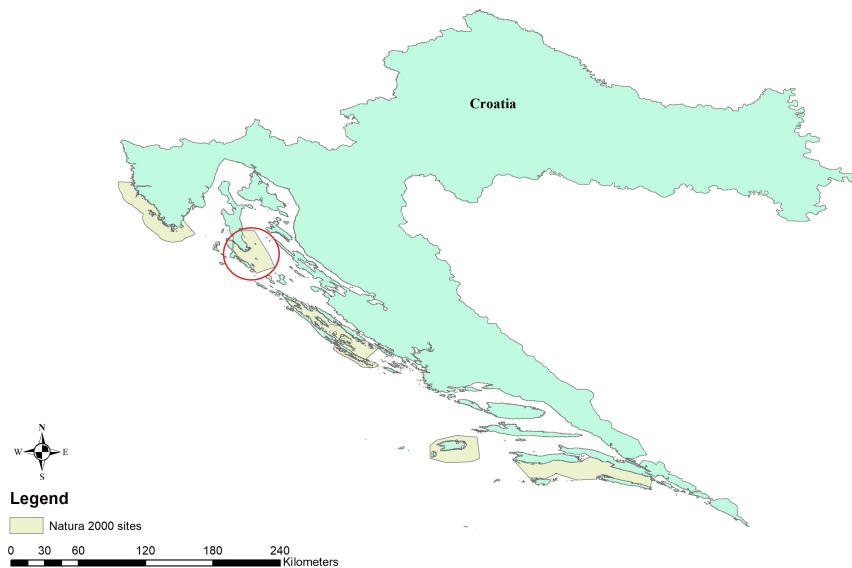


Figure 6: Natura 2000 sites in Croatia related to preservation of bottlenose dolphins habitat. Marked area denotes Cres-Lošinj Natura2000 site.

## 2.2 DATA COLLECTION

Boat based *ad libitum* surveys of the Cres-Lošinj archipelago were conducted between 13<sup>th</sup> June and 17<sup>th</sup> October 2017. All data were collected during daylight and only in good weather conditions. The study design involved detailed data collection protocol. Data sampling were carried out from the 5.85-meter-long RIB research boat equipped with 100 HP, four-stroke outboard Honda engine.

The protocol was created to eliminate potential bias of the observing vessel based on Akkaya Bas et al. 2017. The research boat was maneuvered in a way to minimize negative effects on dolphins; dolphin focal groups were approached from the side and back in the same direction as the movement of the group; speed of the boat was matched to the speed of the focal group and the data was collected as described in the following chapter (Lusseau, 2003a).

Behavior data sampling started when dolphins were sighted, regardless on the presence of other vessels in the vicinity of the dolphin group. First recorded behavioral state (current activity) of the focal group of dolphins is considered as initial state. The focal group is defined as individuals engaged in the same/similar behavior with the group cohesion of about 50 meters. The group behavior state corresponds to the behavioral state of more than 50% of the individuals (Akkaya Bas et al., 2017). Behavior states were defined to be mutually exclusive and cumulatively inclusive; as a whole, they describe the entire behavior budget of



dolphins. Behavior of the focal group of dolphins was sampled to understand the effect of boat interactions at the group level, rather than at the individual level (Lusseau, 2003a; Lusseau and Higham, 2007). If the focal group was out of sight for more than 20 minutes, the next sighting was declared as a new group. In the case of division of the group into subgroups, only the first sighted subgroup behavior was noted and the rest of the groups were ignored. Behavior data sampling was conducted at 3-minute intervals consecutively, in duration of minimum 20 minutes (Akkaya Bas et al., 2017).

The behavioral data sheet is filled out by the observer on the research boat and contained following data (Appendix B):

- **categories of dolphins' behavior states.** The field definition of behavior states was strictly based on discrete, non-subjective parameters such as the dive duration, swimming directionality and speed, and few other variables like physical contact among individuals, visible prey and presence of trawlers. The duration of surfacing intervals, in particular helped to discriminate among apparently similar behavioral patterns, assuming that breathing patterns differ during different behaviors (Bearzi et al., 1999). Categories are divided according to following categories definitions (Table 2),
- **position of the research boat** - determined by Global Positioning System (GPS; GPSmap Garmin 78s, Plus; accuracy  $\pm 7$  m),
- **group size (number of animals) and group composition** according to dolphins' size class - based on visual assessment of sizes as compared to average adult size (Table 3),
- **presence, distance and number of other vessels within 500-meters range** from the group - according to seven vessel categories (Table 4).

Table 2: Categories of dolphin behavioral states (Nimak, 2006).

<b>Behavioral states</b>	<b>Definition</b>
Diving (D)	Pattern characterized by cycles of single long dives, lasting up to several minutes; dives are spaced by clusters of relatively regular number of ventilations; last in the series of ventilations is often a fluke up or tail stock submergence, suggesting a vertical dive; submergence and surfacing usually within same area; dolphins diving often synchronous.
Dive-Travelling (DT)	Pattern that is consistent of both travel and dive; dolphins keep the same general direction under water as during surfacing; usually single long dives accompanied by a pattern of clustered ventilations; respiration patterns can be highly variable and poorly consistent in comparison to dive behavior; groups or sub-groups often synchronous.
Travelling (T)	Consistent directional movement of dolphins, with regular surfacing typically every 10-60 seconds. It can be slow or fast where slow is associated with resting behavior.
Active trawler follow (ATF)	Following wake of operating trawler, at about 150-300 m stern; regular single long dives for several minutes; dives are broken up by a pattern of regular ventilations.
Passive trawler follow (PTF)	Consistent directional movement of dolphins, with regular surfacing typical 10-60 seconds, at about 150-300 m stern.
Socializing (S)	Most group members are in almost constant physical contact with one another; oriented towards one another; no forward movement; display of surface behavior (jumps, leaps, rolling, tail slaps...).

Table 3: Dolphins size classes (Bearzi et al., 1997).

<b>Dolphins size class</b>	<b>Definition</b>
Newborn	Below one-half of an adult length; constantly in close association with an adult; dorsal fin typically low and rounded; dark, lead-grey coloration with visible fetal creases; immature swimming style with stereotyped surfacing pattern when breathing.
Calf	About one-half of an adult; in clear association with an adult, but not as strictly as a newborn; light gray coloration, occasionally brownish, usually with lighter vertical stripings left by fetal creases.
Juvenile	About two-thirds of an adult; usually swimming in association with an adult, but sometimes independently; coloration generally slightly lighter than the adult.
Adult	all dolphins approximately 2.5-3 m long.

Table 4: Vessel categories.

1.	Research boat (RB)
2.	Dolphin-watching boat (DW)
3.	Recreational boat - motor yachts, speed boats (RecB)
4.	Sailing boat - on sails or engine (SB)
5.	Fishing boat (FB)
6.	Local boat – small local boat up to 6 meters with no engine above 10 HP (LB)
7.	Tour boat - big excursion boats (TB)

Distances from the focal group and other boats were estimated by eye using visual references (such as the size of the research boat). Phases when only the research boat was present in the vicinity of a dolphin group were considered as control condition, since strict maneuvering regulations should minimize effect on the dolphins, as explained above. During the presence of other boats within 500 meters' range, impacts were determined as shown in table 5.

Table 5: Impact conditions.

<b>Impact condition</b>	<b>Definition</b>
Control	Only research boat present within 500 meters of the focal dolphin group
Impact DW	Research boat + dolphin-watching boat present within 500 meters of the focal dolphin group
Impact OB	Research boat + other categories of vessels (with the exception of DW boat) within 500 meters of the focal dolphin group

## 2.3 DATA ANALYSIS

### 2.3.1 Behavioral transitions and impact conditions

To find out is there any statistically significant difference in the proportion of transition in dolphin's behavior in dependence to impact condition, Cochran's Q test was applied. Tests were run through SPSS 17.0 for Windows (SPSS Inc. Released 2008. SPSS Statistics for Windows, Version 17.0. Chicago: SPSS Inc.). Data distribution was assessed using the Kolmogorov-Smirnov test for goodness of fit. As the test indicated that data was not normally distributed, a non-parametric Cochran's Q test was applied for statistical analysis.

Cochran's Q test is an extension to the McNemar's test commonly used when analyzing participants (in this case dolphin groups) that have undergone multiple different trials (i.e.

control and impact conditions). It is applied on related samples testing the differences in frequencies or proportions between related sets of data. Cochran's Q test can be used when (all) four assumptions are met:

- I. There is one dependent variable with two, mutually exclusive groups (variable is dichotomous). In this study three-minute scanned samples undergo yes/no division for the variables like dolphin behavior transitions, unchanged behavior states, 'control' and impact conditions. Mutually exclusive means that a participant cannot be in more than one group at the same time. In accordance to that assumption every scanned sample can be either yes or no (1/0) in the given variable but never both (for example focal dolphin group state in one scanned sample can either be in behavior transition category (yes) or in category without behavior change (no)).
- II. There is one independent variable that consists of three or more categorical, related groups. In the present study there are three categories of condition: 'control', 'impact DW' and 'impact OB' condition.
- III. The participants are a random sample from the population of interest, which is in accordance with the data of this study.
- IV. Sample size is sufficiently large for interpretation of the asymptotic p-value produced by Cochran's Q test. In case of inadequate sample size, the asymptotic p-value may not be accurate. In the present study the dataset was composed of 883 scanned samples which are adequate for running the Cochran's Q test (Laerd Statistics; NCSS).

In relation to p-value, Cochran's Q test will provide either a statistically significant result ( $p < 0.05$ ) or a statistically non-significant result ( $p > 0.05$ ). If the result is not statistically significant, it indicates that the proportions under the different conditions are the same in the population. In this case, it is not necessary to follow up results with a post hoc analysis. However, if the outcome is a statistically significant result, a post hoc analysis is required as Cochran's Q test detects the differences in the proportions between related groups without showing which specific groups differed from each other. McNemar's post hoc test was therefore applied to determine between which two related groups the differences are found, as described on Medcalc, easy-to-use statistical software (2018).

### 2.3.2 Variation in behavior in dependence of vessel presence

To quantify variation in behavior of the dolphin group in the dependence of vessel presence, Markov chains analysis was applied. Analyses were performed in R software (Version R 3.0.2 GUI 1.62 Snow Leopard build, 2013, <http://cran.r-project.org>) using RStudio (Version 0.99.484, 2009-2014; Spedicato, 2017; Spedicato et al., 2014; Wickham, 2009; R Core Team, 2016). The data consists of a series of scan samples that were analyzed as behavior state sequences. Since those sequences of discrete time samples are ergodic, they could be treated as a Markov chains. A time series is ergodic when transitions between all states are possible. Accordingly, during this study our behavioral stages represented ergodic sequences of discrete time samples, which could be therefore treated as a Markov chains (Lusseau et al., 2009).

Markov chains measure the dependence of an event based on preceding events. If sequencing events are independent of preceding events, they are considered to be a zero-order Markov chain. If an event depends only on the immediately preceding one, it is a first-order Markov chain. If an event depends on the two most preceding events, it is a second-order Markov chain, and so on. In this study every scanned sequence is treated as an event. In order to access the difference in transition from one scanned sequence to another, depending on the presence of vessels, first-order Markov chain model was chosen. A “transition” occurred when a dolphin group changed from a preceding behavior to succeeding behavioral state, so it’s a proportion of time observed a succeeding behavior following a preceding behavior. These transitions were recorded in two two-way contingency tables considering preceding behavioral state versus succeeding behavioral state. If there was no vessel presence between two scanned samples, transitions were recorded in a control table. In case of vessel presence, transitions between two scanned samples were recorded in an impact table (Lusseau, 2003a; Marley, et al., 2017).

Behavioral transition probabilities matrix (from preceding to succeeding behavior), for control and both impact conditions, were constructed by calculating transition probabilities as:

$$p_{ij} = \frac{a_{ij}}{\sum_{j=1}^3 a_{ij}}, \sum p_{ij} = 1$$

where  $i$  is preceding behavioral state and  $j$  is succeeding behavioral state ( $i$  and  $j$  range from 1 to 3 due to the three behavioral states used in the analysis),  $a_{ij}$  is the number of transitions observed from behavioral state  $i$  to  $j$ , and the  $p_{ij}$  is the transition probability from  $i$  to  $j$  in the Markov chain (Lusseau, 2003a; Marley et al., 2017).

To test the effect of vessel presence on the transition probability of dolphins, both impact conditions and control chains were compared using a chi-square test in a way that the number of transitions from the impact table was treated as observed number of transitions and the number of transitions from the control table was considered as expected number of transitions. Each control transition was also compared to its corresponded impact transition ( $3 \times 3 = 9$  in total) using a 2-sample test for equality of proportions with continuity correction (Akkaya Bas et al., 2017).

To assess the effect of vessel presence on the proportion of time dolphins spent in different behavioral states, i.e. behavioral budgets, long term behavior of the transition matrices were used. Transition matrices are based on ergodic time series and initial behavior states can converge toward a stationary behavioral distribution. This stationary distribution, or steady state, corresponds to the behavioral budget of the population. Stationary behavioral distribution of the transition matrices was calculated for 'control' and both impact conditions. For testing the differences between 'control' and both impact behavioral budgets a chi-square test was used. Also, each behavioral state from 'control' behavioral budget was compared to the corresponding behavioral state from the 'impact DW and 'impact OB' behavioral budget by using 2-sample test for equality of proportions with continuity correction. For the estimated proportion of time spent within each behavioral state, the 95% confidence intervals were calculated (Lusseau et al., 2009; Lusseau, 2003a; Akkaya Bas, 2017).

A cumulative behavioral budget is possible to estimate for each impact condition considering both 'control' and impact behavioral budgets. According to proportion of three-minute scanned samples in a given condition within overall scanned samples (exposure level) it is possible to estimate whether the cumulative behavioral budget significantly differ from 'control' budget. Moreover, by artificially varying the exposure level from 0 to 100% it is possible to model at what level of exposure cumulative behavioral budget becomes significantly different from the 'control' budget.

The cumulative behavioral budget was calculated following Akkaya Bas (2017):

$$\text{Cumulative budget} = (a \times \text{impact budget}) + (b \times \text{control budget})$$

where  $a$  is exposure level, and  $b$  is proportion of samples without boat exposure ( $1 - a$ ), corresponding to control chain. If dolphins had no exposure to vessel presence impact,  $a$  would equal 0, meaning that the cumulative behavioral budget would be the same as the 'control' budget. Conversely, if the dolphins were in the vessel presence through-out all study period,  $a$  would equal 1, and the cumulative behavior budget would be the same as impact budget. To test if the dolphin cumulative behavioral budget differs significantly from the 'control' budget, a chi-square test and 2-sample test for equality of proportions with continuity correction was used, for each behavioral state (Lusseau and Higham, 2004; Akkaya Bas, 2017; Christiansen et al., 2010). To compare estimated exposure of cumulative behavioral budget with the mean proportion of dolphins' exposure to vessels, additional analysis of 'control': 'impact DW': 'impact OB' ratio was made for each day of survey.

## 3 RESULTS

### 3.1 DATA DISTRIBUTION

During the study period, a total of 42 days was spent at sea recording dolphin behavior data. In total 1.014 three-minute samples were collected over the 48 hours that were recorded in 71 sightings. Due to the minimum duration of 20 minutes, 65 sightings were used in the analysis. Since each first behavior state in the sequence is an initial state, 949 three-minute scan samples were considered for analyses. Considering given impact conditions 883 scan samples were used for analyses (samples with dolphin-watching boat and other boats present at the same time were discarded). Moreover, due to sample size constraints Markov chains analysis was carried out on only three behavioral states (diving, dive-travelling and travelling), while the descriptive analysis and Cochran's Q Test was carried out including all six behavioral states.

Dolphin behavior states were not equally distributed (Figure 7); in behavioral budget the most frequent dolphin behavior state was diving and travelling (30% each), followed by dive-travelling (21%), while socializing, active trawler follow and passive trawler follow represented relatively small proportion of dolphins' behavioral budget (8%, 9% and 2%, respectively; Figure 8).

Out of 883 three-minute samples, 515 samples were collected in the 'control' condition, 112 samples were collected in 'impact DW' condition and 256 were collected under the 'impact OB' condition. Results showed that out of 515 three-minute samples collected in the 'control' conditions, 456 samples did not indicate any behavioral change from preceding to succeeding state (no transition) while 59 samples indicated behavioral transition (from one behavior state to another). Hence, proportion of dolphin behavior transition in 'control' condition is 0,114. In 'impact DW' condition there were 92 three-minute samples recorded without transition and 20 samples with behavioral transition, with the proportion of transition 0,178. In 'impact OB' out of 256 recorded three-minute samples, 216 did not show any behavior transition, while in 40 samples there was transition in dolphin behavior, making the proportion of behavior transition 0,156 (Table 6).



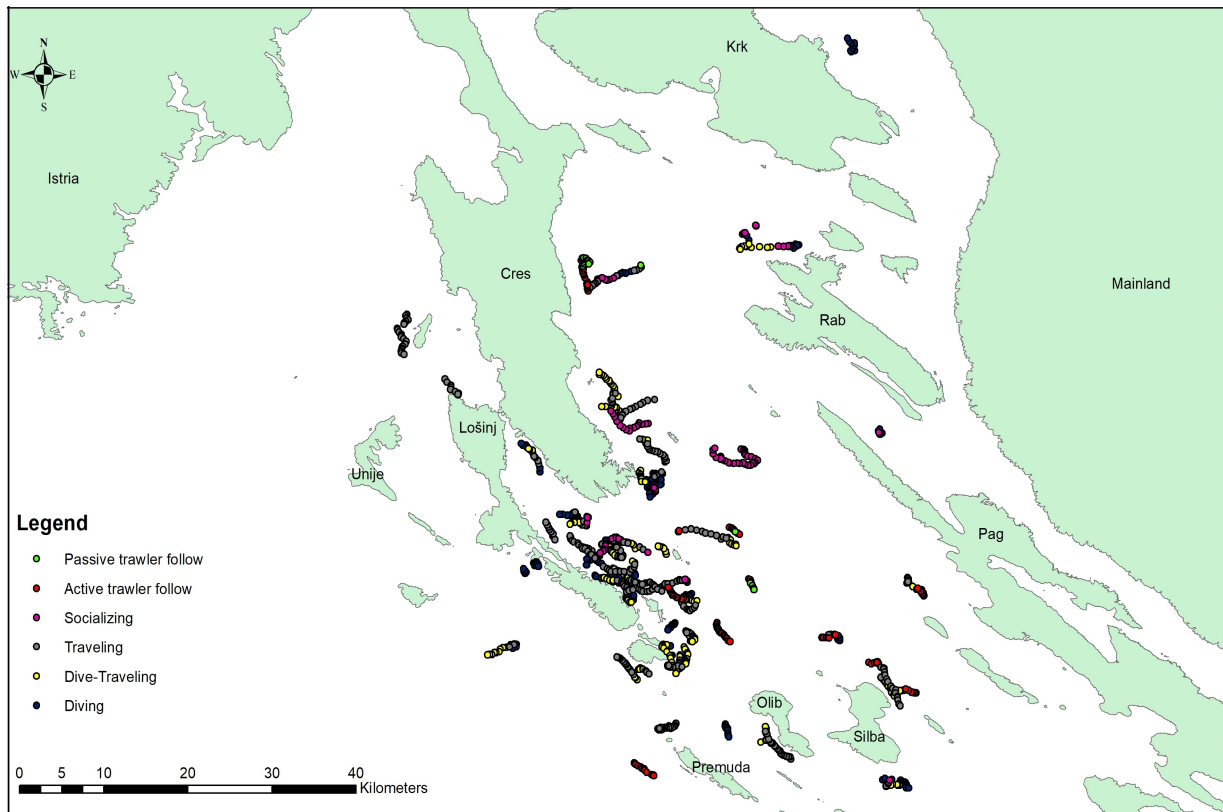


Figure 7: Categories of sampled behavioral states on study area.

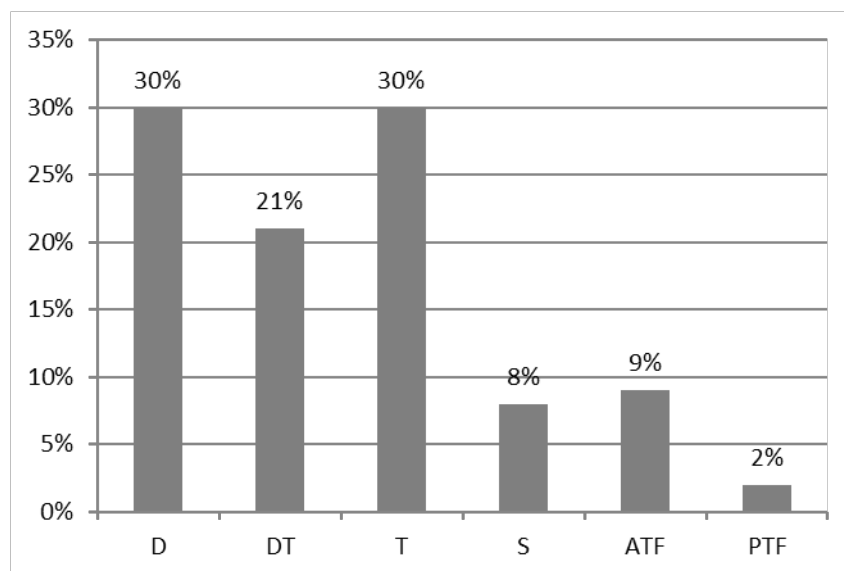


Figure 8: Proportions of each behavioral state in behavioral budget: diving (D), dive-travelling (DT), travelling (T), socializing (S), active trawler follow (ATF), passive trawler follow (PTF).

Table 6: Three-minute scans and proportions of behavior transition scans within ‘control’ and both impact conditions.

Conditions	Total 3-minute scanned samples	Behavioral transitions	
		Number	Proportion
Control (RB)	515	59	0.114
Impact DW (RB + DW)	112	20	0.178
Impact OB (RB + OB)	256	40	0.156

Spatial layout of all conditions is shown in figure 9 with predominance of ‘control’ and ‘impact OB’ three-minute scans over ‘impact DW’ scans.

Out of 65 sightings, 38 started with first approach of the research boat (corresponding to ‘control’ condition) while 8 sightings started with dolphin-watching boat present (‘impact DW’) and 19 sightings with presence of other categories of vessels (‘impact OB’). Analysis of behavior transitions in the first sequence of each sighting (first three-minute scan sample after approaching the research boat), indicate little lower proportion of behavioral transition (proportion of changed/unchanged behavioral state) in ‘control’ condition (0,16/0,84) and ‘impact OB’ condition (also 0,16/0,84), than in ‘impact DW’ condition (0,375/0,625).

Considering different types of vessels which was found with the dolphin group at the beginning of sightings indicate dominance of the two vessel types – dolphin watching boat (in 30% of sightings) and fishing boat (also in 30% of sightings). Other types of vessels were found less frequently with the dolphin group: sailing boat on 15% of times; recreational boats, local boats and combination of different types of boats on 7% and tour boat on only 4% of occasions at the first sequence of sighting.

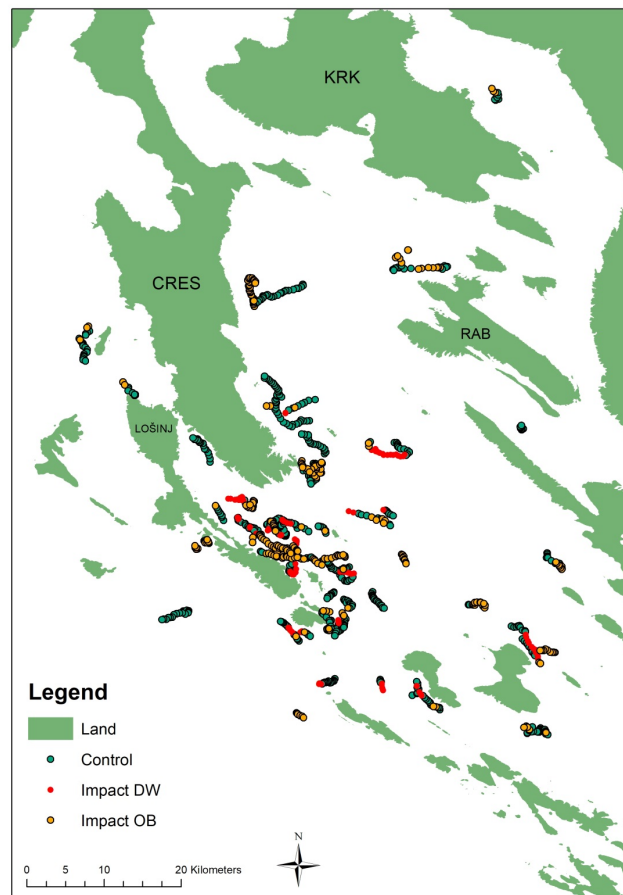


Figure 9: Spatial layout of impact conditions.

The difference in dolphin behavior transition was found considering the type of the vessels found with the dolphin group (in the first three-minute sequence). As mentioned above, in the presence of dolphin watching boat recorded proportion of changed and unchanged behavioral states (transition/no transition) was found to be 0,375/0,625, respectively. In condition when fishing boat was found with the dolphin group there were no changes in dolphins behavior recorded in the first three-minute sequence (Figure 10). Small frequency of occurrence of other types of boats in the first recorded three-minute sequence made it inappropriate for this analysis.

In this study, there were 8 sightings during which only dolphin-watching boat approach to the focal dolphin group. Every eventual behavior transition is considered to be effect of dolphin-watching boat presence (eventual effect of the research boat itself was not considered in this study). During these 8 sightings, in 50% of cases behavior transition occurred, while in another 50% no behavior transition was recorded.

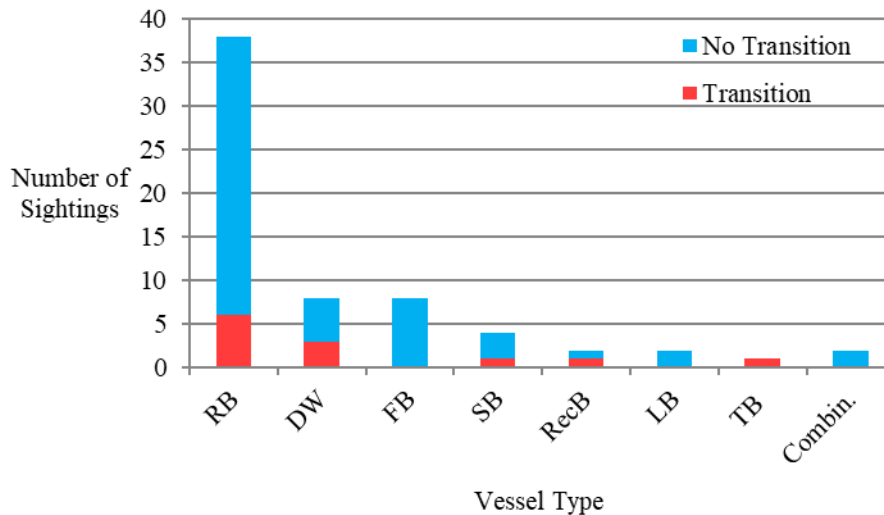


Figure 10: Proportions of behavior transition for every vessel type in the initial 3-minute sequence of every sighting. Blue color represents no transition in dolphin behavior while red color represents transition in behavior. Vessel types are: RB-research boat; DW-dolphin-watching boat; FB-fishing boat; SB-sailing boat; LB-local boat; TB-tour boat and Combin. - combination of more types of vessels.

Considering sightings with approaching of only recreational boats, out of 6 sightings, 2 times there was behavior transition, while 4 times no behavior transition occurred. There were 3 sightings with approaching only the sailing boat (on sails), in which in only one sighting a behavior transition occurred (behavior change from travelling to socializing). Sightings with only the fishing boat present during the whole sighting (bottom trawler already present with the dolphin group at the beginning of sighting) showed the same proportion of behavior transition, namely out of 6 sightings in half of them there was a behavior transition while in half no behavior transition occurred. It should be noted that this behavior changes include transitions between active trawler follow and passive trawler follow. Proportion of sightings with approaching of other categories of vessels was too small for consideration.

Out of 65 sightings, 13 of them was comprising only ‘control’ condition three-minute scans (during the whole sighting no vessel approach to the dolphin group, except the research boat), 9 sightings were recorded completely in ‘impact OB’ condition, while only 2 sightings in ‘impact DW’ condition during the whole sighting. Out of 13 “all control” sightings in 7 of them behavior transitions occurred; in both 2 sightings with only ‘impact DW’ dolphins changed their behavior while out of 9 sightings completely in ‘impact OB’ condition, in 6 of them there were transition in behavior recorded. Due to sample size limitations, it was not

possible to test the effect of different levels (numbers) of boats on dolphin behavior (Christiansen et al., 2010).

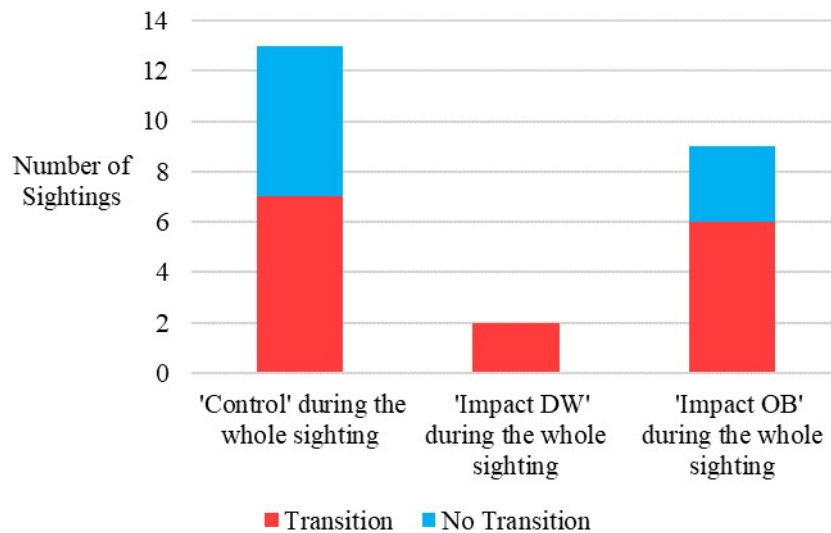


Figure 11: Proportion of behavior transitions in sightings with only 'control', 'impact DW' and 'impact OB' samples during the whole sightings. Blue color represents no transition in dolphin behavior while red color represents transition in behavior.

### 3.2 THE DEPENDENCE OF CHANGES IN DOLPHIN'S BEHAVIOR TO IMPACT CONDITIONS

The dependence of transition in dolphins' behavior to impact condition was tested for overall data. Frequencies of three-minute samples with no-transition in a given condition were compared with frequencies of samples when transition in behavior occurred but out of the given condition. In 'control' condition there were 456 three-minute scans with no transition in behavior and 60 scans with transition in dolphin behavior out of the 'control' condition. Difference was found to be significant (McNemar Test,  $X^2 = 302.374$ ,  $p > 0.001$ ) (Table 7).

For 'impact DW' there was 92 three-minute scans in condition of 'impact DW' without transition in behavior and 99 scans when transition in behavior occurred but not in 'impact DW' condition. Difference is not statistically significant (McNemar Test,  $X^2 = 0.178$ ,  $p = 0.664$ ; Table 7).

Considering 'impact OB' there was 216 three-minute scans without behavioral transition in given condition and 79 scans with transition in dolphin behavior which occurred out of the

‘impact OB’ condition. Difference was found to be significant (McNemar Test,  $X^2 = 62.698$ ,  $p > 0.001$ ; Table 7).

Table 7: The dependence of the transition in dolphins’ behavior to impact condition in overall data. Negative differences represent the number of three-minute scans without behavioral transition in a given condition. Positive differences represent the number of scans when transition in behavior occurred but out of the given condition. Ties represent the number of scans with no behavioral transition out of the given condition. Total represent the number of scans in overall data.

Frequencies		Control	Impact DW	Impact OB
Transition 1/0	Negative Differences <sup>a</sup>	456	92	216
	Positive Differences <sup>b</sup>	60	99	79
	Ties <sup>c</sup>	367	692	588
	Total	883	883	883

a. transition 0 / condition 1

b. transition 1 / condition 0

c. transition 0 / condition 0

Using McNemar's test ‘impact DW’ and ‘impact OB’ condition was additionally tested with ‘control’, considering separately scanned samples with behavioral transition and scanned samples when no transition in behavior occurred.

As shown in the table 8, when **considering only the behavioral transitions**, these have been recorded in 59 three-minute samples in the ‘control’ condition; 20 samples in the condition of ‘impact DW’ and 40 scanned samples in ‘impact OB’ condition. McNemar’s test showed significant difference in proportion of dolphin behavioral transitions considering ‘control’ condition in relation to ‘impact DW’ ( $X^2 = 18.278$ ,  $p > 0.001$ ), while in relation to ‘impact OB’ ( $X^2 = 3.273$ ,  $p = 0.070$ ) difference was not found to be significant (Table 8).

Table 8: Comparison of ‘control’ condition to impact conditions for behavioral transition samples.

a)

Control	Impact DW		Test Statistics	
	0	1	N	
0	40	20	Chi-Square	18.278
1	59	0	p-value	>0.001

b)

Control	Impact OB		Test Statistics	
	0	1	N	
0	20	40	Chi-Square	3.273
1	59	0	p-value	0.070

- a) analysis of ‘control’ condition in relation to ‘impact DW’ condition showed more frequent dolphin behavior transitions (59 transitions) in the ‘control’ condition, than in ‘impact DW’ condition (20 transitions). McNemar’s test showed that the difference is statistically significant.
- b) analysis of ‘control’ condition in relation to ‘impact OB’ again showed more frequent dolphin behavior transitions in ‘control’ condition (59 transitions) than in ‘impact OB’ condition (40 transitions). McNemar’s test showed that the difference is not statistically significant.

McNemar's test of each impact condition and the ‘control’ condition considering only three-minute scanned samples with **no behavioral transition** (unchanged behavioral state), resulted in: 456 scanned samples in the ‘control’ condition, 92 scanned samples in ‘impact DW’ condition and 216 scanned samples in ‘impact OB’ condition. Results showed significantly higher proportion of unchanged states in dolphin behavior in ‘control’ condition compared to both ‘impact DW’ condition ( $X^2 = 240.454$ ,  $p > 0.001$ ) and ‘impact OB’ condition ( $X^2 = 85.001$ ,  $p > 0.001$ ) (Table 9).

Table 9: Comparison of ‘control’ condition to impact conditions for no behavioral transition samples.

a)

Control	Impact DW		Test Statistics	
	0	1	N	
0	216	92	Chi-Square	240.454
1	456	0	p-value	>0.001

b)

Control	Impact OB		Test Statistics	
	0	1	N	
0	92	216	Chi-Square	85.001
1	456	0	p-value	>0.001

- a) analysis of ‘control’ state in relation to ‘impact DW’ showed that dolphin remaining in the same behavioral state occurred more frequent (456 scanned samples) in the ‘control’ condition, than in ‘impact DW’ condition (92 three-minute samples). McNemar’s test showed that the difference is statistically significant.
- b) analysis of ‘control’ state in relation to ‘impact OB’ also showed higher frequency of unchanged behavioral states in ‘control’ condition (456 scanned samples), than in ‘impact OB’ condition (216 three-minute samples). McNemar’s test showed that the difference is statistically significant.

### 3.3 BEHAVIORAL TRANSITION PROBABILITIES AND BEHAVIORAL BUDGETS IN DEPENDENCE TO IMPACT CONDITIONS

According to the study design there were six behavior states recorded, but the low proportion of time dolphin groups spent in states: socializing (8%), active trawler follow (9%) and passive trawler follow (2%) in the overall behavioral budget, precluded usage of these three states in R data processing (Figure 8). To be specific, the program cannot perform analysis if any number in matrices is zero (for example if there was no behavioral change from socializing to active trawler follow). Any transitions involving these three behavioral states were therefore omitted and Markov chains analyses were made considering only the three remaining behavioral states: diving, dive-traveling and traveling (Meissner et al., 2015). Accordingly, ‘impact OB’ in this analysis eliminates fishing boat from consideration given that the excluded behavioral states are directly related to this type of vessel. Markov chains analyses were therefore run on 702 scanned samples: 435 scan samples for control condition; 89 scans for ‘impact DW’ condition and 178 ‘impact OB’ three-minute scans.



Markov chains analysis calculated probabilities for dolphins to change behavior or to stay in the same behavior for every condition ('control', 'impact DW' and 'impact OB' condition) and tested the differences considering 'control' and impact conditions. Analysis showed that when there were no vessels present (except for the research boat) i.e. in 'control' condition, dolphins have 3% possibility to change behavior from dive-travelling to travelling; 5% to change from dive-travelling to diving; 92% probability to stay in dive-travelling state; 3% probability to switch from diving to dive-travelling behavior, and so on, as shown in figure 12.

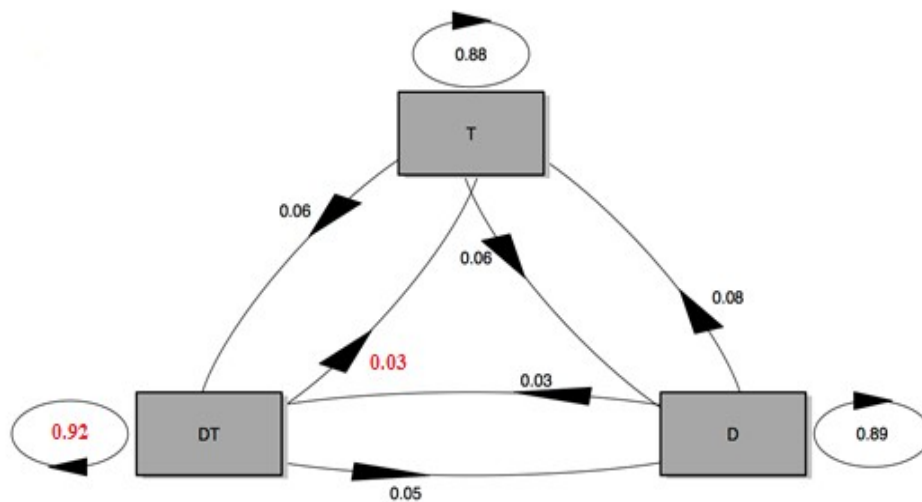


Figure 12: Transition plot for control chain. Behavioral states are Diving (D), Dive-Travelling (DT) and Travelling (T). The values represent probabilities. Significant increase/decrease is marked with red color.

In 'impact DW' condition behavior transitions significantly changed (Goodness-of-fit test,  $\chi^2 = 222.71$ ,  $df = 4$ ,  $p < 0.001$ ) although this effect was not equally distributed among behavioral transitions. Presence of dolphin-watching boat significantly affected ( $p < 0.05$ ) two of nine behavioral transitions. Probability for staying in dive-travelling behavior (Z-test = 11.66,  $p > 0.001$ , control = 92%, impact = 57%) significantly decreased in the presence of dolphin-watching boat, while probability for transition from dive-travelling to travelling (Z-test = 5.29,  $p = 0.02$ , control = 3%, impact = 21%) significantly increased (Figure 13).

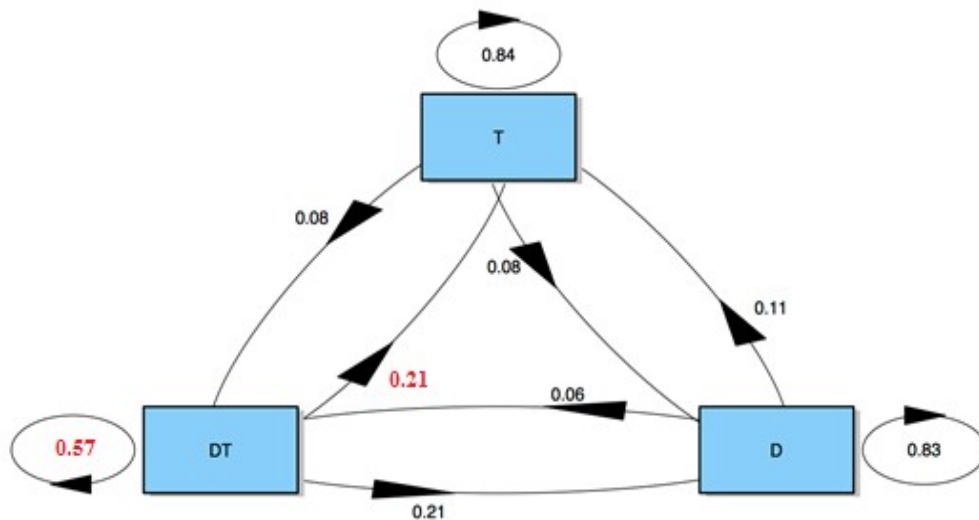


Figure 13: Transition plot for 'impact DW' chain. Behavioral states are Diving (D), Dive-Travelling (DT) and Travelling (T). The values represent probabilities. Significant increase/decrease is marked with red color.

Analysis of 'impact OB' condition also indicate significant effect on the transitions in behavioral states of dolphins (Goodness-of-fit test,  $\chi^2 = 20.21$ ,  $df = 4$ ,  $p > 0.001$ ), although this effect was not significant between specific behavioral states (Figure 14).

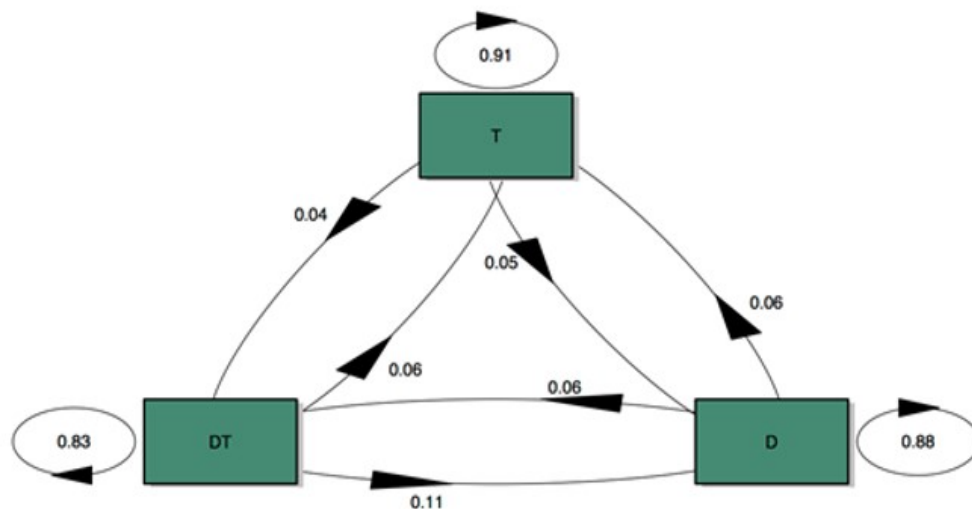


Figure 14: Transition plot for 'impact OB' chain. Behavioral states are Diving (D), Dive-Travelling (DT) and Travelling (T). The values represent probabilities.

Effects of vessel presence on behavior transitions for both impact conditions are shown in figure 15 as a difference in behavior probabilities between 'control' and 'impact' conditions. As shown in figure 15a, the significant difference in behavioral transition probability is

caused by the presence of dolphin-watching boat: probability for remaining in dive-travelling behavior dropped for 35%, while probability to change behavior from dive-travelling to travelling increased for 18%. Presence of other categories of vessels did not induce significant effect between specific behavioral states (Figure 15b).

It's modeled that in 'control' condition dolphins spent approximately the same proportion of time in all three behavioral states (Figure 16). Behavioral budget was significantly affected in 'impact DW' condition (Goodness of fit test,  $\chi^2 = 51.30$ ,  $df = 2$ ,  $p > 0.001$ ). Two out of three behavioral states significantly changed in the presence of dolphin-watching boat: the proportion of time spent dive-travelling was significantly lower (Z-test = 13.51,  $p > 0.001$ , control = 34%, impact = 14%), while proportion of travelling was significantly higher in the 'impact DW' budget (Z-test = 6.01,  $p = 0.01$ , control = 33%, impact = 47%). Proportion of time spent diving (Z-test = 0.95,  $p = 0.33$ , control = 33%, impact = 39%) did not significantly differ between 'control' and 'impact DW' condition (Figure 16).

Behavioral budget of dolphins was also significantly affected by the presence of other categories of vessels (Goodness of fit test,  $\chi^2 = 13.67$ ,  $df = 2$ ,  $p > 0.001$ ). The proportion of time spent dive-travelling was significantly lower in the 'impact OB' budget (Z-test = 7.03,  $p = 0.008$ , control = 34%, impact = 23%). The proportion of time dolphins spent diving (Z-test = 1.99,  $p = 0.16$ , control = 33%, impact = 40%), and the time spent travelling (Z-test = 1.13,  $p = 0.29$ , control = 33%, impact = 38%) did not significantly differ between 'control' and 'impact OB' condition (Figure 16).

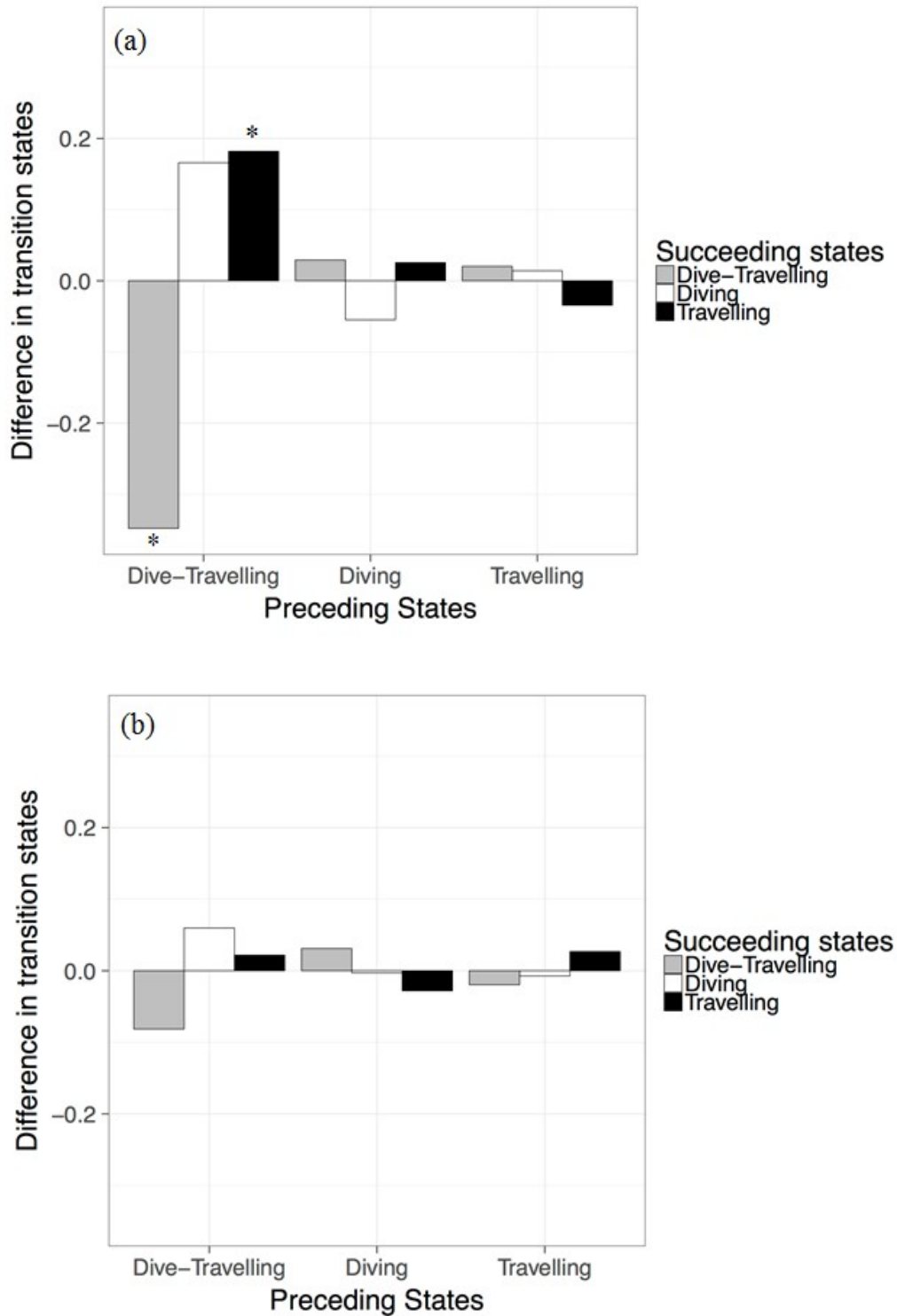


Figure 15: Difference between the transition probability of 'control' and 'impact DW' condition (a), and control and 'impact OB' condition (b). A negative value on the y-axis means that the transition probability of the impact condition was lower than the control one. The significance of the difference between two transition probabilities was assessed using a Z-proportion test. An asterisk (\*) indicates transition with a significant difference ( $P < 0.05$ ).

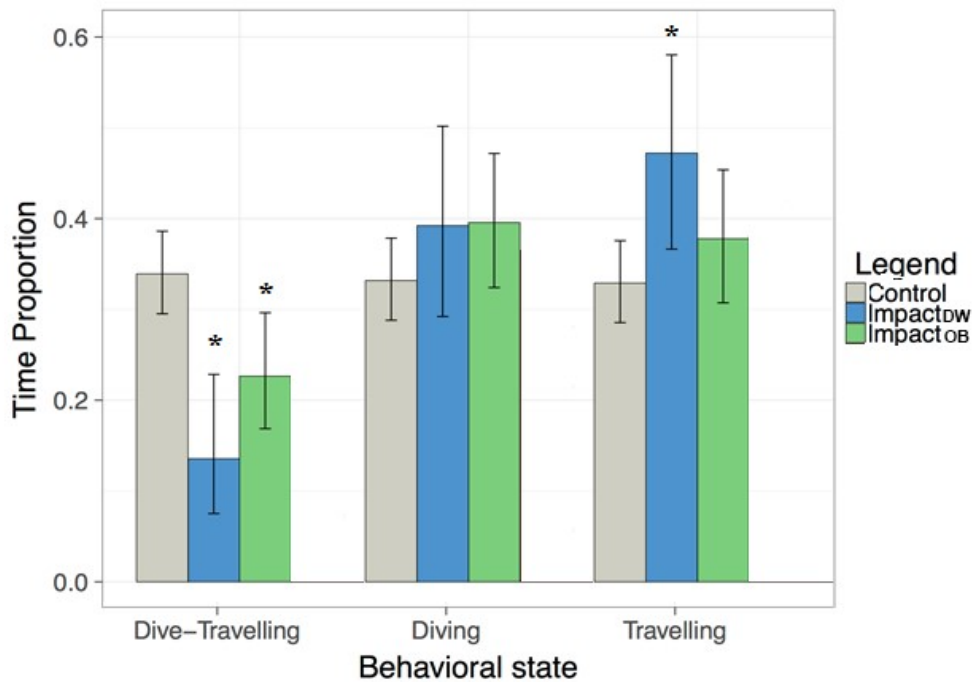
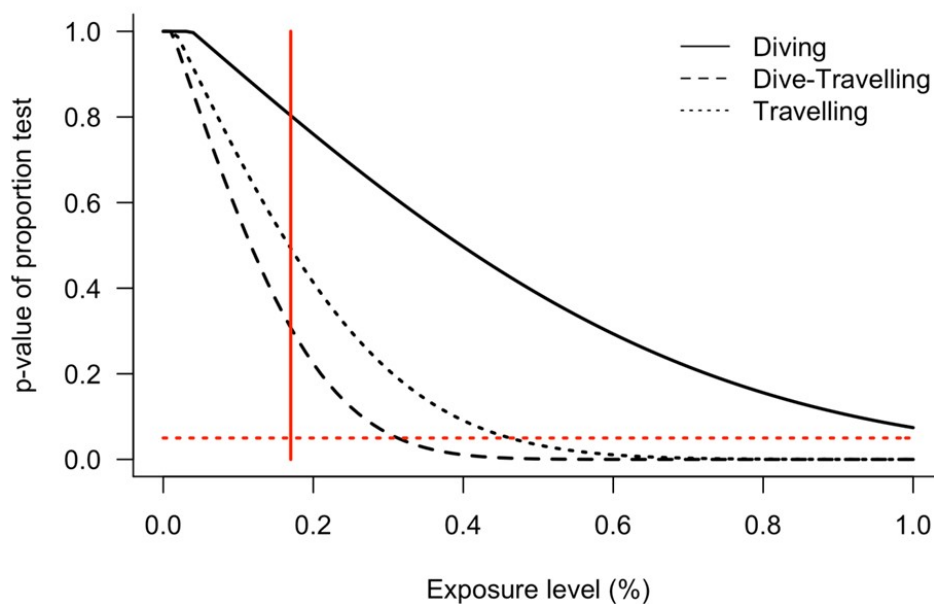


Figure 16: Behavioral budget for dolphin activity: the proportion of time spent in each behavioral state during ‘control’ (grey), ‘impact DW’ (blue) and ‘impact OB’ (green) conditions. Error bars represent 95% confidence intervals. Asterisks indicate significant difference between behavior states in ‘control’ condition compared to each impact condition ( $P < 0.05$ ).

Markov chains modeling resulted with estimated current exposure level of 17% in ‘impact DW’ condition (proportion of ‘impact DW’ scanned samples in overall number of collected samples during the study). Cumulative behavioral budget analyses showed no significant difference from the control behavioral budget of dolphins for estimated exposure level ( $X^2 = 1.26$ ,  $df = 2$ ,  $p = 0.53$ ). Building a linear projection, trend curve of dive-travelling demonstrated a significant effect when exposure level would reach up to 31%, while trend curve of travelling indicated significant differences when dolphin-watching boat exposure would reach up to 45%. Diving state did not show any significant difference between cumulative budgets of ‘impact DW’ and ‘control’ budget, even at 100% of dolphin-watching boat exposure (Figure 17a).

a)



b)

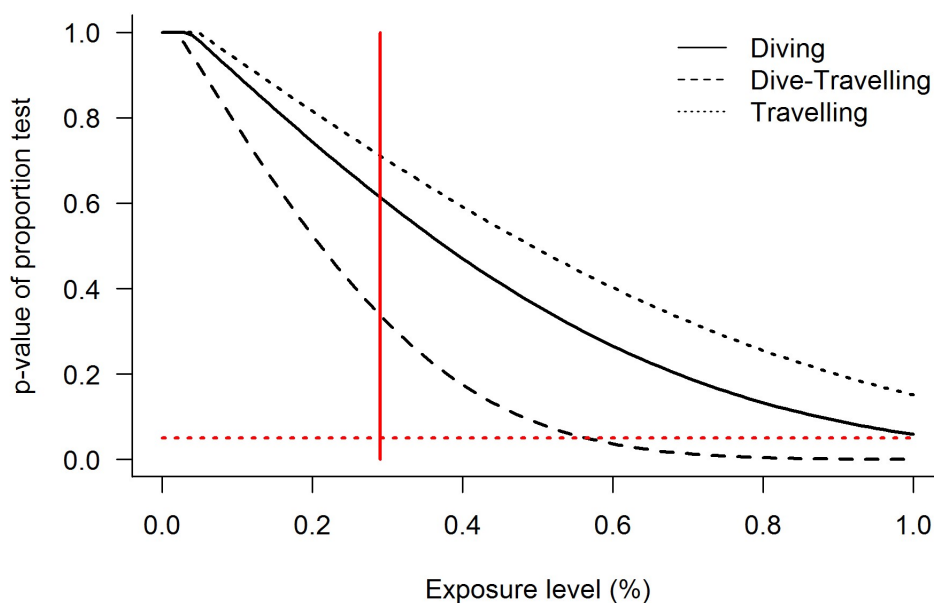


Figure 17: Cumulative budget estimation for 'impact DW' condition (a) and for 'impact OB' condition (b): Effect of dolphin-watching boat on the cumulative behavioral budget during different levels of exposure. The y-axis represents the p-value of the difference between the 'impact DW' cumulative behavioral budget and the 'control' behavioral budget (a) and the difference between the 'impact OB' cumulative behavioral budget and the 'control' behavioral budget (b), for the three behavioral states (see legend) at different vessel exposure levels (x-axis). The dashed red line represents the statistical level of significance ( $p < 0.05$ ). The solid red line indicates the estimated exposure level.

Cumulative budget estimation for 'impact OB' condition also did not show significant difference from 'control' cumulative budget at estimated current exposure level of 29% ( $X^2 = 1.07$ ,  $df = 2$ ,  $p = 0.59$ ). According to data trend, cumulative budget of dive-travelling indicates significant difference at exposure of 55%, while cumulative budgets of diving and travelling showed no difference even at 100% exposure (Figure 17b).

## 4 DISCUSSION

In the last two decades many studies were published with the intention of assessing the effects of marine vessels on cetacean behavior parallel to the rapidly growing cetacean-watching tourism and marine traffic (Nowacek et al., 2009; Lusseau, 2003a; Lusseau and Higham, 2004; Williams et al., 2006; Lusseau et al., 2006; Lusseau et al., 2009; Christiansen et al., 2010; Steckenreuter et al., 2011; Christiansen et al., 2013b; Meissner et al., 2015; Machernis et al., 2018). The majority of literature on vessel-based interactions with marine mammals report similar conclusions on changes in animals' behavior. However, the literature also suggests various factors that may affect animals' behavioral responses to vessels, such as focal group composition, as well as the number of vessels, type of vessel, distance of vessel and the way the vessel approach to the focal group (Machernis et al., 2018). These factors were not considered for the analysis in this study, but could have an effect on results.

There is little published literature suggesting no apparent response by animals to tour vessel interactions. In areas of low-level tourism in Patagonia, Argentina and New Zealand, a neutral response to vessel presence was reported for Commerson's and common dolphins. This neutral response is likely due to very low levels of tourism resulting in less behavioral, physical and acoustic disturbance (Machernis et al., 2018).

To investigate dependence of different types of boats and behavior transitions, sightings which included only one type of vessel approaching to the focal dolphin group (beside the research boat) were analyzed. First of all, results indicated small number of sightings for analysis and secondly no apparent (due to small number of sightings it was not possible to test whether these changes were statistically significant or not) difference in proportions of sightings with behavior transition was found considering impact of a given type of vessel. Research made by Nimak (2006) in the same area found that majority of negative responses of Cres-Lošinj dolphins to vessel presence were caused by recreational vessels (speed boats and yachts) while there were no negative responses to local gill-netters (note that for analysis in this study bottom-trawler fishing boat was considered), but insufficient data set of this study made it inappropriate for comparison. Our result which indicate a higher number of sightings with behavior transition under the impact of only a fishing boat than only a recreational boat raises some questions. In this study all behavior transitions were considered the same although not all transitions in behavior have the same meaning. For example, if dolphin group change their behavior from feeding related behavior (like diving) (Lusseau,



2003a; Lusseau and Higham, 2004) to avoidance related behavior (like travelling) (Machernis et al., 2018) in the presence of vessels, these changes cannot be considered the same as changes of behavior from travelling to socializing (both was recorded during this study). Furthermore, transitions including active trawler follow and passive trawler follow are linked only to presence of trawler boat and require separate treatment in analysis as make time series not ergodic. In other words, transition in dolphin behavior between all states are not possible if there is not trawler boat present. Beside this, considering occasional fishing restrictions proclaimed by the Croatian Ministry, bottom trawler boat doesn't have the same chance to be found, as other types of boats. Therefore, not considering samples which included active trawler follow and passive trawler follow and, consequently fishing boat in Markov chains analysis (as done in this study) give more genuine results.

Each sighting was also analyzed to find possible difference between those without vessel presence (except research boat) during the whole sighting (13 sightings), those with only dolphin-watching boat (2) and sightings with only other boats present during the whole sighting (8). Results indicate higher proportion of behavior transition in 'impact OB' condition and 'impact DW' in relation to 'control' but, due to a small number of samples it is difficult to state if behavior transitions occurred or not as a reaction to boat presence/absence or as a response to some other variable.

Analysis of descriptive statistics highlighted the limitations due to the small data set which precluded useful analyses like differentiation between impacts of different types of vessels, approaching speed and boat maneuvering, number of vessels, distance from the focal group, duration of interaction (Nimak, 2006); as well as the usage of more behavioral states, dolphin group size and composition, and analysis of different behavior transitions. The eventual effect of research boat on dolphin behavior was considered minimal since it is less intrusive than other types of vessels, regarding the size and maneuvering principles (Bejder et al., 2006). Beside this, repeated encounters over a certain period of time can cause habituation of the animals and consequent no behavioral response. Based on this, it might be that certain level of habituation to the research boat occurred during the 30 years of research in Cres-Lošinj archipelago (Nimak, 2006). However, despite protocol compliance, it is also possible that the exposure to the research boat itself may increase the overall effect of additional exposure to other boats (Bejder et al., 2006).

Contrary to what was expected, results of statistical analyses of this study showed low frequency of behavioral transitions (119 transitions out of 883 three-minute samples) of dolphin groups' considering all environmental conditions (presence or absence of a certain type of boats). According to the published data on vessel impact on cetaceans, following the similar protocol, proportions of behavioral transitions within all scanned samples of this study were lower. In the research on vessel traffic disruption of foraging behavior of southern resident killer whales *Orcinus orca* along San Juan Island, Washington (USA), Lusseau et.al. (2009) observed 135 behavioral transitions, out of 373 scanned samples (in 2003), 217 behavioral transitions out of 1058 scanned samples (in 2004) and 251 transitions out of 770 scans (in 2005). Akkaya Bas et.al. (2017) reported 658 behavioral transitions of Black Sea harbor porpoises (*Phocoena phocoena relicta*) within 1403 scanned samples in the Istanbul Strait (Turkey; Lusseau et al., 2009; Akkaya Bas, 2017).

Dependence of changes in dolphin behavior with environmental conditions in the overall data was tested by comparing the frequencies of samples with no-transition in each condition with frequencies of samples with behavioral transition in other conditions. In that way it's possible to see if the eventual behavior transition is linked with certain condition. The differences were found to be significant for 'control' and 'impact OB' condition, but not for 'impact DW' condition, indicating too small proportion of behavioral transitions in 'impact DW' compared to transitions in other conditions.

Frequency of behavior transitions were tested confronting 'control' condition with each impact condition separately. Results showed that dolphins significantly more frequently change their behavior in the presence of dolphin-watching boat ('impact DW' condition) than in the presence of research boat ('control' condition). There is also higher proportion of behavior transition in 'impact OB' condition found in relation to 'control' condition, but this difference is not significant.

Since there is a significant difference in changes in dolphin's behavior i.e. the proportions of dolphin behavior transitions between 'control' and 'impact DW' condition, first hypothesis was rejected. However, significant difference in the proportions of dolphin behavior transitions between 'control' and 'impact OB' condition was not found, therefore regarding 'impact OB' condition, first hypothesis was accepted. Due to the statistically not significant difference in proportion of behavioral changes in 'impact DW' condition compared to other

conditions, and low overall number of samples recorded in the conditions of 'impact DW', this result could be subjected to changes in the number of samples.

Comparisons of 'control' condition in relation to each impact condition for samples when behavioral transition did not occur showed significantly higher proportion of unchanged behavioral states in 'control' condition than in 'impact DW' and 'impact OB' condition. This indicates that dolphins are significantly more likely to stay in the same behavioral state when only a research boat is present in the vicinity of the focal group. Results of statistical analysis in SPSS indicate the need for further sampling in order to obtain larger data set, especially for 'impact DW'.

The insufficient number of scanned samples with certain behavioral states, as mentioned in the results, led to exclusion of those behavioral states from Markov chains analysis and consequently to exclusion of category of vessels that is linked to two excluded behavioral states. Therefore, Markov chain analysis is performed not considering impact of fishing boat within 'impact OB' condition. According to sampling protocol based on Akkaya Bas et al. (2017), this study did not separate impacts of each vessel type (within 'other boats') as it would additionally limit the current data set.

Time-discrete Markov chain analyses are widely applied to estimate the effect of vessel presence on the transition probability between behavioral states and to quantify the effect on the behavioral budget of the cetaceans (Lusseau, 2003a; Lusseau and Higham, 2004; Williams et al., 2006; Stockin et al., 2008; Lusseau et al., 2009; Christiansen et al., 2010; Scarpaci et al., 2014; Christiansen et al., 2013; Meissner et al., 2015; Akkaya Bas, 2017). Sequential observation of focal groups allowed modeling the probability of dolphins switching from one behavioral state to another as a function of vessel presence.

A substantial number of studies on whales are reported with results indicating that whale-watching activities can have short-term behavioral effects on the targeted animals, resulting in changes of activity, swimming speed, movement pattern, diving behavior, group formation, and/or vocalization (Christiansen et al., 2013). According to Christiansen et al. (2013) behavioral disruptions can have cumulative negative effects on an animal's bioenergetic budget, which can lead to long-term negative effects on individual vital rates. Williams et al. (2006) provided indirect evidence that feeding activity is disrupted by the presence of boats, which could lead to a substantial decrease in energy gain opportunities. Whales were significantly less likely to be foraging and significantly more likely to be traveling when

marine vessels were around. This finding is in agreement with previous studies of the northern resident killer whale population (Lusseau et al., 2009) as well as with this study.

Analyzing behavioral transition probabilities resulted with significantly lower probability for dolphins to stay in dive-travelling behavior in the presence of dolphin-watching boat (beside research boat) as well as with increased probability to change dive-travelling behavior to travel. In the presence of other categories of vessels none of the dolphins' behavioral states was affected separately, although there was significant effect on the transitions in behavioral states of dolphins considering overall data. Seems like dolphin-watching boat has the greater impact on probability of changes in certain dolphin behavior. Since dive-travelling is activity related to searching for food, less time spent in this behavior and switching to travelling instead, can have negative consequences for dolphin's welfare (Lusseau and Bejder, 2007; Lusseau et al., 2009; Christiansen et al., 2010; Meissner et al., 2015). According to the results second hypothesis is rejected although the size of the data set should be increased in order to verify the current results.

Behavioral budgets (also commonly referred to as activity budgets) quantify how much time an animal allocates to various behaviors (behavioral states) and are typically used to identify behavioral patterns. Analyzing activity budgets can provide valuable information of an animal's bioenergetics budget and is thus a valuable measure for assessing the effects of marine vessel presence on marine mammals (Christiansen et al., 2013). Even if each small change in animal behavior requires only a small increase in energetic expenditure, the cumulative effect could seriously affect not only individual welfare, but also the population (Fortuna, 2006).

Vessels presence has been shown to increase dolphin travelling behavior at the expenses of foraging, resting or socializing (Meissner et al., 2015). The most commonly documented animal responses to vessel-based tourism are decreased foraging or resting activities, and increased travel behavior (Machernis et al., 2018). Christiansen et al. (2013b) showed that the time minke whales (*Balaenoptera acutorostrata*) spent foraging and surface feeding decreased from 15.3% to 8.8% during interactions with whale-watching boats (Christiansen et al., 2013b). For common dolphins (*Delphinus delphis*) in New Zealand, foraging behavior was documented to decrease by 11.9% (Stockin et al., 2008) and by 12.4% (Meissner et al., 2015) in the presence of a tour vessel. General patterns of decreased foraging behavior in the presence of tour vessels have also been documented for bottlenose dolphins (*Tursiops*

*truncatus*), Risso's dolphins (*Grampus griseus*), Indo-Pacific bottlenose dolphins (*Tursiops aduncus*), pantropical spotted dolphins (*Stenella attenuata*), dusky dolphins (*Lagenorhynchus obscurus*), fin whales (*Balaenoptera physalus*) and killer whales (*Orcinus orca*) (Machernis et al., 2018).

Results of this study showed significant decrease in dive-travelling behavioral budget of Cres-Lošinj archipelago dolphins, in the presence of dolphin-watching boat by 20% and by the other categories of vessels by 11%. Since dive-travelling behavior is related to foraging, this finding is in agreement with previous studies (Lusseau et al., 2009). A decrease in the time spent foraging is likely to result in a reduction in energy procurement due to a decrease in food intake. Therefore, repeated disruptions to this behavior by marine vessels could have long-term negative effects on dolphin survival (Christiansen et al., 2010; Meissner et al., 2015).

Moreover, travelling behavioral budget significantly increased by 14% in 'impact DW' condition. These findings support general conclusion of similar studies that marine mammals generally travel more in the presence of tour vessels, likely as a type of avoidance tactic (Machernis et al., 2018) although by smaller percentage. Steckenreuter et al. (2011) suggested that Indo-Pacific bottlenose dolphins travel 28.8% more when dolphin-watching boats were present (Steckenreuter et al., 2011). Travelling behavior of Indo-Pacific bottlenose dolphins off the south coast of Zanzibar more than doubled in proportion, from 33% to 77%, becoming by far the most dominant activity state during interactions with tourist boats (Christiansen et al., 2010). Other species documented to increase their traveling behavior in the presence of tour vessels include bottlenose dolphins, common dolphins, dusky dolphins, Commerson's dolphins (*Cephalorhynchus commersonii*), Hector's dolphins (*Cephalorhynchus hectori*), fin whales and killer whales (Machernis et al., 2018).

Although above mentioned studies are referring to the effect of boat-based tourism, similar effects have been recorded for vessel traffic interactions that are not specifically engaged in viewing wildlife (e.g., commercial fishing vessels, cruise liners, commercial or recreational boats in transit). Bottlenose dolphins in Florida and Italy also have been found to decrease foraging and increase travelling in the presence of non-tourism-based vessel traffic (Bechdel et al., 2009; Papale et al., 2011). Not all categories of vessels are expected to have the same effect on cetacean. For example, it is known that dolphins are feeding behind the trawlers and use this as one feeding strategy. This behavioral response to fishing boats can be considered

rather positive, although there were negative consequences documented for this feeding strategy (Machernis et al., 2018; Fortuna, 2006). In addition to that, positive correlated distribution patterns of dolphins were recorded considering trawling boats (Nimak, 2006; Bearzi et al., 2008; Radulović, 2016). Throughout in the literature, there is no common understanding of how various terms (i.e., vessel, boat, ship, vessel traffic, etc.) are used to describe a situation. Due to this inconsistency, it can be very challenging to unriddle an animal's response to either vessel-based tour interactions or vessel traffic interactions (Machernis et al., 2018).

Results of this study did not indicate significant impact on travelling behavioral budget of the dolphins while being exposed to other categories of vessels. Again, presence of dolphin-watching boat caused greater impact on behavioral budget of the dolphins.

A bigger data set would be preferable to allow consideration of more behavioral states like socializing for example, which is very important for the reproduction of the population (Garaffo et al., 2007). Since there is a significant difference in the proportion of time dolphins spent in different behavioral states between 'control' and both impact conditions, third hypothesis was rejected.

Markov chains analysis estimated that Cres-Lošinj bottlenose dolphins spent 17% of overall observation time within 500 meters of dolphin-watching boat and 29% of overall observation time in the presence of other categories of marine vessels. Overall observation time (used for Markov chains analysis) implies 37 days in the period from June to October 2017, lasting from 12 to 105 minutes per day, in average 56,9 min/day.

Additional analysis of 'control': 'impact DW': 'impact OB' ratio was made for each day of survey in order to find out the mean proportion of dolphin's exposure to vessels and to compare this calculation with estimated cumulative behavioral budget. Mean value of daily ratio for 'impact DW' condition scans is 0,16, very similar to estimated 17% cumulative budget, while for the 'impact OB' amounts exactly like estimated cumulative budget – 0,29. However, according to Lusseau (pers. comm.) and considering the data distribution, a more valuable central tendency for a more credible reflection of population exposure could be median of the daily ratios rather than the mean. Using the median results in differences in relation to estimated cumulative budget: median value for 'impact DW' is 0 and for 'impact OB' is 0,17, which is significantly different from the overall ratio that was estimated for the

whole dataset (Markov chain's estimation). Such a low median value is not surprising considering the fact that in more than the half of the days of the survey (20 out of 37) there was no dolphin-watching boat present at all. Considering other boats, in 11 days (out of 37) of survey no single other boat was recorded. It's important to note that this "no impact" survey days were sampled throughout the whole observation period, not only in out of season period. Extrapolating this result to the population, one can conclude that even at the peak of the season and high touristic pressure, in the Cres-Lošinj archipelago there are still days without vessel impact on the local bottlenose population.

Although higher than the median value, the results of Markov chains estimation indicate that the current exposure doesn't have a significant effect on cumulative behavioral budget of dolphins. Using this model, it is possible to estimate at what level of vessel impact the cumulative behavioral budget could become significantly affected, given the observed effect size and assuming that such effect size does not vary with daytime exposure rate (Christiansen et al., 2010). When effects were built linearly, dive-travelling behavior in 'impact DW' indicated significant difference from the 'control' behavioral budget when the vessel exposure would reach up to 31%, while travelling behavior was significantly affected when exposure level would make 45%. In other words, it is modeled that if dolphins have been recorded 14% more of the overall observation time in the presence of dolphin-watching boat, cumulative budget of dive-travelling behavior would start to be significantly affected by it. For travelling behavior to be affected increase should be almost for 30%. However, the estimation showed no significant impact on cumulative budget of diving, even if the dolphins were to spend all observation time in the presence of dolphin-watching boat (Akkaya Bas et al., 2017).

Regarding impact of other categories of marine vessels, modeling has shown that if exposure level would rise from current 29% to 55% dive-travelling cumulative budget would be significantly affected. Cumulative budgets of traveling and diving showed no significant change from 'control' cumulative budget even at 100% of exposure. In both impact condition dive-travelling behavior appears to be the most susceptible to changes. Also, dive behavioral state seems to be most resilient on the presence of any category of vessels considering that cumulative budget of diving would not be affected even at 100% exposure in both, 'impact DW' and 'impact OB' condition. Since there is no significant difference in dolphin's cumulative behavioral budget between 'control' and both impact condition, in terms of cumulative budget, third hypothesis is accepted.

## 5 CONCLUSIONS

This thesis analyzed the impact of different categories of marine vessels on behavior of the small bottlenose dolphins' population in Cres-Lošinj archipelago. Impacts were therefore divided in two conditions: condition with the dolphin-watching boat present with the dolphin focal group – ‘impact DW’ condition, and condition with other categories of vessels present with the dolphin focal group (recreational boats, sailing boats, fishing boats, local boats and tour boats with the exception of DW boat) - ‘impact OB’ condition. Results indicate that vessel presence had an effect on the behavior of focal groups of bottlenose dolphins and that there are differences in intensity of impact between two impact conditions. Both impact conditions showed a significant change in dolphin behavior transition probability although not equally. Presence of the dolphin-watching boat significantly decreased probabilities for dolphins to stay in dive-travelling behavior while increased probability for transition from dive-travelling to travel behavior. Presence of other categories of vessels did not induce significant change between certain behaviors. Both impact conditions had an effect on proportion of time dolphins spent in different behavior, but also with the difference in intensity. Presence of the dolphin-watching boat significantly reduced the time dolphins spent dive-travelling and increased the travelling time. Other categories of vessels significantly affected only dive-travelling behavior by reducing it. Lastly it was modeled that Cres-Lošinj dolphins spent 17% of overall observation time between June to October of 2017 exposed to the dolphin-watching boat and 29% in the presence of other categories of marine vessels. However, this estimated current exposure has no significant impact on the cumulative behavioral budget of dolphins. In all analyses dive-travel behavior showed to be the most susceptible one to vessel presence, while diving seems to be the most resilient one in both, ‘impact DW’ and ‘impact OB’ condition.

Despite the application of “Code of conduct”, ‘impact DW’ condition i.e. presence of dolphin-watching boat within 500 m range of a dolphin group showed more prominent impact on dolphin's behavior than presence of other categories of vessels. However, there were a small proportion of recorded three-minute samples in ‘impact DW’ condition in overall data set. Moreover, due to insufficient number of scanned samples with certain behavior, those could not be used in the analyses, as well as some type of vessels. Further sampling is needed to support the current results as well as to create sufficient data set for all the behavioral states to be considered in the analyses. This would also enable differentiation between impacts of



different types of vessels. Results of this study indicate short-term effects of marine vessels on behavior on bottlenose dolphins but still with no significant effect on the cumulative behavioral budget. Therefore, this study highlights the importance of quantitative and qualitative monitoring and provides directions for further research and conservational measures.

## 6 POVZETEK

Pomorski promet je najbolj razširjena antropogena dejavnost v obalnih morjih in na različne načine negativno vpliva na prostoživeče organizme v morju (Marley et al., 2017). Velika pliskavka *Tursiops truncatus*, (Montagu 1821) je vrsta, ki je značilna za obalne habitate in je v primerjavi z drugimi morskimi sesalci v večji meri izpostavljena antropogenim aktivnostim (Nowacek et al., 2001). Poleg povišane stopnje pomorskega prometa so morski sesalci v zadnjem desetletju vse pogosteje izpostavljeni tudi hitro razvijajoči se industriji turističnih ogledov kitov in delfinov v naravnem okolju (Hoyt, 2001). Le-ta se osredotoča na nekaj vrst morskih sesalcev in večinoma poteka v obalnih habitatih (Hoyt, 2001).

Turizem je na hrvaškem eden izmed najpomembnejših stebrov gospodarstva in tako hkrati predstavlja eno izmed največjih potencialnih groženj biodiverziteti obalnih območij in otokov (Mackelworth et al., 2013). V Lošinjsko - Creškem arhipelagu tako zasledimo ekstremno nihanje v pomorskem prometu, s skokovitim povečanjem v poletnih mesecih (Rako et al., 2006). To območje je hkrati pomembno prehranjevalno in razmnoževalno območje velikih pliskavk ter Natura 2000 območje (Rako et al., 2012).

Populacijo velikih pliskavk v Lošinjsko - Creškem arhipelagu preučujejo že od leta 1987 v sklopu projekta Adriatic Dolphin Project (ADP), njena velikost pa je ocenjena na 184 stalno prisotnih živali (Rako, 2006; Radulović 2016). Projekt ADP vodi neprofitna organizacija Blue World Institute of Marine Research and Conservation (BWI), ki se nahaja na otoku Lošinj, Hrvaška, ki je v letu 2013 ustanovila lastno podjetje z omejeno odgovornostjo - Blue World Limited. Glavna dejavnost, s katero se podjetje ukvarja, so vodeni turistični ogledi delfinov v naravnem okolju (Blue World Institute, 2018).

Mnogo raziskav je opisalo odzive delfinov na motnje, ki jih povzročajo plovila, vključno s tistimi, ki izvajajo ogledе morskih sesalcev. Ti odzivi so v glavnem kratkoročne spremembe v vedenju posameznih živali ali skupin. Živali se lahko aktivno poskušajo izogibati plovilom, tako da spremenijo vzorce premikanja v prostoru, podaljšajo intervale potapljanja in povečajo hitrost plavanja. Prav tako lahko zmanjšajo interval med vdih, spremenijo vzorce sinusoidnega premikanja, spremenijo oglašanje in s tem sporazumevanje, skrajšajo intervale hranjenja, počivanja in socializacije in hkrati podaljšajo intervale potovanja (Lusseau, 2003a; Lusseau in Higham, 2004; Arcangeli in Crosti, 2008; Christiansen et al., 2010; Christiansen et al., 2013b; Marley et al., 2017).

Novejše raziskave nakazujejo, da imajo lahko takšni kratkoročni odzivi pomemben vpliv na biologijo vrste ter povzročijo dolgoročne spremembe v življenjskih strategijah osebkov in celotnih populacij (Lusseau in Bejder, 2007). Skrajšan čas prehranjevanja z veliko verjetnostjo vpliva na uspešnost pridobivanja energije zaradi potencialno zmanjšane vnosa hrane, medtem ko skrajšan čas počivanja povzroči fiziološki stres, pospeši srčni utrip in poveča porabo energije ter tako zmanjšuje energetske zaloge živali (Christiansen et al., 2010).

Sočasno se ekološki turizem (ekoturizem) ali zeleni turizem promovira kot dejavnost, ki pripomore k ohranjanju prostoživečih živali. V sklopu ogledov se turisti izobražujejo in ozaveščajo, vodiči pa pri njih skušajo vzpostaviti pozitiven odnos do opazovanih živali (Apps et al., 2017). Apps in sodelovci (2018) so pokazali, da lahko že kratka izkušnja srečanja živali v naravi vpliva na zavedanje, razumevanje, odnos, skrb in vedenje sodelujočega o tej živali in njenem pomenu ter ohranjanju. Prav tako je pomembna ekonomska plat, saj lahko dobro zakonsko urejena in nadzorovana dejavnost opazovanja prostoživečih živali ustvarja nova delovna mesta v okoljih, kjer se izvaja (Notarbartolo di Sciara, 2001).

Namen raziskave je ovrednotenje potencialnih učinkov različnih tipov plovil na vedenje stalno prisotnih osebkov pliskavk v Lošinjско - Creškem arhipelagu.

Območje, vključeno v raziskavo, meri približno 1600 km<sup>2</sup> in se nahaja na vzhodni strani Jadranskega morja. Vključuje del Velebitskega kanala in celotno območje Creško-Lošinjškega arhipelaga – morskega območja med obalo in otoki Krk, Rab, Pag, Cres in Lošinj ter se na južni strani končuje pri otokih Silba in Olib (Slika 5).

*Ad libitum* odprave z raziskovalnim plovilom smo izvedli v obdobju med junijem in oktobrom 2017, po vnaprej določenem raziskovalnem protokolu (Lusseau, 2003a; Lusseau in Higham, 2004; Akkaya Bas, 2017). S sledenjem navodil v protokolu smo poskušali kar najbolj zmanjšati negativne vplive prisotnosti raziskovalnega plovila na proučevano skupino delfinov. Obdobja, ko je ob skupini delfinov le raziskovalno plovilo, smo definirali kot kontrolne pogoje. Le-te smo primerjali z dvema scenarijema:

- »učinek DW«: poleg raziskovalnega je v radiju 500 metrov prisotno tudi plovilo namenjeno ogledu pliskavk (*Dolphin Watching Boat*), in
- »učinek OB«: poleg raziskovalnega je v radiju 500 metrov prisotno tudi eno ali več drugih plovil (*Other Boats*; (Tabela 5).

Vedenje proučevane skupine delfinov smo zbirali v 3-minutnih intervalih. Uporabili smo 6 standardnih oblik vedenja: potapljanje, potovanje s potapljanjem, potovanje, aktivno zasledovanje koče, pasivno zasledovanje koče in socializacija (Tabela 2).

Za analizo podatkov smo uporabili diskretne časovne markovske verige (*Time-discrete Markov chain analysis*). Analizirali smo vpliv prisotnosti plovil na verjetnost spremembe med standardnimi oblikami vedenj in za ovrednotenje učinkov na vedenjske vzorce delfinov (Lusseau, 2003a; Lusseau in Higham, 2004; Williams et al., 2006; Lusseau et al., 2006; Lusseau et al., 2009; Nowacek et al., 2009; Christiansen et al., 2010; Christiansen et al., 2013; Scarpaci et al., 2014; Meissner et al., 2015; Akkaya, 2017; Machernis et al., 2018). Analize podatkov smo naredili v programskem jeziku R (Spedicato, 2017; Spedicato et al., 2014; Wickham, 2009; R Core Team, 2016).

Vzorci vedenja (pogosto tudi vzorci aktivnosti) nam povedo, koliko časa posamezna žival nameni posamezni aktivnosti (obliki vedenja). Z analizo vzorcev vedenja lahko pridobimo informacije o bio-energetskih zalogah in potrebah živali in posledično ovrednotimo vpliv zunanjih dejavnikov (npr. prisotnost plovil) na morske sesalce (Christiansen et al., 2013).

Nizke proporcionalne vrednosti socializacije (8%), aktivnega zasledovanja koče (9%) in pasivnega zasledovanja koče (2%) v vedenjskih vzorcih delfinov so onemogočili uporabo teh stanj v analizi markovskih verig (Slika 8).

Modeliranje verjetnosti vedenjskih sprememb je pokazalo zmanjšanje verjetnosti, da delfini ob prisotnosti plovila za ogled nadaljujejo s potovanjem s potapljanjem, in hkrati zvišanje verjetnosti, da spremenijo obnašanje iz potovanja s potapljanjem v potovanje. Ob prisotnosti drugih plovil nismo zaznali sprememb v pogostosti posameznih oblik vedenja, kljub temu pa je zaznaven učinek na pogostost spreminjanja med posameznimi vedenjskimi stanji.

Potovanje s potapljanjem, aktivnost povezana s prehranjevanjem, se je ob prisotnosti obeh motenj zmanjšala (20% ob prisotnosti plovila za ogled, 11% ob prisotnosti drugih plovil), kar je v skladu s podobnimi raziskavami (Lusseau et al., 2009). Prav tako se je ob plovilu za ogled za 14% povečal čas, ki ga delfini namenijo potovanju. Ob drugih plovilih se ta čas ni statistično značilno razlikoval od kontrole. Te ugotovitve podpirajo sklepe podobnih raziskav, ki ugotavljajo, da morski sesalci ob prisotnosti plovil za ogled več časa namenijo potovanju, najverjetneje zaradi težnje, da se tem plovilom umaknejo (Machernis et al., 2018).

Rezultati modelov nakazujejo, da so bile navadne pliskavke v času naših opazovanj, med junijem in oktobrom 2017, 17 % skupnega časa izpostavljene plovilu za turistično opazovanje delfinov in 29 % skupnega časa drugim plovilom. Prisotnost plovil nima značilnega vpliva na kumulativne vedenjske vzorce navadnih pliskavk. Vse analize so pokazale, da prisotnost plovil najbolj vpliva na potovanje s potapljanjem, medtem ko je potapljanje oblike vedenja, ki je najmanj spremenljiva ob prisotnosti ene ('impact DW') ali druge oblike motnje ('impact OB').

Kljub uporabi »Kodeksa plovbe« ob prisotnosti delfinov, se je izkazalo, da ima plovilo za ogled bolj izrazit vpliv na vedenje živali v primerjavi z drugimi plovili. Pri tem moramo upoštevati, da je vzorec zabeleženih vedenj ob prisotnosti plovila za ogled proporcionalno precej manjši. Prav tako je v celotnem vzorcu število opažanj nekaterih oblik vedenja nezadostno, da bi jih lahko upoštevali v analizi Markovih verig. Potrebovali bi dodatna vzorčenja, da bi lahko preučili vpliv plovil na vse različne oblike vedenja delfinov.

Rezultati te raziskave nakazujejo na kratkoročne spremembe v vedenju delfinov ob prisotnosti plovil, ki pa kumulativno ne vplivajo na celotne vedenjske vzorce. S to raziskavo smo izpostavili pomen kvantitativnih in kvalitativnih metod monitoringa in podali smernice za nadaljnje raziskave ter možne varstvene ukrepe.

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APPENDIX A: Code of conduct

# CODE OF CONDUCT WHILE DOLPHIN WATCHING

Following these simple rules gives you a chance to enjoy watching dolphins in the wild with minimal impact on their behavior.



50 m

**CAUTION**  
Feeding, chasing or swimming with dolphins is **PROHIBITED** by Croatian law.

 This leaflet is supported, in part, by public funds from the Netherlands.

 **Zajedno čuvamo okoliš**  
Sufinancirano sredstvima Fonda za zaštitu okoliša i energetske učinkovitosti

## DO NOT OVERSTAY YOUR WELCOME

Stay with the animals for up to **30 minutes**. Accelerate gradually when leaving.



## GIVE THEM SPACE

Do not come closer than 50m. Let them decide to approach you. If there are other boats around, increase the distance from the group.

## DOLPHINS NEED PRIVACY, JUST LIKE US

They will let you know if you are not welcome! Leave immediately if you hear loud exhalation or observe very small animals, tail slapping, changes in swimming direction or prolonged dives.



## FOLLOW PARALLEL TO THEIR COURSE

Keep the engine in neutral while they are diving. Do not rush to reach them after they surface.

## INTRODUCE YOURSELF POLITELY

Approach slowly from their side. Avoid sudden changes of speed and direction.



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