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Nautical tourism affects common bottlenose dolphin (*Tursiops truncatus* M.) foraging success in a NATURA 2000 site, North-Eastern Adriatic Sea

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Abstract

Several studies indicate that unregulated nautical tourism can have negative implications on cetacean behaviour. In recent years, dolphin watching activities (DWA) have increased off the West coast of Istria, Croatia, a region in which the NATURA 2000 site: “Akvtorij zapadne Istre” has been proposed to be designated for bottlenose dolphins (*Tursiops truncatus* M.). For data collected between 2016 and 2019, we compared dolphin group behaviours from this region during impact (presence of nautical tourism boats-NTBs) and control (absence of NTBs) scenarios, as well as providing descriptive analysis on the displacement of individuals in the presence of NTBs. Throughout the study years, 48.5% of NTBs were observed within 15m of the dolphin focal groups and 97% were observed within 50 m distance. The greatest rates of displacement in dolphin focal groups occurred when NTB numbers were greatest per individual dolphin. Markov chain analyses were used to quantify the short-term effects of NTB presence on dolphin behaviour. In the presence of NTBs, dolphins were more likely to spend time milling and less time foraging. Cumulative behavioural budgets, derived by accounting for the time bottlenose dolphins spent in the presence or absence of NTBs, indicated that vessel exposure levels of 14% and 25% were enough to statistically affect milling and foraging behaviours, respectively. To lessen the lack of sustainable DWA, the implementation of relevant guidelines, e.g., Global Best Practice Guidance for Responsible Whale and Dolphin Watching (50 m *no approach* and 300 m *caution zone*) is therefore crucial to mitigate any long-term consequences the actions of NTBs may have on this key species. To date, 162 bottlenose dolphins have been photo-identified off West coast of Istria and cumulative interference to this population could affect direct ecosystem functioning.

Keywords: Common bottlenose dolphin; dolphin watching; nautical tourism; behavioural transitions; NATURA 2000 site; North-Eastern Adriatic.

Introduction

The northern most part of the Adriatic Sea, between Istria, Croatia, and Italy, has few areas deeper than 50 meters water depth. Influenced by environmental factors such as river discharge and oceanic mixing (generated by wind and oceanic gyres), as well as anthropogenic pressures, this region of the northern Adriatic is ecologically sensitive (Severini, 2013; Ribarič, 2017). For the Common Bottlenose Dolphin (*Tursiops truncatus* M. – hereafter ‘bottlenose dolphin’), it had been reported that a 50% decline in their population during the second half of the 20th century could have been attributed to deliberate killings (Bearzi *et al.*, 2008a) and that such rates of decline may still have been possible from various other anthropogenic actions during the early 2010s (Fortuna, 2006; Bearzi *et al.*, 2008a; 2012; Štrbenac, 2015). Due to their predominantly coastal distribution, it had been

identified that coastal developments, including the construction of new ports and harbours to support a growing nautical tourism industry, could present additional threats to bottlenose dolphins (Ribarič, 2017). Moreover, overfishing and exploitation of fish stocks (Bearzi, *et al.*, 2005), marine traffic and shipping activities and pollution from both industrial development and aquaculture (Aguilar *et al.*, 2000; Fossi & Marsili, 2003; Brotons *et al.*, 2008; Coomber *et al.*, 2016) present other issues. As a result, the IUCN (International Union for Conservation of Nature) Red List of Threatened Species identified the Mediterranean sub-population of bottlenose dolphins as ‘vulnerable’ (Bearzi *et al.*, 2012; UNEP-MAP-RAC/SPA., 2014).

Bottlenose dolphins (and other cetacean species) are afforded protection in accordance with European law. Under Annex IV of the Habitats Directive (Council Directive 92/43/EEC), bottlenose dolphins are European

Protected Species (EPS), where deliberate killing, disturbance or the destruction of their habitat is prohibited. Furthermore, species listed under Annex II of the Habitats Directive, such as the bottlenose dolphin, should be further protected and conserved in designated sites such as Special Areas of Conservation (SACs) or Special Protected Areas (SPAs). In combination, SACs and SPAs form the NATURA 2000 ecological network of protected sites, which is the EU's main policy to protect threatened species and habitats (Ribarič, 2017).

The west coast of Istria in the north-eastern Adriatic Sea represents a home area to a sub-population of approximately 162 bottlenose dolphins (investigated from 2001 onwards), with an average group size (GS) of 9.27 ± 6.53 individuals (Ribarič, 2017). In December 2012, this area was recognised within the NATURA 2000 network recommendations as a candidate SAC (cSAC) for bottlenose dolphins (IUCN-MMPATF 2017, European Environment Agency, 2020). An area of approximately 728 km² extending from the central marine area of Istria, around the promontory of Cape Kamenjak and up to the village Šišan, was proposed as an SAC under the NATURA Network, otherwise known as 'Akvatorij zapadne Istre' (*Aquatorium of West Istria*) (Site Code: 'HR5000032') (Državni zavod za zaštitu prirode, 2015; 2020). Akvatorij zapadne Istre cSAC has been defined however, at present, as a Site of Community Importance (SCI) for the designation of bottlenose dolphins. SCIs are designated as biogeographical regions which are thought to significantly contribute to the maintenance or restoration of a particular species or habitat for favourable conditions. However, despite this area being defined as an SCI by the European Commission, the site is not yet formally designated by the Croatian Government and is a pre-requisite for establishing the SAC. As such, bottlenose dolphins in this region are not yet afforded the protection that SACs provide. In addition, there are currently five more proposals for SAC's designated for bottlenose dolphins in Croatia which will contribute to the NATURA Network, each of which have the SCI status (NATURA 2000, 2020).

High levels of boat traffic associated with nautical tourism have since become of particular concern in this region (Ribarič, 2013; 2017) and there is growing evidence that changes to cetacean behaviour are a response to increased nautical tourism activities (Lusseau, 2003; 2004; Christiansen *et al.*, 2010; Pirodda *et al.*, 2015; Clarkson *et al.*, 2020). Short-term behavioural changes can include changes in vocalisation, changes in movement to avoid vessels (most often observed as increased dive durations, increased swimming speeds and/or increased frequency in changes of the travel direction) (Marley *et al.*, 2017) and changes to behavioural activity (changes in the percentage of the time spent feeding, resting, or socialising) (Christiansen *et al.*, 2010; Meissner *et al.*, 2015; Clarkson *et al.*, 2020). Changes in behavioural activity are most likely to incur energetic costs to animals which experience cumulative disturbances from non-targeted boat traffic (Clarkson *et al.*, 2020) or are targeted by tourism (Williams *et al.*, 2006; Christiansen *et al.*, 2010). Changes in movement to avoid vessels can increase energy

expenditure in marine mammals (Williams *et al.*, 2006; Christiansen *et al.*, 2014) and/or decrease foraging activities and thus, decrease food/energy intake (Christiansen *et al.*, 2013; Wisniewska *et al.*, 2016). Repeated behavioural changes, such as reductions in the percentage of time spent feeding, resting, or socialising, ultimately lead to changes in animal body condition (New *et al.*, 2014; Rolland *et al.*, 2016), which in turn can reduce survival and reproductive rates (Nabe - Nielsen *et al.*, 2014, Christiansen & Lusseau, 2015).

The effects of anthropogenic activities on the behaviour of Istrian bottlenose dolphins are poorly understood, despite the fact their coastal distribution overlaps with increased nautical tourism activities during the summer season (Gomerčić *et al.*, 2008; Ribarič, 2017). At present, Dolphin Watching Boat (DWB) cruises in Istria are frequently organised between May and September, and during the main tourist season (July - August), many tour operators dedicate cruises to dolphin watching twice daily. In addition, non-commercial Personal Boats (PB), as well as DWBs are actively searching for or are deliberately waiting for dolphin groups to surface before directly interacting with them. Large numbers of boat traffic introduce high levels of noise, varying transiting speeds and execute unpredictable changes in the navigating direction. While the government of Croatia is aware that sustainable development objectives are required to be achieved, it has an ambitious target to become the most desirable yachting destination in the Mediterranean (Government of the Republic of Croatia, 2013). Given the regions recommendation as a cSAC within the NATURA 2000, the reactions of bottlenose dolphins through interactions with DWBs and PBs are required to be understood. As such, the present project aims to investigate the short-term behavioural alterations of bottlenose dolphins and following interactions with Nautical Tourism Boats (NTBs - i.e. DWBs and PBs) and make predictions on the cumulative effect of repeated disturbances.

Materials and Methods

Data Collection

Bottlenose dolphin behaviour and NTB data were collected within a study area encompassing approximately 120 km² of Croatia's maritime zone between Poreč (45.2190° N, 13.5834° E) and south of Sv. Ivan lighthouse (45.0333° N, 13.6021° E) (Fig. 1). The study area itself was kept within the Akvatorij zapadne Istre cSAC, except for a 3 NM extension at its northern border. The average water depth in which surveys were undertaken was approximately 32 m. Observations of bottlenose dolphin groups and their proximity to NTBs took place from a 5.5 m long research boat (RB) with a cruise speed of about 12NM h⁻¹ and powered by an 80 HP 4 stroke outboard engine that emits lower sound intensity. The crew were trained, and all were in a standing position on the boat to achieve a greater observation angle towards the sea-surface. A GARMIN GPS MAP 400 series was used



Fig. 1: Survey area off west coast of Istria, in the NE Adriatic Sea (crosshatched). A dark blue area demarcates the NATURA 2000 site HR5000032 Akvatorij zapadne Istre where bottlenose dolphin was identified as the qualifying species for the cSAC. A light blue area on the west side indicates the national park Briuni.

to record GPS positions (Arcangeli & Crosti, 2009). Preliminary data collection for behavioural sampling started in 2015, prior to dedicated survey efforts in consecutive years between April - October 2016, July - August 2017, August 2018, and July - August 2019. Since the study was aimed to investigate NTBs' influence on the animals, there were no predefined transects followed. Instead, field trips were directed to areas of high probability of dolphin observation (Ribarič, 2017). Upon encountering a group of dolphins, the RB followed the recommendations published in the Global Best Practice Guidance for Responsible Whale and Dolphin Watching (GBPRW) (Lewis & Walker, 2018), a guide by the World Cetacean Alliance (WCA). Approaches to any focal group were made from a 60-degree angle adjacent to or in parallel with the dolphins' direction of movement. If this was not possible, the RB remained in an idle position to allow for the dolphin group to continue their activity without disruption. The RB never approached the animals from the front or behind and never prevented the group from continuing in their direction of movement. Under the GBPRW a caution zone of 300 m was presumed. This meant that if the RB was within 300 m distance of the dolphin group, the speed of the RB was reduced to the 'no wake' speed (about 5NM h⁻¹). When required, it was adjusted to the speed of the focal group for data collection.

Dolphin focal groups (as defined by Shane, 1990) were determined to note the interactions between the animals and NTBs. Instantaneous focal group scan sampling was used to define the predominant behaviour of dolphin groups. The behaviour of individuals within a group were recorded by scanning the focal group from one side to the other. If more than 50% of the focal group was engaged in a particular behaviour, then the group was assigned this particular behavioural state. Behavioural states were comprised of travelling, foraging, milling and socialising behaviours, as described by Shane (1990), Lusseau (2003) and Christiansen *et al.*, (2010). The behavioural state of a focal group was recorded at the start of three-minute scan samples, when dolphins were observable at the surface (Altmann, 1974). Samples were discarded if the focal group was no longer observable. If the focal group was not seen for 0.5 - 1 NM of cruising, or after 20 minutes from the last observation, the next observation of a dolphin focal group was recorded as a new group to prevent falsely identifying the new group as the previous.

The number of NTBs present was collected to investigate the cumulative pressure of nautical tourism on the dolphins. DWBs were defined as 10 - 50 m long launch boats built out of wood with conventional shape and usually powered by internal diesel engines (up to 170kW). In the peak season, boats were at capacity, with the largest

DWBs having 120 or more people on board. PBs were defined as 8m long, privately owned, or chartered sport shape boats (mostly made of plastic or rubber). Sailing boats were usually not in interaction with dolphins, unless a natural movement of a dolphin group was close (50 m or less) and if the area was clear of other boats.

Impact scenarios were defined as a dolphin focal group being within a 50 m (*no approach zone*) radius of a single DWB or PB, or within a 300 m (*caution zone*) radius of 3 NTBs. Impact scenarios were defined by the code of conduct as described in Lewis & Walker (2018) and thus, in these scenarios, behavioural states were added to the impact chain for subsequent analyses. Additional parameters for NTB were also collected: (i) the number DWBs and PBs within or outside the *no approach zone* and the *caution zone* to obtain a cumulative nautical pressure on the dolphins, and; (ii) the closest distance of any NTB to the animals. Distances were grouped into 100 - 150 m, 40 - 80 m, 20 - 30 m, 7 - 15 m, 1 - 5 m and 0 m radius (where 0 m indicates NTBs navigating just above submerged dolphins). Distance estimations related to the animals were noted at the beginning of NTBs arrival and throughout the whole encounter. In control scenarios, it was ensured that the distance between the RB and the dolphin group would be no less than 50 m, unless dolphins approached by themselves in which case the boat had a neutral (idle) motor position. Following such protocol minimised the bias of the RB being present (Lusseau, 2003) and avoided potential interactions. On many occasions, the RB encountered a dolphin group before any other boat arrived and thus served as a control parameter.

Average group size, composition, cohesion, and displacement

Average group size was calculated, and dolphin group composition was recorded for the whole research period in both control and impact scenarios. Group structure (adults, juveniles, and calves) was defined similarly to Bearzi *et al.* (1999) and relative group dispersion was delineated as in Bejder *et al.* (1999), where dolphins were defined as cohesive when within 0-2 body lengths of each other and dispersed when > 10 body lengths apart. Displacement of the animals in the presence of NTBs, as well as NTB distance to the animals, was measured with the help of the caliper (JNCC, 2016).

Data Processing

Two-way contingency tables were created between the preceding (behavioural state recorded at *t*) and succeeding (behavioural state at *t* + 3 min) behaviours during both control and impact scenarios. Behavioural samples were only included for analysis when there was a minimum of two behavioural transitions per dolphin focal group encounter. Control chains within the contingency tables were representative of the absence of all marine vessels within a 300 m radius and not just the absence of

DWBs or PBs. Although control chains are representative of the absence of all marine vessels within 300 m, it is important to note that each control chain did not take into consideration the presence of the research vessel in which data was collected upon. In situations where it was not identifiable that DWBs or PBs movements may have an effect of the dolphin focal group to be part of the control or impact chain (i.e., vessels arriving and departing), the data was removed from analysis (Meissner *et al.*, 2015). The sample sizes for socialising and resting behaviours were too small to include them in the analysis and could not be combined with other behaviours for inclusion, thus, they were removed from the analysis.

Effect of NTBs on dolphin behaviour

To quantify the effect of DWB and PB interactions on the behaviour of Istrian bottlenose dolphins, time-discrete Markov Chain analyses were applied (see Akkaya Bas *et al.*, 2017 for details). Markov Chains have been widely used to quantify the effect of marine vessels on cetacean behaviour in the past (Lusseau 2003; 2004; Christiansen *et al.*, 2010; Meissner *et al.*, 2015; Akkaya Bas *et al.*, 2017) and were found to be best suited for this study. Firstly, using two-way contingency tables, a first-order Markov Chain was used to determine the probability of transitioning from the preceding to the succeeding behavioural state, in both the control and impact chain as per Lusseau (2003) and Akkaya Bas *et al.* (2017):

$$p_{ij} = \frac{a_{ij}}{\sum a_{ij}}, \sum p_{ij} = 1$$

where *p_{ij}* represents the transition probability from the preceding behavioural state, *i*, to the following behavioural state, *j* (*i* and *j* range from 1 to 3, as a total of 3 behavioural states were studied, *a_{ij}* is the number of transitions observed from behaviour *i* to *j* and $\sum a_{ij}$ is the total number of observations where *i* is the preceding state (Lusseau, 2003). To test the effect of DWB and PB interactions on bottlenose dolphins, chi-square tests compared impact and control situations (Lusseau, 2003; Christiansen *et al.*, 2010; Akkaya Bas *et al.*, 2017).

To investigate the effect of DWB and PB on the behavioural budgets (the estimated proportion of time spent in a behavioural state based on observations in a time-series) of bottlenose dolphins, Eigen analysis (see Lusseau, 2003; Akkaya Bas *et al.*, 2017 for details) of the contingency tables for both the control and impact chains was performed. Differences between the control and impact behavioural budgets were tested using chi-squared test and 95% confidence intervals were calculated for the estimated proportion of time spent in each state.

Analysis of cumulative behavioural budgets followed that of Akkaya Bas *et al.* (2017, see for details). Cumulative behavioural budgets were derived by accounting for the time bottlenose dolphins spent in the presence (impact) of DWB and PB and the remaining proportion of

time spent without vessels. The exposure level, described as degrees of vessel intensity (i.e. proportion of time spent in DWB or PB presence) was artificially varied from 0 to 100% to see at which point the cumulative behavioural budget in the presence of DWB and PB became significantly different from that of the control budget, in comparison with the current exposure level (see Akkaya Bas *et al.*, 2017 for details).

Results

Between April 2016 and September 2019, 48 survey days were executed with a 96% success rate of bottlenose dolphin encounter. In total, 39.45 hours were spent in the presence of dolphins. NTBs were present in 69.8% (N = 53) sightings. In 51.4% of sightings, NTBs were deliberately waiting on dolphins to enter the survey area. When dolphins surfaced and were sighted by NTBs, they actively approached dolphin groups. 94.7% of waiting events were recorded in the high tourist season, with 63.2% of them performed by DWBs. During 19 bottlenose dolphin sightings, NTBs were already present before the RB arrived. In events when the RB was alone and within the recommended distance to focal groups, it took an average of 25.05 minutes (± 18.5 ; N = 18) for the first NTB to arrive within equal to or less than 50 m from the dolphin group.

The highest number of NTBs recorded as encroaching on a focal group during a sighting event was 38 (4 DWBs, 34 PBs, August 2019). When boats were divided into their individual categories, the highest number of DWBs and PBs identified as encroaching on a focal group within a single sighting was 18 (August 2017) and 34 (August 2019) vessels, respectively. The average number of sightings where the number of NTBs exceeded 15 vessels (N = 13) are shown in Figure 2.

The single greatest ratio of NTBs present against a single adult dolphin (NTB: TT) was observed at 21: 1. When calves were present, the greatest ratio of NTBs per

adult-calf dolphin pair was 19 : 2. Where the number of NTBs was greater than 15, the ratio of NTBs per single dolphin was $> 4: 1$, all occurring during the high tourist season. In each of these cases (N = 15) the average number of DWBs was 10.46 ± 4.79 and that of PBs was 11.06 ± 7.49 boats.

Aggressive navigating, closest boat to animals

Aggressive navigating of NTBs was evident in close proximity to the animals. From 134 events, 97% NTBs had been observed within the *no approach zone* (less than 50 m away from the animals). Within these observations, 38.8% of NTBs were at the distance of 20 - 30 m and almost 48.5% were 1 - 15 m away from the animals. On two occasions (1.5%) NTBs were navigating above a recently submerged dolphin group and were noted as a 0 m distance (Fig. 3). With specific regard to DWBs, $> 95\%$ of DWBs was present in each of the studied high tourist seasons and 18.6% of the same DWBs were navigating aggressively within the *no approach zone*.

Displacement of the dolphins

The displacement of individuals from a dolphin focal group during impact scenarios was observed in 64 events, where all but one displacement (June 2016) happened during the high tourist season. In 76.5% of these events, calves were present within the focal group. The length of an individual dolphins' dive for displacement was estimated at an average of 304 m. When pooling the data into classes by 100 m, most often individual dolphins were estimated to displace themselves 300 m from the focal group (34% of events), followed by 200 m (23% of events), 400 m (16% of events) and 7 times for 100 m or less. In one event (1.6%) a displacement dive was estimated at ~ 500 m. In 11 events (17.2%) it was not possible to determine the group structure prior to displacement

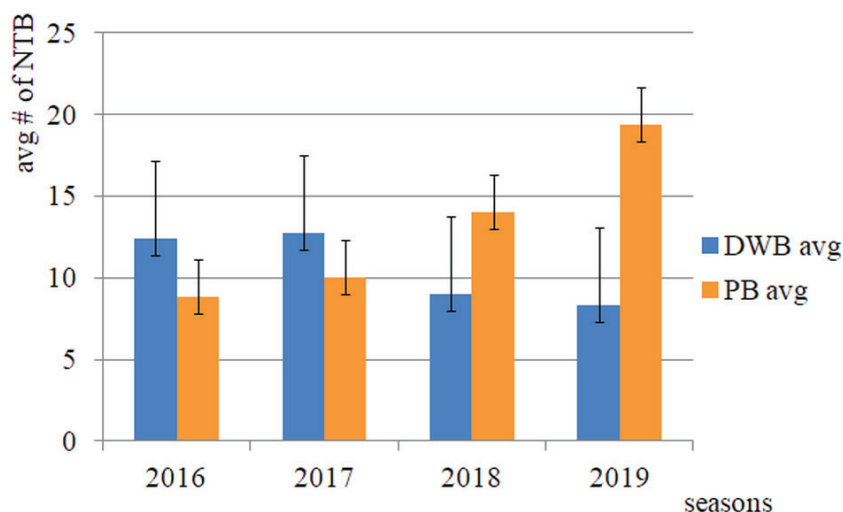


Fig. 2: The average number (avg #) of nautical tourism boats (NTBs) being present at the same time near the bottlenose dolphins, throughout the seasons 2016 - 2019 (N = 13 for data $15 < \text{NTBs}$, 97 % NTBs at ≤ 50 m distance).

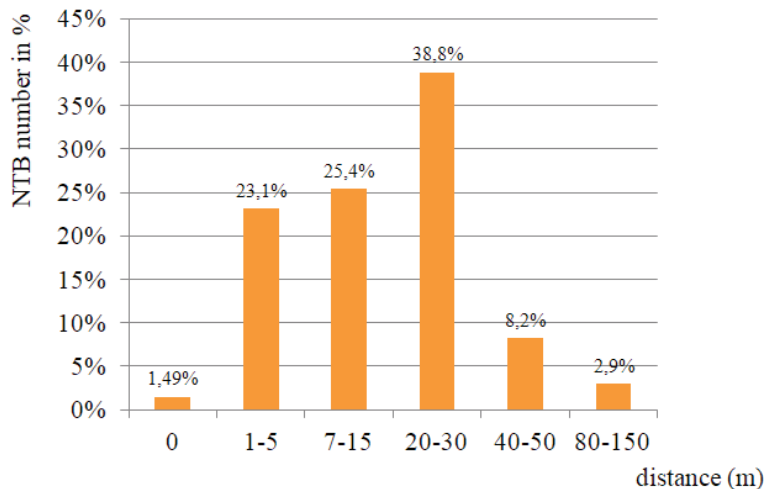


Fig. 3: NTBs distance (in meters) to the bottlenose dolphin focal group, expressed as a percentage. Only 2,9 % (N = 134) of all NTBs were outside the no approach zone (50 m < from the animals). In two cases DWBs were navigating above the just submerged animals.

occurring. Figure 4 shows that displacement dives were the longest (400 - 500 m) when the ratio of NTBs per individual dolphin was greatest (3.52) and when fewer adults were present per individual calf (2.40). By comparison, shorter (200 - 300 m) displacement dives were observed when there were reduced numbers of NTBs present per individual dolphin (1.44) and when more adults per individual calf (2.81) were present in the focal group. However, displacement dives up to 100 m in length were more prevalent than displacement dives which were 200 - 300 m in length. In these events, the numbers of NTBs present per dolphin was greater (2.81) and the number of adults focal group per individual calf were less (1.89). In events where there were several long displacement dives, individual dolphins began to make shorter dive displacements (100 m or less, N = 7) approximately 28.9 minutes on average after the encounter start. No displacement of

individuals from the focal group was noted when only the RB was present.

Average group size and composition

On average, focal group GS in the absence of NTBs (N = 264) was 7.17 ± 6.90 individuals, with 5.26 ± 5.60 adults and 1.81 ± 1.31 of juveniles and calves present. In the presence of NTBs the average GS was reduced to 5.43 ± 3.59 with both the number of adults (3.66 ± 2.26) and juveniles and calves (1.33 ± 1.04) becoming reduced. Dolphin groups were smaller for 24% of observations during impact scenarios, with 30% less adult animals and 26% less juvenile and calves. Group composition was cohesive in 63% (N = 16) when NTBs were already present, before the RB arrived. In 88% of such events calves were

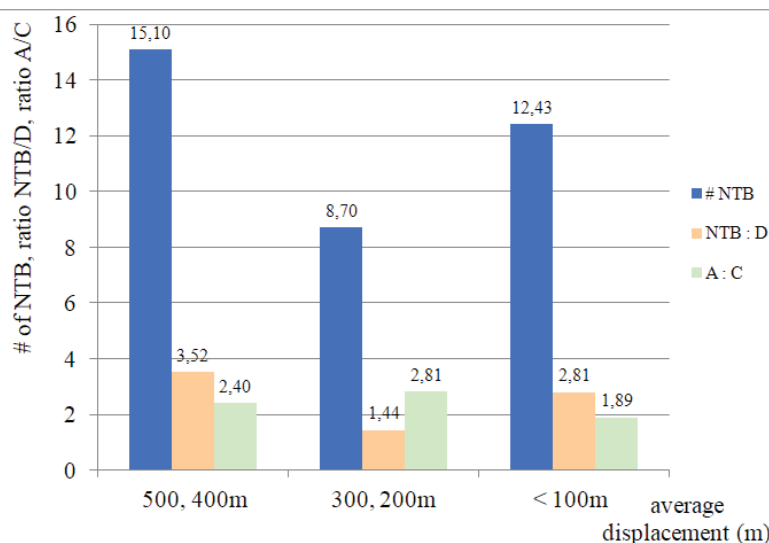


Fig. 4: Displacement (in meters) through the dives. The longest displacements happened with the highest average number of NTBs being present (# NTB) and the highest ratio of NTBs per one dolphin (NTB: D) (N = 20). In the 300, 200 m displacement class the ratio of adult animals per calf (A: C) was the highest (N = 37). In displacements of ≤ 100 m the A: C ratio was the smallest and the ratio of NTB:D was higher again (N = 7).

in a group. On average after 16.9 minutes ($N = 17$) of NTB interactions, one or more adult animals separated from the focal group which were then followed by a certain number of NTBs.

Behavioural Transitions

Markov chain analysis demonstrated that bottlenose dolphin interactions with NTBs influenced the transitions of behavioural states ($X^2 = 191.301$, $df = 4$, $p < 0.001$). This effect was not evenly distributed however, as only 4 transitions showed statistically significant differences ($p < 0.05$) between impact and control situations. Two of the transitions: Foraging \rightarrow Foraging ($p < 0.022$) and Travelling \rightarrow Foraging ($p < 0.034$) decreased when comparing control vs. the impact chains, whilst the other two transitions, Foraging \rightarrow Milling ($p < 0.001$) and Travelling \rightarrow Milling ($p < 0.013$) increased comparing control and impact scenarios because of DWB and PB being present (Fig. 5).

Behavioural Budgets

All three of the behavioural states making up bottlenose dolphin behavioural budgets were significantly affected by the DWBs and PBs ($X^2 = 134.047$, $df = 2$,

$p < 0.001$). In the presence of NTBs, bottlenose dolphins spent a smaller proportion of their time foraging ($p < 0.000$) and travelling ($p < 0.048$) but instead spent more time milling ($p < 0.000$). Foraging was identified as the dominant activity during control scenarios and was the behaviour most impacted upon, decreasing from 61.9% to 23.4%. Travelling - the other activity which decreased between control and impact scenarios, decreased by $\sim 7\%$ from 28.8% to 21.8%. Milling behaviour however, increased by 6 times between control and impact scenarios, becoming the dominant activity in the presence of DWB and PB. Milling behaviour had an increase from 9.2% to 54.6%.

Cumulative Behavioural Budgets

The effects of different levels of NTB intensity on bottlenose dolphin cumulative budget can be seen in Figure 6. Cumulative milling behaviours of dolphins were shown to be significantly affected at 14% of the proportion of time spent in the presence of NTB, whilst it was shown that foraging behaviours were affected at 25%. Travelling behaviours were not identified to be significantly different between control and impact scenarios under any NTB intensity. The results show that exposure to NTBs 14% of the time is already sufficiently large to affect the cumulative behavioural budgets of bottlenose dolphins off the coast of Istria, Croatia. It was identified that the current level of exposure to NTB intensity during the summer months stands at 69.8%.

Discussion

Within the Mediterranean, bottlenose dolphin populations are scattered across many different basins, and on finer scales, localised to specific coastlines where sub-populations have been identified (Borrell *et al.*, 2005; Bearzi *et al.*, 2008a; Ribarič & Herlec 2008; Gnone *et al.*, 2011; Gonzalvo *et al.*, 2014; Ribarič, 2017). Strong-site fidelity can present issues of vulnerability to bottlenose dolphins, particularly in areas which are identified as mass tourist destinations. In Istria, dolphin watching cruises are organised along the coast predominantly between June and September. Outside of the tourist high season in July and August, dolphin watching tours are executed mostly during the weekends and national holidays, although they do not sail out exclusively for dolphin watching. When considering the influence of anthropogenic activities, marine mammal exploitation through eco-tourism has often been cast with positivity when compared with historical culling practices within the Adriatic Sea and lethal whaling activities across other areas of the globe. Regardless, concerns about the effects of dolphin and whale watching activities were raised and studies began to provide evidence of the significant effects that such activities had on cetacean behaviour (Lusseau & Higham, 2004; Stensland & Berggren, 2007; Christiansen *et al.*, 2010; Steckenreuter *et al.*, 2011; Christiansen *et al.*, 2013a; Christiansen

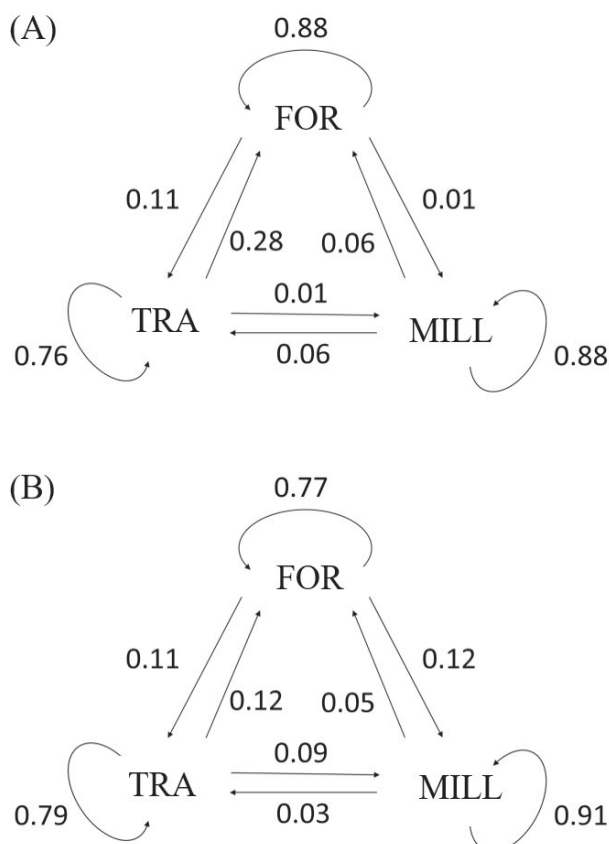


Fig. 5: Markov chains representing transition probabilities between bottlenose dolphin behavioural states of travelling (TRA), foraging (FOR) and milling (MILL) in the (A) absence and (B) presence of nautical tourism boats.

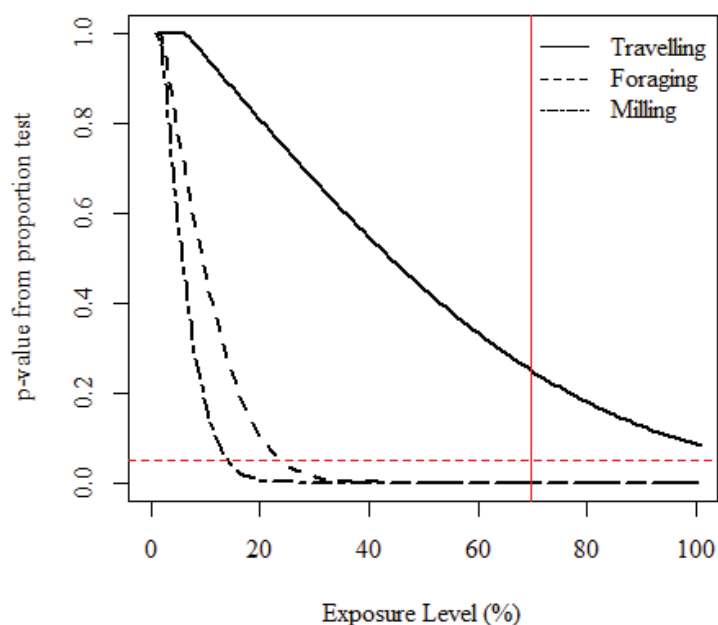


Fig. 6: Effect of marine vessels on the cumulative behavioural budget of bottlenose dolphins during different levels of exposure. The y-axis represents the p-value of the difference between the cumulative behavioural budget and the control behavioural budget for the three behavioural states (see legend) at different vessel exposure levels. The dashed red line represents the statistical level of significance ($p < 0.05$). The solid red line indicates the current exposure level (69.8%) of bottlenose dolphins to nautical tourism boats in the surveyed area of the Akvatorij zapadne Istre cSAC between April and October 2016 - 2019.

et al., 2014; Meissner *et al.*, 2015). Despite this, few studies have documented the potential effects resulting from the interactions between cetaceans and NTBs within the Adriatic Sea (Rako *et al.*, 2013; Clarkson *et al.*, 2020).

The current study demonstrates that the behaviour of bottlenose dolphins within the Akvatorij zapadne Istre cSAC is significantly affected by the presence of NTBs. At present, bottlenose dolphins have high association rates with NTBs during the tourist season, with behavioural observations being made in the presence of NTBs 69.8% of the time. This high association rate significantly altered the short-term differences in behavioural transitions, which were strong enough to alter the behavioural budgets of bottlenose dolphins during the tourist season. Following interactions with NTBs, dolphins increased their milling behaviour, with a significant decrease in their foraging behaviour. Although research throughout the Adriatic Sea has previously indicated that foraging behaviours are increased during spring and summer (Bearzi *et al.*, 1999; Bearzi *et al.*, 2008b; Affinito *et al.*, 2018; Clarkson *et al.*, 2020) and milling behaviours are least observed during the same seasons (Affinito *et al.*, 2018; Clarkson *et al.*, 2020), the current study demonstrates a significant increase in milling behaviour upon interaction with NTBs which cannot be attributed to seasonality.

Milling behaviours are most associated with feeding, socialising, or resting and are often exhibited when bottlenose dolphin adults, juveniles and calves are all present within the same group following a foraging event (Shane *et al.*, 1986; Constantine *et al.*, 2004; Triossi *et al.*, 2013) to build conspecific relationships (Lusseau, 2006; Stanton & Mann, 2012). As the current study demonstrated that foraging behaviour was significantly decreased upon interactions with NTBs, there is the possibility that the

significant increase in milling could be attributed to natural behavioural transitions following disrupted foraging events. It must be noted however, that not all milling behaviours will have followed foraging and the significant increase in milling under NTB presence may correspond to dolphin reactions to disturbance. Studies have shown that dolphin focal groups are more cohesive when NTBs are present (Bejder, 1999) and our observations indicated that Istrian bottlenose dolphin groups were more cohesive when vessels were navigating aggressively and when calves were present. Previously, significant increases in milling behaviour have triggered suggestions for marine mammal protection to be implemented where dolphins were not afforded sufficient protection from disturbance (Constantine *et al.*, 2004).

Significant decreases in foraging behaviour (which include diving and surface feeding behaviours) in bottlenose dolphins have also been reported elsewhere in the Adriatic Sea, despite much lower association rates with NTBs (Clarkson *et al.*, 2020). It is well established that reductions in foraging behaviour reduce the energy intake of dolphins (Williams *et al.*, 2006; Christiansen *et al.*, 2010) and can lead to detrimental implications on the chances of survival and reproductive success if repeated disruptions occur (Lusseau, 2003; Constantine *et al.*, 2004; Lusseau *et al.*, 2006). The likelihood of repeated disruptions is often identified by comparing the current NTB exposure levels with modelled cumulative impacts, which identify the exposure level in which repeated significant effects would occur. Concerningly, the current vessel exposure level (69.8%) during the tourist season is large enough to alter the cumulative budgets of bottlenose dolphin foraging behaviour and exceeds the 25% exposure level which would begin to significantly alter

dolphin foraging behaviour (Fig. 6.). Dolphins in this area of Istria have high site fidelity (Ribarič, 2017), thus repeated disruptions to foraging during the tourist season will persist at the current exposure level and are likely to have long-term negative impacts on exploiting available food resources (Clark & Mangel, 1986; Emlen, 1991; Constantine *et al.*, 2004), inducing limited energy acquisition (Williams *et al.*, 2006; Christiansen *et al.*, 2013b; Meissner *et al.*, 2015).

Further, the presence of NTBs can cause animals to trade off fitness enhancing activities such as foraging to invest time and energy in behavioural responses such as avoidance. In the absence of NTBs, focal group size was larger than when NTBs were present. Reductions in group size in the presence of NTBs could be attributed to observed displacement events, as individual members of the focal group separate themselves from the rest focal group, in order to increase their distance from a source of disturbance (Lusseau, 2003). Individual displacement dives were greater in distance (400 - 500 m) when the number of NTBs present were highest and consequently, focal group size consisted of lower numbers of adult dolphins per individual calf than when less NTBs were present. Although displacement events lessen the cumulative boat disturbance on a single dolphin, they offer the least protection to calves. Thus, the observed increase in milling behaviours attributing to increased cohesiveness in the presence of NTBs, could be an 'anti-predator' mechanism (Bejder, 1999; Heithaus & Dill, 2002; Steckenreuter, 2011) to protect calves as group size becomes smaller. Shorter distanced displacement dives (200 - 300 m) were observed when there was higher ratio of adults per single calf and the cumulative number of NTBs was lower. However, if NTB - dolphin interactions were persistent (~ 30 mins and longer) shorter displacement dives of up to ~ 100 m were observed, even in cases when NTB presence was greater and the number of adult dolphins per individual calf within the focal group was lower. These events were representative of the additional energetic costs avoidance strategies incur, as the animals were notably tired, swam slower (especially when calves were present), had higher ventilation rates and made shallower intermediate exhalation dives, before performing a deeper dive again.

As nautical tourism intensity currently exceeds the exposure levels in which cumulative effects begin to occur, it is possible that dolphins will have to temporarily evade the area during periods when tourism intensity is too high until exposure levels can be reduced (Yazdi *et al.*, 2005) and regulated to a sustainable level to allow the animals to 'co-utilize' the area. Preliminary data that hint on such avoidance were already noted, with the start encounter positions being more distant from the shore as the tourist season grows from June to August (Ilić, 2018; Ribarič, unpubl. data).

Currently, a total of 43 DWBs have been identified to be operating within the Akvatorij zapadne Istre cSAC, whilst the number of PBs being used in the same area are increasing yearly (+14% 2016 - 2017; +40% 2017 -

2018; +38% 2018 - 2019). Despite this area being designated as an SCI by the European Commission, the site is not yet formally designated by the Croatian Government as an SAC (hence, "cSAC" status). As such, bottlenose dolphins in this region are not yet afforded the protection that SACs provide. Changing the status of the site from an SCI / cSAC to an SAC enforces Article 6 (1) of the Habitats Directive onto the site, which states that 'Member States shall establish the necessary conservation measures for the habitats and species of Annexes I and II which are present in the given SACs'. Therefore, while SCI / cSAC have a degree of protection based on Articles 6 (2) - 6 (4), the designation of SACs triggers the implementation of Article 6.1. for the full conservation measures of the Habitats Directive, i.e., all of Article 6, to be applicable. However, regardless of the study area's status, current NTB activities in the Akvatorij zapadne Istre cSAC are not enabling a favourable condition to be maintained for the qualifying species. And thus, it is imperative immediate action is taken to establish appropriate mitigation measures and monitoring.

The necessity to familiarize stakeholders with existing guidelines such as the GBPRW (Lewis & Walker, 2018) will allow them to be implemented in the current DWA activities in Istria. The guidelines suggest 50 m as a *no approach zone* to the animals and 300 m as a *caution zone*. It is suggested that within 300 m, only 3 boats should be present for a maximum of 30 minutes each. If there are more boats within this distance, the maximum time of a single vessel is reduced to 10 minutes and the boat that arrived first should leave the *caution zone* first to give the space to a new vessel. The same distance recommendation to the dolphins is mentioned in the Guidelines for Commercial Cetacean - Watching Activities in the Black Sea the Mediterranean Sea and Contiguous Atlantic Area (RAC/SPA, 2004). In the current study, none of the NTBs in Akvatorij zapadne Istre cSAC respect a 50 m *no approach zone*, making it harder to achieve the illustrative recommendations of ACCOBAMS which suggest a 100 m distance to the animals as the *no approach zone* (ACCOBAMS, 2020). Croatian nautical development is in continuous growth, with demand to offer more anchorage berths for larger vessels (Government of the Republic of Croatia, 2013). Experts from other fields in the country comment that natural resources will importantly influence the nautical development and will represent a vital element of tourism supply and an economic aspect of it (Gračan, 2016). Therefore, if the country wants to develop a nautical tourism that will offer pristine natural heritage as its market advantage, a sustainable co-existence with bottlenose dolphins is one of the milestones that requires prioritisation. This should include the implementation of the aforementioned guidelines which could be available to the DWB captains and to the nautical guests and would allow their quick implementation. Ultimately, preserving the ecosystems top predators will lead to a better ecological balance and functioning of the entire North Adriatic marine ecosystem.

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