POPULATION ECOLOGY, BEHAVIOUR AND CONSERVATION STATUS OF COMMON BOTTLENOSE DOLPHINS *(TURSIOPS TRUNCATUS)* IN THE GULF OF TRIESTE AND ADJACENT WATERS OF THE NORTHERN ADRIATIC SEA

TILEN GENOV

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Population ecology, behaviour and conservation status of common bottlenose dolphins (*Tursiops truncatus*) in the Gulf of Trieste and adjacent waters of the northern Adriatic Sea

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University of
St Andrews

This thesis is submitted in partial fulfilment for the degree of

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October 2021

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I, Tilen Genov, do hereby certify that this thesis, submitted for the degree of PhD, which is approximately 43,973 words in length, has been written by me, and that it is the record of work carried out by me, or principally by myself in collaboration with others as acknowledged, and that it has not been submitted in any previous application for any degree. I confirm that any appendices included in my thesis contain only material permitted by the 'Assessment of Postgraduate Research Students' policy.

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PUBLICATIONS AND CONTRIBUTIONS

The results of Chapter 2 have been published in: *Genov T., Centrih T., Kotnjek P., Hace A. 2019. Behavioural and temporal partitioning of dolphin social groups in the northern Adriatic Sea. Marine Biology 166: 11.* *https://doi.org/10.1007/s00227-018-3450-8*. T. Genov designed the study, obtained funding, collected data in the field, analysed data and wrote the manuscript. T. Centrih helped with collecting data in the field and with exploratory data analysis. P. Kotnjek helped with collecting data in the field and with overall project running. A. Hace helped with collecting data in the field. All authors reviewed the manuscript.

The results of Chapter 3 have not been published yet, but a manuscript will be prepared for submission to a peer-reviewed journal, with T. Genov, M. Vighi and P.S. Hammond as authors. T. Genov designed the study, obtained funding, collected photo-identification data and biopsy samples in the field, analysed data and wrote the chapter. M. Vighi carried out the chemical processing of the samples. P.S. Hammond (PhD supervisor) provided feedback and advice on issues related to statistical analysis.

The results of Chapter 4 have been published in: *Genov T., Jepson P.D., Barber J.L., Hace A., Gaspari S., Centrih T., Lesjak J., Kotnjek P. 2019. Linking organochlorine contaminants with demographic parameters in free-ranging common bottlenose dolphins from the northern Adriatic Sea. Science of the Total Environment 657: 200-212.* *https://doi.org/10.1016/j.scitotenv.2018.12.025*. T. Genov designed the study, obtained funding, collected photo-identification data and biopsy samples in the field, analysed data and wrote the manuscript. P.D. Jepson inspired interest in this topic in the first author, obtained funding for the analysis of the first few samples and helped facilitate collaborations. J.L. Barber carried out the chemical processing of the samples. A. Hace helped collect photo-identification data in the field and helped with obtaining biopsy samples via boat driving. S. Gaspari carried out molecular sex determination of sampled dolphins as part of collaboration on genetic population structure. T. Centrih helped collect photo-identification data in the field and helped with obtaining biopsy samples. J. Lesjak helped with data collection in the field and in locating dolphin groups via land-based surveys. P. Kotnjek helped collect photo-identification data in the field and helped with obtaining biopsy samples via boat driving, and with overall project running. All authors reviewed the manuscript.

The results of Chapter 5 have not been published yet, but a manuscript will be prepared for submission to a peer-reviewed journal, with T. Genov, J. Železnik and P.S. Hammond as authors. T. Genov designed the study, obtained funding, collected photo-identification data in the field, carried out photo-identification matching and processing, analysed data and wrote the chapter. J. Železnik carried out photo-identification matching and processing. P.S. Hammond (PhD supervisor) provided feedback and advice on issues related to mark-recapture analysis. Various team members from Morigenos – Slovenian Marine Mammal Society helped with data collection in the field at different times.

The results of Chapter 6 have been published in: *Genov T., Centrih T., Wright A.J., Wu G.-M. 2018. Novel method for identifying individual cetaceans using facial features and symmetry: A test case using dolphins. Marine Mammal Science 34(2): 514-528.* *https://doi:10.1111/mms.12451*. T. Genov discovered the pattern, designed the study, collected data in the field, extracted images, analysed data and wrote the manuscript. T. Centrih helped collect some of the photographic data in the field and encouraged the formal investigation and publication of initial findings. A.J. Wright encouraged the formal investigation and publication of initial findings and helped design the matching trials. G.-M. Wu carried out data analysis of matching trials. All authors reviewed the manuscript.

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Although this is my PhD thesis, it is also not really ***my*** PhD thesis. As is probably typical for projects of this kind, this work is the result of collaborative efforts and the involvement and hard work of many people. Needless to say, this thesis would not be possible without them and I certainly cannot take all of the credit. I am truly grateful to all.

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Thanks to my collaborators and co-authors on some of the published chapters of this thesis. Processing of samples for pollutant analysis was carried out by Jon Barber at CEFAS, UK, while processing of samples for stable isotope analysis was carried out by Morgana Vighi at the University of Barcelona, Spain. Mick Wu performed the statistical analysis for the dolphin face matching trials. Many thanks also to Andrew Wright, Paul Jepson and Stefania Gaspari. Thank you Jeremy Kiszka, for teaching me how to biopsy dolphins.

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Thank you to my mother and father, for giving me the opportunities to pursue my dreams, and to my grandma for always believing (and reminding me) that I will become a Doctor.

Since I am submitting this at the very last second (is anyone surprised?) I have neither the time nor brain capacity left to include everyone who would likely also deserve to be mentioned. Please forgive me, but I hope you know that you are important to me, and why.

Finally, thank you Tina and Nika, my greatest treasure, and Wesley, you three have been my most important support system during this journey.

ABSTRACT

This thesis aims to explore a combination of distinct but interconnected aspects of dolphin population ecology, behaviour and interactions with human activities in the Gulf of Trieste and adjacent waters of the northern Adriatic Sea. Boat surveys, photo-identification techniques and biopsy sampling between 2003 and 2018 were used to investigate social structure, interactions with local fisheries, isotopic niche variation and levels of organochlorine contaminants, and estimate dolphin abundance. The population was found to be structured into distinct social clusters, two of which displayed marked differences in fisheries-related behaviour and temporal partitioning previously unknown for this species or marine mammals generally. Stable isotope analysis showed isotopic niche differences among social groups. Levels of polychlorinated biphenyls (PCBs) were high, with evidence of maternal offloading of these pollutants to offspring, but no differences among social groups. Abundance estimates suggest that the total abundance of bottlenose dolphins in the study area during 2013–2018 ranged between 161 (95% CI = 153–170) and 245 (95% CI = 219–273). Finally, a novel method for individually identifying dolphins by facial features is described, which can complement existing photo-identification techniques. Together, these results provide a reasonably holistic picture of the dolphin population inhabiting the Gulf of Trieste and provide insights into social, ecological and anthropogenic drivers of its population dynamics. This study extends the available knowledge on Adriatic dolphins and provides a baseline for further studies.

CHAPTER 1: GENERAL INTRODUCTION

1.1 Cetacean population ecology, behaviour and conservation

The ecology, behaviour and conservation status of cetaceans as marine top predators is of interest for a number of reasons. Mammals that live in water, an environment so drastically different to their terrestrial origin, and with anatomical features that are probably the most extremely modified from what might be considered a ‘typical’ mammalian form among all mammals, are inherently interesting in their own right. Essentially, they are mammals which resemble fish but are more related to humans, and they live in an environment that is largely alien to humans. They come in many forms and sizes, and exploit a range of habitats and ecological niches. There is no denying it: cetaceans are fascinating to study. But furthermore, and perhaps more importantly, there are other reasons why they are interesting and important species to study.

As animals living in often complex societies (Mann et al. 2000), having complex communication pathways (Tyack & Miller 2002, Janik et al. 2006, Garland et al. 2011, Quick & Janik 2012, Cholewiak et al. 2013, Janik & Sayigh 2013, King & Janik 2013), and with what is generally perceived as remarkable cognitive abilities, at least for odontocetes (Pack & Herman 1995, Reiss & Marino 2001), they are interesting model species to study the evolution of social complexity and cognitive abilities, which in turn may provide insight into our own (human) evolution of intelligence and social complexity.

Cetaceans and other marine mammals also play an important role in their ecosystems (Bowen 1997). They can be considered as sentinels of ecosystem health (Ross 2000, Wells et al. 2004, Moore 2008, Bengtson Nash et al. 2018), as well as marine ecosystem engineers (Roman et al. 2014). They impact their ecosystems not only through direct predation, but also via the effects of fear in their prey (Wirsing et al. 2008, Heithaus et al. 2012). Recent work has also shown that cetaceans play an important role in Earth’s carbon cycle and in sequestering carbon from the atmosphere (Lavery et al. 2010, Pershing et al. 2010, Lavery et al. 2012, Lavery et al. 2014), which has important implications for climate change mitigation.

Marine biodiversity is rapidly declining due to increased anthropogenic pressure (Worm et al. 2006). This includes numerous species and populations of cetaceans (Reeves et al. 2003). Cetaceans are directly impacted and threatened by a number of human activities, including incidental mortality in fishing gear (Read et al. 2006, Read 2008, Reeves et al. 2013, Brownell et al. 2019), ship and boat strikes (Wells & Scott 1997, Panigada et al. 2006, Douglas et al. 2008), underwater noise (Gordon et al. 2003, Buckstaff 2004, Nowacek et al. 2007, Southall et al. 2007, Weilgart 2007, Clark et al. 2009, Di Iorio & Clark 2009, Williams et al. 2020), direct disturbance (Bejder et al. 2006, Tyne et al. 2015, Tyne et al. 2017, Fumagalli et al. 2018, Fumagalli et al. 2019), chemical pollutants (Reijnders et al. 1999, Law et al. 2012, Jepson et al. 2016, Stuart-Smith & Jepson 2017, Desforges et al. 2018, Murphy et al. 2018), marine debris (Laist 1997, Jacobsen et al. 2010, Simmonds 2012, de Stephanis et al. 2013, Baulch & Perry 2014, Lambert et al. 2020) and climate change (MacLeod 2009, Lambert et al. 2010, Salvadeo et al. 2010, Scheinin et al. 2011). Understanding, documenting and predicting such impacts in an evidence-based framework is clearly important for effective conservation. Effective conservation requires good scientific knowledge of critical aspects of a species ecology. In turn, good science requires appropriate methods and analyses. Obtaining reliable scientific data should be one of the most fundamental parts of any effective conservation strategy.

Finally, cetaceans are popular and charismatic animals that have the ability to capture public attention and can be considered flagship species. While there has been a lot of controversy over whether long-lived mobile top predators are good surrogates for conservation of the habitats they live in, it has been shown that focusing conservation efforts on certain species can be effective if their protection leads to the conservation of wider biodiversity (Walpole & Leader-Williams 2002). Flagship species, for example, are charismatic species that can draw attention to conservation efforts because they appeal to the public and stimulate awareness and action (Caro et al. 2004, King & Beazley 2005). Umbrella species, on the other hand, are species which have such habitat requirements and live over such large areas that protecting them will automatically confer protection to several other species within their ecosystem (Simberloff 1998, Hooker & Gerber 2004). Umbrella species are often used to delineate boundaries of Marine Protected Areas (Caro et al. 2004). The efficacy of charismatic and highly mobile species as surrogates is highly species- and context-dependent, but at least in some settings, conservation based on charismatic top predators can have broader biodiversity benefits (Sergio et al. 2006). Cetaceans are often referred to as both flagship and umbrella species (Hooker & Gerber 2004, Notarbartolo di Sciara 2007), so an improved understanding and conservation of such species should, at least in theory, have wider conservation benefits.

This thesis aims to explore a combination of distinct but interconnected aspects of dolphin population ecology, behaviour and interactions with human activities, to gain new insights into the social and other determinants of within-population structuring, investigate how a coastal population of a marine top predator persists in a highly degraded ecosystem and copes with both natural and anthropogenic changes, and hopefully provide information relevant to the conservation of this species locally and regionally. In particular, some of the questions addressed in this thesis are: 1) How do social factors relate to potential behavioural and habitat use variability, and how might that relate to human activities? 2) What determines dolphin social structure and what may be the consequences of that? 3) How does social structure relate to dietary niche, and how much variability in niche can be attributed to social factors or individual variability? 4) How are social and dietary factors related to the susceptibility to anthropogenic influences and how might that impact the conservation status of a population? 5) Given a range of pressures, how many animals use an area and what can this mean for the population conservation? 6) How can our collective capability to individually identify cetaceans be improved and how can this improve their conservation and our overall understanding of their ecology.

1.2 The common bottlenose dolphin (*Tursiops truncatus*)

The common bottlenose dolphin (*Tursiops truncatus*, Montagu, 1821, hereafter “bottlenose dolphin”, Figure 1.1) is a cosmopolitan species distributed in tropical and temperate waters and likely one of the best (if not *the* best) studied cetaceans (Shane et al. 1986, Leatherwood & Reeves 1990, Pack & Herman 1995, Silber & Fertl 1995, Patterson et al. 1998, Reynolds et al. 2000, Reiss & Marino 2001, Parsons et al. 2003, Thayer et al. 2003, Wells et al. 2004, Natoli et al. 2005, Lusseau 2006b, Tezanos-Pinto et al. 2008, Torres et al. 2008, Bearzi et al. 2009, Wells & Scott 2009, Janik & Sayigh 2013, Wells et al. 2019, Moura et al. 2020). Three sub-species are currently recognised and include the Black Sea bottlenose dolphin (*T. t. ponticus*), the Lahille’s bottlenose dolphin (*T. t. gephyreus*) from coastal waters of the western South Atlantic Ocean, and the nominate subspecies (*T. t. truncatus*), widespread elsewhere (Committee on Taxonomy 2021). In the western North Atlantic two ecotypes, inshore and offshore, have been described, which differ in their mitochondrial and nuclear DNA haplotypes, blood profiles, parasite loads, diet and external morphology (Duffield et al. 1983, Hersh & Duffield 1990, Mead & Potter 1990, 1995, Hoelzel et al. 1998, Wells et al. 1999), with strong evidence to suggest they should be considered at least separate subspecies (Wells et al. 2019). In addition to the common bottlenose dolphin, the genus also includes a second species, the Indo-Pacific bottlenose dolphin (*Tursiops aduncus*). A third species (*Tursiops australis*) has been proposed (Charlton-Robb et al. 2011), but is not currently widely recognised by the scientific community due to limited evidence (Jedensjö et al. 2020, Moura et al. 2020, Committee on Taxonomy 2021). The global population is listed as Least Concern by the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (Wells et al. 2019).

Within the Mediterranean Sea, the bottlenose dolphin is the most widespread cetacean, occurring in all parts of the basin, but for the most part, it is essentially “coastal”, occurring primarily inshore or over the continental shelf (Bearzi et al. 2009, Notarbartolo di Sciara & Birkun 2010). It is largely found all along the Mediterranean Sea coast, including the Strait of Gibraltar and the Alboran Sea (Cañadas et al. 2005, Cañadas & Hammond 2006, De Stephanis et al. 2008), in the Balearic Sea and around the Balearic

Islands (Forcada et al. 2004, Gonzalvo et al. 2014), around Corsica and Sardinia (Lauriano et al. 2004, Díaz López & Shirai 2007, Lauriano et al. 2014), in the Ligurian Sea (Azzellino et al. 2008, Gnone et al. 2011, Lauriano et al. 2014), Tyrrhenian Seas (Blasi & Boitani 2014, Lauriano et al. 2014), around Sicily (Papale et al. 2017), around the Lampedusa Island (La Manna et al. 2010, Papale et al. 2012), in the Ionian Sea (Bearzi et al. 2005, Bearzi et al. 2011b, Bearzi et al. 2016, Gonzalvo et al. 2016), the Adriatic Sea (Bearzi et al. 2004, Fortuna et al. 2018), Aegean Sea (Frantzis et al. 2003, Bonizzoni et al. 2014), the Levantine Sea (Goffman et al. 2000, Scheinin et al. 2014), and off Tunisia (Ben Naceur et al. 2004, Benmessaoud et al. 2013). Most studied populations show a relatively high level of individual site fidelity (Bearzi et al. 1997, Díaz López & Shirai 2008, Genov et al. 2008, Gnone et al. 2011, Gonzalvo et al. 2014, Gonzalvo et al. 2016, Giménez et al. 2018), but "mid-distance" movements have been reported in various areas (Bearzi et al. 2011c, Gnone et al. 2011, Genov et al. 2016) and there is much less information on offshore animals. The Mediterranean subpopulation is currently listed as Vulnerable by the IUCN Red List (Bearzi et al. 2012), however a recent reassessment proposed the new listing as Least Concern (Natoli et al. in press).

Figure 1.1. Common bottlenose dolphins (*Tursiops truncatus*) from the northern Adriatic Sea, showing typical morphological characteristics of the species. Photographs: Tilen Genov, Morigenos.

1.3 The study area

The study area included the entire Gulf of Trieste, as well as waters adjacent to it. The total extent of the study area is shown in Figure 1.2. The Gulf of Trieste is part of the Adriatic Sea, which in turn is part of the Mediterranean Sea. The Gulf of Trieste is the northernmost part of both basins. The Adriatic Sea is composed of a generally shallow northern Adriatic (average depth ~35 m), a somewhat deeper (average ~ 120 m) central Adriatic, and a generally deep (up to about 1200 m) southern Adriatic (Artegiani et al. 1997a). The eastern shore is generally rocky and relatively steep, whereas the western shore is predominantly sandy and gently sloping. The water exchange with the rest of the Mediterranean Sea takes place through the Strait Otranto, which connects Adriatic Sea to Ionian Sea. The predominant Adriatic current moves in an anti-clockwise fashion, moving northward along the eastern shores and southward along the western shores of the basin, with various gyres in all three subregions, as well as local currents influenced by wind and tide (Artegiani et al. 1997b, Mauri & Poulain 2001, Crise et al. 2006). Tidal range in the Mediterranean Sea is generally considered minor, of the order of a few centimeters (Tsimplis et al. 1995), but the northern Adriatic Sea, including the Gulf of Trieste, features some of the largest tidal ranges in the Mediterranean, with extremes of up to 200 cm (Malačič et al. 2000b, Marcos et al. 2009). The Gulf of Trieste typically features relatively strong thermal stratification during summer and more profound mixing of the water column during winter, with various intermediate conditions during spring and autumn (Malej et al. 1995), although wind conditions may perturb the general stratification pattern also during summer (Crise et al. 2006). Sea surface temperature, which features the temperature extremes across the water column, ranges between 6° C in winter and 30° C in summer. General functional groups in the northern Adriatic Sea, including the Gulf of Trieste, include phytoplankton, micro-, meso- and microzooplankton, jellyfish, benthic invertebrates such as polychaetes, gastropods and bivalves, decapods, cephalopods, bentho-pelagic fishes, pelagic fishes, non-piscivorous and piscivorous demersal fishes, large predatory pelagic fishes, elasmobranchs, sea turtles, seabirds and bottlenose dolphins (Libralato et al. 2010). The entire northern Adriatic Sea has experienced substantial fishing pressure over at least the past 5 decades (Coll et al. 2007) and is one of the most intensively trawled areas of the world (Eigaard et al. 2016, Amoroso et al. 2018, Ferrà et al. 2018, Russo et al. 2019).

The Gulf of Trieste is relatively shallow (average depth ~20 m) and characterized by muddy, sandy or rocky substrates, as well as sea grass meadows (Lipej et al. 2000). Three main rivers (and several smaller tributaries) enter the Gulf, making the area subject to substantial freshwater input (Malačič & Petelin 2001). The Gulf includes two large international ports, Koper (Slovenia) and Trieste (Italy), both of which are among the key ports in the region, and a smaller industrial port, Monfalcone (Italy), which serves as one of the main construction sites for cruise ships. In addition to ports, there are several towns and villages around the shores of the Gulf, important for both fishing and tourism. The Gulf of Trieste is probably one of the most heavily human-impacted areas within the Adriatic and Mediterranean Seas.

The entire Gulf, and the northern Adriatic Sea in general, is under considerable pressure from human activities and threats, which include, but are not limited to, shipping (Ferraro et al. 2007, Perkovič et al. 2013), tourism and recreational boat traffic (Codarin et al. 2008, Genov et al. 2008, Picciulin et al. 2010), underwater noise (Picciulin et al. 2008, Codarin et al. 2009), intensive fishing (Pranovi et al. 2000, Casale et al. 2004, Coll et al. 2007, Genov et al. 2008), aquaculture (France & Mozetič 2006, Grego et al. 2009), non-indigenous species (David & Perkovič 2004, David et al. 2007, Lipej et al. 2012), urbanisation, sewage discharge and pollution (Horvat et al. 1999, Malačič et al. 2000a, Faganeli et al. 2003, Mozetič et al. 2008). Fishing in the area dates back at least a few centuries, with early records as far back as 1552, when Slovenian fishermen carried out traditional fishing for little tunny (*Euthynnus alletteratus*) using locally specific traditional boats and nets (Gaberc 1996, Republic of Slovenia 2011). After World War II and to date, common fishing techniques have included mid-water trawling, bottom trawling, purse seining, gill nets and trammel nets, as well as occasional set longlines, pots or traps (Republic of Slovenia 2011). Two fish farms and a number of mussel farms are also present in the area. Four extremely small Marine Protected Areas (MPAs) are present in the Gulf, half of which are without proper management or a management body (Turk & Odorico 2009). The largest of the four is the Strunjan MPA, part of Landscape park Strunjan, about 4 km long (alongshore) and 200 m wide.

The Gulf of Trieste is shared by three countries: Italy, Slovenia and Croatia. The latter two countries were both part of the former Yugoslavia and have had an ongoing border dispute since their independence in 1991, including the maritime border in Piran Bay and adjacent waters.

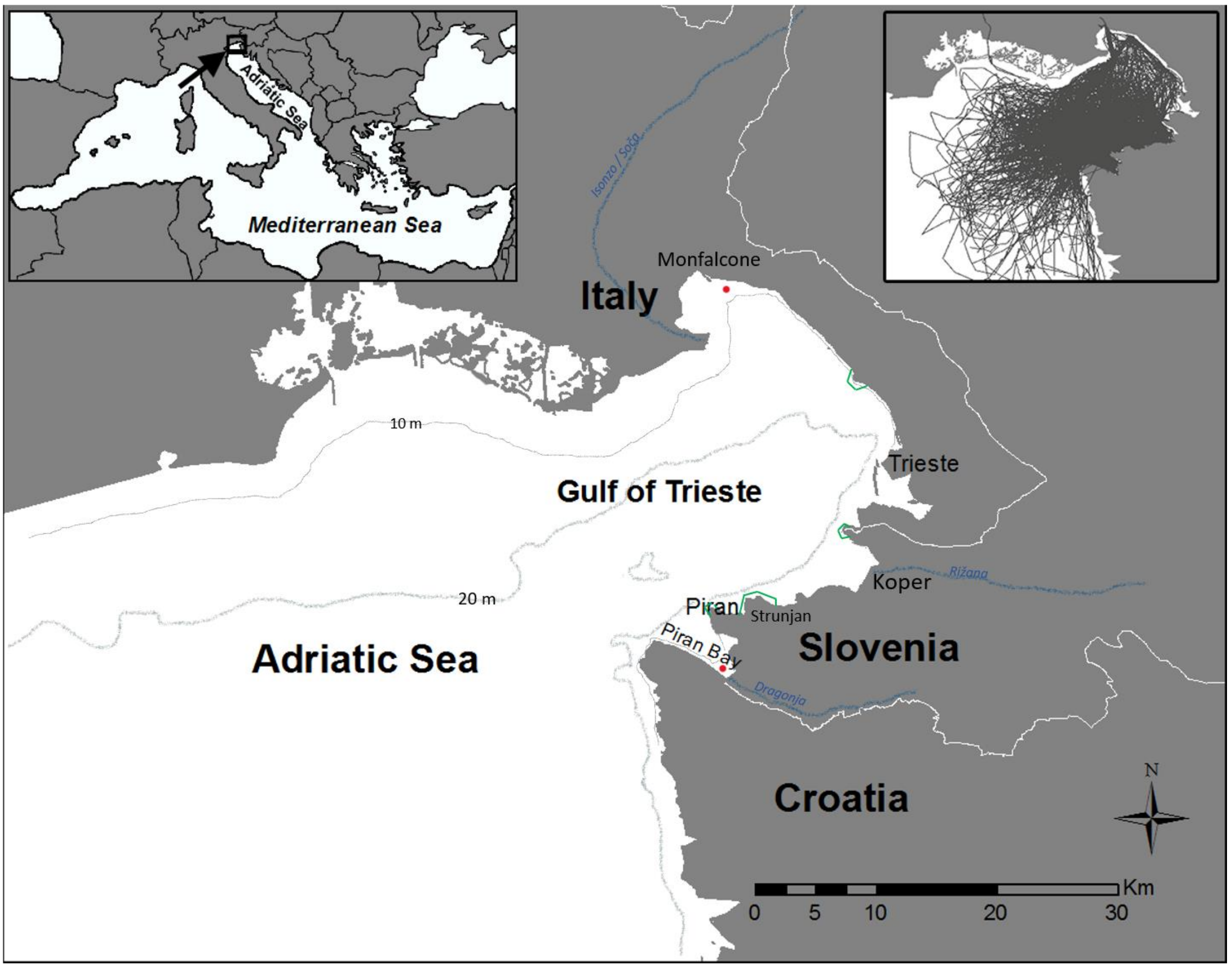


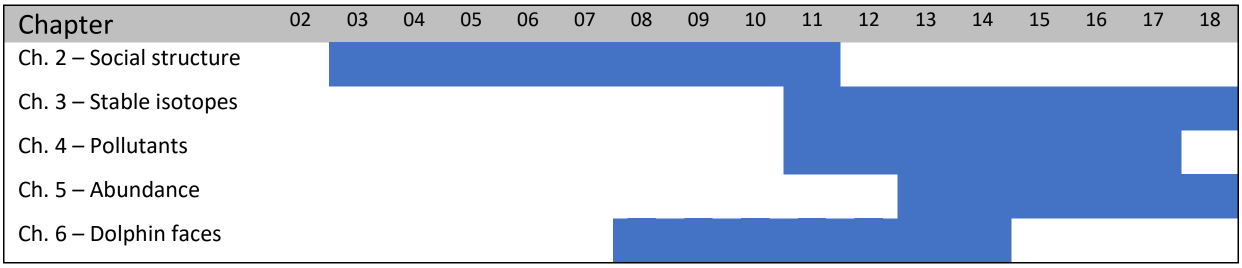
Figure 1.2. Study area in the northern Adriatic Sea, with some locations cited in the text. The upper left inset shows the location of the study area in the Adriatic Sea. The upper right inset shows the survey effort (navigation tracks). The approximate boundaries of local marine protected areas are shown in green. The two red dots depict the locations of the two fish farms. Three main rivers entering the Gulf of Trieste are shown, but smaller streams are omitted.

1.4 General field procedures

All research was carried out under the auspices of Morigenos – Slovenian Marine Mammal Society, a non-profit non-governmental organisation. Surveying consisted of both boat-based and land-based surveys (Genov et al. 2008). Whenever possible, these were carried out simultaneously by two teams, to maximise the likelihood of detecting and subsequently photographing dolphins. Survey conditions were considered favourable if Beaufort sea state was ≤ 2 and there was no heavy fog or precipitation. At least one experienced researcher was present during surveys of each team. The survey effort of each team was kept independent until a sighting was made. If dolphins were spotted from land, this was communicated to the boat team, which went off effort and headed to the location of the animals spotted by the land team. Boat-based surveys were predominantly carried out using rigid-hull inflatable boats (RIB) with outboard engines (from 2008 onwards the same 5.7m RIB with 60 HP outboard engine was used). A relatively constant search speed of 25–30 km/h was maintained during search, with at least one experienced observer present. Surveys did not follow pre-determined transects, but an attempt was made to cover the study areas as homogeneously as possible across a survey season. The spatial and temporal distribution of survey varied according to weather conditions, logistics and dolphin sightings. Information on time, position coordinates using Global Positioning System (GPS), Beaufort sea state, speed, effort status and the presence of any trawlers or bottom-set nets was recorded roughly every 15 min, or whenever the conditions or the direction of the boat changed. Survey data was recorded onto custom designed data sheets and onto a voice recorder (for boat surveys). Land-based surveys were predominantly undertaken from the cliff at St George’s church in Piran using binoculars, and, from 2013 onwards, from the bell tower of the same church using Big Eyes binoculars. During 2002–2012, surveys were conducted in all seasons, but were mostly concentrated in summer months (July–September). From 2013 onwards, surveys were conducted regularly year-round, but were again more concentrated during summer (June–September). Surveys could be carried out in different parts of the day and last from 2 hours to 10 hours, depending on weather conditions, time of year and various other considerations. Typically, especially in summer, surveys would commence around 08:00 in the morning, end around noon as the wind picked up, and resume around 16-17h in the afternoon, until nightfall.

When a dolphin group or an individual was found at sea, a focal group/individual follow commenced (Mann 1999, 2000). "Sighting" was defined as an uninterrupted continuous observation of a dolphin focal group. A dolphin focal group was considered any number of dolphins in visual range of the researchers, observed in apparent association, moving in the same direction or staying in the same area and often, but not always, engaged in the same activity (Genov et al. 2008). Dolphins were followed for variable periods of time, typically between 30 minutes and 2 hours, to allow photographing all individuals in the group. Although the time spent following dolphins could vary due to group size, behaviour and sea conditions, an attempt was made to keep it to a minimum necessary to obtain photographs, in order to minimise potential disturbance. Information on sighting position, time, group size, presence of offspring, initial and end behaviours, and interactions with fisheries or maritime traffic was recorded during each sighting. Respiration sampling and behavioural sampling were carried out during some sightings. Standard photo-identification procedures (Würsig & Jefferson 1990) were carried out during sightings, with an attempt to photograph both sides of dorsal fins of all members of a dolphin group. Photographs were taken using a single-lens reflex camera Nikon F80D equipped with Sigma 70–300 mm zoom lens and ISO 100 or 200 colour transparency films during 2002–2007 and using digital single-lens reflex cameras Canon EOS 30D, EOS 7D and EOS 7D mark II equipped with Canon L USM 70-200 mm f2.8 zoom lens from 2008 onwards. Group size was assessed in the field and later confirmed through photo-identification. Operating trawlers were often opportunistically approached and inspected for possible dolphin presence. From 2011 onwards, biopsy samples (Gorgone et al. 2008, Kiszka et al. 2010b, Noren & Mocklin 2012) were collected for genetic, toxicological, diet and other studies.

Although most methods and general protocols were kept consistent throughout the duration of this project, some of the approaches were refined, surveys became more temporally consistent and additional methodologies (such as biopsy sampling) were introduced. Therefore, due to the continuous and evolving nature of the project, different chapters of this thesis utilise data collected during different periods of time (Figure 1.3).



Chapter	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18
Ch. 2 – Social structure		■	■	■	■	■	■	■	■	■							
Ch. 3 – Stable isotopes										■	■	■	■	■	■	■	■
Ch. 4 – Pollutants										■	■	■	■	■	■	■	
Ch. 5 – Abundance												■	■	■	■	■	■
Ch. 6 – Dolphin faces							■	■	■	■	■	■	■				

Figure 1.3. Timeline of different data periods during 2002–2018 utilised in different chapters.

1.5 Bottlenose dolphins in the Gulf of Trieste and adjacent waters of the northern Adriatic Sea

The bottlenose dolphin is the only regularly occurring marine mammal species in the northern Adriatic Sea (Bearzi et al. 2004). A local population of bottlenose dolphins inhabits the Gulf of Trieste and adjacent waters of the northern Adriatic Sea year-round, where it has been the focus of continuous study and monitoring since 2002 by Morigenos – Slovenian Marine Mammal Society (Genov et al. 2008, Genov 2011). Early mark-recapture abundance estimates based on photo-identification data suggested that between 70 and 100 individuals used this area annually, and most of them carried natural marks suitable for long-term identification (Genov et al. 2008, Genov 2011). Recent year-round data suggested that the population may in fact be somewhat larger and this is explored in detail in Chapter 5. Individual resighting patterns show high site fidelity of dolphins to the area, with a number of animals consistently using the area over the past 18 years.

Group size typically ranges between 1 and 30 animals, but groups consisting of over 60 individuals can be encountered at times (Genov *et al.*, 2008; Morigenos, unpublished data). Immature animals, including newborns, calves and juveniles, are present in 54 % of the encountered groups. This, together with behavioural data, indicates that the animals use these waters for all aspects of their lives, including feeding, resting, socializing and breeding (Genov et al. 2008, Genov 2011). Interactions with fisheries are common (Genov et al. 2008, Kotnjek et al. 2013, Kotnjek 2016) and bycatch occurs occasionally (Hace et al. 2015). Some of the identified prey species of bottlenose dolphins in the area are also commercially targeted by local fisheries.

Dolphin numbers, distribution, habitat use and movement patterns are highly variable from year to year, and dolphins clearly range over an area much larger than the study currently covers. The population is transboundary, as identified individuals regularly use Italian waters, Slovenian waters, Croatian waters, and northernmost part of Adriatic international waters (Genov *et al.*, 2008; Genov 2011; Morigenos, unpublished data).

There is evidence that this local population represents a distinct population unit, rather than being a small part of a panmictic Adriatic population. This is supported by two lines of evidence. Firstly, comparison of photo-identification data with the Cres-Lošinj archipelago in Croatia, where the closest other studied dolphins population resides (Bearzi et al. 1997, Fortuna 2006, Pleslić et al. 2015), suggests that the two local populations do not mix and that they should be considered separate management units (Genov et al. 2009). Secondly, the presence of several local populations and potential management units in the Adriatic Sea is also supported by genetic evidence, with the animals from the Gulf of Trieste forming a distinct local population (Gaspari et al. 2015).

1.6 Legal framework

The bottlenose dolphin is protected in all three countries bordering the northern Adriatic Sea (Italy, Slovenia and Croatia) and by a number of supra-national pieces of legislation. These include international conventions (e.g. Convention on Biological Diversity, Convention on the Conservation of Migratory Species of Wild Animals, the Barcelona Convention - Convention for the Protection of the Marine Environment and the Coastal Region of the Mediterranean, ACCOBAMS - Agreement on the Conservation of Cetaceans of the Black Sea, Mediterranean Sea and Contiguous Atlantic Area, CITES - Convention on International Trade in Endangered Species of Wild Fauna and Flora) and EU legislation such as the Habitats Directive (Council Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora) and the Marine Strategy Framework Directive (MSFD, Directive 2008/56/EC). Ensuring a good conservation status of the species and its habitat is mandated by all of these legislative mechanisms.

1.7 Social structure

Complex social structure is a prominent feature in many mammals, including cetaceans (Mann et al. 2000). It plays an important role in population dynamics and behavioural patterns in these species and may lead to behavioural diversity among individuals and populations (Mann & Sargeant 2003, Cantor & Whitehead 2013). The social structure of bottlenose dolphins has been especially well studied in some areas (Wells et al. 1987, Lusseau et al. 2003, Wells 2003, Lusseau 2006a), but patterns cannot necessarily be generalised to the species globally, especially with many populations remaining poorly studied. Animal social structure can be investigated through social network analysis (Wey et al. 2008, Krause et al. 2009a) and can provide insights into behavioural ecology of different populations. Further background is provided in Chapter 2, which investigates the social structure of the local dolphin population.

1.8 Stable isotopes to investigate diet and ecological niches

Understanding the diet of a species is one of key aspects to understanding its ecology and role in the ecosystem (Bowen et al. 2002). A number of methods are available to study diet in cetaceans, including stomach content analysis, direct observations, analysis of faecal samples and the use of stable isotope signatures (Tollit et al. 2010). Isotopic ratios of certain chemical elements such as carbon and nitrogen reflect the ratios founds in their prey (Hobson & Clark 1992, Hobson 1999, Bearhop et al. 2002), which allows the assessment of the ecological niche width, trophic level and general diet of the consumer to be estimated (Newsome et al. 2007, Tollit et al. 2010, Hopkins & Ferguson 2012, Phillips 2012, Phillips et al. 2014). This approach can be used to study the foraging ecology and trophic relationships of marine mammals (Newsome et al. 2010) and can be very useful when trying to determine potential differences between marine mammal species (Kiszka et al. 2010a, Kiszka et al. 2011) or populations within species (Vighi et al. 2014, Vighi et al. 2016). A population's niche has been found to be composed of a combination of individual niches (Bolnick et al. 2003, Araújo et al. 2011, Bolnick et al. 2011), so this variability can be explored. Further background is provided in Chapter 3, where the isotopic niche variation in this population is investigated.

1.9 The effects of chemical pollutants on cetaceans

In modern times, human activities have introduced over 200,000 synthetic chemicals into the environment and have profoundly altered the levels of naturally occurring elements (Reijnders et al. 1999). These chemicals may enter the environment through air, water and soil. Many are not easily degradable and have been shown to have substantial impacts on various species and ecosystems, including cetaceans. Persistent organic pollutants (POPs) are one example of such chemical compounds, and are also present in the marine environments. They have been used as insecticides for agriculture enhancement and malaria eradication, and for various industrial purposes, including dielectric fluids in transformers and capacitors (Tanabe 2002). Aquatic organisms acquire these pollutants from water, sediment and through prey (Green & Larson 2016). Marine top predators, including marine mammals, primarily acquire them via diet and are known to bioaccumulate POPs, which represent conservation and health concerns for these species and their environment (Tanabe et al. 1994, Aguilar et al. 2002, Vos et al. 2003, Jepson & Law 2016). They are persistent in the environment, lipophilic, bioaccumulate in individuals over time, and biomagnify in marine top predators through trophic transfer (Green & Larson 2016). Their effects can be direct or indirect, and can be manifested at the molecular, individual or community level (Reijnders et al. 1999). They may cause anaemia (Schwacke et al. 2012), immune system suppression (Tanabe et al., 1994) and the subsequent increased vulnerability to infectious disease (Aguilar & Borrell 1994a, Jepson et al. 2005, Randhawa et al. 2015), endocrine disruption (Tanabe et al. 1994, Vos et al. 2003, Schwacke et al. 2012), reproductive impairment (Schwacke et al.

2002) and developmental abnormalities (Tanabe et al. 1994, Vos et al. 2003). These compounds can also directly impact abundance via reduced reproduction or survival (Hall et al. 2006, Hall et al. 2017), with potentially dire consequences (Desforges et al. 2018). Due to their effects on health, survival and reproduction, and because marine mammals reproduce relatively slowly, POPs have the potential to present a serious threat to these animals. Linking organochlorine concentrations with individual-level effects and particularly population-level effects is extremely challenging. Given that predators obtain these compounds from their prey, potential dietary differences among individuals, social groups or populations may lead to differences in contaminant loads. This is further discussed and explored in Chapter 4.

1.10 Abundance to inform conservation

Abundance is one of the key parameters in assessing the conservation status of animal populations (Schipper et al. 2008). Information on abundance is also important for understanding the roles of various species in their ecosystem (Heithaus et al. 2012). Obtaining reliable and unbiased estimates of abundance is typically the initial and crucial step in any effective conservation strategy (Williams et al. 2002), but can often be a difficult task. A number of methods are available for estimating abundance of marine mammals (Hammond et al. 2021), with two most commonly used for cetaceans being line-transect distance sampling and mark-recapture (Hammond 1986, Borchers et al. 2002, Thomas et al. 2002, Evans & Hammond 2004, Hammond 2010). The choice of the methods (as well as models within those methods) depend on the species of interest, the characteristics and size of the study area, as well as logistical and funding considerations. Whatever method is used, it comes with a set of assumptions, violations of which may render the resulting estimates unreliable and potentially useless (Hammond 2010). Mark-recapture techniques applied to photo-identification data are often used for small cetaceans, particularly in relatively small and well-defined areas (Wilson et al. 1999b, Read et al. 2003, Fortuna 2006, Currey et al. 2007, Bearzi et al. 2008a, Silva et al. 2009, Verborgh et al. 2009, Bearzi et al. 2011a, Cantor et al. 2012, Mansur et al. 2012, Cheney et al. 2013, Arso Civil et al. 2019b). The data collected for this purpose can also be used to study other aspects of cetacean biology, ecology and conservations status, including residency patterns, survival, reproduction, movements, social structure and epidermal disease (Whitehead 1995, Bearzi et al. 1997, Wilson et al. 1999a, Haase & Schneider 2001, Ramp et al. 2006, Stevick et al. 2011). Detailed discussion of approaches and the implications of not accounting for the assumptions of the methods is provided in Chapter 5, which addresses abundance estimates of the local dolphin population.

1.11 Cetacean identification methods

Several species of cetacean can be individually identified using natural markings (Hammond et al. 1990), which has been instrumental in our global understanding of their numbers, social structure and movements, among other things (Hammond et al. 1990, Baird 2000, Whitehead & Weilgart 2000, Hammond 2009, Barlow et al. 2011, Connor et al. 2011, Stevick et al. 2011). Individual identification is relevant to both biological and conservation-oriented questions. Scarring, notches, pigmentation, callosity patterns and other long-term natural markings have been used for this purpose. However, given the utility of cetacean identification methods, there is still room to improve them (e.g., Auger-Méthé et al. 2010), especially as technology continues to advance. Chapter 6 explores this, using bottlenose dolphins from the Gulf of Trieste as a case study.

1.12 Thesis overview

In this thesis, I focus on various aspects of dolphin population ecology, behaviour and conservation status, all of which are interconnected. In Chapter 2, I investigate the social structure of the local dolphin population, through social network analysis. Given the results from Chapter 2, Chapter 3 then explores some of the potential drivers (or consequences) of the observed patters in social structure. Chapter 4 focuses on assessing the presence and potential effects of chemical pollutants, which is relevant to conservation but also to issues explored in Chapters 2 and 3. Chapter 5 investigates the population abundance and demographic processes related to it, to inform conservation and enable future monitoring. Chapter 6 extends some of the methodology described in Chapter 5, by describing a novel method of delphinid photo-identification, which may complement existing approaches. Finally, Chapter 7 rounds up the thesis by placing the findings from various chapters into a wider context.

1.13 Ethical statement

The research described in this thesis was approved by the Slovenian Environmental Agency via permit 35601-102/2010-4, as well as by the School of Biology Ethics Committee, University of St Andrews (approval SEC20020).

CHAPTER 2: BEHAVIOURAL AND TEMPORAL PARTITIONING OF DOLPHIN SOCIAL GROUPS IN THE NORTHERN ADRIATIC SEA[1]

2.1 INTRODUCTION

Complex social structure is a prominent feature in many mammals including primates (Chapman & Rothman 2009), elephants (Wittemyer et al. 2005), hyaenas (Smith et al. 2008), bats (Popa-Lisseanu et al. 2008) and cetaceans (Mann et al. 2000), and plays an important role in population dynamics and behavioural patterns. It governs the way the spread or containment of behaviours is facilitated, e.g. through social learning (Heyes 1994, Laland 2004). This may lead to behavioural diversity not only among, but within populations, where different population segments exhibit different behaviours (Mann & Sargeant 2003, Cantor & Whitehead 2013). Understanding this is not only interesting biologically, but may help conservation efforts (Whitehead 2010), because not all population segments necessarily respond to, or interact with, human activities the same way, or at the same time. There is concern over the effects of anthropogenic disturbance to populations, yet it is difficult to assess population-level impacts without understanding what proportion of animals may be affected.

Bottlenose dolphins (*Tursiops* sp.) are well-studied social mammals (Wells et al. 1987, Smolker et al. 1992, Lusseau et al. 2003, Connor et al. 2006, Lusseau 2006a). Most information on their social structure comes from studies in Sarasota, Florida (Wells et al. 1987, Wells 2003) and Shark Bay, Australia (Connor et al. 1999, Mann et al. 2000), but many populations remain poorly studied. They are generally described as fission-fusion species, where group composition changes frequently (Connor et al. 2000), but arguably their social structure varies considerably among populations. For example, dolphins in Florida

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[1] The results of this chapter have been published in:
Genov T., Centrih T., Kotnjek P., Hace A. 2019. Behavioural and temporal partitioning of dolphin social groups in the northern Adriatic Sea. *Marine Biology* 166: 11. https://doi.org/10.1007/s00227-018-3450-8

appear to feature marked sex-age segregation, where males form paired alliances, females form bands and nursery groups, and juveniles form smaller groups (Wells et al. 1987). In Shark Bay, males form hierarchical alliances (Connor et al. 1999, 2011, Randić et al. 2012). At the other end of the spectrum, dolphins in Doubtful Sound, New Zealand, form mixed-sex groups with strong associations not only within, but also between sexes (Lusseau et al. 2003). This shows that patterns cannot be generalised and that our understanding of bottlenose dolphin social structure remains incomplete.

Social network analysis allows groups of social animals to be studied as a network of nodes and ties (Wey et al. 2008, Krause et al. 2009a). When coupled with information on behaviour and interactions with human activities, it is a powerful tool in the study and conservation of social animals. Common bottlenose dolphins (*T. truncatus*) inhabit the Gulf of Trieste and adjacent areas of northern Adriatic Sea, where they have been studied since 2002 (Genov et al. 2008, Genov 2011). This chapter examines the social structure of local dolphins, and assesses whether different population segments show differences in behaviour and interactions with human activities.

2.2 METHODS

2.2.1 Data collection

Data were collected between February 2003 and September 2011 in the Gulf of Trieste and adjacent waters, northern Adriatic Sea (Fig. 2.1). Based on early mark-recapture abundance estimates, about 40-100 dolphins used this area annually, the majority carrying natural marks suitable for long-term identification (Genov et al. 2008, Genov 2011). The study area is described in Chapter 1. Boat surveys, using small rigid-hull inflatable boats (RIB) were complemented with land-based surveys to maximize the probability of encountering and photographically capturing dolphins. Survey coverage varied among years, due to weather, dolphin distribution and logistical constraints (Table 2.1). Each year an attempt was made to survey the entire area as homogeneously as possible. Surveys were done predominantly during summer (July-September), but periodically also in other months. Due to typical summer weather, surveys were commonly done in the morning and early afternoon, ceased in the early afternoon due to wind, and resumed in late afternoon. The southern portion of the Gulf of Trieste, including waters along the Slovenian coast and Piran Bay, was surveyed consistently over the years, and received more coverage than the outer edges of the study area (Fig. 2.1), due to the location of the home port and the land-based observation point. This sub-area, encompassing a roughly 5km radius around the Piran peninsula, was regularly surveyed by both boat-based and land-based surveys, and can be considered 'core study area' for the purposes of some of the results presented later on.

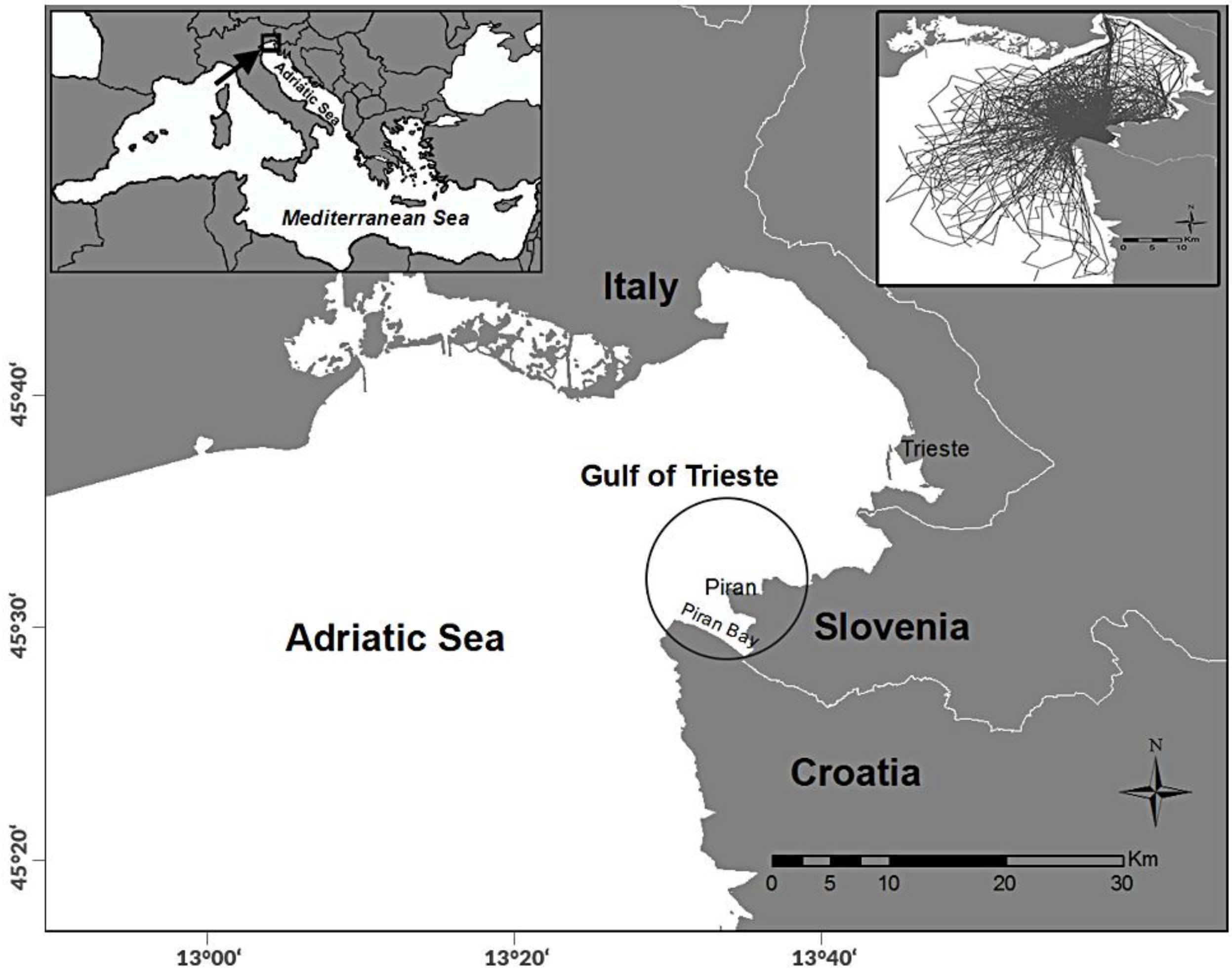


Figure 2.1. Study area in the northern Adriatic Sea, with locations cited in the text. The upper left inset shows the location of the study area in the Adriatic Sea. The upper right inset shows the spatial distribution of boat survey effort (navigation tracks). The circle depicts the 'core study area' where effort was most intense and included both boat-based and land-based surveys (see main text for details).

Photographs of dorsal fins were obtained during focal follows and allowed individual identification (Würsig & Jefferson 1990). Members of a dolphin group were considered associated. Group was defined as dolphins observed behaving in a generally coordinated fashion (moving in the same direction or staying in the same area, usually engaged in the same general activity). In practice this meant that group members were always within about 100m from the nearest other dolphin. Field observations and photo-identification showed that group composition rarely changed during several hours of observation (Genov et al. 2008).

Sex of individuals was determined by any of the following: a) observations of temporally stable adult-calf associations (adults consistently accompanied by calves were assumed to be mothers and therefore females); b) photographs of the genital area during bowriding or aerial behaviour and c) molecular methods from biopsy samples, analysed for genetic studies (Gaspari et al. 2015).

Skin and blubber samples were obtained using a 68 kg draw weight crossbow, using custom made bolts and stainless steel sampling tips with length of 25 mm and internal diameter of 7 mm. Tips were sterilised using 96% ethanol and burning prior to being used. Dolphins were sampled in the dorso-lateral area below the dorsal fin, at distances of 4–10 m. All biopsy attempts were accompanied by concurrent photo-identification. Sampling was only attempted on adults without accompanying offspring. Skin samples were removed and excised with sterilised forceps and surgical scissors, placed in 96% ethanol and stored at −20°C until analysis. Biopsy sampling was conducted under the permit 35601-102/2010-4 issued by the Slovenian Environmental Agency.

Numerous trawlers operated in the area year-round. They could be divided into a) single bottom trawlers and b) pelagic/mid-water pair trawlers. Bottom trawlers were typically 9–15m long, operated alone and trawled nets on the seabed, targeting several demersal species. Pair trawlers were typically 30m long, operated in pairs and trawled nets in mid-water. They mostly targeted European anchovies (*Engraulis encrassicolus*) and sardines (*Sardina pilchardus*). Dolphins interacted with both trawler types (Genov et al. 2008, Kotnjek 2016). Interaction was defined as dolphins following operating trawlers, approximately 200–400m from stern (closer for bottom trawlers and further for pair trawlers, but the exact distance could vary), and typically alternating long dives (>1min) with sequences of short dives (5–30s).

2.2.2 Data restrictions

Only high-quality photographs fulfilling the following criteria were used in analysis: 1) dolphin dorsal fin height taking up >7% of the photographic frame height, 2) sharp photograph, 3) dorsal fin perpendicular to a line between the camera and the fin, 4) dorsal fin unobstructed by another dolphin, water spray or water surface. Any photographs not matching these criteria were excluded from further analyses. Analysis only included individuals with long-term markings visible from both sides of the fin (e.g., nicks, notches). For the time period considered, there were 123 such individuals. However, as individuals with low encounter rates can introduce biases (Chilvers & Corkeron 2002, Whitehead 2008b), only those encountered on ≥ 4 occasions *and* ≥ 2 different years considered. This restricted the analysis to animals with some meaningful level of site fidelity and removed transient individuals, to ensure an accurate representation of the social network. Although most authors limit analyses to animals with some arbitrary number of *total* sightings (Quintana-Rizzo & Wells 2001, Chilvers & Corkeron 2002, Pace et al. 2012), it was decided to further limit this to animals encountered in more than one year. This was because several animals seen multiple times were only seen in a single year and therefore considered visitors/transients. The restriction criteria resulted in 38 individuals used in the analysis (13 females, 12 males and 13 individuals of unknown sex). This subset represents regular individuals ('residents') and was considered representative for this local population for the time period of the study. Thirty two animals (84.2 %) were seen ≥ 5 times and 18 (47.4 %) ≥ 10 times. Mean number of sightings per individual was 14 (SD = 11.3, range = 4–41). Multiple encounters during the same day were only included if they were of different groups (multiple encounters of the same group in the same day were rare, but could occur if, for example, the research team left a group, continued surveying and then encountered the same group while returning to port). Mother-dependent calves were excluded due to non-independence.

Table 2.1. Survey effort between 2003 and 2011, showing boat effort (in km surveyed) and land effort (in hours and minutes surveyed), number of groups observed and number of individuals identified. Numbers in parentheses show the breakdown of survey effort into morning and afternoon, respectively. (Note: Nr. groups observed and individuals identified only refers to the already restricted dataset of individuals included in social network analysis, not all identified dolphins - see Methods).

Year	**Survey effort**		**Nr. groups observed**	**Nr. individuals identified**
	Boat	**Land**		
	km (morning / afternoon)	hours + minutes (morning / afternoon)		
2003	na	44h 47min (26h 2min / 17h 45min)	4	8
2004	na	52h 28min (35h 59min / 16h 29min)	5	13
2005	261 (174 / 87)	33h 23min (20h 50min / 12h 33min)	15	21
2006	219 (153 / 66)	44h 17min (23h 12min / 21h 5min)	14	20
2007	256 (170 / 86)	56h 36min (41h 6min / 15h 30min)	7	21
2008	502 (306 / 196)	65h 37 min (43h 25min / 22h 12min)	18	32
2009	641 (408 / 233)	88h 39 min (55h 52min / 32h 47min)	14	31
2010	607 (358 / 249)	142h 20 min (89h 15min / 53h 5min)	27	19
2011	600 (361 / 239)	148h 25 min (97h 48min / 50h 37min)	11	22
TOTAL	**3086**	**675 h 32 min**	**115**	**38**

2.2.3 Testing association patterns and network analysis

To minimize bias and facilitate comparisons, the half-weight association index (HWI) was used (Cairns & Schwager 1987). Although an attempt was made to photograph all members of each group, this was not always possible, and the HWI accounts for this. It was recently suggested that a new index accounting for gregariousness (HWIG) may be more suitable (Godde et al. 2013). Data on associations were also analysed using HWIG, but this made little difference in results. Therefore, and to facilitate comparisons with previous studies, only HWI results are presented.

Analyses were performed in program SOCPROG 2.4 (Whitehead 2009). To test whether dyads (pairs of individuals) associated more often than expected by chance, the Manly-Bejder permutation technique (Manly 1995, Bejder et al. 1998) with extensions (Whitehead 1999, Whitehead et al. 2005) and corrections (Krause et al. 2009b) was used. Day was used as a sampling period. A total of 20,000 permutations (associations within samples) were generated to ensure stability of *P*-values. Another round of permutations was also performed, with sampling period of 5 days, because permutations are often impossible (or perform poorly) with too few associations within a period.

Standardised lagged association rates (SLAR, Fig. 2.3) were used to estimate the probability of dyads associated at a given time still being associated after a time lag, and assess the stability of associations (Whitehead 1995). Precision (SE) was estimated by jack-knifing on each sampling period (Whitehead 2008a). To test for preferred/avoided associations, SLAR was compared to null association rate, which represents expected values for random associations (Whitehead 1995). A moving average enabled the optimal adjustment between precision and smoothing. Exponential models of social organisation developed by Whitehead (1995) were fitted to SLAR. Model selection was based on minimising the Quasi-Akaike Information Criterion (QAIC) (Burnham & Anderson 2002).

Social differentiation (*S*) was calculated to evaluate the level of variation in dyadic probability of association, i.e. how differentiated the network was (Whitehead 2008a). The correlation between true and estimated association indices (*r*) was calculated to evaluate if data accurately represented the true social network (Whitehead 2008b). To investigate existence of clusters and delineate units within the network, modularity analyses (Newman 2004) were carried out by applying the eigenvector method of Newman (2006), the knot-diagram analyses and the modularity-G (Whitehead 2008a). With this approach the animals were assigned to clusters so that the separation between clusters was maximised (Whitehead 2008a). To evaluate if association rates were similar within/between clusters, and within/between sexes, mean association rates were compared via a two-tailed Mantel test.

To facilitate comparisons with other studies, several network metrics were calculated – HWI, Affinity, Betweenness, Closeness, Clustering coefficient, Eigenvector centrality, Reach and Strength (Wey et al. 2008, Whitehead 2008a) – for the entire network, individual clusters and for individuals (Table 2.2). These are measures of how well-connected and central individuals are (Whitehead 2008a).

A social network diagram (Fig. 2.4) was created using NetDraw 2.123 (Borgatti 2002) to illustrate relationships and network structure. Nodes with highest associations are grouped together, while those with fewer links remain on the periphery. Two diagrams were created, one with all associations, regardless of strength (Fig. 2.4a), and one displaying only those with HWI greater than twice the overall mean (Fig. 2.4b), believed to represent meaningful associations (Durrell et al. 2004, Gero et al. 2005, Wiszniewski et al. 2012).

Social structure was also represented with hierarchical average linkage cluster analysis (dendrogram, Fig. 2.2). Since dendrograms can be over-interpreted, especially if the society is not hierarchically arranged, the strength of cophenetic correlation coefficient (CCC) was used to indicate whether the data interpretation was valid (Whitehead 2008a).

2.2.4 Sex composition

To further examine potential sex segregation, sightings involving at least two known-sex animals were selected (including sightings with no or single known-sex individual would introduce a bias in estimating sex composition). In this sub-sample, the proportion of male-only, female-only and mixed-sex groups was determined.

2.3 RESULTS

2.3.1 Association patterns

In total, 132 dolphin groups were photographed, but the restriction criteria resulted in 115 encounters of 38 individuals included in the analysis. All individuals were observed in the core study area, but could also be encountered elsewhere.

Network metrics are shown in Table 2.2. The correlation between true and estimated association indices (r ± SE = 0.840 ± 0.040, based on bootstrap with 10,000 replications) suggests that the data accurately describe the true social network (Whitehead 2008b). P-values stabilised after about 9000 permutations. Standard deviation of the calculated (observed) associations was significantly higher than that of permuted data (observed SD = 0.236, random SD = 0.228, $P < 0.001$), as was the CV (observed CV = 1.259, random CV = 1.226, $P < 0.001$), indicating that associations were non-random (Gowans et al., 2001; Lusseau et al., 2003). Figure 2.2 shows that most dolphins had preferred associates, with one pair (NUI-TEA) always recorded together. The CCC of 0.96 suggests a good fit and thus a good representation of true social structure (Whitehead 2008a). The SLAR was best described by the so-called 'constant companions and casual acquaintances' model (Fig. 2.3, Table 2.3). SLAR line never reached the null association rate, indicating the absence of random associations, and a high probability of dyads associated even after a prolonged time lag.

Table 2.2. Social network metrics (mean ± SD) of individual social clusters. HWI: half-weight association index, n: number of animals.

Metric	**Cluster A (n = 19)**	**Cluster B (n = 13)**	**Cluster C (n = 6)**	**Overall (n = 38)**
Mean HWI	0.21 ± 0.03	0.19 ± 0.03	0.06 ± 0.03	0.18 ± 0.06
Affinity	7.74 ± 0.14	7.04 ± 0.12	4.21 ± 0.86	6.94 ± 1.29
Betweenness	13.3 ± 16.6	5.04 ± 7.9	8.08 ± 8.46	9.68 ± 13.38
Closeness	52.37 ± 5.36	58.38 ± 4.93	64.67 ± 8.79	56.37 ± 7.28
Clustering coefficient	0.39 ± 0.06	0.45 ± 0.04	0.23 ± 0.03	0.39 ± 0.09
Eigenvector centrality	0.21 ± 0.04	0.09 ± 0.01	0.02 ± 0.01	0.14 ± 0.08
Reach	59.26 ± 9.69	48.82 ± 6.01	9.13 ± 5.25	47.77 ± 19.27
Strength	7.66 ± 1.27	6.95 ± 0.93	2.17 ± 0.96	6.55 ± 2.23

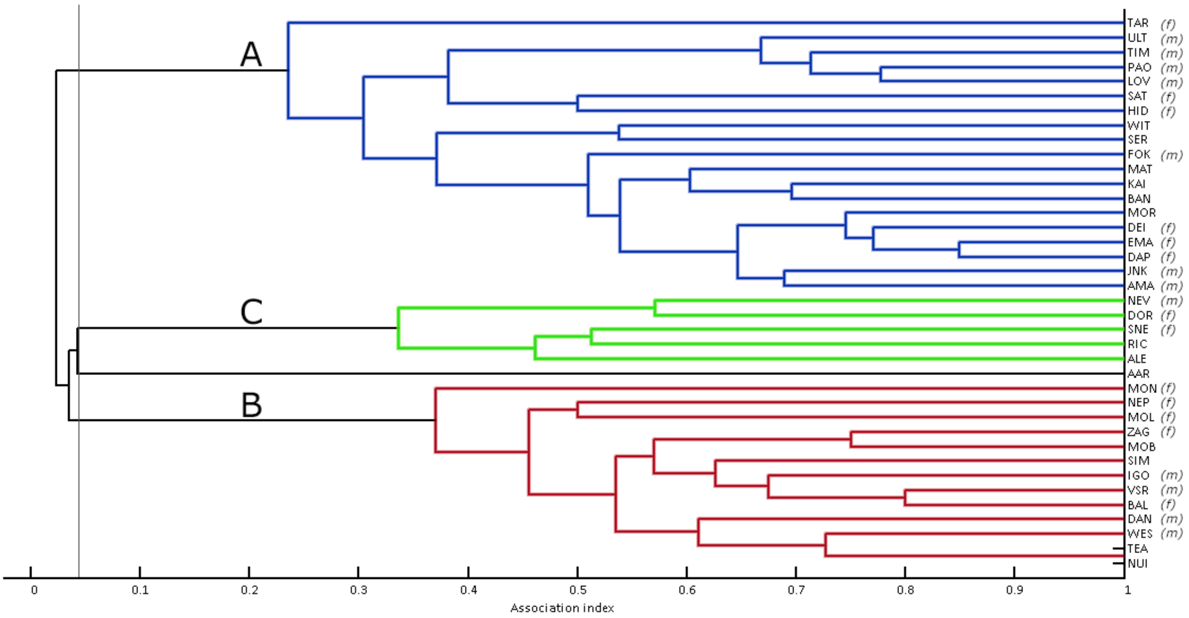


Figure 2.2. Dendrogram produced using average-linkage hierarchical cluster analysis (CCC = 0.96) for 38 common bottlenose dolphins. The clusters A, B and C represent clusters of animals based on modularity analyses with the eigenvector method of Newman (2006). The modularity-G of 0.464 suggests that the best division into clusters is with an association index of 0.043 (thin vertical line). Note that the dolphin AAR is not included in any cluster.

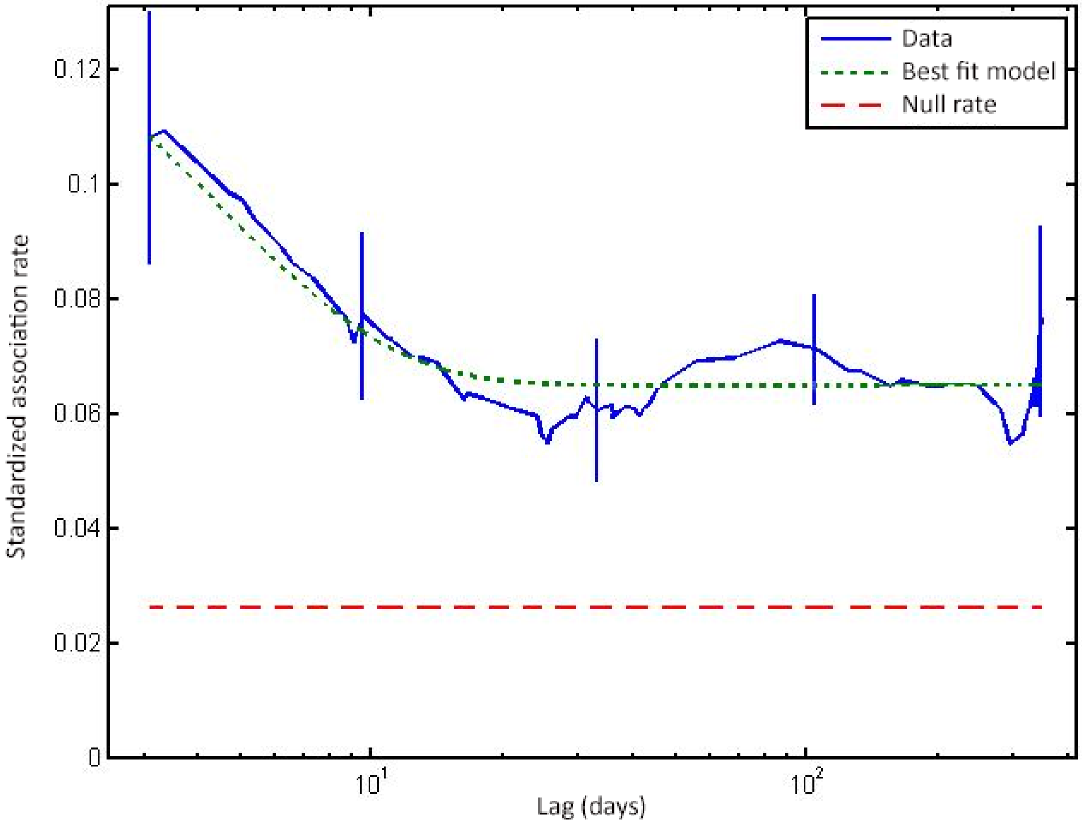


Figure 2.3. Standardized lagged association rate (SLAR) for 38 common bottlenose dolphins. A moving average of 8000 associations was used. Vertical bars indicate standard errors calculated using the temporal jackknife method on each sampling period. The best fit model (dotted line) indicates a social system model of 'constant companions and casual acquaintances'. The null association rate (dashed line) represents the theoretical SLAR if individuals associated randomly.

Table 2.3: Fit of social system models to the standardised lagged association rate (SLAR). τ represents time lag in days. CC – Constant companions, CA – Casual acquaintances. The lowest Quasi-Akaike Information Criterion (QAIC) indicates the best-fitting model, and ΔQAIC (difference between QAIC and that of the best model) indicates the degree of support for the other models.

Model	Formula	Number of parameters	QAIC	ΔQAIC
CC	0.068	1	48,999.91	83.13
CA	$0.069552e^{-0.00002585\tau}$	2	48,999.97	83.19
CC + CA	$0.066285 + 0.091054e^{-0.25144\tau}$	3	48,916.78	0
Two levels of CA	$0.24804e^{-0.85368\tau} + 0.066852e^{0.000003792\tau}$	4	48,943.40	26.63

2.3.2 Division of social network

Although the network was fluid overall, social differentiation estimate using likelihood method (S = 1.076, SE = 0.025) indicates a well-differentiated society (Whitehead 2008a). Average linkage cluster analysis (Fig. 2.2) and network analysis (Fig. 2.4) both showed a clear division into three distinct clusters, with one individual (AAR) not fully belonging in any. Modularity analysis assigned individuals to clusters with significantly higher associations within than between clusters (two-tailed Mantel test: t = 21.25, P = 1.0). Modularity-G division (peak at 0.464) suggests that the best division is with an association index of 0.043. The modularity-G peak suggests that with this division, there is much more total association within clusters than would be expected for randomly determined clusters. Since modularity values > 0.3 suggest a meaningful division (Newman 2004), the value of 0.464 provides compelling evidence of a structured network.

Dolphins formed two main clusters, A (19 individuals) and B (13 individuals), with strong associations, and a smaller cluster C (6 individuals) with much weaker associations (Table 2.2). Mean HWIs were similar between A and B, and lower in cluster C (Table 2.2). Dolphins were predominantly found with other members of the same cluster, although group sizes varied. Cluster A and B dolphins were usually seen in large groups (>10 and up to 45 individuals). Cluster A dolphins rarely associated with those from cluster B (4 out of 115 encounters, or 3.5%). These interactions never involved the majority of both clusters. Instead, while one cluster (either A or B) featured the majority of animals in the group, the other cluster was represented by few individuals (1–4).

Cluster C contained individuals that occasionally interacted with clusters A and B, but were typically seen with other cluster C animals, on their own, or with transient dolphins. They were predominantly found in small groups (2–3) or alone. They had no particularly strong bonds with anyone. Individual AAR did not fit into any cluster well (Fig. 2.2), but was placed in cluster C based on modularity, extremely low mean HWI of 0.01, and an eigenvector value close to zero, and other network metrics.

Of 115 encounters, 55 (47.8 %) included only cluster A animals, 10 (8.7%) included only cluster B animals and 37 (32.2%) included only cluster C animals. Three encounters (2.6%) included a mix of clusters A and B, 6 (5.2%) of clusters A and C, 3 (2.6%) of clusters B and C and 1 (0.9%) of all three clusters.

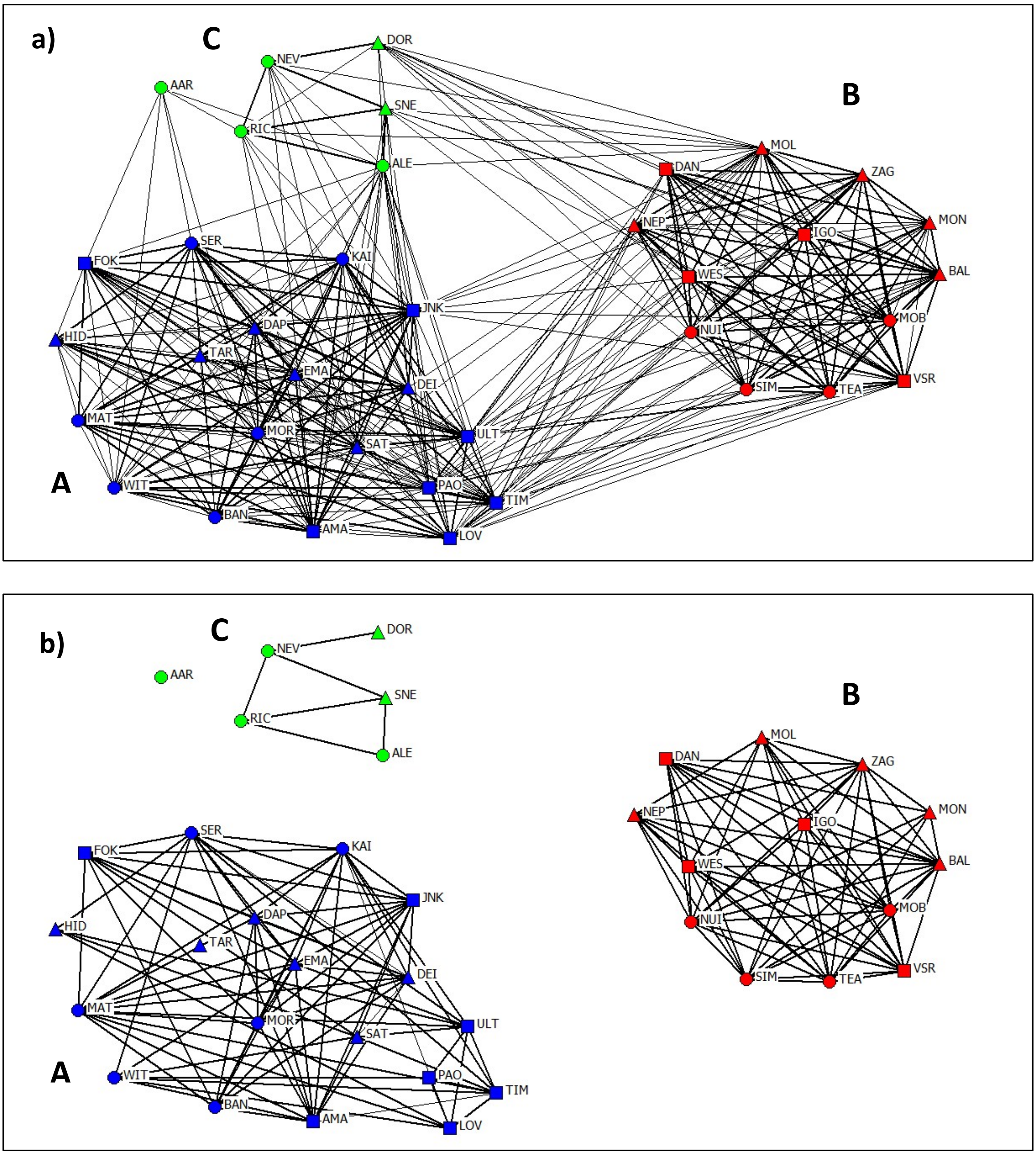


Figure 2.4. Social network diagram of the common bottlenose dolphin population. Nodes represent individuals (■ = males, ▲ = females, ● = unknown sex). Lines between nodes represent associations between dyads and the thickness of lines indicates the strength of relationship (value of an association index between dyads). Division of clusters is based on eigenvector method of Newman (2006) and modularity from gregariousness analyses. Cluster **A** = blue nodes, cluster **B** = red nodes, cluster **C** = green nodes. Note that individual AAR is included in cluster C but does not fit into it well. **a)** All recorded associations between dyads, regardless of strength. **b)** Only associations higher than twice the mean HWI (see main text for details).

2.3.3 Network metrics

Affinity, Clustering coefficient, Eigenvector centrality, Reach and Strength were comparable between clusters A and B, and lower in cluster C. Individuals in A and B had more associates and formed more stable associations with them than those in C. Conversely, cluster C had a higher Closeness, which is a different measure of centrality – as cluster C animals interacted with both A and B, their shortest paths to all other individuals were shorter than for other two clusters. Finally, cluster B had the lowest Betweenness centrality, which is likely a combination of cluster size and how often its members interacted with other clusters.

2.3.4 Sex segregation and sex differences

Twenty five animals were sexed (13 females, 12 males). No evidence of sex segregation was found. Cluster A contained 6 females, 7 males, and 6 unknown sex animals; cluster B contained 5 females, 4 males, and 4 unknown sex animals; and cluster C contained 2 females and 4 unknown sex animals (Fig. 2.4).

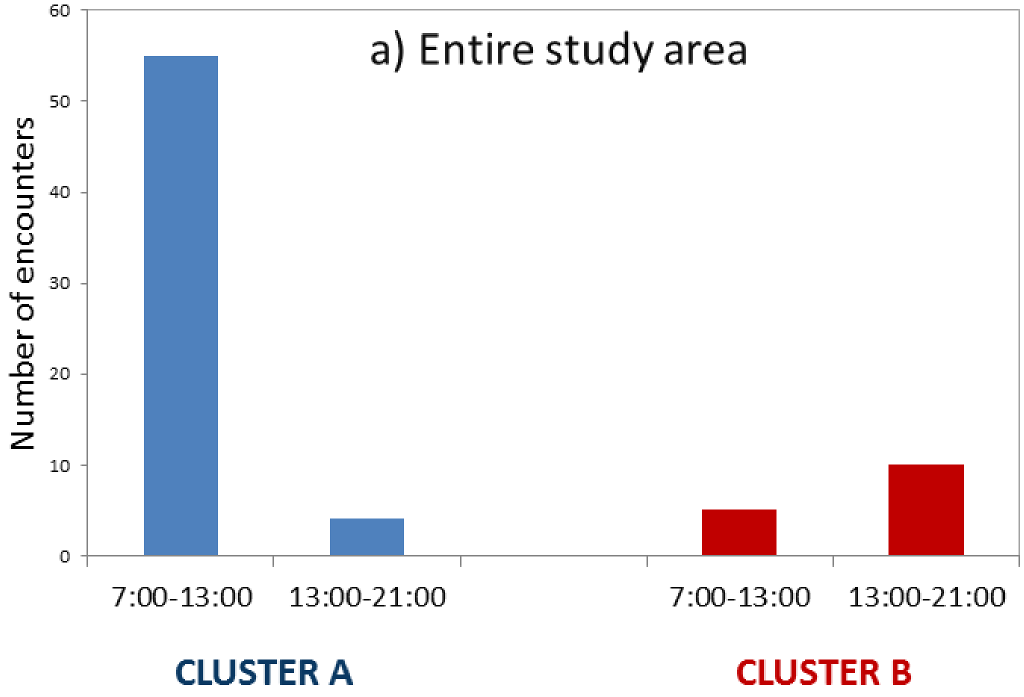
Among groups where sex of at least two animals was known (n = 60), 76.7% were mixed-sex. This is likely an underestimate, as groups classified as 'single-sex', but involving unsexed individuals, could in fact be mixed. Among groups composed only of cluster A dolphins (n = 44), 81.8% were mixed-sex. This could not be estimated for clusters B and C, as the number of encounters with at least two sexed animals was insufficient.

Mean HWI was higher for male-male pairs (HWI ± SD = 0.25 ± 0.07) than male-female pairs (0.21 ± 0.05) and female-female pairs (0.15 ± 0.06), but differences between sexes were not significant (two-tailed Mantel test: t = -0.916, P = 0.16).

2.3.5 Temporal habitat use patterns

Upon examining temporal (diel) occurrence patterns, an interesting trend became apparent. Clusters A and B overlapped spatially, but not temporally. They were almost never seen together, apart from four encounters mentioned earlier. Furthermore, while both regularly used the core study area, they used it at different times of day: cluster A was predominantly sighted in morning hours (07:00–13:00), and cluster B only in late afternoon hours (18:00–21:00). This trend was consistent in the core study area without deviation, although cluster A could be found elsewhere in the afternoon, and cluster B could be found elsewhere in the morning. To test if there was any real pattern, hours of occurrence of the two clusters for the entire study area were first examined. Next, to avoid bias resulting from different spatial preferences of dolphins or the spatial coverage of the survey effort, hours of occurrence in the core study area only (i.e. the area regularly covered by both boat-based and land-based surveys) were examined. For those few occasions when animals from more than one cluster were together, a group was assigned to a given cluster if it was predominantly composed of that cluster. When considering the entire study area and groups composed only or predominantly of cluster A dolphins, 55 encounters (93.2%) were between 08:00 and 13:00, and only 4 encounters (6.8%) after 13:00 ($n = 59$). Looking at the core study area only, all encounters (100%) of cluster A groups were before 13:00 ($n = 18$, Fig.2. 5). When considering the entire study area and groups composed only or predominantly of cluster B dolphins, 5 encounters (33.3%) occurred before 13:00, while the remaining 10 (66.7%) occurred after 18:00 ($n = 15$). Looking at the core study area only, all encounters (100%) of cluster B groups were recorded after 18:00 ($n = 8$; Fig. 5). The temporal use of the entire study area differed significantly between clusters A and B, as did the use of the core study area (Fisher's exact test: $p < 0.001$).

Cluster C groups did not display such patterns. In the entire study area, 23 (62.2%) encounters of cluster C groups were before 13:00, while 14 (37.8%) were after 13:00 ($n = 37$). In the core study area, 20 (66.7%) were before 13:00, while 10 (33.3%) were recorded after ($n = 30$).



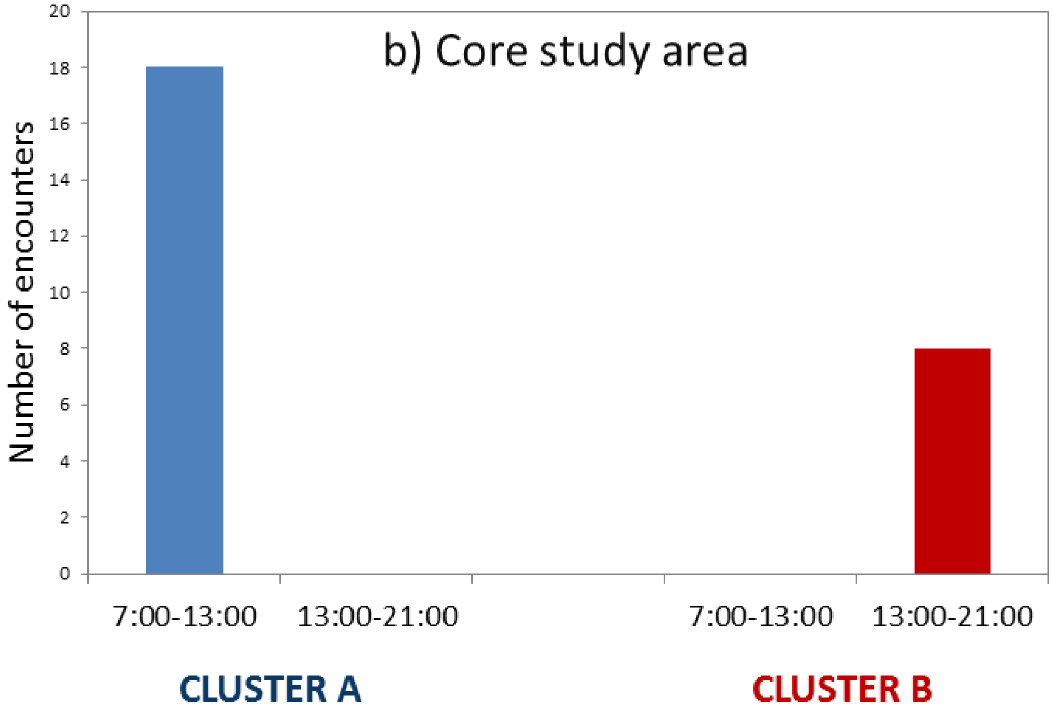


Figure 2.5. Temporal occurrence of clusters A and B in the a) entire study area and b) core study area.

2.3.6 Interactions with trawlers

Forty eight interactions with trawlers were recorded during the study, of which 35 were during dolphin encounters considered in the analysis. Encounters involving trawler interactions accounted for 29.6% of dolphin encounters. Twenty two (62.9 %) of these interactions were with pelagic pair trawlers and 13 (37.1%) with bottom trawlers (one encounter involved interactions with both).

The majority of interactions with trawlers involved cluster A dolphins, with one individual (MOR) present in more than 50% of all interactions (Fig. 2.6). Mean number of interactions with any trawlers per individual in cluster A was 10.6 (SD = 6.1, range = 3–24). Twenty eight interactions involved only cluster A dolphins (82.4%), 4 involved cluster A and cluster C dolphins (11.8%), 1 involved only cluster C dolphins (2.9%) and 1 involved cluster B and cluster C dolphins (2.9%). No interactions involved only cluster B dolphins.

Cluster B dolphins were never observed interacting with pair trawlers, while four individuals apparently interacted with a bottom trawler on one occasion. Mean number of interactions with any trawler per individual in this cluster was 0.31 (SD = 0.48, range = 0–1).

Dolphins from cluster C interacted with trawlers at intermediate level. Only one animal from cluster C (ALE) ever interacted with pair trawlers. This happened on one occasion, when the individual was with cluster A dolphins. On another occasion, the same individual was observed diving (*sensu* Bearzi et al., 1999) with another unidentifiable adult, when active pair trawlers passed by. The animals appeared to ignore them and continued diving in the same location. Other animals from cluster C were either never observed interacting with trawlers, or only observed interacting with bottom trawlers (Fig. 2.6). Mean number of interactions with any trawlers per individual in this cluster was 1 (SD = 0.01, range = 0–3).

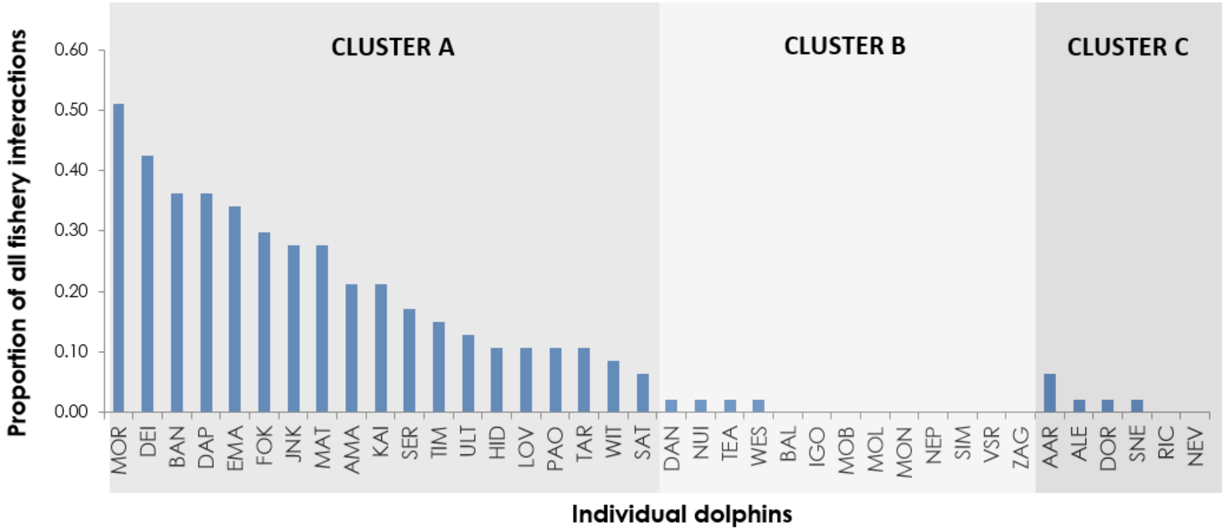


Figure 2.6. Proportion of all dolphin-fishery interactions an individual dolphin was recorded in.

2.4 DISCUSSION

2.4.1 General social structure

Dolphins in the Gulf of Trieste appear to live in two general kinds of social units: a) large mixed-sex groups with strong, long-lasting associations and b) small groups with weaker, temporally unstable associations. This does not appear to be age-dependent. Two largest clusters featured strong bonds, while seldom interacting with the other cluster. This structuring was also evident in the field. These two clusters showed high levels of group stability, which persisted through the study years and beyond (note: these patterns were still highly apparent for the data during 2013–2018, analysed in Chapter 5), although exact group membership could vary. Gregariousness, connectedness and strength of associations (indicated by HWI, Affinity, Clustering coefficient, Eigenvector centrality and Strength) were quite high and relatively similar between the two, as was the number of associates (Reach; Table 2.2). In contrast, these metrics were substantially lower in cluster C, where animals showed no strong association preferences. Because they were occasionally observed with animals from other clusters, their Closeness was highest (Table 2.2).

When including all associations (Fig. 2.4a) the network was reasonably well-connected, with no individual 'bottlenecks' between clusters, which were inter-connected via several but not particularly numerous individuals. Such 'social brokers' (Lusseau & Newman 2004) may maintain population cohesiveness and prevent complete cluster isolation, possibly having disproportionate influence on the population connectedness, as found in killer whales (Williams & Lusseau 2006), macaques (Flack et al. 2005) and squirrels (Manno 2008). However, when considering only 'meaningful' associations, greater than twice the mean HWI (Durrell et al. 2004, Gero et al. 2005, Wiszniewski et al. 2012), structuring becomes striking and clusters completely separated (Fig. 2.4b).

Associations were temporally relatively stable (as supported by SLAR and field observations), although stability varied with different levels of social organisation. Cluster A in particular (but also B) seemed to contain 'core' membership (first-level unit) and other 'tiers' that joined core members to form higher-level units. In such multi-level systems, seen also in African elephants (Wittemyer et al. 2005) clusters can sub-split during times of ecological constraints and fuse again when conditions are favourable or promote cooperation. Sometimes cluster A dolphins were observed forming smaller groups (≤ 10), which then often joined into groups of 30+ animals. Group composition during encounters was also surprisingly stable, more than in the closest other known local population in the Adriatic Sea (Bearzi et al., 1997) or in most well-studied other populations worldwide (Connor et al. 2000, Lusseau et al. 2006). Once encountered, groups were unlikely to change during observations, which could last several hours (Genov et al. 2008). This population is rather small (Genov et al. 2008; Genov 2011, but see also Chapter 5) and some authors hypothesised that community size influences group stability in fission-fusion societies, with smaller communities leading to decreased fission-fusion flexibility (Lehmann & Boesch 2004, Augusto et al. 2012).

In several *Tursiops* populations, social structure involves sex/age segregation (Wells et al. 1987, Connor et al. 2000, Fury et al. 2013). Here, structuring did not appear sex-related, as clusters contained both sexes. No evidence of male alliances was found. Although male-male associations were stronger than male-female or female-female associations, this was not significant, with stronger male-female than female-female associations. Most encountered groups contained both sexes (regardless of season), which suggests that mixed-sex groups were not related to reproductive state. Likewise, although more than half of all groups contained calves, adult-only groups were common. Reproductive state or presence of calves therefore fail to explain these patterns.

Presence of large mixed-sex groups resembles Doubtful Sound bottlenose dolphins in New Zealand (Lusseau et al. 2003). Lusseau et al. (2003) hypothesised that ecological constraints, such as variable productivity, drive social organisation. In such environments, groups may need to rely on individuals with long-term knowledge about spatio-temporal distribution of prey sources, which might explain lack of sex segregation and greater population connectedness (Lusseau et al. 2003). The northern Adriatic Sea is characterised by large spatio-temporal variability in nutrient input and productivity (Fonda Umani et al. 2005, Mozetič et al. 2010, 2012), and the study area contains relatively uniform bottom topography. With lack of major prey-aggregating bottom features, spatio-temporal distribution of prey is likely highly variable, which may promote network connectedness. Clusters A and B both contained individuals which appeared 'older' based on their external appearance. These animals may possess long-term knowledge needed to tackle such constraints, for example by knowing when to be at certain places or when to avoid them, and thus play a key role in their community.

2.4.2 Temporal segregation

Several studies found spatial segregation in *Tursiops* (Chilvers & Corkeron 2001, Chilvers et al. 2003, Lusseau et al. 2006, Fury et al. 2013, Carnabuci et al. 2016). In Moray Firth, Scotland, this segregation appeared season-dependent (Wilson et al. 1997). During summer, part of the population moved into inner parts of the Firth, and was replaced by dolphins from outer parts. However, clusters in this study overlapped spatially, but not temporally, and differences were detected on a daily, rather than seasonal level. Such intraspecific diel temporal partitioning does not appear to have been documented in cetaceans previously, nor in other mammals (Kronfeld-Schor & Dayan 2003), with one exception recorded in the use of running wheel in captive mice (Howerton & Mench 2014). Whether this pattern results from competitive exclusion, avoidance of aggressive interactions, or different foraging tactics, remains unknown. Given that prey resources in the marine environment are patchy and variable, prey resource defence is not a likely explanation (Ramp et al. 2010b). Lack of sex segregation also dismisses access to females as an explanation. Current ongoing work attempts to determine if genetic relatedness correlates with the social partitioning observed here.

Potential confounding factors were considered. If the distribution of cluster A was linked to trawlers, which only operated during certain hours, this would explain the pattern. However, pair trawlers operated in the morning and afternoon, and bottom trawlers operated day-long (including evenings). Cluster A regularly used trawling areas even in the absence of trawlers, with no difference in group composition. More importantly, no trawlers operated in the core study area due to restricitons related to the proximity to the coast. Finally, cluster A dolphins did not *always* follow trawlers, even if trawlers were around. Trawlers therefore fail to explain temporal partitioning.

Lower sample size for cluster B was also considered. Caution is needed when making inferences from small sample sizes, but temporal patterns here appear quite striking. The presence of a temporal (rather than spatial) pattern suggests the observed associations were not an artefact of space use (animals being together just because they use the same space), but due to genuine social preferences. Further, due to long-term and extensive survey effort (Table 2.1, Fig. 2.1), this pattern is unlikely to be an artefact of effort. Surveys in recent years (2013–2018, Chapter 5, as well as 2019–2021, T. Genov, *personal observations*) further support this, with both clusters continuing this pattern, and even occuring in the same area within a single day, but at different times.

Finally, it remains to be determined if segregation is specific to this area, or if it occurs in other areas used by the animals. The range of this local population is unknown (Genov et al. 2016), but evidence from photo-identification (Genov et al. 2009) and genetic markers (Gaspari et al. 2015) suggest it is a distinct unit.

2.4.3 Interactions with trawlers

Two clusters displayed behavioural differences related to trawling. Cluster A dolphins often interacted with pair trawlers and occasionally bottom trawlers, while cluster B dolphins did not (‘trawler dolphins’ vs. ‘non-trawler dolphins’, Chilvers & Corkeron 2001). Fishing has a major impact on cetaceans worldwide, not only through incidental mortality (Read et al. 2006), but also through prey depletion (Bearzi et al. 2008b), habitat degradation (Turner et al. 1999) and ecosystem change (Worm et al. 2006). More subtly, fishing activities can affect, or be affected by, cetacean behaviour. In Queensland, Australia, bottlenose dolphins were found to form two communities, where one fed in association with trawlers and the other did not (Chilvers & Corkeron 2001, Chilvers et al. 2003). Following fishery closure, dolphins restructured and homogenised their network, suggesting that structuring was fishery-induced (Ansmann et al. 2012). The present study shows similarities, but also important differences. First, in the population studied by Ansmann et al. (2012), dolphins fed on discards, while dolphins in this study followed operating trawlers, presumbly feeding actively inside/behind the net (Genov et al. 2008, Kotnjek 2016). Second, structuring in this study was related to temporal rather than spatial segregation, and did not appear only fishery-related. Another study in the Mediterranean Sea related dolphin association patterns to bottom trawling and fish farming, but animals mixed more frequently than in the present study (Pace et al. 2012).

Human activities can likely alter behaviour and social structure of mammals (Rutledge et al. 2010, Ansmann et al. 2012) and this may well be the case here. However, causal links are unclear and it is difficult to ascertain what came first. The inherent social structure itself, and social learning, may lead to differential behaviour and interactions with anthropogenic activities, without these activities changing the social system in the first place. It is interesting to note that the pair trawler fishery in the area closed in 2012. This did not appear to change associations or temporal patterns, but cluster A did appear to increase rates of interactions with bottom trawlers (T. Genov, *personal observations*).

Diet information for this population is limited, but dietary preferences may explain different fishery-related foraging tactics. Both clusters were observed taking mullets (*Mugil*/*Liza* sp., Genov et al., 2008, Morigenos, *unpublished data*) and both regularly fed in the core study area. Their diets therefore apparently overlap, but to an unknown extent. However, the apparent 'switch' of cluster A to bottom trawlers after the closure of pair trawler fishery suggests that behavioural specialisation and hunting techniques, rather than prey preference, may be more likely. Further research on this may provide better insight into the feeding ecology of this population through stable isotope analysis, and this is explored in Chapter 3.

Whether interactions with trawlers increase fitness (by maximising energetic intake and minimising expenditure) or decrease it (through increased bycatch), is unknown. Both clusters produce new offspring and appear stable (see also Chapter 5), and there is no evidence of trawler-related bycatch in this area (there is, however, bycatch in bottom-set trammel nets).

2.4.4 Conclusions

This study showed that local dolphins 1) form distinct social clusters; 2) exhibit temporal partitioning; and 3) differ in interactions with fisheries. It demonstrated how different segments of the same population may behave very differently, and have differing effects on human activities such as fishing (through potential depredation or gear damage). In turn, they may respond differently to anthropogenic pressures, as temporal partitioning may make animals either more or less vulnerable to disturbance from boat traffic.

CHAPTER 3: INTRA-POPULATION ISOTOPIC NICHE VARIATION IN BOTTLENOSE DOLPHINS FROM THE GULF OF TRIESTE

3.1 INTRODUCTION

“You are what you eat.” This statement has been used, and possibly abused, repeatedly in many contexts. But in the context of the topics explored in this chapter, it is largely true. One of the central aspects to understanding the ecology of a species is knowing its diet (Bowen et al. 2002). A number of methods are available to study diet in cetaceans, each with its own advantages and caveats (Tollit et al. 2010). Methods include stomach content analysis, direct observations, analysis of faecal samples and the use of stable isotope signatures. Not all methods are equally representative or available in studies of cetaceans, and generally speaking, unravelling the diet in this taxonomic group can be rather challenging.

The use of stable isotopes is one possible approach to investigating cetacean diet (Tollit et al. 2010), where the isotopic ratios of particular chemical elements (often nitrogen and carbon, but not only) in predators reflect the ratios in their prey (Hobson & Clark 1992, Hobson 1999, Bearhop et al. 2002). The disadvantage of this technique is that it does not allow prey species, prey size or prey mass to be identified or estimated, at least not directly, particularly if different prey species have similar signatures (Tollit et al. 2010). However, the averaged diet of the consumer can be estimated by quantitatively determining the relative contribution of a variety of candidate prey species to diet through mathematical mixing models (Hopkins & Ferguson 2012, Phillips 2012, Phillips et al. 2014, Borrell et al. 2021). Stable isotopes have been used to estimate diet in a number of cetacean species (Todd et al. 1997, Hooker et al. 2001, Ruiz-Cooley et al. 2004, Kiszka et al. 2010a, Kiszka et al. 2014, Scheinin et al. 2014). In particular, when samples used in the analysis are collected through biopsy sampling (Todd et al. 1997, Hooker et al. 2001, Kiszka et al. 2014), researchers have more control over ensuring that their sampling is representative of the population in question.

In addition to determining diet itself, this approach can also be highly valuable when the interest lies in determining whether there are dietary differences or resource partitioning among species or populations within species. For example, stable isotopes have been used to assess trophic relationships among sympatric delphinids (Kiszka et al. 2010a, Kiszka et al. 2011) and to investigate (sometimes in combination with other methods) population structure and potential management units in fin whales (*Balaenoptera physalus*) in the North Atlantic and the Mediterranean Sea (Giménez et al. 2013, Vighi et al. 2016, Gauffier et al. 2020), southern right whales (*Eubalaena australis*) in the Southwest Atlantic (Vighi et al. 2014), killer whales (*Orcinus orca*) in the eastern North Atlantic (Esteban et al. 2016) and common bottlenose dolphins (*Tursiops truncatus*) around the Iberian peninsula (Giménez et al. 2018). Today, stable isotopes are routinely used to study the ecology of marine mammals, including foraging ecology, trophic relationships, habitat use, migration and population structure (Newsome et al. 2010).

One of the key concepts in ecology is that of an ecological niche, which relates to issues such as resource use, competition, community composition, geographic variability and speciation, among others (Schoener 2009, Pocheville 2015). Even though the concept often, or perhaps even typically, applies to species, it can be extended to populations within species, or potentially also different parts within a population (Van Valen 1965). In fact, among-individual variability in behaviour and diet leads to a plethora of mini-niches within a population’s general niche (Bolnick et al. 2003, Araújo et al. 2011, Bolnick et al. 2011). Such variability is directly or indirectly linked to inter- and intra-specific competition, behavioural adaptation and variation in foraging tactics, and may lead to evolutionary divergence and speciation (Bolnick et al. 2007, Bolnick et al. 2011), but is also relevant to population resilience and conservation (Bolnick et al. 2003, Whitehead 2010, Chapter 2).

The concept of the ecological niche can be extended to trophic niche (which may or may not be considered analogous to ecological niche, depending on the framework and definitions used, but these two are not necessarily the same thing) and from there, to isotopic niche, when investigated through stable isotopes (Bearhop et al. 2004, Newsome et al. 2007). As described in Chapter 2, the bottlenose dolphin population inhabiting the Gulf of Trieste was found to be structured into distinct social clusters, two of which displayed strong social, temporal and behavioural partitioning. Given that the two social clusters displayed differences in temporal patterns of habitat use, as well as differential behaviour with respect to fisheries, the next logical question was whether these animals differ in their diet and whether this may help explain the observed social patterns. The aim of this chapter was to examine exactly that, using stable isotopes of carbon and nitrogen, to provide insights into the potential drivers, or consequences, of such partitioning.

3.2 METHODS

3.2.1 Sample collection

Biopsy samples were collected from free-ranging common bottlenose dolphins between 2011 and 2018. Sampling followed standard methodology (Gorgone et al. 2008, Kiszka et al. 2010b) and was carried out exclusively in good weather conditions (Beaufort sea state ≤2, good visibility, no precipitation). Samples of skin (for stable isotope analysis, this chapter) and blubber tissue (for pollutant analysis, see Chapter 4) were obtained using custom made bolts and stainless steel sampling tips (tip length 25 mm, internal diameter 7 mm), made by Ceta Dart, Copenhagen, Denmark. Sampling tips were sterilised using 96% ethanol and burning prior to being used. Bolts with sterile sampling tips were fired into the dorso-lateral area below the dorsal fin (Fig. 3.1), at distances of 4–10 m, using a Barnett Panzer V crossbow with 68 kg draw weight. A high-pressure moulded stopper prevented the tip from penetrating more than about 20 mm and ensured the re-bouncing of the bolt. The floating bolt was retrieved from the water by hand. Skin samples were removed and excised with sterilised forceps and surgical scissors, placed in aluminium foil and stored at −20°C until further analysis.

An attempt was made to sample the animals as representatively as possible across different social clusters (Chapter 2). Sampling across seasons was also attempted, but was largely dictated by weather, logistical and other circumstances, so completely homogeneous sampling coverage across seasons was not feasible. All biopsy attempts were accompanied by concurrent photo-identification (see Chapter 5) of targeted individuals and other dolphins in their group, in order to prevent re-sampling of the same individuals and to allow the integration of information from biopsy samples (in this case stable isotopes) with information from long-term photo-identification. Unlike in photo-identification (Chapter 5), where all animals were photographed regardless of the degree of marking, here an explicit attempt was made to sample identified animals, to be able to link tissue samples to individuals with known sighting histories.

Sampling was only attempted on adults. No sampling was attempted on calves or mothers with calves. Care was taken not to attempt sampling of animals accompanied (followed) by another animal in their slipstream, to prevent potential shots in the head. During each attempt, the behavioural reactions of the target animal and the focal group were recorded, together with information on distance of the target animal, the area hit and the sea state, for a posteriori assessment of reactions to, and potential impact of, biopsy sampling. In short, the animals always reacted to the biopsy hit, but all the reactions were instantaneous and therefore short-lived. Reactions typically consisted of a body flinch, tail flick and dive, with no prolonged behavioural changes. Biopsied individuals or other group members showed no evidence of lasting impact or subsequent boat avoidance, and a number of biopsies individuals could be seen approaching or bowriding the research boat afterwards, either during the same sighting or on a different day. There were no visible differences in reactions regardless of whether the biopsy attempt was a hit or a miss, suggesting that any reactions were likely a result of a surprise and a startle response to a foreign object touching the dolphin or hitting the water next to it, rather than any substantial pain or discomfort. Subsequent monitoring of biopsy wounds via photography showed that all dolphins healed rapidly, with any visual evidence of biopsy disappearing within weeks to months. No infections of the biopsied area were documented.

In addition to samples from biopsies, three samples were obtained from animals entangled in fishing gear (one newborn, one calf and one adult). Due to the freshness of all three carcasses, all three individuals could be identified with certainty as local dolphins known from photo-identification, and therefore included in the analysis. Stranded animals too decomposed to be identified were excluded. Biopsy sampling and sampling of stranded animals was conducted under the permit 35601-102/2010-4 issued by the Slovenian Environmental Agency.

Sex of individuals was determined by any of the following: a) observations of temporally stable adult-calf associations (an adult consistently accompanied by a calf was assumed to its mother and therefore a female); b) photographs of the genital area during bowriding or aerial behaviour and c) molecular methods from biopsy samples, analysed for previous studies (Gaspari et al 2015). Social group membership was determined using social network analysis (Chapter 2) and additional photo-identification data (explained below). Even though the primary motivation for this analysis was the social partitioning between the two largest social groups (Chapter 2), all sampled dolphins were included in the analysis, including clusters A, B and C (Chapter 2), as well as other animals not included in social network analysis in Chapter 2. These were animals that were not included in the social network analysis due to being more transient in nature or because they were not yet photo-identified at the time of social network analysis. However, these animals could be photo-identified at the time of sampling and determined not to be part of any of the three clusters from Chapter 2. Although their social membership was not assessed in the same way as for the clusters from Chapter 2, they were considered as belonging to a separate social group and therefore referred to here as group D for ease of data visualisation and reporting of results. Seasons were defined as spring (March–May), summer (June–August), autumn (September–November) and winter (December–February).

Figure 3.1. Biopsy sample collected from a free-ranging common bottlenose dolphin in the Gulf of Trieste, northern Adriatic Sea. Photograph: Morigenos – Slovenian Marine Mammal Society

3.2.2 Stable isotope analysis

After defrosting (or rinsing with distilled water for samples preserved in ethanol), subsamples of skin were cut into fine pieces with scalpel and scissors, and dried in an oven at 60°C for 48 h. Given that lipid depletion in $\delta^{13}C$ may affect analytical results (DeNiro & Epstein 1977), dried samples were delipidated with a chloroform-methanol (2:1) solution (Bligh & Dyer 1959) and subsequently dried again at 60°C for 48 h. Most samples were weighed before and after the delipidation to determine the lipid content (Evanson et al. 2000).

A 0.5 (± 0.02) mg aliquot of each sample was weighed in tin capsules, automatically loaded, combusted at 1000°C, and analysed in a continuous flow stable isotope ratio mass spectrometer (Flash 1112 IRMS Delta C Series EA Thermo Finnigan, Bremen, Germany). Primary standards for $\delta^{13}C$ and $\delta^{15}N$ were the Vienna Pee Dee Belemnite limestone (V-PDB) and the atmospheric nitrogen, respectively. Secondary standards of known $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios in relation to V-PDB and atmospheric nitrogen were used for calibration of $\delta^{13}C$ and $\delta^{15}N$, to ensure an optimum range of reference values. These were: fructose ($\delta^{13}C$=-11.2‰), polyethylene (IAEA CH7; $\delta^{13}C$=-31.8‰), ammonium sulphate (IAEA N1; $\delta^{15}N$ =+0.4‰ and IAEA N2; $\delta^{15}N$ =+20.3‰), L-glutamic acid (USGS 40; $\delta^{13}C$ =-26.2‰; $\delta^{15}N$ =-4.6‰;), UCGEMA P ($\delta^{15}N$=7.6‰; $\delta^{13}C$=-27.7‰), all distributed by the International Atomic Energy Agency (IAEA). The calibration precision, based on the standard deviation of repeated measurements of the secondary standards, was 0.11 ± 0.11 ‰ for $\delta^{13}C$ and 0.16 ± 0.09 ‰ for $\delta^{15}N$.

Results were expressed in parts per thousand (‰) and following the delta (δ) notation, where the relative variations of stable isotope ratios are calculated as follows:

$$\delta^{15}N \ or \ \delta^{13}C \ (‰) = \left[\left(\frac{R_{sample}}{R_{standard}}\right) - 1\right] * 10^3,$$

where *R* is the heavy-to-light isotope ratio ($^{15}N/^{14}N$; $^{13}C/^{12}C$) in the sample and in the reference standards. Stable isotope analysis was carried out at the *Centres Científics i Tecnològics* of the University of Barcelona (CCiT-UB).

3.2.3 Statistical analysis

In exploratory analysis, boxplots for $\delta^{15}N$ and $\delta^{13}C$ data were plotted to evaluate the relationship with different explanatory variables (year, season, month, social group and sex). Summary statistics (mean, median, standard deviation and range) were calculated for $\delta^{15}N$ and $\delta^{13}C$ across different levels of each explanatory variable (Table 3.1).

Next, to simultaneously test for the potential effects of various variables on stable isotope ratios, a multiple linear regression model was fitted to $\delta^{13}C$ and $\delta^{15}N$ data, respectively. The candidate set of models included all possible combinations of variables sex, social cluster membership (referred to here as "group"), season and year (i.e. all subsets of a fully saturated model). To determine which variables were potentially important in explaining the observed stable isotope ratios, model selection (i.e. the inclusion or exclusion of candidate variables) on a candidate set of models was carried by minimising the Akaike Information Criterion (Akaike 1973) corrected for small sample size (AICc). In case of support for two or more models, model averaging based on Akaike weights was carried out with the *MuMIn* package in R (Bartoń 2009), using a 95% confidence set (Burnham & Anderson 2002, Grueber et al. 2011). Model assessment was done using diagnostics plots for residuals (Zuur et al. 2009, Matthiopoulos 2011). The relative importance of variables was judged by the sum of model weights over all models including each explanatory variable within the 95% confidence set. Data analysis was carried out within program R (R Core Team 2020).

The isotopic niches of identified social groups were estimated, compared and plotted by fitting convex hull areas and bivariate ellipses using the R package *SIBER* (Jackson et al. 2011). Convex hull areas (total area, TA) are a two-dimensional representation of $\delta^{13}C$ and $\delta^{15}N$ isospace and a measure of the total occupied isotopic niche space (Layman et al. 2007). Maximum-likelihood-based Standard Ellipse Area (SEA) and SEA corrected for small sample size (SEA_C) were calculated for each group (Jackson et al. 2011). SEA_C are less influenced by extreme values and contain approximately 40% of data, irrespective of sample size. They can be thought of as representing the same thing as standard deviation represents for univariate data (Jackson et al. 2011). The overlap between SEA_C of different groups was also calculated, expressed as a proportion of the sum of the non-overlapping areas of the ellipses, ranging from 0 (completely distinct ellipses) to 1 (completely coincidental ellipses). The width of the isotopic niche was estimated by fitting Bayesian multivariate normal distributions to ellipse-based metrics for each group (Jackson et al. 2011).

3.3 RESULTS

3.3.1 General results

The number of samples collected across different years, seasons, months, social groups and the two sexes, together with summary statistics for $\delta^{13}C$ and $\delta^{15}N$, broken down by these variables, is provided in Table 3.1. Most samples were collected during spring and summer, when weather conditions were most favourable and when most fieldwork was carried out. The most sampled social groups were A and B, which were encountered most often and which were also of primary interest for this analysis. Animals from group C were typically found in small groups or alone (Chapter 2), which generally made them more difficult to approach and therefore sample. Group D animals were either animals seen relatively rarely or encountered only on a single occasion.

Table 3.1. Summary statistics for nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) values by year, season, month, social group and sex.

	Nitrogen ($\delta^{15}N$)					**Carbon ($\delta^{13}C$)**					
Year	Mean	Median	SD	Min	Max	Mean	Median	SD	Min	Max	n
2011	15.250	14.815	1.344	14.400	17.960	-16.515	-16.520	0.765	-17.300	-15.240	6
2013	16.006	15.930	1.470	14.260	17.800	-16.840	-17.490	1.758	-19.010	-14.900	5
2014	14.515	14.505	0.296	13.970	15.130	-16.748	-16.560	0.435	-17.390	-16.290	12
2015	15.040	14.905	0.768	14.310	16.040	-16.673	-16.375	0.682	-17.690	-16.250	4
2016	14.923	14.815	0.454	14.500	15.560	-16.985	-17.105	0.292	-17.180	-16.550	4
2017	15.065	15.025	0.484	14.390	15.890	-17.081	-17.080	0.489	-17.790	-15.970	10
2018	14.620	14.620	0.000	14.620	14.620	-17.610	-17.610	0.000	-17.610	-17.610	1
Season	Mean	Median	SD	Min	Max	Mean	Median	SD	Min	Max	n
Spring	15.042	14.950	0.494	14.430	16.040	-17.121	-17.180	0.429	-17.690	-15.970	18
Summer	14.654	14.560	0.503	13.970	15.930	-16.422	-16.450	0.562	-17.490	-14.900	13
Autumn	15.445	14.815	1.521	14.260	17.960	-16.658	-16.520	1.244	-19.010	-15.160	8
Winter	15.340	14.520	1.534	14.390	17.110	-17.463	-17.640	0.442	-17.790	-16.960	3
Month	Mean	Median	SD	Min	Max	Mean	Median	SD	Min	Max	n
Jan	15.815	15.815	1.831	14.520	17.110	-17.300	-17.300	0.481	-17.640	-16.960	2
Feb	14.390	14.390	0.000	14.390	14.390	-17.790	-17.790	0.000	-17.790	-17.790	1
Mar	15.141	15.010	0.515	14.660	15.890	-17.114	-16.980	0.251	-17.500	-16.860	7
Apr	15.154	15.130	0.537	14.490	16.040	-17.014	-17.250	0.628	-17.690	-15.970	7
May	14.670	14.680	0.194	14.430	14.890	-17.320	-17.280	0.225	-17.610	-17.110	4
Jun	14.470	14.515	0.308	13.970	14.930	-16.633	-16.490	0.389	-17.490	-16.290	8
Jul	14.500	14.500	0.000	14.500	14.500	-16.550	-16.550	0.000	-16.550	-16.550	1
Aug	15.060	15.000	0.689	14.310	15.930	-15.968	-16.270	0.716	-16.430	-14.900	4
Sep	15.250	14.815	1.344	14.400	17.960	-16.515	-16.520	0.765	-17.300	-15.240	6
Oct	16.030	16.030	2.503	14.260	17.800	-17.085	-17.085	2.722	-19.010	-15.160	2
Group	Mean	Median	SD	Min	Max	Mean	Median	SD	Min	Max	n
A	15.397	15.070	1.056	14.260	17.960	-16.740	-16.895	0.929	-19.010	-14.900	22
B	14.595	14.530	0.379	13.970	15.230	-16.755	-16.575	0.484	-17.490	-15.970	10
C	14.642	14.605	0.150	14.490	14.890	-17.093	-17.145	0.441	-17.610	-16.320	6
D	14.575	14.475	0.267	14.390	14.960	-17.228	-17.295	0.520	-17.790	-16.530	4
Sex	Mean	Median	SD	Min	Max	Mean	Median	SD	Min	Max	n
Female	15.369	15.010	1.014	14.470	17.800	-16.632	-16.765	0.873	-17.690	-14.900	14
Male	14.845	14.655	0.762	13.970	17.960	-16.945	-16.965	0.679	-19.010	-15.240	28

3.3.2 Relationships between explanatory variables and isotope ratios

3.3.2.1 Individual comparisons

There was a lot of variability in both $\delta^{15}N$ and $\delta^{13}C$ ratios across different explanatory variables (Fig. 3.2 – 3.6). Overall, females had higher $\delta^{15}N$ ratios than males, although the highest value belonged to a male and there was substantial overlap (Fig. 3.2). There were no differences between sexes for $\delta^{13}C$.

For $\delta^{15}N$, there were substantial differences among groups. Group A had overall higher ratios than the other three groups, which were very similar compared to one another (Fig. 3.3), although the sample size for groups C and D is relatively small (Table 3.1). Group A also had more variability in $\delta^{13}C$ than other groups, but this may be an artifact of sample size. There was clear overlap among all groups and no substantial differences were found among groups for $\delta^{13}C$ (Fig. 3.3).

Because sample sizes were variable (and often small) for different months, there is little that can be interpreted from Fig. 3.4. No differences were apparent among seasons for $\delta^{15}N$, but substantial differences were apparent for $\delta^{13}C$, particularly between spring and summer and between summer and winter (Fig. 3.5) .

There was substantial variability among years for both $\delta^{15}N$ and $\delta^{13}C$ (Fig. 3.6), , but the small sample sizes in some years preclude any meaningful interpretation.

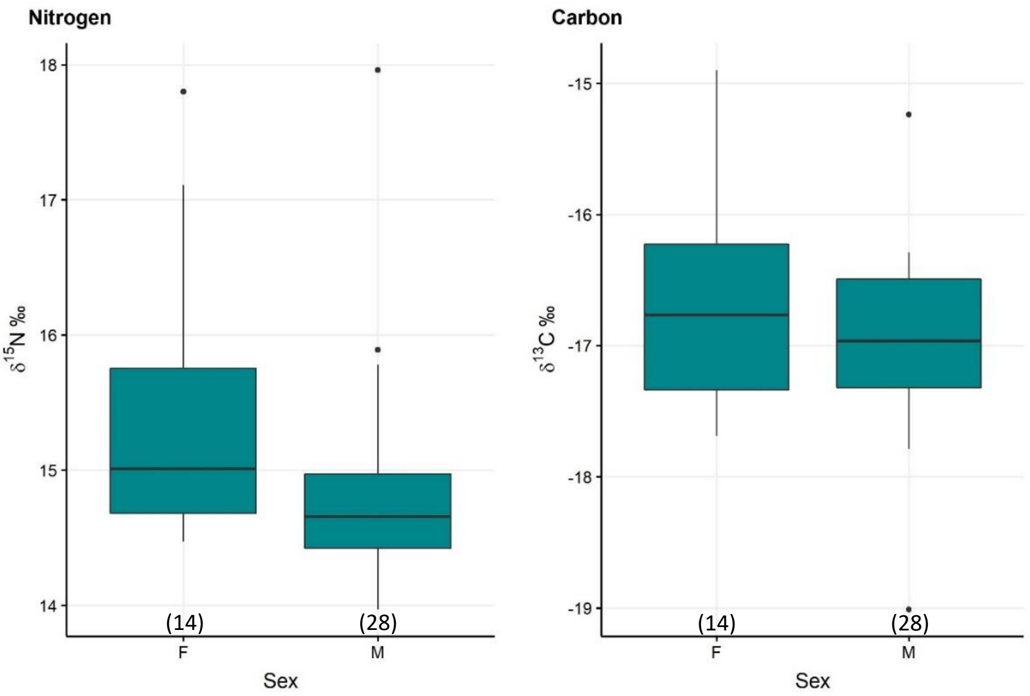


Figure 3.2. Boxplots showing $\delta^{15}N$ and $\delta^{13}C$ between females and males. Sample sizes are shown in parentheses. The boxes show the median (solid line) and the inter-quartile range, while the whiskers extend 1.5 x inter-quartile range from the upper and lower bounds of the box, respectively. Outlying data points beyond the whiskers are plotted individually. The same boxplot characteristics apply to Figs. 3.3–3.6.

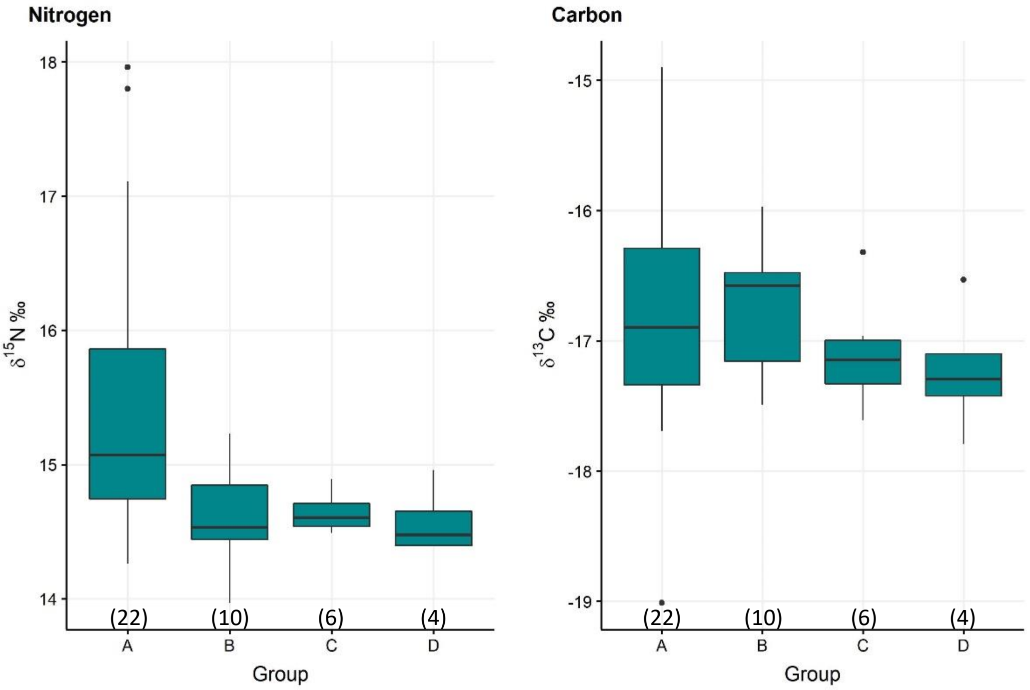


Figure 3.3. Boxplots showing $\delta^{15}N$ and $\delta^{13}C$ across social groups. Sample sizes are shown in parentheses.

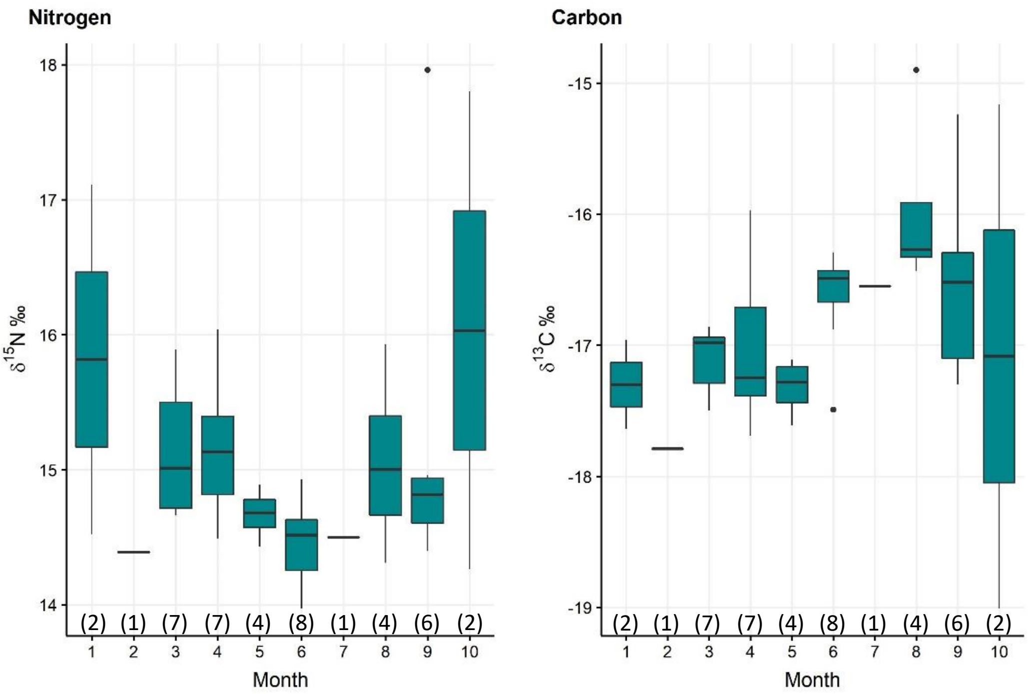


Figure 3.4. Boxplots showing $\delta^{15}N$ and $\delta^{13}C$ across months. Sample sizes are shown in parentheses.

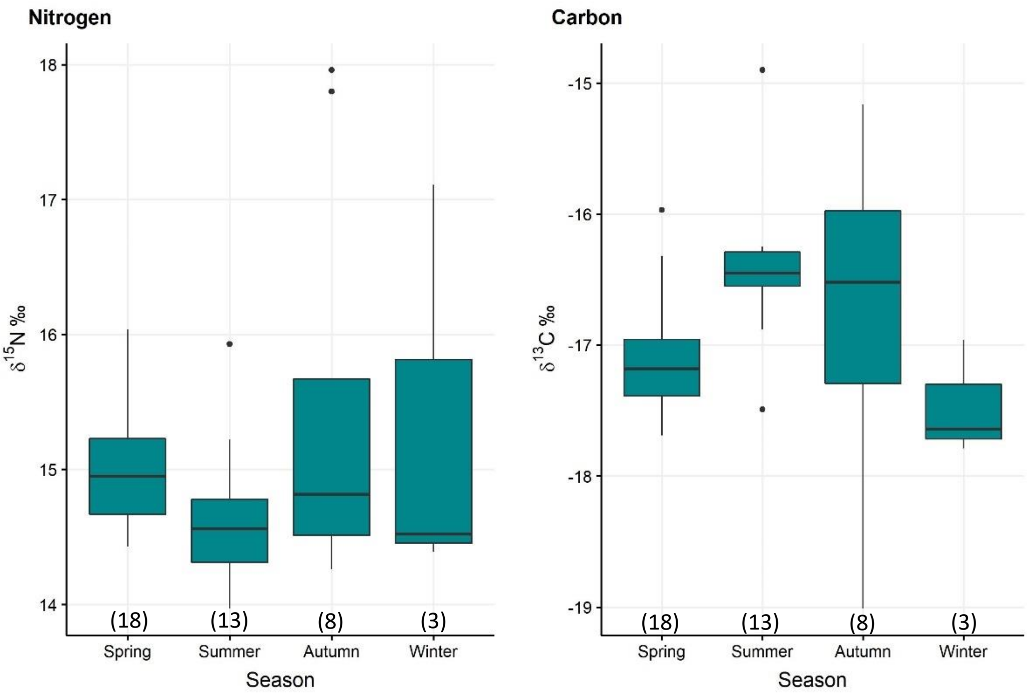


Figure 3.5. Boxplots showing $\delta^{15}N$ and $\delta^{13}C$ across seasons. Sample sizes are shown in parentheses.

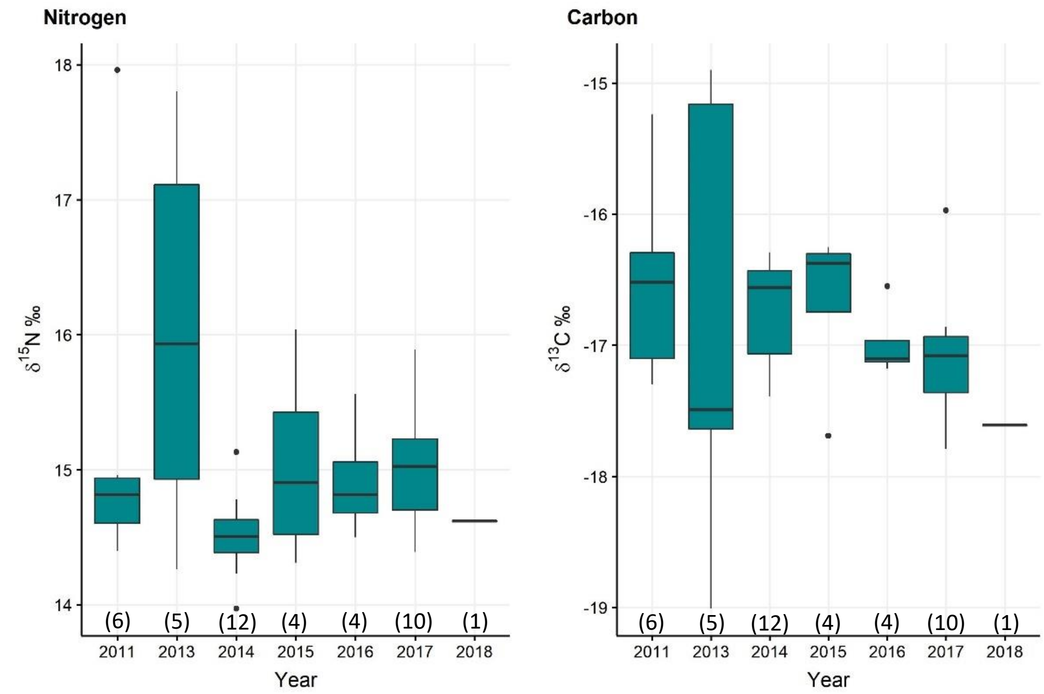


Figure 3.6. Boxplots showing $\delta^{15}N$ and $\delta^{13}C$ across years. Sample sizes are shown in parentheses.

3.3.2.2 Linear regression models

For $\delta^{15}N$, the model containing Group and Season was the most supported model, followed by the model containing only Group and the model containing Group and Sex (Table 3.2). Among all candidate models, variable Group had the highest relative importance (0.74), followed by variables Sex (0.45) and Season (0.38). Variable Year had almost no support (0.03). The 95% confidence set of models included variables Group, Sex and Season, but not Year. Within the 95% confidence set of models, Group, Sex and Season had 0.77, 0.46 and 0.38 relative importance, respectively (Table 3.3). For $\delta^{13}C$, the model containing Season and Sex was the most supported model, followed by the model containing only Season and the intercept-only model (Table 3.2). Among all candidate models, variable Season had the highest relative importance (0.88), followed by variable Sex (0.61). Variables Group (0.03) and Year (< 0.01) had almost no support. The 95% confidence set of models included variables Season and Sex, but excluded Group and Year. Within the 95% confidence set of models, Season and Sex had 0.93 and 0.60 relative importance, respectively (Table 3.3).

The modelling results largely corroborated the patterns from initial data visualisation (Fig. 3.2 – 3.6). For $\delta^{15}N$, the model-averaged mean estimated coefficient for males was lower (β = -0.4222, SE = 0.2859, 95% CI = -0.9826 – 0.1382) than for females, which represented the baseline level (intercept) in the model. Group A (intercept) had highest $\delta^{15}N$ ratios, followed by group B (β = -0.6213, SE = 0.3619, 95% CI = -1.3306 – 0.088), group C (β = -0.7736, SE = 0.3752,95% CI = -1.509 – -0.0382) and group D (β = -1.0309, SE = 0.5685, 95% CI = -2.1452 – 0.0834). Highest $\delta^{15}N$ ratios were in winter (β = 0.0353, SE = 0.5529, 95% CI = -1.0484 – 1.119), followed by autumn (model intercept), spring (β = -0.6163, SE = 0.3863, 95% CI = -1.3735 – 0.14099) and summer (β = -1.0636, SE = 0.4257, 95% CI = -1.898 – -0.2292).

For $\delta^{13}C$, the model-averaged mean estimated coefficient for males was lower (β = -0.4258, SE = 0.2236,95% CI = -0.8641 – 0.0125) than for females (intercept). Highest $\delta^{13}C$ ratios were in summer (β = 0.2201, SE = 0.3044, 95% CI = -0.3765 – 0.8167), followed by autumn (model intercept), spring (β = -0.4865, SE = 0.2884, 95% CI = -1.0518 – 0.0788) and winter (β = -0.9202, SE = 0.4718, 95% CI = -1.8449 – 0.045).

Table 3.2. Model selection results for nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$). Models are ordered by Δ AICc. Δ AICc is the diffence between the AICc of a given model and the AICc of the most supported model. df = degrees of freedom; logLik = log likelihood.

Nitrogen ($\delta^{15}N$)						
#	Model	df	logLik	AICc	Δ AICc	AICc weight
1	Group + Season	8	-44.2995	108.9626	0.000	0.232
2	Group	5	-48.7144	109.0956	0.133	0.217
3	Group + Sex	6	-47.4836	109.3672	0.405	0.189
4	Sex	3	-51.8406	110.3127	1.350	0.118
5	Group + Season + Sex	9	-43.5726	110.7702	1.808	0.094
6	Intercept only	2	-53.6189	111.5454	2.583	0.064
7	Season + Sex	6	-49.1866	112.7732	3.811	0.034
8	Season	5	-51.1509	113.9684	5.006	0.019
9	Year	8	-47.076	114.5157	5.553	0.014
10	Year + Sex	9	-45.6166	114.8581	5.896	0.012
11	Year + Group	11	-42.9659	116.7318	7.769	0.005
12	Year + Group + Sex	12	-41.9719	118.7025	9.740	0.002
13	Year + Season	11	-45.8043	122.4086	13.446	0
14	Year + Group + Season	14	-40.245	124.0455	15.083	0
15	Year + Season + Sex	12	-44.6706	124.0999	15.137	0
16	Year + Group + Season + Sex	15	-39.799	128.0596	19.097	0
Carbon ($\delta^{13}C$)						
#	Model	df	logLik	AICc	Δ AICc	AICc weight
1	Season + Sex	6	-40.0118	94.42358	0.000	0.551
2	Season	5	-41.975	95.61668	1.193	0.303
3	Intercept only	2	-47.2036	98.7148	4.291	0.064
4	Sex	3	-46.3629	99.35731	4.934	0.047
5	Group + Season	8	-40.6299	101.6234	7.200	0.015
6	Group + Season + Sex	9	-39.2388	102.1025	7.679	0.012
7	Group	5	-46.0198	103.7063	9.283	0.005
8	Group + Sex	6	-45.4524	105.3048	10.881	0.002
9	Year	8	-45.2119	110.7874	16.364	0
10	Year + Season + Sex	12	-38.5757	111.91	17.486	0
11	Year + Sex	9	-44.3171	112.2591	17.836	0
12	Year + Season	11	-40.896	112.5919	18.168	0
13	Year + Group	11	-42.6938	116.1876	21.764	0
14	Year + Group + Season	14	-37.507	118.5696	24.146	0
15	Year + Group + Sex	12	-42.0907	118.9401	24.517	0
16	Year + Group + Season + Sex	15	-35.6718	119.8051	25.382	0

Table 3.3. Relative importance of variables in explaining $\delta^{15}N$ and $\delta^{13}C$ ratios, as judged by the sum of model weights over all models including the explanatory variable within the 95% confidence set. Variables are ordered by their relative importance.

<table>
<tr><th colspan="3">Nitrogen ($\delta^{15}N$)</th></tr>
<tr><th>#</th><th>Variable</th><th>Relative importance</th></tr>
<tr><td>1</td><td>Group</td><td>0.77</td></tr>
<tr><td>2</td><td>Sex</td><td>0.46</td></tr>
<tr><td>3</td><td>Season</td><td>0.38</td></tr>
<tr><td>4</td><td>Year</td><td>0 (not in 95% confidence set)</td></tr>
<tr><th colspan="3">Carbon ($\delta^{13}C$)</th></tr>
<tr><th>#</th><th>Variable</th><th>Relative importance</th></tr>
<tr><td>1</td><td>Season</td><td>0.93</td></tr>
<tr><td>2</td><td>Sex</td><td>0.6</td></tr>
<tr><td>3</td><td>Group</td><td>0 (not in 95% confidence set)</td></tr>
<tr><td>4</td><td>Year</td><td>0 (not in 95% confidence set)</td></tr>
</table>

3.3.3 Isotopic niches of social groups

The isotopic niches among groups largely overlapped, but group A had a much wider niche than the rest of the groups (Fig. 3.7 – 3.9). Table 3.4 shows the TA, SEA and SEA_{C} values for each group. Group A stands out in all of the reported metrics and was the group with the widest niche (Fig. 3.10). The SEA_{C} overlap between group pairs is shown in Table 3.5.

Table 3.4. Total hull area (TA), standard ellipse area (SEA) and standard ellipse area corrected for small sample size (SEA_{C}) for each social group and for each sex.

Metric	Group A	Group B	Group C	Group D	Females	Males
TA	9.552	1.077	0.266	0.136	5.267	6.674
SEA	2.843	0.575	0.207	0.166	1.496	2.679
SEA_{C}	2.985	0.647	0.259	0.249	1.553	2.903

Table 3.5. SEA_{C} overlap between pairs of social groups.

	B	C	D
A	0.149	0.087	0.061
B		0.247	0.173
C			0.319

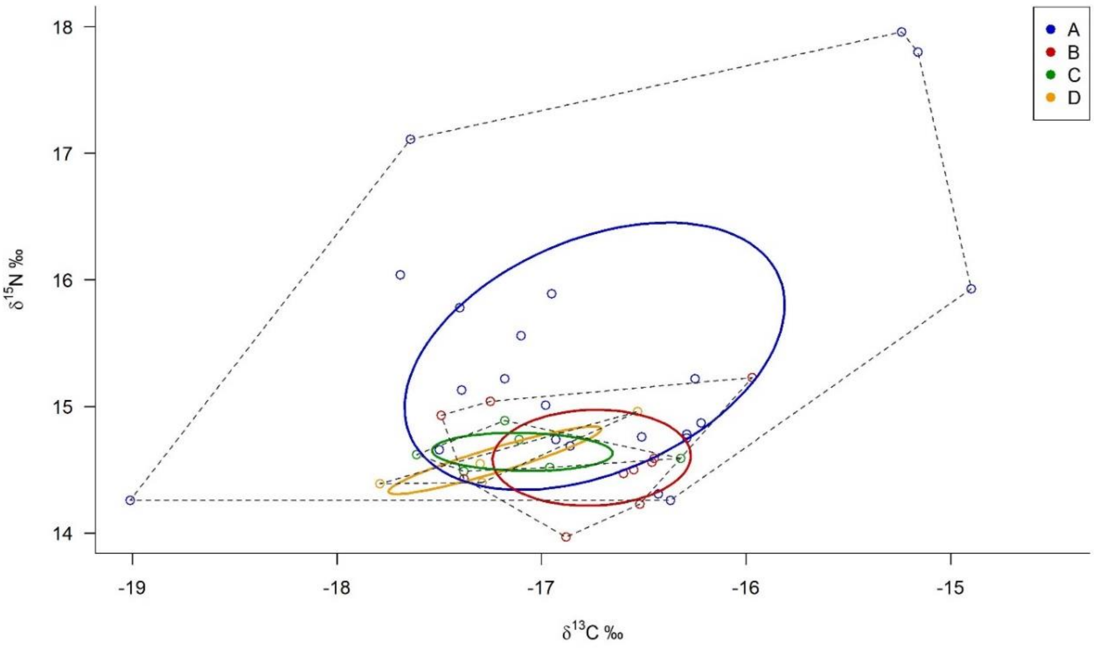


Figure 3.7. Isospace of $\delta^{15}N$ and $\delta^{13}C$ across social groups. The ellipses represent Standard Ellipse Areas (SEA$_C$), encompassing 40 % of the data for each group, while the dotted lines represent the convex hulls (total area, TA) acompassing all data points (empty circles) for each group.

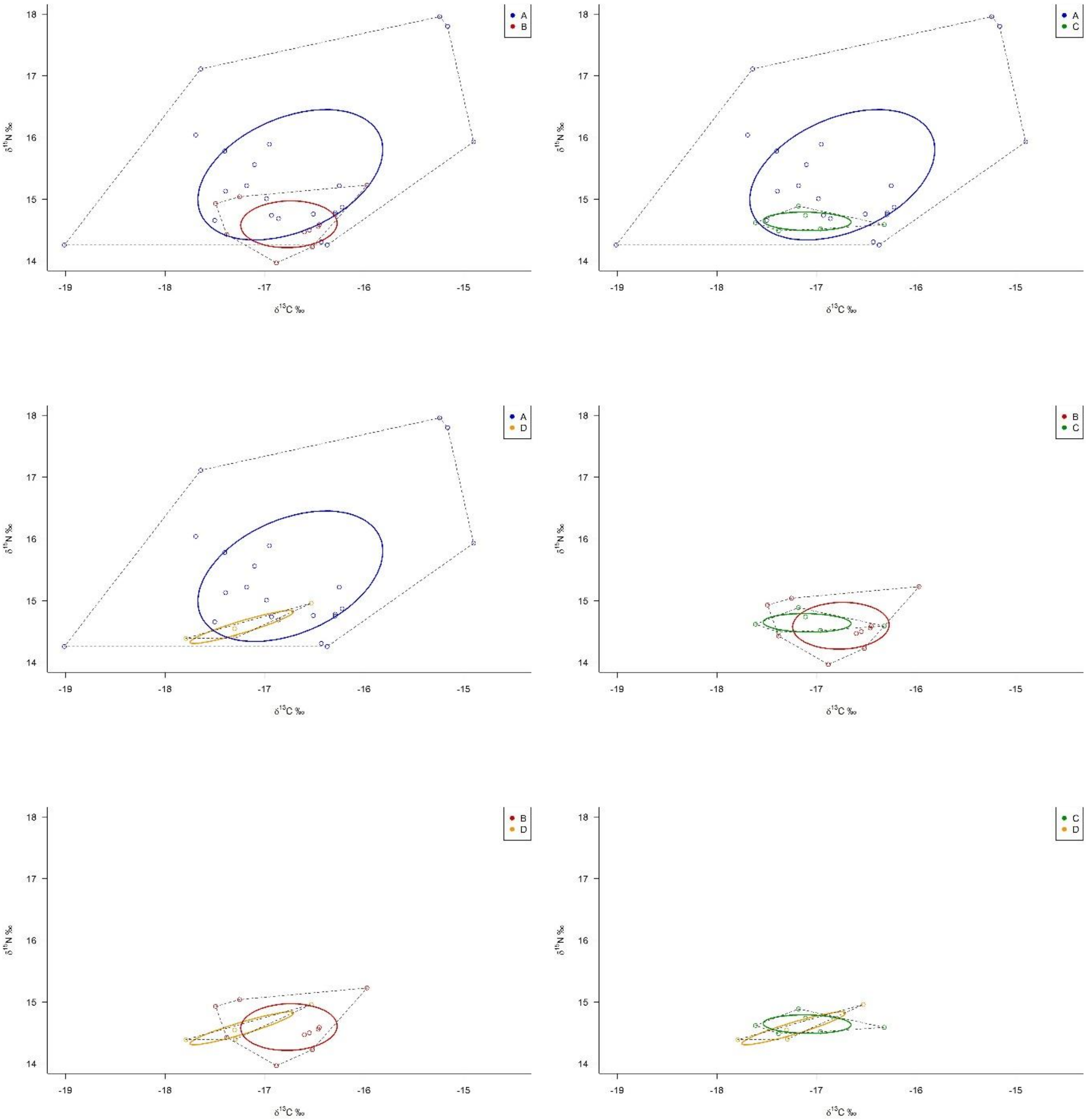


Figure 3.8. Pairwise comparisons of isospace of $\delta^{15}N$ and $\delta^{13}C$ between groups. The ellipses represent Standard Ellipse Areas (SEA$_C$) for each group, while the dotted lines represent the convex hulls acompassing all data points (empty circles) for each group.

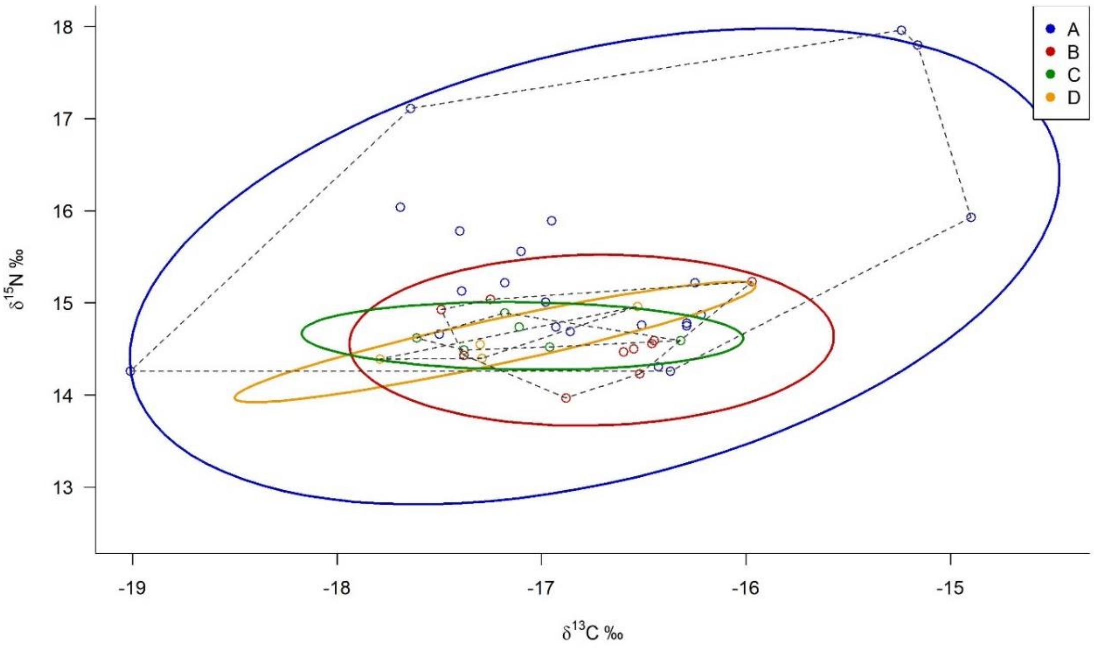


Figure 3.9. 95% isospace of $\delta^{15}N$ and $\delta^{13}C$ across social groups. The ellipses represent Standard Ellipse Areas as in Fig. 3.7, but encompassing 95 % of the data for each group. Note also the different scale of the y-axis compared to Fig. 3.7., due to the wider area of the ellipses. Dotted lines represent the convex hulls (total area, TA) acompassing all data points (empty circles) for each group.

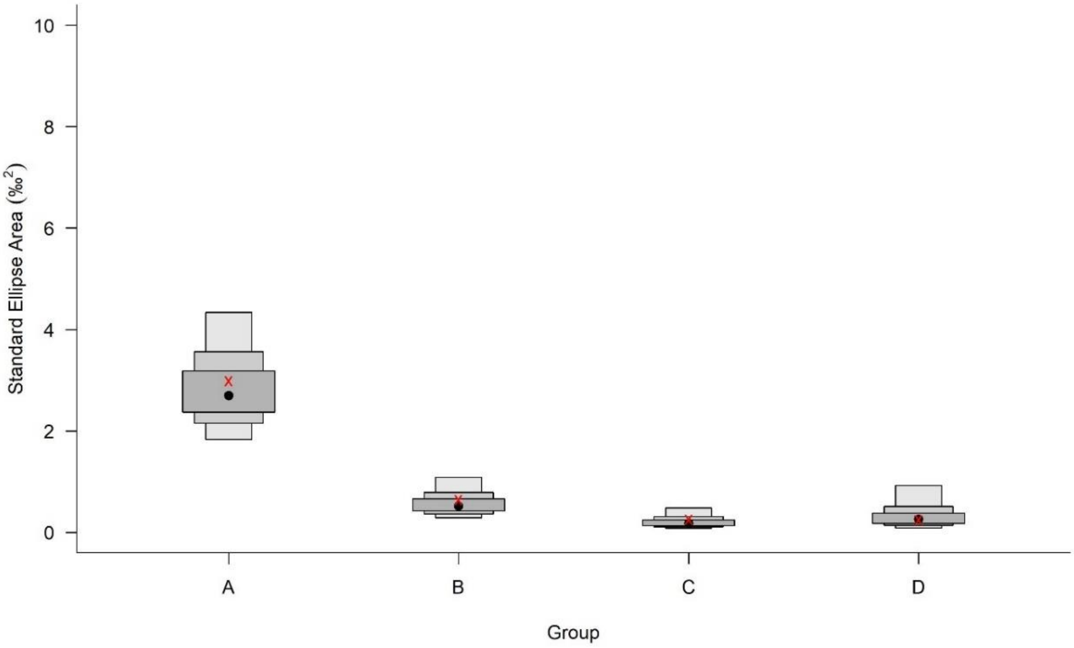


Figure 3.10. Distribution of the standard ellipse areas, based on 10^6 Bayesian model runs, depicting isotopic niche width of different social groups. Black dots represent the mode, while the shaded boxes represent the 50, 75 and 95 % credible intervals from dark to light grey. Red crosses represent the SEA_C estimates based on maximum likelihood.

3.3.4 Isotopic niches of sexes

The isotopic niches between females and males overlapped (Fig. 3.11, Table 3.4, SEA_C overlap = 0.389), but females appear to have an overall wider niche (Fig. 3.12). Table 3.4 shows the TA, SEA and SEA_C values for each sex.

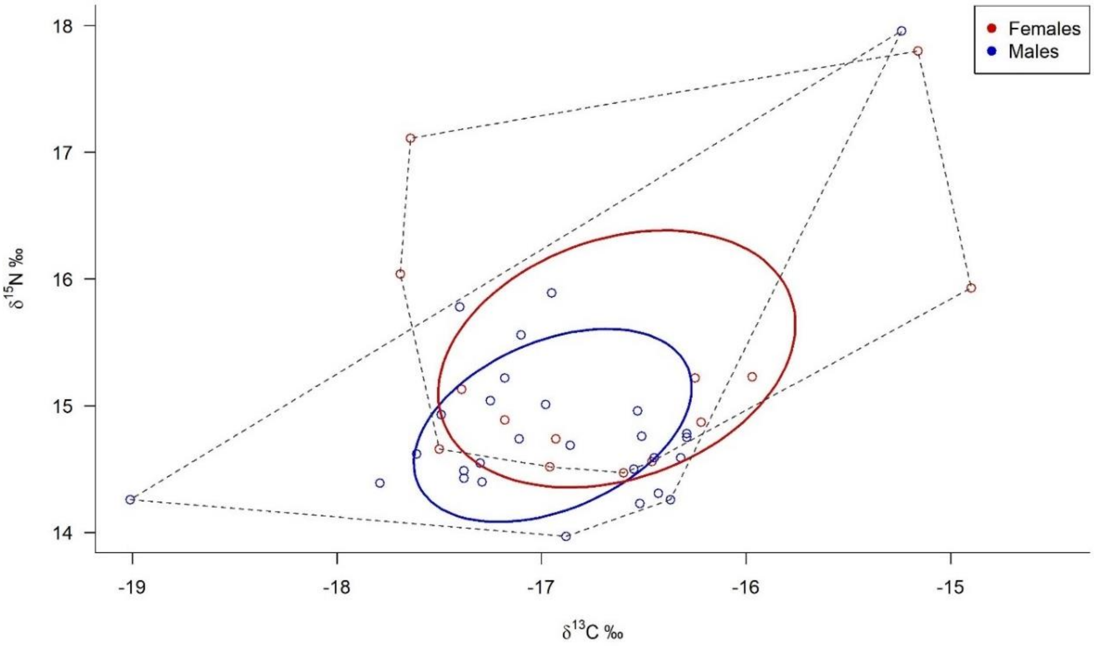


Figure 3.11. Isospace of $\delta^{15}N$ and $\delta^{13}C$ of females and males. The ellipses represent Standard Ellipse Areas (SEA$_C$), encompassing 40 % of the data for each sex, while the dotted lines represent the convex hulls (total area, TA) acompassing all data points (empty circles) for each sex.

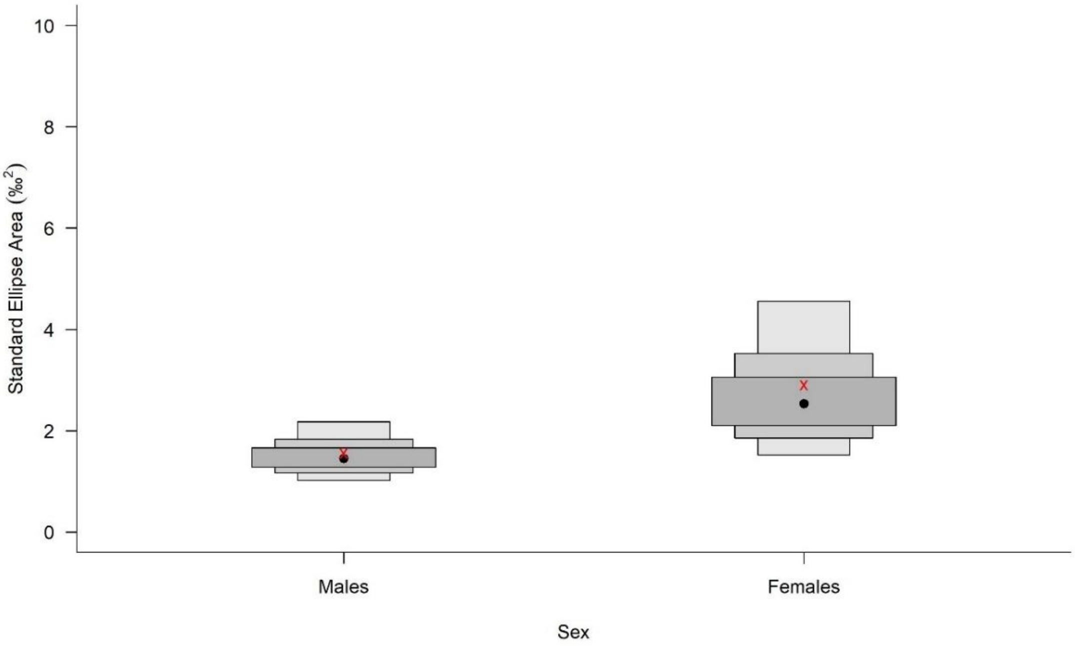


Figure 3.12. Distribution of the standard ellipse areas, based on 10^6 Bayesian model runs, depicting isotopic niche width of both sexes. Black dots represent the mode, while the shaded boxes represent the 50, 75 and 95 % credible intervals from dark to light grey. Red crosses represent the SEA_C estimates based on maximum likelihood.

3.4 DISCUSSION

3.4.1 Potential caveats, limitations and strengths

Although the attempt was made to sample dolphins as representatively as possible across sexes, social groups, seasons and years, this could not be completely achieved. Biopsy sampling of free-ranging delphinids is difficult; likely much more so than biopsy sampling of large whales, simply as a function of size. In order for samples to be obtained, several conditions need to become aligned, including favourable weather (both for survey to happen in the first place, and for biopsy sampling to happen in case the animals are found), animals being found, the "cooperativeness" of the animals, group size (in this study area at least, the difficulty of approaching dolphins close enough for biopsy sampling tends to increase with decreasing group sizes, T. Genov, *personal observation*), group composition (presence of calves may preclude sampling) and other logistical considerations (for example, biopsy sampling was not attempted when other boats were present during high season). Furthermore, if not done correctly, biopsy sampling can have detrimental consequences (Bearzi 2000). This is why all care was taken to conduct biopsy sampling safely, responsibly and to minimise any adverse effects as much as possible (as described in the Methods). In case of unfavourable conditions or in case of doubt (e.g., whether or not an animal is accompanied by another in its slipstream), sampling was not attempted. Moreover, in a few cases (e.g., in the case reported by Genov et al. 2016), the target animal was judged to be in poor body condition and therefore considered not suitable for sampling. As a result of all these factors, the samples are not equally distributed across sexes, social groups or various time periods (Table 3.1). For example, the number of samples for males was double that for females. Males were not preferentially targeted over females, and several animals were of unknown sex at the time of sampling. Nevertheless, the skewed sex ratio is likely driven by the fact that females with accompanying calves were not sampled. Likewise, sample sizes across social groups were highly uneven and therefore some of the results, particularly those related to groups C and D, need to be treated with caution. Still, the sample sizes across groups A and B, which were of primary interest for this work, were relatively large, although not completely even (Table 3.1). This may be partly attributed to differences in how these two social groups use their habitat, and consequently their availability for sampling. As described in Chapter 2, group A was typically encountered during early parts of the day, whereas group B was typically encountered in later parts of the day. This often led to the fact that focal follows of group B were cut short by nightfall (Chapter 2), thereby limiting the opportunities and time available for biopsy sampling.

Controlled experiments with common bottlenose dolphins in captive settings have estimated half-time turnover rates in skin to be 48 ± 19 days for nitrogen and 24 ± 8 days for carbon (Giménez et al. 2016). The reported isotopic values in this study are therefore expected to represent the diet in the four to six weeks prior to sampling and cannot necessarily be generalised to broader time periods.

Even though assessing the diet (Hooker et al. 2001, Kiszka et al. 2014, Borrell et al. 2021) of the social groups and of the population as a whole is one of the overall long-term objectives of the approach described here, the goal for this specific study was to determine the presence or absence of any dietary differences among social groups. The goal at this stage was neither to determine the diet itself, nor to assess prey composition. While various potential prey species from the area have already been sampled and some preliminary results are available (T. Genov and M. Vighi, unpublished data), a large sample size with greater species variability is needed to describe the diet with some confidence. Given that the stable isotope mixing models partition the observed variability among the candidate prey species (Borrell et al. 2021), the candidate set of species should be large enough and representative enough to be meaningful.

This study provides insight into intra-population differences in isotopic niche, using a relatively large overall sample size for a study of this type. Being able to link information on stable isotopes with that collected from observations of known individuals in the field was particularly valuable for interpreting isotope patterns (Jourdain et al. 2020) and integrating that knowledge with other studies (Chapter 2).

Stable isotopes are often used to distinguish among populations and conservation units (Giménez et al. 2018), but as alluded to in the introduction, understanding among-individual variation in behaviour and diet is beneficial in many ways, and may help better understand not only drivers of potential resource partitioning (or lack thereof), but also the consequences of it.

3.4.2 Drivers of stable isotope ratios

The results are largely consistent with what could be expected based on the knowledge of this population, the characteristics of the study area, and about isotopic ecology in general. Lack of strong differences between sexes are to be expected, because common bottlenose dolphins are highly social animals (Shane et al. 1986, Wells 2003, Lusseau et al. 2006) that share habitats and often forage cooperatively (Gazda et al. 2005, Daura-Jorge et al. 2012). Most groups encountered in this area are of mixed sex and were observed foraging together (Chapter 2). It is nevertheless interesting to see that sex was identified as an important variable in the $\delta^{15}N$ models, suggesting that some differences may be present after all. Such differences are more likely for species with different life history, social structure and movement patterns, such as sperm whales (Pirotta et al. 2020).

The fact that the observed patterns of $\delta^{15}N$ and $\delta^{13}C$ ratios were largely driven by different explanatory variables (Table 3.2) is also consistent with general patterns for stable isotopes of these two elements in marine ecosystems. $\delta^{15}N$ is generally thought to increase with increasing trophic level (Newsome et al. 2010), even across large geographical scales (Vanderklift & Wernberg 2010), although a large part of the variation is also related to nitrogen excretion mechanisms and nutritional status (Vanderklift & Ponsard 2003, Montoya 2008, Newsome et al. 2010). It is therefore not surprising that the main variables identified by the $\delta^{15}N$ models included social group and season (in addition to sex, discussed above). The northern Adriatic Sea features considerable spatial and temporal variability in nutrient input and productivity (Fonda Umani et al. 2005, Mozetič et al. 2010, Mozetič et al. 2012) and local fishermen regularly point out that the presence of various fish stocks varies though the year. It is therefore to be expected that dolphins feed on different prey at different times of the year, which is likely reflected in the observed seasonal patterns in the isotope ratios.

The variable social group was most supported as explaining the variation in $\delta^{15}N$ ratios. Even though the standard ellipse area of group A overlapped with other groups, it appears to feed at a generally higher trophic level. The influence of social group in the model is consistent with the differential behaviour of these dolphins with respect to fisheries (Chapter 2). But it is interesting to note that group A (trawler dolphins) was in fact the most generalist one, as it had the widest isotopic niche width (Fig. 3.10), indicating the most diverse diet. This suggests that the group most consistently associated with foraging behind trawlers was not the most specialised, but rather the least specialised, at least with respect to target prey species. However, it should be noted that a behavioural specialisation is not necessarily a dietary specialisation, as interactions with fisheries (even if a highly specialised form of foraging) may still lead to a wide prey base. It may also be that the wide niche for group A reflects differences in diet during different parts of the year.

Within oceanic basins, higher $\delta^{13}C$ values are often associated with productive nearshore regions, whereas lower values are often associated with less productive offshore regions, although specific habitat types also play a role (Newsome et al. 2010). $\delta^{13}C$ values therefore indicate the general habitat of the consumer, such as inshore-neritic vs offshore-oceanic. The lack of differences in $\delta^{13}C$ values among groups is not surprising, because the entire northern Adriatic is essentially a shelf sea and therefore represented by neritic habitats. This appears to explain why social group was an important variable in the $\delta^{15}N$ models, but not in $\delta^{13}C$ models (Tables 3.2 and 3.3). The lack of variation in $\delta^{13}C$ among social groups supports the notion that they generally utilize the same types of habitat despite temporal differences in how they use it.

Overall, these results may reflect different underlying prey preferences among social groups, differences that arise due to behavioural specialisations, temporal variations in prey availability, or all of these factors. These results also suggest an overall generalist population composed of groups and individuals that vary substantially in their diet, as has been shown, for example, for killer whales around Iceland (Samarra et al. 2017). Insights into intra-population variation in isotopic niche is useful for understanding evolutionary mechanisms shaping populations and species. Ecological variation and specialisation have been suggested to be important in promoting genetic divergence in bottlenose dolphins (Louis et al. 2014). The fine-scale genetic structure of this population is not yet understood, but a study investigating this is currently underway (T. Genov, S. Marfurt and M. Krützen, unpublished data) and may help place the results from this chapter and Chapter 2 into proper context. It would also be interesting to see intra-individual variation over longer time scales (Samarra et al. 2017), but this would require repeated sampling of the same individuals through time.

Being a specialist or generalist may predispose the animals to varying levels of impacts of fisheries. More generalist animals may cope better with potential effects of overfishing or other anthropogenic influences on the food web in the long term. For the time being, there is no indication of either of the two large social groups having any fitness benefits of their differing behavioural and habitat use strategies. Perhaps improving the knowledge about potential dietary differences or preferences may help mitigate against certain detrimental fisheries management scenarios in the future. Likewise, it may also help better understand the role of diet in the susceptibility of individuals to persistent organic pollutants, although the demonstrated dietary differences in this population do not appear to translate into differing pollutant loads (Chapter 4).

CHAPTER 4: LINKING ORGANOCHLORINE CONTAMINANTS WITH DEMOGRAPHIC PARAMETERS IN FREE-RANGING COMMON BOTTLENOSE DOLPHINS FROM THE NORTHERN ADRIATIC SEA[2]

4.1 INTRODUCTION

Persistent organic pollutants (POPs) are chemical compounds that occur in the marine environment and have far-reaching consequences for human and ecosystem health. Marine top predators, including marine mammals, are known to bioaccumulate POPs, which represent a conservation and health concerns for these species and their environment (Tanabe et al. 1994, Aguilar et al. 2002, Vos et al. 2003, Jepson & Law 2016). Of these, organochlorines such as polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) are of particular concern, as they are persistent in the environment, highly lipophilic, bioaccumulate in individuals over time, and biomagnify in marine top predators through trophic transfer (Green & Larson 2016). These toxic compounds may cause anaemia (Schwacke et al. 2012), immune system suppression (Tanabe et al., 1994) and the subsequent increased vulnerability to infectious disease (Aguilar & Borrell 1994a, Jepson et al. 2005, Randhawa et al. 2015), endocrine disruption (Tanabe et al. 1994, Vos et al. 2003, Schwacke et al. 2012), reproductive impairment (Schwacke et al. 2002) and developmental abnormalities (Tanabe et al. 1994, Vos et al. 2003) in marine mammals, thereby representing a serious health risk for these top predators. Such health risks are likely to have direct impacts on marine mammal abundance, through reduced reproduction or survival (Hall et al. 2006, Hall et al. 2017). Because of their trophic position, propensity for bio-accumulating organochlorines, and long life span, marine mammals are often considered ecosystem sentinels (Ross 2000, Wells et al. 2004, Moore 2008).

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[2] The results of this chapter have been published in:
Genov T., Jepson P.D., Barber J.L., Hace A., Gaspari S., Centrih T., Lesjak J., Kotnjek P. 2019. Linking organochlorine contaminants with demographic parameters in free-ranging common bottlenose dolphins from the northern Adriatic Sea. *Science of the Total Environment* 657: 200-212. https://doi.org/10.1016/j.scitotenv.2018.12.025

Due to concerns about toxicity and suspected carcinogenicity to humans, their effects on biota and environmental persistence, the use of PCBs and OCPs such as dichlorodiphenyltrichloroethane (DDT) was banned in most of Europe in the 1970s-1980s. Subsequent monitoring of POPs in tissues of several marine mammal species demonstrated their decline in several European seas (Law et al. 2012), including the Mediterranean Sea (Aguilar & Borrell 2005, Borrell & Aguilar 2007). However, a recent European-wide study showed that PCB levels continue to be high in European and Mediterranean cetaceans (Jepson et al. 2016). In particular, very high PCB concentrations were linked to small populations, range contraction, or population declines in some striped dolphin (*Stenella coeruleoalba*), common bottlenose dolphin (*Tursiops truncatus*) and killer whale (*Orcinus orca*) populations (Jepson et al. 2016).

Linking organochlorine concentrations with individual-level effects in wild marine mammals (and especially cetaceans) is challenging at best, while linking them with potential population-level effects is extremely difficult. It is therefore unsurprising that few quantitative approaches for estimating such effects have been developed (Hall et al. 2017). Stranded animals can be a valuable source of samples for pollutant studies in wild populations (Geraci & Lounsbury 2005), and are often the only source of samples used in toxicological analysis (Jepson et al. 1999, Jepson et al. 2005, Law et al. 2012). However, the use of stranded animals, especially in some contexts or in some locations, may introduce substantial biases. For example, stranded animals may not be representative of the population or area of interest, but may originate from other areas, due to winds, currents, or abnormal behaviour prior to stranding (Hansen et al. 2004). Moreover, putrefaction processes, resulting from exposure to the sun, high temperatures, wind and bacterial activity, can lead to altered organochlorine concentrations and potentially misleading results (Borrell & Aguilar 1990). Finally, it has also been suggested that the presence of disease may lead to abnormal rates of pollutant metabolism or excretion (Borrell & Aguilar 1990). On the other hand, blubber biopsy samples (Noren & Mocklin 2012) collected from live, free-ranging cetaceans offer a good alternative for evaluating the toxicological burden of populations (Fossi et al. 2000), especially when linked to long-term re-sighting histories of known individuals (Ross et al.

2000, Ylitalo et al. 2001, Wells et al. 2005). For example, information on pollutant levels can be combined with mark-recapture techniques to estimate the impact of contaminants on survival or reproduction (Hall et al. 2009). Moreover, an appropriate study design can ensure that the sampling is representative of the population or area in question. It was previously recognised that the proper evaluation of pollutants on marine mammals will require efforts directed toward long-term studies of known individuals in wild populations (Hall et al. 2006).

The common bottlenose dolphin is a long-lived marine top predator (Wells & Scott 1999, 2009). In many parts of the world, including the Mediterranean Sea, it is essentially ‘coastal’ and mainly found nearshore (Bearzi et al. 2009). This makes it particularly susceptible to a range of anthropogenic impacts, including the exposure to organochlorine contaminants. This species is regularly present in the Gulf of Trieste and adjacent waters, where it has been continuously studied since 2002 (Genov et al. 2008, Genov et al. 2016). As a coastal, mobile and long-lived top predator with strong site fidelity, it is a particularly good candidate for investigating the effects of organochlorine contaminants, and for regional monitoring of organochlorine pollution.

Chapter 2 described peculiar behavioural and temporal partitioning among dolphin social groups. In Chapter 3, this partitioning was found to be related to differences in isotopic niche width. Given this, there is potential for these differences to be reflected in contaminant loads. This study evaluated organochlorine levels, particularly PCBs, in free-ranging common bottlenose dolphins in relation to demographic parameters, by testing for the effects of sex, parity and social group membership on organochlorine concentrations.

4.2 METHODS

4.2.1 Sample collection

Biopsy samples were collected from free-ranging common bottlenose dolphins between 2011 and 2017. Details about sampling protocols and steps taken to minimise adverse impacts are described in Chapter 3. All biopsy attempts were accompanied by concurrent photo-identification (Würsig & Jefferson 1990) of targeted individuals and other dolphins in their group. This ensured that the identity of the sampled animal was known, in order to prevent re-sampling the same individuals, and to be able to link organochlorine concentrations to various individual-specific parameters known from photo-identification.

In addition to biopsies, one sample was collected from an adult male found entangled in fishing gear – due to the freshness of the carcass, it could be identified with confidence, determined to be one of the local dolphins, and therefore included in the analysis. Stranded animals too decomposed to be identified were not included in the analyses, as they were of unknown origin and may not be representative of the population in question.

4.2.2 Demographic parameters

As in Chapter 3, sex was determined by observations of mother-offspring pairs, photographs of the genital area and molecular methods from biopsy samples (Gaspari et al. 2015).

Parity was assessed based on re-sighting histories and reproductive output of photo-identified females. Females known to have produced at least one offspring during the study period were considered parous. Females never observed with offspring were assumed to be nulliparous. One of these females appeared older based on external appearance, and could potentially be of post-reproductive age, although evidence for reproductive senescence in bottlenose dolphins is limited (Marsh & Kasuya 1986, Wells & Scott 1999, Ellis et al. 2018).

Previous work on social network analyses (Chapter 2) has shown that the local dolphin population is structured into distinct social groups, which exhibit temporal partitioning, differences in behaviour with respect to fisheries and have different dietary niches (Chapter 3).

4.2.3 Chemical analysis

Blubber samples were stored frozen at –20.0 °C. Samples were analysed at the Centre for Environment, Fisheries and Aquaculture Science (CEFAS, UK), using the methods reported in detail in Jepson et al. (2016). In brief, samples were subjected to Soxhlet extraction using of acetone: *n*-hexane 1:1 (*v:v*) and cleaned up and fractionated using alumina (5% deactivated) and silica (3% deactivated) columns, respectively. The total extractable lipid content was determined gravimetrically after evaporation of the solvent from an aliquot of the uncleaned extract. Lipid content varied from 3.4 to 33.8%. PCB concentrations in dolphin samples were determined with an Agilent 6890 GC with µECD. The PCB standard solutions contained the following 27 compounds in iso-octane: Hexachlorobenzene; *p,p'*-DDE; CB101; CB105; CB110; CB118; CB128; CB138; CB141; CB149; CB151; CB153; CB156; CB158; CB170; CB18; CB180; CB183; CB187; CB194; CB28; CB31; CB44; CB47; CB49; CB52; CB66, together with the internal standard CB53. Quantification was performed using internal standards and 11 calibration levels (range 0.5 – 400ng/ml). CEFAS follows a strict QA/QC regime for analysis of samples. The laboratory biannually participates in proficiency testing scheme Quasimeme (Quality Assurance of Information for Marine Environmental Monitoring in Europe) as external quality assurance. All analyses were carried out under full analytical quality control procedures that included the analysis of a certified reference material (BCR349 cod liver oil; European Bureau of Community reference) and a blank sample with every batch samples analysed so that the day-to-day performance of the methods could be assessed. Wet weight analyte concentrations were converted to lipid-normalised concentrations using measured lipid contents. Values below the limit of quantification (LOQ) were reported as <LOQ. LOQs are conservatively set at the lowest calibration standard concentration normalised to the sample multiplier (which varies depending on sample size and lipid content), which gives higher values than the alternative approach based on a S/N ratio of 10 would allow. In addition to the compounds mentioned above, four samples (two males, one female and one animal of unknown sex) were also analysed for *p,p′*-TDE (also known as *p,p'*-DDD) and *p,p′*-DDT. The limited budget available for analysis prevented this being done for the entire sample set.

4.2.4 Statistical analysis

For statistical analysis, congener concentrations below the limit of quantification (LOQ) were set to one-half of the LOQ (Darnerud et al. 2006, Lignell et al. 2009, Law et al. 2012). This approach of treating <LOQ values was compared with two alternative approaches: 1) replacing <LOQ values with zero and 2) keeping <LOQ values at the LOQ value. The choice of the approach had negligible effect on the results, and had no effect on conclusions. This approach was therefore considered the best compromise between underestimating and overestimating toxicological burden.

The values of individual 25 PCB congeners for each sample were summed to obtain the Σ25PCB for each individual. In addition, the sum of priority PCB congeners (28, 52, 101, 118, 138, 153 and 180) listed by the International Council for the Exploration of the Sea (ICES) was also calculated and displayed, for ease of comparison with some of the previous studies. The lipid content of each sample was used to obtain concentrations as mg/kg lipid weight (mg/kg lw).

Tests of normality revealed non-normal distribution of data. Both arithmetic and geometric means across individuals were calculated for Σ25PCB, ΣICES7 and *p,p'*-DDE. HCB values were too low (below the limit of quantification) to allow any useful analysis (Table 4.1). The contribution of each individual PCB congener to the Σ25PCB was also calculated across all individuals.

Effects of sex, parity and social group membership on contaminant concentrations was tested. The Mann-Whitney U test was used to examine differences between males and females, and between apparently nulliparous and parous females. The Kruskal-Wallis test was used to examine differences among social groups. Statistical analysed were carried out in program R (R Core Team 2020).

4.2.5 Assessing toxicity

Two PCB toxicity thresholds or reference values were used, following Jepson et al. (2016). A lower PCB toxicity threshold was used for the onset of physiological endpoints in marine mammals of 17 mg/kg lipid weight (lw) (as Aroclor 1254, Kannan et al. 2000), which was calculated to be equivalent to 9.0 mg/kg lw (Σ25PCB) in Jepson et al. (2016) and in this study. A higher PCB toxicity threshold, the highest reported in marine mammal toxicology studies, of 77 mg/kg lw (as Clophen 50) for reproductive impairment in Baltic ringed seals (*Pusa hispida*, Helle et al. 1976) was calculated to be equivalent to 41 mg/kg lw (as Σ25PCB) in Jepson et al. (2016) and in this study.

4.3. RESULTS

Between 2011 and 2017, samples were obtained from 32 adult dolphins, including 18 males, 9 females and 5 animals of unknown sex (Table 4.1). Six of these samples were included in the study by Jepson et al. (2016). Six females were previously observed with offspring, while three were not.

4.3.1 PCBs

Σ25PCB ranged from 4.13 to 293 mg/kg lipid weight, with an arithmetic mean of 81.5 (95% CI = 57.2 – 105.8) and a geometric mean of 53.4 (95% CI = 36.9 – 77.3, Table 2). Males had significantly higher Σ25PCB concentrations than females (Mann-Whitney U = 155, $P < 0.001$, Fig. 4.3). Furthermore, nulliparous females had significantly higher concentrations than parous ones (Mann-Whitney U = 17, $P < 0.05$, Fig. 4.4). There were no statistically significant differences among social groups (Kruskal-Wallis H = 1.21, P = 0.75, Fig. 4.5).

Figure 4.6 shows female and male PCB concentrations in relation to two toxicity thresholds. Overall, 87.5% of dolphins had PCB blubber concentrations above the toxicity threshold of 9 mg/kg lw for physiological effects in experimental marine mammal studies (Kannan et al. 2000), while 65.6% had concentrations above the highest threshold (41 mg/kg lw) published for marine mammals based on reproductive impairment in ringed seals (Helle et al. 1976). In males, mean Σ25PCB were above the higher of the two thresholds, even when the lower confidence limit is considered (Fig. 6). One male had a Σ25PCB concentration of 293 mg/kg lw. In females, mean Σ25PCB were above the lower toxicity threshold of 9 mg/kg lw, but did not reach the higher one of 41 mg/kg lw, not even when the upper confidence limit is considered (Fig. 4.6). The lower confidence limit of ΣPCB in females was just below the lower toxicity threshold (Fig. 4.6). The ΣICES7 concentrations follow a similar pattern and are presented in Tables 4.1 and 4.2.

Among dioxin-like PCBs, these represented 2.3% (PCB 118, found in 90.6% of samples), 0.8% (PCB 156, found in 75% of samples) and 0.7% (PCB 105, found in 75% of samples) of the total PCB burden, respectively. Concentrations of the PCB congener 28 was below LOQ for all samples. PCB congeners 153, 138, 180, 187, 149 and 170 had the highest mean values across individual dolphins (Table 4.3, Fig. 4.7). Combined, they contributed 77.9% of the total PCB burden. Congeners 44, 31, 28, 18, 141, 49 and 110 had the lowest mean values, with a combined contribution of 2.2% to the total PCB burden (Table 4.3, Fig. 4.7).

4.3.2 DDE and DDT

The concentrations of *p,p'*-DDE ranged from 0.3 to 32.9 mg/kg lw, with an arithmetic mean of 11.6 (95% CI = 8.3 – 14.8) and a geometric mean of 6.7 (95 % CI = 4.2 – 10.7, Table 4.2). As with PCBs, males had significantly higher *p,p'*-DDE concentrations than females (Mann-Whitney U = 152, $P < 0.001$, Table 4.2), and nulliparous females had significantly higher concentrations than parous ones (Mann-Whitney U = 18, $P < 0.05$). Like for PCBs, there were no statistically significant differences among social groups (Kruskal-Wallis H = 1.15, P = 0.76). The values of total DDT (the sum of *p,p'*-DDE, *p,p'*-TDE and *p,p'*-DDT) for four individuals are shown in Table 4.1. For these four samples, the mean contribution of *p,p'*-DDE to total DDT was 89.7% (range = 83.9 – 92.6%), showing that *p,p'*-DDE is the predominant metabolite of total DDT.

4.3.3 HCB

Most HCB values were below the limit of quantification (Table 4.1). Using half the LOQ for calculations, the HCB concentrations ranged from 0.03 to 0.22 mg/kg lw, with an arithmetic mean of 0.09 (95% CI = 0.08 – 0.12) and a geometric mean of 0.09 (95% CI = 0.07 – 0.10, Table 2). Due to these low values, no further analysis was carried out on HCB concentrations.

Table 4.1. Summary of common bottlenose dolphin samples from the Gulf of Trieste (northern Adriatic Sea), analysed in this study. F = female, M = male. Parity is indicated by + (parous) and – (nulliparous). Σ25PCB, ΣICES7, *p,p'*-DDE, DDT and HCB values expressed as mg/kg lipid weight. DDT represents total DDT. LOQ = Limit of quantification. The "<" indicates that the concentration was below the LOQ.

Sample	**Year**	**Sex**	**Parity**	**Source**	**% Lipid**	**Σ25PCB**	**ΣICES7**	***p,p'*-DDE**	**ΣDDT**	**HCB**	**LOQ**
1	2011	M		Biopsy	23.3	64.2	40.9	9.03		<0.098	0.098
2	2011	M		Biopsy	9.7	80.2	50.9	11.3		<0.144	0.144
3	2011	M		Biopsy	16.2	58.7	37.1	8.02		<0.166	0.166
4	2011	M		Biopsy	11.7	139.8	94.8	13.7		0.102	0.071
5	2011	M		Biopsy	19.5	293	190	32.9		0.128	0.066
6	2011	F	+	Biopsy	17.5	29.0	14.9	1.54		<0.091	0.091
7	2013	M		Biopsy	15.2	34.2	21.2	4.49		<0.197	0.197
8	2013	F	+	Biopsy	12.9	7.96	3.96	0.44		<0.341	0.341
9	2013	F	+	Biopsy	10.9	17.9	9.89	0.95		<0.202	0.202
10	2013	M		Biopsy	3.4	23.0	14.4	2.67		<0.414	0.414
11	2014	F	–	Biopsy	10.5	27.2	17.5	9.41		<0.208	0.208
12	2014	F	+	Biopsy	27.9	4.13	2.12	0.25		<0.093	0.093
13	2014	M		Biopsy	6.6	32.2	20.2	16.7		<0.441	0.441
14	2014	M		Biopsy	13.5	43.7	27.0	5.51		<0.228	0.228
15	2014	M		Biopsy	6.9	56.7	35.6	7.72		<0.305	0.305
16	2014	M		Biopsy	23.9	123	81.2	17.5		<0.092	0.092
17	2014	F	–	Biopsy	19.3	30.7	19.2	4.25		<0.124	0.124
18	2014	F	–	Biopsy	33.8	48.9	31.0	6.45		<0.141	0.141
19	2014	M		Biopsy	10.1	131	84.8	21.9		<0.217	0.217
20	2014	M		Biopsy	18.8	65.9	40.7	9.55		<0.333	0.333
21	2014	M		Biopsy	9.3	93.8	60.9	13.5		<0.139	0.139
22	2014	M		Biopsy	14.5	76.8	48.8	10.1		<0.200	0.200
23	2015	M		Bycatch	6.6	152	96.5	25.9		<0.166	0.166
24	2015	M		Biopsy	7.9	111	74.2	16.0	17.3	<0.164	0.164
25	2015	U		Biopsy	7.7	58.3	37.8	8.17		0.195	0.128
26	2016	U		Biopsy	13.7	145	96.6	20.3	22.04	<0.080	0.080
27	2016	F	+	Biopsy	14.4	6.82	3.88	0.54	0.54	<0.104	0.104
28	2016	M		Biopsy	4.4	121	80.3	16.7	18.6	<0.215	0.215
29	2016	U		Biopsy	11.3	150	98.2	23.5		<0.194	0.194
30	2017	U		Biopsy	18.9	157	102	23.5		<0.106	0.106
31	2017	U		Biopsy	11.8	219	144	27.2		<0.126	0.126
32	2017	F	+	Biopsy	25.3	7.64	4.37	0.47		<0.059	0.059

Table 4.2. Σ25PCB, ΣICES7, *p,p*'-DDE and HCB concentrations by sex: mean, median, geometric mean with 95% confidence interval, and range. All values are in mg/kg lipid weight. “Mean” is arithmetic mean. “Geomean” is geometric mean.

Σ25PCB	**N**	**Mean**	**Median**	**Geomean**	**Geomean 95% CI**	**Range (min–max)**
Males	18	94.5	78.5	78.3	58.3 – 105.1	23.0 – 293.0
Females	9	20.0	17.9	14.9	8.5 – 26.1	4.1 – 48.9
Unknown	5	145.7	150	134.1	87.0 – 206.7	58.3 – 219.0
OVERALL	**32**	**81.5**	**61.5**	**53.4**	**36.9 – 77.3**	**4.1 – 293.0**

ΣICES7						
Males	18	61.1	49.9	50.1	37.0 – 67.9	14.4 – 190.0
Females	9	11.9	9.9	8.5	4.6 – 15.4	2.1 – 31.0
Unknown	5	95.7	98.2	88.0	56.8 – 136.3	37.8 – 144.0
OVERALL	**32**	**52.7**	**39.3**	**33.2**	**22.4 – 49.1**	**2.1 – 190.0**

***p,p'*-DDE**						
Males	18	13.5	12.4	11.4	8.5 – 15.3	2.7 – 32.9
Females	9	2.7	0.9	1.3	0.6 – 3.1	0.3 – 9.4
Unknown	5	20.5	23.5	19.0	12.5 – 29.1	8.2 –27.2
OVERALL	**32**	**11.6**	**9.5**	**6.7**	**4.2 – 10.7**	**0.3 – 32.9**

HCB						
Males	18	0.11	0.1	0.1	0.08 – 0.12	0.05 – 0.22
Females	9	0.07	0.06	0.07	0.05 – 0.09	0.03 – 0.17
Unknown	5	0.09	0.06	0.08	0.04 – 0.13	0.04 – 0.20
OVERALL	**32**	**0.09**	**0.09**	**0.09**	**0.07 – 0.10**	**0.03 – 0.22**

Table 4.3. Summary statistics for individual PCB congeners. All values are in mg/kg lipid weight.

PCB congener	Mean	Median	SD	Min	Max	Geomean	Geomean 95% CI
C101	1.35	1.33	0.89	0.05	3.16	0.93	0.64 - 1.35
C105	0.42	0.39	0.27	0.03	0.94	0.32	0.23 - 0.43
C110	0.14	0.10	0.10	0.03	0.35	0.11	0.09 - 0.14
C118	1.57	1.48	1.05	0.05	4.10	1.09	0.75 - 1.57
C128	1.67	1.40	1.31	0.05	5.13	1.01	0.66 - 1.56
C138	14.64	11.05	12.47	0.48	51.33	8.86	5.83 - 13.47
C141	0.10	0.09	0.05	0.03	0.22	0.09	0.07 - 0.1
C149	5.83	4.56	5.51	0.15	27.72	3.42	2.2 - 5.31
C151	2.40	1.92	1.97	0.05	8.21	1.45	0.94 - 2.24
C153	24.30	16.89	21.43	0.76	92.40	14.53	9.55 - 22.11
C156	0.61	0.43	0.56	0.03	2.41	0.39	0.27 - 0.56
C158	0.81	0.64	0.65	0.03	2.77	0.52	0.35 - 0.77
C170	3.52	2.61	2.89	0.25	11.81	2.39	1.69 - 3.37
C18	0.09	0.08	0.05	0.03	0.22	0.08	0.07 - 0.09
C180	9.71	6.34	8.72	0.68	36.96	6.31	4.42 - 8.99
C183	2.25	1.67	1.81	0.15	7.19	1.51	1.06 - 2.15
C187	8.07	6.09	6.76	0.58	30.80	5.45	3.86 - 7.7
C194	1.45	1.31	1.09	0.17	4.47	1.05	0.78 - 1.43
C28	0.09	0.08	0.05	0.03	0.22	0.08	0.06 - 0.09
C31	0.09	0.08	0.05	0.03	0.22	0.08	0.06 - 0.09
C44	0.09	0.08	0.05	0.03	0.22	0.08	0.06 - 0.09
C47	0.57	0.55	0.42	0.03	1.51	0.38	0.26 - 0.56
C49	0.10	0.09	0.05	0.03	0.22	0.09	0.07 - 0.11
C52	0.99	0.91	0.76	0.03	2.71	0.6	0.39 - 0.92
C66	0.68	0.46	0.71	0.03	2.79	0.31	0.19 - 0.52

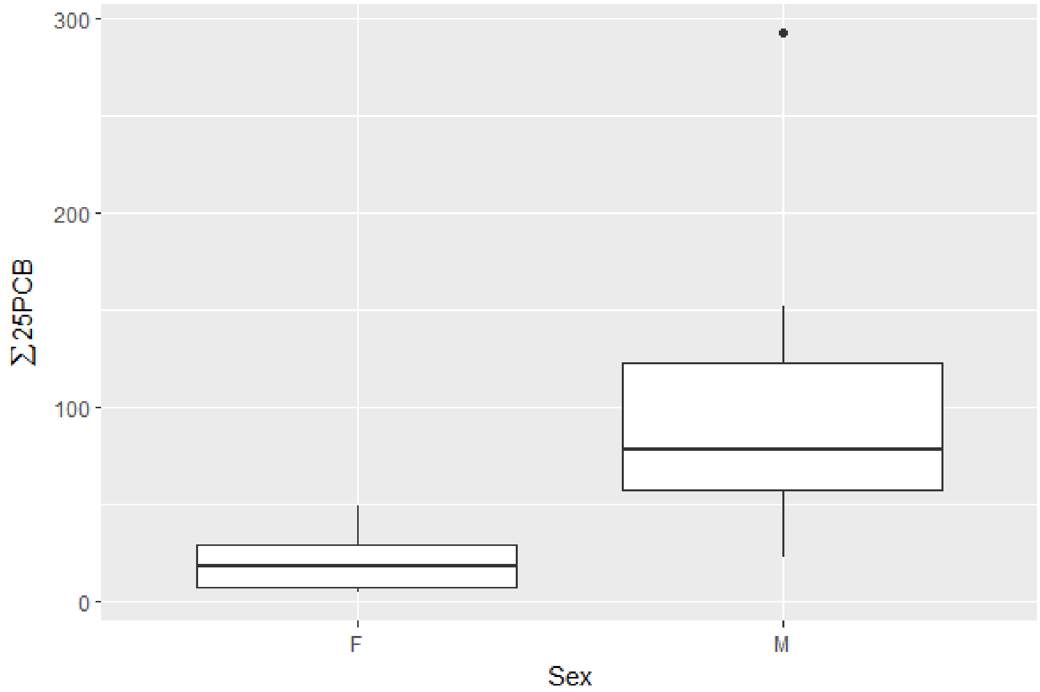

Figure 4.3. Boxplots showing differences in Σ25PCB concentrations (mg/kg lipid weight) between females (F, n = 9) and males (M, n = 18). The difference is statistically significant (Mann-Whitney U = 155, P < 0.001).

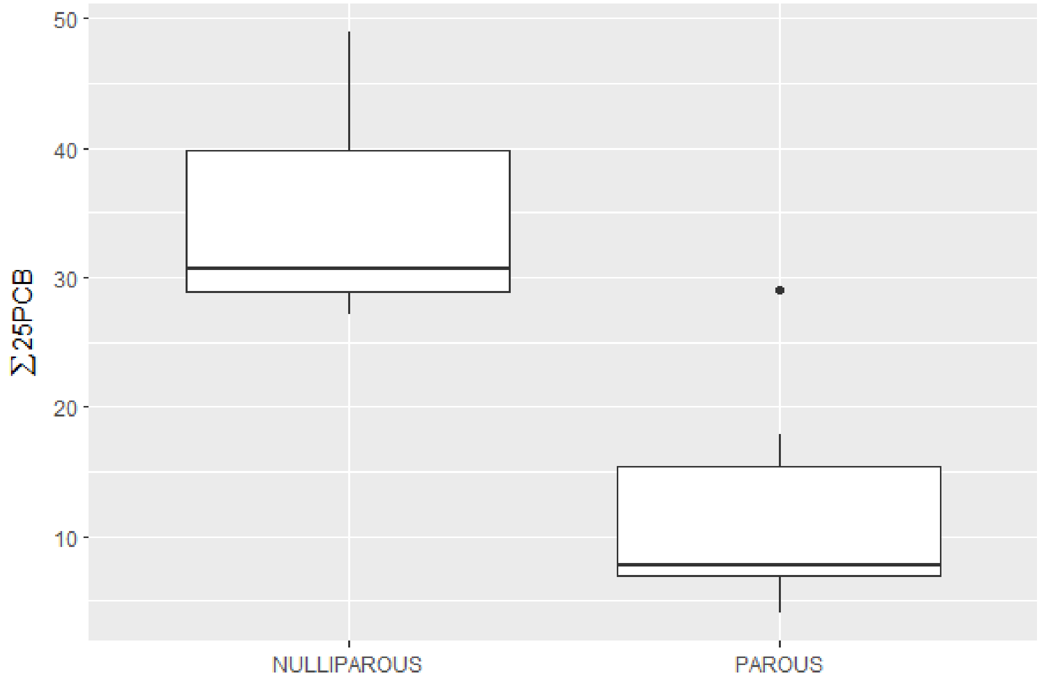

Figure 4.4. Boxplots showing differences in Σ25PCB concentrations (mg/kg lipid weight) between nulliparous (n = 3) and parous (n = 6) females. The difference is statistically significant (Mann-Whitney U = 17, P < 0.05).

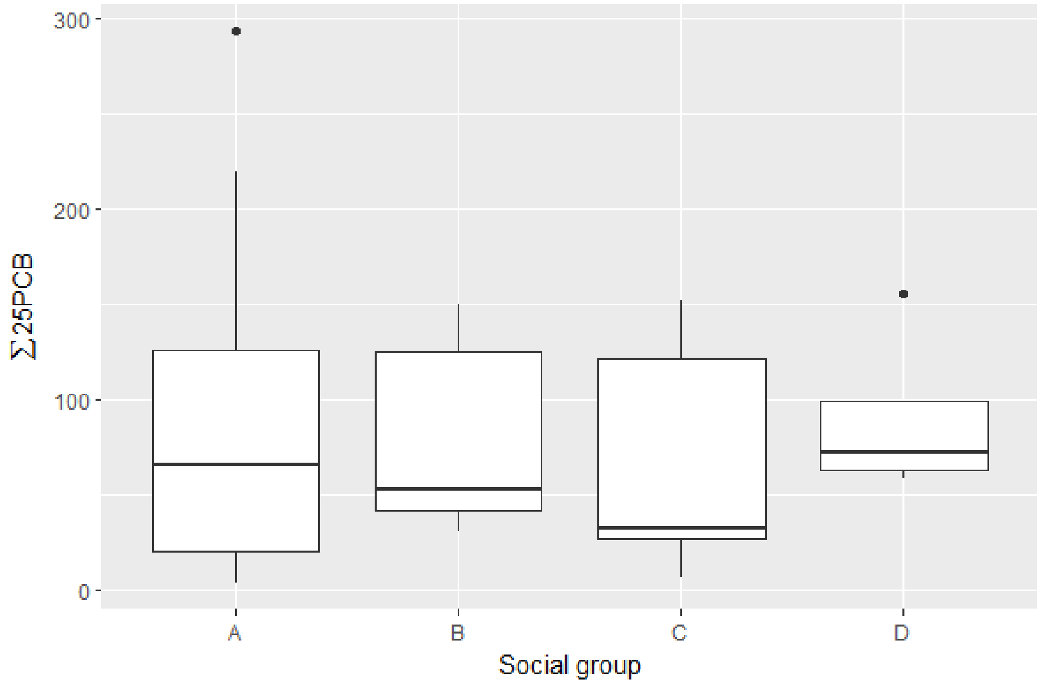

Figure 4.5. Boxplots showing differences in Σ25PCB concentrations (mg/kg lipid weight) among social groups A (n =15), B (n = 8), C (n = 5) and D (n = 4). Differences are not statistically significant (Kruskal-Wallis test, H = 1.24, P = 0.743).

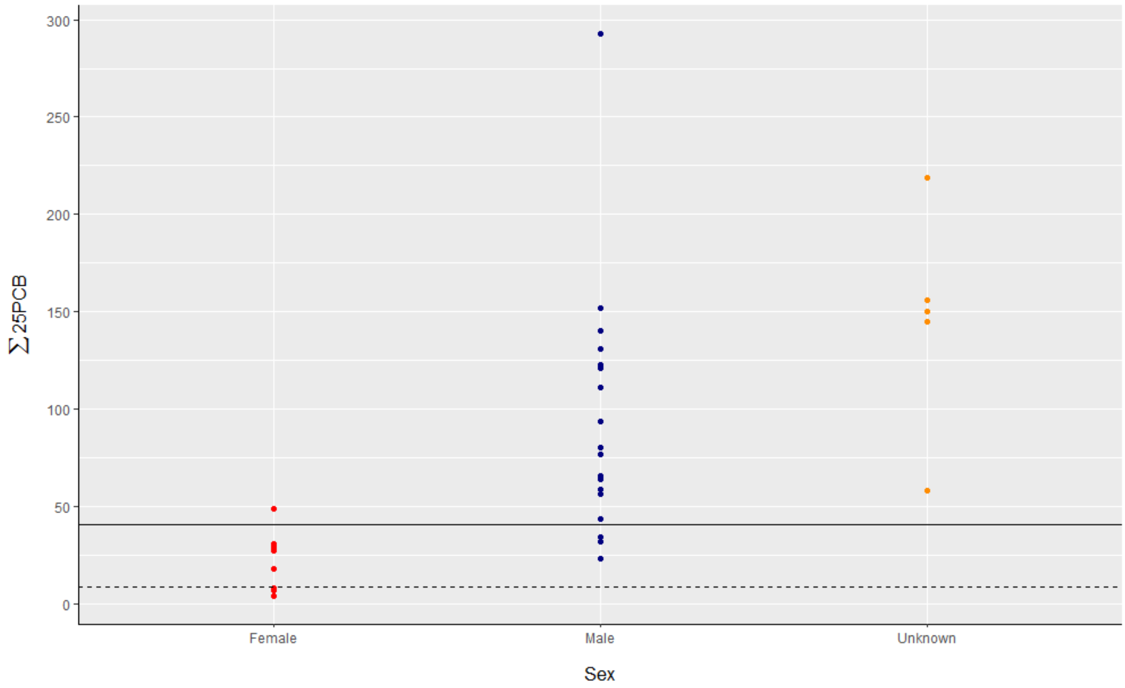

Figure 4.6. Σ25PCB (mg/kg lipid weight) concentrations for females (n = 9), males (n = 18) and unknown sex (n = 5), in relation to published toxicity thresholds. The lower dashed line represents the lower toxicity threshold (9 mg/kg lw) for onset of physiological effects in experimental marine mammal studies (Kannan et al. 2000). The solid

line represents the highest threshold (41 mg/kg lw) published for marine mammals based on reproductive impairment in ringed seals from the Baltic Sea (Helle et al. 1976).

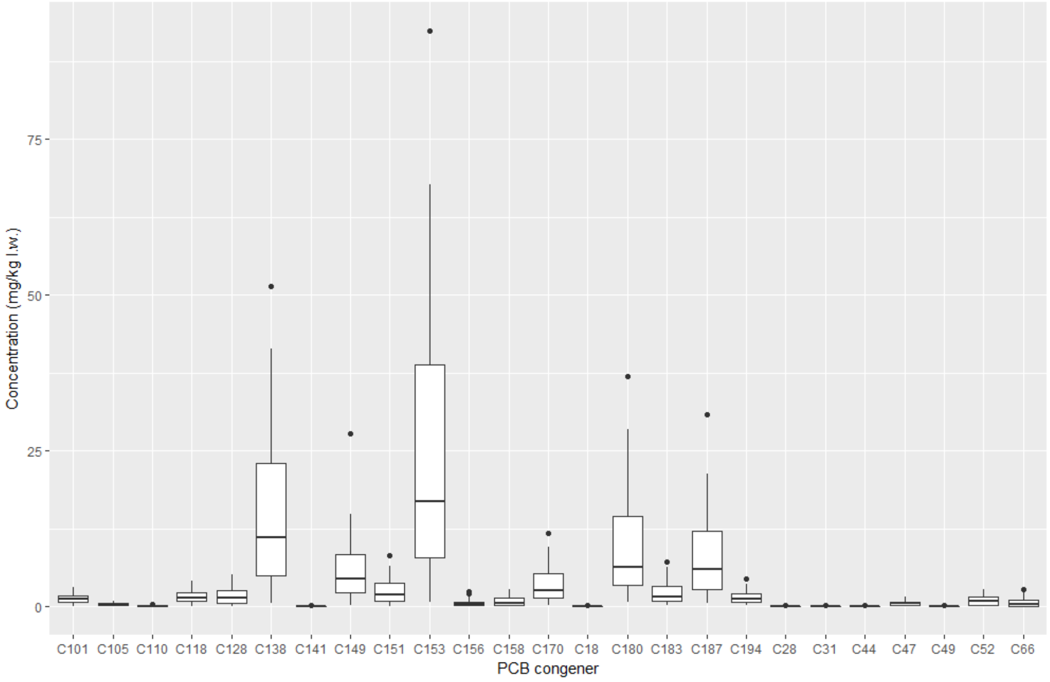

Figure 4.7. Contribution of individual PCB congeners to the total PCB burden.

4.4 DISCUSSION

4.4.1 General considerations

This study assessed the organochlorine levels in free-ranging common bottlenose dolphins from the Gulf of Trieste and adjacent waters in the northern Adriatic Sea. It shows that concentrations vary with sex and reproductive status, but not with social group membership. With the largest sample size analysed in the Adriatic Sea to date, and samples coming from live resident animals with known resighting histories, this study provides an unprecedented insight into the organochlorine burden in Adriatic dolphins. Judging from the literature, this may also represent the largest sample size of live free-ranging animals in the Mediterranean Sea or Europe published for this species to date, and is comparable to some of the world's largest sample sizes analysed (Table 4.4).

To date, a number of studies looked at contaminants in different cetacean species in the Adriatic Sea. Marsili and Focardi (1997) investigated organochlorines in cetaceans stranded around the Italian coasts, but only three samples were from bottlenose dolphins from the northern Adriatic. Storelli and Marcotrigiano (2000) assessed organochlorines from three Risso's dolphins (*Grampus griseus*) stranded in the southern Adriatic. Storelli and Marcotrigiano (2003) and Storelli et al. (2007) assessed organochlorines in bottlenose dolphins stranded on the southern Adriatic Sea coast, but the latter study did not include analysis of blubber tissue. In the same area, Storelli et al. (2012) measured organochlorines in stranded striped dolphins. In the northern Adriatic Sea, on its eastern side, Lazar et al. (2012) analysed different tissues in a single common dolphin (*Delphinus delphis*), a species considered extremely rare in the basin nowadays (Bearzi et al. 2004, Genov et al. 2012). Finally, Herceg Romanić et al. (2014) analysed organochlorine contaminants in various tissues in 13 bottlenose dolphins stranded along the Croatian coast in the northern Adriatic, providing the most comprehensive organochlorine assessment for dolphins in the northern part of the Adriatic Sea until now. All of these studies provided valuable insights, but due to limited sample sizes and the use of stranded animals, the inferences that can be made are somewhat limited.

In the context of this study (i.e. photo-identification and pollutants), cetacean studies typically involve either a) collecting photo-identification data of free-ranging individuals, or b) analysing pollutant concentrations in stranded animals. However, studies combining these two important aspects, the analysis of pollutants in conjunction with long-term photo-identification of live animals (e.g. Ross et al. 2000, Ylitalo et al. 2001, Wells et al. 2005) are still relatively rare. In this study, all sampled animals were photo-identified and are part of a well-known population monitored since 2002 (Chapters 2, 3 and 5), which adds additional value to this dataset. Long-term records of identifiable individuals could be combined with individually-specific organochlorine concentrations, which in turn enabled linking contaminant loads to certain demographic parameters in a known resident dolphin population. In the long term, the continued organochlorine monitoring in conjunction with photo-identification may provide further useful insights and hopefully this study can be expanded in the future by including additional parameters. Such integrated approach offers a lot of potential, as PCBs can be linked to sex, reproductive output and other parameters (Ross et al. 2000, Ylitalo et al. 2001, Wells et al. 2005). Such information is often lacking for wild populations and is of considerable importance for evaluating the impacts of pollutants on marine top predators.

When considering potential caveats, it should be noted that sampling live free-ranging animals meant there was some heterogeneity in the origin of samples with respect to the exact body location, despite the same general body area being targeted. This could potentially affect the resulting organochlorine concentrations, as these may vary across the body parts sampled (Aguilar 1987). However, because the proportion of lipid was quantified and concentrations expressed on a lipid weight basis, the resulting concentrations can be considered unbiased (Aguilar 1987). Moreover, previous studies showed that biopsy samples yield representative details on chlorinated and brominated aromatic compounds in marine mammal blubber, regardless of the quantity and type of blubber sampled, provided that lipid normalization is performed on the resulting concentrations (Ikonomou et al. 2007).

Even though known males were not preferentially targeted over known females, and several animals were of unknown sex at the time of sampling, the skewed sex ratio is likely driven by the fact that females with accompanying calves were not sampled.

4.4.2 PCB concentrations

Relatively high PCB concentrations were detected in the samples. This is in agreement with other studies that showed the continued persistence of PCBs in large marine predators in Europe (Law et al. 2012, Jepson et al. 2016). In a previous European-wide study (Jepson et al. 2016), PCB levels were shown to be high in six Gulf of Trieste bottlenose dolphins, but the sample size from this area was limited. Here, using a larger sample size, this corroborates that concentrations in this population are indeed high in relation to published reference values (Kannan et al. 2000, Jepson et al. 2016). It is probably safe to assume that organochlorine threats to this population are mainly restricted to PCBs, as is the case for other Mediterranean areas (Jepson et al. 2016). Other studies in Europe have shown that following the 1970s-1980s ban the declines of PCBs have been slower than those of DDTs (Aguilar & Borrell 2005) and levels have subsequently reached a plateau in harbour porpoises (*Phocoena phocoena*) around the United Kingdom (Law et al. 2012) and in striped dolphins (*Stenella coeruleoalba*) in the western Mediterranean Sea (Jepson et al. 2016).

The main part of the PCB profile was represented by congeners 153, 138 and 180 (Table 4.3, Fig. 4.7), which is in agreement with other studies from the region (Storelli & Marcotrigiano 2003, Lazar et al. 2012, Herceg Romanić et al. 2014) and elsewhere (Fair et al. 2010, García-Álvarez et al. 2014).

Comparing organochlorine levels across various literature sources is not always straightforward and can in fact be challenging. The reasons for this include different methods of organochlorine quantification, differences in compounds analysed (e.g. the total number and selection of individual PCB congeners), the basis on which the concentrations are expressed (e.g. lipid, wet or dry weight basis - especially if the proportion of lipid or water is not reported), the summary statistics used (e.g., arithmetic mean, geometric mean or median) together with measures of spread (e.g. standard deviation, confidence intervals or range); the sources of samples (controlled live captures, biopsies, bycaught animals or stranded animals), sample size, the sex and age classes included or excluded from the analysis, period of sampling, etc. For these reasons, not all studies are directly comparable.

Still, considering these caveats, some general comparisons can be made (Table 4.4). Looking at a regional perspective, it appears that PCB concentrations in this study are relatively similar to those found in stranded bottlenose dolphins along the eastern Adriatic coast of Croatia (Herceg Romanić et al. 2014), but substantially higher than in stranded bottlenose dolphins along the Adriatic coast of south-eastern Italy (Storelli & Marcotrigiano 2003), stranded along the coast of Israel, eastern Levantine Basin (but note the extremely small samples size, Shoham-Frider et al. 2009) , or biopsied in the Gulf of Ambracia, western Greece (Gonzalvo et al. 2016). Looking at the wider European and Mediterranean picture, concentrations in this study are higher than those found in bottlenose dolphins from Ireland (Berrow et al. 2002, Jepson et al. 2016), but lower than in bottlenose dolphins from western Mediterranean (Borrell & Aguilar 2007, Jepson et al. 2016) and those from Portugal, north-western Spain, Wales, England and Scotland (although note that the patterns are somewhat different between males and females, Table 4.4, Jepson et al. 2016). Based on the above, it appears that within the Mediterranean, generally speaking, PCB concentrations tend to decline from west to east, and from north to south, which is consistent with the general geographical pattern of anthropogenic impacts (particularly pollution and exploitation of marine resources) in the Mediterranean basin (Coll et al. 2012).

On a global scale, the reported concentrations from this study are higher than those found in bottlenose dolphins in Taiwan (Chou et al. 2004), around Canary Islands (García-Álvarez et al. 2014), off Rio de Janeiro, Brazil (Lailson-Brito et al. 2012), Bermuda (Kucklick et al. 2011), Beaufort, North Carolina, USA (Hansen et al. 2004), southern Biscayne Bay, Florida, USA (Kucklick et al. 2011), and along the coasts of Louisiana, Mississippi and northwestern Florida (Kucklick et al. 2011, Balmer et al. 2015), relatively similar to those from Indian River Lagoon, Florida, USA (Fair et al. 2010), Sarasota Bay, Florida, USA (Yordy et al. 2010) and Charleston, South Carolina, USA (Fair et al. 2010, Adams et al. 2014), and lower than in New Jersey (Kucklick et al. 2011), northern Biscayne Bay and Tampa Bay in Florida, USA (Kucklick et al. 2011), and waters of Georgia, USA (Balmer et al. 2011). With respect to other species, bottlenose dolphins from the Gulf of Trieste had higher PCB concentrations than striped dolphins from the southern Adriatic Sea (Storelli et al. 2012), harbour porpoises from the United Kingdom (Law et al. 2012), Guiana dolphins (*Sotalia guianensis*) from north-eastern Brazil (Santos-Neto et al. 2014), common dolphins (*Delphinus* sp.) from New Zealand (Stockin et al. 2007) or northern resident killer whales from British Columbia, Canada (Ross et al. 2000, Ylitalo et al. 2001), but substantially lower than striped dolphins from the western Mediterranean Sea (Jepson et al. 2016), killer whales from the United Kingdom, Canary Islands and the Strait of Gibraltar (Jepson et al. 2016), or southern resident and transient killer whales from the waters of British Columbia, Canada, and the states of Alaska and Washington, USA (Ross et al. 2000, Ylitalo et al. 2001). In addition, male dolphins in this study had higher concentrations than male pilot whales, male sperm whales and male fin whales from the western Mediterranean Sea (Pinzone et al. 2015), while female dolphins in this study had lower concentrations than female pilot whales, similar concentrations as female sperm whales and higher concentrations than female fin whales from the western Mediterranean Sea (Pinzone et al. 2015).

Table 4.4. PCB blubber concentrations in *Tursiops truncatus* across different studies for males, females and both sexes. Whenever possible, reported values pertain to adult animals. All concentrations are in mg/kg, and expressed on lipid weight basis, unless otherwise noted. Concentrations expressed in different units in source literature were converted to mg/kg. Concentrations are shown as either arithmetic mean (A) ± standard deviation, (or with range in parentheses), or geometric mean (G) with 95% confidence intervals in parentheses. Summary statistics were obtained from text or tables of cited sources, or calculated from raw data reported in tables. Note that both the number and choice of individual PCB congeners tested varied across studies. See cited sources for details.

Location	**N**	**Mean**	**M**	**F**	**M-F**	**Source**
Croatia, north-eastern Adriatic Sea	13	A	-	-	97 ± 133	Herceg-Romanić et al. 2014
Italy, southern Adriatic Sea	9	A	30.3	28.8	32.7 (7.3–53)	Storelli & Marcotrigiano 2003
Gulf of Ambracia, western Greece	14	A	23.4 ± 18.0	32.9 ± 43.3	26.9 ± 28.3	Gonzalvo et al. 2016
Israel, eastern Levantine Basin	2	A, wet weight	6.3 ± 2.3	-	-	Shoham-Frider et al. 2009
South-east Spain, western Mediterranean	36	A	336.0 ± 241.1	246.4 ± 183.5	286.6 ± 274.6	Borrell & Aguilar 2007
Spain, western Mediterranean	27	A	182.7 (27.4–399)	193.2 (45.3–601.4)	-	Jepson et al. 2016
Strait of Gibraltar	8	A	324.0 (28.3–879.3)	123.1 (20.8–179.7)	-	Jepson et al. 2016
Gulf of Cadiz, south-west Spain	21	A	247.3 (98.5–445.3)	150 (3.7–426.4)	-	Jepson et al. 2016
Portugal	12	A	85.7 (19.4–164.7)	88.5 (35.0–226.8)	-	Jepson et al. 2016
North-western Spain	11	A	118.9 (5.1–382.2)	34.7 (5.4–82.0)	-	Jepson et al. 2016
Wales, UK	7	A	91.8 (8.2–175.4)	111.9 (9.1–307.5)	-	Jepson et al. 2016
England, UK	10	A	176.9 (22.1–446.6)	91.2 (4.1–358.5)	-	Jepson et al. 2016
Scotland, UK	21	A	96.6 (1.8–698.0)	46.1 (8.5–125.1)	-	Jepson et al. 2016
Shannon Estuary, Ireland	8	A	29.5 ± 21.0	7.1 ± 8.7	23.9 ± 20.8	Berrow et al. 2002
Shannon Estuary, Ireland	8	A	46.9 (13.0–95.1)	11.4 (1.5 21.2)	-	Jepson et al. 2016
Canary Islands	25	A	-	-	47.2 ± 53.9	García-Álvarez et al. 2014
Cape May, New Jersey, USA	3	G	139 (95% CI 62.8–130)	-	-	Kucklick et al. 2011
Beaufort, North Carolina, USA	5	G	53.3 (15.9–52.2)	11.6 (3.3–40.6)	-	Hansen et al. 2004
Charleston, South Carolina, USA	9	G	50.4 (23.6–84.6)	7.9 (2.7–31.2)	-	Hansen et al. 2004
Charleston, South Carolina, USA	47	G	94 (28.6–255)	14.3 (4.5–131)	-	Fair et al. 2010

Charleston, South Carolina, USA	40	G	76.6 (25.9–246)	-	-	Adams et al. 2014
Sapelo area, Georgia, USA	46	G	115.7 (95% CI 91.7–146.1)	48.3 (95% CI 27.3–85.5)	-	Balmer et al. 2011
Mixed area, Georgia, USA	22	G	253.6 (95% CI 177.9–361.5)	45.9 (95% CI 20.8–101.7)	-	Balmer et al. 2011
Brunswick area, Georgia, USA	34	G	509.6 (95% CI 369.0–703.6)	116.5 (95% CI 78.1–173.6)	-	Balmer et al. 2011
Indian River Lagoon, Florida, USA	11	G	20 (14.7–27.9)	9.3 (5.0–17.0)	-	Hansen et al. 2004
Indian River Lagoon, Florida, USA	48	G	79.8 (35–227)	25.5 (1.5–105)	-	Fair et al. 2010
Biscayne Bay – North, Florida, USA	15	G	157 (95% CI 110–224)	-	-	Kucklick et al. 2011
Biscayne Bay – South, Florida, USA	15	G	33.7 (95% CI 23.6–48.2)	-	-	Kucklick et al. 2011
Sarasota Bay, Florida, USA	47	G	98.6 ± 159	4.7 ± 5.4	-	Yordy et al. 2010
Tampa Bay, Florida, USA	5	G	109 (95% CI 58.9–203)	-	-	Kucklick et al. 2011
East of Apalachicola Bay, Florida, USA	20	G	33.1 (95% CI 24.3–45.1)	-	-	Kucklick et al. 2011
St. Joseph Bay to St. Andrews Bay, Florida, USA	38	G	63 (95% CI 50.4–78.9)	-	-	Kucklick et al. 2011
Mississippi Sound, Mississipi, USA	55	G	68 (95% CI 56.4–81.9)	-	-	Kucklick et al. 2011
Barataria Bay, Louisiana, USA	19	G	51.4 (95% CI 38.5–68.6)	-	-	Balmer et al. 2015
Bermuda	3	G	38.8 (95% CI 17.4–86.1)	-	-	Kucklick et al. 2011
Rio de Janeiro State, Brazil	2	A	11.8 ± 2.4	-	-	Lailson Brito et al. 2012
Taiwan	6	A	6.78	2.3	5.4 ± 3.6	Chou et al. 2004

4.4.3 Effects of demographic parameters on PCB concentrations

Males had significantly higher PCB concentrations than females (Fig. 4.3). Animals of unknown sex also had high concentrations, with values more similar to males than to females (Table 4.2, Fig. 4.6). This suggests most of these animals were likely also males. The significant differences between males and females are suggestive of PCB offloading from reproducing females to their offspring via gestation and/or lactation (Borrell et al. 1995, Schwacke et al. 2002, Wells et al. 2005, Weijs et al. 2013). The significant differences in PCB concentrations between nulliparous and parous females (Fig. 4.4) further support this, despite limited sample size. Even though the premise of maternal offloading is well established, particularly based on experimental laboratory or captive studies involving mammals (Kannan et al. 2000) and samples from whaling operations (Aguilar & Borrell 1994b, Borrell et al. 1995), it is informative to be able to demonstrate that this is indeed happening in a wild, free-ranging cetacean population. In Sarasota Bay, Florida, research initiated in the 1970s, combining tagging, photo-identification monitoring and capture-release operations for health assessments, provided an unparalleled opportunity to investigate the relationships between organochlorine levels and life-history and reproductive parameters in the world's best-studied bottlenose dolphin population (Wells et al. 2005). In the eastern North Pacific, long-term identification records of one of the best-studied killer whale populations in the world enabled similar comparisons (Ross et al. 2000, Ylitalo et al. 2001). However, such studies remain relatively rare, especially in the Mediterranean Sea, the largest enclosed sea in the world, with substantial anthropogenic pressure.

There is some evidence of first-born offspring mortality in this dolphin population, as a few of the observed newborns (presumed to be the first offspring of respective females) did not survive to the following year (T. Genov, *personal observations*). This would support the notion that first-borns may receive a very high (possibly lethal) dose of PCBs from their mothers, as females may transfer up to 80% of their burden to offspring (Cockcroft et al. 1989). This may lead to poor first-born survival , with an improved survival of subsequent offspring (Schwacke et al. 2002, Wells et al. 2005). However, this evidence from the study area is limited and circumstantial, so further inferences are not possible. Given the long-term and ongoing monitoring of this population, future work incorporating PCB monitoring, individual re-sighting histories and information on reproductive rates may provide further insight into the temporal accumulation of PCBs by females and the possible links between pollutant loads and recruitment, as recommended by Hall et al. (2006).

Even though this dolphin population is structured into several social groups that display differences in behaviour and feeding strategies in relation to fisheries (Chapter 2), as well as diet (Chapter 3), it appears that PCBs pose a threat to these animals regardless of social group membership and associated dietary differences (Fig. 4.5).

4.4.4 Potential toxicological effects

The vast majority of animals in this study exceeded the lower toxicity threshold (Kannan et al. 2000), with more than 50% also exceeding the higher threshold (Helle et al. 1976, Fig. 4.6). As discussed by Jepson et al. (2016), the lower toxicity threshold may in fact overestimate the true PCB risk to cetaceans, but PCB levels reported here nevertheless provide a compelling case for the inherent PCB toxicity risk to these animals. In previous studies, high PCB levels were linked to pathological findings consistent with immunosuppression and increased susceptibility to disease, including macro-parasitic and bacterial pneumonias, high lung and gastric macro-parasite burdens, and generalised bacterial infections in harbour porpoises (Jepson et al. 2016). In Mediterranean striped dolphins, high levels of PCBs were associated to increased mortality during a morbillivirus epizootic outbreak, possibly due to immunosuppression (Aguilar & Borrell 1994a).

These results are of concern, particularly in combination with other known or suspected threats to this population, including marine litter, disturbance from boat traffic, underwater noise, frequent interactions with fisheries, overfishing and occasional bycatch (Genov et al. 2008, Hace et al. 2015, Genov et al. 2016, Kotnjek et al. 2017, Genov 2020, Genov et al. 2021). Hopefully, the quantification of organochlorine concentrations and establishing links with various demographic parameters as presented here, will enable placing the effects of contaminants in context with other anthropogenic stressors (Hall et al. 2017).

4.4.5 DDE and DDT

Only PCB concentrations, but not DDT, could be determined in the samples, except for four samples referred to above. DDE concentrations could be determined as they were obtained as a 'side product' of PCB analyses. In these four samples, DDE was the majority component of the total DDT, representing 89.7% (Table 4.1). Biotransformation processes of DDT in vertebrates largely end up as DDE (Aguilar & Borrell 2005). Unless there is a recent source, DDE tends to be the highest concentration DDT metabolite present (Storelli et al. 2004, Pinzone et al. 2015), and can be used as an indicator of DDT contamination (but see Kljaković-Gašpić *et al*. 2010 on possible recent input). These results are similar to several other studies and indicative of DDT ageing (Lailson-Brito et al. 2012, Adams et al. 2014, García-Álvarez et al. 2014, Gonzalvo et al. 2016). This suggests that DDE (and hence DDT) levels are relatively low, as is the case in the western Mediterranean Sea and around the United Kingdom (Aguilar & Borrell 2005, Borrell & Aguilar 2007, Law et al. 2012). In the Eastern Mediterranean Sea, however, levels of DDTs appear higher than those of PCBs (Shoham-Frider et al. 2009, Gonzalvo et al. 2016). For HCB, the extremely low levels in this study, consistent with studies on other biota from the Adriatic Sea (Storelli et al. 2004), suggest that recent environmental input of this compound is negligible (Borrell & Aguilar 2007).

4.4.6 Future monitoring perspectives

These results represent a useful baseline for future research and monitoring. With ongoing studies of this dolphin population and new insights into its ecology, future sampling may provide a better understanding of population-level impacts of pollutants. It should be noted that concentrations in top predators with high lipid stores inevitably lag behind any reductions in environmental concentrations (and those in prey), due to the slow depuration of POPs out of the population (through the legacy from female to calf, as well as the cycling of POPs in the marine environment). Nevertheless, this approach may represent a monitoring tool in relation to EU legislation such as the Habitats Directive and the Marine Strategy Framework Directive (MSFD). The presence of pollutants in tissues of marine biota is already included as a Descriptor 8 of MSFD, while marine mammals are one of the indicators of the "Good Environmental Status" under Descriptor 1 of MSFD. Jepson and Law (2016) proposed that at a European policy level, PCB levels in relation to established toxicity thresholds should also be used to assess "Favourable Conservation Status" of marine mammals under the EU Habitats Directive.

Even though biopsy sampling took place within Slovenian waters, due to the extensive spatial survey coverage (Chapter 1) and the fact that sampled dolphins have been re-sighted throughout the study area, the reported organochlorine levels can likely be considered representative of this part of the Adriatic Sea. Furthermore, individual dolphin re-sighting frequencies have shown that the sampled individuals are part of a resident population inhabiting this area over the long term (Chapters 2 and 5) while both photo-identification (Genov et al. 2009) and genetic data (Gaspari et al. 2015) suggest that this population is distinct. This adds confidence to the notion that these concentrations are representative of this particular area, rather than being a result of acute PCB exposure elsewhere (Phillips & Segar 1986).

Molluscs have typically been used as model species to monitor contaminants in the Gulf of Trieste, elsewhere in the Adriatic Sea (Kljaković-Gašpić et al. 2010), and other parts of the world (Phillips & Segar 1986, Farrington et al. 2016). This is primarily due to their widespread distribution, abundance, sessile nature, tolerance to various types of stress, and the ability to accumulate a wide range of contaminants (Phillips & Segar 1986, Kljaković-Gašpić et al. 2010), but probably also due to ease of access to the animals. However, while molluscs may be better indicators for local point sources of contamination, cetaceans may be more representative over larger spatial and temporal scales. Dolphins are long-lived predators that integrate contaminant concentrations over time. They have been shown to be incapable of metabolizing certain PCB congeners, making them accumulate these compounds more readily than other mammals or taxa of comparable life history (Aguilar & Borrell 2005). Moreover, being highly mobile, they are likely better regional rather than local indicators, due to their propensity to move around more. Finally, as top predators, they are likely representative of the ecosystem as a whole (Borrell & Aguilar 2007).

4.4.7 Conclusions

It is important to review current methods of PCB mitigation in the marine environment, at a European and international level. In Europe, much greater compliance with the Stockholm Convention is urgently needed by many EU member states, in order to significantly reduce PCB contamination of the marine and terrestrial environment by 2028 (Jepson et al. 2016, Jepson & Law 2016, Stuart-Smith & Jepson 2017). Measures may include the safe disposal or destruction of large stocks of PCBs and PCB-containing equipment, limiting the dredging of PCB-laden rivers and estuaries, reducing PCB leakage from old landfills, limiting PCB mobilization in marine sediments, and regulating demolition of PCB-containing precast buildings such as tower blocks built in the 1950s–1980s (Jepson et al. 2016, Jepson & Law 2016, Stuart-Smith & Jepson 2017).

The results presented in this chapter show that PCB levels are relatively high in northern Adriatic dolphins, and may be high enough to potentially cause population-level effects in this population. This study provides important baseline data of a considerable sample size, against which future trends can be assessed. It demonstrates that POP monitoring combined with long-term photo-identification and population ecology studies can be highly informative for assessing the impacts of organochlorine pollution.

CHAPTER 5: ABUNDANCE OF COMMON BOTTLENOSE DOLPHINS (*TURSIOPS TRUNCATUS*) IN THE GULF OF TRIESTE

5.1 INTRODUCTION

Abundance is one of the key parameters in assessing the conservation status of animal populations, understanding their effects on other species and for understanding the effects of other species (including humans) on them. Information on abundance represents crucial information in both ecology and conservation, as it can inform the knowledge of the role any given species plays in its ecosystem (Heithaus et al. 2012), and enable the assessment of conservation status (Schipper et al. 2008). Obtaining reliable and unbiased estimates of abundance is typically the first step towards assessing population status, detecting trends, defining conservation and management objectives, and provides a baseline for carrying out monitoring (Williams et al. 2002). Abundance estimates are one of the basic prerequisites for evaluating whether human activities are having detrimental effects on populations (Cooke 1994, Wade 1998, Taylor et al. 2000, Punt & Donovan 2007).

Although a variety of methods exist for estimating cetacean abundance (see Hammond et al. (2021) for a recent overview), two most commonly employed techniques include the line-transect distance sampling and mark-recapture (also known as capture-recapture, capture-mark-recapture, mark-release-recapture and capture-mark-reencounter) techniques (Hammond 1986, Borchers et al. 2002, Thomas et al. 2002, Evans & Hammond 2004, Hammond 2010). While both methods have their advantages and disadvantages, depending on the target species, the study area and other considerations, distance sampling is potentially logistically and financially more challenging, typically more suited for larger areas (e.g., at the level of the entire North Sea or European Atlantic Shelf waters, Hammond et al. 2013) and less likely to perform well in small areas with low animal densities. Conversely, if animals are individually and naturally marked, mark-recapture techniques (Borchers et al. 2002, Amstrup et al. 2005) using individual identification data from photographs, i.e. photo-identification (Würsig & Würsig 1977, Würsig & Jefferson 1990), can be employed and allow one to estimate the number of animals using a defined area over a given study period (Hammond et al. 1990). This may be especially well suited for relatively small study areas. The abundance of common bottlenose dolphins and other small cetaceans in relatively small and well-defined areas has typically been estimated via mark-recapture (Wilson et al. 1999b, Read et al. 2003, Fortuna 2006, Currey et al. 2007, Bearzi et al. 2008a, Silva et al. 2009, Verborgh et al. 2009, Bearzi et al. 2011a, Cantor et al. 2012, Mansur et al. 2012, Cheney et al. 2013, Arso Civil et al. 2019b). Furthermore, photo-identification data allow one to build a long-term data series on individual animals and to obtain other information on population ecology, such as residency patterns (Bearzi et al. 1997, Parra et al. 2006, 2008a, Espécie et al. 2010) survival rates (Hammond 1990, Ramp et al. 2006, Currey et al. 2009a, Verborgh et al. 2009, Arso Civil et al. 2019a), reproductive rates (Haase & Schneider 2001, Arso Civil et al. 2017, Robinson et al. 2017, Rossi et al. 2017), movements (O'Brien et al. 2010, Bearzi et al. 2011c, Stevick et al. 2011, Genov et al. 2012, Robinson et al. 2012, Genov et al. 2016), social structure (Wells 1991, Smolker et al. 1992, Whitehead 1995, 1997, Chilvers & Corkeron 2002, Lusseau et al. 2003) and epidermal disease (Wilson et al. 1999a, Van Bressem et al. 2009, Burdett Hart et al. 2010, Gonzalvo et al. 2015). Assessing vital rates such as survival and reproductive rates in particular, is highly important in assessing and conserving animal populations (Heppell et al. 2000, Williams et al. 2002), including marine mammals (Baker et al. 2010). Studying these parameters has been very informative in investigating the status and threats in some cetacean populations (Slooten et al. 1992, Ramp et al. 2006, Currey et al. 2009c, Silva et al. 2009, Ramp et al. 2010a, Beasley et al. 2013).

In the most general sense, mark-recapture techniques can be divided into open population and closed population models (Amstrup et al. 2005), which differ in one key assumption. Open population models assume (or allow for) population change (resulting from births, deaths, and permanent immigration and emigration) between sampling occasions (Cormack 1964, Jolly 1965, Seber 1965). Closed population models, on the other hand, assume that the population is “closed” to any changes between sampling occasions (Otis et al. 1978, Borchers et al. 2002). This has implications for the appropriateness of particular analyses and study design, but the major advantage of closed population models is that they allow heterogeneity in capture probabilities to be accounted for (Chao et al. 1992, Pledger 2000). Heterogeneity in capture probabilities refers to animals having unequal probabilities of being captured in any given sampling occasion, due to individual differences in behaviour, movements, habitat preferences, etc. This violates a key assumption of equal capture probabilities in the basic closed population models and, if not accounted for, can lead to underestimation of population abundance (Hammond 2010). Assumptions of mark-recapture are treated in more detail below in Methods. Closed-population models also tend to provide better precision and are routinely used for estimating the abundance of marine mammals (Hammond 2010).

Another potential issue in mark-recapture studies is the presence of temporary emigration, where animals temporarily leave the study area, rendering themselves unavailable to be captured (Kendall et al. 1995, Kendall et al. 1997). This, if not accounted for, is likely to lead to lower capture probabilities and consequently to overestimation of population abundance. The so-called robust design models (Pollock 1982, Kendall et al. 1995, Kendall et al. 1997), which can be thought of as a combination of open and closed population models, allow abundance and survival to be estimated simultaneously whilst taking into account temporary emigration from the study area. Within this framework, the study period consists of primary sampling occasions (typically years), among which the population is assumed to be demographically open, and secondary sampling occasions within the primary ones, among which the population is assumed to be demographically closed.

Common bottlenose dolphins in the Gulf of Trieste and adjacent waters have been continuously studied since 2002, primarily by means of photo-identification (Genov et al. 2008). Little information exists on their occurrence in the area prior to this, with no data on their numbers. Such information on this particular population is of interest to managers at both national and regional levels in relation to meeting various conservation objectives stipulated by national, European and global legislative frameworks. For example, there have been growing concerns about the impact of bycatch on this population (Genov 2020, T. Genov, unpublished data), which requires reliable estimates of abundance to place this threat into proper context. The objectives of this chapter were to provide robust estimates of annual abundance and apparent survival.

This population is transboundary, inhabiting national waters of Italy, Slovenia and Croatia (Genov et al. 2008), so information on its size is relevant to various stakeholders at various levels. For example, the population is known to use the entire extent of Slovenian national waters (Genov 2011), so knowing how many animals use Slovenian waters (or what proportion of a wider population this represents) is important to managers from the national point of view, even though the animals range beyond national waters. Such information is relevant to Slovenia's international reporting, such as to the European Commission or international agreements, as well as the implementation of the provision of legislative pieces such as the EU Habitats Directive or the Marine Strategy Framework Directive.

From Italy's perspective, this population uses the entire marine area of Friuli Venezia Giulia, one of 20 regions of Italy, and one of five autonomous regions with special statute (Article 116, Constitution of Italy), which has legislative power related to a number of matters not expressly defined in country-level law. The implementation of various pieces of legislation, including environmental matters, lies with such autonomous regions (Article 117, Constitution of Italy). For example, the Italian state has delegated the identification and approval of conservation measures and management plans related to Natura 2000, as well as the management of sites, to regions (Lai 2020), so information on the abundance of bottlenose dolphins is of interest for Italy's implementation of EU directives at the regional levels.

The dolphin population from this area also inhabits the waters of a third EU country, Croatia (Genov 2011). Even though Croatia's overall marine territory is much larger and there is no specific jurisdiction related specifically to the area covered by this dolphin population, it is nevertheless likely that such information can still be informative from a national point of view. Despite the rationale from different national points of view described above, this clearly only relates to subsections of the overall range of the study population, while the mark-recapture analysis in this chapter pertains to the overall study area. Nevertheless, in case of Slovenia and Italy’s Friuli Venezia Giulia, the abundance of bottlenose dolphins in the entire Gulf of Trieste and adjacent waters is largely representative of the abundance in each of those respective parts of the study area. Moreover, if needed, data can also be partitioned to explore potential fine-scale differences among subareas. From a regional (e.g., at the level of the Adriatic Sea), perspective, information on local abundance patterns from various areas may be informative in relation to abundance in the overall region and may feed into information obtained from regional surveys.

First abundance estimates were provided by Genov et al. (2008) and Genov (2011). However, during the early years (2002–2012), surveys were primarily concentrated in summer months (July-September), with sporadic and unequal survey effort throughout the rest of the year. Moreover, the temporal and spatial distribution of survey effort was highly variable, making estimates highly variable and often difficult to interpret. In addition, the estimates were produced within the program CAPTURE, based on models described by Otis et al. (1978). Even though CAPTURE provides a relatively quick and simple way of estimating abundance, it lacks the capabilities of newer approaches and software utilities, including the use of information criteria, multi-model inference and model averaging (Burnham & Anderson 2002).

From 2013 onwards, survey effort has been carried out throughout the year and became more intensive and consistent, both spatially and temporally. As a result, the temporal resolution and spatial coverage of photo-identification data increased, leading to higher reliability and representativity of data.

Therefore, this chapter focuses on data during 2013–2018, to estimate the abundance of common bottlenose dolphins in the Gulf of Trieste.

5.2 METHODS

5.2.1 Study area and data collection

The study area included the entire Gulf of Trieste, as well as waters directly adjacent to it (Fig. 5.1), and is described in Chapter 1. Dedicated boat surveys from a 5.8m inflatable boat with an outboard engine and land-based surveys from a high vantage point were carried out between January and December of each year during 2013–2018. Land-based surveys using both normal and high-powered binoculars ("Big eyes") were carried out to increase the likelihood of detecting dolphins and therefore maximising the likelihood of photographic captures. The search effort of the boat and land observers was kept independent, until a sighting was made. In case of sightings from land, the boat went off effort and headed to the location of the animals as observed by the land team. During boat surveys, a searching speed of 25–30 km/h was maintained. Navigation, environmental and survey effort data were recorded every 15 minutes, or whenever survey conditions or boat direction changed. Upon sighting dolphins, the animals were approached slowly and a focal follow (Mann 1999, 2000) commenced. At this point, time, GPS position, behaviour, environmental data, group size and presence of calves were recorded. A sighting was defined as an uninterrupted continuous observation of a dolphin focal group. A dolphin focal group was defined as any number of dolphins in visual range of the researchers, observed in apparent association, moving in the same direction or staying in the same area and often, but not always, engaged in the same type of behaviour (Genov et al. 2008). Group size was assessed in the field and later confirmed through photo-identification. Dolphins were followed until photographs of all group members had been obtained, the visibility deteriorated due to weather or late hour, or the group was lost.

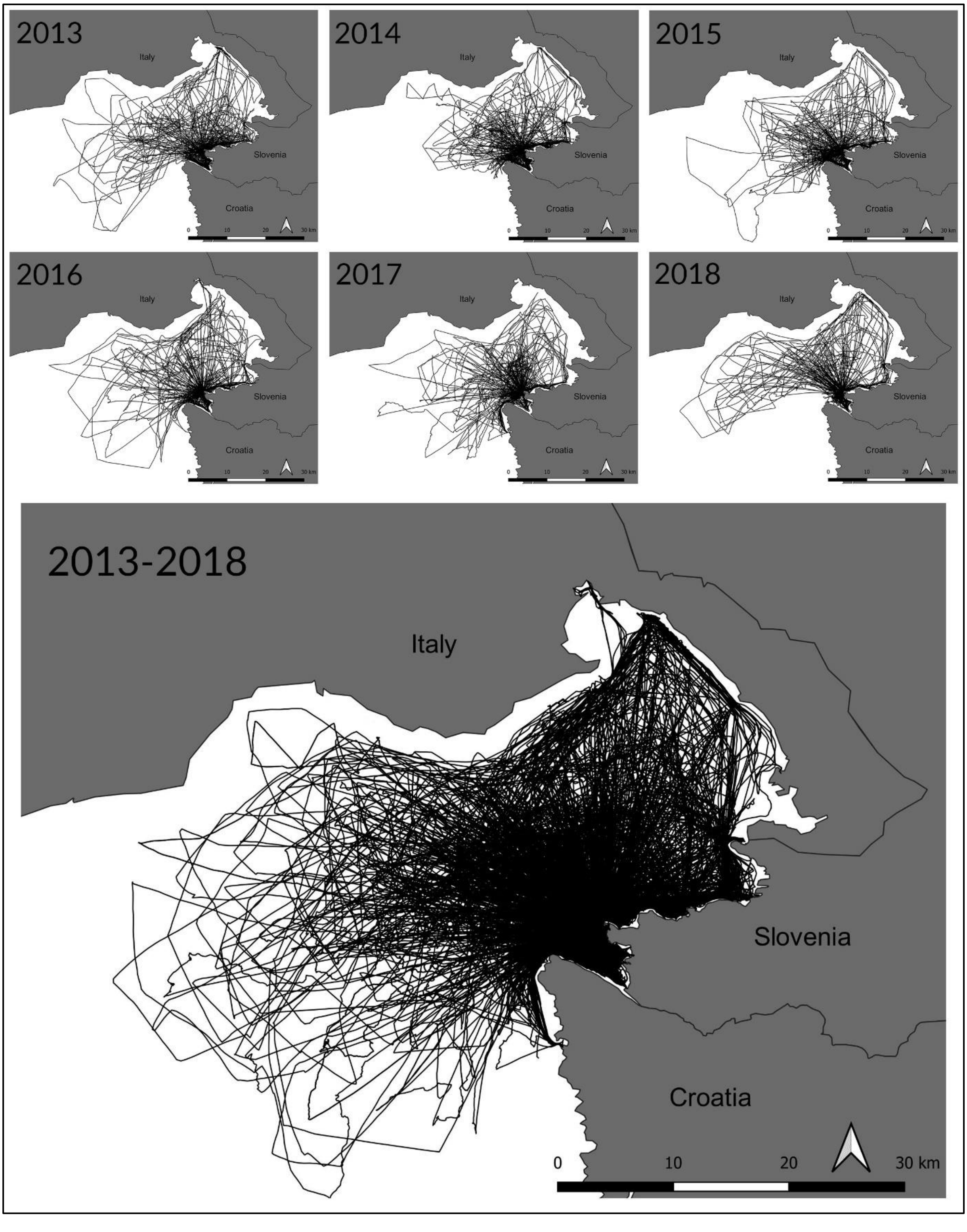

Figure 5.1. Spatial distribution of boat-based survey effort in different years and across the study period.

During each sighting, standard photo-identification procedures (Würsig & Jefferson 1990) were carried out. Individual dolphins were identified via natural markings on their dorsal fins, including nicks, notches, scars and fin shape and pigmentation patterns (Bearzi et al. 1997, Wilson et al. 1999b, Bearzi et al. 2008a, Genov et al. 2008). Photographs of dorsal fins were obtained using digital single-lens-reflex cameras Canon EOS 30D, EOS 7D and EOS 7D mark II with a telephoto lens Canon L USM 70-200 f2.8. An attempt was made to obtain high quality dorsal fin shots of all animals in the group, regardless of their behaviour, age class or degree of marking. While permanent markings visible from both sides of the fin (e.g., nicks, notches) were primary features used to identifying marked individuals, pigmentation patterns and short-term skin marks were only used as secondary features to distinguish individuals within a sighting and for confirming matches in case of substantial changes in primary features.

Occasionally, dolphin sightings were reported by the public (including local residents, fishermen, bar owners, various boat operators, the maritime police, etc.) in real time. Whenever possible, the research team responded to such reports and often located the animals. Data collected as a result of such successful responses were included in the analysis, but non-verified sightings reports alone or any photographs provided by the public were not.

5.2.2 Data processing

For each sighting, a record of all identified dolphins (including calves and poorly marked individuals) was constructed, by creating a sub-folder for each identifiable individual within a sighting. This included all dolphins that could be distinguished from others within that sighting, even if they carried only subtle or temporary markings, which would not otherwise allow them to be recognisable over the long term. All photographs of a given individual were then screened for quality. To avoid potential errors and unequal capture probabilities resulting from variable quality of photographs, only photographs that met the following criteria were used for analysis: 1) dolphin dorsal fin height taking up >7% of the photographic frame height, 2) sharp photograph, 3) dorsal fin perpendicular to a line between the camera and the fin,

4) dorsal fin unobstructed by another dolphin, water spray or water surface. Any photographs not matching these criteria were excluded from further analyses. Only animals with long-term markings (considered "*marked*") were used in mark-recapture analyses, as the inclusion of poorly marked or unmarked animals may increase the likelihood of errors. An animal was considered marked if it carried several large notches, one large and well-defined notch, multiple small nicks or a peculiar dorsal fin shape (some examples are given in Figure 5.2), irrespective of whether it was an adult, a juvenile or a calf. Since these markings are visible from both sides of the fin, there was no need to stratify photographs into left- and right-hand sides. Animals with no nicks or notches, only one small nick or two barely visible nicks were considered "unmarked" (Fig. 5.3), even if they could be distinguished from other dolphins within the same sighting based on these features or the presence of temporary markings (e.g., tooth rakes). It was deemed that such individuals could not be reliably identified across different months and even less so across years, so they were not included in the mark-recapture analysis.

Such individuals were, however, incorporated into the final estimates of abundance via a separate step in analysis. Because high quality photographs allowed animals to be distinguished from one another within the same sighting even in the absence of long-term markings, a binomial Generalized Linear Model (GLM) with a logit link function was fitted to data on the number of marked and unmarked individuals in each sighting, to obtain overall and annual estimates of the proportion of marked animals (mark rate) with the associated standard errors (Cheney et al. 2013, Arso Civil et al. 2019b). Calendar year was treated as a factor explanatory variable to obtain annual mark rate estimates. These estimates of mark rate were used to account for unmarked animals in final estimates of abundance (see section 5.2.5, below).

In photo-identification of small cetaceans, an animal may transition from an "unmarked" state to a "marked" state, i.e. the animal becomes marked during a particular study year, and because high-quality photographs may allow "unmarked" individuals to be recognisable in some sightings, sometimes such a change is known and observed. Conversely, identified animals may die during the course of a year and some of those deaths may be known because they are recorded (e.g. Genov et al. 2016). In both cases, these events within a year violate the assumption of demographic closure and can bias the resulting estimates. In this study, two instances of change of marked status, and three cases of known deaths occurred. To account for this, animals dying in a particular year were excluded from capture histories of that entire year. On the other hand, animals that became marked during a course of a year were only included in the capture histories in the following year.

Figure 5.2. Examples of individuals considered marked. Photographs: Morigenos – Slovenian Marine Mammal Society

Figure 5.3. Examples of individuals considered unmarked. Photographs: Morigenos – Slovenian Marine Mammal Society

5.2.3 Modelling framework

Mark-recapture techniques were applied to photo-identification data to estimate the number of animals using the study area during the study period (Hammond 1986, 2010). No assumption was made about what proportion of the total population this represented, although there is evidence these animals represent a somewhat distinct unit (Genov et al. 2009, Gaspari et al. 2015). Data on photo-identified individuals were used to build individual capture histories, using calendar month as a sampling occasion (secondary sampling occasions in the robust design framework, see below). In other words, all captures of an individual dolphin within one month were pooled to represent either a presence or an absence of that individual in that particular month. This was considered a good balance between three key factors: a reasonable number of sampling occasions in a year, maximising capture probabilities within each sampling occasion, and sufficient time for the population to mix between sampling occasions (Hammond 2010). Data were also organised into calendar years (primary sampling occasions in the robust design framework, see below).

As a first exploratory step, closed population models with variations in capture probabilities were fitted to data of each year, to obtain annual estimates of abundance and assess the potential sources of unequal capture probabilities, if any. Several candidate models were considered for the initial candidate set, in which capture probabilities were either held constant (the null model, M_0), allowed to vary with capture occasion (time-varying capture probabilities, model M_t), allowed to vary individually among animals (i.e., are heterogeneous, model M_h), or the combination of the above (M_{th}). Because photo-identification is unlikely to lead to a behavioural response following first capture (Hammond 2010), models incorporating a behavioural response were not considered. Therefore, capture probability (p_i) was set to be equal to re-capture probability (c_i) in all models.

The potential presence of heterogeneity in capture probabilities was investigated by fitting a) finite mixture models with two mixtures (Pledger 2000, Pledger & Phillpot 2008), referred to here as the "finite mixture models" and b) models where individual capture probabilities are considered to come from a logit-normal distribution and modelled as individual random effects using Gaussian-Hermite quadrature integration (Huggins 1989, 1991, Coull & Agresti 1999, Dorazio & Royle 2003, Gimenez & Choquet 2010, White & Cooch 2017), referred to here as the "random effects models" (also known as continuous mixture models). If heterogeneity of capture probabilities is present, it is expected that the estimates incorporating heterogeneity will be noticeably higher than estimates derived from models that do not.

Then, as the primary analysis, Pollock's robust design (Pollock 1982, Kendall et al. 1995, Kendall et al. 1997) was used to obtain annual estimates of abundance (and compare them to aforementioned methods), as well as probabilities of apparent annual survival and temporary emigration. The estimated parameters from closed population models and robust design models are shown in Table 5.1. Calendar years were used as primary sampling occasions (also sometimes referred to as “sessions” in the literature), while calendar months were used as secondary occasions within primary occasions. Here, as in closed population models, capture probabilities were either held constant, allowed to vary with time (in this case on the primary or secondary sampling occasion levels), or allowed to vary among individuals (again using two approaches for incorporating heterogeneity). The potential presence of temporary emigration was investigated by the inclusion of models that assumed either a constant or time-varying Markovian temporary emigration, constant or time-varying random temporary emigration, or no temporary emigration. To ensure identifiability of model parameters, a constraint was included in the model by making the last two γ' parameters and the last two γ'' parameters (Table 5.1) equal to each other (Kendall et al. 1997).

Model selection was carried out based on an information theoretic approach, by minimising the Akaike Information Criterion (Akaike 1973) corrected for small sample size (AICc). In cases where more than one model had support from the data, model averaging based on Akaike weights was used, based on a 95% confidence set (Burnham & Anderson 2002, Grueber et al. 2011). Models utilising two different approaches to estimating heterogeneity (mixture vs. random effects models) are not directly comparable with information criteria such as the AIC, because they are based on two different types of likelihood (the finite mixture models are constructed under full maximum likelihood, whereas the random effects models are constructed under the conditional likelihood approach first developed by Huggins (1989, 1991)). Therefore, these models were compared in terms of the derived abundance estimates to potentially provide useful insights, but could not be selected in favour of one another in a model selection framework.

Initially, the candidate model set for the robust design models included both heterogeneity in capture probabilities and temporary emigration. However, these models produced spurious estimates (see

Results) and were therefore not considered further. As a second step, a separate set of models incorporating either heterogeneity or temporary emigration were fitted, to investigate the effect of either of these two mechanisms on abundance and apparent survival. To assess any potential bias resulting from ignoring either of these two mechanisms in abundance estimates, estimates from the best model from each set were then compared to the most supported "null" model, among candidate models with either constant or time-varying capture probabilities and survival, but with no heterogeneity or temporary emigration. This allowed assessment of the direction and magnitude of potential bias resulting from not accounting for either of the two sources of unequal capture probability.

All mark-recapture models were built and run within program R (R Core Team 2020), using the *RMark* package (Laake 2013), an R-based interface for program MARK (White & Burnham 1999, White 2008). Other data analysis (including Generalized Linear Models for proportion of marked animals) were carried out in base R (R Core Team 2020). Visualisation of results was carried out using the *ggplot2* package (Wickham 2016).

Table 5.1. Parameters estimated (or derived in case of $\widehat{N}_t$) within the modelling framework.

Parameter	Notation	Description
Survival	φ_t	Probability of survival between two primary sampling occasions *t*
Gamma prime	γ'_t	Probability of being outside the study area at time *t*, given being outside the study area at time *t-1*
Gamma double prime	γ''_t	Probability of being outside the study area at time *t*, given being in the study area at time *t-1*
Capture probability	p_{ts}	Probability of capture at primary sampling occasion *t* and secondary sampling occasion *s*, conditional on being alive and in the study area
Recapture probability	c_{ts}	Probability of recapture at primary sampling occasion *t* and secondary sampling occasion *s* conditional on being alive and in the study area
Mixture probability	π_A	Probability of belonging to one of two mixtures (in the case of finite mixture models)
Individual variation in capture probability	σ_p	Standard deviation of the continuous distribution of the individual variation in capture probability *p* (in the case of random effects models)
Individuals never captured	f_0	The number of individuals never captured
Abundance estimate	$\widehat{N}_t$	Estimate of abundance in primary sampling occasion *t* (this parameter is derived from the estimated parameter f_0)

5.2.4 Assumptions

Conventional mark-recapture models require a number of underlying assumptions to be met. In case of the robust design, these are a combination of assumptions for closed population models and the classical Jolly-Seber open population method. The assumptions are: 1) the population is demographically closed (i.e. there are no births, deaths, immigration or emigration) within primary sampling occasions (in this case years) and open among them; 2) marks are unique, cannot be lost and are correctly recorded and reported; 3) capture does not affect survival or future catchability; 4) all animals have an equal probability of survival between capture occasions and of being captured within each sampling occasion; 5) temporary emigration (if present) is assumed to be either completely random, Markovian (i.e. conditional on previous state), or based on a temporary response to first capture (the latter is unlikely in cetacean photo-identification studies and was therefore not considered). Violation of these assumptions can lead to serious biases in abundance estimates (Hammond 1986, 2010). Accordingly, each of these assumptions was carefully considered in the study design.

The biology of the species, the type of “marking” and “capture” (in this case photographing natural marks), field methodology, and quality assurance (using only high-quality photographs) allowed most of these assumptions to be met. For example, because bottlenose dolphins are a slow-reproducing species with a delayed sexual maturity and high survival rates (Wells & Scott 1999), any births or deaths are unlikely to substantially affect the number of marked animals in the population within a primary sampling occasion (year). Furthermore, this population features a relatively high site fidelity and re-sighting rates (Genov et al. 2008, Genov et al. 2019a, Genov et al. 2019b), and appears to be demographically (Genov et al. 2009) and genetically distinct (Gaspari et al. 2015), so any substantial permanent immigration or emigration is unlikely. The assumption of demographic closure within primary sampling occasions was therefore likely met to a large degree. Because photo-identification is a non-invasive method, it is not expected to affect survival and future catchability of the animals

(Hammond 2010). Because only high-quality photographs were used, the assumption related to uniqueness, loss and reporting of marks was also met. Finally, because the temporal and spatial distribution of survey effort meant that sampling was representative for the region of interest and because all animals of any given group were photographed whenever possible, this increased the likelihood of equal capture probabilities among individuals.

Nevertheless, in most biological populations, and dolphins in particular, two potential causes may lead to violations of part of the 4th assumption, which states that all animals have an equal probability of being captured within each sampling occasion. First, environmental factors such as temperature, temporal and spatial distribution of prey and other biological factors (affecting the animals) and weather (affecting survey effort) may lead to differences in capture probabilities among sampling occasions. Second, individual differences in behaviour, habitat preferences and movement patterns may lead to unequal capture probability among different individuals, referred to as *heterogeneity* of capture probabilities (Amstrup et al. 2005). Such heterogeneity is common in biological populations, but failure to account for it may lead to severely biased abundance estimates, which tend to be too low and with confidence intervals that are too narrow (Wilson et al. 1999b, Amstrup et al. 2005, Hammond 2010). In addition to these two causes, temporary emigration out of the study area is also possible, and should be accounted for as far as possible. As noted earlier, certain models allow heterogeneity, time-varying capture probabilities and temporary emigration to be concurrently accounted for during analysis (see below).

5.2.5 Accounting for unmarked animals

The estimates described above ($\widehat{N}$) refer to *"marked*" animals. However, because the population also included "unmarked" animals (including unmarked or poorly marked calves, juveniles and adults), the estimates of the marked part of the population need to be corrected by incorporating information on unmarked animals. To do this, the total population size ($\widehat{N}_{tot}$) was obtained by dividing the abundance estimates of marked animals ($\widehat{N}$) from the best model(s) by the estimate of the proportion of marked animals ($\widehat{\theta}$) in the population (Wilson et al. 1999b):

$$\widehat{N}_{tot} = \frac{\widehat{N}}{\widehat{\theta}}$$

The variance was estimated using the delta method:

$$var\left(\widehat{N}_{tot}\right) = \widehat{N}_{tot}{}^{2}\left(\frac{var(\widehat{N})}{\widehat{N}^{2}} + \frac{var(\widehat{\theta}}{\widehat{\theta}^{2}}\right)$$

Confidence intervals were constructed assuming a log-normal distribution of abundance estimates (Hammond 2010). The lower and upper confidence intervals were calculated as N/C and N*C, respectively, where:

$$C = exp\left(1.96\sqrt{\ln\left(1 + CV_{\widehat{N}_{tot}}{}^{2}\right.}\right)$$

5.3 RESULTS

5.3.1 General results

A total of 392 sightings with successful photo-identification were recorded between 2013 and 2018 (Fig. 5.4), with between 45 and 91 sightings annually, leading to between 8 and 11 secondary sampling occasions per year (Table 5.2). In total, 282 marked individuals were photo-identified across the study period. Between 96 and 152 marked individuals were photo-identified in any given year, with a mark rate varying between 0.747 (SE = 0.014) and 0.836 (SE = 0.014), with an overall mean of 0.8 (SE = 0.006). The number of times an individual was seen ranged between 1 and 102, with a mean of 13.2 (SD = 18.2, median = 4, mode = 1), with 27.3 % of individuals observed only once (Fig. 5.5). The mean number of months an individual was seen was 6.9 (SD = 8.2, median = 3, mode = 1, range = 1–37, Fig. 5.6). The mean number of years an individual was seen in was 2.6 (SD = 2, median = 2, mode = 1, range = 1–6, Fig. 5.7), with 17.7% of individuals seen in all six years and 46.1 % seen only in a single year. On an annual basis, individuals were seen between 0 and 33 times in a given year (Fig. 5.8).

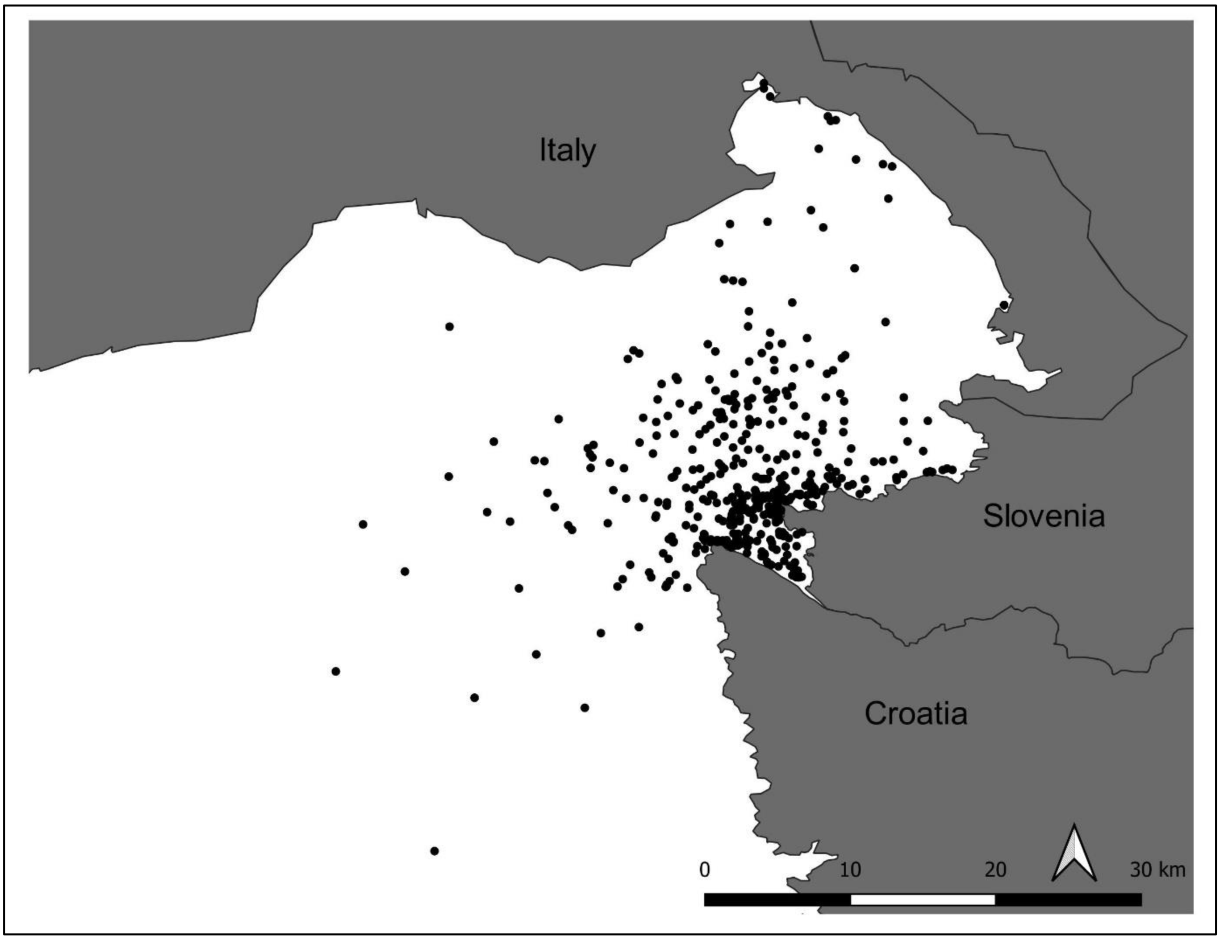

Figure 5.4. Spatial distribution of dolphin sightings across the study period.

Table 5.2. Summary of photo-identification effort and the number of photo-identified animals across years.

Year	Sightings with photo-identification	Secondary sampling occasions (months)	Marked individuals	Newly identified individuals
2013	45	8	120	120
2014	68	11	125	46
2015	66	10	127	30
2016	61	9	122	41
2017	91	8	152	40
2018	61	9	96	5
TOTAL	392	55	282	

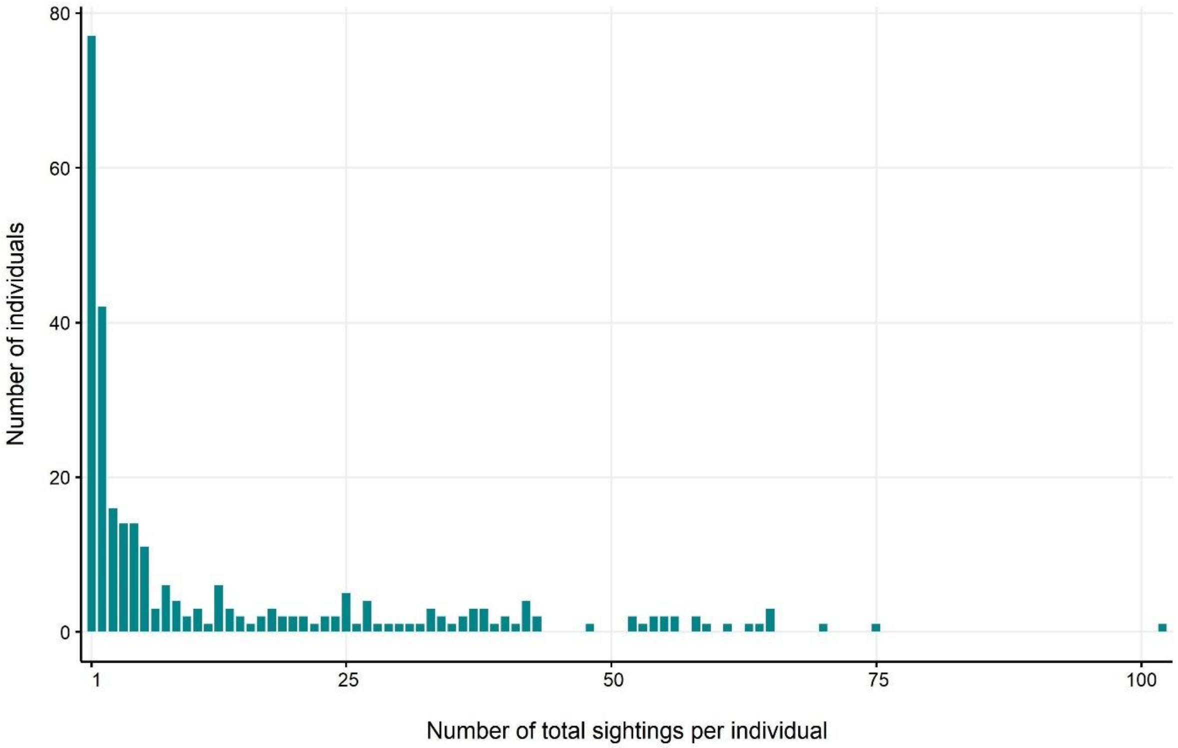

Figure 5.5. Frequency distribution of the number of times each individual was seen across the entire study period (2013–2018).

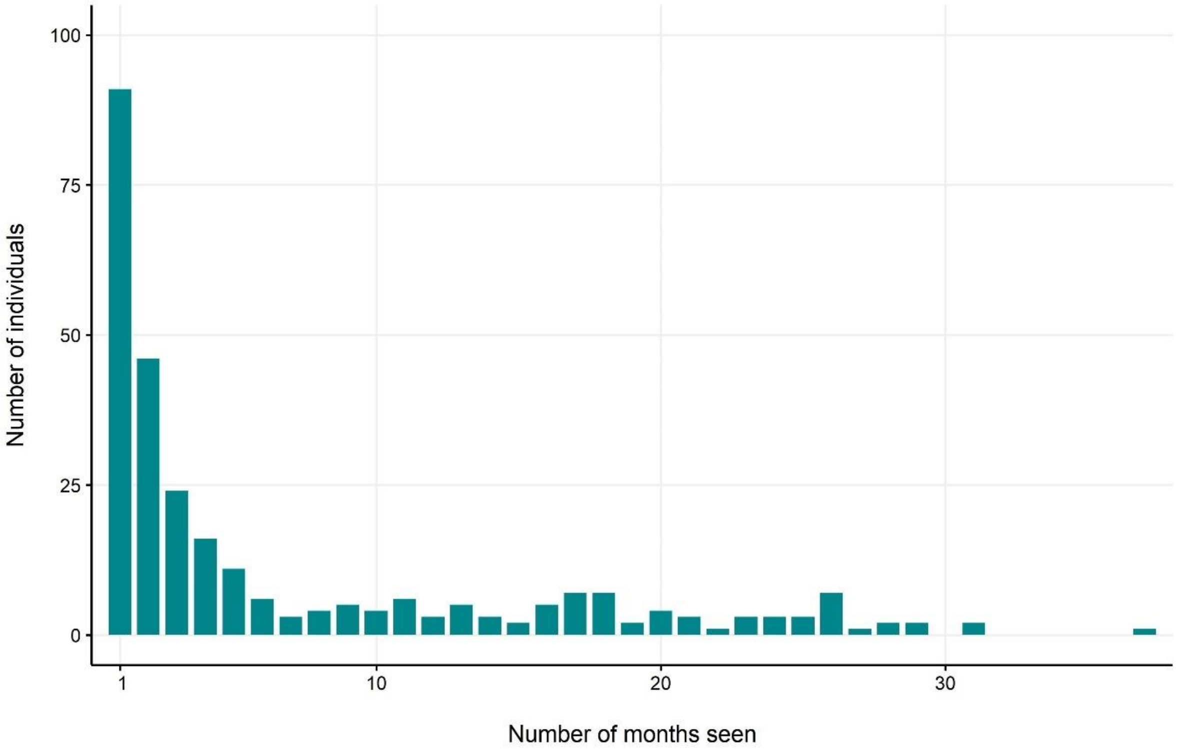

Figure 5.6. Frequency distribution of the number of months each individual was seen in across the study period (2013–2018).

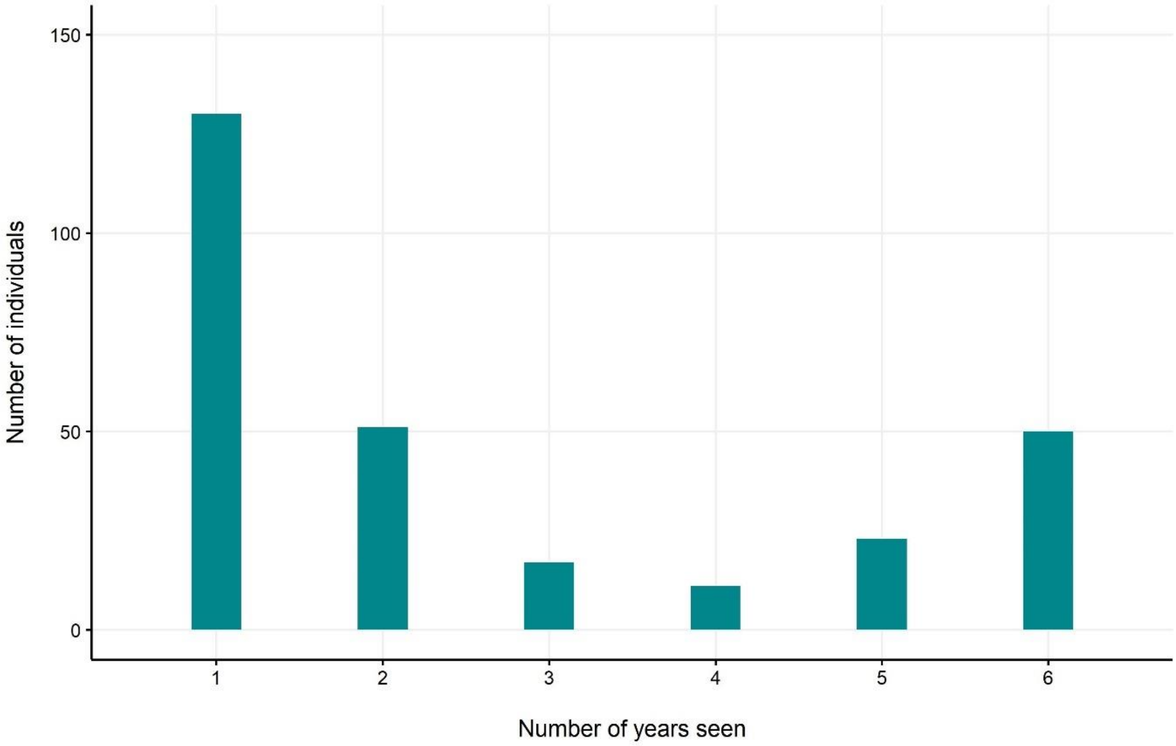

Figure 5.7. Frequency distribution of the number of years in which each individual was seen.

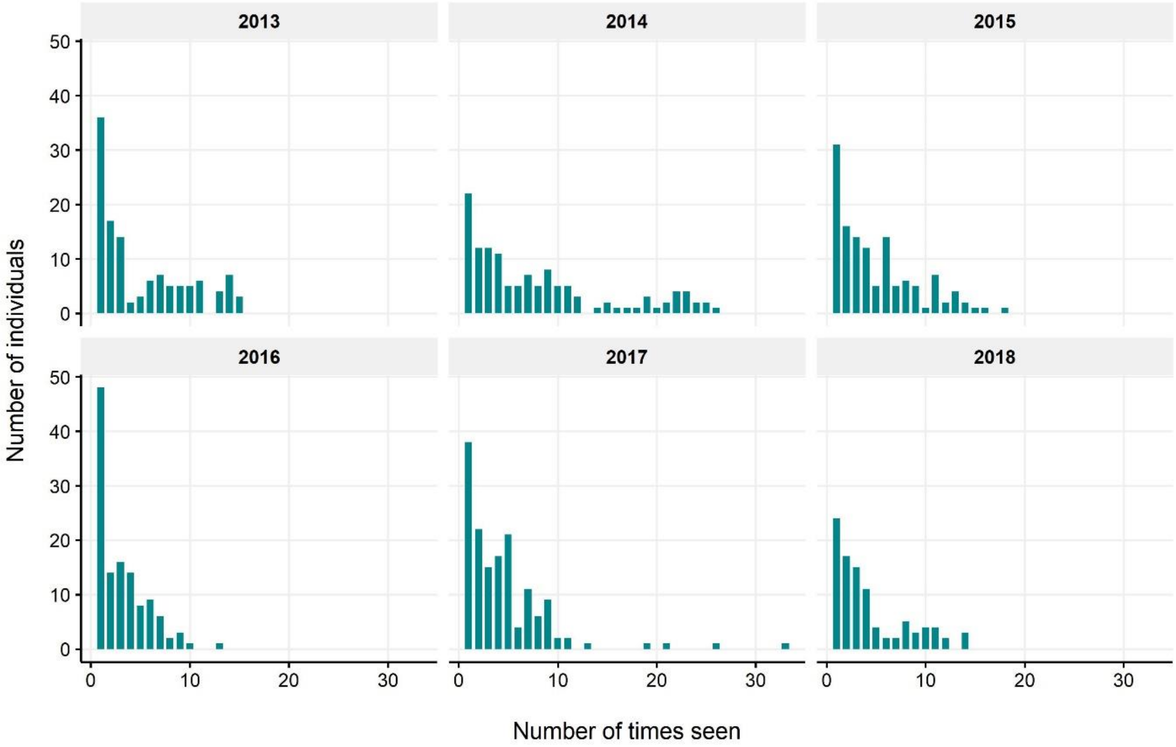

Figure 5.8. Frequency distribution of the number of times each individual was seen in any given year.

The annual discovery curves, showing the cumulative number of identified marked individuals over time (Fig. 5.9) suggest that most individuals were eventually captured in each year, giving further support to the notion of demographic closure within secondary sampling occasions. Even though the flattening of the curves varied among years, all curves appear to gradually start reaching an asymptote, suggesting demographic closure, but the number of individuals that this represented varied greatly, particularly between 2017 and 2018 (Table 5.2, Fig. 5.9). In 2018, the first half of the year notably featured repeated sightings of the social cluster B (see Chapter 2) and occasionally other dolphins, but not cluster A. The entire social cluster A was not seen until about midway through the year, when most of its members were encountered in a single sighting, which is evident from the marked increase in the cumulative number of identified individuals at that point. Relatively few additional individuals were captured after this. The discovery curves across the entire study period (Fig. 5.10 and 5.11) suggest that the majority of the local population had been captured.

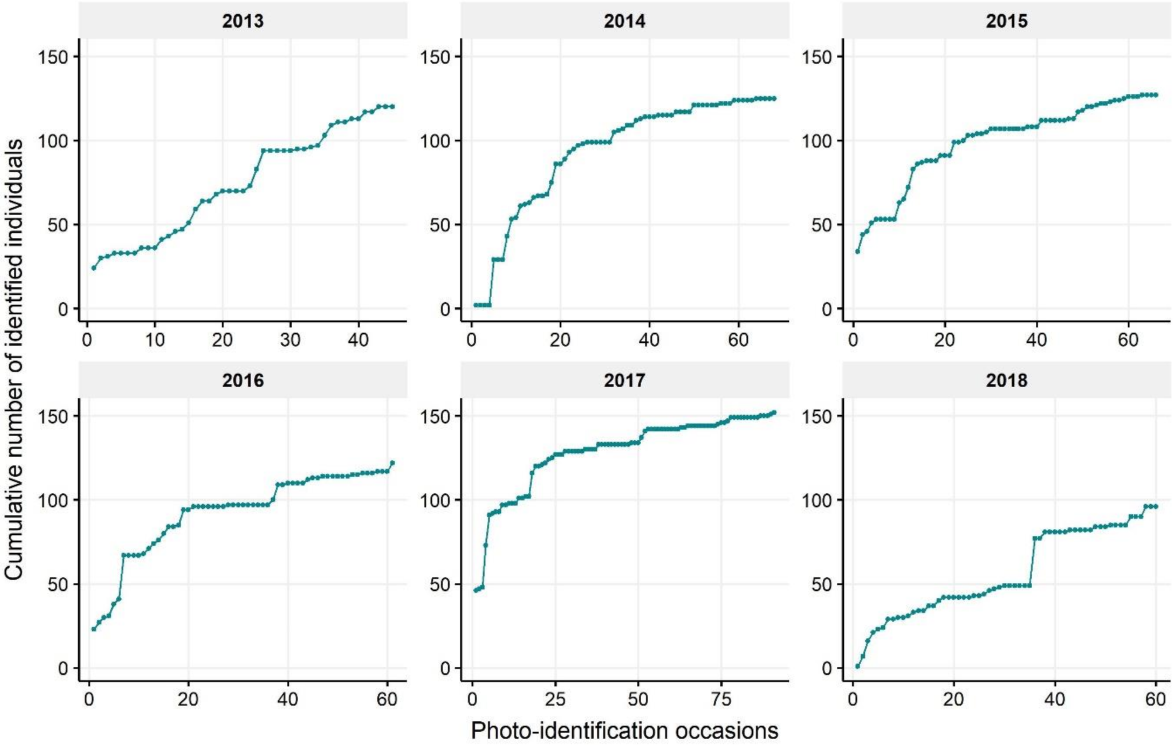

Figure 5.9. Annual discovery curves, showing the cumulative number of identified marked individuals over time.

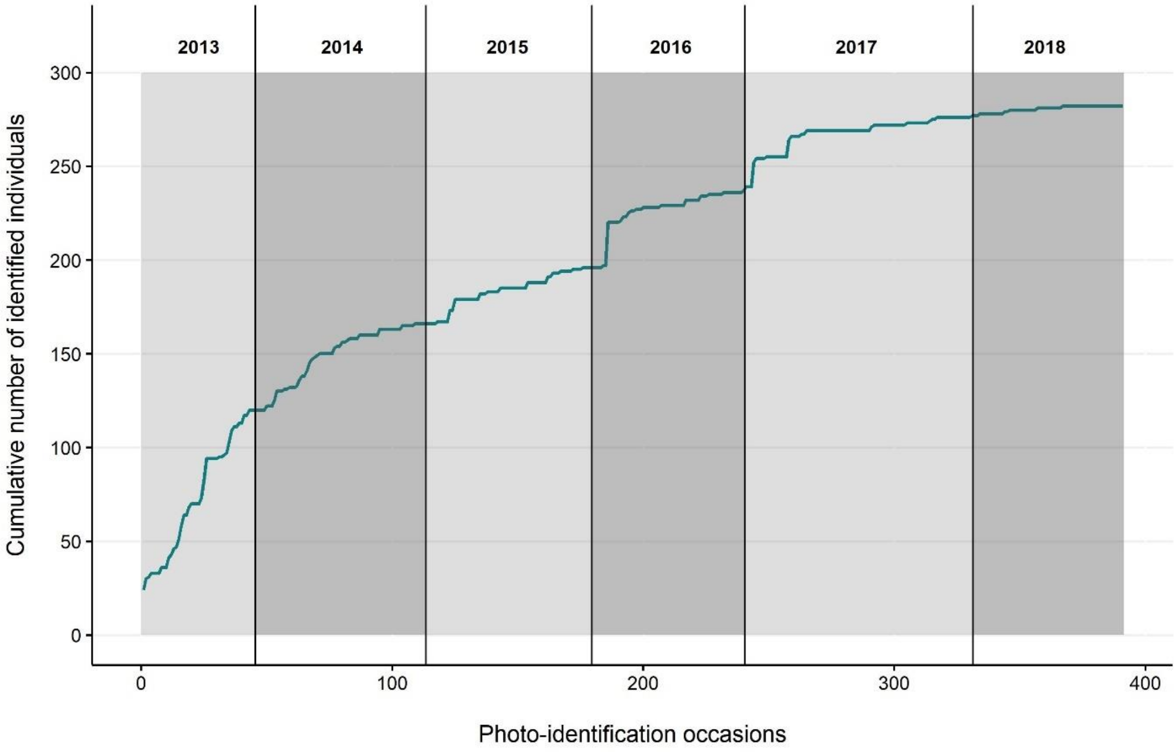

Figure 5.10. Discovery curves curve for the entire study period, showing the cumulative number of identified marked individuals over time.

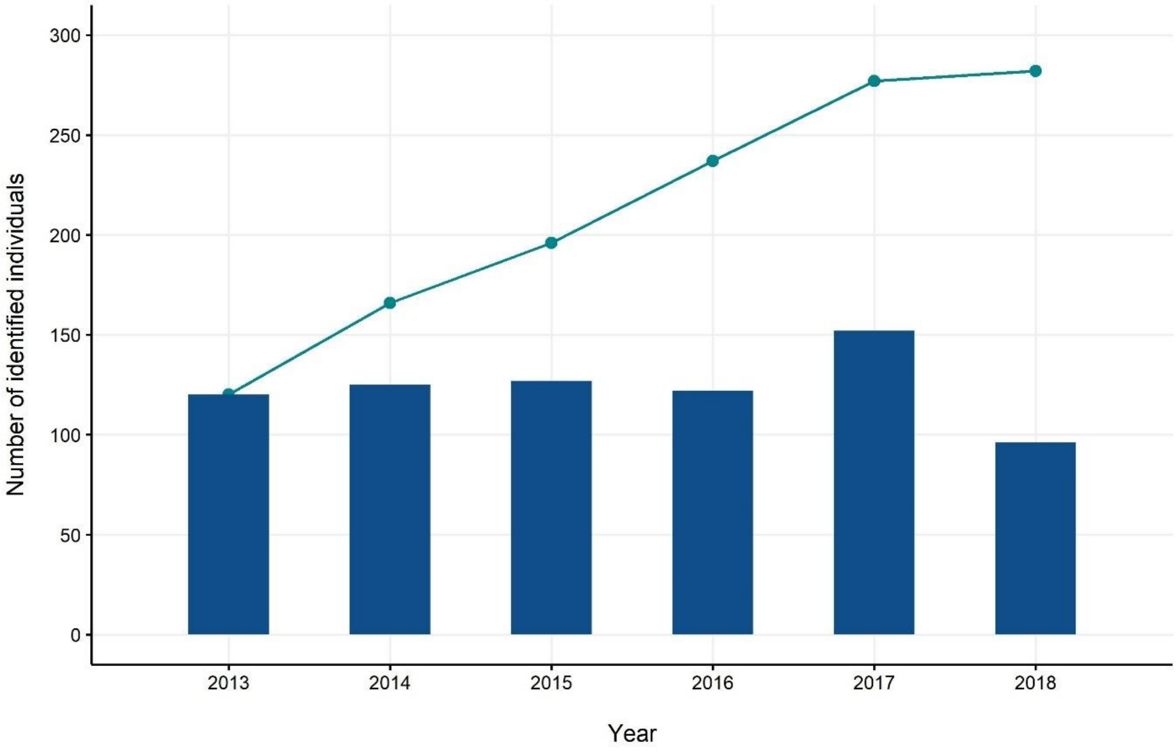

Figure 5.11. Number of identified individuals in each year, together with a cumulative discovery curve across time.

5.3.2 Modelling results

5.3.2.1 Annual closed models

Model selection strongly favoured models that incorporated both time variation and heterogeneity in capture probabilities (i.e. model M_{th}) in all years except 2018. In that year the model with only time-varying capture probabilities (M_t) received marginally more support than the model M_{th} in the full likelihood approach (Table 5.3), and somewhat less support in the Huggins approach (Table 5.4). The resulting abundance estimates between the two most supported models were similar, but the estimates accounting for heterogeneity were somewhat higher (Fig. 5.12), suggesting that heterogeneity was indeed present. Because of this, and to keep estimates among years consistent for the purposes of comparison and inferring any trend, the heterogeneity model was favoured and no model averaging was attempted for year 2018. Figure 5.13 and Table 5.5 show the annual abundance estimates from M_{th} models using the two modelling approaches. In general, the Huggins approach produced somewhat higher estimates, with wider confidence intervals (Fig. 5.13, Table 5.5). In some years, the difference in mean estimates was more pronounced (2013 in particular, but also 2014), while in others it was negligible (2015 and 2018, but also 2016, Fig. 5.13), but there was clear overlap of confidence intervals.

Table 5.3. Results of model selection for annual closed models using finite mixture models (full likelihood). Models are ordered by Δ AICc. Δ AICc is the difference between the AICc of a given model and the AICc of the most supported model. Model variations: π, p(t) = capture probabilities vary between two mixtures and by capture occasion; p(t) = capture probabilities vary by capture occasions; π, p(.) = capture probabilities vary between two mixtures but not by capture occasion; p(.) = constant capture probabilities. These models can be thought of as models M_{th}, M_t, M_h and M_0, respectively.

Year	#	Model	Parameters	AICc	Δ AICc	AICc weight	Deviance
2013	1	π, p(t)	11	-57.53725	0	1.000	96.55955
	2	p(t)	9	23.2762	80.81345	0.000	181.462
	3	π, p(.)	4	219.11797	276.65523	0.000	387.45137
	4	p(.)	2	262.08917	319.62642	0.000	434.45191
2014	1	π, p(t)	14	371.0422	0	1.000	426.2718
	2	p(t)	12	505.7757	134.7336	0.000	565.0851
	3	π, p(.)	4	684.7981	313.7559	0.000	760.3074
	4	p(.)	2	768.9437	397.9015	0.000	848.4734
2015	1	π, p(t)	13	278.5318	0	1.000	389.4101
	2	p(t)	11	310.3163	31.78449	0.000	425.2745
	3	π, p(.)	4	480.6458	202.114	0.000	609.7822
	4	p(.)	2	498.2581	219.7263	0.000	631.4167
2016	1	π, p(t)	12	164.7347	0	1.000	290.2063
	2	p(t)	10	190.2807	25.54603	0.000	319.8375
	3	π, p(.)	4	260.9755	96.2408	0.000	402.6981
	4	p(.)	2	280.1112	115.3765	0.000	425.8594
2017	1	π, p(t)	11	89.61673	0	1.000	290.1187
	2	p(t)	9	104.858	15.24127	0.000	309.43
	3	π, p(.)	4	274.6273	185.0106	0.000	489.3155
	4	p(.)	2	281.3853	191.7686	0.000	500.0966
2018	1	p(t)	10	147.574	0	0.596	256.4749
	2	π, p(t)	12	148.3548	0.780784	0.404	253.147
	3	π, p(.)	2	259.1627	111.5887	0.000	384.3076
	4	p(.)	4	262.2335	114.6594	0.000	383.3457

Table 5.4. Results of model selection for annual closed models using random effects models (Huggins). Models are ordered by Δ AICc. Δ AICc is the difference between the AICc of a given model and the AICc of the most supported model. Model variations: σ, p(t) = capture probabilities vary among individuals and by capture occasion; p(t) = capture probabilities vary by capture occasions; σ, p(.) = capture probabilities vary among individuals but not by capture occasion; p(.) = constant capture probabilities. These models can be thought of as models M_{th}, M_t, M_h and M_0, respectively.

Year	#	Model	Parameters	AICc	Δ AICc	AICc weight	Deviance
2013	1	σ, p(t)	9	858.5892	0	1.000	1016.775
	2	p(t)	8	933.4274	74.83814	0.000	1093.651
	3	σ, p(.)	2	1137.756	279.167	0.000	1310.119
	4	p(.)	1	1171.799	313.2097	0.000	1346.17
2014	1	σ, p(t)	12	1324.146	0	1.000	1383.455
	2	p(t)	11	1465.578	141.4319	0.000	1526.923
	3	σ, p(.)	2	1642.819	318.6731	0.000	1722.349
	4	p(.)	1	1728.284	404.1376	0.000	1809.819
2015	1	σ, p(t)	11	1256.673	0	1.000	1371.631
	2	p(t)	10	1288.265	31.59198	0.000	1405.258
	3	σ, p(.)	2	1457.954	201.2811	0.000	1591.112
	4	p(.)	1	1475.678	219.005	0.000	1610.843
2016	1	σ, p(t)	10	1092.263	0	1.000	1221.82
	2	p(t)	9	1118.848	26.58525	0.000	1250.442
	3	σ, p(.)	2	1189.183	96.91937	0.000	1334.931
	4	p(.)	1	1208.554	116.2908	0.000	1356.31
2017	1	σ, p(t)	9	1308.865	0	1.000	1513.437
	2	p(t)	8	1329.3	20.43465	0.000	1535.902
	3	σ, p(.)	2	1496.587	187.7221	0.000	1715.299
	4	p(.)	1	1505.494	196.6294	0.000	1726.212
2018	1	σ, p(t)	10	830.6613	0	0.699	939.5622
	2	p(t)	9	832.3452	1.68395	0.301	943.2932
	3	σ, p(.)	1	943.7279	113.0667	0.000	1070.882
	4	p(.)	2	944.5524	113.8911	0.000	1069.697

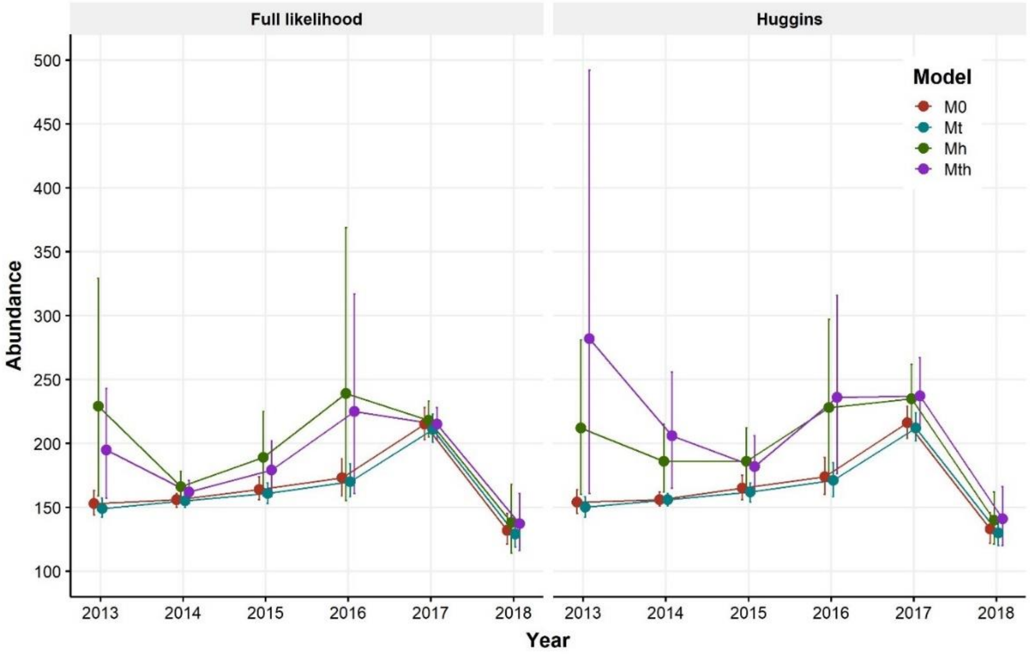

Figure 5.12. Comparison between competing models and two modelling approaches. The plot shows abundance estimates and 95 % confidence intervals of the total number of animals in different years, using either the finite mixture models (full likelihood) or random effects models (Huggins), and with variations in capture probabilities for each. Note that the treatment of heterogeneity (models M_h and M_{th}) differs between finite mixture models and random effects models.

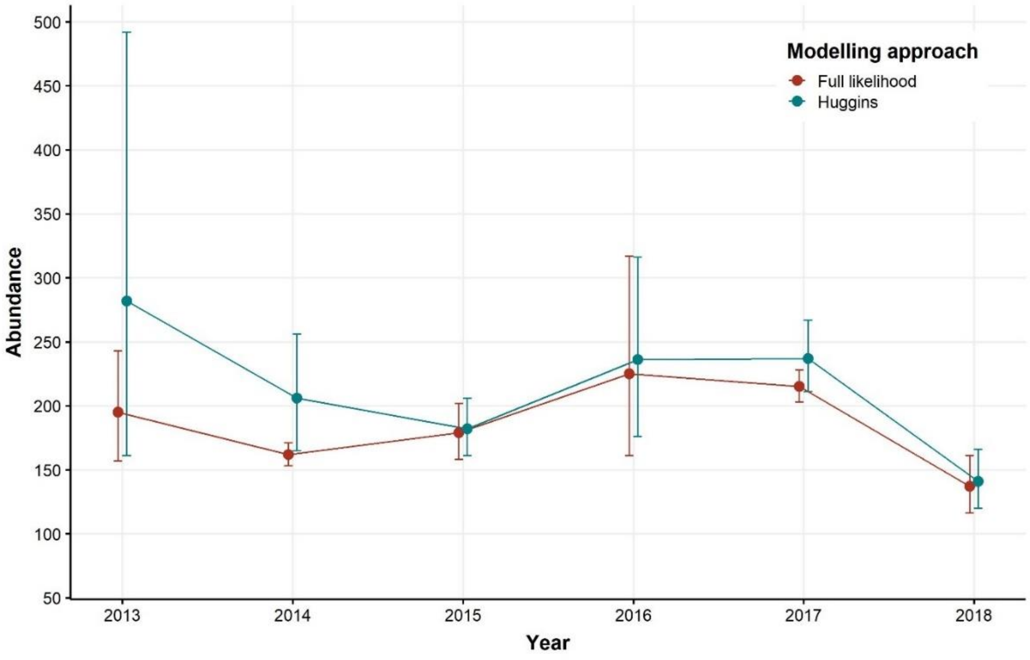

Figure 5.13. Abundance estimates and 95 % confidence intervals of the total number of animals in different years from models with time variation and heterogeneity in capture probabilities (M_{th}), using the finite mixture models (full likelihood) and random effects models (Huggins).

Table 5.5. Annual estimates of total abundance of bottlenose dolphins in the Gulf of Trieste between 2013 and 2018, based on the M_{th} closed model using finite mixture models (full likelihood) and the mixed effects models (Huggins).

Full likelihood						
Year	$\widehat{N}$	$\widehat{N}$ (95% CI)	Mark rate (with SE)	$\widehat{N}_{tot}$	$\widehat{N}_{tot}$ (95% CI)	$\widehat{N}_{tot}$ (CV)
2013	163	139–215	0.836 (0.014)	195	157–243	0.11
2014	131	127–141	0.811 (0.011)	162	153–171	0.03
2015	145	133–171	0.81 (0.014)	179	158–202	0.06
2016	176	141–273	0.783 (0.019)	225	161–317	0.17
2017	160	155–171	0.747 (0.014)	215	203–228	0.03
2018	110	100–140	0.810 (0.017)	137	116–161	0.08
Huggins						
Year	$\widehat{N}$	$\widehat{N}$ (95% CI)	Mark rate (with SE)	$\widehat{N}_{tot}$	$\widehat{N}_{tot}$ (95% CI)	$\widehat{N}_{tot}$ (CV)
2013	235	159–457	0.836 (0.014)	281	161–492	0.29
2014	167	143–220	0.811 (0.011)	206	165–256	0.11
2015	147	135–173	0.81 (0.014)	182	161–206	0.06
2016	185	149–264	0.783 (0.019)	236	176–316	0.15
2017	177	163–205	0.747 (0.014)	237	211–267	0.06
2018	114	103–141	0.810 (0.017)	141	120–166	0.08

5.3.2.2 Robust design models

With the initial candidate model set, which included models that accounted for both heterogeneity in capture probabilities and temporary emigration, the models apparently fitted successfully. However, upon examining the model output, it became clear that several of the parameters were in fact poorly estimated, resulting in standard errors so large that they essentially rendered the estimates uninformative. In other words, the width of the confidence intervals for these parameters (e.g., 0.0002–

0.9999) suggested that the model had difficulty estimating them and that they were therefore neither useful nor reliable. This was true for both the finite mixture models (full likelihood) and the random effects models (Huggins). Because of this the results were not considered to be reliable and therefore no further inferences were made from these models. In light of this, as a second step, two sets of models were fitted separately, with one incorporating heterogeneity in capture probabilities (with no temporary emigration) and the other incorporating temporary emigration (with no heterogeneity), as described in the Methods.

In the first set, for the finite mixture models approach (full likelihood), models not accounting for heterogeneity essentially had no support (Table 5.6). Two most supported models both included time-varying capture probabilities (at both the primary and secondary occasion level) as well as heterogeneity in capture probabilities varying by year, but differed in survival being either constant or time-dependent (Table 5.6). The third most supported model, which together with the first two comprised the 95% confidence set for model averaging, had constant survival, time-varying capture probabilities and constant heterogeneity in capture probabilities. Similarly, for the random effects models approach (Huggins), models ignoring heterogeneity had no support (Table 5.6). The two most supported models featured time-varying capture probabilities (at both the primary and secondary occasion level) and a constant among-individual heterogeneity in capture probabilities, and again differed only in survival being constant or varying among years. These two models comprised the 95% confidence set for model averaging. None of the models in the heterogeneity set that did not allow capture probabilities to vary by both primary and secondary capture occasions (p_{txs}) had any support and were therefore not considered further. Therefore, only models including this temporal variability were included in the second set investigating temporary emigration.

Table 5.6. Results of model selection for robust design models with heterogeneity in capture probabilities, using finite mixture models (full likelihood) and mixed effects models (Huggins). Models are ordered by Δ AICc. Δ AICc is the difference between the AICc of a given model and the AICc of the most supported model. Model variations: $\varphi(.)$ = constant survival probability among years; $\varphi(t)$ = survival probability varies by year; p(.) = constant capture probabilities; p(t) = capture probabilities vary by year; p(txs) = capture probabilities vary by year and secondary capture occasion; π(.) = capture probabilities vary between two mixtures, probability of belonging to one of two mixtures is constant (full likelihood only); π(t) = capture probabilities vary between two mixtures, probability of belonging to one of two mixtures varies by year (full likelihood only); $\sigma(.)$ = capture probabilities vary among individuals, at a constant level across years (Huggins only); $\sigma(t)$ = capture probabilities vary among individuals, at a level that varies among years (Huggins only). No temporary emigration is included in this candidate model set.

Full likelihood						
#	Model	Parameters	AICc	Δ AICc	AICc weight	Deviance
1	$\varphi(.)\ p(txs)\ \pi(t)$	70	2016.157	0	0.485	4724.667
2	$\varphi(t)\ p(txs)\ \pi(t)$	74	2016.563	0.405528	0.396	4716.444
3	$\varphi(.)\ p(txs)\ \pi(.)$	65	2019.877	3.719785	0.075	4739.122
4	$\varphi(t)\ p(txs)\ \pi(.)$	69	2020.961	4.803596	0.044	4731.622
5	$\varphi(t)\ p(txs)$	66	2316.512	300.3546	0	5033.614
6	$\varphi(.)\ p(txs)$	62	2324.969	308.8119	0	5050.627
7	$\varphi(.)\ p(t)\ \pi(t)$	20	3182.391	1166.233	0	5995.747
8	$\varphi(t)\ p(t)\ \pi(t)$	24	3184.275	1168.118	0	5989.444
9	$\varphi(.)\ p(t)\ \pi(.)$	15	3191.915	1175.757	0	6015.458
10	$\varphi(t)\ p(t)\ \pi(.)$	19	3192.847	1176.69	0	6008.245
11	$\varphi(.)\ p(.)\ \pi(t)$	16	3223.626	1207.469	0	6045.137
12	$\varphi(t)\ p(.)\ \pi(t)$	20	3247.245	1231.088	0	6060.602
13	$\varphi(.)\ p(.)\ \pi(.)$	11	3320.033	1303.876	0	6151.689
14	$\varphi(t)\ p(.)\ \pi(.)$	15	3326.008	1309.851	0	6149.552
15	$\varphi(t)\ p(t)$	17	3370.017	1353.86	0	6189.493
16	$\varphi(.)\ p(t)$	13	3376.731	1360.573	0	6204.334
17	$\varphi(t)\ p(.)$	12	3422.862	1406.705	0	6252.493
18	$\varphi(.)\ p(.)$	8	3437.701	1421.544	0	6275.418
Huggins						
#	Model	Parameters	AICc	Δ AICc	AICc weight	Deviance
1	$\varphi(.)\ p(txs)\ \sigma(.)$	57	7699.298	0	0.714	10435.6
2	$\varphi(t)\ p(txs)\ \sigma(.)$	61	7701.132	1.833818	0.286	10428.92
3	$\varphi(t)\ p(txs)\ \sigma(t)$	66	7728.292	28.99331	0	10445.39
4	$\varphi(.)\ p(txs)\ \sigma(t)$	62	7740.777	41.47885	0	10466.43
5	$\varphi(t)\ p(txs)$	60	8000.754	301.4559	0	10730.68
6	$\varphi(.)\ p(txs)$	56	8009.061	309.763	0	10747.49
7	$\varphi(.)\ p(t)\ \sigma(t)$	13	8873.961	1174.663	0	11701.57
8	$\varphi(t)\ p(t)\ \sigma(t)$	17	8875.177	1175.878	0	11694.65
9	$\varphi(.)\ p(t)\ \sigma(.)$	8	8880.202	1180.904	0	11717.92
10	$\varphi(t)\ p(t)\ \sigma(.)$	12	8881.259	1181.961	0	11710.89
11	$\varphi(t)\ p(.)\ \sigma(t)$	12	8893.262	1193.964	0	11722.89
12	$\varphi(.)\ p(.)\ \sigma(t)$	8	8902.417	1203.119	0	11740.14
13	$\varphi(t)\ p(.)\ \sigma(.)$	7	8907.657	1208.359	0	11747.39
14	$\varphi(.)\ p(.)\ \sigma(.)$	3	8913.011	1213.712	0	11760.79
15	$\varphi(t)\ p(t)$	11	9052.902	1353.603	0	11884.56
16	$\varphi(.)\ p(t)$	7	9059.487	1360.188	0	11899.22
17	$\varphi(t)\ p(.)$	6	9105.832	1406.534	0	11947.58
18	$\varphi(.)\ p(.)$	2	9120.64	1421.341	0	11970.42

In the second set, for the finite mixture models approach (full likelihood), models not accounting for temporary emigration had no support (Table 5.7). The two most supported models both included time-varying survival and Markovian temporary emigration, but differed in Markovian temporary emigration being either constant or time-dependent (Table 5.7). However, upon examining the output, the second-best model, with Markovian temporary emigration, did not estimate some of the temporary emigration parameters and was therefore dropped from further inference due to uncertain reliability. The third most supported model differed from the best model in having constant survival. These three models comprised the 95% confidence set for model averaging but, due to the issues with the second model, only the first and third model were used for model averaging. For the random effects models approach (Huggins), the model selection results were virtually identical to the full likelihood approach (Table 5.7) because no heterogeneity was included in either of the two candidate model sets. However, due to the different type of likelihood, the number of parameters, AICc and deviance values differ from those of the full likelihood approach.

Table 5.7. Results of model selection for robust design models with temporary emigration, using finite mixture models (full likelihood) and mixed effects models (Huggins). Models are ordered by Δ AICc. Δ AICc is the diffence between the AICc of a given model and the AICc of the most supported model. All models in the candidate set included time-varying capture probabilities by both primary and secondary occasion (p(txs)). Model variations: $\varphi(.)$ = constant survival probability among years; $\varphi(t)$ = survival probability varies by year; temporary emigration (random or Markovian) is either constant (.) or time-varying (t). No heterogeneity in capture probability is included in this candidate model set. Note that results between the two approaches are virtually identical, because no heterogeneity is included in either of the two candidate model sets.

Full likelihood							
#	Model	Temporary emigration	Parameters	AICc	Δ AICc	AICc weight	Deviance
1	$\varphi(t)\ p(txs)\ \gamma'(.)\ \gamma''(.)$	Markovian (.)	68	2282.014	0	0.525	4994.825
2	$\varphi(t)\ p(txs)\ \gamma'(t)\ \gamma''(t)$	Markovian (t)	74	2282.462	0.448151	0.420	4982.343
3	$\varphi(.)\ p(txs)\ \gamma'(.)\ \gamma''(.)$	Markovian (.)	64	2287.393	5.378988	0.036	5008.778
4	$\varphi(.)\ p(txs)\ \gamma'(t)\ \gamma''(t)$	Markovian (t)	70	2289.063	7.048924	0.015	4997.573
5	$\varphi(t)\ p(txs)\ \gamma'(t)=\gamma''(t)$	Random (t)	70	2292.36	10.34592	0.003	5000.87
6	$\varphi(t)\ p(txs)\ \gamma'(.)=\gamma''(.)$	Random (.)	67	2294.638	12.62406	0.001	5009.596
7	$\varphi(.)\ p(txs)\ \gamma'(t)=\gamma''(t)$	Random (t)	66	2296.909	14.8946	0	5014.011
8	$\varphi(.)\ p(txs)\ \gamma'(.)=\gamma''(.)$	Random (.)	63	2297.602	15.58803	0	5021.125
9	$\varphi(t)\ p(txs)$	None	66	2316.512	34.4975	0	5033.614
10	$\varphi(.)\ p(txs)$	None	62	2324.969	42.95484	0	5050.627
Huggins							
#	Model	Temporary emigration	Parameters	AICc	Δ AICc	AICc weight	Deviance
1	$\varphi(t)\ p(txs)\ \gamma'(.)\ \gamma''(.)$	Markovian (.)	62	7966.921	0	0.527	10692.58
2	$\varphi(t)\ p(txs)\ \gamma'(t)\ \gamma''(t)$	Markovian (t)	68	7967.394	0.472558	0.416	10680.2
3	$\varphi(.)\ p(txs)\ \gamma'(.)\ \gamma''(.)$	Markovian (.)	58	7972.259	5.337683	0.037	10706.44
4	$\varphi(.)\ p(txs)\ \gamma'(t)\ \gamma''(t)$	Markovian (t)	64	7973.917	6.996146	0.016	10695.3
5	$\varphi(t)\ p(txs)\ \gamma'(t)=\gamma''(t)$	Random (t)	64	7977.39	10.46935	0.003	10698.77
6	$\varphi(t)\ p(txs)\ \gamma'(.)=\gamma''(.)$	Random (.)	61	7979.586	12.66487	0.001	10707.38
7	$\varphi(.)\ p(txs)\ \gamma'(t)=\gamma''(t)$	Random (t)	60	7981.891	14.96989	0	10711.81
8	$\varphi(.)\ p(txs)\ \gamma'(.)=\gamma''(.)$	Random (.)	57	7982.525	15.60425	0	10718.83
9	$\varphi(t)\ p(txs)$	None	60	8000.754	33.83329	0	10730.68
10	$\varphi(.)\ p(txs)$	None	56	8009.061	42.14036	0	10747.49

The most supported "null" model (without heterogeneity or temporary emigration), in the candidate set of models in which survival and capture probabilities were either held constant or allowed to vary with time, was one with time-varying survival and capture probabilities that varied by both primary and secondary capture occasions, in both the full likelihood and the Huggins approach (Table 5.8). This model was used to assess the magnitude and direction of potential bias resulting from either not accounting for heterogeneity or not accounting for temporary emigration.

Table 5.8. Results of model selection for the most supported "null" model (without heterogeneity or temporary emigration), using finite mixture models (full likelihood) and random effects models (Huggins).

Full likelihood						
#	Model	Parameters	AICc	Δ AICc	AICc weight	Deviance
1	$\varphi(_{t})\ p(_{txs})$	66	2316.512	0	0.986	5033.614
2	$\varphi(.)\ p(_{txs})$	62	2324.969	8.457344	0.014	5050.627
3	$\varphi(_{t})\ p(_{t})$	17	3370.017	1053.506	0	6189.493
4	$\varphi(.)\ p(_{t})$	13	3376.731	1060.219	0	6204.334
5	$\varphi(_{t})\ p(.)$	12	3422.862	1106.351	0	6252.493
6	$\varphi(.)\ p(.)$	8	3437.701	1121.189	0	6275.418
Huggins						
#	Model	Parameters	AICc	Δ AICc	AICc weight	Deviance
1	$\varphi(_{t})\ p(_{txs})$	60	8000.754	0	0.985	10730.68
2	$\varphi(.)\ p(_{txs})$	56	8009.061	8.307067	0.015	10747.49
3	$\varphi(_{t})\ p(_{t})$	11	9052.902	1052.147	0	11884.56
4	$\varphi(.)\ p(_{t})$	7	9059.487	1058.732	0	11899.22
5	$\varphi(_{t})\ p(.)$	6	9105.832	1105.078	0	11947.58
6	$\varphi(.)\ p(.)$	2	9120.64	1119.886	0	11970.42

Not accounting for temporary emigration had virtually no effect on the resulting estimates, as the model incorporating it was nearly identical to the null model, regardless of the modelling approach (Fig. 5.14). Not accounting for heterogeneity, however, led to lower abundance estimates (Fig. 5.14), providing strong support for the model incorporating heterogeneity. Moreover, when comparing AICc values among the best heterogeneity model, the best temporary emigration model and the best null model, the model including heterogeneity was by far the most supported one, regardless of the modelling approach (full likelihood vs. Huggins, Table 5.9). Given this, the best models incorporating heterogeneity (using both the full likelihood and the Huggins approach, respectively) were considered the best robust design models. The 95% confidence set of the most supported heterogeneity models was therefore used for model averaging and further inference.

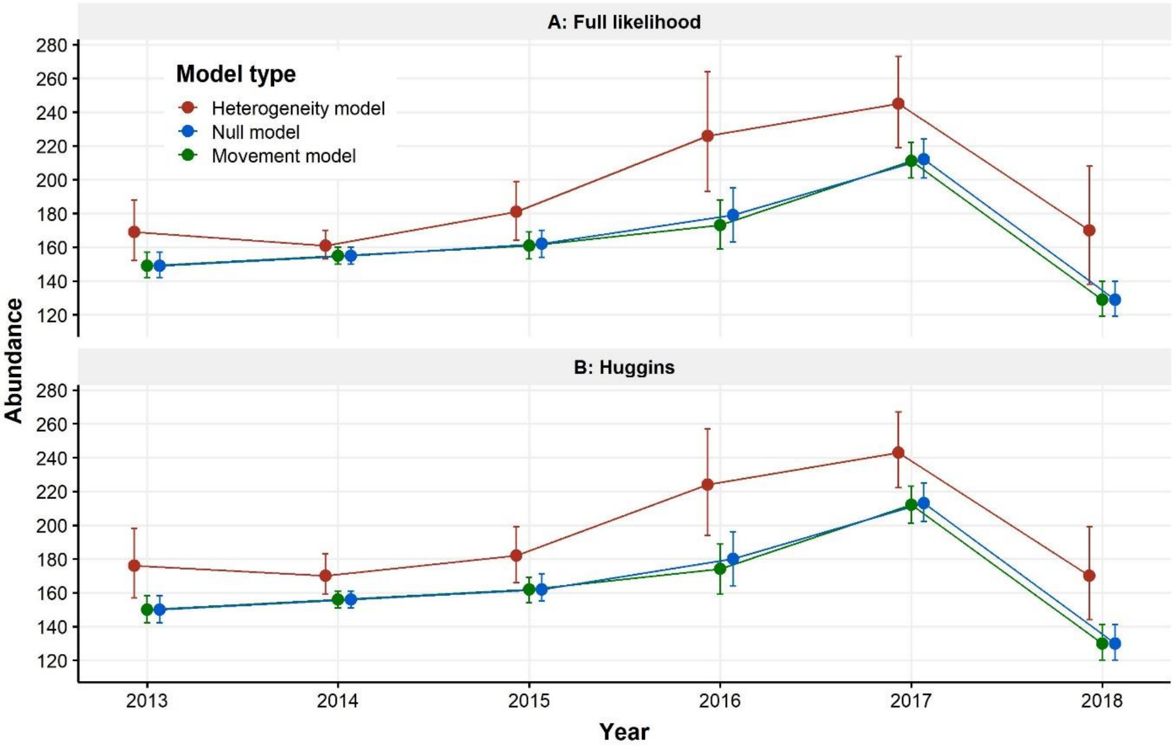

Figure 5.14. Comparison of the null model with heterogeneity and temporary emigration models in terms of the resulting abundance estimates and 95 % confidence intervals for both the finite mixture models (full likelihood) and the random effects models (Huggins).

Table 5.9. Comparison between the best heterogeneity model, best temporary emigration model and best null model.

Full likelihood						
#	Model	Parameters	AICc	Δ AICc	AICc weight	Deviance
1	Best heterogeneity model	70	2016.157	0	1	4724.667
2	Best temporary emigration model	68	2282.014	265.8571	0	4994.825
3	Best null model	66	2316.512	300.3546	0	5033.614
Huggins						
#	Model	Parameters	AICc	Δ AICc	AICc weight	Deviance
1	Best heterogeneity model	57	7699.298	0	1	10435.6
2	Best temporary emigration model	62	7966.921	267.6227	0	10692.58
3	Best null model	60	8000.754	301.4559	0	10730.68

5.3.2.3 Annual closed models vs. robust design models

Estimates from annual closed models and the model-averaged best robust design models for the finite mixture models (full likelihood) and the random effects models (Huggins), respectively, were relatively consistent, but the robust design models were generally more precise (Fig. 5.15 and 5.16) and were used for final inferences about population size.

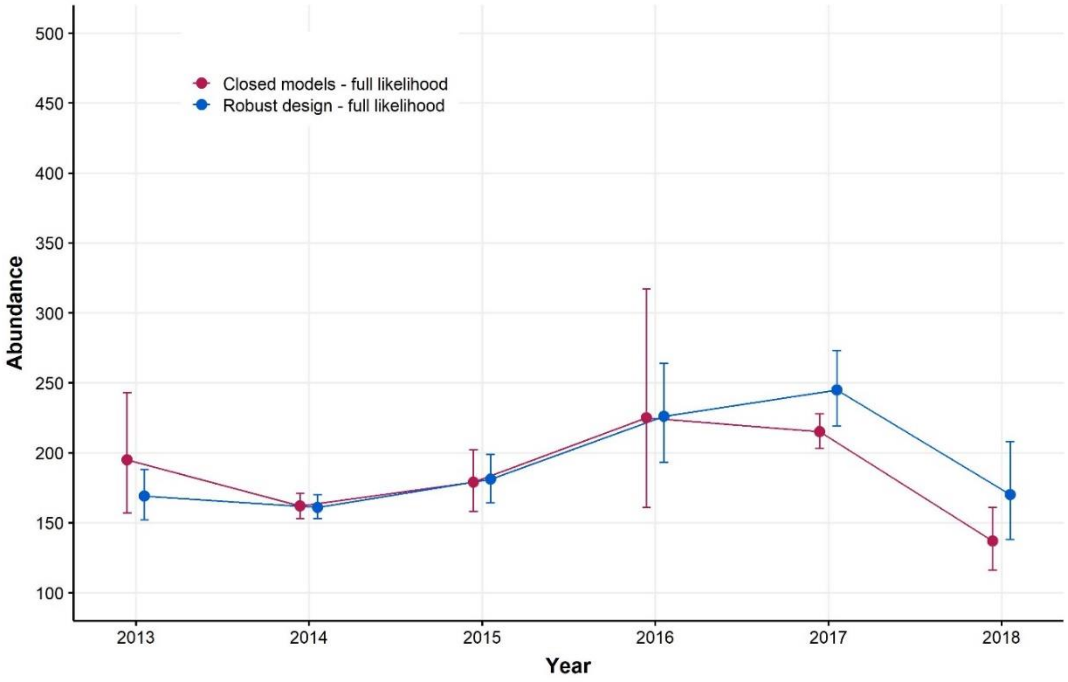

Figure 5.15. Comparison between the most supported annual closed models and the most supported robust design model, utilising the finite mixtures approach (full likelihood).

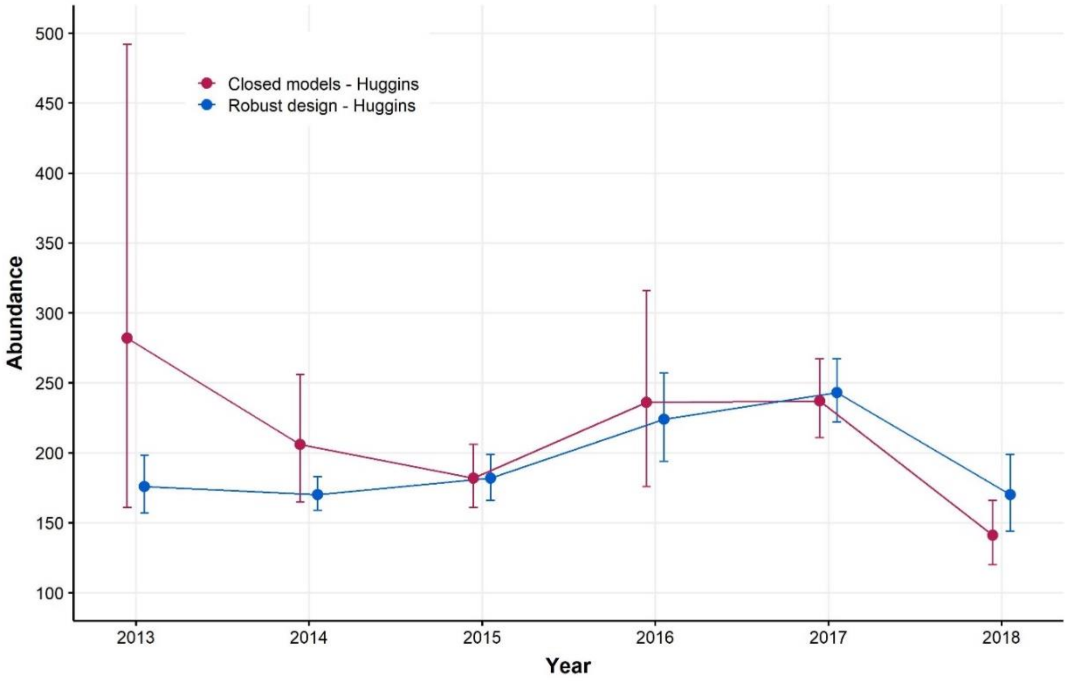

Figure 5.16. Comparison between the most supported annual closed models and the most supported robust design model, utilising the mixed effects approach (Huggins).

5.3.2.4 Abundance of dolphins in the study area

The model-averaged estimates of the best robust design models incorporating heterogeneity in capture probabilities for both the full likelihood and the Huggins approach, respectively, are shown in Table 5.10 and Fig. 5.17. Estimates between the two approaches were very consistent. In general, the Huggins estimates were more precise, but this was not true in all years. Both the overall lowest and highest estimates (considering both the mean estimates and the 95% confidence interval) were produced by the full likelihood approach. Overall, and taken together, these results suggest that the annual total abundance of bottlenose dolphins in the study area ranged between 161 (95% CI = 153–170) and 245 (95% CI = 219–273).

Table 5.10. Model-averaged annual estimates of total abundance of bottlenose dolphins in the Gulf of Trieste between 2013 and 2018, based on the most supported robust design models using finite mixture models (full likelihood) and the mixed effects models (Huggins).

Full likelihood						
Year	$\hat{N}$	$\hat{N}$ (95% CI)	Mark rate (with SE)	$\hat{N}_{tot}$	$\hat{N}_{tot}$ (95% CI)	$\hat{N}_{tot}$ (CV)
2013	141	131–161	0.836 (0.014)	169	152–188	0.06
2014	131	127–140	0.811 (0.011)	161	153–170	0.03
2015	147	137–164	0.81 (0.014)	181	164–199	0.05
2016	177	156–209	0.783 (0.019)	226	193–264	0.08
2017	183	169–205	0.747 (0.014)	245	219–273	0.06
2018	137	120–167	0.810 (0.017)	170	138–208	0.1
Huggins						
Year	$\hat{N}$	$\hat{N}$ (95% CI)	Mark rate (with SE)	$\hat{N}_{tot}$	$\hat{N}_{tot}$ (95% CI)	$\hat{N}_{tot}$ (CV)
2013	147	135–168	0.836 (0.014)	176	157–198	0.06
2014	138	131–151	0.811 (0.011)	170	159–183	0.04
2015	147	138–163	0.81 (0.014)	182	166–199	0.05
2016	175	156–203	0.783 (0.019)	224	194–257	0.07
2017	182	169–200	0.747 (0.014)	243	222–267	0.05
2018	137	121–163	0.810 (0.017)	170	144–199	0.08

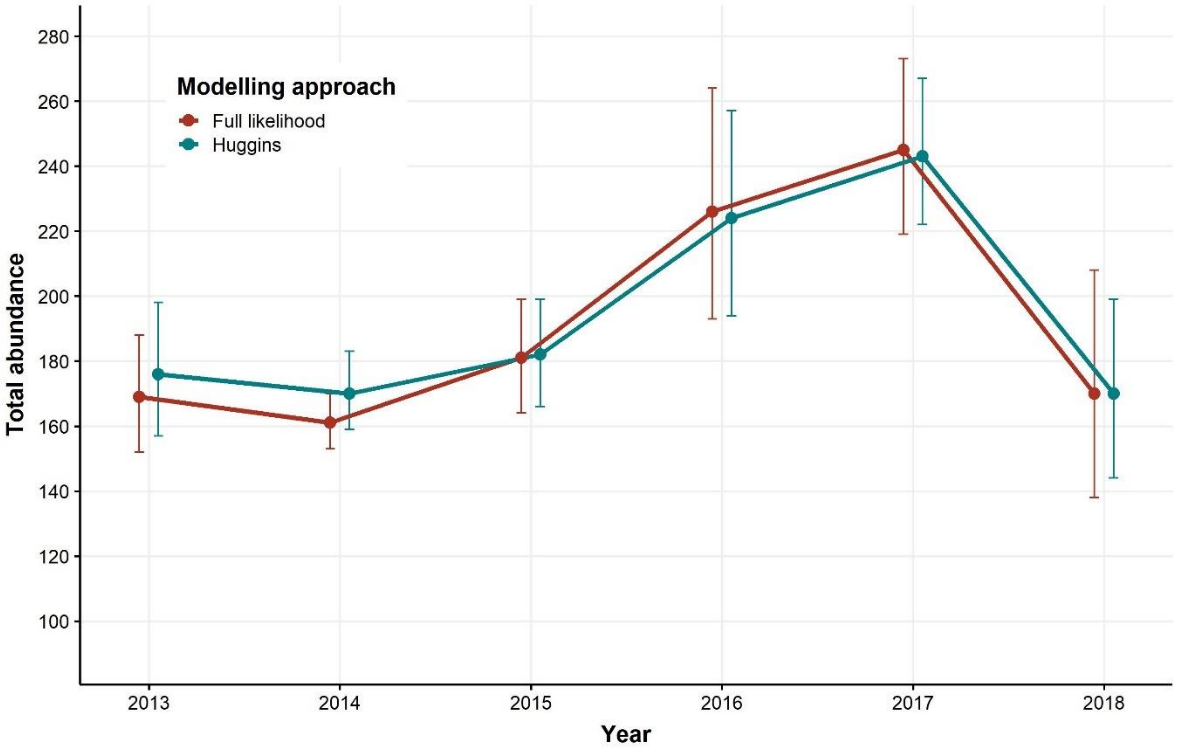

Figure 5.17. Final best estimates of the abundance of animals in different years for both the finite mixture models (full likelihood) and the random effects models (Huggins), from the most supported heterogeneity models.

5.3.2.5 Apparent survival, temporary emigration and capture probabilities

The estimates of annual apparent survival, temporary emigration parameters and annual capture probabilities are shown in Table 5.11. Annual estimates of apparent survival ranged between 0.784 (95% CI = 0.622–0.889) and 0.81 (95% CI = 0.727–0.873) for the full likelihood models and between 0.769 (95% CI = 0.661–0.85) and 0.804 (95% CI = 0.735–0.859) for the Huggins models. The probability of emigration (γ'') was low for both modelling approaches, as was the probability of immigration ($1 - \gamma'$).

Table 5.11. Model-averaged estimates of apparent survival, temporary emigration and capture probability for bottlenose dolphins in the Gulf of Trieste between 2013 and 2018, based on the most supported robust design models using finite mixture models (full likelihood) and the mixed effects models (Huggins). For each year, the apparent survival refers to survival from that year to the next (note that there is no information on survival for year 2018, as it was the last year of the study). $\hat{\phi}$ = estimate of apparent survival; $\widehat{\gamma'}$ = estimate of the probability of being outside the study area in a given year, given being outside the study area in the previous year (constant across years); $\widehat{\gamma''}$ = estimate of the probability of being outside the study area in a given year, given being outside the study area in the previous year (constant across years). $\hat{p}$ = estimated capture probability. Note that estimates of apparent survival and capture probabilities are model-averaged based on the most supported heterogeneity models, whereas temporary emigration parameters are based on the most supported temporary emigration models.

Full likelihood				
Year	$\hat{\phi}$ (95% CI)	$\widehat{\gamma'}$ (95% CI)	$\widehat{\gamma''}$ (95% CI)	$\hat{p}$ (95% CI)
2013	0.755 (0.629–0.848)	–	–	0.32 (0.3–0.34)
2014	0.805 (0.738–0.858)	–	0.107 (0.023–0.382)	0.384 (0.367–0.401)
2015	0.81 (0.729–0.871)	0.784 (0.128–0.989)	0.107 (0.023–0.382)	0.273 (0.252–0.294)
2016	0.81 (0.727–0.873)	0.784 (0.128–0.989)	0.107 (0.023–0.382)	0.243 (0.221–0.265)
2017	0.784 (0.622–0.889)	0.784 (0.128–0.989)	0.107 (0.023–0.382)	0.229 (0.21–0.248)
2018	–	0.784 (0.128–0.989)	0.107 (0.023–0.382)	0.156 (0.139–0.173)
Huggins				
Year	$\hat{\phi}$ (95% CI)	$\widehat{\gamma'}$ (95% CI)	$\widehat{\gamma''}$ (95% CI)	$\hat{p}$ (95% CI)
2013	0.769 (0.661–0.85)	–	–	0.227 (0.197–0.257)
2014	0.8 (0.742–0.847)	–	0.109 (0.022–0.404)	0.273 (0.248–0.299)
2015	0.804 (0.735–0.859)	0.792 (0.122–0.991)	0.109 (0.022–0.404)	0.214 (0.192–0.236)
2016	0.804 (0.735–0.859)	0.792 (0.122–0.991)	0.109 (0.022–0.404)	0.139 (0.12–0.159)
2017	0.788 (0.687–0.863)	0.792 (0.122–0.991)	0.109 (0.022–0.404)	0.252 (0.226–0.277)
2018	–	0.792 (0.122–0.991)	0.109 (0.022–0.404)	0.138 (0.115–0.16)

5.4 DISCUSSION

5.4.1 Study strengths and limitations

The study design, study area coverage, the choice of the modelling framework, the choice of capture occasions and quality assurance (with respect to photographic quality), as well as the biology of the species, ensured that the model assumptions were met, providing high confidence in the results. For example, one of the most crucial assumptions is that of demographic closure within primary capture occasions (Borchers et al. 2002, Amstrup et al. 2005, White 2008, Hammond 2010). The slow-reproducing nature of this species (Wells & Scott 1999), existing information about site fidelity (Genov et al. 2008, Genov et al. 2019a, Genov et al. 2019b) and previous evidence on connectivity (or rather lack thereof) from both photo-identification (Genov et al. 2009) and genetics (Gaspari et al. 2015) already suggested that the assumption of closure was likely to be met. This was further corroborated by discovery curves (Fig. 5.9 and 5.10). Care was taken to ensure, as much as possible, that all sources of bias were removed or accounted for. This also included accounting for animals that either became marked or died during the course of the study. From mark-recapture assumptions, it is implicit that this be the case. However, this is not typically addressed (or referred to) explicitly. In photo-identification of small cetaceans, an animal may transition from an "unmarked" state to a "marked" state, i.e. the animal becomes marked during a particular study year. Moreover, because high-quality photographs may allow "unmarked" individuals to be recognisable in some sightings, sometimes such a change is known and observed. This change in mark status likely happens relatively often in photo-identification studies of small cetaceans and may have implications on how such events are considered in data analysis and how they may impact results. Because the animal is known and identified, it is tempting to include all of its records in capture histories. However, an animal becoming marked during a year violates the assumption of demographic closure, as it essentially represents recruitment into the marked part of the population. This artificially reduces capture probability and leads to an overestimate of population size. Conversely, identified animals may die during the course of a year and some of those deaths may be known because they are recorded (e.g., Genov et al. 2016). An animal dying during the year would also violate the assumption of population closure, and would lead to a decrease in capture probabilities, which in turn would lead to abundance being overestimated for that year (despite the fact that the population has just been reduced by that animal that died). Moreover, the survival in the robust design framework assumes recruitment and deaths occur *between* primary sampling occasions (years), not *within* them. In this study, explicitly removing animals dying in a particular year from capture histories of that entire year, and only adding animals that became marked during a course of a year in the capture histories of the following year, further ensured consistency and contributed to assumptions being met. Potential violations of assumptions that could not be avoided or minimised by survey design were explicitly addressed in the modelling, which included time-varying capture probabilities, individual heterogeneity in capture probabilities and the effect of temporary emigration.

Ideally, all of these sources of unequal capture probabilities could be investigated and accounted for in the same models. However, the most complex models, those incorporating all of these effects, led to poor estimates of parameters and were considered unreliable. With respect to modelling heterogeneity, White & Cooch (2017) suggested that a minimum of 5 capture occasions is likely necessary to achieve reasonable performance for either the finite mixture or the random effects models approach. Here, this was achieved, with the number of secondary capture occasions ranging between 8 and 11 for a given year, and indeed both approaches could successfully be implemented for modelling heterogeneity. It may be that the combined heterogeneity-movement models were too complex, with too many parameters to be fitted, for the available data. Nevertheless, a multi-tiered approach was informative and useful in providing an insight into the mechanisms affecting capture probabilities and obtaining robust abundance estimates.

One obvious limitation of this study was that the extent of spatial survey effort was limited by the fact the survey vessel was a small boat and the need to return to a home port after each survey. This likely meant that not all of the range of the studied animals was sampled. No assumption was made about what proportion of the total population the abundance estimates represented, although there is evidence these animals represent a somewhat distinct unit (Genov et al. 2009, Gaspari et al. 2015). However, the geographical limits of this population are not well defined, with evidence that it ranges beyond the current study area (Genov et al. 2008, Genov et al. 2016, Morigenos, unpublished data). Estimates considered here therefore relate to the population inhabiting the area, not to a wider putative biological population (Hammond et al. 2021).

Survey effort was also complicated by the fact that this is a transboundary area. While surveys in the waters of Slovenia and Italy (both EU member states within the Schengen area) could be carried out without problems, Croatia only joined the EU in mid-2013 and is still not within the Schengen area. This caused logistical problems with respect to cumbersome and time-consuming border crossing procedures. In addition, in June 2017 the Arbitral Tribunal in the Hague delivered its final ruling in the arbitration between the Republic of Slovenia and the Republic of Croatia, following an ongoing border dispute, determining the maritime (and land) boundary between the two countries. The Republic of Croatia challenged that ruling, resulting in a continued dispute, which has since increased the difficulty of carrying out surveys in the disputed area. In practice, this often meant that dolphin groups could not be approached, or that focal follows had to cease.

5.4.2 Accounting for unequal capture probabilities

Both heterogeneity in capture probabilities and temporary emigration, if not accounted for, may cause bias in estimates of abundance, in opposite directions. Heterogeneity, if present but not accounted for, causes underestimation of abundance (Borchers et al. 2002), whereas temporary emigration, if present but not accounted for (unless completely random, in which case it only affects precision), causes overestimates in abundance (Kendall 1999).

The lack of strong evidence for temporary emigration was somewhat surprising, as the general perception over the entire course of this research project (2002–2021) was that animals range beyond the currently surveyed area and are not always present within it. This alone would not necessarily be reflected in temporary emigration causing a bias, as animals may move in and out of the study area within a given primary occasion (year) and still be captured at some point. In may be that this is in fact what is happening. However, several individuals in this population have demonstrated absences of several years between re-sightings (T. Genov, unpublished data). The notion of temporary emigration is further supported by the discovery curve in year 2018 (Fig. 5.9), where well-known individuals that have been known since the very beginning of the project were absent for the first half of the year. The fact that the number of photo-identified marked animals differed among years, would also generally support this. Nevertheless, even though model selection did favour models with temporary emigration included, the resulting estimates essentially did not differ from the null model (Fig. 5.14). Moreover, the probability of emigration (γ'') was low for both modelling approaches, as was the probability of immigration ($1 - \gamma'$), suggesting there isn't much temporary emigration and immigration occurring. This, together with low precision for these estimates (Table 5.11), indicates there was not much signal in the data and may be why these parameters were difficult to estimate by the models. Therefore, temporary emigration, even if present, did not have an effect on estimates of abundance, neither in magnitude nor precision, and appears to be a minor driver in patterns of abundance and capture probabilities for this population during the study period.

Temporary emigration (at least as modelled within the robust design framework) operates on an inter-annual basis. Individual heterogeneity, on the other hand, manifests itself across capture occasions, including within years and within secondary capture occasions. Indeed, heterogeneity appeared to play a much more prominent role in this dataset. Its presence is strongly supported not only by the results of model selection, but also by the fact that models in which heterogeneity was explicitly modelled produced estimates that were higher than those not accounting for it (Fig. 5.14). In all years and in both approaches for detecting and accounting for heterogeneity, the model with time-varying and individually-varying capture probabilities was by far the most supported model (Table 5.6), also when compared to the best temporary emigration and null models (Table 5.9). This is hardly surprising, as capture probabilities in a wild dolphin population are expected to vary through time, as well as by individual animals. Individual heterogeneity is typically a feature in mark-recapture studies of bottlenose dolphins (Wilson et al. 1999b, Gonzalvo et al. 2016, Arso Civil et al. 2019b) and in fact of most cetaceans (Hammond et al. 2021). The use of the study area by the dolphins (as far as relevant to the abundance estimates) appears to be more variable continuously – throughout the year, rather than among years, therefore favouring heterogeneity models over temporary emigration models.

This study used two different approaches to estimating and accounting for heterogeneity, by exploring both finite mixture models and random effects models. In cetacean studies, finite mixtures are typically used (e.g., Arso Civil et al. 2019b). However, there is little reason to believe the animals in the population form only two distinct groups with substantially different underlying capture probabilities. It is more conceivable that there is more individual variability, or that there are more than two groups. Even in this study, where two largest social clusters differ dramatically in their behaviour and temporal movement patterns (Chapter 2), there is little reason to believe this translates into a pattern of just two groups with distinct capture probabilities. Even though both clusters do likely differ in their capture probabilities in relation to time of day and perhaps overall, members of both clusters were seen regularly and were captured in a large proportion of secondary capture occasions, which likely renders their capture probabilities to be similar overall, over a given year and across years. Moreover, other social clusters do exist in this population (Chapter 2), which again suggests that the two mixtures approach is unlikely to be representative of biological reality. It may be that the frequent use of finite mixtures approach is more driven by practicality and necessity rather than biological soundness, as it may be difficult or even impossible to fit more complicated models with the amount and type of data typical of marine mammal studies.

Nevertheless, reassuringly, both approaches in this study produced very consistent results. This may suggest that either two mixtures was indeed the right way to model individual heterogeneity and that the random effects approach provided no additional benefits, or that even in the absence of two clear groups, the two approaches both work well. This may be because the finite mixtures adequately capture the inherent variability, even if the underlying process does not originate from discrete groups (Pledger 2005). Social network analysis (Chapter 2) did show that the population was composed of two major social clusters, which could represent the two mixture groups. However, as alluded to earlier, despite their behavioural and habitat use differences, both social clusters were seen consistently, which is unlikely to lead to two distinct sets of capture probabilities. On the other hand, both of these clusters may in fact form the same discrete group, while the smaller cluster C (the so-called “freelancers”), as well as animals not included in the initial social analysis but present during the 2013-2018 mark-recapture study, may represent the other discrete group.

Nevertheless, the idea of two largest social clusters having different capture probabilities cannot be dismissed entirely. As described in Chapter 2, the cluster A was largely the “morning group”, whereas the cluster B was largely the “evening group”. What this meant in practice was that photographs of the entire group were more difficult to obtain for cluster B, because sightings were limited by daylight and were often cut-off by nightfall (Chapter 2). This could lead to overall lower capture probabilities for cluster B. Testing this explicitly, by using individual known covariates (in this case group membership), was beyond the scope of this analysis. The time mis-match between the data analysed in Chapter 2 (2003-2011) and those analysed in this chapter (2013-2018) meant that in order to accurately assign social group membership to individuals, social network analysis would need to be repeated for the period 2013–2018. This will be attempted as a follow-up of the analyses presented here in the future.

5.4.3 Apparent survival

Estimates of apparent survival were low compared to other populations of this species (Wells & Scott 1990, Fortuna 2006, Currey et al. 2009a, Silva et al. 2009, Arso Civil et al. 2019a). This was surprising, given the high site fidelity and re-sighting rates for a large proportion of photo-identified individuals. However, these results are consistent with genetic evidence of this population being a source population for other local populations in the region (S. Gaspari, T. Genov and A. Moura, unpublished data).

5.4.4 Conservation implications

The abundance estimates were relatively consistent across time. There was no obvious trend, although the relatively short study period (6 years) precludes meaningful trend assessment, and trends are generally (and notoriously) difficult to detect in marine mammal populations even with longer time series (Taylor & Gerrodette 1993, Wilson et al. 1999b, Forney 2000, Taylor et al. 2000, Thompson et al. 2000, Williams & Thomas 2009, Jewell et al. 2012).

When considering these estimates, together with their measures of precision (Table 5.10), all of the mean estimates and lower 95 % confidence interval limits (for both modelling approaches), as well as the upper 95 % confidence interval limits for all years except 2016 and 2017, suggest that the population abundance is below 250 animals. A population with fewer than 250 mature individuals would qualify as

Endangered under IUCN criterion D (IUCN 2012). As mentioned above, the geographical limits of this population are not well defined, so whether or not these estimates pertain to a coherent ecological or conservation unit, remains to be determined.

Generally, and particularly when considering the size of the study area, the abundance estimates for this study are relatively comparable to estimates for this species elsewhere in the northern Adriatic Sea (Pleslić et al. 2015, Bearzi et al. 2021), other Mediterranean areas (Bearzi et al. 2008a, Gnone et al. 2011, Gonzalvo et al. 2016, Papale et al. 2017) and other parts of Europe and the North Atlantic (Wilson et al. 1999b, Silva et al. 2009, Berrow et al. 2012, Cheney et al. 2013, Louis et al. 2015, Arso Civil et al. 2019b, Nykänen et al. 2020), based on similar methodology.

In a recent assessment (Genov 2020), 39 % of death causes of bottlenose dolphins in Slovenia between 2002 and 2020 were attributed (either confirmed or strongly suspected) to fisheries. More specifically, 17 % of death causes were confirmed bycatch, while 22 % were attributed to other types of fisheries-related mortality, including larynx strangulation (Genov 2020). These estimates do not account for the neighbouring Italian and Croatian waters and may therefore be underestimates. In terms of numbers, between 0 and 5 animal deaths annually (confirmed or strongly suspected) were fisheries-related, while 0 to 2 annually were confirmed bycatch. With a very simplified calculation (Genov 2020) and in light of the results of this chapter, simple preliminary assessments can be made. When accounting for all (suspected or confirmed) fishery-related mortality, and assuming population estimates from Table 5.10 and all the stranded animals belonging to this population, annual fisheries-related mortality is 0–3.1 %. When accounting only for confirmed cases of bycatch, and assuming population estimates from Table 5.10 and all the stranded animals belonging to this population, annual fisheries-related mortality is 0–1.2 %.

CHAPTER 6: NOVEL METHOD FOR IDENTIFYING INDIVIDUAL CETACEANS USING FACIAL FEATURES AND SYMMETRY: A TEST CASE USING DOLPHINS[3]

6.1 INTRODUCTION

Dolphins all look the same. Or do they? The identification of individuals plays a major role in our understanding of the biology, ecology, behavior, and population dynamics in various animal species, including cetaceans (Hammond et al. 1990, Whitehead 2008a, Hammond 2009). Being able to tell individuals apart can provide invaluable insight into basic biological and scientific questions, but is also highly relevant to science-based conservation. Hence, given the importance of individual identification, it appears vital to improve our ability to identify individuals, even in those species where (perhaps limited) recognition methods already exist. Furthermore, it seems imperative to find ways to identify individuals in those species we currently consider non-amenable to individual identification.

It is widely known that several species of cetacean can be individually identified using natural markings (Hammond et al. 1990). Scarring, notches, pigmentation, and callosity patterns, other long-term natural markings, as well as human-inflicted markings (*e.g.*, freeze-brands or signs of sublethal boat strikes (Irvine et al. 1981, Wells & Scott 1997) are used to identify individuals of various species (*e.g.*, Würsig and Würsig 1977, Baird 2000, Whitehead 2001, Ramp et al. 2006, Barlow et al. 2011, Genov et al. 2012).

A number of efforts have been made to find different ways of identifying cetacean individuals. For example, while humpback whales (*Megaptera novaeangliae*) are primarily identified using photographs of tail flukes (Katona & Whitehead 1981, Hammond 1986, Barlow et al. 2011), researchers have also used photographs of dorsal fins (Katona & Whitehead 1981, Blackmer et al. 2000, Barendse et al. 2011).

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[3] The results of this chapter have been published in:
Genov T., Centrih T., Wright A.J., Wu G.-M. 2018. Novel method for identifying individual cetaceans using facial features and symmetry: A test case using dolphins. *Marine Mammal Science* 34(2): 514-528. doi:10.1111/mms.12451

This is especially useful in situations when animals do not fluke up, either due to the local environmental conditions (*e.g.*, depth), their behavior, or both (Blackmer *et al*. 2000). Furthermore, researchers have relatively recently applied photo-identification (hereafter 'photo-ID') to species that were generally thought not well suited to this method, *e.g.*, narwhals (*Monodon monoceros*, Auger-Méthé et al. 2010) and striped dolphins (*Stenella coeruleoalba*, Bearzi et al. 2011). Given the value of individual identification to both science and conservation, efforts to find new ways of identifying marine mammals may be important.

The development of digital photography has further facilitated a wider use of photographs in wildlife research, including photo-ID of cetaceans (Markowitz et al. 2003). This advance allows researchers to take extended image sequences in an effort to make sure that a quality dorsal fin or fluke picture is obtained, without the concern of wasting expensive film. Consequently, it is no longer uncommon for an entire surfacing sequence of a cetacean to be photographed, from the moment the head or rostrum breaks the surface, to the moment the peduncle or tail disappears again. As a result, more images of dolphin heads can be captured now than ever before.

One candidate identification method is the use of facial features and facial symmetry. Individuals across several taxa exhibit unique facial features, that can be recognized by humans and/or by conspecifics (Tate et al. 2006). In this study, an experiment was designed to evaluate the usefulness of facial features and facial symmetry to identify individual wild bottlenose dolphins as a case study, based on photographic data collected in the Gulf of Trieste. A long-term photographic dataset and human observers in matching trials were used to investigate: a) if individual animals could be distinguished by facial features; and b) if these features were consistent on left and right sides. Additionally, one of the aims was also to determine if such features were long-lasting and if calves, which tend to have 'clean' dorsal fins and are generally recognized through association with their mothers, could potentially be re-identified using this method after weaning.

6.2 METHODS

6.2.1 Data collection and evaluation

This study focused on free-ranging bottlenose dolphins inhabiting the Gulf of Trieste and adjacent waters (Chapters 2–5). The study area and research protocols are described in detail in Genov et al. (2008) and in Chapters 1, 2 and 5. Photographs of dolphins were collected between 2002 and 2014. In 2008 the use of color transparency film was replaced by digital photography. Between then and 2014, over 56,000 digital photographs were taken, resulting in the identification of about 150 individuals.

Sequences of photographs containing both dolphin faces and dorsal fins were selected, from which 2,318 images of faces were extracted, matching them to individuals known from dorsal fins from the same sequences. A total of 31 dolphins (29 adults and 2 calves) that had photographs of both left and right sides of the face could be reliably matched to photo-identified dorsal fins.

6.2.2 Matching trials

Twenty seven biologists (people with some form of a biology degree and with biological research being their main occupation) were asked to match photographs of dolphin faces. Fourteen were *experienced* in dolphin dorsal fin photo-ID (but not familiar with the animals from this population), meaning they had direct and extensive experience with photographing and matching wild dolphins. Thirteen were *inexperienced* with cetacean photo-ID or any cetacean-related work, as their work focused on non-cetacean species (reptiles, amphibians, invertebrates, plants, etc.). Each participant received three folders with dolphin face photographs (A, B, C). Folder A contained *left*-side photographs of 20 different dolphins. Folder B contained *left*-side photographs of 10 of those dolphins, but represented by different photographs, taken either during separate surfacing sequences or in different days. Folder C contained *right*-side photographs of the same 10 animals as in folder B. In other words, 10 animals from folder A had matches in both B and C, but the other 10 had no match. The identity of all animals, as verified using dorsal fins, was known to the investigator, but not to the participants. The choice of these 20 dolphins was dictated by the availability of photographs. Participants were first asked to decide which animals from folder A had matches to animals from folder B and to pair them accordingly. They were then asked to pair the 10 animals assigned to folder B to the animals from folder C. A total of 20 participants (12 experienced and 8 inexperienced) completed the tests (two experienced and five inexperienced participants failed to follow the instructions provided and were excluded from subsequent analyses). Note that the aim of this exercise was not to obtain a large sample size or ensure statistical rigor, but rather to assess the potential viability of this technique.

6.2.3 Statistical analysis

The scores of the participants were compared with the probability distribution of scores from random matching. The design of the matching trials required a hierarchical statistical model to establish the null distribution of scores. For the first task, matching 20 left faces (A) to 10 left faces (B), the probability distribution of scores was calculated using a hierarchical process: 1) selecting 10 out of 20 photographs to be matched, and 2) matching the 10 selected photographs with the 10 reference images in B. The probability of selecting *n* correct photographs from A has a binomial distribution, with an expectation of 0.5 (10/20).

$$P(n \mid N_B, N_A) \sim Binomial(N_B, p = N_B / N_A) \qquad \textit{Equation 1}$$

When the correct 10 photographs have been included in the matching set (n = N_B), the probability of getting $k \in \{0, \dots, N_B\}$ matches is equivalent to Montmort's matching problem (de Montmort 1713) and solved by equation 2.

$$P(k \mid N_B) = \frac{1}{k!} \sum_{j=0}^{N_B - k} \frac{(-1)^j}{j!} \qquad \textit{Equation 2}$$

In this study however, most participants did not select all 10 correct photographs to be matched. The probability of getting *k* matches for $n < N_B$ was solved recursively for $n \in \{ N_B -1, \dots , 0 \}$ using equation 3.

$$P(k \mid N_B, n) = (1 - k/n) \times P(k \mid N_B, n+1) \ + \ (k/n) \times P(k+1 \mid N_B, n+1) \qquad \textit{Equation 3}$$

The overall probability of getting *k* matches is therefore the probability of selecting *n* correct photographs X the probability of getting *k* matches given *n*.

$$P(k \mid N_B, N_A, n) = P(n \mid N_B, N_A) \times P(k \mid N_B, n) \qquad \textit{Equation 4}$$

For the second task (matching the 10 left faces selected in task 1 to the 10 right faces in folder C), the probability distribution of getting *k* matches was calculated separately for each individual using equation 3. This is because *n* was determined by the number of photographs selected in the first task and was not the same for all individuals. The overall performance of the participants was assessed using the joint probability of their scores. The performance of experienced and inexperienced participants was compared using the mean log-likelihood of their test score and bootstrapping (10,000 random samples with replacement).

6.3 RESULTS

Facial features appeared to be distinctive enough to allow participants to distinguish individual dolphins (Table 6.1). For the first task, the expected score for random matching was 5/20, with 10/20 being above the 95% confidence interval. Participants matching left face photographs of dolphins did substantially better than expected by chance (Fig. 6.2, joint probability $P < 0.0001$). Experienced participants performed far better than inexperienced ones (lower mean log-likelihood in all 10,000 bootstrap samples). The symmetry between left and right sides of individual dolphin faces (Fig. 6.1) was also confirmed by matching trials. Participants matched photographs of left and right faces better than expected by chance ($P < 0.0001$). Here, the score expected for random matching depended on the outcome of the previous trial and is shown for each observer in Fig. 6.6. Only one experienced participant did not do significantly better than random (Fig. 6.6: "Experienced B", $P = 0.154$). Again, experienced participants did better than inexperienced ones on average (in 98.5% of bootstrap samples). Three experienced participants were able to correctly assign all matches, regardless of which side of the face was in the photographs.

Table 6.1. Matching results by experienced and inexperienced participants for left (same) side matching, right (opposite) side matching and overall. Results are shown as median number of correct scores, with range provided in the parentheses. Median % shows the median percentage of correct scores, with range provided in the parentheses.

	Experienced participants (*n* = 12)		**Inexperienced participants (*n* = 8)**	
	median [range]	median % [range]	median [range]	median % [range]
Left/same side (*n* = 20)	18 [14–20]	90 [70–100]	15 [10–15]	75 [50–75]
Right/opposite side (*n* = 20)	17.5 [9–20]	87.5 [45–100]	13 [10–17]	65 [50–85]
Overall (*n* = 40)	35 [23–40]	87.5 [57.5–100]	28 [20–31]	70 [50–77.5]

Figure 6.1. Left and right side face photographs of 16 bottlenose dolphins showing individually distinct facial features. Three-letter names codes represent individual IDs. Note that, with the exception of scarring, features are consistent on both sides. The identity of these dolphins (i.e. the match between left and right sides, as well as uniqueness from other individuals) was verified via conventional dorsal-fin photo-identification.

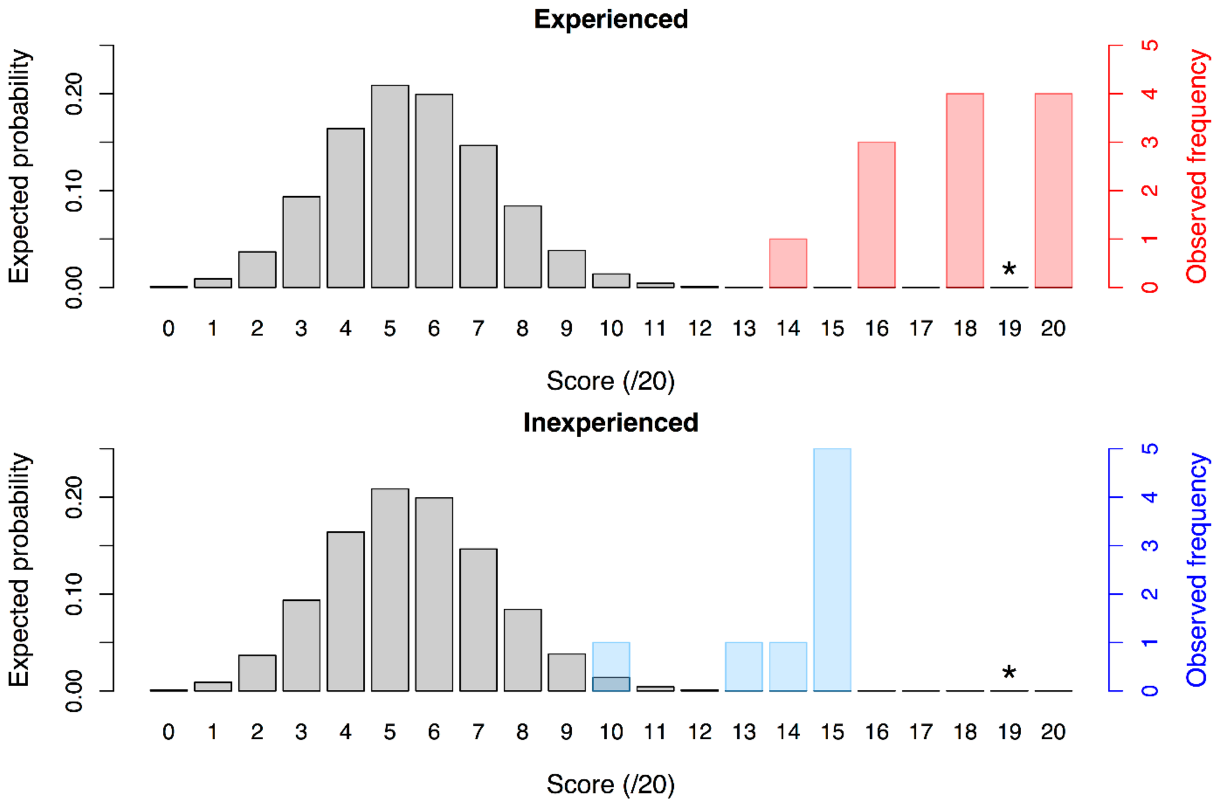

Figure 6.2. Probability distribution of scores in the first dolphin face matching trial (Left-Left). Grey bars show the probability distribution of scores expected by chance, and the asterisks (*) indicate impossible scores. The performance of participants *experienced* (N=12) and *inexperienced* (N=8) in cetacean photo-ID is shown in red (upper panel) and blue (lower panel), respectively.

Long time series (5 years or more) of face photographs were available for three individual dolphins. In these three individuals, facial features appeared to be stable and recognizable over at least 8–9 years (Fig. 6.3). Two calves showed temporal stability of their facial features over a minimum of 32 and 10 months, respectively (Fig. 6.4). Furthermore, the two calves appeared to resemble their respective mothers more than they did other dolphins, but the current sample is limited (Fig. 6.5).

Figure 6.3. A selection of three individual dolphins showing long-term stability in facial features. Individual DAP is represented by both sides. Note that the oldest picture of each individual is a scanned slide transparency, hence the poorer photo quality.

Figure 6.4. Faces of two dolphin calves, showing consistency of facial features over time, as well as differences between the two animals.

Figure 6.5. Two females (nicknamed Daphne and Hidro) and their respective calves, showing facial similarities between mothers and calves.

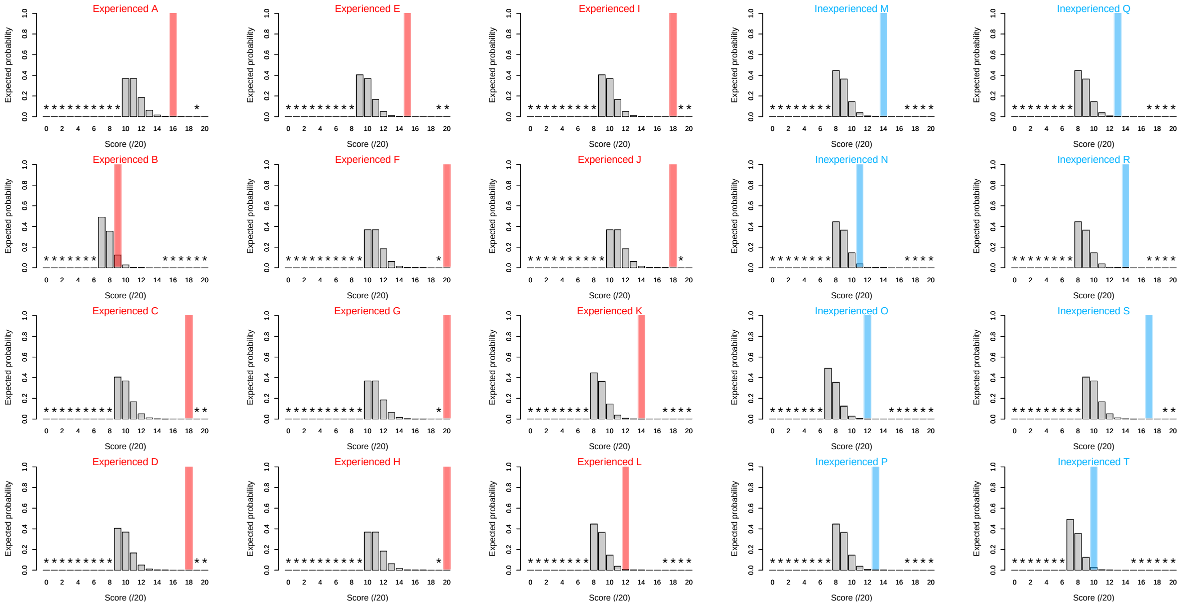

Figure 6.6. Scores for the second dolphin face matching trial (Left-Right) by individual participant. Grey bars show the probability distribution of scores expected by chance specific to each participant, and the asterisks (*) indicate impossible scores. Scores of participants experienced and inexperienced in cetacean photo-ID is shown in red and blue, respectively.

6.4. DISCUSSION

These results suggest that: a) bottlenose dolphins can be distinguished and identified by facial features; and b) these features are consistent across the left and right sides and therefore symmetrical. In addition, these preliminary findings also suggest that such facial features are long-lasting, and may potentially allow calves to be re-identified after weaning.

Symmetry, including facial symmetry, is common in many species of mammals and other animals (Finnerty 2005). Symmetry is also a salient visual property that has been shown to be detected efficiently and rapidly by humans (Wagemans 1997). Moreover, in humans, faces are among the most important visual stimuli, especially in social contexts (Leopold & Rhodes 2010), and it has been shown that humans visually process images of animals and animal faces just as fast as those of human faces (Rousselet et al. 2003, 2004). Therefore, it is perhaps not surprising that participants were successful at assigning matches, even across the two sides of dolphin faces. This was despite anatomical differences, such as the marked lateral (rather than frontal) domination of facial features, in the dolphins as compared to humans. Given that facial recognition of individuals is a special ability in many social animals, including humans, its use may be more applicable than previously thought. It was left to the participants to decide which particular facial features they used to identify individuals. They could not use scarring, as some photos were too far apart in time to allow this, and they also matched opposite sides of the face. As far as the author is concerned, when he first discovered that several dolphins appeared identifiable by faces, the 'identifying feature' was simply a 'holistic' image of the dolphin's face, rather than any single type of feature (T. Genov, *personal observation*).

The incidental nature of the face photographs meant that their quality varied greatly. Consequently, trials included high quality photographs, as well as those of lower quality (one that would not pass the quality screening for standard mark-recapture analyses, see Chapter 5). Despite this, participants were successful in assigning matches. The use of more high-quality photos would likely further increase the success rate. Moreover, conscious attempts to obtain head shots, in addition to dorsal fin shots, would greatly increase the number of such photographs, without compromising standard photo-ID, and this is now being done when in the field. Researchers with even larger and longer-term photo-ID data sets are encouraged to further explore and test this in the future. In fact, inspired by this study, this was recently successfully tested in bottlenose dolphins from Cardigan Bay, Wales (Spencer 2019). Images of stranded and captive dolphins may also contribute to the future corroboration of this method.

These results may have broader implications. Studies have shown that faces are an important category of visual stimuli in all major vertebrate groups (although some aspects of facial perception appear to be limited to primates and a few other social mammals) and are thought to have possibly evolved to facilitate complex social communication (Leopold & Rhodes 2010). Face recognition has been documented in primates (Pascalis & Bachevalier 1998), to which the cognitive abilities of delphinids are often compared, as well as in sheep (Kendrick et al. 2001). Mirror self-recognition is well documented in bottlenose dolphins (Reiss & Marino 2001) and some other delphinids (Delfour & Marten 2001), demonstrating a capacity to use visual cues to recognize themselves and other individuals. Bottlenose dolphins have good vision (Herman et al. 1975, Pack & Herman 1995) and a long-term social memory (Connor et al. 2000, Bruck 2013), making it possible that they also use visual cues, such as unique facial features, to recognize each other at close quarters in the wild, despite relying primarily on acoustic recognition (Janik et al. 2006, Quick & Janik 2012).

Perhaps more importantly, the ability to distinguish individual animals by permanent features other than dorsal fin markings can help document changes in dorsal fin marks over time (Hammond 1986, Gowans & Whitehead 2001). Such changes are generally commonplace in bottlenose dolphin populations (Wilson et al. 1999b, Bearzi et al. 2008, Chapter 5) and may cause researchers to miss fin matches. Unlike regular dorsal fin marks, facial features do not appear to change, or are at least not prone to external influences such as social interactions. This additional ‘marking’ can reduce the likelihood of false negative or false positive errors (Stevick et al. 2001), or help estimate the rate of such errors *via* double-marking experiments (Barrowman & Myers 1996, Gowans & Whitehead 2001, Barendse & Best 2011). Furthermore, when animals carry more than one mark type, individuals can be cross-classified across marks, and the effect of mark type on the recapture probability modelled (Smout et al. 2011). Facial identification can also provide an additional way to identify animals with ‘unmarked’ fins and may help estimate the number or proportion of ‘clean’ fins in the sample (*e.g.*, in an encounter or in a population). In studies of abundance, social behavior or habitat use, it is often beneficial to be able to identify every individual within a group, and having more tools available to do so would be advantageous. Facial recognition can also work in conjunction with other markings such as body scars and coloration to help confirm identifications, but the demonstrated symmetrical property of dolphin faces is of particular value, as identifications (and matches) can be done irrespective of which body side is displayed.

Additional means of identifying individuals may also help reduce the number of ‘lost’ calves that leave their mothers before they are recruited into the marked part of the population. The ability to follow dolphins through several generations is highly valuable for long-term studies (Wells 1991, Mann 2000, Arso Civil et al. 2017) and tracking of individuals from weaning to adulthood can provide information on primiparity, recruitment and juvenile survival (Paterson et al. 2013). However, calves are typically unmarked and do not often acquire identifiable dorsal fin marks prior to becoming independent. Valuable information on inter-generational links is thus often lost in the absence of additional genetic studies. Nevertheless, dolphin calves are known for their tendency to ‘pop’ their heads out of the water upon surfacing, making them particularly suitable for this type of additional identification (Fig. 6.4). Accordingly, obtaining a face shot at a later time would aid in the re-identification of calves following weaning, offering greater potential for cross-generational analyses. Even though the tendency of calves to ‘pop’ their heads out of the water will likely decrease after weaning, facial recognition may still increase the probability of subsequent matches, especially when used in combination with other markings such as coloration, scarring and potential subtle nicks in the fin. Moreover, in this study population head exposure is rather common in adults (T. Genov, *personal observations*). There was some indication of similarity between at least some mothers and calves (Fig. 6.5), suggesting potential inheritance of specific traits, but the limited sample size precludes any further inferences.

It would also be very useful to determine if males could be differentiated from females. While there is some indication that this may be possible (T. Genov, *personal observations*), sex information on a large enough number of individuals is currently not available to test the premise. Sexual dimorphism in skull morphology is present in some populations of bottlenose dolphins, but not in others (Turner & Worthy 2003), so potential sex differences might be a topic of further study.

To be clear, it is not suggested that facial recognition could or should replace standard dorsal fin identification, as there are a number of limitations. First, facial recognition may carry some bias as not all animals regularly lift their heads out of the water upon surfacing. This will vary by species, populations within a species, individuals within a population, and also different behaviors of a single individual. Perhaps this approach may be especially useful for studies that collect photo-ID photographs underwater (Herzing 1997). But as noted above, head exposure is rather common in this study population. Second, the faces of some individuals were clearly more distinct than others, and it remains to be determined what proportion of the population is sufficiently 'marked' in such a way to be consistently identified and whether this approach can be scaled to the entire population. Presumably, the size of the population may influence the ability to reliably tell individuals apart, although this issue will pertain to dorsal fin identification as well. In any population, the combination of population size and the distinctiveness of the markings used will dictate the utility of the identification method. It should be noted here that the reliability of dorsal fin matching has not been explicitly or widely tested either. It is generally assumed it is reliable, but it is not without pitfalls (Quick et al. 2017). Therefore, explicitly testing the reliability of dorsal fin matching (as was done for dolphin faces here) would be useful to put different methods in perspective. Still, it is believed that facial recognition could complement standard photo-ID, especially in the age of digital cameras and high definition videography. It is thus hoped that this work can provide inspiration for a range of future studies. Computer-assisted matching, applied more and more widely to cetaceans (Gope et al. 2005, Auger-Méthé et al. 2011) and several other taxa (Arzoumanian et al. 2005, Hiby et al. 2009), could also potentially be applied to these features, much like automated recognition of humans faces (Camastra & Vinciarelli 2008) and chimpanzees (Loos & Ernst 2013).

Even though potential facial differences remain to be documented in other species (something to investigate as the next step), this approach may have implications for the study of other delphinids, other cetaceans and possibly other taxa. For example, it may prove very useful in species that do not typically acquire fin marks, or in species such as right whale dolphins (*Lissodelphis borealis* and *L. peronii*), which lack dorsal fins entirely, but often expose their heads (Lipsky 2009). Facial features may be one of the few ways to identify individuals in these species at all. Face identification may also be particularly suited to humpback dolphins (*Sousa* sp.), as they typically surface in a very characteristic manner, with the beak breaking the water surface first, followed by the head (Parra & Ross 2009). Therefore, future research should look into whether our findings apply to other cetaceans and other animal groups.

Finally, the ability to recognize dolphins from face photographs may also have implications in post-mortem studies. For instance, Atlantic humpback dolphins (*Sousa teuszii*) are frequently bycaught in artisanal fishing nets (Collins 2015). Their carcasses are often taken as ‘marine bushmeat’ (Clapham & Van Waerebeek 2007) for human consumption and thus unavailable to scientists. In some instances however, arrangements with local fishermen have allowed researchers to gain access to at least the heads of the dolphin (T. Collins, *pers. comm.*). These heads are usually fresh enough to allow face identification attempts for bycaught dolphins, and may help researchers trace bycaught dolphins to photo-identified individuals (T. Collins, *pers. comm.*).

Previously, lateral photographs of faces have been used to identify individuals in sea turtles (Reisser et al. 2008, Schofield et al. 2008). In those studies, however, photographs and identification were restricted to the right-side of the head, with no investigation into potential symmetry of those features. The utility of the method was therefore reliant on obtaining photographs of the appropriate side, while this is not the case in the present study.

This method was recently successfully used to match a bottlenose dolphin between Netherlands and France (Hoekendijk et al. 2021).

CHAPTER 7: GENERAL DISCUSSION

7.1 Thesis overview

Chapter 1 provided the general context for the remainder of the thesis. Chapter 2 used social network analysis to describe unique social partitioning, in which two social clusters differ in temporal patterns in habitat use and fisheries-related behaviour. From there, chapter 3 investigated the potential drivers of such partitioning, by investigating the within-population isotopic niche variation and determined substantial among-group variability in diet. Chapter 4 extended this by assessing potential differences in contaminant loads among different social clusters within the population, demonstrating high levels of and maternal offloading of polychlorinated biphenyls (PCBs), but without differences related to social group membership. Chapter 5 provided robust estimates of abundance for this population, which will be helpful in determining the status of the species in this region and inform future conservation strategies. Chapter 6 described a novel method of identifying bottlenose dolphins, which may complement existing photo-identification methods and prove useful for species that may not be individually identifiable by conventional methods.

Almost 20 years ago, when this study began in earnest, it started with very basic questions. In an area where the general perception, even among biologists, was that dolphins were just occasional or accidental visitors (a misunderstanding which still occasionally manages to find its way into some official texts), the initial aims were to assess the spatial and temporal patterns of occurrence of these animals, if any. In Chapter 1, I pointed out that cetaceans are fascinating to study and this dolphin population has lived up to and exceeded that expectation, providing the basis for some very interesting scientific findings.

Chapters 2 and 5 address commonly asked research questions using established methodology, but Chapter 2 described social patterns that have not been described for this species previously, nor for other mammals (to the extent that can be inferred from the literature), with two largest social groups overlapping spatially, but essentially time-sharing their habitat. It showed that dolphins in the Gulf of Trieste appear to live in two general kinds of social units: a) large mixed-sex groups with strong, long-lasting associations and b) small groups with weaker, temporally unstable associations, which does not appear to be age-dependent. No evidence of sex segregation or male alliances was found. The year-round occurrence of mixed-sex groups with calves suggest that mixed-sex groups were not related to reproductive state and that reproductive state does not explain these patterns, and temporal patterns also do not appear to be driven by differences in fisheries-related behaviour. These findings add to the knowledge about the behavioural and social plasticity in this species. Common bottlenose dolphins and several other species of cetacean, are known to live in complex societies and share cultures (Mann et al. 2000, Rendell & Whitehead 2001, Wells 2003, Lusseau 2006a, Connor et al. 2011, Quick & Janik 2012, Brakes et al. 2019). While this has been well established, the population investigated here has added an additional layer of complexity to this knowledge and has contributed a small but important piece of the puzzle into our global understanding of these patterns. Based on the increasing body of literature worldwide (Lusseau et al. 2003, Gero et al. 2005, Lusseau et al. 2006, Daura-Jorge et al. 2012, Blasi & Boitani 2014), as well as new information from Chapter 2, it is becoming clear that bottlenose dolphin social structure is remarkably varied and does not fit the typical mould once thought for this species (Leatherwood & Reeves 1990). The genus *Tursiops* is extremely plastic across the globe, in terms of morphology, behaviour and habitats in which it occurs (Duffield et al. 1983, Mead & Potter 1995, Rossbach & Herzing 1997, Connor et al. 2000, Wang et al. 2000a, b, Mann & Sargeant 2003, Randić et al. 2012, Jedensjö et al. 2020), so there is likely much more to learn about the social lives of these animals.

Results from Chapter 3, using a relatively large overall sample size for a study of this type, provide additional insight into some of the potential mechanisms of these patterns, showing intra-population differences in isotopic niche. Being able to link information on stable isotopes with that collected from observations of known individuals in the field was particularly valuable for interpreting isotope patterns and integrating that information with other analyses. Together, these results suggest an overall generalist population composed of individuals with variability in prey preferences or behavioural specialisations. Together, chapters 2 and 3 demonstrate substantial plasticity within this population, in terms of both behaviour and diet.

With the largest sample size for a study of this kind in Europe to date, Chapter 4 demonstrated relatively high PCB concentrations in the study population and successfully linked demographic parameters from individual photo-identification histories with pollutant concentrations in blubber tissue. It also demonstrated maternal offloading of pollutants to offspring through two lines of evidence (sex and parity), which is not commonly achieved in free-ranging cetacean populations (Ross et al. 2000, Ylitalo et al. 2001, Wells et al. 2005). The results presented in this chapter show that PCB levels are relatively high in northern Adriatic dolphins, and may be high enough to potentially cause population-level effects in this population. This is of concern particularly in combination with other stressors. The impacts of PCBs may be especially pertinent in case of potential catastrophic events leading to increased population mortality, e.g. due to oil spills (Schwacke et al. 2014), as they may supress population recovery, given their effects on reproduction (Tanabe et al. 1994, Schwacke et al. 2002, Vos et al. 2003).

Chapter 5 tested related but different approaches to estimating abundance from mark-recapture and provided robust abundance estimates relevant to future monitoring and conservation planning. For example, these estimates provide a baseline for placing the threat from bycatch into population context. In addition, results also suggested little substantial movement in and out of the study area, providing further support for the notion of strong site fidelity and potential discreteness of this population, as well as low apparent survival probability, which provides further support for metapopulation dynamics inferred from recent genetic evidence (S. Gaspari, T. Genov, A. Moura et al., unpublished data).

The novel method of identification based on facial features, described in chapter 6 offers new possibilities for studying cetaceans through photo-identification. It has since been replicated in Cardigan Bay in Wales (Spencer 2019) and recently successfully used to match a bottlenose dolphin between Netherlands and France (Hoekendijk et al. 2021). This new approach can be used to complement existing photo-identification techniques, help reduce identification errors (Stevick et al. 2001) through double-marking (Barendse & Best 2011) and improve cross-generational knowledge, which is useful for long-term studies (Mann 2000, Mann & Sargeant 2003, Arso Civil et al. 2019a).

7.2 Informing conservation

As reviewed in Chapter 1, marine mammals, their habitats and other species living in the same ecosystems are directly impacted and threatened by a number of human activities, These include, but are not limited to, incidental mortality in fishing gear (Read et al. 2006, Read 2008, Reeves et al. 2013, Brownell et al. 2019), ship and boat strikes (Wells & Scott 1997, Panigada et al. 2006, Douglas et al. 2008), underwater noise (Gordon et al. 2003, Buckstaff 2004, Nowacek et al. 2007, Southall et al. 2007, Weilgart 2007, Clark et al. 2009, Di Iorio & Clark 2009, Williams et al. 2020), direct disturbance (Bejder et al. 2006, Tyne et al. 2015, Tyne et al. 2017, Fumagalli et al. 2018, Fumagalli et al. 2019), chemical pollutants (Reijnders et al. 1999, Law et al. 2012, Jepson et al. 2016, Stuart-Smith & Jepson 2017, Desforges et al. 2018, Murphy et al. 2018), marine debris (Laist 1997, Jacobsen et al. 2010, Simmonds 2012, de Stephanis et al. 2013, Baulch & Perry 2014, Lambert et al. 2020) and climate change (MacLeod 2009, Lambert et al. 2010, Salvadeo et al. 2010, Scheinin et al. 2011). As pointed out by Read (2010), these animals have intrinsic, societal and economic value, which makes their conservation of general interest.

The bottlenose dolphin is one the most widespread marine mammals in Mediterranean and European waters (Natoli et al. 2005, Bearzi et al. 2009, Nykänen et al. 2019). It is a protected species in Europe, both nationally and internationally, under a number of legislative mechanisms, including the Convention on Biological Diversity, Convention on the Conservation of Migratory Species of Wild Animals, the Barcelona Convention, ACCOBAMS, CITES, the Habitats Directive and the Marine Strategy Framework Directive. These mechanisms all mandate ensuring a good conservation status for the species over the long term. Obtaining reliable, robust and precise abundance estimates plays a small but important role in informing conservation actions and improving management of human activities affecting cetaceans and their ecosystems (Williams et al. 2002, Currey et al. 2009b, Hammond 2010, Hammond et al. 2013, Authier et al. 2017, Hammond et al. 2021). Abundance estimates, even if local, can provide useful information that can help feed into wider assessments. For example, the knowledge of cetacean abundance and status in the Mediterranean is generally fragmented (Boisseau et al. 2010, Notarbartolo di Sciara & Birkun 2010, Frantzis et al. 2011, Authier et al. 2017, Mannocci et al. 2018, Bearzi & Genov 2021), but for the recent Red List assessments of the bottlenose dolphin and the common dolphin (*Delphinus delphis*) in the Mediterranean Sea (Bearzi et al. in press, Natoli et al. in press), data from various sources, including local abundance estimates, were instrumental in inferring the status of the species and for making assessments. The abundance estimates from Chapter 5 add this small piece of the puzzle for the Adriatic Sea, so that fine-scale local estimates (Pleslić et al. 2015, Bearzi et al. 2021, Chapter 5) and large-scale regional estimates (Fortuna et al. 2018) can be placed in proper conservation context for this species.

In particular, bycatch levels for this population were recently flagged as potentially being more serious than previously thought (Genov 2020), so robust abundance estimates are an essential prerequisite for putting any bycatch mortality into a population viability context (Wade 1998, Taylor et al. 2000, Hamner et al. 2014, Punt et al. 2020, Hammond et al. 2021, Wade et al. 2021), including in relation to estimates of bycatch risk elsewhere in the Adriatic Sea (Đuras Gomerčić et al. 2009, Fortuna et al. 2010).

The data presented in Chapter 5 further corroborate the notion that Slovenian waters represent an important part of habitat for bottlenose dolphins (Genov et al. 2008), which may help support management decisions in the future. For example, the new Maritime Spatial Plan of the Republic of Slovenia (Decree on Maritime Spatial Plan of Slovenia, Official Gazette 116/21) envisages construction of an artificial island in Slovenian waters, which would have detrimental effects on a number of marine biodiversity elements in this area, and is strongly opposed by the majority of marine biology and marine conservation experts. Since the proposed island would severely infringe on the habitat of the local dolphin population, the abundance data (Chapter 5), together with the existence of unique social partitioning in this area (Chapter 2), may help inform the decision-making on such proposals.

In addition, Slovenia has not designated any Natura 2000 sites for bottlenose dolphins, predominantly on the grounds of this population being transboundary. The European Union Habitats Directive (Council Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora) is probably one of the most influential and important pieces of legislative text related to biodiversity conservation in Europe. Member States of the European Union are required to undertake measures for the conservation of a number of species and habitats, to ensure their long-term viability. This includes the identification of Sites of Community Importance (SCI), defined under Article 1(k) as sites that 'contributes significantly to the maintenance or restoration at a favourable conservation status of a natural habitat type in Annex I or of a species in Annex II'. The identified SCI represent the framework from which Member States designate Special Areas of Conservation (SACs) (European Environment Agency 2021), defined under Article 1(l) as ‘a Site of Community Importance where necessary measures are applied to maintain, or restore, to favourable conservation status, the habitats or population of the species for which the site is designated’. SACs form a network of protected sites across the European Union called Natura 2000, which has become a cornerstone of EU biodiversity policy. However, implementation of the Habitats Directive can be challenging, especially in marine habitats or for highly mobile species that regularly transgress political borders. The common bottlenose dolphin is one species for which the provisions of the Habitats Directive apply to, and for which a favourable conservation status is to be maintained, because the species is listed both in Annex II and in Annex IV of the Directive. EU member states are required to take measures for its conservation, including the establishment of Natura 2000 sites. However, for countries with a small extent of national waters, such as Slovenia (with a coastline length of only 46 km), this may be challenging with respect to mobile species such as dolphins. Because animals regularly traverse national borders, the usefulness of any spatially-based measures will only be effective if coordinated with neighbouring states. Information presented in this thesis, showing regular presence of the species and providing robust abundance estimates, may help inform future conservation action in this regard.

The Gulf of Trieste, together with waters adjacent to it, is a heavily human-impacted area, subject to shipping (Ferraro et al. 2007, Perkovič et al. 2013), tourism and recreational boat traffic (Codarin et al. 2008, Genov et al. 2008, Picciulin et al. 2010), underwater noise (Picciulin et al. 2008, Codarin et al. 2009), intensive fishing (Pranovi et al. 2000, Casale et al. 2004, Coll et al. 2007, Genov et al. 2008), aquaculture (France & Mozetič 2006, Grego et al. 2009), non-indigenous species (David & Perkovič 2004, David et al. 2007, Lipej et al. 2012), urbanisation, sewage discharge and pollution (Horvat et al. 1999, Malačič et al. 2000a, Faganeli et al. 2003, Mozetič et al. 2008). However, it is nevertheless also remarkably rich in biodiversity (Žiža et al. 2001, Lipej et al. 2003, Bonaca & Lipej 2005, Guidetti et al. 2005a, Guidetti et al. 2005b, Genov et al. 2008, Libralato et al. 2010, Mozetič et al. 2012, Koce 2018). The studies presented in this thesis (Chapters 2, 3 and 4, in particular) provide insights into how a species such as the bottlenose dolphin is able to cope with a myriad of pressures in such an environment. Hopefully, this can lead to a better understanding of these mechanisms, but also to improved overall conservation of this region. Moreover, because charismatic marine megafauna can provide conservation benefits to other components of biodiversity (Hooker & Gerber 2004, Sergio et al. 2006), improved understanding and conservation of this species may also have wider conservation benefits in the long term.

Pressures and threats to biodiversity are multiple and varied. For any given species, they sometimes operate as a single and clearly identified threat, such as bycatch in the case of the vaquita (*Phocoena sinus*) in the upper Gulf of California (Rojas-Bracho et al. 2006, Gerrodette et al. 2011, Brownell et al. 2019) or predominant threats such as bycatch and hunting in the case of the Atlantic humpback dolphin (Collins 2015). Much more frequently, however, threats to species and ecosystems manifest themselves not singularly, but through synergistic and cumulative effects (Turvey et al. 2007, Coll et al. 2012, Maxwell et al. 2013, Simmonds 2018, Stelzenmüller et al. 2018). This is also the case for the bottlenose dolphin population from the Gulf of Trieste, which faces a range of anthropogenic pressures, including fisheries interactions and bycatch (Genov et al. 2008, Hace et al. 2015, Genov et al. 2016, Kotnjek 2016, Kotnjek et al. 2017, Genov 2020), disturbance from boat traffic and underwater noise (Genov & Kotnjek 2007, Genov et al. 2021). Chapter 4 adds chemical pollutants to this list and provides robust data that place this additional threat into a wider conservation context with other anthropogenic stressors (Hall et al. 2017, Stelzenmüller et al. 2018). This, together with a better understanding of the foraging ecology and trophic niche of this population (Chapter 3), and robust abundance estimates (Chapter 5), provides a basis for assessing these threats in a population-level effects of cumulative stressors framework (Maxwell et al. 2013, National Academies of Sciences 2016, Simmonds 2018).

7.3 Long-term perspective

This thesis is derived from various pieces of work conducted during a long period of study, and not all chapters pertain to the same time periods. For example, the first data chapter involved the analysis of data collected during the first nine years of the study. Chapters 3 and 4 involved the subsequent collection of samples for genetic, toxicological and diet analyses. Chapter 6 utilised the intermediate portion of the data, from the time when digital photography started being used in this study, to 2014, when the data analysis of the photographic material for that chapter began. Chapter 5 focused on data collected during the final six years, which were of better quality linked to year-round monitoring, which was not previously feasible due to limitations with logistics and funding. Despite the temporal variability in datasets used, these results provide an overall picture of this population with breadth and depth, with results being complementary. Together, these studies form pieces of a puzzle, which is becoming highly interesting scientifically, but is also becoming increasingly important for conservation.

7.4 Further work

Further work on photo-identification is recommended, planned and ongoing, to be used for abundance and survival estimation, and allow for long-term monitoring, because longer time series allow for better assessment of potential trends. In a similar study, Wilson et al. (1999b) estimated that > 8 years would be required to detect a population trend at a 90% confidence level, based on estimates with similar precision to their study (which is the case here). Given the three additional years of photo-identification data available after the last year analysed in Chapter 5, this time frame now appears to be reached. Additional years of data may also help improve model fit and potentially better estimate some of the parameters from Chapter 5, which may be used in a Population Viability Analysis context (Thompson et al. 2000, Vermeulen & Bräger 2015). In relation to this, expanding the study area to better capture the range of the population, would also be desirable (as well as integrate data from nearby areas, e.g., Pleslić et al. 2015, Bearzi et al. 2021, see below). Better spatial coverage of the population range would likely lead to higher capture probabilities (Arso Civil et al. 2019b). In addition, animals may shift their distribution (Wilson et al. 2004), so wider spatial coverage may help detect any such changes, rather than inferring them to be population declines.

In relation to the spatial dimensions of occurrence and habitat use, the long-term sighting and focal follow data now also allow for spatial modelling of habitat use (Cañadas & Hammond 2006, Bonizzoni et al. 2014, Arso Civil et al. 2019b, Bonizzoni et al. 2019), which may help identify important habitats within the study area and inform the potential designation of Natura 2000 sites or marine protected areas (Hooker & Gerber 2004, Gerrodette & Eguchi 2011), or the extension of existing marine protected areas (Genov & Železnik 2021b).

Increasing the sample size for stable isotope analysis may provide further cues into niche partitioning among social groups or individuals (Chapter 3). Moreover, the repeated sampling of the same individuals (some of which were sampled many years ago) would allow the long-term integration of such information and provide insights into long-term dietary variability, as well as the accumulation levels of pollutants across sexes, age classes and reproductive status. In addition, individual information on pollutant levels can be linked with information on epidermal conditions obtained from individual photo-identification (Wilson et al. 1999a, Van Bressem et al. 2009, Gonzalvo et al. 2015). In a recent photographic assessment, 30 % of examined individuals featured skin lesions (McLean 2019).

Additional studies, currently ongoing, are attempting to build on the findings presented in this thesis and provide further insights into the biology and ecology of this population, in order to contribute to local and regional conservation, but also contribute to global science. Ongoing efforts (S. Bonizzoni, J. Železnik, G. Bearzi and T. Genov, unpublished data) aim to better understand the movement patterns and connectivity of dolphins in the region through photo-identification (Bearzi et al. 2011c), which can help better understand the patterns inferred from genetic markers (Gaspari et al. 2015) and place local abundance estimates (Chapter 5) into regional conservation context (Gnone et al. 2011, Cheney et al. 2013).

Samples collected through biopsy sampling (Chapters 3 and 4) have also been genotyped (T. Genov, S. Marfurt and M. Krützen, unpublished data) to study intra-population genetic structure and kindship, and infer potential drivers or consequences of social and diet partitioning (Chapters 2 and 3), in order to better understand the potential ecological or evolutionary drivers of the observed patterns (Möller et al. 2006, Wiszniewski et al. 2010, Diaz-Aguirre et al. 2018). The same samples are also being used to study population structure and meta-population dynamics at a regional level (S. Gaspari, T. Genov, A. Moura et al., unpublished data), to build on previous studies in the region. In relation to the observed social structure patterns, future work aims to investigate the acoustic repertoire of this population (Quick & Janik 2008, Quick & Janik 2012, King & Janik 2013) and determine potential differences in vocal behaviour among social groups, with preliminary data already collected (J. Železnik, T. Genov and S. King, unpublished data).

With respect to informing local conservation, recent work assessed the importance of the Strunjan marine protected area for the local dolphins population (Genov & Železnik 2021a) and used passive acoustic monitoring to investigate behaviour and habitat use around fish farms (Železnik et al. 2019) and assess underwater noise levels (Genov et al. 2021). Last but not least, the novel photo-identification approach described in Chapter 6 and replicated by Spencer (2019) should be tested on other species of small cetacean.

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APPENDICES:

Chapters published in peer-reviewed publications

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ORIGINAL PAPER

Behavioural and temporal partitioning of dolphin social groups in the northern Adriatic Sea

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Abstract

Complex social structure is a prominent feature in several mammal species. Such structure may lead to behavioural diversity not only among populations, but also within a single population, where different subsets of a population may exhibit different types of behaviour. As a consequence, understanding social structure is not only interesting biologically, but may also help conservation and management efforts, because not all segments of a population necessarily respond to or interact with human activities in the same way, or at the same time. In this study, we examined the social structure of common bottlenose dolphins (*Tursiops truncatus*) in the Gulf of Trieste and adjacent waters (northern Adriatic Sea), based on a 9-year dataset, using social network metrics and association indices. We assessed whether different segments of the population show differences in behaviour and interactions with fisheries. Dolphin social network was structured into distinct social clusters of mixed sexes. We found no evidence of male alliances. The two largest social clusters overlapped spatially, but not temporally, as they used the same area at different times of day. Such diel temporal partitioning does not appear to have been documented in cetaceans previously. The two clusters also differed in ways they interact with fisheries, as one regularly interacted with trawlers, while the other did not. This study demonstrates how different segments of animal populations can interact differently with human activities and in turn respond differently to anthropogenic impacts.

Introduction

Complex social structure is prominent in many mammals including primates (Chapman and Rothman 2009), elephants (Wittemyer et al. 2005), hyaenas (Smith et al. 2008), bats (Popa-Lisseanu et al. 2008) and cetaceans (Mann et al. 2000), and plays an important role in population dynamics and behavioural patterns. It governs the way the spread or containment of behaviours is facilitated, e.g. through social learning (Heyes 1994; Laland 2004). This may lead to behavioural diversity not only among, but within populations, where different population segments exhibit different behaviours (Mann and Sargeant 2003; Cantor and Whitehead 2013). Understanding this is not only interesting biologically, but may help conservation efforts (Whitehead 2010), because not all population segments necessarily respond to, or interact with, human activities the same way, or at the same time. There is concern over the effects of anthropogenic disturbance to populations, yet it is difficult to assess population-level impacts without understanding what proportion of animals may be affected.

Bottlenose dolphins (*Tursiops* sp.) are well-studied social mammals (Wells et al. 1987; Smolker et al. 1992; Lusseau et al. 2003; Connor et al. 2006; Lusseau 2006). Most information on their social structure comes from studies in Sarasota, Florida (Wells et al. 1987; Wells 2003) and Shark Bay, Australia (Connor et al. 1999; Mann et al. 2000), but many populations remain poorly studied. They are generally described as fission–fusion species, where group composition changes frequently (Connor et al. 2000), but we argue that their social structure varies considerably among populations. For example, dolphins in Florida appear to feature marked sex-age segregation, where males form paired alliances, females form bands and nursery groups and juveniles form smaller groups (Wells et al. 1987). In Shark Bay,

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males form hierarchical alliances (Connor et al. 1999, 2011; Randić et al. 2012). At the other end of the spectrum, dolphins in Doubtful Sound, New Zealand, form mixed-sex groups with strong associations not only within, but also between sexes (Lusseau et al. 2003). This shows that patterns cannot be generalised and that our understanding of bottlenose dolphin social structure remains incomplete.

Social network analysis allows groups of social animals to be studied as a network of nodes and ties (Wey et al. 2008; Krause et al. 2009a). When coupled with information on behaviour and interactions with human activities, it is a powerful tool in the study and conservation of social animals. Common bottlenose dolphins (*T. truncatus*) inhabit the Gulf of Trieste and adjacent areas of northern Adriatic Sea, where they have been studied since 2002 (Genov et al. 2008, 2017). Here, we examine the social structure of local dolphins and assess whether different population segments show differences in behaviour and interactions with human activities.

Materials and methods

Data collection

Data were collected between February 2003 and September 2011 in the Gulf of Trieste and adjacent waters, northern Adriatic Sea (Fig. 1). Based on mark–recapture abundance estimates, about 40–100 dolphins use this area annually, the majority carrying natural marks suitable for long-term identification (Genov et al. 2008; Genov 2011). The study area, survey methods and photo-identification procedures are described in detail in Genov et al. (2008). In short, boat surveys were complemented with land-based surveys to maximise the probability of encountering and photographically capturing dolphins. Survey coverage varied among years, due to weather, dolphin distribution and logistical constraints (Table 1). Each year we attempted to

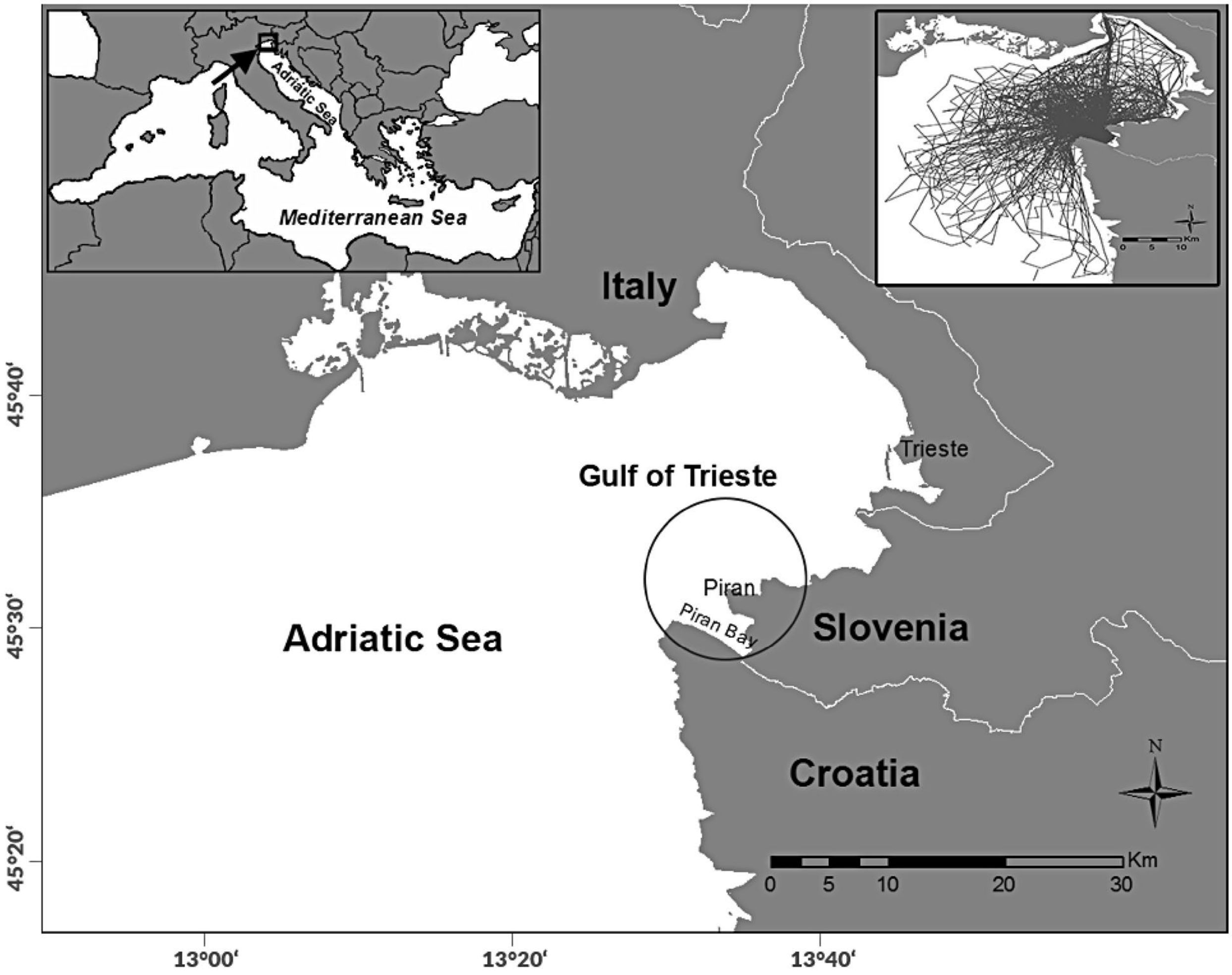

Fig. 1 Study area in the northern Adriatic Sea, with locations cited in the text. The upper left inset shows the location of the study area in the Adriatic Sea. The upper right inset shows the spatial distribution of boat survey effort (navigation tracks). The circle depicts the 'core study area' where effort was most intense and included both boat-based and land-based surveys (see main text for details)

Table 1 Survey effort between 2003 and 2011, showing boat effort (in km surveyed) and land effort (in hours and minutes surveyed), number of group observed and number of individuals identified

Year	Survey effort		Nr. groups observed	Nr. individuals identified
	Boat [km (morning/afternoon)]	Land [hours + minutes (morning/afternoon)]		
2003	na	44 h 47 min (26 h 2 min/17 h 45 min)	4	8
2004	na	52 h 28 min (35 h 59 min/16 h 29 min)	5	13
2005	261 (174/87)	33 h 23 min (20 h 50 min/12 h 33 min)	15	21
2006	219 (153/66)	44 h 17 min (23 h 12 min/21 h 5 min)	14	20
2007	256 (170/86)	56 h 36 min (41 h 6 min/15 h 30 min)	7	21
2008	502 (306/196)	65 h 37 min (43 h 25 min/22 h 12 min)	18	32
2009	641 (408/233)	88 h 39 min (55 h 52 min/32 h 47 min)	14	31
2010	607 (358/249)	142 h 20 min (89 h 15 min/53 h 5 min)	27	19
2011	600 (361/239)	148 h 25 min (97 h 48 min/50 h 37 min)	11	22
Total	3086	675 h 32 min	115	38

Numbers in parentheses show the breakdown of survey effort into morning and afternoon, respectively. (Nr. groups observed and individuals identified only refers to the already restricted dataset of individuals included in social network analysis, not all identified dolphins—see "Materials and methods")

survey the entire area as homogeneously as possible. Surveys were done predominantly during summer (July–September), but periodically also in other months. Due to typical summer weather, surveys were commonly done in the morning and early afternoon, ceased in the early afternoon due to wind and resumed in late afternoon. Southern portion of the Gulf of Trieste, including waters along the Slovenian coast and Piran Bay, was surveyed consistently over the years and received more coverage than the outer edges of the study area (Fig. 1), due to the location of the home port and the land-based observation point. This subarea, encompassing a roughly 5 km radius around the Piran peninsula, was regularly surveyed by both boat-based and land-based surveys and can be considered 'core study area' for the purposes of some of the results presented later on.

Photographs of dorsal fins were obtained during focal follows and allowed individual identification (Würsig and Jefferson 1990). Members of a dolphin group were considered associated. Group was defined as dolphins observed behaving in a generally coordinated fashion (moving in the same direction or staying in the same area, usually engaged in the same general activity). In practice this meant that group members were always within about 100 m from the nearest other dolphin. Field observations and photo-identification showed that group composition rarely changed during several hours of observation (Genov et al. 2008).

Sex was determined by (a) observations of mother–calf pairs (adults consistently accompanied by calves were assumed to be mothers and, therefore, females); (b) photographs of the genital area or (c) molecular methods from biopsy sampling carried out opportunistically within Slovenian waters (permit 35601-102/2010-4 by the Slovenian Environmental Agency). Skin and blubber samples were obtained using a 68-kg draw weight crossbow, using custom-made bolts and stainless steel sampling tips with length of 25 mm and internal diameter of 7 mm. Tips were sterilised using 96% ethanol and burning prior to being used. Dolphins were sampled in the dorso-lateral area below the dorsal fin, at distances of 4–10 m. All biopsy attempts were accompanied by concurrent photo-identification. Sampling was only attempted on adults without accompanying offspring. Skin samples were removed and excised with sterilised forceps and surgical scissors, placed in 96% ethanol and stored at − 20 °C until analysis. Samples were analysed as described in Gaspari et al. (2015).

Numerous trawlers operated in the area year-round. They can be divided into (a) single bottom trawlers and (b) pelagic/mid-water pair trawlers. Bottom trawlers were typically 9–15 m long, operated alone and trawled nets on the seabed, targeting several demersal species. Pair trawlers were typically 30 m long, operated in pairs and trawled nets in mid-water. They mostly targeted European anchovies (*Engraulis encrassicolus*) and sardines (*Sardina pilchardus*). Dolphins interacted with both trawler types (Genov et al. 2008). Interaction was defined as dolphins following operating trawlers, approximately 200–400 m from stern (closer for bottom trawlers and further for pair trawlers, but the exact distance could vary), and typically alternating long dives (> 1 min) with sequences of short dives (5–30 s).

Data restrictions

Only high-quality photographs (sharp image, fin perpendicular to the camera lens, entire fin visible and not obstructed by water spray or other animals, fin height < 7% of the frame height) were used. Association patterns were analysed for well-marked individuals only (123 individuals). However, as individuals with low encounter rates can introduce biases

(Chilvers and Corkeron 2002; Whitehead 2008b), we only considered those encountered on ≥ 4 occasions *and* in ≥ 2 different years. This restricted the analysis to animals with some meaningful level of site fidelity and removed transient individuals, to ensure an accurate representation of the social network. Although most authors limit analyses to animals with some arbitrary number of *total* sightings (Quintana-Rizzo and Wells 2001; Chilvers and Corkeron 2002; Pace et al. 2012), we further limited this to animals encountered in more than 1 year. This was because several animals seen multiple times were only seen in a single year and, therefore, considered visitors/transients. Our restriction criteria resulted in 38 individuals used in the analysis. This subset represents regular individuals ('residents') and is considered representative for this local population. Thirty-two animals (84.2%) were seen ≥ 5 times and 18 (47.4%) ≥ 10 times. Mean number of sightings per individual was 14 (SD 11.3, range 4–41). Multiple encounters during same day were only included if they were of different groups. Mother-dependent calves were excluded due to non-independence.

Testing association patterns and network analysis

To minimise bias and facilitate comparisons, the half-weight association index (HWI) was used (Cairns and Schwager 1987). Although an attempt was made to photograph all members of each group, this was not always possible and the HWI accounts for this. It was recently suggested that a new index accounting for gregariousness (HWIG) may be more suitable (Godde et al. 2013). We also analysed associations using HWIG, but found little difference in results. Therefore, and to facilitate comparisons with previous studies, only HWI results are presented.

Analyses were performed in program SOCPROG 2.4 (Whitehead 2009). To test whether dyads (pairs of individuals) associated more often than expected by chance, we used the Manly-Bejder permutation technique (Manly 1995; Bejder et al. 1998) with extensions (Whitehead 1999; Whitehead et al. 2005) and corrections (Krause et al. 2009b). We used day as a sampling period. We generated 20,000 permutations (associations within samples) to ensure stability of P values. We also performed another round of permutations, with sampling period of 5 days, because permutations are often impossible (or perform poorly) with too few associations within a period.

We used standardised lagged association rates (SLAR, Fig. 3) to estimate the probability of dyads associated at a given time still being associated after a time lag and assess the stability of associations (Whitehead 1995). Precision (SE) was estimated by jack-knifing on each sampling period (Whitehead 2008a). To test for preferred/avoided associations, we compared SLAR to null association rate, which represents expected values for random associations (Whitehead 1995). A moving average enabled the optimal adjustment between precision and smoothing. Exponential models of social organisation developed by Whitehead (1995) were fitted to SLAR. Model selection was based on minimising the Quasi-Akaike Information Criterion (QAIC) (Burnham and Anderson 2002).

We calculated the social differentiation (S) to evaluate the level of variation in dyadic probability of association, i.e. how differentiated the network was (Whitehead 2008a). We calculated the correlation between true and estimated association indices (r) to evaluate if data accurately represented the true social network (Whitehead 2008b). To investigate existence of clusters and delineate units within the network, we carried out modularity analyses (Newman 2004) by applying the eigenvector method of Newman (2006), the knot-diagram analyses and the modularity-G (Whitehead 2008a). With this approach the animals were assigned to clusters so that the separation between clusters was maximised (Whitehead 2008a). To evaluate if association rates were similar within/between clusters, and within/between sexes, we compared mean association rates via two-tailed Mantel test.

To facilitate comparisons with other studies, we calculated several network metrics—HWI, Affinity, Betweenness, Closeness, Clustering coefficient, Eigenvector centrality, Reach and Strength (Wey et al. 2008; Whitehead 2008a)—for the entire network, individual clusters and for individuals (Table 2). These are measures of how well-connected and central individuals are (Whitehead 2008a).

A social network diagram (Fig. 4) was created using NetDraw 2.123 (Borgatti 2002) to illustrate relationships and network structure. Nodes with highest associations are grouped together, while those with fewer links remain on the periphery. We created one diagram with all associations, regardless of strength (Fig. 4a), and one displaying only those with HWI greater than twice the overall mean (Fig. 4b), believed to represent meaningful associations (Durrell et al. 2004; Gero et al. 2005; Wiszniewski et al. 2012).

Social structure was also represented with hierarchical average linkage cluster analysis (dendrogram, Fig. 2). Since dendrograms can be over-interpreted, especially if the society is not hierarchically arranged, we used strength of cophenetic correlation coefficient (CCC) to indicate whether the data interpretation was valid (Whitehead 2008a).

Sex composition

To further examine potential sex segregation, we selected sightings involving at least two known-sex animals (including sightings with no or single known-sex individual would introduce a bias in estimating sex composition). In this

Table 2 Social network metrics (mean ± SD) of individual social clusters

Metric	Cluster A (*n* = 19)	Cluster B (*n* = 13)	Cluster C (*n* = 6)	Overall (*n* = 38)
Mean HWI	0.21 ± 0.03	0.19 ± 0.03	0.06 ± 0.03	0.18 ± 0.06
Affinity	7.74 ± 0.14	7.04 ± 0.12	4.21 ± 0.86	6.94 ± 1.29
Betweenness	13.3 ± 16.6	5.04 ± 7.9	8.08 ± 8.46	9.68 ± 13.38
Closeness	52.37 ± 5.36	58.38 ± 4.93	64.67 ± 8.79	56.37 ± 7.28
Clustering coefficient	0.39 ± 0.06	0.45 ± 0.04	0.23 ± 0.03	0.39 ± 0.09
Eigenvector centrality	0.21 ± 0.04	0.09 ± 0.01	0.02 ± 0.01	0.14 ± 0.08
Reach	59.26 ± 9.69	48.82 ± 6.01	9.13 ± 5.25	47.77 ± 19.27
Strength	7.66 ± 1.27	6.95 ± 0.93	2.17 ± 0.96	6.55 ± 2.23

HWI half-weight association index, *n* number of animals

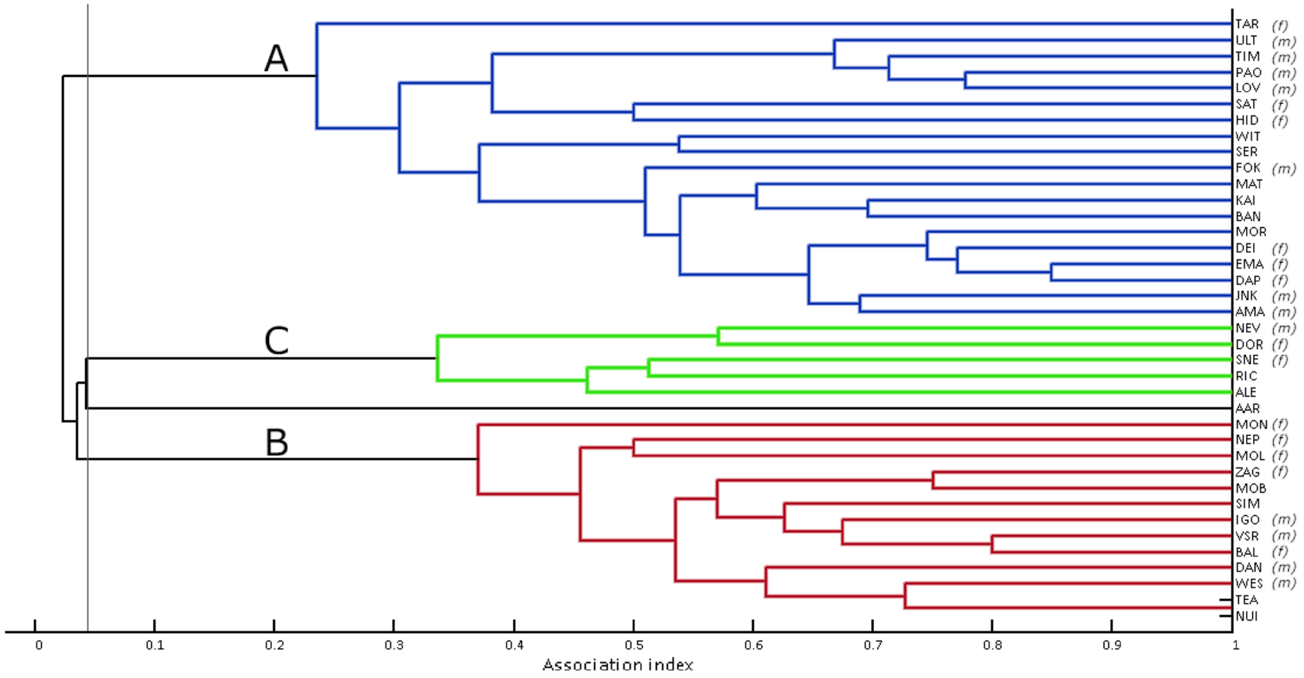

Fig. 2 Dendrogram produced using average-linkage hierarchical cluster analysis (CCC = 0.96) for 38 common bottlenose dolphins. The clusters A, B and C represent clusters of animals based on modularity analyses with the eigenvector method of Newman (2006). The modularity-G of 0.464 suggests that the best division into clusters is with an association index of 0.043 (thin vertical line). Note that the dolphin AAR is not included in any cluster

sub-sample, we determined the proportion of male-only, female-only and mixed-sex groups.

Results

Association patterns

We photographed 132 dolphin groups, but the restriction criteria resulted in 115 encounters of 38 individuals included in analysis. All individuals were observed in the core study area, but could also be encountered elsewhere.

Network metrics are shown in Table 2. Correlation between true and estimated association indices ($r \pm \mathrm{SE} = 0.840 \pm 0.040$, based on bootstrap with 10,000 replications), suggests that the data accurately describe the true social network (Whitehead 2008b). *P* values stabilised after about 9000 permutations. Standard deviation of the calculated (observed) associations was significantly higher than that of permuted data (observed SD = 0.236, random SD = 0.228, $P < 0.001$), as was the CV (observed CV = 1.259, random CV = 1.226, $P < 0.001$), indicating that associations were non-random (Gowans et al. 2001; Lusseau et al. 2003). Figure 2 shows that most dolphins had preferred associates, with one pair (NUI-TEA) always recorded

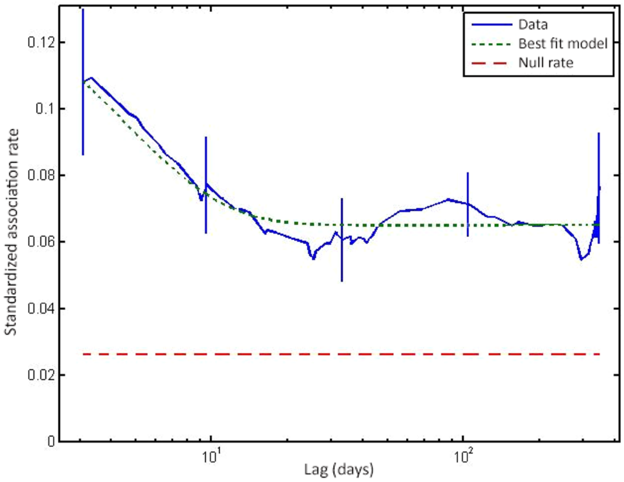

Fig. 3 Standardized lagged association rate (SLAR) for 38 common bottlenose dolphins. A moving average of 8000 associations was used. Vertical bars indicate standard errors calculated using the temporal jackknife method on each sampling period. The best fit model (dotted line) indicates a social system model of 'constant companions and casual acquaintances'. The null association rate (dashed line) represents the theoretical SLAR if individuals associated randomly

together. CCC of 0.96 suggests a good fit and thus a good representation of true social structure (Whitehead 2008a). SLAR was best described by the so-called 'constant companions and casual acquaintances' model (Fig. 3, Table 3). SLAR line never reached the null association rate, indicating the absence of random associations and a high probability of dyads associated even after a prolonged time lag.

Division of social network

Although the network was fluid overall, social differentiation estimate using likelihood method (*S* 1.076, SE 0.025) indicates a well-differentiated society (Whitehead 2008a). Average linkage cluster analysis (Fig. 2) and network analysis (Fig. 4) both showed a clear division into three distinct clusters, with one individual (AAR) not fully belonging in any. Modularity analysis assigned individuals to clusters with significantly higher associations within than between clusters (two-tailed Mantel test: $t=21.25$, $P=1.0$). Modularity-G division (peak at 0.464) suggests that the best division is with an association index of 0.043. The modularity-G peak suggests that with this division, there is much more total association within clusters than would be expected for randomly determined clusters. Since modularity values > 0.3 suggest a meaningful division (Newman 2004), the value of 0.464 provides compelling evidence of a structured network.

Dolphins formed two main clusters, A (19 individuals) and B (13 individuals), with strong associations, and a smaller cluster C (6 individuals) with much weaker associations (Table 2). Mean HWIs were similar between A and B and lower in cluster C (Table 2). Dolphins were predominantly found with other members of the same cluster, although group sizes varied. Cluster A and B dolphins were usually seen in large groups (> 10 and up to 45 individuals). Cluster A dolphins rarely interacted with those from cluster B (4/115 encounters, or 3.5%). These interactions never involved the majority of both clusters. Instead, while one (either A or B) featured the majority of animals, the other was represented by few (1–4).

Cluster C contained individuals that occasionally interacted with clusters A and B, but were typically seen with other cluster C animals, on their own, or with transient dolphins. They were predominantly found in small groups (2–3) or alone. They had no particularly strong bonds with anyone. Individual AAR did not fit into any cluster well (Fig. 2), but was placed in cluster C based on modularity, extremely low mean HWI of 0.01, an eigenvector value close to zero, and other network metrics.

Of 115 encounters, 55 (47.8%) included only cluster A animals, 10 (8.7%) included only cluster B animals and 37 (32.2%) included only cluster C animals. Three encounters (2.6%) included a mix of clusters A and B, 6 (5.2%) of clusters A and C, 3 (2.6%) of clusters B and C and 1 (0.9%) of all three clusters.

Network metrics

Affinity, Clustering coefficient, Eigenvector centrality, Reach and Strength were comparable between clusters A

Table 3 Fit of social system models to the standardised lagged association rate (SLAR)

Model	Formula	Number of parameters	QAIC	ΔQAIC
CC	0.068	1	48,999.91	83.13
CA	$0.069552e^{-0.00002585\tau}$	2	48,999.97	83.19
CC + CA	$0.066285+0.091054e^{-0.25144\tau}$	3	48,916.78	0
Two levels of CA	$0.24804e^{-0.85368\tau}+0.066852e^{0.000003792\tau}$	4	48,943.40	26.63

τ represents time lag in days. The lowest Quasi-Akaike Information Criterion (QAIC) indicates the best-fitting model, and ΔQAIC (difference between QAIC and that of the best model) indicates the degree of support for the other models

CC constant companions, *CA* casual acquaintances

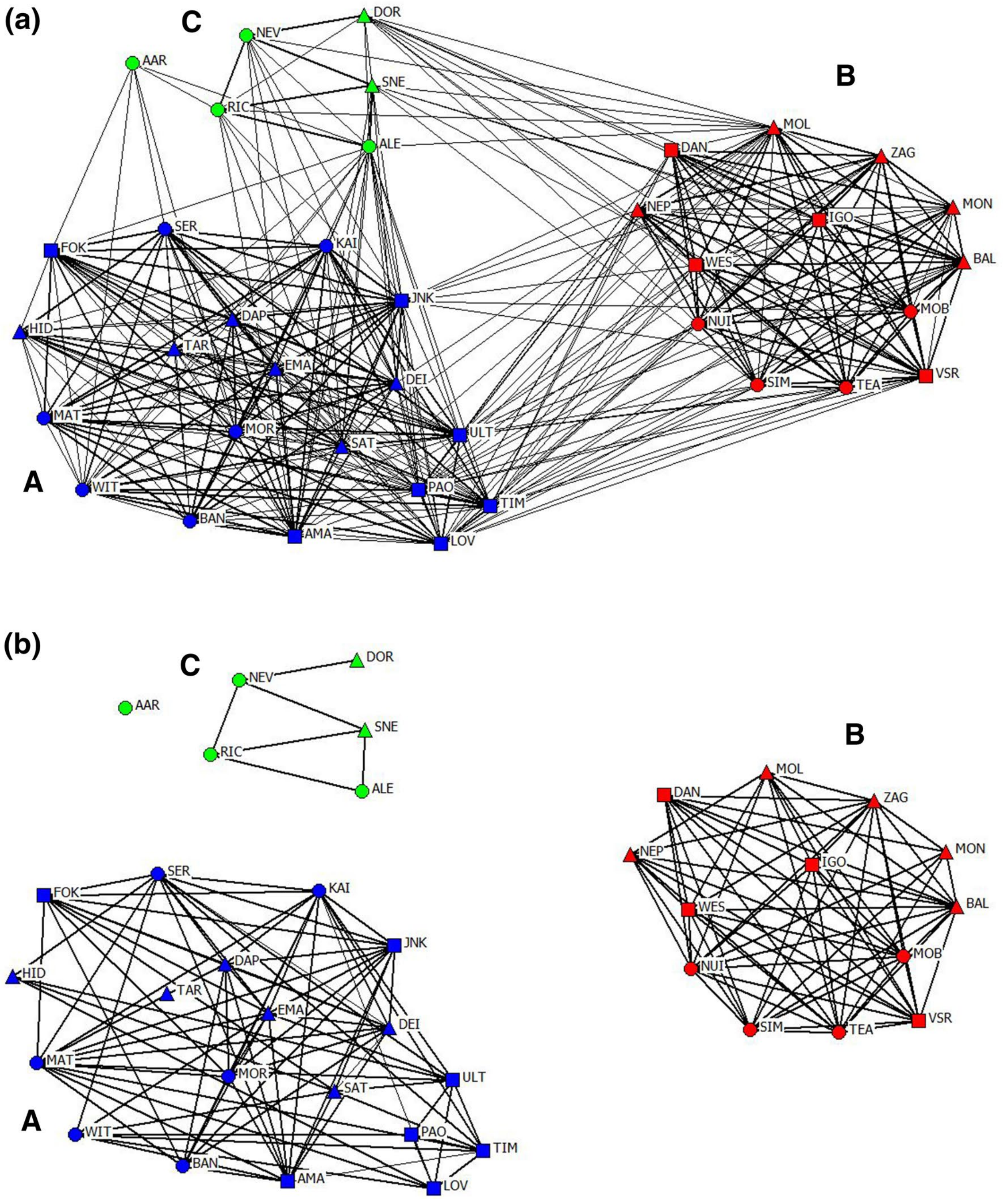

Fig. 4 Social network diagram of the common bottlenose dolphin population. Nodes represent individuals (filled square = males, filled triangle = females, filled circle = unknown sex). Lines between nodes represent associations between dyads and the thickness of lines indicates the strength of relationship (value of an association index between dyads). Division of clusters is based on eigenvector method of Newman (2006) and modularity from gregariousness analyses. Cluster A = blue nodes, cluster B = red nodes, cluster C = green nodes. Note that individual AAR is included in cluster C but does not fit into it well. **a** All recorded associations between dyads, regardless of strength. **b** Only associations higher than twice the mean HWI (see main text for details)

and B and lower in cluster C. Individuals in A and B had more associates and formed more stable associations with them than those in C. Conversely, cluster C had a higher Closeness, which is a different measure of centrality—as cluster C animals interacted with both A and B, their shortest paths to all other individuals were shorter than for other two clusters. Finally, cluster B had the lowest Betweenness centrality, which is likely a combination of cluster size and how often its members interacted with other clusters.

Sex segregation and sex differences

Twenty-five animals were sexed (13 females, 12 males). We found no evidence of sex segregation. Cluster A contained 6 females, 7 males, and 6 unknown sex animals; cluster B contained 5 females, 4 males, and 4 unknown sex animals; and cluster C contained 2 females and 4 unknown sex animals (Fig. 4).

Among groups where sex of at least two animals was known ($n = 60$), 76.7% were mixed-sex. This is likely an underestimate, as groups classified as 'single-sex', but involving unsexed individuals, could in fact be mixed. Among groups composed only of cluster A dolphins ($n = 44$), 81.8% were mixed-sex. We could not estimate this for clusters B and C, as the number of encounters with at least two sexed animals was insufficient.

Mean HWI was higher for male–male pairs ($\text{HWI} \pm \text{SD} = 0.25 \pm 0.07$) than male–female pairs (0.21 ± 0.05) and female–female pairs (0.15 ± 0.06), but differences between sexes were not significant (two-tailed Mantel test: $t = -0.916$, $P = 0.16$).

Temporal habitat use patterns

When we examined temporal (diel) occurrence patterns, an interesting trend became apparent. Clusters A and B overlapped spatially, but not temporally. They were almost never seen together, apart from four encounters mentioned earlier. Furthermore, while both regularly used the core study area, they used it at different times of day: cluster A was predominantly sighted in morning hours (07:00–13:00) and cluster B only in late afternoon hours (18:00–21:00). This trend was consistent in the core study area without deviation, although cluster A could be found elsewhere in the afternoon and cluster B could be found elsewhere in the morning. To test if there was any real pattern, we first looked at hours of occurrence of the two clusters for the entire study area. Next, to avoid bias resulting from different spatial preferences of dolphins or the spatial coverage of our survey effort, we looked at hours of occurrence in the core study area only (i.e. the area regularly covered by both boat-based and land-based surveys). For those few occasions when animals from more than one cluster were together, we assigned a group to a given cluster if it was predominantly composed of that cluster. When considering the entire study area and groups composed only or predominantly of cluster A dolphins, 55 encounters (93.2%) were between 07:00 and 13:00, and only 4 encounters (6.8%) after 13:00 ($n = 59$). Looking at the core study area only, all encounters (100%) of cluster A groups were before 13:00 ($n = 18$, Fig. 5). When considering the entire study area and groups composed only or predominantly of cluster B dolphins, 5 encounters (33.3%) occurred before 13:00, while the remaining 10 (66.7%) occurred after 18:00 ($n = 15$). Looking at the core study area only, all encounters (100%) of cluster B groups were recorded after 18:00 ($n = 8$; Fig. 5). The temporal use of the entire study area differed significantly between clusters A and B, as did the use of the core study area (Fisher's exact test: $P < 0.001$).

Cluster C groups did not display such patterns. In the entire study area, 23 (62.2%) encounters of cluster C groups were before 13:00, while 14 (37.8%) were after 13:00 ($n = 37$). In the core study area, 20 (66.7%) were before 13:00, while 10 (33.3%) were recorded after ($n = 30$).

Interactions with trawlers

Forty-eight interactions with trawlers were recorded during the study, of which 35 were during dolphin encounters considered in the analysis. Encounters involving trawler interactions accounted for 29.6% of dolphin encounters. Twenty-two (62.9%) of these interactions were with pelagic pair trawlers and 13 (37.1%) with bottom trawlers (one encounter involved interactions with both).

Majority of interactions with trawlers involved cluster A dolphins, with one individual (MOR) present in more than 50% of all interactions (Fig. 6). Mean number of interactions with any trawlers per individual in cluster A was 10.6 (SD 6.1, range 3–24). Twenty-eight interactions involved only cluster A dolphins (82.4%), 4 involved cluster A and cluster C dolphins (11.8%), 1 involved only cluster C dolphins (2.9%) and 1 involved cluster B and cluster C dolphins (2.9%). No interactions involved only cluster B dolphins.

Cluster B dolphins were never observed interacting with pair trawlers, while four individuals apparently interacted with a bottom trawler on one occasion. Mean number of interactions with any trawler per individual in this cluster was 0.31 (SD 0.48, range 0–1).

Dolphins from cluster C interacted with trawlers at intermediate level. Only one animal from cluster C (ALE) ever interacted with pair trawlers. This happened on one occasion, when the individual was with cluster A dolphins. On another occasion, the same individual was observed diving (*sensu* Bearzi et al. 1999) with another unidentifiable adult, when active pair trawlers passed by. The animals appeared to ignore them and continued diving in the same location. Other animals from cluster C were either never observed interacting with trawlers, or only observed interacting with bottom trawlers (Fig. 6). Mean number of interactions with any trawlers per individual in this cluster was 1 (SD 0.01, range 0–3).

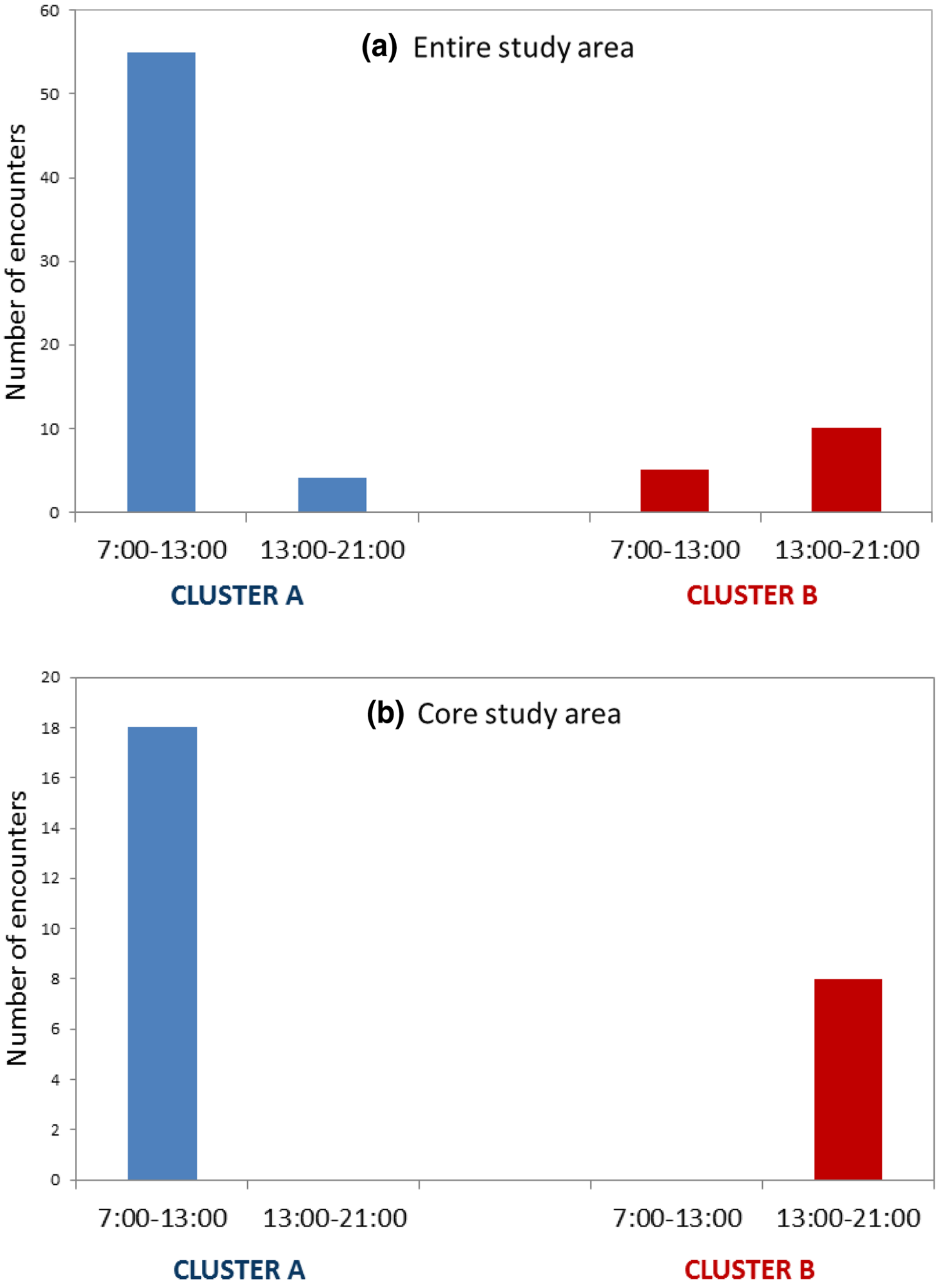

Fig. 5 Temporal occurrence of clusters A and B in the **a** entire study area and **b** core study area

Discussion

General social structure

Gulf of Trieste dolphins appear to live in two general kinds of social units: (a) large mixed-sex groups with strong, long-lasting associations and (b) small groups with weaker, temporally unstable associations. This does not appear to be age-dependent. Two largest clusters featured strong bonds, while seldom interacting with the other cluster. This structuring was also evident in the field. These two clusters showed high levels of group stability, which persisted through the study years and beyond (T. Genov, *personal observation*), although exact group membership could vary. Gregariousness, connectedness and strength of associations (indicated by HWI, Affinity, Clustering coefficient, Eigenvector centrality and Strength) were quite high and relatively similar between the two, as was the number of associates (Reach; Table 2). In contrast, these metrics were substantially lower in cluster C, where animals showed no strong association preferences. Because they were occasionally observed with animals from other clusters, their Closeness was highest (Table 2).

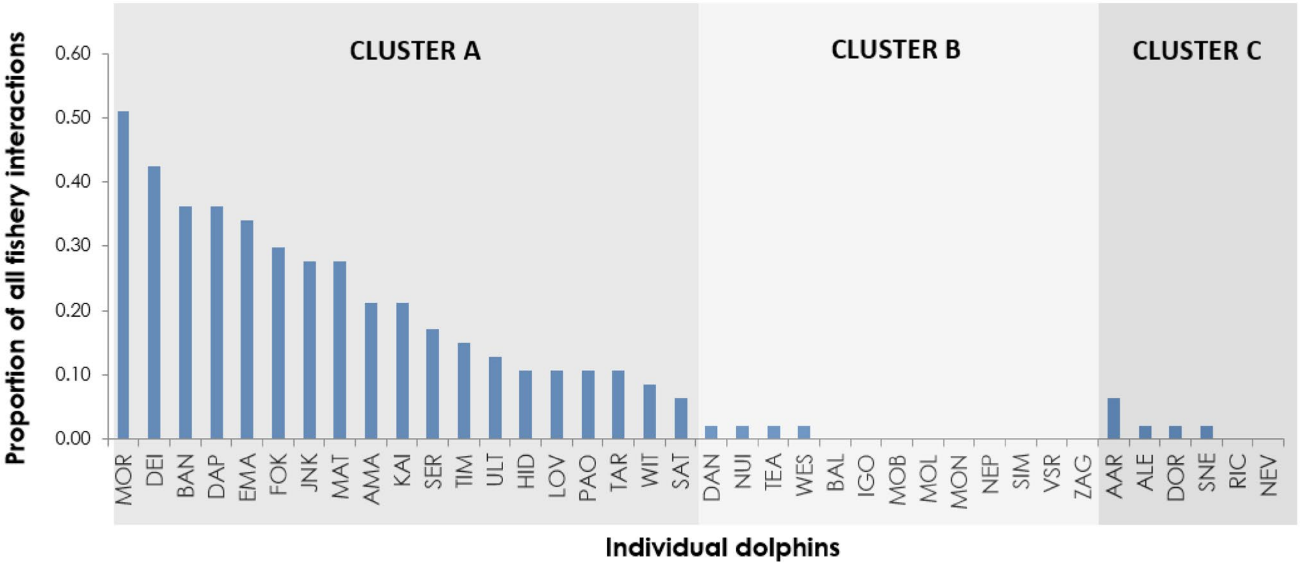

Fig. 6 Proportion of all dolphin–fishery interactions an individual dolphin was recorded in

When including all associations (Fig. 4a) the network was reasonably well-connected, with no individual 'bottlenecks' between clusters, which were inter-connected via several but not particularly numerous individuals. Such 'social brokers' (Lusseau and Newman 2004) may maintain population cohesiveness and prevent complete cluster isolation, possibly having disproportionate influence on the population connectedness, as found in killer whales (Williams and Lusseau 2006), macaques (Flack et al. 2005) and squirrels (Manno 2008). However, when considering only 'meaningful' associations, greater than twice the mean HWI (Durrell et al. 2004; Gero et al. 2005; Wiszniewski et al. 2012), structuring becomes striking and clusters completely separated (Fig. 4b).

Associations were temporally relatively stable (as supported by SLAR and field observations), although stability varied with different levels of social organisation. Cluster A in particular (but also B) seemed to contain 'core' membership (first-level unit) and other 'tiers' that joined core members to form higher-level units. In such multi-level systems, seen also in African elephants (Wittemyer et al. 2005) clusters can sub-split during times of ecological constraints and fuse again when conditions are favourable or promote cooperation. We sometimes observed cluster A dolphins forming smaller groups (≤ 10), which often joined into groups of 30 + animals. Group composition during encounters was also surprisingly stable, more than in the closest other known local population in the Adriatic Sea (Bearzi et al. 1997) or in most other populations worldwide (Connor et al. 2000; Lusseau et al. 2006). Once encountered, groups were unlikely to change during observations, which could last several hours (Genov et al. 2008). This population is rather small (Genov et al. 2008; Genov 2011) and some authors hypothesised that community size influences group stability in fission–fusion societies, with smaller communities leading to decreased fission–fusion flexibility (Lehmann and Boesch 2004; Augusto et al. 2012).

In several *Tursiops* populations, social structure involves sex/age segregation (Wells et al. 1987; Connor et al. 2000; Fury et al. 2013). Here, structuring did not appear sex-related, as clusters contained both sexes. We found no evidence of male alliances. Although male–male associations were stronger than male–female or female–female associations, this was not significant, with stronger male–female than female–female associations. Most encountered groups contained both sexes (regardless of season), which suggests that mixed-sex groups were not related to reproductive state. Likewise, although more than half of all groups contained calves, adult-only groups were common. Reproductive state or presence of calves, therefore, fails to explain these patterns.

Presence of large mixed-sex groups resembles Doubtful Sound bottlenose dolphins in New Zealand (Lusseau et al. 2003). Lusseau et al. (2003) hypothesised that ecological constraints, such as variable productivity, drive social organisation. In such environments, groups may need to rely on individuals with long-term knowledge about spatio-temporal distribution of prey sources, which might explain lack of sex segregation and greater population connectedness (Lusseau et al. 2003). The northern Adriatic is characterised by large spatio-temporal variability in nutrient input and productivity (Fonda Umani et al. 2005; Mozetič et al. 2010, 2012), and our study area contains relatively uniform bottom topography. With lack of major prey-aggregating bottom features, spatio-temporal distribution of prey is likely highly variable, which may promote network connectedness. Clusters A and B both contained individuals which appeared 'older' based on their external appearance. These animals may possess long-term knowledge needed to tackle such constraints and thus play a key role in their community.

Temporal segregation

Several studies found spatial segregation in *Tursiops* (Chilvers and Corkeron 2001; Chilvers et al. 2003; Lusseau et al. 2006; Fury et al. 2013; Carnabuci et al. 2016). In Moray Firth, Scotland, this segregation appeared season-dependent (Wilson et al. 1997). During summer, part of the population moved into inner parts of the Firth and was replaced by dolphins from outer parts. However, clusters in our study overlapped spatially, but not temporally, and we found differences on a daily, rather than seasonal level. Such intraspecific diel temporal partitioning does not appear to have been documented in cetaceans previously, nor in other mammals (Kronfeld-Schor and Dayan 2003), with one exception recorded in the use of running wheel in captive mice (Howerton and Mench 2014). Whether this pattern results from competitive exclusion, avoidance of aggressive interactions, or different foraging tactics, remains unknown. Given that prey resources in the marine environment are patchy and variable, prey resource defence is not a likely explanation (Ramp et al. 2010). Lack of sex segregation also dismisses access to females as an explanation. We are currently working to determine if genetic relatedness correlates with the social partitioning observed here.

We considered potential confounding factors. If the distribution of cluster A was linked to trawlers, which only operated during certain hours, this would explain the pattern. However, pair trawlers operated in the morning and afternoon, and bottom trawlers operated day-long (including evenings). Cluster A regularly used trawling areas even in the absence of trawlers, with no difference in group composition. More importantly, no trawlers operated in the core study area. Finally, cluster A dolphins did not *always* follow trawlers, even if trawlers were around. Trawlers, therefore, fail to explain temporal partitioning.

We also considered lower sample size for cluster B. Caution is needed when making inferences from small sample sizes, but temporal patterns here appear quite striking. The presence of a temporal (rather than spatial) pattern suggests the observed associations were not an artefact of space use (animals being together just because they use the same space), but due to genuine social preferences. Further, due to long-term and extensive survey effort (Table 1, Fig. 1), this pattern is unlikely to be an artefact of effort. Surveys in recent years (2012–2017, analysis pending) further support this, with both clusters continuing this pattern, and even occurring in the same area within a single day, but at different times (Morigenos, unpublished data).

Finally, it remains to be determined if segregation is specific to this area, or if it occurs in other areas used by the animals. The range of this local population is unknown (Genov et al. 2016), but evidence from photo-ID (Genov et al. 2009) and genetics (Gaspari et al. 2015) suggest it is a distinct unit.

Interactions with trawlers

Two clusters displayed behavioural differences related to trawling. Cluster A dolphins often interacted with pair trawlers and occasionally bottom trawlers, while cluster B dolphins did not ('trawler dolphins' vs. 'non-trawler dolphins', Chilvers and Corkeron 2001). Fishing has a major impact on cetaceans worldwide, not only through incidental mortality (Read et al. 2006), but also through prey depletion (Bearzi et al. 2008), habitat degradation (Turner et al. 1999) and ecosystem change (Worm et al. 2006). More subtly, fishing activities can affect, or be affected by, cetacean behaviour. In Queensland, Australia, bottlenose dolphins were found to form two communities, where one fed in association with trawlers and the other did not (Chilvers and Corkeron 2001; Chilvers et al. 2003). Following fishery closure, dolphins restructured and homogenised their network, suggesting that structuring was fishery-induced (Ansmann et al. 2012). Our study shows similarities, but also important differences. First, in the population studied by Ansmann et al. (2012), dolphins fed on discards, while our dolphins followed operating trawlers, presumably feeding actively inside/behind the net (Genov et al. 2008; Kotnjek 2016). Second, structuring in our study related to temporal rather than spatial segregation, and did not appear only fishery-related. Another study in the Mediterranean Sea related dolphin association patterns to bottom trawling and fish farming, but animals mixed more frequently than ours (Pace et al. 2012).

Human activities can likely alter behaviour and social structure of mammals (Rutledge et al. 2010; Ansmann et al. 2012) and this may well be the case here. However, causal links are unclear and it is difficult to ascertain what came first. The inherent social structure itself, and social learning, may lead to differential behaviour and interactions with anthropogenic activities, without these activities changing the social system in the first place. It is interesting to note that the pair trawler fishery in our area closed in 2012. This did not appear to change associations or temporal patterns, but cluster A did appear to increase rates of interactions with bottom trawlers (Morigenos, unpublished data).

Diet information for this population is limited, but dietary preferences may explain different fishery-related foraging tactics. Both clusters were observed taking mullets (*Mugil*/*Liza* sp., Genov et al. 2008, Morigenos, unpublished data) and both regularly feed in the core study area. Their diets, therefore, overlap, but to an unknown extent. However, the apparent 'switch' of cluster A to bottom trawlers after the closure of pair trawler fishery suggests that behavioural specialisation and hunting techniques, rather than prey preference, may be more likely. Our further research aims to provide better insight into the feeding ecology of this population through stable isotope analysis.

Whether interactions with trawlers increase fitness (by maximising energetic intake and minimising expenditure) or decrease it (through increased bycatch), is unknown. Both clusters produce new offspring and appear stable, and there is no evidence of trawler-related bycatch in this area.

Conclusions

We show that local dolphins (1) form distinct social clusters; (2) exhibit temporal partitioning; and (3) differ in interactions with fisheries. We demonstrate how different segments of the same population may behave very differently and have differing effects on human activities such as fishing (through potential depredation or gear damage). In turn, they may respond differently to anthropogenic pressures, as temporal partitioning may make animals either more or less vulnerable to disturbance from boat traffic.

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Data availability The dataset analysed during the current study is available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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Linking organochlorine contaminants with demographic parameters in free-ranging common bottlenose dolphins from the northern Adriatic Sea

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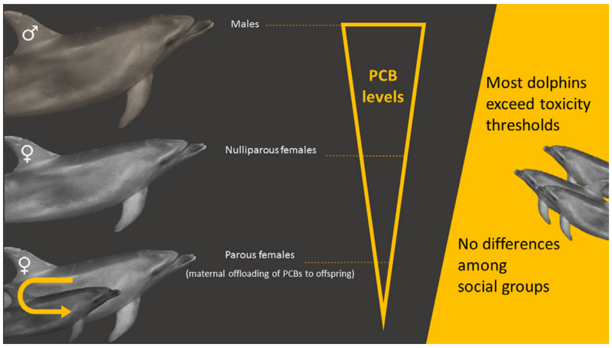
HIGHLIGHTS

- Male bottlenose dolphins have significantly higher PCB concentrations than females.
- Nulliparous females have significantly higher concentrations than parous ones.
- There are no differences among social groups.
- Majority of animals exceed the toxicity thresholds.
- Pollutant concentrations can successfully be linked with demographic parameters.

GRAPHICAL ABSTRACT



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ABSTRACT

Marine top predators, including marine mammals, are known to bio-accumulate persistent pollutants such as polychlorinated biphenyls (PCBs), a serious conservation concern for these species. Although PCBs declined in European seas since the 1970s–1980s ban, considerable levels still persist in European and Mediterranean waters. In cetaceans, stranded animals are a valuable source of samples for pollutant studies, but may introduce both known and unknown biases. Biopsy samples from live, free-ranging cetaceans offer a better alternative for evaluating toxicological burdens of populations, especially when linked to known histories of identified individuals. We evaluated PCB and other organochlorine contaminants in free-ranging common bottlenose dolphins (*Tursiops truncatus*) from the Gulf of Trieste (northern Adriatic Sea), one of the most human-impacted areas in the Mediterranean Sea. Biopsies were collected from 32 male and female dolphins during 2011–2017. All animals were photo-identified and are part of a well-known population of about 150 individuals monitored since 2002. We tested for the effects of sex, parity and social group membership on contaminant concentrations. Males had significantly higher organochlorine concentrations than females, suggesting offloading from reproducing females to their offspring via gestation and/or lactation. Furthermore, nulliparous females had substantially higher concentrations than parous ones, providing further support for maternal offloading of contaminants. Overall, 87.5% of dolphins had PCB concentrations above the toxicity threshold for physiological effects in experimental

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marine mammal studies (9 mg/kg lw), while 65.6% had concentrations above the highest threshold published for marine mammals based on reproductive impairment in ringed seals (41 mg/kg lw). The potential population-level effects of such high contaminant levels are of concern particularly in combination with other known or suspected threats to this population. We demonstrate the utility of combining contaminant data with demographic parameters such as sex, reproductive output, etc., resulting from long-term studies.

1. Introduction

Persistent organic pollutants (POPs) are chemical compounds that occur in the marine environment and have far-reaching consequences for human and ecosystem health. Marine top predators, including marine mammals, are known to bioaccumulate POPs, which represent conservation and health concerns for these species and their environment (Tanabe et al., 1994; Aguilar et al., 2002; Vos et al., 2003; Jepson and Law, 2016). Of these, organochlorines such as polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) are of particular concern, as they are persistent in the environment, highly lipophilic, bioaccumulate in individuals over time, and biomagnify in marine top predators through trophic transfer (Green and Larson, 2016). These toxic compounds may cause anaemia (Schwacke et al., 2012), immune system suppression (Tanabe et al., 1994) and the subsequent increased vulnerability to infectious disease (Aguilar and Borrell, 1994a; Jepson et al., 2005; Randhawa et al., 2015), endocrine disruption (Tanabe et al., 1994; Vos et al., 2003; Schwacke et al., 2012), reproductive impairment (Schwacke et al., 2002) and developmental abnormalities (Tanabe et al., 1994; Vos et al., 2003) in marine mammals, thereby representing a serious health risk for these top predators. Such health risks are likely to have direct impacts on marine mammal abundance, through reduced reproduction or survival (Hall et al., 2006; Hall et al., 2017). Because of their trophic position, propensity for bioaccumulating organochlorines, and long life span, marine mammals are often considered ecosystem sentinels (Ross, 2000; Wells et al., 2004; Moore, 2008).

Due to concerns about toxicity and suspected carcinogenicity to humans, their effects on biota and environmental persistence, the use of PCBs and OCPs such as dichlorodiphenyltrichloroethane (DDT) was banned in most of Europe in the 1970s–1980s. Subsequent monitoring of POPs in tissues of several marine mammal species demonstrated their decline in several European seas (Law et al., 2012), including the Mediterranean Sea (Aguilar and Borrell, 2005; Borrell and Aguilar, 2007). However, a recent European-wide study showed that PCB levels continue to be high in European and Mediterranean cetaceans (Jepson et al., 2016). In particular, very high PCB concentrations were linked to small populations, range contraction, or population declines in some striped dolphin (*Stenella coeruleoalba*), common bottlenose dolphin (*Tursiops truncatus*) and killer whale (*Orcinus orca*) populations (Jepson et al., 2016).

Linking organochlorine concentrations with individual-level effects in wild marine mammals (and especially cetaceans) is challenging at best, while linking them with potential population-level effects is extremely difficult. It is therefore unsurprising that few quantitative approaches for estimating such effects have been developed (Hall et al., 2017). Stranded animals can be a valuable source of samples for pollutant studies in wild populations (Geraci and Lounsbury, 2005), and are often the only source of samples used in toxicological analysis (Jepson et al., 1999; Jepson et al., 2005; Law et al., 2012). However, the use of stranded animals, especially in some contexts or in some locations, may introduce substantial biases. For example, stranded animals may not be representative of the population or area of interest, but may originate from other areas, due to winds, currents, or abnormal behaviour prior to stranding (Hansen et al., 2004). Moreover, putrefaction processes, resulting from exposure to the sun, high temperatures, wind and bacterial activity, can lead to altered organochlorine concentrations and potentially misleading results (Borrell and Aguilar, 1990). Finally, it has also been suggested that the presence of disease may lead to abnormal rates of pollutant metabolism or excretion (Borrell and Aguilar, 1990). On the other hand, blubber biopsy samples (Noren and Mocklin, 2012) collected from live, free-ranging cetaceans offer a good alternative for evaluating the toxicological burden of populations (Fossi et al., 2000), especially when linked to long-term re-sighting histories of known individuals (Ross et al., 2000; Ylitalo et al., 2001; Wells et al., 2005). For example, information on pollutant levels can be combined with mark-recapture techniques to estimate the impact of contaminants on survival or reproduction (Hall et al., 2009). Moreover, an appropriate study design can ensure that the sampling is representative of the population or area in question. It was previously recognised that the proper evaluation of pollutants on marine mammals will require efforts directed toward long-term studies of known individuals in wild populations (Hall et al., 2006).

The common bottlenose dolphin is a long-lived marine top predator (Wells and Scott, 1999, 2009). In many parts of the world, including the Mediterranean Sea, it is essentially “coastal” and mainly found nearshore (Bearzi et al., 2009). This makes it particularly susceptible to a range of anthropogenic impacts, including the exposure to organochlorine contaminants. This species is regularly present in the Gulf of Trieste and adjacent waters, where it has been continuously studied since 2002 (Genov et al., 2008; Genov et al., 2016; Genov et al., 2017). As a coastal, mobile and long-lived top predator with strong site fidelity, it is a particularly good candidate for investigating the effects of organochlorine contaminants, and for regional monitoring of organochlorine pollution.

In this study, we evaluated organochlorine levels, particularly PCBs, in free-ranging common bottlenose dolphins in relation to demographic parameters, as part of a long-term investigation into their ecology, behaviour and conservation status in the Gulf of Trieste and adjacent waters in the northern Adriatic Sea. In particular, we tested for the effects of sex, parity and social group membership on organochlorine concentrations, in one of the most heavily human-impacted areas within the Mediterranean Sea.

2. Material and methods

2.1. The study population

The Gulf of Trieste, together with its surrounding waters (Fig. 1), is probably one of the most heavily human-impacted areas within the Adriatic and Mediterranean Seas, due to shipping, fishing, industrialisation, tourism, aquaculture and agriculture (Horvat et al., 1999; Faganeli et al., 2003; David et al., 2007; Mozetič et al., 2008; Codarin et al., 2009; Grego et al., 2009). The dolphin population inhabiting these and surrounding waters (Fig. 1) has been the focus of a long-term study and monitoring by Morigenos – Slovenian Marine Mammal Society since 2002, primarily through boat-based surveys and photo-identification, and is now relatively well studied (Genov et al., 2008; Genov, 2011; Genov et al., 2016; Genov et al., 2017). The population is present within the area year-round (Genov et al., 2008; Genov, 2011) and appears to be demographically and genetically distinct (Genov et al., 2009; Gaspari et al., 2015). The annual abundance estimates range between about 70 and 150 animals (Genov, 2011;

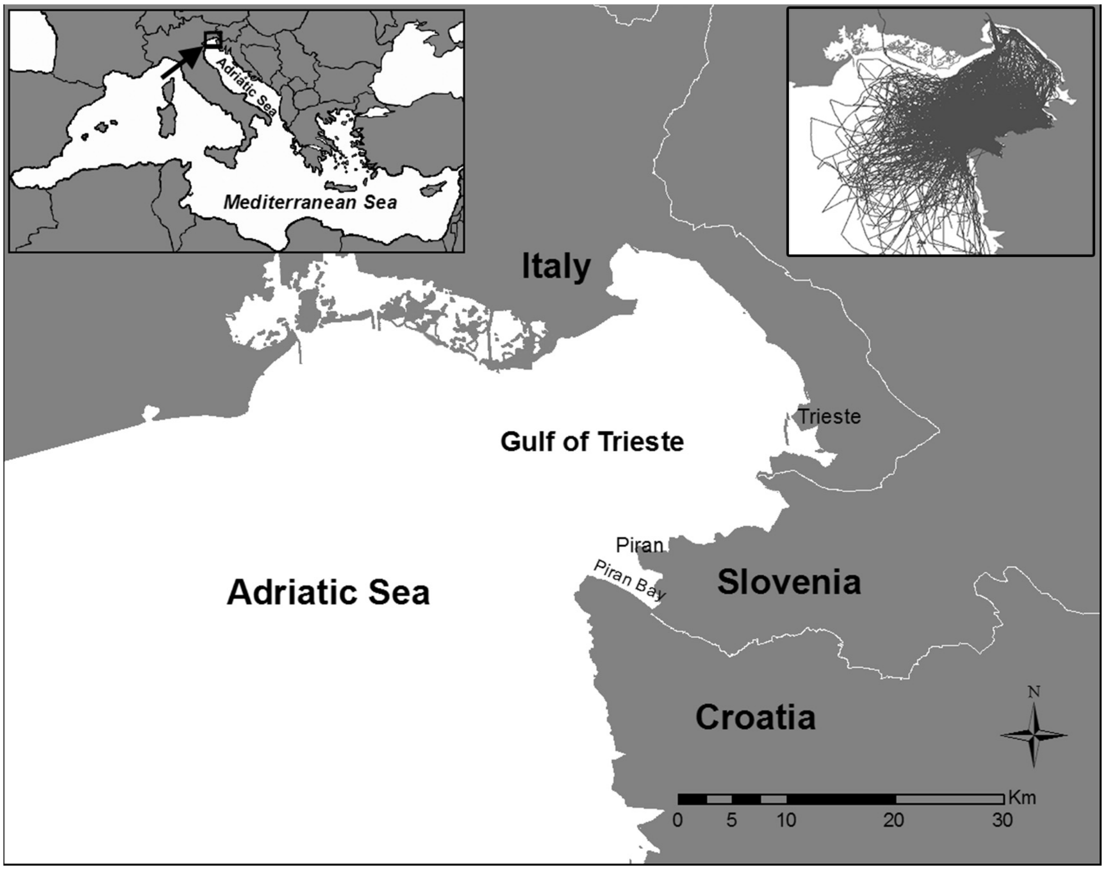

Fig. 1. Study area in the northern Adriatic Sea. The upper left inset shows the location of the study area in the Adriatic Sea. The upper right inset shows the survey effort (navigation tracks).

Morigenos, *unpublished data*). Most encountered individuals have extensive re-sighting histories over the study period, and several are of known sex and reproductive output.

2.2. Sample collection

Biopsy samples were collected from free-ranging common bottlenose dolphins between 2011 and 2017. Sampling followed standard methodology (Gorgone et al., 2008; Kiszka et al., 2010) and was carried out exclusively in good weather conditions (Beaufort sea state ≤2, good visibility, no precipitation). Samples of skin and blubber tissue were obtained using custom made bolts and stainless steel sampling tips (tip length 25 mm, internal diameter 7 mm), made by Ceta Dart, Copenhagen, Denmark. Sampling tips were sterilised using 96% ethanol and burning prior to being used. Bolts with sterile sampling tips were fired into the dorso-lateral area below the dorsal fin (Fig. 2), at distances of 4–10 m, using a Barnett Panzer V crossbow with 68 kg draw weight. A high-pressure moulded stopper prevented the tip from penetrating more than about 20 mm and ensured the re-bouncing of the bolt. The floating bolt was retrieved from the water by hand. Blubber samples were removed and excised with sterilised forceps and surgical scissors, placed in aluminium foil and stored at −20 °C until chemical analysis.

Sampling was only attempted on adults. No sampling was attempted on offspring or mothers with offspring. Care was taken not to attempt sampling of animals accompanied (followed) by another animal in their slipstream, to prevent potential shots in the head. All biopsy attempts were accompanied by concurrent photo-identification (Würsig and Jefferson, 1990) of targeted individuals and other dolphins in their group. This ensured that the identity of the sampled animal was known, in order to prevent re-sampling the same individuals, and to be able to link organochlorine concentrations to various individual-specific parameters known from photo-identification. During each attempt, the behavioural reactions of the target animal and the focal group were recorded, together with information on distance of the target animal, the area hit and the sea state. Biopsy sampling was conducted under the permit 35601–102/2010–4 by the Slovenian Environmental Agency.

In addition to biopsies, one sample was collected from an adult male found entangled in fishing gear – due to the freshness of the carcass, it could be identified with confidence, determined to be one of the local

Fig. 2. Biopsy sample collected from a free-ranging common bottlenose dolphin in the Gulf of Trieste, northern Adriatic Sea. Photo: Ana Hace, Morigenos.

dolphins, and therefore included in the analysis. Stranded animals too decomposed to be identified were not included in the analyses, as they were of unknown origin and may not be representative of the population in question.

2.3. Demographic parameters

Sex of individuals was determined by a) observations of temporally stable adult-offspring associations (adults consistently accompanied by offspring were assumed to be mothers and therefore females); b) photographs of the genital area during bowriding or aerial behaviour and c) molecular methods from biopsy samples. For molecular sex determination, DNA was extracted with phenol/chloroform and ethanol precipitation from tissue samples preserved in 95% ethanol. Sex was determined through differential amplification of the zinc finger gene regions present in the X and Y chromosomes (ZFX and ZFY, respectively), as described by Bérubé and Palsbøll (1996).

Parity was assessed based on re-sighting histories and reproductive output of photo-identified females. Females known to have produced at least one offspring during the study period were considered parous. Females never observed with offspring were assumed to be nulliparous. One of these females appeared older based on external appearance, and could potentially be of post-reproductive age, although evidence for reproductive senescence in bottlenose dolphins is limited (Marsh and Kasuya, 1986; Wells and Scott, 1999; Ellis et al., 2018).

Previous work on social network analyses has shown that the local dolphin population is structured into distinct social groups, which exhibit temporal partitioning, differences in behaviour with respect to fisheries and may have different feeding preferences (Centrih et al., 2013, 2014; Genov et al., 2014, 2015; Genov et al., in press).

2.4. Chemical analysis

Blubber samples were stored frozen at −20.0 °C. Samples were analysed using the method reported in detail in Jepson et al. (2016). In brief, samples were subjected to Soxhlet extraction using acetone: *n*-hexane 1:1 (*v:v*) and cleaned up and fractionated using alumina (5% deactivated) and silica (3% deactivated) columns, respectively. The total extractable lipid content was determined gravimetrically after evaporation of the solvent from an aliquot of the uncleaned extract. Lipid content varied from 3.4 to 33.8%. PCB concentrations in dolphin samples were determined with an Agilent 6890 GC with μECD. The PCB standard solutions contained the following 27 compounds in isooctane: Hexachlorobenzene; *p,p'*-DDE; CB101; CB105; CB110; CB118; CB128; CB138; CB141; CB149; CB151; CB153; CB156; CB158; CB170; CB18; CB180; CB183; CB187; CB194; CB28; CB31; CB44; CB47; CB49; CB52; CB66, together with the internal standard CB53. Quantification was performed using internal standards and 11 calibration levels (range 0.5–400 ng/ml). CEFAS follows a strict QA/QC regime for analysis of samples. The laboratory biannually participates in proficiency testing scheme Quasimeme (Quality Assurance of Information for Marine Environmental Monitoring in Europe) as external quality assurance. All analyses were carried out under full analytical quality control procedures that included the analysis of a certified reference material (BCR349 cod liver oil; European Bureau of Community reference) and a blank sample with every batch samples analysed so that the day-to-day performance of the methods could be assessed. Wet weight analyte concentrations were converted to lipid-normalised concentrations using measured lipid contents. Values below the limit of quantification (LOQ) were reported as <LOQ. LOQs are conservatively set at the lowest calibration standard concentration normalised to the sample multiplier (which varies depending on sample size and lipid content), which gives higher values than the alternative approach based on a S/N ratio of 10 would allow. In addition to the compounds mentioned above, four samples (two males, one female and one animal of unknown sex) were also analysed for *p,p'*-TDE (also known as *p,p'*-DDD) and *p,p'*-DDT. The limited budget available for analysis prevented us from doing this for the entire sample set.

2.5. Statistical analysis

For statistical analysis, congener concentrations below the limit of quantification (LOQ) were set to one-half of the LOQ (Darnerud et al., 2006; Lignell et al., 2009; Law et al., 2012). We compared this approach of treating <LOQ values with two alternative approaches: 1) replacing <LOQ values with zero and 2) keeping <LOQ values at the LOQ value. The choice of the approach had negligible effect on the results, and had no effect on conclusions. We therefore considered this approach the best compromise between underestimating and overestimating toxicological burden.

The values of individual 25 PCB congeners for each sample were summed to obtain the Σ25PCB for each individual. In addition, the sum of priority PCB congeners (28, 52, 101, 118, 138, 153 and 180) listed by the International Council for the Exploration of the Sea (ICES) was also calculated and displayed, for ease of comparison with some of the previous studies. The lipid content of each sample was used to obtain concentrations as mg/kg lipid weight (mg/kg lw).

Tests of normality revealed non-normal distribution of data. Both arithmetic and geometric means across individuals were calculated for Σ25PCB, ΣICES7 and *p,p'*-DDE. HCB values were too low (below the limit of quantification) to allow any useful analysis (Table 1). The contribution of each individual PCB congener to the Σ25PCB was also calculated across all individuals.

We tested for the effects of 1) sex, 2) parity (whether a female has previously had a calf or not) and 3) social group membership on contaminant concentrations. The Mann-Whitney *U* test was used to examine differences between males and females, and between nulliparous and parous females. The Kruskal-Wallis test was used to examine differences among social groups. Statistical analysed were carried out in program R (R Core Team, 2017).

2.6. Assessing toxicity

Two PCB toxicity thresholds or reference values were used, following Jepson et al. (2016). A lower PCB toxicity threshold was used for the onset of physiological endpoints in marine mammals of 17 mg/kg lipid weight (lw) (as Aroclor 1254, Kannan et al., 2000), that was calculated to be equivalent to 9.0 mg/kg lw (Σ25PCB) in Jepson et al. (2016) and in this study. A higher PCB toxicity threshold, the highest reported in marine mammal toxicology studies, of 77 mg/kg lw (as Clophen 50) for reproductive impairment in Baltic ringed seals (*Pusa hispida*, Helle et al., 1976) was calculated to be equivalent to 41 mg/kg lw (as Σ25PCB) in Jepson et al. (2016) and in this study.

3. Results

Between 2011 and 2017, samples were obtained from 32 adult dolphins, including 18 males, 9 females and 5 animals of unknown sex (Table 1). Six of these samples were included in the study by Jepson et al. (2016). Six females were previously observed with offspring, while three were not.

3.1. PCBs

Σ25PCB ranged from 4.13 to 293 mg/kg lipid weight, with an arithmetic mean of 81.5 (95% CI = 57.2–105.8) and a geometric mean of 53.4 (95% CI = 36.9–77.3, Table 2). Males had significantly higher Σ25PCB concentrations than females (Mann-Whitney U test, $U = 155$, $P < 0.001$, Fig. 3). Furthermore, nulliparous females had significantly higher concentrations than parous ones (Mann-Whitney U test, $U = 17$, $P < 0.05$, Fig. 4). There were no statistically significant differences among social groups (Kruskal-Wallis test, $H = 1.21$, $P = 0.75$, Fig. 5).

Table 1
Summary of common bottlenose dolphin samples from the Gulf of Trieste (northern Adriatic Sea), analysed in this study. F = female, M = male, U = unknown sex. Parity is indicated by + (parous) and – (nulliparous). Σ25PCB, ΣICES7, *p,p'*-DDE, DDT and HCB values expressed as mg/kg lipid weight. DDT represents total DDT. LOQ = Limit of quantification. The "<" indicates that the concentration was below the LOQ.

Sample	Year	Sex	Parity	Source	% Lipid	Σ25PCB	ΣICES7	*p,p'*-DDE	ΣDDT	HCB	LOQ
1	2011	M		Biopsy	23.3	64.2	40.9	9.03		<0.098	0.098
2	2011	M		Biopsy	9.7	80.2	50.9	11.3		<0.144	0.144
3	2011	M		Biopsy	16.2	58.7	37.1	8.02		<0.166	0.166
4	2011	M		Biopsy	11.7	139.8	94.8	13.7		0.102	0.071
5	2011	M		Biopsy	19.5	293	190	32.9		0.128	0.066
6	2011	F	+	Biopsy	17.5	29.0	14.9	1.54		<0.091	0.091
7	2013	M		Biopsy	15.2	34.2	21.2	4.49		<0.197	0.197
8	2013	F	+	Biopsy	12.9	7.96	3.96	0.44		<0.341	0.341
9	2013	F	+	Biopsy	10.9	17.9	9.89	0.95		<0.202	0.202
10	2013	M		Biopsy	3.4	23.0	14.4	2.67		<0.414	0.414
11	2014	F	–	Biopsy	10.5	27.2	17.5	9.41		<0.208	0.208
12	2014	F	+	Biopsy	27.9	4.13	2.12	0.25		<0.093	0.093
13	2014	M		Biopsy	6.6	32.2	20.2	16.7		<0.441	0.441
14	2014	M		Biopsy	13.5	43.7	27.0	5.51		<0.228	0.228
15	2014	M		Biopsy	6.9	56.7	35.6	7.72		<0.305	0.305
16	2014	M		Biopsy	23.9	123	81.2	17.5		<0.092	0.092
17	2014	F	–	Biopsy	19.3	30.7	19.2	4.25		<0.124	0.124
18	2014	F	–	Biopsy	33.8	48.9	31.0	6.45		<0.141	0.141
19	2014	M		Biopsy	10.1	131	84.8	21.9		<0.217	0.217
20	2014	M		Biopsy	18.8	65.9	40.7	9.55		<0.333	0.333
21	2014	M		Biopsy	9.3	93.8	60.9	13.5		<0.139	0.139
22	2014	M		Biopsy	14.5	76.8	48.8	10.1		<0.200	0.200
23	2015	M		Bycatch	6.6	152	96.5	25.9		<0.166	0.166
24	2015	M		Biopsy	7.9	111	74.2	16.0	17.3	<0.164	0.164
25	2015	U		Biopsy	7.7	58.3	37.8	8.17		0.195	0.128
26	2016	U		Biopsy	13.7	145	96.6	20.3	22.04	<0.080	0.080
27	2016	F	+	Biopsy	14.4	6.82	3.88	0.54	0.54	<0.104	0.104
28	2016	M		Biopsy	4.4	121	80.3	16.7	18.6	<0.215	0.215
29	2016	U		Biopsy	11.3	150	98.2	23.5		<0.194	0.194
30	2017	U		Biopsy	18.9	157	102	23.5		<0.106	0.106
31	2017	U		Biopsy	11.8	219	144	27.2		<0.126	0.126
32	2017	F	+	Biopsy	25.3	7.64	4.37	0.47		<0.059	0.059

Fig. 6 shows female and male PCB concentrations in relation to two toxicity thresholds. Overall, 87.5% of dolphins had PCB blubber concentrations above the toxicity threshold of 9 mg/kg lw for physiological effects in experimental marine mammal studies (Kannan et al., 2000), while 65.6% had concentrations above the highest threshold (41 mg/kg lw) published for marine mammals based on reproductive impairment in ringed seals (Helle et al., 1976). In males, mean Σ25PCB were above the higher of the two thresholds, even when the lower confidence limit is considered (Table 2, Fig. 6). One male had a Σ25PCB concentration of 293 mg/kg lw. In females, mean Σ25PCB were above the lower toxicity threshold of 9 mg/kg lw, but did not reach the higher one of 41 mg/kg lw, not even when the upper confidence limit is considered (Table 2, Fig. 6). The lower confidence limit of ΣPCB in females was just below the lower toxicity threshold (Table 2, Fig. 6). The ΣICES7 concentrations follow a similar pattern and are presented in Tables 1 and 2.

Among dioxin-like PCBs, these represented 2.3% (PCB 118, found in 90.6% of samples), 0.8% (PCB 156, found in 75% of samples) and 0.7% (PCB 105, found in 75% of samples) of the total PCB burden, respectively.

Table 2
Σ25PCB, ΣICES7, *p,p'*-DDE and HCB concentrations by sex: mean, median, geometric mean with 95% confidence interval, and range. All values are in mg/kg lipid weight. "Mean" is arithmetic mean. "Geomean" is geometric mean.

	N	Mean	Median	Geomean	Geomean 95% CI	Range (min–max)
Σ25PCB						
Males	18	94.5	78.5	78.3	58.3–105.1	23.0–293.0
Females	9	20.0	17.9	14.9	8.5–26.1	4.1–48.9
Unknown	5	145.7	150	134.1	87.0–206.7	58.3–219.0
Overall	32	81.5	61.5	53.4	36.9–77.3	4.1–293.0
ΣICES7						
Males	18	61.1	49.9	50.1	37.0–67.9	14.4–190.0
Females	9	11.9	9.9	8.5	4.6–15.4	2.1–31.0
Unknown	5	95.7	98.2	88.0	56.8–136.3	37.8–144.0
Overall	32	52.7	39.3	33.2	22.4–49.1	2.1–190.0
p,p'-DDE						
Males	18	13.5	12.4	11.4	8.5–15.3	2.7–32.9
Females	9	2.7	0.9	1.3	0.6–3.1	0.3–9.4
Unknown	5	20.5	23.5	19.0	12.5–29.1	8.2–27.2
Overall	32	11.6	9.5	6.7	4.2–10.7	0.3–32.9
HCB						
Males	18	0.11	0.1	0.1	0.08–0.12	0.05–0.22
Females	9	0.07	0.06	0.07	0.05–0.09	0.03–0.17
Unknown	5	0.09	0.06	0.08	0.04–0.13	0.04–0.20
Overall	32	0.09	0.09	0.09	0.07–0.10	0.03–0.22

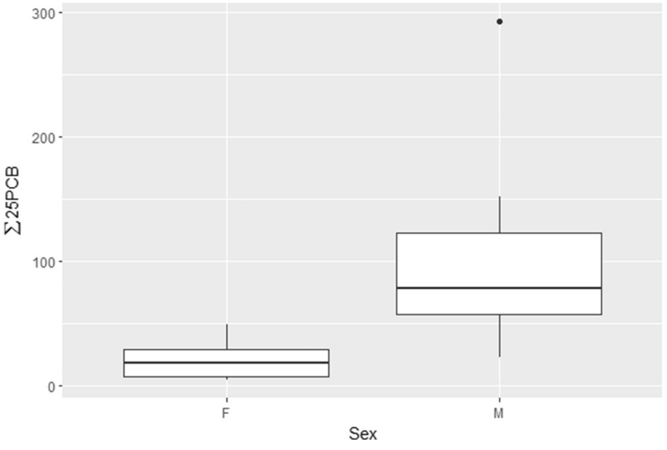

Fig. 3. Boxplots showing differences in Σ25PCB concentrations (mg/kg lipid weight) between females (F, $n = 9$) and males (M, $n = 18$). The difference is statistically significant (Mann-Whitney U test, $U = 155$, $P < 0.001$).

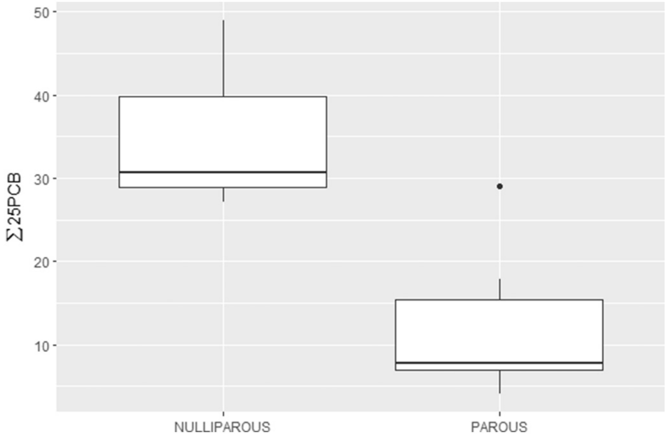

Fig. 4. Boxplots showing differences in Σ25PCB concentrations (mg/kg lipid weight) between nulliparous ($n = 3$) and parous ($n = 6$) females. The difference is statistically significant (Mann-Whitney U test, $U = 17$, $P < 0.05$).

Concentrations of the PCB congener 28 was below LOQ for all samples. PCB congeners 153, 138, 180, 187, 149 and 170 had the highest mean values across individual dolphins (Table 3, Fig. 7). Combined, they contributed 77.9% of the total PCB burden. Congeners 44, 31, 28, 18, 141, 49 and 110 had the lowest mean values, with a combined contribution of 2.2% to the total PCB burden (Table 3, Fig. 7).

3.2. DDE and DDT

The concentrations of *p,p'*-DDE ranged from 0.3 to 32.9 mg/kg lw, with an arithmetic mean of 11.6 (95% CI = 8.3–14.8) and a geometric mean of 6.7 (95% CI = 4.2–10.7, Table 2). As with PCBs, males had significantly higher *p,p'*-DDE concentrations than females (Mann-Whitney U test, $U = 152$, $P < 0.001$, Table 2), and nulliparous females had significantly higher concentrations than parous ones (Mann-Whitney U test, $U = 18$, $P < 0.05$). Like for PCBs, there were no statistically significant differences among social groups (Kruskal-Wallis test, $H = 1.15$, $P = 0.76$). The values of total DDT (the sum of *p,p'*-DDE, *p,p'*-TDE and *p,p'*-DDT) for four individuals are shown in Table 1. For these four samples, the mean contribution of *p,p'*-DDE to total DDT was 89.7% (range = 83.9–92.6%), showing that *p,p'*-DDE is the predominant metabolite of total DDT.

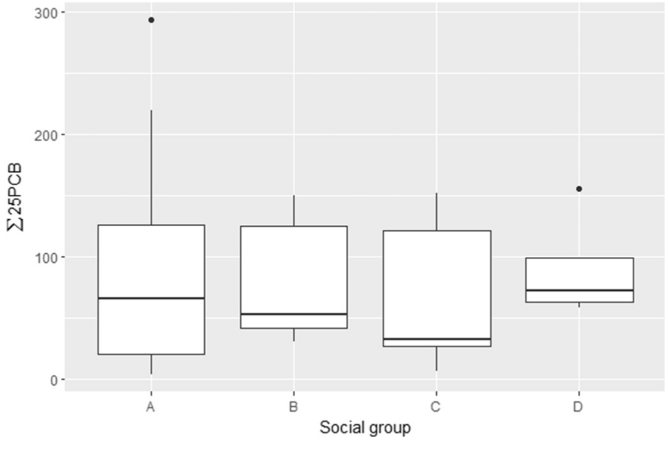

Fig. 5. Boxplots showing differences in Σ25PCB concentrations (mg/kg lipid weight) among social groups A ($n = 15$), B ($n = 8$), C ($n = 5$) and D ($n = 4$). Differences are not statistically significant (Kruskal-Wallis test, $H = 1.24$, $P = 0.743$).

3.3. HCB

Most HCB values were below the limit of quantification (Table 1). Using half the LOQ for calculations, the HCB concentrations ranged from 0.03 to 0.22 mg/kg lw, with an arithmetic mean of 0.09 (95% CI = 0.08–0.12) and a geometric mean of 0.09 (95% CI = 0.07–0.10, Table 2). Due to these low values, no further analysis was carried out on HCB concentrations.

4. Discussion

We assessed the organochlorine levels in free-ranging common bottlenose dolphins from the Gulf of Trieste and adjacent waters in the northern Adriatic Sea. We show that concentrations vary with sex and reproductive status, but not with social group membership. With the largest sample size analysed in the Adriatic Sea to date, and samples coming from live resident animals with known resighting histories, this study provides an unprecedented insight into the organochlorine burden in Adriatic dolphins. Judging from the literature, this may also represent the largest sample size of live free-ranging animals in the Mediterranean Sea or Europe published for this species to date, and is comparable to some of the world's largest sample sizes analysed (Table 4).

To date, a number of studies looked at contaminants in different cetacean species in the Adriatic Sea. Marsili and Focardi (1997) investigated organochlorines in cetaceans stranded around the Italian coasts, but only three samples were from bottlenose dolphins from the northern Adriatic. Storelli and Marcotrigiano (2000) assessed organochlorines from three Risso's dolphins (*Grampus griseus*) stranded in the southern Adriatic. Storelli and Marcotrigiano (2003) and Storelli et al. (2007) assessed organochlorines in bottlenose dolphins stranded on the southern Adriatic Sea coast, but the latter study did not include analysis of blubber tissue. In the same area, Storelli et al. (2012) measured organochlorines in stranded striped dolphins. In the northern Adriatic Sea, on its eastern side, Lazar et al. (2012) analysed different tissues in a single common dolphin (*Delphinus delphis*), a species considered extremely rare in the basin nowadays (Bearzi et al., 2004; Genov et al., 2012). Finally, Herceg Romanić et al. (2014) analysed organochlorine contaminants in various tissues in 13 bottlenose dolphins stranded along the Croatian coast in the northern Adriatic, providing the most comprehensive organochlorine assessment for dolphins in the northern part of the Adriatic Sea until now. All of these studies provided valuable insights, but due to limited sample sizes and the use of stranded animals, the inferences that can be made are somewhat limited.

In most cases, cetacean studies typically involve either a) collecting photo-identification data of free-ranging individuals, or b) analysing pollutant concentrations in stranded animals. However, studies combining these two important aspects, the analysis of pollutants in conjunction with long-term photo-identification of live animals (e.g. Ross et al., 2000; Ylitalo et al., 2001; Wells et al., 2005) are still relatively rare. In our study, all sampled animals were photo-identified and are part of a well-known population of about 150 individuals monitored since 2002 (Genov et al., 2008; Genov et al., 2009; Genov et al., 2016; Genov et al., 2017), which adds additional value to this dataset. It allowed us to combine long-term records of identifiable individuals with individually-specific organochlorine concentrations, which in turn enabled us to link contaminant loads to certain demographic parameters in a known resident dolphin population. In the long term, the continued organochlorine monitoring in conjunction with photo-identification may provide further useful insights and we hope to be able to expand on this in the future by including additional parameters. Such integrated approach offers a lot of potential, as PCBs can be linked to sex, reproductive output and other parameters (Ross et al., 2000, Ylitalo et al., 2001, Wells et al., 2005). Such information is often lacking for wild populations and is of considerable importance for evaluating the impacts of pollutants on marine top predators.

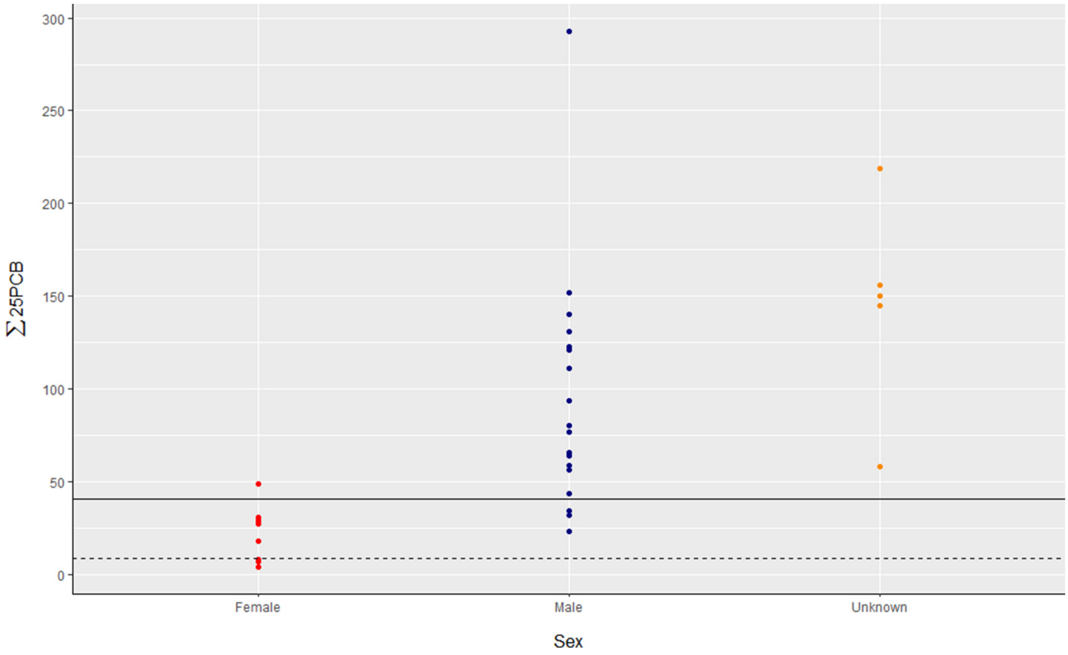

Fig. 6. Σ25PCB (mg/kg lipid weight) concentrations for females ($n = 9$), males ($n = 18$) and unknown sex ($n = 5$), in relation to published toxicity thresholds. The lower dashed line represents the lower toxicity threshold (9 mg/kg lw) for onset of physiological effects in experimental marine mammal studies (Kannan et al., 2000). The solid line represents the highest threshold (41 mg/kg lw) published for marine mammals based on reproductive impairment in ringed seals from the Baltic Sea (Helle et al., 1976).

When considering potential caveats, it should be noted that sampling live free-ranging animals meant there was some heterogeneity in the origin of samples with respect to the exact body location, despite the same general body area being targeted. This could potentially affect the resulting organochlorine concentrations, as these may vary across the body parts sampled (Aguilar, 1987). However, because we quantified the proportion of lipid and expressed the concentrations on a lipid weight basis, the resulting concentrations can be considered unbiased (Aguilar, 1987). Moreover, previous studies showed that biopsy samples yield representative details on chlorinated and brominated aromatic compounds in marine mammal blubber, regardless of the quantity and type of blubber sampled, provided that lipid normalization is performed on the resulting concentrations (Ikonomou et al., 2007).

Even though known males were not preferentially targeted over known females, and several animals were of unknown sex at the time of sampling, the skewed sex ratio is likely driven by the fact that females with accompanying calves were not sampled.

Table 3
Summary statistics for individual PCB congeners. All values are in mg/kg lipid weight.

PCB congener	Mean	Median	SD	Min	Max	Geomean	Geomean 95% CI
C101	1.35	1.33	0.89	0.05	3.16	0.93	0.64–1.35
C105	0.42	0.39	0.27	0.03	0.94	0.32	0.23–0.43
C110	0.14	0.10	0.10	0.03	0.35	0.11	0.09–0.14
C118	1.57	1.48	1.05	0.05	4.10	1.09	0.75–1.57
C128	1.67	1.40	1.31	0.05	5.13	1.01	0.66–1.56
C138	14.64	11.05	12.47	0.48	51.33	8.86	5.83–13.47
C141	0.10	0.09	0.05	0.03	0.22	0.09	0.07–0.1
C149	5.83	4.56	5.51	0.15	27.72	3.42	2.2–5.31
C151	2.40	1.92	1.97	0.05	8.21	1.45	0.94–2.24
C153	24.30	16.89	21.43	0.76	92.40	14.53	9.55–22.11
C156	0.61	0.43	0.56	0.03	2.41	0.39	0.27–0.56
C158	0.81	0.64	0.65	0.03	2.77	0.52	0.35–0.77
C170	3.52	2.61	2.89	0.25	11.81	2.39	1.69–3.37
C18	0.09	0.08	0.05	0.03	0.22	0.08	0.07–0.09
C180	9.71	6.34	8.72	0.68	36.96	6.31	4.42–8.99
C183	2.25	1.67	1.81	0.15	7.19	1.51	1.06–2.15
C187	8.07	6.09	6.76	0.58	30.80	5.45	3.86–7.7
C194	1.45	1.31	1.09	0.17	4.47	1.05	0.78–1.43
C28	0.09	0.08	0.05	0.03	0.22	0.08	0.06–0.09
C31	0.09	0.08	0.05	0.03	0.22	0.08	0.06–0.09
C44	0.09	0.08	0.05	0.03	0.22	0.08	0.06–0.09
C47	0.57	0.55	0.42	0.03	1.51	0.38	0.26–0.56
C49	0.10	0.09	0.05	0.03	0.22	0.09	0.07–0.11
C52	0.99	0.91	0.76	0.03	2.71	0.6	0.39–0.92
C66	0.68	0.46	0.71	0.03	2.79	0.31	0.19–0.52

4.1. PCB concentrations

We detected relatively high PCB concentrations. This is in agreement with other studies that showed the continued persistence of PCBs in large marine predators in Europe (Law et al., 2012; Jepson et al., 2016). In a previous European-wide study (Jepson et al., 2016), PCB levels were shown to be high in six Gulf of Trieste bottlenose dolphins, but the sample size from this area was limited. Here, using a larger sample size, we corroborate that concentrations in this population are indeed high in relation to published reference values (Kannan et al., 2000; Jepson et al., 2016). It is probably safe to assume that organochlorine threats to this population are mainly restricted to PCBs, as is the case for other Mediterranean areas (Jepson et al., 2016). Other studies in Europe have shown that following the 1970s–1980s ban the declines of PCBs have been slower than those of DDTs (Aguilar and Borrell, 2005) and levels have subsequently reached a plateau in harbour porpoises (*Phocoena phocoena*) around the United Kingdom (Law et al., 2012) and in striped dolphins in the western Mediterranean Sea (Jepson et al., 2016).

The main part of the PCB profile was represented by congeners 153, 138 and 180 (Table 3, Fig. 7), which is in agreement with other studies from the region (Storelli and Marcotrigiano, 2003; Lazar et al., 2012; Herceg Romanić et al., 2014) and elsewhere (Fair et al., 2010; García-Álvarez et al., 2014).

Comparing organochlorine levels across various literature sources is not always straightforward and can in fact be challenging. The reasons for this include different methods of organochlorine quantification, differences in compounds analysed (e.g. the total number and selection of individual PCB congeners), the basis on which the concentrations are expressed (e.g. lipid, wet or dry weight basis - especially if the proportion of lipid or water is not reported), the summary statistics used

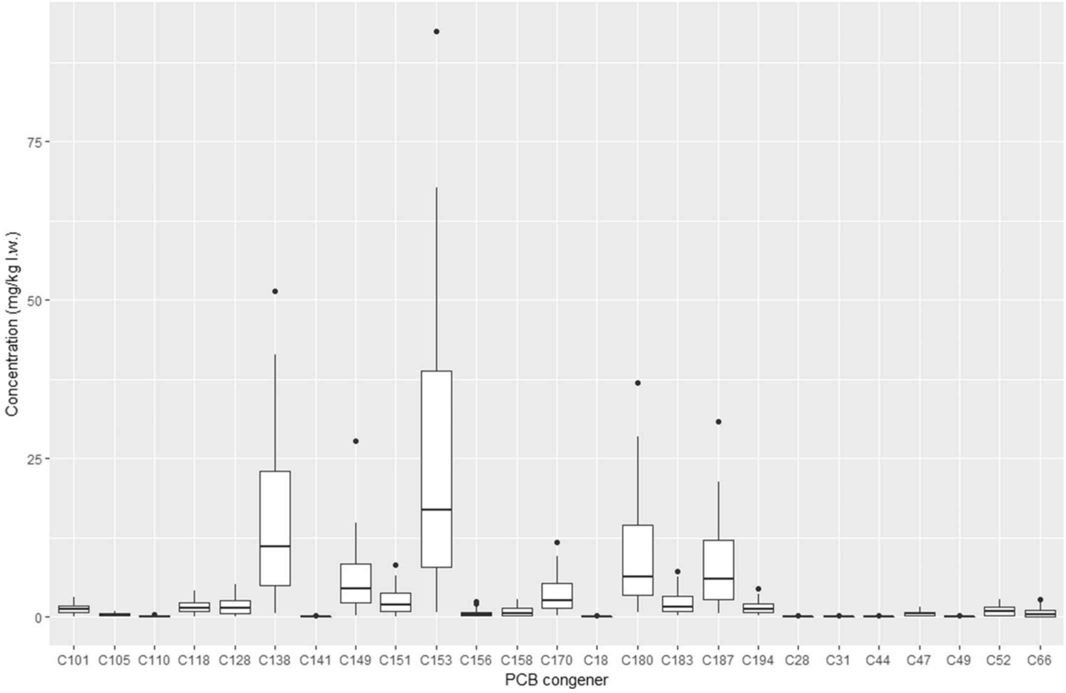

Fig. 7. Contribution of individual PCB congeners to the total PCB burden.

(e.g. arithmetic mean, geometric mean or median) together with measures of spread (e.g. standard deviation, confidence intervals or range); the sources of samples (controlled live captures, biopsies, bycaught animals or stranded animals), sample size, the sex and age classes included or excluded from the analysis, period of sampling, etc. For these reasons, not all studies are directly comparable.

Still, considering these caveats, some general comparisons can be made (Table 4). Looking at a regional perspective, it appears that PCB concentrations in our study are relatively similar to those found in stranded bottlenose dolphins along the eastern Adriatic coast of Croatia (Herceg Romanić et al., 2014), but substantially higher than in stranded bottlenose dolphins along the Adriatic coast of south-eastern Italy (Storelli and Marcotrigiano, 2003), stranded along the coast of Israel, eastern Levantine Basin (but note the extremely small samples size, Shoham-Frider et al., 2009), or biopsied in the Gulf of Ambracia, western Greece (Gonzalvo et al., 2016). Looking at the wider European and Mediterranean picture, concentrations in our study are higher than those found in bottlenose dolphins from Ireland (Berrow et al., 2002; Jepson et al., 2016), but lower than in bottlenose dolphins from western Mediterranean (Borrell and Aguilar, 2007; Jepson et al., 2016) and those from Portugal, north-western Spain, Wales, England and Scotland (although note that the patterns are somewhat different between males and females, Table 4, Jepson et al., 2016). Based on the above, it appears that within the Mediterranean, generally speaking, PCB concentrations tend to decline from west to east, and from north to south, which is consistent with the general geographical pattern of anthropogenic impacts (particularly pollution and exploitation of marine resources) in the Mediterranean basin (Coll et al., 2012).

On a global scale, our reported concentrations are higher than those found in bottlenose dolphins in Taiwan (Chou et al., 2004), around Canary Islands (García-Álvarez et al., 2014), off Rio de Janeiro, Brazil (Lailson-Brito et al., 2012), Bermuda (Kucklick et al., 2011), Beaufort, North Carolina, USA (Hansen et al., 2004), southern Biscayne Bay, Florida, USA (Kucklick et al., 2011), and along the coasts of Louisiana, Mississippi and northwestern Florida (Kucklick et al., 2011; Balmer et al., 2015), relatively similar to those from Indian River Lagoon, Florida, USA (Fair et al., 2010), Sarasota Bay, Florida, USA (Yordy et al., 2010) and Charleston, South Carolina, USA (Fair et al., 2010; Adams et al., 2014), and lower than in New Jersey (Kucklick et al., 2011), northern Biscayne Bay and Tampa Bay in Florida, USA (Kucklick et al., 2011), and waters of Georgia, USA (Balmer et al., 2011). With respect to other species, our bottlenose dolphins had higher PCB concentrations than striped dolphins from the southern Adriatic Sea (Storelli et al., 2012), harbour porpoises from the United Kingdom (Law et al., 2012), Guiana dolphins (*Sotalia guianensis*) from north-eastern Brazil (Santos-Neto et al., 2014), common dolphins (*Delphinus* sp.) from New Zealand (Stockin et al., 2007) or northern resident killer whales from British Columbia, Canada (Ross et al., 2000; Ylitalo et al., 2001), but substantially lower than striped dolphins from the western Mediterranean Sea (Jepson et al., 2016), killer whales from the United Kingdom, Canary Islands and the Strait of Gibraltar (Jepson et al., 2016), or southern resident and transient killer whales from the waters of British Columbia, Canada, and the states of Alaska and Washington, USA (Ross et al., 2000, Ylitalo et al., 2001). In addition, male dolphins in our study had higher concentrations than male pilot whales, male sperm whales and male fin whales from the western Mediterranean Sea (Pinzone et al., 2015), while female dolphins in our study had lower concentrations than female pilot whales, similar concentrations as female sperm whales and higher concentrations than female fin whales from the western Mediterranean Sea (Pinzone et al., 2015).

4.2. Effects of demographic parameters on PCB concentrations

Males had significantly higher PCB concentrations than females (Fig. 3). Animals of unknown sex also had high concentrations, with values more similar to males than to females (Table 2, Fig. 6). This suggests most of these animals were likely also males. The significant differences between males and females are suggestive of PCB offloading from reproducing females to their offspring via gestation and/or lactation (Borrell et al., 1995; Schwacke et al., 2002; Wells et al., 2005; Weijs et al., 2013). The significant differences in PCB concentrations between nulliparous and parous females (Fig. 4) further support this, despite limited sample size. Even though the premise of maternal offloading is well established, particularly based on experimental laboratory or captive studies involving mammals (Kannan et al., 2000) and samples from whaling operations (Aguilar and Borrell, 1994b; Borrell et al., 1995), it

Table 4
PCB blubber concentrations in *Tursiops truncatus* across different studies for males, females and both sexes. Whenever possible, reported values pertain to adult animals. All concentrations are in mg/kg, and expressed on lipid weight basis, unless otherwise noted. Concentrations expressed in different units in source literature were converted to mg/kg. Concentrations are shown as either arithmetic mean (A) ± standard deviation, (or with range in parentheses), or geometric mean (G) with 95% confidence intervals in parentheses. Summary statistics were obtained from text or tables of cited sources, or calculated from raw data reported in tables. Note that both the number and choice of individual PCB congeners tested varied across studies. See cited sources for details.

Location	N	Mean	M	F	M-F	Source
Croatia, north-eastern Adriatic Sea	13	A	–	–	97 ± 133	Herceg Romanić et al., 2014
Italy, southern Adriatic Sea	9	A	30.3	28.8	32.7 (7.3–53)	Storelli and Marcotrigiano, 2003
Gulf of Ambracia, western Greece	14	A	23.4 ± 18.0	32.9 ± 43.3	26.9 ± 28.3	Gonzalvo et al., 2016
Israel, eastern Levantine Basin	2	A, wet weight	6.3 ± 2.3	–	–	Shoham-Frider et al., 2009
South-east Spain, western Mediterranean	36	A	336.0 ± 241.1	246.4 ± 183.5	286.6 ± 274.6	Borrell and Aguilar, 2007
Spain, western Mediterranean	27	A	182.7 (27.4–399)	193.2 (45.3–601.4)	–	Jepson et al., 2016
Strait of Gibraltar	8	A	324.0 (28.3–879.3)	123.1 (20.8–179.7)	–	Jepson et al., 2016
Gulf of Cadiz, south-west Spain	21	A	247.3 (98.5–445.3)	150 (3.7–426.4)	–	Jepson et al., 2016
Portugal	12	A	85.7 (19.4–164.7)	88.5 (35.0–226.8)	–	Jepson et al., 2016
North-west Spain	11	A	118.9 (5.1–382.2)	34.7 (5.4–82.0)	–	Jepson et al., 2016
Wales, UK	7	A	91.8 (8.2–175.4)	111.9 (9.1–307.5)	–	Jepson et al., 2016
England, UK	10	A	176.9 (22.1–446.6)	91.2 (4.1–358.5)	–	Jepson et al., 2016
Scotland, UK	21	A	96.6 (1.8–698.0)	46.1 (8.5–125.1)	–	Jepson et al., 2016
Shannon Estuary, Ireland	8	A	29.5 ± 21.0	7.1 ± 8.7	23.9 ± 20.8	Berrow et al., 2002
Shannon Estuary, Ireland	8	A	46.9 (13.0–95.1)	11.4 (1.5–21.2)	–	Jepson et al., 2016
Canary Islands	25	A	–	–	47.2 ± 53.9	García-Álvarez et al., 2014
Cape May, New Jersey, USA	3	G	139 (95% CI 62.8–130)	–	–	Kucklick et al., 2011
Beaufort, North Carolina, USA	5	G	53.3 (15.9–52.2)	11.6 (3.3–40.6)	–	Hansen et al., 2004
Charleston, South Carolina, USA	9	G	50.4 (23.6–84.6)	7.9 (2.7–31.2)	–	Hansen et al., 2004
Charleston, South Carolina, USA	47	G	94 (28.6–255)	14.3 (4.5–131)	–	Fair et al., 2010
Charleston, South Carolina, USA	40	G	76.6 (25.9–246)	–	–	Adams et al., 2014
Sapelo area, Georgia, USA	46	G	115.7 (95% CI 91.7–146.1)	48.3 (95% CI 27.3–85.5)	–	Balmer et al., 2011
Mixed area, Georgia, USA	22	G	253.6 (95% CI 177.9–361.5)	45.9 (95% CI 20.8–101.7)	–	Balmer et al., 2011
Brunswick area, Georgia, USA	34	G	509.6 (95% CI 369.0–703.6)	116.5 (95% CI 78.1–173.6)	–	Balmer et al., 2011
Indian River Lagoon, Florida, USA	11	G	20 (14.7–27.9)	9.3 (5.0–17.0)	–	Hansen et al., 2004
Indian River Lagoon, Florida, USA	48	G	79.8 (35–227)	25.5 (1.5–105)	–	Fair et al., 2010
Biscayne Bay – North, Florida, USA	15	G	157 (95% CI 110–224)	–	–	Kucklick et al., 2011
Biscayne Bay – South, Florida, USA	15	G	33.7 (95% CI 23.6–48.2)	–	–	Kucklick et al., 2011
Sarasota Bay, Florida, USA	47	G	98.6 ± 159	4.7 ± 5.4	–	Yordy et al., 2010
Tampa Bay, Florida, USA	5	G	109 (95% CI 58.9–203)	–	–	Kucklick et al., 2011
East of Apalachicola Bay, Florida, USA	20	G	33.1 (95% CI 24.3–45.1)	–	–	Kucklick et al., 2011
St. Joseph Bay to St. Andrews Bay, Florida, USA	38	G	63 (95% CI 50.4–78.9)	–	–	Kucklick et al., 2011
Mississippi Sound, Mississipi, USA	55	G	68 (95% CI 56.4–81.9)	–	–	Kucklick et al., 2011
Barataria Bay, Louisiana, USA	19	G	51.4 (95% CI 38.5–68.6)	–	–	Balmer et al., 2015
Bermuda	3	G	38.8 (95% CI 17.4–86.1)	–	–	Kucklick et al., 2011
Rio de Janeiro State, Brazil	2	A	11.8 ± 2.4	–	–	Lailson-Brito et al., 2012
Taiwan	6	A	6.78	2.3	5.4 ± 3.6	Chou et al., 2004

is informative to be able to demonstrate that this is indeed happening in a wild, free-ranging cetacean population. In Sarasota Bay, Florida, research initiated in the 1970s, combining tagging, photo-identification monitoring and capture-release operations for health assessments, provided an unparalleled opportunity to investigate the relationships between organochlorine levels and life-history and reproductive parameters in the world's best-studied bottlenose dolphin population (Wells et al., 2005). In the eastern North Pacific, long-term identification records of one of the best-studied killer whale populations in the world enabled similar comparisons (Ross et al., 2000; Ylitalo et al., 2001). However, such studies remain relatively rare, especially in the Mediterranean Sea, the largest enclosed sea in the world, with substantial anthropogenic pressure.

There is some evidence of first-born offspring mortality in our dolphin population, as a few of the observed newborns (presumed to be the first offspring of respective females) did not survive to the following year (T. Genov, *pers. obs.*). This would support the notion that first-borns may receive a very high (possibly lethal) dose of PCBs from their mothers, as females may transfer up to 80% of their burden to offspring (Cockcroft et al., 1989). This may lead to poor first-born survival, with an improved survival of subsequent offspring (Schwacke et al., 2002; Wells et al., 2005). However, this evidence from our study area is limited and circumstantial, so further inferences are not possible. Given the long-term and ongoing monitoring of this population, future work incorporating PCB monitoring, individual re-sighting histories and information on reproductive rates may provide further insight into the temporal accumulation of PCBs by females and the possible links between pollutant loads and recruitment, as recommended by Hall et al. (2006).

Even though this dolphin population is structured into several social groups that display differences in behaviour as well as feeding strategies in relation to fisheries (Centrih et al., 2013; Genov et al., 2015; Genov et al., in press), it appears that PCBs pose a threat to these animals regardless of social group membership and potential associated dietary differences (Fig. 5).

4.3. Potential toxicological effects

The vast majority of animals in our study exceeded the lower toxicity threshold (Kannan et al., 2000), with >50% also exceeding the higher threshold (Helle et al., 1976, Fig. 6). As discussed by Jepson et al. (2016), the lower toxicity threshold may in fact overestimate the true PCB risk to cetaceans, but PCB levels reported here nevertheless provide a compelling case for the inherent PCB toxicity risk to these animals. In previous studies, high PCB levels were linked to pathological findings consistent with immunosuppression and increased susceptibility to disease, including macro-parasitic and bacterial pneumonias, high lung and gastric macro-parasite burdens, and generalised bacterial infections in harbour porpoises (Jepson et al., 2016). In Mediterranean striped dolphins, high levels of PCBs were associated to increased mortality during a morbillivirus epizootic outbreak, possibly due to immunosuppression (Aguilar and Borrell, 1994a).

Our results are of concern, particularly in combination with other known or suspected threats to this population, including marine litter, disturbance from boat traffic, frequent interactions with fisheries, overfishing and occasional bycatch (Genov et al., 2008; Hace et al., 2015; Genov et al., 2016; Kotnjek et al., 2017). Hopefully, the quantification of organochlorine concentrations and establishing links with various demographic parameters as presented here, will enable placing the effects of contaminants in context with other anthropogenic stressors (Hall et al., 2017).

4.4. DDE and DDT

We were only able to determine PCB concentrations, but not DDT in our samples, except for four samples referred to above. DDE concentrations could be determined as they were obtained as a “side product” of PCB analyses. In these four samples, DDE was the majority component of the total DDT, representing 89.7% (Table 1). Biotransformation processes of DDT in vertebrates largely end up as DDE (Aguilar and Borrell, 2005). Unless there is a recent source, DDE tends to be the highest concentration DDT metabolite present (Storelli et al., 2004; Pinzone et al., 2015), and can be used as an indicator of DDT contamination (but see Kljaković-Gašpić et al., 2010 on possible recent input). Our results are similar to several other studies and indicative of DDT ageing (Lailson-Brito et al., 2012; Adams et al., 2014; García-Álvarez et al., 2014; Gonzalvo et al., 2016). This suggests that DDE (and hence DDT) levels are relatively low, as is the case in the western Mediterranean Sea and around the United Kingdom (Aguilar and Borrell, 2005; Borrell and Aguilar, 2007; Law et al., 2012). In the Eastern Mediterranean Sea, however, levels of DDTs appear higher than those of PCBs (Shoham-Frider et al., 2009; Gonzalvo et al., 2016). For HCB, the extremely low levels in our study, consistent with studies on other biota from the Adriatic Sea (Storelli et al., 2004), suggest that recent environmental input of this compound is negligible (Borrell and Aguilar, 2007).

4.5. Future monitoring perspectives

Our results represent a useful baseline for future research and monitoring. With ongoing studies of this dolphin population and new insights into its ecology, future sampling may provide a better understanding of population-level impacts of pollutants. It should be noted that concentrations in top predators with high lipid stores inevitably lag behind any reductions in environmental concentrations (and those in prey), due to the slow depuration of POPs out of the population (through the legacy from female to calf, as well as the cycling of POPs in the marine environment). Nevertheless, this approach may represent a monitoring tool in relation to EU legislation such as the Habitats Directive and the Marine Strategy Framework Directive (MSFD). The presence of pollutants in tissues of marine biota is already included as a Descriptor 8 of MSFD, while marine mammals are one of the indicators of the “Good Environmental Status” under Descriptor 1 of MSFD. Jepson and Law (2016) proposed that at a European policy level, PCB levels in relation to established toxicity thresholds should also be used to assess “Favourable Conservation Status” of marine mammals under the EU Habitats Directive.

Even though biopsy sampling took place within Slovenian waters, the extensive spatial survey coverage (Fig. 1) and the fact that sampled dolphins have been re-sighted throughout the study area shown in Fig. 1 (Genov et al., 2008), the reported organochlorine levels can likely be considered representative of this part of the Adriatic Sea. Furthermore, individual dolphin re-sighting frequencies have shown that the sampled individuals are part of a resident population inhabiting this area over the long term (Genov et al., 2008; Genov, 2011), while both photo-identification (Genov et al., 2009) and genetic data (Gaspari et al., 2015) suggest that this population is distinct. This adds confidence to the notion that these concentrations are representative of this particular area, rather than being a result of acute PCB exposure elsewhere (Phillips and Segar, 1986).

Molluscs have typically been used as model species to monitor contaminants in the Gulf of Trieste, elsewhere in the Adriatic Sea (Kljaković-Gašpić et al., 2010), and other parts of the world (Phillips and Segar, 1986; Farrington et al., 2016). This is primarily due to their widespread distribution, abundance, sessile nature, tolerance to various types of stress, and the ability to accumulate a wide range of contaminants (Phillips and Segar, 1986; Kljaković-Gašpić et al., 2010), but probably also due to ease of access to the animals. However, while molluscs may be better indicators for local point sources of contamination, cetaceans may be more representative over larger spatial and temporal scales. Dolphins are long-lived predators that integrate contaminant concentrations over time. They have been shown to be incapable of metabolizing certain PCB congeners, making them accumulate these compounds more readily than other mammals or taxa of comparable life history (Aguilar and Borrell, 2005). Moreover, being highly mobile, they are likely better regional rather than local indicators, due to their propensity to move around more. Finally, as top predators, they are likely representative of the ecosystem as a whole (Borrell and Aguilar, 2007).

5. Conclusions

It is important to review current methods of PCB mitigation in the marine environment, at a European and international level. In Europe, much greater compliance with the Stockholm Convention is urgently needed by many EU member states, in order to significantly reduce PCB contamination of the marine and terrestrial environment by 2028 (Jepson et al., 2016; Jepson and Law, 2016; Stuart-Smith and Jepson, 2017). Measures may include the safe disposal or destruction of large stocks of PCBs and PCB-containing equipment, limiting the dredging of PCB-laden rivers and estuaries, reducing PCB leakage from old landfills, limiting PCB mobilization in marine sediments, and regulating demolition of PCB-containing precast buildings such as tower blocks built in the 1950s–1980s (Jepson et al., 2016, Jepson and Law, 2016, Stuart-Smith and Jepson, 2017).

Our results show that PCB levels are relatively high in northern Adriatic dolphins, and may be high enough to potentially cause population-level effects in this population. We provide important baseline data of a considerable sample size, against which future trends can be assessed. We demonstrate that POP monitoring combined with long-term photo-identification and population ecology studies can be highly informative for assessing the impacts of organochlorine pollution.

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Novel method for identifying individual cetaceans using facial features and symmetry: A test case using dolphins

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Abstract

Individual identification plays a major role in our understanding of the biology, ecology and behavior in cetaceans. Being able to tell individuals apart can provide invaluable insight into basic biological and scientific questions, but is also highly relevant to science-based conservation. Given the importance of individual identification, it appears vital to improve existing identification methods and find new ones. Here, we present a novel identification method of using facial information, with common bottlenose dolphins (*Tursiops truncatus*) as a case study. In dolphins, dorsal fins are typically used for identification, but not all individuals or species are sufficiently marked. We show that facial features in bottlenose dolphins are long-term and consistent across the left and right sides, and may enable calves (which tend to have unmarked fins) to be re-identified after weaning, thus increasing cross-generational knowledge. This novel method can complement dorsal fin identification by helping document mark changes over time and reduce false negative or positive errors. It also shows that current identification methods can still be improved. With increasing prevalence of digital photography and computer-aided matching, it may become more viable to use 'unconventional' means of identification. We encourage other researchers to explore their photographic records for similar discoveries.

Key words: individual identification, facial recognition, facial symmetry, photo-identification, bottlenose dolphin, mark-recapture, delphinids, cetaceans, novel method.

The identification of individuals plays a major role in our understanding of the biology, ecology, behavior, and population dynamics in various animal species, including cetaceans (Hammond *et al.* 1990, Whitehead 2008, Hammond 2009).

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Being able to tell individuals apart can provide invaluable insight into basic biological and scientific questions, but is also highly relevant to science-based conservation. Hence, given the importance of individual identification, it appears vital to improve our ability to identify individuals, even in those species where (perhaps limited) recognition methods already exist. Furthermore, it seems imperative to find ways to identify individuals in those species we currently consider nonamenable to individual identification.

It is widely known that several species of cetacean can be individually identified using natural markings (Hammond *et al.* 1990). Scarring, notches, pigmentation, and callosity patterns, other long-term natural markings, as well as human-inflicted markings, *e.g.*, freeze-brands or signs of sublethal boat strikes (Irvine *et al.* 1981, Wells and Scott 1997), are used to identify individuals of various species (*e.g.*, Würsig and Würsig 1977, Baird 2000, Whitehead 2001, Ramp *et al.* 2006, Barlow *et al.* 2011, Genov *et al.* 2012).

A number of efforts have been made to find different ways of identifying cetacean individuals. For example, while humpback whales (*Megaptera novaeangliae*) are primarily identified using photographs of tail flukes (Katona and Whitehead 1981, Hammond 1986, Barlow *et al.* 2011), researchers have also used photographs of dorsal fins (Katona and Whitehead 1981, Blackmer *et al.* 2000, Barendse *et al.* 2011). This is especially useful in situations when animals do not fluke up, either due to the local environmental conditions (*e.g.*, depth), their behavior, or both (Blackmer *et al.* 2000). Furthermore, researchers have recently applied photo-identification to species that were generally thought not well suited to this method, *e.g.*, narwhals (*Monodon monoceros*; Auger-Méthé *et al.* 2010) and striped dolphins (*Stenella coeruleoalba*; Bearzi *et al.* 2011). Given the value of individual identification to both science and conservation, efforts to find new ways of identifying marine mammals may be important.

The development of digital photography has further facilitated a wider use of photographs in wildlife research, including photo-ID of cetaceans (Markowitz *et al.* 2003). This advance allows researchers to take extended image sequences in an effort to make sure that a quality dorsal fin or fluke picture is obtained, without the concern of wasting expensive film. Consequently, it is no longer uncommon for an entire surfacing sequence of a cetacean to be photographed, from the moment the head or rostrum breaks the surface, to the moment the peduncle or tail disappears again. As a result, more images of dolphin heads can be captured now than ever before.

One candidate identification method is the use of facial features and facial symmetry. Individuals across several taxa exhibit unique facial features, that can be recognized by humans and/or by conspecifics (Tate *et al.* 2006).

Here, we designed an experiment to evaluate the usefulness of facial features and facial symmetry to identify individual wild common bottlenose dolphins (*Tursiops truncatus*; hereafter bottlenose dolphins) as a case study (Genov *et al.* 2015). This species is regularly present in the Gulf of Trieste and adjacent waters in the northern Adriatic Sea, where it has been the focus of a long-term study by Morigenos, Slovenian Marine Mammal Society since 2002 (Genov *et al.* 2008). We used a long-term photographic data set and human observers in matching trials to investigate (1) if individual animals could be distinguished by facial features and (2) if these features were consistent on left and right sides. Additionally, we tried to determine if such features were long-lasting and if calves, which tend to have "clean" dorsal fins and are generally recognized through association with their mothers, could potentially be re-identified using this method after weaning.

METHODS

Data collection and evaluation

This study focused on free-ranging bottlenose dolphins inhabiting the Gulf of Trieste and adjacent waters (northern Adriatic Sea). The study area and research protocols are described in detail in Genov *et al.* (2008). Up to 150 individuals use this area annually, most of which carry dorsal fin markings suitable for long-term identification (Genov *et al.* 2008, Genov 2011). Photographs of dolphins were collected between 2002 and 2014. In 2008 the use of color transparency film was replaced by digital photography. Since then, over 56,000 digital photographs were taken, resulting in the identification of about 150 individuals.

We selected sequences of photographs containing both dolphin faces and dorsal fins and extracted photographs of faces (2,318 images), matching them to individuals known from dorsal fins from the same sequences. We found 31 dolphins (29 adults and 2 calves) that had photographs of both left and right sides of the face and could be reliably matched to photo-identified dorsal fins.

Matching trials

We asked 27 biologists to match photographs of dolphin faces. Fourteen were *experienced* in dolphin dorsal fin photo-ID (but not familiar with our study animals), and 13 were *inexperienced* with cetacean photo-ID or any cetacean-related work. Each participant received three folders with dolphin face photographs (A, B, C). Folder A contained *left*-side photographs of 20 different dolphins. Folder B contained *left*-side photographs of 10 of those dolphins, but represented by different photographs. Folder C contained *right*-side photographs of the same 10 animals as in folder B. In other words, 10 animals from folder A had matches in both B and C, but the other 10 had no match. The identity of all animals, as verified using dorsal fins, was known to the authors, but not to the participants. The choice of these 20 dolphins was dictated by the availability of photographs. Participants were first asked to decide which animals from folder A had matches to animals from folder B and to pair them accordingly. They were then asked to pair the 10 animals assigned to folder B to the animals from folder C. A total of 20 participants (12 experienced and 8 inexperienced) completed the tests. Note that the aim of this exercise was not to obtain a large sample size or ensure statistical rigor, but rather to assess the potential viability of this technique.

Statistical analysis

We compared the scores of the participants with the probability distribution of scores from random matching. The design of the matching trials required a hierarchical statistical model to establish the null distribution of scores. For the first task, matching 20 left faces (A) to 10 left faces (B), the probability distribution of scores was calculated using a hierarchical process: (1) selecting 10 out of 20 photographs to be matched, and (2) matching the 10 selected photographs with the 10 reference images in B. The probability of selecting n correct photographs from A has a binomial distribution, with an expectation of 0.5 (10/20).

$$P(n|N_B, N_A) \sim \text{Binomial}(N_B, p = N_B|N_A) \quad (1)$$

When the correct 10 photographs have been included in the matching set ($n = N_B$), the probability of getting $k \in \{0,\ldots,N_B\}$ matches is equivalent to Montmort's matching problem (de Montmort 1713) and solved by Equation 2.

$$P(k|N_B) = \frac{1}{k!}\sum_{j=0}^{N_B-k}\frac{(-1)^j}{j!} \tag{2}$$

In this study however, most participants did not select all 10 correct photographs to be matched. The probability of getting k matches for $n < N_B$ was solved recursively for $n \in \{N_B - 1,\ldots,0\}$ using Equation 3.

$$P(k|N_B, n) = (1 - k/n) \times P(k|N_B, n + 1) + (k/n) \times P(k + 1|N_B, n + 1) \tag{3}$$

The overall probability of getting k matches is therefore the probability of selecting n correct photographs × the probability of getting k matches given n.

$$P(k|N_{B,}N_A, n) = P(n|N_B, N_A) \times P(k|N_B, n) \tag{4}$$

For the second task (matching the 10 left faces selected in task 1 to the 10 right faces in folder C), the probability distribution of getting k matches was calculated separately for each individual using Equation 3. This is because n was determined by the number of photographs selected in the first task and was not the same for all individuals. The overall performance of the participants was assessed using the joint probability of their scores. The performance of experienced and inexperienced participants was compared using the mean log-likelihood of their test score and bootstrapping (10,000 random samples with replacement).

RESULTS

Facial features appeared to be distinctive enough to allow participants to distinguish individual dolphins (Table 1). For the first task, the expected score for random matching was 5/20, with 10/20 being above the 95% confidence interval. Participants matching left face photographs of dolphins did substantially better than

Table 1. Matching results by experienced and inexperienced participants for left (same) side matching, right (opposite) side matching and overall. Results are shown as median number of correct scores, with range provided in the parentheses. Median % shows the median percentage of correct scores, with range provided in the parentheses.

	Experienced participants (n = 12)		Inexperienced participants (n = 8)	
	Median (range)	Median % (range)	Median (range)	Median % (range)
Left/same side (n = 20)	18 (14–20)	90 (70–100)	15 (10–15)	75 (50–75)
Right/opposite side (n = 20)	17.5 (9–20)	87.5 (45–100)	13 (10–17)	65 (50–85)
Overall (n = 40)	35 (23–40)	87.5 (57.5–100)	28 (20–31)	70 (50–77.5)

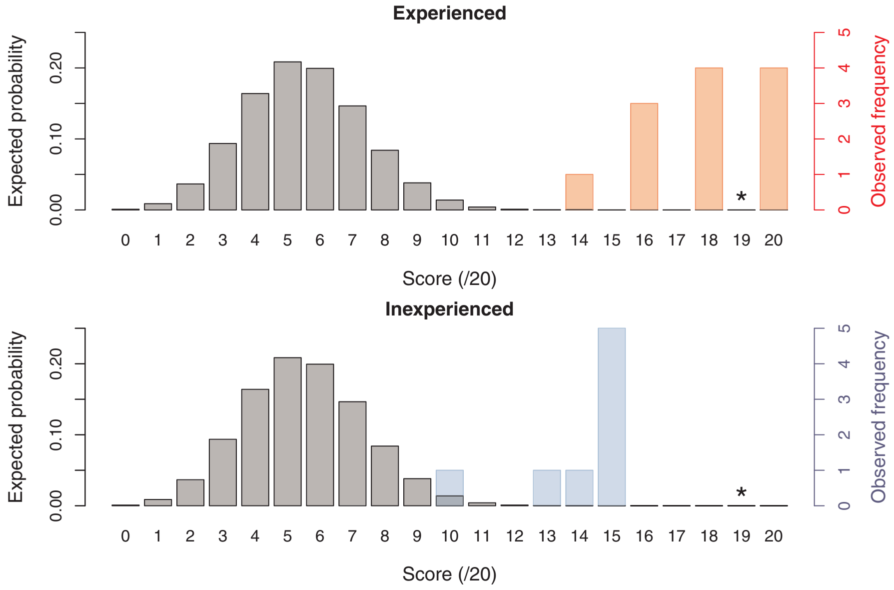

Figure 1. Probability distribution of scores in the first dolphin face matching trial (Left-Left). Gray bars show the probability distribution of scores expected by chance, and the asterisks (*) indicate impossible scores. The performance of participants *experienced* (n = 12) and *inexperienced* (n = 8) in cetacean photo-ID is shown in red (upper panel) and blue (lower panel), respectively.

expected by chance (Fig. 1, joint probability $P < 0.0001$). Experienced participants performed far better than inexperienced ones (lower mean log-likelihood in all 10,000 bootstrap samples). The symmetry between left and right sides of individual dolphin faces (Fig. 2) was also confirmed by matching trials. Participants matched photographs of left and right faces better than expected by chance ($P < 0.0001$). Here, the score expected for random matching depended on the outcome of the previous trial and is shown for each observer in the Figure S1. Only one experienced participant did not do significantly better than random (Fig. S1: "Experienced B," $P = 0.154$). Again, experienced participants did better than inexperienced ones on average (in 98.5% of bootstrap samples). Three experienced participants were able to correctly assign all matches, regardless of which side of the face was in the photographs.

We had long time series (5 yr or more) of face photographs for three individual dolphins. In these three individuals, facial features appeared to be stable and recognizable over at least 8–9 yr (Fig. 3). Two calves showed temporal stability of their facial features over a minimum of 32 and 10 mo, respectively (Fig. 4). Furthermore, the two calves appeared to resemble their respective mothers more than they did other dolphins, but the current sample is limited (Fig. 5).

Discussion

Our results suggest that (1) bottlenose dolphins can be distinguished and identified by facial features and (2) these features are consistent across the left and right


ARM
ARM
PAO
PAO
CAT
CAT
RIC
RIC
DAP
DAP
SAT
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GEO
TAR
TAR
HID
HID
WIF
WIF
KAI
KAI
WIT
WIT
NEV
NEV
ZAC
ZAC

Figure 2. Left and right side face photographs of 16 bottlenose dolphins showing individually distinct facial features. Three-letter name codes represent individual IDs. Note that, with the exception of scarring, features are consistent on both sides. The identity of these dolphins (*i.e.*, the match between left and right sides, as well as uniqueness from other individuals) was verified *via* conventional dorsal-fin photo-identification.

sides and therefore symmetrical. In addition, our preliminary findings also suggest that such facial features are long-lasting, and may potentially allow calves to be reidentified after weaning.

Symmetry, including facial symmetry, is common in many species of mammals and other animals (Finnerty 2005). Symmetry is also a salient visual property that has been shown to be detected efficiently and rapidly by humans (Wagemans 1997). Moreover, in humans, faces are among the most important visual stimuli, especially in social contexts (Leopold and Rhodes 2010), and it has been shown that humans visually process images of animals and animal faces just as fast as those of human faces (Rousselet *et al.* 2003, 2004). Therefore, it is perhaps not surprising that participants were successful at assigning matches, even across the two sides of dolphin faces. This was despite anatomical differences, such as the marked lateral (rather than frontal) domination of facial features, in the dolphins as compared to humans. Given that facial recognition of individuals is a special ability in many social animals, including humans, its use may be more applicable than previously thought. We left it to the participants to decide which particular facial features they used to identify individuals. They could not use scarring, as some photos were too far apart in time to allow this, and they also matched opposite sides of the face. As far as the authors are concerned, when one of us (TG) first discovered that several dolphins appeared identifiable by faces, the "identifying feature" was simply a "holistic" image of the dolphin's face, rather than any single type of feature.

The incidental nature of the face photographs meant that their quality varied greatly. Consequently, trials included high quality photographs, as well as those of lower quality (one that would not pass our quality screening for standard mark-recapture analyses). Despite this, participants were successful in assigning matches. The use of more high-quality photos would likely further increase the success rate. Moreover, conscious attempts to obtain head shots, in addition to dorsal fin shots, would greatly increase the number of such photographs, without compromising standard photo-ID. We encourage researchers with even larger and longer-term photo-ID data sets to further explore and test this in the future. Images of stranded and captive dolphins may also contribute to the future corroboration of this method.

These results may have broader implications. Studies have shown that faces are an important category of visual stimuli in all major vertebrate groups (although some aspects of facial perception appear to be limited to primates and a few other social mammals) and are thought to have possibly evolved to facilitate complex social communication (Leopold and Rhodes 2010). Face recognition has been documented in primates (Pascalis and Bachevalier 1998), to which the cognitive abilities of delphinids are often compared, as well as in sheep (Kendrick *et al.* 2001). Mirror self-recognition is well documented in bottlenose dolphins (Reiss and Marino 2001) and some other delphinids (Delfour and Marten 2001), demonstrating a capacity to use visual cues to recognize themselves and other individuals. Bottlenose dolphins have good vision (Herman *et al.* 1975, Pack and Herman 1995) and a long-term social memory (Connor *et al.* 2000, Bruck 2013), making it possible that they also use visual cues, such as unique facial features, to recognize each other at close quarters in

Figure 3. A selection of three individual dolphins showing long-term stability in facial features. Individual DAP is represented by both sides. Note that the oldest picture of each individual is a scanned slide transparency, hence the poorer photo quality.

the wild, despite relying primarily on acoustic recognition (Janik *et al.* 2006, Quick and Janik 2012).

Perhaps more importantly, the ability to distinguish individual animals by permanent features other than dorsal fin markings can help document changes in dorsal fin

Figure 4. Faces of two dolphins calves, showing consistency of facial features over time, as well as differences between the two animals.

Figure 5. Two females (nicknamed Daphne and Hidro) and their respective calves, showing facial similarities between mothers and calves.

marks over time (Hammond 1986, Gowans and Whitehead 2001). Such changes are generally commonplace in bottlenose dolphin populations (Wilson *et al.* 1999, Bearzi *et al.* 2008) and may cause researchers to miss fin matches. Unlike regular dorsal fin marks, facial features do not appear to change, or are at least not prone to external influences such as social interactions. This additional "marking" can reduce the likelihood of false negative or false positive errors (Stevick *et al.* 2001), or help estimate the rate of such errors *via* double-marking experiments (Barrowman and Myers 1996, Gowans and Whitehead 2001, Barendse and Best 2011). Furthermore, when animals carry more than one mark type, individuals can be cross-classified across marks, and the effect of mark type on the recapture probability modelled (Smout *et al.* 2011). Facial identification can also provide an additional way to identify animals with "unmarked" fins and may help estimate the number or proportion of "clean" fins in the sample (*e.g.*, in an encounter or in a population). In studies of abundance, social behavior or habitat use, it is often beneficial to be able to identify every individual within a group, and having more tools available to do so would be advantageous. Facial recognition can also work in conjunction with other markings such as body scars and coloration to help confirm identifications, but the demonstrated symmetrical property of dolphin faces is of particular value, as identifications (and matches) can be done irrespective of which body side is displayed.

Additional means of identifying individuals may also help reduce the number of “lost” calves that leave their mothers before they are recruited into the marked part of the population. The ability to follow dolphins through several generations is highly valuable for long-term studies (Wells 1991, Mann 2000) and tracking of individuals from weaning to adulthood can provide information on primiparity, recruitment and juvenile survival (Paterson *et al.* 2013). However, calves are typically unmarked and do not often acquire identifiable dorsal fin marks prior to becoming independent. Valuable information on intergenerational links is thus often lost in the absence of additional genetic studies. Nevertheless, dolphin calves are known for their tendency to “pop” their heads out of the water upon surfacing, making them particularly suitable for this type of additional identification (Fig. 4). Accordingly, obtaining a face shot at a later time would aid in the reidentification of calves following weaning, offering greater potential for cross-generational analyses. Even though the tendency of calves to pop their heads out of the water will likely decrease after weaning, facial recognition may still increase the probability of subsequent matches, especially when used in combination with other markings such as coloration, scarring and potential subtle nicks in the fin. Moreover, in our study population head exposure is rather common in adults (TG, personal observation). There was some indication of similarity between at least some mothers and calves (Fig. 5), suggesting potential inheritance of specific traits, but the limited sample size precludes any further inferences.

It would also be very useful to determine if males could be differentiated from females. While there is some indication that this may be possible (TG, personal observation), we currently do not have sex information on a large enough number of individuals to test the premise. Sexual dimorphism in skull morphology is present in some populations of bottlenose dolphins, but not in others (Turner and Worthy 2003), so potential sex differences might be a topic of further study.

To be clear, we are not suggesting that facial recognition could or should replace standard dorsal fin identification, as there are a number of limitations. First, facial recognition may carry some bias as not all animals regularly lift their heads out of the water upon surfacing. This will vary by species, populations within a species, individuals within a population, and also different behaviors of a single individual. Perhaps this approach may be especially useful for studies that collect photo-ID photographs underwater (Herzing 1997). But as noted above, head exposure is rather common in our study population. Second, the faces of some individuals were clearly more distinct than others, and it remains to be determined what proportion of the population is sufficiently “marked” in such a way to be consistently identified. Still, we believe that facial recognition could complement standard photo-ID, especially in the age of digital cameras and high definition videography. We thus hope that our work can provide inspiration for a range of future studies. Computer-assisted matching, applied more and more widely to cetaceans (Gope *et al.* 2005, Auger-Méthé *et al.* 2011) and several other taxa (Arzoumanian *et al.* 2005, Hiby *et al.* 2009), could also potentially be applied to these features, much like automated recognition of humans faces (Camastra and Vinciarelli 2008) and chimpanzees (Loos and Ernst 2013).

Even though potential facial differences remain to be documented in other species (something we aim to investigate as the next step), our approach may have implications for the study of other delphinids, other cetaceans and possibly other taxa. For example, it may prove very useful in species that do not typically acquire fin marks, or in species such as right whale dolphins (*Lissodelphis borealis* and *L. peronii*), which lack dorsal fins entirely, but often expose their heads (Lipsky 2009). Facial features may be one of the few ways to identify individuals in these species at all. Face identification may also be particularly suited to humpback dolphins (*Sousa* sp.), as they typically surface in a very characteristic manner, with the beak breaking the water surface first, followed by the head (Parra and Ross 2009). Therefore, future research should look into whether our findings apply to other cetaceans and other animal groups.

Finally, the ability to recognize dolphins from face photographs may also have implications in postmortem studies. For instance, Atlantic humpback dolphins (*Sousa teuszii*) are frequently bycaught in artisanal fishing nets (Collins 2015). Their carcasses are often taken as "marine bushmeat" (Clapham and Van Waerebeek 2007) for human consumption and thus unavailable to scientists. In some instances, however, arrangements with local fishermen have allowed researchers to gain access to at least the heads of the dolphin.[2] These heads are usually fresh enough to allow face identification attempts for bycaught dolphins, and may help researchers trace bycaught dolphins to photo-identified individuals.[2]

Previously, lateral photographs of faces have been used to identify individuals in sea turtles (Reisser *et al.* 2008, Schofield *et al.* 2008). In those studies, however, photographs and identification were restricted to the right-side of the head, with no investigation into potential symmetry of those features. The utility of the method was therefore reliant on obtaining photographs of the appropriate side, while this is not the case in our study.

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TG conceived the ideas and study; TG and TC collected the data; TG and AJW designed matching trials; TG performed trials with participants; TG and GMW analyzed the data; TG led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication. The authors declare no conflict of interest.

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Supporting Information

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Figure S1. Scores for the second dolphin face matching trial (Left-Right) by individual participant. Gray bars show the probability distribution of scores expected by chance specific to each participant, and the asterisks (*) indicate impossible scores. Scores of participants experienced and inexperienced in cetacean photo-ID are shown in red and blue, respectively.