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MAGISTRSKO DELO (MASTER'S THESIS)

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REPRODUKTIVNA EKOLOGIJA VELIKE PLISKAVKE,  
*TURSIOPS TRUNCATUS*, V TRŽAŠKEM ZALIVU  
(REPRODUCTIVE ECOLOGY OF THE COMMON  
BOTTLENOSE DOLPHIN, *TURSIOPS TRUNCATUS*, IN  
THE GULF OF TRIESTE)

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Magistrsko delo

(Master's thesis)

**Razmnoževalna ekologija velike pliskavke, *Tursiops truncatus*, v  
Tržaškem zalivu**

(Reproductive ecology of the common bottlenose dolphin, *Tursiops truncatus*, in the Gulf  
of Trieste)

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Izvleček: Namen te raziskave je izboljšanje poznavanja razmnoževalne ekologije rezidentne populacije velike pliskavke v Tržaškem zalivu in okoliških vodah, kjer je vpliv človeka zelo velik. Glede na to, da na tem območju že 20 let poteka prepoznavanje posameznih delfinov, so na voljo podatki, ki omogočajo oceno razmnoževalnih parametrov ter primerjave z drugimi populacijami. Na podlagi zgodovine opažanj ter podrobne analize fotografij samic in njihovih mladičev v obdobju med letoma 2013 in 2020 so bili izračunani sezonskost kotitev, rodnost, reproduktivni uspeh samic, interval med dvema kotitvama ter smrtnost mladičev. Kot je bilo pričakovano, so rezultati pokazali, da se rojstva večinoma dogajajo v poletnih mesecih, ko je višja temperatura morja potencialno bolj ugodna za preživetje mladičev. Povprečna rodnost 0,02 je manjša v primerjavi z drugimi populacijami, medtem ko je preživetje 80 % podobno. Med vsemi starostnimi skupinami mladičev je bila največja smrtnost pri tistih, ki so mlajši kot eno leto. Nekatere samice so se pokazale kot bolj uspešne kot druge, z večjim deležem mladičev, ki so preživeli do minimalne starosti osamosvojitve. Obdobje med dvema zaporednima kotitvama se pokazalo različno od drugih populacij, s podobnim povprečjem treh let, ampak brez velike razlike v vrednosti glede na preživetje prejšnjega mladiča te samice, kar je pogosto dejavnik, ki vpliva na dolžino tega obdobja. Pridobljeni rezultati lahko podajo boljši vpogled v stanje lokalne populacije, in prispevajo k načrtovanju učinkovitega varstva te vrste.

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Abstract: The aim of this study was to improve the knowledge of the reproductive ecology of the resident common bottlenose dolphin population in the Gulf of Trieste and adjacent waters, an area of a high anthropogenic pressure. Since the identification of individuals in this area has been conducted for 20 years, the resulting data enable the estimation of reproductive parameters and comparisons with other populations. Sighting histories and analyses of photographs of females and their calves in the period between 2013 and 2020 were used to assess birth seasonality, crude birth rate, reproductive success of females, inter-birth interval and calf mortality. As expected, the results showed that births mostly occur during summer months, when warmer water potentially favours calf survival. The average crude birth rate of 0.02 is lower compared to other populations, while the survival rate of 80% is similar. Among all offspring age classes, calves younger than 1 year had the highest mortality. Some females showed to be more successful than the others, with the higher percentage of the calves surviving to the minimum age of weaning. The inter-birth interval was different than in other populations, with a similar average of 3 years, but no big difference in value when considering the fate of the previous calf, which has usually been shown to be a factor that affects how long it takes for a female to give birth again. These results provide useful insights into the status of the local population, and may contribute to the planning of the effective conservation of this species.

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# 1 INTRODUCTION

## 1.1 Common bottlenose dolphin

### 1.1.1 Ecology and distribution

The common bottlenose dolphin, *Tursiops truncatus*, is a marine mammal from the family Delphinidae (Table 1) and one of the best studied species of cetaceans in the world (Arnason et al. 2004; Wells and Scott 2009). It is characterized by its robust body, short and rounded beak, sickle-shaped dorsal fin and body coloration which includes dark grey upper parts and white ventral parts (Figure 1) (Podestà and Affronte 2015). This species belongs to the suborder Odontoceti, also known as the toothed whales, and has approximately a hundred robust teeth (Arnason et al. 2004; Podestà and Affronte 2015). The length of the adults varies by geographic location, but it usually ranges from approximately 2.5 m to about 3.8 m (Wells and Scott 2009). Males and females are usually morphologically distinguished only by their genital area (Wells and Scott 2009; Mann et al. 2000).

Table 1: Classification of *T. truncatus* (Notarbartolo di Sciara 2002; Vislobokova 2013)

Kingdom	Animalia
Phylum	Chordata
Class	Mammalia
Superorder	Cetartiodactyla
Order	Cetacea
Suborder	Odontoceti
Family	Delphinidae
Genus	<i>Tursiops</i>

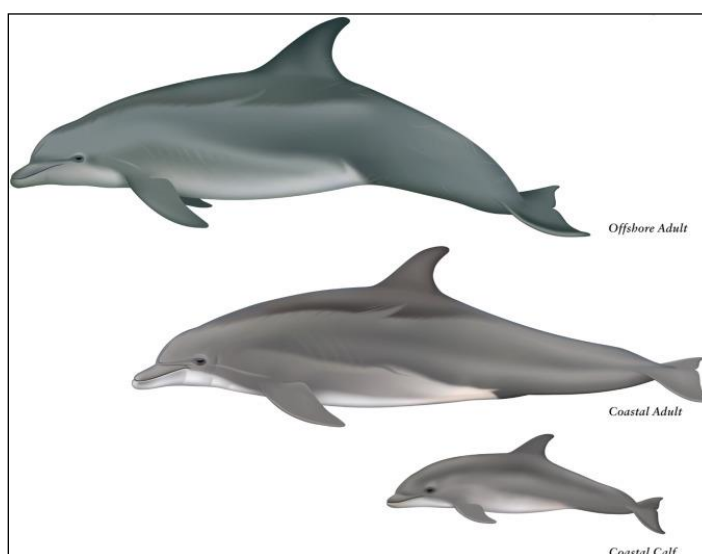


Figure 1: Appearance of *T. truncatus* individuals (Wells and Scott 2018)

The common bottlenose dolphin is a cosmopolitan species present in most of the world's warm temperate and tropical seas (Figure 2). It is primarily coastal species, but it can also be found in offshore waters (Wells and Scott 2009). Along with that come some variations in the characteristics between the individuals from different habitats within ocean basins (Hersh and Duffield 1990). For example, Duffield et al. (1983) concluded that Atlantic coastal and Pacific offshore ecotypes can be distinguished due to significantly higher values of hemoglobin concentration, packed cell volume and red blood cell counts in the offshore type. In addition, skull and body measurements of Northwest Atlantic bottlenose dolphins indicate that offshore individuals are larger, have wider skulls and proportionately smaller flippers than the coastal ones, reflecting different thermal and maneuverability requirements (Hersh and Duffield 1990).

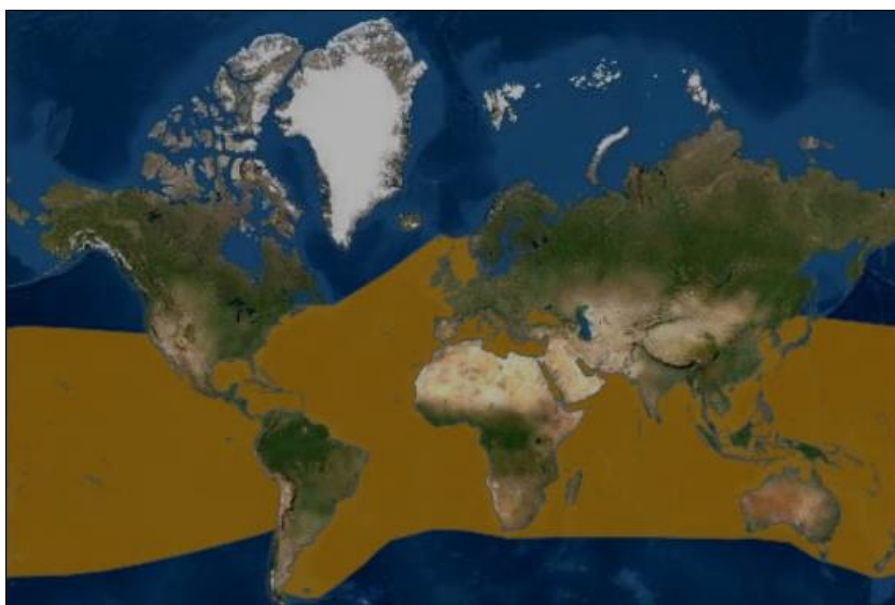


Figure 2: Geographic range of *T. truncatus* indicated by an orange field (<https://www.iucnredlist.org/species/22563/156932432#geographic-range>)

In the North Pacific, individuals are found as far north as the southern Okhotsk Sea, the Kuril Islands and central California (Wells and Scott 2009; Maldini et al. 2010). In the North Atlantic, they are spotted inshore off New England in the summer and offshore as far north as Nova Scotia (Wells and Scott 2009). The species is present as far south as Tierra del Fuego, South Africa, Australia, and New Zealand (Cockcroft et al. 1991; Haase and Schneider 2001; Wells and Scott 2009; Jaiteh et al. 2013). The range of distribution appears to be limited by temperature directly or indirectly, through prey distribution (Wells and Scott 2009). This species also inhabits coastal and shelf waters of the Mediterranean basin, where it occurs around many of the region's islands and archipelagos (Figure 3) (Bearzi et al. 2008).



Figure 3: Geographic range of *T. truncatus* Mediterranean subpopulation indicated by the orange field (<https://www.iucnredlist.org/species/16369383/50285287>)

The diet and feeding behaviour of *T. truncatus* has been described in many regions of its distribution (Barros and Wells 1998; Blanco et al. 2001; Milmann et al. 2016). The opportunistic character of the diet arises from local availability of prey. It is mostly based on cephalopods and various fish with individuals showing a consistent preference for sciaenids, scombrids and mugilids (Barros and Wells 1998; Blanco et al. 2001; Wells and Scott 2009). Although the individuals may seem to be generalists concerning their prey, the common bottlenose dolphins might show some degree of specialization within the population. For example, feeding areas of some dolphin groups differ due to the individuals' sex and size, with lactating females and calves feeding in the near-shore zones, and resting females and adult males feeding farther offshore (Wells and Scott 2009).

*T. truncatus* has a characteristic social structure. The individuals live in dynamic fission-fusion societies, where they associate in small groups with a temporary composition that changes on a daily or hourly basis (Connor et al. 2000). On the other side, there are some examples in which those groups are more stable, forming distinct social clusters, as is the case in the resident population of the Gulf of Trieste (Genov et al. 2019a). Common bottlenose dolphin is a vocal mammalian species and vocal communication is a very important part of its social interactions. It enables the group members to interact more effectively, as individuals provide identity information using their signature whistles when they encounter other groups in the sea (Connor et al. 2000; Díaz López 2011; Quick and Janik 2012; King et al. 2013).

### 1.1.2 Anthropogenic threats

Although *T. truncatus* is a common species in numerous world's regions, many of the populations are small and potentially locally threatened (Tezanos-Pinto et al. 2014; Brough et al. 2016). Today, at the global scope of assessment, the species is listed as "Least Concern" by the criteria of IUCN, International Union for Conservation of Nature (Wells et al. 2019). In addition, since 2021 the Mediterranean subpopulation is also listed as "Least Concern", after being listed as "Vulnerable" since 2012 (Bearzi et al. 2012; Natoli et al. 2021). As they occur in coastal waters, individuals are exposed to a variety of anthropogenic activities, including fishery, urbanization, marine traffic and tourism (Bearzi et al. 2008; Wells et al. 2008). Some of them cause injuries which may lead to dolphins' death or interfere with their reproduction, which is, from a conservation perspective, a serious concern (Bearzi et al. 2008; Wells et al. 2008).

#### 1.1.2.1 Fishery

Fishery poses an important ongoing threat causing incidental mortality in fishing gear, reduced amounts and modified composition and distribution of dolphins' key prey (Bearzi et al. 2008). In the research of Wells et al. (2008) conducted along the west coast of Florida, the results indicated several kinds of injuries due to fishery that seem to have high probability of leading to mortality. Ingestion of fishing gear, when it includes hooks becoming embedded in the throat, the "goosebeak" or esophagus, and line wrapped around "goosebeak" were found to be eventually fatal in all cases examined. In addition, multiple wraps of line around dolphin's body (Figure 4) may lead to deep lacerations, loss of appendages, infections, and damaged mobility (Wells et al. 2008). Fisheries might also cause short- to long-term changes in dolphins' behaviour, causing lower reproductive rates (Bearzi 2002).



Figure 4: *T. truncatus* individual entangled in fishing nets in southern Brazil (Fruet et al. 2016)



### 1.1.2.2 Marine traffic and underwater noise

Due to the increase in marine traffic at the local and global scale, interactions with marine vessels also present a growing threat for cetaceans, changing their behaviour and habitat use and causing injuries by strikes (Bearzi et al. 2008; Wells et al. 2008; Bas et al. 2017). For example, in the research conducted by Bas et al. (2017) in the Istanbul Strait, important route for oil transportation, results showed negative effects of marine traffic on the behaviour of *T. truncatus*. More precisely, there was an increase in diving behaviour, while important surface-feeding, resting and socializing behaviours were reduced. Those changes might lead to the decreased energy intake and lower pregnancy rates, leaving long-term negative impact on the females' reproductive success and the whole population (Bas et al. 2017). In addition, maritime traffic, as well as seismic surveys, contribute to the underwater noise, interfering with vocal communication among individuals and their social interactions, including mating (Notarbartolo di Sciara 2016; Sciacca et al. 2016; Williams et al. 2020). Some of the observed effects also include changes in foraging behaviour, diving, swim speed and respiration, as well as increased stress, hearing damage and strandings (Weilgart 2007).

### 1.1.2.3 Pollution

Common bottlenose dolphins have also been considered to be directly affected by heavy metal and organochlorine pollution, as they inhabit coastal areas, a repository for toxic agents from industrial, agricultural, and urban sources (Storelli and Marcotrigiano 2000; Jepson et al. 2016). Bioaccumulation of contaminants is a serious concern for the health of the species and conservation of the environment (Storelli and Marcotrigiano 2000; Bearzi et al. 2008; Genov et al. 2019b). For example, high levels of organochlorine compounds can also be found in calves, due to their transfer through female's milk, and in neonates probably because of transplacental transfer (Storelli and Marcotrigiano 2000; Wells et al. 2005). Concentrations of the pollutants may also be connected with demographic parameters (Wells et al. 2005; Genov et al. 2019b).

## 1.1.3 Research of reproductive ecology

*T. truncatus* is a long-lived mammal that produces just one infant at a time. In its mating system no monogamous bonds were observed, meaning that in no cases were all of female's calves sired by a single male (Wells 2003). Calves are usually born after 12 month gestation period, most often during late spring or summer months, as is observed in the populations from Doubtful Sound in New Zealand, Patos Lagoon estuary in the Southwestern Atlantic Ocean, and Tyrrhenian Sea (Haase and Schneider 2001; Wells and Scott 2009; Fruet et al. 2015; Blasi et al. 2020). Such a seasonality in births might be associated with warmer sea surface temperatures during that period, as well as with higher food abundance (Urian et al. 1996; Fruet et al. 2015). The lactation lasts for about 19 months, while resting period, in

which females accommodate for seasonal breeding, lasts for about 5 months (Harrison and Ridgway 1971; Perrin and Reilly 1984).

After they are born, dolphin calves immediately swim and follow their mothers. Mothers take care of their offspring for the first three to four years of their life, during which they nurse, protect, discipline, play with and maintain proximity to the calves (Figure 5) (Haase and Schneider 2001; Hill et al. 2007). Depending on the calf's age and type of the locomotion, there are three primary swimming positions in relation to its mother (Figure 6) (Shoele and Zhu 2015). In a "neonate position", the calf swims slightly above mother's flank, probably because of the lacking ability to control its buoyancy. Within a few hours after being born, calf relocates to the "echelon" position, which is within 10 cm of the mother's flank and close to her dorsal fin. The last one is an "infant" position, which includes swimming under the mother's tail section at very close proximity and is mostly present in older calves (Shoele and Zhu 2015). This position enables them to increase their swimming performance at reduced locomotor effort in comparison with periods of solitary swimming (Noren and Edwards 2011).



Figure 5: Female jumping out of the water with her calf (Photo: Morigenos)



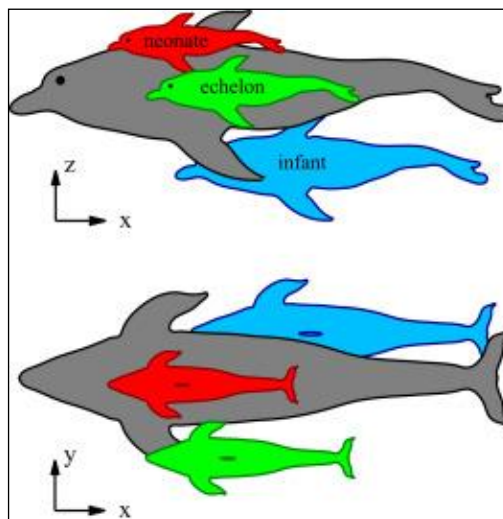


Figure 6: Side and top view of the calf's swimming positions in relation to its mother (Shoeler and Zhu 2015)

Each female dolphin seems to have a unique mothering style. For example, some of them are very protective, while some of them let their calves explore around (Hill et al. 2007). There are also some cases of interactions and associations between infants and non-mothers, known as allomaternal behaviour (Mann and Smuts 1998). This kind of association might be costly to an infant compared with the one with its mother, but beneficial when compared with being alone. For example, there is an increased possibility of disease transmission. On the other hand, there are some benefits such as the development of social relationships, provided protection from predator attacks, varied social experience leading to short- and long-term gains in skills, and hydrodynamic advantages of echelon swimming (Mann and Smuts 1998).

Long-term monitoring of the *T. truncatus* populations and their demographic characteristics has been regularly conducted in different areas, including New Zealand, coastal waters of southern Brazil, North Sea, and Mediterranean Sea (Henderson et al. 2014; Fruet et al. 2015; Robinson et al. 2017; Rossi et al. 2017; Blasi et al. 2020). The rate at which population abundance changes is largely dependent on animals' ability to reproduce (Fruet et al. 2015). Continuous recordings of individuals' occurrence enable the analysis of population's reproductive traits and parameters, including females' minimum age at first reproduction, crude birth rate, calf mortality and survival, female fecundity and reproductive success, inter-birth interval and birth seasonality (Henderson et al. 2014; Fruet et al. 2015; Blasi et al. 2020). Because of the species' cosmopolitan distribution there are variations in reproductive output along its range, following different group size, protection from predators and conspecifics and mate choice (Wells 2003; Fruet et al. 2015; Brough et al. 2016; Rossi et al. 2017; Blasi et al. 2020). Offspring care and survival are influenced by a variety of other factors, including social ranking, reproductive status, and previous experience of the mother (Mann and Smuts 1999; Hill et al. 2007; Brough et al. 2016). For example, Robinson et al. (2017) concluded that the individual heterogeneity in reproductive success could be

attributed to some females, that is, “good mothers”, being more successful and more important than the others. The disappearance of these females might leave a great effect on the overall reproductive output of the population. That is why the identification of demographic groups that contain those females may be very desirable to conservation efforts for the protection of the species (Robinson et al. 2017).

The determination of mortality and fertility within the population is required in order to understand its life history (Rossi et al. 2017). Knowledge of reproduction parameters provides more information about the population viability and helps in conservation management of the species (Rossi et al. 2017). Although *T. truncatus* is a well studied species in many regions of the world, information about its reproduction in the Mediterranean is limited, with an exception of a few studies, including those in the Ligurian and Tyrrhenian Sea (Rossi et al. 2017; Blasi et al. 2020). As a result, this area has enormous potential for conducting such research. More data on population viability and a better knowledge of the space and time in which the births are most common can help in the future protection of this species in the Mediterranean.

#### **1.1.4 Photo-identification and capture-mark-recapture techniques**

Photo-identification is a non-invasive method for the identification of individuals, commonly used in marine mammal research (Würsig and Jefferson 1990). It is based on the photographs of animals' natural markings taken when they come to the surface to breathe (Young et al. 2011). It is important that primary marks used for the identification are persistent and recognizable over time in order to minimize the risk of misidentification during data analysis (Würsig and Jefferson 1990). These marks should also prevail throughout the population to improve sample representativeness (Auger-Méthé et al. 2010). Depending on the species, different features are used for identification. In most cases, individuals are identified by nicks and notches in the trailing edges of their dorsal fins, as well as the shape of the fins (Figure 7) (Urian et al. 2014). In the studies of killer whales, *Orcinus orca*, and northern bottlenose whales, *Hyperoodon ampullatus*, the saddle and the mottled patch are very reliable and usable marks (Gowans and Whitehead 2001; Baird and Stacey 2011). The individuals of humpback whales, *Megaptera novaeangliae*, are primarily identified by the light and dark pigmentation patches on their tail flukes (Constantine et al. 2007), while the principal marks that are used in the photo-identification of Amazon river dolphin, *Inia geoffrensis*, are the pigmentation patterns on the dorsal ridge (Gomez-Salazar et al. 2011).

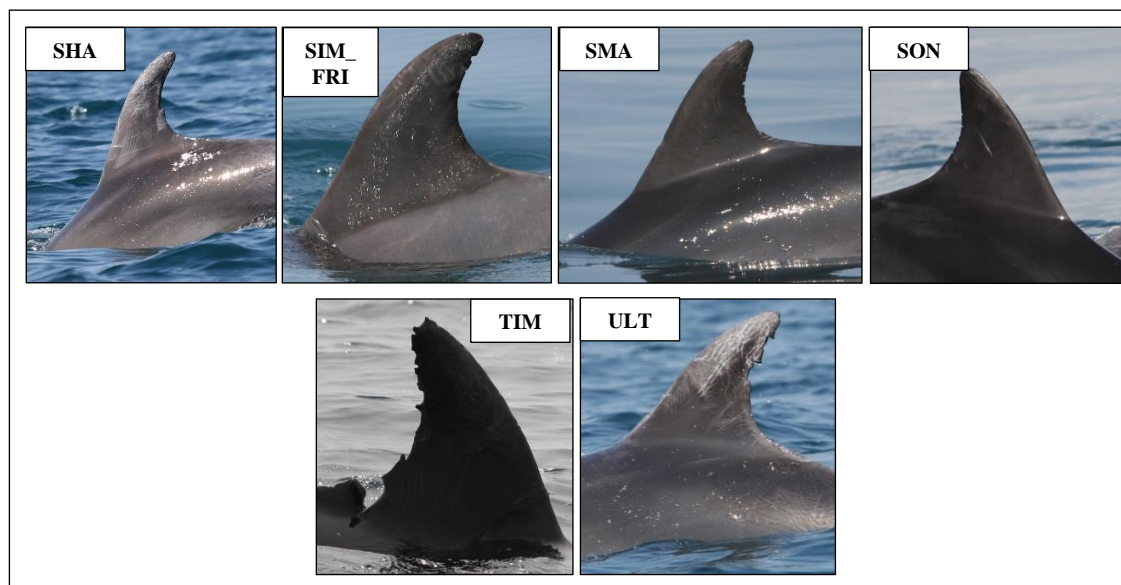


Figure 7: Dorsal fin identification photographs of six different *T. truncatus* individuals from the Gulf of Trieste (Photo: Morigenos)

The reliability of scrapes and scratches varies among the cetacean species. In common bottlenose dolphins, these marks are not very reliable since they tend to disappear in the period of a few weeks to a year (Wilson et al. 1999; Benedetto Leone et al. 2019). In Cuvier's beaked whales, *Ziphius cavirostris*, medium scrapes and large stripes are long-lasting, while in white-beaked dolphins, *Lagenorhynchus albirostris*, they seem to be reliable for studies up to 5 years (Rosso et al. 2011; Bertulli et al. 2015).

Photo-identification is very helpful in understanding cetacean behaviour, interactions, and other aspects of their ecology. For example, some studies, such as the one of Kügler and Orbach (2014), have explored the origins of distinctive marks on individuals, which include intraspecific interactions, predation attempts and anthropogenic impact such as entanglement in fishing gear and vessel strikes. In the long-term monitoring it is important that the field research and photo-identification are conducted continuously and frequently in order to detect any changes in the dorsal fins in the meantime.

Genov et al. (2018) also presented a method which uses photographs of unique facial features and symmetry in the identification of individuals. The first case study used common bottlenose dolphins from the resident population in the Gulf of Trieste. Big advantage of this method is that facial features are long-term and consistent, so they may be a great help in documenting the changes of dorsal fin marks and confirming identification by dorsal fins (Genov et al. 2018). Calves usually tend to have dorsal fins without distinctive marks and are generally identified through association with their mothers (Fruet et al. 2015; Robinson et al. 2017). The novel method might potentially enable calves to be re-identified after weaning, especially because they usually “pop“ their heads out of the water upon surfacing, making it possible to take quality photographs of their facial features (Figure 8) (Genov et al. 2018).



Figure 8: Calf upon surfacing in association with its mother (Photo: Morigenos)

Longitudinal photo-identification studies enable continuous monitoring of individuals and, eventually, determination of population parameters, such as abundance and survival (Fruet et al. 2011). Capture-mark-recapture methods (Pradel 1996; Lettink and Armstrong 2003) are frequently employed in the estimation of cetacean abundance using photo-identification and various statistical models (Fruet et al. 2011; Urian et al. 2014). The general principle of the method is to mark captured individuals during the first capture session and then to record the proportion of marked animals in subsequent recapture session (Petit and Valiere 2005). In the case of cetacean research, individuals are captured by taking their photographs and they are marked naturally. If they are recaptured, they are already documented in the photo-ID catalogue (Genov et al. 2008). There are three primary assumptions regarding identification data: marks are unique, recognised without error, and do not change or disappear (Urian et al. 2014).

## 1.2 Adriatic Sea

Adriatic Sea is a prolonged and semi-enclosed sea in the central Mediterranean with its major axis in the northwest-southeast direction, and surface area of approximately 138,000 km<sup>2</sup> (Artegiani et al. 1997; Viličić 2014). It makes up 4.6% of the total Mediterranean Sea's surface and is mostly shallower than 200 m. Adriatic Sea is considered to have three parts (Figure 9) with its depth increasing towards the 70 km wide and 789 m deep Otranto Strait in the south, where it opens into the Ionian Sea (Orlić et al. 1992; Viličić 2014). The first, northern part of the Adriatic, including the Gulf of Trieste, is shallow and slowly sloping towards the second part, the central Adriatic, just south of the Ancona-Zadar line. The middle part has an average depth of 140 m, but also includes the Pomo Depression reaching 260 m. Important feature of the third, southern part of the Adriatic is the South Adriatic Pit with the depth greater than 1200 m (Orlić et al. 1992; Artegiani et al. 1997; Giani et al. 2012; Viličić 2014). The eastern coast is rocky and generally high, while the western coast is low and mainly sandy (Artegiani et al. 1997). Rivers in the northwestern part of the Adriatic represent

an important source of nutrients and freshwater, with the Po river being major one among them and having a significant effect on the circulation (Orlić et al. 1992; Viličić 2014).



Figure 9: Map of the Adriatic Sea and its three parts (Kovačić et al. 2012): Northern Adriatic – N, Central Adriatic – C, Southern Adriatic - S

### 1.2.1 Northern Adriatic Sea

The northern Adriatic Sea is the shallowest and northernmost region of the Mediterranean, with the depth reaching less than 60 m (Giani et al. 2012). It is bounded by the Italian peninsula in the west and the Balkans in the east. Its northern and western coasts are mainly sandy and the nearby land is flat. On the other side, its eastern coast is rough and montane, including different islands, bays, inlets, and coves (Poulain et al. 2001). Its southern boundary is open and topographically not clearly specified (Boicourt et al. 1999). The biggest part of its coastal area are lagoon-river delta systems with numerous transitional water bodies - unpredictable aquatic systems characterised by big environmental fluctuations. The largest of them is the Lagoon of Venice, but there are also many estuaries, wetlands, embayments and ponds with different connections to the sea. These productive ecosystems provide important ecosystem services, such as protection of the shoreline, fishery resources, improvement of the water quality and habitats for both resident and migratory organisms (Abbiati et al. 2010).

The northern Adriatic Sea is an area of great importance for the common bottlenose dolphin (Fortuna et al. 2014), as well as one of the most important Mediterranean feeding habitats for the loggerhead sea turtle, *Caretta caretta* (Margaritoulis et al. 2003; Lazar et al. 2004). In addition, it is the only area in the Mediterranean Sea in which we can find biogenic formations known as trezze or tegnue, the living environments composed of rock formations (Casellato et al. 2007). They have an important ecological role in the northern Adriatic as it



is the only solid structure on a muddy base that predominates in the open waters of that area. They represent important places for feeding and reproduction of many fish and demersal invertebrate species, especially the ones that are exposed to major fishing pressure (Casellato et al. 2007).

Data from many previous years of research showed some modifications in physical and chemical oceanographic characteristics of the northern Adriatic. Those modifications are caused by both natural and anthropogenic pressures, such as climate change, eutrophication, inputs of continental freshwater and nutrients, overexploitation of resources and habitat destruction, altogether having impact on region's biota (Abbiati et al. 2010; Giani et al. 2012). In addition, the anthropogenic impact in the northern Adriatic Sea is bigger than in other parts of the Adriatic because of the high river discharges (Cozzi and Giani 2011). Bearzi et al. (2004) summarized information about the changes that occurred over several decades, showing that until the early 1970s the northern Adriatic was considered relatively uncontaminated ecosystem. At that time algal blooms also became a growing concern, especially because of the much greater intensity, frequency and geographic extension than in any other part of the Mediterranean. In addition, sea-surface temperatures and salinity slightly increased, while seawater transparency and oxygen saturation in bottom layers decreased, causing increased primary production and modifications in the distribution of key prey for common bottlenose and common dolphins, *Delphinus delphis* (Bearzi et al. 2004). The cumulative effects of all of the above, together with the increased commercial exploitation of both demersal and pelagic fish stocks, have led to the northern Adriatic being far from what it once was.

### 1.2.2 Presence of cetaceans

Mediterranean cetacean fauna includes ten species that are regularly present throughout the region (Table 2): fin whale, *Balaenoptera physalus*; sperm whale, *Physeter macrocephalus*; Cuvier's beaked whale, *Ziphius cavirostris*; long-finned pilot whale, *Globicephala melas*; Risso's dolphin, *Grampus griseus*; common bottlenose dolphin, *T. truncatus*; striped dolphin, *Stenella coeruleoalba*; common dolphin, *D. delphis*; rough-toothed dolphin, *Steno bredanensis*, and Black Sea harbour porpoise, *Phocoena phocoena relicta* (Notarbartolo di Sciara 2002; Notarbartolo di Sciara and Tonay 2021). Current knowledge shows that *T. truncatus* is today the only cetacean species that is regularly present in the entire Adriatic. On the other side, for example, striped dolphin, Risso's dolphin and Cuvier's beaked whale are present in different densities in the southern Adriatic, sperm whales visit the area occasionally, and fin whales occur seasonally in all three parts of the Adriatic, including the Gulf of Trieste (Lipej et al. 2004; Genov 2011; Holcer et al. 2014).

Table 2: List of cetacean species regularly present in the Mediterranean (Štrbenac 2015; Notarbartolo di Sciara and Tonay 2021; [www.iucnredlist.org](http://www.iucnredlist.org)): Endangered – EN, Vulnerable – VU, Critically Endangered – CR, Least Concern – LC, Near Threatened – NT, Central and Southern Adriatic – CSA, SA – Southern Adriatic

Species	Classification	Presence in the Mediterranean	Status of the Mediterranean subpopulations	Presence in the Adriatic Sea
<i>B. physalus</i>	Mysticeti, Balaenopteridae	regular	EN	seasonally regular in CSA
<i>P. macrocephalus</i>	Odontoceti, Physeteridae	regular	EN	rare
<i>Z. cavirostris</i>	Odontoceti, Ziphiidae	regular	VU	regular in SA
<i>G. melas</i>	Odontoceti, Delphinidae	regular in the western Mediterranean	EN – inner Mediterranean CR – Strait of Gibraltar	not occurring
<i>G. griseus</i>	Odontoceti, Delphinidae	regular	EN	regular in SA
<i>T. truncatus</i>	Odontoceti, Delphinidae	regular	LC - Mediterranean CR – Gulf of Ambracia	regular
<i>S. coeruleoalba</i>	Odontoceti, Delphinidae	regular	LC – Mediterranean EN – Gulf of Corinth	regular in SA
<i>D. delphis</i>	Odontoceti, Delphinidae	regular	EN – inner Mediterranean CR – Gulf of Corinth	rare
<i>S. bredanensis</i>	Odontoceti, Delphinidae	regular in the Levantine Sea	NT	not occurring
<i>P. phocoena relicta</i>	Odontoceti, Phocoenidae	regular in the Northern Aegean Sea	/	not occurring

*D. delphis* used to be very common in the Mediterranean in the past, but it has undergone a dramatic decline across most of the basin by the end of 1970s and is considered regionally extinct nowadays (Bearzi et al. 2003; Holcer et al. 2014). In the northern Adriatic Sea, the common dolphin was thought to be the most abundant cetacean during most of the 20th century. In contrast, in the Gulf of Trieste, as a part of the northern Adriatic, there are only four cases of the individuals' occurrences in the recent times, according to the review made by Genov et al. (2021). Today, common dolphins remain relatively abundant only in the Alborà Sea (Bearzi and Genov 2021). The exact reasons and mechanisms of this decline are still not well understood, but intentional culling, incidental mortality in fishing gear, habitat degradation and prey reduction have been mentioned as likely causes (Bearzi et al. 2004; Bearzi and Genov 2021; Genov et al. 2021).

### **1.2.3 Resident population of *T. truncatus* in the Gulf of Trieste**

Systematic and continuous research of *T. truncatus* in the Gulf of Trieste and adjacent waters (including Italian, Slovenian and Croatian waters) started in 2002 (Genov et al. 2008). The local population consists of about 161–245 individuals, based on annual mark-recapture models during 2013–2018 (Genov 2021). Population's social network is structured into three distinct social clusters, two of which are overlapping spatially, but not temporally, which has never been documented in cetaceans before (Genov et al. 2019a). More precisely, one cluster is predominantly sighted in the morning hours, while the other one is sighted in the late afternoon hours (Genov et al. 2019a). Research by Genov et al. (2019a) also showed behavioural differences in the interactions with trawlers between two social groups. The “morning group“ often interacted with pair trawlers and occasionally with bottom trawlers, while the “evening group“ never did. In conclusion, those social groups may have different relations with human activities, for example through different impacts on fisheries catch, as well as responses to anthropogenic pressures, such as intense boat traffic (Genov et al. 2019a). The individuals from the third social cluster occasionally interact with the dolphins from the “morning group“ and the “evening group”, but they are typically observed with the other dolphins from that cluster, on their own or with transient individuals. They do not have strong bonds with anyone and are mostly found in small groups consisting of two to three dolphins.

### **1.3 Goals of the research**

This thesis aims to improve the knowledge about the reproductive ecology of the resident *T. truncatus* population in the Gulf of Trieste and adjacent waters. The first objective of the thesis is to estimate the population's reproductive parameters, while the second objective is the comparison of the results with the studies from other populations around the world. This may also provide insights into the status of the local population and the northern Adriatic as an ecosystem.

Based on the available knowledge, the first assumption is the higher birth frequency occurring in the warmer part of the year. Such a pattern is already known from the other population studies, including the one from the Tyrrhenian Sea, where most of the births occur in June and July (Blasi et al. 2020). The second assumption is that the inter-birth interval is approximately 3 years, as reported in other populations. The third assumption is that the age at first calf production is similar to those from other studies.



## 2 MATERIAL AND METHODS

### 2.1. Study area

The study area included the entire Gulf of Trieste (45°39'59.99" N, 13°34'59.99" E), as well as the waters off north-western Istria.

The Gulf of Trieste is a part of the Gulf of Venice located in the northeastern corner of the northern Adriatic Sea, to which it is connected through its western side (Figure 10). It is approximately 25 km x 25 km wide and with the average depth of around 20 m. The maximum depth of approximately 38 m is recorded in the waters off Piran (Orlando-Bonaca and Lipej 2009; Cosoli et al. 2013). This area also hosts two of the largest cargo shipping ports in the Adriatic Sea – one in the city of Koper and one in Trieste. The bottom of this area is mostly muddy and sandy with the occasional appearance of hard substrates and seagrass meadows of *Cymodocea nodosa* and *Posidonia oceanica*, important nursery areas for coastal fisheries (Peduzzi and Vukovič 1990; Turk et al. 2002). Seasonal variations of water temperatures range between 8 and 24 °C at the surface, and between 8 and 20 °C in the bottom layer. The salinity is typically marine between 33 and 38.5 PSU in the surface layer, and between 36 and 38.5 PSU near the bottom (Ogorelec et al. 1991).

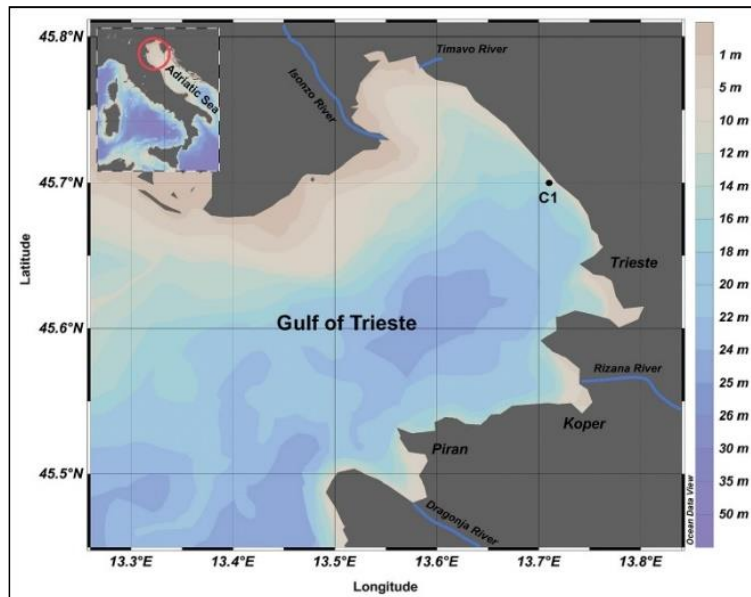


Figure 10: Map showing the Gulf of Trieste and its position in the Adriatic Sea (Franzo et al. 2019)

The Gulf of Trieste is considered one of the most polluted areas in the Mediterranean Sea (Olivotti et al. 1986; Horvat et al. 2010; Cozzi et al. 2014). The major allochthonous source of freshwater and nutrients are the river loads, causing modifications in hydrology, biogeochemistry and productivity of the coastal zone (Cozzi et al. 2012). Urban and

industrial sewages discharge great amounts of nutrients and organic matter causing the diffusion of faecal coliforms and heavy metals (Cozzi et al. 2008). This area is also one of the most mercury-polluted areas in the world because of the past mining activity in the Idrija region in the western Slovenia (Horvat et al. 1999; Horvat et al. 2010; Faganeli et al. 2014). In addition, the bay is under great anthropogenic pressure due to intensive human activities, such as maritime traffic, tourism, urbanization, fishery, and mariculture.

## 2.2 Field surveys

Field surveys and data collection have been conducted since July 2002 until today. The data used for the analysis in this thesis was collected over a period of 8 years, between 2013 and 2020, inclusive. Although the surveys were carried out in all seasons, they were mostly concentrated in the summer, from June to August, due to general weather conditions.

The surveys were often conducted simultaneously by two teams – one on the boat and one on the land with an independent search effort. Boat-based surveys were undertaken using inflatable boat with outboard engine, while land-based surveys were carried out using binoculars on higher observation points, such as 47.2 m high bell tower of the St. George's Church in Piran with extensive view of the Gulf of Trieste. Field surveys on the boat were conducted as described in Genov et al. (2008).

Surveys were conducted only if the conditions were considered good, i.e. the sea state of Beaufort scale was maximum of 2 and at least one experienced observer was present on the research boat. "Sighting" was defined as a continuous and uninterrupted observation of a dolphin focal group – any number of dolphin individuals that were in the visual range of the researchers on the boat, moving in the same direction or staying in the same area, observed in some kind of association and often participating in the same activity (Genov et al. 2008). The group was carefully approached and slowly followed for as long as needed for data collection, causing minimal disturbance for the animals. The exact location of the boat and dolphin group was recorded using Global Positioning System, GPS, compass or, in the case of land-based surveys, local land marks. During field work the size of the group was estimated, as well as the number of present calves, which was confirmed later during photo and video analysis. Photo-identification was carried out in the field. The photographs of dorsal fins were taken with an attempt of taking pictures of both sides of the fins of all the dolphins present in the group. In addition to the planned routes, operating trawlers were opportunistically approached in order to record the potential presence of dolphins that often feed around them (Genov et al. 2008, Bonizzoni et al. 2022).

## 2.3 Photo-identification

All the photographs taken in the field were analysed, compared to those taken during previous sightings and sorted into photo-identification catalogue as described in Genov et al. (2008). The individuals were identified by the shape of their dorsal fins and natural marks, such as scars, nicks, notches, and tooth rakes. All of the identified dolphins were given names or numeric identity codes as a reference. Their names are written in the form of abbreviations (the first 3 letters of the name, or a numeric code consisting of a year when first identified and the consecutive identification number below).

The quality of the photographs played a major role in this part of the study, as did the degree of marking and distinctiveness of individuals' dorsal fins. The quality of each photograph was assessed by assigning a quality value ranging from 1, for the lowest quality, to 3, for the highest quality. The quality value depended on the focus and exposure of the subject, as well as on the size and orientation of the fin relative to the frame. The photographs of the quality 3 met four criteria: the position of the dorsal fin was parallel to the camera, the height of the fin in the photo was at least 7% of the height of the frame, the photograph was focused and without any water splashes in it. Poorly-marked, unmarked and well-marked individuals for which only poor quality photographs were taken were not considered identified.

The individuals' gender was opportunistically determined if there were photographs of the genital area taken during aerial behaviour or bowriding. In addition, if the adults were consistently and closely accompanied by an offspring, they were also considered females. The adults were recognized by their large and robust bodies, dark skin colour, and numerous marks on the dorsal fins and the bodies (Blasi et al. 2020). The offspring were identified by their smaller size, coloration, behaviour and association with adults, as described in Bearzi et al. (1997).

For each year two Excel tables were made containing the dolphins that were sighted. One of the tables contains sighting history based on the photographs of all qualities on which the individuals are still sufficiently recognized. The other table is based only on the photographs with the quality value 3. For each separate sighting of the year, the dolphin was marked either as present (1) or not present (0). The same was done for the offspring that were clearly with their mothers.

### 2.3.1 Identification of the calves

Due to their young age and protection from older females, calves usually lack marks on their dorsal fins that could be used for identification (Mann and Smuts 1998; Robinson et al. 2017). That is why the calves are typically tracked from close associations with their mothers. Some temporary marks on calves' dorsal fins and bodies, such as lesions, scars and scratches, enable short-term recapture of maturing individuals (Robinson et al. 2017). In

addition, during their maturation calves gain some natural marks which allow their identification without their mother's presence, after which they also gain their name or numeric identity code as a reference.

## 2.4 Data analysis

The data analyzed in this thesis was provided by Morigenos – Slovenian Marine Mammal Society. It included sighting histories of all *T. truncatus* individuals in the Gulf of Trieste collected in the period between January 2013 and September 2020. For the purpose of the better understanding and interpretation of the results, the data on certain dolphin individuals obtained in the period before 2013 was also used. All calculations, tables and graphs for displaying the results were made in Excel.

The sighting history based on the photographs of all qualities was used as data in this thesis. All females sighted at least once in close association with a calf were selected and their sighting histories, as well as the photographs taken during field surveys, were further analyzed. The sightings in which the female was observed with the calf were specially marked in order to track the periods in which their new calves were born or the existing ones potentially died.

The photographs of the offspring were examined to determine their approximate age. There were two methods combined by which this was done:

- 1) The analysis of the visible characteristics which indicated whether the calf was a newborn, young of the year or older than one year. The most prominent neonate features are foetal folds, long stripes that are running down their body (Figure 11). These folds arise from the blubber of the calf being compacted in-utero, but they fade over some time. For example, Haase and Schneider (2001) concluded that foetal folds last for approximately 8 months in their study area, Doubtful Sound. Newborn dolphins are also mostly recognized by their small size, which is less than one-third of the mother's body, floppy dorsal fin and tail fluke, and pale coloration (Bearzi et al. 1997; Robinson et al. 2017; Blasi et al. 2020). For the calves that were not newborns, a characteristic that mostly indicated the approximate age was the size relative to the size of the female with which they were associated (Table 3).



Figure 11: Female with her calf with obvious foetal folds indicating that it is a newborn (Photo: Morigenos)

2) The analysis of information on life history for the individuals that were already sighted. Some of the females that were analyzed were seen every or almost every year of the study period. Since calves are considered to stay in close association with their mothers for at least 2 to 3 years since their birth (Haase and Schneider 2001; Grellier et al. 2003; Hill et al. 2007), this made it possible to track their offspring over the years. For example, knowing that the female had a newborn in the previous year, the larger size of the calf in the photographs from the coming year confirmed that it was the same calf. After the analysis of several such cases, we were able to fine-tune the approach on how to recognize the offspring of different ages in the studied population (Table 3).

Table 3: Age classes and associated characteristics in the resident population in the Gulf of Trieste

Age class	Characteristics
Newborn dolphin, younger than 1 month	Foetal folds, size less than one-third of the mother's size, floppy dorsal fin, pale coloration
Young of the year, dolphin younger than 1 year	Around one-third of the mother's size
Calf aged between 1 and 2 years	Around two-thirds of the mother's size
Juvenile, dolphin older than 3 years	Obviously larger calf, almost reaching full size

#### 2.4.1 Selection of individuals for the analysis

All the females that were seen at least once with a calf, assuming that they were the mothers, were selected from the sighting history data for each year. For each of these reproductively active females it was determined in how many years they were sighted during the study period, as well as how many times in each year. The same was done for their offspring. This

was important in order to select those individuals to be used for further analysis and determination of reproductive parameters.

There are several potential pitfalls in this type of research. For example, estimates of inter-birth intervals tend to be positively biased because some births might be missed when dolphins are not seen every year, but also negatively biased when the length of the study does not allow the longest birth intervals to be observed (Arso Civil et al. 2017). Another type of error might appear if the calf is assigned to a female other than the mother (e.g. allomaternal care). In addition, if some females are not regularly sighted, biases may arise in estimated mortality and survival rates, as the calf may have died before the female is encountered following the birth (Arso Civil et al. 2017). That is why it is important to select those dolphins that are regularly sighted, i.e. they meet the conditions that were set for the analysis in the study. In that way the attempt is made to obtain the most accurate representation of data and description of reproductive ecology of the studied population.

After the detailed study of the females' sighting histories, two conditions were set. In the further analysis, except when calculating crude birth rate, only the females sighted in at least 4 years and at least 4 times in each year were used (further addressed as “selected females”). The first reason for setting such conditions is that, during the initial review of the sighting histories, it was observed that some females from the studied population would disappear from the study area in the year when their young were born. Some research showed (Haase and Schneider 2001; Robinson et al. 2017) that, if the calf died within the first year of its life, female would be able to reproduce again in the following breeding season, meaning that it could have new calves every two years (more precisely, 4 years between 2013 and 2020), since the gestation period lasts for about 12 months (Wells 2003; Wells and Scott 2009). That way the abovementioned females would also be sighted every two years, that is, in 4 years during the study period. The second reason for the set conditions is that, in the research conducted by Genov et al. (2019a) on the same population as in this study, individuals had to be observed on at least 4 occasions to be considered in the data analysis, which is why the same criterion was used here.

For each of the selected females the occurrence rates across years and across sightings were calculated by the following ratios:

the occurrence rate (%) across years = number of female sightings / years of sightings

the occurrence rate (%) across sightings = number of female sightings / total number of sightings



### 2.4.2 Birth seasonality

In order to investigate the birth seasonality and the peak birth period, it was important to assess approximate month and, accordingly, the date of birth of the calves. When the sighting histories did not allow determination of the birth period within a month, the range over which the birth of the calf possibly happened was estimated as the period between the last mother's sighting without the calf and the first sighting with a calf (later addressed as “possible months of birth”). The objective was to see if there is a seasonal pattern in reproduction, i.e. if there are times of the year when the births occur more frequently. According to the criteria that were proposed by Henderson et al. (2014), birth months were assigned if one of the conditions was met:

- 1) the mother was seen without the calf in the month of the first sighting of the calf
- 2) the mother was seen without the calf in the month prior to the first sighting of the calf
- 3) the photographs of the calf suggested it was a newborn

According to the third condition, the calves of the females that were not selected for detailed analysis could also be used if their neonate traits were obvious in the photos, suggesting they were born within a month.

The date of calf's birth was estimated as the midpoint between the date of the mother's last sighting without a newborn and the date of her first sighting with the new calf. The estimated date of birth was not included in the analysis if this period was longer than 45 days, as proposed by Fruet et al. (2015).

### 2.4.3 Crude birth rate

Unlike other reproductive parameters in this thesis, annual crude birth rate was determined for the period between 2013 and 2018 since there is no data on the population's total abundance for 2019 and 2020 yet. In addition, this reproductive parameter was determined through two approaches – one including only the selected females, and the other one including all the females that were sighted with a calf at least once during the study period. In the first approach, the first step was the analysis of the photographs and the sighting histories of females and their calves in order to assign their years of birth. Some photographs showed that the calf was obviously a newborn, meaning that it was born in the year when the photograph was taken. In some examples, the mother was seen without her calf within the same year prior to the calf's first sighting, allowing us to assign that year as the year of birth of that calf. That type of data, which enables determination of the exact year of calf's birth, is the most accurate for the calculation of the crude birth rate. But, in some other cases, the assessed birth period of the calf extended over the period that included more than one calendar year. Since this was the case for most calves, both of the years were considered as the potential years of birth and the calf was included in the potential annual crude birth rate

determination for each of those years. In the second approach, for the females that were not regularly sighted during the study period, only the calves whose photographs showed obvious neonate traits were considered to be born in the certain year. The crude birth rate was then calculated by the following ratio:

$$N_b/\hat{N}_T$$

where  $N_b$  represents the total number of documented births in that year, and  $\hat{N}_T$  represents the estimation of the total abundance of the studied population, including newborn dolphins of the year. The latter was provided by Genov (2021) and based on the most supported robust design models using finite mixture models (full likelihood). The results of the aforementioned approaches were then compared.

#### **2.4.4 Reproductive success of the females**

The number of calves per female during the study period was determined. A distinction was made between the number of all the calves, including the ones that were born before the beginning of the study, and the number of the calves that were clearly born between 2013 and 2020.

A female was considered to have reproduced successfully if her calf survived from birth to the minimum age of weaning (Robinson et al. 2017), which is 2 years (Grellier et al. 2003; Fruet et al. 2015).

#### **2.4.5 Inter-birth interval (IBI)**

Inter-birth interval, IBI, refers to the period between successive births of an individual female (Campbell 2016). In the study of dolphins' reproductive traits, IBI is determined as the period between the first sighting of the mother with one calf and the first sighting of the mother with a new calf. The length of that period was determined for the selected females that had two or more calves during study period. This made it possible to determine the minimum, maximum and mean inter-birth interval for each female.

In addition, the inter-birth intervals were compared in relation to the fate of each female's previous calves:

- 1) IBIs for females whose previous calves died within the first year of their lives
- 2) IBIs for females whose previous calves died between 1 and 2 years of age
- 3) IBIs for females whose previous calves died between 2 and 3 years of age
- 4) IBIs for females whose previous calf survived and reached 3 years of life



#### 2.4.6 Calf mortality and survival

In order to determine calf mortality and survival, it was important to record their first and the last sighting. Calves are considered to be in close association with their mothers for at least 2 to 3 years after birth (Haase and Schneider 2001; Grellier et al. 2003; Hill et al. 2007). In most studies of reproductive ecology, the calf was presumed dead if the mother was steadily sighted alone within 12 months since their last sighting together, during the first 3 years of calf's life. Although we considered the minimum age of weaning to be 2 years, the abovementioned criterion was also used in the determination of calf mortality between 2 and 3 years of age. Together with the analysis of the inter-birth interval, the obtained results would give us information about the weaning period in the studied population, as well as if the mortality rate of the calves between 2 and 3 years of age should be determined by means of association with their mother. In the calculation of mortality rate, the calves that were directly observed dead were also included. The potential month of the death was also determined if one of the following criteria was met, as was the case in Fruet et al. (2015):

- 1) the mother was seen repeatedly without the calf in the same month in which the calf had last been seen
- 2) the mother was seen repeatedly without the calf in the month following the month in which the calf had last been seen

The annual mortality rate was calculated as the following ratio:

$$\frac{\text{number of calves that have died in a given year}}{\text{total number of calves that were in association with their mother in the same year}}$$

After mortality rate was calculated, the survival rate could be determined. The survival rate was calculated separately for offspring age classes: for the calves that were younger than 1 year, the ones that were between 1 and 2 years old, and the calves whose age was between 2 and 3 years.

### 3 RESULTS

In the period between 2013 and 2020, based on the photographs of all qualities, there were altogether 482 *T. truncatus* sightings that included at least one dolphin, either male or female, and provided data for the analysis of the reproductive ecology of the resident population in the Gulf of Trieste (Table 4). The highest number of sightings (20.5%) occurred in 2017, followed by 2015 (16.0%). The lowest number of observations (5.0%) was recorded in 2020, which was caused by smaller number of conducted field surveys due to the numerous limitations related to the COVID-19 pandemic. In addition, the highest number of sightings was recorded during summer, in August (23.0%) and July (20.3%). On the other hand, only one sighting (0.2%) was recorded in November and December each. When considering only quality 3 photographs, there were 441 sightings, which meant that only 8.5% of all sightings did not meet the highest photographic quality criteria.

In 47.3% of all sightings (n=228), based on photographs of all qualities, there was at least one female-calf pair in the dolphin group (Table 4). The highest number of mother-calf sightings occurred in 2015, making up 71.4% of all the sightings in that year. In contrast, in 2013 there were only 11 out of 45 sightings (24.4%) involving mother-calf pairs.

Table 4: Number of sightings for each month and year of the study based on the photographs of all qualities (MC pairs = mother-calf pairs)

Year	Month												In total	MC pairs sightings
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec		
2013	2	0	1	0	1	10	5	14	7	5	0	0	45	11
2014	4	4	4	6	4	11	13	14	10	1	1	1	73	47
2015	4	2	6	2	1	16	12	27	5	2	0	0	77	55
2016	0	1	6	6	13	14	16	6	2	1	0	0	65	30
2017	0	5	14	5	8	15	19	28	5	0	0	0	99	29
2018	9	10	10	5	4	5	18	6	5	0	0	0	72	34
2019	0	5	1	0	1	0	8	7	5	0	0	0	27	9
2020	2	2	1	0	0	1	7	9	2	0	0	0	24	13
In total	21	29	43	24	32	72	98	111	41	9	1	1	482	228

When considering photographs of all qualities, 92 females from 228 sightings were seen at least once in association with a calf and considered its mother. 26 of these females (28.3%) were seen in at least 4 years and at least 4 times in each year, with a mean of 7.3 years (range = 5-8) and a mean of 54.8 sightings (range = 24-119) (Table 5). The total number of calves that were assigned to selected females was 64, out of which 49 (76.6%) were born during the study period, while 15 were born before 2013 and sighted as older calves until 2020. Fifteen females were observed every year of the study period. The most commonly observed

female was Daphne (DAP), which was present in 24.7% of 482 sightings, followed by Bella (BEL) occurring in 19.1% of the sightings. The minimum number of years in which the selected females were sighted was 5, as was the case with females 2014-037, Ket (KET), and TNT (TNT). The mean number of years in which the female was seen in association with a calf was 5.2, ranging from 2 in the case of the females CO2 (CO2) and TNT to maximum of 8 years, which was observed with females 2013-114, Lori (LOR), and Neptun (NEP).

Table 5: Females sighted regularly and selected for the analysis based on the photographs of all qualities (ordered from the highest to the lowest occurrence rate across sightings)

Female ID	Occurrence rate (%) across sightings	Occurrence rate (%) across years	Number of years in association with a calf
DAP	24.7	100	5
BEL	19.1	100	5
EMA	15.8	100	6
HID	14.9	100	3
SAT	14.5	100	7
CO2	13.5	100	2
SMA	13.3	100	6
NEP	12.2	100	8
NAR	12.2	100	6
TAR	11.6	100	6
LOR	11	100	8
NET	10.8	100	5
SAN	10.4	100	7
2013-019	10.4	100	3
KAL	10.4	87.5	5
2013-101	10.2	87.5	7
MON	10.2	87.5	5
SON	10	87.5	6
2013-086	9.1	87.5	3
RAD	8.9	87.5	6
KET	8.7	62.5	5
2013-114	8.5	100	8
2014-008	7.5	87.5	4
2018-171	7.1	87.5	5
TNT	5.8	62.5	2
2014-037	5	62.5	3

Since some females were accompanied with their offspring in all 8 years of the study, such examples were used to track the patterns of mother-calf size ratio, as well as the duration of their close association through detailed analysis of their photographs (Figure 12; Figure 13).



Figure 12: Female Sataya with her three calves over the study period. (A) Newborn in 2014. (B) Newborn in 2016. (C) Calf of 2/3 of mother's size in 2017, the same one that was a newborn in 2016. (D) The calf in 2018, the same one as in 2016 and 2017. (E) The calf in 2019, the same one as in 2016, 2017 and 2018. (F) Newborn in 2020, characterized by foetal folds. (Photo: Morigenos)

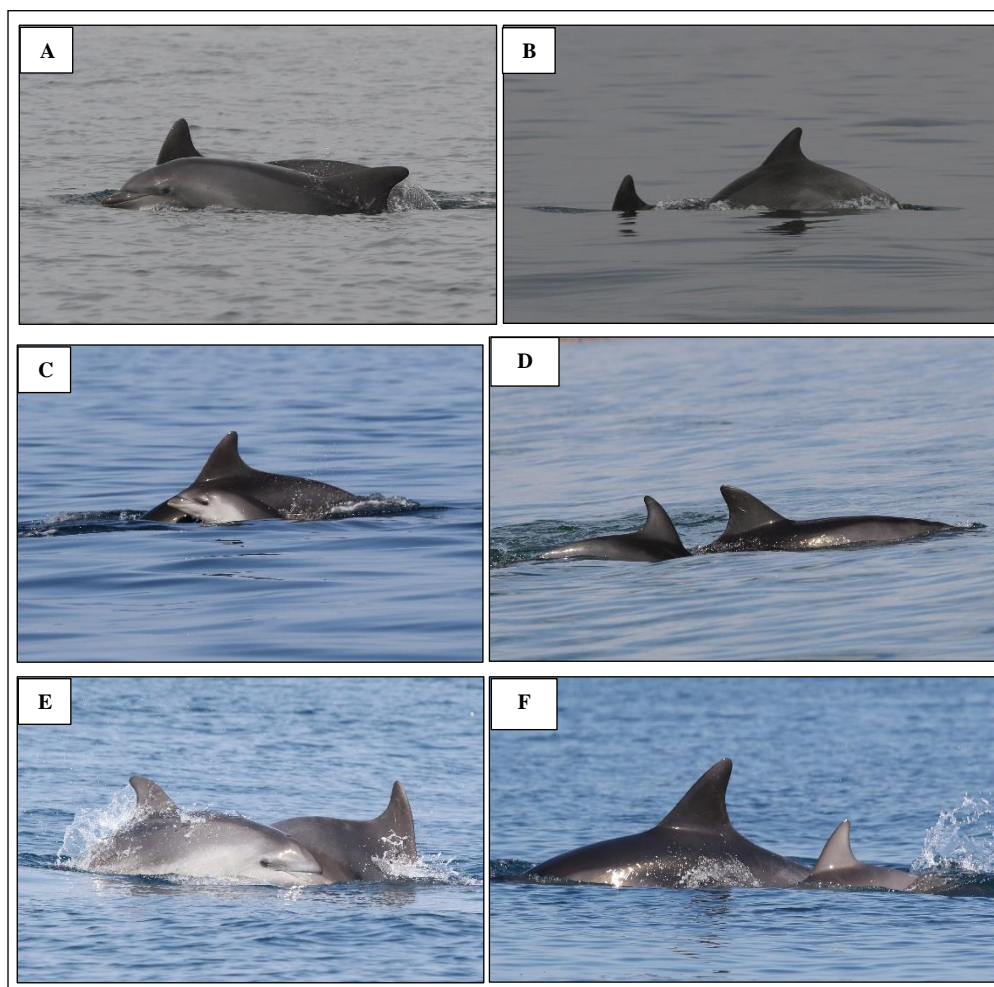


Figure 13: Female Sonja with her three calves over the study period. (A) Older calf in 2014, bigger than 2/3 of mother's size. (B) Big calf in 2015, the same one as in 2014. (C) New calf in 2017. (D) Calf of about 1/3 of mother's size in 2018, the same one that was born in 2017. (E) Big calf in 2019, the same one from the previous two years. (F) Newborn in 2020. (Photo: Morigenos)

### 3.1 Analysis based on the photographs of all qualities

#### 3.1.1 Birth seasonality

For each of the 49 calves born between 2013 and 2020 the birth period was estimated. In some cases, the estimated range reached almost a year, for example for 2013-101's second calf, which is considered to have been born either in 2018 or 2019. In the case of 21 other calves, their sighting histories enabled the estimation of the birth period within a maximum of 3.5 months. For 12 of those dolphins the possible birth period extends over 2 months, for 4 calves the exact month of birth was determined, while for 3 of them even the date of birth was assessed. The highest number of possible and assigned births occurred during summer months, between June and August, which together make up 80.4% of all the potential and assigned months of births (Figure 14). Conversely, only 1 birth possibly occurred in February and March each, while none were estimated to occur between September and January with this method.



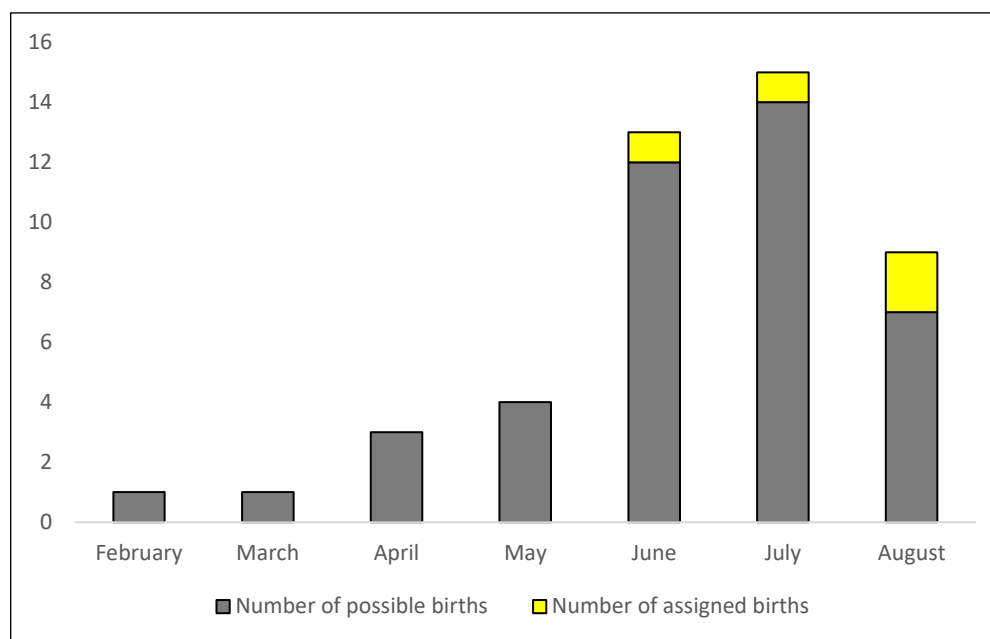


Figure 14: Number of possible and assigned births that occurred between February and August of the study period

### 3.1.2 Crude birth rate

#### 3.1.2.1 Analysis based on the selected females and calves

When considering only the calves born by 26 selected females, in the period between 2013 and 2018 a total of 37 dolphins were born and assigned to 24 different females. For some of those dolphins, data enabled the determination of the exact year of birth. That was the case with individuals whose mothers were seen without them within the same year prior to the calves' first sighting or, alternatively, when the photographs indicated that the calves were born within the year of their first sighting. Another 12 dolphins were definitely or potentially born during 2019 and 2020, years that were not included in the determination of the crude birth rate due to lack of available abundance estimates (see Material and methods), while 15 calves were considered to be born before the beginning of the study. Out of 49 calves born between 2013 and 2020, for 34 (69.4%) of them the exact year of birth was assigned (Table 6). The highest number of these births occurred in 2017 (20.6%), while only one birth (2.9%), the one of Lori's calf, occurred in 2013.

The potential annual crude birth rate was determined by considering all of the potential births for each year, having in mind occurrence of deviations from the actual values. For example, the sighting histories suggested that Hidro's calf was born between August 2017 and July 2018, making both years the potential years of birth. Accordingly, 2013 was the year with the lowest crude birth rate (0.03), but this was not much lower than the value of 0.04 for 2015 and 2016. The highest crude birth rate was recorded in 2014 with the value 0.07. The

average number of births per year was 8.5 (SD=2.51) for the period between 2013 and 2020, while the average crude birth rate was 0.02 (SD=0.012) for the period between 2013 and 2018.

Table 6: Annual crude birth rates between 2013 and 2018 considering selected females. \*Data from Genov (2021); newborn dolphins of the year included in the estimation.

Year	Number of certainly assigned births	Number of assigned and potential births	Annual mean total abundance estimate*	(Potential) crude birth rate
2013	1	5	169	0.03
2014	6	11	161	0.07
2015	5	7	181	0.04
2016	5	8	226	0.04
2017	7	13	245	0.05
2018	2	9	170	0.05
2019	3	7	N/A	N/A
2020	5	8	N/A	N/A

### 3.1.2.2 Analysis based on all females and calves

As mentioned before, 92 females were seen at least once in association with a calf during the study period. Since 66 of these females were not observed regularly, it was not possible to assess the birth period of their calves based on the sighting histories without substantial errors. That is why the birth year was determined only for the calves whose photographs showed prominent neonate traits and which were clearly born within a year in which the photograph was taken. These are further addressed as “additional births”, the births that were given by females that were not regularly sighted and selected in the analysis considering set criterion (see Material and methods). For all the years except 2014 and 2020 some additional births were assigned (Table 7). The highest number of additional births was assigned to 2016 (n=3).

Table 7: Number of additional births for each year between 2013 and 2020 for the dolphins that were not regularly seen during the study period

Year	Number of additional births	Females having calves
2013	2	2013-075, 2013-092
2014	/	/
2015	2	2015-021, 2015-062
2016	3	2014-018, 2015-016, 2016-040
2017	2	2014-021, 2016-057
2018	2	2015-006, 2018-161
2019	2	2014-021, 2019-012
2020	/	/

When considering all the females that certainly had newborns between 2013 and 2020, the mean annual number of births was 10.1 (SD=2.47). The mean annual crude birth rate, when considering assigned and potential births for each year, was 0.06 (SD=0.01). The highest number of births (n=15) occurred in 2017, while the highest crude birth rates were in 2014 and 2018 with the value 0.07 (Figure 15). The lowest crude birth rate (0.04) was recorded in 2013. From 2013 to 2014 there was an increase in crude birth rate, followed by two years of decrease, with another increase in 2017 and 2018.

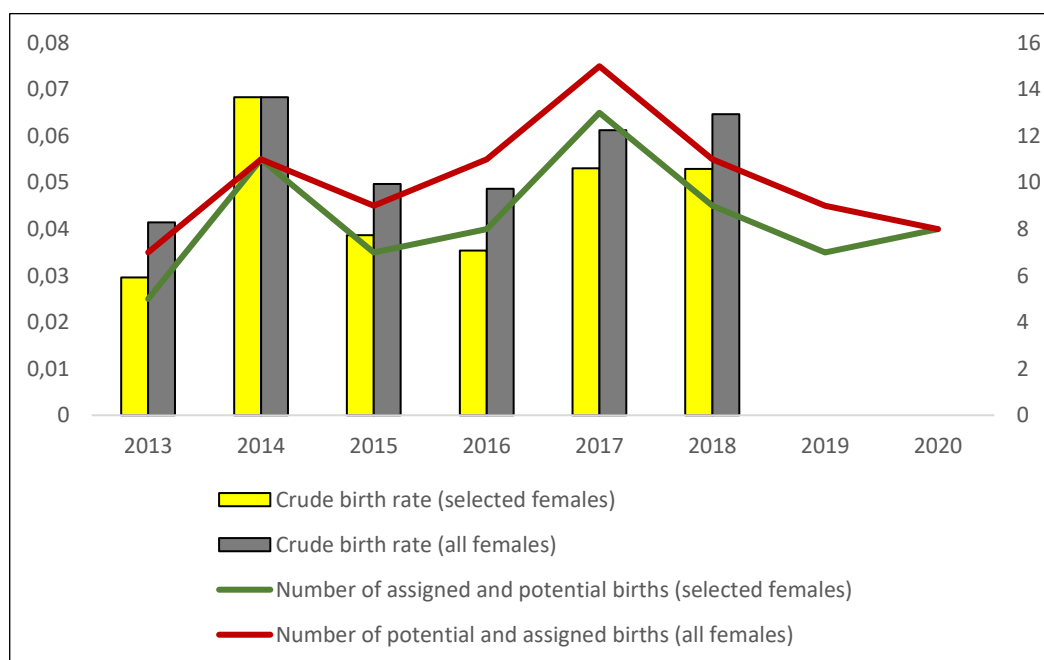


Figure 15: Comparison of annual crude birth rates when considering only selected females and all the females whose calves' birth year could be assessed (left y-axis: crude birth rate; right y-axis: number of assigned and potential births)



### 3.1.3 Reproductive success of females

The fate of 64 calves that were assigned to 26 females was tracked during the study period. 49 of these calves were born between 2013 and 2020. On average, around 2 calves were born per female over the 8 years of the study (mean=1.9; SD=0.91), ranging from 1 (females 2013-101, 2014-008, 2014-037, 2018-171, CO2, KAL, KET, NET, SMA, TNT, DAP) to 4 in the case of Sandra (SAN). When considering the calves that were born before 2013, every female was associated with approximately 2 calves during the study period (mean=2.5; SD=0.76).

Female reproductive success was calculated as a proportion of calves successfully raised until the minimum age of weaning (2 years) in the number of calves that were born before 2019 and have a known fate, meaning that it is known whether the calf survived until that age. This calculation also included the calves who were born before the study period and which, based on their sighting histories, certainly survived until the minimum age of weaning. The mean reproductive success was 66.7%. Six out of 26 females (23.1%) successfully raised all of their calves until the minimum age of weaning (Figure 16). Among those females, individual 2014-008 had only one calf, while 2018-171, KAL, Ket (KET), Nari (NAR) and Smash (SMA) had 2 calves each. 23 more calves also survived until the minimum age of weaning, 11 of them died when they were younger than 2 years, and the fate of 8 calves is unknown due to insufficient data that could give us information about their survival (Figure 16). In addition, 11 calves were younger than 2 years and still alive when the study ended in 2020. Conversely, considering only the number of calves that were successfully raised until the age of 2 years, female Sandra had the highest reproductive success, since 3 of her calves survived until that age, while the fourth one, born in 2020, was still alive by the end of the study.

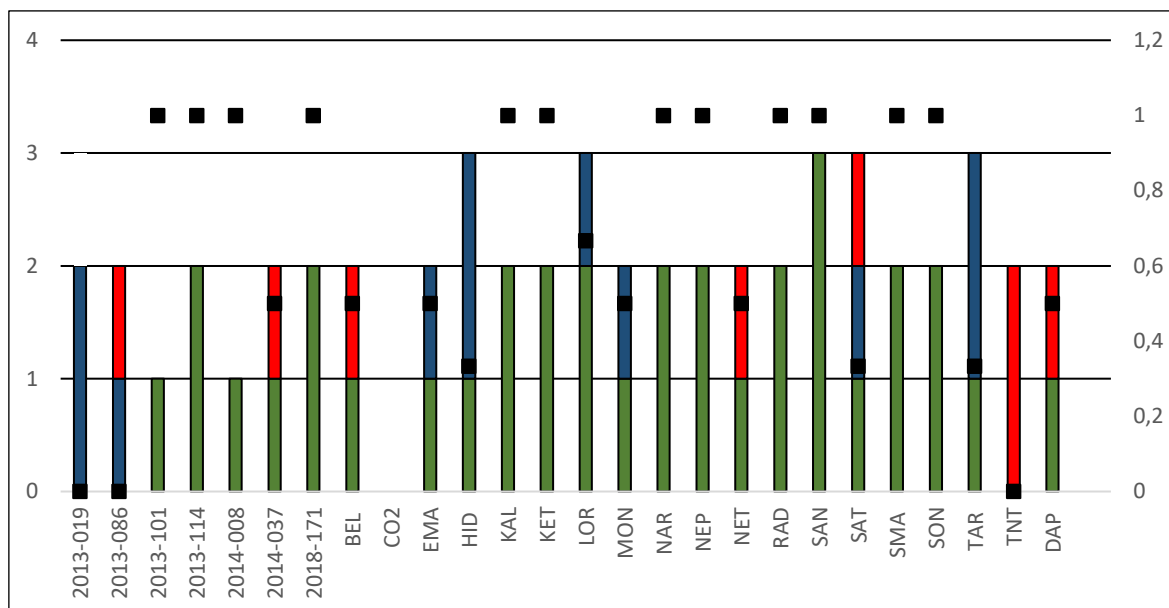


Figure 16: Calves' fate representing females' reproductive success (■ -died before reaching the minimum age of weaning (age>=2yr); ■ -unknown fate; ■ -successfully weaned; black box - female reproductive success in percentages; left y-axis: number of calves; right y-axis: female reproductive success in percentage)

### 3.1.4 Inter-birth interval

Out of 26 selected females, 24 (92.3%) had two or more calves during the study period. For 15 of them there was sufficient data to determine 21 inter-birth intervals (Figure 17). Mean inter-birth interval for the population is 2.8 years (SD=0.60), with the range between 2 to 3.4 years.

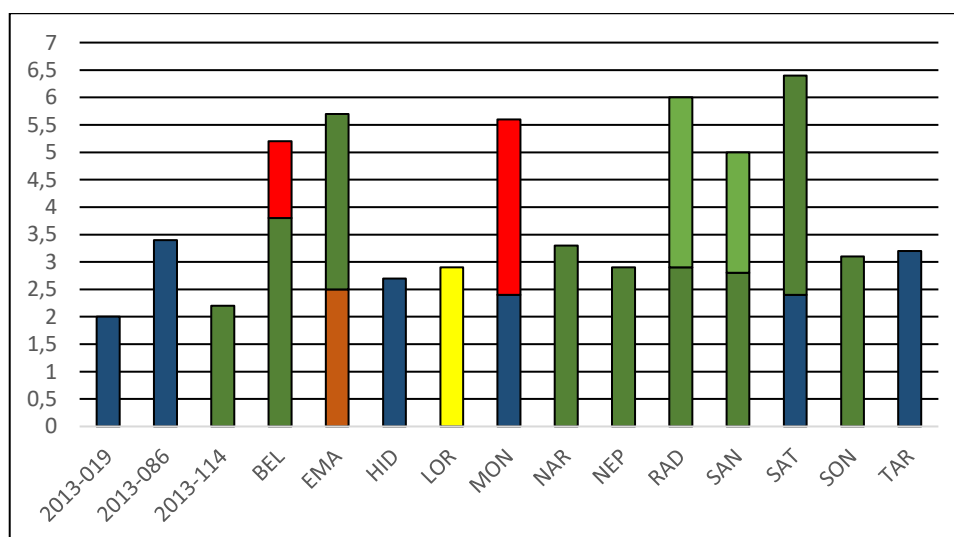


Figure 17: Mean inter-birth intervals during the period 2013–2020 (■ -previous calf died before reaching 1 year of age; ■ -previous calf survived and reached 3 years of age; ■ -fate of the previous calf is unknown; ■ -previous calf died before reaching 2 years of age; ■ -previous calf died between 2 and 3 years of age)

Concerning only the inter-birth intervals in cases when the previous calf survived for more than 3 years, the mean interval was 3.0 years (SD=0.56) (Table 8). For the females whose previous calf died within the first year of life, the mean inter-birth interval was 2.7 years (SD=0.53) (Table 8).

Table 8: Inter-birth intervals in relation to different fates of the previous calves

The fate of the previous calf	Number of calves	Min.	Max.	Mean	Median	SD	SE
Died within the first year of life	6	2.0	3.4	2.7	2.55	0.53	0.22
Died between 1 and 2 years of age	1	2.5	2.5	2.5	2.5	N/A	N/A
Died between 2 and 3 years of age	1	2.9	2.9	2.9	2.9	N/A	N/A
Previous calf reached 3 years of age	11	2.2	4.0	3.0	3.1	0.56	0.17
Unknown fate	2	1.4	4.6	3.1	3.2	1.60	0.93

### 3.1.5 Calf mortality and survival

Out of 64 calves whose fate was tracked during the study period, sighting histories of 49 of them (76.6%) enabled us to conclude if they survived or died. In the period between 2013 and 2020, a total of 13 calves (age < 3 years) (26.5%) were considered to have died. One of them, Tarja's first calf from the study period, was directly observed dead. It was younger than 1 year, had neonate traits and was found entangled in fishing gear on 6 Jan 2013.

Nine calves (69.2%) of 7 different mothers (2013-019, 2013-086, Moni, Sataya, Tarja, Hidro and Lori) died before they reached the first year of their life (Figure 18). Females 2013-019 and Tarja were the ones that lost two of their calves within their first year. Two calves (15.4%), Emanuela's and Hidro's, died when they were older than 1 and younger than 2 years, while another two, Tarja's and Lori's, died when they were between 2 and 3 years old. The highest number of deaths occurred in 2018 and 2019 (30.8%), while there were no calf deaths recorded in 2017.

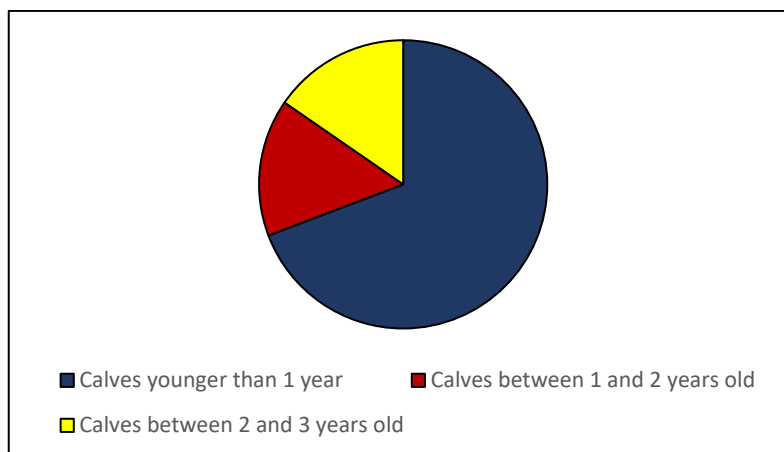


Figure 18: The proportion of different offspring age classes in the number of dead calves between 2013 and 2020

The highest total calf mortality rate was recorded in 2019 (0.36), while in 2017 the mortality rate was 0, meaning that all of the calves survived (Figure 19). Second lowest mortality of the calves was during 2013 when only one out of 8 calves with the known fate died (0.13). In the study period between 2013 and 2020, the mean annual total mortality rate was 0.16 (SD=0.11), meaning that on average 84% of calves of known fate survived.

When considering different offspring age classes, the mean annual mortality rate of the calves younger than 1 year was 0.20 (SD=0.16), for the calves between 1 and 2 years old 0.09 (SD=0.18), and for the calves between 2 and 3 years of age 0.25 (SD=0.38). In 2018, 92.3% of all the calves younger than 1 year survived, while in 2014 their mortality rate was the highest during the study period (0.43).

For 6 calves the month of death was assessed with the help of their sighting histories. Three calves died during summer months – two of them in July and the third one in August. Two cases of calves' deaths were assigned to September, while one calf is considered to have died in February.

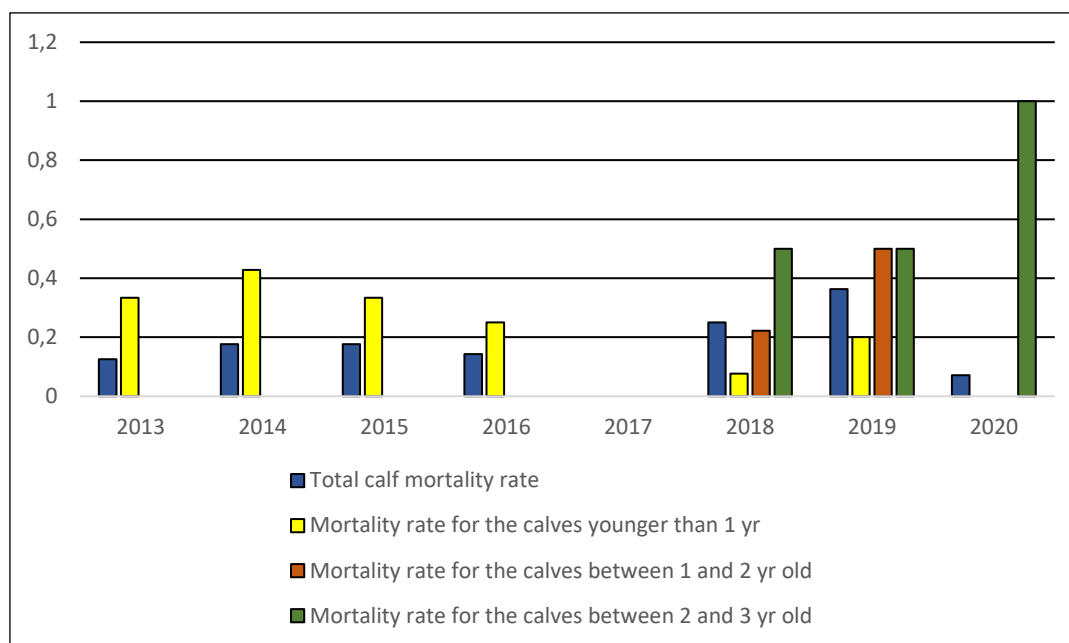


Figure 19: Calf mortality rates for each year between 2013 and 2020

### 3.1.6 Age at the first calf production

In the study sample of this research, among all the analyzed dolphins, there was one individual which could provide data about the age at the first reproduction, since it was tracked since birth and gave birth to a calf during study period. Female CO2 was observed in every year of the study period, first as Hidro's calf in 2013 and since 2019 as the mother (Figure 20). According to prior data, CO2 was born in 2012, while the date of her first sighting with her own calf was 1st September 2019. According to that, CO2's age at the first calf production was 7 years, meaning that the sexual maturity must have been attained by 6 years of age. In addition, CO2 was first sighted away from her mother in 2015, when Hidro was observed with her new calf.

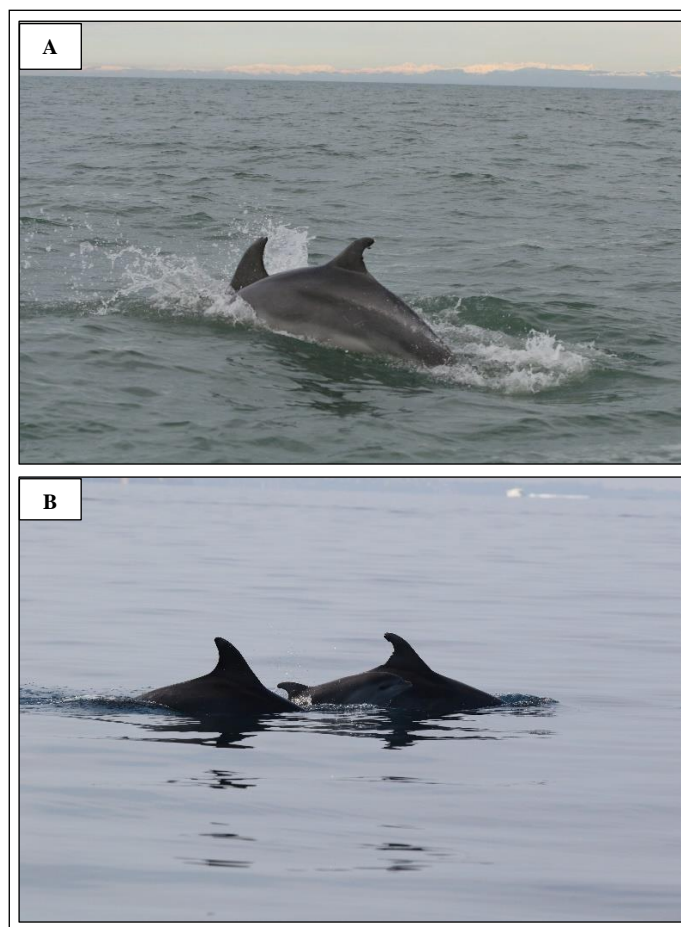


Figure 20: (A) Female CO2 as a calf in association with her mother Hidro. (B) Female CO2 with her own newborn calf in 2019. (Photo: Morigenos)

### 3.1.7 Acquirement of the natural markings and further observations

During study period there were 4 cases in which the tracked calves acquired natural markings between two sightings. At that moment they were given a code as a reference and it became possible to monitor their presence in the population regardless of the presence of their mother (Figure 21; Figure 22).

Daphne's second calf from the study period, now known as 2016-070, was born in 2014 and gained natural markings in 2016, when it was about 2 years old. Until 2017, individual 2016-070 was always sighted with its mother, after which its presence was not observed anymore. Another calf that acquired natural marking in 2016 was individual 2016-068, Rada's calf born in 2014. Since that year, when the calf was about 2 years old, individual 2016-068 was sometimes sighted without the presence of the mother until the end of the study period. In addition, in a year following the first calf's independent sighting, in 2017, Rada's other calf was born. Two of the calves, Neptun's calf 2018-175 and Smash's calf 2018-184, started being recognized by natural marks in 2018. Both of them were born in 2016, after which individual 2018-175 was sighted without Neptun in 2020, when she already had a new calf

from 2019. The other one, 2018-184, was first sighted without mother's presence in 2018, while Smash had no more new calves.

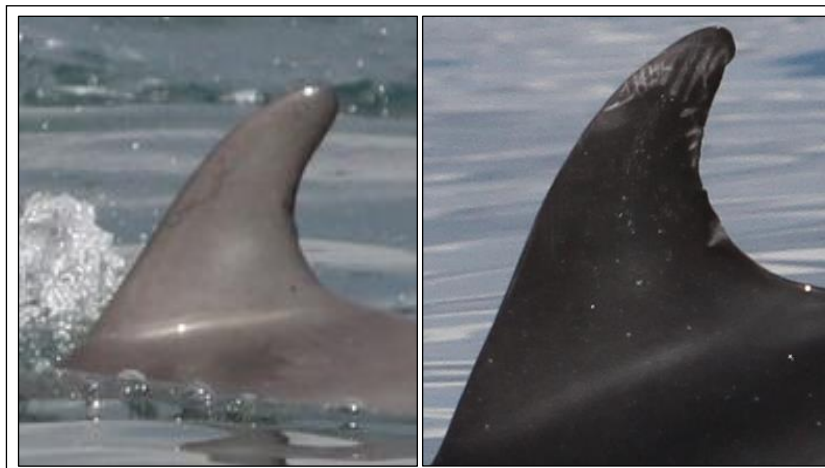


Figure 21: 2016-068's dorsal fin before and after gaining natural marks and name as a reference (Photo: Morigenos)



Figure 22: 2018-184's dorsal fin before and after gaining natural marks and name as a reference (Photo: Morigenos)

### 3.1.8 Reproductive peculiarities of the “evening group”

Having in mind the existence of three different social clusters in the analyzed population (Genov et al. 2019a), some peculiarities regarding reproductive behaviour of the evening group were noticed. Three females from this social group (BAL, MON and TNT) have disappeared the year in which they very likely gave birth to a new calf. More precisely, these three females were sighted in the year prior to the year of birth of a new calf and were observed again two years later, when the calf was about  $2/3$  of the mother's size, indicating that it was between 1 and 2 years old. This kind of pattern was not observed in the rest of the individuals from the study, including the ones from the “morning” group.



## 4 DISCUSSION

### 4.1 Births in studied population

#### 4.1.1 Birth seasonality

The results showed the seasonality in births in the resident dolphin population of the Gulf of Trieste, since the peak period of births occurred during late spring and summer months, from June to August. More precisely, the highest number of the births occurred in July, making up 32.6% of all the potential and assigned births. The first assumption of births occurring in the warmer part of the year is therefore supported. Although no births were registered to occur in the period between September and January with the used method, it does not mean that they certainly did not happen. For example, Nari's first calf from the study period was estimated to be born between August and January, but the range of 6 potential months of birth is too wide to draw firm conclusions. Besides, much less fieldwork was carried out in the winter months and consequently there were fewer sightings which might allow us to observe some births during colder part of the year. Similar case was in the study of Robinson et al. (2017) in which dedicated boat surveys were conducted only in the period between May and October. Therefore, we should not completely rule out the birth of calves during the winter. Other studies also showed the seasonality of births in *T. truncatus* populations (Urian et al. 1996). For example, in the Doubtful Sound in New Zealand the calving mostly happened in the period between December and February, in austral late spring and early summer (Haase and Schneider 2001). Similarly, in Moray Firth in the North Sea, 94% of all the calves were born from July to September (Robinson et al. 2017). Calving along the Texas coast had the peak in March, while along the west coast of Florida the peak was in May, with the births occurring year-round (Urian et al. 1996). The results from this thesis match with the results from another Mediterranean population, the one from the southern Tyrrhenian Sea, which also showed the peak of birth period during late spring and early summer, in June and July (Blasi et al. 2020).

Birth seasonality might be connected with the higher sea temperatures, since the warmer water is thermally more suitable for the calf and its mother since it might help thinly insulated calves avoid thermal stress (Mann et al. 2000; Adamczak et al. 2021). For example, the seasonality of births in Doubtful Sound was very similar in shape to the plot of the mean water temperature with both maximum values in January (Haase and Schneider 2001). In the Moray Firth, the peak also coincided with the annual peak of sea temperatures (Robinson et al. 2017). On the contrary, there was no direct relationship between the sea temperature and birth frequencies in the case of Texas' and Florida's populations (Urian et al. 1996). In that case, warmer water may not be directly connected with the timing of birth, but it contributes to the higher food availability, which is of great importance for pregnant and lactating females under bigger nutritional stress (Urian et al. 1996; Oftedal 1997). During

the warmest months, there is also reduced energy demand on lactating females and the newborns need less energy in order to regulate their body temperatures (Adamczak et al. 2021). In conclusion, in studied species local environmental conditions obviously play an important role in the determination of period when the births take place (Urian et al. 1996).

#### 4.1.2 Crude birth rate

In case of the studied population, crude birth rate calculation brought certain difficulties due to which interpretation of results cannot be straightforward. Some calves were assigned to two calendar years as birth years, whereby we must be aware of deviations from the actual rate values from the beginning. Therefore, the results for each year are interpreted as “potential”. During 2013-2018 period, the highest crude birth rate was recorded in 2014 when 6 new dolphins were certainly and 5 new ones were potentially born, and in 2018, when 2 dolphins were certainly and 7 new ones were potentially born. In 2015, the crude birth rate was 0.05, i.e. 26.5% lower than in the previous year. 2015 was also the year with the highest number of mother-calf pairs sightings. After a slight decrease in 2016, the crude birth rate started increasing again, reaching 0.07 in 2018. Crude birth rates were the lowest in 2013. It seems that, in general, when regarding this reproductive parameter, the population was in a better condition at the end of the research than at the point from which the research started. For example, the crude birth rate in 2016 was the second lowest in the study period, but still increased by 19.5% when compared to 2013. High number of births in certain years might reflect a synchronization of female estrus, as was suggested by Bearzi et al. (1997) in the research of Kvarnerić *T. truncatus* community, where most births occurred at two-year intervals. Such a strategy may optimize foraging efficiency and enable the concentration of calf care and protection by bringing most of them together (Bearzi et al. 1997). Such results might also be a consequence of reproductive maturation of several females during the same season, as was the case in the research of Haase and Schneider (2001). However, this was not the case in our study because all the calves born in, for example, 2014 and 2018 were born by females who already had calves before.

Mean crude birth rate of 0.022 is lower in comparison with other *T. truncatus* populations. For example, Haase and Schneider (2001) recorded the average crude birth rate of 0.069 in the population of Doubtful Sound, while in the population from Brazil this rate was 0.090 (Fruet et al. 2015). As was mentioned before, it is important to be aware of the potential biases made during this analysis, especially when regarding all the females from the study period. Out of 66 females with calves that were not regularly sighted but were included in the determination of the crude birth rate, for only 11 new dolphins the birth year was determined between 2013 and 2018. In reality, this number is bigger since new dolphins appeared even towards the end of the study period, but they were not sighted frequently enough to estimate the birth period which could enable their inclusion in the crude birth rate calculation. This is an example of one of the main difficulties in this type of research, and it

is necessary to find better ways to avoid large biases. In our case, one possible solution would be to consider only selected females in the calculations, as well as to set stricter criteria for their selection, e.g. a higher minimum number of sightings per year. One of the solutions might also be grouping parts of the year in the best possible way regarding our data, where the birth rate would not be calculated on an annual basis, but for each grouped period.

## 4.2 Reproductive success

Out of all the calves for which it was possible to conclude if they survived until the minimum age of weaning, 75.6% were considered to be successfully weaned. The differences in the reproductive success between particular females were also recorded, with two different perspectives. For example, when considering the percentages of successfully weaned calves in the total number of calves that were born before 2019, 12 female dolphins were reproductively the most successful. This criterion is not the most relevant since it may attribute higher success to females with a single surviving calf, as is the case with 2014-008. If we consider the total number of successfully weaned calves per every female, the most successful mother was definitely Sandra, since 3 of her calves survived until the age of 2 years, while the fourth one, born in 2020, was still alive by the end of the study. According to that, the least successful mother was 2013-019, whose both calves died before reaching the minimum age of weaning.

As described in Robinson et al. (2017), the variability in females' reproductive success might be influenced by many factors, including maternal age and size, previous breeding experience, dominance of the mother, survival of the previous calves, and different social factors. The individual heterogeneity in this reproductive parameter might indicate that some mothers are more important for the viability of the population than others. In the case of the population in the Gulf of Trieste, Sandra would be an example of a female that provides a high value to the population size, but more research over a longer period of time is required to make this kind of conclusions. Other females may have some other important roles, connected with social structure or antipredator strategy.

## 4.3 Inter-birth interval

In common bottlenose dolphins, the gestation period lasts for 12 months, while the lactation lasts for about 19 months (Harrison and Ridgway 1971; Perrin and Reilly 1984). With an added resting period of approximately 5 months in order to accommodate for seasonal breeding, an expected inter-birth interval (IBI) of this species would be about 3 years. Our results showed that in the Gulf of Trieste the mean calving interval was 2.8 years, which supports the second assumption, but with a lower range (range = 1.4-4.9) than in some other populations, which is possibly caused by the length of the study period. The minimum IBI, recorded for the female whose previous calf died within the first year of its life, was 2 years. It means that *T. truncatus* individuals from the studied population are capable of giving birth

approximately every 2 years, assuming that the existing calf died prematurely. It also shows that the females can reproduce and conceive in the breeding season that is immediately following the death of the previous calf. In the population from the Doubtful Sound, the mean calving interval was also 3 years, ranging from 2 to 5 years (Haase and Schneider 2001). In the population from the Moray Firth, the mean value of the IBI was about 3.8 years, but with the wider range, from 2 to 9 years (Robinson et al. 2017). Mitcheson (2008) recorded the mean calving interval in the population from northeastern Scotland with the value 3.2 years, while the mean IBI in the population of eastern Scotland was 3.7 (range = 2-9; Arso Civil et al. 2017). When comparing the results with the results from other Mediterranean populations, the average inter-birth interval in the Gulf of Trieste was also similar to the one from the Ligurian Sea (mean=3.0 years, Rossi et al. 2017), as well as to the one from the Tyrrhenian Sea, where the recorded average value for 5 dolphins was about 3.5 years, ranging from 2 to 7 years (Blasi et al. 2020).

In some research, the inter-birth interval was shown to be influenced by the fate of the female's previous calf. For example, Haase and Schneider (2001) showed that in the Doubtful Sound population, if the calf lived for more than 1 year, calving interval increased to 3 to 5 years, reflecting mother's parental investment in previous offspring. On the other side, if the calf died within the first year of its life, the interval was from 2 to 3 years long. Robinson et al. (2017) also confirmed that the IBIs were significantly lower in females that were experiencing calf loss. In the studied population from this thesis, the results showed a not very common pattern in which IBI was around 3 years regardless the fate of the previous calf. For example, the average IBI for the females whose previous calf survived for more than 3 years was also 3 years. In comparison, if the previous calf died when it was younger than 1 years, the average IBI was 2.7 years. This kind of pattern was also recorded in the population from the Southwestern Atlantic Ocean, which was explained as a consequence of the minimal predatory pressure and abundant and predictable feeding resources in the studied area, which enable females to reduce maternal investment without the addition of substantial costs for calves' survival after weaning (Fruet et al. 2012; Fruet et al. 2015).

Reproductive senescence is an age-related decline of reproductive output and success which is evident in many mammalian species, including common bottlenose dolphins (Karniski et al. 2018; Comizzoli and Ottinger 2021). Karniski et al. (2018) concluded that increasing inter-birth intervals indicate slowing reproductive rate with age, although some of the increases might be a consequence of spontaneous abortions and unrecorded stillbirths. In addition, longer IBIs are largely driven by declining maternal condition which causes lower ability to conceive or complete another pregnancy before she dies (Karniski et al. 2018). Since recorded inter-birth intervals in the Gulf of Trieste were about 3 years, prolonged periods without new calves, for example 6 years in the case of Daphne, might indicate aforementioned conditions, but it should be more investigated in the future with a bigger

sample size and prolonged study duration. The possible reason for this might also be missed birth of Daphne's calf during these 6 years.

#### **4.4 Calf mortality and survival**

Annual total calf mortality rates showed that 2019 was the year when the largest proportion of the calves of a known fate had died. It was also the year in which the deaths of all offspring age classes were recorded, as well as in 2018. Most of the calves that died were younger than 1 year. The mean survival rate for this age class was 80%, which is comparable with the results from other *T. truncatus* populations. Wells and Scott (1990) found that in the Florida's Sarasota population 80.3% of the calves survive their first year. Very similar survival rate of 80.0% was also recorded in Doubtful Sound (Haase and Schneider 2001), while in Brazil the survival rate was 84.0% (Fruet et al. 2015). In the Mediterranean, for example in the Ligurian Sea, 75.0% of 1-year-old calves survive (Rossi et al. 2017), while in the Tyrrhenian Sea the recorded survival rate was as much as 87.5% (Blasi et al. 2020). As was the case with crude birth rate, there may be potential biases in these results, since there is a high level of uncertainty in the determination of whether a calf survived, was weaned and became independent from its mother, or whether it died and the new calf was born before the end of the calving period (Blasi et al. 2020).

As the results showed, the highest mortality risk occurs within the first year of life, especially in the first few months. The causes of the calf mortality in the northern Adriatic, as well as in the Mediterranean in general, still have to be further studied. Shark attacks and infanticide appear to be rare in this area, at least according to available data, but some potential causes of death might be connected with modifications in diet, age and malnutrition of the mother because of low prey concentrations in the habitat (Mann et al. 2000; Bianucci et al. 2002; Rossi et al. 2017; Blasi et al. 2020). There are also some anthropogenic threats for the *T. truncatus* calves, including boat strikes and bycatch (Wells et al. 2008; Tezanos-Pinto et al. 2014), which was also documented in our study as the cause of death of one calf during the study period.

#### **4.5 First calf production**

As reported in Results, one mother was tracked since her birth and therefore provided us with data on the age at the first calf production, which was 7 years. Although this is only one concrete case that cannot be generalized to the whole population, this indicates earlier sexual maturation in comparison with most other populations, because of which the third assumption cannot be confirmed. For example, in the Moray Firth two primiparous females gave birth at age 6, even though the estimated age at the first calf production was between 6 and 10 years (Robinson et al. 2017). Fruet et al. (2015) recorded the first calf production at the age of 8 and 10. Later sexual maturation at 9 to 14 years of age was observed in common bottlenose dolphins elsewhere, for example in the northeastern Pacific and northwestern

Atlantic (Perrin and Reilly 1984; Mead and Potter 1990). The age of the first pregnancy in the Tyrrhennian Sea was 7.5 years (Blasi et al. 2020). During the first reproduction, females are unexperienced and their calves are considered to be more vulnerable to predators. In addition, those females may not be big and fat enough to nurse a growing calf, which should still be more investigated (Robinson et al. 2017).

#### **4.6 Additional reproductive characteristics of the population**

As the studied population is characterized by the existence of three different clusters, there are potentially some differences in their reproductive ecology, as is the case with the different interactions with trawlers. Females' disappearance from the study area in the year of giving birth, recorded only in the "evening group", was also observed in the population from the Tyrrhenian Sea (Blasi et al. 2020). Females might have left the study area temporarily in order to give birth in better habitat for rearing the calf. In the further research, it would be useful to study which area the females use during that period and why only "evening group" females would behave that way, but not the "morning group" ones.

Another prominent feature of the reproductive ecology of the studied population is connected with the shorter range of the inter-birth intervals. The results showed that, even when the calf survived for more than 3 years, the mother already had a new calf, meaning she conceived when the previous calf was about 2 years old. It means that the calves were already independent and not associated with the mother even after such a short period of time, at the age of about 3 years. This conclusion might present a base for further study of calf mortality, e.g. dolphins between 2 and 3 years of age can be included in the analysis of the survival and mortality rate, but not by means of their association with their mothers. It might be expected that the calf of that age is not observed with the mother, not because it is dead, but because it is already independent. It is therefore problematic to draw conclusions because of the difficulties with the recognition of the calves if they are no longer in association with the mother before acquiring natural markings.

#### **4.7 Advantages and disadvantages of the study**

In some studies of the *Tursiops* reproductive ecology, the analyzed data was collected over a long period of time (Steiner and Bossley 2008; Henderson et al. 2014). For example, the study in New Zealand's Doubtful Sound (Henderson et al. 2014) was based on the data from 17-year-long period and the population examined was so small in size and distribution that the probability of detecting adult individuals was very high. In this thesis some shortcomings might occur due to the shorter period in which data was collected. Biases may appear because of potentially missed births in females that were not sighted each year. Despite that, the data was collected consistently and new calves were recorded every year, making it possible to determine some important reproductive parameters of the population. In order to obtain more data for each year, it would be useful to increase the number of boat surveys, as well as to distribute them or sampling effort as evenly as possible throughout the year.



## 5 CONCLUSION

As this was the first study on the reproductive ecology of the *T. truncatus* population in the Gulf of Trieste, it provides an initial insight into its reproductive parameters and the potential viability of the population. For example, new calves were observed in every year of the research, making it a good starting point from the conservation aspect. Estimates of the total population abundances for the years after 2018 are yet to be made so further trend in the crude birth rates could be tracked. Calf mortality rates were also similar to the ones from other populations with the need to investigate more potential causes of death, not only in the northern Adriatic, but also in the wider Mediterranean. As the bycatch of the calves has been recorded (Genov 2020), and some very possible causes of death include other anthropogenic activities (Wells et al. 2008; Tezanos-Pinto et al. 2014), it would be useful to determine which areas are of the greatest importance for mother-calf pairs in the Gulf of Trieste in order to minimize anthropogenic pressure there. There is also a need to make more reliable estimates of the relationship between dolphin's age and body length, which can be done using photogrammetry, a method which enables making measurements from photographs (Cheney et al. 2017), and can be conducted both from the boat and using drones. Some reproductive peculiarities might also be further explored, including the differences between the social clusters (Genov et al. 2019a) of the explored population.

In studying the reproductive ecology there are always some shortcomings, since dolphins are free-ranging animals. Some births might have been missed and the recorded absence of some individuals in certain years does not necessarily mean that they were not using the study area. Another possible limitation of this work comes from the consideration of every female associated with the calf as its mother, which may not be the case because of the documented allomaternal behaviour in dolphins (Mann and Smuts 1998). The awareness of the possible disadvantages of this type of research will contribute to further improvements to reduce potential biases. There is also a need for a larger data set from a longer study period in order to make conclusions about the reproduction of the population in general. This preliminary analysis could be included in the population viability analysis to support future conservation strategies aiming to increase conservation and management efforts to protect viability of this important marine species.

## 6 POVZETEK V SLOVENSKEM JEZIKU

Velika pliskavka, *Tursiops truncatus*, je morski sesalec iz družine Delphinidae in ena izmed najbolj raziskanih vrst kitov (Cetacea) (Arnason in sod. 2004; Wells in Scott 2009). Gre za kozmopolitsko vrsto, prisotno v priobalnih in pelaških delih večine zmerno toplih in tropskih morij (Wells in Scott 2009). Velika pliskavka ima zanimivo socialno strukturo, v kateri posamezniki živijo v tako imenovanih »fission-fusion« skupnostih, kjer se združujejo v skupine različnih velikosti in pogosto spreminjajo njihovo sestavo (Connor in sod. 2000). Kljub tem pogostim spremembam so ponekod skupine bolj stabilne, kot je pri rezidentni populaciji Tržaškega zaliva (Genov in sod. 2019). Gre za vrsto, pri kateri je zvočna komunikacija pomemben del socialnih interakcij (Quick in Janik 2012; King in sod. 2013).

Kljub pogostosti velike pliskavke na številnih območjih so mnoge populacije majhne in lahko tudi lokalno ogrožene (Tezanos-Pinto in sod. 2014; Brough in sod. 2016). Sredozemska subpopulacija je od leta 2021 po kriterijih Mednarodne zveze za varstvo narave (IUCN) opredeljena kot »najmanj ogrožena« (»Least Concern«) (Bearzi in sod. 2012; Natoli et al. 2021). Posamezni primerki so pogosto vključeni v interakcije z ribiškimi aktivnostmi, zato v nekaterih primerih pride do zapletanja v ribiške mreže in zaužitja delov ribiške opreme (Bearzi 2002; Wells in sod. 2008). Občasno prihaja do trkov delfinov s plovili, ki lahko privedejo do telesnih poškodb ali smrti (Wells in sod. 2008). Povečevanje podvodnega hrupa zaradi človeških dejavnosti moti komunikacijo med osebki ali onemogoča prehranjevanje (Notarbartolo di Sciara 2016; Sciacca in sod. 2016). Posamezni osebki so tudi neposredno prizadeti zaradi onesnaženja s kemičnimi onesnaževali, saj naseljujejo obalna območja, ki so odlagališča strupenih snovi iz industrijskih, kmetijskih in mestnih virov (Storelli in Marcotrigiano 2000; Jepson in sod. 2016).

Velika pliskavka je dolgoživ sesalec, ki skoti le enega mladiča naenkrat, najpogosteje v poznih pomladnih ali poletnih mesecih, kot je zabeleženo v populacijah ob Novi Zelandiji, v jugozahodnem Atlantskem oceanu in Tirenskem morju (Haase in Schneider 2001; Wells 2003; Wells in Scott 2009; Fruet in sod. 2015; Blasi in sod. 2020). Samica je običajno breja 12 mesecev, mladiči pa po rojstvu takoj zaplavajo in sledijo materam, ki skrbijo za njih v prvih treh do štirih letih (Hill in sod. 2007; Wells in Scott 2009). Pogoste so tudi interakcije med mladiči in samicami, ki niso njihove matere ter predstavljajo t. i. »varuške« (Mann in Smuts 1998).

Dolgoročno spremljanje populacije omogoča analizo razmnoževalnih lastnosti in znakov življenjskih strategij delfinov določenega območja, vključno s starostjo samic ob prvi kotitvi, rodnostjo, smrtnostjo in preživetjem mladičev, sezonskostjo rojstev, reproduktivnim uspehom samic ter intervalom med kotitvami (Henderson et al. 2014; Fruet et al. 2015; Blasi et al. 2020). Poznavanje razmnoževanih parametrov zagotavlja več informacij o viabilnosti populacije in pomaga pri ohranjanju vrste (Rossi et al. 2017). Razmnoževalna ekologija

velike pliskavke z izjemo nekaterih raziskav, na primer v Ligurskem in Tirenskem morju (Rossi in sod. 2017; Blasi in sod. 2020), v Sredozemlju še ni dobro preučena, zato je na tem območju še ogromno potenciala za izvajanje takšnih raziskav. Tržaški zaliv v severnem Jadranu je plitvo območje, ki velja za enega najbolj onesnaženih delov Sredozemlja (Olivotti in sod. 1986; Cozzi in sod. 2014), v katerem je prisoten velik antropogeni pritisk. Raziskave velike pliskavke na tem območju sicer potekajo že 20 let, zato so na voljo številni podatki, ki omogočajo potencialno določanje različnih razmnoževalnih parametrov ter primerjave z drugimi območji.

Osnovni vhodni podatki so vključevali zgodovino opažanj vsake identificirane samice (angl. „sighting history“) v obdobju med letoma 2013 in 2020, skupaj s podatkom, ali je bila samica opažena sama ali z mladičem. Podatki v okviru te naloge so bili zbrani z metodo, imenovano fotoidentifikacija, pri kateri se posamezne osebkke identificira na podlagi fotografij naravnih oznak na hrbtnih plavutih. Posamezne osebkke prepoznamo po edinstvenih brazgotinah, zarezah in oblikah hrbtne plavuti. Ta metoda omogoča pregled pogostosti in natančnega datuma pojavljanja identificiranih osebkov. Hrbtne plavuti mladičev so na splošno brez posebnih oznak, po katerih bi jih lahko prepoznali in spremljali, zato jih identificiramo s pomočjo samice, s katero so v tesnem stiku (Robinson in sod. 2017).

V obdobju med letoma 2013 in 2020 je bilo opaženo 92 samic z mladiči. Da pa bi bili rezultati čim bolj natančni in reprezentativni, je bilo v analizo parametrov, razen v primeru rodnosti, vključenih le 26 samic, ki so bile redno opazovane v raziskovalnem obdobju. Največje število rojstev je bilo zabeleženo v toplejših mesecih, med junijem in avgustom, kot je bilo zabeleženo tudi v drugih populacijah. Rodnost je bila ocenjena za obdobje med letoma 2013 in 2018 (obdobje, za katero so na voljo tudi ocene velikosti celotne populacije). Njena povprečna vrednost (0,02) je manjša v primerjavi z drugimi populacijami, z vrhuncem v letu 2014, upadom v letu 2015 in ponovno rastjo od leta 2016 do leta 2018. Rezultati so tudi pokazali večji reproduktivni uspeh določenih samic v primerjavi z drugimi iz populacije. Nekatere samice so vse mladiče izgubile še preden so le-ti dosegli minimalno starost odstavitve, nekatere samice pa so vse mladiče uspele obvarovati do tega obdobja. Povprečna dolžina obdobja med dvema kotitvama je bila 2,8 let, kar je bilo pričakovano, glede na to, da je samica breja 12 mesecev, laktacija traja okoli 19 mesecev, nekaj mesecev pa je potrebno za »počitek« (Harrison in Ridgwa 1971; Perrin in Reilly 1984; Haase in Schneider 2001). Takšna povprečna vrednost je zabeležena tudi v drugih populacijah velike pliskavke, ampak z večjim razponom, ker je vrednost odvisna od usode prejšnjega mladiča. To pomeni, da v preučevani populaciji mladiči ostanejo krajši čas v stiku z materjo, samice pa lahko skotijo novega mladiča vsaki dve leti, če predhodni mladič umre. Povprečno preživetje mladičev velike pliskavke z vrednostjo 0.80 je podobno preživetju mladičev iz drugih delov sveta, največja smrtnost pa je bila pri mladičih, ki so bili mlajši kot eno leto. Zgodovina

opažanj ene izmed samic je pokazala, da je le-ta skotila prvega mladiča pri starosti 7 let, kar je manjša starost kot pri večini drugih populacijah.

Pri interpretaciji rezultatov je potrebne nekaj previdnosti. V določevanju obdobja med dvema kotitvama lahko pride do odklonov, saj nekatera rojstva niso nujno zabeležena, ali pa raziskava ne traja dovolj dolgo, da bi zabeležili najdaljši možni interval. Če nekatere samice niso redno opažene, lahko pride do napak v določevanju smrtnosti in preživetja, ker je mladič morda poginil preden je samica opažena po kotitvi. Ker je to prva analiza razmnoževalne ekologije velike pliskavke v Tržaškem zalivu, lahko služi kot osnova za nadaljnje raziskave, vključno z ugotavljanjem morebitnih razlik med socialnimi skupinami ter vpliva človeških aktivnosti na razmnoževalne parametre.

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