



# DOLPHINS WITHOUT BORDERS

Final report

Tethys Research Institute, 30 April 2019



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## 1. Executive Summary

A one-year project, “Dolphins Without Borders” (DWB), to support the conservation of common bottlenose dolphins *Tursiops truncatus* within the Italian waters of the Pelagos Sanctuary, was jointly funded in 2018 by the Prince Albert II of Monaco Foundation and by the Pelagos Sanctuary. The project was conducted in collaboration amongst the Italian organisations Tethys Research Institute, Fondazione Acquario di Genova ONLUS, CE.TU.S. and Università di Sassari, and the French NGO GIS3M (“Group d’Intérêt Scientifique pour les Mammifères Marins de Méditerranée”). The main goal of DWB was to extend to the Italian portion of Pelagos actions that had been previously implemented in French waters by GIS3M. DWB contributed to accrue updated and comprehensive information on bottlenose dolphin ecology and conservation status in the north-western Mediterranean. Efforts conducted within DWB, which began in May 2018 and ended in April 2019, facilitated advances in local knowledge of the species’ abundance, distribution, movement patterns, habitat selection, threats and population structure within the waters off Liguria and Tuscany. In Summer 2018 research conducted by Tethys, Fondazione Acquario di Genova and CE.TU.S. resulted in >12,500 km of sea surveys on effort, yielding 101 sightings of bottlenose dolphins (for a total of 249 individuals), over 170 hours of behavioural and photo-identification data collection, and 132 minutes of acoustic recordings. Results included a population estimate (based on mark/recapture of photo-identified individuals) of 200 (95% C.I. 191-232). Dolphins were widely distributed across the shallow waters of the study area within the 100 m isobath, and exhibited a tendency for wide west – east movements across their habitat, from western Liguria to Versilia and the Tuscan Archipelago, confirmed by repeated re-sightings of identified individuals across the range, with a mean travelled distance per individual of 125 km (range: 3,5-464 km; median: 86 km). By contrast, connectivity between coastal waters off continental Italy and continental France was more modest, with only 4 matches between the Italian and French catalogues. This, however, does not indicate the existence of separate populations, as no population structure was detected in genetic analyses; these revealed the presence of a single population in the northwestern Mediterranean, distinct from a population in the Gibraltar/Cadiz area (although some level of gene flow was evident between the two). The above findings will be the subject of conference presentations (e.g., to the World Marine Mammal Conference, Barcelona, December 2019) and one or more scientific papers to be developed in the coming months. Based on the results of these studies, the current coverage of Natura2000 sites to protect bottlenose dolphins within the Pelagos Sanctuary, Italian side, appeared to be inadequate both in terms of their locations and in terms of their area extent; suggestions for the improvement of the network are included in this report. During the course of the project three training/information workshops were organised: in Caprera (Sardinia) on 28 February (30 participants), in Isola d’Elba (Tuscany) on 5 March (50 participants), and in Genoa on 13 March 2019 (40 participants). Significant attention was paid to lay the foundations for the continuation of monitoring efforts by existing MPA/National Park management bodies and local research groups, on the basis of shared data collection protocols, thereby enhancing the future sustainability of obtained results. Finally, a DWB FaceBook page and a website were created. The latter (<https://www.dolphinswithoutborders.net>) in Italian and French, includes not only basic information on the project activities but also an extensive list of scientific bibliography relevant to bottlenose dolphins research and conservation in the Pelagos Sanctuary. The site also contains a download section where a brochure on the project can be obtained, as well as a form to report bottlenose dolphin sightings.

## 2. Administrative details

**DOLPHINS WITHOUT BORDERS** Final report

- Study required and financed by:

**Permanent Secretariat of the Pelagos Agreement**

Tour Odéon B1 – 36, avenue de l'Annonciade, 98000 Monaco

and

**Prince Albert II of Monaco Foundation**

Villa Girasole", 16, Boulevard de Suisse, 98000 Monaco

- Project leader:

Simone Panigada, PhD. Tethys Research Institute - President

- With the participation of

**Tethys Research Institute**

**GIS3M**

**Fondazione Acquario di Genova**

**CE.TU.S.**

**Università di Sassari**

**SeaMe Sardinia**

- Reference of the project 2018-09

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J.Gonzalvo / Tethys Research Institute

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### 3. Specific Goal 1: Supporting the conservation of common bottlenose dolphins *Tursiops truncatus* in the Pelagos Sanctuary

#### 3.1. Activity A1. Field campaigns

Field work campaigns were conducted in summer 2018 in the Italian continental waters of the Pelagos Sanctuary to collect photo-identification and ecological data. The northern area was covered by the Tethys Research Institute, the north-eastern area by Fondazione Acquario di Genova ONLUS, and the eastern area by CE.TU.S. Brief background information on the three actors dealing with this dolphin monitoring initiatives are presented in the following section.

- The Cetacean Sanctuary Research (CSR), by **Tethys Research Institute - TRI**, is a long-term project established in 1990. It is focussed on the ecology, behaviour, feeding habits and conservation of all the cetacean species living in the Pelagos Sanctuary, including bottlenose dolphins, and the main research techniques used are: visual and acoustic surveys, photo-identification, vocalizations recordings and other acoustic data collection, behavioural sampling and respiration patterns, photogrammetry of sperm whales, faecal sampling.
- **Fondazione Acquario di Genova - FAdG** was founded in 2003 to promote public awareness and education to aquatic environment conservation, management and responsible use. This mission is carried out through communication campaigns and scientific research. *Delfini Metropolitan* started with support from FAdG in 2001 and was adopted by FAdG since 2003. This is a long-term project developed with the main objective of assessing the presence, abundance and conservation status of the bottlenose dolphins along the coast of the Pelagos Sanctuary. Since 2009, geo-referred and photographic data are regularly compared with data produced by different research groups operating along the Italian and French coasts of the Sanctuary. Moreover, FAdG developed a Web-GIS platform to support data aggregation and integrated analysis: Intercet ([www.intercet.it](http://www.intercet.it)) for Regione Liguria within GIONHA (Governance and Integrated Observation of marine Natural Habitat) a project funded by the EU Cross Border Cooperation Programme.
- **CE.TU.S. Cetacean Research Center** was founded in Viareggio in 1999 by marine biologists and engineers. It conducts research and data collection about cetaceans inside the Pelagos Sanctuary, specially focussing on bottlenose dolphins inhabiting Tuscany waters. CE.TU.S. sea survey campaigns occur year-round between La Spezia and Livorno (core area in front of Viareggio) and in the north of Tuscany archipelago (Gorgona, Capraia and Elba islands). It participates in regional, national and international project in collaboration with Tuscany Administration, ARPAT, CIBM, Italian and European Research Centers and others. Moreover, CE.TU.S. also conducts actions on environmental education and provides trainings opportunities to university students. Since 2008 it is the responsible for the Information Point O.T.B (Tuscany Observatory for Biodiversity).

#### Methods

Tethys Research Institute data were collected during dedicated boat surveys, conducted between June and September 2018, aboard “Pelagos”, a motor sailing vessel (21 m long). Surveys were conducted *ad libitum* without following any predesigned routes. Nevertheless, data were predominantly collected along North-to-South transects surveyed at a mean cruising speed of about 6 knots. Fieldwork protocols were standardized and all the observations were made under favourable sea and weather conditions. The searching effort was interrupted when wind exceeded Beaufort 3 (wind speed higher than  $5.4 \text{ m s}^{-1}$ ). During the visual searching effort, two trained and experienced observers were

positioned, one at each side of the vessel at a height of approximately 3 m above the sea surface. Concurrently, an acoustic survey was conducted by using an hydrophone array (H1c, version 2018 - 110m cable polyurethane jacketed and Kevlar reinforced with two wideband hydrophones and separate preamps). Effort, environmental conditions and sighting data were recorded regularly by using dedicated software PAMGUARD 1.15.14. Searching and sea state status were recorded every 30 min, or whenever changes in any variables defining survey conditions occurred.



Similarly, both FAdG (*Delfini Metropolitan*) and CE.TU.S. Research Center conducted surveys exclusively in good sea conditions (<4 on the Douglas Scale), following a random track within their respective study areas. All the geo-referred data were recorded through GPS device: effort track, sighting position of the target species (start and end sighting point), sighting track.

During the sighting, the following data were collected by all three partners: species involved in the observation, number of individuals, presence and number of offspring (calf/newborn), association with other species (e.g., birds, fish), association with human activity (e.g., fishing boats, pleasure boats). Photographic data for individual photo-identification were collected with digital reflex cameras equipped with zoom lenses.

**Photo-identification** is based on long-term natural marks such as nicks and notches in the dolphin's dorsal fins, as well as on any additional marks that could be observed in other parts of the body. At each dolphin sighting, researchers aimed at obtaining as many good photographic images as possible of every individual present, trying to avoid biases towards any particular individual. During the analysis, all digital photos are being selected using consistent criteria (i.e. entire dorsal fin visible, fin perpendicular to camera, high sharpness and resolution, no water spray masking fin profile). The best images of every dolphin during each sighting is then selected and compared with a catalogue of identified individuals. Whenever a match is not found, the individual must then be given a unique identification code and added to the catalogue. Identifications and details relating to dolphin group/sighting membership have been recorded on a database to construct individual sighting histories.



## Results

### Research effort

Fieldwork conducted by all three partners across 203 daily surveys resulted in 12,514 km of navigation at sea on effort, yielding a total of 101 sightings of bottlenose dolphins. The direct observations of dolphin groups lasted over 170 hours and a total of 15,930 digital images were taken during photo-identification effort. See table 1 below for detail.

| Research team                           | CSR-Tethys | FAdG         | CE.TU.S.     | TOTAL        |
|---|------------|--------------|--------------|--------------|
| Months at sea                           | 4          | 4            | 11 (Feb-Dec) | 19           |
| Days at sea                             | 63         | 27           | 113          | 203          |
| Survey effort (km)                      | 6,198      | 1,211        | 5,105        | 12,514       |
| Sightings of <i>T. truncatus</i>        | 12         | 25           | 64           | 101          |
| Sightings of other species              | 182        | -            | 3            | 185          |
| Time spent with <i>T. truncatus</i>     | 6 h 52 min | 106 h 29 min | 57 h 06 min  | 170 h 27 min |
| Photos taken                            | 2,140      | 5,000        | 8,790        | 15,930       |
| <i>T. truncatus</i> acoustic recordings | 132 min    | -            | -            | 132 min      |

Table 1. Research effort conducted by all three DWB partners responsible for conducting fieldwork.

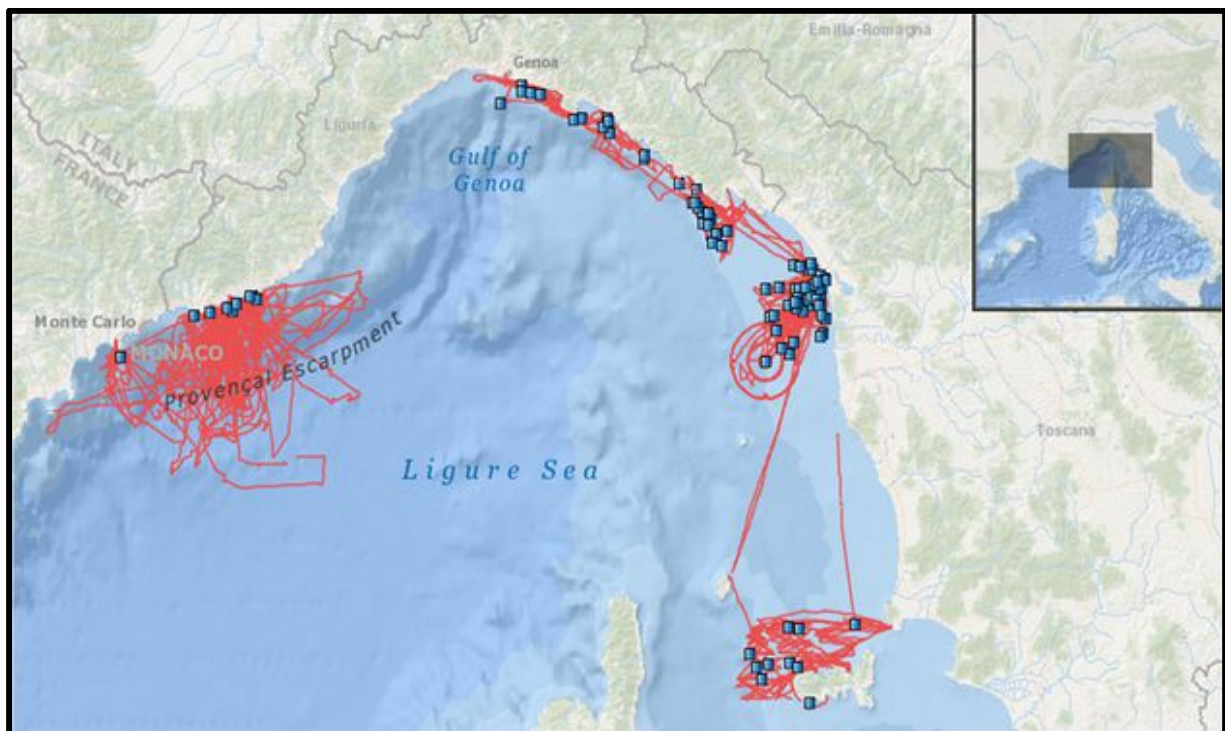


Figure 1. Total research effort conducted by all three DWB partners responsible for conducting fieldwork. Blue squares represent bottlenose dolphin sightings locations and red tracks the navigation conducted on survey effort.

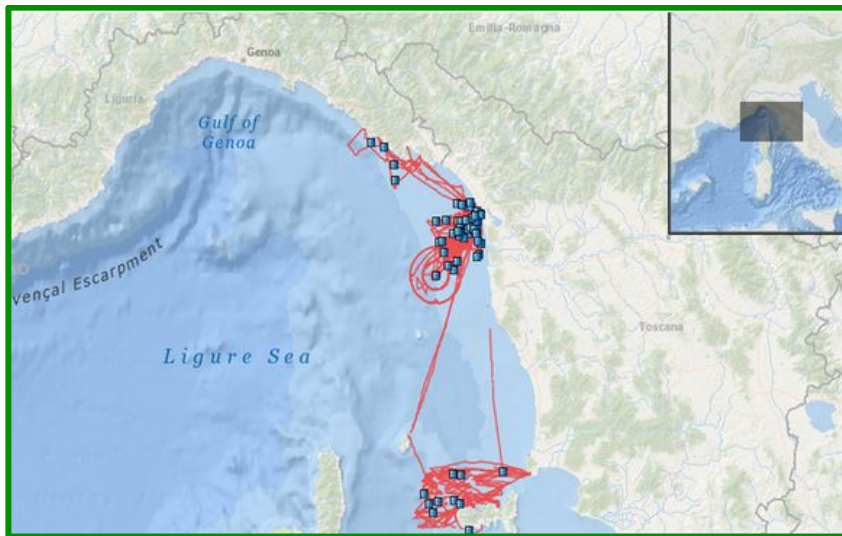
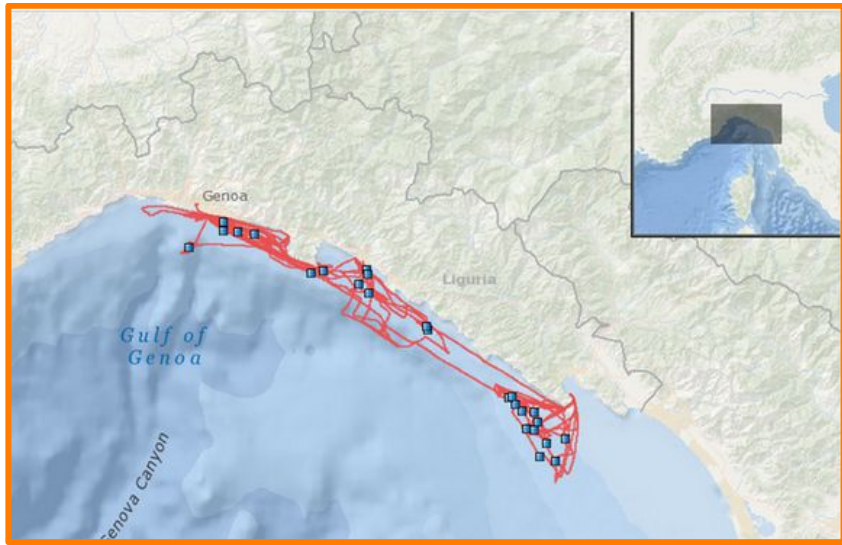
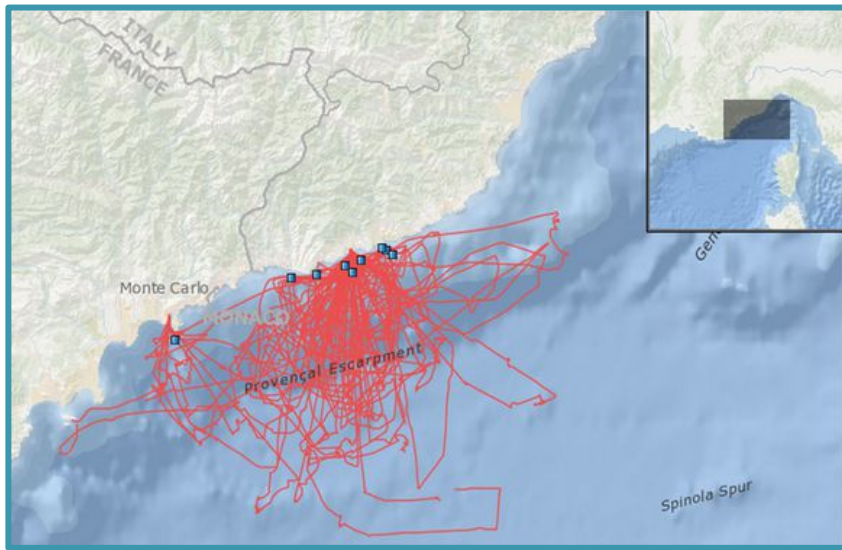


Figure 2. Research effort conducted by CSR-Tethys (top), **Fondazione Acquario di Genova** (middle) and **CE.TU.S. Cetacean Research Centre** (bottom).



**Assessment of encounter rates (ER) throughout the project area.**

The study area was divided into 3312 cells of about 9x7 km by means of GIS tools (ESRI GIS, Spatial Analyst and 3D Analyst extensions, Fig 3). Searching effort was evaluated as kilometres of track line per cell unit. Only the effort in favourable conditions was considered (i.e. wind conditions lower than 3 according to the Beaufort scale).

The encounter rate for every dataset considered was calculated for each cell as the number of sightings per km surveyed under favourable condition. The overall encounter rate was then calculated merging all the datasets (Fig. 3).

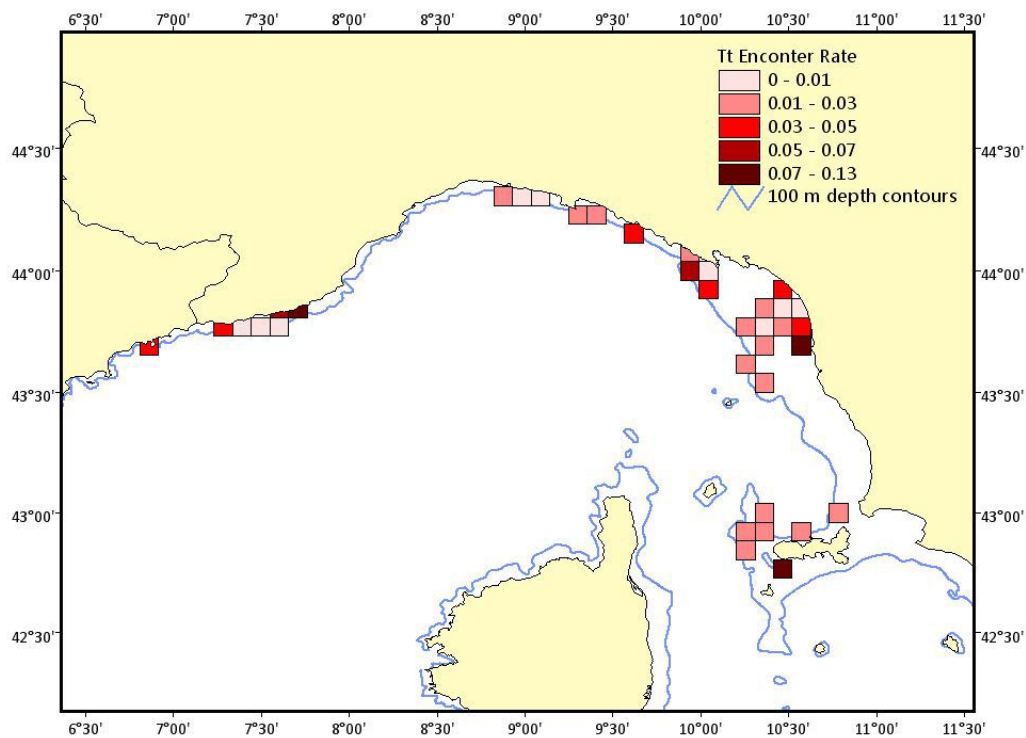


Figure 3. Common bottlenose dolphin Encounter Rate.

| Research Group | Positive effort (km) | N. Tt sightings | E.R.   |
|----------------|----------------------|-----------------|--------|
| TRI            | 3165                 | 12              | 0.0038 |
| CE.TU.S.       | 4302                 | 62              | 0.0144 |
| DM FAdG        | 1237                 | 25              | 0.0202 |
| Total          | 8703                 | 99              | 0.0114 |

Table 2. Summary of the positive research effort made available from the three institutes. Total number of sightings and the overall encounter rates are also shown.

The three areas surveyed by the three institutes have different encounter rates (Kruskal-Wallis H: 6.02;  $P < 0.05$ , see Fig.20). Particularly, while the encounter rates between CE.TU.S. and FAdG (DM Project) are comparable (Mann-Whitney U: 18;  $P > 0.05$ ), the encounter rate in the TRI area seems to be significantly lower. This is also consistent with the available abundance estimates based on photoID records (Nuti et al., 2006; Gnone et al., 2011; De Santis et al., 2018).

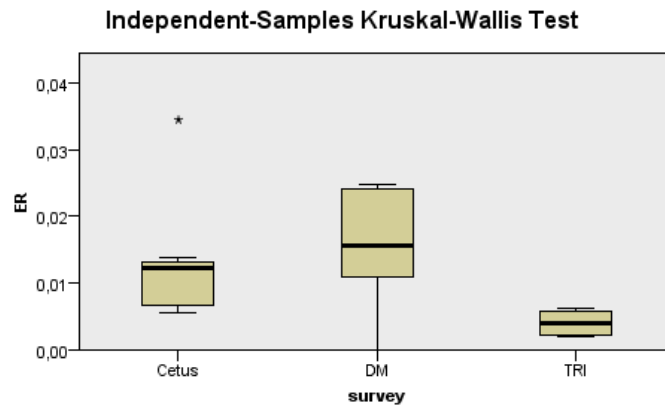


Figure 4. Bottlenose dolphin Encounter Rate by organisation.

The temporal variability of bottlenose occurrence has been investigated by analysing the encounter rate among months (Fig. 5).

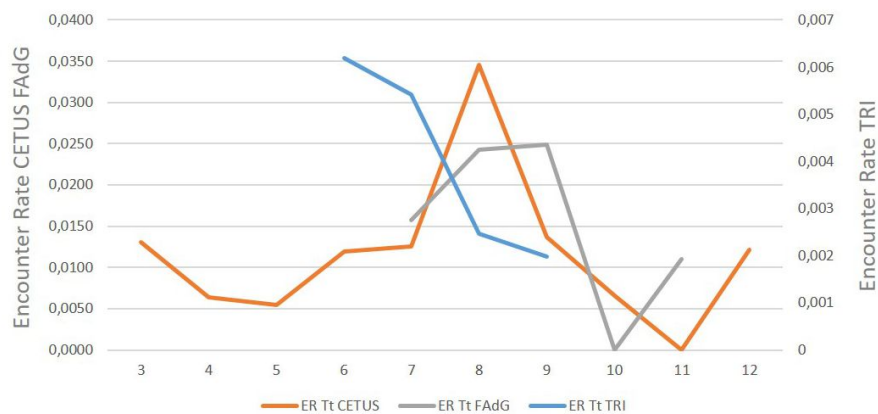


Figure 5. Bottlenose dolphins Encounter Rate per month by research team.

The main difference seems to be the lack of correlations in the monthly pattern being FAdG and CETUS more similar ( $r = -0.998$ ;  $P < 0.05$ ) while the encounter rates in the TRI area shows a negative correlation with FAdG suggesting a possible temporal shift in the use of the three areas.

**Probability map of the species presence obtained from the analysis of presence/absence data.**

Binary logistic regression analysis (Afifi and Clark. 1996; Guisan and Zimmermann. 2000) was used to correlate presence/absence data of the species to the physiographic (i.e. depth and slope predictors). All the analyses were performed using the IBM SPSS Statistics package (version 25). Depth data obtained through the GEBCO One minute Digital Atlas were gridded by means of the Spatial Analyst extension GIS software. Slope was also calculated by using the Spatial Analyst tool according to Burrough (1986). Static predictors considered as potential covariates for each cell are listed in Table 8. Absence cell (or pseudo-absence) was considered any cell with effort without sighting of bottlenose dolphin.

| Cell physical characteristics |
|-------------------------------|
| Mean Depth (m)                |
| Minimum Depth (m)             |
| Maximum Depth (m)             |
| Standard deviation Depth (m)  |
| Depth Range (m)               |
| Mean Slope (%)                |
| Minimum Slope (%)             |
| Maximum Slope (%)             |
| Standard deviation Slope (%)  |
| Slope Range (%)               |

**Table 3.** Static predictors considered as potential covariates in the presence/absence model.

As the presence/absence data set was zero-inflated, the number of absence cells was balanced to the number of presence cells through a random extraction of absence cells (e.g. the balanced number of absence cells was extracted through the Mersenne Twister random number generator, Matsumoto and Nishimura, 1998).

In order to select the best set of predictors. a forward stepwise method based on the Wald statistic was used (Hosmer & Lemeshow. 2000). The variable with the largest probability greater than the specified threshold value is removed and the model is re-estimated. The procedure stops when no more variables meet entry or removal criteria or when the current model is the same as a previous model. The parameters and corresponding statistics of the model selected through the stepwise procedure are shown in Table 4.

|            | B     | S.E. | Wald   | df | Sig. | Exp(B) | 95% C.I. for EXP(B) |       |
|------------|-------|------|--------|----|------|--------|---------------------|-------|
|            |       |      |        |    |      |        | Lower               | Upper |
| Depht_mean | -.052 | .014 | 13.519 | 1  | .000 | .949   | .923                | .976  |
| Depth_max  | .019  | .006 | 11.854 | 1  | .001 | 1.019  | 1.008               | 1.031 |
| Constant   | 2.340 | .682 | 11.769 | 1  | .001 | 10.378 |                     |       |

**Table 4.** Bottlenose dolphin presence/absence model using physiographic features (i.e depth mean and max) as predictors.

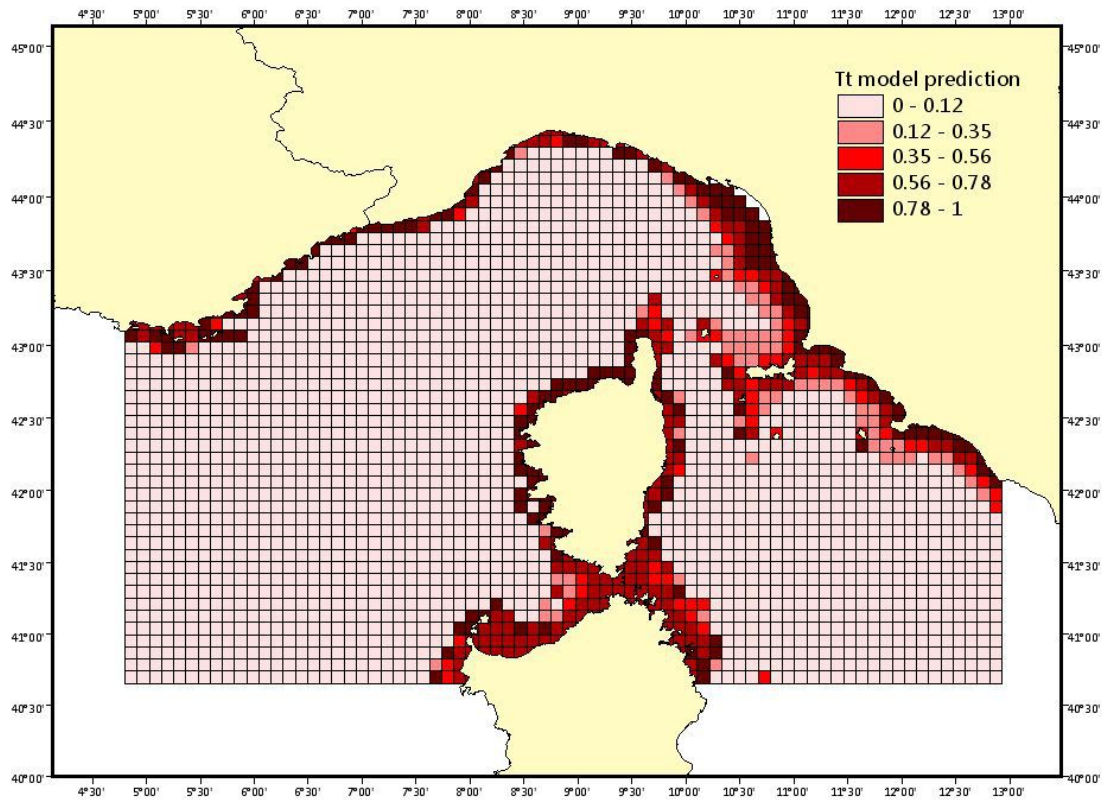
**NOTE:** The following statistics are shown: **B:** unstandardized regression coefficient; **S.E.:** Standard Error of B; **Wald** statistic for the included parameter; **df:** degrees of freedom; **Sig:** level of significance; **Exp(B):** estimated odds ratio; **95% C.I.** Confidence Interval for exp(B).

| Observed |      |                    | Predicted |          | Percentage correct |
|----------|------|--------------------|-----------|----------|--------------------|
|          |      |                    | Tt01      |          |                    |
| Step 2   | Tt01 | Absence            | 29        | 7        | 80.6               |
|          |      | Overall percentage |           | Presence | 4                  |
|          |      |                    |           |          | 84.3               |

a. Cut value is ,500

**Table 5.** Confusion Matrix of the model using bathymetric feature as predictors (See Table 4).

Figure 6 shows the probability map of the species presence within the Pelagos Sanctuary based on the predictors shown in Table 4. These results are consistent with the ones obtained in a previous study by Azzellino and colleagues (2012).



**Figure 6.** Probability map of the species presence within the Pelagos Sanctuary based on the topographic predictors (as shown in Table 4).

### Abundance estimates

A standardized protocol with specific guidelines was followed by the three data providers (TRI-CETUS - FAdG) to select and classify the photos resulting in a unified catalogue. The standardization of photographic procedures concerned the preparation of the images, the selection of those to be included in the photo-identification catalogue, the cropping of the images (Figure 7) and the assignment of the scores concerning photographic quality and distinctiveness of the individual marks (see Airoidi et al, 2015). A unified photo-id catalogues of well-marked individuals was considered considering both sides right and left.



Figure 7. Preparation and cropping of the common bottlenose dolphin images.

| Research Team                 | Species                   | N. of Sightings | N. individuals | Study period        |
|-------------------------------|---------------------------|-----------------|----------------|---------------------|
| Tethys Research Institute     | <i>Tursiops truncatus</i> | 12              | 52             | June-September 2018 |
| Fondazione Acquario di Genova | <i>Tursiops truncatus</i> | 25              | 90             | July-November 2018  |
| CE.TU.S.                      | <i>Tursiops truncatus</i> | 56              | 107            | March-October 2018  |

Table 6. Summary of the photo-identification catalogues by organisation. The shared photo-identification catalogue resulted in a total of 185 photo-identified individuals.

In order to apply mark-recapture methods, a dataset of capture histories per encounter was created using the individual photo-identification records. A capture was defined as an individual identification within an encounter.

Given the uneven photographic effort, mark recapture methods were not applied to the whole dataset and data were pooled based on the homogeneity of the photographic effort. Only the summer months (June to September) had enough recaptures to allow robust abundance estimates through mark recapture methods based on the “month” as primary sampling interval (Figure 8).

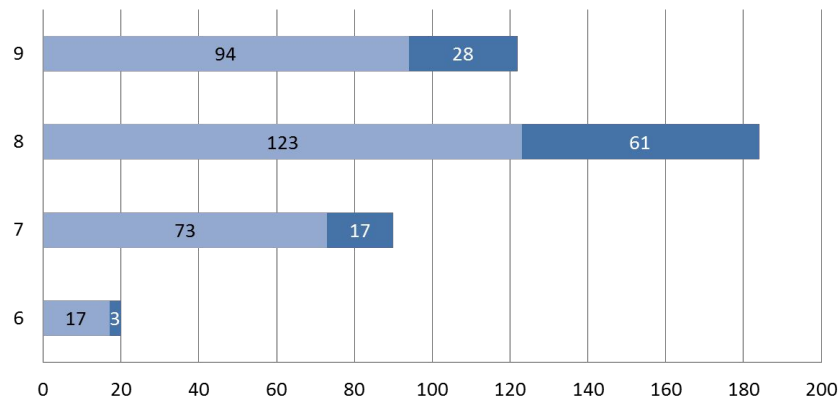


Figure 8. Number of total capture and recaptures (the latter reflected in dark blue) of photo-identified individuals by month (6-June; 7-July; 8-August; 9-September)

Individuals sighted up to 2 times represented the 60% of the total captures. 1 individual has been sighted 10 times by CE.TU.S (ID CODE: A331) between June and September in the eastern part of the Pelagos Sanctuary.

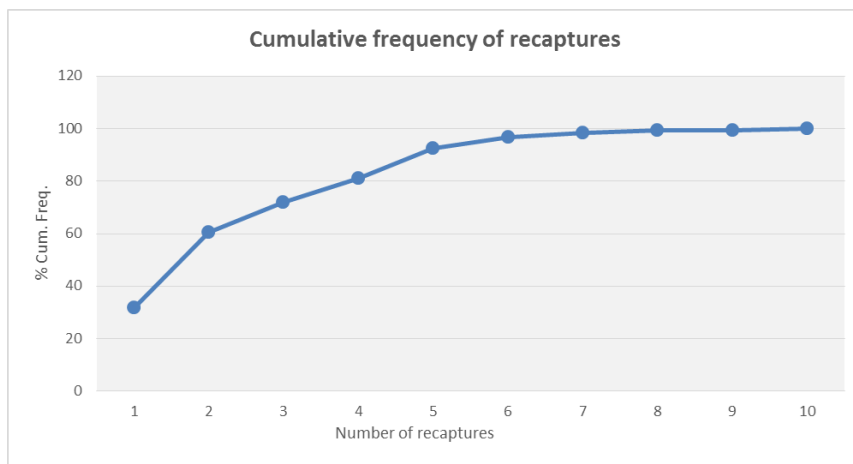


Figure 9. Cumulative frequency of recaptures.

Estimates of abundance were obtained, assuming closed population models. Time dependent models were tested with closed population models. Capture histories were analysed using the CAPTURE application run within program MARK (Mark and Recapture Parameter Estimation) v.8.0 developed by the Department of Fishery and Wildlife, Colorado State University (White and Burnham, 1999). This application has 9 available models that test for 3 sources of variation in sightings probabilities (Otis et al., 1978): that of (i) a time response, which considers that a sighting probability varies from sampling

period to sampling period but that all animals within each sampling period have the same probability of being sighted (Mt), plus one additional model where probability of capture remains constant (M0). In program MARK a testing procedure is available allowing the user to compare alternative models to assess which effects are operating, and then estimate population size using the most appropriate model. As far as the closed population models are concerned, the time model (Mt), assuming that sighting probability varies from sampling period to sampling period but the same probability of being sighted for all animals captured within each sampling period, was selected as most appropriate in most of the situations. Mark-recapture models based on the closed population assumption (Mt Darroch and Mt Chao models) outlined that bottlenose dolphin abundance in the period June-July 2018 was in average around 90 individuals (95% C.I. 83-124) peaking up to 170 individuals in average (95% C.I. 162-192) in the period August-September. The overall period estimate (June to September) provided an estimate of about 200 individuals (95% C.I. 191-232).

| Period           | Capture occasions | Captures | Recaptures | Model               | N          | SE   | lower 95%CI | upper 95%CI | p-hat prob. Capture    |
|------------------|-------------------|----------|------------|---------------------|------------|------|-------------|-------------|------------------------|
| June-July        | 2                 | 77       | 13         | M(o)                | <b>153</b> | 29.6 | 114         | 235         | 0.29                   |
|                  | 2                 | 77       | 13         | <b>M(t) Darroch</b> | <b>93</b>  | 9.6  | 83          | 124         | 0.18 0.79              |
|                  | 2                 | 77       | 13         | <b>M(t) Chao</b>    | 94         | 10.8 | 83          | 130         | 0.18 0.78              |
| Period           | Capture occasions | Captures | Recaptures | Model               | N          | SE   | lower 95%CI | upper 95%CI | p-hat prob. Capture    |
| August-September | 2                 | 150      | 66         | M(o)                | <b>176</b> | 8.3  | 163         | 195         | 0.62                   |
|                  | 2                 | 150      | 66         | M(t) Darroch        | <b>172</b> | 7.5  | 162         | 192         | 0.71, 0.54             |
|                  | 2                 | 150      | 66         | M(t) Chao           | <b>173</b> | 7.7  | 163         | 193         | 0.71, 0.55             |
| Period           | Capture occasions | Captures | Recaptures | Model               | N          | SE   | lower 95%CI | upper 95%CI | p-hat prob. Capture    |
| June-September   | 4                 | 173      | 94         | M(o)                | <b>303</b> | 8.0  | 192         | 223         | 0.37                   |
|                  | 4                 | 173      | 94         | <b>M(t) Darroch</b> | <b>193</b> | 6.1  | 185         | 209         | 0.09, 0.38, 0.64, 0.48 |
|                  | 4                 | 173      | 94         | <b>M(t) Chao</b>    | <b>206</b> | 10.3 | 191         | 232         | 0.08, 0.35, 0.60, 0.45 |

Table 7. Population Size Estimates (N) based on MR estimators from Photo-identification of Common Bottlenose Dolphins in the Pelagos Sanctuary.

No adjustment was applied to these estimates to take into account the unmarked or poorly marked individuals (e.g. Williams et al., 1993) since the data to calculate the proportions of well-marked and poorly marked individuals were not available from all the partners.

### Distribution and seasonality

Research effort conducted by all three partners across 203 daily surveys collected between March and December 2018 (see pages 6-7 for detail on methods), resulted in more than 8700 km of positive effort, resulting in a total of 101 sightings of bottlenose dolphins. A unified dataset was created and sightings were georeferenced by using GIS software (QGIS 2.18.x) (Figure 10). Position was not available for 2 sightings belonging to CE.TU.S dataset. Therefore, only 99 sightings have been considered for the distribution analysis.

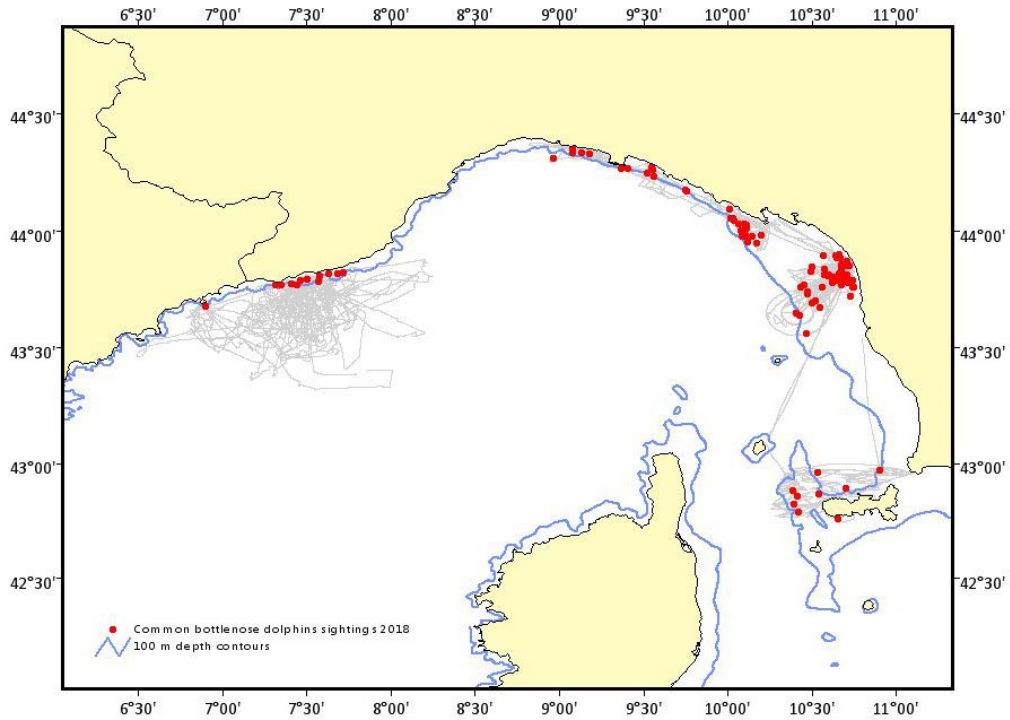


Figure 10. Tracks (in grey) and sightings (in red) collected by the three data providers. The 100 m bathymetry is also shown (blue line).

Most of the sightings occurred in shallow waters within the 100 m isobath. Higher sightings number has been recorded in July and August (Table 8; Figure 11).

| Month | Number of Sighting |          |      |
|-------|--------------------|----------|------|
|       | TRI                | CE.TU.S. | FAdG |
| 3     |                    | 2        |      |
| 4     |                    | 3        |      |
| 5     |                    | 3        |      |
| 6     | 3                  | 4        |      |
| 7     | 5                  | 11       | 4    |
| 8     | 3                  | 26       | 11   |
| 9     | 1                  | 9        | 9    |
| 10    |                    | 2        |      |
| 11    |                    |          | 1    |
| 12    |                    | 2        |      |

Table 8. Summary of the Common bottlenose sightings by research team



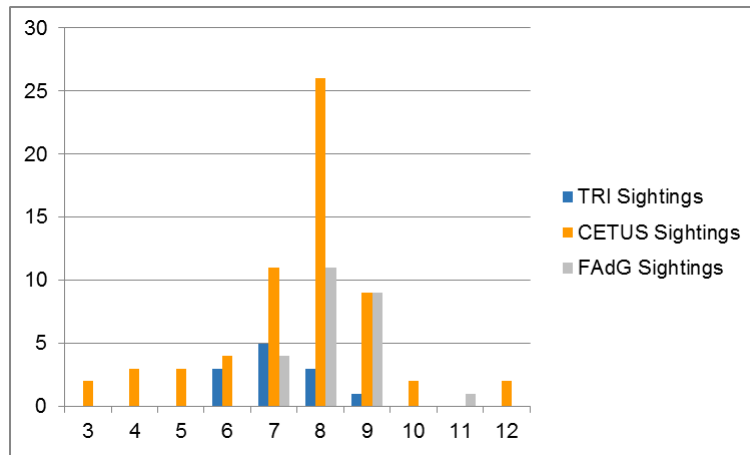


Figure 11. Bottlenose dolphin sightings per month by each research team.

### Home range and movements

Movements of bottlenose dolphins were analysed by comparing photo-identification data collected by each one of the three research teams. 53 different individuals were found shared among the three partner catalogues. Tethys catalogue has 25 individuals in common with the FAdG catalogue. The same number of individuals has been found as common with FAdG and CETUS. Only 2 individuals were present in all the three catalogues (i.e. Tt7 and Tt20). These were adult females sighted with newborn calves between July and September. Six Individuals reported in both TRI and FAdG were sighted with newborn calves while other 6 were seen with older calves (see summary Table 10).

| Catalogue          | Species                   | Individuals in common |
|--------------------|---------------------------|-----------------------|
| TRI - FAdG         | <i>Tursiops truncatus</i> | 25                    |
| TRI - CETUS        | <i>Tursiops truncatus</i> | 7                     |
| FAdG - CETUS       | <i>Tursiops truncatus</i> | 25                    |
| TRI - FAdG - CETUS | <i>Tursiops truncatus</i> | 2                     |

Table 9. Number of individual shared among the three photo-id catalogues (Tethys, Fondazione Acquario di Genova and CE.TU.S.)

| ID Number | TRI          | FAdG                        | CETUS                          | Notes                |
|-----------|--------------|-----------------------------|--------------------------------|----------------------|
| Tt01      | June, July   |                             | August, September              | with Calf            |
| Tt02      | June, July   |                             | August, September              | Calf of Tt1          |
| Tt03      | June, July   | August, September           |                                | with Newborn         |
| Tt04      | June, July   | August, September           |                                |                      |
| Tt05      | June, July   | August                      |                                | with Calf            |
| Tt06      | June, July   | August, September           |                                |                      |
| Tt07      | July         | August                      | September                      | with Newborn         |
| Tt08      | July         | July, September             |                                | with Newborn         |
| Tt09      | July, August | September                   |                                | with Newborn         |
| Tt10      | July         | September                   |                                |                      |
| Tt11      | July, August | September                   |                                |                      |
| Tt12      | July         | August, September, November |                                |                      |
| Tt13      | July         | August, September           |                                | with Newborn         |
| Tt14      | July         | August                      |                                | with Newborn         |
| Tt15      | July, August | September                   |                                | with Calf            |
| Tt16      | July         | August, September           |                                |                      |
| Tt17      | July, August | September                   |                                |                      |
| Tt18      | July         | August                      |                                |                      |
| Tt19      | July         | August, September           |                                | with Calf            |
| Tt20      | July         | September                   | July, August                   | with Newborn         |
| Tt21      | July, August | August                      |                                |                      |
| Tt22      | July         | September                   |                                |                      |
| Tt23      | July, August |                             | September                      |                      |
| Tt24      | July         | August                      |                                | with Calf, Collision |
| Tt25      | July, August | August, September           |                                |                      |
| Tt26      | August       | August                      |                                |                      |
| Tt27      | August       | September                   |                                | with Calf            |
| Tt28      | August       | September                   |                                | with Calf            |
| Tt29      | August       |                             | March, September               | with Newborn         |
| Tt30      | July         |                             | August, September              |                      |
| Tt31      |              | August                      | August                         |                      |
| Tt32      |              | September                   | September                      |                      |
| Tt33      |              | August, September           | August                         |                      |
| Tt34      |              | September                   | July                           |                      |
| Tt35      |              | August, September           | August                         |                      |
| Tt36      |              | November                    | April, August                  | with Calf            |
| Tt37      |              | September, November         | July, August                   | with Calf            |
| Tt38      |              | August                      | July, August, September        | with Newborn         |
| Tt39      |              | August, September, November | August                         | with Calf            |
| Tt40      |              | August                      | July, August, September        |                      |
| Tt41      |              | September                   | July, August, September        | with Calf            |
| Tt42      |              | September                   | April, July, August, September |                      |
| Tt43      |              | August                      | July, August                   |                      |
| Tt44      |              | August, September           | August                         | with Newborn         |
| Tt45      |              | August, September           | August, September              |                      |
| Tt46      |              | August                      | August                         |                      |
| Tt47      |              | August                      | March, July, September         |                      |
| Tt48      |              | August                      | August                         |                      |
| Tt49      |              | August, September           | August                         |                      |
| Tt50      |              | August, September, November | August, September              |                      |
| Tt51      |              | September                   | April, August, September       |                      |
| Tt52      |              | August, September           | August, September              |                      |
| Tt53      |              | September                   | August                         | with Newborn         |

Table 10. Summary of bottlenose sightings by organisation.

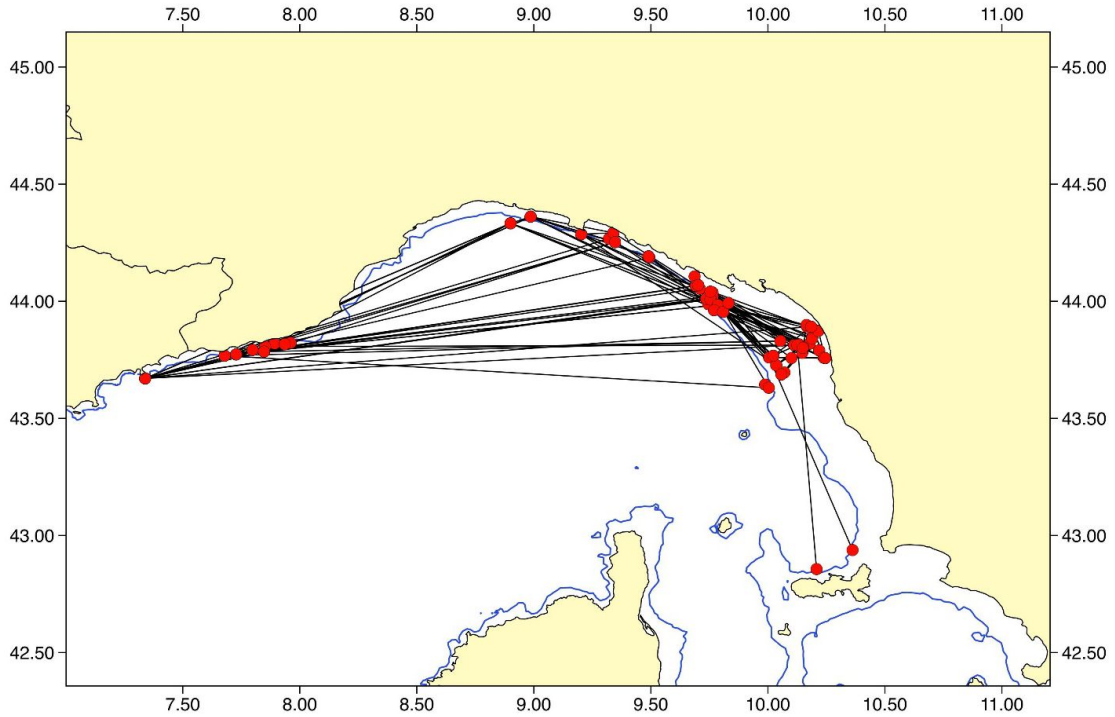


Figure 12. Overall movements of bottlenose dolphins across the study area

It appears quite clear that west to east movements across the study area are quite common for this population (see Figure 12 above). Most of the movements in fact were in the order of 215-220 km.

Table 11 shows the movement statistics concerning the whole dataset. The displacements showed in this study are larger than what was found by Gnone et al., 2011, where the average displacement were of the order of 50 km. This may possibly be the effect of the larger weight in this dataset of the northwestern individuals which may possibly travel over longer distances with respect to the other investigated dolphin groups.

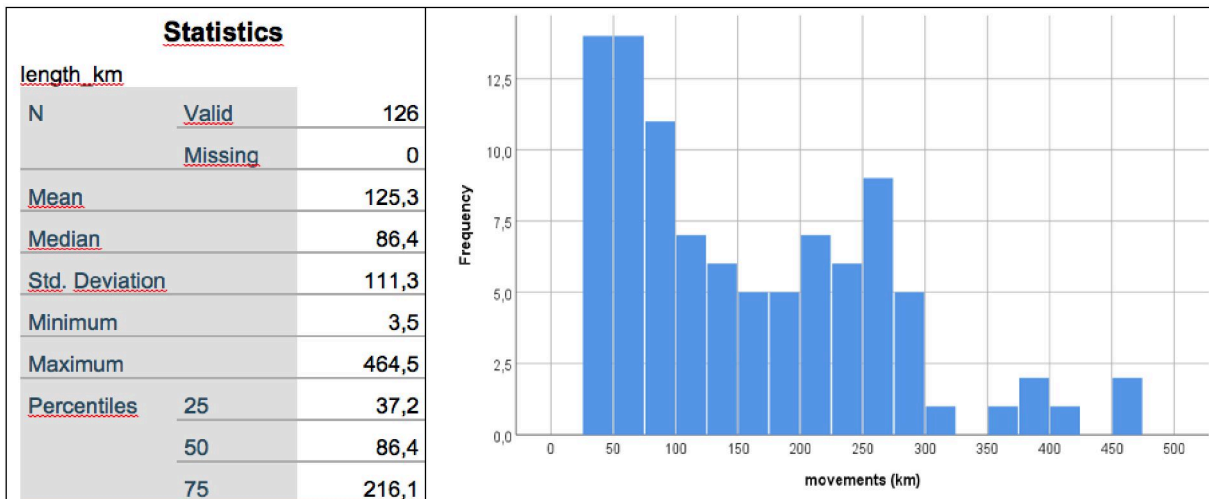


Table 11. Summary statistics of bottlenose dolphin movements.

| Descriptives |                                  |             |            |  |
|--------------|----------------------------------|-------------|------------|--|
|              |                                  | Statistic   | Std. Error |  |
| lentgh_km    | Mean                             | 221.21      | 17.901     |  |
|              | 95% Confidence Interval for Mean | Lower Bound | 185.29     |  |
|              |                                  | Upper Bound | 257.13     |  |
|              | 5% Trimmed Mean                  | 211.49      |            |  |
|              | Median                           | 215.00      |            |  |
|              | Variance                         | 16984.13    |            |  |
|              | Std. Deviation                   | 130.323     |            |  |
|              | Minimum                          | 35          |            |  |
|              | Maximum                          | 705         |            |  |
|              | Range                            | 670         |            |  |
|              | Interquartile Range              | 166         |            |  |
|              | Skewness                         | 1.246       | 0.327      |  |
|              | Kurtosis                         | 2.658       | 0.644      |  |

Table 12. Statistics of movements for all the 53 photo-identified individuals that were found to be in common between the three partners.

Figure 13 shows the histogram and the box plot of the movements of the 53 individuals found share between the three study areas.

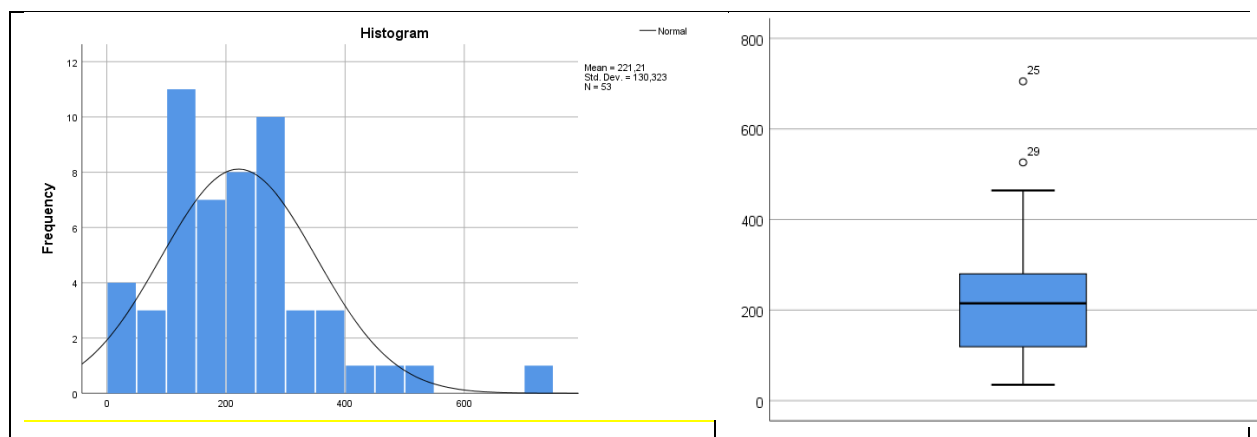


Figure 13. Histogram (left) and box plot (right) of the movements in km of the 53 individual bottlenose dolphin. The Box plot shows the distribution quartiles and the outliers (i.e. movements falling at more than 1,5 times the interquartile range from the 75th percentile).

These estimates are probably conservative since it is reasonable to believe that in their longitudinal movements the animals tend to follow their preferred habitat. That would probably lead to longer movements (i.e. in the order of 250-300 km). In the following maps are shown some of the most significant movements tracks concerning both mother and calf pairs and single individuals.

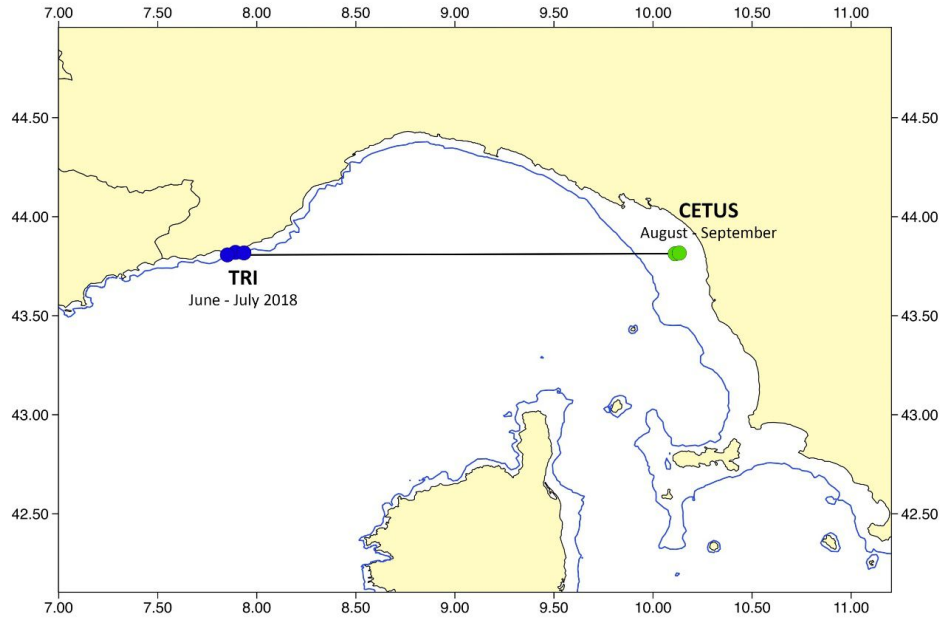


Figure 14. Movements of Tt01. Mother – Calf pair covering a linear distance of about 260 km in about 1 month.

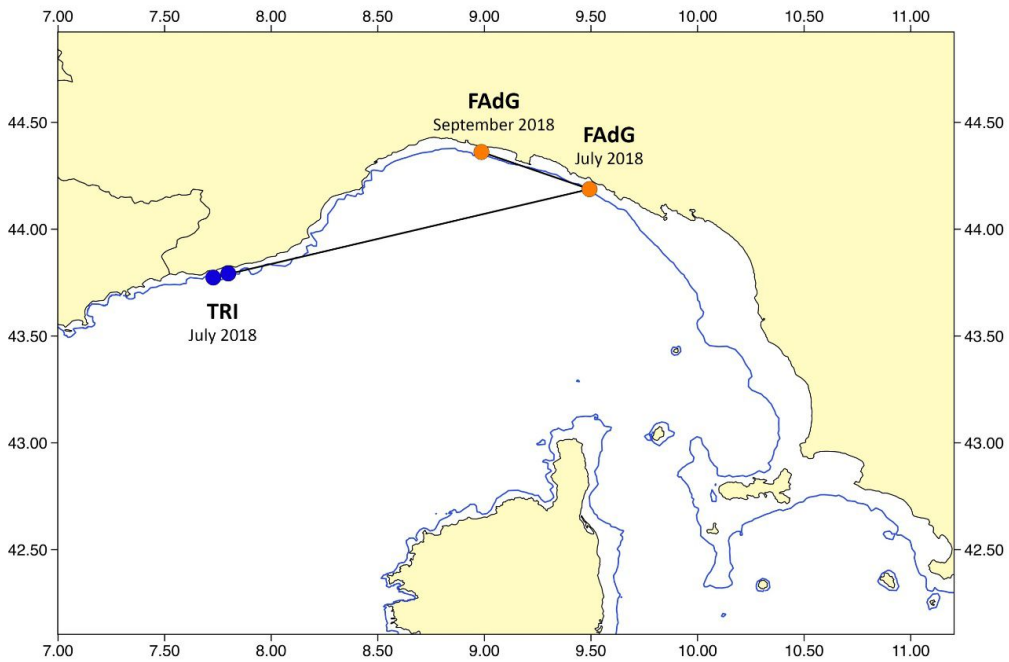
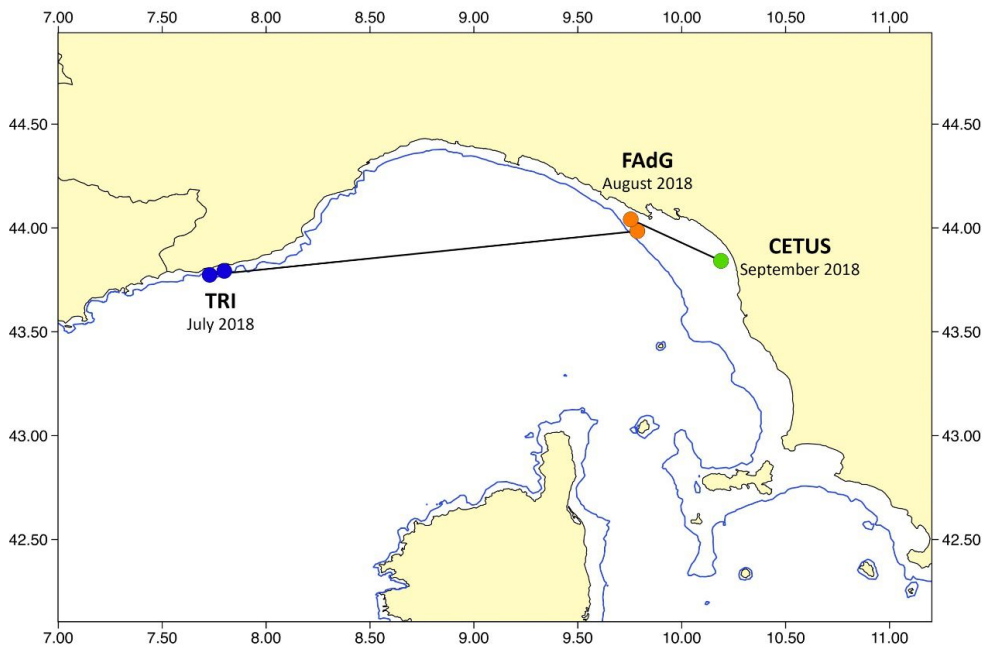
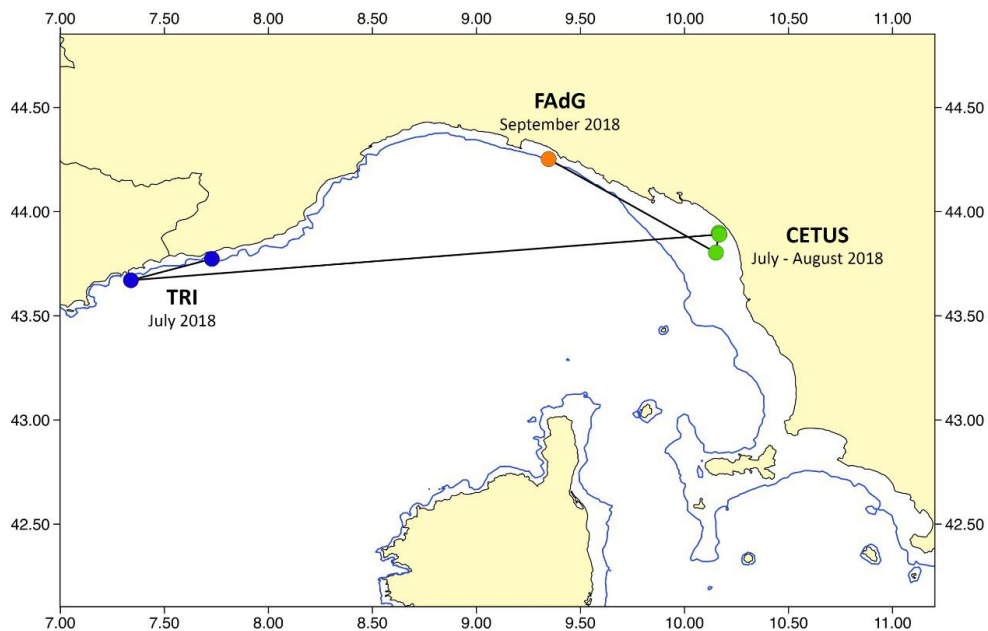


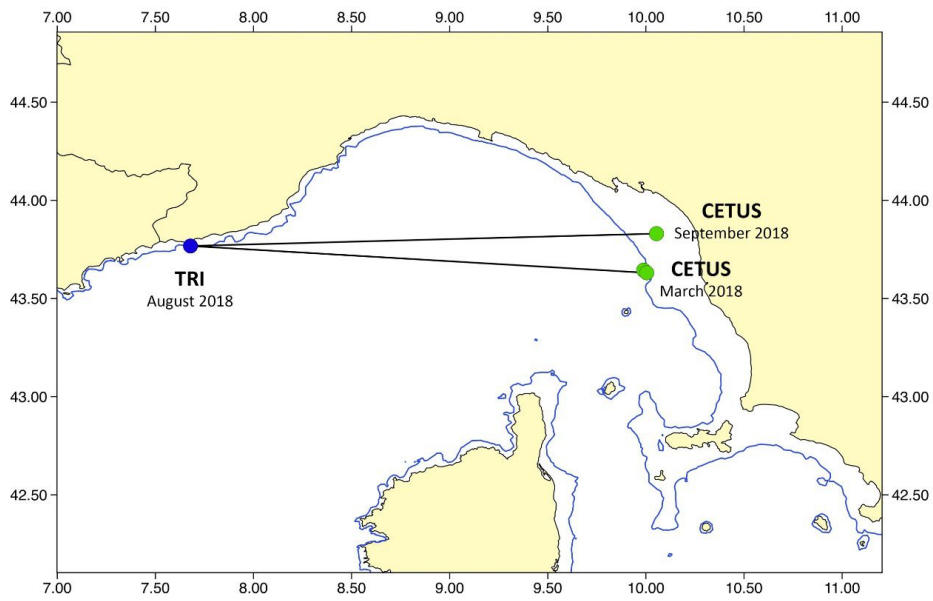
Figure 15. Movements of Tt08. Mother – Newborn pair seen in July in the western portion of the Sanctuary, then resighted in July and September after moving westwards, covering a linear distance of about 270 km in 7 days.



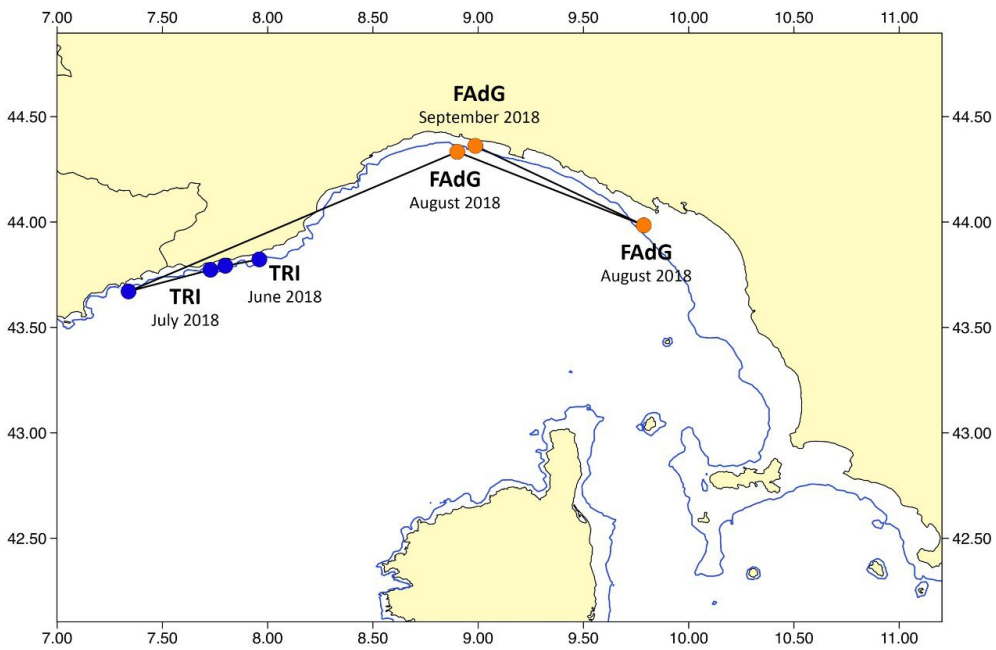
**Figure 16.** Movements of Tt07. Mother – Newborn pair sighted in July by TRI in the western portion of the Sanctuary, in August by FAdG and then September in the CETUS study area, covering a linear distance of about 300 km.



**Figure 17.** Movements of Tt20. Mother – Newborn pair sighted in July by TRI in the western portion of the Sanctuary, in July and August by CETUS and then in September in the FAdG study area, covering a linear distance of about 475 km.



**Figure 18.** Movements of Tt29. Mother – Newborn pair sighted in March by CETUS, in August by TRI in the western portion of the Pelagos Sanctuary and then again in the CETUS study area in September, covering a linear distance of about 526 km.



**Figure 19.** Movements of Tt03. Mother – Calf pair sighted in June - July by TRI and in August - September by FAdG, covering a linear distance of about 464 km.

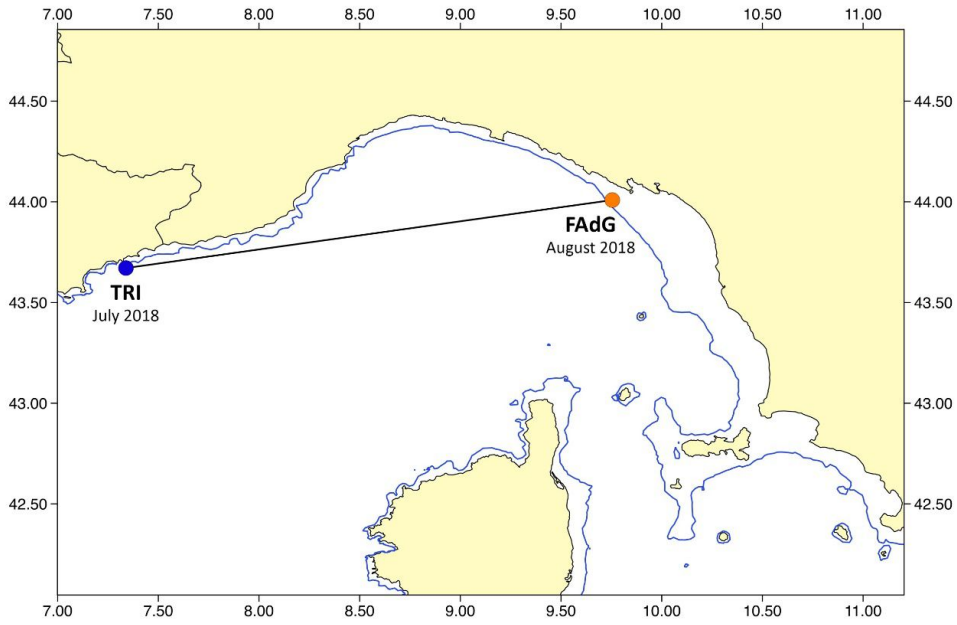


Figure 20. Movements of Tt24. Mother – Calf pair sighted in July by TRI and in August by FAdG, covering a linear distance of about 272 km. The mother has the dorsal fin sliced.

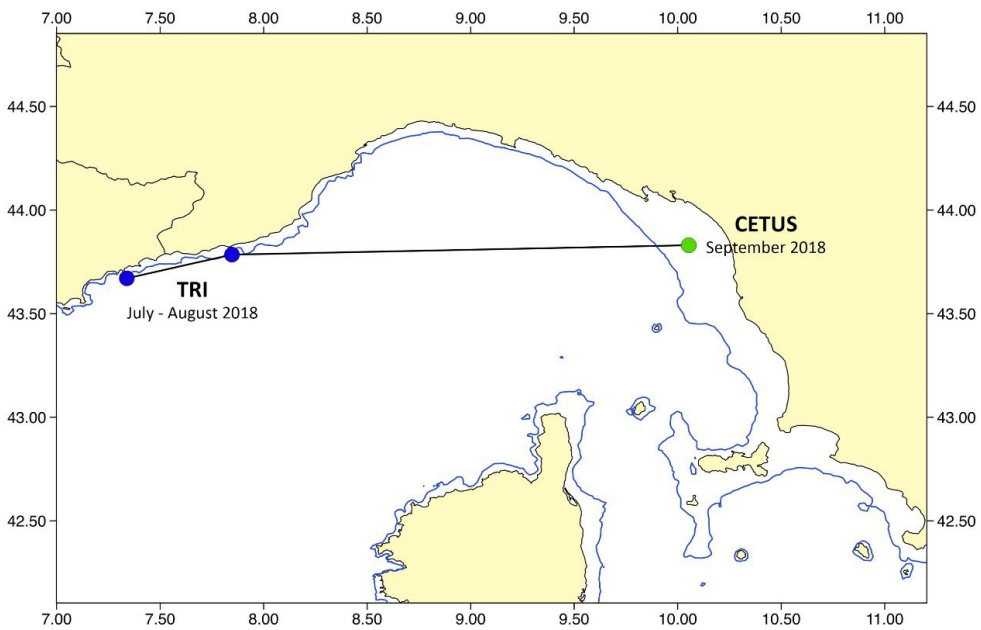


Figure 21. Movements of Tt23. Adult covering a linear distance of about 304 km.



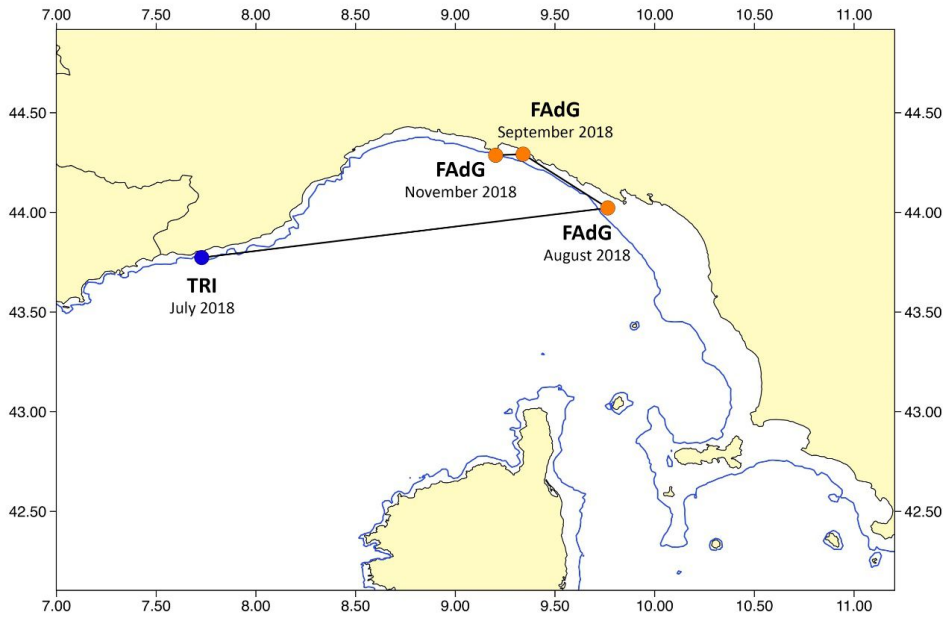


Figure 22. Movements of Tt12. Adult sighted in July by TRI and then in August, September and November by FAdG, covering a linear distance of about 300 km.

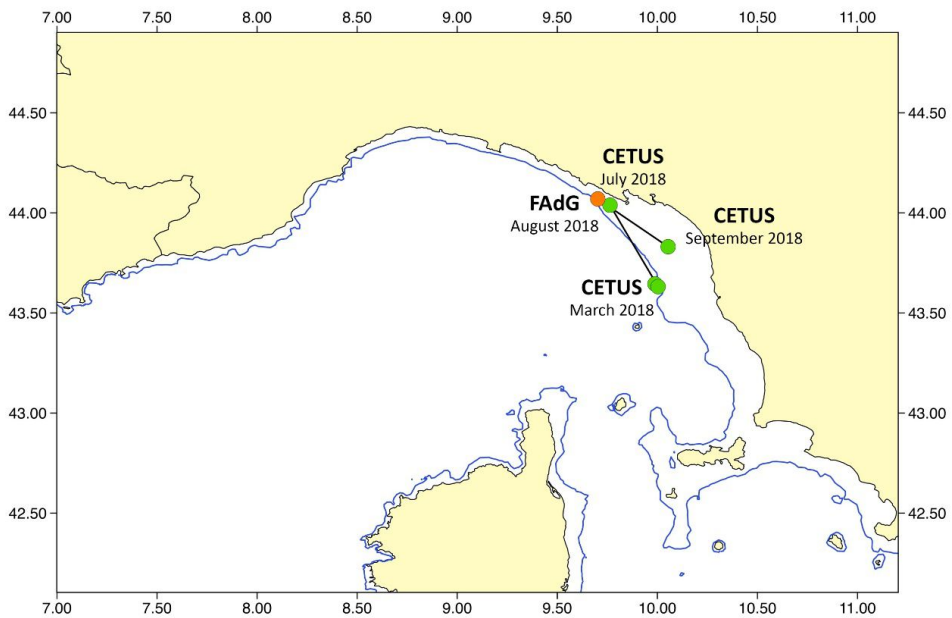
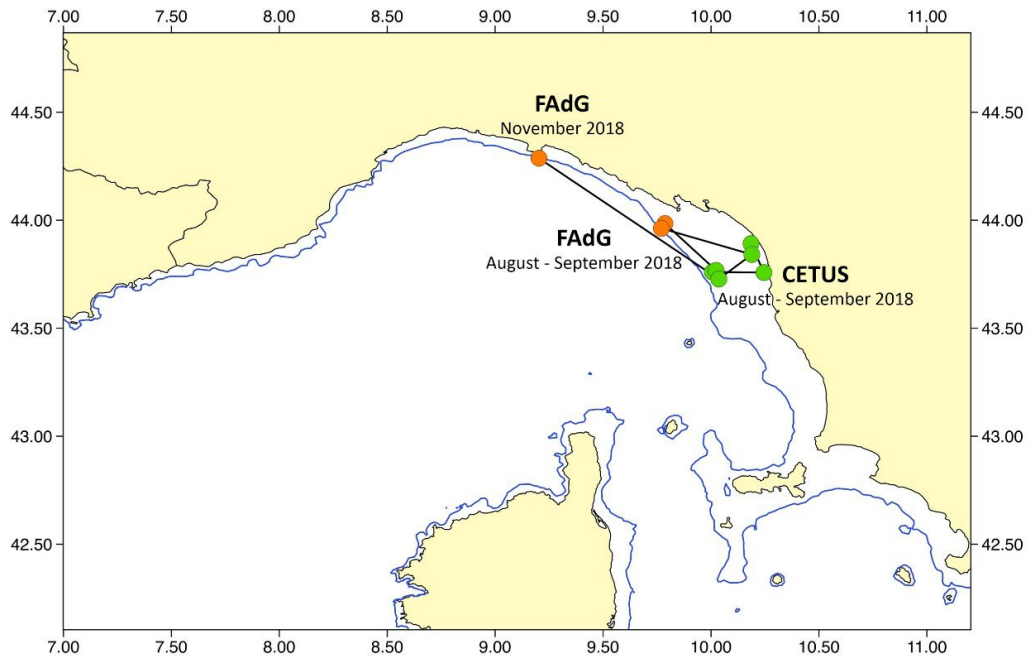


Figure 23. Movements of Tt47. Adult sighted in July by CETUS in March and July, by FAdG in August, and then again by CETUS in September, covering a linear distance of about 110 km.



**Figure 24.** Movements of Tt50. Adult sighted in both of CETUS and FAdG’s study area in August and September, and then in November by FAdG, covering a linear distance of about 265 km.

The photo-ID data collected within the DWB project were also matched with the photo-ID data collected along the French Mediterranean coast and Corsica within the GDEGeM project in 2014 (381 individuals), and only 4 positive matches were found. These are all related to individuals moving between Liguria and the eastern portion of the French coast, while no matches were found between Corsica and Tuscany, despite the close proximity of these two areas (Fig. 25, 26, 27 and 28). Conversely, it is very likely that matches will be found between southern Corsica and northern Sardinia once a photo-id catalogue from the latter will be available for comparison.

These results are in agreement with previous study from Carnabuci et al. (2016) and seem to confirm quite stable discontinuities in the connectivity of the Pelagos bottlenose dolphin network. As suggested also by Carnabuci and co-authors, these discontinuities (not reflected in the genetic analyses reported here) seem to retrace the ecological breaks and may be the consequence of a (cultural) specialization of the dolphins on the residency habitat.



Figure 25. Sighting points of individual Intercet 6 (TTDM320 - TTGC012)

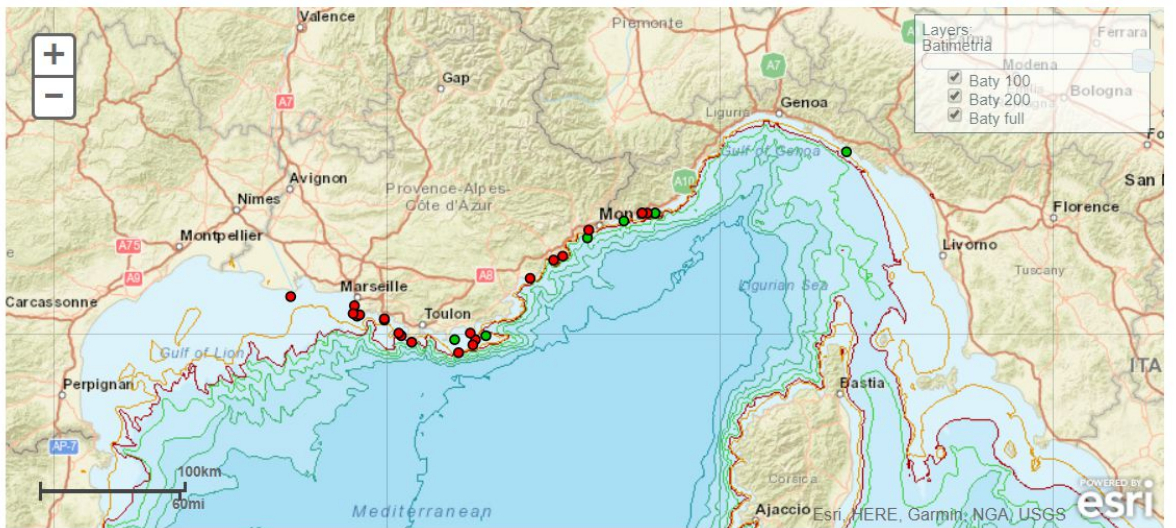


Figure 26. Sighting points of individual Intercet 8 (TTDM276 - TTGC020 - TTRI059)

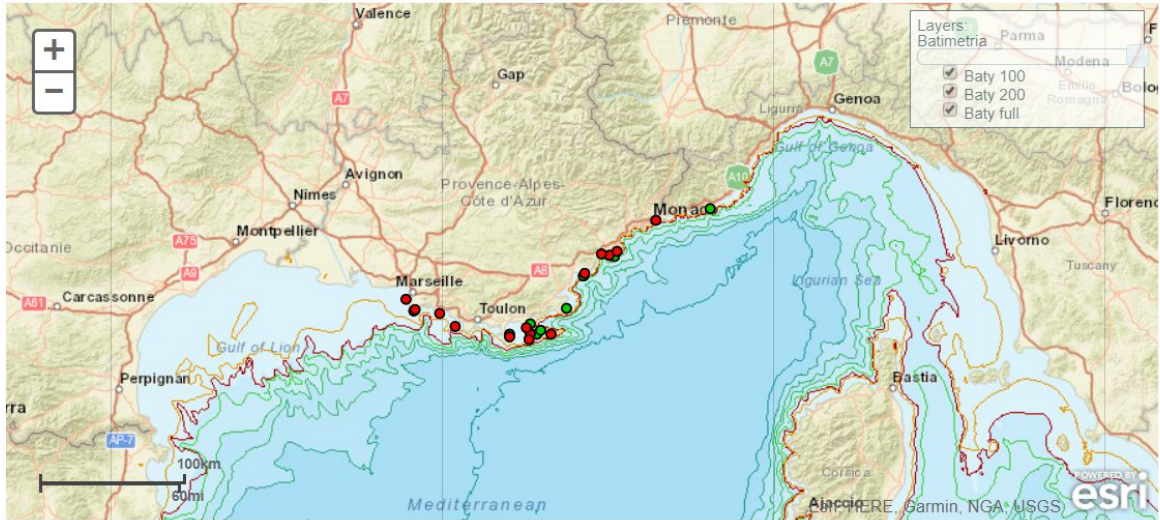


Figure 27. Sighting points of individual Intersect 363 (TTTRI230 - TTGC001)



Figure 28. Sighting points of individual Intersect 364 (TTTRI085 - TTGC117)

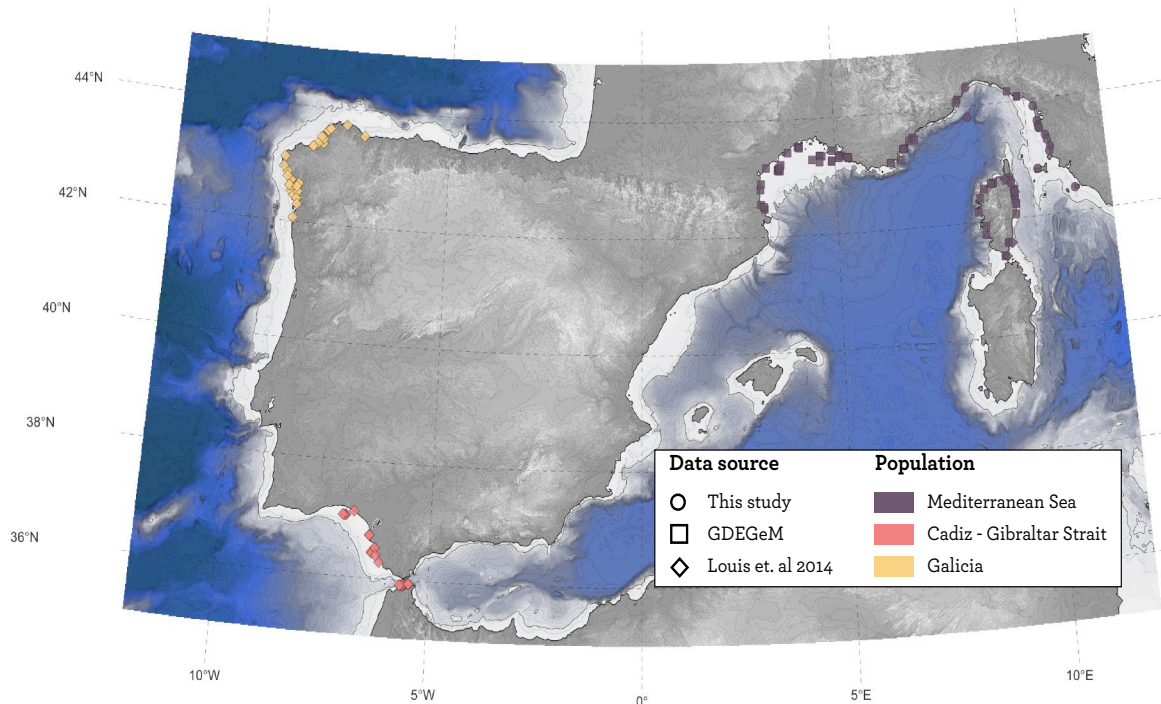
### 3.2. Activity A2. Genetic analyses of biological samples.

No remote biopsy sampling took place during campaigns at sea. Nevertheless, 13 bottlenose dolphin samples collected during stranding events in the Pelagos Sanctuary, were stored at the Mediterranean Marine Mammal Tissue Bank (MMMTB)<sup>1</sup> of the University of Padua and have been made available to DWB project (see Table 13 below). These samples have been transferred to GIS3M and analysed together with samples derived from the GDEGeM project to investigate population structure and levels of gene flow within the project area. For the sake of comparison and in order to gain some wider perspective, 75 individuals already analysed by Marie Louis (see for instance REF1 and REF2) have been included in the following analyses: 31 individuals from Galicia, 40 individuals from the Cadiz to Gibraltar Strait area and 4 individuals from Corsica. These individuals are a mix of both stranded animals and biopsy samples on living animals (See figure 29 for the geographical location of these additional individuals).

| MMMTB code  | Species                   | Stranding location       | Date stranded | Stored samples                                 | Conservation Status |
|-------------|---------------------------|--------------------------|---------------|--|---------------------|
| 42906 (IZS) | <i>Tursiops truncatus</i> | Albisola Superiore (SV)  | 13.05.2018    | Various  | 3                   |
| 312         | <i>Tursiops truncatus</i> | Rosignano Marittima (LI) | 22.09.2013    | Nervous Central System - Lung                  | 2                   |
| 308         | <i>Tursiops truncatus</i> | Castagneto Carducci (LI) | 20.03.2013    | NCS - Lung - Liver - Kidney                    | 3                   |
| 223         | <i>Tursiops truncatus</i> | San Vicenza (LI)         | 15.03.2012    | NCS - Lung - Liver - Kidney                    | N/A                 |
| 207         | <i>Tursiops truncatus</i> | Tirrenia (PI)            | 19.06.2011    | Lung - Liver - Kidney - Spleen - Heart         | 3                   |
| 190         | <i>Tursiops truncatus</i> | Spiaggia di Mandras (SS) | 10.04.2010    | NCS  | 2                   |
| 168         | <i>Tursiops truncatus</i> | Livorno (LI)             | 17.12.2009    | Lung - Liver - Kidney - Skin - Muscle          | 2                   |
| 155         | <i>Tursiops truncatus</i> | Cecina (LI)              | 07.10.2008    | Lung - Kidney - Muscle                         | 3                   |
| 152         | <i>Tursiops truncatus</i> | Antignano (LI)           | 14.03.2008    | Liver - Skin - Muscle                          | 4                   |
| 151         | <i>Tursiops truncatus</i> | Antignano (LI)           | 14.03.2008    | Lung - Liver - Kidney - Skin - Muscle          | 4                   |
| 131         | <i>Tursiops truncatus</i> | Viareggio (LI)           | 27.09.2007    | Lung - Liver - Kidney - Spleen - Skin - Muscle | 4                   |
| 130         | <i>Tursiops truncatus</i> | Marciana Marina (LI)     | 03.10.2007    | Liver - Kidney - Skin - Muscle                 | 3                   |
| 118         | <i>Tursiops truncatus</i> | Monte Argentario (GR)    | 17.05.2007    | Lung - Liver - Kidney - Muscle                 | 4                   |

Table 13. Bottlenose dolphin samples collected during stranding events in the Pelagos Sanctuary made available to DWB by The Mediterranean Marine Mammal Tissue Bank (MMMTB) of the University of Padua.

<sup>1</sup> MMMTB was established in 2002 within the ACCOBAMS agreement. The mission of MMMTB is to collect samples from marine mammals stranded along the Italian coastline or in other countries facing the Mediterranean basin for comparative anatomical studies, pathology and ecotoxicology investigations.



**Figure 29:** location of individuals sampled in this project (circles) during the GDEGeM project (squares) and by Marie Louis (Louis et. al 2014, diamonds). The colours indicate the origin of the samples: the 3 groups identified here are the ones we used to compute diversity indices for genetic analyses.

### Mitochondrial DNA analyses

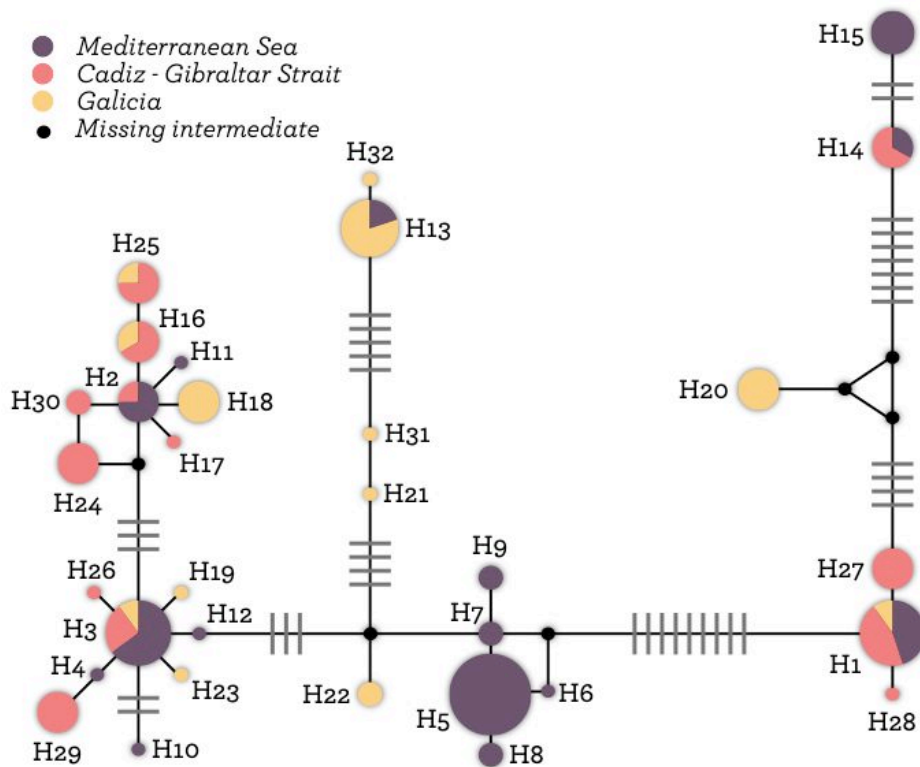
A 682 base pairs fragment of the mitochondrial D-loop gene was sequenced for 11 of the 13 individuals listed in table 13, and 63 individuals from the GDEGeM project. We followed the protocol described in Louis et al. (2014) for DNA extractions and PCR amplifications using primers Dlp1.5 (5'-TCACCCAAAGCTGRARTTCTA-3') (Baker et al. 1998) and Dlp8G (5'-GGAGTACTATGTCCTGTAACCA-3') (as reported in Dalebout et al. 2005). Together with the 74 sequences previously obtained by Louis et al. (2014), a total of 149 individuals have been compared. All sequences have been cleaned up and aligned in Bioedit (Hall 1999), and descriptive statistics have been computed in DNAsp (Rozas & Rozas, 1999).

Genetic diversity indices are presented in table 14. Taken as a whole, Mediterranean individuals have characteristics very similar to those of other populations, both in term of number of haplotypes (NH) and number of segregating sites (S). However, gene diversity (h), nucleotide diversity ( $\pi$ ), and genetic divergence within the population (k) seem to be slightly smaller among the Mediterranean individuals than in Galicia and the Cadiz-Gibraltar Strait region. Tajima's D was found non-significant at both the scale of the 3 populations and of the whole dataset suggesting a neutrally evolving population of constant size.

|                   | $N$ | $N_H$ | $S$ | $h$   | $\pi$  | $D$   | $k$   |
|-------------------|-----|-------|-----|-------|--------|-------|-------|
| Mediterranean Sea | 78  | 15    | 29  | 0.789 | 0.0107 | 0.609 | 7.271 |
| Cadiz-Gibraltar   | 40  | 13    | 26  | 0.905 | 0.0125 | 1.326 | 8.499 |
| Galicia           | 31  | 13    | 27  | 0.839 | 0.0122 | 0.833 | 8.34  |
| Overall           | 149 | 32    | 37  | 0.905 | 0.0129 | 0.778 | 8.811 |

**Table 14.** Mitochondrial diversity indices. For each population and the whole dataset, the following statistics are provided: number of individuals successfully sequenced ( $N$ ), number of haplotype ( $N_H$ ), number of segregating sites ( $S$ ), gene diversity ( $h$ ), nucleotide diversity ( $\pi$ ), Tajima's  $D$  (all values are non-significant), mean number of nucleotide difference between pairs of sequences ( $k$ ).

The genealogical relationships among the 32 haplotypes have been examined using a median-joining network constructed in Network v. 5.0.1.1 (Bandelt et al. 1999). The geographic distribution of haplotypes and their phylogenetic relationships (Fig. 30) shows that 2 of the most frequent haplotypes (H1 and H3) are shared across the 3 regions studied. Five haplotypes are shared between 2 of the 3 regions studied (H2, H13, H14, H16 and H25). However, The most frequent haplotype (H5, 32 individuals) and 4 other haplotypes closely related to it are found only within the Mediterranean group. Likewise, most haplotypes found in more than one copy in our dataset are private alleles (e.g. H15, H18, H24, H27, H29...). This suggesting a very limited gene flow between the three regions examined here.



**Figure 30.** Median-joining network picturing the phylogenetic relationships between 32 mitochondrial haplotypes. Each coloured circle represents a unique mitochondrial haplotype, its size being proportional to its frequency in the dataset. The colours are the same as in figure 1 and represent the origin of the samples. Black dots indicate missing evolutionary intermediates not sampled in this work. Grey segments indicate the number of mutations between two haplotypes (only when the number of mutations is greater than one).

## Microsatellite analyses

All individuals of the present study have been genotyped using a set of 25 nuclear microsatellite loci (Table 15). The loci have been chosen in order to allow cross-studies comparisons. Indeed, the same set of loci was used by Louis *et al.* (2014) on a much larger scale, and a subset of 12 of these markers was used to analyze 40 individuals from the GDEGeM project. Since we obtained the raw genotypes for the 13 individuals of this project, we will only present here preliminary results based on individuals from the GDEGeM project analysed together with individuals from the Cadiz to Gibraltar Strait region and from Galicia.

| Markers  | Reference                                 | Primers 5' - 3' (R and F)                                   | Motif  | Allele size ranges | Annealing T° in °C |
|----------|---|---|--|--------------------|--------------------|
| EV37     | Valsecchi & Amos 1996 - Vollmer 2011      | AGCTTGATTTGGAAGTCATGA<br>GTTTTAGTAGAGCCGTGATAAAGT<br>GC     | (AC) <sub>24</sub>   | 196-250            | 55                 |
| KMW12a   | Hoelzel <i>et al.</i> 1998                | CCATACAATCCAGCAGTC<br>CACTGCAGAATGATGACC                    | (CA) <sub>n</sub>  | 144-168            | 46                 |
| MK5      | Krutzen <i>et al.</i> 2001 - Vollmer 2011 | CTCAGAGGGAAATGAGGCTG<br>GTTTTGTCTAGAGGTCAAAGCCTTC<br>C      | (TG) <sub>13</sub> CT(TG) <sub>2</sub> CA(TG) <sub>2</sub> (TA) <sub>2</sub> (T<br>G) <sub>4</sub> | 205-243            | 55                 |
| MK6      | Krutzen <i>et al.</i> 2001 - Vollmer 2011 | GTCCTCTTTCCAGGTGTAGCC<br>GCCCACTAAGTATGTTGCAGC              | (GT) <sub>17</sub>   | 145-191            | 55                 |
| MK8      | Krutzen <i>et al.</i> 2001 - Vollmer 2011 | TCCTGGAGCATCTTATAGTGGC<br>GTTTCTCTTTGACATGCCCTCACC          | (CA) <sub>23</sub>   | 87-117             | 55                 |
| MK9      | Krutzen <i>et al.</i> 2001 - Vollmer 2011 | CATAACAAAGTGGGATGACTCC<br>GTTTTTATCTGTGGCTGCAGTG            | (CA) <sub>17</sub>   | 166-182            | 55                 |
| Tur4_87  | Nater <i>et al.</i> 2009                  | CCCATATGATGCCTTTGTAAGTCC<br>AATTCCTTGTAACAAACCTCTTTAT<br>CT | (GATA) <sub>8</sub>  | 182-202            | 61                 |
| Tur4_98  | Nater <i>et al.</i> 2009                  | GTCCCAGAACTTAGCACACTGTC<br>CAACTGGGGTCCAAAGAAAGAAG          | (GATG) <sub>10</sub>   | 172-204            | 63                 |
| Tur4_128 | Nater <i>et al.</i> 2009                  | ACGTGCGCATGTCTTTGTCTTAT<br>CTTTGGACGGGAGTAGAACCTA           | (GATA) <sub>11</sub>   | 280-304            | 62                 |
| Tur4_142 | Nater <i>et al.</i> 2009                  | GGCCCCCTTTCCATCCTCA<br>CCAGCCCCAAATCACGAGT                  | (GATA) <sub>9</sub>  | 320-340            | 61                 |
| TexVet5  | Rooney <i>et al.</i> 1999 - Vollmer 2011  | GATTGTGCAATGGAGACA<br>GTTTTGAGATGACTCCTGTGGG                | (CA) <sub>24</sub>   | 201-223            | 55                 |
| TexVet7  | Rooney <i>et al.</i> 1999 - Vollmer 2011  | TGCACTGTAGGTTGTTAGCAG<br>CTTAATFGGGGCGATTTCAC               | (CA) <sub>12</sub>   | 162-178            | 55                 |
| Ttr04    | Rosel <i>et al.</i> 2005                  | CTGACCAGGCACTTTCCAC<br>GTTTGTTCACAGGATTTTAGTGC              | (CA) <sub>25</sub>   | 106-128            | 60                 |
| Ttr11    | Rosel <i>et al.</i> 2005                  | CTTTCAACCTGGCCTTTCTG<br>GTTTGGCCACTACAAGGGAGTGAA            | (CA) <sub>21</sub>   | 194-226            | 62                 |
| Ttr19    | Rosel <i>et al.</i> 2005                  | TGGGTGGACCTCATCAAATC<br>GTTTAAGGGCTGTAAGAGG                 | (CA) <sub>17</sub>   | 174-202            | 60                 |
| Ttr34    | Rosel <i>et al.</i> 2005                  | GCACATGAGTATGTGGACAGG<br>GTTTCCTCCTGGGAGTGTCTCT             | (CA) <sub>19</sub>   | 182-204            | 58                 |
| Ttr48    | Rosel <i>et al.</i> 2005                  | AAGAGGATGCAAATGGCAAG<br>GTTTGGTAAGAAAATACCAAAGTC<br>C       | (CA) <sub>18</sub>   | 132-144            | 58                 |
| Ttr58    | Rosel <i>et al.</i> 2005                  | TGGGTCTTGAGGGGTCTG<br>GTTTGCTGAGGCTCCTTGTGG                 | (CA) <sub>17</sub>   | 168-196            | 60                 |



|        |                          |  |                    |         |    |
|--------|--------------------------|--|--------------------|---------|----|
| Ttr63  | Rosel <i>et al.</i> 2005 | CAGCTTACAGCCAAATGAGAG<br>GTTTCTCCATGGCTGAGTCATCA         | (CA) <sub>34</sub> | 86-140  | 60 |
| TtrFF6 | Rosel <i>et al.</i> 2005 | AAGTAAGTGCTCCTTTGACTGG<br>GTTTGGCAGAGAGATATTAGGACA<br>GC | (CA) <sub>20</sub> | 134-174 | 54 |
| Tut01  | Louis <i>et al.</i> 2014 | CTGTTGTTGCCTCAATTTGC<br>CCCATAGGACATATCCCACA             | (TG) <sub>11</sub> | 117-125 | 56 |
| Tut02  | Louis <i>et al.</i> 2014 | CATTTGTTGGGAAGCTGTTG<br>AGTGGGTTGACACATTCCCT             | (AC) <sub>11</sub> | 181-209 | 56 |
| Tut05  | Louis <i>et al.</i> 2014 | GTATGCCTTGCTTTGGTGC<br>TGGGAGGTATGTCTGCAATAA             | (AC) <sub>13</sub> | 154-166 | 56 |
| Tut08  | Louis <i>et al.</i> 2014 | AAGTTCCTAATTTCCCACCCA<br>ACTTGTGTTTGCCTGCCTGT            | (AC) <sub>15</sub> | 149-175 | 56 |
| Tut09  | Louis <i>et al.</i> 2014 | TAGGCTGGCAGAACAACAAG<br>TGATTGTTTTCCTTCCTCGT             | (AC) <sub>15</sub> | 149-167 | 56 |

**Table 15.** Characteristics of the 25 microsatellite markers used in this study.

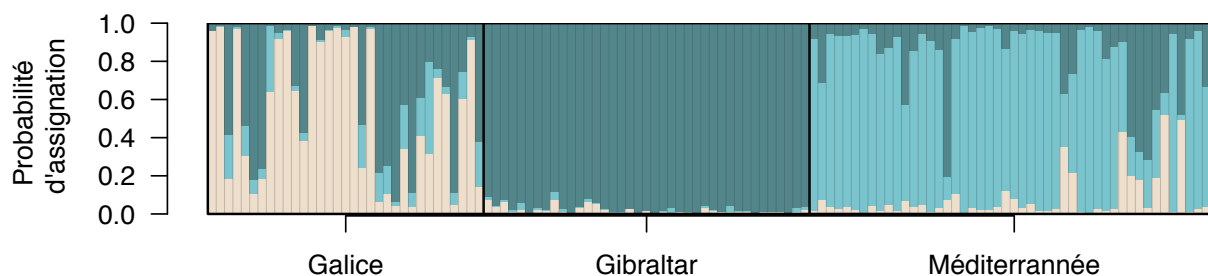
We mostly focused on the analysis of population structure. We used three distinct approaches to identify the most likely genetic structure within the study area.

First, we used the Structure v2.3.4 (Pritchard *et al.* 2000) to identify the most likely number of populations at Hardy-Weinberg equilibrium using only the multilocus genotypes. With this method, probabilities to belong to each of the putative population is computed for each individual

Second, we used TESS v2.3 (Durand *et al.* 2009). This software uses both the multilocus genotype of individuals and their geographic location in the study area in a “landscape genetics” framework. The objective is to (i) identify the most likely number of population at Hardy-Weinberg equilibrium in the study area, and (ii) to locate these populations (and their boundaries) spatially.

Third, we used adegenet v2.0.1 (Jombart, 2008), to compute a Discriminant Analysis on the Principal Components (DAPC). Once again, this method tries to (i) identify the most likely number of clusters in a dataset and (ii) assign each individual to one of the cluster based on its genotype. Contrary to the 2 previous methods, this approach does not make any assumption about the evolution model of the markers used, about the admixture model or the Hardy-Weinberg equilibrium. Here, we only try to minimise the genotypic variance within clusters and to maximise the genotypic variance between clusters using classical multivariate statistics methods.

The 3 approaches used here yielded the same results: we observe only 3 populations on the study area, namely Galicie, Cadiz – Gibraltar, and the Mediterranean population (see figures 31, 32 and 33). This suggests that there is no apparent fine scale sub-structure within the Mediterranean Basin for bottlenose dolphins.



**Figure 31.** Results produced by Structure v2.3.4 (Pritchard *et al.* 2000). For each individual analysed, a vertical bar indicates the probability to belong to each of the 3 populations identified by the software.

Figure 31 shows that most individuals from the Mediterranean group are assigned to the same putative population. However, 4 individuals have a strong probability to belong to the Gibraltar population. This might indicate the existence of migration from the Gibraltar area to the Mediterranean Basin. Likewise, 3 individuals have an equal probability to belong to two populations. This might indicate an individual having one parent from each of the two populations (first generation hybrids). So even if the Mediterranean population has a distinct genetic signature, it is not fully isolated from neighbouring populations. This is confirmed by the results produced by TESS (figure 32). For instance, the bottom probability map groups all individuals from the Cadiz and Gibraltar Strait area. Yet, a few individuals from both Galicia and Corsica seem to have non-zero probabilities to belong to the same population.

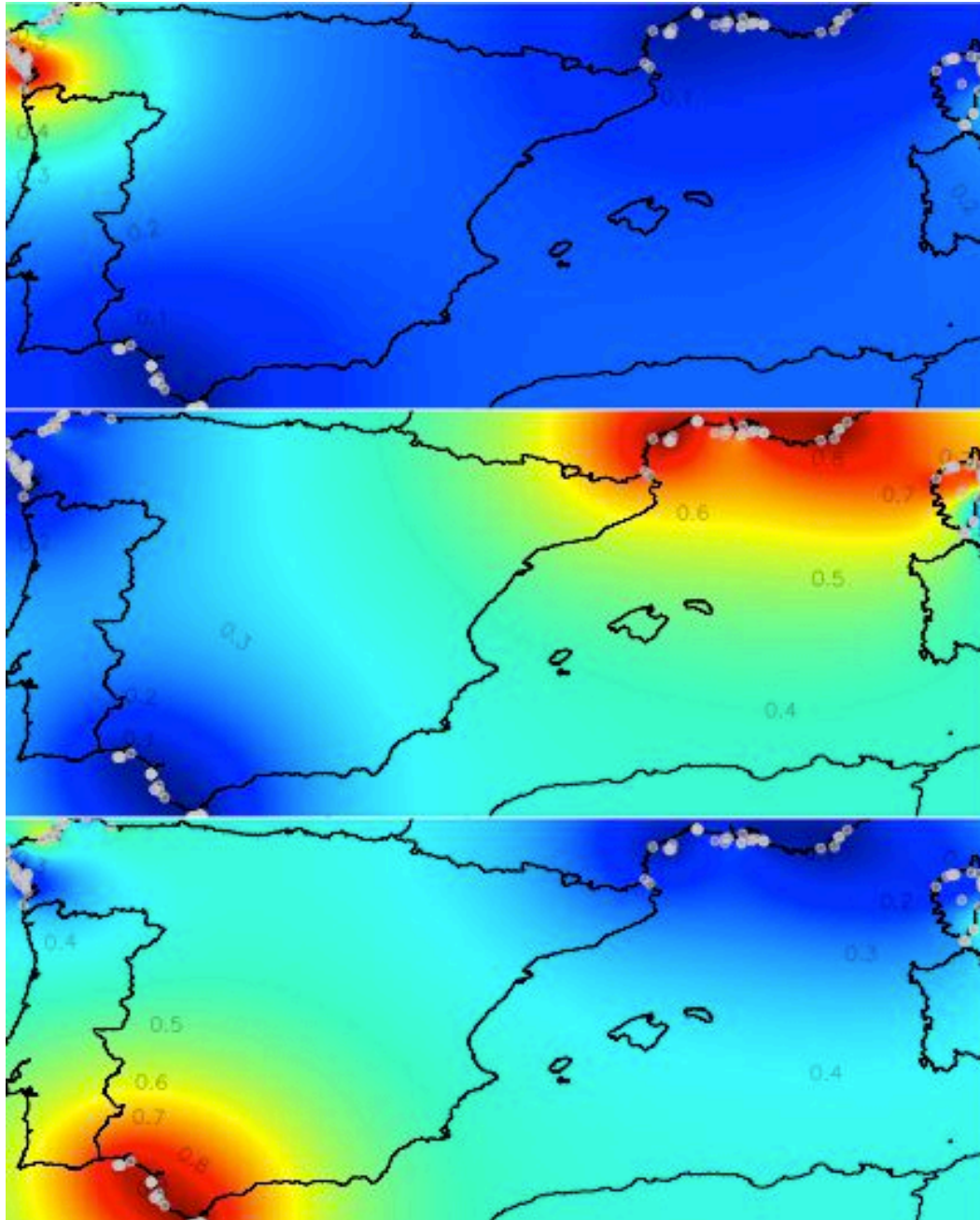
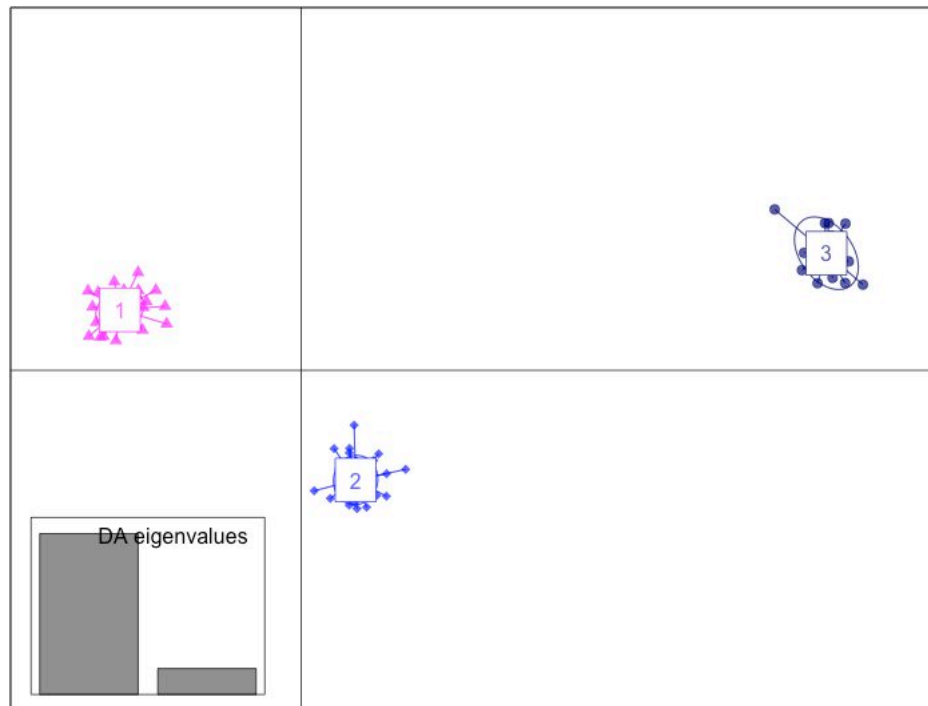


Figure 32. Results produced by TESS v2.3 (Durand et al. 2009). The most likely number of populations identified by the software is 3, hence 3 probability maps have been produced. On each map, blues indicate a low probability to belong to the population, and oranges/reds indicate a high probability to belong to the population.

Finally, the DAPC results confirm the existence of 3 and only 3 groups of individuals. The differentiation index  $\rho_{ST}$  (computed following the method of Michalakis & Excoffier (1996) in genepop v4.2, Rousset 2008) confirms that gene flow exists between the 3 locations studied (Table 16). The  $\rho_{ST}$  is analogous to the  $F_{ST}$  but it takes into account the size of alleles, and therefore their genealogical relationships. Here, we see an absence of differentiation between the Mediterranean population and the Cadiz – Gibraltar Strait population, a moderate (and significant) differentiation between the Cadiz – Gibraltar Strait population and the Galicia population, and a strong (and significant) differentiation between the Mediterranean population and the Galicia population.



**Figure 33.** DAPC results showing the existence of 3 distinct groups of individuals: the Mediterranean population (pink, 1), the Cadiz – Gibraltar Strait population (light blue, 2) and the Galicia population (dark blue, 3).

|                          | <b>Galicia</b> | <b>Cadiz - Gibraltar</b> |
|--------------------------|----------------|--------------------------|
| <b>Cadiz – Gibraltar</b> | 0.070*         | -                        |
| <b>Mediterranean Sea</b> | 0.266*         | 0.151                    |

**Table 16.** pairwise  $\rho_{ST}$  between the 3 populations identified by TESS. Stars indicate a significant  $\rho_{ST}$  ( $p < 0.05$ )

### 3.3. Activity A3. Survey of existing knowledge

A list of about 152 scientific peer-reviewed papers relevant to the biology, ecology and conservation of bottlenose dolphins in the Mediterranean, paying particular attention to the Pelagos Sanctuary reality, was produced and is available at the DWB website (see point 5.4 below) providing also the possibility of searching through it by keywords/authors <https://www.dolphinswithoutborders.net/bibliografia/>

### 3.4. Activity A4. Publications in the scientific literature



The following abstract has been submitted and will be presented at the World Marine Mammal Conference WMMC19 <https://www.wmmconference.org>.

In December 2019, the Society for Marine Mammalogy and the European Cetacean Society will jointly host the WMMC19 in Barcelona, Catalonia, Spain. This conference will bring together scientists, managers, policy-makers, educators and students from

across the globe to discuss the world's most exciting science and most pressing marine mammal conservation issues. It provides the best possible platform to present the results of DWB.

#### ***Analysis of the movements of common bottlenose dolphin (*Tursiops truncatus*) within the Pelagos Sanctuary (North-Western Mediterranean Sea).***

Valentina De Santis<sup>1</sup>, Caterina Lanfredi<sup>1,2,3</sup>, Arianna Azzellino<sup>1,2,3</sup>, Sabina Airoidi<sup>1</sup>, Michela Bellingeri<sup>4</sup>, Guido Gnone<sup>4</sup>, Silvio Nuti<sup>5</sup>, H  l  ne Labach<sup>6</sup>, Giuseppe Notarbartolo di Sciarra<sup>1</sup>, Joan Gonzalvo<sup>1</sup>

(1) Tethys Research Institute, Viale G. B. Gadio 2, 20121, Milano, Italy

(2) Politecnico di Milano, Civil & Environmental Engineering Department (DICA), Piazza Leonardo da Vinci 32, 20132, Milano, Italy

(3) CoNiSMa (Inter-University National Consortium for Marine Sciences), Piazzale Flaminio 9, 00196, Roma, Italy

(4) Fondazione Acquario di Genova Onlus, Area Porto Antico, Ponte Spinola, 16128, Genova, Italy

(5) CE.TU.S., Museo della Marineria, via Peschiera 9, 55049, Viareggio (LU), Italy

(6) GIS3M Le Kalliste, 1 avenue Cl  ment Monnier 13960 Sausset-les-pins, France

*Common bottlenose dolphin (*Tursiops truncatus*) research and conservation actions were implemented in the Pelagos Sanctuary, the largest marine protected area (87,500 km<sup>2</sup>) for Mediterranean marine mammals, within the framework of project "Dolphins Without Borders". Studies were conducted in three areas with a water surface, respectively (west to east), of 11,000, 2,200 and 8,150 km<sup>2</sup>, between Nice and Elba Island. During 203 visual surveys, totaling about 8700 km of research effort under positive conditions (sea state < 4 on the Douglas scale), bottlenose dolphins were sighted 101 times. The unified photo-identification catalogue resulted in a total of 185 well-marked individuals. Of these, 53 (28%) matched with at least one of the other two catalogues. Only 2 dolphins (1%), both mother/newborn pairs, sighted between July and September 2018, were included in all the three catalogues. The maximum displacement distance was measured for each dolphin sighted at least twice (n=143): mean and median displacements were respectively 105, and 81 km, with 20% of the dolphins showing a displacement higher than 188 km, while the maximum displacement recorded was 272 km. These movements are longer than those reported in a similar study carried out in the same area, suggesting a possible extension of the dolphins' home range over time. The photo-ID data collected within this project were also matched with those collected along the French Mediterranean coast and Corsica within the GDEGeM project (2014, 381 individuals), resulting in only 4 matches, all involving individuals moving between Liguria and the eastern portion of the French coast. This result seems to confirm that there are quite stable discontinuities in the connectivity of the Pelagos bottlenose dolphin network, in agreement with previous studies.*

In addition, a manuscript (in preparation) will be submitted for publication to a peer-reviewed journal based on DWB findings, discussing future conservation strategies for bottlenose dolphins in the Pelagos Sanctuary.

### 3.5. Activity A5. Suggestions for the establishment of Natura2000 sites

Thirteen Natura2000 sites including bottlenose dolphins in their marine waters have been established by Italy within the boundaries of the Pelagos Sanctuary. In Fig. 34 these sites were overlaid with the modeled predicted probability of bottlenose dolphins presence in the area.

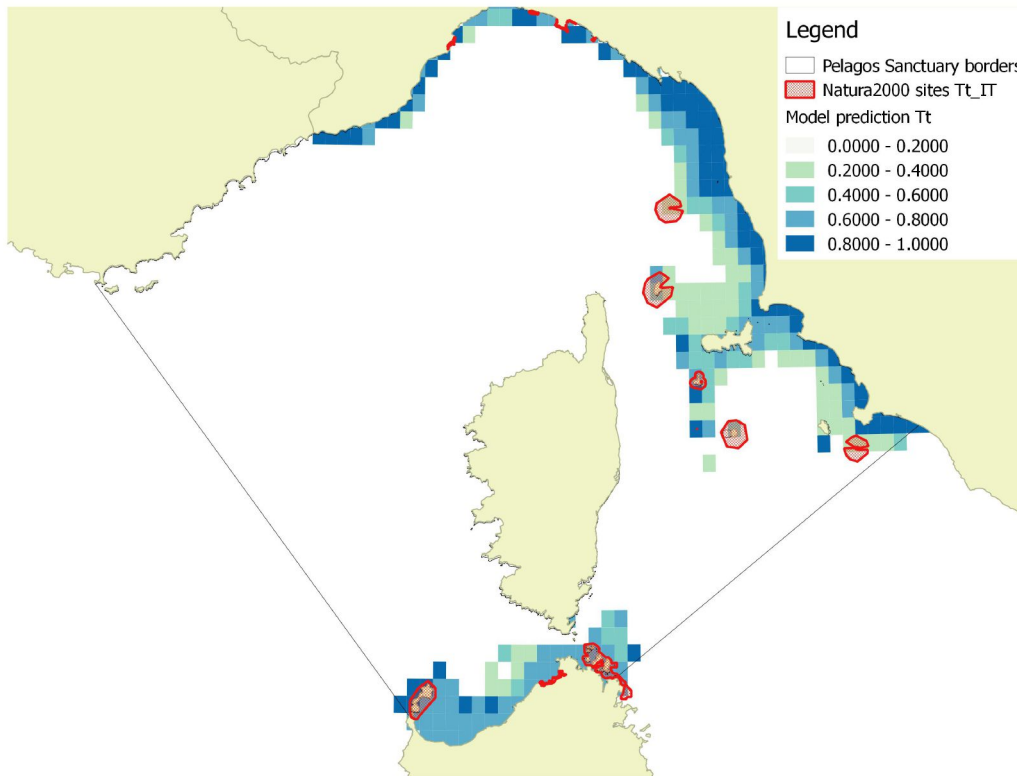


Figure 34. Marine Natura2000 sites including bottlenose dolphins in their species list overlaid with modeled probability of bottlenose dolphin presence (cf. Figure 6) within the Italian waters of the Pelagos Sanctuary.

Even a cursory examination of Fig. 34 reveals that the network of Natura2000 sites within the Pelagos Sanctuary established to conserve bottlenose dolphins is inadequate, both in terms of where the sites are located, and in terms of the extent of their surface area. In many cases (e.g., Gorgona, Capraia, Montecristo, Giannutri) the sites are located at the outer boundary of the predicted bottlenose dolphin habitat, their location mostly justified by the concurrent presence of protected areas the establishment of which had little to do with bottlenose dolphin habitat. Large portions of suitable bottlenose dolphin habitat (e.g., the wide continental shelf off Versilia in northern Tuscany, the Gulf of Asinara off northwestern Sardinia) are devoid of Natura2000 sites. Furthermore, in general, the marine portions of all sites extend offshore to an extremely limited distance and as such fail to adequately cover bottlenose dolphin habitat.

Based on the results of this study we recommend that the following areas be considered for the establishment of Natura2000 sites to protect bottlenose dolphins in the Pelagos Sanctuary (Italian waters):

- portions of the narrow corridor between the coastline and the 200 m isobath from Ventimiglia to La Spezia;
- a wider area between a line connecting Porto Venere to Gorgona and the Italian mainland;
- the waters surrounding Elba Island;

- the entire coastal area of northern Sardinia comprised within the Pelagos Sanctuary, between the 200 m isobath west of Isola dell'Asinara, east across the Bocche di Bonifacio to include the entire Arcipleago della Maddalena and the shallowest portion of the Caprera Canyon.

It should be further considered that although the remit of this project is to recommend the consideration of new Natura2000 sites for bottlenose dolphins within the boundaries of the Pelagos Sanctuary, suitable bottlenose dolphin habitat by no means stops outside such boundaries. In particular we recall that the presence of bottlenose dolphins is well known along the east coast of Sardinia (e.g., in Golfo Aranci and the waters of the "Isola di Tavolara – Capo Codacavallo" MPA, as well as in the coastal waters of Latium south of Fosso Chiarone. Accordingly, suitable Natura2000 sites should also be considered to protect bottlenose dolphin habitat further to the south of the Pelagos boundaries, also keeping in mind that bottlenose dolphins found there are almost certainly part of the same population residing within Pelagos.

## 4. Specific Goal 2: Strengthening the links among Pelagos and MPAs and National Parks existing within the Sanctuary boundaries

### 4.1. Activity A6. Training programmes

In order to strengthen links among Pelagos and the many MPAs and National Parks existing within the Sanctuary boundaries, MPA managers and relevant stakeholders were contacted in January 2019 and informed about training programmes addressed to their personnel to be executed between February and March 2019 in the context of DWB (See Annex 1 with letter sent to Italian Ministry of Environment and Protection of the Territory and the Sea, General Directorate for the Protection of Nature and the Sea a few weeks before contacting MPA managers). Three training sessions were organized in Sardinia, Elba and Genoa in order to facilitate participation of most relevant stakeholders. A complete list of all participants in these three training events is facilitated in Annex 2. A small photo album is provided in Annex 3.

#### Caprera 28 February 2019

**Dolphins Without Borders**

**Workshop sul TURSIOPE nel Santuario Pelagos**  
**Giovedì 28 Febbraio 2019**  
*Stagnali, Caprera, locali del C.E.A., Parco Nazionale Arcipelago di La Maddalena*

**PROGRAMMA**  
9:30 START/WELCOME 9:45 Introduzione/presentazione dei partecipanti 10:00 Dr. Giuseppe Notarbartolo di Sciarra. Progetto Dolphins Without Borders, *vision*, obiettivi, partner coinvolti; il Santuario Pelagos.  
10:20 Dr. Luca Bittau. Il Tursiope, biologia, ecologia, monitoraggio e conservazione della specie nel Santuario Pelagos e in Sardegna.  
11:00-11:30 COFFEE BREAK 11:30 Dr. Joan Gonzalvo. Foto-identificazione del tursiope e Piattaforma Intercet  
12:15 Dr. Giuseppe Notarbartolo di Sciarra. *Stakeholders engagement* e sinergie verso obiettivi comuni (es. Marine Conservation)  
12.45-14:00 PAUSA PRANZO - RINFRESCO  
14:00-16:00 Tavola di discussione sulle tematiche del workshop  
16:00 Chiusura workshop

Ente Ospitante: Parco Nazionale Arcipelago di La Maddalena

This was a full day training organized by the local non-governmental organization SeaMe Sardinia and the Tethys Research Institute, with logistic support from La Maddalena Archipelago National Park, who kindly provided its facilities to host the event, which took place in the National Park Environmental Education Center (CEA) of Stagnali in the island of Caprera. The objective of the workshop was to enhance and consolidate the networking among the stakeholders dealing with the protection and conservation of the bottlenose dolphin and its habitat in the Pelagos Sanctuary waters off Sardinia

The workshop was addressed to government institutions (bodies dealing with the management of the environment), MPAs management bodies, national parks, NGOs and local research groups, whale and dolphin watching companies, Italian Coastguard personnel, Sardinia Regional Rangers and other law enforcement officials working at sea. Contacts were previously taken with municipalities of la Maddalena and Palau, both partners of the Pelagos Sanctuary in Sardinia.

About 30 people attended the workshop in representation of the following bodies and associations operating in Sardinia: La Maddalena Archipelago National Park; Tavolara-Punta Coda Cavallo Marine Protected Area; La Maddalena and Palau Municipalities; La Maddalena Coast Guard; Naval Body of Carabinieri; Naval Body of Guardia di Finanza; Sardinian Regional Rangers CFVA from both La Maddalena and Palau command stations; ISPRA; Whale Watching Sardinia; Futurismo; Cooperativa Isule; CRAMA Asinara association.

The programme covered the following topics:

- DWB project and implication for cetacean conservation;
- biology and ecology of the bottlenose dolphin, with particular reference to the local population, and the need for conservation of the species;
- monitoring techniques and protocols for the study of cetaceans and introduction to INTERCET platform, in order to provide basic tools for the continuation of monitoring efforts by participants.

Presentations were followed by a plenary session with an open round-table discussion, focussed primarily on raising awareness, networking and stakeholder engagement. During the discussion, participants declared their interest in contributing to the proposed monitoring and reporting network developed in the context of DWB project.

Law-enforcement bodies participating in the workshop manifested the convenience of having simple protocols to gather more effectively information on bottlenose dolphins during their patrols at sea. The development of citizen-science programs was discussed, as well as the importance of collaboration between research groups, NGOs and public bodies (parks and MPAs) aimed at the study and monitoring of the bottlenose dolphin, to manage data on a regional scale, scientifically elaborate them and share their data through common database platforms such as INTERCET.

The stakeholders present brought up for discussion the main threats faced by bottlenose dolphins in the region. These reportedly included: a) interaction with fisheries (e.g.: by-catch, overfishing, noise pollution due to boat traffic, domestication); b) disturbance from boat traffic and strikes; c) dolphin domestication (the Golfo Aranci case). Participants declared the need for precise establishment of management rules (including penalties) in the regulations of the MPAs/National Parks. Some fishermen representatives complained about the difficulty of adapting small-size fishing boats into "pesca-turismo" activities. These difficulties derived primarily from needing a new boat (i.e., large money investment) and from reluctance by many fishermen to convert to this activity, mostly due to cultural/tradition reasons. Whale and dolphin watching operators complained about the difficulty in setting-up and continuing with their whale/dolphin watching activity due to the lack of relevant legislation and regulatory recognition of this activity in Italy.



Dolphins Without Borders [www.dolphinswithoutborders.net](http://www.dolphinswithoutborders.net)

## PROGETTO D.W.B. Dolphins Without Borders

in collaborazione con

### SEMINARIO FORMATIVO

MARTEDI 5 MARZO 2019  
ORE 9.30 – 13.00  
ISOLA D'ELBA  
SEDE DEL PARCO ARCIPELAGO TOSCANO  
LOC. ENFOLA

Direzione Marittima di Livorno

Parco Arcipelago Toscano

Progetto INTERNAZIONALE per la salvaguardia dei tursiopi nel Santuario Pelagos

Progetto coordinato da

Progetto finanziato da

TETHYS since 1988

GIS 3M

FONDAZIONE

CE.T.U.S.

SEA ME

PRINCE ALBERT II OF MONACO FOUNDATION

Pelagos

This second Dolphin Without Borders workshop was organized by CE.TU.S. and was conducted in a Conference Room, kindly granted by the Tuscan Archipelago Park, in Enfola, PortoFerraio, Elba.

The workshop was addressed to Tuscan MPA management bodies, law enforcement officials, local research groups and other stakeholders with the aim of providing basic information to contribute to a cetacean monitoring network specifically focussed on bottlenose dolphins. 50+ people participated in the workshop in representation of Tuscan Archipelago Park, Coast Guards, Carabinieri/Forestry, Penitentiary Police, Mare Libero Association, Ambiente Mare Association, Coop. Pelagos (Park guides), Marina di Campo Diving Club, Cavo Diving Club, as well as some local naturalists.

During the workshop, the following topics were addressed:

- The Pelagos Sanctuary and the its cetacean diversity;
- the role of Italian Coast Guards in environmental protection;
- biology, ecology, threats of the bottlenose dolphin and on-going research in the Pelagos Sanctuary;
- cetacean monitoring and photo-identification techniques;
- INTERCET online platform for cetacean monitoring and data sharing;
- engagement of stakeholders and synergies towards the achievement of common objectives.

The presentations were delivered, in order of intervention, by the president of the Tuscan Archipelago Park who presented the park's environmental protection projects; by Dr. Bonelli, Marine Biologist, who introduced the activities conducted by the naturalistic guides working with tourists; and by Dr. Nuti (CE.TU.S) who presented the DWB project, the cetaceans species present in Pelagos Sanctuary waters, paying particular attention to the biology and ecology of bottlenose dolphins, the research techniques at sea and the INTERCET platform.

Presentations were followed by a round table encouraging all participants to actively comment on their perception on the conservation needs of bottlenose dolphins in the area. All institutions representatives declared their willingness to participate in a dolphin monitoring network. The brochure and sightings reporting forms, described in detail in section 5.3 below, downloadable from the DWB website were presented and distributed among participants and representatives of the municipalities. In particular, the Portoferraio Harbour Master and the Tuscan Archipelago Park Director, both confirmed their determination to collaborate with DWB and on the sighting reporting network. Likewise, local diving centres manifested their interest in providing dolphin data and in following the basic

guidelines on how to behave when encountering dolphins at sea present in the DWB brochure.

Additionally, on 15<sup>th</sup> April 2019, a DWB presentation was delivered by CE.TU.S. at the Department of Marine Biology of the University of Pisa, Talking about the Pelagos Sanctuary and its cetacean diversity, research techniques and threats faced by these marine mammals.

Genoa, 13<sup>th</sup> March 2019

Dolphins Without Borders

## PROGETTO Dolphins Without Borders (DWB)

### Programma

- 09:00 Saluti di benvenuto
- 09:15 Presentazione del progetto DWB, partner e obiettivi
- 09:30 Il ruolo della Guardia Costiera nella protezione ambientale
- 09:45 Il Santuario Pelagos e i cetacei che lo abitano
- 10:20 Coffe break
- 10:40 Il Tursiopo
  - Descrizione, biologia ed ecologia della specie
  - Problematiche a livello mediterraneo con focus sul Santuario Pelagos
  - Studi in corso nel Santuario sulla specie
- 11:10 Tecniche di monitoraggio e foto-identificazione
- 11:50 Piattaforma INTERCET
- 12:10 Coinvolgimento degli stakeholder e sinergie verso obiettivi comuni
- 12:30 Coffe break
- 12:50 Tavola rotonda
- 13:50 Chiusura convegno e saluti



Acquario di Genova, 13 marzo 2019



Progetto finanziato da



The third and last DWB workshop, took place in the Nautilus room of Acquario di Genova and was organized by Tethys Research Institute and Fondazione Acquario di Genova.

The workshop was addressed to Ligurian MPA management bodies, law enforcement officials, local research groups and other stakeholders. It counted with the participation of about 40 people representing the following bodies and associations: Portofino Marine Protected Area; Bergoggi Marine Protected Area; C.I.R.C.E. (Inter-University Research Center on Cetaceans); University of Genoa; ARPAL (Regional Agency for Environmental Protection in Liguria); Experimental Zooprophyllactic Institute of Piedmont, Liguria and Valle d'Aosta; Coast Guard; Whale Watch Genova - Golfo Paradiso; Costa Balenae Association; Battibaleno Association. Personnel from Tethys Research Institute, Fondazione Acquario di Genova, SeaMe Sardinia Association and CE.TU.S. participated also in representation of the DWB partners. The topics addressed were the same covered in the previous DWB workshop held in Elba Island.

In this case, during the round-table discussion, all participants declared their willingness to contribute to a monitoring and reporting network, capable of integrating in a functional system each single effort. However, several problems emerged during the discussion; many of the bodies involved complained about the lack of resources to carry out research and monitoring activities on a continuous basis and the difficulty in having contacts with the institutions in charge of environmental management and conservation, at national and regional level. Reportedly, this lack of feedback, together with some problems of coordination between the bodies involved (which may lead to overlapping efforts without necessarily increasing the productivity of significant results), makes difficult the production and execution of concrete and effective research and conservation actions. Moreover, lack of attention by the institutions was pinpointed as the main cause preventing a proper enhancement of natural resources, which should be a fundamental component for effective conservation action. There was unanimous agreement among participants on the need of keeping, and possibly increasing, marine environment conservation efforts, not only through public awareness actions but also by pressing on the institutions for a more concrete and operational commitment.

## 4.2. Activity A7. Contacts established with selected Italian municipalities

In order to raise awareness within the wider public on the conservation of the marine environment, taking advantage of the popularity of these charismatic mammals, contacts were established with selected Italian municipalities that are partners of the Pelagos Sanctuary, including the following: San Remo, Chiavari and Genova (Liguria); Viareggio-Livorno, Isola Elba (Tuscany); and Maddalena and Porto Torres (Sardinia). Education and public awareness materials were produced in Italian, 6,000 copies of the DWB brochure were printed and distributed among these municipalities, marinas, recreational ports, yacht clubs and to Italian Naval League during the three training workshops presented above.

Moreover, 123 Italian municipalities partners of the Pelagos Sanctuary (75 from Liguria, 32 from Tuscany and 16 from Sardinia) were formally contacted to be informed about DWB project, the existence of the website including information on the project and downloadable materials (see below). The letter sent to all these municipalities is included in Annex 4.

## 4.3. Activity A8. Awareness materials

DWB educational material can be downloaded as .pdf files from <https://www.dolphinswithoutborders.net>.

These include:

- ✓ DWB brochure (Annex 4)
- ✓ DWB sightings reporting form; including guidelines on how to behave when encountering dolphins at sea and basic species guide (Annex 5)

## 4.4. Activity A9. Project Webpage and FaceBook Page.

DWB website (<https://www.dolphinswithoutborders.net>) is on-line in Italian and in French.

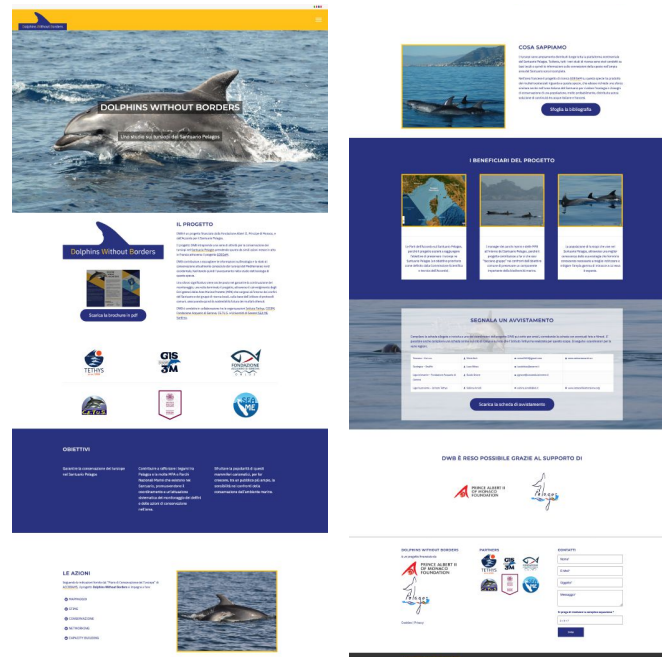


Figure 35. Two different screen shots illustrating the design and structure of the DWB website (Italian version) <https://www.dolphinswithoutborders.net>

A DWB FaceBook page was also set up in early 2019 and is used to share in this popular social media platform news relevant to the research and conservation of bottlenose dolphins in the Pelagos Sanctuary (and not only), as well as, will be referring occasionally to DWB related activities (e.g., training and capacity building initiatives). <https://www.facebook.com/dolphinswithoutborders/>

## 5. Concluding Considerations

The DWB project implemented actions for common bottlenose dolphins *Tursiops truncatus* monitoring and conservation in the Pelagos Sanctuary waters taking into account the actions that were already implemented in France through the GDEGeM project (2013-16), funded by MAVA Foundation. DWB continued to accrue information on bottlenose dolphin ecology and conservation status available from the north-western Mediterranean to improve ecological knowledge of the species. In addition to the direct monitoring efforts (sea surveys) presented in this report, significant effort was also devoted to lay the foundations for the continuation of monitoring efforts by available MPA management bodies and local research groups, on the basis of shared data collection protocols, thereby enhancing the future sustainability of obtained results. The project's goals included: a) to support the conservation of bottlenose dolphins in the Pelagos Sanctuary; b) taking advantage of the popularity of these charismatic mammals to help raising awareness within the wider public on the conservation of the marine environment; and c) strengthening the links among Pelagos and the many MPAs and National Parks existing within the Sanctuary boundaries by promoting a sustained coordination and systematic implementation of dolphin monitoring and conservation actions in the area.

Bottlenose dolphins are widely distributed over the continental shelf of the Pelagos Sanctuary, where their presence has been known for a long time. However, since a few years ago, their ecology and abundance was poorly known. In recent years an increase in research effort and coordination between groups have finally produced a better understanding of the bottlenose dolphin conservation status in the Pelagos area. The project DWB has progressed along this line, reinforcing the cooperation with the French side of the Sanctuary and beyond (i.e., the GDEGeM project) and contributing to fill the knowledge gaps on the species in the research area. These findings are essential to understand the conservation needs of the Pelagos bottlenose dolphin population as a whole and to plan an effective protection plan.

DWB strived to apply in the study area (which coincides with the eastern portion of Sub-area 5 identified by the ACCOBAMS "Conservation Plan for the Conservation of the Bottlenose Dolphin") the indications provided by the ACCOBAMS Conservation Plan. These include: a) contributing to the identification and mapping of primary dolphin habitat within the Italian portion of the Pelagos Sanctuary, b) strengthening the population estimates based on photo-ID efforts and research cruises, c) investigating existing pressure factors that might affect connectivity between the different primary dolphin areas, and existing threats to the local populations, d) promoting networking among the various research, conservation and management communities (including MPA managers), in particular through data sharing by all the different ongoing research projects by taking advantage of the INTERCET platform, and e) enhancing capacity building within the above mentioned communities.

Indexes of relative abundance, if periodically measured and obtained from standardised observations, may support the assessment of the bottlenose dolphin population status and trends. It is very important the observation being standardised and based on the existing know how. It is now well assessed that the species presence in this study area shows differences in terms of encounter rate and it may be increasing in some areas (e.g. De Santis et al., 2018) so a regular monitoring at specific relevant locations (e.g. AMPs) may greatly improves the understanding about the status of this species.

A simplified method to assess the relative abundance of the species would be to standardise the effort of the observations to avoid the need of its computation.

Observations could be land based or boat based.

Land-based watch site locations can be chosen based on the following criteria:

- Good field of view (to allow large area of water to be surveyed from a single place)
- Accessibility and suitability for access
- Height

Typically, land-based watches may last two to four hours and should be made at fixed times (e.g. 09:00-13:00; or 15:00-19:00). Dolphin numbers, positions, behaviours and direction of movement can be recorded onto data sheets during scan samples (e.g. Altmann, 1973) throughout every watch. Scans should be made using 10x50 binoculars and/or a bigeye telescope with a 30x eyepiece.

Surveying and photographing cetaceans from small boats may allow to collect photographic data and better estimates of dolphin group sizes and composition. Standardised boat-based surveys need to be regularly undertaken. Boat-based surveys should be made within a predefined survey area (e.g. 400 or 500 km<sup>2</sup>) and the boat routes should be designed to cover the largest portion of the area within a fixed period of observation (e.g. 5 days boat survey made of 4 daily cruising hours).

A minimum of two surveys could be made during the summer (April to September) and during the winter (October to March) and conducted in sea states of Beaufort 3 or less in order to minimise the effects of sea state on the probability of sighting and photographing of dolphins. The surveys should be conducted at a steady speed of about 10 km/h and lookout should be maintained throughout for three or four hours.

During a dolphin encounter all the animals need to be photographed, and the time and position of the sighting recorded.

Provided that the effort is standardised for both land-based and boat-based observations, synthetic indexes of abundance may be the number of dolphin encounters/sightings or the mean number of dolphins per each encounter/sighting.

The main beneficiaries from DWB were: a) the Parties to the Pelagos Sanctuary Agreement, because this project supported reaching the goal of conserving bottlenose dolphins within Pelagos (a priority goal as defined by the Scientific and Technical Committee of the Agreement); b) the managers of the marine parks and protected areas comprised within the Pelagos Sanctuary boundaries; and c) ultimately, but most importantly, the population of bottlenose dolphins living in the Pelagos Sanctuary, because a better understanding of its ecological traits provides ammunition to better address and mitigate the range of threats it is exposed to.

## **6. Acknowledgments**

Tethys Research Institute is grateful for the work carried out by participants in Cetacean Sanctuary Research CSR citizen science program. Many thanks to Roberto Raineri and Paolo Pinto, skippers of the vessel “Pelagos”, who have provided an invaluable support during fieldwork, and to Maddalena Jahoda and Elena Politi for contributing to the DWB communication phase. The Milan Civic Aquarium and Hydrobiological Station provided logistical support.

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## 8. Annexes



**Annex 1** Letter sent to the *Italian Ministry of Environment and Protection of the Territory and the Sea, General Directorate for the Protection of Nature and the Sea*, in December 19<sup>th</sup> 2018, to keep them informed about DWB training and capacity building initiatives.



**ISTITUTO TETHYS ONLUS**

*Organizzazione non-profit per lo studio e la tutela dell'ambiente marino*

Milano, 19 dicembre 2018

Cortese Attenzione:

D.ssa Maria Carmela Giarratano

Ministero dell'Ambiente e della Tutela del Territorio e del Mare

Direzione Generale per la Protezione della Natura e del Mare

Gentile Direttore,

Le scrivo per informarla della nostra intenzione di contattare gli Enti gestori di alcune Aree Marine Protette e Parchi Nazionali, sulla base degli impegni assunti da noi e dai nostri Partners nell'ambito del progetto "Delfini Senza Frontiere" (Dolphins without Borders) co-finanziato dall'Accordo Pelagos.

Gli Enti che intendiamo contattare sono i seguenti:

- Parco Nazionale dell'Arcipelago Toscano
- Parco Nazionale Arcipelago della Maddalena
- Parco Nazionale dell'Asinara
- Area Marina Protetta dell'Isola di Bergeggi
- Area Marina Protetta di Portofino
- Area Marina Protetta delle Cinque Terre
- Area Marina Protetta delle Secche della Meloria.

L'obiettivo di questa azione consisterà nel prendere accordi per l'attuazione di quanto stabilito nel progetto approvato:

- "Training programmes of personnel from Italian MPAs and National Parks in the project area that have expressed their availability to participate. Entities to be contacted include: Arcipelago Toscano, Isola dell'Asinara, Arcipelago della Maddalena, Isola di Bergeggi, Portofino, Cinque Terre, Secche della Meloria", e
- "A framework of management guidelines and rules, based on risk assessments, will be developed in cooperation with available MPA management bodies and the Pelagos Sanctuary, to support bottlenose dolphin conservation in the area. These will include standardized bottlenose dolphin conservation measures, including systematic monitoring, which we suggest should be incorporated into the MPAs' management plans".

Qualsiasi suggerimento e supporto da parte sua nell'adempimento delle azioni sopra elencate sarà grandemente apprezzato.

L'occasione mi è gradita per inviare i miei migliori saluti e auguri,

Simone Panigada

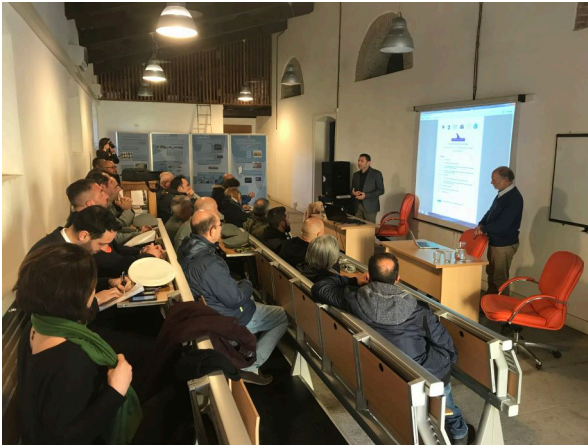
Presidente

**Annex 2** Names, affiliations and e-mail (as provided) of participants in the three DWB training sessions.

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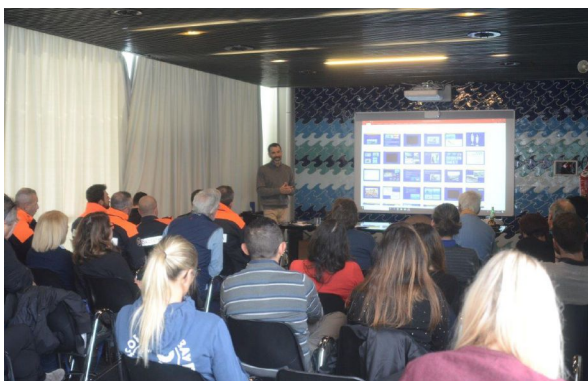
**Annex 3** Photo album of the three DWB training workshops held in Sardinia, Elba and Genoa.



Caprera, 28 February 2019



Elba Island, 5 March 2019



Genoa, 13 March 2019

## **Annex 4** Communication sent to 123 Italian municipalities partners of the Pelagos Sanctuary informing them about DWB project\_

*Alla cortese attenzione del*

*Referente Comunale per il Partenariato del Santuario Pelagos*

*Comuni aderenti alla Carta di Partenariato del Santuario Pelagos*



Gentile Referente,

con questo messaggio desideriamo portare alla sua attenzione l'iniziativa "Dolphins Without Borders" (DWB), augurandoci che possa essere di interesse per il Comune da lei rappresentato, nell'ambito delle attività inerenti al Partenariato con il Santuario Pelagos.

DWB - un progetto finanziato dalla Fondazione Albert II Principe di Monaco e dall'Accordo per il Santuario Pelagos - contempla una serie di attività per la conservazione dei tursiopi nel Santuario Pelagos prendendo spunto da simili azioni messe in atto recentemente in Francia attraverso il progetto GDEGeM della Società GIS3M. DWB contribuisce a raccogliere le informazioni sull'ecologia e lo stato di conservazione attualmente conosciuto dei tursiopi del Mediterraneo nord occidentale, facilitando quindi l'avanzamento nello studio dell'ecologia di questa specie. Uno sforzo significativo viene anche posto nel garantire la continuazione del monitoraggio, una volta terminato il progetto, attraverso il coinvolgimento degli Enti gestori delle Aree Marine Protette (MPA) che sorgono all'interno dei confini del Santuario e dei gruppi di ricerca locali, sulla base dell'utilizzo di protocolli comuni, assicurando quindi una maggiore efficacia e la sostenibilità futura dei risultati ottenuti.

Il progetto si propone anche di contribuire al rafforzamento dei legami tra il Santuario Pelagos e le molte aree marine protette e parchi nazionali marini esistenti all'interno dei confini del Santuario stesso, e inoltre di approfittare della popolarità dei tursiopi, mammiferi altamente carismatici, che possono aiutarci a far crescere nel pubblico più vasto la sensibilità nei confronti della conservazione dell'ambiente marino.

Sul sito di DWB: <https://www.dolphinwithoutborders.net> potrà trovare alcune informazioni di base sul progetto e sulle sue finalità. Potrà anche accedere a una esauriente raccolta bibliografica sulle conoscenze scientifiche sul tursiope in Mediterraneo. Potrà infine scaricare una scheda per segnalare gli avvistamenti e un dépliant sul progetto stesso in pdf

Per qualsiasi ulteriore informazione non esiti a contattarci:

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DWB è condotto in collaborazione tra le organizzazioni Istituto Tethys, GIS3M, Fondazione Acquario di Genova, CE.TU.S. e Università di Sassari/SEA ME Sardinia. Anche a nome delle suddette organizzazioni la ringraziamo per l'attenzione e le inviamo i nostri migliori saluti

Giuseppe Notarbartolo di Sciara

Joan Gonzalvo

Istituto Tethys



finanziato da





## IL TURSIOPÈ (*Tursiops truncatus*)

È il delfino più conosciuto dal pubblico; massiccio, grigio tendenzialmente uniforme, è lungo circa 3 metri e pesante fino a 300 kg. Normalmente vive in piccoli branchi ma capita a volte che individui saltari si spingano vicino alle spiagge o fino nei porti.

Nel Santuario è ampiamente diffuso nelle acque costiere di Toscana, Sardegna e Corsica; più frequente nella parte orientale, ma recentemente viene avvistato con regolarità anche nei pressi della costa del Ponente ligure.

È una specie molto adattabile; frequenta sia le acque basse e limacciose che gli ambienti rocciosi e il nitrato di una gran varietà di pesci e di cefalopodi. Le sue abitudini costiere lo rendono vulnerabile a tutti i contaminanti di origine antropica; altre minacce sono le reti da pesca e la scarsità di cibo a causa dell'eccessivo sfruttamento da parte dell'uomo.

## DELFINI SENZA FRONTIERE

I tratti di mare che ospitano tursiopi rientrano nelle aree di studio di diversi gruppi di ricerca, di tre parchi nazionali e quattro aree marine protette. I delfini però non conoscono i confini, da cui l'esigenza di coordinare gli sforzi per gli studi e la loro conservazione.

**Lo scopo del progetto** "Dolphins Without Borders" è ottenere un quadro complessivo della loro ecologia, biologia e conservazione.

**I metodi utilizzati** comprendono foto-identificazione e analisi genetiche. La condivisione dei dati fra i vari gruppi contribuisce al miglioramento delle rispettive competenze.

**Il progetto**, che complementa un analogo programma francese e si svolge nell'arco di un anno tra 2018 e 2019, vede il coinvolgimento di **Liguria, Toscana e Sardegna**, con 180 giorni complessivi in mare e una serie di training specifici. Lo scopo finale è avanzare delle **proposte mirate** per la conservazione dei tursiopi sul territorio, attuando nel contempo programmi di informazione e sensibilizzazione del pubblico in collaborazione con i Comuni aderenti alla **Carta del Partenariato** per il Santuario Pelagos.

[dolphinswithoutborders.net](http://dolphinswithoutborders.net)


IL PROGETTO  
È FINANZIATO DA




## IL SANTUARIO PELAGOS

Area marina protetta, proposta in primo luogo da Tethys, e istituita nel 2001 grazie a un accordo internazionale tra Francia, Italia e Principato di Monaco, si estende su 87.500 km<sup>2</sup> e comprende Costa Azzurra, Corsica, Principato di Monaco, Liguria, Toscana e Sardegna e un'ampia zona d'alto mare.

Particolari condizioni climatiche e geomorfologiche rendono queste acque ricche di nutrimento e di importanza cruciale per tutte le specie di cetacei che frequentano regolarmente il Mediterraneo.



## CONOSCIUTI UNO AD UNO

Attraverso la fotoidentificazione ogni tursiopo può essere riconosciuto individualmente dalla forma della pinna dorsale e da eventuali tacche e altri segni. Questo permette non solo di conoscere la storia dei singoli delfini, le associazioni fra di loro, gli spostamenti, ma anche di stimare il numero di animali.

### PARTNERS DWB

Progetto coordinato da:



Con la partecipazione di:

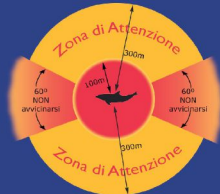


Massimo Demma (design) • Biemo Follì (grafici)  
Maddalena Jarchio (text) • Fondazione Acquario di Genova (foto)

## INCONTRI IN MARE


In mare è importante seguire alcune semplici **regole\***, per non disturbare o ferire i delfini.

- non puntare mai direttamente verso il branco, ma mantenere una **rotta parallela** a quella degli animali;
- mantenere una velocità non superiore a 5 nodi;
- non avvicinarsi a più di 100 m; a questa distanza mettere il motore in folle;
- fare attenzione a eventuali animali in immersione, non visibili alla superficie;
- spegnere **ecoscandagli** e fishfinder;
- non **separare** mai i piccoli dagli adulti;
- **alternarsi**, una alla volta, con altre barche eventualmente presenti entro 1.300 m;
- non **toccare** i delfini, né gettare loro del cibo;
- non **entrare in acqua** in loro presenza.



Chi avvista un delfino in **difficoltà** o spiaggiato, chiami il 11530 o si metta in contatto direttamente con la Guardia Costiera o la Capitaneria, che provvederanno a inviare personale esperto.

\* In accordo con le linee guida di ACCOBAS (Accordo per la Conservazione dei Cetacei in Mar Nero, Mediterraneo e aree contigue, Albania) e dell'agente Pelagos



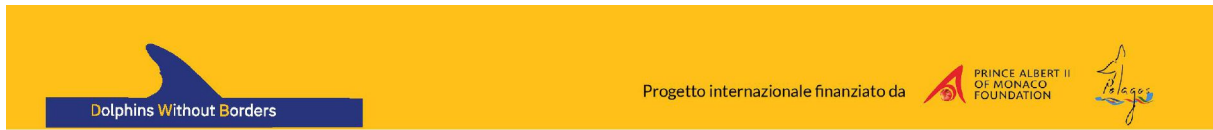
**Dolphins Without Borders**

## INSIEME

PER I TURSIOPÈ DEL SANTUARIO PELAGOS

Delle otto specie di cetacei regolari in Mediterraneo, il più facile da incontrare sottocosta è il tursiopo, un delfino presente sulla piattaforma continentale del Santuario Pelagos.

Come tutti i mammiferi marini, ha un ruolo importante: non solo va tutelato nell'interesse della biodiversità del Pianeta, ma può essere anche un prezioso indicatore dello stato di salute dei nostri mari. Per questo i tursiopi del Santuario Pelagos sono al centro di un progetto di cooperazione tra i maggiori esperti di cetacei del Mediterraneo.



## CODICE DI CONDOTTA IN CASO DI AVVISTAMENTO



In mare è importante seguire alcune semplici regole, per non disturbare o ferire i delfini.

- non puntare mai direttamente verso il branco, ma mantenere una rotta parallela a quella degli animali;
- mantenere una velocità non superiore a 5 nodi;
- non avvicinarsi a più di 100 m; a questa distanza mettere il motore in folle;
- fare attenzione a eventuali animali in immersione, non visibili alla superficie;
- spegnere ecoscandagli e fishfinder;
- non separare mai i piccoli dagli adulti;
- alternarsi, una alla volta, con altre barche eventualmente presenti entro i 300 m;
- non toccare i delfini, né gettare loro del cibo;
- non entrare in acqua in loro presenza.

## COME SEGNALARE UN AVVISTAMENTO

Compilare la scheda allegata e inviarla a uno dei coordinatori del progetto DWB qui sotto per email, corredando la scheda con eventuali **foto** o **filmati**.

E' possibile anche compilare una scheda online sul sito di Cetus e sul sito che l'Istituto Tethys ha realizzato per questo scopo.

|                    |                                     |                |  |  |
|--------------------|-------------------------------------|----------------|--|--|
| Toscana            | CETUS                               | Silvio Nuti    | <a href="mailto:cetus2019@gmail.com">cetus2019@gmail.com</a>               | <a href="http://www.cetusresearch.eu">www.cetusresearch.eu</a>                 |
| Sardegna           | SEAME                               | Luca Bittau    | <a href="mailto:lucabittau@seame.it">lucabittau@seame.it</a>               |  |
| Liguria<br>levante | FONDAZIONE<br>ACQUARIO DI<br>GENOVA | Guido Gnone    | <a href="mailto:ggnone@costaedutainment.it">ggnone@costaedutainment.it</a> |  |
| Liguria<br>ponente | ISTITUTO<br>TETHYS                  | Sabina Airoidi | <a href="mailto:sabina.airoidi@iol.it">sabina.airoidi@iol.it</a>           | <a href="http://www.cetaceifaiattenzione.org">www.cetaceifaiattenzione.org</a> |



## SCHEDA DI AVVISTAMENTO

NOME \_\_\_\_\_ EMAIL \_\_\_\_\_

Nome barca \_\_\_\_\_

DATA \_\_\_\_\_ ORA \_\_\_\_\_

LUOGO DELL'AVVISTAMENTO \_\_\_\_\_

LAT \_\_\_\_\_ LONG \_\_\_\_\_

MARE \_\_\_\_\_ CIELO \_\_\_\_\_ VENTO \_\_\_\_\_

DURATA AVVISTAMENTO \_\_\_\_\_ DISTANZA AVVISTAMENTO \_\_\_\_\_

SPECIE AVVISTATA \_\_\_\_\_

N. INDIVIDUI AVVISTATI \_\_\_\_\_ PRESENZA DI PICCOLI \_\_\_\_\_ QUANTI \_\_\_\_\_

isolato       a poppa       confidenti       dietro rete       salti   
 in gruppo       a prua       diffidenti       dietro scia

NOTE



## COME IDENTIFICARE I CETACEI

Balenottera comune



Capodoglio



Globicefalo



Grampo



Tursiope



Delfino comune



Stenella striata



Disegni: Massimo Demma - [www.massimodemma.it](http://www.massimodemma.it)