

# An index based on the biodiversity of cetacean species to assess the environmental status of marine ecosystems

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## 1. Introduction

The EU environmental policies during the last three decades have focused on determining adverse and undesirable changes to the natural system as the result of human activities and then, if such changes are detected, management responses are then foreseen to alleviate those adverse changes. The Marine Strategy Framework Directive (hereinafter MSFD) and before the Water Framework Directive (WFD) might be both considered as components of a suite of environmental controls linked on their own to the Directives for Environmental Impact Assessment, Strategic Environmental Assessment, Nitrates control and the Habitats and Species and Wild Birds Directives (Borja et al., 2010). The MSFD establishes a framework for the development of marine strategies designed to achieve the “Good Environmental Status” (GES) in the marine environment, by the year 2020, using 11 qualitative

descriptors. The descriptors are not objectives per se; rather, they describe features of the ecosystem that are widely considered as important, either from a conservation (e.g., biodiversity, food web) or threat (e.g., non-indigenous species, marine litter) perspective that may be useful in developing a specific set of management objectives. Therefore, the MSFD requires the assessment of the functioning of each objective in relation to pressures. Based on this knowledge, appropriate programs of measures might be enforced to control the pressures that significantly affect the marine environmental status. Understanding the mechanism and/or the hierarchical pathways through which specific activities affect descriptor indicators is an essential step in the process of managing their potential impact. This assessment is further complicated by the fact that specific impacts may result from activities associated with numerous sectors (Ban et al., 2010). Thus, the link between sectors, the pressures they generate and the effects that those pressures have on the components of the ecosystem, need to be clearly understood if the impact of a sector and its activities is to be reduced or mitigated to avoid detrimental effects to the ecological characteristics of the ecosystem.

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There are numerous human activities that have the potential to negatively impact marine ecosystems (Halpern et al., 2007), many of which are common to several sectors functioning in Europe's regional seas. MSFD identifies 18 specific pressures, which could be placed into one of eight general pressure groupings based on their shared impact characteristics such as whether the pressure caused physical damage (e.g., abrasion or selective extraction), physical loss (e.g., smothering or sealing) or contamination (e.g., introduction of synthetic compounds) (see Annex III of the Directive [EC 2008] for the full list of pressures and impacts).

Among the other MSFD descriptors, descriptor 4 (D4) addresses the marine food webs and states “All elements of the marine food webs, to the extent that they are known, occur at normal abundance and diversity and levels capable of ensuring the long-term abundance of the species and the retention of their full reproductive capacity”.

It is well known that human activities may cause direct or indirect changes in food webs (Layman et al., 2005; Raffaelli, 2005). Events such as overexploitation (Pauly et al., 1998), pollution (Boon et al., 2002), eutrophication (Cloern, 2001), habitat fragmentation and destruction (Layman et al., 2007; Melian and Bascompte, 2002), invasions of species (Vander Zanden et al., 1999) and anthropogenic climate change (Kirby and Beaugrand, 2009; Muren et al., 2005) all pose potential threats to the structure and dynamics of food webs, acting at variable spatial scales and affecting food webs in different ways (Moloney et al., 2010).

To successfully identify and then monitor all these processes is extremely challenging. To date, ecologists have proposed several quantitative indicators to describe the status of marine ecosystems. However, strengths and weaknesses of the different indicators are usually only partially known. In many cases, due to the gaps in our knowledge about the relationship of the ecological status with the existing pressures, these indicators fail to support the setting of management objectives and do not allow the provision of scientific advice on how these objectives might be achieved. This is particularly true for indicators based on multiple species. In spite of that, marine food web indicators are becoming increasingly important as a factor in conservation management, particularly concerning the assessment of the ecological risk deriving from human activities (de Ruiter et al., 2005; Sala and Sugihara, 2005). In contrast to the single-species approaches, a system-level approach is in fact considered attractive since both, direct and indirect effects of disturbance are integrated into a single interaction network (Raffaelli, 2005). However, due to the high functional diversity in marine ecosystems and to the food-web complexity, practical applications remain quite rare. Whilst an ecosystem perspective is increasingly used in fisheries management to study ecosystem responses to different stressors and to assure sustainable use of resources (e.g. Coll et al., 2008), similar holistic approaches to evaluate the combined influences of other anthropogenic stressors on food webs are still lacking.

In this study, the biodiversity of the cetacean community is proposed as MSFD D4 indicator (e.g. indicator 4.3.1 Abundance trends of functionally important selected groups/species) and reference points are provided to correlate the environmental status derived by this indicator with the pressures affecting the study area (i.e. naval traffic, pollution, fishing pressure etc.).

### 1.1. The MSFD D4 descriptor and cetacean species

The D4 indicators stipulated in the Commission Decision (European Commission, 2010; 2010/477/EU), following extensive review by the JRC/ICES Task Group (TG4) on food webs (Rogers et al., 2010), address three criteria related to food web structure and energy transfer between different components (Table 1).

**Table 1**  
Criteria and associated indicators for the MSFD Descriptor 4 (food webs).

Attribute	Criterion	Indicator
Energy flow in the food web	Productivity of key species or trophic group (4.1)	Performance of key predator species using their production per unit biomass (4.1.1)
Structure of the food web (size)	Proportion of selected species at the top of the food web (4.2)	Large fish (by weight) (4.2.1.)
Structure of the food web (abundance)	Abundance/distribution of key trophic groups/species (4.3)	Abundance trends of functionally important selected groups/species (4.3.1)

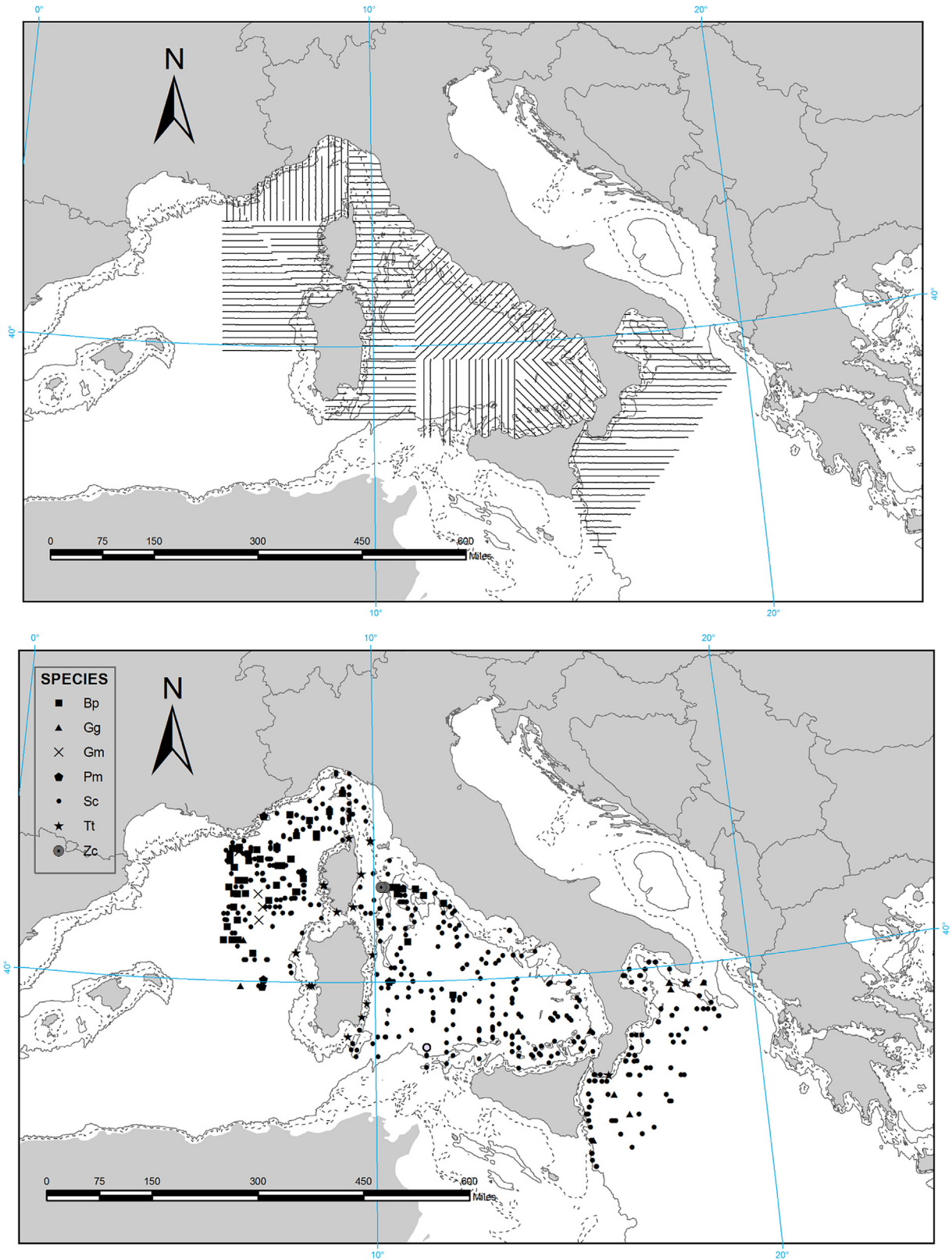
**Table 2**  
List of criteria for selecting key species/groups for indicator 4.3.1 “Abundance/distribution of key trophic species” as proposed by the Commission Decision (2010/477/EU).

Criterion	Indicator	Selection criteria for key trophic groups/species
Abundance/distribution of key trophic groups/species (4.3)	Abundance trends of functionally important selected groups/species (4.3.1)	(i) Groups with fast turnover rates (ii) Groups/species that are targeted by human activities or that are indirectly affected by them (iii) Habitat-defining groups/species (iv) Groups/species at the top of the food web (v) Long-distance anadromous and catadromous migrating species (vi) Groups/species that are tightly linked to specific groups/species at another trophic level

As shown in Table 1, whereas criterion 4.1 and its associated indicator 4.1.1 is proposed mainly as a proxy measure of energy flow within marine food webs, structural properties of food webs are covered by criteria 4.2 and 4.3 (Table 1). Given that



**Fig. 1.** Study area: The three subregions under the Italian jurisdiction are shown.



**Fig. 2.** Aerial surveys. Upper section: survey transects; lower section: sightings. *Bp*: fin whales, *Pm*: sperm whales, *Gg*: Risso's dolphins, *Gm*: long-finned pilot whales, *Sc*: striped dolphins, *Tt*: common bottlenose dolphins and *Zc*: Cuvier's beaked whales.

many food webs components are also relevant to other MSFD descriptors (e.g. D1, biodiversity, D3, commercial fish species and D6; seafloor integrity), it might be expected that indicators used for D4 overlap those used for the other Descriptors. The

criteria for selecting key species/groups to calculate indicator 4.3.1, are stated in the previously mentioned Commission Decision and relate to many possible food web components (Table 2).

Marine mammals, and particularly cetacean species, respond to most of these criteria. Bănaru et al. (2013) in their study about the trophic structure in the Gulf of Lions marine ecosystem have found that dolphins are *keystone* species. Power et al. (1996) defined *keystone* as species with a structuring role within ecosystems and the food webs that interconnect in spite of a relatively low biomass and hence food intake. Keystone species strongly influence and are strongly influenced by the abundances of other species and the ecosystem dynamic (Piraino et al., 2002) and may reasonably be considered key species for the indicator 4.3.1.

## 2. Material and methods

### 2.1. Study area

This study concerns the three Mediterranean subregions, which have been considered for the Initial Assessment of the Italian marine waters, namely the Western Mediterranean Sea, the Adriatic Sea and the Ionian and Central Mediterranean Sea (Fig. 1).

Fin whales (*Balaenoptera physalus*), sperm whales (*Physeter macrocephalus*) Risso's dolphins (*Grampus griseus*), longfinned pilot whales (*Globicephala melas*), striped dolphins (*Stenella coeruleoalba*), common bottlenose dolphins (*Tursiops truncatus*) and Cuvier's beaked whales (*Ziphius cavirostris*) are known as regularly occurring species (Boisseau et al., 2010) in the Mediterranean Sea. Short-beaked common dolphins (*Delphinus delphis*), although also occurring, are much rarer (Bearzi et al., 2003).

For the purpose of the analysis the study area was covered by grid of 2490 cell units with a size of 17 × 22 km. Such a grid has been used for all the presented spatial analysis.

### 2.2. Used data set

For the purpose of this study two different types of data were used: sighting data deriving from the aerial surveys funded by the Italian Ministry of the Environment, and conducted respectively in the Pelagos Sanctuary (see Panigada et al., 2011a), in the Tyrrhenian, and Ionian Seas (see Panigada et al., 2011b; Lauriano et al., 2011); and the strandings data available from the Italian Stranding Network.

#### 2.2.1. Sightings from the aerial surveys

Surveys were designed following the 'distance sampling' method to estimate abundance (Buckland et al., 2001; see Fig. 2). In all the surveys, the study area was subdivided into strata, following bathymetric criteria and the available knowledge of cetacean presence and distribution. Due to the seasonality of fin whale presences, surveys in subarea 1 and 2 were conducted both in the high presence season (i.e. summer) and in the low presence season (i.e. winter). Parallel line transects, 10 or 15 km apart, with a random starting point were determined using the program Distance ver. 5.0 (<http://www.ruwpa.st-and.ac.uk/distance/>), to allow for homogeneous coverage probability. Survey methods are described in detail in Panigada et al., 2011a. Table 3 shows the main features of the aerial survey data set.

#### 2.2.2. Strandings data

Data on cetacean strandings along the Italian coasts have been regularly collected on a national basis between 1986 and 2005 by the Centro Studi Cetacei. The network managed the monitoring of Italian coasts and the study of the strandings, bycatches and ship collisions. Since 2006 the Italian database of strandings (BDS) is the national archive of these data and provides information about the date of the event, its location, data of the specimen such as species, gender and, length. The considered records, updated to 2012, hold

information about toxicological and parasitological investigations, description of samples collected and the institute where the samples are stored. The considered data set contains little more than 4000 records, belonging to 14 species. These historical strandings of Mediterranean species along Italian coastline are available on line <http://mammiferimarini.unipv.it> (Podestà et al., 2006, 2009).

### 2.2.3. Observed biodiversity: DAR index based on sightings/strandings data

The ratio between Dominance<sub>Sp</sub> (i.e. number of sightings of the dominant species over total number of sightings. The dominant species is the one with the highest frequency in the cell unit) and the Relative Abundance<sub>All Spp</sub> (i.e. total number of sightings normalised over the maximum abundance value recorded in the study area) considering all the species was chosen as biodiversity index.

$$\text{DAR} = \frac{\text{Dominance}_{\text{Sp}}}{\text{Rel. Abundance}_{\text{All spp}}}$$

Such an index was preferred to other known diversity indexes since it relies on dominance and relative abundance which are both concepts well known to researchers studying cetacean species. Moreover dominance and relative abundance can be easily determined based on cetacean census data.

DAR index is inversely correlated to diversity. It increases in low biodiversity conditions, where largely dominant species are present and determine almost entirely the abundance, and decreases when the biodiversity increases and the presence of dominant species is balanced by the presence of other species.

Fig. 3 shows how the DAR index increases as dominance increases and decreases as the relative abundance increases. A sensitivity analysis of DAR index was performed and its variability was compared with the one of the most well known Shannon's diversity index (see Supplementary material S1 and S2).

### 2.2.4. Expected biodiversity based on habitat availability

Presence/absence habitat models using physiographic predictors as covariates were used to estimate the presence probability of the six species of cetaceans regularly occurring in the study area (due to the fact we had very few sightings and stranding records of long finned pilot whale the species was not considered in this study) and to obtain the theoretical biodiversity (i.e. expected) based on such habitat availability. Most of the used models were developed based on long-term data series (see Azzellino et al., 2012 for reference). The habitat availability for the species Cuvier's beaked whale was instead obtained from a much shorter data series. However, model accuracy in this case was validated evaluating the model performance in an area different from the calibration (see Azzellino et al., 2011 for details). The physiographic predictors for the study area have been obtained through the GEBCO One minute Digital Atlas and gridded by means of a GIS software. Sea bed slope was calculated according to Burrough (1986).

Based on the habitat model predictions, a map was obtained for the expected biodiversity. Predictions were produced for every cell unit (Fig. 4) and the 75% probability was assumed as threshold value for the species presence.

### 2.2.5. Anthropogenic pressures

Ship traffic, pollution, and impact of fisheries were considered to explain the patterns of the biodiversity deviations from the expected. Particularly, the maritime traffic density was derived from the results of PASTA-MARE project, pollution data were obtained from the EIONET Archive (European Environment Information and Observation Network) which concerns sediments and biota, and the fishery impact of was evaluated through the statistics available



**Table 3**  
 Characteristics of the aerial surveys data set. Sighting codes: *Bp*: fin whales, *Pm*: sperm whales, *Gg*: Risso's dolphins, *Gm*: long-finned pilot whales, *Sc*: striped dolphins, *Tt*: common bottlenose dolphins, *Zc*: Cuvier's beaked whales, and *Un*: Undetermined.

Area	Period	Surface of study area (approx.)	Sightings
Pelagos Sanctuary	Winter 2009	90,000 km <sup>2</sup>	Total sightings: 131; Sc: 114; Tt: 7; Bp: 1; Pm: 1; Zc: 1; Un: 7
Pelagos Sanctuary	Summer 2009	90,000 km <sup>2</sup>	Total sightings: 336; Sc: 280; Tt: 8; Bp: 24; Gg: 4; Gm: 5; Pm: 5; Zc: 4; Un: 6
Pelagos Sanctuary – Sardinia Tyrrhenian Sea:	Summer 2010	230,000 km <sup>2</sup>	Total sightings: 259; Sc: 187; Tt: 11; Bp: 48; Gg: 3; Gm: 5; Pm: 4; Un: 1
Central	Summer 2010	98,000 km <sup>2</sup>	Total sightings: 97; Sc: 90; Tt: 1; Gg: 2; Zc: 1; Un: 3
Southern	Autumn 2010–Winter 2011		
Ionian Sea	Spring 2010	110,000 km <sup>2</sup>	Total sightings: 82; Sc: 69; Tt: 2; Gg: 6; Un: 5

from Osservatorio Nazionale Pesca (2011) and from the FAO year-books (<http://www.fao.org/fishery/statistics/en>).

### 2.2.6. Levels of contamination and genetic individual variability as a measure of animal stress

The level of contamination of the most frequent cetacean species occurring in the study area and other measure of animal stress were also taken into account. Particularly, the level of contamination from organochlorines (e.g. HCB, DDTs and PCBs) in 73 striped dolphins stranded in 1985–2011 along the Italian coasts (see Marsili et al., 1997; Marsili, 2000 as reference for the method) and the standardised observed heterozygosity (e.g. St.het\_Obs, after Coltman and Slate, 2003) in striped and common bottlenose dolphins were taken into account. Standardised observed heterozygosity (st\_het\_Obs) as a measure of individual variability, was obtained by genotyping: 15 microsatellite loci for 305 striped dolphins; and 12 microsatellites loci for 116 common bottlenose dolphins. This measure of individual variability is based on a score for each locus weighted by the average heterozygosity at that locus (Coltman et al., 1999). The proportion of heterozygous typed loci/mean heterozygosity of typed loci was used. This method assigns equal weight to all loci examined, regardless of their allelic frequencies, and assumes a linear relationship between locus-specific heterozygosity and number of alleles.

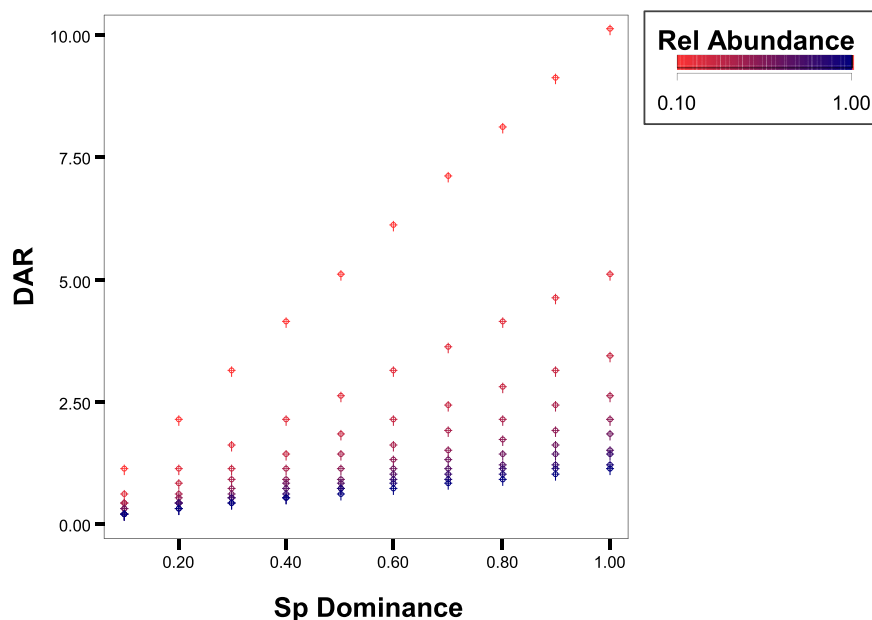
### 2.3. Statistical analysis

#### 2.3.1. Multivariate methods

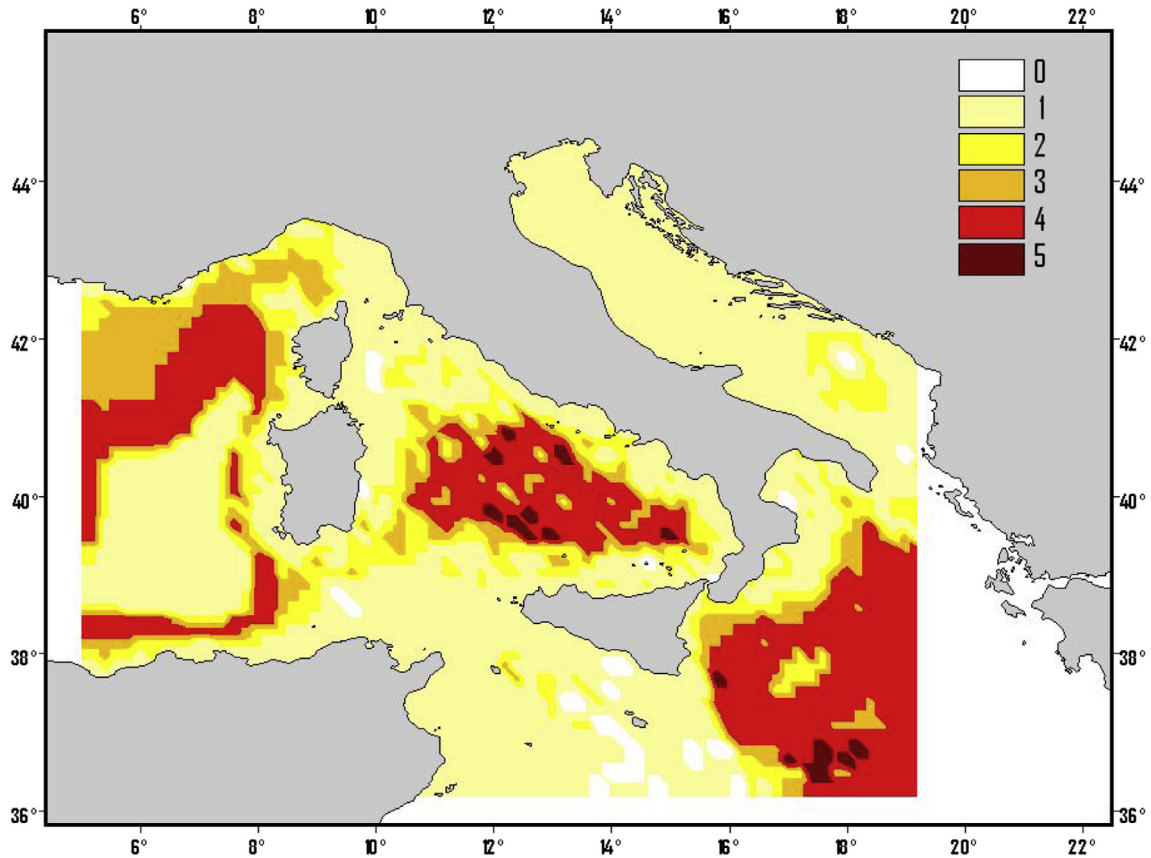
Different multivariate techniques were used to analyse the data: Principal Component Analysis (PCA), Factor Analysis (FA) and Cluster Analysis (CA) (Afifi and Clark, 1996).

#### 2.3.2. Principal Component Analysis/Factor Analysis

Principal Component Analysis and Factor Analysis were chosen to reduce the dimensionality of the pollution data included in the EIONET data set. PCA extracted the eigenvalues and eigenvectors from the covariance matrix of the original variances. Factor Analysis (FA) was obtained through the rotation of the extracted PCA eigenvalues and eigenvectors allowing to reduce the contribution of the less significant parameters within each component. The Varimax rotation criterion was used to rotate the PCA axes allowing the rotated varifactors to maintain the orthogonality. The number of components or factors to retain was chosen on the basis of the “eigenvalue higher than 1” criterion (i.e. all the components/factors that explained less than the variance of one of the original variables were discarded). That allowed to select few components to describe the whole data set with minimum loss of original information.



**Fig. 3.** DAR Index sensitivity analysis: Colour scale shows the Relative Abundance scale (i.e. increasing from lighter to darker). DAR index increases as species Dominance (i.e. Sp Dominance) increases and decreases as Relative Abundance increases. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



**Fig. 4.** Map of the expected biodiversity (i.e. number of expected species). Species presence predictions are produced based on physiographic predictors through the species habitat models.

### 2.3.3. K-means Cluster Analysis

A K-means Cluster Analysis (CA) was used to analyse the habitat similarities among the cell units. The cell areas of potential habitat for the six species were the input of the analysis. The Euclidean Distance was chosen as distance measure:

$$d_2(\mathbf{x}_i, \mathbf{x}_j) = \sqrt{\sum_{k=1}^q (\mathbf{x}_{ik} - \mathbf{x}_{jk})^2}$$

CA was run twice. The final cluster centroids obtained from the first run were used as initial centres of the second run.

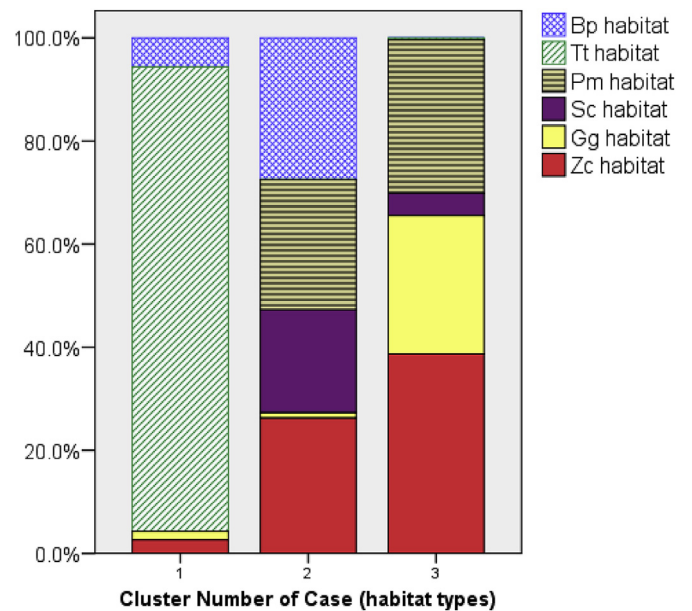
### 2.3.4. Hypothesis testing

Statistical significance of the differences was assessed by non-parametric hypothesis testing. Particularly Kruskal–Wallis test was used to compare K independent samples and the Mann–Whitney test was used for the comparison of 2 independent samples. The correlation between biodiversity deviations from the expected and the anthropogenic pressures were quantified through the Pearson's correlation coefficient or the Spearman's rank correlation coefficient depending on variable skewness.

## 3. Results

### 3.1. Classification of the habitat types

The K-means Cluster Analysis applied to the areas of potential habitat (i.e. areas with species presence probability higher than 75%) of the six species in the 2490 cell units. CA allowed the



**Fig. 5.** Characteristics of the habitat types identified through the K-mean Cluster Analysis. *habitat type 1*: coastal habitat, *habitat type 2*: pelagic habitat, *habitat type 3*: shelf break and continental slope habitat. Abbreviations: *Bp habitat*: typical habitat of the species fin whale, *Pm habitat*: typical habitat of the species sperm whale, *Gg habitat*: typical habitat of the species Risso's dolphin, *Gm habitat*: typical habitat of the species long-finned pilot whale, *Sc habitat*: typical habitat of the species striped dolphin, *Tt habitat*: typical habitat of the species common bottlenose dolphin and *Zc habitat*: typical habitat of the species Cuvier's beaked whale.

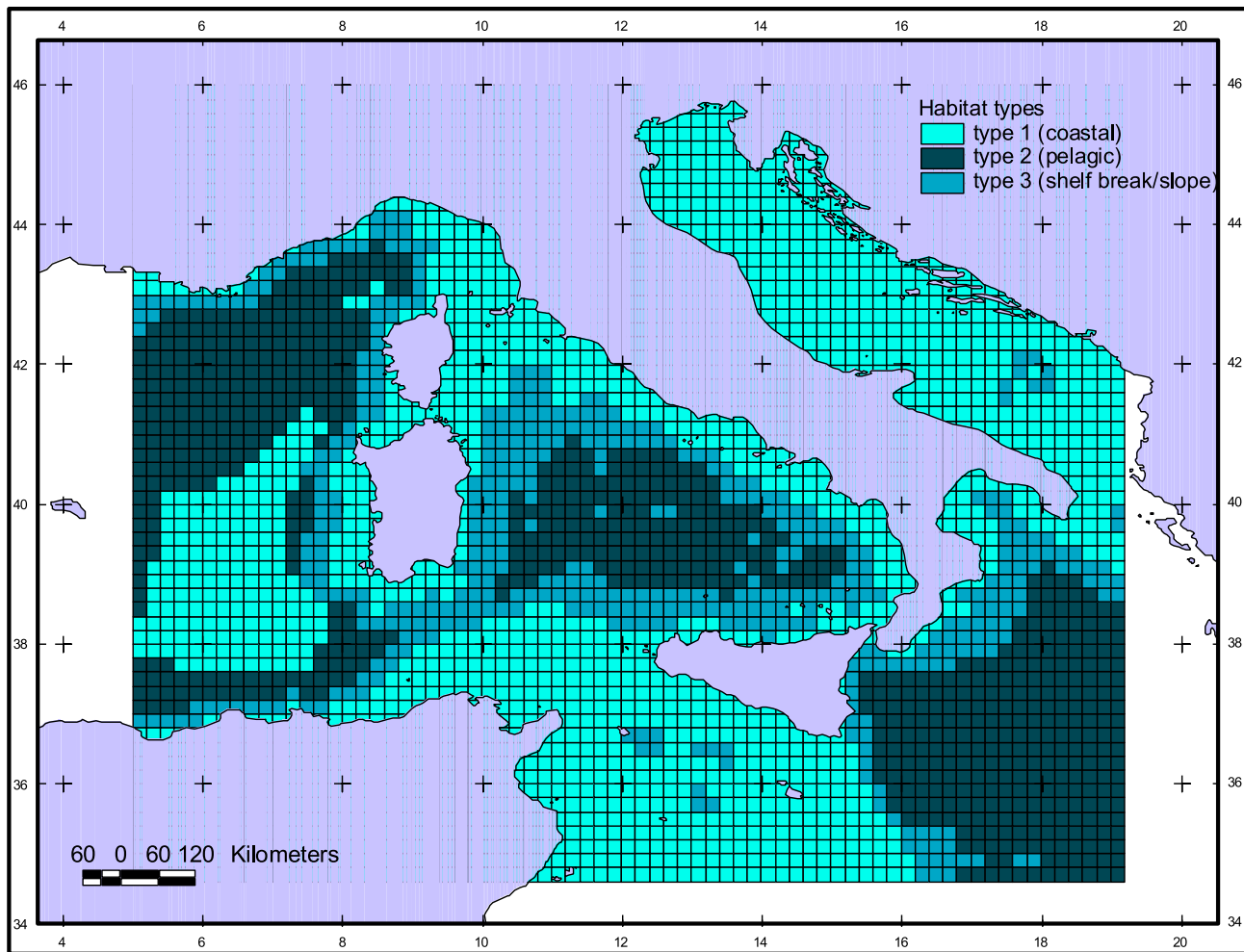


Fig. 6. Map of the three habitat types identified through K-means CA.

identification of 3 categories of habitats (Fig. 5): *habitat type 1*: coastal habitat (bottlenose dolphin habitat is dominant), *habitat type 2*: pelagic habitat (almost homogeneous presence of striped dolphin, fin whale, sperm whale and Cuvier's beaked whale habitats), *habitat type 3*: shelf break and continental slope habitat (sperm whale, Risso's dolphin and Cuvier's beaked whale habitats prevail). The map of the habitat types is shown in Fig. 6. The average physical characteristics of the habitat types are shown in Table 4.

### 3.2. Biodiversity assessment through the DAR index

The biodiversity was assessed through the DAR index, and calculated based on the sightings available from the aerial surveys. For the purpose of the comparative analysis, the study area was subdivided into the subareas shown in Fig. 7 and, to avoid the over-

representation of the area with the highest effort, only the 2010 campaign (see Table 3) was used to evaluate the DAR index in the subareas 1 and 2. Data from aerial surveys were not available for subareas 0 and 6 but they were considered in the analysis of the strandings.

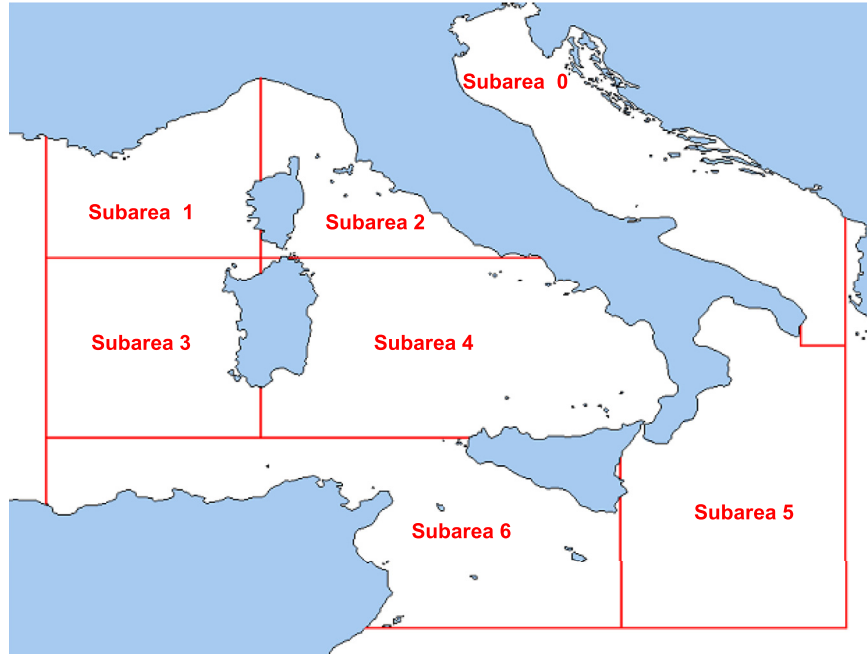
DAR indexes of the different subareas were tested through a Kruskal Wallis test which revealed that the 5 subareas had significantly different biodiversity values (chi-square: 23.1, df: 4,  $P < 0.001$ ). A multiple comparison Mann-Whitney test where the Bonferroni correction (i.e.  $P/k$  comparisons) was applied, revealed that subareas 1, 2 and 3 are homogenous in terms of biodiversity ( $P > 0.0125$ ) as subareas 4 and 5 ( $P > 0.0125$ ). On the other hand, subareas 4 and 5 have a significantly lower biodiversity than subareas 1, 2, 3 (Mann-Whitney test  $P < 0.001$ , see Fig. 8).

### 3.3. Expected biodiversity based on habitat availability

Grounding on the fact that subareas 1, 2 and 3 were found to have the highest biodiversity values, only these subareas were considered to attribute a DAR index value to the habitat types described in the paragraph 3.1. All the sightings available from the aerial surveys conducted in the area (see Table 3) were used to calculate the DAR index to be associated to the habitat types. Table 5 shows the results of these evaluations.

Table 4  
Physical characteristics of the habitat types.

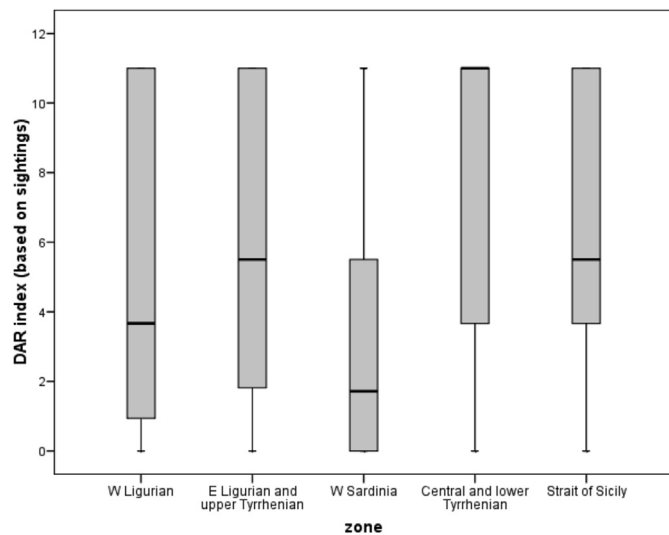
Habitat types		Mean	Std. deviation	Median	Minimum	Maximum
Type 1	Depth (m)	300	657	50	20	2800
	Slope (m/m)	1.4	0.8	1.9	0.0	2.0
Type 2	Depth (m)	2961	475	2790	2021	4000
	Slope (m/m)	1.8	0.4	2.0	0.0	2.0
Type 3	Depth (m)	1554	485	1521	422	3044
	Slope (m/m)	2.0	0.1	2.0	0.9	2.0



**Fig. 7.** Map of the 7 subareas. *Subarea 0:* Adriatic Sea *Subarea 1:* Western Ligurian Sea; *Subarea 2:* Eastern Ligurian and upper Tyrrhenian Sea; *Subarea 3:* West Sardinia; *Subarea 4:* Central and Southern Tyrrhenian Sea; *Subarea 5:* Ionian Sea and Gulf of Taranto; *Subarea 6:* Strait of Sicily.

Based on these estimates it was possible to obtain the expected biodiversity as function of the availability of potential habitats for all the subareas. The expected DAR index for each subarea was evaluated as the weighted mean of the DAR indexes of the habitat types as function of their percent coverage of the total area (see Table 6).

As shown in Table 6 some relevant deviations between the actual and the expected biodiversity were observed for subareas 2 (Eastern Ligurian and upper Tyrrhenian) 4 (Central and lower Tyrrhenian) and 5 (Ionian). Particularly observed DAR indexes in these subareas are higher than the expected values, possibly revealing a loss of biodiversity.



**Fig. 8.** DAR index distribution of the 5 subareas. For the sake of comparison only the 2010 survey data were used for subareas 1 (Western Ligurian Sea) and 2 (Eastern Ligurian Sea and upper Tyrrhenian Sea). The boxes show the median, the quartiles, the minimum and maximum values.

### 3.4. DAR index evaluated on strandings

With the exception of very few studies (Haelters et al., 2006; Hart et al., 2006; Pierce et al., 2007; Camphuysen, 2010; Peltier et al., 2012) to date only few attempts have been made to infer information on marine species distribution from stranding data. Strandings, in fact, are generally thought to be disjointed, both spatially and temporally, from the open sea habitats and the corresponding uses of the species. However, it has been proved that the proportions of species in the stranding records well reflect the relative abundance of live animals of the species living in the respective region (Pyenson, 2010, 2011). Also in our study area such correspondence between the proportion of the species frequencies in the strandings and in sighting records is confirmed (see Fig. S1).

Following this line of thought, the DAR index was applied to the stranding records available for the Italian coasts (see Fig. 9). The used data series spans from 1986 up to 2012 and consists of 4222 records.

The same grid used for the sightings has been used to analyse the strandings data. The differences among subareas have been tested using Kruskal Wallis test that resulted to be significant (chi-square: 24.7, df: 6,  $P < 0.001$ ) only when the Adriatic subarea was included (see Fig. 10).

Excluding the Adriatic Sea, which was shown to have a lower biodiversity (i.e. higher DAR index), all the other subareas were shown to have the same level of biodiversity (chi-square: 8.61, df: 5,  $P: 0.126$ ).

**Table 5**

DAR index of the three habitat types. The index is estimated based on the sightings available from all the aerial conducted in subareas 1, 2 and 3.

Habitat type	N	Mean	Median	Minimum	Maximum	Percentiles		
						Valid	25	50
Type 1	62	4.73	3.67	0.00	11.00	0.00	3.67	11.00
Type 2	160	5.99	5.50	0.00	11.00	2.26	5.50	11.00
Type 3	115	7.35	11.00	0.00	11.00	3.67	11.00	11.00



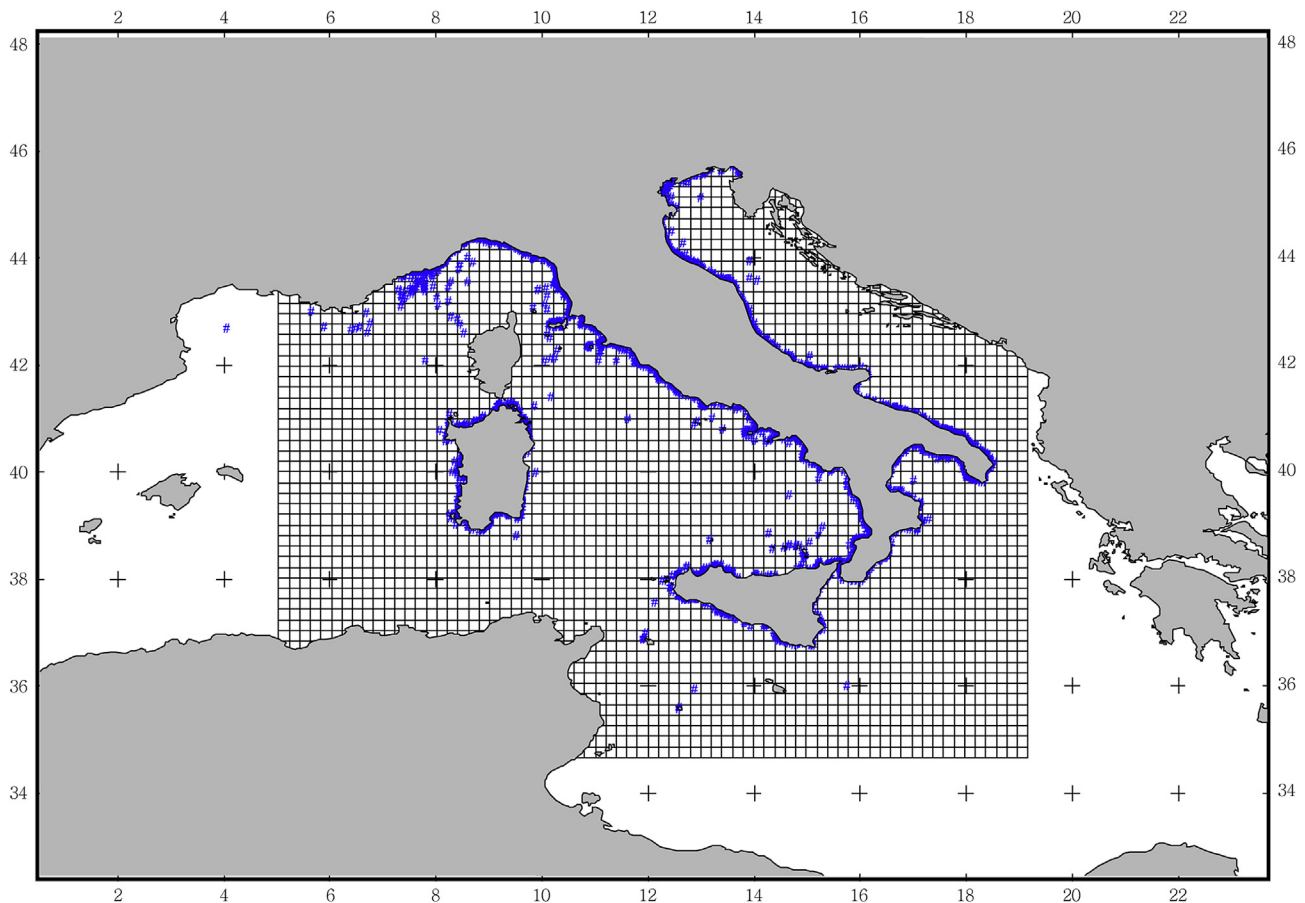
**Table 6**  
 Observed and expected DAR index of the subareas. The observed DAR index is the median value of cell units in the subarea calculated based on the sightings (see paragraph 3.2). The Expected DAR index is the weighted mean of the DAR indexes of the habitat types as function of their percent coverage of the total area. In bold the observed DAR values higher than the expected.

Zone	% Over total area			Expected DAR index	Observed DAR index
	Habitat type 1	Habitat type 2	Habitat type 3		
(0) Adriatic	98.7	0.4	0.9	3.51	—
(1) W Ligurian	44.3	42.9	12.7	3.64	3.67
(2) E Ligurian and upper Tyrrhenian	88.3	1	10.7	3.62	<b>5.50</b>
(3) Sardinia	63.2	26	10.9	3.63	1.72
(4) Central and lower Tyrrhenian	35.3	29.3	35.3	3.91	<b>11.00</b>
(5) Ionian	24.4	56.6	19	3.72	<b>5.50</b>
(6) Strait of Sicily	90.7	5.2	4.2	3.55	—

As Fig. 11 shows, both the correlation matrix and the corresponding scatter plots, reveal a high coherence between the Expected DAR index, evaluated based on the habitat availability and the Observed DAR index, calculated on sightings ( $r: 0.908$ ). On the other hand, a sort of inverse correlation can be seen between DAR indexes evaluated either on sightings or on strandings.

That seems to be the direct consequence of the Tyrrhenian and Sardinia subareas where the DAR indexes evaluated on strandings suggest an inverse pattern than the indexes evaluated on sightings (e.g. DAR indexes calculated on strandings respectively suggest a lower biodiversity for Sardinia and a higher biodiversity for Tyrrhenian Sea). Off course it must be remarked that while the stranding records span over several years, the evaluation based on sightings is almost instantaneous. Reasonably the DAR index

evaluated on strandings reflects the biodiversity occurring over the whole period. That is the case of the Western Sardinia and Central and lower Tyrrhenian areas where the DAR index variability in time reveals a slightly higher biodiversity in the Tyrrhenian area (see Fig. S2), although the difference is not statistically significant. Table 7 shows the Spearman's rank correlations of DAR index and the index components (i.e. *Scdom*: dominance of the species striped dolphin; *Ttdom*: dominance of the species bottlenose dolphin and *RelAbund*: Relative Abundance considering all the species) evaluated on strandings versus the time series. It can be observed that both *Ttdom* and *RelAbund* show a positive trend with the year while the time trend is inverse concerning DAR index (see Table 7). These trends are also shown in Fig. 12.



**Fig. 9.** Map of the available stranding records (time series for 1986–2012).

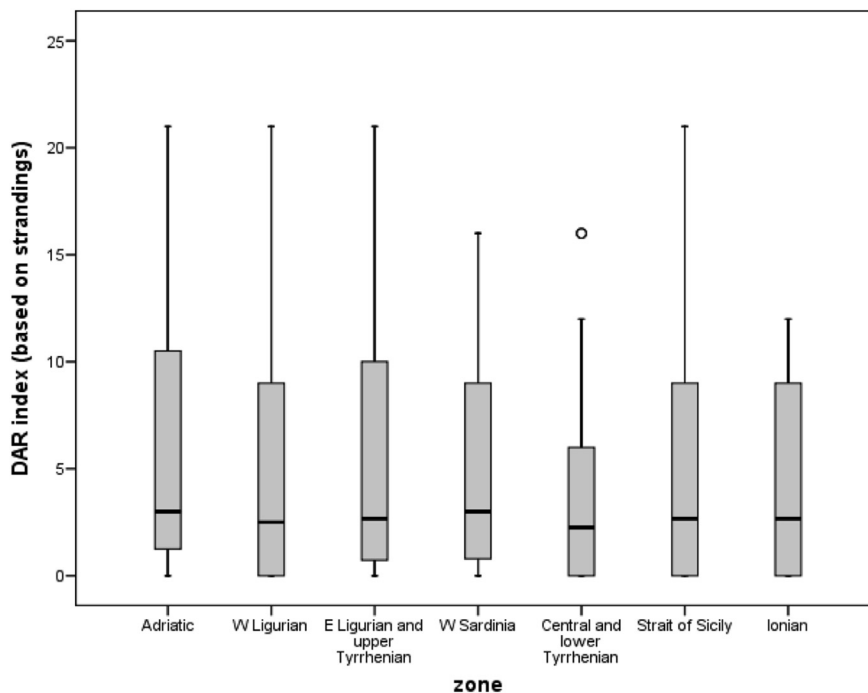


Fig. 10. DAR index calculated on stranding records of the 7 subareas. The box shows the median, the quartiles, the minimum and maximum values.

### 3.5. Biodiversity and human pressures

In order to correlate the biodiversity pattern of the different areas with the existing pressures, some pre-processing of the data was required. Particularly, Principal Component Analysis and Factor Analysis (hereinafter PCA and FA) was needed to reduce the dimensionality of the pollution data included in the EIONET data set, and some spatial processing was needed either to interpolate ship traffic densities and to associate the fishing pressure indicators to every subarea.

#### 3.5.1. PCA/FA of the EIONET data set

PCA was applied to both the EIONET sediment and the biota data set. As far as sediments were concerned PCA extracted 7

components, globally explaining 88.9% of the total variance. No rotation criterion was applied since the extracted principal components were clean enough to be interpreted. As shown in Table 8, most of the variance is explained by PAHs and PCBs, respectively constituting the first and the second component, globally explaining little less than 60% of the explained variance. Metals were separated on different components.

Factor Analysis was instead applied to the biota data set. The Varimax rotation of the principal components allowed in this case to have the pollutants well distributed over the components and it facilitated the interpretation (see Table 9). As for sediments, 7 components were extracted, globally explaining 79.5% of the total variance. Factor loadings revealed that PAHs and PCBs are more linked in the biota, most of the compounds lying on the first rotated

Correlations			Expected DAR Index (sightings)	Observed DAR Index (sightings)
Expected DAR Index (sightings)	Pearson Correlation			
	Sig. (2-tailed)			
	N			
Observed DAR Index (sightings)	Pearson Correlation	.908*		
	Sig. (2-tailed)	.033		
	N	5		
Observed DAR Index (strandings)	Pearson Correlation	-.767*	-.858	
	Sig. (2-tailed)	.044	.063	
	N	7	5	

\*. Correlation is significant at the 0.05 level (2-tailed).

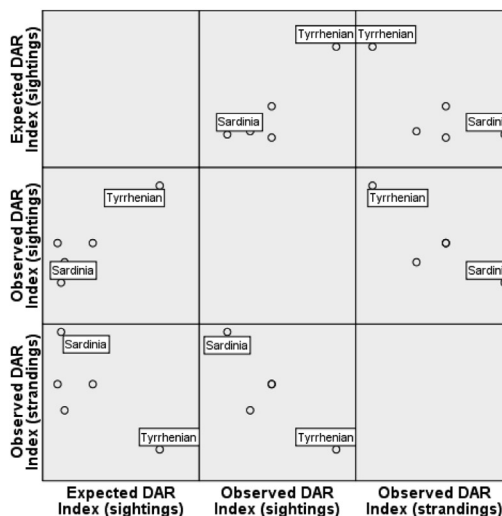


Fig. 11. Correlation analysis of DAR indexes: Expected (i.e. evaluated based on habitat availability), Observed (sightings) (i.e. evaluated on sightings) and Observed (strandings) (i.e. evaluated on strandings). Scatter plots are also shown. It can be observed that the subareas Sardinia and Tyrrhenian have a string leverage for all the correlations.

**Table 7**  
Correlation analysis between DAR index and DAR index components (i.e. *Scdom*: dominance of striped dolphin; *Ttdom*: dominance of bottlenose dolphin and *RelAbund*: Relative Abundance considering all the species) evaluated on the strandings and the corresponding time series. The correlations are shown in terms of Spearman's rank correlation coefficient.

	Year	Scdom	Ttdom	RelAbund	DARindex	
Spearman's rho	Year	Correlation coefficient				
		Sig. (2-tailed)				
Scdom		N				
	Scdom	Correlation coefficient	-0.034			
		Sig. (2-tailed)	0.171			
Ttdom		N	1593			
	Ttdom	Correlation coefficient	0.148 <sup>a</sup>	-0.408 <sup>a</sup>		
		Sig. (2-tailed)	0.000	0.000		
RelAbund		N	1593	1593		
	RelAbund	Correlation coefficient	0.399 <sup>a</sup>	0.134 <sup>a</sup>	0.199 <sup>a</sup>	
		Sig. (2-tailed)	0.000	0.000	0.000	
DARindex		N	1593	1593	1593	
	DARindex	Correlation coefficient	-0.067 <sup>a</sup>	0.511 <sup>a</sup>	0.270 <sup>a</sup>	-0.260 <sup>a</sup>
		Sig. (2-tailed)	0.008	0.000	0.000	0.000
	N	1593	1593	1593	1593	

<sup>a</sup> Correlation is significant at the 0.01 level (2-tailed).

component. Pesticides such as DDT and its residues and beta-HBH strongly correlate with the third rotated component which explained alone little more than 10% of the variance. Metals fell on different components also in this case, but their reciprocal correlations were found different from the correlations found in sediments (e.g. in biota data set Cr, Cu and Zn are weakly correlated and lie on the same component of the Tributyltin compounds, whereas Cd and Hg that were separated on different components in the sediment analysis, correlate both with the seventh biota component explaining 4% of the total variance).

The extracted components from both the sediment and the biota data set, were used to summarise the pollution information for the different subareas.

### 3.5.2. Spatial interpolation of the ship traffic densities

As explained in the Methods, maritime traffic density data were derived from the results of PASTA-MARE project. These data were interpolated by using an Inverse Distance Weighted algorithm (IDW, Webster and Oliver, 2001), which assumes that each input point has a local influence that diminishes with distance. So the points closer to the processing cell have greater weight than more distant points. The graduated colour scale shown in Fig. 13 is the result of the IDW interpolator applied to the analysis grid.

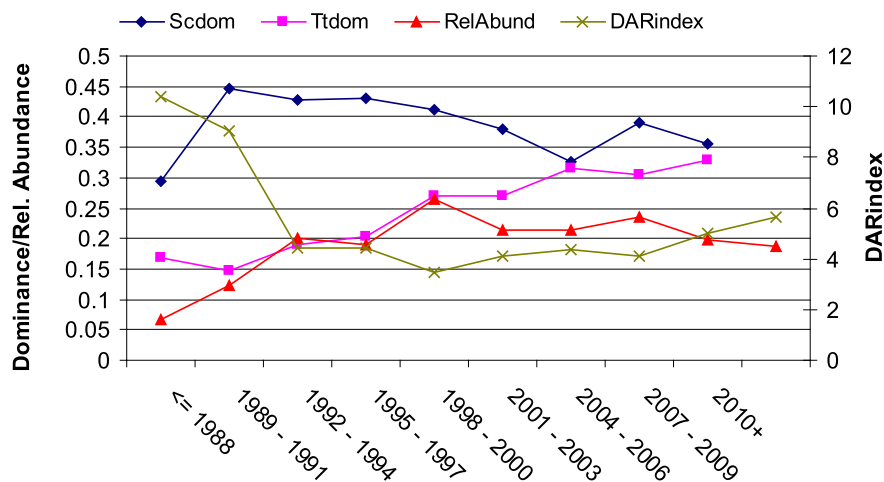
Based on these values, an average naval density was attributed to every subarea.

### 3.5.3. Fishery impacts

FAO statistics could only be associated to the whole study area, so no pre-processing of these data was attempted to disaggregate these statistics to the subarea level. On the other hand, the statistics available from Osservatorio Nazionale Pesca (2011) were more detailed and available at the scale of the Italian regions (see Table 10), so they could be processed and associated to the subareas. Particularly, the statistics available from the Osservatorio Nazionale Pesca were attributed to each subarea as function of the coastline length of each Italian region falling within the subarea borders.

### 3.5.4. Biodiversity deviations from the expected and correlation with pressures

As it was described in the paragraph 3.3, some relevant deviations of the actual biodiversity from the one expected were found in some subareas. The deviations of the DAR index, observed and expected (see Table 6), were correlated with all the indicators of human pressures described so far. The resulting correlation matrix is shown in Table 11.



**Fig. 12.** DAR index and components trends over the studied period. *Scdom*: dominance of striped dolphin; *Ttdom*: dominance of bottlenose dolphin and *RelAbund*: Relative Abundance considering all the species.

**Table 8**

Principal Component Analysis of the Sediments EIONET data set: the factor loadings of the 7 components extracted are shown. Factor loadings higher than 0.5 and lower than -0.5 are shown in bold. The percent and cumulative variance explained by each components are also shown.

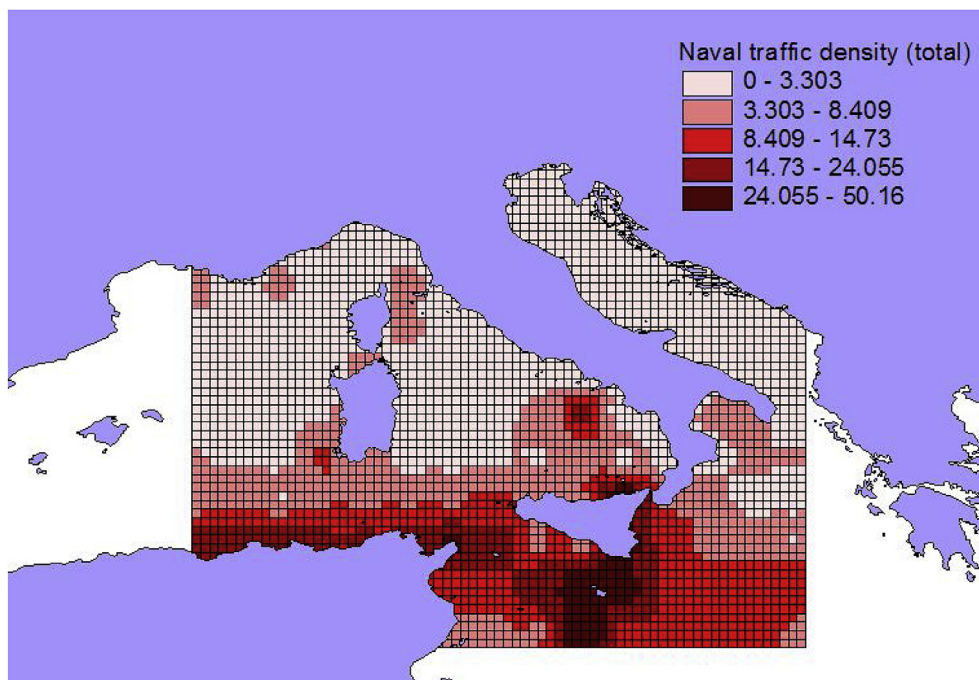
	Components						
	1	2	3	4	5	6	7
Aldrin	-0.111	-0.017	-0.111	-0.113	-0.384	0.483	<b>0.697</b>
Anthracene	<b>0.933</b>	-0.229	0.017	0.020	0.041	-0.015	-0.024
Benzo(a)pyrene	<b>0.946</b>	-0.262	-0.030	-0.078	-0.028	0.051	-0.109
Benzo(b)fluoranthene	<b>0.943</b>	-0.266	-0.032	-0.131	0.035	0.086	-0.076
Benzo(g,h,i)perylene	<b>0.937</b>	-0.274	-0.044	-0.128	0.002	0.088	-0.105
Benzo(k)fluoranthene	<b>0.898</b>	-0.308	-0.006	-0.166	0.182	0.059	0.009
Cadmium and its compounds	0.044	0.216	<b>0.923</b>	-0.067	-0.145	0.007	-0.021
Chromium and its compounds	<b>0.629</b>	-0.130	0.028	0.309	-0.296	-0.472	0.259
Copper and its compounds	<b>0.545</b>	<b>0.585</b>	0.210	0.338	0.128	0.016	0.051
Dieldrin	<b>0.864</b>	-0.203	-0.067	-0.006	-0.291	0.128	-0.112
Fluoranthene	<b>0.947</b>	-0.262	-0.037	-0.064	-0.078	0.029	-0.077
indeno(1,2,3-cd)pyrene	<b>0.928</b>	-0.285	-0.030	-0.154	0.087	0.061	-0.052
Lead	<b>0.609</b>	0.092	0.329	0.114	0.437	-0.095	0.307
Mercury	0.104	0.041	0.323	0.274	<b>0.529</b>	0.075	0.059
Naphthalene	0.136	-0.192	0.026	-0.411	<b>0.646</b>	-0.057	0.280
Nickel	<b>0.601</b>	-0.172	0.061	0.404	-0.221	-0.448	0.310
Organic carbon	0.091	0.331	<b>0.596</b>	-0.349	-0.250	0.169	-0.123
PCB138	0.417	<b>0.879</b>	-0.209	-0.063	0.037	0.013	0.006
PCB153	0.475	<b>0.850</b>	-0.208	-0.059	0.028	0.007	0.009
PCB169	0.338	<b>0.909</b>	-0.204	-0.047	0.053	-0.032	0.024
PCB52	0.323	-0.132	-0.007	0.416	0.028	<b>0.658</b>	0.138
PCB77	<b>0.884</b>	0.332	-0.153	0.003	-0.200	-0.014	-0.099
Polychlorinated biphenyls	0.407	<b>0.878</b>	-0.220	-0.060	0.025	0.027	0.023
Tributyltin compounds	-0.024	0.004	0.016	<b>0.772</b>	0.128	0.232	-0.288
Zinc and its compounds	0.195	0.199	<b>0.923</b>	-0.044	-0.128	0.016	-0.014
<b>% of Variance</b>	<b>39.68</b>	<b>17.62</b>	<b>10.18</b>	<b>6.59</b>	<b>5.95</b>	<b>4.94</b>	<b>4.02</b>
<b>Cumulative %</b>	<b>39.68</b>	<b>57.30</b>	<b>67.48</b>	<b>74.08</b>	<b>80.02</b>	<b>84.96</b>	<b>88.99</b>

**Table 9**

Factor Analysis of the Biota EIONET data set: the factor loadings of the 7 components extracted are shown. Factor loadings higher than 0.5 and lower than -0.5 are shown in bold. The percent and cumulative variance explained by each rotated components are also shown.

Components	1	2	3	4	5	6	7
Aldrin	-0.010	<b>0.793</b>	0.057	-0.053	0.113	0.171	-0.005
Anthracene	0.183	<b>0.671</b>	0.145	0.161	0.076	-0.017	-0.029
Benzo(a)pyrene	<b>0.847</b>	0.356	0.019	0.200	-0.093	-0.047	0.029
Benzo(b)fluoranthene	<b>0.888</b>	0.338	-0.001	0.128	-0.073	-0.009	0.032
Benzo(g,h,i)perylene	<b>0.812</b>	0.486	0.092	0.208	-0.063	-0.029	0.035
Benzo(k)fluoranthene	<b>0.758</b>	<b>0.532</b>	0.029	0.188	-0.076	-0.051	0.028
beta-HCH	0.032	0.108	<b>0.899</b>	0.012	-0.157	0.086	-0.013
Cadmium	-0.019	-0.017	0.012	0.013	0.020	-0.024	<b>0.802</b>
Chromium	0.131	0.292	0.227	<b>0.581</b>	0.107	0.367	0.112
Copper	0.460	0.277	0.260	<b>0.684</b>	0.088	0.188	0.025
DDD, o,p'	-0.005	0.197	0.486	0.306	<b>0.650</b>	-0.009	-0.004
DDD, p,p'	<b>0.721</b>	-0.025	0.280	0.109	<b>0.560</b>	0.061	-0.010
DDE, p,p'	<b>0.563</b>	-0.016	<b>0.654</b>	0.172	0.337	0.123	-0.011
DDT, o,p'	0.066	0.280	<b>0.747</b>	0.147	0.219	0.044	-0.018
DDT, p,p'	0.292	0.173	<b>0.817</b>	0.123	0.353	0.043	-0.010
Dieldrin	0.025	0.186	0.114	0.066	<b>0.936</b>	0.018	-0.015
Fluoranthene	<b>0.644</b>	<b>0.640</b>	0.081	0.179	-0.029	-0.036	0.015
Hexachlorobenzene (HCB)	<b>0.521</b>	0.200	<b>0.543</b>	-0.044	0.424	0.085	-0.003
Indeno(1,2,3-cd)pyrene	0.193	0.247	0.012	0.444	-0.121	-0.109	-0.094
Lead	0.359	0.048	0.045	0.149	-0.112	0.328	-0.004
Mercury	0.103	0.029	-0.041	-0.020	-0.037	-0.034	<b>0.799</b>
Naphthalene, chloro derivatives	0.048	<b>0.821</b>	0.190	0.168	<b>0.821</b>	-0.038	0.038
Nickel	0.058	0.080	0.035	0.010	0.021	<b>0.847</b>	-0.064
PCB138	<b>0.969</b>	-0.037	0.069	0.088	0.100	0.113	0.022
PCB153	<b>0.935</b>	-0.061	0.148	0.098	0.135	0.143	0.018
PCB169	-0.004	<b>0.871</b>	0.117	0.025	0.108	0.143	0.004
PCB52	<b>0.899</b>	-0.016	0.191	0.068	0.255	0.111	0.010
PCB77	<b>0.963</b>	-0.068	-0.021	0.080	0.006	0.084	0.029
Polychlorinated biphenyls	<b>0.904</b>	-0.079	0.320	0.078	-0.003	0.131	0.019
Tributyltin compounds	0.119	-0.078	0.025	<b>0.822</b>	0.228	0.115	0.008
Zinc	0.229	0.103	0.219	<b>0.505</b>	0.120	<b>0.676</b>	-0.022
<b>% of Variance</b>	<b>29.78</b>	<b>13.49</b>	<b>11.34</b>	<b>7.72</b>	<b>7.64</b>	<b>5.30</b>	<b>4.25</b>
<b>Cumulative %</b>	<b>29.78</b>	<b>43.28</b>	<b>54.62</b>	<b>62.34</b>	<b>69.98</b>	<b>75.28</b>	<b>79.53</b>





**Fig. 13.** Spatial interpolation of the naval traffic density data. No differentiation is given in this map between types of ships (e.g. passengers ship, tankers, cargos etc.).

As it can be observed the only strong and significant correlation between DAR deviations and human pressures is the one with fishery ( $r: 0.896$ ).

It is also worthwhile to point out that DAR deviations might be correlated also with the pollution component  $Cr\_Cu\_Zn\_TBT$  which was extracted from the biota data set. However, such correlation is not significant at the 5% significance level and the coarse scale of this analysis does not allow to test whether this correlation is real or just the side effect of the fact that this pollution component is correlated on its own with fishery ( $r: 0.922$ ).

### 3.5.5. Strandings' biodiversity and correlations with fishery pressure

As it was described in the paragraph 3.4, the DAR index calculated on the stranding time series and two out of the three index components (i.e.  $Ttdom$ : dominance of the species bottlenose dolphin and  $RelAbund$ : Relative Abundance considering all the species) showed trends with time. Particularly the trend was positive for both  $Ttdom$  and  $RelAbund$  and inverse for the DAR index itself.

**Table 10**

Statistics of the total landings (tons) available from Osservatorio Nazionale Pesca (2011).

Regions	t/y
Liguria	4461
Toscana	9059
Lazio	5739
Campania	14,144
Calabria	10,063
Puglia	32,305
Molise	2199
Abruzzo	11,449
Marche	25,360
Emilia Romagna	17,635
Veneto	19,625
Fiuli Venezia Giulia	3676
Sardegna	9573
Sicilia	45,037

Concurrently, the fishery statistics available from the FAO year-books suggest for the same period a dramatic change in the fishery catches over the Italian seas (see Fig. 14).

The correlation analysis of DAR index, and its components with the fishery catches outlined the inverse correlation of  $Ttdom$  with the fishery catches ( $r: -0.754, P < 0.05$ ) suggesting an overall recovery of the species bottlenose dolphin associated with the decrease of the fishery catches. No significant correlation of this kind was outlined instead for DAR index and the other components.

### 3.5.6. Levels of contamination and genetic individual variability as a measures of animal stress

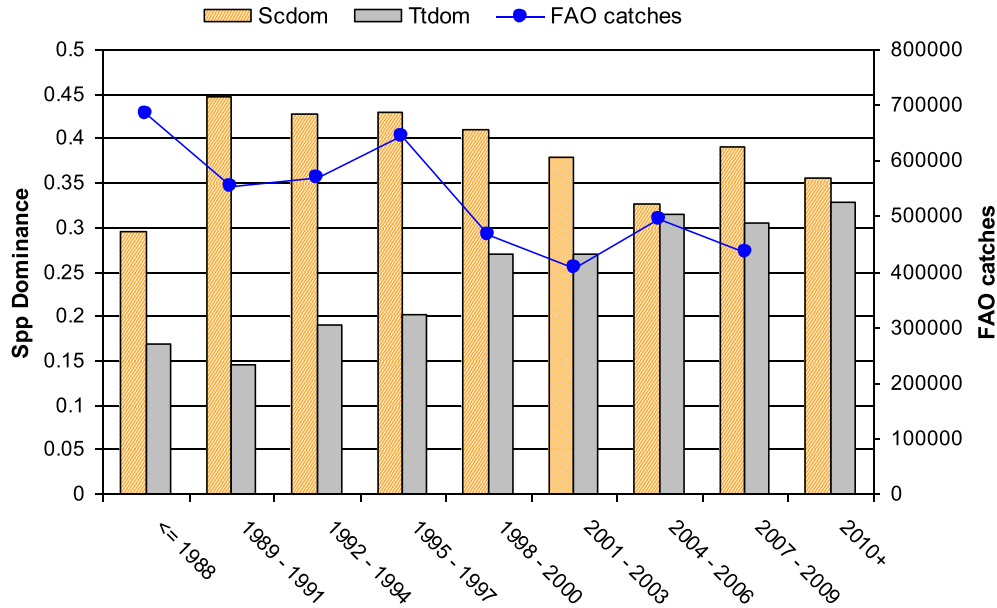
No clear correlation was found between the biodiversity status and pollution indicators, even though significant differences were found in the background contamination of the different subareas ( $P < 0.05$ ). To assess whether such differences were visible, at least in the animal contamination levels, the available information about organochlorines presence in the tissues of the striped dolphins stranded along the Italian coasts was tested versus the subarea. Table 12 shows the contamination statistics of the considered sample. It can be observed that whereas subareas 2 (i.e. Eastern Ligurian and upper Tyrrhenian Seas) and 4 (i.e. central and southern Tyrrhenian Sea) are well represented in the study sample, sample sizes are much smaller for the other subareas and do not allow the full testing of the differences. However, the Kruskal Wallis test applied to all the subareas with the exception of subarea 1, although not showing significant differences ( $P > 0.05$ ), suggests at least for DDTs (chi-square: 6.44, df: 3,  $P: 0.092$ ) and PCBs (chi-square: 6.41, df: 3,  $P: 0.093$ ) that a bigger sample might reveal whether these differences truly exist. The bigger subsamples (i.e. subareas 2 and 4) were tested also through a Mann–Whitney  $U$  test which revealed a significant difference of the PCBs concentration between the two areas ( $U: 102; P < 0.05$ ). Particularly PCBs contamination was significantly lower in the stranded specimens of the upper Tyrrhenian Sea area with respect to the specimens of the central and southern Tyrrhenian subarea. The temporal pattern of the contamination was also analysed revealing for subarea 2, and

**Table 11**  
Correlation matrix between DAR Deviations and human pressure indicators.

	DAR Deviations	Naval Traffic	Fishery	IPA_Ni	PCBs	Cd_Zn	TBT	Napth	Pcb52	HCb	DARstr	BenzolPA_PCBs	Aldrin_Napht_Anthr	HCH_DDT	Cr_Cu_Zn_TBT	DDD_Dieldrin	Ni_Zn	Cd_Hg
DAR Deviations	Pearson Sig.																	
Naval Traffic	Pearson 0.266 Sig. 0.665																	
Fishery	Pearson 0.896 <sup>a</sup> Sig. 0.040	0.616 0.269																
IPA_Ni	Pearson -0.299 Sig. 0.625	-0.470 0.424	-0.573 0.312															
PCBs	Pearson 0.314 Sig. 0.607	0.313 0.608	0.542 0.346	-0.980 <sup>b</sup> 0.003														
Cd_Zn	Pearson -0.251 Sig. 0.683	-0.529 0.359	-0.237 0.702	-0.291 0.635	0.451 0.446													
TBT	Pearson 0.040 Sig. 0.948	-0.368 0.543	0.064 0.918	-0.296 0.629	0.462 0.434	0.919 <sup>a</sup> 0.028												
Napth	Pearson -0.318 Sig. 0.601	-0.804 0.101	-0.576 0.309	0.690 0.197	-0.541 0.346	0.473 0.421	0.469 0.425											
Pcb52	Pearson 0.180 Sig. 0.772	0.192 0.757	0.410 0.493	-0.927 <sup>a</sup> 0.023	0.976 <sup>b</sup> 0.005	0.625 0.260	0.619 0.266	-0.370 0.540										
HCb	Pearson -0.269 Sig. 0.662	-0.026 0.967	-0.423 0.478	0.662 0.224	-0.773 0.125	-0.761 0.135	-0.893 <sup>a</sup> 0.041	-0.030 0.961	-0.859 0.062									
BenzolPA_PCBs	Pearson -0.303 Sig. 0.620	-0.472 0.422	-0.575 0.310	1.000 <sup>b</sup> 0.000	-0.979 <sup>b</sup> 0.004	-0.283 0.644	-0.288 0.639	0.696 0.192	-0.924 <sup>a</sup> 0.025	0.655 0.230	-0.074 0.890							
Aldrin_Napht_Anthr	Pearson 0.093 Sig. 0.882	-0.229 0.710	0.176 0.777	-0.451 0.445	0.599 0.286	0.896 <sup>a</sup> 0.040	0.985 <sup>b</sup> 0.002	0.310 0.611	0.737 0.156	-0.955 <sup>a</sup> 0.011	-0.177 0.737	-0.340 0.456						
HCH_DDT	Pearson -0.523 Sig. 0.366	-0.526 0.363	-0.737 0.156	0.415 0.488	-0.433 0.467	-0.146 0.815	-0.487 0.405	0.150 0.810	-0.441 0.457	0.693 0.194	0.826 <sup>a</sup> 0.043	0.450 0.310	-0.193 0.678					
Cr_Cu_Zn_TBT	Pearson 0.834 Sig. 0.079	0.479 0.414	0.922 <sup>a</sup> 0.026	-0.416 0.487	0.433 0.467	-0.039 0.951	0.328 0.591	-0.260 0.672	0.371 0.539	-0.576 0.309	-0.488 0.326	-0.131 0.780	0.068 0.068	0.068 0.885				
DDD_Dieldrin	Pearson -0.285 Sig. 0.642	-0.662 0.224	-0.622 0.262	0.971 <sup>b</sup> 0.006	-0.913 <sup>a</sup> 0.030	-0.129 0.836	-0.170 0.784	0.785 0.116	-0.844 0.072	0.578 0.307	-0.026 0.962	0.626 0.133	0.328 0.473	0.627 0.132	0.604 0.151			
Ni_Zn	Pearson 0.607 Sig. 0.278	0.565 0.321	0.684 0.203	0.066 0.916	-0.105 0.867	-0.413 0.490	-0.026 0.967	-0.117 0.851	-0.165 0.790	-0.123 0.844	-0.544 0.265	-0.246 0.595	-0.073 0.876	-0.785 <sup>a</sup> 0.037	-0.385 0.394	-0.578 0.174		
Cd_Hg	Pearson -0.672 Sig. 0.214	-0.694 0.194	-0.909 <sup>a</sup> 0.032	0.852 0.067	-0.807 0.098	0.046 0.942	-0.151 0.808	0.736 0.156	-0.698 0.190	0.571 0.314	0.390 0.444	0.806 <sup>a</sup> 0.029	-0.318 0.487	0.776 <sup>a</sup> 0.040	-0.243 0.599	0.556 0.195	-0.593 0.160	

<sup>a</sup> Correlation is significant at the 0.05 level (2-tailed).

<sup>b</sup> Correlation is significant at the 0.01 level (2-tailed).



**Fig. 14.** Time series of the FAO catches concerning Italian seas, and the DAR components *Scdom* (i.e. dominance of the species striped dolphin) and *Ttdom* (i.e. dominance of the species bottlenose dolphin).

possibly also for subarea 4 – which unfortunately is characterised by a much lower sample size – a significant increasing trend of the organochlorines concentration spanning over the period 1988–2011 ( $r > 0.4$ ,  $N: 39$ , HCBs and PCBs  $P < 0.01$ , DDTs  $P < 0.05$ ).

Following the rationale that populations living in impacted environments present a reduced individual genetic variability, (Fossi et al., 2013) we tested across the subareas the standardised observed heterozygosity (*St.het\_Obs*) of striped and common bottlenose dolphins. While no significant difference was found among subareas for common bottlenose dolphins (KW chi-square: 4.98, df: 4,  $P: 0.29$ ), Adriatic striped dolphins were found to have a significantly lower standardised observed heterozygosity (KW-with Adriatic subarea: chi-square: 10.55, df: 3,  $P < 0.014$ ; KW-without Adriatic subarea: chi-square: 2.32, df: 2,  $P: 0.317$ ).

#### 4. Discussion

Marine food webs are becoming increasingly important as a factor in conservation management, with particular focus on assessing and minimising ecological risk from human activities (de

Ruiter et al., 2005; Sala and Sugihara, 2005). In contrast to single-species approaches, a system-level approach is attractive since both direct and indirect effects of disturbance can be considered in a single interaction network (Raffaelli, 2005). However, the high functional diversity in marine ecosystems and the high food-web complexity, make practical applications still a challenge in many situations. The MSFD Descriptor 4 (D4) concerns “functional aspects such as energy flows and the structure of food webs” (European Commission, 2010; 2010/477/EU). In this study the biodiversity of the cetacean community is proposed as MSFD D4 indicator and its practicality and specificity to a manageable anthropogenic pressure is evaluated, as required by the MSFD. Rombouts et al. (2013) have recently provided a thorough analysis of the indicators proposed for the MSFD and have underlined the fact that in the case of the food web indicators, attention should be focused on the functional importance of abundance. According to these authors, functional group abundance is often less variable than the one of single species because variability in the abundances of the group’s constituent species averages out. In practice, the use of functional groups is often favoured over indicator species since

**Table 12**  
Concentration ( $\text{ng g}^{-1}$  d.w.) of organochlorines (i.e. HCB, DDTs and PCBs) in the tissue of stranded striped dolphins.

Zona	N	Mean	Median	Std. deviation	Minimum	Maximum	Percentiles			
							25	50	75	
Adriatic Sea	HCB	3	352.7	296.0	120.5	271.0	491.0	271.0	296.0	491.0
	DDTs	3	112080.0	61409.0	130240.8	14790.0	260041.0	14790.0	61409.0	260041.0
	PCBs	3	105966.0	85806.0	71812.6	46388.0	185704.0	46388.0	85806.0	185704.0
W Ligurian Sea	HCB	1	182.0	182.0		182.0	182.0	182.0	182.0	182.0
	DDTs	1	9874.0	9874.0		9874.0	9874.0	9874.0	9874.0	9874.0
	PCBs	1	28294.0	28294.0		28294.0	28294.0	28294.0	28294.0	28294.0
E Ligurian and upper Tyrrhenian Sea	HCB	47	520.7	186.0	1479.5	2.4	10091.0	45.3	186.0	413.9
	DDTs	47	45126.0	29001.7	51023.5	560.0	218636.6	9656.2	29001.7	55371.1
	PCBs	47	79374.4	52970.2	100443.3	1596.5	573262.0	11791.0	52970.2	94006.4
Central and lower Tyrrhenian Sea	HCB	8	968.1	288.5	1769.0	45.0	5281.0	112.8	288.5	857.8
	DDTs	8	85273.9	65229.0	76175.2	4446.0	207821.0	27489.8	65229.0	167339.3
	PCBs	8	180213.4	136666.5	173722.7	14037.0	534694.0	43635.5	136666.5	287733.8
Ionian Sea	HCB	2	1380.0	1380.0	1302.5	459.0	2301.0	459.0	1380.0	2301.0
	DDTs	2	206739.5	206739.5	202550.0	63515.0	349964.0	63515.0	206739.5	349964.0
	PCBs	2	273810.0	273810.0	276765.8	78107.0	469513.0	78107.0	273810.0	469513.0

indices of species abundance are frequently subject to large inter-annual variation, often due to natural physical dynamics rather than anthropogenic stressors (de Jonge, 2007). On the contrary, indicators based on functional traits of key groups combined with information of species distributions in communities are in this respect more efficient and are becoming increasingly common (Bremner, 2008; Vandewalle et al., 2010; de Bello et al., 2010) in assessing, for example, community response to sewage pollution (Charvet et al., 1998; Tett et al., 2008), anoxia (Rakocinski, 2012), fishing (Bremner et al., 2004) and climate change (Beaugrand, 2005). Marine mammals, and particularly cetacean species, were found to be *keystone* species (Bănaru et al., 2013), having, in spite of the relatively low biomass, a structuring role within the ecosystem and the food webs that interconnect. In this sense they can definitely be considered a functional group according to the MSFD definition. In addition to that, cetaceans are in many situations the better known component of pelagic ecosystems: their habitat preferences are generally well documented in literature (Aissi et al., 2008; Azzellino et al., 2008, 2011, 2012; Cottè et al., 2010; Gannier et al., 2002; Gannier and Epinat, 2008; Gannier and Praca, 2007; Gnone et al., 2011; Gannier, 2006; Gordon et al., 2000; Laran and Gannier, 2008; Moulins et al., 2007; Panigada et al., 2008; Praca and Gannier, 2008; Praca et al., 2009) and the information about their occurrence, distribution and relative abundance are more easily available and accessible than for other pelagic species. Under this rationale, we developed the proposed biodiversity index which is a function of two different concepts: a) the dominance of the most common species and b) the relative abundance in a certain area. The main advantage of the proposed index is the fact that it may be estimated also based on the habitat availability for the different species of interest, providing a reference value for the biodiversity that could be expected in a certain area depending on the habitat characteristics. This theoretical biodiversity value can be compared with the actual biodiversity that can be on its own inferred from monitoring campaigns. We applied the index both to sightings and strandings. Although we found the two data series not directly comparable either in terms of time scale and spatial scale, we believe that DAR index applied to strandings, may provide a historical perspective and the ground of the relevant patterns outlined by the same index applied to sightings. The DAR index applied to the whole time series (1986–2012) available for strandings, in fact, revealed an overall increase of the biodiversity status in all the Italian seas, presumably due to the recovery of bottlenose dolphin populations that was found correlated with the decrease of the fishery catches.

We believe this research proves that the deviations between theoretical biodiversity, depending on habitat availability, and the actual biodiversity may be used to detect the impacts of human activities. Despite of the low sample size of this preliminary investigation, significant correlations were found, in fact, between the proposed biodiversity index and the indicators of human pressures. The index itself and its components (i.e. dominance and relative abundance) were in fact proven to respond to the spatial pattern of the human drivers and pressures present in the study area (e.g. Tyrrhenian and Ionian Seas being more impacted than other subareas), as well as to the temporal pattern of the activity that was identified as the most impacting (e.g. the relative increase of the bottlenose dolphin dominance correlated with the temporal decrease of the fishery catches). Although, this preliminary investigation clearly suggests that among the existing pressures fishery might be by far the most significant in terms of impact, no clear correlation was found between the biodiversity status and the other pressure indicators (i.e. pollution and naval traffic). It may be speculated that the effects of these pressures are detectable only on a much finer scale and only after removing the

effect of fishery. Furthermore, it should be underlined in this respect that the information available on more direct indicators of the health status of these populations (i.e. body contamination or the loss of individual genetic variability) is still too sporadic to offer a clear picture of the real situation. Fossi et al. (2003) documented a difference in the organochlorines contamination of cetacean living in the Ligurian Sea with respect to Ionian Sea (Greece) and Tyrrhenian Sea (Aeolian islands).

Marsili et al. (2004) confirmed these results and underlined the fact that stranded individuals had higher contamination levels than free-ranging cetaceans. Some toxicological stress has been recently documented (Fossi et al., 2013) in cetacean populations living in the Pelagos Sanctuary area, that were found more contaminated than populations living in the Ionian Sea and the Strait of Gibraltar. However, sample sizes in these studies are generally low and both, sex and age effect (see Marsili et al., 1997; Marsili et al., 2004) may possibly have masked or enhanced some differences. The analysis of the individual genetic variability appears promising to improve our understanding of the health status of these populations. We believe that these preliminary results suggest striped dolphins being more vulnerable than bottlenose dolphins and indicate the Adriatic Sea as an area to be further investigated also in this respect. It should be also considered that if some threats (e.g. chemical and possibly noise pollution) may affect the entire ecosystem and thus potentially all species considered here, some other threats may be less inclusive affecting several but not necessarily all the concerned species (e.g. fisheries, depending on target species and fishing mode) or even affecting very few species (e.g. ship collision). The effects that such threats produce on a group-level diversity index could be different. Further studies should definitely address these aspects to improve the understanding of the DAR index capability to respond to these ecosystem alterations.

## 5. Conclusions

- > A cetacean biodiversity index is proposed as GES descriptor in the MSFD framework concerning the functional aspects of marine ecosystems (i.e. energy flows and structure of food webs).
- > Deviations from the biodiversity that could be expected based on habitat availability and the actual biodiversity may be correlated with indicators of human pressures (e.g. naval traffic, pollution, fishing pressure etc.).
- > Although not directly comparable, DAR index can be evaluated also based on strandings, providing a historical perspective. The DAR index applied to the strandings time series (1986–2012) revealed an overall increase of the biodiversity status in all the Italian seas, presumably due to the recovery of bottlenose dolphin populations that was found correlated with the decrease of the fishery catches.
- > This preliminary analysis suggests that among the existing anthropic pressures, fishery is by far the most significant in terms of impact.
- > No clear correlation was found between the biodiversity status and the other pressure indicators (i.e. pollution and naval traffic).
- > More direct indicators of the health status of cetacean populations (i.e. body contamination or the loss of individual genetic variability) may provide significant insights about pollution impacts. However, the information available is still too sporadic to offer a clear picture of the situation.
- > Determination of the individual genetic variability of the populations living in different areas may be a promising approach to improve our understanding of the health status of the dominant species. These preliminary results suggest that among the



dominant species, striped dolphins might be more vulnerable than bottlenose dolphins and indicate the Adriatic Sea as an area to be further investigated.

> Further and dedicated studies should be addressed to better understand the effects of the less impacting pressures.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found on line.

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