Are shipwrecks a real hazard for the ecosystem in the Mediterranean Sea?

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ABSTRACT

The aim of the present study was to evaluate the hazard from shipwrecks on communities by a holistic approach taking into account different effects on biological communities. Multibeam and Remotely Operated Vehicles surveys recorded ecological assessment of fish and benthic species on three shipwrecks flooded during the Second World War on *Maërl* beds habitats in the strait of Sicily. Pollution levels of a wide range of chemicals of ecotoxicological concern were also measured in sediments and in fish species from different trophic levels. Statistical analysis evidenced significant differences among pollutant levels between both sediments and fish collected in shipwreck sites and controls. Concerning fish, significant effects due to the vessel's cargo type and flooding position are recorded. In spite of that, our results underline that shipwrecks are also a hotspots of biodiversity and a habitat for preservation strategies in marine ecosystems that need to be monitored.

1. Introduction

International Maritime Organization (IMO) (Nairobi, 2007), defined a wreck as "*a sunken or stranded ship or any part of a sunken or stranded ship, that, for various reasons (structural failure, fire, shifting of cargo, accidental collision, sinking voluntary acts of war or terrorism), is partially or totally submerged by the waters of the sea*" and as estimated by the Parliamentary Assembly of the Council of Europe they are the biggest sources of ocean pollution (Parliamentary Assembly, Resolution No. 1869/2012).

In spite of this, it has suggested we need to define which of them could be environmental hazards in the future (REMPEC, 2004).

In the southern Mediterranean Sea and in particular in the strait of Sicily, shipwrecks are dramatically increasing due to the uncontrolled flow of migrants from African and Middle East coastlines towards Europe. This represents first of all a severe humanitarian problem for European country but shipwrecks could also affect deep-sea environments including priority habitats.

It was proved that the effects of the presence of a wreck at sea could cause a marine hazard, directly as source of pollution (ICRAM, 2007), and indirectly (Sprovieri et al., 2013), but it is not yet well known.

Shipwrecks' role on Mediterranean marine ecosystems have so far received relatively little attention (Sprovieri et al., 2013; Consoli et al., 2015; Sinopoli et al., 2015), and only few studies regard the biological and chemical effects caused by known wrecks (Jewett et al., 1999; Guidetti et al., 2000; Viarengo et al., 2007).

Previous studies performed on shipwrecks consider a single flooded structure, approaching the problem from a single point of view and considering separate effects on benthic species, fish species, and environmental levels of induced pollution. The lack of literature is due to the fact that shipwreck research in open-sea water is not an easy task to undertake and requires study of the existing literature, the consultation of official and unofficial data sources, interviews with fishing operators, and the collection of information that are not always easy to access.

In the Mediterranean Sea, among possible geographical areas suitable to such studies, Lampedusa represents a key study area concerning

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shipwrecks thanks to its location within the strait of Sicily that is considered a strategic economic area of the Mediterranean Sea. Furthermore, the strait is characterized by the presence of rhodoliths and *Maërl* beds: the conservation value of these ecologically fragile systems in European waters is recognized under EU legislation (Annex V of the 'Habitats Directive' 92/43/EEC, 1992, regulation CE 1967/2006, MSFD 2008/56/EC) and international conventions (*Maërl* constitute a priority habitat according to the SPA/BIO protocol of the Barcelona Convention; OSPAR convention, 1998).

This study proposes a holistic approach based on multivariate statistics on three open-sea shipwrecks flooded close to Lampedusa at − 70 m of depth on *Maërl* beds habitat and characterized by different potential polluting cargoes including weapons, arms and military instruments. A control site settled in the strait of Sicily was also considered to compare results with those obtained on the shipwrecks. The aim of this study is to evaluate effects induced by these three shipwrecks on the biodiversity of demersal fish and benthic assemblages, correlating the observed effects to sediment pollution levels and trophic web pollution.

2. Materials and methods

2.1. Logic model applied & shipwrecks studied

Searching for shipwrecks is a complex task to undertake and this research started from a complex files studying the analyses of the official bibliography on the sinking of merchant and military ships around the Sicilian sea, since the Second World War (WWII). On the basis of the literature and on numerous interviews with fisherman, diving centers and experts, a large number of possible targets on which to perform field studies were selected. Among these possible targets, only three shipwrecks sank during the WWII fit into the following *a priori* selection criteria: i) comparable flooded data; ii) not recent flooded data; iii) similar water depth; iv) similar bottom type (*Maërl* beds habitats); v) different pollution type (*i.e.* different cargo type or different vessel position after flooding). Criteria i); iii); iv) are imposed for standardization reasons (respectively to standardize shipwrecks' surface exposure time, depth effects on communities, and the effects of shipwreck presence on similar *Maërl* beds habitat type substratum. Criteria ii) is imposed to allow the climax of communities before samplings. On the contrary, criteria v) is considered as a factor of pollution variability of interest in multivariate statistical analyses. Oceanographic cruises were performed by the R/V ASTREA (ISPRA) starting from March 2010 to find wrecks based on the possible targets suggested by the interviews. Localized targets were georeferenced and insert in a GIS software (Arc Map Desktop 10, Esri). A multibeam approach was followed to find targets and perform three-dimensional reconstruction of each wreck. Remotely Operated Vehicles (ROV) inspections were performed and collected videos were analyzed to obtain checklists of fish and benthic species that colonize the shipwreck's solid substrates and to calculate abundances and richness compared to controls. Furthermore, pollution levels were evaluated in both sediments and fish species (*Diplodus vulgaris*, *Serranus cabrilla*, *Spondyliosoma cantharus*) sampled in a significant statistical number close to the shipwrecks and in Control sites. Fish species owing to different trophic levels were collected to evaluate possible enrichment of pollutants along the trophic web. Biometrics of each collected fish species were recorded as well as gender and developmental stage of specimens.

2.2. Multibeam and ROV surveys

A Kongsberg EM 2040 Multibeam Sonar interfaced to SIS (Seafloor Information System, Kongsberg) software were used to collect bathymetric measures during the surveys with the ASTREA Research Vessel. The system operated at a frequency of 300 kHz. The R/V was equipped with a Differential Global Position System RTK. A SBE probe collected sound velocity profile data. Data were acquired with a 40% lateral overlap and processed in order to remove spikes due to the navigation system problem and/or to the acquisition system. Collected data were analyzed using HIPS version 7.1 software package (CARIS).

The first application of the ROV approach in marine environments what concerned with engineering and archeological purposes (AA.VV., 2008) but recently ROV was successfully applied in several marine biological and ecological studies (Bergstrom et al., 1987; Hamner and Robison, 1992; AA.VV., 2008). ROV represents a consolidated method to perform biological diversity studies in deep-sea environments if supported by a standard method of data analysis (Karpov et al., 2006). Furthermore, ROV technique is frequently applied to obtain abundance/density indices on fish species (Grassle et al., 1975; Adams et al., 1995; Cailliet et al., 1999; Andaloro et al., 2012; Consoli et al., 2015). A ROV "Pollux" equipped with a digital camera (Nikon, D80, 10 megapixels) a strobe (Nikon, SB 400) and a high definition video camera (Sony, HDR, HC7) was used to collect the data. The ROV was equipped with an underwater acoustic tracking position system (LinkQuest Inc., Tracklink 1500 MA). The communities associated (fish species and macrobenthos) with the three investigated wrecks were surveyed according to Consoli et al. (2015). Shipwrecks were entirely plotted and investigated in the fore and aft directions and, when possible, penetrated the inner parts of shipwreck. Videos collected were analyzed to record fish species and their abundance according to the methodology proposed by Consoli et al. (2015). Specific abundances (standardized at 1 h) and specific richness were calculated at each shipwreck for fish species. Some elements of macrobenthos unambiguously distinguishable *via* images were classified following the literature (Lousy, 2004; Trainito, 2004). Presence/Absence for a large number of benthic species was assessed. Identifications were performed at the taxonomic level allowed by the method used for determinations.

2.3. Sediment & fish species collection

Sediments and fish species were collected in three replicates both around shipwrecks and control sites located as represented in Fig. 1. Sediment samples were collected using a 0.1 m^2 *van Veen* grab (18 l total volume), immediately near the shipwreck and at a distance of 50 m and 100 m to evaluate micro spatial scale variability on sediment pollution. On board an undisturbed superficial layer (0–5 cm) of sediment was collected, homogenized and frozen (− 20 °C) until chemical analyses. Cosmopolite, benthonic and ubiquitous fish species

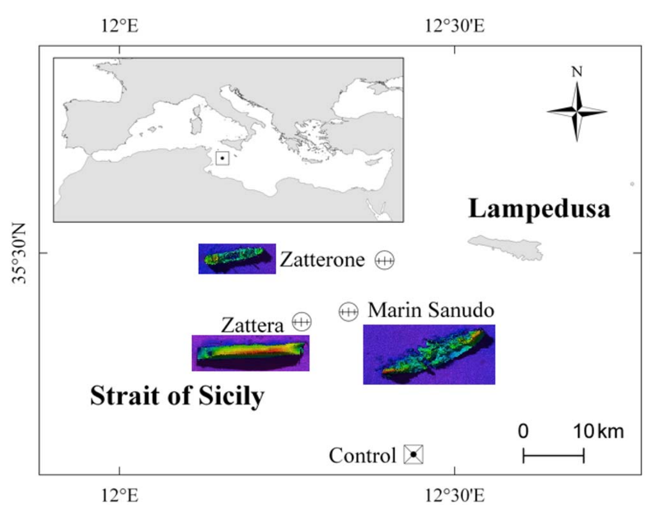


Fig. 1. Geographical localization of Shipwreck sites and Control site (Sicily strait). The square represents the study area localization within the Sicily strait at a smaller geographical scale. At higher geographical scale the relative position among shipwrecks (Zattera, Zatterone and Marin Sanudo) and Controls is highlighted. More details on multibeam's shipwreck reconstructions are reported in Fig. S1 (Supplementary Materials).

(*Spondyliosomacantharus*, *Diplodus vulgaris*, *Serranus cabrilla*) owing to different trophic levels were selected for sampling in a statistically significant number of individuals. Fish species were collected by pole and line near the shipwreck. On board each fish specimen was identified and frozen (− 20 °C) for biological and chemical analysis. In the laboratory, total length (mm) and total weight (g) of each fish specimen was determined. Specimens' gender and developmental stages were also recorded. From each individual a homogenize aliquot of both liver and dorsal muscle was extracted, and stored for chemical analysis.

2.4. Target pollutants

Chemicals of specific interest were selected to trace pollution in sediments and fish species. Trace elements (Al, As, Ba, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, V, Zn) were analyzed to evaluate possible environmental enrichments from cargoes and vessels. PAHs (acenaphthene, acenaphthylene, anthracene, dibenzo(*a,h*)anthracene, benzo(*b*)-fluoranthene, benzo(*a*)pyrene, benzo(*g,h,i*)perylene, benzo(*k*)fluoranthene, chrysene, dibenzo(*a,h*)anthracene, fluoranthene, indenopyrene, naphthalene, phenanthrene, pyrene, perylene) were analyzed to evaluate pollution from fuels. PCBs (congeners 28, 52, 77, 81, 101, 118, 126, 128, 138, 153, 156, 169, 180) and organochlorine pesticides (OCPs: β-HCH, γ-HCH, Eptachlor, Aldrin, Eptachlor epoxide, Oxychlordane, *cis*-Chlordane, *trans*-Nonachlor, 2,4′-DDT, 4,4′-DDT, 2,4′-DDE, 4,4′-DDE, 2,4′-DDD, 4,4′-DDD, *cis*-Nonachlor, Mirex, Dieldrin, and Endrin) were selected to evaluate possible releases from batteries and cargoes. Furthermore, even if TBTs were known during the WWII they were also excluded due to the scarce application until the end of WWIIas antifouling. In fact, at the time, Cu- and Zn-based paints were more commonly used in underwater paints.

2.5. Laboratory analyses

Trace elements were determined after mineralization of 0.5 g dried sediment using a mixture of H_2O_2 and HNO_3 (4:1 v/v), according to the US-EPA 6020A method. Limits of detection were lower than 1 mg/kg d.w. for Al, Fe, and lower than 0.02 mg/kg d.w. for all the other analyzed trace elements.

Persistent organic pollutants (μg/kg dry weight, d.w.) extractions were performed according to ICRAM (2001). Quantifications of PCBs and OCPs were performed following US-EPA 8082A/2007 standard method, PAHs were determined according to US-EPA Method 8270D. Concerning organic pollutants, results were expressed on a dry weight basis (d.w.). The lowest sensitivity threshold for the persistent organic pollutant analyzed was 0.1 μg/kg d.w.

Quality control was performed by SRMs 1941b (Organics in marine sediment) purchased by National Institute of Standard & Technology; PACS-2 (Marine Sediment Reference Materials for Trace Metals and other Constituents) purchased by National Research Council Canada, Institute for National Measurement Standards; TORT-2 (Lobster Hepatopancreas Reference Material for Trace Metals) purchased by National Research Council Canada, Institute for National Measurement Standards. PCBs and POCs ^{13}C labelled internal standards were used to spike fish tissues. Recoveries of all elements were included within standard deviation certified and associated to the average values of SRM. Limits of quantification were determined according to the Association of Analytical Communities (Shrivastava and Gupta, 2011).

2.6. Statistical analysis

Univariate statistics were performed using GraphPad Prism® Software Inc., version 5.0. Average, minimum, maximum and standard deviation values were calculated for each parameter and *t*-test and F-test were calculated to compare respectively average and variance of pairs of parameters of specific interest. Multivariate statistical analysis was performed using the PRIMER6® & PERMANOVA + ® software packages (Clarke and Warwick, 2001; Anderson et al., 2008). Data were transformed (square root, log(x + 1)) and normalized to calculate Euclidean matrix of similarity and to perform Principal Component Analysis (PCA) and non-metric multidimensional scaling (*nm*-MDS) on chemicals levels in sediments and fish tissues. Values below the limits of detection were considered equal to one-half of the limit. Presence/Absence (benthic species) or abundance (fish species) data were used to calculate Bray-Curtis similarity matrices on biological data (fish and benthic species). Cluster analysis projections were superimposed to the *nm*-MDS to evaluate level of significance of observed segregations. Multivariate ANOSIM (ANalysis Of SIMilarity) one-way test were performed to calculate significances running 9999 permutations and imposing different factors of interest. The relationships between the abundance of fish species and presence/absence of benthic species were investigated using the Pearson's correlation test.

3. Results

3.1. Multibeam surveys

The oceanographic survey allows the localization of three shipwrecks named “Zattera”, “Zatterone”, and “Marin Sanudo”. Fig. 1 represents the geographical position of the three targets and Control site in the strait of Sicily. Principal shipwrecks' features are detailed in Supplementary materials (Table S1). Concerning criteria of selection *a priori* defined in this study, the three shipwrecks flooded during WWII in a sandy bottom substratum characterized by *Maërl* formations and at a water depth of-70 m. In Fig. 1, a snapshot on multibeam reconstruction is reported for the three shipwrecks while a detailed multibeam reconstruction is reported in Supplementary materials (Fig. S1). As observed by the figure, Zattera was flooded on its upper-work while the other two vessels' position after flooding was the cruising position.

3.2. Biodiversity assessment

In Table 1, abundance of fish species is reported for each shipwreck. Categorization of different species ecology (pelagic, P; necto-benthonic, NB; benthonic, B) and preferential diet (Pi = piscivorous, Pl = planktivorous, Be = benthivorous) are also indicated. Control sites are characterized by zero presence of listed species and are not reported in Table 2. Overall, 17 fish taxa belonging to 8 families were recorded in the study area and 5 taxa were common to all shipwrecks. Two protected species were also identified: *Sciaena umbra*, in “MarinSanudo” and “Zattera”, and *Epinephelus marginatus* in “Zatterone”; these two species are included in the Annex III of the SPAMI protocol and the Appendix III of the Berna Convention, and the latest is also considered endangered for the RED LIST IUCN.

Moreover, “MarinSanudo” shows the highest abundance and fish species richness. On the contrary, “Zattera” shows the lowest abundance and fish species richness. Abundance in “Zattera” is over eight times lower than “MarinSanudo”, while species richness is less than half. Species distribution is associated to the shipwreck structure and dominant species *Anthiasanthias* and *Chromischromis* are principally distributed in the upper part of the shipwreck.

Results obtained by *nm*-MDS multivariate statistical analyses, performed on fish species for each shipwreck, are reported in Fig. 2, with the superimposed Cluster analysis. A clear difference among Control and shipwrecks is observed for fish species. Furthermore, the Cluster analysis performed evidences that “Zattera” is only 20% similar to the other two shipwrecks. F-test and *t*-test ($p < 0.01$) performed between pairs of shipwrecks evidenced significant differences in averages and variances of fish species abundance between “Zattera” and the other two vessels.

Concerning benthos biodiversity in “MarinSanudo”, 58 taxa from 8 different phyla are recorded. Some threatened and endangered species

Table 1
Abundances of fish species. Abundances in Control sites are zero for all considered species and are not included in this table. Ecology (P = pelagic, NB = Necto Benthonic, B = benthonic) and diet preference (Pi = piscivorous, Pl = planktivorous, Be = benthivorous) categories of considered species are evidenced. Total abundances and Species Richness are, also, calculated per each shipwreck.

Taxa		Marin Sanudo	Zattera	Zatterone	Ecologic category	Diet preference
Carangidae	*Seriola dumerili*	63	300	32	NB	Pi
Labridae	*Coris julis*	4	0	11	NB	Be
	Thalassoma pavo	1	0	2	NB	Be
Mullidae	*Mullus surmuletus*	9	0	0	B	Be
Pomacentridae	*Chromis chromis*	2130	265	1200	NB	Pl
Sciaenidae	*Sciaena umbra*	8	5	0	NB	Pi
Scorpaenidae	*Scorpaena scrofa*	3	0	0	B	Be
Serranidae	*Anthias anthias*	4633	522	5265	NB	Pl
	Epinephelus marginatus	0	0	2	NB	Pi
	Mycteroperca rubra	1	0	0	NB	Pi
	Serranus cabrilla	10	5	2	NB	Pi
Sparidae	*Dentex dentex*	19	0	24	NB	Pi
	Diplodus puntazzo	0	0	5	NB	Be
	Diplodus sargus	0	0	3	NB	Be
	Diplodus vulgaris	56	4	3	NB	Be
	Pagrus auriga	3	0	0	NB	Be
	Spondyliosoma cantharus	1	0	0	NB	Be
Total abundance		6876	801	6515	–	
Species richness		14	6	11	–	

are recorded as well as: ASPIMAnnex II protected species as *S. spinulosus*, *I. foetida*, *A. polypoides* (Porifera), *H. lichenoides* (Bryozoa), ASPIM Annex II and Berna Convention, Appendix II protected species as well as *Aplysina* sp. (Porifera), *O. ophidianus* (Echinodermata), ASPIM Annex III and Berna Convention, Appendix III protected species as well as *S. agaricina* (Porifera), *S. officinalis* (Porifera), *P. elephas* (Crustacea), and CITES Annex II as well as *Cariophyllia* spp. (Cnidaria). In "Zattera", 26 taxa from 7 different phyla are recorded while in "Zatterone" 10 benthic species are recorded. A detailed checklist on presence/absence of benthonic species is reported in Supplementary material (Table S2). Images of assemblages of benthonic species and associated fish are showed in Supplementary materials (Fig. S2).

Results obtained by *nm*-MDS multivariate statistical analyses, performed on benthic species presence/absence data for each shipwreck, are reported in Fig. 3 with the Cluster Analysis superimposed. Also in this case, a clear difference among Control and shipwrecks is observed for benthic species. The Cluster analysis evidences that the three vessels are only 20% similar to each other, with "MarinSanudo" and "Zatterone" showing 50% of similarity. Furthermore, *t*-test and F-test results support an effect on abundance and biodiversity of fish species due to the vessel position after flooding.

3.3. Chemical pollution of sediments

In sediment samples, Cd results below the limit of quantification in each sampling site (both shipwrecks and Control sites) while POPs resulted very close to their LOQ for a large part of considered OCPs (β-HCH, γ-HCH, Eptachlor, Aldrin, Eptachlor epoxide, Ossichlordane, *cis*-Chlordane, *trans*-nonachlor, 2,4′-DDE, 4,4′-DDE, 2,4′-DDD, 4,4′-DDD, *cis*-nonachlor, Mirex). Some PAHs (benzo(*a*)pyrene, naphthalene) range within LOQ and 1 μg/kg. For these compounds, a statistical comparison among sampling sites does not make sense. On the contrary, the larger part of measured trace elements and some POPs are recorded at levels higher than LOQ.

Measured levels of Al and Fe on *Maërl* beds in this study are respectively 0.43% (0.23% SD) and 0.66% (0.21% SD). Measured levels for trace elements of some ecotoxicological concern and POPs result scaled as follows (average ± standard deviation):

Mn (63.78 ± 20.45 mg/kg) > Hg (17.12 ± 9.20 mg/kg) > V (15.86 ± 4.16 mg/kg) > Ba (14.61 ± 5.58 mg/kg) > Zn (12.66 ± 9.43 mg/kg) > Pb (10.43 ± 6.52 mg/kg) > Cr (8.58 ± 2.37 mg/kg) > As (7.74 ± 3.70 mg/kg) > Cu (3.13 ± 0.54 mg/kg) > Ni (2.03 ± 0.56 mg/kg) > Co (0.84 ± 0.30 mg/kg) > Endrin (3.63 ± 1.77 μg/kg) > Dieldrin (3.00 ± 2.74 μg/kg) > ΣPCBs (0.65 ± 0.54 μg/kg).

"Zatterone" shows Al (0.70%) and Fe (0.90%) levels that are significant highest than other shipwrecks (above 3.5 times for Al and 1.5 times for Fe). The latter are, on the contrary, not significantly different from controls. The same trend is recorded for Ba Co, Hg, Mn, Ni (over twice as high in "Zatterone" than other shipwrecks and controls). The opposite trend is reported for As, Pb, Zn, with higher levels in "MarinSanudo" than recorded in the other shipwrecks and controls while, Cr, Cu and V are comparable in considered shipwreck sites but higher than the controls. The levels of OCPs, Dieldrin and Endrin are higher in shipwrecks than controls but show an opposite trend and the first is higher in "MarinSanudo" while the second is higher in "Zatterone". On the contrary, ΣPCBs is twice as high in control sites than shipwrecks.

The Principal Component Analysis performed on pre-treated chemical data of sediments (Fig. 4) evidences that the first three components (36.7%, 23.1%, and 18.6%) account for the 78.4% of the total variance. The first two axes account for over 50% of the whole variability observed in this matrix. Chemicals considered in this study evidence different associations. The coefficients in the linear combinations of variables making up PC's evidenced a strong positive correlation with the first axis for As (0.285), Cr (0.253), V (0.265) while a strong negative correlation is reported for Mn (−0.256), and OCPs from 3 to 10. On the contrary, the second axis shows a strong positive relation to PCBs (0.208) and some OCPs (1; 2; 13; 16). A strong negative correlation with Al (−0.356), Fe (−0.274), Ba and Co (−0.314), Hg (−0.270), Ni (−0.353) is reported. Principal component scores evidence that Controls are inversely related to the first axis (lower levels of As, Cr, V), "MarinSanudo" is positively related to the second axis (higher levels of PCBs, OCPs), while "Zatterone" is negative related to the second axis (higher levels of Al, Fe, Ba, Co, Hg, Ni).

The vessel position after flooding does not significantly affect sediment pollution (ANOSIM test not significant).

The ANOSIM test performed on the factor "distance from the vessel" evidenced not significant differences (Global R of 0.078, p = 33.3%), showing that pollutant levels could be considered similar within 150 m distance from the vessel. The ANOSIM test performed on the factor "impact" evidenced higher, but not significant, (Global R = 0.377, significance level of sample statistic = 6.0%) suggesting possible but not statistically supported effects on sediment pollution due to the

Table 2
Pollution of trophic web. Average (standard deviation, range mg/kg d.w.) of measured pollutants recorded in each species of target sites grouped separately for each tissues. Only pollutants levels that resulted significant higher (*t*-test) in target sites than Control are reported. For values recorded in Controls see par. 3.4. NS = *t*-test not significant. Values reported in bold are significantly lower than Controls. Trace elements are expressed as mg/kg d.w. while POPs are expressed as µg/kg d.w.

2a. Pollutants levels in muscle tissues

	Zattera (*S. cantharus*)		Zattera (*D. vulgaris*)		Zattera (*S. cabrilla*)		Zatterone (*S. cantharus*)		Zatterone (*D. vulgaris*)		Zatterone (*S. cabrilla*)		Marin Sanudo (*S. cantharus*)		Marin Sanudo (*D. vulgaris*)		Marin Sanudo (*S. cabrilla*)	
	Average ± SD	Min-max	Average ± SD	Min-max	Average ± SD	Min-max	Average ± SD	Min-max	Average ± SD	Min-max	Average ± SD	Min-max	Average ± SD	Min-max	Average ± SD	Min-max	Average ± SD	Min-max
As	11.51 ± 2.58	9.68–13.34	1.44 ± 0.94	0.50–2.38	1.33 ± 1.14	0.50–3.73	5.49 ± 2.00	3.09–10.01	37.55 ± 49.14	2.80–72.30	NS	NS	3.79 ± 1.59	1.60–6.29	3.70 ± 2.17	0.59–7.93	NS	NS
Cd	NS	NS	0.10 ± 0.09	0.05–0.21	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Cr	**0.10**	**0.10–0.10**	**0.10**	**0.10–0.10**	0.22 ± 0.32	0.10–0.95	**0.15 ± 0.16**	**0.10–0.66**	**0.1**	**0.10–0.10**	2.22 ± 4.84	0.10–12.08	**0.1**	**0.10–0.10**	**0.1**	**0.10–0.10**	NS	NS
Cu	**0.05**	**0.05–0.05**	0.82 ± 0.67	0.05–1.28	NS	NS	NS	NS	5.91 ± 8.09	0.19–11.63	NS	NS	NS	NS	NS	NS	NS	NS
Hg	0.25	0.25	0.25	0.25–0.25	0.25	0.25–0.25	NS	NS	0.27 ± 0.06	0.22–0.31	0.25 ± 0.14	0.08–0.50	NS	NS	0.38 ± 0.40	0.04–1.29	NS	NS
Mn	**0.05** ± 0.01	**0.05–0.06**	NS	NS	0.24 ± 0.16	0.11–0.57	NS	NS	0.85 ± 1.13	0.05–1.65	0.40 ± 0.35	0.06–0.88	NS	NS	NS	NS	NS	NS
Mo	NS	NS	NS	NS	NS	NS	NS	NS	0.15 ± 0.18	0.02–0.28	NS	NS	NS	NS	NS	NS	NS	NS
Ni	NS	NS	NS	NS	0.09 ± 0.14	0.04–0.41	NS	NS	NS	NS	0.84 ± 1.66	0.04–4.19	NS	NS	NS	NS	NS	NS
Se	NS	NS	NS	NS	NS	NS	NS	NS	7.75 ± 9.89	0.75–14.74	NS	NS	NS	NS	NS	NS	**0.25 ± 0.18**	**0.01–0.53**
V	NS	NS	NS	NS	NS	NS	NS	NS	0.30 ± 0.37	0.04–0.57	NS	NS	NS	NS	0.20 ± 0.50	< 0.01–1.62	NS	NS
Zn	NS	NS	NS	NS	NS	NS	NS	NS	32.45 ± 42.36	2.50–62.41	NS	NS	NS	NS	NS	NS	8.13 ± 3.90	4.23–14.37
PCB	**0.20 ± 0.06**	**0.15–0.24**	**0.03 ± 0.03**	**< 0.01–0.05**	**0.30 ± 0.14**	**0.12–0.48**	2.91 ± 7.13	0.18–25.45	**1.93 ± 0.02**	**1.91–1.95**	**1.51 ± 0.81**	**0.74–2.86**	**1.24 ± 0.80**	**0.32–3.22**	NS	NS	NS	NS
αHCH	NS	NS	NS	NS	NS	NS	1.0 ± 0.1	0.7–1.0	0.8 ± 0.3	0.5–1.0	1.0 ± 0.1	0.9–1.0	0.9 ± 0.2	0.6–1.0	0.9 ± 0.2	0.5–1.0	NS	NS
β-HCH	NS	NS	NS	NS	NS	NS	NS	NS	1.7 ± 1.1	0.9–2.5	NS	NS	NS	NS	NS	NS	2.0 ± 1.9	0.5–5.9
γ-HCH	NS	NS	NS	NS	NS	NS	NS	NS	2.8 ± 3.1	0.6–5.0	NS	NS	NS	NS	NS	NS	2.8 ± 2.6	1.0–7.9
Aldrin	NS	NS	NS	NS	NS	NS	NS	NS	2.1 ± 1.6	1.0–3.3	2.6 ± 1.2	1.7–3.5	NS	NS	NS	NS	NS	NS
Oxychlor.	NS	NS	NS	NS	NS	NS	2.3 ± 1.4	1.0–5.4	3.1 ± 2.9	1.0–5.1	NS	NS	NS	NS	4.6 ± 3.3	1.0–10.6	NS	NS
Dieldrin	NS	NS	NS	NS	NS	NS	5.4 ± 11.7	0.6–38.8	**1.4 ± 0.5**	**1.0–1.7**	NS	NS	NS	NS	3.7 ± 4.6	0.9–12.2	3.5 ± 3.4	1.0–9.5
Endrin	NS	NS	NS	NS	NS	NS	NS	NS	4.0 ± 2.9	2.0–6.0	NS	NS	5.5 ± 5.0	< 0.1–17.7	NS	NS	5.3 ± 3.6	1.0–10.4
ΣDDD	NS	NS	NS	NS	NS	NS	1.5 ± 0.8	< 0.1–3.0	1.7 ± 1.7	0.5–2.9	NS	NS	NS	NS	1.6 ± 1.5	0.8–5.9	1.9 ± 1.2	1.0–3.6
cis-Nonachlor	NS	NS	NS	NS	NS	NS	NS	NS	2.0 ± 1.4	1.0–2.9	4.5 ± 4.9	1.0–7.9	NS	NS	NS	NS	1.9 ± 2.5	0.6–7.5
ΣDDT	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	1.9 ± 1.3	1.0–2.9	NS	NS	1.4 ± 0.8	0.1–2.5	1.6 ± 0.9	1.0–3.6
Methossichlor	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**0.8 ± 0.2**	**0.7–1.0**	**0.9 ± 0.2**	**0.5–1.0**	**1.6 ± 1.0**	**0.7–3.9**	**1.5 ± 0.8**	**0.6–2.7**

2b. Pollutants levels in liver tissues

Liver	As	Cd	Co	Cr	Cu	Hg	Mo	Ni	Pb	Se	V	Zn
Zattera (*S. cantharus*)												
Average ± SD	9.02 ± 0.63	0.81 ± 0.15	0.16 ± 0.01	NS	4.60 ± 0.72	NS	0.17 ± 0.04	0.18 ± 0.19	0.15 ± < 0.01	3.10 ± 0.02	NS	32.49 ± 6.67
Min-max	8.57–9.46	0.70–0.91	0.150.17	NS	4.09–5.11	NS	0.14–0.19	0.04–0.31	0.15–0.15	3.09–3.12	NS	27.78–37.20
Zattera (*D. vulgaris*)												
Average ± SD	19.36 ± 17.73	NS	0.08 ± 0.06	NS	2.71 ± 3.66	1.37 ± 0.19	0.12 ± 0.12	0.11 ± 0.02	0.15 ± < 0.01	NS	0.48 ± 0.65	30.77 ± 26.41
Min-max	7.38–39.73	NS	0.05–0.15	NS	0.58–6.94	1.19–1.57	0.05–0.26	0.09–0.12	0.15–0.15	NS	0.10–1.23	12.43–61.04
Zattera (*S. cabrilla*)												
Average ± SD	2.58 ± 0.67	0.48 ± 0.34	0.06 ± 0.02	NS	1.84 ± 0.54	0.71 ± 0.62	0.16 ± 0.06	0.55 ± 0.94	0.15 ± < 0.01	NS	NS	24.72 ± 2.90
Min-max	1.61–3.53	0.05–1.03	0.05–0.11	NS	1.39–2.94	0.25–1.71	0.10–0.28	0.04–2.44	0.15–0.15	NS	NS	20.42–29.79
Marin Sanudo (*S. cantharus*)												
Average ± SD	6.71 ± 1.74	NS	0.23 ± 0.12	0.16 ± 0.21	7.19 ± 2.60	**0.08 ± 0.06**	0.12 ± 0.03	0.18 ± 0.035	NS	3.11 ± 0.52	0.79 ± 0.58	32.42 ± 7.24
Min-max	4.43–9.61	NS	0.12–0.50	0.01–0.65	2.88–10.80	**0.01–0.18**	0.07–0.17	0.01–0.94	NS	2.26–3.89	0.14–1.97	21.81–41.07
Marin Sanudo (*D. vulgaris*)												
Average ± SD	7.59 ± 2.32	NS	0.21 ± 0.11	0.40 ± 0.56	9.21 ± 3.96	0.78 ± 0.39	0.31 ± 0.17	0.36 ± 0.51	NS	4.07 ± 2.56	1.51 ± 0.74	40.55 ± 18.57
Min-max	4.94–12.72	NS	0.01–0.37	0.01–1.58	3.17–17.75	0.20–1.66	0.21–0.78	0.01–1.74	NS	2.58–11.15	0.60–3.19	23.53–88.80
Marin Sanudo (*S. cabrilla*)												
Average ± SD	1.98 ± 1.10	**0.12 ± 0.06**	NS	0.11 ± 0.23	NS	NS	NS	NS	NS	2.88 ± 1.43	0.33 ± 0.51	NS

(continued on next page)

Table 2 *(continued)*

2b. Pollutants levels in liver tissues

Liver	As	Cd	Co	Cr	Cu	Hg	Mo	Ni	Pb	Se	V	Zn
Min-max	1.23–4.31	**0.01–0.20**	NS	0.01–0.63	NS	NS	NS	NS	NS	1.60–5.76	0.01–1.25	NS
Zatterone (*S. cantharus*)												
Average ± SD	5.81 ± 3.54	NS	0.14 ± 0.03	0.17 ± 0.25	7.06 ± 2.89	0.12 ± 0.08	NS	NS	NS	3.17 ± 0.69	0.72 ± 0.50	32.48 ± 7.36
Min-max	0.01–14.49	NS	0.09–0.19	0.01–0.81	3.15–11.45	0.01–0.25	NS	NS	NS	2.23–4.28	0.19–2.04	22.98–46.18
Zatterone (*D. vulgaris*)												
Average ± SD	8.88 ± 0.64	0.67 ± 0.22	0.18 ± 0.03	NS	9.99 ± 3.12	0.60 ± 0.23	0.26 ± 0.07	0.10 ± 0.01	NS	3.17 ± 0.04	2.05 ± 1.34	46.75 ± 11.26
Min-max	8.42–9.33	0.52–0.83	0.16–0.21	NS	7.78–12.19	0.43–0.76	0.21–0.31	0.10–0.11	NS	3.14–3.20	1.10–3.00	38.79–54.71
Zatterone (*S. cabrilla*)												
Average ± SD	2.92 ± 1.86	NS	NS	NS	NS	NS	0.12 ± 0.06	0.26 ± 0.47	0.10 ± 0.07	NS	**0.13 ± 0.12**	21.52 ± 3.82
Min-max	1.61–6.46	NS	NS	NS	NS	NS	0.03–0.20	0.01–1.22	0.01–0.15	NS	**0.01–0.35**	15.80–25.01

presence of the shipwrecks. The low significance could be related to a time-dependent dilution effect of pollution due to the years passed since the flooding occurred.

3.4. *Chemical pollution & trophic web*

In Supplementary material (Table S3), principal features of fish species considered to evaluate tissue and trophic web pollution are detailed reported. In Table 2, the average (standard deviation, range) of measured pollutants is reported grouping data per species. Results of target sites are also grouped according to tissue (Table 2a – Muscle, Table 2b – Liver). Only pollutant levels resulted significantly higher (*t*-test) in target than Control sites are summarized.

Concerning trace elements measured in Controls, average (SD) values recorded in fish species' muscle tissues result as follows (data expressed as mg/kg d.w.): As (0.89 ± 3.84); Cd (0.02 ± 0.01); Co (0.05 ± 0.01); Cr (0.48 ± 0.18); Cu (0.36 ± 0.08); Hg (0.13 ± 0.08); Mn (0.15 ± 0.18); Mo (0.05 ± NC); Ni (0.10 ± 0.05); Pb (0.15 ± NC); Se (0.62 ± 0.11); V (0.04 ± 0.04); Zn (4.03 ± 2.02).

On the contrary, liver tissues report the following levels (data expressed as mg/kg d.w.): As (0.80 ± 0.79); Cd (0.37 ± 0.29); Co (0.02 ± 0.02); Cr (0.08 ± 0.16); Cu (1.01 ± 0.55); Hg (0.32 ± 0.29); Mn (0.87 ± 0.49); Mo (0.05 ± 0.04); Ni (0.03 ± 0.06); Pb (0.01 ± NC); Se (2.33 ± 1.35); V (0.26 ± 0.37); Zn (13.09 ± 7.23).

Concerning POPs measured in Controls, the average (SD) values recorded in fish species' muscle tissues result as follows (data expressed as μg/kg d.w.): ΣPCBs (3.02 ± 4.05); α-HCH (0.98 ± 0.17); β-HCH (1.02 ± 0.31); γ-HCH (1.66 ± 1.86); Eptachlor (0.97 ± 0.07); Aldrin (1.32 ± 0.69); Eptachlor Epoxide (1.00 ± NC); Oxichlordane (1.21 ± 0.55); *cis*-Chlordane (1.03 ± 0.10); *trans*-Chlordane (1.05 ± 0.35); *trans*-Nonachlor (1.00 ± NC); Dieldrin (2.90 ± 1.73); ΣDDE (0.87 ± 0.19); Endrin (3.01 ± 1.76); ΣDDD (0.94 ± 0.40); *cis*-Nonachlor (1.16 ± 0.46); ΣDDT (0.93 ± 0.37); Methoxychlor (6.30 ± 17.51); Mirex (1.03 ± 0.62); ΣPAHs (8.52 ± 2.21).

On the contrary, levels measured in liver tissues result as follows (data expressed as μg/kg d.w.): ΣPCBs (29.71 ± 5.32); *α*-HCH (3.54 ± 0.09); *β*-HCH (18.32 ± 2.54); γ-HCH (9.43 ± 3.81); Eptachlor (2.65 ± 0.90); Aldrin (9.31 ± 0.78); Eptachlor Epoxide (2.59 ± 0.07); Oxychlordane (5.29 ± 0.32); *cis*-Chlordane (9.31 ± 0.33); *trans*-Chlordane (6.44 ± 0.91); *trans*-Nonachlor (3.62 ± 0.97); Dieldrin (18.44 ± 5.92); ΣDDE (5.28 ± 0.79); Endrin (12.81 ± 6.80); ΣDDD (11.09 ± 1.33); *cis*-Nonachlor (7.21 ± 0.99); ΣDDT (3.56 ± 0.95); Methoxychlor (15.23 ± 12.01); Mirex (16.31 ± 2.44); ΣPAHs (25.99 ± 7.76).

Results obtained by *nm*-MDS multivariate statistical analyses performed on pollutant levels in fish species are reported in Fig. 5, results obtained by the Cluster Analysis are also highlighted. Different species are indicated as follows:1 = *S. cantahrus*, 2 = *S. cabrilla*, 3 = *D. vulgaris*. Results are grouped by tissue (Fig. 5a – Muscle; Fig. 5b - Liver).

Dataset collected on liver and muscle tissues were analyzed separately due to the significant differences among levels in considered matrices. The ANOSIM test performed on the factor "impact" evidenced significant differences between impacted sites and Control both for muscle (Global R of 0.226, significance level of sample statistic of 0.02%) and liver (Global R of 0.117, significance level of sample statistic of 2.9%) tissues. The Pairwise test evidences not significant differences between "MarinSanudo" and Zatterone concerning pollutants in liver tissues while no differences are recoded between "Zattera" and "Zatterone". On the contrary, in muscle tissues, significant differences are recorded between MarinSanudo, Zattera and Zatterone pairs (Global R of 0.226, significance level of sample statistic of 0.02%).

ANOSIM tests were performed for both datasets to evaluate the relative contributions to other possible factor of interest on fish

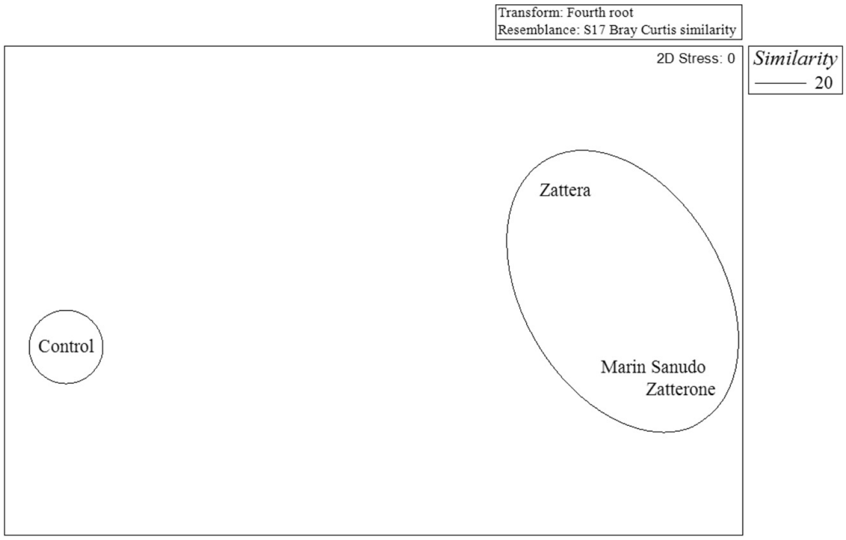


Fig. 2. *nm*-MDS performed on fish abundances dataset superimposed to the Cluster analyses. Data are grouped per shipwreck, controls included. Notes: Multivariate statistics are performed on Bray-Curtis similarity matrix calculated after transformation of abundance data. Cluster analysis is superimposed and a similarity level of 20% is evidenced with black circles.

pollution (*i.e.* sex, life stage, species). ANOSIM test (impact *vs* specie, impact *vs* sex, impact *vs* life stage) concerning muscles, were plotted. Results evidence significant differences among species (Global R of 0.422, significance level of sample statistic of 0.01%). Significant difference is recorded among sex (Global R of 0.159, significance level of sample statistic of 1.0%), while not significant differences are recorded for life stage (Global R of 0.075, significance level of sample statistic of 24.8%).

ANOSIM test (impact *vs* specie, impact *vs* sex, impact *vs* life stage), concerning liver, were plotted. Results evidence significant differences among species (Global R of 0.473, significance level of sample statistic of 0.01%). In this case, the Pairwise test performed does not show differences between *S. cantharus* and *D. vulgaris* (Global R of 0.200, significance level of sample statistic of 7.3%). However, low significant differences are recorded in relation to life stage (Global R of 0.387, significance level of sample statistic of 0.1%), while not significant values are reported for sex (Global R of -0.053, significance level of sample statistic of 63.4%).

4. Discussion

4.1. General effects of artificial structures

Shipwrecks are artificial structures that represent key microhabitats for fish species in open seawater (Ogden and Ebersole, 1981) able to attract numerous benthonic and necto-benthonic species (Bohnsack and Sutherland, 1985). Research has evidenced that species richness is directly related to the habitat complexity and to the large number of trophic niches and recoveries (Luckhurst and Luckhurst, 1978; MacPherson, 1994; Garcìa Charton and Pérez-Ruzafa, 2001; La Mesa and Vacchi, 2005). Results obtained by this study evidence shipwrecks increase biodiversity of both fish and benthic species with significant difference compared to controls. Previous studies evidenced that shipwrecks could improve biodiversity close to coastal zones (Jones and Thomson, 1978; Arreola-Robles and Elorduy-Garay, 2002; Massin et al., 2002), acting as fish aggregating devices. Even if literature supports the positive effect on biodiversity of artificial structure, this research contributes to improve knowledge on their effects on marine ecosystems associating biodiversity data to possible threats due to release of pollutants by flooded vessels. The available researches on biodiversity associated with shipwrecks are focused on coastal marine areas, low water depth and concerns the Atlantic Ocean (Edgar et al., 2003; Massin et al., 2002; Marshall and Edgar, 2003; Zintzen et al., 2006). In the Mediterranean Sea, although the effects on fish diversity of other artificial structures, such as extractive platforms and artificial reefs, have been widely investigated (Fabi et al., 2002a, 2002b, 2004; Consoli et al., 2007, 2013; Andaloro et al., 2011a, 2011b, 2012; Scarcella et al., 2011), the only data on the role played by shipwrecks are available from Consoli et al. (2015), Sinopoli et al. (2015) and Sprovieri et al. (2013).

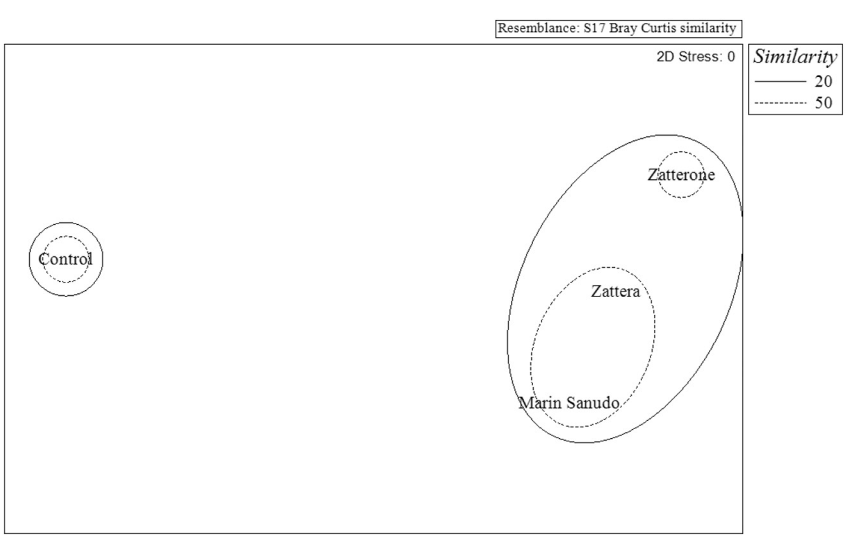


Fig. 3. *nm*-MDS performed on benthic dataset superimposed to the Cluster analyses. Data are grouped per shipwreck, controls included. Notes: Multivariate statistics are performed on Bray-Curtis similarity matrix calculated after transformation of presence/absence data. Cluster analysis is superimposed and a similarity level of 20% is evidenced with black circles.

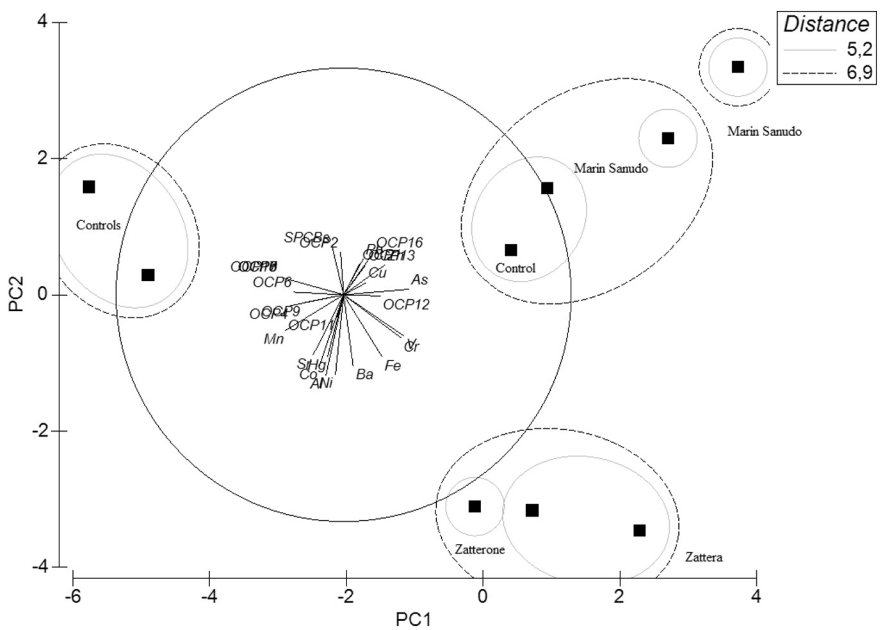


Fig. 4. Principal component Analyses performed on pollutants in sediments.

4.2. Shipwrecks: effects on biodiversity

Fish and benthic species represent useful indicators of ecosystem quality evidencing the occurrence of impacts of human and natural origin (MSFD2008/56/EC; Warwick and Clarke, 1991; Thomson et al., 2003; Simboura and Reizopoulou, 2008).

Greater species richness, as well as the several exclusive fish species found at the three investigated vessel-reefs, suggest these artificial habitats are providing unique habitat, which may not be found on surrounding natural ones. The main mechanism invoked to explain the aggregation effect of the wreck is a higher habitat complexity in comparison to control sites located on natural soft bottoms. More than a simple favorable microhabitat in hostile nude open-water ecosystems (Deudero et al., 1999; Deudero, 2001; Andaloro et al., 2002), shipwrecks act in more complex ways inducing a positive tigmotrophic effect (Badalamenti et al., 2000; Consoli et al., 2015), refuge, reef, and trophic effect (Laur and Haldorson, 1996; Edgar et al., 2003). Results regarding dominant trophic group are comparable to previous studies carried out on oil platforms (Rilov and Benayahu, 2000) and on shipwrecks (Lindquist and Pietrafesa, 1989; Consoli et al., 2015) where the authors reported planktivores to be the most numerous species. Artificial structures that show a vertical development allow these species (*A. anthias* and *C. chromis*) to feed reducing the risk to be preyed upon (Dulčić, 2007; Gonçalves et al., 2004). These species provide a positive feedback on trophic nutrients in sediments and shipwrecks bottoms due to their dejections (Pinnegar et al., 2007). Upper parts of shipwreck are characterized by the presence of predators of benthic vagile species as well as *Diplodus* spp. and *C. julis* (Stergiou and Karpouzi, 2002). Moreover, the presence of upper predators such as *E. marginatus* and *D. dentex* suggests the achievement of the highest ecological successional stage in "MarinSanudo". Artificial structures could have a significant role to enhance recruitments rates (Rilov and Benayahu, 2002). It is important to underline that shipwrecks, which are an ideal target for recreational fishermen, could contribute to the overexploitation of some high-value fish species, such as *Mycteroperca rubra*, *D. dentex*, *Diplodus* spp., attracted by the artificial hard substrate of the vessel-reefs (Consoli et al., 2015).

4.3. Shipwrecks: effects on protected species

Results of this study evidence as shipwrecks could represent hotspot of biodiversity also for a large number of ASPIM, Berna Protocol, and IUCN Endangered or Protected associated species. On overall, 67 species (17 fish and 50 benthic macroinvertebrates) were observed on shipwrecks including IUCN Red list protected and allochtone species underlining that this artificial structures could support protection strategies. Moreover, even if it is not yet included in any protection lists, the cnidarians *Elisella paraplexauroides* is a key species for marine ecosystems and it was recorded by the literature as distributed only in the Occidental part of the Mediterranean Sea (Vafidis et al., 1994). Even if a previous study evidenced the presence of this species in the strait of Sicily, close to Pantelleria Island (Angiolillo et al., 2012), the present study represents the most oriental record of this species in Mediterranean Sea. This result improves knowledge on the global distribution of this species considered Atlantic-Mediterranean.

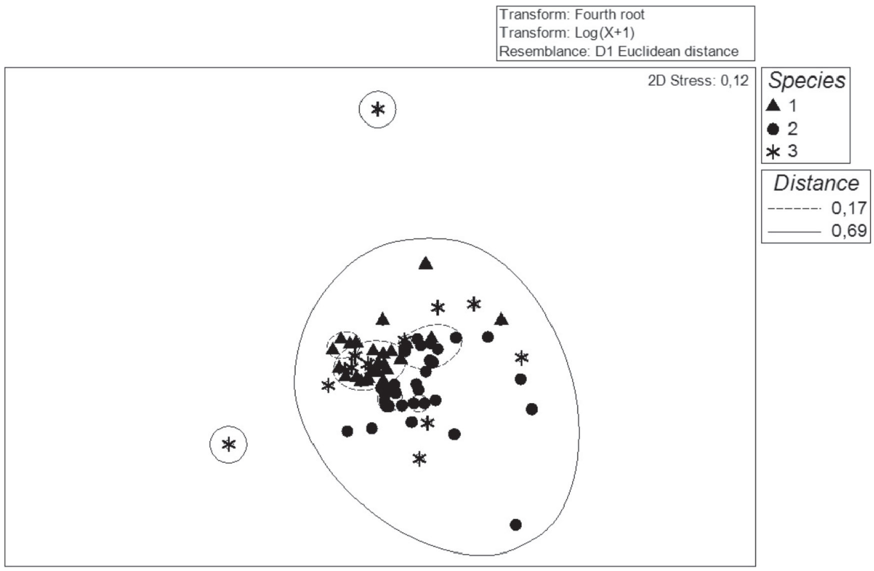
4.4. Shipwrecks: effects of pollution on sediments

Pollutants could be released by shipwrecks during and immediately after flooding due to the release of carried cargo and fuels but for a longer period by the destructuring process due to oxidation phenomena. Pollutants that are released immediately are in huge quantities and could reach a widespread surface; nevertheless, the continuous release of fewer quantities due to oxidation phenomena closed to the flooded area in deep-sea marine ecosystems could also represent a significant danger for communities (Landquist et al., 2013). Results obtained by this research evidence as persistent organic pollutants that could be measured in Mediterranean benthic habitats after over than 60 years from flooding, are few congeners among the large number of measured chemicals.

Only Endrin, Dieldrin (OCPs) and ΣPCBs are recorded in this study. In spite of that, ΣPCBs levels are higher in controls than in shipwrecks sites and this is probably due to the dry air deposition contributes to the measured levels of PCBs in *Maërl* sediments than to the contribute due to shipwrecks. Indeed, high PCBs levels are often associated to marine sediments that represent their final sink and are due to coastal contributes driven by marine currents and to dry air deposition phenomena driven by winds and atmospheric phenomena (Stemmler and Lammel, 2009).

Concerning trace elements, this research allows better defining natural range in *Maërl* beds habitats. Trace element levels in Mediterranean Sea are relatively better defined than organic pollutants due to the occurrence of large areas affected by geomorphological and volcanic anomalies (*i.e.* Aeolian Archipelago, and Etna only to cite

a. Data are grouped per tissue (muscle), control excluded.



b. Data are grouped per tissue (liver), control excluded.

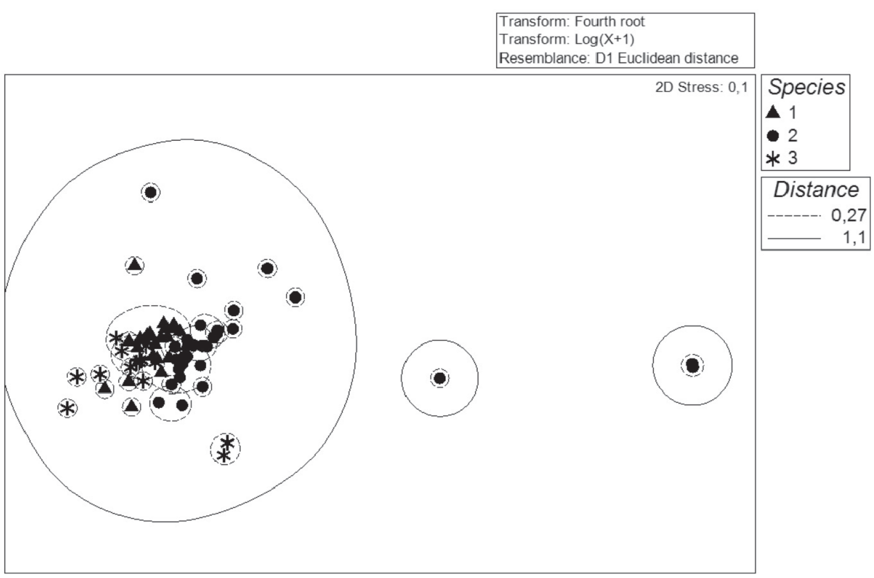


Fig. 5. *nm*-MDS performed on pollutant levels in fish tissues with superimposed Cluster analyses. Notes: Multivariate statistics are performed on Euclidean similarity matrix calculated after transformation and normalization of concentration data. Cluster analysis is superimposed and similarity levels are evidenced with black circles.
a. Data are grouped per tissue (muscle), control excluded.
b. Data are grouped per tissue (liver), control excluded.

geographical sites closed to the study area). Trace elements in sediments could be notably higher than those reported in this study closed to volcanic emission active sources but also inside the geomorphological anomalies (Andaloro et al., 2012; Renzi et al., 2011a, 2015 and citations therein). Furthermore, the largest part of literature well defines trace elements closed to coastal areas (*e.g.* harbors) and transitional water ecosystems that are significantly affected by human activities and terrestrial inputs resulted often higher than values recorded in the present study (Renzi et al., 2011b; Renzi et al., 2012 and citations therein). Hg that is notably higher than many levels measured in coastal Mediterranean Sea (Renzi et al., 2011b; Renzi et al., 2012 and citations therein) could represent an exception. For these reason to perform comparisons among literature data and values recorded in this study could be misunderstandings and, in our opinion, the better comparison site is represented by the study controls. Statistics performed on sediments evidence a clear difference in trace elements and persistent organic pollutants in sediments due to shipwreck type. It is to notice that the fingerprint on sediment chemical composition appears after long period from flooded. This occurrence is probably due to the cargo typology (weapons and heavy vehicles in Marin Sanudo *vs* goods in Zattera and Zatterone) and by the degradation of the vessel than in the case of Zatterone led to the release of higher levels of some trace elements than others and, in particular, of Al, Fe, Ni that are principal constituents of the vessel itself.

4.5. Shipwrecks: effects on pollution of trophic webs

Levels of pollution measured in muscles and liver of sampled species are low compared to values recorded in the same species collected closed to Sicilian volcanic emission sources (Andaloro et al., 2011a, 2011b). Furthermore, a large number of considered chemicals are not significantly different comparing shipwreck sites and controls. In spite of that, some pollutants show significant differences in species collected closed to shipwrecks compared to controls. Concerning levels in muscles, Marin Sanudois significantly different from the other two vessels. Measured levels in Zatterone of As, Cr, Cu, Se, Zn, PCBs and quite almost pesticides resulted higher than species sampled closed to other vessels. This occurrence could not be reliable neither to vessel position after flooding nor to cargo typology and is probably due to other factor of pollution that could not be isolated by this study. Not significant difference concerning *S. cantahrus* and *D. vulgaris* could be due to different biology of these species that produces different accumulation rates of pollutants in muscles rather than to trophic level and behavior that are similar. Lower levels associated to *S. cabrilla* compared to other considered species should support low pollutants emissions from shipwrecks. In fact, *S. cabrilla* shows a different reef-association degree compared to other considered species. *S. cabrilla* is a sedentary fish, considered a strictly reef-associated species, differently from the two sparids that are benthopelagic fishes no strictly associated to shipwrecks even if attracted. Life stage of fish is not able to affect significantly pollutants levels in muscles, while gender is able to affect levels in tissues and this is probably due to the fact that rather than life stages, levels in tissues are generally affected by metabolic rates differences among male and female (eggs production). Collected data are not sufficient to evidence metabolic differences among young and old females.

As expected, being liver, the main site of accumulation of the contaminants of interest, liver samples show higher pollutants levels than muscles, in both sites (Corsolini et al., 2008; Guerranti et al., 2016). Furthermore, shipwreck sites show highest levels in liver than controls for almost all trace elements. Even in this case, highest values are associated to benthopelagic fishes supporting a significant contribute to pollution not exclusively due to shipwrecks.

4.6. Straightness and weakness

This research allows better defining possible positive and negative effects of shipwrecks on *Maërl* bed habitats biodiversity in Mediterranean Sea. Shipwrecks studies were performed with different approaches during the years (Jones and Thomson, 1978; Arreola-Robles and Elorduy-Garay, 2002; Massin et al., 2002; Duffy-Anderson et al., 2003) evidencing as the use of ROV could be an useful and standardized technique (Karpov et al., 2006) to approach deep sea habitats, becoming the better technique deeper than 70 m (AA.VV., 2011). To the best of our knowledge, this study represent the first attempt to approach to the problem represented by shipwrecks on target MSFD 2008/56/EC habitats, as *Maërl* beds habitats are, proposing a holistic approach taking into account, fish, benthic biodiversity, allochtone and protected species, sediment and trophic web pollution. Such researches are expansive; time consuming, and needs of a lot of economical and human resources and a large knowhow that it is difficult to assess. Furthermore, the shipwrecks research in open and deep-sea water is not easy to do. This is a necessary preamble to justify some laciness associated to this study. Exposure time could significantly affect vessel decomposition (Crochet, 1991) and, for this reason, we try to standardize this factor by the selection of shipwrecks of WWII. Also, the water depth is standardized at -70 m. In spite of that, some other variables able to affect in some way observed results, could not be standardized. This is the case of some factors as well as: type of material used for vessel, the vessel position after flooding in respect of the bottom and in respect of main local currents, distance from the coastline, physical-chemical features of water column, local trophism. These factors could affect oxygenation rates, microbial dynamics and degradation rates of the vessel structure and consequently, pollutants released by cargo and vessels. It has been reported by the literature that the presence of artificial structure can affect currents, water fluxes, sediment resuspension, oxygen, and nutrients distribution (Ogawa and Kuroki, 1982; Lindquist and Pietrafesa, 1989). Upwelling processes could be also affected (Sheng, 2000), affecting secondary productivity (Genin et al., 1986; Leichter and Witman, 1997) and ecology of some species (Leichter and Witman, 1997). Effects on living communities and sediments could be observed closed to the structure (distance from wreck < 20 m) or larger (distance from wreck > 200 m) related to dimension and morphology of the shipwrecks (Ambrose and Anderson, 1990). Dimension and morphology of shipwrecks are not *a priori* standardizable. Concerning the type of material used for the vessel structure and the range of pollutants that could be associated to the shipwrecks, we decided to perform an indirect standardization by selecting WWII flooded vessels. In fact, recent materials and recent chemicals of some ecotoxicological concerns were of any concern at that time. The use of TBT as antifouling paint for vessels was not yet diffuses during WWII and we can assume with a certain confidence that this compound could not affect considered shipwrecks. Concerning biodiversity, ROV approach that is the only suitable in shipwrecks area for safety reasons, underestimates crypto-benthonic species (Andaloro et al., 2012; Consoli et al., 2015) as Gobiidae and Blennidae. Furthermore, underestimation of fish abundance can be also caused by light reduction and water turbidity (Bortone and Mille, 1999) that affect the ROV technique. As last remark, some of benthic taxa could not be identified by this approach due to the presence of microscopic differences among species that requires the direct sampling for laboratory analyses.

5. Conclusions

The results achieved underline that shipwrecks, despite the impact they have on the seabed in physical terms and in terms of chemical contaminants, with the passing of time following the sinking event, they become also a hotspots of biodiversity and a habitat for preservation strategies in marine ecosystems that need to be monitored.

Results of our study confirm that every shipwreck represents a specific example to analyze in order to improve the knowledge of their potential sink of hazard for the marine environment. This study provides information on shipwrecks regarding risk for impediment to navigation or they may reasonably be expected to result in major harmful consequences to the marine ecosystem, and, all data acquired can be used also to satisfy the general objectives of the Nairobi International Convention on the Removal of Wrecks, 2007. Moreover, the knowledge of the state of the wreck preservation, the depth and the characterization of the seabed and associated fauna may contributed to determine the threatened on maritime coastal including fishing activity, tourist attractions, health and conservation of marine living resources and to support management plans to prevent marine hazard.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.marpolbul.2017.06.084.

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