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Understanding the causes of mortality and contaminant loads of stranded cetacean species in Sardinian waters (Italy) using Bayesian Hierarchical Models

Maria Grazia Pennino [a,b,c,*], Marie-Christine Rufener [b,d], Joan Giménez [e], Fiammetta Berlinguer [f], Enrico Bollo [g], Simonetta Appino [f], Daniele Zucca [h], Giannina Chessa [i], Andrea Rotta [f]

[a] Instituto Español de Oceanografía (IEO, CSIC), Centro Oceanográfico de Vigo, Subida a Radio Faro, 50-52, 36390 Vigo, Pontevedra, Spain
[b] Statistical Modeling Ecology Group (SMEG), Spain
[c] Fishing Ecology Management and Economics (FEME) - Universidade Federal do Rio Grande do Norte – UFRN, Depto. de Ecologia, Natal, RN, Brazil
[d] Technical University of Denmark, Institute for Aquatic Resources, Kemitorvet, Building 201, 2800 Kgs. Lyngby, Denmark
[e] Institut de Ciències Del Mar (ICM-CSIC), Passeig Marítim de La Barceloneta 27-49, 08003 Barcelona, Spain
[f] Dipartimento di Medicina Veterinaria, Università degli Studi di Sassari, Via Vienna 2, 07100 Sassari, Italy
[g] Dipartimento Scienze Veterinarie, Università degli Studi di Torino, Largo Paolo Braccini 2, 10095 Grugliasco, Torino, Italy
[h] Institute for Animal Health, Veterinary School, University of Las Palmas de Gran Canaria, Canary Islands, Spain
[i] Istituto Zooprofilattico Sperimentale della Sardegna "G. Pegreffi", Via Duca degli Abruzzi, 8, 07100 Sassari, Italy

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ABSTRACT

Cetacean strandings represent unique opportunities to collect biological material from these wild animals and obtain information on their population statuses. Here, we apply biological and pathological perspectives to analyze stranded cetaceans collected along the Sardinian coast (Italy) between 2006 and 2011. We quali-quantitatively explore the primary causes of deaths, and use Bayesian Hierarchical Models (BHMs) to explore the potential effects of cetacean sex, age, body length, and month, year, and stranding location on Dicloro Difenil Tricloroetano (DDT) and Polychlorinated Biphenyl (PCB) contaminant loads. Although natural causes, such as bacterial and virus infections, were identified to be the main causes of death among the stranded cetaceans, fisheries also played an important role among the anthropogenic causes of death. The BHMs revealed that both contaminants were positively related to the length, sex and age of the cetaceans, and that higher concentrations of these contaminants were mainly found in larger and older individuals. Despite the scattered nature of these data, the present study contributes valuable insights into the major causes of death of stranded cetaceans, and adds to growing worldwide efforts to biomonitor cetaceans.

1. Introduction

Given that cetaceans have long lifespans and feed at high trophic levels, they are good sentinel species to monitor marine ecosystems (Moore, 2008; Bossart, 2011). That said, studying these animals is generally difficult given that they are highly mobile and spend most of their time either underwater or offshore, depending on the species. Therefore, despite the high potential they offer as sentinel species, their lifestyle causes some cetacean populations to remain unknown, and information about their distributions, abundance, feeding habitats, health, and contaminant loads, etc., is scattered.

Strandings occur when marine mammals, including cetaceans, either float ashore alive and become stranded in shallow waters, or die at sea and their carcasses are beach cast post-mortem. Strandings represent a unique opportunity to collect biological and pathological data from wild animals that would otherwise be impossible to obtain. Depending on the state of a given beach-cast animal carcass, it may, for example, be possible to assess whether the cause of death was natural or anthropogenic (Hernández-Mora et al., 2012). Given their wide habitat range, cetaceans are particularly vulnerable to anthropogenic threats, such as

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* Corresponding author at: Instituto Español de Oceanografía (IEO, CSIC), Centro Oceanográfico de Vigo, Subida a Radio Faro, 50-52, 36390 Vigo, Pontevedra, Spain.

E-mail address: grazia.pennino@ieo.es (M.G. Pennino).

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fishing activities (Pennino et al., 2015; Giménez et al., 2021), acoustic pollution (Weilgart, 2007), and maritime traffic (David, 2002; Pennino et al., 2016). Moreover, in recent years, new infectious diseases in cetaceans have been associated with high exposure to chemical pollutants (e.g., Jensen et al., 2010; Burge et al., 2014). An analysis of samples collected from stranded cetaceans can, therefore, help assess the impacts of detrimental activities, such as chemical use (Dierauf and Gulland, 2001). Additionally, stranding records can contribute toward improving our knowledge of various ecological aspects, such as the migratory range of these species, and to gathering information on changes in both population mortality patterns and age structure (Bogomolni et al., 2010).

Data analyses (i.e., spatio-temporal analysis, characterization of contaminant loads), of samples collected from stranded cetaceans have already provided insights into ocean health (Leeney et al., 2008; Lasek-Nesselquist et al., 2008). Nevertheless, despite the wide-ranging use and application of this type of data (i.e., location and cause of strandings), when it comes to untangling the causes and consequences of cetacean strandings these data are usually scarce and irregular (i.e., design-based surveys are hardly applied given the random nature of strandings). This lack of data is even more problematic when attempting to study the pathological causes of strandings, given that such studies require well-preserved carcasses. These circumstances impose considerable challenges when it comes to applying conventional statistical analyses, given that such analyses usually demand large sample sizes to enhance the power of statistical tests (Moore and Read, 2008; Krzywinski and Altman, 2013; McNeish, 2016). Consequently, when dealing with data-poor scenarios, such as information derived from stranded cetaceans, trustworthy estimates might come into question. Bayesian inference has been shown to be a natural choice when data are scarce, as it neither relies on large sample sizes, nor on specific design-based sample surveys and assumptions (Dennis, 1996). These kinds of models have provided satisfactory results due to their intuitive quantification of uncertainty (Rufener et al., 2017), thus they are potentially adequate when analyzing sparse data.

Here, we analyze the causes of death of cetaceans stranded off Sardinia Island (Italy) over a 6-year period (2006–2011). Strandings were recorded quali-quantitatively, and the causes of death were related to either natural or anthropological factors. In addition, to use all available information, we used Bayesian Hierarchical Models (BHMs) to infer the effects of persistent organic pollutants (POPs) on stranded individuals according to their age, sex, body length, time-period and spatial location.

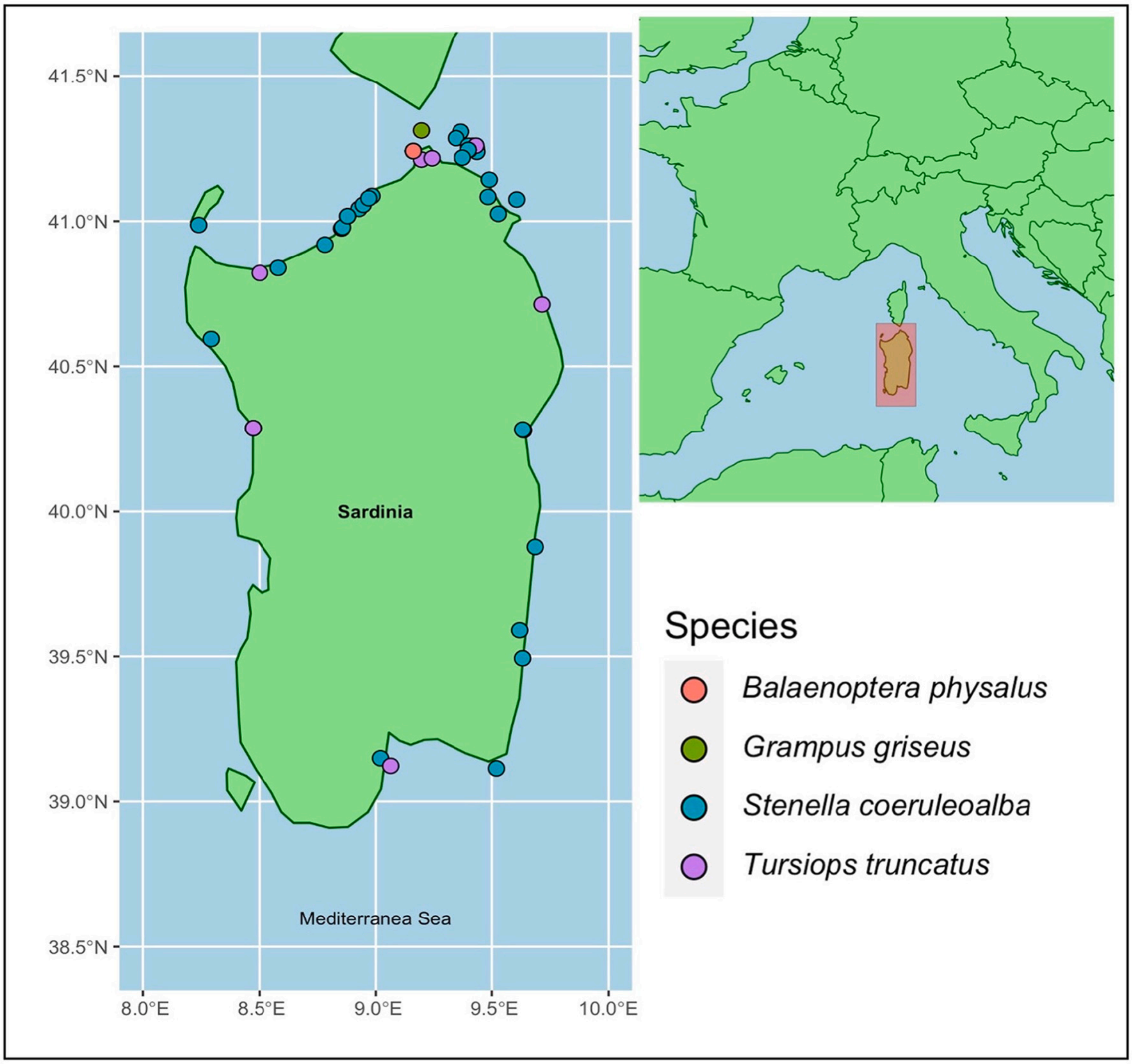

Fig. 1. Map of the study region, with Sardinia Island highlighted in red (left panel), and the cetacean stranding locations pointed out according to the identified species (right panel). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2. Material and methods

2.1. Study area

This study was carried out along the coastal waters of Sardinia Island (Italy), located in the western Mediterranean Sea (Fig. 1). The surface of the island is ~24,000 km^2 and its total coastal length is 1896 km, of which 24% (458 km) is composed of low, sandy or pebbly shores (Atzeni et al., 2000). Most of the coastal area is bordered by large and medium-sized cities, fisheries harbors and industrial areas. The entire coast is encompassed by important tourist destinations; thus, it is subject to considerable added human pressure during the summer. As a result, diverse human activities (i.e., fishery and marine traffic) cause current and potential threats to cetacean populations, such as changes their distributions and behavior (Pennino et al., 2015, 2016).

2.2. Sample collection

Strandings were attended to by experienced personnel from the Department of Veterinary Medicine at the University of Sassari, between the years of 2006 and 2011. Over this period, 48 strandings were recorded, and 27 necropsies were carried out on the stranded animals, all of which followed the standardized protocol proposed by Kuiken and García-Hartmann (1991). Based on body length and gonadal appearance (Geraci and Lounsbury, 1993), individuals were grouped into three age categories: young, sub-adult, and adult. Furthermore, stranded cetaceans were classified into one of five decomposition states, namely: very fresh, fresh, moderate autolysis, advanced autolysis, or very advanced autolysis (Kuiken and García-Hartmann, 1991).

The cause of death could only be determined on carcasses classified as either 'very fresh' or 'fresh' when a complete necropsy was possible. The causes of death were then classified as either natural or anthropogenic. In those cases where a necropsy was not possible, a gross description was used to categorize the cause of death as detailed as possible.

2.3. Laboratory processing

Tissue samples that were 10% buffered, formalin fixed, and paraffin embedded, were collected from all organs of necropsied subjects and submitted to routinary Haematoxylin-Eosin stain for histological examination. When these histological examinations found nervous tissue suggestive of demyelination, a Luxol Fast Blue (Luxol fast Blue-SIGMA Aldrich) stain was then performed to better characterize the cause of death.

To test the presence of cetacean morbillivirus (CeMV), an Immunohistochemistry (IHC) was performed using a monoclonal antibody directed against the NP protein of the Canine Distemper Virus (Onderstepoort strain). Luxol Fast Blue Stains was used to carry out IHC staining on central nervous system samples that showed mild demyelination, and to further test positivity for glial cells.

Finally, DDT (Dicloro Difenil Tricloroetano) and Polychlorinated Biphenyl (PCB) contaminants were extracted to test for contaminant loads. To that end, two different techniques were used following three consecutive steps: (1) extraction, (2) solid phase cleanup, and (3) instrumental analysis.

2.4. Extraction

In the first phase, organochlorine pesticide samples (1 g) were ground together with 4.5 g of Hydromatrix in a beaker and transferred into a 22 mL extraction cell. The extraction cell was placed in a Dionex ASE-200 and extracted in 1 cycle at 70 °C with petroleum ether-dichloromethane (2/1, *v*/v) at a pressure of 1500 psi. The heating time was set to 5 min, the static time was set to 2 min, the flush volume was set at 60%, and the purge time was set to 60 s. Extracts were collected in a 50 mL collection flask, then transferred via a funnel containing anhydrous sodium sulphate to a 150 mL evaporating flask concentrated with a rotary vacuum evaporator, until reaching roughly 1 mL.

Similarly, with respect to polychlorinated biphenyls samples, 1 g was spiked with 50 μL of surrogate standard solution (PCB29, PCB128) at 1 μg/mL and mixed with 4.5 g of Hydromatrix, and then transferred into a 22 mL extraction cell and extracted in Dionex ASE-200 in 1 cycle at 100 °C with hexane-acetone (80/20, *v*/v) at a pressure of 1500 psi. The heating time was set to 5 min, the static time was set to 2 min, the flush volume was set at 60%, and the purge time was set to 60 s. The extracts were collected in a 50 mL collection flask, then transferred via a funnel containing anhydrous sodium sulphate into a 150 mL evaporating flask concentrated with a rotary vacuum evaporator until reaching approximately 1 mL.

2.5. Solid phase cleanup

For organochlorine pesticides, the Florisil® cartridge (2 g) was conditioned with 4 mL of petroleum ether-dichloromethane (2/1, v/v) under high vacuum. The extract was loaded onto the conditioned cartridge and eluted with 12 mL of petroleum ether-dichloromethane (2/1, v/v) at approximately 1 drop per second. Eluted samples were dried with a gentle stream of nitrogen gas and reconstituted to 1 mL of isooctane.

For the polychlorinated biphenyls*, the* Florisil® cartridge (2 g) was conditioned with 4 mL of isooctane. This extract was then loaded onto the conditioned cartridge and eluted with 12 mL of isooctane at approximately 1 drop per second. Eluted samples were dried with a gentle stream of nitrogen gas and reconstituted to 1 mL of isooctane.

2.6. Instrumental analysis

Analyses were carried out on organochlorine pesticides with an Agilent Technologies 7890A Gas Chromatography system with a μECD electron capture detector and a 7693 series autosampler/injector. An Agilent HP5 column (length = 30 m, inside diameter 0.32 mm and film thickness 0.25 μm) was used. The injector and detector temperatures were held at 250 °C and 300 °C, respectively. The starting GC oven temperature was set at 80 °C for 1 min, then increased at increments of 30 °C/min until reaching 190 °C and, in the second step, at increments of 6 °C/min until reaching 300 °C and maintained for 4 min. Ultrapure grade Helium (purity 99.999%) with a constant flow rate of 1.2 mL/min was used as a carrier gas. 1.0 μL of the sample was injected by autosampler in the spitless mode. All quantifications were performed using an internal standard (PCB100).

Tissues were analyzed for the polychlorinated biphenyls using an Agilent Technologies 6890 N gas chromatography system coupled with an Agilent Technologies 5975 mass selective detector and a 7683B series autosampler/injector. A capillary column Agilent HP5 (length = 30 m, inside diameter 0.32 mm and film thickness 0.25 μm) was used. The injector temperature was 250 °C. Helium was used as a carrier gas, with a flow rate 1.0 μL of the sample and injected by autosampler into the splitless mode. The GC oven temperature was set to 80 °C at the start, for 1 min, then increased at increments of 30 °C/min until reaching 190 °C and, in the second step, at increments of 6 °C/min until reaching 300 °C, and maintained for 4 min. The mass spectrometer settings used were: ionization energy, 70 eV and ion source temperature 280 °C. The analyses were operated in SIM mode. All quantifications were performed using an internal standard (PCB100) and two surrogate compounds (PCB 29, PCB 128).

2.7. Statistical analysis

PCB and DDT contaminants were extracted and quantified in 22 of the 48 stranded cetaceans, consisting of 20 striped dolphins (*Stenella* *coeruleoalba*) and 2 bottlenose dolphins (*Tursiops truncatus*). Statistical analysis was based on these 22 individuals.

To evaluate the effects of spatio-temporal and demographic characteristics on the concentration of both contaminants, Bayesian Hierarchical models (BHMs) were used. Similar to a Generalized Linear Model (GLM), the response variable, Y_i, in BHMs may be any member of the exponential probability distribution family with a mean $\mu_i = E(Y_i)$ linked to a structured additive predictor η through a link function $g(\cdot)$, such that $g(\mu_i) = \eta_i$:

$$Y_i \sim N(\mu_i, \tau)$$

$$g(\mu_i) = \eta_i = \beta_0 + \Sigma^{M}{}_{m=1}\, \beta_m\, X_{mi};\, i = 1, \ldots, n$$

where Y is the response variable for either PCB or DDT contaminants for each individual i following a log-normal distribution; τ is the precision parameter (inverse of the variance); η_i is the linear predictor for either PCB or DDT contaminants; β_0 is a scalar representing the intercept; and β_m is the coefficient vector quantifying the effect of some predictors X_m on the response variable.

Overall, seven predictors were considered, among which three corresponded to demographic characteristics (sex, length, and age), and four to spatio-temporal characteristics (longitude, latitude, month, and year) (Table 1). It is worth noting that the spatial coordinates refer to the stranding location and are only spatial proxies of where contaminants could be accumulated. Predictor variables were evaluated using both forward and backward approaches.

To compare the goodness-of-fit of each model, three different measures were computed: (1) the Watanabe-Akaike information criterion (WAIC), (2) the Root Mean Square Error (RMSE), and (3) the adjusted coefficient of determination (R^2). WAIC can be considered an improvement of the Deviance Information Criterion (DIC) and is better suited than the Akaike Information Criterion (AIC), which is usually used within frequentist modeling approaches (Spiegelhalter et al., 2002). Unlike the DIC, which is conditioned on a point estimate and is not fully Bayesian, the WAIC is a fully Bayesian measure and uses the entire posterior distribution to carry out inferenced on the parameters; hence, estimations are more precise (Watanabe, 2010). The RMSE, in turn, consists of the standard deviation of the residuals and, thus, measures how much the observed values deviate from the predicted values. The R^2, on the other hand, expresses the percentage of variability in the response variable that was explained by the model.

Standard graphical checks of the model's residuals were also done. Whereas residual normality was evaluated by means of Quantile-Quantile plots, homogeneity was assessed through a residual vs. predicted plot. Moreover, as linearity is expected between the observed and predicted values, the Pearson's correlation coefficient (ρ) was used to validate the models. Thus, the best (and most parsimonious) model was chosen based on the compromise between low WAIC values, low RMSE values, high R^2 values, and a significant Pearson coefficient (*p*-value $\leq$0.05).

Table 1
Summary of the explanatory variables considered in the HBMs.

Characteristics	Explanatory variables	Description	Units
Demographic	Sex	Sex of the individual (categorical)	Female, Male
	Length	Length of the individual (continuous)	cm
	Age	Age of the individual (categorical)	Adult, sub-adult, young
Spatial	Longitude	Longitude at which stranding was recorded (continuous)	Decimal degrees
	Latitude	Latitude at which stranding was recorded (continuous)	Decimal degrees
Temporal	Month	Month at which stranding was recorded (categorical)	January–December[a]
	Year	Year at which stranding was recorded (categorical)	2006–2009[a]

[a] None of the 22 cetaceans considered were recorded in June and November. Additionally, none were recorded between the years 2010 and 2011.

Given the use of Bayesian inference, it should be noted that parameter estimates were achieved through marginal posterior distributions by means of the Integrated Nested Laplace Approximation (INLA) methodology (Rue et al., 2009) and its respective package (http://www.r-inla.org) implemented in the R software (R Core Team Development, 2017). Within INLA, posterior distributions are approximated numerically through the Laplace operator, unlike conventional Markov Chain Monte Carlo (MCMC) techniques that rely on stochastic simulations (Rufener et al., 2017). For complex and highly dimensional latent Gaussian models, such as those used herein, such a framework provides much faster and more accurate parameter estimates, and avoids the convergence issues that typically underlay MCMC (Rue et al., 2009). Although conventional convergence checks do not apply within the INLA framework, the accuracy of the Laplace approximation can be evaluated through the Kullback-Leider divergence value (kld). The smaller the kld value, the better, and it should ideally converge to the value of zero (Wang et al., 2018).

Given that no previous information on the model's parameters were available at the time this study was conducted, default Gaussian priors with a mean of 0 and a variance of 100 were assigned for all fixed-effect parameters, following recommendations from Held et al. (2010). Gaussian priors represent approximations to non-informative priors that are designed to have little influence on the posterior distribution and are, thus, akin to the frequentist approach.

3. Results

3.1. Descriptive results

Of the 48 cetaceans included in this study, 97.92% ($n = 47$) belonged to the odontocete species, among which 76.59% ($n = 36$) were striped dolphins (*S. coeruleoalba*), 21.27% ($n = 10$) were bottlenose dolphins (*T. truncatus*), and 2.13% ($n = 1$) was a 147 cm long young female Risso's dolphin (*Grampus griseus*). Only one individual belonged to the mysticete group, namely a 1078 cm sub-adult female fin whale (*Balaenoptera physalus*). With respect to the demographic characteristics, the sample set included 21 males (7 youngs, 7 sub-adults, and 7 adults), 22 females (4 youngs, 4 sub-adults, and 14 adults), and 5 animals of undetermined sex (1 youngs and 4 adults) (Table S1 in the Supplementary material). The body length of the bottlenose dolphins ranged between 90 and 335 cm (mean = 200 ± 79.41 cm), whereas for the striped dolphins it varied between 90 and 220 cm (mean = 163.19 ± 44.28 cm).

With respect to the temporal variability, our results revealed that the highest number of strandings was recorded in February ($n = 8$), April ($n = 6$), and October ($n = 6$), and the lowest number of strandings was recorded in July ($n = 1$) and December ($n = 1$) (Fig. 2A). Furthermore, over the 6-year sample period, the highest number of strandings was registered in 2007 ($n = 14$), followed by 2008 ($n = 13$), whereas the lowest number of strandings was recorded in 2006 ($n = 2$) (Fig. 2B). With respect to spatial variability, most strandings occurred in the northern areas of Sardinia, especially along the coastal areas of the Maddalena Archipelago (Fig. 1).

3.2. Causes of death

It was only possible to conduct full necropsies on 27 of the 48 stranded cetaceans, and elaborate detailed descriptions of the possible causes of death for only 26 individuals. It is important to note that the

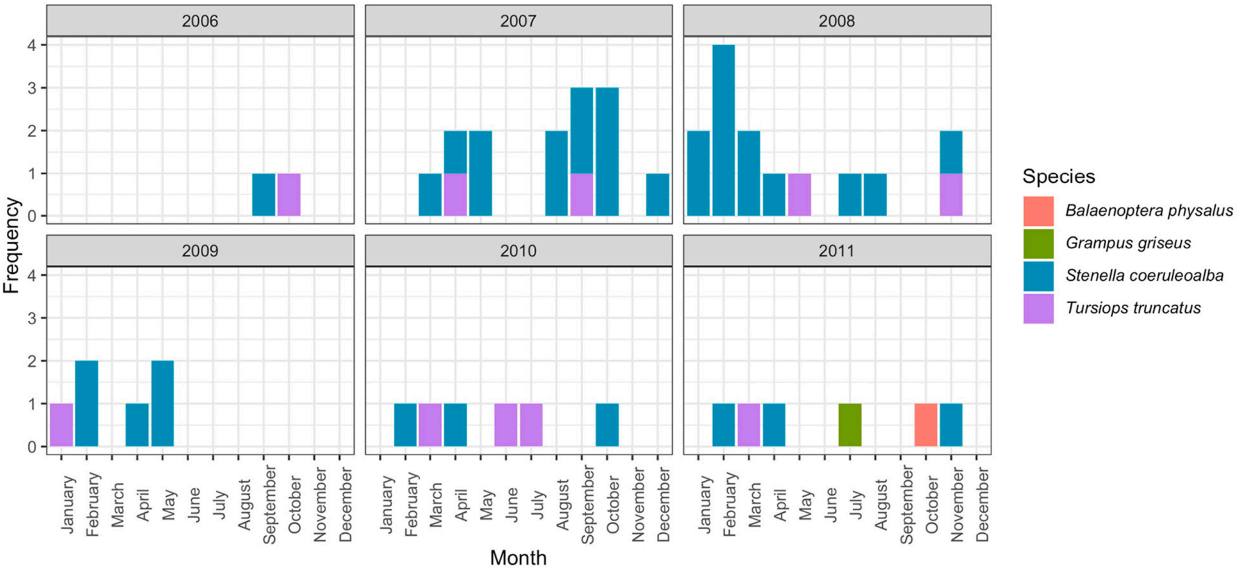

Fig. 2. Number of recorded strandings on Sardinia Island, by month (A) and year (B).

evidence only points to possible causes of death, and it is not possible to confirm with absolute certainty that these were, in fact, the final causes of death. Likely natural causes of mortality were identified for 16 of the 27 dolphins, i.e., the majority of all identified causes of death (59.26%). Among them, the most frequent diseases identified were associated with bacterial pneumonia and sepsis/bacteremia secondary to pyoderma (29.63%, $n = 8$). Parasitic infections were also recurrent. Specifically, three individuals (11.11%, 2 striped and 1 bottlenose dolphin) were infected with *Anisakis* sp. and *Skrjabinalius* spp., and two striped dolphins (7.41%), which presented mild demyelination through LUXOL FAST BLUE STAIN, tested positive for cetacean morbillivirus (CeMV) IHC staining in the glial cells. Lastly, a moderate degree of neuron necrosis with hypoxia and brain edema was detected in 2 bottlenose dolphins (7.41%), and one individual striped dolphin presented verminous gastritis.

Anthropogenic interactions were the most probable causes of mortality in 10 of the 27 dolphins that underwent full necropsies (37.04%). The most common human interaction found to affect cetaceans was entanglement in fishing nets (18.52%, $n = 5$), specifically four individual bottlenose dolphins and one striped dolphin were found entangled in monofilament nets. Additionally, four striped dolphins were found to have traumatic lesions and fractures of the cranium, possibly due to vessel collisions. Finally, the death of one striped dolphin was found to be potentially related to the ingestion of a plastic bag.

3.3. *Persistent organic pollutant (POP) effects on demographic and spatio-temporal characteristics*

PCB and DDT contaminants could be extracted and quantified among 22 of the 48 stranded cetaceans, among which 20 individuals were striped dolphins (*S. coeruleoalba*) and 2 were bottlenose dolphins (*T. truncatus*).

The recorded concentrations of PCB ranged from 0.59 to 75.85 mg/kg, whereas DDT ranged from 0.45 to 79.58 mg/Kg.

Several BHMs were tested to unravel the contaminant load variability across demographic and spatio-temporal predictors. As shown in Table 2, the final model selected for both response variables (i.e., PCB and DDT) agreed on the same combination of predictor variables, whereas the relevant predictors in the best and most parsimonious model included cetacean length and the interaction term between sex and age.

Table 2
Model comparison of the most relevant tested models for both PCB and DDT contaminants.

Model	PCB			DDT		
	WAIC	R^2	RMSE	WAIC	R^2	RMSE
1 + Length + Sex[a] + Age[a] + Long + Lat + Month[a] + Year[a]	75.64	0.86	0.40	75.34	0.88	0.40
1 + Length + Sex + Age + Long + Lat + Month	88.58	0.57	0.71	91.16	0.58	0.75
1 + Length + Sex + Age + Long + Lat	72.38	0.38	0.86	76.36	0.36	0.93
1 + Length + Sex + Age + Long:Lat	72.51	0.38	0.85	76.48	0.36	0.93
1 + Length + Sex + Age + Long	70.90	0.36	0.87	74.80	0.35	0.94
1 + Length + Sex + Age	69.20	0.32	0.89	73.71	0.29	0.98
1 + Length + Sex:Age	**70.29**	**0.40**	**0.84**	**71.65**	**0.45**	**0.87**
1 + Length + Sex	71.36	0.09	1.04	74.93	0.07	1.12
1 + Length	69.72	0.07	1.05	73.14	0.05	1.13

The selected model is highlighted in bold (Long = Longitude; Lat = Latitude). The best (and most parsimonious) model was chosen based on the compromise between low WAIC values, low RMSE values, high R^2 values, and significant Pearson coefficient (p-value ≤ 0.05).

[a] Reference level for categorical variables: Sex (female), Age (adult), Month (April), Year (2006).

For PCB, only length was a relevant predictor and was found to be positively related to cetacean size (posterior mean = 1.8, $CI_{95\%} = [0.38, 3.29]$). This means that PCB concentrations increase as the animals grow in size. The remaining predictors, i.e., sex, age, and the interaction term between sex and age, were not found to be relevant, given that the 95% credible interval covered the zero value entirely (Fig. 3). With respect to the DDT model, slightly different contributions of each predictor variable were found compared to the PCB model. Whereas length and the interaction term between age and sex were found to be relevant variables with respect to explaining the DDT concentration variability, neither sex, nor age on their own were found to be relevant variables. Similar to PCB, length was also positively related to DDT (posterior mean = 1.81, $CI_{95\%} = [0.30, 3.31]$), meaning that larger animals had higher DDT concentrations. With respect to the interaction term, higher estimated DDT concentrations were found to occur in younger male individuals compared to the reference level (adult females) (sub-adult male posterior mean = 0.88, $CI_{95\%} = [-1.95, 3.70]$; young male

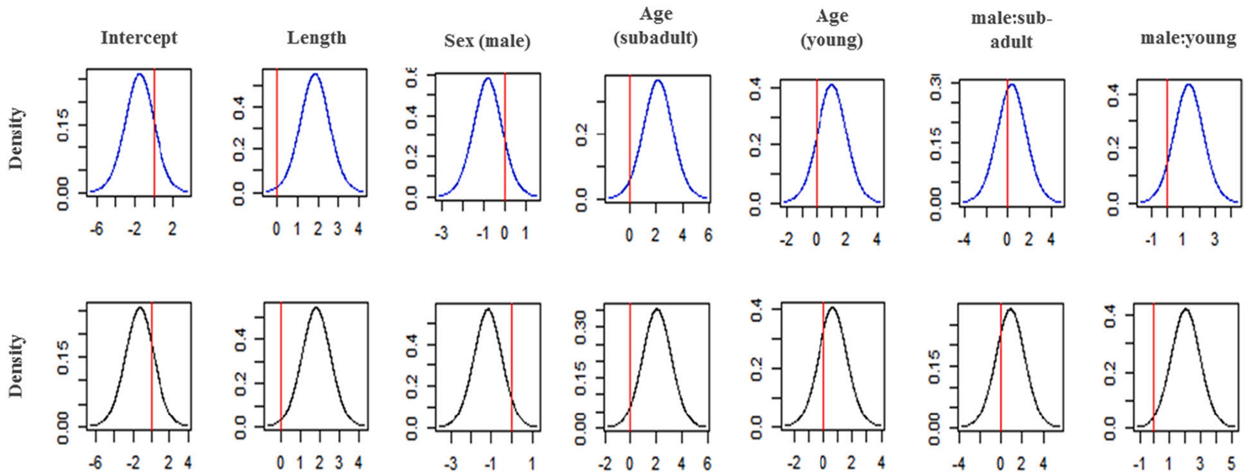

Fig. 3. Graphical summary of the marginal posterior distributions of the parameters, where the upper (blue curves) and lower (black curves) panels refer to the PCB and DDT models, respectively. Note that the vertical red line at zero delimits the hypothesis of $\Theta = 0$, where Θ is the evaluated parameter. In Bayesian inference, if 95% of the density distribution is concentrated far from this line, then one has enough evidence that $\Theta \neq 0$, i.e., the evaluated parameter is statistically relevant. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

posterior mean = 2.04, $CI_{95\%}$ = [0.11, 3.96]) (Fig. 3). This means that young and sub-adult males presented higher DDT concentrations than adult females.

Despite the small sample sizes, the Q-Q plots showed a reasonably normal distribution for the residuals of both selected models. For all models, kld values converged to zero, indicating that the models converged.

Furthermore, the predicted versus observed values were positively and significantly correlated in both the PCB and DDT models, indicating, therefore, that they are suitable to explain the mean tendencies of each response variable.

4. Discussion

In this study, the primary causes of death of cetaceans stranded in Sardinia Island waters between 2006 and 2011 were identified. It is possible to assert that the anatomo-pathological results, such as the presence of mild lesions caused by the morbillivirus, the high evidence of parasitic lesions, and the presence of traumatic lesions, together with the presence of pollutants, are signs of overall environmental degradation. In this study, the causes of death were found to come from both anthropogenic and natural causes, the latter of which are seemingly the main reasons for cetacean strandings. Both bacterial and viral infections were found to be the main agents responsible for natural deaths among the cetaceans, causes that have also commonly been reported among cetacean populations elsewhere (Bogomolni et al., 2010). Here, death causes from these diseases were found to have affected sub-adults more than adults, and no records were found among young individuals. Nevertheless, it is not clear whether this difference is related to the development stage of the immune system (i.e., sub-adults could have slower immune responses than adults), or whether heavily parasitized young individuals died at sea and were not stranded.

Parasites identified in these cases included *Anisakis* sp. and *Skrjabinalius* spp. Identification of these parasites to the genus level has resulted in a better understanding of both the presence and extent of infections, however, further studies are needed to improve identification at the species level. The Cetacean Morbillivirus (CeMV), specifically, is considered to be the most pathogenic virus among cetaceans (Bellière et al., 2011) and over the period 2006–2008 there had been a Dolphin Morbillivirus (DMV) epidemic among Mediterranean striped dolphins (Di Guardo et al., 2013). Although this period had the highest mortality rate, only two striped dolphin individuals were found to have been affected by this disease in the present study. DMV appears to be circulating in the Western Mediterranean Sea in cyclic epidemics (Di Guardo and Mazzariol, 2013) and individuals with higher pollutant levels are more susceptible to this virus due to immunosuppression caused by high pollutant levels (Aguilar and Borrell, 1994; Di Guardo and Mazzariol, 2013).

With respect to anthropogenic causes of death, fishery interactions were among the most frequent, especially for bottlenose dolphins. This fact is likely due to the coastal home range of this species, which exposes them to fishing activities. In fact, several studies have already identified this type of interaction in both the Northern Sardinia area (Pennino et al., 2013, 2015) and other western Mediterranean areas, such as the Catalan coast (Cuvertoret-Sanz et al., 2020). Fishing interactions, together with prey depletion due to fisheries, is one of the major threats facing cetaceans today. Several populations have declined due to unsustainable bycatch in fishing gears. The high bycatch rate in the New Zealand gillnet fisheries, for example, has provoked a drastic population decline of Māui dolphins (*Cephalorhynchus hectori maui*) to only a few animals (55 individuals, 95% CI 48–69; Hamner et al., 2014). Similarly, the vaquita (*Phocoena sinus*) population from the Gulf of California (Mexico) has nearly been driven to extinction by illegal gillnet fisheries that target totoaba (*Totoaba macdonaldi*) but have high bycatch rates of vaquita (Jaramillo-Legorreta et al., 2019).

Moreover, in the present study, traumatic lesions and cranium fractures were the second highest cause of mortality among the reported strandings. As a result of the introduction of high-speed ferries in Sardinian waters, maritime traffic has significantly increased over the past decades. This has contributed to a higher number of collisions with cetaceans, which are particularly at risk as some of the main maritime transportation routes traverse areas that overlap with the habitat of cetaceans (Pennino et al., 2016). Laist et al. (2001) have previously pointed out that the majority of cetaceans affected by vessel collisions are calves or juveniles, as they spend more time at the surface compared to adults, who spend more time feeding at depth, and/or have more ability to avoid collisions. Similar results were also found in the present study, where 3 out of 4 striped dolphins that died in ship collisions were sub-adults.

Among the stranded cetaceans, a striped dolphin appeared to have died from ingesting a plastic bag. Though most interactions among debris and marine mammals are related to entanglement, there are some sparse records of cetacean deaths related to the ingestion of debris (Denuncio et al., 2011; De Stephanis et al., 2013). Notwithstanding, it is possible to mitigate both the entanglement and ingestion of debris among cetaceans by establishing community education programs, proper management of old fishing gears, and diversification of artisanal fisheries into other lucrative and more sustainable activities (e.g., dolphin-watching).

Additionally, more formal policy and management actions may be needed to decrease fishing-gear and marine traffic interactions in this area, and in particular the Northern Sardinia waters included in the Pelagos Sanctuary. The Pelagos sanctuary is sometimes considered to be a “park on paper”, as it fails to provide real protection for the cetaceans that inhabit those waters (Notarbartolo di Sciara et al., 2008). In fact, results of this study indicate that anthropogenic activities have been causing cetacean deaths in the southernmost limit of the sanctuary. It should be stressed, however, that the total number of strandings that this study identified to be associated with human activities in Sardinian waters is probably underestimated, given that several previous studies have reported higher cetacean interactions with different anthropogenic activities in the same area (Pennino et al., 2013, 2015, 2016). A detailed drift model of cetacean carcasses like the one carried out in Atlantic French waters (Peltier et al., 2012, 2014) would help to determine the proportion of dead animals arriving to the coast. In addition, this kind of model can determine the precedence of bycatch individuals, which could help determine high risk areas at sea and whether individuals living in different areas present dissimilar amounts of contaminant loads.

With respect to POP contaminants, positive relationships between cetacean body length and PCB and DDT concentrations were found. Similar results were also reported for striped dolphins, pilot whales, harbor porpoises, and common and bottlenose dolphins (e.g., Pompe-Gotal et al., 2009; García-Alvarez et al., 2015), among other cetacean species. The use of the body length can be used as a proxy of the age, so longer individulas correspond to older individuals, which, in turn, bioaccumulate higher concentrations of contaminants in their bodies (e.g., Aguilar et al., 1999).

Contamination loads are often found to be different between male and female cetaceans (e.g., Borrell, 1993; Tanabe et al., 1986). In this study, the interaction between sex and age was only found to be relevant for DDT concentration levels. Specifically, the findings of this study revealed young and sub-adult males to have higher DDT concentrations than adult females. These differences could be due to the fact that organochlorine loads in cetaceans tend to increase with age during the juvenile stage, both in males and females, given that the uptake of contaminants usually exceeds metabolization and excretion. However, in adult males, this trend continues and their contaminant levels increase with age, whereas in sexually mature females, concentrations often decrease (Aguilar and Borrell, 1988) because they transfer appreciable quantities of these pollutants to their offspring during pregnancy and, to a larger extent, during lactation (Borrell, 1993; Aguilar et al., 1999).

5. Conclusion

Categorizing the causes of death is a valuable tool to monitor the mortality trends of cetacean species. Despite the small sample size, this study contributes to a growing worldwide effort to biomonitor cetaceans, not only to assess the health status of these animals, but also to determine anthropogenic impacts on them. In this study, Bayesian inference permitted that precise information on contaminant accumulation be gathered using only a few stranded animals that were collected in the area over a prolonged time-period. Despite the limited number of individuals used to perform the statistical analysis, the Bayesian approach provided both results that are consistent with other similar studies, and good performing models, despite limited information. This highlights the utility of the Bayesian approach when dealing with data-limited areas. Nevertheless, we encourage the establishment and the financial maintenance of stranding networks not only in Sardinia Island, but Mediterranean-wide. Long-term monitoring of cetacean death causes and contaminant loads is essential to detecting changes in the marine environment, given that cetaceans can act as sentinel species of ocean heath (Bossart, 2006). Thus, systematic biomonitoring of cetaceans, together with standardized necropsy and disease protocols, should be centralized and continued, in order to improve our knowledge of the threats facing cetaceans and, consequently, improve their conservation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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