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Master in Terrestrial Ecology and Biodiversity Management

Universitat Autònoma de Barcelona

**Disentangling the main causes and potential origin of
an unusual razorbill (*Alca torda*) die-off in the
Mediterranean Sea: an interdisciplinary approach**

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Joan Navarro Supervisor (ICM)	Sara Dallarés Supervisor (UAB)	Anna Soler Academic mentor (UAB)

This study was conducted from February to September 2023 in the Institut de Ciències del Mar (ICM - CSIC) and in the Faculty of Veterinary Medicine of the UAB (Universitat Autònoma de Barcelona). The manuscript has been written following the author guidelines of the journal Aquatic Conservation: Marine and Freshwater Ecosystems.

The student, Ángela Loring Salmerón, has contributed to the following aspects of the study:

Components of the study	Student contribution
Project design	B
Information gathering	A
Anatomopathological study	A
Sample processing	A
Data analysis	B
Graphical work	B
Writing	A

A, entirely performed by the student; B, partially performed by the student; C, entirely performed by others.

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Abstract:

An unprecedented wreck of razorbills (*Alca torda*) occurred along the Mediterranean Sea during winter 2022-2023. To determine the potential causes of death and origin of these birds, 46 dead razorbills collected on the coast of Malaga (Spain) were analyzed. Necropsies revealed poor body condition and starvation signs in most birds. Influenza virus was excluded through PCR testing. However, a high prevalence (58%) of gastrointestinal nematodes was found, primarily *Contracaecum variegatum*, associated with esophageal and proventricular ulcers in 60% of razorbills. Stable isotope analysis of feathers showed that most of the birds likely were from North Sea colonies, although 33% of the individuals presumably were from other breeding locations. Persistent Atlantic storms likely triggered this wreck by impeding foraging and forcing migration of weakened razorbills. Although starvation induced by harsh weather seemed to be the primary cause of mortality, the high parasite loads and associated digestive ulcers found in most razorbills may have acted synergistically to further weaken these already emaciated birds. The interplay of these factors offers a better understanding of the multifaceted drivers of mass seabird wreck events.

Keywords: *Contracaecum*, migration, origin, razorbill, stable isotopes, starvation, ulcers, wreck.

1. Introduction

Over the past few decades, the increase in frequency of extreme climate events has profoundly impacted certain species and ecosystems that are particularly susceptible to climate change, especially those with limited capacity for recovery or adaptation (Pörtner et al., 2022). These extreme weather events include drastic temperature variations, strong winds, heavy rains, large wave cyclones or marine heatwaves, and significantly alter ecosystem structure, phenology and species distributions (Piatt et al., 2020).

Within the marine ecosystem, predators such as seabirds are one of the most affected organisms by these extreme weather events (Dias et al., 2019; Orgeret et al., 2022). They play a crucial role as environmental indicators due to their capacity to reflect conditions over extensive spatial and temporal scales at a population level (Furnes et al., 1997). However, our understanding of vital rates of these marine species, such as survival and recruitment probabilities, remains limited, primarily due to their extended periods of immaturity during which they spend considerable time at sea before returning to breeding colonies (Croxall, 1991). It is important to note that the responses of seabirds to weather events can have profound impacts on their population dynamics (Votier et al., 2008).

Mass mortality events, usually known as "wrecks", are episodes during which many dead, injured or exhausted seabirds are washed out on coasts with no apparent cause (Glencross et al., 2021). Accurately assessing the magnitude of these mortality events poses challenges since the individuals that can be counted represent only a fraction of the total because a significant number of individuals perish at sea (Votier et al., 2005; Mesquita et al., 2015; Morley et al., 2016). In many cases, immature seabirds are usually the most affected age-group due to their inexperience during

the post-breeding dispersion (Votier et al., 2008; Lavers et al., 2020). In the absence of apparent pathologies, wreck events over a short period of time involving weak and dying animals are mainly attributed to starvation, which can be a consequence of various factors, including unusual severe weather conditions that hinder foraging, changes in distribution or abundance of prey, anomalous oceanographic conditions, or a combination of these factors (Piatt et al., 1997).

Auks (family Alcidae) are the seabirds most affected by these massive wrecks. These species with narrow thermal ranges are highly exposed to adverse weather events as they breed in northern circumpolar latitudes where storms, cyclones and climate variations caused by climate change are more notorious (Clairbaux et al., 2021; Orgeret et al., 2022). In the North Atlantic Ocean, particularly during winter months, how these wreck events affect particular species, such as the razorbill (*Alca torda*) or Atlantic Puffins (*Fratercula arctica*), is well-documented (Underwood & Stowe, 1984; Tasker et al., 1994; Camphuysen et al., 1999; Heubeck et al., 2009; Morley et al., 2016; Anker-Nilssen et al., 2017). However, it is typically challenging to attribute these mass mortality events to a single cause, and it is crucial to exclude other factors such as infectious diseases like the avian influenza virus. Indeed, 2022 and 2023 were two devastating years for many wild bird species in the northern hemisphere due to a highly pathogenic strain of this virus (European Food Safety Authority, 2022).

Razorbills are distributed through boreal and low-arctic Atlantic waters, with colonies being found from the eastern coast of North America and Canada to northern Europe and northwestern Russia (Figure 1), with approximately a total of 838,000-1,660,000 breeding individuals. The vast majority of the world's population breeds in Europe, where Iceland's breeding colonies represent approximately 41% of the total population, followed by the UK, Ireland, Sweden and Finland (BirdLife International, 2021). As a migratory seabird, routes followed by razorbills often span

unsuitable habitats for feeding, so they present fat stores to provide energy for long-distance flights (Lavers et al., 2020). Shortly after migration, birds with depleted fat stores are more vulnerable to environmental stresses and, consequently, could die in their winter home range outside of the breeding season (Gwinner et al., 2012). Although razorbills are reported as migrant species in the Mediterranean Sea (De Juana & García, 2015), their wintering population numbers are comparatively much lower than on the Atlantic coasts (Carboneras, 1988; De la Cruz et al., 2022). Records of dead individuals along the Mediterranean coast are generally considered local incidents at a small scale, frequently associated with bycatch (Espí et al., 2012), although numbers of affected individuals stay uncertain (Belda & Sanche, 2001; Cooper et al., 2003; Karris et al., 2016).

In the winter of 2022, an unprecedented large number of razorbills arrived to the Mediterranean Sea, involving reports of both alive and dead individuals on all Mediterranean coasts. Between November 2022 and January 2023 hundreds of razorbills were spotted close to the coast and in ports in different countries such as Spain, France, Italy, Greece or Tunisia, as well as in Croatia where it was the first record for this species. This recent event was mainly recorded by citizens and ornithological organizations, according to which its leading cause seems to be persistent Atlantic storms which pushed razorbills considerably further south and east off the British Islands (Balestrieri et al., 2023) but no in-depth studies were carried out to discard other potential causes or the exact origin of the dead razorbills affected in this remarkable incident. Therefore, further studies are needed.

In the present study, we aimed to unravel this wreck's leading factors and identify the potential origin of the razorbills found in the western Mediterranean Sea during the winter of 2022-2023. To address this research question, we embraced an interdisciplinary approach, including epidemiology (specifically, avian influenza virus testing and the identification and quantification

of gastrointestinal parasites), pathology (encompassing necropsies and histological examination of lesions) and ecology (covering trophic ecology, human interactions, and stable isotopes analysis). This comprehensive approach was applied to a sample of 46 razorbills collected in the southeastern Iberian Peninsula, specifically along the Málaga coast within the Alboran Sea.

2. Materials and methods

2.1. Sampling and necropsy procedures

Forty-six dead razorbills were collected from the coasts of Málaga (Alboran Sea; western Mediterranean Sea) between the end of November and the beginning of December of 2022 (Figure 1). Only fresh individuals were collected, discarding the most decomposed or depredated ones. All bodies were frozen at -20° C until necropsies and further analyses were done.

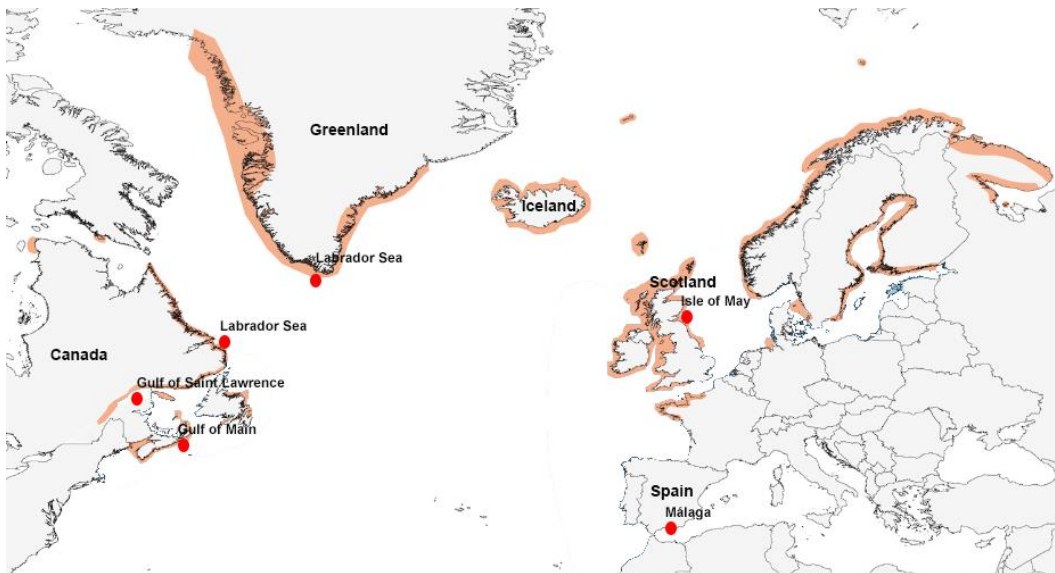


Figure 1. Breeding distribution map (in orange) of razorbills (*Alca torda*) (based on Lavers et al., 2020). The locations of the colonies used to infer the origin of the individuals collected for this

study in November/December 2022 (Málaga, Spain) based on the stable isotopic information are also indicated.

Avian influenza was tested prior to necropsies by analysing cloacal swabs of each individual. A reverse transcription-polymerase chain reaction (RT-PCR) for an influenza virus matrix gene-specific PCR primer set designed for a region conserved in all type A influenza virus matrix genes was used (Spackman et al., 2002), a highly sensitive and specific molecular technique. PCR analyses were conducted in the Animal Health Research Centre (CReSA) in Bellaterra, Spain.

Before carrying out the necropsies, the total head length (including the bill), bill length, maximum and minimum bill depth and right tarsus length were measured using a digital calliper (± 0.01 mm). The right-wing length was measured with a metal ruler (± 0.1 mm) (Supplementary, Figure 1). Total body weight was not reliable due to the amount of sand and water present in the body of the razorbills; therefore, it was not recorded. Bill development was used as the main criterion to determine the age of the individuals (see Supplementary, Figure 2), followed by gonads development and the presence and size of the bursa of Fabricius. Sex was determined based on direct observation of their gonads during necropsy, when possible (Camphuysen, 1995; Van Franeker, 1983). In addition, feather samples from the cheek of each individual were collected and stored in dry paper envelopes (Glew et al., 2018) for further stable isotope analyses.

Necropsies were performed systematically following Van Franeker (2004) protocol modified for this study (Supplementary, Necropsy form). Briefly, this protocol included instructions for the determination of the moult status, biometric measures, weight, age, sex, and measurements of sexual organs and bursa of Fabricius. In addition, a macroscopic evaluation of the overall physical and organ status was conducted to identify possible gross signs of the cause of death, such as

hemorrhages, traumas or drowning signs. Also, the corporal condition of each individual was scored following a four-point scale (0-3) for each of the three following parameters: the presence and quantity of subcutaneous and abdominal fat, and the volume of the pectoral muscle, following Van Franeker (1983). The sum of these three scores yielded an overall corporal condition index (CCN) ranging between 0 and 9; correspondence between scores and body condition categories was as follows: 0-1: mortally emaciated; 2-3: critically emaciated; 4-6: moderate body condition; and 7-9: good body condition (Supplementary, Figure 3).

The complete gastrointestinal tract (from the pharynx to the cloaca when possible) was extracted from forty-five individuals (one was too autolytic) and frozen until content examination. For future studies, tissue samples of muscle, heart, lung, spleen, kidney and liver of each individual were also collected and frozen.

2.2. Gastrointestinal analysis

After thawing gastrointestinal tracts, the esophagus and stomachs of each individual were opened with a longitudinal incision, and their contents were examined for remains of prey, parasites, pathological signs, and macroplastics (>0.5 cm) and fishing gears as signs of human interactions.

The otoliths found in the stomachs were cleaned with distilled water, measured and photographed for species identification using AFORO database (Tuset et al., 2008; <http://aforo.cmima.csic.es/>).

Gastrointestinal parasites (nematodes) were collected and washed in physiological saline solution before being stored in 70% ethanol. Then, some representatives of each distinct morphological types were selected and gradually cleared through a graded glycerin series and mounted in pure glycerin for the species identification by light microscopy using a Leica DM 2000 (Wetzlar,

Germany) microscope. Identification was performed following specialized taxonomic keys and morphological descriptions based on the morphology and size of cephalic structures (lips), deirids or male spicules (Nagasawa et al., 1999; Anderson et al., 2009; Mutafovchiev et al., 2010).

Any pathological sign on the digestive tract was annotated and photographed. Ulcerative lesions in the mucosa of the esophagus or proventriculus were classified into four categories attending to the longitudinal extent of the surface damage: 0 for no lesions present, 1 for one small lesion (<0.5 cm), 2 for one medium (0.5-1 cm) or multiple lesions, and 3 for big or deep ulcers (>1 cm) (Figure 2).

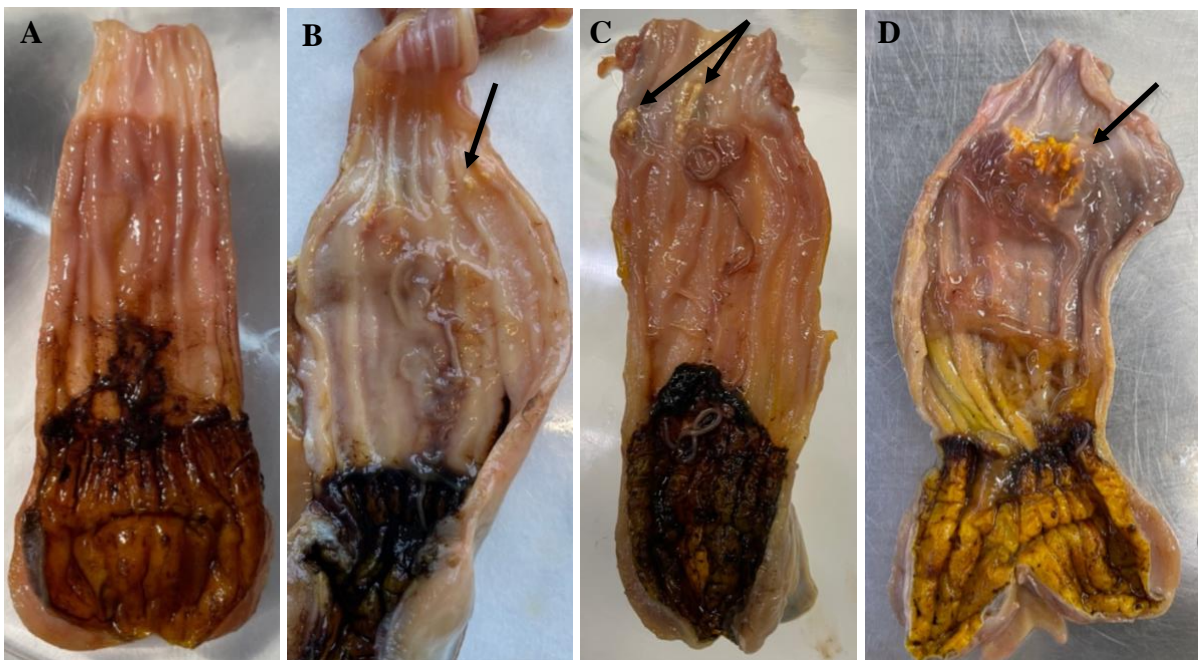


Figure 2. Gastric tracts of razorbills found dead in Malaga in the winter 2022. Ulcerative lesions categorization by extent of damaged surface between 0 and 3. A) 0 = No macroscopic lesions; B) 1 = Small single lesion (<0.5 cm); C) 2 = Medium or multiple small/medium lesions (0.5-1 cm); D) 3= Big or deep ulcers (>1 cm). Lesions indicated by black arrows.

Specific tissue samples of pathological portions were collected and fixed in formaldehyde 10% for further histological analysis. Then, fixed tissue samples were embedded in paraffin, sectioned and stained with Hematoxylin and Eosin for microscopical inspection.

2.3. Stable isotope analysis

Cheek feathers of each individual were cleaned, dehydrated in an oven for 24h and placed on 1.5 ml tubes with two stainless steel beads to disrupt the tissues through high-speed shaking with a TissueLyser (Qiagen) until a soft powder was obtained. Then, 0.3–0.4 mg of each individual sample was packed into tin capsules for isotopes determination in the Stable Isotope laboratory (LIE-EBD) of the Biological Station of Doñana (Sevilla, Spain). Samples were combusted at 1020 °C using a continuous flow isotope-ratio mass spectrometry system (Thermo Electron) by means of a Flash HT Plus elemental analyser interfaced with a Delta V Advantage mass spectrometer. Stable isotope ratios were expressed in the standard δ -notation (‰) relative to Vienna Pee Dee Belemnite ($\delta^{13}\text{C}$) and atmospheric N_2 ($\delta^{15}\text{N}$). Laboratory standards were previously calibrated with international standards supplied by the International Atomic Energy Agency (IAEA, Vienna). The standard deviation (\pm SD) was ± 0.1 and $\pm 0.2\text{‰}$ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively.

Carbon ($\delta^{13}\text{C}$) isotopic values are representative of the primary carbon source and show a number of spatial gradients in marine ecosystems such as inshore *vs.* offshore, pelagic *vs.* benthic, and latitude (Hobson, 2008). Nitrogen ($\delta^{15}\text{N}$) values show a consistent stepwise enrichment by trophic level and reflect the nitrogen pool supporting primary producers at the base of different food webs (Jennings & Warr, 2003). Stable isotopic values of cheek feathers were used to identify the breeding origin of the individuals as they do not moult them until adulthood during the pre-breeding season (December-March), reflecting in juveniles and immatures birds the isotopic

values of the breeding area (Linnebjerg et al., 2013; Glew et al., 2018). The stable isotope values of the sampled razorbills were compared with the published $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of razorbills from different breeding colonies (Bond & Diamon., 2007; Lavoie et al., 2012; Linnebjerg et al., 2013; Pratte et al., 2017; St John Glew et al., 2019.). These reference isotopic values were used to infer the potential origin of the collected razorbills. When published values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were obtained from other tissues than feathers, a correction factor was applied following Hobson et al. (1992) in order to make them comparable to present data. The same was done when the median and standard error (SE) were used instead of the mean and standard deviation (SD).

2.4. Data analysis

Before statistical analyses, all variables were tested for normality and homoscedasticity using the Shapiro-Wilk and Levene's tests, respectively, as well as visual methods such as histograms or Q-Q (quantile-quantile) plots. Differences in razorbills biometric measurements according to age (i.e. juveniles vs. immatures) and sex were tested using one-way ANOVA tests. Then, a multidimensional scaling (MDS) or principal coordinates analysis (PCoA) was applied to biometric data to visualize the ordination of individual birds with respect to the factors age and sex. To statistically evaluate differences in the combination of biometric attributes between age groups and sexes, a permutational analysis of variance (PERMANOVA; "vegan" package) was also carried out, obtaining p-values under unrestricted permutation of raw data (9999 permutations). Both analyses were applied on a Bray-Curtis similarity matrix derived from biometric data and using individual razorbills as replicate samples. Additionally, a similarity percentage (SIMPER) analysis was used to check which biometric measures contributed most to the differentiation between juveniles/immatures and males/females. Principal Component Analysis (PCA) was also applied to biometric data to establish which variable explained most of

the variability in the biometric dataset along the first component. The selected variable was thus chosen as representative of biometric features of razorbills on subsequent analyses.

To quantify the parasites load in the razorbills, prevalence (P, as the percentage of razorbills infected with at least one parasite of a particular species), mean abundance (MA, as the total number of parasites found divided by the total number of hosts examined) and mean intensity (MI, as the total number of parasites found divided by the number of infected hosts (Bush et al., 1997)) were calculated for the species of nematodes recovered from gastrointestinal tracts.

Moreover, the relationships between the most representative biometric measure obtained in the PCA and parasite abundance was analyzed with a Spearman's correlation test. Additionally, potential differences in parasite abundance and depending on age, sex, CCN and ulcerative lesions categories were tested using a Generalized linear models (GLMs). Significant associations among razorbill health factors like CCN, parasites, lesions presence and categories of ulcerative lesions, as well as birds age (juvenile/immatures) and sex (male/female) were tested by Chi-square tests. Finally, a multiple-factor analysis (MFA) was used to assess possible associations among all the different health and biological variables. This multivariate data analysis displays the relationships among sets of variables (both quantitative and qualitative), which are structured into groups, and enables the identification of individuals in relation to these variables. Thus, the differences within groups are minimized, whilst differences between groups are maximized. MFA was conducted using the following explanatory variables: the most representative biometric measure previously identified in the PCA, CCN, ulcerative lesions categories, and parasite abundance. All data analysis was performed using R version 4.2.0.

3. Results

3.1. Biological features of collected razorbills:

Of the 46 razorbills collected, 87% were juveniles/first-year birds and 13% immatures/second-year birds. None of them were sexually mature, being 38% females and 48% males. Seven individuals could not be sexed due to poor carcass status. Mean and standard deviation of the biometric measures are displayed in Table 1.

Table 1. Biometric data of 46 razorbills (*Alca torda*) found dead in Malaga coasts (Spain) between November and December 2020. Mean followed by standard deviation, in millimeters, are shown according to age and sex.

Age/Sex	n	Bill length (mm)	Bill depth at gonys (mm)	Bill depth at base (mm)	Head length (mm)	Wing length (mm)	Tarsus length (mm)
JUVENILES	40	31.92 ± 1.57	16.53 ± 1.10	14.86 ± 0.78	90.85 ± 2.10	187.10 ± 5.03	31.32 ± 1.04
Females	15	31.21 ± 1.18	16.11 ± 1.20	14.52 ± 0.66	89.83 ± 1.35	186.07 ± 3.71	30.75 ± 0.78
Males	19	32.43 ± 1.73	16.84 ± 0.93	15.11 ± 0.69	91.94 ± 2.06	188.53 ± 5.45	31.87 ± 0.98
Unsexed	6	32.05 ± 1.48	16.60 ± 1.18	14.90 ± 1.12	89.93 ± 2.30	185.17 ± 6.05	31.02 ± 1.07
IMMATURES	6	32.74 ± 1.47	18.58 ± 0.92	16.44 ± 0.63	91.68 ± 2.29	190.83 ± 15.30	32.12 ± 1.62
Females	2	31.80 ± 1.33	18.80 ± 0.24	16.83 ± 0	89.73 ± 1.38	200.00 ± 0	31.10 ± 0.15
Males	3	33.15 ± 1.78	18.17 ± 1.21	16.07 ± 0.75	92.50 ± 2.52	195.00 ± 0	32.03 ± 1.70
Unsexed	1	33.40	19.40	16.80	93.10	201.00	34.40

Bill length (ANOVA, $F_{(1, 37)} = 6.69$, $p < 0.05$), head length (ANOVA, $F_{(1, 37)} = 14.51$, $p < 0.01$) and tarsus length (ANOVA, $F_{(1, 37)} = 13.55$, $p < 0.01$) presented significant differences between sexes, being the three measures larger in males than in females. Wing length (ANOVA, $F_{(1, 44)} = 27.13$, $p < 0.01$), bill depth at gonys (ANOVA, $F_{(1, 44)} = 18.79$, $p < 0.01$), and bill depth at base (ANOVA, $F_{(1, 44)} = 21.98$, $p < 0.01$) presented significantly higher values with age, being immatures significative larger than juveniles regarding these measures. Other contrasts involving biometric measure yielded non-significant results ($p > 0.05$).

According to the MDS or PCoA, that provides an ordination of individual razorbills based on biometric data and according to the factors age and sex (Figure 3), the 57.8% of the variability of the data is explained by the first two axes. This result evidences that immature or second-year birds are larger than juveniles, since they are closer to vectors representing biometric variables (Figure 3A). The same pattern can be appreciated for males, as they tend to be larger than females (Figure 3B).

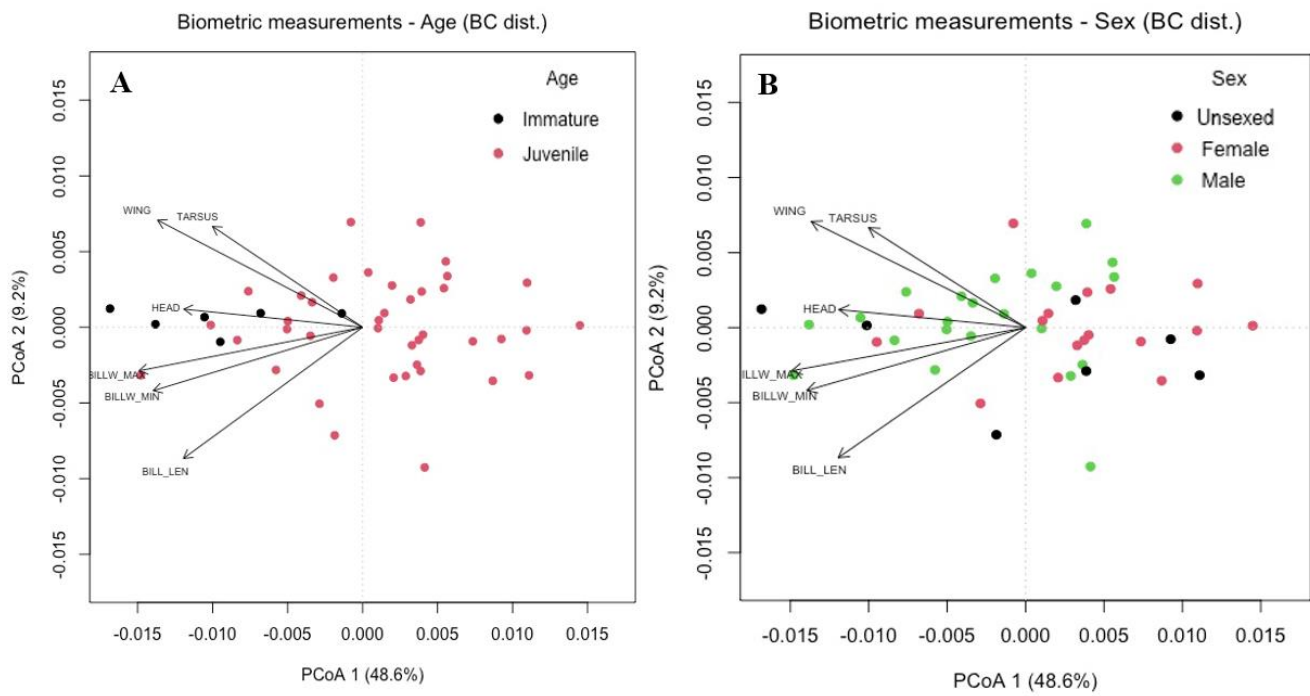


Figure 3. Multi-dimensional scaling (MDS) showing ordination of individual razorbills collected in Malaga coasts (Spain) between November and December 2020 based on biometric measurements with respect to age (A) and sex (B) using Bray-Curtis distribution (BC dist.).

PERMANOVA tests applied to evaluate the effect of age/sex on biometric measures, also showed a significative difference in these both according to age (PERMANOVA, Pseudo- $F_{(1, 37)} = 10.43$, $p_{(perm)} < 0.01$) and sex (PERMANOVA, Pseudo- $F_{(1, 37)} = 6.52$, $p_{(perm)} < 0.01$).

In addition, the SIMPER analysis allowed the identification of the three biometric measures that contributed most to the dissimilarity between age and sex groups. Wing length and head length were the first and second most contributing variables for both age and sex discrimination. In third place, bill depth at gonys was the most differentiating biometric variable between ages (juveniles/immatures) and bill length between sexes (males/females).

PCA analysis displaying associations among biometric measures (Figure 4) explained 92.30 % of the variability of the data in the first two axes (81.4% and 10.9% to the first and second dimensions, respectively).

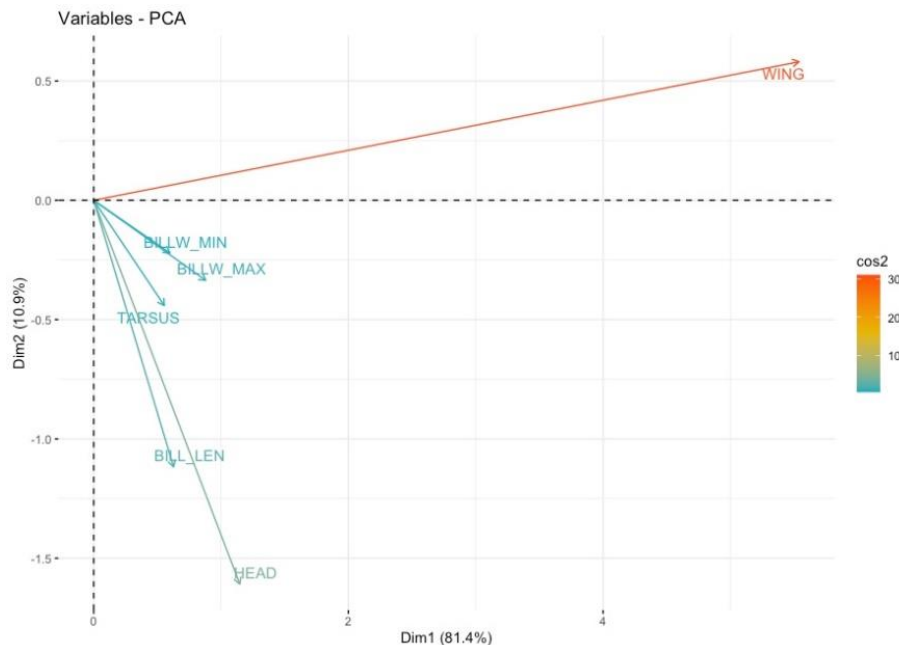


Figure 4. Principal Component Analysis (PCA) performed on razorbills biometric measurements.

Overall, wing length was the most explicative variable contributing 90.68% to the first dimension. On the second component, head length was the most important variable, contributing a 57.11% in the second dimension. Consequently, right wing length was used as a representation of razorbill biometric attribute in further analyses.

3.2. Necropsies results

All razorbills analyzed showed a very poor body condition; 89% had subcutaneous and mesenteric fat deposits entirely consumed and severe atrophy of the pectoral muscles, scoring between 0 and 1 as "mortally emaciated" in the CCN. The 9% were categorized between 2 and 3 as "critically emaciated", and only one individual was assigned a "moderate body condition" with a score of 4. During necropsies, findings were compatible with drowning signs only in three individuals. In these cases, observations included swollen, intensely congested and edematous lungs and slightly frothy fluid in the lower trachea and bronchi. There were no other signs or lesions suggesting pathologies. All individuals tested negative for Influenza virus.

Significant associations were found between CCN and razorbills age ($\chi^2_{(2, 45)} = 7.36$, $p = 0.02$), with immature birds showing a better corporal condition than juveniles. However, CNN association with sex gave not significant results (χ^2 , $p > 0.05$).

3.3. Gastrointestinal analysis

Of the 45 stomachs examined, 73% were completely empty (not counting parasites), 16% contained small anthropogenic items ($> 5\text{mm}$) such as fine plastic threads, one (2%) contained four hooks and pieces of fishing lines. In four animals (9%) otoliths were found, corresponding to *Atherina boyeri* (in two animals), *Spondylosoma cantharus* and *Sardinella aurita* (both in a single animal).

Gastrointestinal parasites found consisted of nematodes and displayed a global prevalence of 58%. They were identified as adults and fourth-stage larvae (L4) of *Contracaecum variegatum* (Order: Rhabditia, Family: Anisakidae) (abundance ranging from 1 to 34 worms per host) and adults of

Cosmocephalus obvelatus (Order: Rhabditia, Family: Acuariidae) (maximum abundance of only 2 individuals) (Table 2, Figure 5). There was no coinfection of both of parasites in any bird.

Table 2. Nematodes found in digestive tracts of razorbills collected in Malaga coasts (Spain) between November and December 2020. Prevalence (P (%)), mean abundance (MA), mean and standard deviation intensity (MI) are provided.

	<i>Contracaecum variegatum</i>	<i>Cosmocephalus obvelatus</i>
P (%)	53%	4%
MA	3.53 ± 6.75	0.07 ± 0.33
MI	6.11 ± 7.99	0.11 ± 0.43

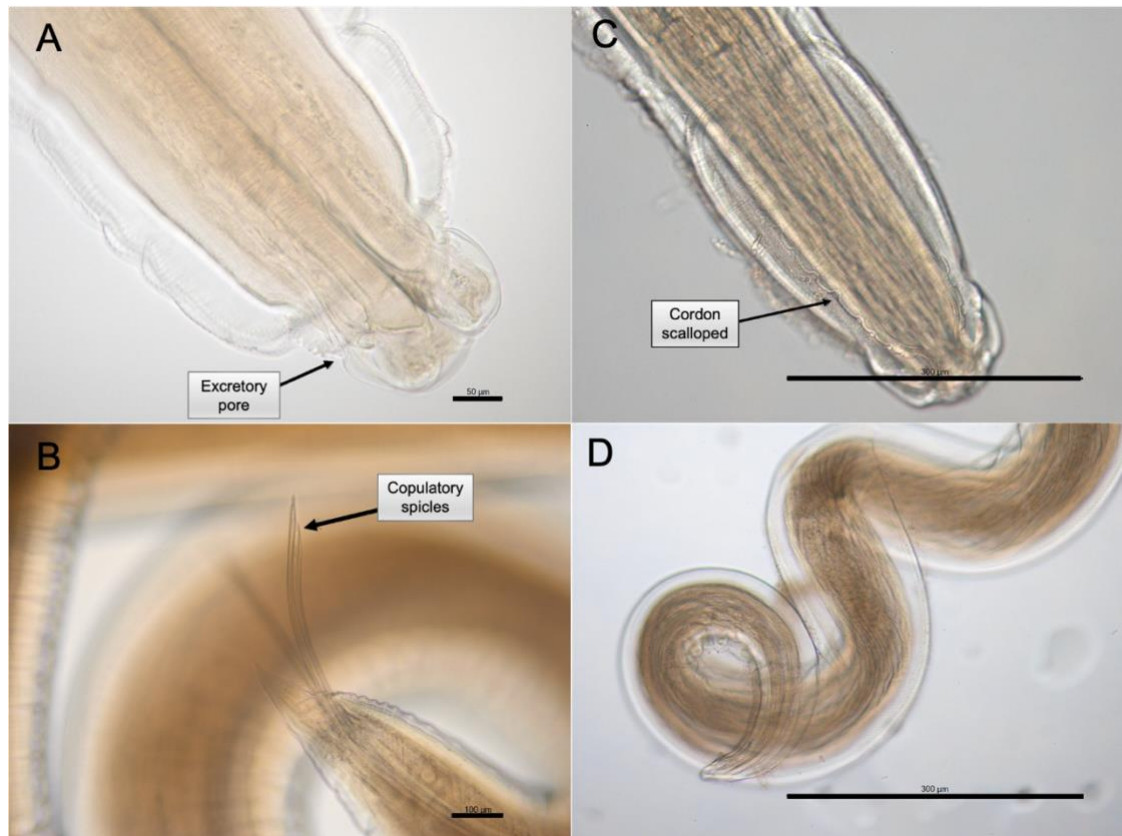


Figure 5. Nematodes found in digestive tracts of razorbills collected in Malaga coasts (Spain) between November and December 2020. *Contracaecum variegatum* head (A) and male spicules (B). *Cosmocephalus obvelatus* head (C) and male tail (D).

Spearman's correlation tests showed a significant negative association between parasite abundance and wing length (Spearman, $r_s = -0.37$, $p < 0.05$), as well as for tarsus length (Spearman, $r_s = -0.31$, $p < 0.05$). For the other biometric measures; head, total bill length, bill depth at gonys and bill depth at base not significant correlations were found (Spearman, $p > 0.05$). In contrast, the correlation between CCN and each of the biometric measures (wing length, tarsus length, head length, total bill length, bill depth at gonys and bill depth at base), and CCN with parasite abundance were not significant (Spearman, $p > 0.05$) (Figure 6).

No differences in parasites presence and abundance was found between sexes, age-groups or corporal condition categories (mortally emaciated, critically emaciated and moderate body condition) (χ^2 , $p > 0.05$, GLM, $p > 0.05$).

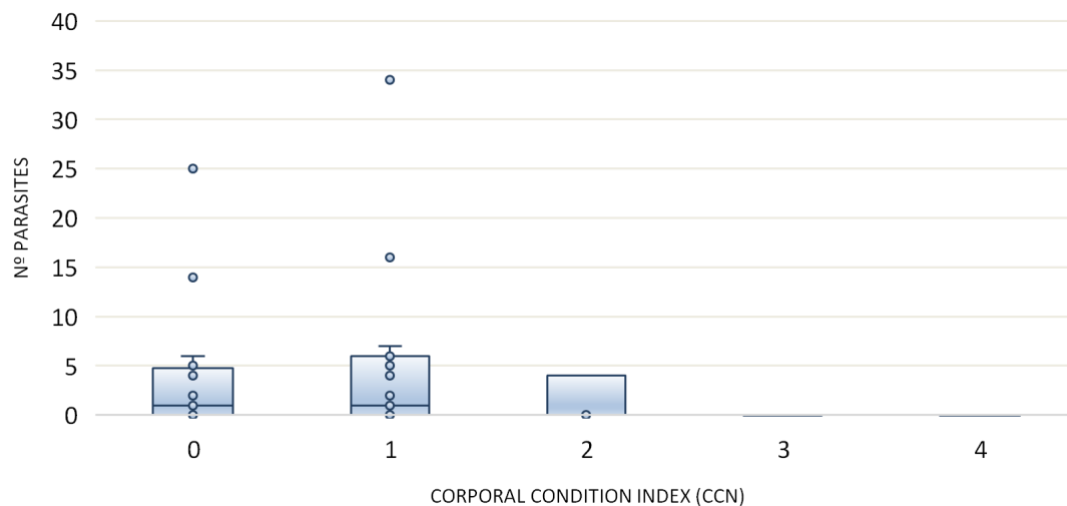


Figure 6. Number of parasites associated with corporal condition score obtained from the sum of points given to corporal condition score (CCN), where 0 - 1 is mortally emaciated, 2 - 3 is critically emaciated and 4 is moderate body condition.

Twenty-five individuals scored mortally emaciated were parasitized (62.5% regarding to the all individuals). One out of four critically emaciated had parasites (25%). The only animal which scored moderate body condition was not parasitized.

Ulcerative lesions were found in esophageal and proventricular mucosa in 60% of the animals. This lesions macroscopically presented a yellowish granulomatous proliferative aspect with necrotic areas associated with mucosal loss normally in the esophagus-proventriculus transition (See Figure 2 and 7). Adult parasites and larvae (L4) were free in the tract or closed to lesions but not attached. In some animals, the mucosa surface of the isthmus, at the junction between the proventriculus and gizzard, showed a reddish-brown color and digested blood was found in the lumen.

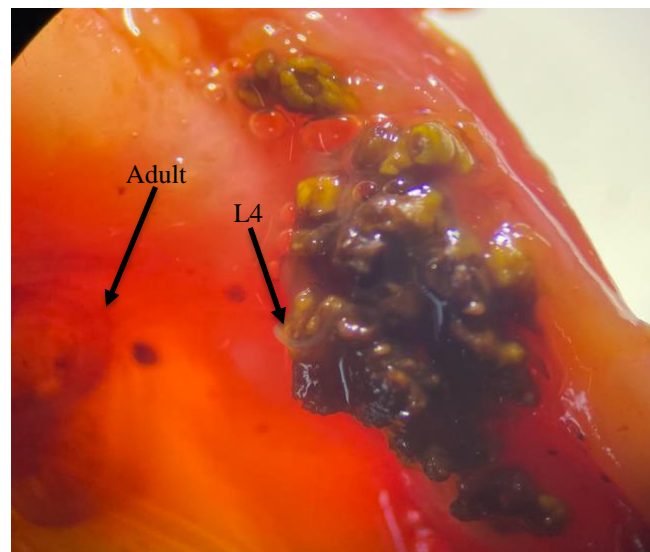


Figure 7. Proliferative lesion in the esophagus mucosa associated with two stages (L4 and adult) of the nematode *Contracaecum variegatum*. in a razorbill collected on Málaga (Spain) in the die-off event on Mediterranean coasts in Winter 2022/2023.

The histology of selected portions did not provide helpful information about the etiopathogenesis of the lesions due to poor tissue conservation; no parasites were observed embedded within the walls of the proventriculus nor the esophagus. Ulcers presented extensive superficial areas of necrosis by coagulation surrounded by an abundant inflammatory infiltrate and large number of bacteria associated with superficial desquamation of the mucosa and submucosa. In severe cases, the lamina propria was also affected. Lesion presence and lesion extent in association with sex or age gave no significant results (χ^2 , $p > 0.05$) and with corporal condition categories neither (χ^2 , $p > 0.05$).

The 49% of all the individuals had lesion and parasites together (Figure 8). Significant relations were found between lesion presence ($\chi^2(1, N=45)= 15.55$, $p < 0.01$) and lesion extent ($\chi^2(3, N=45)= 16.87$, $p < 0.01$) with the parasite's presence.

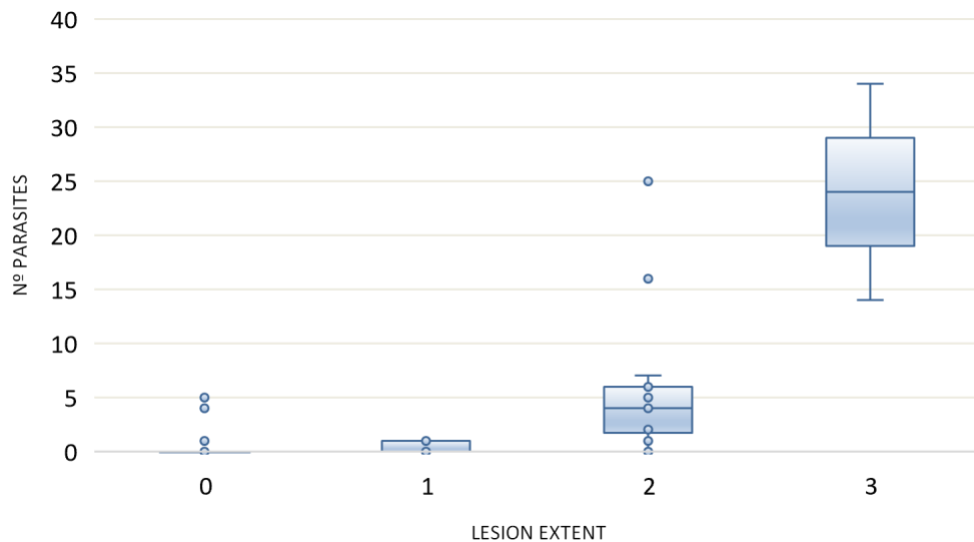


Figure 8. Number of parasites associates with lesion extent divided in four categories: 0 for no lesions present, 1 for one small lesion (<0.5cm), 2 for one medium (0.5-1cm) or multiple lesions, and 3 for big or deep ulcers (>1cm).

No macroscopic lesions were observed in 18 individuals (40%), 14 of them (78%) were free of parasites. Small lesions (scored as 1 attending to the extent of the surface damage categorization, see section 2.2.), were presented in five individuals (11%), two of them (40%) were not parasitized. Medium or multiple small/medium lesions (scored as 2) were found in 20 animals (44%), three of them (15%) were not parasitized. Lastly, big or deep lesions (scored as 3) were present in two animals (4%), both of them showing a high parasitic load (14 and 34 parasites, respectively).

Furthermore, significant differences in parasites abundance were detected among razorbills with different lesions extent categories (GLM, $F = 16.15$, $p < 0.01$). Specifically, nematode abundance was significantly higher in individuals with category 3 lesions than in those with all other lesion categories, as well as in auks with category 2 lesions than in those with category 0.

3.4 Association among factors

The MFA testing associations among the most representative biometric measure (i.e. wing length), CCN, ulcerative lesion extent, and parasite abundance explained a 52.2% of the total variance in the first two components, being 31.1% and 21.1% the first and second axis, respectively (Figure 9). Wing, parasite abundance and ulcerative lesion extent were correlated with the first component, whereas CCN was correlated with the second component. Regarding associations among variables, parasite abundance and ulcerative lesion extent were positively correlated while wing length was negative correlated to the formers (Figure 9A).

Male and immature individuals were associated to the biometric variable "wing length" as a result of their bigger size when compared to juveniles and females. Thus, the differentiation according to sex and age occurred along the first component, in turn closely related to the biometric variable "wing", as noted above. In addition, females and juveniles tend to be more associated to lesion and

parasite abundance, both also had a negative correlation with wing as a result of their smaller size or slower growth (Figure 9B).

Unsexed individuals, whose gonads were impossible to observe due to autolysis, displayed a negative correlation with CCN, indicative of their extremely poor body condition mainly owed to degradation.

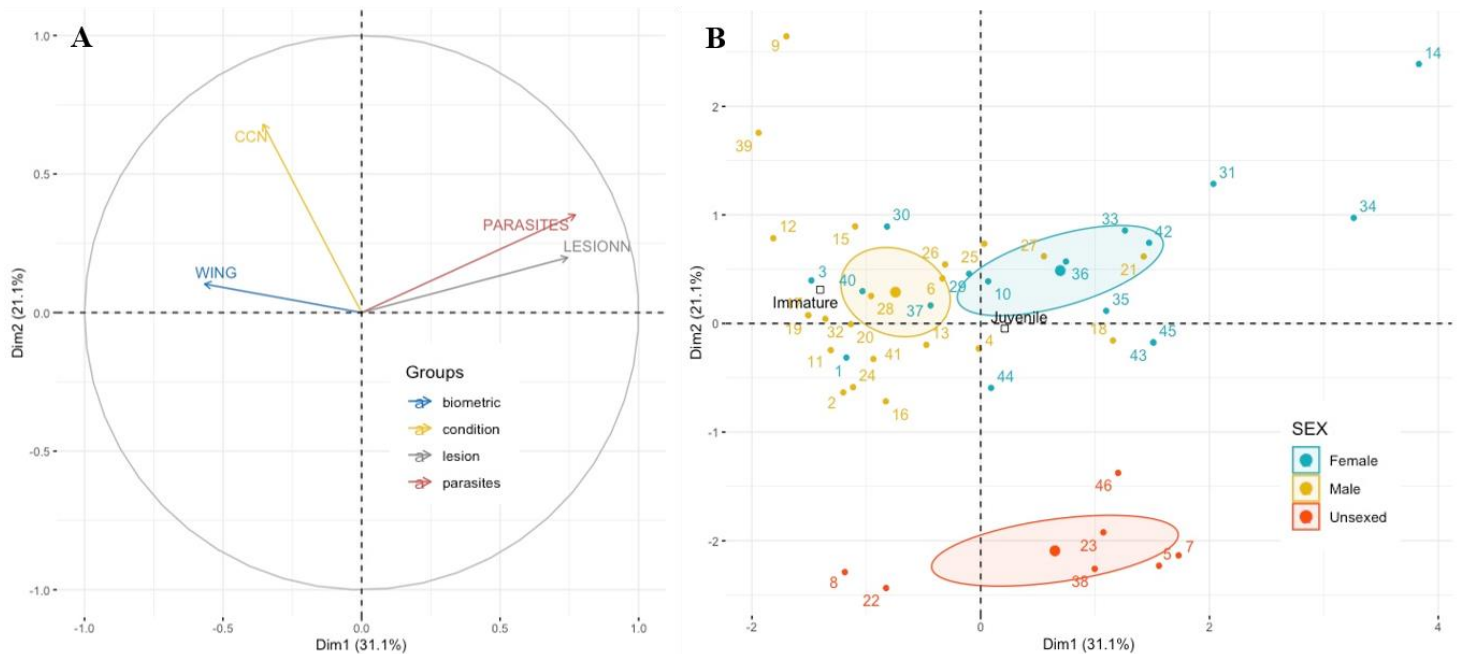


Figure 9. Multi-factorial Analysis (MFA) displaying associations between the most representative variables of the razorbills mortality of Malaga coast (Spain); WING length for biometrics, CCN for corporal condition index, LESIONN for lesion extent and PARASITES for parasite abundance (A) correlated with sexes and age-groups (B). In the latter, ellipses represent 95% confidence intervals around mean group values.

3.5. Stable isotopes results

The values of $\delta^{13}\text{C}$ ranged between -6.53 and -18.83‰ (mean= -17.43‰, SD= 0.67) and the $\delta^{15}\text{N}$ between 14.47 and 17.71‰ (mean= 15.87‰, SD= 0.83). These isotopic values compared to the ones obtained from published data of different geographical areas from razorbills range distribution, showed that most of the individuals found in Málaga are distributed mainly throughout the area that represents North Sea isotopic values. However, some individuals (33%) exhibited isotope values distinct from all sampled breeding colonies, with a few birds appearing isotopically closer to the Labrador Sea or Gulf of St. Lawrence in Canada (Figure 1 and 10).

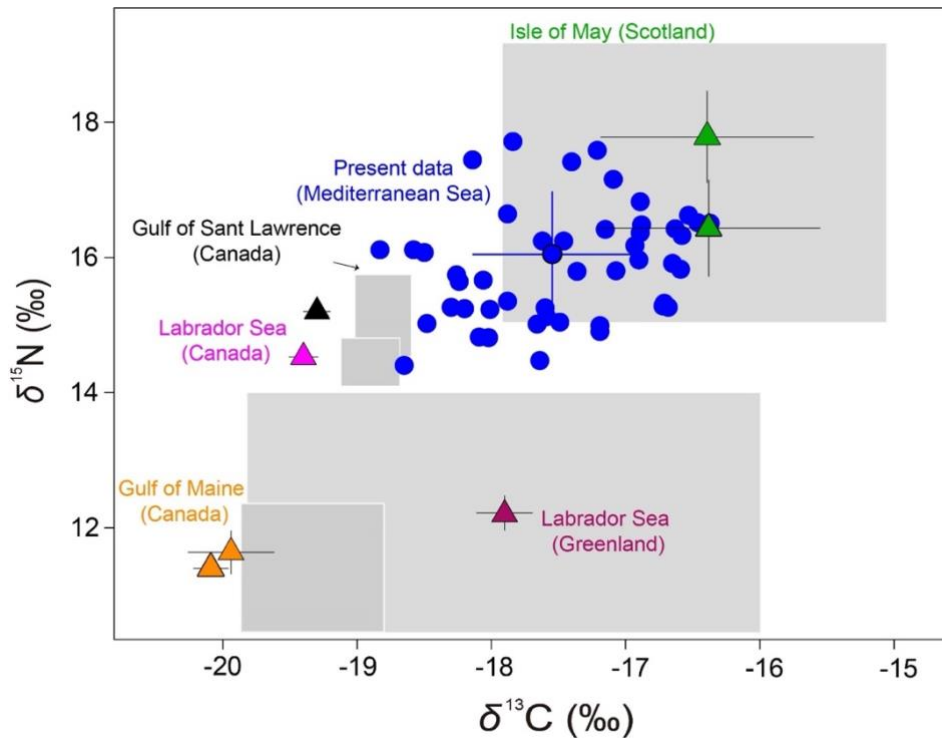


Figure 10. Mean and standard deviation of stable isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of razorbills from Malaga coast in Spain (blue dots) compared with the published values of razorbills from different breeding colonies (triangles) and modified when other tissue different than feather was used (grey areas) to make them comparable and infer the potential origin of the animals.

4. Discussion

This study provides novel insights into the synergistic impacts among starvation, parasitism, and digestive tract lesions as possible drivers of a mass razorbill wreck event in the Mediterranean Sea using an interdisciplinary approach combining epidemiology, pathology and ecology. The results revealed that the irruption and subsequent wreck of razorbills in the Mediterranean Sea during winter 2022-2023 predominantly affected juvenile birds in extremely poor condition, as evidenced by specimens collected on the Málaga (Spain). Influenza virus was ruled out as a causative agent, as all individuals tested negative. However, findings revealed a high prevalence of gastrointestinal parasites associated with some grade of lesion on the digestive tract in nearly half of the examined birds, with higher abundance correlated significantly with more extensive proventricular and esophageal lesions. The frequent absence of stomach contents, further evidenced the lack of recent foraging, coupled with stable isotope results that concluded that the majority of the animals could be originated from North Sea colonies, thus demonstrating the physiological stress to which these animals have been subjected and evidencing the hypothesis of weakness and starvation as the primary cause of death. All this likely triggered by a persisting adverse weather as a contributing factor to the forced migration of already weakened animals. The present findings align with previous investigations of seabird wrecks and provide further insight into the multifaceted influences driving such episodes.

4.1. Potential causes of the wreck

Seabird wrecks may be indicative of changes in prey stock abundance, distribution or availability, as well as of unexpected meteorological features (e.g. strong wind storms, irregular rainfall, air/water currents, big waves) which result in largely opportunistic movements in response to the severe conditions. These climatic conditions experienced by seabirds during

cyclones do not generally increase their energy requirements, and previous results suggest that seabird mortality during winter cyclones is more likely caused by starvation due to the unavailability of prey and/or their incapacity to feed (Clairbaux et al., 2021). Alterations in the food supply driven by weather can significantly restrict seabird populations and substantially impact their overall fitness and survival, particularly during long-distance migrations (Glencross et al., 2021).

The particular episode studied in the present study seemed to have been triggered by persistent Atlantic storms at razorbills home range distribution during the end of October and the beginning of November 2022 (Balestrieri et al., 2022). The harsh weather conditions maintained for almost two weeks forced the displacement of the birds in extreme physiological conditions. Winter cyclones seem to be one of the most common leading causes for seabird wrecks in the North Atlantic Ocean and climate models' predictions suggest an increase in the frequency of the strongest ones alongside with global warming (Clairbaux et al., 2021). It is needed to emphasize that the weather event which triggered this recent event is not fully addressed here, although it is an important factor behind this mass irruption of razorbills in Mediterranean coasts for winter 2021/2022.

In the present study, Influenza virus was first discarded as the primary cause of the wreck, although it had never been detected in razorbills before; it was described in 2011 in another auk species, the Common Murres (*Uria aalge*), in Newfoundland and Labrador, Canada (Wille et al., 2014). Seabird colonies have multiple species in close proximity, so movements of the virus between them are probable. Moreover, because of the highly pelagic movements of seabirds, they could also be important in the spread of the virus across the globe, playing an important role in the global dynamics of the virus. Furthermore, in 2022, a highly pathogenic avian influenza virus epidemic

struck Europe (Giralt Paradell et al., 2023), with 410 virus detections in wild birds in various European countries, including Iceland for the first time, and affecting at least 45 different species of wild birds, involving also seabirds (European Food Safety Authority, 2022).

During necropsies, special attention was paid to drown signs as a possible cause of death; it is common for seabirds to be victims of bycatch in fishing gear (Belda et al., 2001; Karris et al., 2016). These kinds of wrecks are locally important and must be discarded as it is not easily attributed if the cause of death was not found during the anatomopathological analysis (i.e. hooks). Anthropogenic interaction might not be a relevant contributor to the cause of death in Malaga razorbills since a small fraction of the analyzed animals displayed compatible signs of drowning and only one had four hooks in the stomach.

The majority of razorbills sampled were juveniles on their first southward migration, corroborating the greater vulnerability of immature birds during initial seasonal movements when navigational skills and foraging abilities are limited (Lavers et al. 2020). Although birds size range was small in the present study, a certain association between smaller individuals with higher parasite loads and more extensive lesions, as visualized in the MFA, could further point to the higher vulnerability of these younger individuals. Moreover, the absence of prey in the stomach evidenced the lack of recent foraging and starvation hypothesis. Most razorbills examined were extremely emaciated, with no subcutaneous or mesenteric fat deposits and severely reduced pectoral muscle mass. Although present results suggested starvation as the primary cause of death, other factors may have contributed to this mortality event. Indeed, extremely poor body condition is associated with hemorrhages of the digestive system due to inanition (Hocken, 2000; Dorrestein, 2009). During necropsies, 'hemorrhagic enteritis' is frequently detected, although it is not a 'real' enteritis and should be considered a hemorrhagic diathesis (bleeding into the gut), causing in all cases some

grade of anemia. This pathologic process can be observed in small birds (like passerines) that have been anorexic for more than 24 hours (Dorrestein, 2009). Moreover, alcids need to consume fish equivalent to at least 50% of their body weight daily, if unable to find adequate food, they will begin to lose body weight (Piatt et al 2020). Severe food scarcity lasting three to five days can lead to death by starvation in these birds (Diamond et al., 2020). The recurrent digested blood found in several razorbills of this study and the absence of gastrointestinal content, evidenced the lack of recent foraging demonstrating the physiological stress to which these animals have been subjected. Gastrointestinal hemorrhages have also been linked with mild to chronic exposure to oil contamination in seabirds (Jauniaux et al., 1998), even though none of the analyzed razorbills had signs of external oiling, heavy metals analysis was not carried out in this study.

In addition, the ulcerative lesions found in the esophagus and the proventriculus, in association with the nematode *C. variegatum* on half of the sampled animals, could probably worsen the anemia and consequently the health status of the birds by the continuous blood loss through the lesions. Both of the nematode species found in this study (*Contracaecum variegatum* (53%) and *Cosmocephalus obvelatus* (4%)) have been described in razorbills before (Muzaffar & Jones, 2004).

Nematode parasites are well studied in seabirds; in normal circumstances with a good health condition, they are generally ubiquitous (Fagerholm & Overstreet, 2008) and mostly non-pathogenic. However, external agents such as the physiological status of the host or environmental factors can influence the effects of this parasites; thus, cumulative effects likely play a significant role in how parasites influence seabirds at both the individual and population level (Khan et al., 2019).

The anisakid genus *Contracaecum* is known to infect a broad range of hosts across a wide geographic distribution (Barus, 1978). For some species, fish-eating birds are definitive hosts in their life cycle, and regurgitated infected prey from the parents to the chicks are a prevalent direct route of infection (Fagerholm & Overstreet, 2008). Adult nematodes typically parasitize the proventriculus without severely impacting healthy seabirds, as they feed on the host's diet (Anderson, 2000; Fagerholm & Overstreet, 2008). However, juvenile worms embed in the proventricular, esophageal, or intestinal walls, being more pathogenic in heavy infections (Granroth-Wilding et al., 2017; Fagerholm & Overstreet, 2008). *Contracaecum* sp. is capable to produce erosive, yellowed ulcerative lesions, inflammation, tissue necrosis and hemorrhages in the proventriculus and esophagus of immunocompromised animals (Granroth-Wilding et al., 2014; Fagerholm & Overstreet, 2008), which may lead to severe anemia and secondary bacterial infections at attachment parasite sites, causing septicemia and eventual death in these animals already weakened by starvation due to environmental factors (Fagerholm & Overstreet, 2008).

In general, published studies of pathological alterations induced by *Contracaecum* nematodes in seabirds are limited. Similar parasite-associated lesions have been barely documented; in white pelican (*Pelecanus erythrorhynchos*) in California (Liu & Edward, 1971), in little penguins (*Eudyptula minor*) in Australia (Obendorf & McColl, 1980), in Humboldt penguins (*Spheniscus humboldti*) in Chile (Yáñez et al., 2012) and in great cormorants (*Phalacrocorax carbo*) in Poland (Rokicki et al., 2011) and in Japan (El-Dakhly et al., 2012). High prevalence of *Contracaecum* infection associated to ulcers was also reported in razorbills affected by the Prestige oil spill collected in northwest Spain (Ledesma, 2013).

The positive association in this study between parasite abundance and ulcer severity could indicate nutritional and migratory stress exacerbated by parasitism in the studied razorbills. However, the

lack of correlation between parasitism and emaciation suggests nematodes were not the primary driver of poor body condition. Rather, the high parasite burdens potentially magnified the repercussions of the physiological stress to which razorbills were subjected. While not directly responsible for the wreck, the parasites and the resulting digestive lesions may have acted synergistically to worsen the health status of birds weakened by inadequate nutrition and demanding migration. For this, the pathology presented in this study provides additional evidence that *Contracaecum* could induce significant gastrointestinal lesions in debilitated birds. Further investigation is needed to better characterize the pathogenicity of these nematodes among susceptible seabird populations.

4.2. Potential origin of the stranded razorbills

The stable isotope analysis revealed that while those of most razorbills matched values from the British Islands as expected, some individuals exhibited distinct isotope signatures not aligning with the known breeding reference colonies. A more extensive isoscape mapping of the source colonies in the North Atlantic could have aided for the geographical assignment and allowed stronger inferences about the heterogeneous origins of these wrecked birds; unfortunately, the scarcity of available published data has prevented from carrying out a more complete isotopic assessment. Labrador Sea in Canada seems a potential origin for at least one sampled razorbill; although transatlantic migration records are scarce, long-distance movements up to 4,130 km have been documented mainly in prebreeding razorbills (Lloyd, 1974; Lavers et al., 2007; Lavers et al., 2020), fitting with the young age classes affected in this event. However, band recoveries around the Iberia Peninsula are almost entirely from British colonies (De Juana & García, 2015), and little movement data exists for North American populations (Lavers et al., 2020). Expanding tracking studies and isotope sampling of more breeding colonies could help clarify connectivity between

western and eastern Atlantic razorbill populations. Stable isotopes can be a powerful tool to elucidate the origin of migrant seabirds and to better understand their routes and movements. Moreover, knowing the exact origin of the wrecked animals could help to comprehend what really triggers this kind of events.

The severe weather conditions that may have provoked this mortality event were originated in northern North Atlantic regions like Canada and north Greenland by late October. They subsequently spread southward, severely impacting also South Greenland, Iceland and the British Isles by early November (www.wetterzentrale.de; accessed on 25 August 2023). As a large proportion of razorbills distribution range was affected by this cyclone, it is difficult to elucidate the possible origin of the 33% of the birds, which did not overlap with the colonies with available data.

Parasites can also serve as host population markers and help infer the origin of their hosts, especially those with restricted distributions. However, even cosmopolitan parasites like the nematodes found in Malaga's razorbills, may vary in prevalence, abundance or intensity among locations across wide ranges (Pasternak et al., 2007). Unfortunately, this approach was not applicable to assign the origin of stranded razorbills in the present case because there is a lack of data for the two identified nematode species in this alcid; *C. variegatum* and *C. obvelatus*. Prey species might also indicate provenance if endemic to certain areas. But the fish otoliths found – *A. boyeri*, *S. cantharus*, and *S. aurita* inhabit both the North Atlantic Ocean and the Mediterranean Sea (<https://www.fishbase.se/search.php>), obscuring geographic association. Thus, parasites and diet did not provide definitive clues to trace the dead razorbills' breeding origin.

The significance of the actual event lies not only in the unprecedented irruption and subsequent wreck of razorbills washed out in various Mediterranean countries, but in the southward migration in such numbers they accomplished. More reports of this kind had been studied before for this specie and other auks in northern areas of their distribution range, generally closer to their breeding colonies in the North Atlantic Ocean (Heubeck et al., 2011; Morley et al., 2016).

However, wrecks in more southerly waters, such as the Mediterranean Sea, have been rarely noted. Although the Mediterranean Sea hosts razorbills in winter, numbers are typically far lower than on Atlantic coasts (De Juana & García, 2015). There is only a single report of a similar event for razorbills on North America coast, in Florida during the winter of 2012, when several razorbills wrecked out southern of their normal distribution for North American colonies due to a marine heatwave as a possible consequence of the effects of Hurricane Sandy (Diamond et al., 2020).

5. Conclusions

The unprecedented wreck of razorbills in Málaga in winter 2022 predominantly involved juvenile birds that were in extremely poor condition. Necropsies revealed signs of starvation, with empty stomachs and depleted fat and muscle reserves, indicating lack of recent foraging. Although severe weather preventing adequate feeding appeared to be the primary driver of mortality, high burdens of gastrointestinal parasites, especially *C. variegatum*, were prevalent and associated with esophageal and proventricular lesions in most razorbills. While not the primary cause of death, these parasites and digestive ulcers likely acted synergistically to further weaken the already emaciated birds. Still, some key limitations condition the conclusions that can be drawn from these patterns. The sample size, although substantial for seabird mortality events, still constrained

statistical power and prevented from making definitive conclusions. Even so, the interplay and cumulative impacts of nutritional stress, inclement weather, parasitism, and subsequent gastrointestinal ulcers offer insights into the multifaceted factors underpinning mass mortality events in seabird populations. Stable isotope analysis showed that the majority of razorbills likely originated from North Sea colonies, although some individuals may have come from other locations, probably northwestern colonies, being Canada a possible origin. The study of this wreck highlights the vulnerability of juvenile razorbills and other seabirds from across their northern Atlantic range to changing environmental conditions, highlighting the need for continued monitoring of these sentinel species in the context of climate change.

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