DRAFT STANDARD FORM C PRELIMINARY CRUISE REPORT

Cruise name/number:	OCEARCH – Spain 2024/F2024-004
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Brief description of scientific objective:

The primary scientific objective of this research cruise was to survey the northwestern and northern coast of Spain (Bay of Biscay) for the presence of white sharks (*Carcharodon carcharias*) with a goal to temporarily capture, examine, biologically sample, satellite and acoustic tag, and release one or more animals. The secondary objective of the cruise was to survey (environmental DNA) the area for white sharks and other shark and ray species and temporarily capture, examine, biologically sample, satellite or acoustic tag, and release, 5 other shark species of interest including: porbeagle shark (*Lamna nasus*), blue shark (*Prionace glauca*), shortfin mako shark (*Isurus oxyrinchus*), common thresher shark (*Alopias vulpinus*), an bluntnose sixgill shark (*Hexanchus griseus*). The overarching goal of the project is to identify potential habitat connections between the Mediterranean white shark population and isolated individuals rarely seen in the Bay of Biscay. We also sought to explore shark and ray biodiversity in the region and identify potential habitat connectivity for other large shark species. A detailed preliminary summary of the research efforts as of the completion of the cruise are included in the Supplemental Materials.

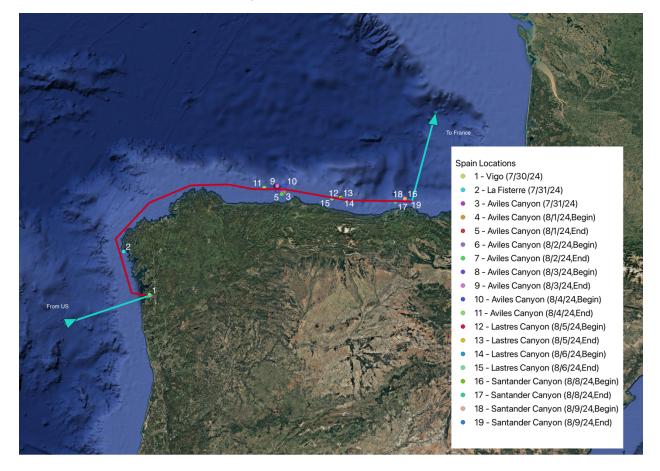
Update on anticipated dates for delivery of final results:

Metadata:	Sample types collected: Fishing effort (hooks, hook hours, sharks caught),
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	eDNA samples, shark morphometrics, blood, muscle, fin clips, feces,
	microbiome (mucus/cloacal swab), ectoparasites, ultrasound. Samples
	will be used for projects as listed in the Supplemental Materials (section
	1.5). Results expected 12/31/2025
Raw Data:	12/31/2025
Processed Data:	6/30/2026
Data Analysis:	12/31/2026
WODC Data Registration (if	NA
applicable):	

SUPPLEMENTAL MATERIALS

1.1 MV OCEARCH Cruise Track:

The MV OCEARCH arrived in Vigo, Spain on 27 July 2024. Research efforts began on 31 July 2024 and ended 8 August 2024. Regions of focus included La Fisterre, Aviles Canyon, Lastres Canyon and Santander Canyon. Figure 1 below is a map of locations of drift fishing efforts and the cruise track:

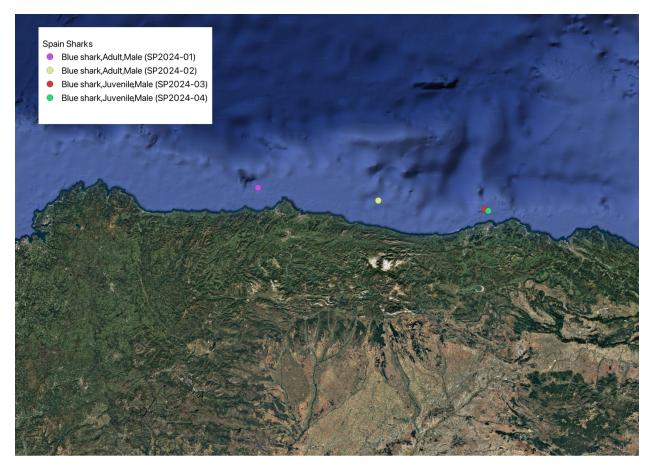


1.2: Fishing Effort Summary:

Fishing efforts included 9 drift fishing days at 4 locations as noted in the figure in section 1.1. This included 221.2 hook hours with 0.018 catch per unit effort (catch/hook hours). Four blue sharks (*Prionace glauca*) were caught and sampled with one animal receiving a fin-mount satellite tag (SPOT) and three receiving roto type numerical tags. A summary of shark metadata is below in Table 2.

Species	Date	Location	Sex	Total Length (cm)	Maturity	Animal ID
Prionace glauca	8/2/2024	43° 44.308N; 6° 06.588W Male 289 Aviles Canyon		Adult	SP2024-01	
Prionace glauca	8/5/2024	43° 38.81N; 4° 54.958W Lastres Canyon	Male	319	Adult	SP2024-02
Prionace glauca	8/8/2024	43° 35.0N; 3° 51.62W Male 144 Santander Canyon 144		Juvenile	SP2024-03	
Prionace glauca	8/9/2024	43° 34 378N 3° 49 474W Santander Canyon	Male	165	Juvenile	SP2024-04

Locations of each shark capture along the Northern coast of Spain (Bay of Biscay) are depicted in Figure 2:



1.3 Biological Sampling efforts:

A total of 111 individual biological samples (blood, muscle, fin clip, microbiome (surface mucus/cloacal swabs), parasites) were retained from the 4 blue sharks for the research projects as outlined in the Science Brief (See section 1.5). A summary of biological samples types collected from each animal is presented in Table 2 below. All samples were provided to the collaborating Spanish research team at the conclusion of the expedition.

Animal ID	Blood	Muscle	Fin	Microbiome	Feces	Parasites	Ultrasound
SP2024-01	Yes	Yes	Yes	Yes	No	Yes	Yes
SP2024-02	Yes	No	Yes	No	No	Yes	Yes
SP2024-03	Yes	Yes	Yes	Yes	Yes	No	Yes
SP2024-04	Yes	Yes	Yes	Yes	Yes	No	Yes

1.4 Environmental DNA Sampling Efforts:

Environmental DNA sampling efforts were performed resulting in the collection of 9 samples from 9 locations on 9 days of drift fishing.

Date	Latitude Start	Latitude End	Longitude Start	Longitude End
7/31/2024	42°52'36"N	43°43'98"N	43°43'98"N	43°43'98"N
8/1/2024	43°43'725''N	43°43'328''N	6°04'406W	6°04'406W
8/2/2024	43°43'731''N	43°42'036''N	6°06'518''W	6°06'518''W
8/3/2024	43°45'105''N	43°49'013''N	6°12'140''W	6°12'140''W
8/4/2024	43°48'954''N	43°47'48"N	6°12'140''W	6°21'80''W
8/5/2024	43°38'81"N	43°38'053''N	4°56'89''W	4°56'551''W
8/6/2024	43°37'881''N	43°38'245''N	4°54'958''W	5°01'027''W
8/8/2024	43°38'053''N	43°36'7''N	3°40'235''W	3°35'2''W
8/9/2024	43 38.053N	43 36.7N	3 40.235	3 35.2 W

1.5 Research Science Brief

The following research projects and primary research investigators were supported by this research cruise and the sampling efforts.

1. Study of the connectivity between marine deep water ecosystems based on elasmobranch populations (DEEPCON) C. Rodriguez-Cabello, F. Sánchez Delgado — IEO-CSIC Oceanographic Centre of Santander

C. González-Pola, — IEO-CSIC Oceanographic Centre of Gijón

The aim of this project is to investigate the degree of isolation among the fish populations inhabiting deep sea ecosystems as well as their dependence on particular characteristics of their habitat. This study tries to increase the knowledge of connectivity and dependence among these ecosystems which is necessary for defining a coherent network of Marine Protected Areas, following the Natura 2000 and the European Marine Strategy. In particular, the study areas are the Aviles Canyon (SIC) and Le Danois Bank (MPA), both located in the Cantabrian Sea (www.ecomarg.com). The main objective is to know the movement patterns of deep water sharks applying conventional plastic tags and satellite tracking technology using ARGOS floats to infer the trajectories. At the same time biological data is recorded to estimate biological parameters (length, sex, maturity) and trophic ecology studies to assess their trophic habits and habitat dependence by means of direct stomach observation and isotope analysis.

2. Habitat characterization, migration patterns and spatio-temporal interaction of the Basque fleet with pelagic sharks in the Bay of Biscay

M. Erauskin-Extramiana, A. Salgado — Center for Marine Research AZTI

It has recently been shown that the Bay of Biscay is an area with a high presence of juvenile blue sharks, being a migration area from the north on their way to the Canary Islands and the Azores. This area of passage makes the Basque coast a suitable place to see these animals, and in recent years, an increase in shark watching and swimming activities has been observed. The Bay of Biscay is an interesting area for the blue sharks but also for other pelagic shark species. The general objective of the study is to characterize the dynamics and migration patterns of blue sharks (Prionace glauca) and shortfin makos (Isurus oxyrinchus) in the Bay of Biscay and to identify areas of interaction with the fleet (incidental catches) to develop management measures that will lead to a reduction in fishing mortality of these species. However, the study also aims to include other pelagic shark species. Although this is the main objective, the project is a multidisciplinary study that involves genetic tools, habitat modelling, historical fishing catch data analysis, and monitoring among others.

3. Project Galeus: building a comprehensive shark database along the Spanish coast

J. B. Goncalves-Neto – University of Cádiz

Á.R. Domínguez-Bustos – University of Cádiz

The objective of this pilot project is to initiate a database through photographic and visual surveys of the main shark species along the Spanish coast. This aims to create a database for the correct identification of captured shark species. The methodology includes the creation of an image bank, identification of specimens, morphometric measurements. social media data base and future conservation actions with the dissemination of material about the species present in the Northeast Atlantic.

4. Probing white shark presence with the metaprobe: advancing eDNA sampling technology D. March, P. Gabasa, D. Ruiz-García — University of Valencia

Environmental DNA (eDNA) technology offers a powerful tool for monitoring marine biodiversity by detecting the presence of species from genetic material shed into the environment. The metaprobe is a low-cost, efficient eDNA sampler designed to upscale aquatic monitoring. It consists of a 3D-printed perforated sphere containing custom-made gauze rolls to trap eDNA. Unlike traditional methods, the metaprobe eliminates the need for onboard filtration of water samples, simplifying field operations and reducing logistical constraints. This study aims to integrate the eDNA metaprobe with experimental fishing methods to enhance shark species detection. Our specific objectives are 1) to assess the feasibility and effectiveness of integrating the eDNA metaprobe system with experimental drumline fishing methods, and 2) to detect the presence of shark species, particularly great white sharks, even if they are not physically caught or observed. This integration aims to support robust data collection for biodiversity monitoring and conservation efforts.

5. Insights into shark reproductive ecology from North Atlantic waters M. Valls, P. García-Salinas, P. Morón-Elorza — Fundación Oceanogràfic M. Muñoz-Baguero — Universidad CEU Cardenal Herrera

Knowledge of the biology and reproductive ecology of sharks is crucial for understanding their population dynamics and developing effective conservation plans. In this study, our objective is to correlate hormonal parameters obtained from blood and reproductive fluids with information derived from ultrasounds, morphology, and sexual maturity stages in both male and female sharks captured. This integrated approach will provide insights into the significance of capture areas for the overall population of these animals, thereby informing conservation priorities and strategies.

<u>6. Analysis of sperm dynamics and morphology in white sharks: understanding reproductive insights through environmental variations</u>

P. García-Salinas, M. Valls — Fundación Oceanogràfic M. Muñoz-Baquero — Universidad CEU Cardenal Herrera

Elasmobranchs possess spermatozoa with unique morphology and dynamics. Understanding the processes that have shaped the evolution of these cells allows us to infer reproductive aspects of great interest. The aim of the study is to be able to analyze the dynamics of rotation, advancement, and directionality of white shark spermatozoa. Variations in the quality of these cells could be indicative of altered states in the animals. To this end, sperm obtained from male individuals will be studied under different environmental conditions, recording the behavior of the cells as well as their general morphology. This information will be compared with morphological characteristics and physicochemical and hormonal patterns of the animals, in order to establish relationships among these variables.

7. Advancements in cryopreservation techniques for white shark sperm: evaluating cryoprotectant efficacy and sperm auality

P. García-Salinas, M. Valls — Fundación Oceanogràfic M. Muñoz-Baquero — Universidad CEU Cardenal Herrera

Recent years have seen an upsurge in the study and application of techniques for the control of elasmobranch reproduction. These techniques include artificial insemination, for which quality sperm is required. Unfortunately, techniques that allow the storage of quality sperm, over a prolonged period of time, are scarce. The present study aims to obtain white shark sperm samples for cryopreservation. To this end, once the samples have been obtained, they will be subjected to various dilution and mixing procedures, with internal (DMSO, methanol) and external (egg yolk) cryoprotectants. Subsequently, they will be subjected to a freezing process using liquid nitrogen vapor, which will allow the samples to be kept at -196° C. The samples will be evaluated before and after freezing by their cell mobility and the integrity of their plasma membranes, establishing which protocol is more effective in the species.

<u>8. Microbiome signatures in white sharks: establishing baseline data and health correlations</u> M. Muñoz-Baquero — Universidad CEU Cardenal Herrera

P. García-Salinas, M. Valls — Fundación Oceanogràfic

The microbiome significantly impacts shark health and indicates environmental interactions, with unique microbiomes in different internal fluids linked to biological functions. This study aims to determine the microbiome signatures of reproductive fluids (seminal plasma in males and lower reproductive tract in females) and compare them with blood microbiomes in white sharks (*Carcharodon carcharias*) and other species. Focusing on healthy individuals for baseline data, diseased animals will also be sampled to explore microbiome-health interactions. This study will expand knowledge of shark microbiota, distinguishing it from environmental components, and explore variations related to age, size, sex, health, and geography.

<u>9. What can microbiome communities tell us about shark fitness?</u> T. Bañeras, A. Espino Ruano — Las Palmas de Gran Canaria University

H. Toledo — Angel Shark Project: Canary Islands

Elasmobranchs play an essential role in oceanic food chains as apex species. Despite this, they face numerous threats, most of which are anthropogenic, such as overfishing and habitat destruction, leading to a drastic decline in their populations in recent decades. In most animals, especially humans, it has been documented that the microbiota is closely linked to the physiology, immunity, and ecology of the hosts. The microbiota-host relationship can be very close, highly specific, and represent an essential characteristic in the resistance of wild animals to potential toxins and infectious diseases, as well as in the assessment of shark fitness. The composition of these microbiomes can be shaped by various factors, including diet, life stage, and environmental conditions. Fish in general, and elasmobranchs in particular, are no exception to this rule. We know there are differences in body microbiota depending on the species or their ecological status, although the relationship between sharks and their commensals is not entirely clear. Understanding the microbiomes of sharks in their natural environment can help manage and assess shark fitness in a less invasive way (using sterile swabs). This study aims to test four different hypotheses: 1) Shark microbiomes are distinct from the surrounding seawater environment and from different shark body parts (skin, teeth/mouth, cloaca, and gills); 2) Microbes vary in community composition among shark species; 3) Tooth and mouth microbiomes have the potential to transfer pathogenic microbes to humans via shark bites; and 4) Assess shark fitness through the microbiome community, abundance, and diversity.

10. Study of ectoparasites of great white sharks in the North East Atlantic

J. Penadés-Suay – Associació LAMNA

M. Valmaseda-Angulo – University of Valencia

Pelagic sharks are commonly infected by different kinds of ectoparasites, which tend to be generalists and infect more than one species of shark. Ectoparasitic communities on great white sharks of the North East Atlantic are still poorly described, giving us the opportunity to elaborate a field guide for their identification which would be useful for future campaigns. Ectoparasites can act as 'natural tags' showing where these migratory species have been before, while also giving insights into sex or age differences in shark distribution. Given that most ectoparasites are generalists, the study of other pelagic shark species in the area is also of great interest for these studies. The main objective of our project is to collect both qualitative (presence, species identification, infection site) and quantitative data (prevalence, abundance, intensity of infection), to compare it with host characteristics (sex, age, size, health condition). The importance of the study of ectoparasites lies not only in unravelling their role in intricate marine ecosystems, but also in recognizing the challenges inherent in their study, especially in species as elusive as sharks.

- 11. Integrating a health assessment toolkit and new technologies to assess post-capture stress in pelagic sharks
- D. March, P. Gabasa, D. Ruiz-García University of Valencia
- P. Morón-Elorza, P. García-Salinas, M. Valls Fundación Oceanogràfic
- A. Newton OCEARCH
- N. Queiroz CIBIO/InBIO
- B. Finucci National Institute of Water and Atmospheric Research

Capture-induced stress and its physiological repercussions threaten both commercially targeted and discarded species, with incidental capture often resulting in mortality. Bycatch mitigation plans aim to reduce fisheries mortality, but the lack of data on post-release mortality rates and their drivers hampers effective strategy evaluation. Qualitative health assessment indices are commonly used to assess the health condition of chondrichthyans at the time of release, providing estimates into the probabilities of post-release mortality. We aim to assess the response of pelagic sharks to stress associated with research capture and tagging and evaluate the effectiveness of a health assessment toolkit developed by our team. This toolkit measures physical, behavioral, and physiological health indicators, supplemented by cameras and high-resolution accelerometers attached to the gear, to monitor the response of sharks and the fishing operation. This holistic analysis can reveal species-specific stress responses and their effects on post-capture mortality, which is essential for developing targeted management strategies to mitigate discard mortality and ensure the sustainability of shark populations.

12. Trophic ecology of pelagic sharks analyzed from cloacal swabs and stable isotopes of sharks living in the eastern North Atlantic and the Mediterranean Sea

E. Fernández-Corredor, J. Navarro — Institut de Ciències del Mar, Barcelona

Understanding the trophic ecology of pelagic sharks is crucial for predicting their resilience to shifts in resource availability and for managing key prey species that support their populations. Studies assessing shark diets usually rely on stomach contents analysis, involving invasive methods like gastric lavage or lethal sampling, which is not desirable for threatened species. This project aims to address the significant knowledge gap regarding what these sharks eat in the eastern North Atlantic and Mediterranean Sea by using non-lethal techniques. DNA metabarcoding of cloacal swabs from the sampled sharks will allow us to identify the dietary components of these sharks by analyzing the genetic material found in their digestive tracts. Fin samples will be taken from the captured sharks and stable isotope (C and N) analysis will be performed to assess the diet assimilation with stable isotope mixing models and to compare the trophic niche of the different species. By uncovering their feeding habits, we can better understand their ecological roles.

<u>13. Impact of Anthropogenic Contaminants on North-Atlantic Shark Species</u> O. Novillo Sanjuan — Technical University of Denmark (DTU)

Shark populations are declining across the globe due to several anthropogenic factors, including pollution of the seas. Of the multiple pollutants that can affect sharks, we find plastics and novel contaminants that are related to a range of manmade materials. Perfluoroalkyl substances (PFAS) and plasticizers are of special concern due to their ubiquity in the environment and their potential harmful effects, including endocrine disruption and immune suppression. In this study, we will analyze tissues of North Atlantic shark species in search of contaminants of concern, with the aim of knowing the extent to which these top predators are exposed to these substances in the open ocean. The results obtained will help us to understand whether the analyzed contaminants constitute a risk for sharks' survival and, furthermore, will help us to evaluate the risk humans have by consuming these species. Additionally, this study will allow us to set baseline concentrations and to compare these findings with other species to see if top predators have significantly different concentrations of pollutants in their tissues due to potential bioaccumulation.