UNIVERSITÀ POLITECNICA DELLE MARCHE

Dipartimento di Scienze della Vita e dell'Ambiente DISVA

PHD COURSE IN LIFE AND ENVIRONMENTAL SCIENCES

Report Form for PhD student annual evaluation (XXXVII and XXXVIII cycles)

Name of PhD student: Chiara Gambardella

Title of PhD research: Large Marine Predators in the Mediterranean Sea: a losing game or there is still room for their recovery?

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Research lab name: Laboratorio di Zoologia

Cycle:
[ ] XXXVI
[x] XXXVIII

PhD Curriculum::
[x] Marine biology and ecology
[ ] Biomolecular Sciences
[ ] Civil and environmental protection

DISVA instrumentation labs/infrastructure eventually involved in the project:
[ ] Actea Mobile Laboratory
[ ] Advanced Instrumentation lab
[ ] Aquarium
[ ] MassSpec lab
[ ] MaSBiC
[ ] Simulation/informatics lab
[ ] Other. Please, indicate: .....

ABSTRACT (1000 characters, including spaces):
Ecological information on large species such as the Mako shark (Isurus oxyrinchus, Rafinesque 1810) and the White shark (Carcharodon carcharias, Linneaus 1758) are getting a higher demand seen their status of "Critically Endangered" in the Mediterranean Sea and their importance in sightings and bycatch in several fisheries. Nevertheless, data is still limited and scattered in time and space. In this framework, the aims of this PhD project are to implement the use of opportunistic data in species distribution models paired with the use of environmental DNA and genetic approaches, as well as citizen science, which all together can result in a better understanding of habitat use and connectivity of large shark populations, offering promising results in monitoring pelagic environments, as happened with the white shark in the Mediterranean Sea, which is often misidentified with the mako and occupy the same ecological niche.

Part 1. Scientific case of the PhD Research (2 to 3 pages, including figures)

- BACKGROUND

Large marine predators, such as pelagic elasmobranchs, are among the most charismatic organisms of the marine realm. Nevertheless, in the Mediterranean Sea, there is still a lack of information on these species, often resulting in inadequate management and population declines (Dulvy et al., 2016). This loss of large predators is mainly due to pressing overexploitation, habitat degradation and coastal urbanization (Estes et al., 2011). Indeed, organisms at the top of the trophic web usually have life-history traits (e.g., late age and maturity and low fecundity) which make them more vulnerable to these anthropogenic stressors (Myers et al., 2007). Overexploitation of predators can lead to shifts in community composition, mid-level consumer releases and cascading effects (Myers et al., 2007) Even if the real extent of these changes is still debated, it is largely recognized that top predators are essential in shaping ecosystems. Yet, many species still do not benefit from focused studies or monitoring activities to infer their abundance and distribution, especially in the Mediterranean Sea. Most of the data is present only for species of commercial interest (e.g., *Thunnus thynnus*, Atlantic Bluefin Tuna, BFT) and/or come from outdated assessments and many species are still classified as Data Deficient in the IUCN Red List; while others are facing an elevated risk of extinction or are only described historically (such as *Carcharodon carcharias*, white shark and *Isurus oxyrinchus*, mako shark)(Rigby et al., 2019; Soldo et al., 2016). Information about the presence of most elasmobranch species comes from opportunistic sightings, which, if well used and combined with citizen science activities, can represent a powerful data source to keep track of their distribution, abundance trends, and habitat use (Bargnesi et al., 2022). Also new molecular approaches are enriching data collection with cost-effective and non-invasive tools such as environmental DNA, which can be easily broadened to citizen scientists (Jenrette et al., 2023). These data can also update knowledge on genetics and population connectivity (Gargan et al., 2017; Lafferty et al., 2018; Maiello et al., 2023; Mariani et al., 2021; Truelove et al., 2019) possibly highlighting differences in sub-populations in different sectors (e.g. the Atlantic population, the Pacific population,…). All this information is crucial in informing ecosystem-based management and conservation actions. The combined use of new technologies and good management practices have already demonstrated that it is possible to gain information on large pelagic species, as it happened with the White Shark Chase(Jenrette et al., 2023).

- SCIENTIFIC AIMS

The aims of this PhD project are:

I) Advance knowledge on spatiotemporal pattern of distribution of large pelagic species- with a special focus on the Shortfin Mako, to inform management and conservation, and raise awareness towards their importance in maintaining ecosystem structures
II) Sequence the mitochondrial genome of the shortfin mako in the Mediterranean Sea and create species-specific primers for environmental DNA detections and understand genetic differences between other sub-populations (e.g. Atlantic shortfin mako)
III) Understand the relationships between oceanographic variables and habitat use of two lamnids occupying the same niche

- WORKPLAN AND RESEARCH ACTIVITIES

WP 1. Which are the spatiotemporal patterns of Shortfin Mako in the Mediterranean Sea? Are there any differences in the distribution according to season and/or age?

Objective.

The objective is to investigate the spatiotemporal distribution and habitat preferences of the shortfin mako in the Mediterranean Sea. The species is frequently caught in several pelagic fisheries, which have severely depleted this population to “Critically Endangered” levels.

I synthesized occurrence records available for the species since the 1800s from the scientific literature, online databases, social media, fishermen reports, and port monitoring to develop species distribution models (SDMs) with GLM, GAM and Point Process Model approach.

Methods.

I started creating a database of occurrences, collecting data from multiple sources. For each occurrence I created an ID, and I recorded the date in dd/mm/yyyy format, the location, the country, the GSA, the latitude and longitude coordinates (where present) and a coordinate rank number, for which every observation is weighted on the accuracy of the coordinates. The rank is a number from 1 to 5, with 1 as lowest resolution (large area) and 5 the highest (precise coordinates from literature or directly from source). Other variables are: source of observation, type (fishery/sighting), sex, total length (TL in cm), weight (W in grams) and eventual notes.

Then, I aggregated in a new column the season, the historical period – stacking my observation in 1800-1900/1900-1990/1990-2023 and the Age class (Adult- ADL, Juvenile- JUV, and Young of the Year-YOY) according to Mollet et al. (2000). Whereas the size was not available but there was a picture, I estimated the size using ImageJ software, and then according to the age classification in (Mollet et al., 2000) I added the Age class.

Expected/Obtained Results.

The preliminary analyses led to the compilation of a dataset of 607 records. Occurrence records were not uniformly distributed in time; with 91% of the data coming after 1990. The highest frequency of occurrence was detected in the Sicilian Channel and Tyrrhenian Sea, while the majority of historical data before 1990 occurred in the Adriatic Sea, with the highest values between 1800 and 1900. These patterns reflected changes in observation efforts as well as biological processes. We will investigate the ecological and environmental drivers of their occurrence within the Mediterranean Sea. To deal with the statistical nature of these opportunistic data, we will develop presence-only SDMs with different approaches, from Generalized Additive Models to Point Process Models (PPMs). Our approach contributes to obtaining reliable data on the distribution patterns of shortfin makos in the Mediterranean Sea, with promising results to identify essential areas for the conservation of this species, as well as to the development of efficient analytical approaches to analyse opportunistic occurrence records, which are increasingly available from citizen science programs and online social network platforms. The same approach has been used for the White Shark (Moro et al., 2020) during the White shark chase, and it is currently under further development using a Bayesian hierarchical PPM method.

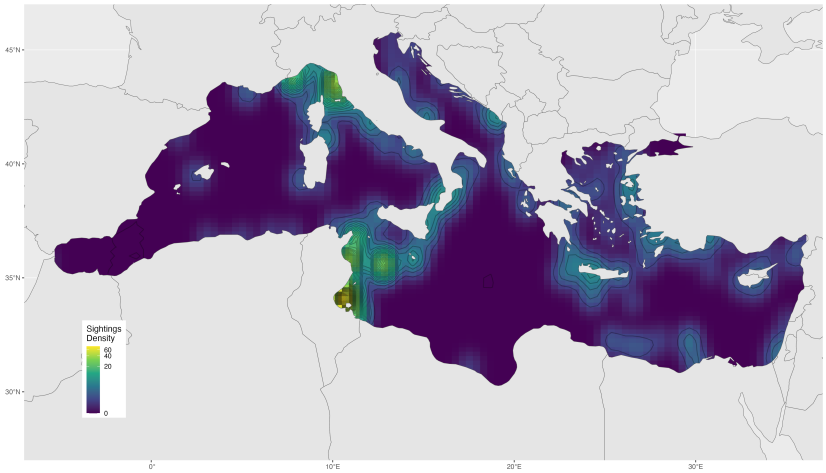

Figure 1- Kernel Density of 607 Shortfin Mako occurrences in the Mediterranean Sea

WP 2. Is the Shortfin Mako Mediterranean population distinct from the Atlantic one?

Objective.

The models highlight core areas of occurrence, where to focus research efforts. The fieldwork activities include:

- Collaboration with commercial fishermen in order to collect information on seasonality and catches
- Engagement of recreational fishermen for sightings communication and water sample collection for eDNA analyses
- Participate in scientific expeditions (White Shark Chase - WSC) to collect data for the White shark and side data on Shortfin Mako from methodologies such as pelagic BRUVs and eDNA water collection

During WSC 2023 I collected muscular samples of mako sharks from bycatch in the Pelagie archipelago and one sighting of an adult mako shark in the same area from the pelagic BRUVs.

My objective for this work package is to (i) extract and sequence the mDNA of *I .oxyrinchus* caught in the Mediterranean Sea (ii) develop an eDNA assay to detect the species' presence with non-invasive tools and usable by a large amount of people (iii) understand the connectivity with the Atlantic sub-population. The overarching goal is to provide both the full sequencing of the genome of the Mediterranean shortfin mako, and a new tool to monitor the population in Mediterranean waters, in order to understand movement patterns and distribution and inform management and conservation efforts.

Moreover, the collaboration with other international partners within different sectors of the Mediterranean Sea will help in collecting new tissue samples for investigating connectivity. As for now, we are collecting 2 samples from Turkey and 1 sample from Spain.

Methods.

We collected tissue samples from 4 individuals of YOY shortfin mako, caught as bycatch by a swordfish mesopelagic longline. We extracted DNA using the Qiagen DNeasy Blood & Tissue Kit, with the corresponding protocol, and then we verified the amount of DNA contained in each sample. We extracted in total four replicates of red muscle per sample, with a total of 16 resulting samples, which can be used both for sequencing and as a trial with existing generic primers (e.g. MiFish-U and MiFish-E, from literature) (Miya et al., 2020; Taguchi et al., 2011).

We also designed specific primers starting from the available sequence of the CYTB region from GenBank.

Expected/Obtained Results.

Samples will be sequenced for the mitochondrial DNA in order to possibly highlight differences in the Mediterranean sub-population, comparing the resulting sequences with existing ones from other sectors. Moreover, we will create a Mediterranean species-specific primer for eDNA detections. We will test existing primers (from Taguchi et al., 2011) designed to amplify the mDNA and also test primers specifically designed starting from the CYTB sequence.

WP 3. What is the ecological segregation of two lamnids- the shortfin mako and the white shark, in the Mediterranean Sea? What is the link with oceanographic variables?

Objective.

The data collected for both species can be refined with information at a higher resolution, in order to understand more about the habits and ecology of the white shark and mako shark and how they can overlap.

Methods.

The dataset of occurrences - for both the mako and the white shark, contains latitude/longitude coordinates that can be used to extract punctual values of oceanographic variables from Copernicus website.

Then I will carry an exploratory analysis to highlight interesting patterns on oceanographic preferences that can be used to understand if there are differences according season or life stage, and if they overlap between the two species, underlying which are the ecological interactions among two important lamnids of the Mediterranean Sea.

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Part 2. PhD student information on the overall year activity (courses/seminars/schools, mobility periods, participation to conferences)

List of attended courses/seminars/schools

1. Technology Transfer And Innovation – D. Iacobucci UNIVPM

2. Latex Informatic Course- F. Spinozzi UNIVPM

3. Assessing Oxidative Stress In Biological Systems- E. Damiani UNIVPM

4. Institutional Seminar SZN M. Pernice "Can algae support our transition to a sustainable bioeconomy?"

5. Institutional Seminar SZN J. Claudet "One Planet, One Ocean: Challenges for ocean conservation in the decade to come."

6.Communicating with Science - M. Signore SZN Course

7. Presenting with ZEN - M. Signore SZN Course

8. Basic notions in numerical biology and ecology – P. Licandro SZN Course

9. Institutional Seminar SZN A. D. Mazaris “Spatial prioritization for charismatic marine megafauna under climate change"

10. Institutional Seminar SZN S. Coelho “Brown algal development, reproduction and evolution”

List of periods spent abroad

1. Ferretti Lab, Virginia Tech- Department of Fish and Wildlife Conservation, 01/09/2023-28/11/2023
2. White Shark Chase – Tunisia and Lampedusa (04/05/2023-2/06/2023)

List of conferences/workshops attended and of contributions eventually presented

1. Workshop "LA RICERCA PUBBLICA ITALIANA A SUPPORTO DELLA CONSERVAZIONE DEGLI ELASMOBRANCHI DEL MEDITERRANEO" Naples, SZN, 28 Feb-1 Mar 2023

Part 3. PhD student information on publications

If not yet published, please indicate the publication status (submitted, accepted, in preparation...)

List of publications on international journals

J1. J.F. Jenrette, J.L. Jenrette, N.K. Truelove, S. Moro, N.I. Dunn, T.K. Chapple, A.J. Gallagher, **C. Gambardella**, R. Schallert, B.D. Shea, D.J. Curnick, B.A. Block, and F. Ferretti. "Detecting Mediterranean white sharks with environmental DNA." (2023) In Frontiers in Ocean Observing: Emerging Technologies for Understanding and Managing a Changing Ocean. Oceanography 36 https://doi.org/10.5670/oceanog.2023.s1.28

J2. Roveta C.; Pulido Mantas T.; Calcinai B.; Di Camillo C. G.; **Gambardella C.**; Gregorin C.; Coppari M.; Marrocco T.; Puce S.; Riccardi A.; Cerrano C. "Photogrammetry, from the land to the sea and beyond: a unifying approach to study terrestrial and marine environments", (2023) ISPRS Journal of Photogrammetry and Remote Sensing 10.3390/jmse11040759

J3. C. Cattano, **C. Gambardella**, D. Grancagnolo, E. Principato, G. Aglieri, G. Turco, F. Quattrocchi, M. Milazzo, "Multiple interannual records of young-of-the-year identify an important area for the protection of the Shortfin Mako, *Isurus oxyrinchus*" - Marine Environmental Research ***ACCEPTED***

List of publications on conference proceedings

List of other publications (books, book chapters, patents)

[14/10/2023]

Student signature

Supervisor signature