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PROJECT TITLE: Combining 'omics tools and passive acoustics: a new integrative approach to get at key processes of a marine soniferous predator exploiting trawling fishery and to infer its trophic spectrum

SCIENTIFIC BACKGROUND

Increased anthropogenic pressure as a consequence of the progressive and intensive use of coastal marine habitats has led to the need of identifying sensitive species as indicators of impacted/rapidly changing systems. Having strong preferences for shallow waters and well-known ecological importance in coastal ecosystems as a flexible top-predator (top-down effect), nutrient translocator (bottom-up effect), driver of behavioral-mediated processes, and "umbrella" species (Kiszka *et al*, 2022), the bottlenose dolphin (*Tursiops truncatus*, hereafter BD) is often used as a model to assess the status and detect changes in different coastal habitats worldwide.

The extreme BD adaptability allows it to consume different food resources depending on local availability, including the opportunistic exploitation of fisheries' activities (e.g. following bottom trawlers or hunting on nets set by small-scale fisheries; Pace *et al*, 2012). These interactions may result in a competitive overlap between harvested resources and dolphins' prey, and be mutually harmful, as nets depredation by dolphins can lead to economic loss, and dolphins can be lethally bycatched or injured (Li Veli *et al*, 2023). Nonetheless, foraging behind trawlers on dead/injured/disoriented prey entangled in the net remains a less demanding activity in terms of energy expenditure than other strategies (Pace *et al*, 2012).

The BD is classified as an "ecologically relevant" species for the European Marine Strategy Framework Directive (MSFD, 2008/56/EC). As suggested by Neri *et al* (2023), it would be desirable to formally include BD trophic ecology (especially opportunistic feeding during commercial trawling fishery) in the monitoring protocols of the MSFD to increase the knowledge of coastal biodiversity of an investigated area, address appropriate conservation measures and set effective management actions. BD is usually studied through surveys conducted by experts aboard research vessels, and methods such as passive acoustics and 'omics tools like environmental DNA (eDNA) are increasingly being used to improve the efficiency of the research effort. Both are extremely useful to investigate BD trophic spectrum when feeding behind trawlers as 1) BD is soniferous, highly vocal and expresses great acoustic variability/complexity during such opportunistic interaction (Rako-Gospić *et al*, 2021), and 2) eDNA metabarcoding can effectively infer fish diversity and bottom trawling catch composition (Maiello *et al*, 2022; Qu *et al*, 2020).
It follows that BD may even change its acoustic behaviour based on the presence/abundance of specific prey, although this phenomenon has been scarcely investigated until now.

In addition, the opportunistic feeding behind trawlers may alter the species' distribution within an area, thus requiring a modelling approach able to 1) produce crucial information for conservation, and 2) incorporate biotic factors, such as predator-prey interactions, as they seem to be just as important as abiotic factors in marine predators' distribution modelling (Roberts *et al*, 2023).

AIMS AND METHODOLOGY

Aims of this PhD project:

1) To test the efficacy of a novel integrated approach, consisting of the combination of passive acoustic monitoring (PAM) and eDNA sampling, to investigate BD trophic spectrum and the intensity of opportunistic feeding behind trawling vessels in relation to catch composition
2) To develop a model for the BD distribution which incorporates the potential prey abundance/diversity as inferred by the integrative eDNA metabarcoding and PAM approach

The following hypotheses will be tested:

1) eDNA metabarcoding can effectively detect BD presence in the trawling vessel areas while informing on catch composition
2) BD acoustic repertoire, and its expression pattern, change in relation to the potential prey inside/near the trawling net
3) Prey availability and diversity, together with environmental variables, is a good predictor of BD distribution in the area

Data will be collected on a BD geographic unit in the central Tyrrhenian Sea usually exploiting bottom trawling fishery (Pace *et al*, 2021,2022a,b), both onboard fishing vessels and a research vessel (in collaboration with DBA-Sapienza University) following trawlers operating in the area. In particular;

- eDNA will be sampled using the “metaprobes” developed by Maiello *et al* (2022), which will be 1) placed in the trawl nets and 2) towed by the research boat;
- BD acoustic emissions during interactions with trawling vessels will be recorded in boat-based surveys using a towed array of two hydrophones and a digital sound interface.

Data will be analyzed as follows:

- Samples will be metabarcoded using a specific mitochondrial 12S rRNA molecular marker, in order to obtain taxa composition of each sampling site. BD detections through eDNA will be then compared with visual and acoustic occurrences both from fishing and research vessels. The fourth root of the number of reads will be used as proxy of fish biomass
- Emission rates and acoustic parameters of different sounds in the acoustic repertoire will be measured in Raven Pro, imported in R using the Rraven package and then modelled through a functional analysis newly developed R interface (Labriola *et al*, unpublished) and other tools.
- Bottlenose dolphins’ distribution in the area will be modeled as suggested by Roberts *et al* (2023), by building both a generalized additive model (GAM) and a random forest model, with the probability of presence in each square cell the area is divided into as the dependent variable and the following independent variables: co-occuring fish

species/families' biomass, environmental parameters (e.g depth, SST, SSS, chlorophyll-a, distance from the coast, season-month). Predictive performance of the models will be assessed by means of the true skill statistics (TSS) and by measuring the area under the ROC curve, which is obtained by plotting true-positive rate (sensitivity) against the false positive rate (1-specificity).

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