BIOSSA Sphyrna Odyssey Mission: France to Brazil

Multimodal study of cetaceans using passive acoustics, satellite images and DNAe: culture and environment

Scientific Director:

- Pr. Hervé GLOTIN, Chaire IA, CNRS, LIS
  Université de Toulon
  Laboratoire LIS - CS 60584
  83041 Toulon cedex 9
  Phone : 04 94 21 54 42
  Email : h.glotin@gmail.com

- Pr. Renata SOUSA-LIMA,
  Federal University of Rio Grande do Norte, UFRN Lab, Rio Grande do Norte, Lagoa Nova, Natal - State of Rio Grande do Norte, Brésil
  Phone :
  Email : sousalima.renata@gmail.com

Offshore Operations Coordinator:

- M. Fabien DE VARENNE, Sailor, operations manager
  SEA PROVEN, ZI de Chaffenay
  53240 Saint-Jean-sur-Mayenne
  Phone : 07 60 03 20 63
  Email : fbv@seaproven.com

- M. Antoine THEBAUD, Coordinateur
  SEA PROVEN, ZI de Chaffenay
  53240 Saint-Jean-sur-Mayenne
  Phone : 06 79 19 09 61
  Email : at@seaproven.com

Project owner:

- Sara VIEIRA, Présidente et fondatrice de l'Association Sphyrna-Odyssey
  2 imp de la Croix du Sud
  56860 Séné
  Phone : 07 68 02 53 98
  Email : sara.erler@gmail.com

Sphyrna Odyssey Mission & 3D passive acoustic interoceanic survey

Drone 'Sphyrna' S70 (21m) and S55 (17m) in Villefranche Bay, for acoustic acquisition of cetacean signals during the Sphyrna Odyssey 2029-2020 mission.

1. Main goals

1.1. Introduction

Over 70% of our planet is covered by oceans and seas, and more than half of these bodies of water are more than 3,000 m deep. The oceans are the planet's largest biological environment, home to almost every group in the plant and animal kingdoms, from bacteria and microscopic algae to mammals. The oceans thus play an essential role in regulating the climate, balancing the water cycle, absorbing carbon dioxide from the atmosphere and producing oxygen, and are a major energy and food resource. Upwellings of cold, nutrient-rich ocean water drive high levels of primary productivity, in which zones of high zooplankton density form and provide rich feeding habitats for fish, seabirds and cetaceans (Butler et al., 2002; Croll et al., 2005; Joiris, 2011; Tynan et al., 2005; Baines & Reichelt 2014).

Marine mammals are among the most threatened vertebrates on earth, with 37% considered endangered by the IUCN (Albouy et al., 2020). However, monitoring marine mammals remains difficult, generally due to their low abundance, long-distance migration, large geographic range and elusive behavior (Hays et al., 2016). Whales are an important indicator of ecosystem health. They play an essential role in the structure, maintenance and balance of marine food webs.

The ocean is not homogeneous; it is made up of oceanic fronts and eddies where cetaceans nest. Recent work (Cotté 2011; Glotin 2020) shows that oceanic eddies and associated structures (fronts, filaments) have a strong influence on predator activity through enhanced marine productivity, in zooplankton and fish communities, as well as in squid communities. Some cetacean species are thought to use the physical structures of seawater to optimize their approaches to prey capture. This research is essential to better understand the distribution of these superpredators as a function of currents, water column stratification, bathymetry and seasons. Chlorophyll A, temperature, salinity and ocean currents are among the fundamental parameters for determining ocean fronts, key to ocean biodiversity. Variations in temperature, salinity and other parameters in marine ecosystems lead to rapid changes in the chemical, physical and biological composition of organisms (Otsuka 2016). It should be noted that variations in these environmental parameters are linked to seasonality. Temperature is a fundamental parameter for assessing the characteristics of water masses, as it plays a critical role in biological cycles as well as in sound speed variability. In addition, plankton blooms are caused by high nutrient inputs; wind stress along the continents causes upwelling of cold (oxygen-rich), nutrient-laden waters, leading to the development of greater marine biomass. Since this phenomenon occurs seasonally, the combination of favorable conditions requires nutrient-rich, well-oxygenated water with sufficient light energy for photosynthesis to occur (Rai 2014).

Management and conservation issues are addressed through the identification of areas of particular importance, which requires the acquisition of basic information by various methods on species distribution and dynamics. Indeed, Cetaceans comprise one of the taxonomic groups that lack basic information, mainly regarding their ecological function in the aquatic ecosystems (Silva et al., 2021), making it difficult to establish effective conservation plans and mitigation strategies in the face of environmental impacts (Zerbini et al. 2004; Ott et al. 2009; Siciliano et al. 2012).

Using complementary and effective tools to detect and monitor threatened, rare marine mammal species is essential to better guide their conservation (Pikitch, 2018). Gathering information from bioacoustics, satellite images, environmental DNA (eDNA) and chemical measurements, based on data collection obtained during the "Sphyrna Odyssey" oceanographic missions, gives us a better understanding of ocean health. This knowledge is essential to help conserve marine biodiversity. The combination of the four above-mentioned approaches is necessary, as they are complementary and operate at different scales.

These types of data can be difficult to obtain for cetaceans, given that oceanographic missions could be difficult to access, certain methods notably passive acoustics can be limited by weather conditions in certain areas (acoustic signal masked by noise), and individual detection by satellite image due to cloudiness, wave reflections and foam ridges, and these missions are logistically costly.

The passive acoustic approach has been increasingly used since the 1970s, as it enables the presence and abundance of cetaceans to be detected, the species to be identified and located, individuals to be tracked over a long period, connectivity between populations to be quantified, seasonal distribution patterns to be determined, the risk of collisions to be reduced, groups to be recognized by regional dialects, and behavior to be studied and behavioral questions to be answered using ethoacoustics. Marine fauna use the whole range of acoustic waves to detect and locate obstacles and prey, or to communicate between individuals.
This approach has the advantage of being non-intrusive, and thanks to the 'Sphyrna' sub-surface marine drones - each equipped with hydrophones for acoustic surveys - allows access to remote, offshore areas where it is difficult to carry out surveys. The acoustic recording system records the underwater sound environment within a radius of 6,000 meters down to a depth of 2,000 meters. In addition, the high stability of the UAVs reduces noise disturbance to on-board instrumentation, and they have long autonomy, enabling acoustic observations to be made over long periods of time in a given area.

Satellite data obtained by the European Space Agency (ESA), the European Copernicus program, provides unique environmental monitoring. They provide an effective means of monitoring ocean conditions, such as surface chlorophyll-a levels, sea surface temperatures (SST), temperature gradient and ocean currents, which serve as indicators of cetacean habitat quality (Burtenshaw et al., 2004). Satellite-derived chlorophyll-a concentrations reveal the spatial and temporal variability of algal blooms, in order to identify areas of interest for mysticetes whose main food source is plankton. Furthermore, the use of the highest resolution satellite imagery will enable cetaceans to be counted at the water's surface from space, and greatly improve the effectiveness of whale census methods for assessing species distribution, abundance and movement from satellite imagery.

Furthermore, all species inevitably leave genetic traces in their environment, and the resulting environmental DNA (eDNA) reflects the species present in a given habitat. eDNA metabarcoding; based on the recovery of DNA naturally released by organisms into their environment, is increasingly used to detect micro- and macro-organisms in aquatic environments (Ruppert et al., 2019). More case studies are needed, however, to demonstrate its ability to detect invisible, elusive, threatened and rare species in marine ecosystems (Juhel et al., 2020).

The combination of environmental DNA tools and the acoustic approach makes it possible to monitor the abundance and distribution of species and to identify cetaceans. Indeed, eDNA can identify the presence or absence of species, but depends on oceanic conditions (strong currents, winds, etc.), while passive acoustics gives an instantaneous measurement within a radius of a few km for low frequencies. These two approaches are therefore complementary for passive, long-distance cetacean monitoring.

1.2. Context and main goals

Launched in 2018, the main objective of the oceanographic missions 'Sphyrna Odyssey' is to promote a better knowledge of the oceans (including listening and monitoring of cetaceans) from new exploration tools that combine the use of autonomous surface vessels developed by the company SEAPROVEN and passive listening technologies developed by the laboratory LIS of the University of Toulon.

These particularly innovative scientific missions have already allowed the realization of many high-quality missions in the Mediterranean Sea, in 2018, 2019 and 2020, in a vast maritime space located between Genoa and the Balearic Islands, along the Ligurian current and on large submarine canyons, privileged hunting areas for deep-sea diving cetaceans. A strong point of this device is to produce 3D representations of abyssal activities of large sounders, as here sperm whales "drilling" during abyssal waters, on January 14, 2020 off Monaco (**Fig. 1**, Glotin et al., 2020).

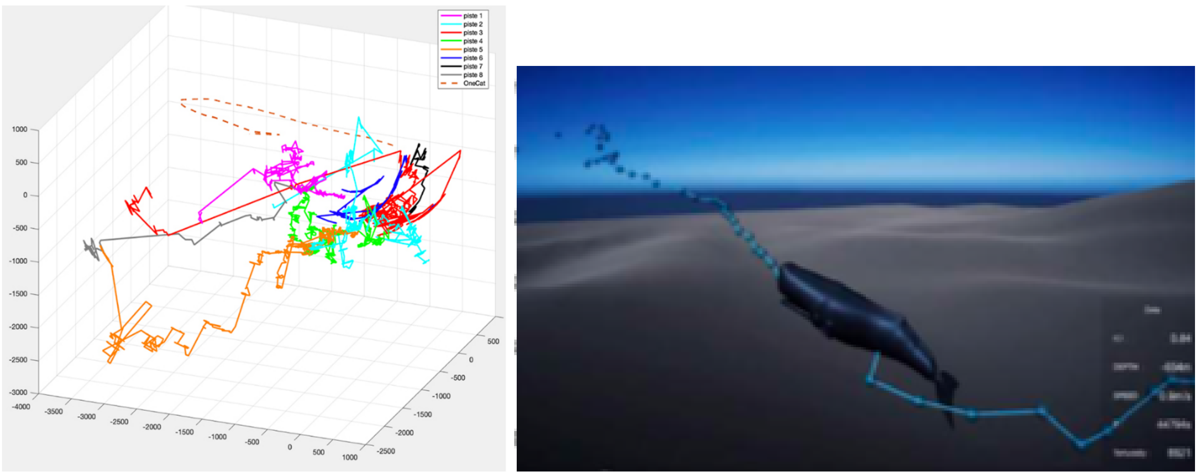


Figure 1. *3D tracks of 5 or 7 sperm whales off Monaco, during 2 hours, hunting in collaboration in the abysses (-1000 to -2000m), a color = a probe, the boat is on the surface (axes in m) and illustration of a sperm whale trajectory calculated by the LIS from Sphyrna signals on the surface in August 2018* (Glotin et al., 2020).

This innovative tracking technique, in 3 dimensions and continuously, allowed for very rich recordings, notably for their frequency quality, but also because a large part of the listening was done in winter over long periods of time.

Placed under the scientific direction of Professor Hervé Glotin (Toulon University, France) and Professor Renata Sousa-Lima (Rio Grande da Norte University, Brazil) world-renowned specialists in underwater bioacoustics, our missions are dedicated to the bioacoustic monitoring of cetaceans, to the collection of a wide range of scientific data as well as to the validation of various protocols in the field of the Sea-Space relationship. The oceanographic mission 'Sphyrna Odyssey' in Madeira, Canary Islands, Cape Verde and in Brazil will be mainly dedicated to the bioacoustic monitoring of cetaceans and to the detection of their vocal productions (vocalizations, codas, whistles) - emitted in different frequency ranges -, from a Sphyrna (Autonomous Laboratory Vessel - ALV), - equipped with 5 hydrophones (H1 to H5) for the acoustic surveys

We will complement the acoustic measurements with environmental DNA sampling (identification of cetacean species), with the capture of chemical parameters (T°C, chlorophyll a, salinity) and with satellite imagery protocols (environmental parameters: chlorophyll a, T°C and detection of cetaceans: distribution and abundance, and determination of cetacean areas of interest), in order to better understand the distribution of living organisms in their environment. The objective is also to correlate spatial and surface observations to assess the marine environment and cetacean populations in order to finely study cetacean behavior (life cycles) as a function of oceanographic structures, bathymetry and environmental parameters.

2. **Mission Planning**: Total transect from France to Brazil

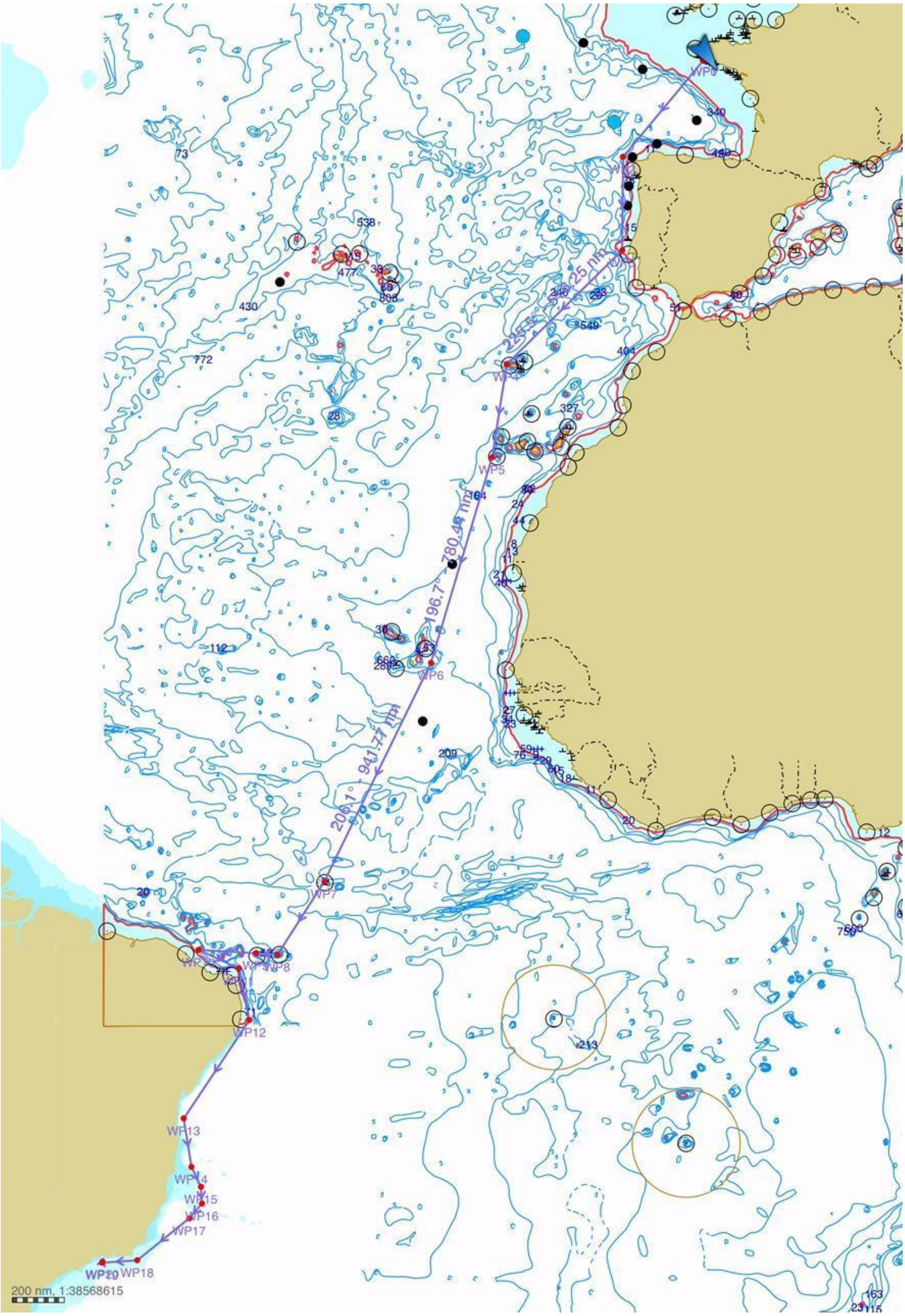


Figure 2. *Total transect from France to Brazil with stopovers in Brittany, Madeira, Canaries Islands and Cape Verde.*

The waters of Brittany, Madeira, Canary Islands and Cape Verde are considered a hotspot for marine biodiversity, especially regarding cetacean species. Indeed, the variations in the oceanographic, hydrological, and topographic features of the oceans create a wide heterogeneity of habitats, favoring the high diversity of cetaceans that is observed in the Northeast Subtropical Atlantic Ocean (Baines & Reichelt 2014). However, there is little information about the relative abundance of each species in the different parts of the Macaronesian archipelago (Moore et al., 2003).

We will carry out a mission in Brittany to test the two 'Sphyrna S20' UAVs by making acoustic acquisitions (Study location, **Appendix 2**), followed by four different missions:

Mission 1 in Macaronesia, in the archipelagos of Madeira and the Canaries.

Mission 2 in Macaronesia in the Cape Verde archipelagos.

Mission 3 in North Brazil

Mission 4 in South Brazil

For all four missions, we will be carrying out acoustic acquisitions to study cetaceans, environmental DNA sampling, and chemical measurements of the water (chlorophyll a, T°C, pH, salinity).

2.1. Mission Sphyrna 1

In Madeira Island, at least 9 species, were found to use the Madeiran waters on a regular basis, such as the Atlantic spotted dolphin (*Stenella frontalis*), the short-beaked common dolphin (*Delphinus delphis*), the bottlenose dolphin (*Tursiops truncatu*s), and others featured in the Red List of the International Union for Conservation of Nature as Endangered, Vulnerable, and Data Deficient. In addition, 10 species were found to use the Madeira waters for travelling, feeding, resting, socializing and calving, which suggests that the southern and southeastern waters of Madeira Island constitute an area of interest for cetaceans (**Fig. 3-6**, Alves et al., 2018)

At present, 30 species of cetaceans (Jefferson et al., 2015), have been recorded in the Canary Islands (23 odontocetes and 7 mysticetes). The Canary Islands are an oceanic volcanic archipelago in the Northeast Subtropical Atlantic Ocean, comprising eight islands with a total surface area of 7,273 km2, a coastline of approximately 1,581 km, and an exclusive economic zone (EEZ) of approximately 494,192 km2.

The complex oceanographic characteristics of the Canary Islands are determined by a combination of factors, for example, the filaments—nutrient-rich waters—which originated in the upwelling system of the Northwestern African shores (Cape Juby, Cape Ghir, and Cape Bojador) that reach the Canary Islands. These filaments have an essential biological function, as they transport fish, cephalopods, and crustacean larvae—food for marine mammals—from the African coast to the coastal waters of the Canary Islands (Rodríguez et al., 1999, 2004; Bécognée et al., 2009; Baines & Reichelt 2014; Landeira et al., 2017).

Long-term monitoring can provide essential information for identifying areas of high marine mammal abundance, as well as key information on their resident or migratory status in the Canary and Madeira Islands. In this sense, it seems essential to monitor cetacean species around these

Islands, as well as to promote new initiatives to protect migratory routes and marine ecosystems (Baines & Reichelt 2014).

2.1.1. Study sites in Madeira

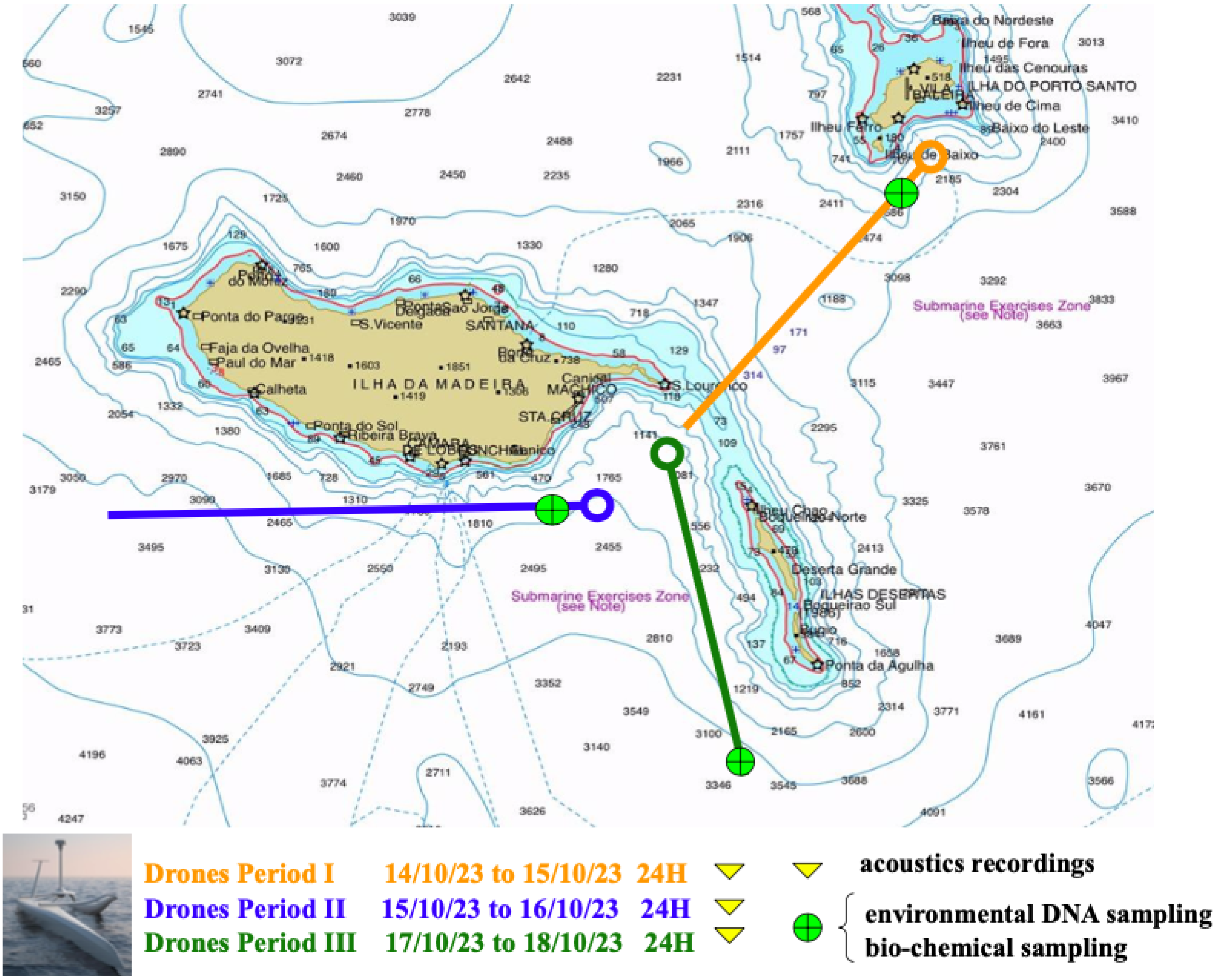


Figure 3. *Map of the Madeira archipelagos with Madeira Island, Porto Santo Island (North of Madeira Island) and Desarta Grande Island (South of Madeira archipelago), 5 days of data acquisition.* The trajectories of the Sphyrna drones for acoustic acquisitions correspond to the orange, blue and green lines. DNA sampling and chemical measurements are represented by a green circle.

2.1.1.1. Presence and distribution of cetaceans in Madeira

Table 1. *Updated list of cetacean species recorded in the waters of the archipelago of Madeira. Classification according to* Rice (1998) *and common names according to approved IWC list. Type of record: S - sighting; C - catch - stranding. First recorded: first record (year) according to the first published scientific or historical reference that reports the species; references in brackets are non-peer-reviewed. Last recorded: last known record (year) of the species based on all types of data until December 2012. Symbols legend: * new confirmed record; + new record based only on visual observations; ! species described in previous years for Madeira archipelago* (Freitas et al., 2012).

	Scientific name	English name	Type of record	First published scientific or historical reference	First recorded	Last recorded
	Sub-order Mysticeti					
	Family BALAENIDAE					
!	*Eubalaena glacialis* Müller, 1776	Northern Atlantic right whale	C	Maul & Sergeant (1977)	1967[1]	1967
	Familia BALAENOPTERIDAE					
*	*Balaenoptera musculus* (Linnaeus, 1758)	Blue whale	S	this note	1964	2009
!	*Balaenoptera physalus* (Linnaeus, 1758)	Fin whale	S, C, ST	Gordon *et al.* (1995)	1990[2]	2012
*	*Balaenoptera borealis* Lesson, 1828	Sei whale	S	this note	2002	2011
*	*Balaenoptera acutorostrata* Lacépède, 1804	Common minke whale	S, ST	this note	1956	2010
!	*Balaenoptera edeni* Anderson, 1878	Bryde's whale	S, ST	Alves *et al.* (2010)	2005[3]	2012
*	*Megaptera novaeangliae* (Borowski, 1781)	Humpback whale	S, C	this note	1957	2012
	Sub-order Odontoceti					
	Family PHYSETERIDAE					
!	*Physeter macrocephalus* Linnaeus, 1758	Sperm whale	S, C, ST	Townsend (1935)	≤1920[4]	2012
	Family KOGIIDAE					
	Kogia breviceps (Blainville, 1838)	Pygmy sperm whale	S, C, ST	Maul & Sergeant (1977)	1941	2012
*	*Kogia sima* (Owen, 1866)	Dwarf sperm whale	ST	this note	2000	2000
	Family ZIPHIIDAE					
*	*Ziphius cavirostris* G. Cuvier, 1823	Cuvier's beaked whale	S, ST	this note	1992	2011
!	*Mesoplodon bidens* (Sowerby, 1804)	Sowerby's beaked whale	C, ST	Maul & Sergeant (1977)	1941	2012
	Mesoplodon densirostris Blainville, 1817	Blainville's beaked whale	S, ST	Harmer (1924)	1917	2012
*	*Mesoplodon europaeus* (Gervais, 1855)	Gervais' beaked whale	ST	this note	2007	2007
+	*Hyperoodon ampullatus* (Forster, 1770)	Northern bottlenose whale	S	this note	2001	2008
	Family DELPHINIDAE					
!	*Orcinus orca* (Linnaeus, 1758)	killer whale	S	(Sarmento, 1948), Hammond & Lockyer (1988)	≤1948	2012
	Globicephala macrorhynchus Gray, 1846	Short-finned pilot whale	S, C, ST	(Sarmento, 1936; Nobre, 1938), Fraser (1950)	1936	2012
+	*Globicephala melas* (Traill, 1809)	Long-finned pilot whale	S	(Pais, 1993), this note	1984	1993
*	*Pseudorca crassidens* (Owen, 1846)	False killer whale	S, ST	this note	1997	2011
	Steno bredanensis (G. Cuvier *in* Lesson, 1828	Rough toothed dolphin	S, C	Reiner (1981)	1968	2012
*	*Grampus griseus* (G. Cuvier, 1812)	Risso's dolphin	S, ST	(Sarmento, 1948), this note	≤1948	2012
	Tursiops truncatus (Montagu, 1821)	Bottlenose dolphin	S, ST	(Sarmento, 1936), Maul & Sergeant (1977)	1933-34	2012
	Stenella frontalis (G. Cuvier, 1829)	Atlantic spotted dolphin	S, ST	Freitas *et al.* (1998)	1997	2012
!	*Stenella coeruleoalba* (Meyen, 1833)	Striped dolphin	S, ST	Freitas *et al.* (1998)	1995[5]	2012
	Delphinus delphis Linnaeus, 1758	Common dolphin	S, ST	(Sarmento, 1936), Maul & Sergeant (1977)	1932	2012
*	*Lagenodelphis hosei* Fraser, 1956	Fraser's dolphin	S	this note	2003	2010
+	*Peponocephala electra* (Gray, 1846)	Melon-headed whale	S	this note	2005	2005
*	*Feresa attenuata* Gray, 1874	Pigmy killer whale	S	this note	2009	2009
	Family PHOCOENIDAE					
!	*Phocoena phocoena* (Linnaeus, 1758)	Harbour porpoise	C	(Sarmento, 1936), Matias (1988)	1905	1905

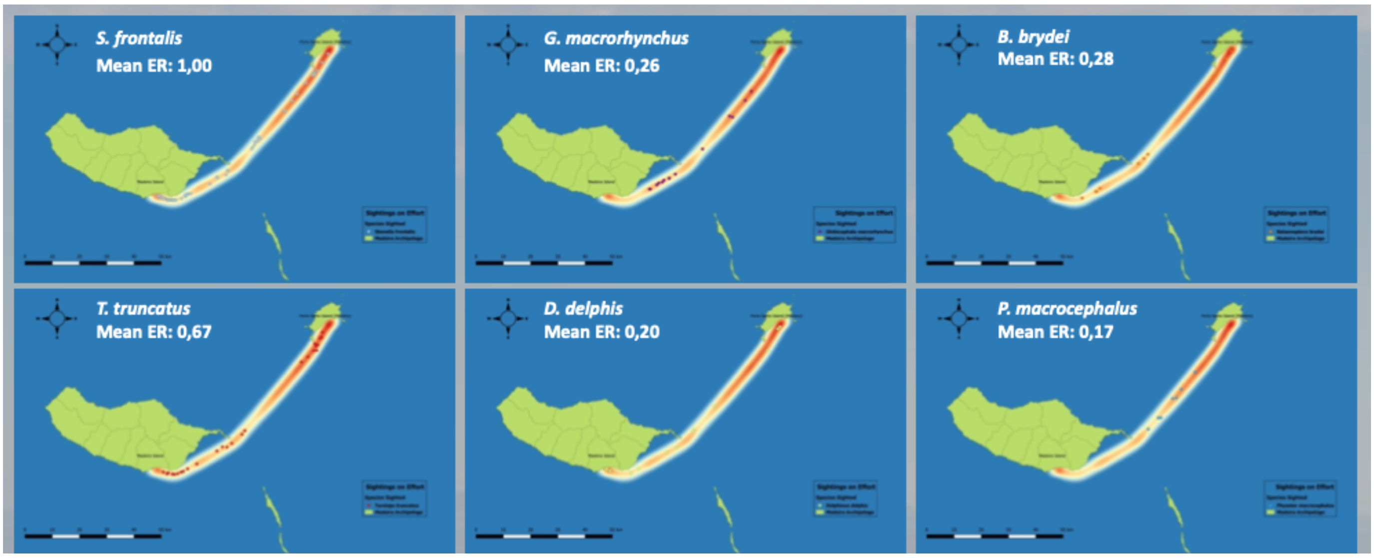


Figure 4. *High presence and distribution of cetaceans between Madeira and Porto Santo.*

Furthermore, the waters between Madeira and Porto Santo show a relatively high cetacean presence and distribution between these two islands **(Fig. 4)**. This area is also reported as a higher used corridor for vessels (Cunha 2013), it might represent a "sensitive" zone of potential presence for cetaceans.

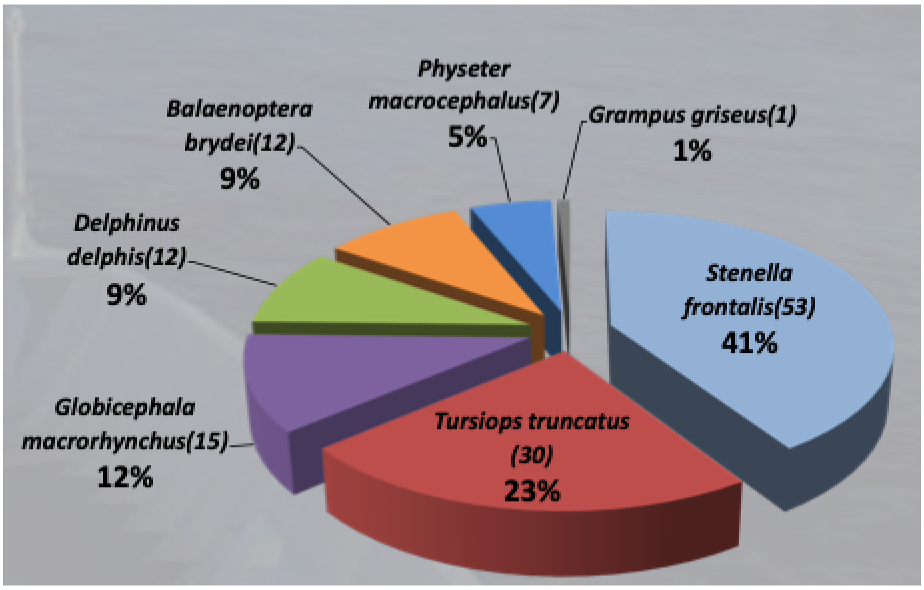


Figure 5. *Percentage ratio of sighted species* (Sambolino et al., 2017).

2.1.2. Study sites in Canary Islands

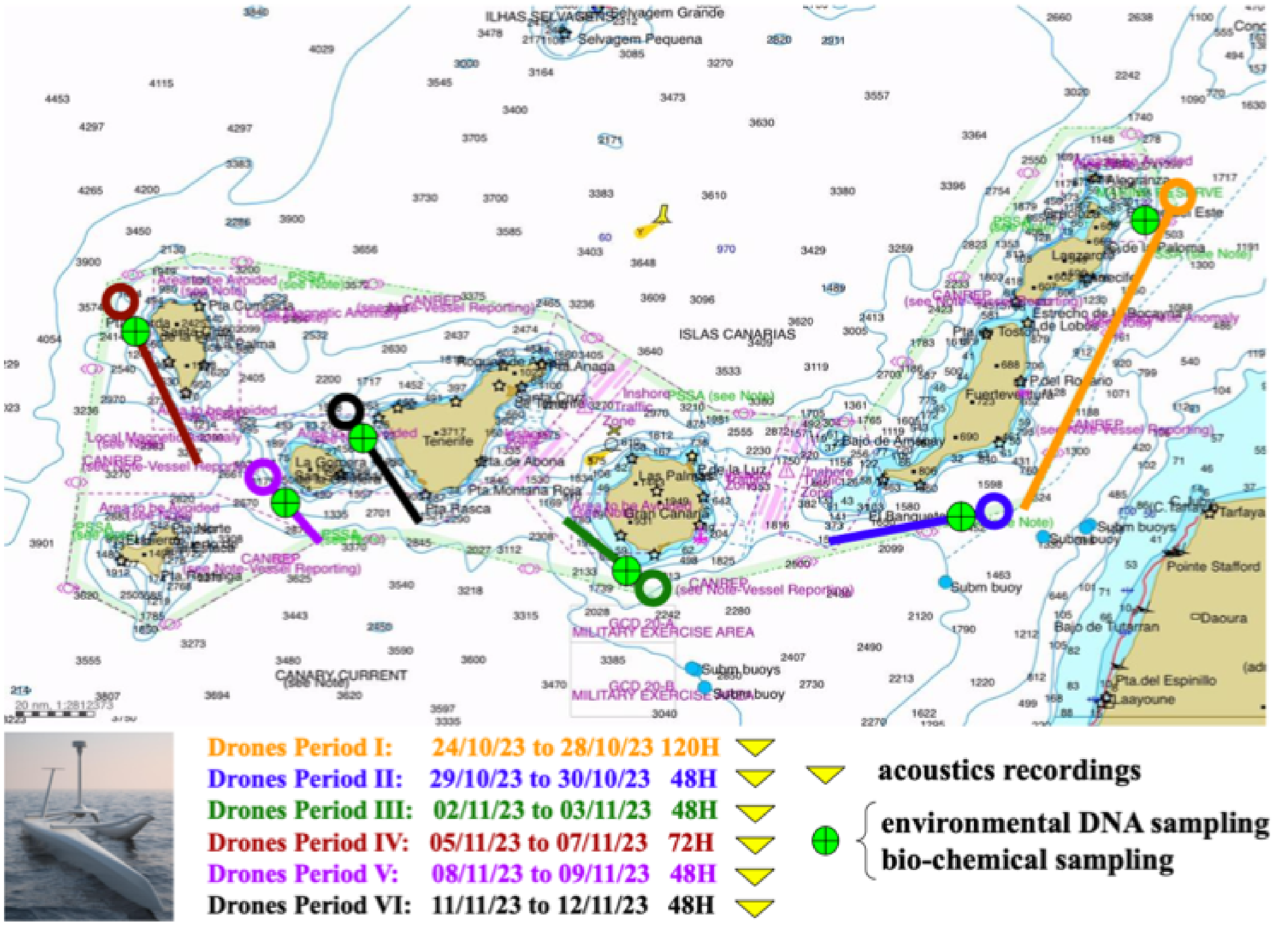


Figure 6. *Map of the Canary archipelagos with Fuerteventura, La Gomera, Grande Canarie, El Hierro, Lanzarote, La Palma, Tenerife, 19 days of data acquisition.* The trajectories of the Sphyrna drones for acoustic acquisitions correspond to the orange, blue, green, red, purple and dark lines. DNA sampling and chemical measurements are represented by a green circle.

2.1.2.1. Cetaceans distribution and environmental variables in Canary

The temporal variation of the total cetacean sightings per unit effort (SPUE; sighting/surveyed days) **(Fig. 7)** show that Odontoceti are the dominant cetacean species in Canarian waters than Mysticeti *(Herrera et al., 2021)*. The four most frequently sighted cetacean species were three odontocetes (*G. macrorhynchus, T. truncatus, and S. frontalis*) and one Mysticeti (*Balaenoptera edeni*) *(Herrera et al., 2021).*

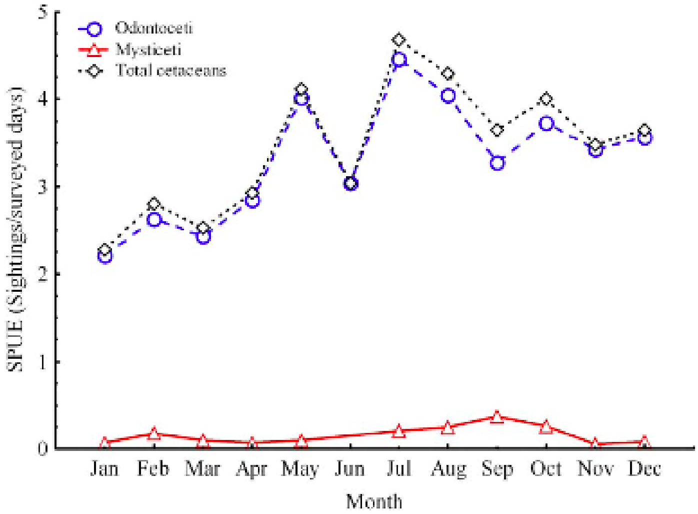


Figure 7. *Temporal variation of total cetacean sightings per unit effort (SPUE; sighting/surveyed days) in the Canary Islands during the study period (Jan. 2007–Dec. 2018). Black line: Total cetacean sightings; blue line: Odontoceti; red line: Mysticeti* (Herrera et al., 2021).

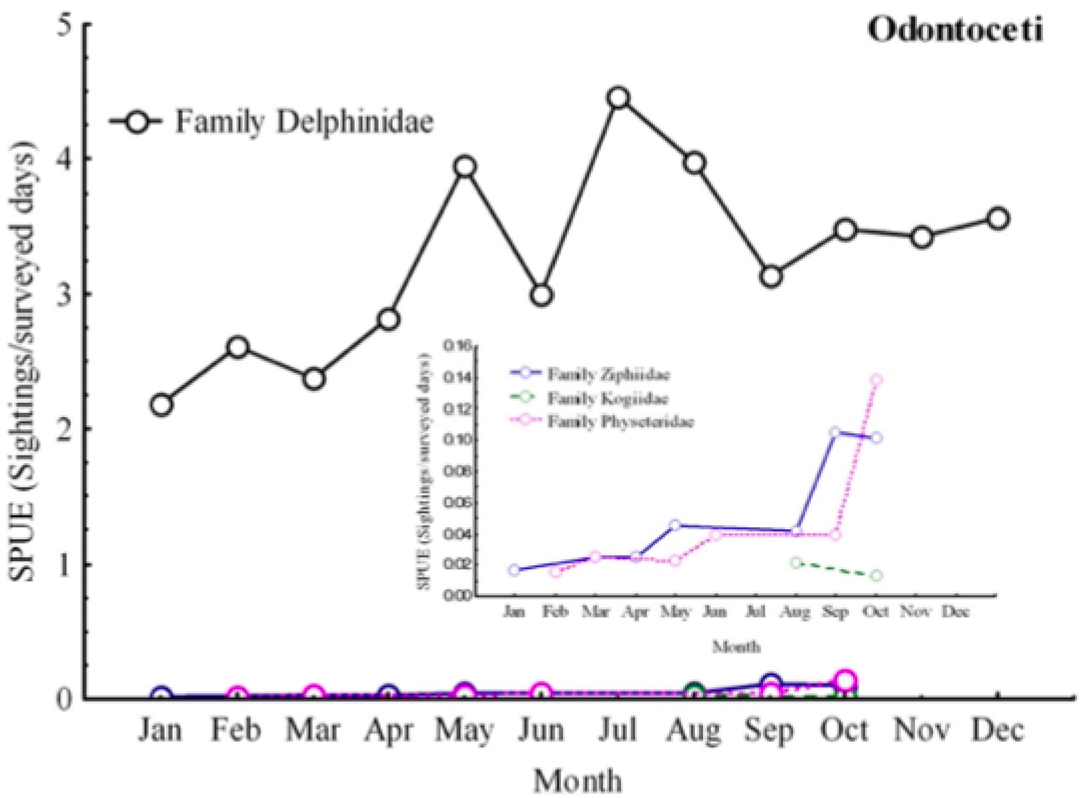


Figure 8. *Temporal variation of total Odontoceti sightings per unit effort (SPUE; sighting/surveyed days) in the Canary Islands during the study period (Jan. 2007–Dec. 2018). Blue line: Family Zipphiidae; green line: Family Kogiiidae; pink line: Family Physeteridae; black line: Family Delphinidae* (Herrera et al., 2021).

The Delphinidae (G. macrorhynchus, T. truncatus, and S. frontalis) is the most frequently sighted cetacean family, with a minor presence for species belonging to other Odontoceti families **(Figure 8)**.

Regarding the SPUE of the sub-order Mysticeti in Canarian waters, only one family, Balaenopteridae, has been observed. In addition, very few species have been observed throughout the different months, *Balaenoptera edeni* being the main species observed with a high SPUE during the summer months **(Figure 9)**.

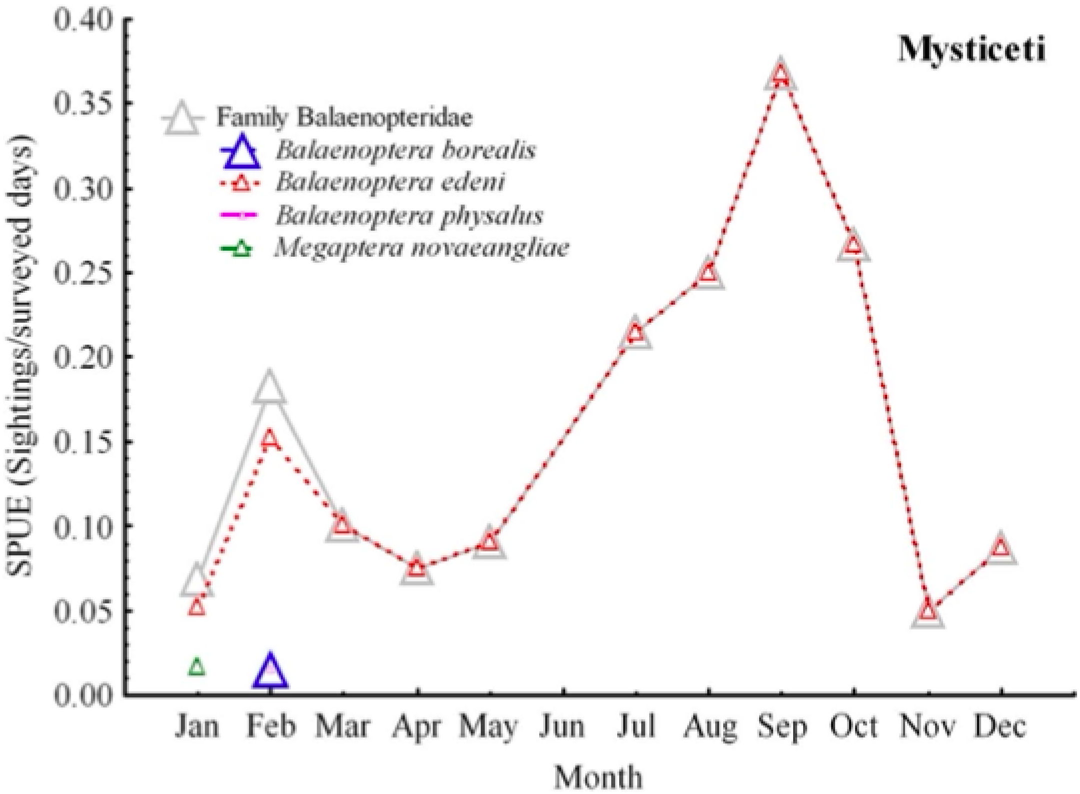


Figure 9. *Temporal variation of total cetacean sightings per unit effort (SPUE; sighting/surveyed days) in the Canary Islands during the study period (Jan. 2007–Dec. 2018). Gray triangle: Family Balaenopteridae; blue triangle: Balaenoptera borealis; red triangle: Balaenoptera edeni; pink triangle: Balaenoptera physalus; green triangle: Megaptera novaeangliae* (Herrera et al., 2021).

In terms of spatial distribution, 18 species of cetaceans are seen around the Canary Islands (Figure 6). These animals can be sighted frequently in areas close to the coast, mainly on the leeward side of the islands, coinciding with warmer temperatures (SST; ◦C) and high chlorophyll-a values (Chl-a; mg·m−3).

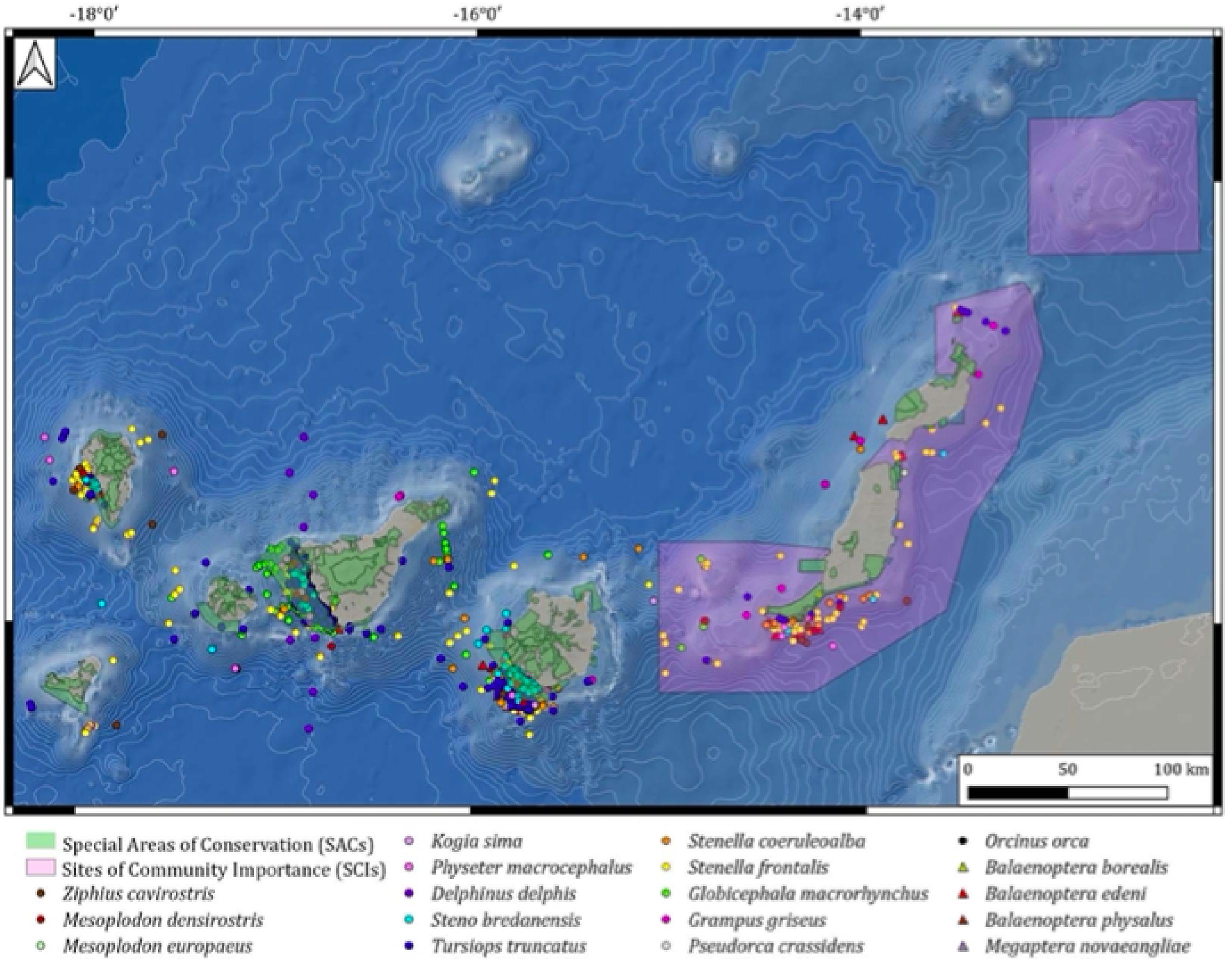


Figure 10. *Cetacean spatial distribution around the Canary archipelago (Odontoceti: circles; Mysticeti: triangles), including the Special Areas of Conservation (SACs; green color) and the Sites of Community Importance (SCIs; pink color)* (Herrera et al., 2021).

Of the 18 species of cetaceans in Canary waters, ten are labeled as "Least Concern," four as "Data Deficient," two as "Vulnerable," one as "Near Threatened," and one as "Endangered" in the International Union for Conservation of Nature (IUCN) red list (Supplementary Table 1; IUCN, 2020). Looking at the four most frequently sighted species in the Canary Islands waters, the odontocetes G. macrorhynchus, T. truncatus, and S. frontalis, and the mysticete Balaenoptera edeni are labeled as "Least Concern (LC), unlikely to become extinct in the near future" in the IUCN; however, the species T. truncatus and G. macrorhynchus, at the national level (Spain) and at the regional level (Canary Islands), are labeled as "Vulnerable." *(Herrera et al., 2021).*

2.2. Mission Sphyrna 2

The Cape Verde Islands **(Fig. 11)** area archipelago consisting of 10 islands and several islets situated in the Atlantic Ocean c. 500 km west of Senegal, West Africa. The total land area is 4033 km2. These volcanic islands emerge steeply from depths of about 4000 m. The climate is dry and tropical, but conditions are strongly influenced by the cool Canary current from the north, and strong northeasterly crosswinds blow constantly at sea. The winds cause rough seas and often make navigation around the Cape Verde islands difficult and hazardous. This explains, in part, the lack of information about cetaceans occurring in the archipelago (Reiner et al., 1996).

More than 17 species of whales and dolphins are reported (Reiner et al., 1996; Jann et al., 2003, Moore et al., 2003). It seems that the humpback whale (*Megaptera novaeangliae*) population of North Atlantic use the Cape Verde archipelago as a breeding area (Jann et al., 2003). Sperm whales are still fairly common around the island of Cape Verde (Moore et al., 2003).

2.2.1. Study sites in Cape Verde

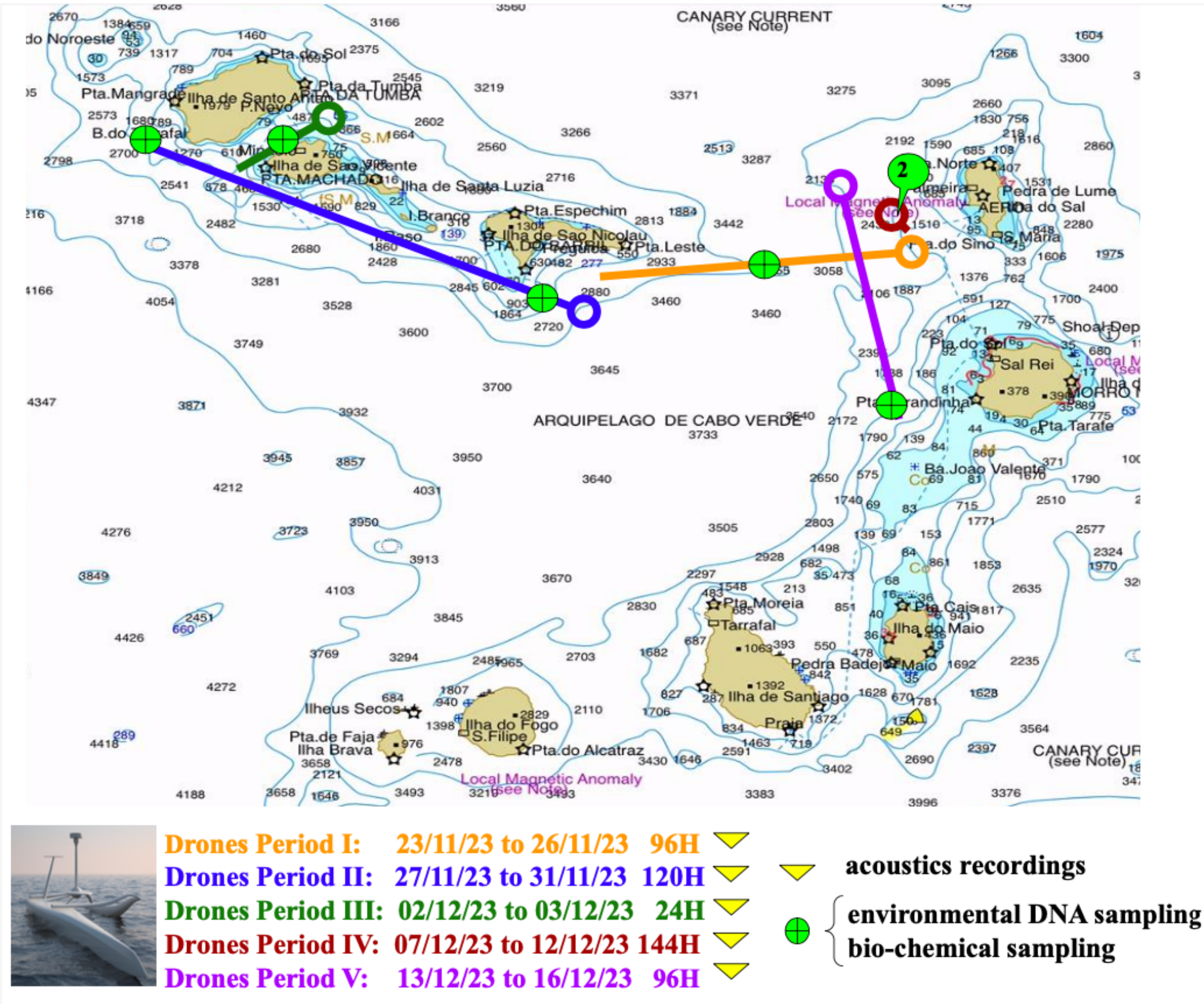


Figure 11. *Map of the Cape Verde Islands Archipelago with Boa Vista, Sal (Humpback calving area in the Bay of Mordeira), Sao Nicolau, Sao Vicente and Santa Antao, 25 days of data acquisition*

The trajectories of the Sphyrna drones for acoustic acquisitions correspond to the orange, blue, green, red and purple lines. DNA sampling and chemical measurements are represented by a green circle.

2.2.1.1. Presence and distribution of cetaceans in Cape Verde

Table 2. *Number of acoustic detections and sightings, and sighted group size ranges, for each cetacean species detected in the Cape Verde region between 7 November and 27 December 2000 (Moore et al., 2003).*

Name	Common name	Species code for Figure 2	No. acoustic detections	No. sightings	Group size range for sightings
Stenella frontalis	Atlantic spotted dolphin	Sf	0	5	3–50
Tursiops truncatus	Bottlenose dolphin	Tt	0	2	10–300
Balaenoptera physalus	Fin whale	Bp	0	7	1–12
Megaptera novaeangliae	Humpback whale	Mn	0	1	1
Globicephala sp.	Pilot whale	Gsp	0	4	1–40
Stenella attenuata	Pantropical spotted dolphin	Sa	0	2	30–100
Steno bredanensis	Rough-tooth dolphin	Sb	0	1	15
Physeter macrocephalus	Sperm whale	Pm	16	25	1–3
	Unidentified dolphin	Ud	11	30	1–3
	Unidentified small whale	Usw	0	2	1

Species detections are summarized in Table 2.
On passage to the Cape Verde Islands from La Gomera, Canary Islands, 180 miles north-east of the island of Sal on 17 November 2000, rough-toothed dolphins (*Steno bredanensis*) were observed once (Moore et al., 2003)..

Around the islands, pantropical spotted (*Stenella attenuata)* and Atlantic spotted (*S. frontalis*) dolphins were also identified, although their distinction is difficult in the field. They were found primarily in waters <500 m deep. Bottlenose dolphins (*Tursiops truncatus*) were observed once on the NW tip of the island of Santiago. Pilot whales (*Globicephala sp.*) were observed to the west and south of the island of Sal. On the basis of a previous report (*Hazovoet & Wenzel, 2000*), these were likely to be short-fin pilot whales (*G. macrorhynchus*), but since both this species and long-fin pilot whales (*G. melas*) have been reported from strandings in the region we cannot rule out *G. melas* (Moore et al., 2003).

The most common species detected around the Cape Verde Archipelago was the sperm whale. These were commonly found in waters deeper than 500 m, north and west of Sal, and Boavista, and between Boavista and São Nicolau (Moore et al., 2003).

Fin whales were sighted in coastal waters just NW of the island of Boavista. Seven sightings of groups ranging in size from one to twelve individuals, were made from *Rosita* on 14 December 2000 and 15 December 2000 in a water temperature of 23 to 25°C and a depth of 50 to 60m. The fin whale sightings all occurred between 16.19° and 16.30°N, and 22.92° and 22.97°W (Moore et al., 2003).

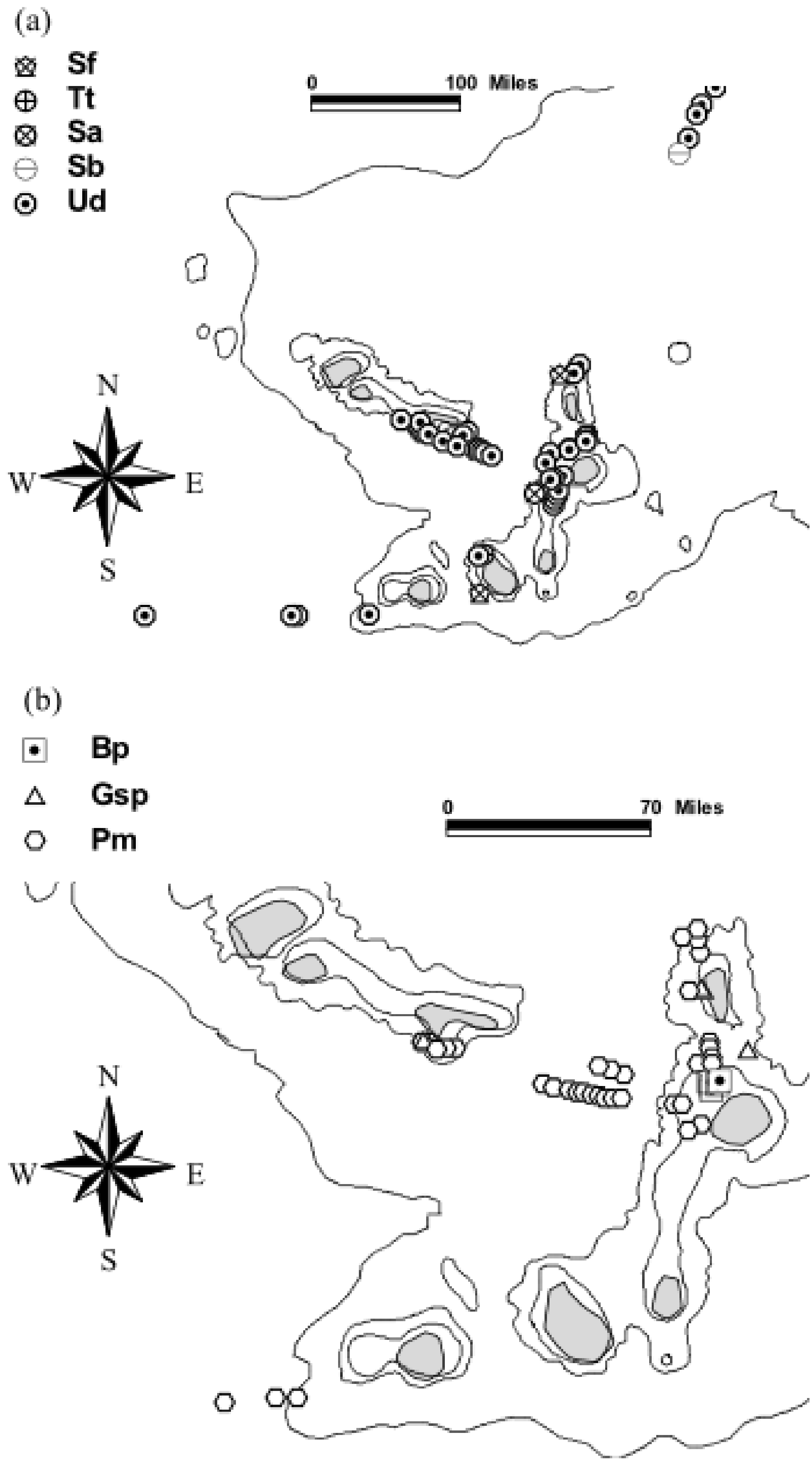


Figure 12. *Cetacean observations in Cape Verde waters between 7 November and 27 December 2000: (a) dolphins, (b) whales. See Table 1 for species key* (Moore et al., 2003).

2.3. Mission Sphyrna 3

Brazil has one of the world's most extensive coastlines, spanning almost 8000 km (Ab'Saber 2001). The biological diversity of these ecosystems has been substantially impacted by increasing anthropogenic changes in the marine, and coastal regions, threatening the survival of many species and even entire communities (Amaral and Jablonski 2005; Costa et al. 2005).

Knowledge about the existing cetaceans diversity in the coastal, and oceanic regions of Brazil is essential to understand the functioning of its different ecosystems, as well as to ensure the conservation of their living resources (e.g., Longo and Amado Filho 2014). The current knowledge about the cetaceans in these regions is still insufficient to guarantee their conservation, although there have been important research programs in the Brazilian oceanic regions in the last decades (e.g., REVIZEE, Archipelago Program and Oceanic Islands).

Brazilian waters are a remarkable cetacean diversity (>50% of the global diversity). Currently, there are confirmed records of 47 cetacean species in Brazil, out of the 90 that are recognized worldwide (Pinedo et al. 1992, 2002; Zerbini et al. 1997, 2004; ICMBio 2011a, 2011b; Hrbek et al. 2014; Cypriano-Souza et al. 2016; Bastida et al. 2018). Eight of them are classified as threatened, and eight are considered "data deficient" (DD) in the Brazilian Red List (ICMBio 2018). Moreover, six species are classified as globally threatened and 12 as "data deficient" by the IUCN (2020).

Many factors can influence the structure of cetacean communities. Cetacean distribution has been related to several environmental factors such as depth, slope, sea surface temperature, abundance of prey and others (*e.g.*, Selzer & Payne, 1988; Baumgartner, 1997; Jaquet & Gendron, 2002; Rossi-Santos et al., 2003). The habitat requirements of each species, allied with other aspects of their biology (*e.g.*, diet), may determine how different species partition their habitat. Understanding the differences on spatial and habitat use among the species of a community of marine predators may help plan strategies of conservation of their habitats, such as marine protected areas zoning, fisheries regulation and others (Rossi-Santos et al., 2003).

2.3.1. Focus on the study areas in Brazil

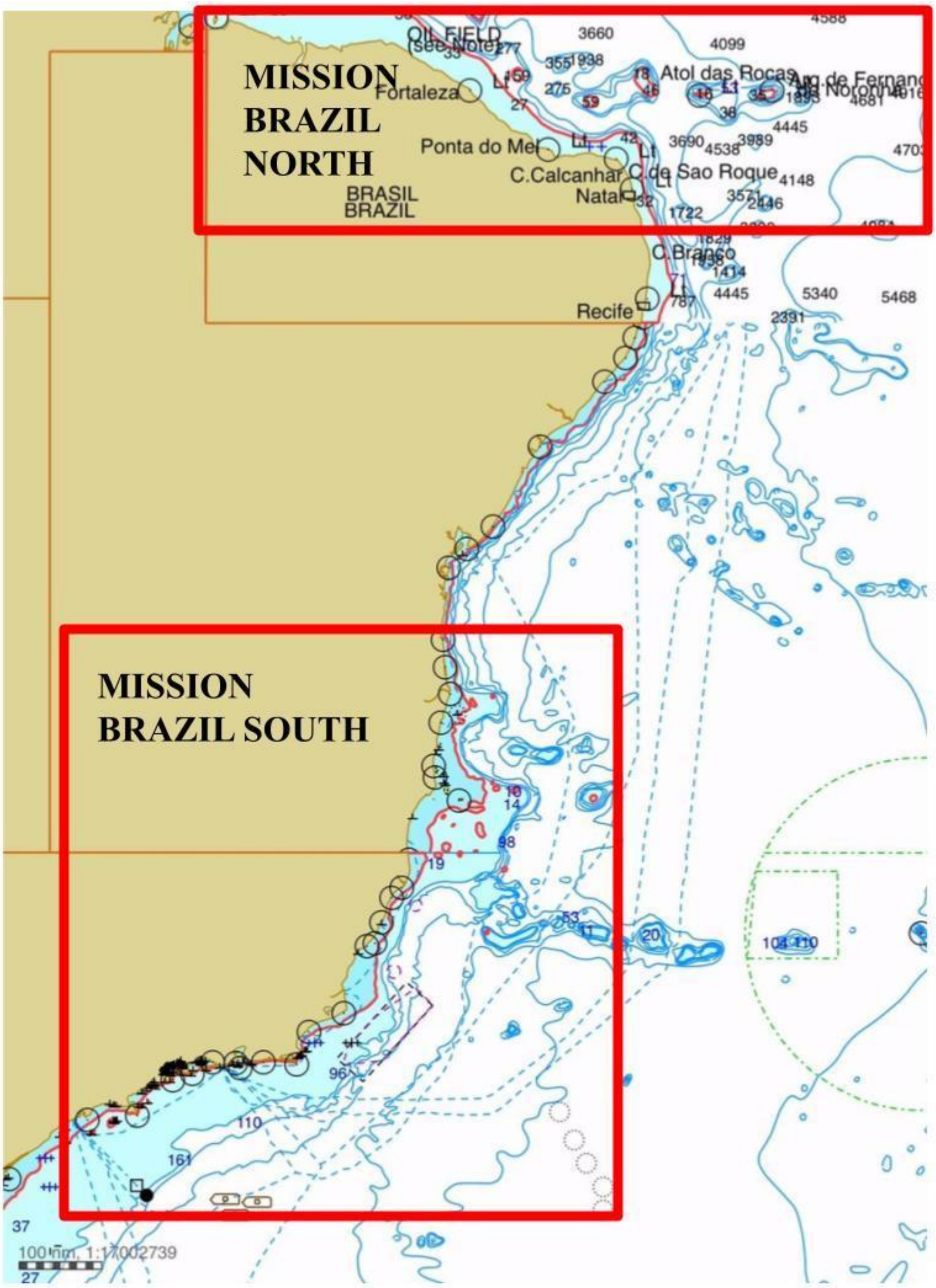


Figure 13. *The two study sites in Brazil; North and South sectors.*

2.3.2. Study sites in BRAZIL North

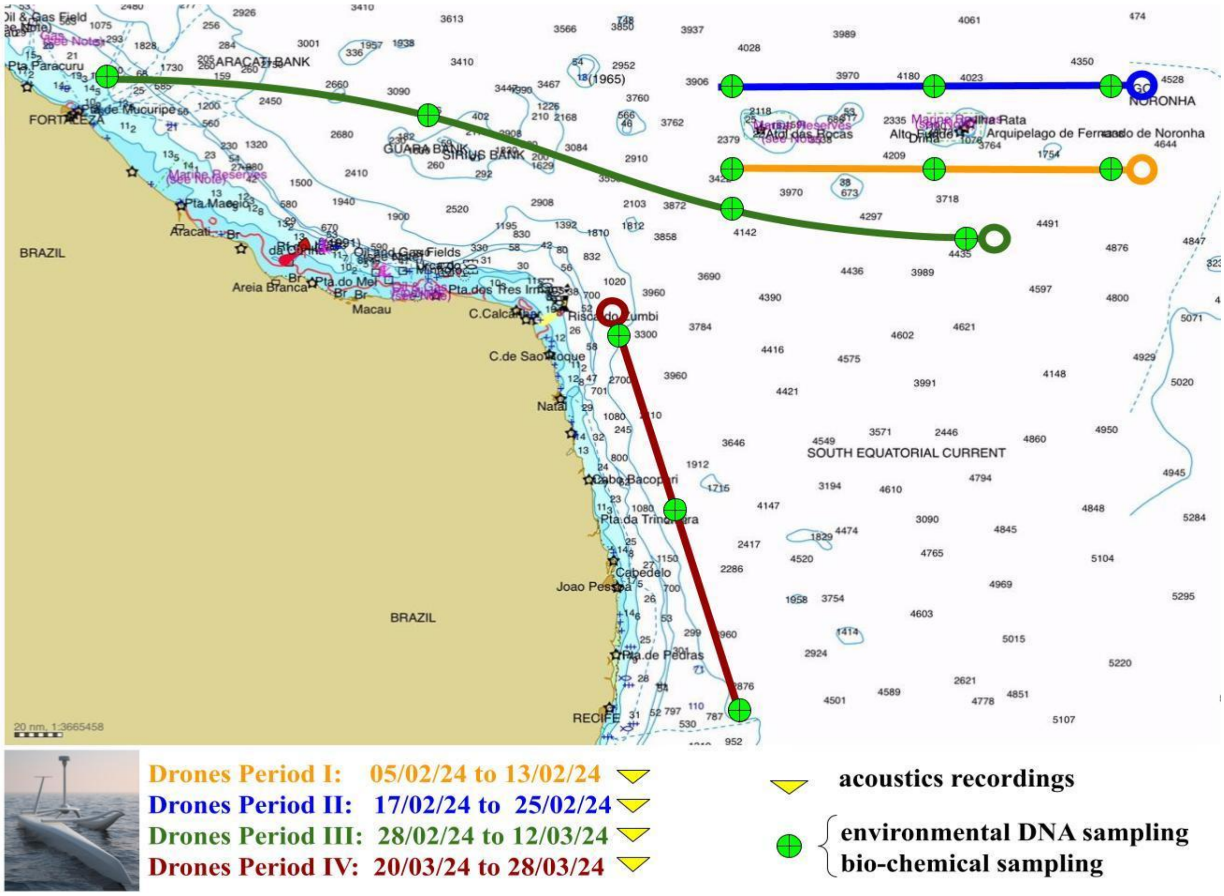


Figure 14. Map of the Brazil North mission with São Pedro and São Paulo Rocks Island, Atol das Rocas Island, Fernando de Noronha Island and the Canyons submerged of Rio Grande da Norte, 2 months of data acquisition. The trajectories of the Sphyrna drones for acoustic acquisitions correspond to the orange, blue, green and red lines. DNA sampling and chemical measurements are represented by a green circle.

2.3.2.1. São Pedro and São Paulo Rocks

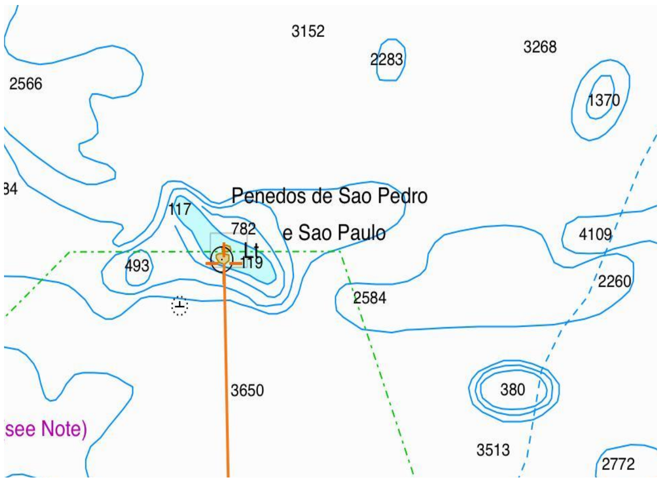


Figure 15. Map of the São Pedro and São Paulo Rocks Island.

2.3.2.2. Atol das Rocas, hot spot of HW

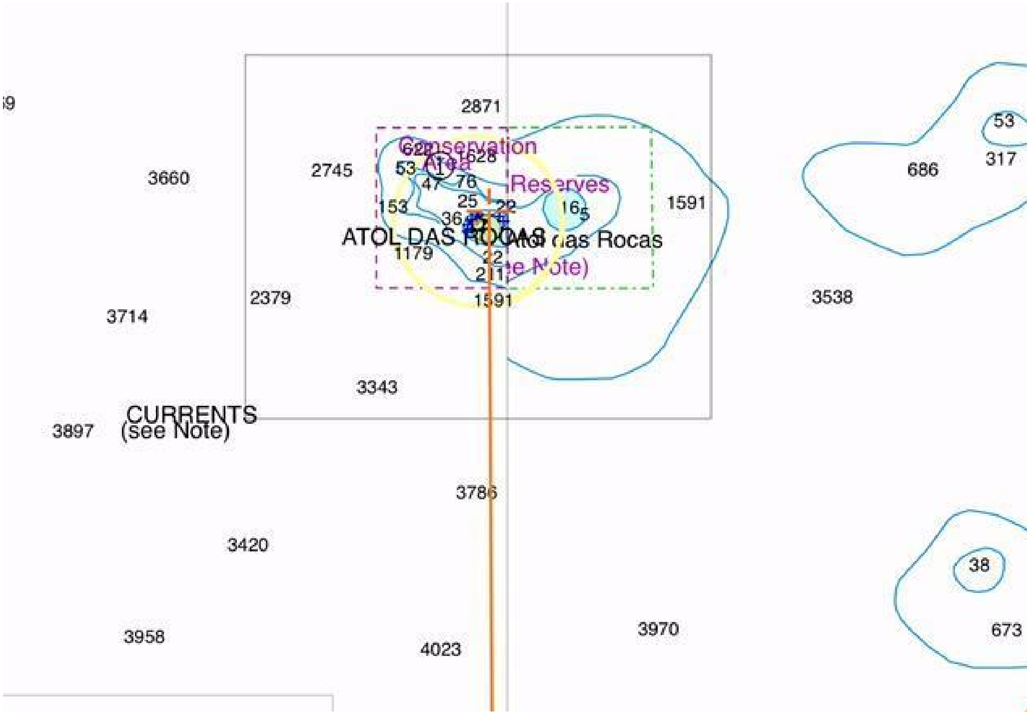


Figure 16. Map of the Atol das Rocas Island.

2.3.2.3. Fernando de Noronha

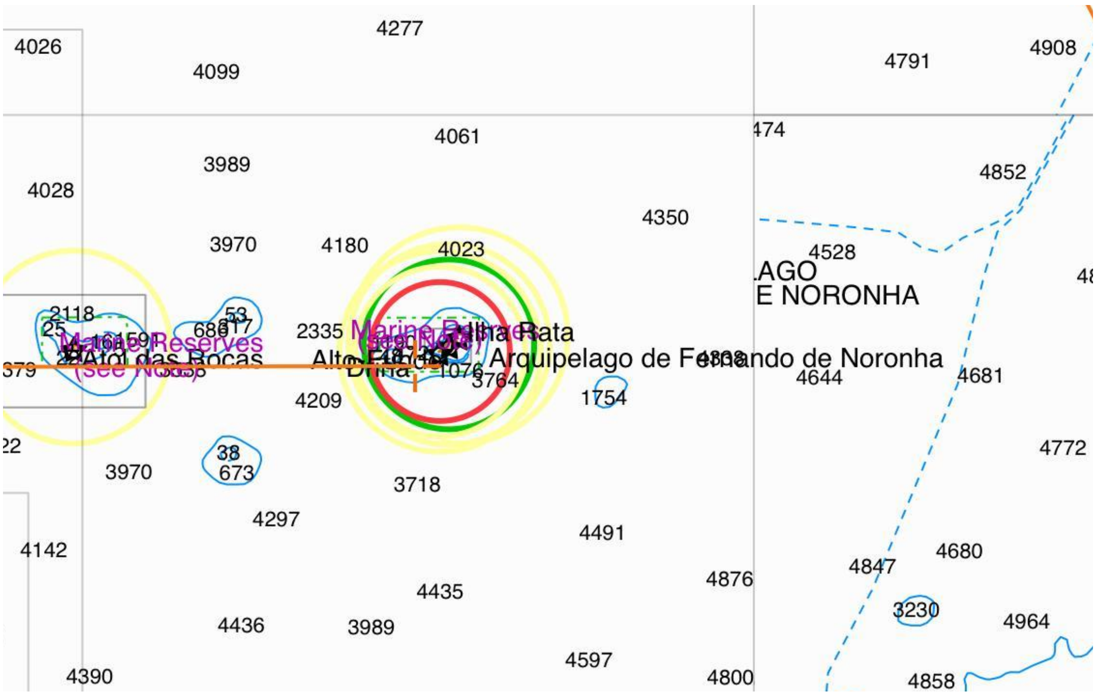


Figure 17. Map of the Fernando de Noronha Island.

2.3.2.4. Canyons submerged of Rio Grande da Norte

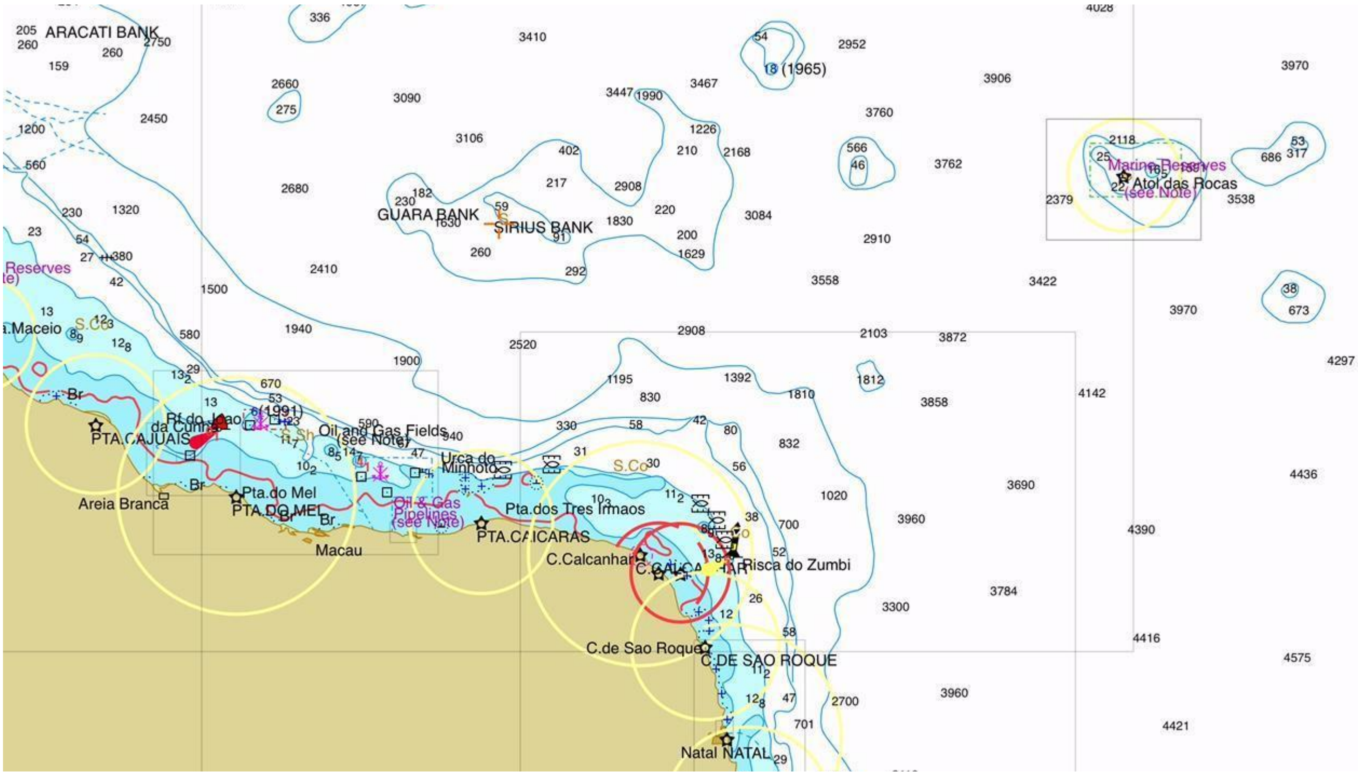


Figure 18. *Map of the Canyons submerged of Rio Grande da Norte.*

2.4. Mission Sphyrna 4

2.4.1. Study sites in BRAZIL South

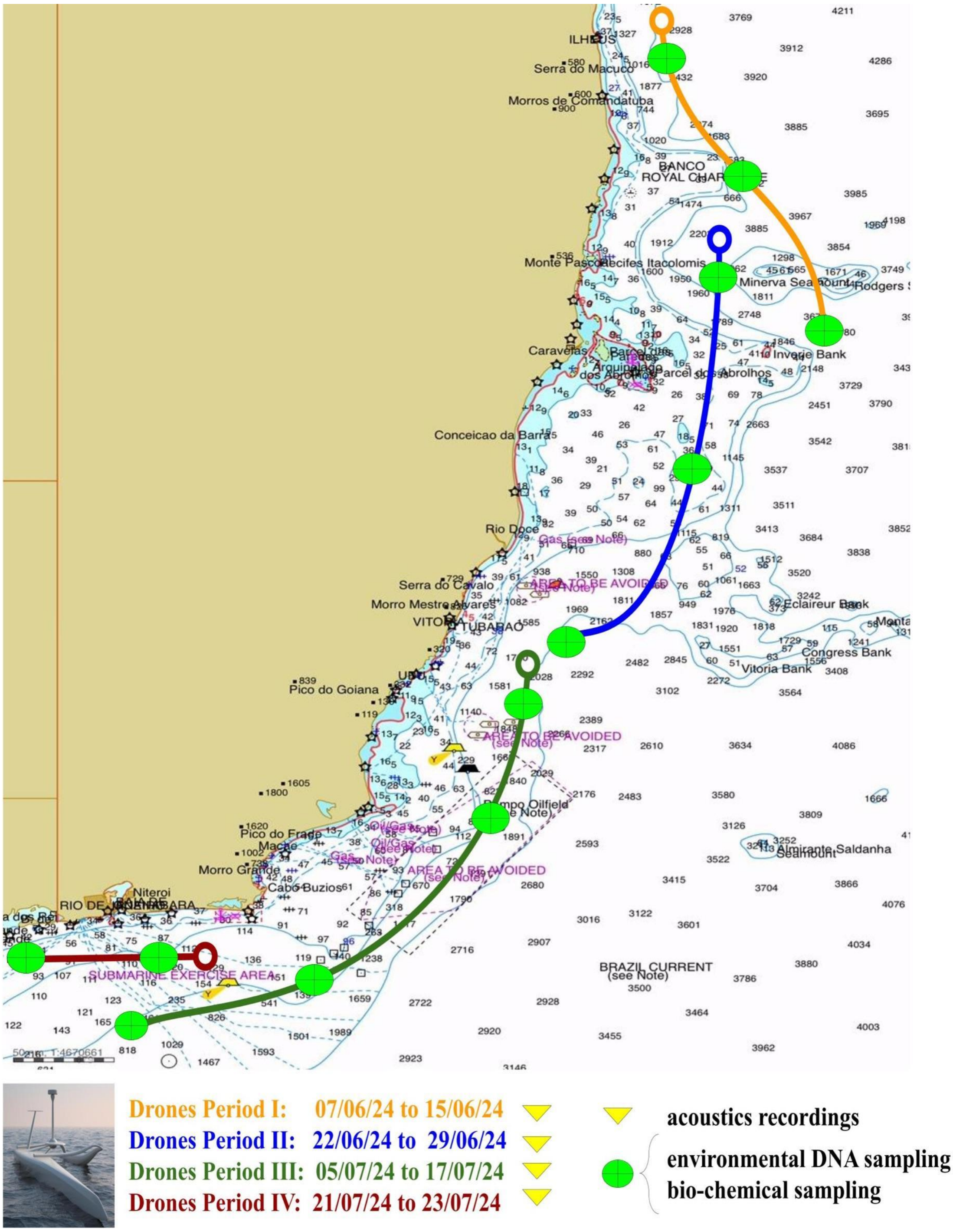


Figure 19. *Map of the Brazil South mission with Ilheus, Abrolhos Bank Island, Trindade Island, Cagarras Island, 2 months of data acquisition. The trajectories of the Sphyrna drones for acoustic acquisitions correspond to the orange, blue, green and red lines. DNA sampling and chemical measurements are represented by a green circle.*

2.4.1.1. Ilheus

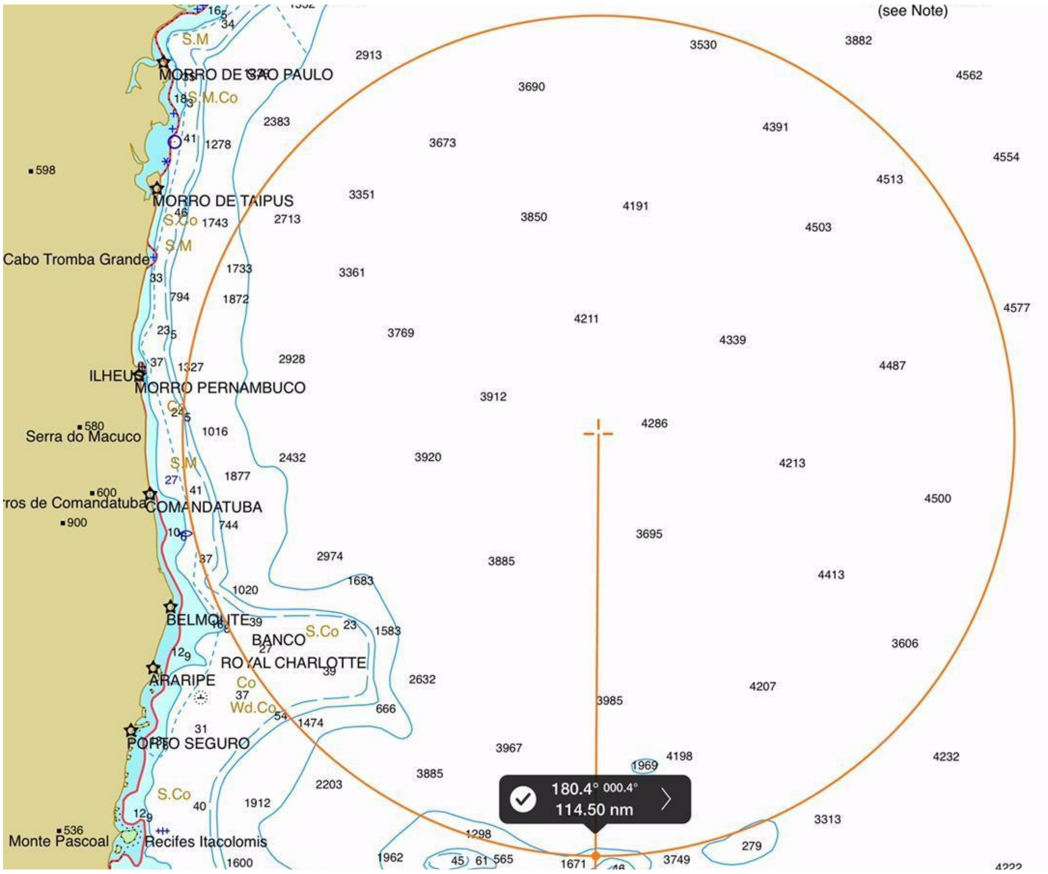


__Figure 20.__ Map of Ilheus, departure point for the South Brazil mission, with a high diversity of cetacean species

2.4.1.2. Abrolhos Bank

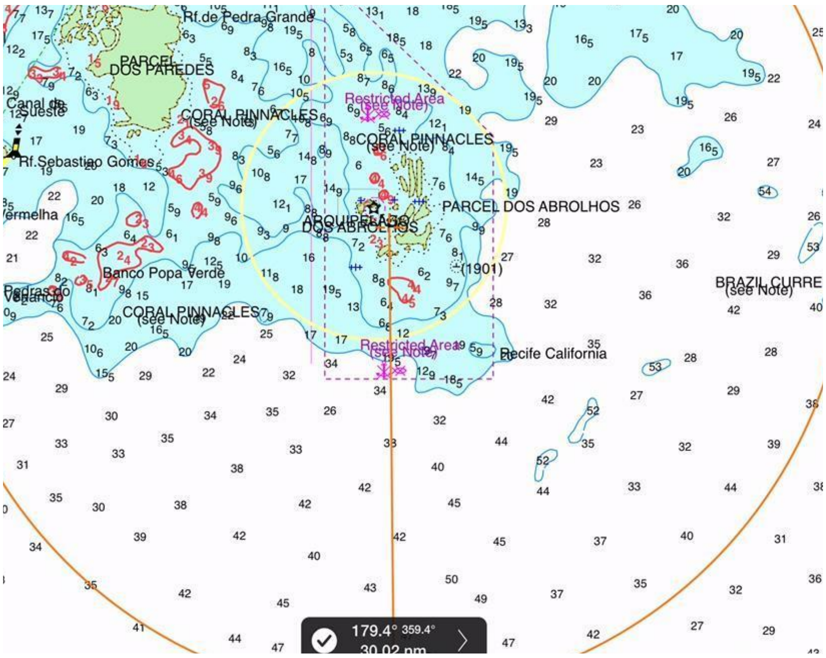


__Figure 21.__ Map of the Abrolhos Bank Island

2.4.1.3. Trindade

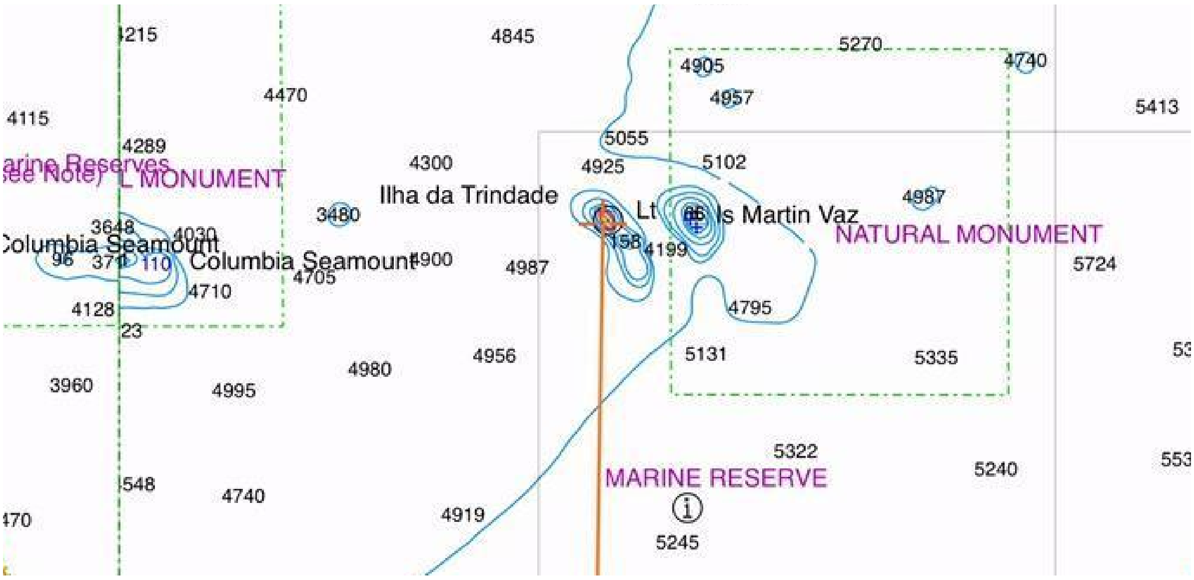


Figure 22. *Map of Trindade Island.*

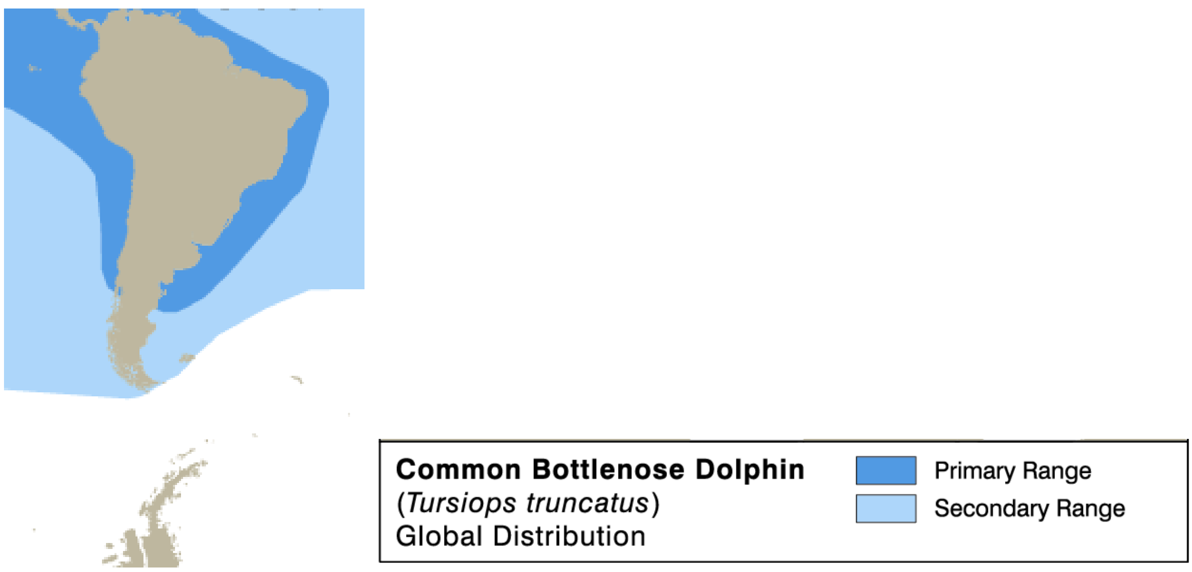
2.4.1.4. Cagarras

Figure 23. *Map of the Cagarras Island.*

2.4.1.5. Presence and distribution of cetaceans in Brazil

We figure in the following the distribution of the cetacean species present in Brazil :

Species in BRAZIL
• Blue whale • Bottlenose dolphin • Bryde's whale • Common Dolphin • False killer whale • Humpback whale • Orca • Minke whale • Globicephale • Free whales • Sei whale • Sperm whale • Longirostre dolphin • Spotted dolphin • Striped dolphin



Blue Whale
(*Balaenoptera musculus*)
Global Distribution

Primary Range
Secondary Range
Overlap of Pygmy Blue Whale

Bryde's Whale
(*Balaenoptera edeni*)
Global Distribution

Primary Range
Secondary Range

Common Dolphin
(*Delphinus delphis*)
Global Distribution

Primary Range
Possible Range

Fin Whale
(*Balaenoptera physalus*)
Global Distribution

Primary Range

Humpback Whale
(*Megaptera novaeangliae*)
Global Distribution

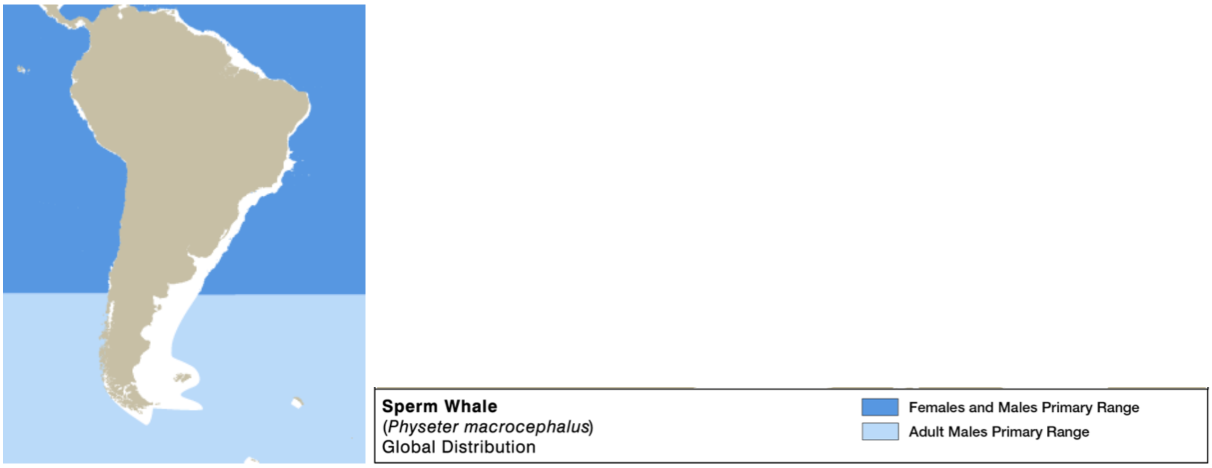
Primary Range
Secondary Range
Possible Range

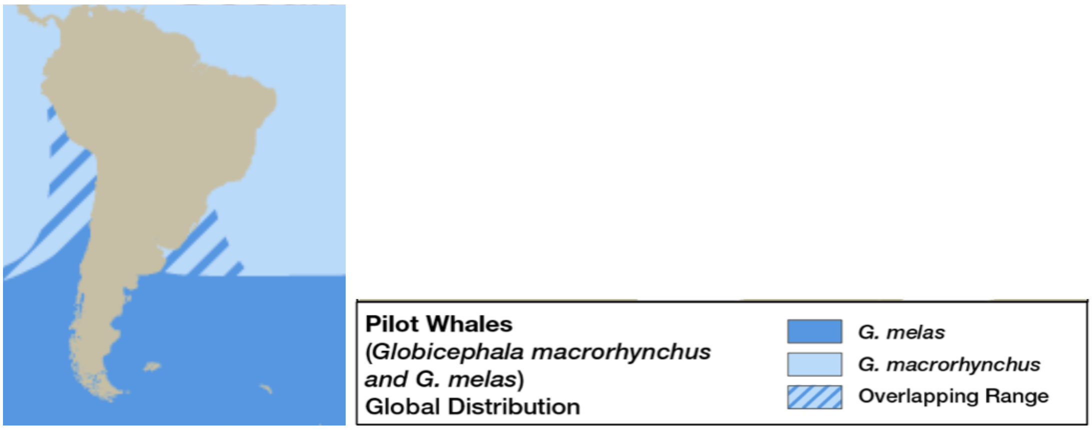
Killer Whale
(*Orcinus orca*)
Global Distribution

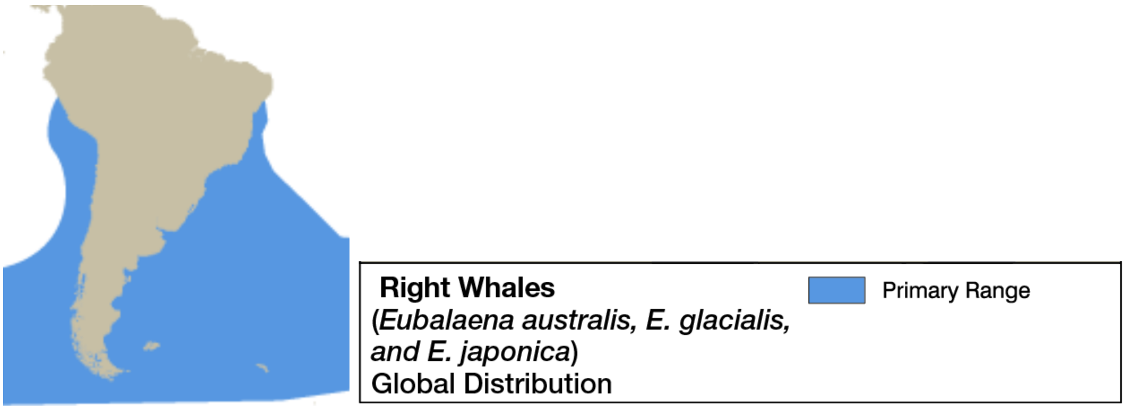
Primary Range
Secondary Range

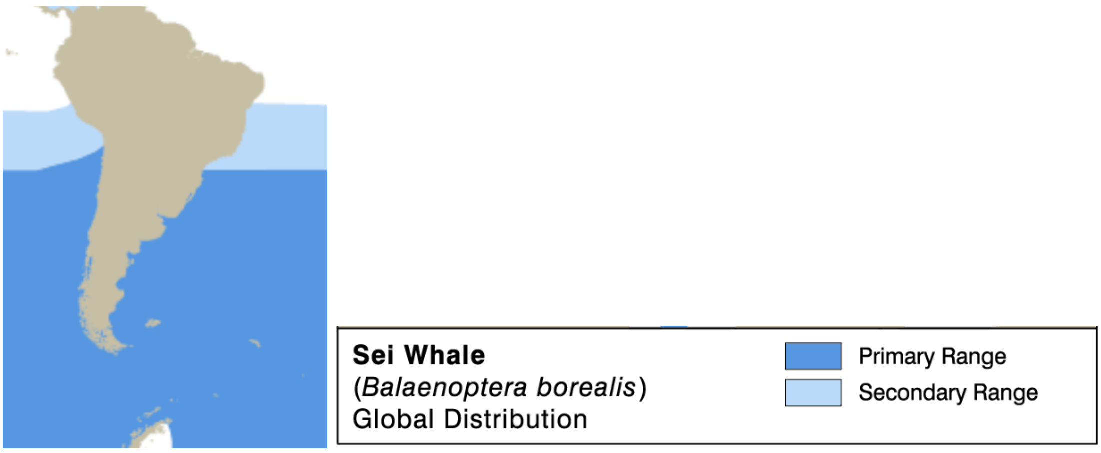
Common Minke and Antarctic Minke Whales
(*Balenoptera acutorostrata, B. bonaerensis*)
Global Distribution

Primary Range *B. acutorostrata*
Primary Range *B. bonaerensis*
Overlapping Ranges


Sperm Whale
(Physeter macrocephalus)
Global Distribution
Females and Males Primary Range
Adult Males Primary Range


Pilot Whales
(Globicephala macrorhynchus
and G. melas)
Global Distribution
G. melas
G. macrorhynchus
Overlapping Range


Right Whales
(Eubalaena australis, E. glacialis,
and E. japonica)
Global Distribution
Primary Range


Sei Whale
(Balaenoptera borealis)
Global Distribution
Primary Range
Secondary Range

Spinner Dolphin
(*Stenella longirostris*)
Global Distribution

Primary Range of *S. l. roseiventris*
Primary Range of *S. l. longirostris*
Primary Range of *S. l. orientalis*
Primary Range of *S. l. centroamericana*

Atlantic Spotted Dolphin
(*Stenella frontalis*)
Global Distribution

Primary Range
Secondary Range

Striped Dolphin
(*Stenella coeruleoalba*)
Global Distribution

Primary Range
Possible Range

3. Four approaches to the behavioral study of cetaceans in their environment

3.1. ‘Sphyrna’ marine surface drone: acoustic monitoring of cetaceans.

Designed and developed by the Sea Proven Naval Design Office (expert in marine robotics), based in Laval, in Mayenne, the Sea Proven company designs marine subsurface drones. The Sphyrna, also called "Autonomous Laboratory Vessels", such as the Sphyrna S70 **(Fig. 25)**, are equipped with an oceanic autonomy. The Sphyrna are designed to reduce waste at all stages of its life cycle ("Eco-Conception" approach). These autonomous vessels, designed to observe deep ocean layers, are operated from a base ship, the 'SOLAR Odyssey' **(Fig. 24)**. This base ship (18 meters), hosts the scientific team and also allows the storage of samples, DNA in particular. It can receive real-time signals from the UAVs via WIFI up to a distance of 7 nautical miles (nearly 13 kilometers).

The remote-controlled marine drones are equipped with hydrophones (fixed to the keel) for acoustic surveys **(Fig. 26)**. These drones are capable of ensuring a continuous presence at sea 7 days a week, in complete autonomy and without the need for crew relief. This performance results from a very low energy consumption. They thus allow to carry out surveys for the surveillance and protection of offshore maritime areas at a lower cost.

Figure 24. *Photos of the base ship 'Solar Odyssey' (Trimaran (L=18m)) accommodating 2 people.*

Figure 25. *A Sphyrna surface marine drone (17m)*

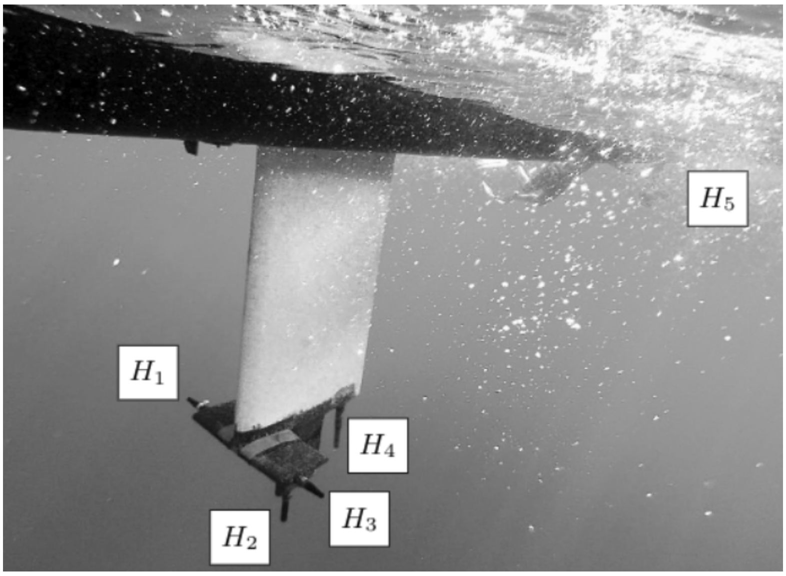


Figure 26. *Acoustic antenna fixed under the keel of the Sphyrna UAVs, 2 meters below the surface. The inter-hydrophone distances are H1H2 to H3H4 : 591,703, 630, 590, 350, 629 mm, H5 is 7m at the stern*

The passive acoustic monitoring technique allows for non-intrusive studies, operates independently of weather and lighting conditions, and allows access to remote areas where it is difficult to conduct studies (even for a short term). This approach has been increasingly used since the 1970s. It allows to detect the presence of cetaceans, identify species, locate and track individuals in their acoustic fields, quantify connectivity between cetacean populations, determine seasonal distribution patterns, species presence and relative abundance, reduce the risk of collisions, respond to behavioral questions by passive bioacoustics, and recognize groups of animals by regional and cultural dialects.

Throughout the mission, acoustic data will be acquired using 13 hydrophones (1C75 and 12 C57) from Cetacean Research; 5 hydrophones fixed under the keel of the 'Sphyrna 24' drone **(Fig.27-28)** and 2 hydrophones from the 'Solar Odyssey' base ship (directional boom and 30 m depth). The use of hydrophones placed under the 'Sphyrna' subsurface marine UAVs enables us to listen live and in real time to everything that evolves underwater at depths of over 2,000 meters. This approach makes it possible to understand and apprehend the natural behaviors (lifestyle, hunting, socialization, mating, etc.) of these animals. They enable us to study marine animals in great detail, without having to approach or disturb them.

<u>CETACEAN RESEARCH Hydrophones type C57</u>
Bandwidth :
Linear Frequency Range (±3dB) [kHz] 0.015 to 45
Usable Frequency Range (+3/-12dB) [kHz] 0.008 to 100

Sensitivity: Transducer Sensitivity* [dB, re 1V/µPa] -187 / Preamplifier Gain [dB] 20 / Effective Sensitivity* [dB, re 1V/µPa] -167

Dynamic:
SPL Equiv. Self Noise at 1kHz [dB, re 1µPa/√Hz] 46 (Sea State Zero) / RMS Overload Acoustic Pressure [dB, re 1µPa] 171 to 188 / Transducer Sensitivity + Preamplifier Gain = Effective Sensitivity, Sampling rate (Max S/sec): 512 kHz Fe, 24 bits

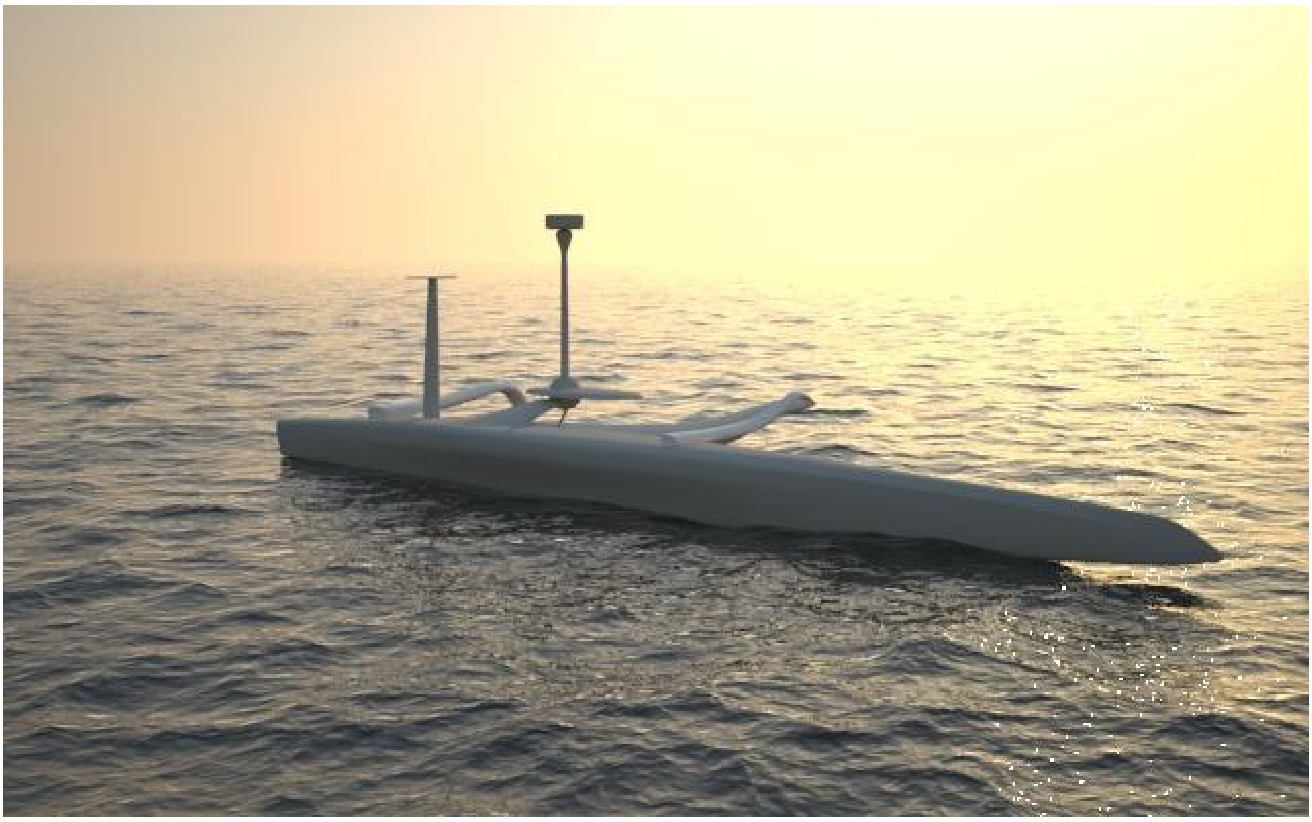


Figure 27 . *Computer-generated image of the S24 drone (7m25), profile view (actual size), fitted with 5 hydrophones at keel level. Design completed, production scheduled for July-August.*

Figure 28. *Computer-generated image of the S24 drone (6m20), front view (actual size). Design completed, production scheduled for July-August.*

Acoustic recordings will be made using a high temporal resolution sound card called Jason also called Qualilife-Sound. An equipment manufactured by the SMIOT platform of the University of Toulon. It allows recording 5 channels with a very high sampling rate (1 million points per second, 5 x 1 mHz). It is thus a unique card in the world for an embedded system. These underwater investigation tools are developed and produced for advanced environmental measurements by the SMIoT technology platform **(Fig. 29)**. Connected to five hydrophones placed under the hull, this device allows triangulating the sound to locate its origin. Thus, we can know which animal emits which click (1 per second approximately) and deduce the trajectory.

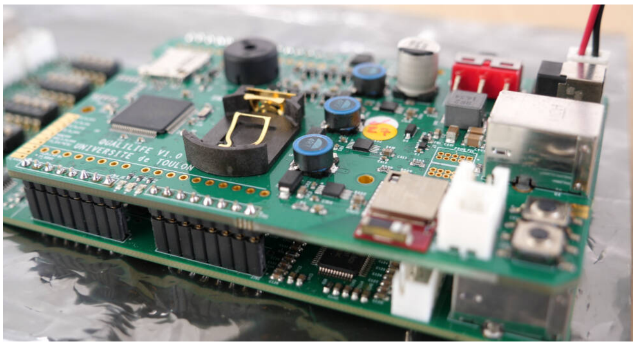


Figure 29. *Qualilife JASON' acquisition card allowing to record 5 channels with a very high sampling rate.*

3.2. Population genomics and environmental DNA

In addition, the recent use of environmental DNA (eDNA), is a passive monitoring method to study changes in the biological composition of communities in different ocean regions. eDNA is defined as DNA that can be extracted from environmental samples. We conducted eDNA measurements during the Sphyrna Odyssey 2020 mission **(Fig. 30 and Fig. 33)**. Seawater samples (and thus potentially eDNA) will be taken before, during and after visual and/or acoustic observations of the target species of this project, or outside of any observations in order to test the relevance of the eDNA tool to acoustic detections.

Figure 30. *Extraction of eDNA by seawater filtration, performed during the Sphyrna Odyssey 2020 mission by Sara. The eDNA was obtained by filtering a volume of sea water through filters (porosity 0.45 µm)*

These eDNA measurements are made by filtering seawater in areas close to animal passage, allowing identification of individuals whose presence had been validated by an acoustic signal. Species monitoring at the eDNA level is rapid, cost-effective, and provides a standardized collection of data on the distribution and relative abundance of species, families, and subpopulations **(Fig. 31)**. The target species for the eDNA protocol will focus on mysticetes and odontocetes.

Figure 31. 'Argaly' filtration pump*; filter with a surface area of 600 square centimeters and a pore diameter of 0.45 microns, for recovering DNA from aquatic organisms.*

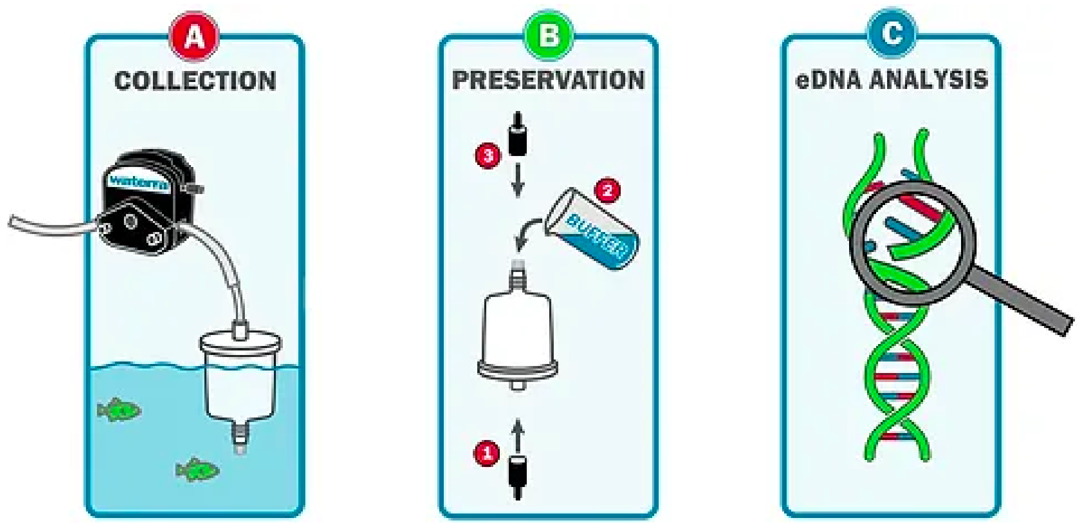


***Figure 32 . 'Argaly'** seawater filtration to recover DNA from aquatic organisms.*

The pump (CE certified) is connected to a battery that holds a charge for up to 12 hours, with only 4 hours needed for recharging. A flowmeter at the pump outlet lets you know the volume filtered at all times **(Fig. 32)**.

All included in a waterproof case with shoulder strap, so you can take samples with your hands free and your feet in the water, if necessary.

The capsules feature a polyethersulphone filter with a surface area of 600 square centimeters and a pore diameter of 0.45 microns, enabling the recovery of DNA from aquatic organisms.

These filters can be used in highly turbid waters, and filtered water volumes can reach up to 100 liters. The filtration technique proposed by ARGALY, with filtration upstream of the pumping system, eliminates the need to change all the hoses every time you take a sample and limits the risk of contamination.

One end of the hose is placed at the capsule outlet, and the other end is connected to a diaphragm pump. The filtered water is then discharged via a second hose at the pump outlet. By way of example, 30 minutes filtration in clear water yields around 40 liters of water.

Once filtration is complete, the capsule is drained of any residual water, then filled with DNA preservation buffer. Watertight caps are used to seal the capsule. After vigorous shaking of the capsule, the preservation buffer is either collected in a Falcon tube or preserved in the capsule at room temperature until dispatch to the laboratory.

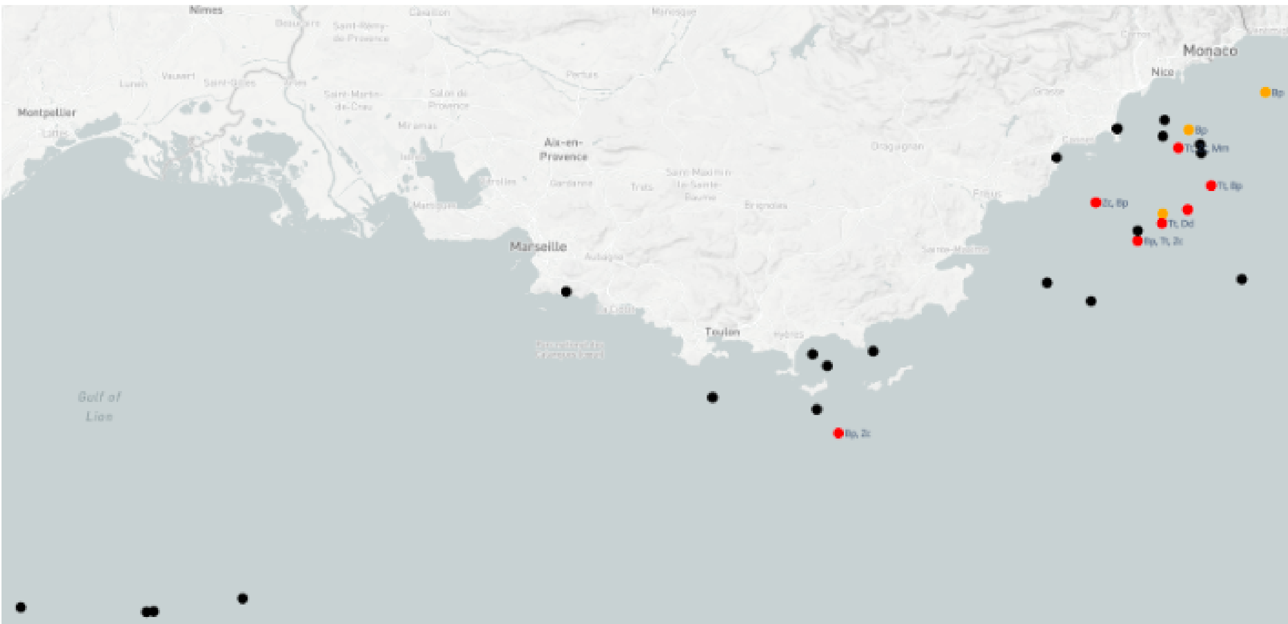


Figure 33. *EdNA map showing species detections in Sphyrna Odyssey Depart Sphyrna EdNA samples processing;. black dots:current samples Orange dots: detection of one species; Red: more than one species Zc:.Ziphius c .; Bp: Balaenoptera p .; Tt: Tursiops t .; Dd: Delphinus d .; Sc: Stenella c .; Mm: Monachus m.* (Glotin 2020).

3.3. Satellite data

ESA satellite data provide unique monitoring of the environment. They radically change the way we understand, manage and protect our environment. Satellite imagery allows on the one hand to locate large-scale ocean fronts, mesoscale eddies (10-100 km) and part of the sub-scale structures (<10 km, filaments) at the ocean surface (Bon 2015). In synergy with in situ measurements and/or with the output of ocean models such as Mercator-Ocean products, they provide information on the structure of these faults as well as a detailed analysis of environmental parameters (temperature and salinity gradient over the water column, chlorophyll a levels, and ocean currents in the water column), to understand cetacean activity and behavior as a function of oceanographic structures and bathymetry. Indeed, areas with oceanic fronts are rich in nutrients, and therefore in prey, which facilitates the hunting of cetaceans and reduces their energy expenditure in their search for food.

Furthermore, the use of satellite imagery with the highest resolution allows us to count cetaceans from the water surface from space. These photographic records are significant enough (with a high level of iconicity) to capture the distinctive shapes of the different species such as their fins. The satellite images obtained allow a better understanding of the trajectories of the animals (Guirado 2019) which is useful to avoid collisions in shipping lanes **(Fig. 34).**

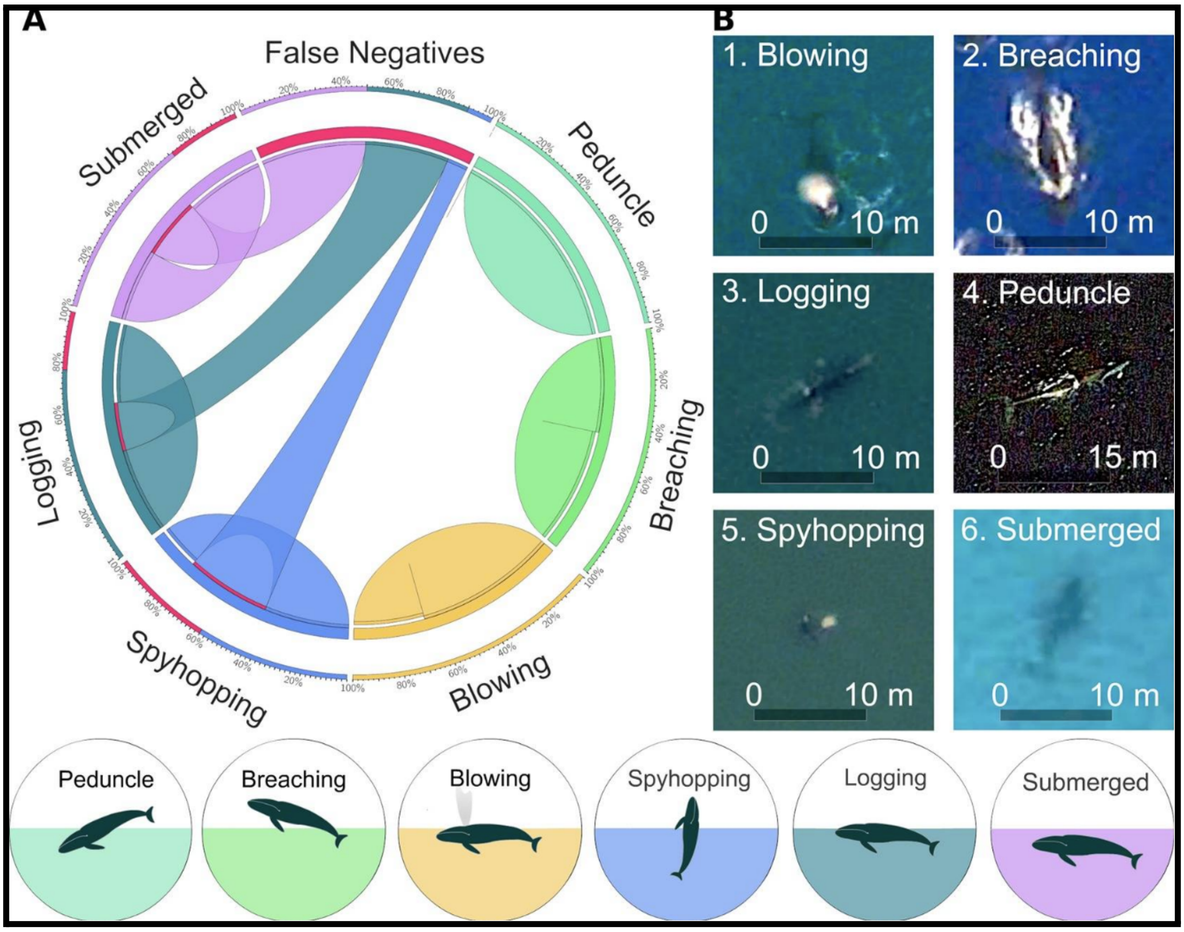


Figure 34. *Whale behavior and image detection performance of whale presence from satellites* (Guirado 2019)*.(A) The distribution of false negatives (undetected whales, in red) and true positives across whale postures. Whales subjected to blowing, breaching, and stalk postures were better detected. (B) Example images for each behavior from the hotspots detected at the highest zoom. This thesis will evaluate which behaviors are useful for training better image classifiers.*

3.4. Biological, chemical, physical analysis

Other data sets will be obtained, such as temperature variations, salinity and other parameters in marine ecosystems that lead to rapid changes in their chemistry, physical and biological composition. Chemical measurements of water quality, using a multiparameter chemical probe (Aquaread AP-7000, **Fig.35)**, allow the collection of data on physical, chemical and biological characteristics. The probe's sensors can measure the following hydrological variables: temperature; salinity; pH; dissolved oxygen; chlorophyll; colored dissolved organic matter; total dissolved solids; electroconductivity; redox potential; blue-green algae; specific gravity; depth; refined oils (p40 Glotin 2020).

Figure 35. *Multiparameter chemical probe (Aquaread AP-7000), with sensors included in Sphyrna (https://www.aquaread.com/portofolio/ap-7000/).*

These characteristics are important to take into account because they determine the distribution of living organisms in the ocean and allow a better understanding of the links between organisms, ecosystems and environmental parameters. Indeed, chlorophyll (a pigment essential for algal photosynthesis) is an indicator of the total biomass of phytoplankton. Chlorophyll concentrations in surface waters show seasonal variability: phytoplankton development depends on light energy, nutrient concentration, water mass stability and zooplankton consumption intensity. Crustaceans, small fish, some large fish species, and whales consume them as their primary food source. Phytoplankton provide over 200 billion tons of food per year. Thus, a decrease in the phytoplankton population can have serious repercussions on the entire marine ecosystem.

3.5. Correlation of surface and space observations

The correlation and calibration of bathymetry, currents, temperature, chlorophyll content, plankton blooms, water color and information related to water pollution (hydrocarbons), coming from space via the Sentinel and SWOT satellites and from the surface of the water via the Sphyrna, will allow the determination of conditions favoring the presence of cetaceans and thus make it possible to precisely target our listening by passive bioacoustics in favorable areas.

Thus, the crossing of these spatial data and data obtained from Sphyrna, by the accumulation of environmental variables and oceanic vortex, will allow us to characterize the seasonal or permanent areas of presence of cetaceans, to highlight the trophic chains related to these observations, and to identify the favorable conditions for reproduction, and therefore to protect these areas. In addition, analyses of areas with high density of maritime traffic in sites where cetaceans are present will evaluate the risk of collision and stranding to avoid these events in the future.

The high density of CNES data also allows us to deepen our bioacoustic research, obtained by the Sphyrna drones, on the distribution of clicks and vocalizations of cetaceans in the water column (= number and types of clicks and vocalizations according to depth) linked to temperature, salinity and currents in order to establish data crossovers between the peak of bioacoustic activity of cetaceans with peaks of temperature and salinity (p182 Glotin 2020).

Indeed, water temperature and salinity have a significant impact on sound propagation in water. The echolocation system used by cetaceans relies on inference about sound propagation to navigate and find prey, and is likely to be adapted to the characteristics of the water.
The results of a mission in the Mediterranean Sea show the movements of sperm whales, with a 3D model of temperature, salinity and currents. According to measurements made on January 14, 2020, the temperature, according to ESA records, is 1.5 ° C higher at the surface compared to 300-500 m, and there is a difference of 2.4 ° C between the surface and the bottom. The temperature gradient with depth **(Fig. 36-37)** shows a higher density of sperm whale tracks in the 300-500 m range.

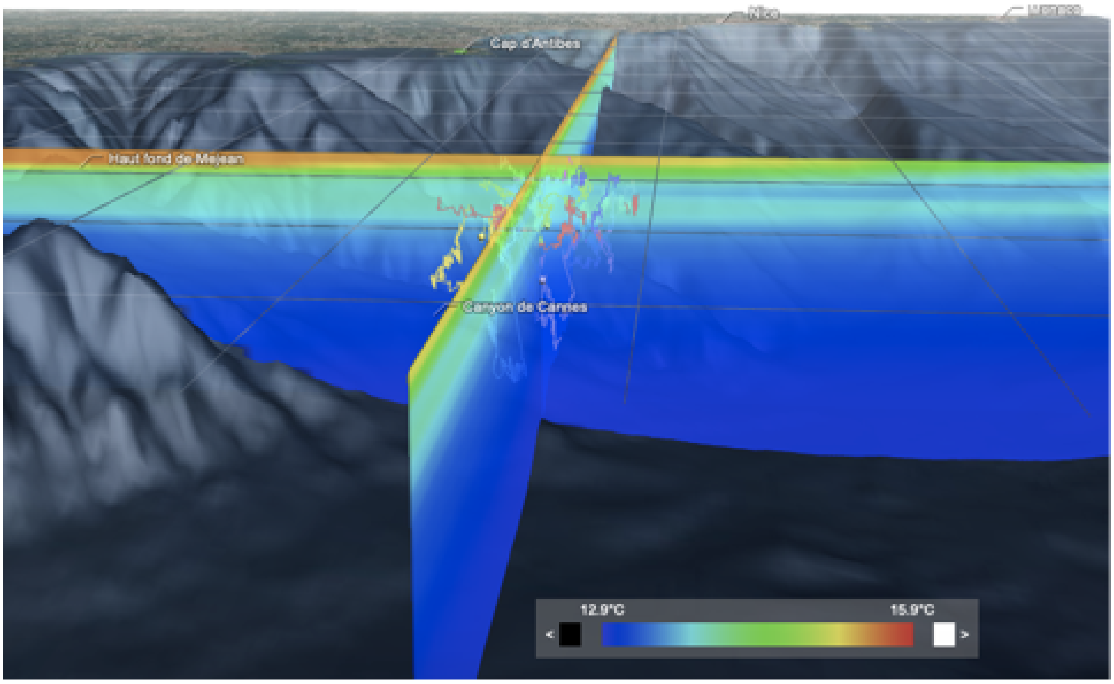


Figure 36. *3D temperature gradient as of January 14, 2020, computed by LIS MIO, at the surface (top), and in a vertical cross-section of the sperm whale hunting area computed from the SPhyrna mission 3D record (bottom) - Sphyrna Odyssey Mission (Glotin 2020).*

For salinity, the map (Fig.4) shows values ranging from 38.0 to 38.7 PSU over the entire water column, with a maximum between 300 and 500 m depth.

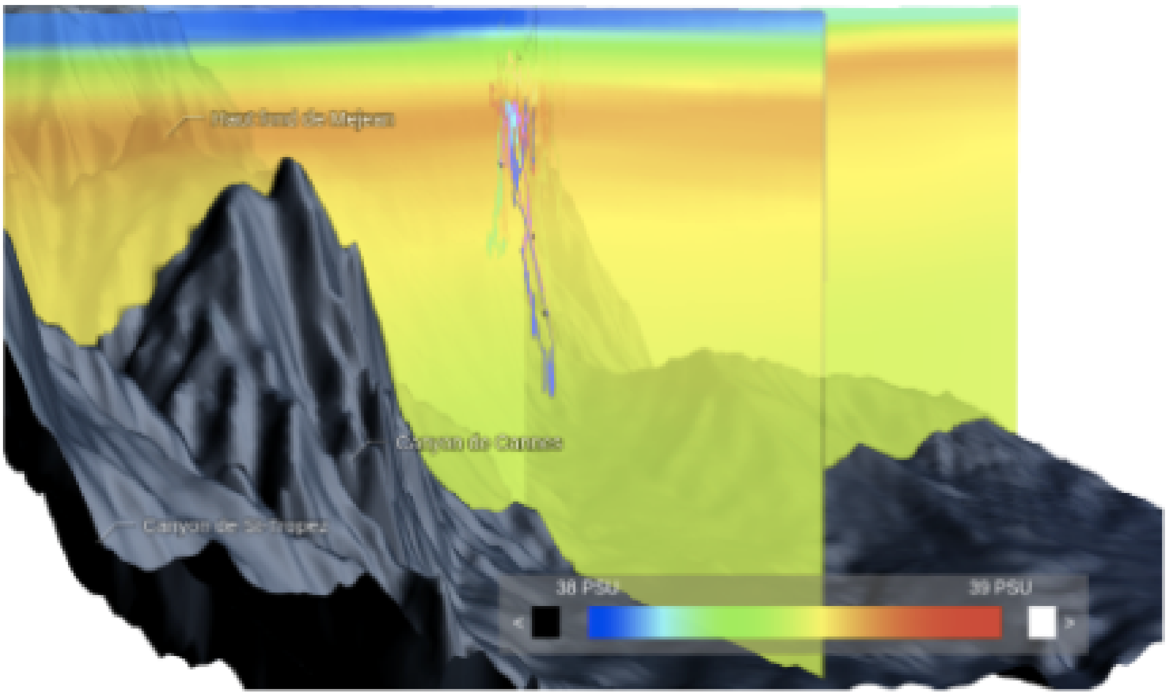


Figure 37. *The salinity calculated by our model on January 14, 2020 is highest offshore (top), and maximum between -300 and -500m at 38.7 PSU (bottom) (Glotin 2020).*

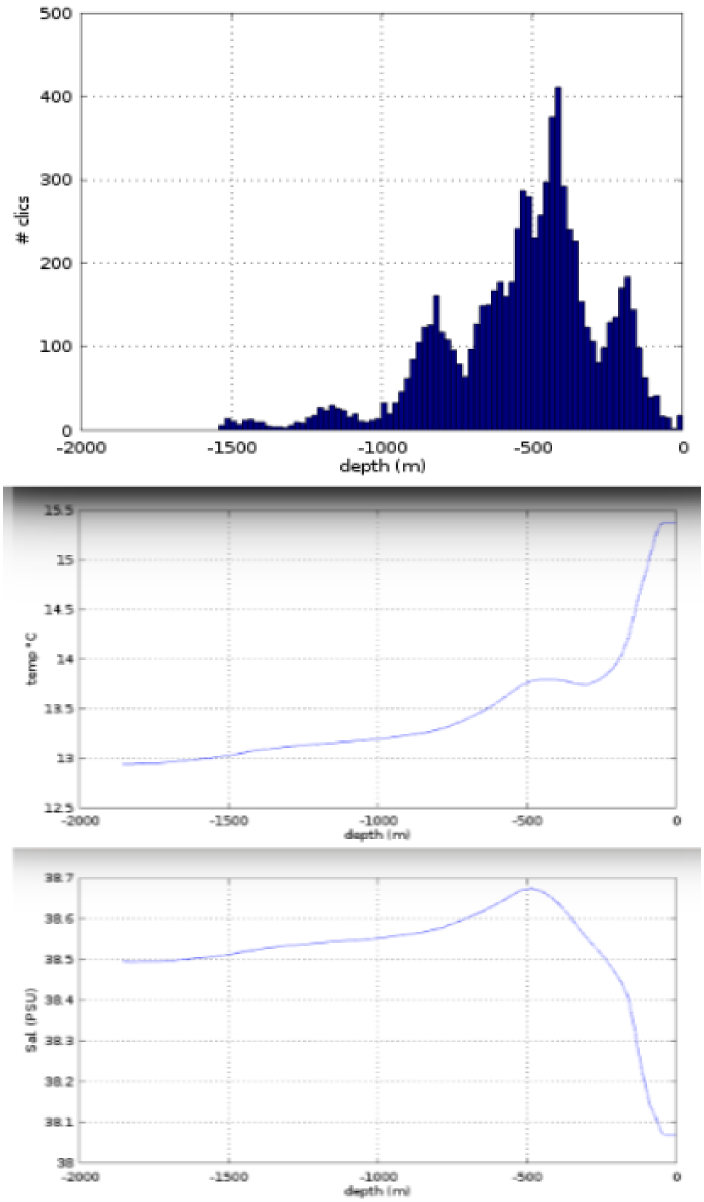


Figure 38. *Crossing between: (Top) the distribution in the water column of sperm whale clicks (= number of clicks as a function of depth), (middle) the temperature profile, (bottom) the salinity. The peak of sperm whale bioacoustic activity is at -400m, in the middle of the temperature plateau (-300 to -500m). A second peak of sperm whale presence is located at -500 m, at the salinity peak (Glotin 2020).*

In conjunction with the effect of temperature and salinity, estimates of currents throughout the water column on the distribution of sperm whales **(Fig. 38)** show that the current is maximum at the surface at 0.65 m/s. Further down the water column, the current velocity decreases until it cancels (at -600 m). The current velocity decreases very rapidly where cetaceans are present (p177 Glotin 2020).

The current is therefore likely to be used by cetaceans to optimize swimming effort during hunting and travel **(Fig. 39-40)**. This research is essential to better understand the distribution of cetaceans at sea and the analysis of their movements. In addition, in high traffic areas, these studies allow to predict cetacean activities according to temperature, salinity, currents, bathymetry, eddies, and thus to prevent the risk of collision with maritime traffic which is very high in the Mediterranean. Sea. Numerous research projects are being carried out to better predict and avoid these collisions.

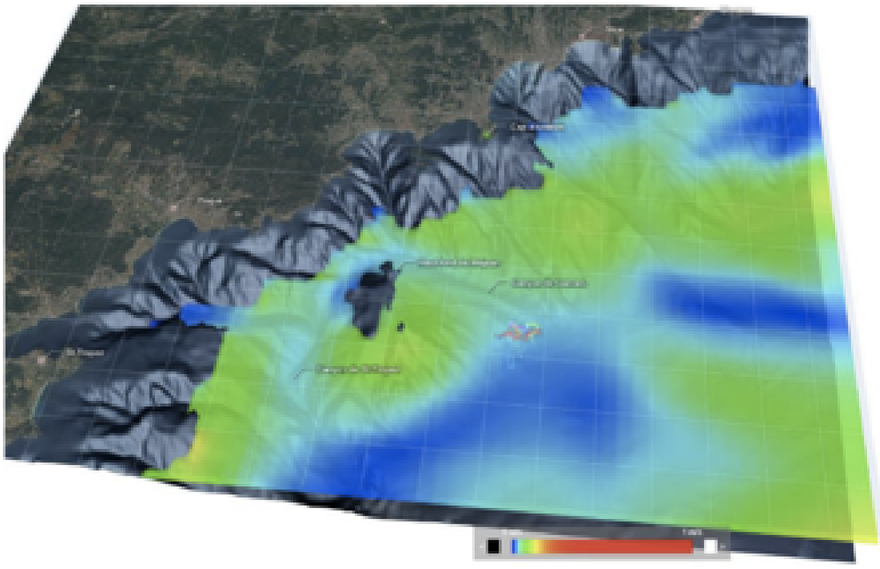


Figure 39. *Representation of the current expected velocity on January 14, 2020 seen from -400 m and the whale track (middle). At the surface, the expected speed is maximum at 0.65 m / s, but a smaller speed occurs. We see that sperm whales dive in this ocean front between waters with weak versus strong currents (Glotin 2020).*

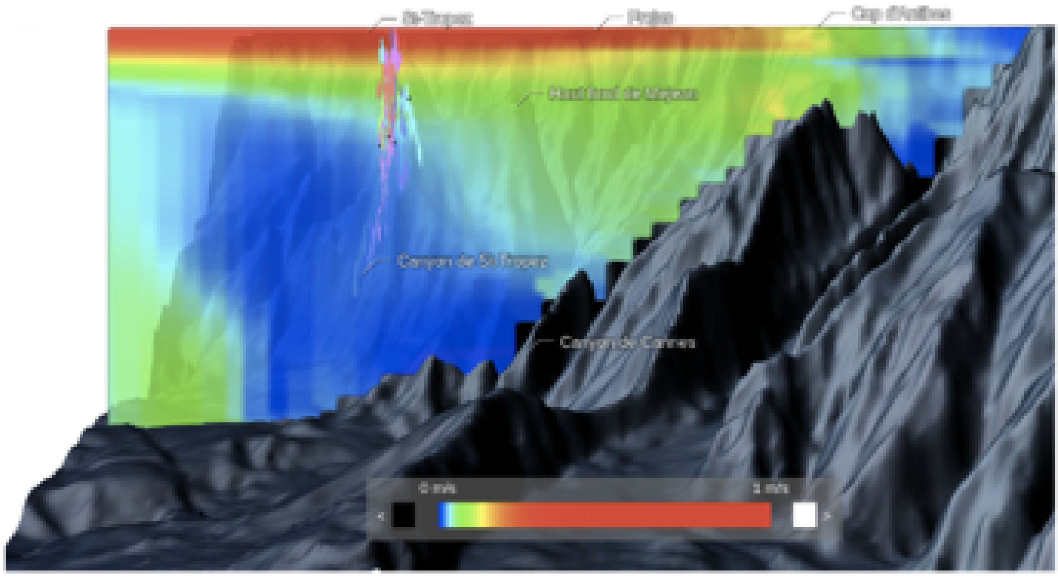


Figure 40. *Representation of the expected current under vertical section on January 14, 2020. We see that sperm whales dive in this ocean front between weak versus strong currents, as the area is favorable for upwelling due to nutrient mixing (Glotin 2020).*

This research would predict the probability distribution of whales based on eddies predicted from CNES data. These data are of great interest for tracking how species respond to their environment, which is most needed for effective conservation efforts, and for assessing the impact of conservation interventions (Kays 2020).

4. Schedule (Dates are subject to adaptations due to climate or operational reasons)

- **Test mission: BRITTANY mission**

24/08/23 to 30/08/23: 7-day mission in Brittany, in the Bay of Audierne, testing two Sphyrna 20 drones for acoustic acquisition (Cf. Appendix 2, p. 58)

- **MISSION 1: MACARONESIA mission**

4/09/23 to 12/09/23: MISSION DEPARTURE: Departure L'Orient, Arrival Madeira
13/09/23 : Equipment preparation (Assembly of the 'Sphyrna 25' + deployment of eDNA equipment and chemical probe)

➢ **14/09/23 to 18/09/23 : MADEIRA mission**

Acoustic + DNAe + Chemical acquisitions
19/09/23: Equipment storage
20/09/23 to 22/09/23: Madeira to Canary Islands route

➢ **24/09/23 to 12/10/23: CANARY ISLAND mission**

Acoustic + DNAe + Chemical acquisitions
13/10/23: Equipment storage
14/10/23 to 21/10/23: Departure Canary Islands, arrival Cape Verde
22/10/23: Equipment preparation (assembly of the 'Sphyrna 25' + deployment of eDNA equipment and chemical probe)

- **MISSION 2: MACARONESIA mission**

➢ **23/10/23 to 16/11/23: CAPE VERDE mission**

Acoustic + DNAe + Chemical acquisitions
17/11/23 : Equipment storage
18/11/23 to 29/11/23 : Departure Cape Verde, arrival Brazil
01/12/23 to 31/01/24: Data analysis at UFRN BRAZIL laboratory

- **MISSION 3: BRAZIL mission**

➢ **01/02/24 to 31/03/24: MISSION NORTH BRAZIL**
Acoustic +DNAe + Chemical acquisition mission
➢ **01/04/24 to 31/05/24:** Data analysis at UFRN BRAZIL laboratory

- **MISSION 4: BRAZIL mission**

➢ **01/06/24 to 31/07/24: SOUTH BRAZIL MISSION**
Acoustic +DNAe + Chemical acquisition mission

➢ 01/08/24 to 30/09/24: Data analysis at UFRN BRAZIL laboratory
01/10/24: Departure from Brazil

5. References

1) **Amaral ACZ, Jablonski S (2005).** Conservation of marine and coastal biodiversity in Brazil. Conserv Biol 19:625–631. https://doi.org/10.1111/j.1523-1739.2005.00692.

2) **Ab'Saber AN (2001).** Litoral do Brasil. Meta Livros, São Paulo

3) **Albouy, C., Delattre, V., Donati, G., Frölicher, T. L., Albouy-Boyer, S., Rufino, M., Pellissier, L., Mouillot, D., & Leprieur, F. (2020).** Global vulnerability of marine mammals to global warming. Scientific Reports, 10, 1–12. https://doi.org/10.1038/s41598-019-57280-3

4) **Alves, F., Ferreira, R., Fernandes, M., Halicka, Z., Dias, L., & Dinis, A. (2018).** Analysis of occurrence patterns and biological factors of cetaceans based on long-term and fine-scale data from platforms of opportunity: Madeira Island as a case study. *Marine Ecology*, *39*(2), e12499.

5) **Baines, M. E., and Reichelt, M. (2014).** Upwellings, canyons and whales: an important winter habitat for balaenopterid whales off Mauritania, northwest Africa. *J. Cetacean Res. Manage.* 141, 57–67.

6) **Bastida RO, Rodríguez D, Secchi E, da Silva V (2018).** Mamíferos Aquáticos da América do Sul e Antártica. Vázquez Mazzini Editores, Buenos Aires

7) **Baumgartner, M. F. (1997).** The distribution of Risso's dolphin (Grampus griseus) with respect to the physiography of the northern Gulf of Mexico. *Marine Mammal Science*, *13*(4), 614-638.

8) **Butler, A., Althaus, F., Furlani, D. and Ridgway, K. (2002).** Assessment of the conservation values of the Bonney upwelling area: a component of the Commonwealth Marine Conservation Assessment Program 2002– 2004. Report to Environment Australia. 73pp. [Available from: *http://www.environment.gov.au*].

9) **Bécognée, P., Moyano, M., Almeida, C., Rodríguez, J. M., Fraile-Nuez, E., Hernández-Guerra, A., et al. (2009).** Mesoscale distribution of clupeoid larvae in an upwelling filament trapped by a quasi-permanent cyclonic eddy off Northwest Africa. *Deep Sea Res. Part I Oceanogr. Res. Pap.* 56, 330–343. doi: 10.1016/j.dsr.2008.10.008

10) **Burtenshaw, J. C., Oleson, E. M., Hildebrand, J. A., McDonald, M. A., Andrew, R. K., Howe, B. M., & Mercer, J. A. (2004).** Acoustic and satellite remote sensing of blue whale seasonality and habitat in the Northeast Pacific. *Deep Sea Research Part II: Topical Studies in Oceanography*, *51*(10-11), 967-986.

11) **Cotté, C., d'Ovidio, F., Chaigneau, A., Lévy, M., Taupier-Letage, I., Mate, B., & Guinet, C. (2011).** Scale‐dependent interactions of Mediterranean whales with marine dynamics. *Limnology and Oceanography*, *56*(1), 219-232.

12) **Croll, D.A., Marinovic, B., Benson, S., Chavez, F.P., Black, N., Termullo, R. and Tershy, B.R. (2005).** From wind to whales: trophic links in an upwelling ecosystem. *Mar. Ecol. Prog. Ser.* 289: 117–30. [Final report to the Monterey Bay National Marine Sanctuary Contract no. 50ABNF500153].

13) **Costa LP, Leite LR, Mendes SL, Ditchfield AD (2005).** Mammal Ccnservation in Brazil. Conserv Biol 19:672–679

14) **Cunha, I. (2013).** Marine traffic and potential impacts towards cetaceans within the Madeira EEZ: a pioneer study (Master's thesis). Biology Department, University of Porto, Portugal.

15) **Cypriano-Souza AL, Meirelles ACO, Luz V, Bonatto SL (2016).** Rare or cryptic? The first report of an Omura's whale (Balaenoptera omurai) in the South Atlantic Ocean. Marine Mammal Sci 33:80– 95. https://doi.org/10.1111/mms.12348

16) **Freitas, L., Dinis, A., Nicolau, C., Ribeiro, C., & Alves, F. (2012).** New records of cetacean species for Madeira Archipelago with an updated checklist.

17) **Glotin H., Thellier N., Best P., Poupard M., Ferrari M., Vieira S., Giés V., ... Sarano F., Benveniste J., Gaillard S., de Varenne F. (2020).** Sphyrna-Odyssey 2019-20, Découvertes Etho-acoustiques de Chasses Collaboratives de Cachalots en Abysse & Impacts en Mer du COVID19, http://sabiod.org/pub/SO1.pdf, 197p, Univ. de Toulon, CNRS

18) **Hays, G. C., Ferreira, L. C., Sequeira, A. M. M., Meekan, M. G., Duarte, C. M., Bailey, H., Bailleul, F., Bowen, W. D., Caley, M. J., Costa, D. P., Eguíluz, V. M., Fossette, S., Friedlaender, A. S., Gales, N., Gleiss, A. C., Gunn, J., Harcourt, R., Hazen, E. L., Heithaus, M. R., ... Thums, M. (2016).** Key questions in marine megafauna movement ecology. Trends in Ecology and Evolution, 31, 463–475. https://doi.org/10.1016/j.tree.2016.02.015

19) **Herrera, I., Carrillo, M., Cosme de Esteban, M., & Haroun, R. (2021).** Distribution of cetaceans in the canary islands (Northeast Atlantic ocean): implications for the natura 2000 network and future conservation measures. *Frontiers in Marine Science*, *8*, 669790.

20) **Hrbek T, Silva VMF, Dutra N, Gravena W, Martin AR, Farias IP (2014).** A new species of river dolphin from Brazil or: how little do we know our biodiversity. PLoS ONE 9:e83623. https://doi.org/10.1371/ journal.pone.0083623

21) **IUCN (2020).** The IUCN Red List of Threatened Species. Version 2020- 2. http://www.iucnredlist.org. Accessed 11 March 2020

22) **Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) (2011a).** Plano de Ação Nacional para Conservação dos Mamíferos Aquáticos: Pequenos cetáceos. Brasília: Instituto Chico Mendes de Conservação da Biodiversidade. https://www.icmbio.gov.br/portal/ images/stories/docs-pan/pan-pequenos-cetaceos/1-ciclo/pan- pequenos-cetaceos-livro.pdf. Accessed 07 September 2020

23) **Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) (2011b).** Plano de Ação para a Conservação dos Mamíferos Aquáticos – Grandes Cetáceos e Pinípedes. In: Rocha-Campos et al. (Orgs). Brasília: Instituto Chico Mendes de Conservação da Biodiversidade. https://www.icmbio.gov.br/portal/faunabrasileira/ plano-de-acao-nacional-lista/861-plano-de-acao-para-conservacao- dos-grandes-cetaceos-e-pinipedes Accessed 07 September 2020

24) **Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) (2018).** Listas de espécies ameaçadas do Brasil.

25) **Jaquet, N., & Gendron, D. (2002).** Distribution and relative abundance of sperm whales in relation to key environmental features, squid landings and the distribution of other cetacean species in the Gulf of California, Mexico. *Marine biology*, *141*, 591-601.

26) **Jefferson, T. A., Webber, M. A., and Pitman, R. L. (2015).** *Marine Mammals of the World: A Comprehensive Guide to Their Identification*, 3rd Edn (San Diego, CA: Academic Press).

27) **Joiris, C. (2011).** A major feeding ground for cetaceans and seabirds in the south-western Greenland Sea. *Polar Biol.* 34: 1,597–607.

28) **Juhel, J. B., Marques, V., Fernandez, A. P., Borrero‐Pérez, G. H., Martinezguerra, M. M., Valentini, A., ... & Pellissier, L. (2021).** Detection of the elusive Dwarf sperm whale (Kogia sima) using environmental DNA at Malpelo island (Eastern Pacific, Colombia). Ecology and Evolution, 11(7), 2956.

29) **Landeira, J. M., Brochier, T., Mason, E., Lozano-Soldevilla, F., Hernández-León, S., and Barton, E. D. (2017).** Transport pathways of decapod larvae under intense mesoscale activity in the Canary-African coastal transition zone: implications for population connectivity. *Sci. Mar.* 81, 299–315. doi: 10.3989/scimar.0459 9.06a

30) **Moore, M., Steiner, L., & Jann, B. (2003).** Cetacean surveys in the Cape Verde Islands and the use of cookiecutter shark bite lesions as a population marker for fin whales. *Aquatic Mammals*, *29*(3), 383-389.

31) **Otsuka A., Feitosa F., Flores-Montes M., Silva A. (2016)** Dynamics of chlorophyll A & oceanographic parameters in the coastal zone: Barra das Jangadas-Pernambuco, Brazil. J. of Coastal Research, 32(3), 490-499

32) **Ott PH, Tavares M, Moreno IB, Oliveira LR, Danilewicz D (2009).** Os cetáceos do Arquipélago de São Pedro e São Paulo. In: Mohr LV, Castro, JWA, Costa, PMS, Alves, RJV (Orgs) Ilhas Oceânicas Brasileiras: da Pesquisa ao Manejo. MMA/Secretaria de Biodiversidade e Floresta, Brasília, pp 283-300

33) **Pikitch, E. K. (2018).** A tool for finding rare marine species. *Science*, *360*(6394), 1180-1182.

34) **Rodríguez, J. M., Hernández-León, S., and Barton, E. D. (1999).** Mesoscale distribution of fish larvae in relation to an upwelling filament off Northwest Africa. *Deep Sea Res. Part I Oceanogr. Res. Pap.* 46, 1969–1984. doi: 10.1016/ s0967- 0637(99)00036- 9

35) **Rodríguez, J. M., Barton, E. D., Hernández-León, S., and Arístegui, J. (2004).** The influence of mesoscale physical processes on the larval fish community in the Canaries CTZ, in summer. *Prog. Oceanogr.* 62, 171–188. doi: 10.1016/j.pocean. 2004.07.006

36) **Rai S., Rajashekhar M. (2014)** Effect of pH, salinity & temperature on the growth of six species of marine phytoplankton. J. Algal Biomass, 5(4), 55-59

37) **Ruppert, K. M., Kline, R. J., & Rahman, M. S. (2019).** Past, present, and future perspectives of environmental DNA (eDNA) metabarcoding: A systematic review in methods, monitoring, and applications of global eDNA. Global Ecology and Conservation, 17, e00547. https:// doi.org/10.1016/j.gecco.2019.e00547

38) **Rice, D. W. (1998).** Marine mammals of the world. *Systematics and distribution*.

39) **Reiner, F., Santos, M. E. D., Wenzel, F. W., & Whale, A. (1996).** Cetaceans of the Cape Verde archipelago. *Marine Mammal Science*, *12*(3), 434-443.

40) **Sambolino, A., Alves, F., Correia, A. M., Ferreira, R., Carvalho, P., Silva, G., & Dinis, A. (2017).** Monitoring cetaceans in the Madeira Archipelago from a ferry along a fixed transect.

41) **Selzer, L. A., & Payne, P. M. (1988).** The distribution of white‐sided (Lagenorhynchus acutus) and common dolphins (Delphinus delphis) vs. environmental features of the continental shelf of the northeastern United States. *Marine Mammal Science*, *4*(2), 141-153.

42) **Siciliano S, Moura JF, Filgueiras HR, Rodrigues PP, Leite NO Jr (2012).** Sightings of humpback whales on the Vitória-Trindade chain and around Trindade Island, Brazil. Braz J Oceanogr 60:455–459. https://doi.org/10.1590/S1679-87592012000300016

43) **Tynan, C.T., Ainley, D.G., Barth, J.A., Cowles, T.J., Pierce, S.D. and Spear, L.B. (2005).** Cetacean distributions relative to ocean processes in the northern California Current System. *Deep-Sea Res. I* 52: 145– 67.

44) **Longo LL, Amado Filho GM (2014).** O conhecimento da fauna marinha bentônica brasileira através dos tempos. História, Ciências, Saúde- Manguinhos 21:995–1010. https://doi.org/10.1590/S0104- 59702014000300011

45) **Pinedo MC, Rosas FCW, Marmontel M (1992).** Cetáceos e Pinípedes do Brasil. UNEP/FUA, Manaus

46) **Pinedo MC, Barreto AS, Lammardo MP, Andrade ALV, Geracitano L (2002).** Northernmost records of the spectacled porpoise, Layard's beaked whale, Commerson's dolphin and Peale's dolphin in the southwestern Atlantic Ocean. Aquat Mamm 28:32–37

47) **Rossi-Santos, M. R., Wedekin, L. L., & Engel, M. H. (2003).** Behavioral ecology of the Sotalia guianensis dolphins in the extreme southern Bahia region. *Revista de Etologia*, *5*, 200-201.

48) **Wenzel, F. W., Jann, B., Allen, J., Carrillo, M., Hanquet, S., Katona, S. K., ... & Stevick, P. T. (2003).** Migration of a humpback whale (Megaptera novaeangliae) between the Cape Verde Islands and Iceland. *J. Cetacean Res. Manage.*, *5*(2), 125-129.

49) **Zerbini NA, Secchi ER, Siciliano S, Simões-Lopes PC (1997).** A review of the occurrence and distribution of whales of the Genus Balaenoptera along the Brazilian coast. Report Int Whaling Commis 47:407–417

50) **Zerbini AN, Secchi ER, Bassoi M, Dalla Rosa L, Higa A, Sousa L, et al.(2004).** Distribuição e abundância relativa de cetáceos na Zona Econômica Exclusiva da região sudeste-sul do Brasil. Série de Documentos Revizee-Score Sul. http://www.mma.gov.br/o-ministerio/organograma/item/7605-programa-revizee-s%C3%A9rie-de-documentos. Accessed 11 March 2020

51) Zerbini AN, Secchi ER, Bassoi M, Dalla Rosa L, Higa A, Sousa L, et al.(2004) Distribuição e abundância relativa de cetáceos na Zona Econômica Exclusiva da região sudeste-sul do Brasil. Série de Documentos Revizee-Score Sul. http://www.mma.gov.br/o-ministerio/organograma/item/7605-programa-revizee-s%C3%A9rie-de-documentos. Accessed 11 March 2020

APPENDIX 1 : Sphyrna 20 data sheet

Sphyrna 20 - General presentation

Name	Sphyrna 20
Constructeur	Sea Proven (France)
Type	USV - Prao
Longueur	6m20 (20 pieds)
Largeur	1m52
Tirant d'air	1m90
Tirant d’eau	1m
Déplacement	100kg
Propulsion	Électrique (hélice) 420W Vélique
Vitesse de travail	2 kts
Vitesse max	10 kts
Masse à vide	35kg
Masse sans charge utile	85kg
Capacité batterie	915Wh
Portée transmissions	Suivi position par satellite Lien direct 500m

Equipements

Navigation

Positionnement GPS	Smartone solar	Advanced tracking
Station météo	Ulp	Calypso instruments

Commandes et propulsion

Motorisation	AQ1020	Apisqueen
Safran	D840wp	Hitec
Voile	Voile rigide 1 m^2	Sea Proven

Transmissions

Datas/ordres de mission	Tandem	FrSky

Système d'automatisation

Calculateur central	Pixhawk4	HolyBro
Calculateurs secondaires	Controllino mega	Controllino

Génération d'énergie

Solaire	0,7 m^2 panneaux souples	Energie mobile

Energie

Batterie	Lithium-ion 30V 915Wh	Torqeedo

Autres

Capteur de son	5 hydrophones	Cetacean research

Utilisations

- Commande temps réel à distance
  - A vue
  - Aux instruments sur PC opérateur
- Waypoints
  - Se dirige vers un ou une série de waypoints GPS
  - Reste dans un périmètre autour d'un WP
  - Points de ralliement
  - Zones d'exclusions/inclusions
- Patterns

APPENDIX 2: 7-day mission in Brittany, in the Bay of Audierne, testing two Sphyrna 20 drones for acoustic acquisition.