biodiversa+
European Biodiversity Partnership

EUROPEAN PARTNERSHIP

Monitoring European Rocky Reef Fishes

EuRockFish Pilot report

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What is Biodiversa+

The European Biodiversity Partnership, Biodiversa+, supports excellent research on biodiversity with an impact for policy and society. Connecting science, policy and practice for transformative change, Biodiversa+ is part of the European Biodiversity Strategy for 2030 that aims to put Europe's biodiversity on a path to recovery by 2030. Co-funded by the European Commission, Biodiversa+ gathers partners from research funding, programming and environmental policy actors in European and associated countries to work on 5 main objectives:

1. Plan and support research and innovation on biodiversity through a shared strategy, annual joint calls for research projects and capacity building activities
2. Set up a network of harmonised schemes to improve monitoring of biodiversity and ecosystem services across Europe
3. Contribute to high-end knowledge for deploying Nature-based Solutions and valuation of biodiversity in the private sector
4. Ensure efficient science-based support for policy-making and implementation in Europe
5. Strengthen the relevance and impact of pan-European research on biodiversity in a global context.

More information at: https://www.biodiversa.eu/

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List of acronyms

FRB	French Foundation for Biodiversity Research
OFB	French Biodiversity Agency (Office français de la Biodiversité)
NEA	Norwegian Environment Agency
FB	Fundación Biodiversidad
IEO	Instituto Espanol de Oceanografía
TAGEM	Ministry of Agriculture and Forestry
MoEP	Ministry of Environmental Protection
MoE of DK	Ministry of Environment of Denmark
AU	Aarhus University
MSFD	Marine Strategy Framework Directive
OSPAR	Oslo-Paris Convention
HELCOM	Helsinki Convention
RSC	Regional Sea Convention
MPA	Marine Protected Area
UVC	Underwater Visual Census
BRUV	Baited Remove Underwater Stereo Video

Executive summary

The European Biodiversity Partnership, Biodiversa+, has established a series of pilots as a proof of concept for its tasks leading to the establishment of a transnational network of biodiversity monitoring systems. In line with one of the priorities of Biodiversa+, the pilot “EuRockFish: Toward a European Rocky Reef Fish Monitoring Network” was launched in January 2024.

The aims of the pilot study are to:

- develop and test homogenized protocols for two traditional methods (visual census by scuba and video) and a novel complementary method (eDNA)
- validate the methodological framework that combines the three sampling methods
- optimize and harmonize sampling designs among methods and partners

This report focuses on all the aspects of the development of the EuRockFish pilot study from logistical aspects to protocols used. A figure presenting the complementarity between the three methods is presented.

The pilot study was coordinated by the OFB (French Biodiversity Agency), and was conducted with six active partners: Norway (Norwegian Environment Agency - Institute of Marine Research), France (PatriNat (OFB-MNHN), Denmark (Ministry of Environment - Aarhus University), Spain (Fundación Biodiversidad - Instituto Espanol de Oceanografía), Turkey (Ministry of Agriculture and Forestry, TAGEM) and Israel (Ministry of Environmental Protection - Israel Oceanographic and Limnological Research).

1. Context and relevance of a transnational biodiversity monitoring scheme

1.1. Context and aim

Biodiversity in European seas is facing growing pressures: climate change, pollution (i.e., contaminants and eutrophication), invasive species and unsustainable exploitation of resources are altering the structure and functioning of marine ecosystems. According to the United Nations, humanity is destroying biodiversity “at lightning pace”, with around one million species of plants and animals now threatened with extinction. Some 75 % of land-based ecosystems and two-thirds of marine environments have been significantly altered by human activity (IPBES, 2019). Understanding how marine ecosystems respond to these changes requires long-term, standardised monitoring efforts that can detect shifts in species composition, abundance, and ecological functioning.

In this context, the European partnership Biodiversa+ has launched the EuRockFish pilot study to build a robust and harmonised monitoring scheme for reef fish. The term “reef fish” refers here to fish assemblages associated with infralittoral (euphotic zone, usually <50m) and circalittoral (mesophotic zone, <150m) hard bottoms (rocky and boulder reefs, and complex 3D artificial structures as offshore wind farms). Reef fish communities play key roles in coastal ecosystems functioning and provide several goods and services to human communities (e.g., resources for food, tourism, economic income; Sanchez et al. 2015).

Currently, monitoring methods vary greatly across countries. Some rely on invasive gears (Cornelis *et al.* 2024), others on visual surveys (UVC, underwater Visual Census; Azurro *et al.* 2013) and still others on molecular tools (eDNA, Boulanger *et al.* 2021). This wide range of distinct, though complementary, methods make comparison, synthesis and pan-European assessment difficult to reach (Harvey *et al.* 2001, Bosh *et al.* 2017).

The primary aim of the Eurockfish pilot study, focusing on reef fish, is to tackle the critical gap represented by the lack of interoperable and non-invasive monitoring methods that can reliably deliver comparable data across diverse European seas. So as to answer this gap, Eurockfish pilot study offered to develop, test and deploy standardised protocols for monitoring reef fish, focusing on 3 complementary methods: UVC, Baited Remote Underwater Video (BRUV) and eDNA metabarcoding.

1.2. Link to public policies and monitoring frameworks

Reef fish are key biodiversity components of European coastal ecosystems, there is an urgent need to use standardised monitoring to assess rocky reef communities through the time and the space (i.e., to assess their spatio-temporal variability). Indeed, it is essential to have a good understanding of the functioning of rocky reef fish communities to accurately assess anthropogenic impacts on fish in both shallower infralittoral and deeper circalittoral reefs. Multi-depth assessment is important since vertical zonation affects natural fish distribution as well as intensity-levels of anthropogenic threats and putative impacts on fish assemblages. Monitoring and assessments of human impacts will allow prioritisation of management measures, and ultimately to achieve and maintain good ecological status of European reef fish and the functionalities of their habitats. By developing interoperable, non-invasive, and standardised protocols for monitoring fish reef communities, EuRockFish provides essential data and methodological tools that can be integrated into regional and national monitoring programmes. This alignment also strengthens coherence between scientific research and policy implementation.

In this respect, improving the monitoring of European reef fish using a combination of traditional and novel methods will produce new knowledge to fill in the taxa gap and enable better reporting of reef fish ecological status within the framework of several regional sea conventions (RSCs), including the Oslo-Paris convention (OSPAR), the Barcelona convention, the Helsinki convention (HELCOM), and the European Union Marine Strategy Framework Directive (MSFD). With regard to the MSFD, the EuRockFish pilot study could contribute to define the appropriate methods to monitor reef fish according to the environmental context. The establishment of a harmonised methodological framework is a first - but essential - step in order to setting up a monitoring network at the pan-European scale and to collect metrics for the D1C2, D1C3 and D1C4 criteria of the MSFD (but see Table 1 for a more exhaustive view of the link between the public policies descriptors and the EuRockFish pilot study).

Table 1: Facets of fish ecological status to be assessed during the EuRockFish pilot study

Facets of fish ecological status to be assessed		Descriptors		
Level	**Type of metrics**	**MSFD and Barcelona**	**OSPAR**	**HELCOM**
Population	Abundances and biomasses of populations	D1C2	FC1	Abundance of key species
Population	Population structure (including body-size distribution)	D1C3	FC2	
Population	Biogeographic patterns in distribution of populations	D1C4		
Population	Fish status x Habitat status : *Population renewal is not hampered by degraded quality nor reduced extent of species habitats (including essential fish habitats such as nursery and spawning grounds)*	D1C5		
Assemblage	Fish diversity in terms of taxonomic, phylogenic and functional indices	D4C1		
Assemblage	Relative abundances per functional guilds	D4C2,		Abundance of coastal fish key functional groups
Assemblage	Body-Size distribution per functional guilds	D4C3	FW3	
NIS	Arrival of new Non-Indigenous-Species (NIS) introduced by human activities	D2C1	Trends in arrival of new NIS introduced	Trends in New Records of NIS
NIS	Abundances and distribution of Non Indigenous Species (NIS)	D2C2		

In addition, assessing reef fish ecological status may contribute to the assessment of the functionalities (e.g., nursery, spawning areas) of rocky benthic habitats (such as shallower algal forests and deeper animal forests), which have to be assessed under the MSFD, Habitats Directive (92/43/CEE) and RSCs. Finally, European reef fish may be targets of conservation/management plans at territorial scales (e.g., Marine Protected Areas, Offshore Wind Farms). It is worth noting that integrating MPA monitoring within MSFD and RSCs monitoring strategies is fundamental since: (1) MPAs could be considered as reference for ecological status, and (2) mutualizing monitoring efforts optimise resources.

1.3. European/transnational dimension

Despite the conservation and management challenges and the regulatory frameworks stated above, European reef fish remain poorly studied and monitored at the European level. To fill this gap, there is an

urgent need to foster closer collaboration between reef fish monitoring practitioners (primarily researchers and managers of MPAs) and marine policy experts involved from European nations bordering both European and adjacent seas. Such collaboration is crucial to advance the harmonisation of sampling protocols, monitoring designs and bioindicators at the European scale. Establishing common standards will ensure the production of high quality, comparable data and harmonisation of long-term time-series, thereby strengthening the scientific basis for ecosystem assessments and policy implementation.

This initiative ultimately aims to operationalize an observational monitoring network capable of collecting interoperable data under a unified monitoring strategy, thereby providing improved support for marine fish-related policies such as MSFD, RSCs, MPAs and OWFs. It also helps to better monitor changes in the distribution range of reef fish as well as to assess the spread of Non-Indigenous-Species (NIS) in European and contiguous seas, as a consequence of climate change (MSFD Descriptor 2).

In some regions, time-series are already available. Modifying existing protocols could pose challenges, making pan-EU standardisation difficult to achieve. However, harmonisation remains feasible through the inter-calibration of existing protocols, which would facilitate results comparisons, and support a gradual transition toward standardised monitoring practices at the European scale, without disrupting ongoing time-series.

In this report, we first present an overview of the existing monitoring schemes linked to the EuRockFish pilot study. We then focus on the following aspects of the EuRockFish study: (1) the activities undertaken during the project, (2) the governance, coordination and (3) the project budget. This section is followed by a dedicated chapter detailing the sampling design, the protocols applied and the preliminary analysis conducted throughout the study. Finally, the report concludes this report with a brief discussion of the key issues and aspects that warrant further investigation.

2. Current biodiversity monitoring landscape

Over the years, several attempts have been made to harmonise marine biodiversity monitoring and centralise data. By example, at the national level, Good Environmental Status reporting for MSFD and RSCs has led to an increase in reef fish monitoring efforts. However, collaboration between stakeholders of different countries to harmonise monitoring protocols and data collection remains limited. More recently, MBON Europe has sought to formalise a European marine biodiversity monitoring network, supporting long-term monitoring and improving the standardisation of methods as well as the integration of both publication and data. However, as no dedicated program currently exists to harmonise reef fish monitoring across Europe, synergies remained to be explored.

Metabarcoding on eDNA currently has emerged as a novel and promising approach, attracting considerable attention and leading to the creation of several working groups, including the European working group DNAqua-Net (https://dnaqua.net/), as well as consortia, such as VigiLife (Giraud *et al.* 2024). These initiatives aim to advance the use of eDNA sequencing-based methods for aquatic biodiversity monitoring, by establishing a robust framework and generating new tools to maximise the potential of this technique (Rey *et al.* 2021). EuRockFish will build upon these efforts, to optimise sampling protocols and bioinformatic pipelines, thereby improving knowledge on reef fish assemblages.

Regarding data storage and sharing, several information systems have emerged in recent years, such as GBiF, the Global Biodiversity Information Facility, or OBIS, the Ocean Biodiversity Information System. Such facilities are highly valuable for Eurockfish, as they will facilitate the dissemination of knowledge generated by the EuRockFish project and ensure that it remains readily accessible for further research.

Hence, at your knowledge, the network established by EuRockFish represents the first initiative aiming to harmonise the monitoring of rocky reef fish assemblages using a combination of visual and genetic methods. It paves the way for similar efforts, complementing existing monitoring programs for MSFD D1-FC, while creating a framework for data acquisition, analysis, and a reference database for eDNA metabarcoding.

3. Proof of concept: the pilot study

3.1. Activities and timeline

3.1.1. Generalities

Several activities have been/will be implemented during the project, in accordance with the tasks identified in Biodiversa+ Work Package 2 (WP2)

(1) Develop consensual protocols to monitor fish assemblages in infralittoral and circalittoral reefs, based on two traditional methods (underwater visual census by scuba diving and baited remote underwater stereo video stations) and a complementary novel method (metabarcoding on eDNA). The definition of the localities and monitoring sites will also be discussed to optimise the sampling effort.
(2) Conduct the fieldwork in one or two localities in each partner's country to test the monitoring protocols in the field and collect new data.
(3) Process the newly acquired visual data and send the eDNA samples to the selected external supplier for eDNA extraction and bioinformatic analyses, prior to data centralisation by the pilot coordinator for analysis.
(4) Evaluate the potential and added value of methods combination to assess fish assemblages' responses to natural and anthropogenic forcings, detect the presence of NIS, and validate a methodological framework that combines traditional visual methods with novel sampling techniques to provide complementary metrics on the ecological status of fish assemblages and populations.
(5) Based on critical evaluation, statistical power analyses, and feedback from the active contributors, propose updates to the protocols and monitoring strategy to support the long-term monitoring of rocky reef fish in European and adjacent seas.
(6) Synthesise results, and produce a methodological guideline for European reef fish monitoring under the MSFD, RSCs and MPAs.
(7) Share data according to FAIR principles and publish a data paper (GBIF or OBIS)

3.1.2. Temporal list of activities

Spring 2024

- Procure necessary equipments, based on the guidelines provided by the pilot coordinator
- Prepare for the field surveys
- Select monitoring sites: up to 2 localities representing a gradient of anthropogenic pressure, in close collaboration with the pilot coordinator
- Organization of in-person workshop in Marseille with all partners

Summer 2024 and/or summer 2025

- Conduct field surveys in each country (expertise required), deploying at least one visual method (UVC or BRUV), in addition to water sampling for eDNA metabarcoding.

Fall 2024 until/or fall 2025

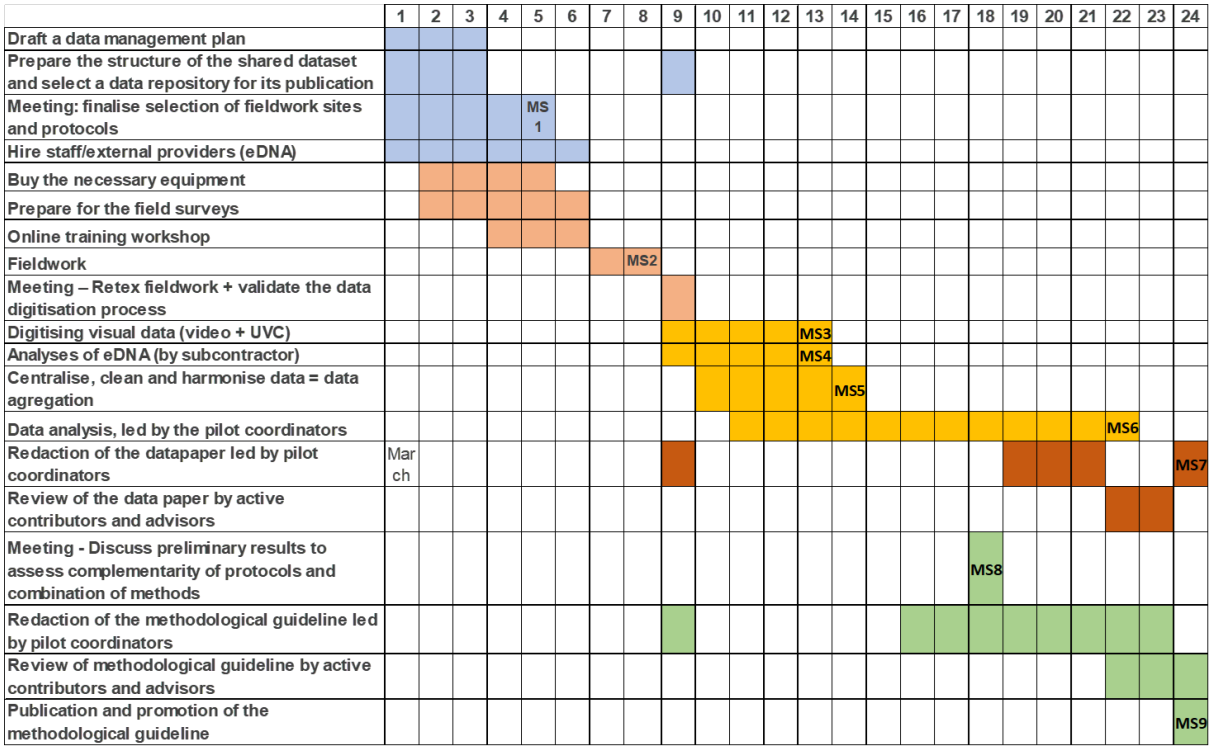
- Digitise collected data
- Centralise the data in a dedicated dataset (managed by coordinator)
- The coordinator (in collaboration with all other partners) is responsible for overall project coordination, data analysis, and reporting between winter 2024 and end of December 2025.
- Active contributors and advisors will assist reviewing and finalising the reports by the end of December 2025.

2026 (upcoming)

- The coordinator will complete the data analysis and publication in close collaboration with all partners.
- The coordinator will lead the preparation of a methodological analysis for monitoring rock reef fish.

To properly implement the protocols that will be refined during the proposal, it is necessary to assemble a team of experts in each participating country. Additional resources will also be required to conduct the monitoring, with associated costs covered by Biodiversa+ funding.

Initial timeline (Gantt chart)



3.1.3. Milestones

- MS1: Finalised protocols and sites selection
- MS2: At least 80% of sites monitored using the selected combination of methods
- MS3: all visual data are processed (UVC + video)
- MS4: eDNA data and biomolecular analyses delivered by subcontractor

- MS5: data aggregation process is over and the final dataset including 15 fish-related metrics is available for analysis
- MS6: Results from analyses to evaluate the effectiveness of the methodological framework
- MS7: publication of the data paper
- MS8: Guideline plan collectively drafted
- MS9: Methodological guideline is published.

Field work is postponed to 2025 for FB.

3.1.4. Updated timeline (considering the one-year extension)

Context of the extension

The Biodiversa+ EuRockFish pilot (sub-task 2.6.6) focuses on monitoring rocky reef fish and analysing the complementarity of three different methods to more accurately assess infralittoral and circalittoral reef fish communities at the European scale. This pilot study included a major fieldwork campaign involving 6 partners from Norway, Denmark, France, Spain, Turkey and Israel. Fieldwork is constrained to the period between June and September due to the seasonality/phenology of target fish species, ensuring the reproducibility of data. Initially, these campaigns were planned for Spring and Summer 2024, allowing time in 2025 for data analyses and the development of a robust methodological guideline for monitoring rocky reef fish communities.

However, the fieldwork campaign in Spain and Israel had to be postponed to 2025 for different reasons:

- In Israel, the geopolitical context in 2024 resulted the closure of the sea area for safety reasons, preventing the Israeli team from conducting fieldwork.
- In Spain, administrative delays in the shipment of equipment Meant that only the Mediterranean Sea survey could be conducted in 2024, while the Atlantic Ocean survey had to be postponed.

As a result, the two fieldwork campaigns in Israel and Spain have been scheduled for Spring and Summer 2025, with data digitization beginning immediately afterward. eDNA analyses require approximately five months, while digitization of UVC and BRUV data tales at least three months. As a consequence, the complete dataset is only expected by the end of 2025. Therefore, an additional year was required to analyse the entire EuRockfish dataset and to provide a comprehensive and robust methodological guideline incorporating all collected data.

Updated timeline

Tasks	Initial timeline	Updated timeline
Prepare the structure of the shared dataset and select a data repository for publication	Jan-March 2024	Sep 2024 – Jan 2025
Fieldwork	July – Aug 2024	July – Aug 2024 and June – July 2025
Digitising visual data (UVC + video):	Sep 2024 – Jan 2025	July – Oct 2025
eDNA analyses (by subcontractor)	Sep 2024 – March 2025	Sep 2025 – Jan 2026
Data centralisation, cleaning and harmonisation data	extended duration Oct 2024 – Nov 2026	

Data analyses (led by the pilot coordinator)	extended duration Oct 2024 – Nov 2026	
Preparation of the data paper (led by the pilot coordinator)	June – Dec 2025	Oct – Dec 2026
Review of the data paper (active contributors and advisors)	Nov – Dec 2025	Nov – Dec 2026
Preparation of the methodological guideline (led by the pilot coordinator)	Sep 2024	March – Nov 2026
Publication and dissemination of the methodological guideline	Dec 2025	Dec 2026

3.2. Governance and coordination

The mandate of the coordinator is not nominative. Yet, as far as possible, continuity is expected in the person or persons representing the Biodiversa+ partner acting as pilot coordinator (OFB).

Each sub-pilot coordinator possesses both coordination/chairing skills, as well as in-depth knowledge and expertise on the topic addressed by the pilot study. When necessary, to cover both the strategic coordination and thematic expertise, the role of pilot coordinator may be shared between two individuals (either from the same or different organisations).

Specifically, the tasks of the coordinator have included:

- Drafting the pilot work plan
- Planning and monitoring the activities carried out within the pilot, in collaboration with the general coordinator and active contributors
- Maintaining an overarching view on the pilot study
- Preparing and chairing the meetings of the pilots working groups
- Reporting on pilot activities and drafting lessons learned, including synthesising the main outcomes of the pilot study, using quantitative data where relevant.
- Reporting progress to the Operational Teams for the activity report and other reporting obligations to the European Commission.

Similarly, the mandate of an active contributor is not nominative. Active contributors ensure continuity of the person or persons representing the Biodiversa+ Partner acting in this role.

The tasks of the active contributors have included:

- Providing feedback on the pilot study work plan
- Actively co-developing and co-implementing the activities of the pilot in which they are involved (see planned deliverables and milestones, and activity list below).
- Reporting progress to the pilot coordinator and Operational Team for activity reports and other reporting obligations to the European Commission.
- Participating in the activities listed below.

3.3. Budget

3.3.1. Initial budget

Partner	Nb PM	PM (€)	PM description	Travel & subsistence (€)	Equipment (€)	Other goods, works and services (€)	TT Other direct costs (€)	Description	Subcontracting costs (€)	Indirect (15% flat rate)	TOTAL with indirect costs (€)
OFB (coord.)	24	164 208.00	8 PM for the coordination during 2 years 16 PM for data analysis	5 000.00	-	2 000.00	7 000.00	Travel & subsistence: 5000 to organize a workshop / attend meeting. Other goods, works and services: 2000 to organize a workshop and communicate.	307 600.00	25 681.20	504 489.20
OFB	15	102 630.00	6 PM for fieldwork, 1 PM UVC data digitization and curation, 4 PM BRUV video derush, 4 PM pilot activities	13500.00	30 500.00	44 900.00	88 900.00	Travel and subsistence: 1500 to attend a workshop and 12000 to travel to field sites. Equipment: 12000 for underwater pumps, 9500 for BRUV camera stereo and 9000 for diving equipment for 3 people. Other goods works and services: 500 for sending samples, 400 for baits, 2000 for UVC consumables, 2000 for tank filling, 40000 for boat renting.	-	28 729.50	220 259.50

NEA	15	118 275.00	6 PM for fieldwork, 1 PM UVC data digitisation and curation, 4 PM BRUV video derush, 4 PM pilot activities	13 500.00	30 500.00	44 900.00	88 900.00	Travel and subsistence: 1500 to attend a workshop and 12000 to travel to field sites. Equipment: 12000 for underwater pumps, 9500 for BRUV camera stereo and 9000 for diving equipment for 3 people. Other goods works and services: 500 for sending samples, 400 for baits, 2000 for UVC consumables, 2000 for tank filling, 40000 for boat renting.	-	31 076.25	238 251.25
FB	15	49 200.80	6 PM for fieldwork, 1 PM UVC data digitisation and curation, 4 PM BRUV video derush, 4 PM pilot activities	13 500.00	30 500.00	44 900.00	88 900.00	Travel and subsistence: 1500 to attend a workshop and 12000 to travel to field sites. Equipment: 12000 for underwater pumps, 9500 for BRUV camera stereo and 9000 for diving equipment for 3 people. Other goods works and services: 500 for sending samples, 400 for baits, 2000 for UVC consumables, 2000 for tank filling, 40000 for boat renting.	-	20 715.00	158 815.00

TAGEM	15	22 500.00	6 PM for fieldwork, 1 PM UVC data digitisation and curation, 4 PM BRUV video derush, 4 PM pilot activities	13 500.00	30 500.00	44 900.00	88 900.00	Travel and subsistence: 1500 to attend a workshop and 12000 to travel to field sites. Equipment: 12000 for underwater pumps, 9500 for BRUV camera stereo and 9000 for diving equipment for 3 people. Other goods works and services: 500 for sending samples, 400 for baits, 2000 for UVC consumables, 2000 for tank filling, 40000 for boat renting.	-	16 710.00	16 710.00
MoEP	15	84 465.00	6 PM for fieldwork, 1 PM UVC data digitization and curation, 4 PM BRUV video derush, 4 PM pilot activities	13 500.00	30 500.00	44 900.00	88 900.00	Travel and subsistence: 1500 to attend a workshop and 12000 to travel to field sites. Equipment: 12000 for underwater pumps, 9500 for BRUV camera stereo and 9000 for diving equipment for 3 people. Other goods works and services: 500 for sending samples, 400 for baits, 2000 for UVC consumables, 2000 for tank filling, 40000 for boat renting.	-	26 004.75	199 369.75

MoE of DK	15	154 590.00	6 PM for fieldwork, 1 PM UVC data digitization and curation, 4 PM BRUV video derush, 4 PM pilot activities	13 500.00	30 500.00	44 900.00	88 900.00	Travel and subsistence: 1500 to attend a workshop and 12000 to travel to field sites. Equipment: 12000 for underwater pumps, 9500 for BRUV camera stereo and 9000 for diving equipment for 3 people. Other goods works and services: 500 for sending samples, 400 for baits, 2000 for UVC consumables, 2000 for tank filling, 40000 for boat renting.	-	36 523.50	280 013.50

3.3.2. Reshuffled budget

NB: No reshuffle for Denmark and Israel

OFB (as coordinator):

- *Travel & Subsistence* and *Other goods, works and services*: For the practical workshop, OFB will pay for an all-inclusive service provision (boat, meeting room, administrative fees for arranging partners accommodations). The 2 categories “travel & subsistence” and “Other goods, works and services” are therefore merged together.

- *Subcontracting costs*: FRB will manage the call for tender. However, it will be too late to order the sampling kits for the partners that start the fieldwork in July: 25 000 € (on the 307 600 €) remain at OFB to buy the sampling kits for Demnark, France and Spain (the analyses will be covered by the call for tender).

OFB (as partner):

- Equipment:
  - ***Underwater pumps****: 22 000€ instead of 12 000€.* In France OFB will use deep diving techniques that allow to dive deeper than other partners. We will take advantage for sampling eDNA deeper. But we need to reduce as much as possible the time at depth. We need to purchase a second UW pump in order to reduce the time needed to sample while being underwater by scuba.
  - ***Diving equipment****: 2 800€ instead of 9 000€.* OFB owns already most of the necessary diving equipment. OFB needs to buy less equipment (3000€ instead of 9000€)
  - ***BRUVs****: 0€ instead of 9 500€.* OFB already has the BRUV systems. This allows to save money for this task and buy a second eDNA UW pump.
- Other goods, works and services:
  - ***Diving equipment:*** *9 100€ instead of 0€.* OFB requires services for annual maintenance:
  - ***Tank filling:*** *600€ instead of 2 000€.* OFB already has tank filling facilities that allow to reduce costs.
- Travel and subsistence:
  - ***Travel expenses****:* 11 347.5€ instead of 12 000€. OFB will rent for a truck during the whole field campaign (7764 €) + rent accommodation
  - ***Workshop:*** 2 152.5€ instead of 1 500€. For the practical workshop, every single partner (including OFB) will have to pay the all-inclusive service provisioner, 715.5€/person for bed and breakfast, lunch and dinner. OFB as coordinator will come with 3 persons.

FB (Spain):

- Equipment:
  - ***Underwater pump*** 12 230.91€ instead of 12 000€. The budget for the pump and transportation to Spain. Supplier's final budget is higher than initial budget.
  - ***Diving equipment****:* 0€ instead of 9 000€. IEO already has diving equipment. This allows to save money on this task and overall to contribute to buy BRUV equipment and hardware, software.
  - ***Camera stereo****:* 24 500€ instead of 9 500€. In the IEO we do not have the BRUV equipment, the quote is higher than initially planned, and also requires hardware and software for the calibration and processing of the images which further increases the budget (14000 €), as it was not contemplated in the original budget.

- Other goods, works and services:
    - ***UVC consumables****: 0€ instead of 2 000€.* IEO already has diving consumables. This allows to save money on this task and overall to contribute to buy BRUV equipment and hardware, software.
    - ***Tank filling****: 1 500€ instead of 2000€*. In some of the locations, IEO will have tank filling facilities that will reduce costs. This allows you to save money on this task (-€500). and generally, contribute to the purchase of a BRUV.

MoE of Denmark:

- Equipment:
    - ***Underwater pumps***: 12 500€ instead of 12 000€. Underwater pumps are a bit more expensive than expected
    - ***Diving equipment***: 9 000€ instead of 3 000€. Aarhus University (AU) already owns most of the necessary diving equipment and therefore needs less to cover the expected costs
    - ***Camera stereo:*** 22 000€ instead of 9 500€. AU has no BRUV equipment at all, yet. the full BRUV equipment is much more expensive than expected
- Other goods, works and services:
    - ***Underwater pump****:* 7 000€ instead of 0€. In case the call for FRB is late and FRB cannot provide AU with sampling kits, AU could pay for the eDNA sampling kits.
    - ***Tank filling:*** 0€ instead of 2 000€. AU has tank filling facilities for free.
    - ***Boat renting:*** 31 000€ instead of 40 000€. AU has boat facilities less expensive than average-partner budget planification
- Travel and subsistence:
    - ***Travel expenses:*** 9 000€ instead of 12 000€. AU expenses related to fieldwork will be less expensive than average-partner budget planification

TAGEM (Turkey):

- Equipment:
    - ***Diving equipment:*** 0€ instead of 9 000€. TAGEM already owns most of the necessary diving equipment
    - ***Camera stereo****:* 18 500€ instead of 9 500€. TAGEM has no BRUV equipment at all, yet. The full BRUV equipment is much more expensive than expected.
- Other goods, works and services:
    - ***UVC consumables****: 2 400€ instead of 2 000€.* TAGEM does not need budget for bait, hence we moved the bait budget to UVC consumables.
    - ***Bait:*** *0€ instead of 400€.* TAGEM does not need budget for bait
    - **Boat renting with pilot**: 3 8000 € instead of 40 000€. Boat renting is less expensive than expected.
- Travel and subsistence:
    - ***Workshop:*** *3 500€ instead of 1 500€.* Participation in the workshop is more expensive than expected.

3.4. Protocols and logistics

3.4.1. Sampling design

The sampling strategy follows a nested design from regional seas to station with a final number of replicates varying among approaches (i.e., UVC, BRUVs and eDNA; see Figure 1).

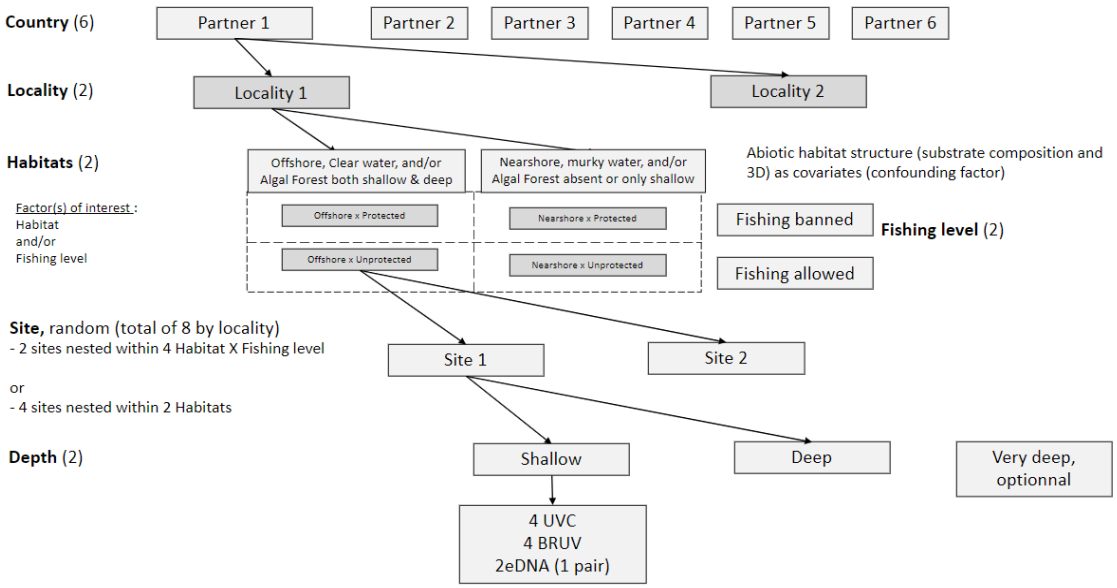


Figure 1 : Nested sampling design of EuRockFish pilot study.

3.4.2. Localities

In each country, two localities are defined. These two localities should be sufficiently geographically distant to integrate spatial variability at the scale of the country's shore. For each locality, height rocky sites are selected according to an environmental gradient (water mass, benthic anthropogenic pressure, etc.; see Figure 1). As example, the sites can be crossed coast/offshore and inside/outside reserve gradient (see Figure 2).

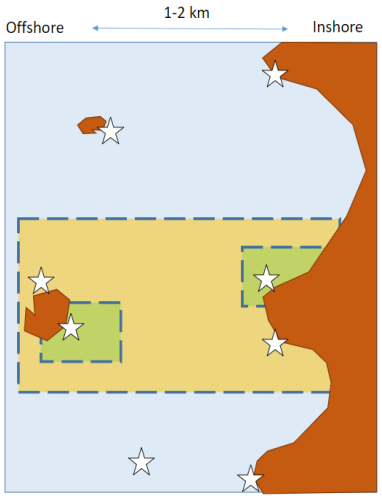


Figure 2 : Example of sites (star symbols) location design across a coast/offshore and an inside/outside reserve gradient (green square: protected area; yellow square: not protected area)

Rocky sites

The different sampling sites are selected to be as homogeneous as possible in their seascape characteristics. They ideally include different depth strata (shallow: around - 8m, deep: around - 18 m and optionally, very deep: around – 38m) and an average slope of 45°. If the slope is very gentle, it is recommended to select a pair of nearby rocky flat semi-sites: one shallow semi-site and one deep semi-site (and if possible, one very deep semi-site).

Depth strata (station)

In each site, two depth strata are targeted at – 8 m and – 18 m ± 2m (chart datum) respectively for shallow and deep stratum. If possible, a very deep stratum at – 38m (chart datum) can be added, at least for BRUV and eDNA. Indeed, as bathymetry interact with anthropogenic and environmental factors (e.g., spearfishing pressure is higher in shallow water, turbidity affects vertical distribution of macrophytes, changes in temperature and wind regimes affect thermocline, etc.), it is necessary to include different strata in the sampling design.

Spatial arrangements of replicates

For each station, UVC, BRUV and eDNA methodology are deployed as follows:

- UVC: four replicates (i.e., transects) are carried out for each bathymetry strata. The starting point of a transect is random but (1) the whole transect will be rock and bathymetry ranges within the depth stratum, and (2) any point of one transect is at least 15 m apart from any other transects.
- BRUV: four replicates (i.e., BRUVs) are deployed in each depth stratum. As far as possible, BRUV units are at least 100 m apart from each other.
- eDNA: two replicates (i.e., two filters) are sampled for each depth stratum (corresponding to four samples per site). Although it is possible to sample both filters at the same time, it is preferable to sample one filter at a time, by dropping the pump twice.

3.4.3. Sampling design according to the partner country

The real number of replicates can vary among partner countries depending on their specific constraints (site and depth visibility, methodological feasibility, etc). The Table 2 summarizes the sampling design of the EuRockFish study and the Figure 3 locates the sampled sites for each country.

Table 2 : Sampling design in the EuRockFish pilot study

Ocean/Sea			ATLANTIC OCEAN (ATL)			MEDITERRANEAN SEA (MED)		
Country	Norway	Denmark	France	Spain	Spain	France	Turkey	Israel
Locality	2 random	Salty vs brackish	ATL & MED	ATL & MED	ATL & MED	ATL & MED	MED vs Black Sea	North and South
Main factor(s)	Lobster reserve X In / offshore	In / offshore	In / offshore	NTZ X In / offshore	NTZ X In / offshore	NTZ	NTZ X In / offshore	NTZ X In / offshore
Shallow	-8m	-8m	-8m	-8m	-8m	-8m	-8m	-8m
Deep	-18m	-18m	-18m	-18m	-18m	-18m	-18m	-18m
Very deep			-38m (4 sites)	-38m (4 sites)	-38m (4 sites)	-38m (4 sites)		-50m (BRUV only)

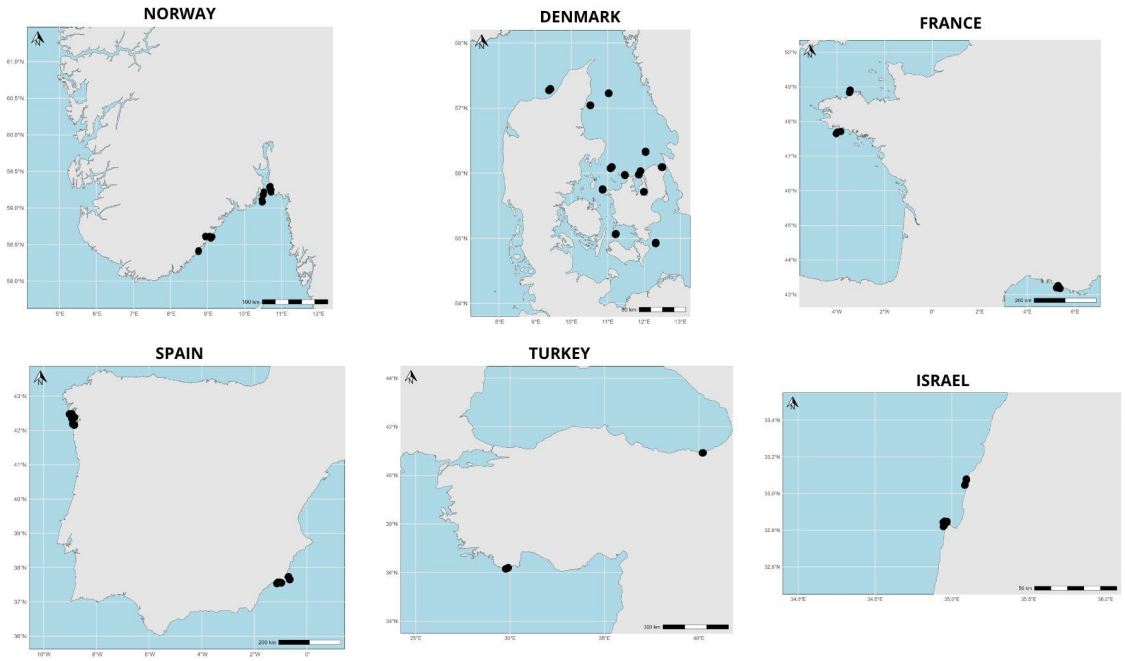


Figure 3 : Location of sampled sites of each partner country during the EuRockFish pilot study.

Example of organization to sample one site

- *Case 1:* Two divers and 2 (or 3) other agents are available. In this case, the divers are in charge of diving and the other agents handle BRUVs.

Morning	Afternoon
○ Dropping eDNA in Station 2 ○ Dropping BRUVs in Station 1 ○ Retrieving eDNA in Station 2 ○ Diving in Station 2 ○ Retrieving BRUVs in Station 1	○ Dropping eDNA in Station 1 ○ Dropping BRUVs in Station 2 ○ Retrieving eDNA in Station 1 ○ Diving in Station 1 ○ Retrieving BRUVs in Station 2

- *Case 2:* Only two divers and one boat diver are available. In this case, it is unsecure to do the plan presented in Case 1 because divers will have to handle BRUVs themselves increasing the risks of decompression sickness. It is so preferable to plan three days for sampling two sites.

	Morning	Afternoon
Day 1	Site 2: UVC replicates and 1 eDNA replicate / station	Site 2: UVC replicates and 1 eDNA replicate / station
Day 2	BRUVs replicates in site 1 and site 2	
Day 3	Same than *Day 1*, UVC and eDNA replicates in Site 1 and 2 for completing	

3.4.4. Protocols

NB: The detailed version of the three protocols is available in Appendices A, B and C..

Underwater visual census (UVC)

Underwater visual census is a conventional method largely used to assess the diversity of rocky reef fish since many years (D'agata et al., 2014; McClanahan et al., 2007). One or two divers count and identify all fishes encountered along a transect of a given length. The size of each individual is also visually estimated. In the context of the EuRockFish pilot study, a standardised transect length of 30 meters has been defined.

Baited Remove Underwater Videos (BRUVs)

A system composed of two stereo-video cameras with a bait is put on the seafloor or hung in the water for minimum one hour (to have a minimum one hour viewing). Then, all the videos are analysed to define the maximum number of individuals of each species in a single frame. We also measure the size of these individuals with *EventMeasure* software.

Metabarcoding on environmental DNA (eDNA)

A 30-litre sample of seawater is filtered by eDNA underwater pump during 30 minutes in depth and on surface. Then, the filters are sent to the subcontractor laboratory for extraction, amplification and eDNA identification. A list of species and/or OTU (Operational Taxonomic Unit) is obtained for each filter.

3.5. Data and sample management

The full workflow for the data and samples that were collected in the field study are presented in the Data Management Plan of the EuRockFish pilot study. By consequence, we listed here only the main steps of the workflow for the data and samples collected in the field.

3.5.1. Data aggregation and analysis

Once the fieldwork is over:

(1) Active contributors (or their third parties) have to digitise the visual data (video and UVC) in the format that be agreed upon the dedicated meeting. The datasets have then been sent to the pilot coordinator
(2) Active contributors send eDNA samples for extraction and biomolecular analysis to the selected subcontractor
(3) The subcontractor processes the eDNA samples. The subcontractor oversees eDNA extraction, eDNA amplification using selected primers and bioinformatic analysis. The results will be presented as a matrix with all MOTUs in addition to the list of detected species.
(4) The pilot coordination centralises, cleans and harmonises data.
(5) The pilot coordination leads data analyses.
(6) Active contributors and advisors are expected to provide their feedback on the analyses and results (by email and/or during dedicated meetings).

3.5.2. Data valorisation/publication

The pilot coordination will lead the writing of the data paper(s). Data will be stored, standardised and published following FAIR principles. Active contributors and advisors are expected to review the data paper before publication.

3.5.3. Analyses

As data analysis is still ongoing at the time of writing this report, only very preliminary results from the Marseille site (Mediterranean Sea, France) are presented here. Further analyses for the other sites and methods will be carried out during 2026.

3.5.4. Study case: Capacity to the three methods to characterize fish communities

Example of Mediterranean Sea, France – Preliminary results

We analyzed the capacity of the three methods to characterize fish communities and eventually, to detect changes in the composition of rocky reef fish communities between protected area (two sites located within the Calanques National Park, Marseille, France) and unprotected areas (two sites located outside the National Park) at three different depth strata: (shallow (-8m), deep (-18m), and very deep (> -30m)).

Underwater Visual Census (UVC)

For UVC, the results demonstrate that this method effectively differentiates rocky reef fish communities across depth strata, particularly between the shallow and very deep zones (PERMANOVA, p-value < 0.001; see Figure 4). Based on this result, we further assessed the capacity of UVC to discriminate variations in fish community composition according to protection level (protected versus unprotected areas) within each depth stratum.

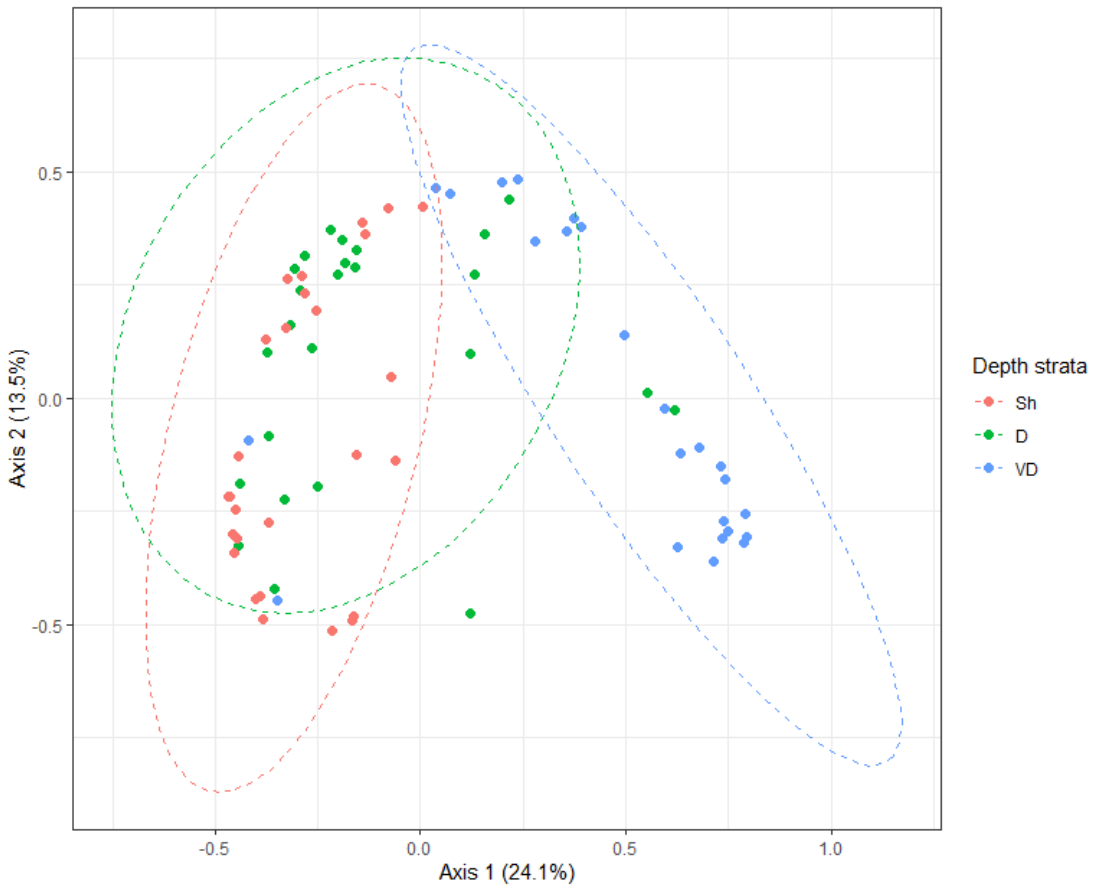


Figure 4 : β-diversity analysis of the fish communities in the Mediterranean Sea (France) between different depth strata. Principal Coordinates Analysis (PCoA), distance metric: Bray-Curtis. Sh: shallow strata, D: deep strata, VD: very deep strata.

In the shallow stratum, our results show that UVC detects differences in fish community composition between protected area and unprotected areas (PERMANOVA, p-value<0.001). A similar pattern is observed in the very deep strata, albeit to a lesser extent (PERMANOVA, p-value = 0.01). In contrast, no significant differences were detected in the deep stratum (PERMANOVA, p-value =0.13).

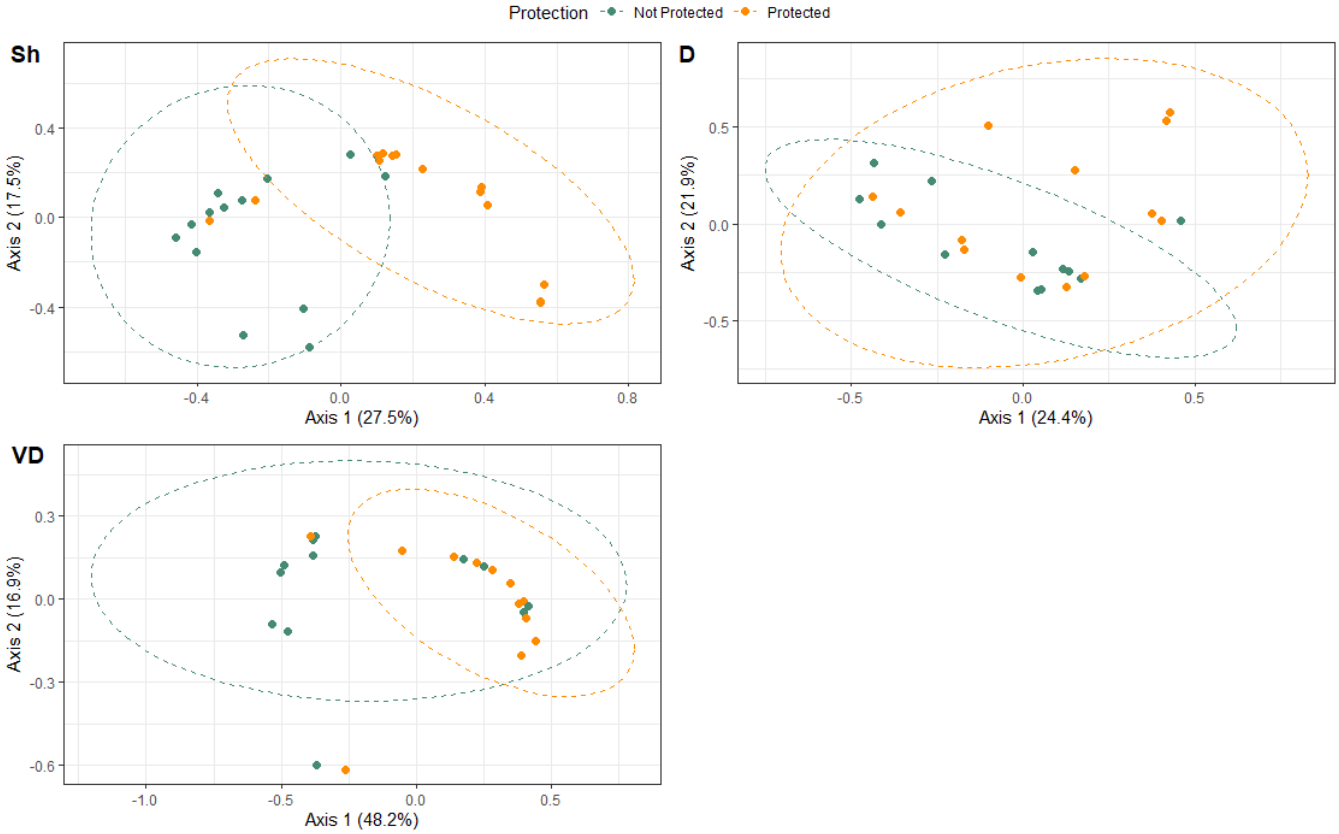


Figure 5: Principal Coordinates Analysis (PCoA; distance metric: Bray-Curtis) of fish community between not protected sites and protected sites for each depth strata (UVC). Sh: shallow strata, D: deep strata, VD: very deep strata

Baited Remove Underwater Stereo-Video (BRUV)

NB: The time required to derush all video from BRUV is considerably longer than initially anticipated. It is imperative to ensure that the necessary human resources are in place prior to planning a significant fieldwork campaign involving BRUV.

Preliminary results indicate that BRUV can distinguish differences in fish community composition between the very deep stratum (>-30m) and the shallow or deep strata (PERMANOVA, p-value<0.001; see Figure 6).

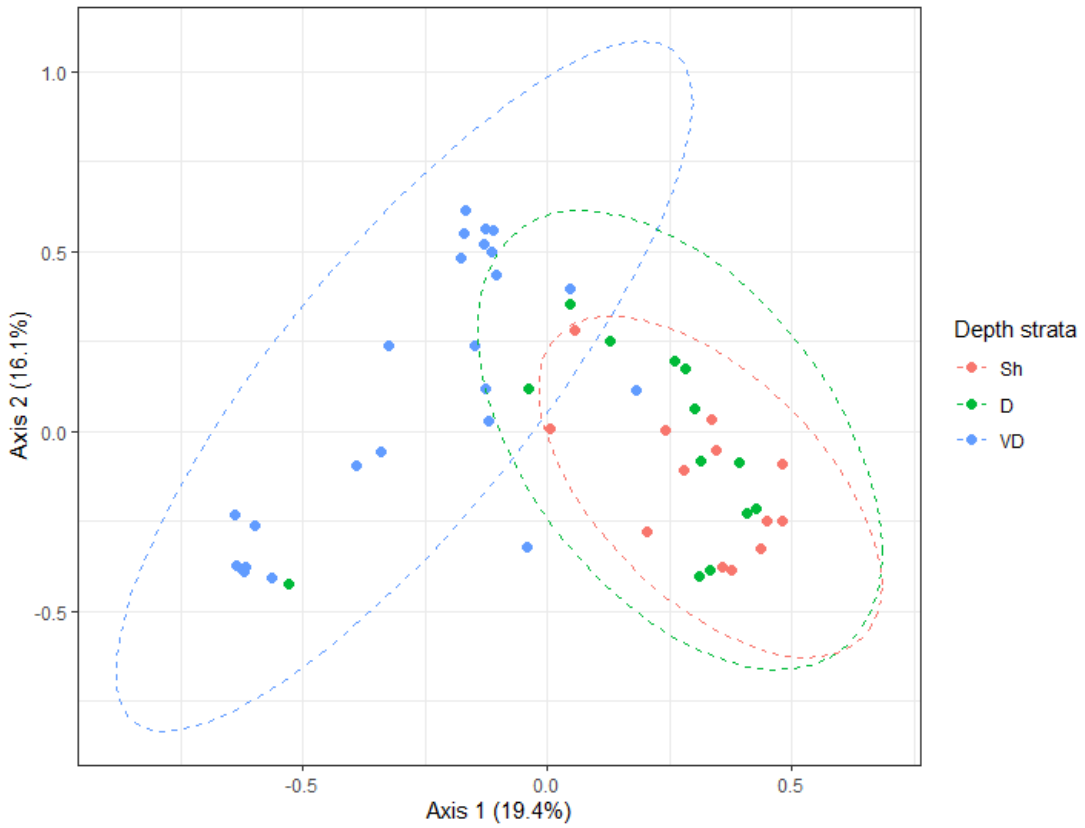


Figure 6 : Principal Coordinates Analysis (PCoA; distance metric: Bray-Curtis) of fish community between not protected sites and protected sites (BRUV). Sh: shallow strata, D: deep strata, VD: very deep strata

However, BRUVs appear to have a limited ability to discriminate differences in community composition between protected and unprotected area, both in shallow (PERMANOVA, p-value = 0.29) and very deep strata (PERMANOVA, p-value = 0.22), in contrast to their strong discriminative in the deep strata (PERMANOVA, p-value<0.001; Figure 7).

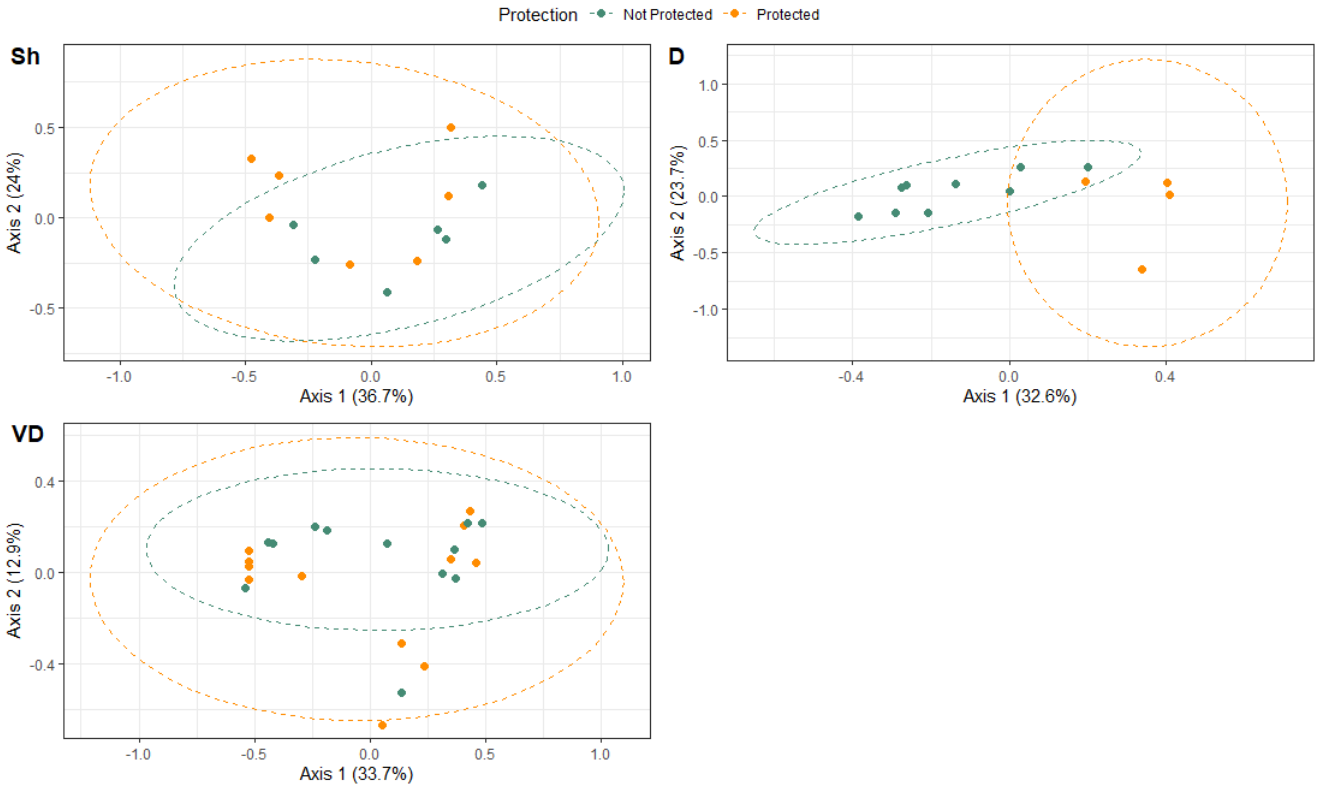


Figure 7: Principal Coordinates Analysis (PCoA; distance metric: Bray-Curtis) of fish community between not protected sites and protected sites for each depth strata (BRUV). Sh: shallow strata, D: deep strata, VD: very deep strata

Environmental DNA metabarcoding (eDNA)

Preliminary results indicate that eDNA has limited capacity to discriminate fish communities based on depth strata and protection status (PERMANOVA (distance metric=Jaccard), p-value=0.92 and p-value=0.60 respectively). However, the number of taxa detected by eDNA (i.e., taxonomic richness) at each site is significantly higher than that detected by the two other methods (i.e., UVC and BRUVs; Kruskal test, both p-values <0.001; and see Figure 8).

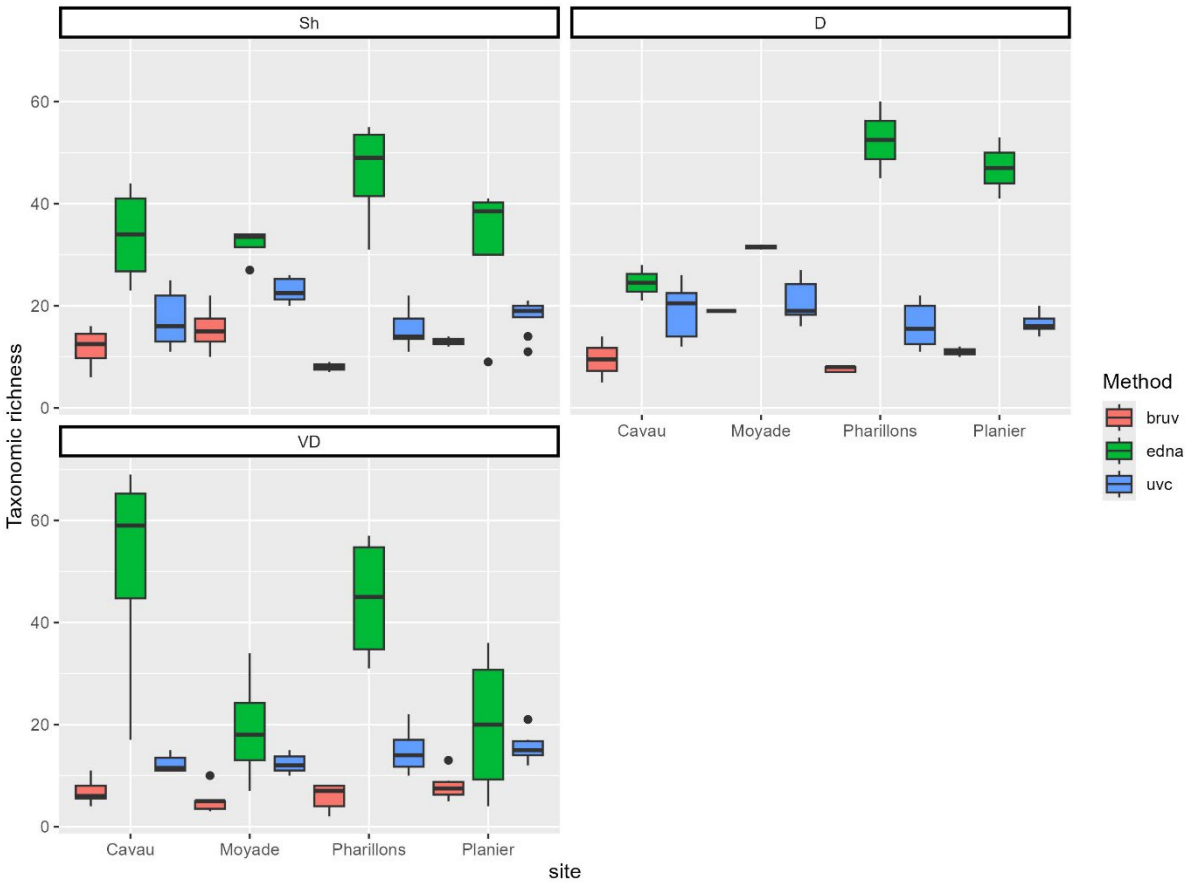


Figure 8 : Taxonomic richness in function of depth category for each method in the different sampled sites of Mediterranean Sea (Marseille, France). Sh: shallow stratum, D: deep stratum, VD: very deep stratum.

3.5.5. Conclusion

According to our preliminary results, UVC and BRUVs (unlike eDNA metabarcoding) appear to be effective in detecting changes in fish community composition across depth strata and protection level of the sampled area. However, the eDNA metabarcoding method provides a broader and more comprehensive overview of fish diversity. Although this has to be confirmed, this method is likely better suited for detecting non-indigenous species (NIS) as well as rare and cryptic species (Boulanger *et al.* 2021). Therefore, it is recommended that one of the observational methods (i.e., UVC or BRUV) be combined with eDNA metabarcoding to monitor rocky reef fish communities in a way that captures both community-level changes and the occurrence of rare species. To support the selection of the most appropriate combination of methods, we have summarised the main advantages and limitations of each

approach in Figure 9. However, it is important to emphasize that these findings are preliminary and must be confirmed through further analyses including additional study sites.

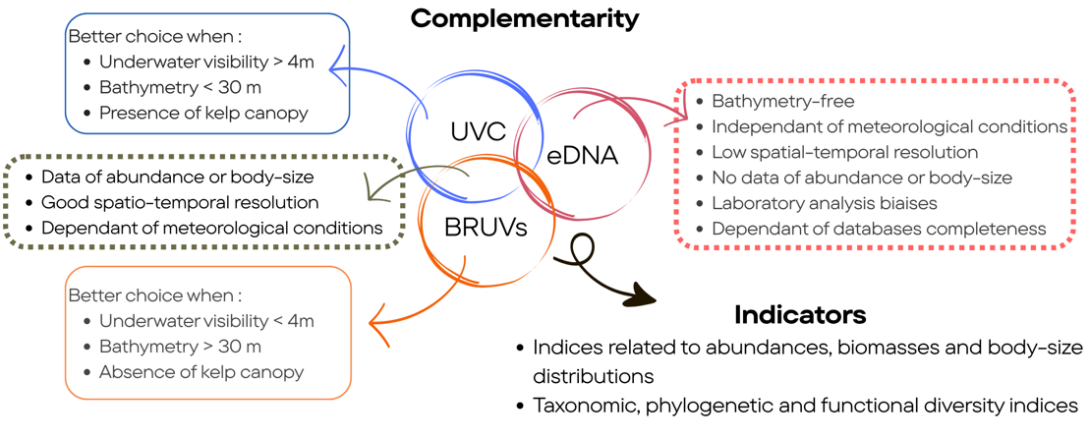


Figure 9 : Complementary of methods (UVC, BRUVs and eDNA) used in the EuRockFish pilot study. UVC: Underwater Visual Census, BRUV: Baited Remote Underwater Stereo-Video, eDNA: Environmental DNA.

3.6. Difficulties and solutions

3.6.1. Administrative/Regulatory constraints

Professional diving:

According to the country considered, the legislation for professional diving is not the same. For example, in Denmark, there is only one diver (linked to the boat with a “safety rope”), in Spain, there are three divers per transect and in France, there are two divers per transect).

Solution: We cross-calibrated the traditional protocols with those developed during EuRockFish to ensure that the data were harmonised and comparable.

BRUVs in Parc National des Calanques (France) :

Deploying BRUVs from the boat in *Parc National des Calanques* (Calanques National Park, France) is forbidden due to the presence of *Posidonia* seabed.

Solution: It is necessary to dive with the BRUVs to delicately place it on the rock/sand boundary of the seafloor. Moreover, it is sometimes necessary to dive to verify BRUVs position to ensure effective sampling.

eDNA call:

Initially all the funding for eDNA (consumables and analysis) was given to OFB. However, due to administrative issues, it was impossible for the OFB to launch the call in 2024. *Solution:* We need to transfer a part of the eDNA budget to the FRB to open the eDNA call (and to have eDNA consumables in time for the fieldwork).

eDNA samples:

Issue: Some legislations (notably Turkish legislation) are very restrictive regarding the sending of eDNA samples outside of Turkey. We spent a lot of time and had to make numerous trips between different departments in order to send the samples.

Budget reshuffling:

When we initially planned the budget for the project, the distribution of funds between the different categories (e.g., UVC equipment, BRUVs, equipment for BRUVs) was the same for all partners. However, as not all partners had initially the same level of equipment (see the Budget section), the budget has been reshuffled. The issue is that the Biodiversa+ Operational Team was unable to guarantee approval of this reshuffle before the end of the project. This uncertainty has created a fear of non-reimbursement among some partners, leading to a slowdown in purchasing some products.

3.6.2. Scientific difficulties

BRUVs and invasive species:

In some countries, invasive species (such as lionfish) are strongly attracted to the bait and behave aggressively towards it. Consequently, the videos only show invasive species, which 'hide' the presence of any other potential species.

Solution: Use bait that is less attractive to invasive species to detect other species.

Video time analysis:

The time and the human resources necessary for de-rushing video was underestimated during the conception of the project.

The development of algorithms (e.g., deep learning) to analyse videos using artificial intelligence (AI) could be an interesting way to explore in order to optimise the derushing process.

3.6.3. Logistical difficulties

Fieldwork postponed

Initially, the pilot included an important fieldwork campaign for 6 partners in Norway, Denmark, France, Spain, Turkey and Israel. The fieldwork campaign was constrained between June and September due to the seasonality/phenology of fishes and to ensure reproducibility of data. More precisely, these campaigns were initially planned during the Spring and Summer 2024 in order to keep time in 2025 to analyse the data and thus, to provide a robust methodological guideline for monitoring rocky reef fish communities. However, the fieldwork campaign in Spain and Israel had to be postponed to 2025 for different reasons:

- In Israel, the geopolitical context in 2024 resulted the closure of the sea area for safety reasons, preventing the Israeli team from conducting fieldwork.
- In Spain, administrative delays in the shipment of equipment meant that only the Mediterranean Sea survey could be conducted in 2024, while the Atlantic Ocean survey had to be postponed. By consequence, only the fieldwork in the Mediterranean Sea could be conducted in 2024, while the fieldwork in the Atlantic Ocean had to be postponed.

Solution: the two fieldwork campaigns in Israel and Spain are be planned in the Spring and Summer 2025

However, despite these difficulties and the fact that some partners have already conducted long-term monitoring protocols for rocky reef fish with their own protocols, we succeeded to develop standardised protocols which have no impact on running time-series and benefit from existing programs while harmonising data collection and analysis at the European level.

4. Conclusion

Despite the well-established ecological importance of reef fish as key components of European coastal biodiversity, they remain poorly studied and inconsistently monitored at the European scale. There is therefore an urgent need to strengthen our understanding of these ecosystems to improve the assessment and reporting of reef fish ecological status within the framework of several regional sea conventions (RSCs), including the Oslo-Paris convention (OSPAR), the Barcelona convention, the Helsinki convention (HELCOM), and the European Union Marine Strategy Framework Directive (MSFD).

In this context, the network launched by EuRockFish represents the first coordinated effort to harmonise the monitoring of rocky reef fish assemblages using a combination of visual and genetic methods. This initiative sets the foundation for future large-scale, standardised monitoring programmes and observatories.

During the first two years of the EuRockFish, administrative, logistic and legal challenges were identified. Alongside these, methodological limitations and practical issues have been discussed in this report, marking a key step forward toward the establishment of a transnational monitoring framework for rocky reef fish biodiversity.

Once the full data becomes available and is fully analysed, several essential questions will be addressed (though not necessarily resolved):

- Which methods can be used complementarily to assess changes in rocky reef fish biodiversity? Under what environmental conditions?
- What sampling frequency is the most appropriate (e.g., annually, bi-annually…).
- Is seasonal sampling necessary to account for intra-annual variability in species distribution (e.g., spring or/and autumn sampling)?
- How many sites should be sampled to ensure representative coverage?
- What additional measurements, if any, would be required for a more comprehensive set of EBVs?

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Appendix A: Underwater Visual Census protocol

Forewords

This document aims to describe the protocol to use the implement UVC transect methodology to assess rocky reef fish communities and give some generalities and justifications of several methodological choices. More precisely, fishes are identified and counted in-situ through diving along transect and fish sizes are visually estimated. *In fine*, the objective is to finely monitor the rocky fish assemblage at the reef and local (depth strata and seascape) scale through a measurement of the fish densities, biomass, diversity, and size structure of populations.

1. Operational protocol

1.1. Overview of the process

The full sampling protocol can be divided into several steps from onboard (before diving) to the comeback at the laboratory (see Figure 1 for a global overview).

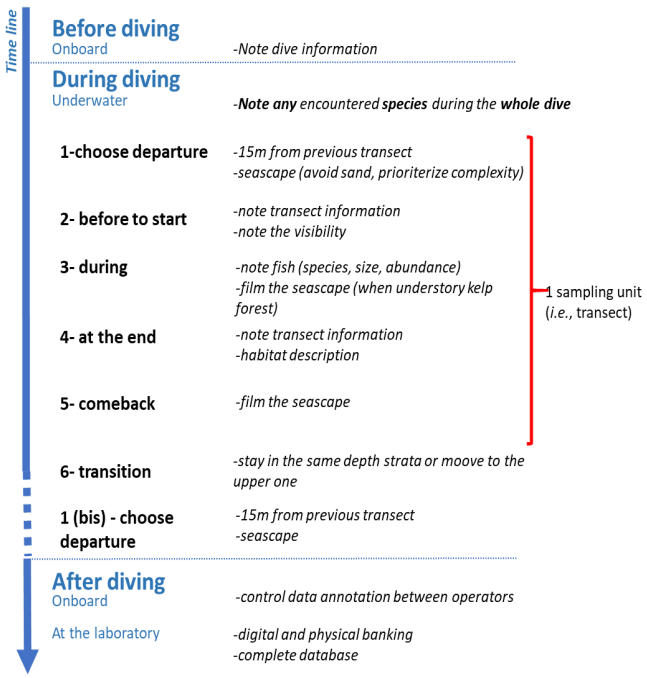


Figure 10 : Overview of the major steps of UVC transect protocol.

1.2. Before the transect sampling

Before the transect (*i.e.,* before diving), some parts of the field sheet (see Appendix C) should be completed. The information to complete are:

- **Obs** (Observer): initials of observer/operator name
- **Site**: Name of the site, composed of the location and type of site (reef, flat, seagrass meadow).
- **Date**: Sample date format dd/mm/yyyy (or yyyy/mm/dd)

- **Hour**: Hour of immersion
- **Tide** (*for tidal environment only*): the level of water (over the chart datum)
- Compute the **targeted depth** for each transect (Tide corrected depth + level of water)

1.3. Transect sampling

1.3.1. Starting point

In each site, the **starting point** of a transect is chosen **randomly** within the predefined **bathymetric stratum** (*e.g.,* -8 ± 2m, 18m ± 2m according to chart datum) in respecting a distance of at least **15 meters away** from any preceding transect.

The **direction** of the route is chosen so as to **stay** as long as possible **within** the **bathymetric stratum**. In the initial heading leads to an exit from the bathymetric stratum, a new direction is taken. In a site with complex topography, it is assumed that a transect is not always a straight line.

During the transect, the **seascape configuration** can be also taken into consideration. **Sand** is to be **avoided** as much as possible. In the case of multiple possibilities, the more complex configuration is prioritized (*e.g.,* between boulders and bedrocks, boulders are prioritized; between cliff and flat bedrock, the transition point should be followed). In the particular case of cliff configuration, it is recommended to reach the nearest cliff toe or top line (while staying in the same depth strata).

1.3.2. Before transect start

Before to start the sampling, the "2 & 4 - at transect start and end" part of the field sheet (see Appendix C) should be completed with information related to the unit (*i.e.*, transect):

- **N°Tr**: Number of the transect. The number of the transect among the whole transects from 1 to the maximum achieved replicates (based on the chronological order by example). For a given transect, this number should be identical between the different observers of a same group.
- **Target**: target depth (as indicated on dive computer) is calculated before the dive, by adding the water height (*i.e.,* "Tide" (take the height 30 min after the start of the dive) and the corrected depth of the targeted stratum (*e.g.*, targeted stratum of 18m chart datum + 2.5m of water height --> 20.5m target depth)).
- **T start**: time at the start of the transect (could be the time since the dive or directly the hour).
- **Role**: role of the diver: W (for wide) or N (for narrow) (see section *Roles*).

Transect size

The precise protocol can vary among localities especially concerning the size of the transect, closely related to turbidity conditions and detection of fishes (see Table 1). Transects of 5m width and 25m long are especially found in in Mediterranean Sea with clear water. On the other side, transects of 4m width and 30m long are mainly found in the more turbid waters of Atlantic Ocean.

Table 1: Examples of transect size respective to localities (ocean/sea and countries). DK: Denmark, FR: France, SP: Spain, TR: Turkey, IL: Israel.

	Location	Width (m)	Length (m)	Surface (m^2)
Atlantic Ocean	DK	4	30	120
	FR	4	30	120
	SP	5	50 (25 x 2)	125 x 2

Mediterranean Sea	SP	5	50 (25 x 2)	125 x 2
	FR	5	25	125
	TR	5	25	125
	IL	4	30	120

Subdivision of transect

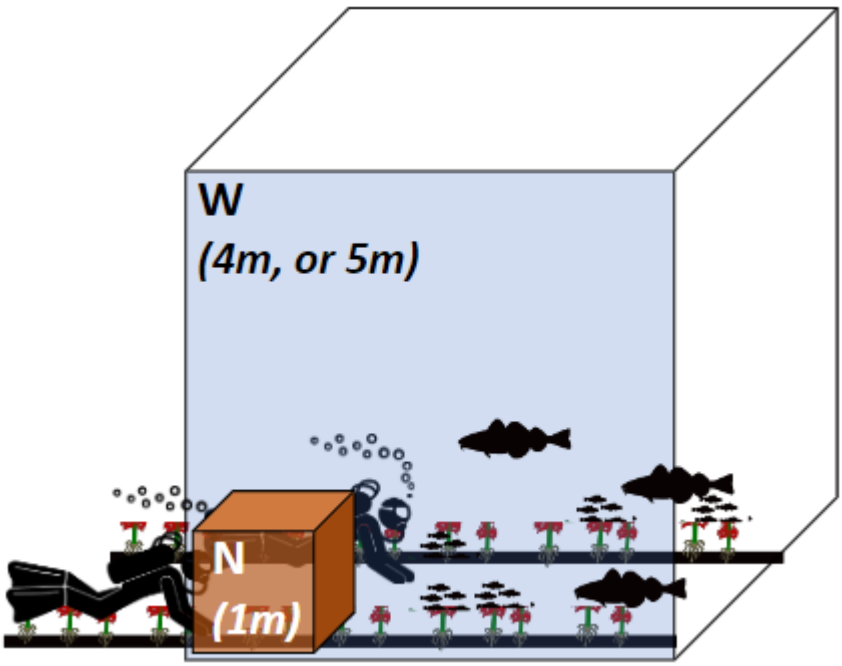
Transects are divided into subsamples to more precisely link fish assemblages to habitat characteristics (when habitat description is carried out) and to secure data collection for interrupted counting (*e.g.* strong current, dive security reasons). More precisely, for 4m wide, transects are divided into portions of 10m long (40m^2 each). For 5m wide, transects are divided at 8, 17, 25, 33, 42 and 50 m for convenience.

Roles

We presented here the case where two operators sampled together the same transect.

For a given transect, each of two operators is assigned to a specified role as follows:

- **Role W (Wide):** the observer W counts **all the individuals** present in a transect **4 meters wide** (2m on both sides; **or 5m wide**, see Table 1). He counts **all species** but preferentially looks for necto-benthic species (Labridae, Gadidae, Mugilidae, Moronidae, etc.). He scans the area up to at least 4 meters (or 5m, see Table 1), in front of him with a horizontal look.
- **Role N (Narrow):** the observer N is positioned next to the observer W, slightly behind (**laterally offset out** of the observer W, see Figure 2). He counts **all the individuals** present in a transect **1 meter wide** (*i.e.,* 0.5 m on both sides). Although he counts **all the species**, the observer N preferentially looks for crypto-benthic species (*e.g.*, blennies, gobies, trypterigions, lepadogasters). He engages the head or at least the **look in all the holes and crevices**. For correctly achieve this role, a diving light is essential.



Cases with one or three divers

One diver:

In the case there is **only one diver**, he combines at the same time the W and N role (far scan, near scan and hole scan) in starting by the W-role. Then, he comes back to the start of the transect and follows again the transect doing the N-role counting and completing the field sheet as a new one (*i.e.,* complete transect unit information before to start, fish assemblage information during transect sampling and transect unit + habitat at transect end). In some particular case, the divers is connected to the boat with a safety cable. In this case, the diver does the W and the N role in the same time.

Three divers:

In the case there is three divers, two divers execute the same protocol than explained above for two divers, and the third one notes the habitat characteristics.

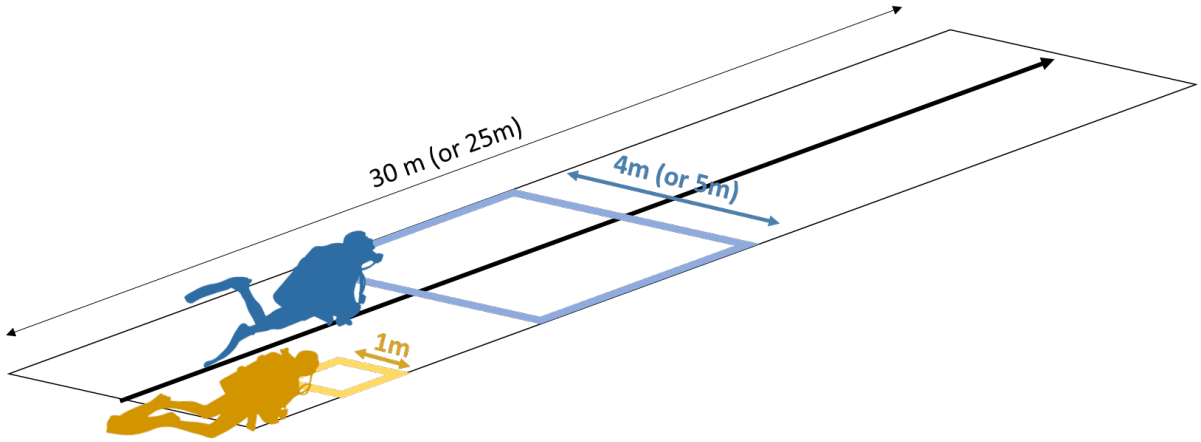


Figure 11: Relative position of the two operators (W: wide and N: narrow) and their corresponding sampling area to count within. The bottom part shows the offset position of N diver relatively to W one.

At each new transect, the two operators **reverse their roles**, in order to prevent effect confusion between role and observer. All fish species, cephalopods and crustaceans of interest (*e.g.*, European lobsters, spiny lobsters, edible crab) must be noted by the two operators (only the way to search for individuals vary between the two operators).

Visibility

To estimate the visibility, a Secchi disk could be used. The Secchi disk is set up at the anchorage point when the first transect begins. Visibility data is taken at the end of the dive, on the way back from the first transect. One of the operators is placed at point 0 on the tape, and the other moves away beyond where they think they'll see the Secchi disk and slowly approaches. As soon as he/she sees it, he/she notes the distance marked by the measuring tape and then retrieves the measuring tape from the transect. The visibility is noted on the field sheet and is defined as the horizontal underwater visibility in meters.

BOX A: Case of kelp forest (*Laminaria spp.* with more than 3 individuals/m²)

In kelp forest, the position of divers is modified to allow a better count of fishes (see Figure 308D0C9EA79F9BACE118C8200AA004BA90B02000000080000000E0000005F005200650066003200310033003800350031003400300035000000). Thus, the role of the two operators is defined as follows:

- **Role Wide (W):** The observer W is positioned **above the canopy**, vertically to observer N, and **counts all individuals above and under the canopy** (if he succeeds to sight fish from its above position), in a transect **4 meters wide** (*i.e.,* 2m on each side and other of the observer). It must have a mixed search strategy, to look for both small individuals hidden **in kelp blades** (*e.g.,* juvenile pollacks and swimming gobies) as well as large individuals often swimming higher **in the water column**. (*e.g.,* adults of pollacks, sea bass and mullet). It therefore alternates between near scans and far scans.
- **Role Narrow (N):** The observer N is positioned in the **understory** and **counts all** detectable individuals **under the canopy**, in a transect **1 meter wide** (*i.e.*, 0.5m on either side of the observer). The observer must have a mixed **search strategy**, to look for **both crypto-benthic** species often with holes (he engages the head or at least the look in all the holes and crevices) **and necto-benthic** species often swimming close to the depths under the canopy. The observer therefore alternates between (1) scanning the area immediately in front of him, inspecting all the holes, and (2) scanning horizontally up to the limit of visibility linked to the kelp stipes. The diving light is essential. The observer N sets the direction and the observer W follows him, unrolling the tape.

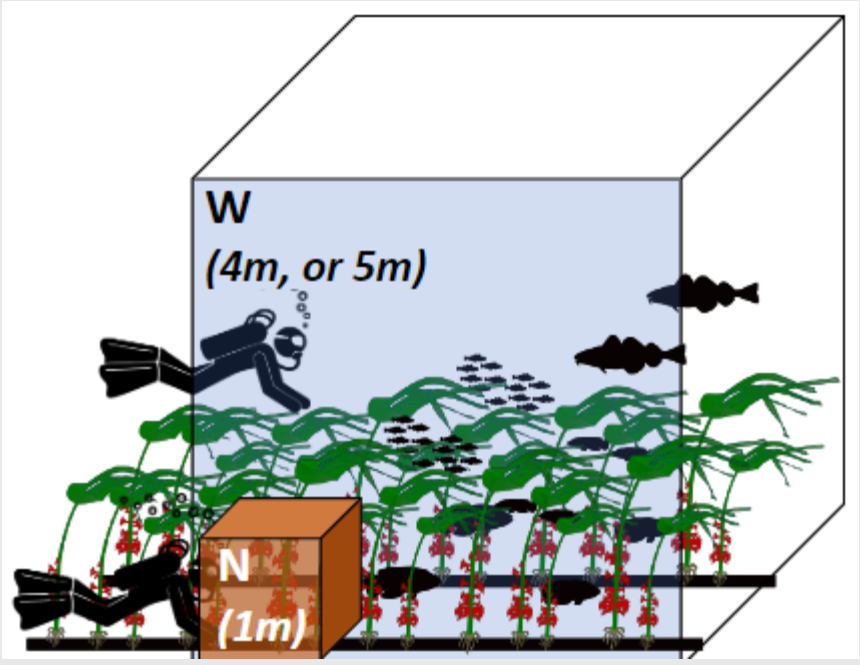


Figure 12 : Relative position of divers (W: wide and N: narrow) for kelp forest habitat and their corresponding sampling area to count within.

1.3.3. During transect sampling

During the sampling, all fishes (as well as cephalopods and several crustaceans of interest) should be counted and identified and their size estimated. All these information is noted in the "3-during transect" part of the field sheet as follows:

- Each line corresponds to one species
- For each species, the estimated size is noted in centimeters and the abundance is added as an exponent.

Example: If you see in chronological order: (1) a group of wrass (*Labrus bergylta*) with two individuals of 12 cm and 1 individual of 25 cm, (2) a group of pollacks (Pollachius pollachius) with 50 individuals of 8 cm and (3) one individual of sea bass (*Dicentrarchus labrax*) of 40 cm and, (4) another group of wrass (*Labrus bergylta*) with one individual of 12cm, two particular individuals with spotted color of 30cm and 5 individuals of 30cm, the lines on your field sheet were be as:

Labrus bergylta	12^2 / 25 / 12 / 30^2 (S) / 30^5
Pollachius pollachius	50^8
Dicentrarchus labrax	40

NB: To facilitate the notes on the field, the most frequent species can be pre-noted and pre-ordered (or grouped) according to their behavior (*e.g.,* very mobile, mobile and cryptic) in the first column and some cells can remain empty to annotate new species. Moreover, other information like phenotypes can be added just after in parenthesis just after the abundance and size annotation (*e.g.*, spotted wrasse: (S), male/female: (M)/(F)).

Fish count

When the abundance of the individual(s) is between 1 and 9, fishes are counted individually. Then, they are counted in tens from 10 to 90; per hundred from 100 to 900; in thousands from 1000 to 9000. An exception each time, the value of 15 between 10 and 20, the value of 150 between 100 and 200 and the value of 1500 between 1000 and 2000 are considered. To summarize, the different abundance could be:

1, 2, 3, 4, 5, 6, 7, 8, 9,

10, **15**, 20, 30, 40, 50, 60, 70, 80, 90

100, **150**, 200, 300, 400, 500, 600, 700, 800, 900

1000, **1500**, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000

Fish size

The total length (from mouth to end of the caudal fin) is estimated visually at the centimeter level when possible and more brought for bigger fish (accounting for 10% error in visual estimation).

Within kelp forest the N operator (within the understory) should film the understory seascape with a camera fixed on the hand, the chest or the front if any other possibility.

NB: If possible, it would be helpful to have an additional diver dedicated to recording environmental data on the sheet and filming each transect on the return journey (particularly in areas with dense macroalgal forests).

1.3.4. At the transect ending

At the end of the transect, the part of the field sheet with unit information should be completed as follow:

- **T end**: time at the end of the transect (could be the time since the dive or directly the hour).
- **Pmin and Pmax**: respectively the minimum and maximum depths taken during the transect. This requires regularly looking at your dive computer, especially when you see that you are reversing the progression of your depth (*e.g.,* when passing basins and hills). *NB:* The two observers of a group can have different values due to their relative position one to the other.

Habitat characteristics

Each category of habitat characteristics is scored semi-quantitatively from 0 to 5 (modified from SACFOR scale):

- 0 = the category was not observed in the course. NB: you can leave the box empty instead of writing 0
- 1 = the category was rarely observed within the transect, the surface area covered by this category ranges in] 0; 5% [
- 2 = the category was not frequently observed, [5 %; 35% [
- 3 = the category covered around half of the transect, [35 %; 65% [
- 4 = the category was dominating [65 %; 95% [
- 5= the category was (almost) the unique category observed [95 %; 100%]

The different levels inside each category are mutually exclusive implying that the sum for each category should be 5. Thus, for a type of category (benthic belts, slope or substrate), depending on the micro-habitat diversity of the environment, all categories can be rated 1 if the environment is very heterogeneous, or at the opposite extreme, only one category can be rated 5 and 0 for all the others if the environment is very homogeneous.

Category 1: Benthic belt (mutually exclusive, sum should be 5).

- Upper infralittoral
- Lower infralittoral
- Upper circalittoral
- Lower circalittoral
- Barren

Category 2: Slope (mutually exclusive, sum should be 5).

- Flat = around 0°
- Slope = around 45°
- Cliff = around 90°
- Overhang = slope > 90°
- Cavities = completely shaded hole into which one can insert at least one's head (~50cm diameter)

Category 3: Substrate categories (mutually exclusive, sum should be 5)

- Bedrock = bedrock
- Boulder (> 25cm)
- Pebble and gravel (< 25cm)
- Sand and mud (< 1mm)
- Boulder/boulder = boulder over boulder
- Boulder/bedrock = boulder over bedrock

- Boulder/sand = boulder over sand

1.3.5. During returning

Case A: Two operators by transect

The first operator swims back following the tape and **film the seascape** with a camera facing horizontally with a little angle (no more than 20° from the horizontal axis) at 1m from the seafloor. For kelp forest, the N-operator filmed the understory seascape during the counting. During the reloading, the operator swims at 1m from the canopy and take another video of the seascape. Having at least 2 videos (1 understory and 1 canopy) for seascape description.

Case B: Only one operator by transect

The operator has to do the **seascape filming** in a first return (letting the transect in place) following the transect as the N (narrow) role counting.

1.3.6. Transition to the next sampling unit

Two cases are possible to transit to the next sampling unit:

- stay in the same depth strata: move away (minimum 15m) from the previous transect while keeping the same depth strata.
- change depth strata: go to the upper depth strata and keep a minimum distance of 15m from the previous transect.

1.3.7. During the whole dive

Any species found at the scale of the whole dive should be noted (as “X” or “present”) even if it occurs out of the sampling area and/or between sampling units. Any other complementary information (thermocline, halocline, current conditions) is mentioned as comment.

1.3.8. After the transect sampling

It is important to quickly (onboard or on land) intercompare your sampling notes with your partner in order to fill empty information you missed or correct interrogations you had during the dive.

Moreover, it is strongly recommended to:

(1) Secure the data collection taking a picture (numeric copy) **and** rewriting on another paper sheet (physic copy) before cleaning the field sheet for the next sampling (it is also possible to remove the original field sheet for physic copy and replace by a new one for the next sampling).

(2) Bank your data on a database file and store either numeric and physic copies.

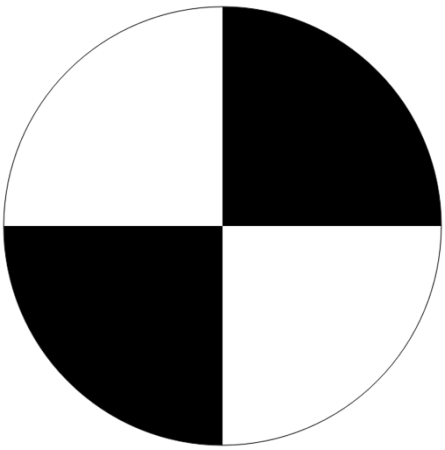
(3) Bank the seascape videos and carry on the seascape

(4) Rename the video file with the transect code as follows: country_method_project_locality_site_date_immersion-time_dive-team_depth_replicate (*e.g.,* FR_UVC-transect_EuRockfish_Marseille_Moyade_16h15_PTVD_18_1-1).

Appendices

Appendix A: Material check list

- field note board with field sheet and pencil
- spare pencil
- diving equipment
- diving lamp
- decameter
- camera
- Secchi disk (see below, to print size 20 x 20cm). It is possible to print it on polyester photographic paper and fix it with tape on a PVC plate.



Appendix B: Build note board

The design proposed as a kind of book (see Figure 4) offered the possibility to bring multiple field sheet fixed on rigid PVC plates. Take care about the material (need to be rigid, robust, positive weight preferable) used for the plates. Those last can be attached together with piece of bungee cord (better than inox ring or plastic hose clamp) on the side. A larger bungee cord can allow to maintain close the whole book. A robust pencil (and eventually a magic eraser) can be attached as well as a carabiner (to avoid unfortunate loss).

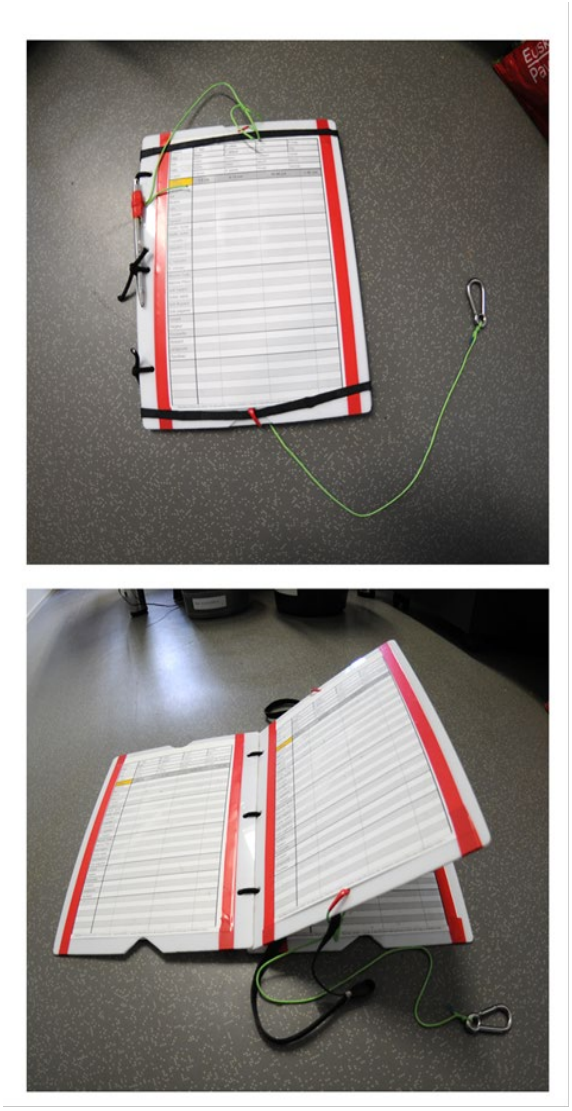


Figure 13 : Field board design

Appendix C: Edit field sheet

Figure 5 is a proposed A4 format sheet design. One sheet allows to do 2 units (*i.e.,* transects). Moreover, we recommended this reference for the field sheets: Xerox Premium NeverTear A4 120µ (or 90µ) matte white tear-resistant synthetic paper 100 sheets.

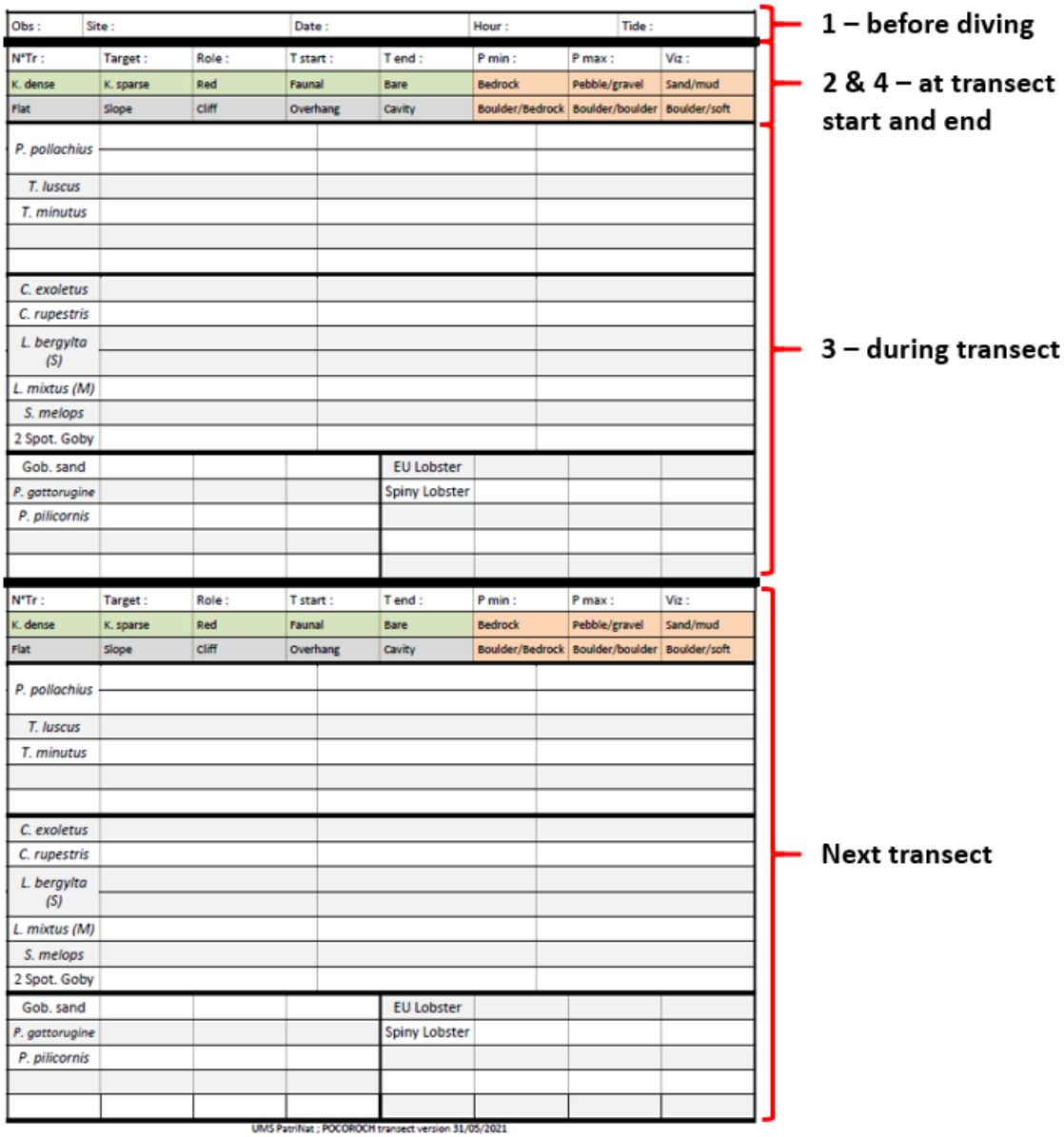


Figure 14 : Field sheet design for fish counting with UVC transect protocol.

The sheet is divided into different parts containing several information associated to the whole diving session (who / when / where) and to the sample unit (which protocol / which unit characteristics / which fishes / in which habitat) that you will complete at different steps of the dive: before diving, at transect start, during the transect and, at transect end.

Appendix D: Training before sampling

Transect width estimation and forward speed

Fishes are counted in a defined area visually estimated which is important to calibrate. For this issue it is proposed to use decameters spaced at wide (4 or 5m, see Figure 6 left part) and narrow (1m, see Figure 6 right part) widths to feel the width estimation. Forward speed can be calibrated at the same time to reach ~0.3m/s (9min for 30m long transect and 7min30s for 25m long - but don't hesitate to accelerate the rhythm for the wide role if you see that fishes run away).

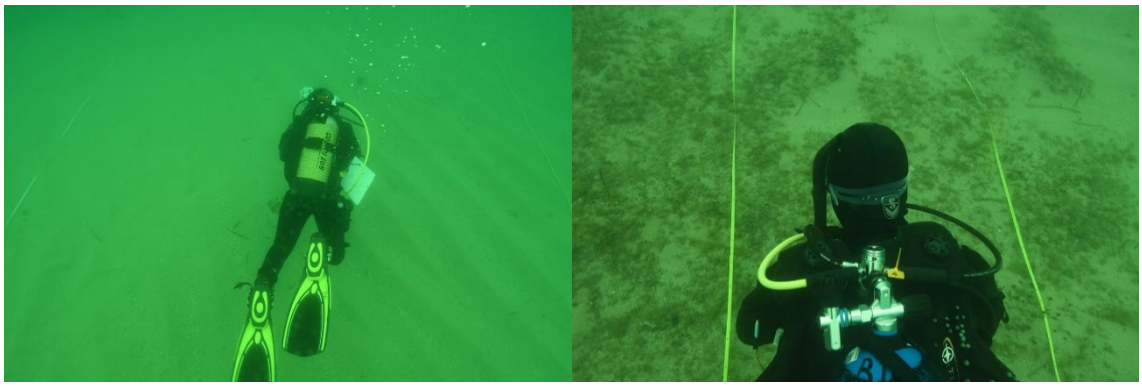


Figure 6: Pictures of a width estimate training for wide (left part) and narrow (right) roles

Fish size estimation:

The visual estimation of fish needs to be pre-calibrated through the measurement of fish silhouettes of different shapes with known sizes (see Figure 7). The comparison of observed versus real size leads to highlight over or lower size estimation and to correct it.

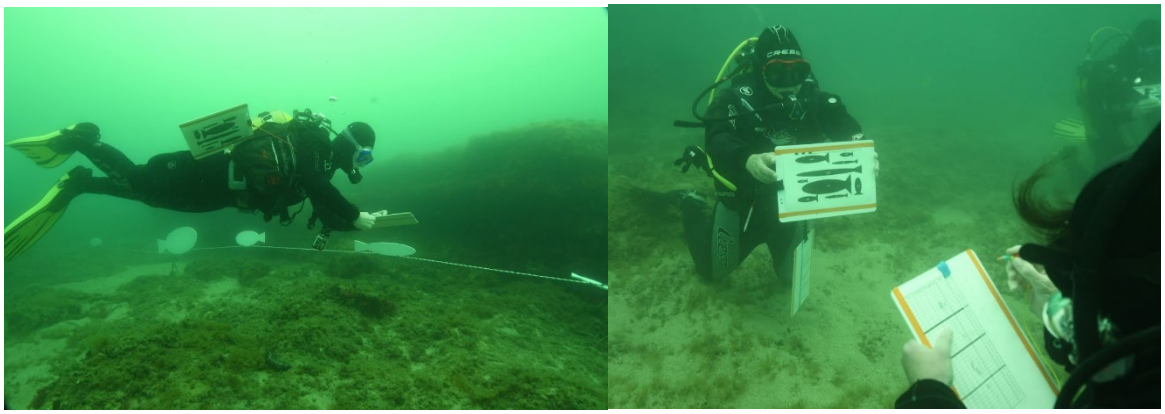


Figure 7: Pictures of a fish size estimate training ((c) Valentin Danet, OFB-Patrinat

Fish recognition:

Species lists vary a lot among localities. It is important to look at the species list present and at the criteria of identification to take care underwater. Several books such *as Patrick Louisy - Marine fish identification guide: Europe and Mediterranean* can bring help for this issue.

Appendix E: Metadata

A metadata table is available in a dedicated separate file. Here is an overview of all variables to note. An entry file is also available in a separate file and must be complete after each dive.

Table 2 : Read me sheet of the metadata file

variable	example	format	unit	definition
country	FR	text	NA	The country of the partner: DK (Denmark), NO (Norway), FR (France), ES (Spain), TR (Turkey), IL (Israel)
laboratory	MNHN	text	NA	The name of the partner laboratory
date	2023-10-23	date	yyyy-mm-dd	The date of the dive
utc	2	numeric	NA	UTC (Coordinated Universal Time) : 1 (UTC+1), 2 (UTC+2), 3 (UTC +3)
immersion_time	13:14	time (24h)	hh:mm	The time of the immersion
locality	Atlantic	text	NA	The ocean or sea name of the sampling area. Need to be constant all along the campaign (same capital letters, same space or underscore…)
region	Brittany	text	NA	The name of the region. Need to be constant all along the campaign (same capital letters, same space or underscore…)
subregion	7 iles	text	NA	The name of the subregion. Need to be constant all along the campaign (same capital letters, same space or underscore…)
site	Vieille	text	NA	The name of the site. Need to be constant all along the campaign (same capital letters, same space or underscore…)
subsite	Vieille north	text	NA	The name of the subsite. Need to be constant all along the campaign (same capital letters, same space or underscore…)
site_code	Viei_no	text	NA	Code name for the site. Need to be constant
site_latitude	48.687564	numeric	decimal coordinate	Latitudinal coordinate of the site in decimal degree WGS84. Must be constant for a same site.
site_longitude	2.330703	numeric	decimal coordinate	Longitudinal coordinate of the site in decimal degree WGS84. Must be constant for a same site.
immersion_latitude	48.687564	numeric	decimal coordinate	Latitudinal coordinate of the immersion point in decimal degree WGS84. For a same site this coordinate can differ during the whole campaign.
immersion_longitude	2.330703	numeric	decimal coordinate	Longitudinal coordinate of the site in decimal degree WGS84. For a same site

				this coordinate can differ during the whole campaign.
nb_immersion	1	numeric	NA	the immersion number of the day
total_nb_transect	6	numeric	NA	the total number of transect sampled during the dive
nb_transect_Sh	2	numeric	NA	the number of shallow (Sh) sampling unit carried out at depth stratum 8m (chart datum)
nb_transect_D	2	numeric	NA	the number of deep (D) sampling unit carried out at depth stratum 18m (chart datum)
nb_transect_Vd	2	numeric	NA	the number of very deep (Vd) sampling unit carried out at depth stratum 38m (chart datum)
dive_time	62	numeric	min	the duration of the dive
divers	Quentin Ternon and Valentin Danet	text	NA	the complete names of the different divers
boat_captain	Julien Guillaudeau	text	NA	the name of the boat captain
boat	Emeraude Explorer	text	NA	the name of the boat use to go on field
swell	0.5	numeric	meter	the swell condition in meters
current	0.2	numeric	m/s	the maximum current speed encountered during the dive
sea_level	6.5	numeric	meter	The level of the sea 30min after immersion (need to be anticipate before immersion) considering tide or any other change in sea level change
maximum_depth	31.2	numeric	meter	The maximum depth encountered during the dive
immersion_comments	strong current at the begining and important raining episode	text	NA	Any comments about the weather, particular condition or event just before or during the diver
id_immersion	FR_2023-10-23_Brittany_Vieille_1	country_date_region_site_nb-immersion	NA	Name of the immersion which be used after to link transect unit to immersion metadata

Appendix B: Stereo Baited Remote Underwater Video protocol

1. Overview

1.1. Context and Objectives

Stereo baited remote underwater video (stereo-BRUV) is a method to characterize the abundance, diversity, body-size structure and biomass of fish assemblage. BRUV can also be used for behavioral studies. Stereo-BRUVs can be effective in turbid areas and in deep areas, places where divers cannot necessarily go. Consequently, BRUV is suitable in a large array of environments, including euphotic and mesophotic rocky reefs.

This document aims to describe stereo-BURVs protocol to be used during the EuRockFish project, as regards hardware build, calibration and deployment as well video analyses, through SeaGIS softwares.

1.2. Timeline

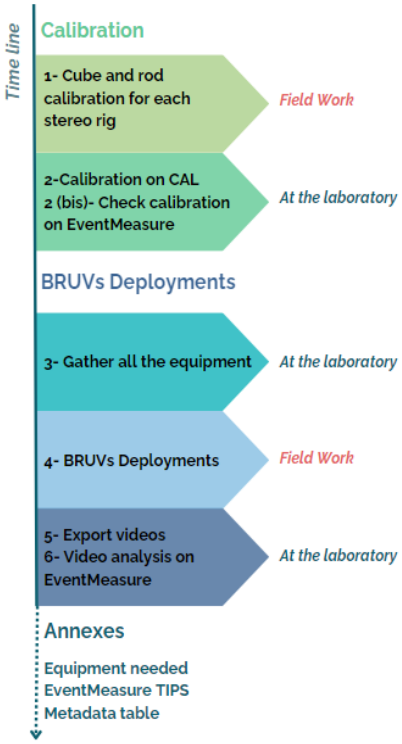


Figure 1: Overview of the major steps of BRUVs protocol ©Lauriane Guérin

1.3. Calibration

All stereo systems need to be calibrated to ensure correct measurement of individuals. This first step is essential and should ideally be carried out before the stereo-BRUVs deployment on the field.

This operation involves video recording of a **1m cube**, positioned in different ways during the recording process. White dots are placed all over the cube, with a known distance between them, to calibrate the

measurement that can be made by the stereo system. The calibration cube is positioned approximately from **1.5m to 3 m away from the cameras and oriented in 20 different positions** (a combination of cube rotation and cube orientation in relation to the camera). Subsequently, a **rod** with known measurements is placed in different positions and orientations relative to the cameras, to verify the correct calibration of the systems and estimate the error of future fish size measurements. The calibration videos produced will be analyzed **using CAL and EventMeasure software**. This calibration stage provides calibration files (.Cam) for the left and right cameras on each stereo rig. This step is essential for subsequent fish measurements.

2. Procedure

2.1. Field procedure

- **What and when?** All stereo rigs must be calibrated. Ideally, calibration is carried out before the stereo-BRUVs are deployed.
- **Where?** If it's possible the best place to calibrate stereo rigs is in the same water that you will deploy it to respect the same density water. In France, for example, we calibrate our stereo rigs in a natural pool filled with seawater. If your conditions don't allow you to calibrate the rigs in a natural environment, *e.g.,* turbid water or poor visibility, you can do so in a swimming pool.
- **How long?** For 8 stereo rigs, it takes half a day in the field to carry out all the manipulations on the cube and the adjustment bar. Then there's the time needed to prepare filed work, rinse the equipment and analyze the videos.
- **How many people?** Two people are needed for the calibration. It is easier to manipulate the cube in pairs and it is better for security, particularly if you are in a natural environment. In practice, one person is in-charge of the camera and recording and the other is in-charge of the cube, the bar and the flash of synchronization.

Set up the cube and stereo rig

> Check the **camera settings** (See Appendix 4.1.1.)

To standardize the calibration of all stereo rigs, we try to maintain the same distance between the rig and the calibration cube. To do this, you can use ground markers such as stones, and a decameter. The **distance between the calibration cube and cameras is 3 meters** but it can be reduced if visibility does not allow it. The most important thing is to keep the same distance

> ***Check with the camera that all the white dots on the cube are visible.*** Making sure that the image appears on the cameras. Take care of the sunlight and avoid to be against the light.

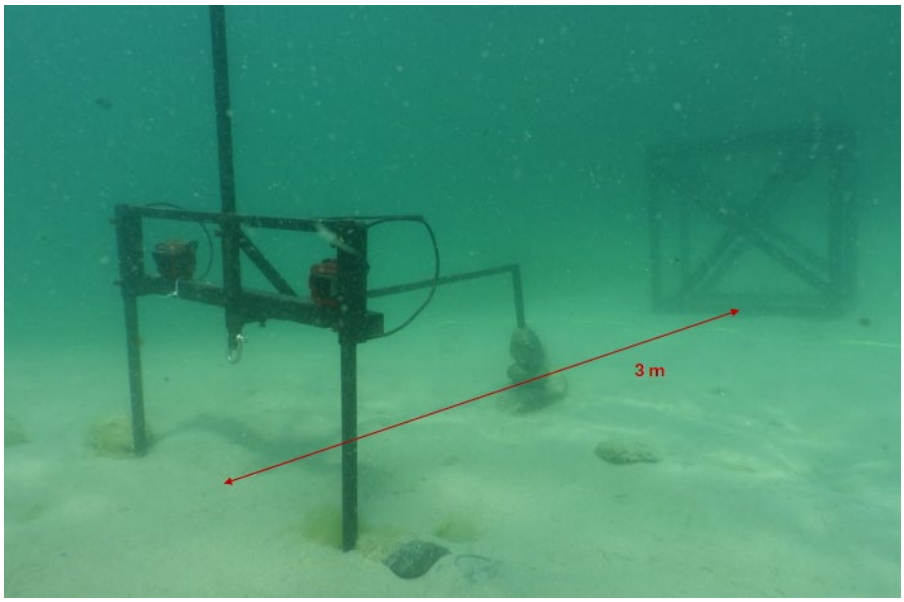


Figure 2: Calibration set up ©Lauriane Guérin

Synchronize cameras

> Write down the numbers of the camera pair and the BRUV unit on the **slateboard**, and place it in front of the camera.

> Use a **flashlight** about 1 m away in front of the camera. This is very important so that the right and left videos can be synchronized later.

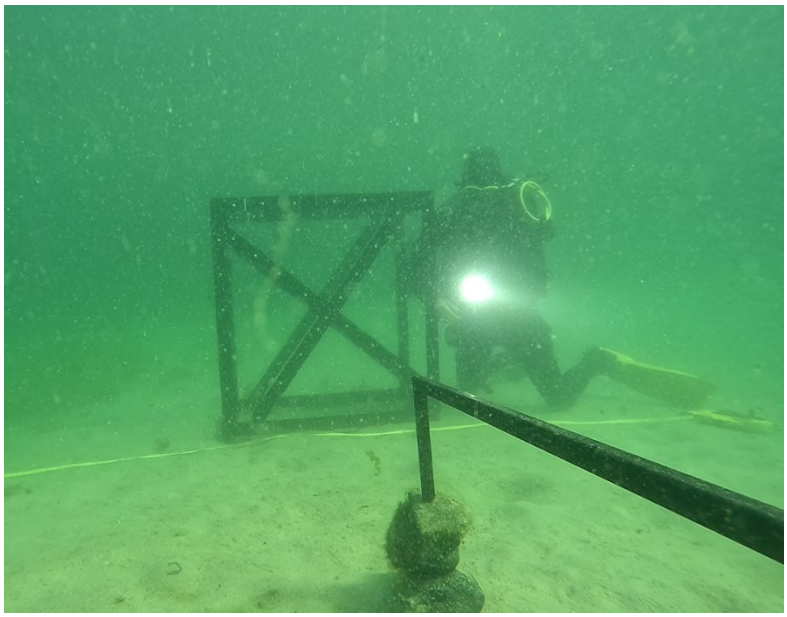


Figure 3: Flash synchronization ©Lauriane Guérin

Calibration

> **Turn the cube** around as shown in the recording imagery-picture sequence guide of the CAL software. There are **20 positions** to do. *Note: A white mark (index mark) at point number 100 helps you remember every position.*

Appendix B: Stereo Baited Remote Underwater Video protocol

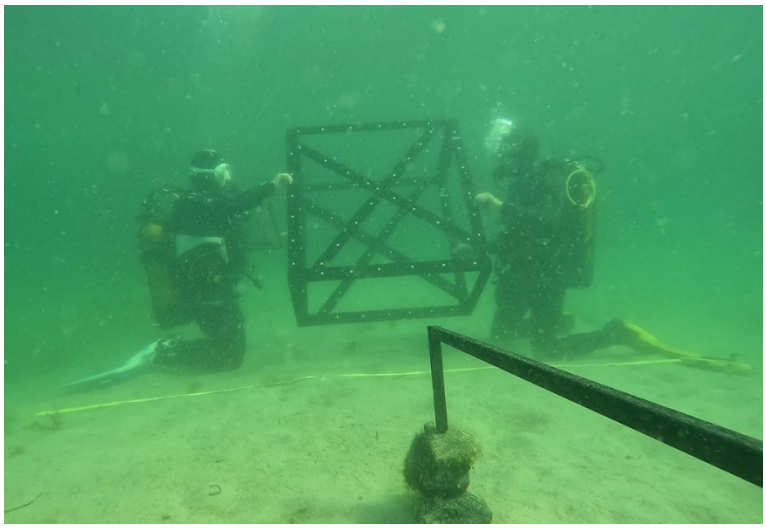


Figure 4 : Cube Calibration (©Lauriane Guérin)

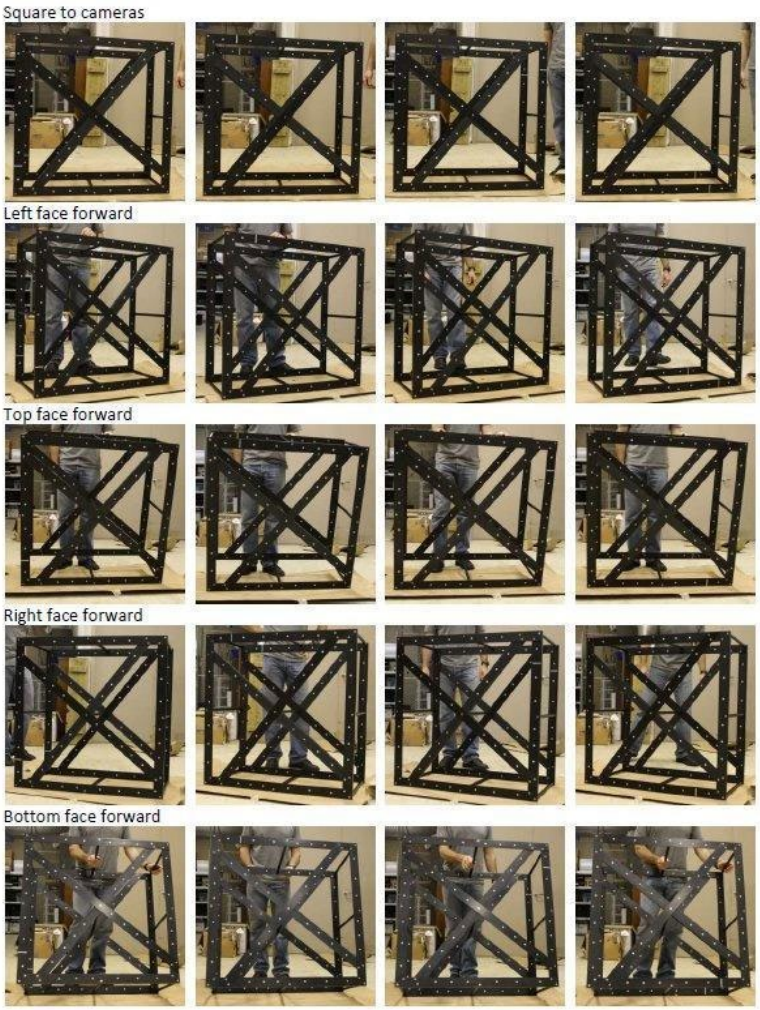


Figure 5: Imagery-picture sequence guide of the CAL software (©SeaGIS)

Check of the Calibration

When the cube picture sequence is complete, one person goes into the field of view of the camera and shows the calibration rod at varying angles.

> Turn the rod through the **4 angles** and play with the **3 rod's inclination**: flat, forward, backward.

> The person **repeats the sequence in 4 different positions**: central, close on the left side, on the right side. This image sequence is used to check the calibration. Turning the rod allows you to determine in which part of the camera's field of view the measurement is most reliable.

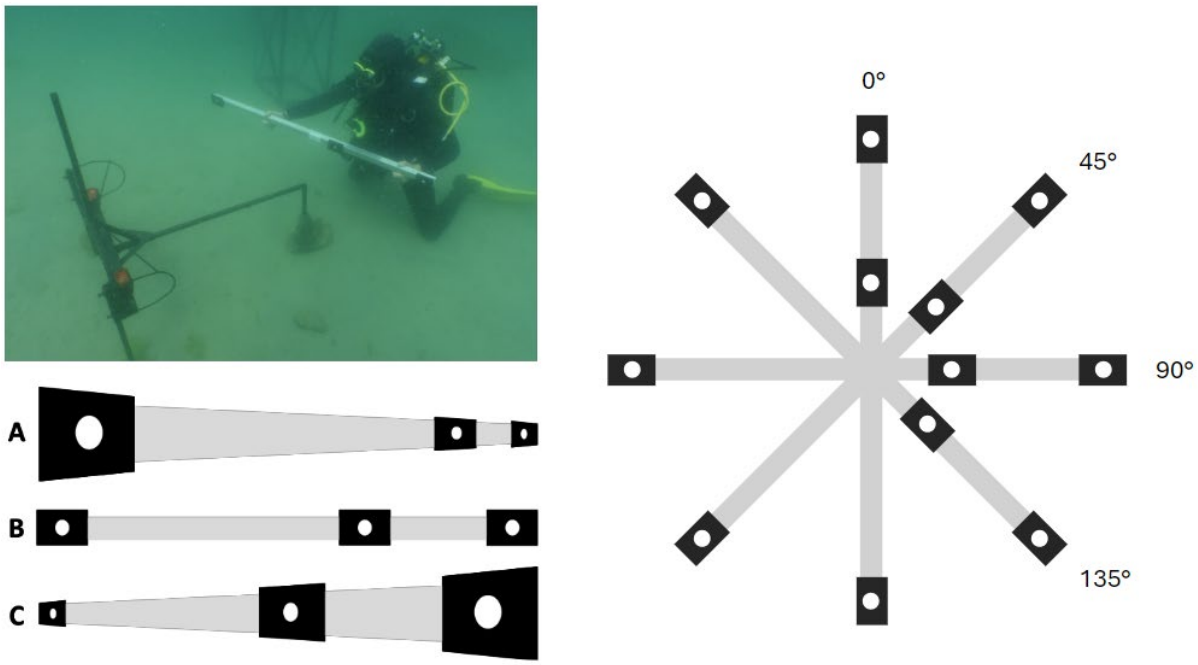


Figure 6: (top left) rod image-sequence made by the diver on the field (close position), (right) diagram showing different rod calibration angles and (down left) rod's inclination backward (A), flat (B), forward (C) ©Lauriane Guérin

Post Field Work

When you return from the field, rinse the cube, stereo-BRUVs and calibration rod with fresh water.

After calibration, do not touch the housing. In case of doubt, you can mark the relative position of housing in relation to the hardware, and if anything moves, you'll have to recalibrate. In case of doubt, you can perform a check just by passing the calibration bar (see associated section 2.1.6.). The remobilization of the camera (successive removal and reinsertion) has been checked and has little impact on measurement error.

Save the videos to your hard disk, in a "calibration_date_place_BRUVnumber" folder. Please note that left and right videos should be saved in the same. As a precaution, it is recommended to copy the files to a second hard disk.

2.2. Post-processing procedure

The post-processing is carried out on the computer using **Seagis CAL software (version 4.10).**

Create the folder

The folder “calibration_date_place_BRUVnumber” that you will use should contain the following files :

- Cube .PtsCAL file
- GoPro Hero567 Black Wide Water.CamCAL (Or the correct GoProHero version)
- Video/s from the left camera
- Video /sfrom the right camera

Open CAL software and create a new project file

> In the upper left corner, **click “Project” select “New Project”**. This will allow CAL to prompt and guide you throughout the calibration process. **Name and save the project**.

> **Choose the camera file** for the left side and right side. Choose the same CamCAL file for both the left and the right side.

> Next, load the calibration cube file (.PtsCAL).

Note: there is two different calibration cubes (144 points and 257 points), be careful to have de right file

> **Set the picture directory** to the folder where you have the videos. You will then be prompted to give a name and save the project. Next, **load the left video**, and confirm the movie sequence configuration. **Same for the right side**.

Synchronize videos

Synchronize the video by **finding the flash**. then clicking on the “**Lock**” box (in the upper left corner) when you are sure that you have found the frame that is the same for the left and right camera.

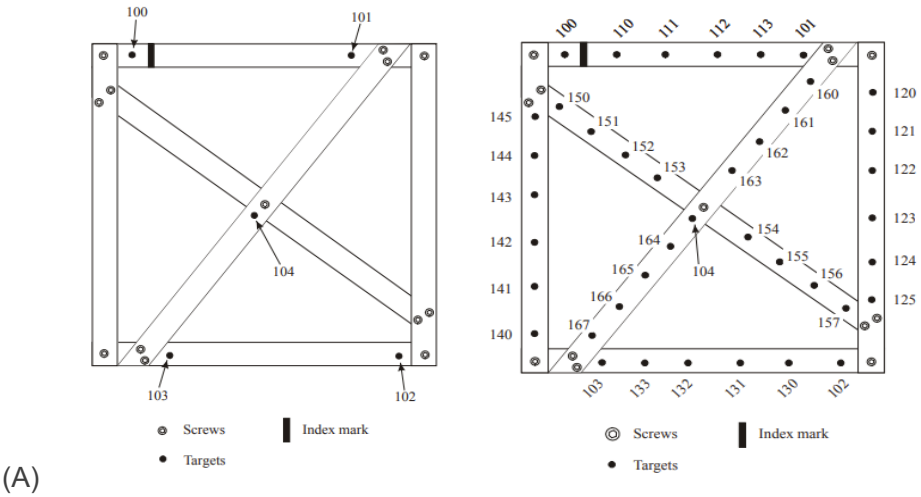
Start the calibration

> Find the picture for the first sequence, and click on **points 100, 101, 102 and 103** (in sequence).

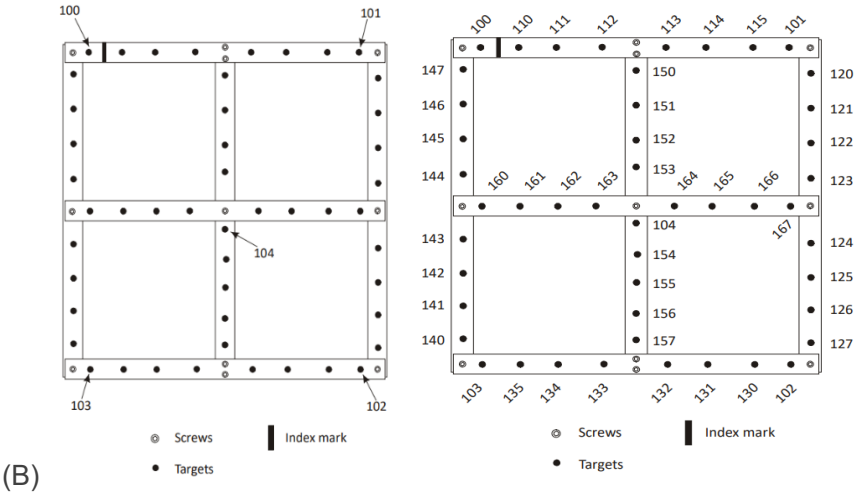
> Always start on the left camera.

> **Zoom in to see the points better** by hovering the pointer to the middle of the area you want enlarged and pressing the Ctrl button. The zoom will depend on the value that is given in the Zoom tab on the upper left side. Pressing the Shift key will generate a square around the pointer which will help you find the exact center of the target.

> The **rest of the points should be automatically detected**. The points you clicked on will have a green color, and the points that CAL suggested will have a blue color. If they do not get detected, click on “**Resect**”.



(A)



(B)

Figure 7: (A) Cube 144 points, (B) Cube 267 points (©SeaGIS)

> Play the movie to find the best picture for the next sequence and do the same procedure.

Note: In the **first image**, the pointing is done **to the left AND to the right**. Then, the software detects automatically points to the right. But sometimes you still need to switch back to the manual.

> In total, there should be 40 records in the Image File List.

Remove marks that are wrongly labeled.

> Click on Measurement > Intersect all points and then delete all the rejected points (colored red) by clicking on Measurement > Delete rejected point measurements.

> Click on Adjustment > Compute bundle adjustment, then “Accept results”.

> The more points per recording, the more accurate the calibration. Whenever possible, try to **approach 60 points per recording**.

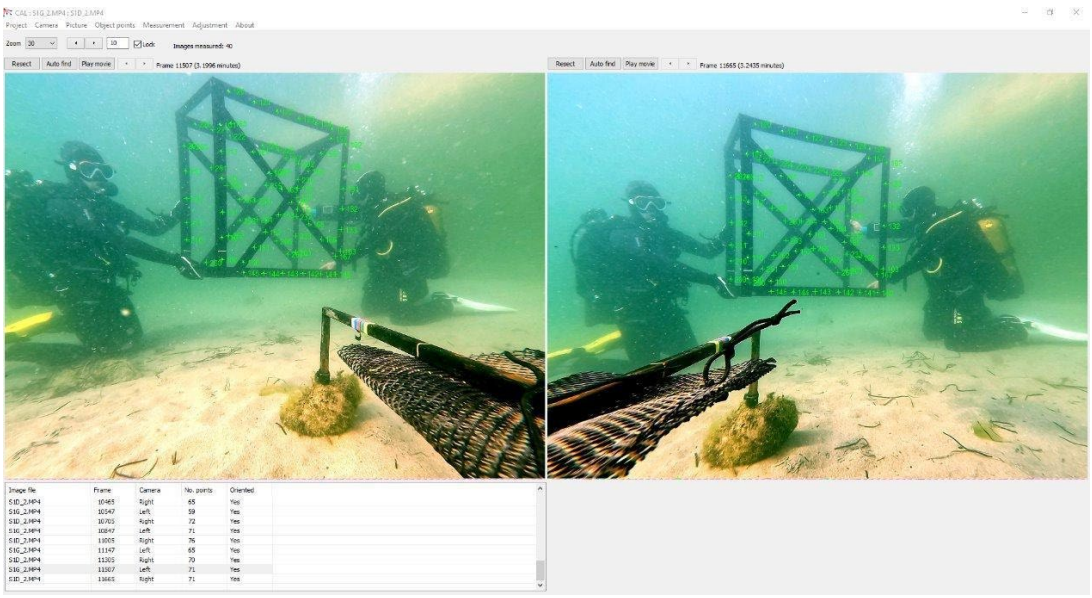


Figure 8: Calibration on CAL Software (©OFB-PatriNat)

Export calibration file

Export calibration file of right and left camera to use it in *EventMeasure* software

> Click on **Measurement** > **Stereo constraints** > **Configure stereo constraints** and **click on "Automatic"** to automatically pair the left and right measures. Click OK.

> Go the **Stereo Constraints** > **View Relative Orientation** to make sure that the measurements are within the recommended values:

- Base separation: distance between the two cameras
- Phi: camera convergence angle (left and right have approximately the same value, left Phi is a negative number)
- Kappa: camera inclination (0 is perfect)

If the values are a bit off, it might get better by going back to Measurements, resecting the images and re-computing the bundle adjustments.

> When you are happy with the stereo constraints, **click on the "Export stereo camera files" to generate the .Cam files for the left and right side**. Follow the prompts/instructions given by the program. It is good practice to label it with the unit's name (for example, BRUV1 Left.Cam) so that it can easily be paired with the video recordings from the same unit afterwards.

The new .Cam files are now ready to be used for analysis with *EventMeasure* software.

Checking the Calibration on *EventMeasure* software

To check if the calibration was successful, run the same video pair with *EventMeasure* software as you would with an analysis.

Comparison between the actual measurement (manufacturer's data) and the measurement obtained by the software enables us to quantify the measurement error associated with each stereo support (+/- 3 mm from actual length is an acceptable length).

Starting a new *EventMeasure* observation file (EMObs)

> Measurement > New measurement file

> Measurement > Information fields > fill in info from the notes/video.

Load pictures

> Picture > Set picture directory > find the right directory where the video and .Cam files are located

> Picture > Load picture (Left side) and define movie sequence

> Stereo > Load picture (Right side) and define movie sequence

Load camera-files (.Cam)

> Stereo > Cameras > Left > Load camera file (Left.cam) > eg: “Ruv 2 left.CAM” > Open

> Stereo > Cameras > Right > Load camera file (Right.cam) > eg: “Ruv 2 right.CAM” > Open

Edit field values

> Play video to check info on field-notes > «Close and update position»

> Measurements > Information fields > Edit field values (check fieldnotes).

Synchronize videos

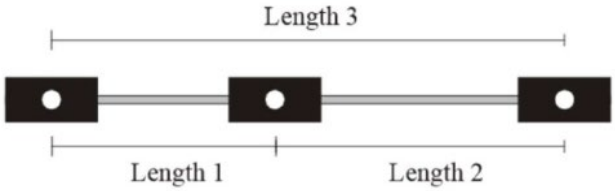
> Find the flash/clap > Click on the “Lock” box in the upper left corner when you are sure that you have found the frame that is the same for the left and right camera.

3D measurements

> On the left frame, click on the two points at the ends of the distance you want to measure. Then do it on the right frame and you have your 3D measurement.

Note: When you click on a point on the left frame, a **red dotted** line appears on the right frame. It's called the epipolar line. It's a projection of the left point onto the right frame, made by the calibration performed beforehand.

> To record measurements, note in **remarks** the position of the diver, the ID length and the orientation of the calibration rod.



Length ID	Distance (mm)
Length 1	451
Length 2	765
Length 3	1217

Figure 9: Actual measurements of the calibration rod, note: Check your calibration rod measurements, it could be different ©OFB-PatriNat

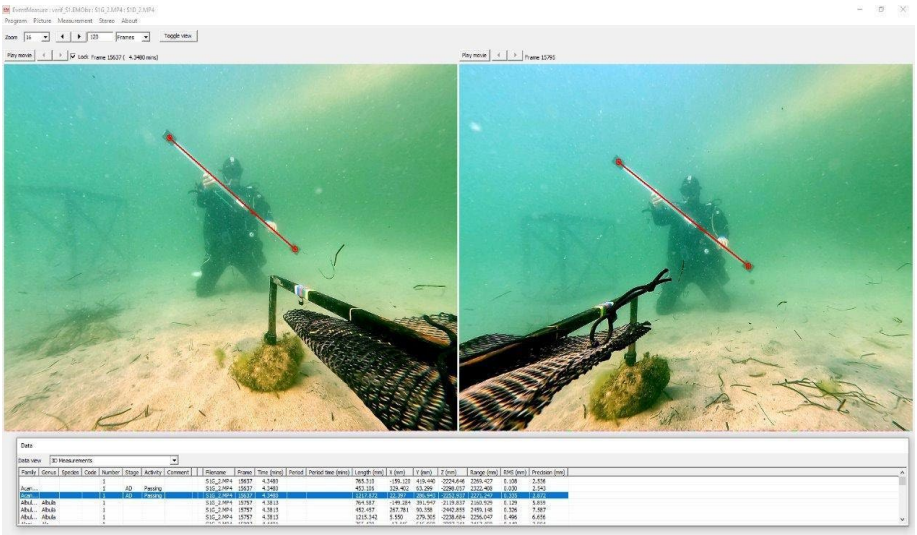


Figure 10: Calibration checking on EventMeasure software ©OFB-PatriNat

Verification of your measurements

Note: Low precision/RMS value over 11.0 = high insecurity; consider removing measurements

> The measurement of the targets on the distance bar should be as close as possible to the actual length as indicated in the SEAGIS calibration hardware manual. **A deviation of +/- 10% is acceptable** up to 3m in front of the cameras.

> Do a few measurements with the calibration rod to **figure out where the best measurement can be made for your set-up**. (The best measurements would be in the middle area of the camera, those that are in the periphery are usually a bit off)

Export your data

> Measurement > Save (EMOBs file)

> Generating a .csv summary file:

> Program > Batch text file output > A pop up screen will appear > Make sure that you have the correct path to the input and output directory

> Select outputs you want by to adding a true (1 or T or True) in the *Data* column next to the appropriate output type > Process > You will get a message if all the files have been processed > The output file should be in the folder you indicated

3. BRUVs deployments

3.1. Pre-fieldwork

Assemble stereo rigs

> Attach each camera housing to its designated rig (See Appendix 4.1).

> Each stereo rig and camera are labeled. Respect this configuration to keep the same rigs calibration.

Prepare camera

> Make sure the cameras are charged and SD card are empty

> Check cameras settings (See Appendix 4.1)

Note: Take care of your systems in particularly against sun

Prepare bait

Note: France use 800g of fresh sardines per stereo-rig

Prepare mooring

3.2. Field Work

Boat storage

Figure 11: Boat storage, French example (A, B), Norwegian example (C) ©OFB-PatriNat, Havforskningsinstituttet

Mooring

> Attach the support to the mooring buoy with the right length of rope (*if it's too long, don't hesitate to coil it*).

> Attach following the mooring system employed depending on habitat sampled (See Appendix 4.1).

BRUV deployments

> **Switch on cameras and divelight** (try turning on the left and right cameras at the same time)

> Before immersion, pass the **slate board** in front of the two cameras (information of site, stereo rig ID, hour, date)

> **Synchronize** cameras by a **flash** (flash is easy to synchronize videos on the software)

Immerse each system from the boat with the rope that is tied to a buoy to find it back

> BRUVs immersion

> **Pull the rope once** to reposition the stereo system to the bottom

> Let it sit for one hour past half (to have hour of video recording)

Note 1: Multiple stereo-BRUVs are deployed concurrently (See Sampling protocol)

Note 2: In kelp forests, stereo rigs under and above the canopy are not deployed in the same place to limit bait bias.

Metadata

> **Note all metadata you can on board** (GPS localisation, date, immersion hour, ...), See metadata table (Appendix 4.3.).

BRUV recovery 1h30 later

Light-weight stereo-BRUVs can be retrieved by hand. But for heavier stereo-BRUVs Vessels fitted with a swinging davit arm, or pot-tipper and winch are ideal.

> Switch off light and cameras

> **Check your equipment** (batteries, general condition, ...)

> When you return, be sure to **rinse everything**.

3.3. Video analysis

Export video recordings

Note: Export video recordings on two external hard disks for safety.

> All video recordings must be labeled properly and placed in their respective folders, together with the correct .Cam files.

Example:

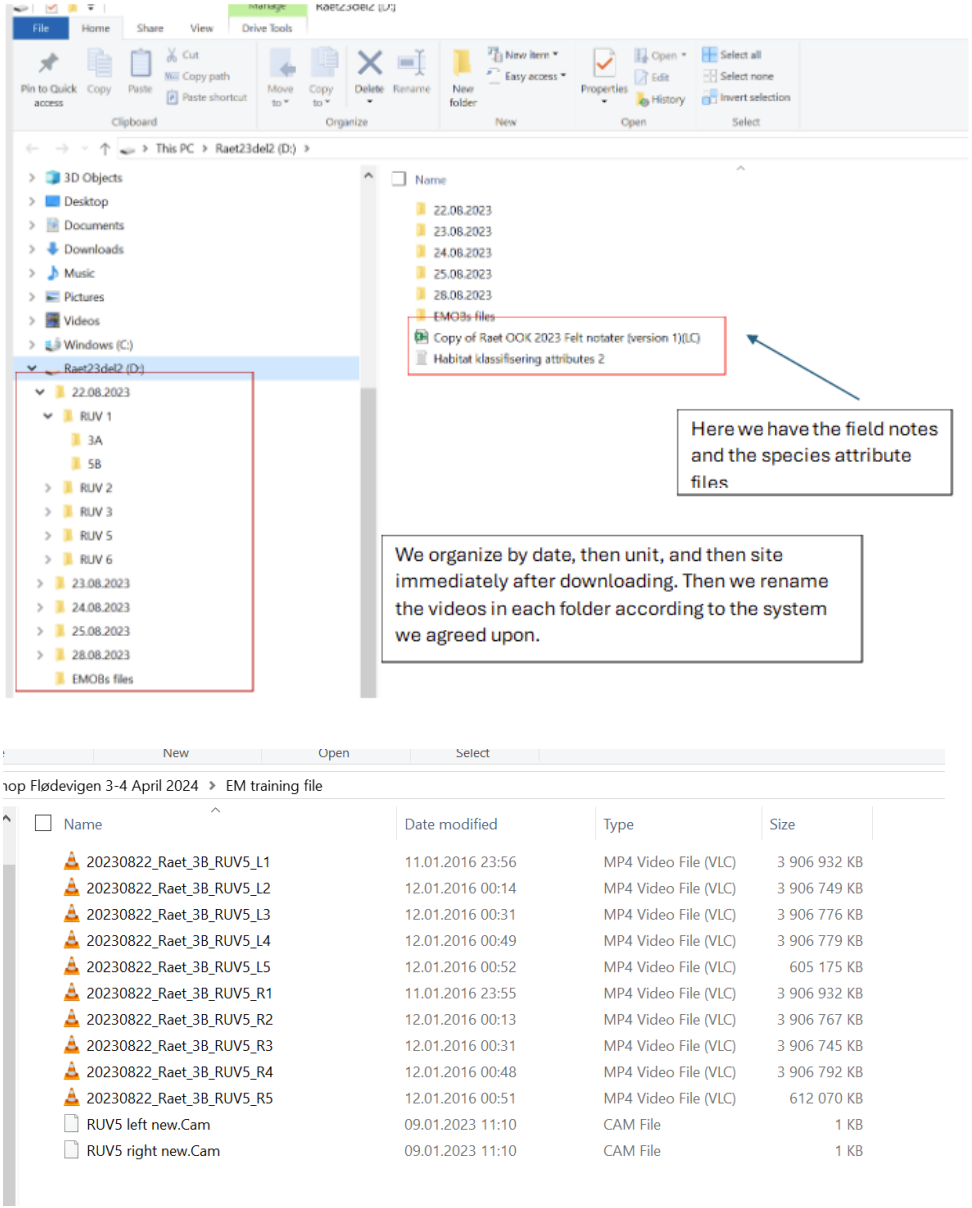


Figure 12: Video recording storages © Havforskningsinstituttet

> Here is the **file name nomenclature**: "Country_Date_Region_Site_Depth-category_BRUV-type_BRUV-number_LeftorRight_ videosequence"

> Remember to have the **ISO alpha-2 country code** at the start of the file (*e.g.*, Norway: NO, France: FR, Spain: ES, Turkey: TR, Israel: IL)

Note: Left and Right videos must be in the same folder to be viewed simultaneously in the software.

Video analysis on EventMeasure software

Gate the latest software version (6.23) from the web: SeaGIS software download

It is highly recommended to work from an external hard drive. Make sure the EM dongle is plugged in as this is the key to open the software.

Note: Count an average of 4 hours of analysis for one hour of video

Complete the metadata table: pre-check your videos

Note: It is recommended to **perform this step before analyzing the videos**. It allows to estimate the number of videos for analysis and thus the time necessary to do it.

Find the metadata table in Appendix 4.3

Which videos should be analyzed?

- **Time:** One of the videos (left or right) must be at least **one hour long**. This is necessary to ensure equal annotation time for MaxN. If the second video has a duration of less than one hour, there will simply be fewer measurements taken.
- Settle condition: The rig must face the bottom.
- **Visibility**: We must at least see the bait (**1 meter minimum**) on one of the videos left or right). If only one of the videos has a visibility of less than 1 m, the annotations are made on the other video and no measurements are taken.

Note about dive light: If the lack of light doesn't affect visibility, you can analyze the video. But to control this bias: indicate in the metadata table whether the divelight was on, off or faded out in the middle of the video.

How do you prioritize which videos to analyze?

- **Habitat:** Start by analyzing **hard bottom videos**. Soft bottom videos are analyzed at the end.
- **Settle condition:** When rigs are oriented towards the bottom but are in a vertical position: only analyze them if you have few analyzable videos. The volume of water is not the same in a vertical or horizontal position.

Note: Don't forget to enter the landing time. If the fish count doesn't start as soon as the rig is landed (e.g., suspended solids), the bait will already disperse and attract the fish.

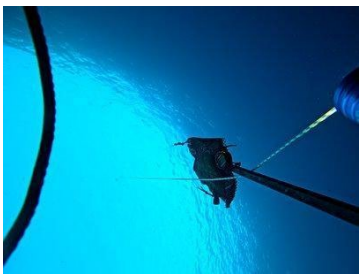
(A)  (B)  (C) 

Figure 13: Examples of settle conditions: (A) bad visibility (0, we don't see the bait), (B) soft bottom and settle condition ok, (C) bad settle condition (rig towards the surface) ©Lauriane Guérin, OFB-PatriNat

> Make a **screenshot** for each replicate to complete the habitat description (Figure 14).

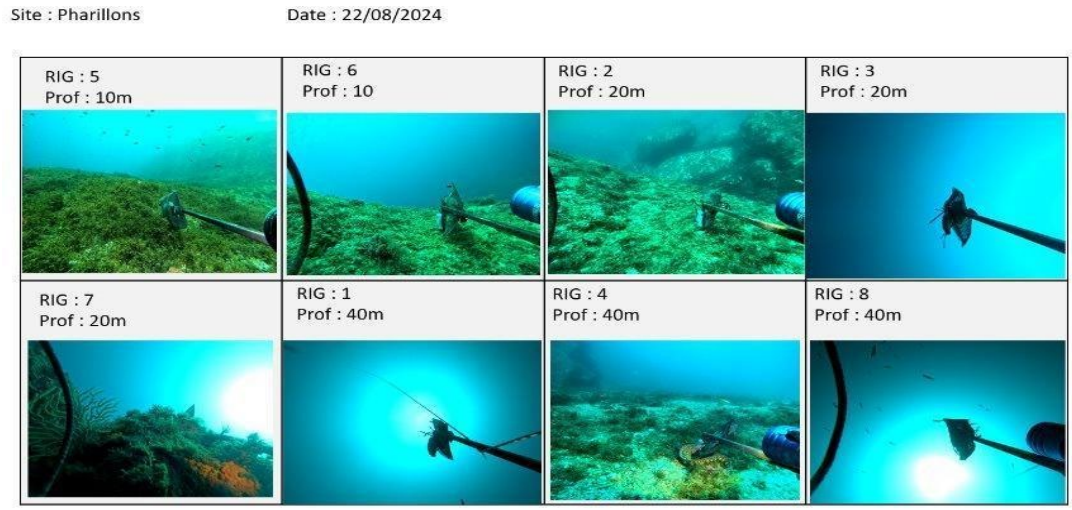


Figure 14: Screenshots of habitat replicates and recording in Powerpoint slides ©OFB-PatriNat

Starting a new Event Measure observation file (EMObs)

> Open a new measurement file

> Measurement > New measurement file

Note: Keep a record of your video analysis time. Record your analysis start in an Excel table or directly on EventMeasure software, don't forget to add it in the metadata table at the end of the analysis.

Load pictures

> Picture > Set picture directory > **find the right directory** where the video and .Cam files are located

> **Picture** > **Define movie sequence** > Add files à Marker L1-L5 (check video order) > Open > Ok

> Picture > Load picture (left side)

> **Stereo** > Picture > **define movie sequence** > Add file > choose all right files; R1-R5 (check video order) > Open > Ok

> Stereo > Picture > **Load picture (right side)** > R1 > Open > Ok

Load the camera-files (.Cam)

> Stereo > Cameras > Left > **Load camera file (Left.cam)** > *e.g.,* "R2 left.CAM" > Open

> Stereo > Cameras > Right > **Load camera file (Right.cam)** > *e.g.*, "R2 right.CAM" > Open

The camera-files (.Cam) were created during the calibration process. You have one file per camera (left and right) per stereo rig.

Edit field values

Play video to check info on field-notes > «Close and update position»

> Measurements > Information fields > **Edit field values** (check fieldnotes).

- **OpCode:** Country_Date_Region_Site_Depth-category_Bruv-type_Bruv-number

(eg. FR_2023-10-23_Brittany_Vieille_VD_C_R1*)*

- **Tape Reader:** The name of the person doing the analysis
- **Depth** (e.g., 36)
- **Comment:** corrected depth, visibility, obstructions, etc.
- **Date:** format Year-Month-Day *(e.g., 2023-10-23)*

> If you don't have the same field names: Measurements > Information fields > **Edit field names.** You can also **edit filed values** later > Measurement > Information fields > Edit field values

Note: Be careful not to put any commas here, because we will generate a .csv file.

Import your species list

> Measurements > Attributes > **Edit/ load species files**

> Your species list must be in **txt format**. You only have to import it once. Your list will already be saved in the software for future analyses.

Note 1: Genus and species names are in separate columns.

Note 2: If you find that you're missing a species from the list when you make your annotations, you need to add it back to your original document and re-import the species list.

Synchronize your videos

> Finding the accurate **flash moment on the left video** à *Update* position

> Finding the accurate **flash moment on the right video** à Update position

> Lock frames

Save your Event Measure observation file (EMObs)

> Measurement > Save

> EM file should be saved with the "OpCode" as filename

Note: If you quit EventMeasure, the software suggests saving. Usually, when you open the file again, it will go back to where it left off. But in some cases, you might have to repeat the process of setting the file directory, loading and synchronizing the videos. Moreover, sometimes the software crashes. That's why, when you're making your annotations, **remember to save your work regularly**.

Starting analysis

> Play movie and find the moment when the rig hits bottom: fill the "**landing_time**" and the "**settlement_time**" in the metadata table.

Note: Observation doesn't begin as soon as you land. You need to wait until the support is properly installed and the particles have settled. As this time is not limited, it **should be noted.**

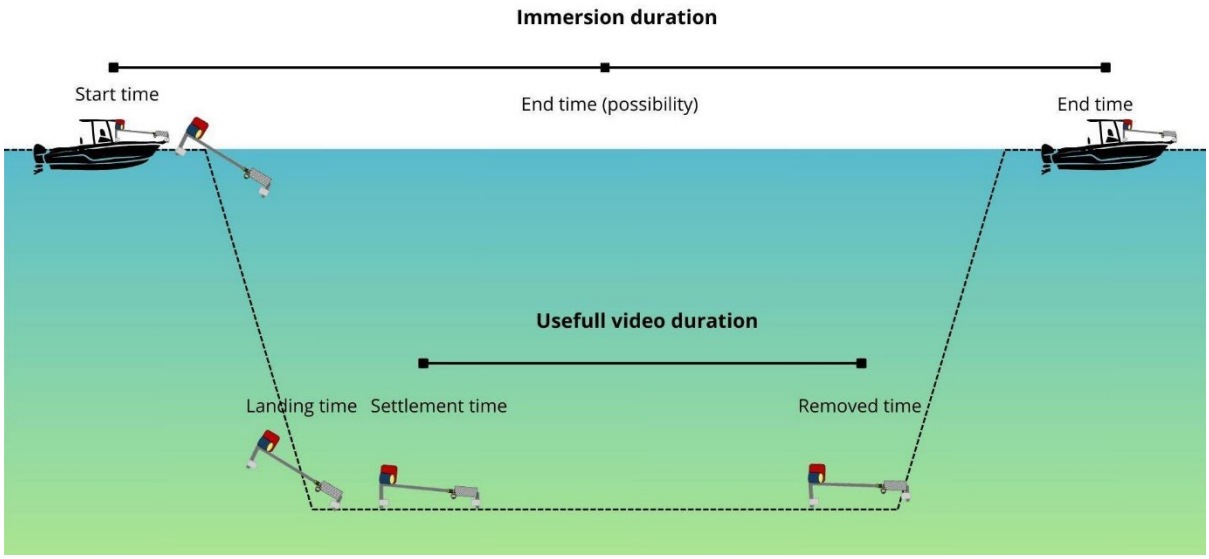


Figure 15: BRUVs deployment milestones ©Lauriane Guérin, OFB-PatriNat

Add a new period start

After the support hits the bottom, wait a few minutes for the particles to settle. Then you can begin your 1-hour observation/annotation period.

> Rightclick on left video > Period definitions > **Add new period start** > Name it P1

Note: There is only once per video, not on each video part!

Find the MaxN

MaxN is the maximum number of individuals for each species.

> Watch the whole video.

> For **each species, record the number of individuals**. Annotate up to MaxN but if you count less than MaxN, don't record the count.

> Count all the fish on the freezer frame, even some that are cut.

Note: There is no visibility limit. Count all the fish as long as they can be identified at least at family level. Distance will then be used as covariate in the analyses.

>Count also other species of interest: target crustaceans, cephalopods and vertebrates.

> To register a fish: Zoom in > «Ctrl», and right click the mouse > Rightclick on left video > Add a point à Choose species/ stage/ number etc

Note 1: It is possible to add a point on every fish or modify the number of one annotation to register a shoal of fish for example

Note 2: It is possible to register fish with the bounding boxes : hold down the “Shift” and “R” keys, then frame the fish.

To **add other headers**: > Measurements > Attributes > Attribute headers

At the end of analysis (after one hour of video):

> Rightclick on left video > Period definitions > **“Period end P1”**

> Measurement > Save (EMOBs file)

- Fish measurements and fish behaviors on the MaxN

Aim: Measure the maximum fish you can on the MaxN.

> Start with the MaxN frame and follow the fish in the video if they are not well positioned to find the frame the measure could be easier.

To measure a fish:

> Zoom in > «Ctrl», and right click the mouse

> **On the left video** > Left click on the **fish's snout** > Left click on the **fish fork** (to get the fork length)

> **Do the same operation on the right video** > Left click on the fish's snout > Left click on the fish fork

> **Register the measure** > Select fish species > Add remarks, if necessary > You can find fish length in the 3D measurements table

Note 1: Low precision/**RMS value over 11.0 = high insecurity**; consider removing measurements after during analysis

Note 2: When you click on a point on the left frame, a **red dotted line** appears on the right frame. It's called the epipolar line. It's a projection of the left point onto the right frame, made by the calibration performed beforehand.

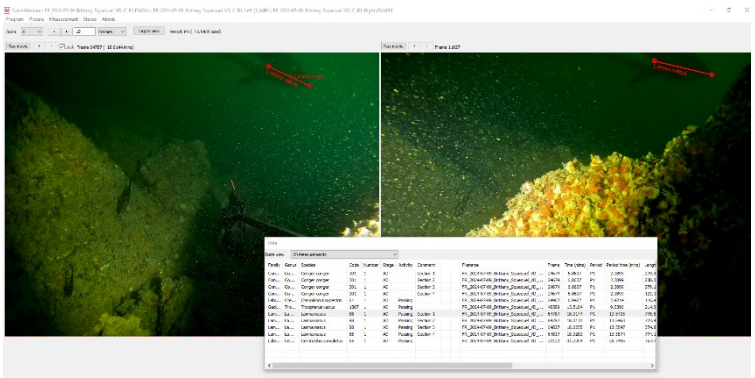
***TIPS**:* Make sure to have at least 1 3D Measurement in the file so that you can easily go back to a point/measurement in a synchronized video.

Special case: measuring individuals in sections

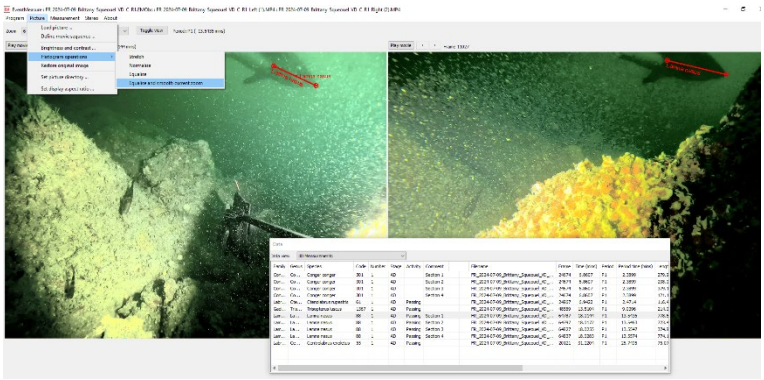
Some fish cannot be measured in the conventional way for many reasons. By example, some are too large and cannot be seen in their entirety on a single frame (*e.g.,* sharks) or cannot be measured in one linear measurement (*e.g.,* serpentiform fish like conger eel or moray eel).

In this case, it is necessary to make S=sections measurements on the same frame or on several different frames. To do this, use reference points on the animal (*e.g.,* fins). If these reference points are difficult to see, play with the software settings to make them stand out (Figure 16, B). When recording, enter the species (give to the individual a number if there are several of the same species, *e.g.,* Conger 1, Conger 2), and the section number in the "comment" attribute (Section 1, Section 2, etc.). Start from the head for Section 1.

(A)



(B)



(C)

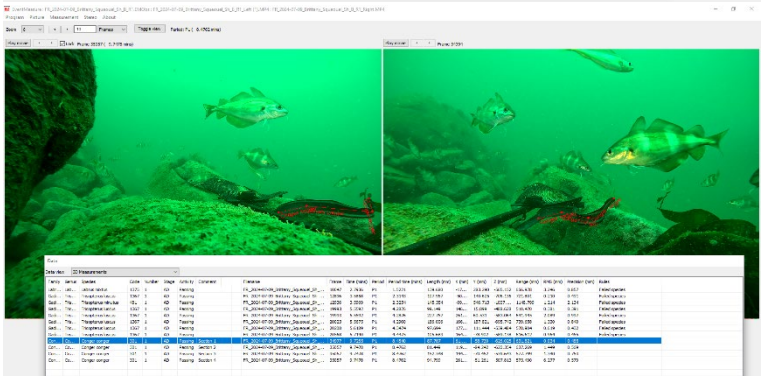


Figure 16: Examples of sections measurements (A) first section in the first frame, (B) software settings, (C) 3 sections in a frame © Lauriane Guérin, OFB-PatriNat

Habitat description

>See Habitat protocol

Export your data

> Keep a record of your video analysis time. Record your analysis duration in the metadata table.

> Measurement > Save (EMOBs file)

> **Generating a .csv summary file**: > Program > Batch text file output > A pop up screen will appear > Make sure that you have the correct path to the input and output directory

> Select all outputs by adding a true (1 or T or True) in the *Data* column next to the appropriate output type > Process *(NB: a message appears if all the files have been processed)* > The output file should be in the folder indicated.

Note: Saving all EM projects in a single folder means that when exporting data, all projects can be exported in a single table (for each output).

Appendices

Equipment needed

List of furniture

- For calibration:
  - Stereo rigs assembled (see Appendix 4.1.2.)
  - 1 calibration cube.
  - 1 calibration bar
  - 1 divelight
  - 1 slateboard
- For field sampling:

> General:

- Flashlight
- Slateboard

> For one replicate:

- Stereo rig assembled (see section 4.1.2. for hardware design)
- Mooring equipment (see section 4.1.3. for mooring design)
- One dive light per benthic stereo rig is used to compensate for the lack of natural light. Even in bright underwater environments, **use a dive light to standardize the protocol**.
- Bait (France use 800 g of fresh sardines for each stereo rig)

> Camera settings:

Resolution	2.7k (2704 x 2028 pix.)
Format	4 :3
Mode	Wide
Shutter speed	1/ 120
FPS	60
ISO	Auto
Color	Natural
White balance	6500 K
Sharpness	High
Smooth	Off

> For video analysis:

- Hard disks (recommendation: copy files on two different hard disks)
- SeaGIS licence
- CAL software (version 3.54)
- EventMeasure software (version 5.61)

TIPS: with a computer mouse, annotations will be easier.

- VLC multimedia player (recommendation)

Hardware design

Homemade French stereo rigs

- Generalities

Homemade French stereo rigs are in the form of a **stainless-steel tripod**. **Two Isotas housings** (submersible to a depth of 200 m) are positioned **50 cm** apart and oriented at a **convergence angle of 10°** (classically found in the literature). A **plastic basket containing the bait** (crushed sardines) is **positioned 60 cm from the cameras** (distance between the camera housing and the bait pocket). A **depth gauge** positioned just before the mesh basket is used to monitor immersion parameters (precision of 10 cm for the depth gauge, and 1°C for the temperature, immersion time in minutes). **Coloured scotches** (white, black, red, green, blue and yellow) are also positioned to let the possibility to calibrate the colours afterwards.

Note: One assembled stereo rig has: 2 GoPro with their protective/ waterproof housing, each GoPro has an empty SD card and a recharged battery

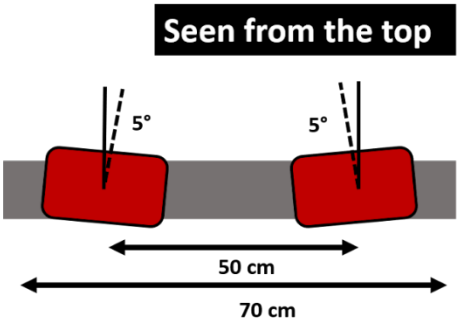


Figure 17: General features of homemade French stereo rigs © OFB-PatriNat

- Adaptation against habitat

France developed **polyvalent stereo rigs**. **Height-adjustable** systems can be deployed in different habitats. The bait sight is also removable. It can be rotated when stereo rigs are deployed in **kelp forests**.

A) Classic benthic stereo rig

Classic benthic stereo rigs are **weighted** with 5 x 2kg sinkers to ensure stability. The **height** of the support feet is set at **50 cm**. The **bait height** is set at **20 cm** long stand allows the cameras to be oriented 30° downwards (to best observe nearby benthic species). A **dive light** is positioned between the cameras to illuminate the scene in low-light conditions, without creating a particle halo.

B) Stereo rigs in kelp forests

In France there are some kelp forests. In this type of habitat, dropping classic benthic stereo rigs is not optimal (kelps block camera field of view). That's why we developed new stereo rigs. To **connect the UVC protocol** we imagined **two different stereo rigs for kelp forests:** one positioned on the floor under the kelps (Benthic stereo rig understory), the other just above the canopy (Stereo rig above the canopy).

➢ Benthic stereo rig kelp understory

These stereo rigs are **weighted with 5 x 2kg sinkers** to ensure stability. The **height** of the support feet is set at **20 cm**. The **bait is set on the floor**, by rotating the 3rd support feet, stand that allows the cameras to be oriented downwards (to best observe nearby benthic species). A **dive light** is positioned between the cameras to illuminate the scene in low-light conditions, without creating a particle halo.

> This set up makes it possible to film under the kelps.

➢ Stereo rig above the kelp canopy

Feet of the stereo rigs are removed, and the **bait foot is rotated** like the benthic stereo rig understory support. To hold the system in place and resist the current, **15 kg of weights** are attached to the underside of the support and **1 buoy weighing 12 kg** ensures its buoyancy. The length of the **rope** used to attach the weights is around **1m** but **can be adapted to suit the height of the kelp**. This means you need to know the site in advance.

> This set up makes it possible to film above the kelp canopy

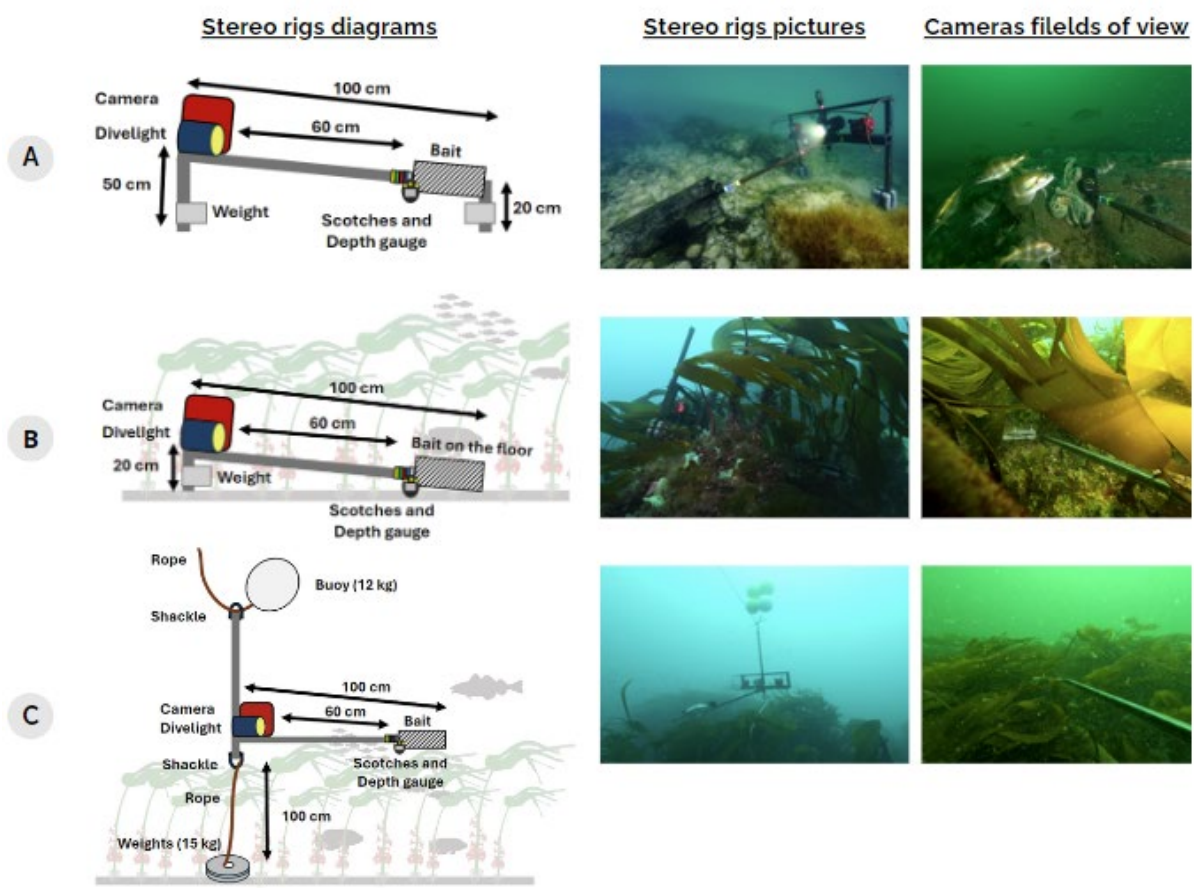


Figure 18: Homemade French Stereo rigs set ups, Classic benthic stereo rig (A), Benthic stereo rig kelp understory (B), Stereo rig above the kelp canopy (C) © Lauriane Guérin, OFB-PatriNat

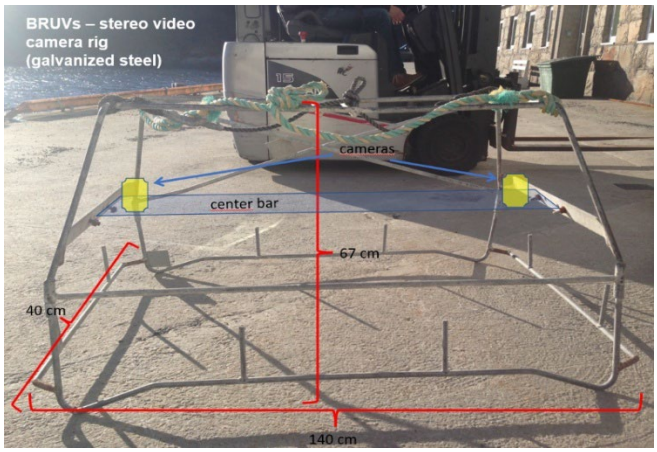


Figure 19: Actual rig used by IMR, front view, housing without camera ports © Havforskningsinstituttet

Stereo rigs are made of **galvanized steel** (can be made in a local welding shop). The center bar holds two cameras **5-7° angled** towards each other. **Weight can be loaded on if needed** (strong currents etc., but rarely used so far in Norway). The **bait bag** mounted in the end of the bait pole with the use of steel clips. The bag is made of double layered chicken wire.

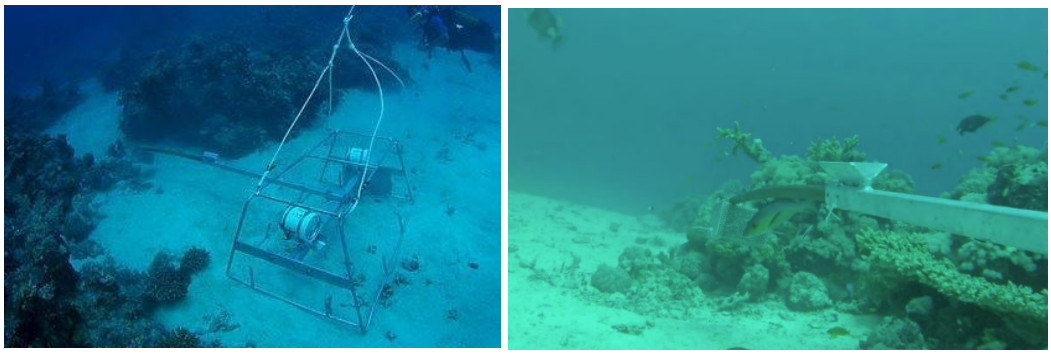


Figure 20: (Left) SeaGIS stereo rig immerged in Soudan, (Right) Left camera field of view © Havforskningsinstituttet

Extra-long underwater casings provide room for an **external power bank**. This makes it possible to set out **4-5 stations** (1 hour video recording for each) **without charging** and downloading in between stations. The housing has to be fastened to the rig so it doesn't move (sits in a bracket on the bar and attached securely with hose clamps by example)

Note: The biggest drawback of using external power banks is that since the cameras have to be physically connected to the battery by cable, the waterproof nature of the camera is compromised (see picture below of current IMR set up). It is therefore imperative that proper care is taken with mounting the camera ports (i.e., check that there is no cable in the way of the seal, the O ring is clean, and greased properly).

Figure 21: Camera housing details: (Left) Side view of GoPro camera with angled cable connected to the power bank (Middle) Side view of underwater housing with safely clip (Right) Behind the camera port is the powerbank that is secured in a custom-made foam holder © Havforskningsinstituttet

If bought from SeaGIS, the camera ports are custom made for given model(s) of GoPros. The **camera is attached to the glass front** and care must be taken that it is **not moved between calibrations**.

Figure 22: (Left) Front view of GoPro 11 mounted on the camera port, with the plastic lid and extra battery pack. (Right) Side view of camera port, with the cover removed to allow the cable connection (cable outlet for the model is located under the battery (white) © Havforskningsinstituttet

Mooring

For deployment, each support is attached to a **rope** and connected to a **labelled mooring buoy.**

> This makes it possible to **signal** the submerged stereo rig and to **find it** once it has been launched.

> The length of the rope depends on the target depth.

> In addition to the main buoy, you can add a **second, smaller buoy to assess the current** (if the small buoy remains on the surface, it means there isn't much current on the bottom; on the other hand, if the small buoy sinks, it means the rope is taut and there is current on the bottom).

Special case for the support above the kelp canopy: an additional buoy, which will be submerged, is attached to the stereo rig (See Figure 14).

Tips on *EventMeasure* Software

- **Zoom**: You can choose the zoom magnification from 2 to 50 by clicking on the arrow in the left-hand corner. To zoom in on a point in the image, press shift key and move your mouse forward. To zoom out, press shift key and move your mouse backwards.
- **Jump to frame**: Right click and choose the frame number you want. You can also jump frames and minutes with the double arrow on the left corner near the zoom.
- **Rate**: When you play movie, you can change the playback speed with the rate arrows.
- **Export movie**: Right click > Export movie à Choose the length of your movie clip Select the file directory and the name file (AVI File only) > Compressor Codec > Ok
- **Export Jpeg:** Right click and export Jpeg.
- **Brightness and contrast:** You can improve the quality of your image in > Picture > Brightness and contrast ... A little box appears. If you close it your modifications are cancelled.
- **Sequence definition:** GoPro films 1 hour in several video sections. Defining the movie sequence at the start of the analysis allows you to select all the video sections concerned and avoid video editing.
- **Come back easy on your synchronized point:** When at least one of the measurements has been taken, you can easily find the synchronization without having to search for the clapper or flash. To do this, click on the measurement and check “lock”.

There's a recurring problem: when you return to your EM project, you need to redefine the “picture directory”. This is enough to restore synchronization without having to do it all over again.

Metadata

A metadata table is available in a dedicated separate file. Here is an overview of all variables to note.

Table 1: Read me sheet of the metadata file © Quentin Ternon, Lauriane Guérin, (OFB-PatriNat)

	variable	example	format	unit	definition
ID of each replicate	country	FR	text	NA	The country of the partner : DK (Denmark), NO (Norway), FR (France), ES (Spain), TR (Turkey), IL (Israel)
	laboratory	MNHN	text	NA	The name of the partner laboratory
	date	2023-10-23	date	yyyy-mm-dd	The date of the BRUV immersion
	locality	Atlantic	text	NA	The ocean or sea name of the sampling area. Need to be constant all along the campain (same capital letters, same space or underscore...)
	region	Brittany	text	NA	The name of the region. Need to be constant all along the campain (same capital letters, same space or underscore...)
	subregion	7 iles	text	NA	The name of the subregion. Need to be constant all along the campain (same capital letters, same space or underscore...)
	site	Vieille	text	NA	The name of the site. Need to be constant all along the campain (same capital letters, same space or underscore...)
	subsite	Vieille north	text	NA	The name of the subsite. Need to be constant all along the campain (same capital letters, same space or underscore...)
	depth_category	VD	text	NA	Depth categories defined in sampling strategy : S (shallow), D (deep) and VP (very deep)
	bruv_type	C	text	NA	The type of BRUV (especially in FR) : C (circalittoral), A (above the kelp canopy), B (below the
	bruv_number	R1	text	NA	The ID number of the BRUV : Rig (R) number (1)
	file_name	FR_2023-10-23_Brittany_Vieille_VD_C_R1	country_date_region_site_depth-category_bruv-type_bruv-numbe	NA	File name of the video which be used after as the name of the OpCode in EventMeasure
Co-variates from the field	operators	Quentin Ternon, Pierre Thiriet, Valentin Danet	text	NA	Names of field operators
	utc	2	numeric	NA	UTC (Coordinated Universal Time) : 1 (UTC+1), 2 (UTC+2), 3 (UTC +3)
	start_time	13:14	time (24h)	hh:mm	The time of the BRUV immersion
	end_time	14:36	time (24h)	hh:mm	The time of the BRUV removing
	immersion_duration	82	numeric	min	The duration of BRUV immersion
	latitude	48.83876	numeric	decimal	Latitudinal coordinate of the immersion point of the BRUV in decimal degree WGS84
	longitude	-3.47615	numeric	decimal	Longitudinal coordinate of the immersion point of the BRUV in decimal degree WGS84
	real_depth	21	numeric	meter	The real depth at witch the BRUV is immersed
	depth_stratum	18	numeric	meter	The target depth (chart datum) stratum
	sea_level	3.6	numeric	meter	The sea level during BRUV immersion
	corrected_depth	17,4	numeric	meter	Corrected depth = real depth - sea level
	surface_temperature	21.6	numeric	°C	The temperature at the surface
	bottom_temperature	19	numeric	°C	The temperature at the BRUV settlement area
	immersion_comment	strong curent and 0.5m swell	text	NA	Any comment related to the immersion of the BRUV (weather, warning message, BRUV condition...)
	camera_type	gopro hero 11	text	NA	The camera model
	fps	60	numeric	frame/s	The image rate in camera settings
	video_format	4_3	text	NA	The image format in camera settings
	video_mode	wide	text	NA	The image mode in camera settings
	resolution	2.7K	text	pixel	The resoution of the image in pixels in camera settings
	shutter	1_120	text	s	The shutter speed in camera settings

Solution for Preventing GoPro Battery Overheating in BRUV housing.

Equipment Needed:

- Ziploc bags (double-layered)
- Ice
- Cooler box
- Circular foam insulation pieces

Instructions:

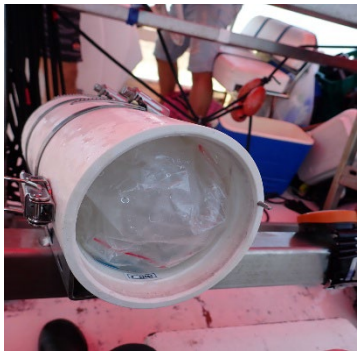
Before each BRUV deployment, prepare a half-filled Ziploc bag with ice, using two bags placed one inside the other, to prevent condensation.

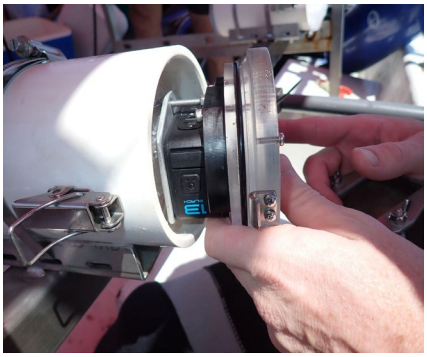
Inside the BRUV housing, first place the circular insulation piece (foam), and then position the ice bag on top of it.

Carefully close the housing with the GoPro inside, ensuring that the ice bag is in direct contact or as close as possible to the camera.

This setup prevents the GoPro from overheating and shutting down, allowing continuous video recording until the battery is fully depleted.

Note: For every new BRUV deployment, replace it with a fresh ice bag.





Appendix C: Environmental DNA sampling protocol

Overview

The objective of the study is to print the diversity of rocky reef fish present at the scale of the rocky reef and its surroundings. A sampling of water is pumped in-situ and filtered either 1) dropping a pump from a surface, 2) diving with the pump or 3) from the boat along a radial transect. The extraction of the DNA materials, sequencing process and comparison to reference library theoretically allow to have the list of the species present in the sampled area.

This document aims to describe the protocol used in the EuRockFish pilot study to implement eDNA methodology and gives some generalities and justifications of several methodological choices.

Materials

The eDNA pump used in this project is the eDNA pump V4 from Subspace (https://www.subspace.ch/dna-underwater-pump).

Figure 15 : Subspace eDNA sampling pump V4 (©Subspace Pictures)

For sampling kits, we used single-use VigiDNA® sampling kits consisting of a tube with an inlet filter, a low-porosity sterile filtration capsule (0.2 µm), a conservation buffer (CL1), and a pair of disposable gloves.

Figure 16 : VigiDNA® sampling kits. On left: Tube with an inlet filter and disposable gloves. On right: Filtration capsules, stoppers and conservation buffer (© SPYGEN)

Before sampling

On land

Figure 3 shows the materials required for the sampling process.

Figure 17 : Checklist of the material you need for one eDNA sampling

- Put gloves and clean your gloves and the basin with the disinfectant wipe
- Clean the plastic bag (inside and outside), tube kit, filter kit and scissors with a new wipe
- Open the tube kit with the scissors and put on the pair of gloves inside
- Take the strainer part of the tube and put it inside one digit of a glove. Make a node and let the kit in the basin (see Figure 4)
- Open the filter kit and present the inlet part outside the bag
- Take the tube kit, catch the pipe extremity and put it to the inlet part of the filter (to do it more easily, apply a pressure at the extremity of the tube to flatten it and progressively slide it to fit the shape on the inlet part of the filter).
- Put the filter inside the last glove of the tube kit and make a node (see Figure 4)

- Put in safety place the filter kit box inside the plastic bag with two plastic plugs and the adhesive label (which is in the filter kit).

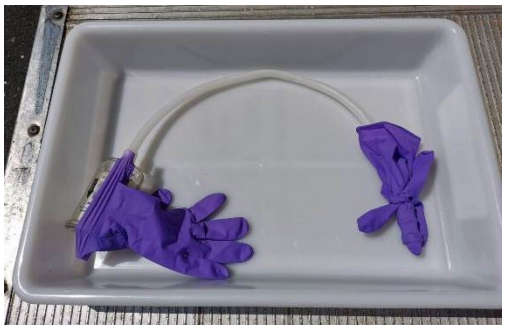


Figure 18 : Sampling setup ready to install

On the sampling site

- Put on a pair of gloves and bleach the pump
- Install the set of tube and filter on the pump: open the head jaw and put the pipe inside (take care of the direction of spinning and flow direction of the filter: they must be in the same direction than the water flow/entrance). Use the two plastic pieces to correctly install the tube (to avoid excessive bending of the tube, see Figure 3). Make sure you start with the head closest to the motor to make it easier to install the next set.

Figure 19 : Installation of the tube and the filter on the pump

During sampling

Three different options are possible to eDNA sampling: Dive sampling, drop sampling and/or radial sampling.

Option 1 : Dive sampling

- Remove the protecting gloves from the strainer and the filter
- Bring the pump until the place of interest (depth strata and rocky seascape)
- Activate the depth gauge, switch on the pump and activate the start button.
- Control the correct start listening to the motor working and feeling the water flow at the outlet part of the filter.
- Note the start time on the field sheet (see Appendix C).
- Hang the bottom part of the pump to you with a rope (the pump should be floating 1-2 meters away from you, with the head part oriented towards the surface).
- After 30 min of filtering, deactivate the start button and switch off the pump
- Note the end time on the field sheet (see Appendix C)
- Bring the pump on board and turn off the depth gauge

Option 2 : Drop sampling

- Remove the protecting gloves from the strainer and the filter
- Active the depth gauge
- Switch on the pump
- Activate the timer button and control that the red light become green (start the timer)
- Note the hour you activate the time
- Hang the pump to the weight or anchor to the rope loop at the bottom part of the pump
- Hang the pump to the surface buoy to the rope loop at the top (head) part of the pump
- Drop the pump and note i) the hour of dropping, ii) the depth displayed on the dashboard and iii) the coordinates.
- After end of filtering, bring the pump on board and note the hour of removing
- Deactivate the timer button, switch off the pump and turn off the depth gauge

Option 3 : Surface/Radial sampling

- Install and fix the pump at the front of the boat
- Remove the protecting gloves from the strainer and the filter
- Immerge the two tubes with their respective inlet filters.
- Switch on the pump and activate the start button
- Control the correct start listening to the motor working and feeling the water flow at the outlet part of the filter
- Note the start time on the field sheet and the GPS coordinates (GPS coordinates of the boat, see Appendix C)
- Proceed with the boat along the pre-defined radial transect at a speed of 3 or 4 knots
- After 30 min of filtering, deactivate the start button and switch off the pump
- Note the end time on the field sheet and the GPS coordinates (GPS coordinates of the boat, see Appendix C)
- Deactivate the timer button and switch off the pump.

After sampling

On board

- Put on a pair of gloves
- First switch on the pump and activate it with the start button in the air to fill out the residual water
- Remove the whole set
- Put the filter in a glove, then put it back in the plastic bag and write with a marker the date, site and hour of sampling

On land (easier to be two)

- Put gloves and clean your gloves and the basin with the disinfectant wipe
- Clean the plastic bag (inside and outside), buffer solution kit, the previous opened filter kit, marker and scissors with a new disinfectant wipe
- Put on a new pair of gloves and take the filter out of the plastic bag
- Put one plastic plug at the outlet part of the filter
- Fill the filter with the buffer solution until reach 95% full (all the filtering membrane need to be immersed). The use of a funnel is strongly recommended.
- Put the second plastic plug, turn the filter and remove the first plug.
- Put the funnel and fill the filter with the rest (5%) of the buffer solution
- Put the second plug back on
- Stick the adhesive label of the filter box on the filter
- Shake strongly the filter in all directions (to put in solution the genetic material)
- Put the filter inside the box arrow facing down (see Figure 4) and check that the ID on the box matches the ID on the sticked label.
- Write on the box several information (date, hour start and end of filtering, site, depth, replicate number, etc.)

Figure 20 : Relative orientation of the filter to the box for storing and invoice

At the laboratory

The filters must be gathered and placed vertically in a shaded, temperate place (ideally in a fridge). They must be sent to the sequencing laboratory within the next month, as good sequencing results cannot be guaranteed after one month.

Complete the metadata file with all required information.

APPENDICES

APPENDIX A: Underwater pump

Before using, some modifications to the pump's hardware of the pump (see Figure 5) may be necessary notably because:

- The pump must have neutral buoyancy underwater. Float parties must be added to the pump until neutral buoyancy.

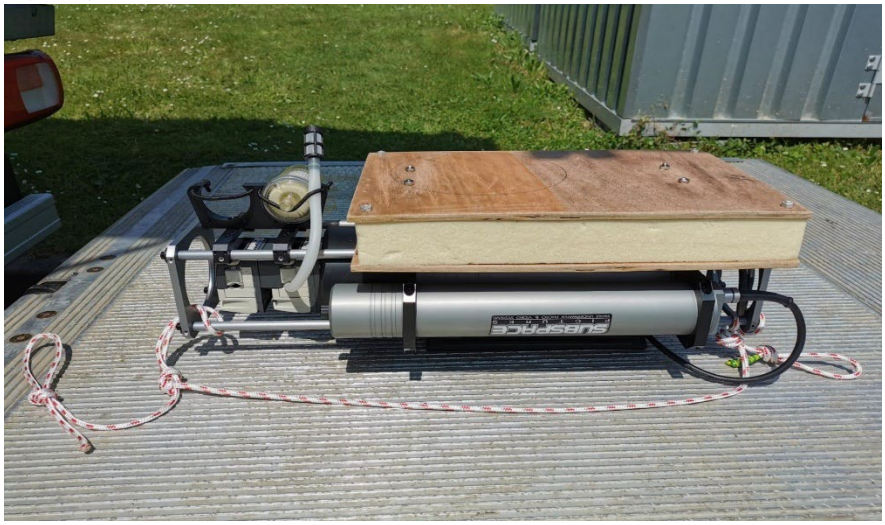


Figure 21: Modified underwater pump (rope with loops and floating panel)

- A rope with two loops on each extremity must be added to the hardware (aluminum cage) to fix any other stuff to the pump (to prevent any physical constraint on the underwater pump). A little buoy (no more than 1kg of buoyancy) has to be attached at the top (head) part in order to maintain vertically the pump with the head towards the surface water (to limit sediment entrance during filtration and physical shock with the substrate).

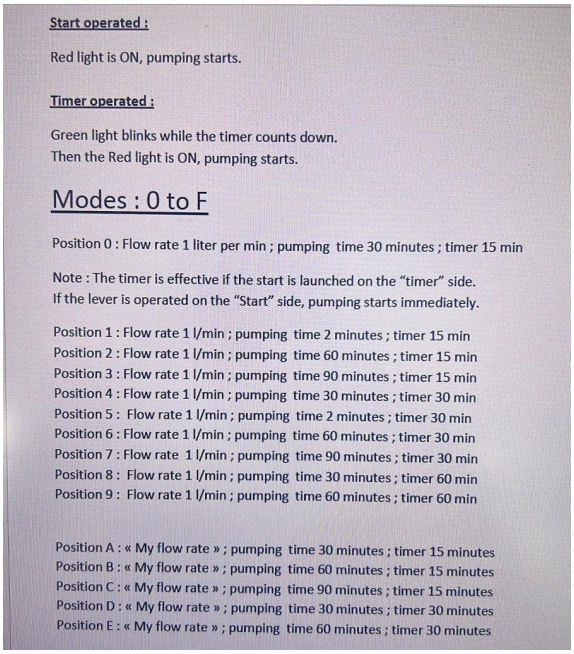
Advices/ Tips for the pump

- Draw an arrow on the head of the pump to indicate the sense of rotation of the pump (help for pipe installation).
- The flow should be standardized to 1L/min (30L for 30min of sampling) for intercomparison and sample quality. Use a flowmeter to control and fix it on the flow regulator. Use a trash pipe and filter on the pump. The flow meter is added to the outlet of the filter and flow regulator is turned until 1L/min. Use the screw to fix the regulator positioning. A predefined mode at 1L/min flow is already available so, you can use it but a control must be carried out in all cases.

Functioning of the pump

- Choose the correct mode (see Figure 6 and the matches between characters and modes). For a flow rate of 1L/min with a pumping time of 30min and a timer at 15min or 30min depending on the timing on board, select mode "0", "4"," A" or "D".
- Switch on the pump turning the 1st button to on (a red light must light up).
- In case of drop sampling: start the timer by turning the 2nd button to "timer" (a green light must light up) and then drop the underwater pump.

- In case of dive sampling: wait until being in the sampling area during the dive and start the pump turning the 2nd button to “start” (you must hear the pump functioning and feel with your hand the flow at the outlet of the filter).



Start operated :

Red light is ON, pumping starts.

Timer operated :

Green light blinks while the timer counts down.
Then the Red light is ON, pumping starts.

Modes : 0 to F

Position 0 : Flow rate 1 liter per min ; pumping time 30 minutes ; timer 15 min

Note : The timer is effective if the start is launched on the “timer” side.
If the lever is operated on the “Start” side, pumping starts immediately.

Position 1 : Flow rate 1 l/min ; pumping time 2 minutes ; timer 15 min
Position 2 : Flow rate 1 l/min ; pumping time 60 minutes ; timer 15 min
Position 3 : Flow rate 1 l/min ; pumping time 90 minutes ; timer 15 min
Position 4 : Flow rate 1 l/min ; pumping time 30 minutes ; timer 30 min
Position 5 : Flow rate 1 l/min ; pumping time 2 minutes ; timer 30 min
Position 6 : Flow rate 1 l/min ; pumping time 60 minutes ; timer 30 min
Position 7 : Flow rate 1 l/min ; pumping time 90 minutes ; timer 30 min
Position 8 : Flow rate 1 l/min ; pumping time 30 minutes ; timer 60 min
Position 9 : Flow rate 1 l/min ; pumping time 60 minutes ; timer 60 min

Position A : « My flow rate » ; pumping time 30 minutes ; timer 15 minutes
Position B : « My flow rate » ; pumping time 60 minutes ; timer 15 minutes
Position C : « My flow rate » ; pumping time 90 minutes ; timer 15 minutes
Position D : « My flow rate » ; pumping time 30 minutes ; timer 30 minutes
Position E : « My flow rate » ; pumping time 60 minutes ; timer 30 minutes

Figure 22: On the left: Functioning button of the pump (ON/OFF, Start/Timer, Flow regulator, Predefined mode control, light). On the right: Quick operated sheet.

APPENDIX B: Materials check list

- ✓ underwater pump with two heads
- ✓ spare battery
- ✓ 3 pipe kits (2 sampling + 1 spare)
- ✓ 3 filter kits (2 sampling + 1 spare)
- ✓ 3 buffer solution kits (2 sampling + 1 spare)
- ✓ basin
- ✓ bleach solution in sprayer
- ✓ disinfectant wipes
- ✓ marker and scissors
- ✓ nitrile glove box
- ✓ zip-lock plastic bags
- ✓ note board with field sheet

APPENDIX C: Field sheet

project_name :		habitat_type :		site :		T°_surface :	
date :		method :		subsite :		T°_bottom :	
depth_min_diving :		depth_min_echo :		time_start :		GPS_start :	
depth_max_diving :		depth_max_echo :		time_end :		GPS_end :	
depth_mean_diving :		depth_mean_echo :					

Filter 1	spygen_code :		Filter 2	spygen_code :	
	comments :			comments :	

project_name :		habitat_type :		site :		T°_surface :	
date :		method :		subsite :		T°_bottom :	
depth_min_diving :		depth_min_echo :		time_start :		GPS_start :	
depth_max_diving :		depth_max_echo :		time_end :		GPS_end :	
depth_mean_diving :		depth_mean_echo :					

Filter 1	spygen_code :		Filter 2	spygen_code :	
	comments :			comments :	