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Disclaimer

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List of abbreviations

AAC: Adriatic Advisory Committee
AF - Application Form
AP - Associated Partner
AQ - Aquaculture
AS - Adriatic Sea
AO – Associated Organization
CM - Communication Manager
FM - Financial Manager
IAS - Invasive Alien Species
PSC - Project Steering Committee
LPMT – Lead Partner Project Management Team
MoU – Memorandum of Understanding
PCL - Project Communication Leader
PCM - Project Communication Manager
PFM - Project Financial Manager
PM - Project Manager
PP - Project Partner
RP - Reporting Period
QM - Quality Manager
QP - Quality Plan
SSF - Small Scale Fisheries
WP - Working Package
WPL - Work Package Leaders

Project executive summary

BRAVE is a European Territory Cooperation project funded under the 2nd call for Operations of Strategic Importance of the Interreg VIA Italy-Croatia 2021 – 2027 Programme, Priority 2 "Green and resilient shared environment", Specific Objective 2.2 "Enhancing protection and preservation of nature, biodiversity and green infrastructure, including in urban areas, and reducing all forms of pollution". The project duration is 36 months (01.07.2025-30.06.2028) and the overall objective is to enhance species knowledge by ensuring ecosystemic functionality and tackling biodiversity loss in the Adriatic Sea (AS), through monitoring activities, creation of a smart digital platform and innovative planning in response to environmental change.

BRAVE intends to address the following common challenges that can have strong environmental (competition for resources and substitution of native species), social (conversion of work activities and change in citizens' habits) and economic (fisheries and aquaculture sectors) impacts:

Invasive Alien Species (IAS): The proliferation of non-native species poses a significant threat to ecosystems, human health, and economic viability

Biodiversity Loss: The AS is experiencing a decline in biodiversity due to IAS, climate and environmental change, habitat degradation, pollution, and overexploitation

Data Fragmentation: Inconsistencies in existing databases hinder our ability to assess the overall health of the AS

Data Communication Gap: Difficulties in dissemination and use of environmental data by nonexperts or those involved in monitoring and managing environmental data, increased by the challenging coordination between public bodies, research centres, business and citizens.

To achieve the goals, 13 project partners from the Italian and Adriatic Regions and 2 associated organizations will jointly implement strategic and operational actions.

Introduction

Work Package 1 (WP1) Knowledge Framework and Data Harmonisation defines the knowledge governance architecture of the BRAVE project. It establishes the strategic framework through which environmental information generated across the Adriatic Sea basin is organised, interpreted and mobilised to support coordinated cross-border action.

The Adriatic Sea is characterised by interconnected ecological systems and cumulative pressures that transcend administrative boundaries. However, environmental data are typically collected, managed and reported within national or sector-specific structures. WP1 addresses this structural fragmentation by creating the conditions for a shared transnational understanding of environmental status, trends and risks.

Rather than focusing solely on technical integration, WP1 strengthens the governance dimension of environmental knowledge. It promotes coherence between freshwater and marine assessments, reinforces alignment with EU regulatory frameworks such as the Water Framework Directive and the Marine Strategy Framework Directive, and enhances the science–policy interface across the project area.

A central component of WP1 is the development of the BRAVE Smart Digital Platform, conceived as a shared analytical environment supporting data visualisation, comparison and interpretation. However, WP1 extends beyond the creation of a digital tool. It ensures that environmental knowledge generated through pilot actions and existing monitoring systems contributes to long-term cooperation, adaptive management and evidence-based policy processes at Adriatic scale.

Through this strategic orientation, WP1 provides the enabling framework that connects data generation, analytical tools and mitigation measures, ultimately supporting the resilience and adaptive capacity of the Adriatic Sea ecosystem.

Within the broader framework established by WP1, Activity 1.1 Development of Common Data Collection Protocols focuses on the definition and validation of common data collection protocols supporting the development and effective functioning of the BRAVE Smart Digital Platform.

The activity responds to the need for harmonised, interoperable and operationally feasible data collection approaches capable of integrating traditions. By establishing minimum data requirements, shared structures and standardisation principles, Action 1.1 enables diverse data heterogeneous datasets generated across different sectors, environmental contexts and monitoring streams—ranging from chemical status monitoring to biological, habitat-based and risk-oriented observations—to be structured in a comparable and compatible manner.

The protocol deliverables developed under this activity provide a shared methodological framework for data collection, ensuring consistency across pilot actions while allowing partner-specific adaptations reflecting different environmental settings and technical capacities. Each protocol defines essential variables, metadata standards and validation procedures necessary to ensure reliability, traceability and quality control of the collected information.

Validation is carried out through pilot actions implemented under real-world operational conditions, involving different types of operators, technologies and environmental contexts. This process ensures that the resulting framework functions not only as a project-specific instrument, but as a transferable and adaptable reference applicable to other Adriatic contexts beyond the pilot phase.

In this way, Action 1.1 operationalises the strategic objectives of WP1, translating the project's governance vision into concrete, tested methodologies that support transnational environmental assessment and coordinated mitigation measures.

The general objectives of Activity 1.1 is designed to develop a harmonised framework for data collection by setting shared principles, defining core requirements, and identifying minimum standards to be applied across all activities. It also aims to guarantee that data produced in different environmental domains and pilot areas remain reliable, consistent, and fully traceable.

In addition, the activity ensures that data collection procedures are compatible with the functional specifications of the BRAVE Smart Digital Platform, while promoting the operational testing and validation of agreed protocols through pilot actions and simulation-based exercises.

By achieving these objectives, Activity 1.1 supports the shift from fragmented monitoring practices toward an integrated and interoperable system, enabling collected data to be effectively used for analysis, visualisation, and informed, evidence-based decision-making.

Objectives of the Data Collection standard 5

Deliverable 1.1.7 defines the Common Data Collection Standard for evaluating unwanted effects of target fish and mollusc species production in the project area with focus on mitigation. This protocol aims to strengthen the sustainability and resilience of aquaculture systems by improving the monitoring, assessment, and management of environmentally and economically important species.

By standardising environmental monitoring and data collection in aquaculture systems, the project enables a comprehensive analysis of the relationships between environmental conditions, biodiversity changes, and farm productivity, including the spread of invasive species and emerging risks. This standard provides the necessary data structures to populate the Smart Digital Platform.

Partner-specific objectives:

Marche Region (PP4): To provide a scalable framework on the collection of data related to unwanted effects affecting mussel farming systems, with particular attention to mitigation measures, capable of being extended to larger regions throughout the Adriatic Sea.

Istria County (PP7): Feasibility assessment of controlled reproduction and early life-stage development of shellfish and crustaceans in recirculating aquaculture systems (RAS) for climate-resilient aquaculture.

Zadar County (PP9): 1) Inventory and population structure of economically important marine bivalve mollusc species in Zadar County, and identification of habitats where these species can be preserved in the long term through the application of Regulation (EU) 2024/1991. 2) Definition of controlled trophic, physical and chemical conditions in a recirculating aquaculture system (RAS) for Mediterranean scallop broodstock conditioning and spawning induction protocols.

RERA S.D. (PP10): 1) Assessment of the condition of commercially important shellfish in Kaštela Bay – analysing their value for local communities and identifying critical areas for

conservation and sustainable exploitation 2) Assessing the impact of mariculture on ichthyofaunal biodiversity through visual observation and e-DNA barcoding for the identification of invasive and non-native species

Dubrovnik and Neretva County (PP13): An overview of the condition of farmed bivalves in Mali Ston bay in relation to the annual production quantities, biomass of standing stocks and under biotic and abiotic environmental conditions over a one-year period.

Importance of the Data collection standard 5 for the BRAVE Project

PP4: This protocol focuses on the collection of data related to unwanted effects affecting mussel farming systems, with particular attention to mitigation measures.

Mussel farms are highly sensitive to environmental variability and coastal pressures, which can directly impact production performance and sustainability. By addressing unwanted effects through a structured and standardised data collection approach, this protocol supports the identification of critical interactions between environmental conditions, farming practices and mitigation strategies.

The protocol contributes to the BRAVE project by generating strategic datasets that inform adaptive management approaches and feed the Smart Digital Platform with operationally relevant information.

PP7: This protocol focuses on the assessment of controlled reproduction and early life-stage development of selected shellfish and crustacean species under recirculation aquaculture systems (RAS), as a response to increasing environmental variability and climate change impacts in the Adriatic Sea.

Natural recruitment of several economically important species is increasingly affected by environmental stressors such as temperature anomalies, salinity fluctuations and habitat degradation. Controlled systems offer a potential tool to mitigate these effects by enabling stable conditions for spawning, larval development and early survival.

The protocol builds upon previous experience from the ARGOS project, where experimental rearing systems were established, and aims to further evaluate the feasibility and operational applicability of such systems.

The activity contributes to the BRAVE project by generating structured data on biological performance (e.g. larval survival, growth, reproduction success) under controlled

environmental conditions, supporting the development of sustainable aquaculture practices and potential restoration approaches.

The results will provide a basis for defining practical procedures for controlled reproduction and contribute to the development of climate-resilient and sustainable production models in the Adriatic region.

PP9: 1) Assessing economically interesting species of bivalve molluscs in the area of Zadar county with a special emphasis on protected areas and habitats listed in the Regulation (EU) 2024/1991 of the European Parliament and of the Council on nature restoration.

Given that the Adriatic Sea represents a large unique ecosystem, all information collected in any part of the Adriatic Sea is important for the entire Adriatic Sea. Shellfish exploitation is carried out in certain areas, however, the presence of certain species in the Adriatic Sea far exceeds the fishing area. The inventory of economically important species and the identification of their habitat can be important for determining the areas from which these bivalves can be reintroduced to areas that are of interest for exploitation. Preservation of small populations can thus contribute to general resilience for species that are under fishing pressure or influenced by changes in the Adriatic Sea environment.

Therefore, assessing economically interesting species of bivalve molluscs in Zadar County has two objectives: the first objective is to contribute to the knowledge of the distribution of bivalve molluscs in the Adriatic Sea and to identify possible habitats in which these species can be preserved in the long term, with special emphasis on protected areas, and the second objective is to specifically identify the species covered by Regulation 2024/1991 and the habitats in which these species are found.

2) Feasible study on environmental change effects mitigation on shellfish cultivation in recirculating aquaculture systems and solutions for restoration by reproduction of Mediterranean scallop in captivity.

The Mediterranean scallop is an economically interesting species that prefers colder seas and is therefore potentially threatened by global warming. A complete cultivation cycle, including spawning and the rearing of larvae to juvenile bivalves, has been fully developed for related species, including the closely related great scallop (*Pecten maximus*). However, the successful transfer of acquired knowledge from the cultivation of related species has not yet been documented. Therefore, the cultivation of this species represents a significant

challenge as an important opportunity for aquaculture development but also for the restoration of habitats in which this bivalve could survive in the long term. By developing its breeding in captivity, the prerequisites for successful aquaculture, in terms of food production and ecosystem service, are achieved.

Within the framework of the ARGOS Project, a pilot project entitled "SET UP experimental rearing of species through recirculating technologies" was carried out, through which a RAS (Recirculating Aquaculture System) was designed and tested.

In the present pilot project, the knowledge and experience related to scallop conditioning, maturation, and spawning will be further refined and applied. Equipment enabling larval rearing was also procured under the ARGOS Project; therefore, if successful spawning is achieved, larval rearing experiments will be continued.

Furthermore, through the BRAVE project, equipment for continuous monitoring of key environmental parameters (temperature, dissolved oxygen, pH), as well as equipment to improve bivalve feeding efficiency within the recirculation system, will be procured. This will enable more comprehensive and higher-quality data collection necessary for guiding the future development of Mediterranean scallop spawning.

If successful settlement occurs, initiate experimental grow-out of settled bivalves in a designated farming area, based on a partnership agreement with an authorized aquaculture producer.

PP10: 1) Kaštela Bay is a semi-enclosed marine system unique within the Adriatic Sea due to its ecological and hydrographic characteristics. Historically, this area was exposed to intense industrial pressure (primarily chemical industry and cement industry production). In addition, wastewater from the surrounding area (eight small towns) was discharged directly into the bay. Following the closure of industrial facilities and the redirection of sewage discharge outside the bay, environmental conditions began to recover in the recent period. Today, tourism represents the main economic activity in the area.

Shellfish harvesting in this region has historically been well developed, with exploitation carried out through manual collection. Intensive exploitation and illegal fishing have led to the degradation of shellfish populations in the bay. Therefore, it is of utmost importance to assess the current state of shellfish stocks and provide recommendations for future fisheries management and resource protection measures.

2) Mariculture represents an important and growing sector of the Croatian economy, with numerous fish and shellfish farms located along the coast. Continuous development is associated with both potential and actual impacts on the marine environment. Therefore, this activity is subject to permanent monitoring. Results indicate that, alongside negative effects, certain positive impacts can also be observed in the vicinity of fish cages.

The aim of this project activity is to investigate the impact of marine organism farming on natural fish populations in the immediate vicinity of cages using non-invasive methods (visual census and e-DNA barcoding), and to provide recommendations for future mariculture monitoring programs that should include long-term tracking of changes in fish populations.

PP13: Due to increasingly changing and often extreme environmental conditions, production success of farmed bivalves has been unstable over previous years, with extreme mortality events occurring in summer months. This pilot action focuses on assessing the quantity (biomass) and condition of farmed bivalves at several locations in Mali Ston bay (protected marine reserve and Natura 2000 site) in relation to abiotic environmental parameters over the course of a year.

The objective is to assess the effect of environmental conditions on the fitness of bivalves not just during extreme conditions, but throughout the year as the ability of bivalves to withstand unfavourable conditions might be synergistically collapsed by multiple stressors.

The species observed will be the two main species produced in the area: Mediterranean mussel *Mytilus galloprovincialis* and European flat oyster *Ostrea edulis*.

Existing data

To support the implementation of the protocols described above, it is first necessary to review the environmental datasets and databases already available within the Adriatic context. The following chapter therefore provides an overview of existing data sources relevant to the BRAVE project.

Existing environmental datasets represent an essential foundation for the development and validation of the BRAVE data collection protocols. Reviewing these sources allows the partnership to identify available information, assess its relevance for the project objectives, and determine how it can be integrated into the BRAVE Smart Digital Platform.

Italian projects and databases

VALSCENT - Valorizzazione delle produzioni ittiche della Laguna attraverso analisi Sensoriali siCio-EcoNomice e relativa ricaduta sul Territorio

This project focuses on the valorization of lagoon fish production through socio-economic and sensory analysis. By examining the resulting impacts on the territory, it provides insights into how the production of target fish species can be better managed to align with local socio-economic needs and environmental sustainability.

Experimental Rearing through Recirculating Technologies (RAS) Previous activities within the ARGOS project included the development and testing of recirculating aquaculture systems (RAS) for selected marine species. These tests provided initial insights into system design and species response under controlled environments, forming a basis for future mitigation strategies against environmental stressors.

Croatian projects and database

In Croatia, monitoring of aquaculture impacts on the marine environment is mandatory; however, it primarily focuses on seawater chemistry parameters rather than on biological communities living around fish cages.

Recent research shows that, in addition to physicochemical impacts, mariculture significantly affects fish populations around cages. This is mainly due to the attraction of wild fish to farming areas because of increased food availability, as well as the fact that these

areas are generally excluded from fishing activities. Furthermore, mariculture facilities function as FADs (Fish Aggregating Devices).

Therefore, alongside the Italian datasets, several national data sources in Croatia provide information relevant to the production and health of target species in the project area:

Aquarium Pula Experimental Rearing Practical knowledge has been generated through experimental work on the rearing of mussel larvae (*Mytilus galloprovincialis*) and European lobster (*Homarus gammarus*). These activities provide critical data on larval development, survival conditions, and system performance, which are essential for developing mitigation measures against high mortality rates in early life-stages.

The **IOF** Institute of Oceanography and Fisheries has conducted several studies suggesting measurable impacts of mariculture, and in recent years there has been increasing use of non-invasive research methods.

Scientific Literature and Technical Reports on Target Species

Scientific documentation provides detailed information on the environmental requirements, spawning conditions, and early life-stage dynamics of target species, including oysters and scallops. These resources help identify current gaps in standardized experimental protocols and the integration of experimental results with management data to improve production resilience.

PHARE Project

Limited investigations were conducted within the framework of the PHARE project "Assessment of demersal fish and shellfish stocks commercially exploited in Croatia" during the period 2007–2009.

BEST ADRIA (HRZZ IP-2019-04-1956; Croatian Science Foundation)

Monitoring that includes production quantities and bivalve condition similar to that proposed in this pilot action has only been performed sporadically, with last data originating from 2021 (*Žurga et al., 2024*) as part of the BEST ADRIA research project (HRZZ IP-2019-04-1956; Croatian Science Foundation) and possibly 2010 before that through some projects funded by Dubrovnik Neretva county and the Croatian Ministry of Science, Education and Sports at that time.

Mariculture assessment study in Mali Ston Bay (Pećarević et al., 2020)

A review study was performed by the University of Dubrovnik and funded by Dubrovnik Neretva county in 2020 titled Mariculture assessment study in Mali Ston Bay (*Pećarević et al., 2020*) which, among other things, included an overview of all research related to mariculture performed in the pilot site to date.

Monitoring of oyster larvae concentrations

Also funded by the county, Monitoring of oyster larvae concentrations throughout the year in the pilot area has been performed almost continuously from 2000 to 2024, with the addition of measuring some environmental parameters such as temperature and salinity (*Bolotin and Bratoš Cetinić, 2020; Bolotin, unpublished data*).

Common projects and databases (IT+HR)

Several initiatives operate across both sides of the Adriatic, contributing to the shared knowledge base required for the sustainable management of biological resources and the mitigation of production-related impacts:

ARGOS Project – Marche Region Mussel Farm Survey (Interreg IT-HR)

Socio-economic surveys

Building on the experience of the ARGOS project, an extensive socio-economic and operational survey was conducted for mussel farms across the Marche Region. This previous activity resulted in a comprehensive overview of farm structures and production practices, establishing a strong collaboration network with operators that supports the validation of new harmonized data collection protocols under real-world conditions.

Ecological Carrying Capacity

The ARGOS project serves as a main cross-border reference for assessing the ecological carrying capacity of production areas like Mali Ston Bay. By defining specific sampling stations and parameters, it ensures that aquaculture production remains synergistic with the ecosystem's ability to support it, preventing over-exploitation and habitat degradation.
Standardization of Aquaculture Protocols and Nature Restoration Existing work has defined protocols and collected data on aquaculture at the local level, specifically case studies on shellfish and finfish (Deliverable D4.2.2). This effort is aligned with Regulation (EU) 2024/1991 on nature restoration, which emphasizes the standardization of data as a basis for a shared approach to managing Adriatic biological resources and mitigating the impacts of economic activities on marine health.

BIOBASED - Development support of blue biorefinery in the Adriatic Sea (Interreg IT-HR)

The project BIOBASED (Interreg IT-HR) focused on assessing marine-based integrated multitrophic aquaculture (IMTA) systems at two points in Mali Ston bay. The IMTA is based on cocultivation of bivalves (*M. galloprovincialis*) and macroalgae *(Ulva lactuca*) on floating longline farms. Temperature, salinity, mussel microbiota, suspended particulate matter, chlorophyll and production parameters are followed.

SENSEI (Smart European Networks for Sensing the Environment and Internet quality) – Horizon Europe - HORIZON-RIA - HORIZON Research and Innovation Actions

The European space agency project **SENSEI**, currently in progress, focuses on remote sensing of marine environmental parameters in coastal areas with aquaculture presence and developing suitability maps for native oyster farming, with the pilot location being Bistrina bay - a small part of the larger Mali Ston bay area.

However, significant gaps remain in:

- standardised experimental protocols across species
- comparable datasets on larval survival and reproduction success
- integration of experimental and environmental data
- transferability of RAS systems to operational aquaculture conditions

These limitations highlight the need for harmonised data collection and validation through pilot-scale experimental systems within the BRAVE framework.

New Data

While existing datasets provide an important baseline for understanding environmental conditions in the Adriatic Sea basin, the BRAVE project also generates additional data through pilot actions implemented by project partners. These new datasets complement existing monitoring systems and support the validation of the common data collection protocols developed under Activity 1.1, contributing to the development of the BRAVE Smart Digital Platform.

Pilot Sites: Description

PP4: General activities description *"Pilot testing applied in 1 or 2 aquaculture farms: environmental data collection through the use of multi-parameters smart sensors; biological and productivity data collection through the use of an ad hoc collection data protocol; bioinformatic elaboration of all data collected to correlate environmental changes with the farms productivity".*

The pilot action implemented by PP4 generates new primary data related to unwanted effects affecting mussel farming systems.
This activity capitalises on the experience gained within the ARGOS project, where PP4 conducted an extensive socio-economic and operational survey of mussel farms in the Marche Region, resulting in a consolidated knowledge base and a well-established collaboration network with farm operators.

New data include:

- static information on farm characteristics (e.g. location, depth, typology);
- operator-based observations of environmental conditions;
- biological and production indicators relevant to mussel farming;
- information on mitigation measures adopted by farm operators.

A substantial portion of the data is collected directly by mussel farmers using standardised data collection sheets developed within the project. These datasets are complemented by environmental data acquired through multiparametric probes where available. All data are structured for integration into the BRAVE Smart Digital Platform.

PP7: General activities description *"Assessment of monitoring and mapping biodiversity of economically interesting species of shellfish, mussels, oysters, scallops, large crabs and bivalve species, keeping shellfish and crabs in recirculation to overcome and mitigate the effects of climate*

change and developing technology for spawning economically important shellfish and crabs (Feasibility studies)".

The data collection protocol will be implemented using established scientific methods to determine the presence of bivalve species and to assess the structure of their populations at selected habitats. Data will be collected through a review of previously published sources, direct field investigations, and the incorporation of local fishery knowledge.

Knowledge concerning the occurrence of bivalve species in Zadar County remains fragmented; the currently available data span an extended temporal period and are of limited reliability. This pilot activity will critically assess and validate existing information while generating new, consolidated, and scientifically robust data on the distribution, population structure, and habitat associations of economically important bivalve species in Zadar County.

By integrating these findings with georeferenced habitat data listed under Regulation (EU) 2024/1991, the activity will strengthen the evidence base for the conservation of sensitive habitats and support the identification of potential source populations for the restoration of degraded habitats.

All data are structured for integration into the BRAVE Smart Digital Platform.

PP9: General activities description "1) *Assessment of economically interesting species of bivalve molluscs in the area of Zadar county with a special emphasis on protected areas, and habitats listed in the Regulation (EU) 2024 /1991 and 2) feasibility study on environmental change effects mitigation on shellfish cultivation in recirculating aquaculture systems and solutions for restoration by reproduction of Mediterranean scallop in captivity".*

The environmental data collection protocol will be implemented using equipment to be procured for continuous monitoring of environmental parameters in the RAS system (temperature, dissolved oxygen, pH), as well as equipment (ammonia and nitrite concentrations) operated by an external collaborator responsible for conducting the experiment.

The success of conditioning will be assessed based on biometric measurements and scallop condition index values, while the success of spawning preparation will be evaluated by the relative quantity of fertilized eggs obtained. In the case of successful spawning, the success of larval rearing will be assessed through survival over time.

PP10: General activities description *"1) Assessment of the condition of economically important shellfish in Kaštela Bay, analysing the value for local communities, and identifying critical areas for conservation and sustainable exploitation (in-situ research and stakeholder interviews for preserving biodiversity) and 2) Assessing the impact of mariculture on ichthyofaunal diversity by visual observation and e-DNA barcoding for identification of invasive and non-native species (focus on behavioural patterns changes and physiology of the species)".*

1) At the beginning of the project activities, all available existing data on shellfish in the Kaštela Bay area will be compiled in order to describe historical changes.
   The main data collection will be carried out through interviews with professional and other fishers engaged in shellfish harvesting. Additionally, depending on available opportunities, limited biological sampling in the field is planned. Professional fishers would conduct the sampling, while scientists would perform detailed analyses of the collected biological material.
2) During the research, data will be collected using non-invasive methodologies, including autonomous diving with visual census and seawater analysis through e-DNA barcoding.
   E-DNA barcoding will provide qualitative information on species richness in the area, while quantification will be carried out using visual census methods.

PP13: General activity description *"Identification of interaction of farmed bivalve species with abiotic/biotic environmental conditions to inform operators on current/emerging risk factors. Assessment of bivalve biomass+condition, qualitative and quantitative composition of biofouling organisms with focus on invasive species".*

This pilot action will establish protocols for monitoring of abiotic environmental and production parameters and implement them in the pilot area for the duration of one year. Dedicated monitoring equipment will be procured that will allow continuous measurement of temperature and salinity at multiple stations and depths for the duration of the activity, which is novel for the pilot site. These will be complemented by hand-held probes for pH, chlorophyll and dissolved oxygen taken at the same sites on a monthly schedule. Sea water samples will be taken seasonally for laboratory analysis of nutrients.

Assessment of production quantities / standing stocks will be performed with questionnaires developed for the project and cross referenced national statistics based on reported production quantities. Condition index of bivalves, calculated as dry weight (g) / dry

shell weight (g) × 100 will be analysed on subsamples of mussels and oysters taken from the bay seasonally.

Pilot Sites: Context & Justification

To ensure the operational feasibility of the protocols and the relevance of newly generated datasets, pilot actions are implemented at selected testing locations across the Adriatic basin. The following section describes the context and justification for the selected pilot sites.

PP4

Pilot actions are implemented in at least 3-5 mussel farming sites distributed along the Marche Region coastline.

The selected sites are characterised by:

- different coastal settings where mussel farming is established;
- variability in environmental conditions, including potential influence of freshwater inputs and local hydrodynamics;
- different operational configurations of farming systems;
- exposure to a range of coastal pressures typical of the Adriatic Sea.

These sites are critical for validating the data collection protocol under real-world operational conditions, involving farm operators as data collectors. Testing the protocol across multiple sites with different environmental and operational settings supports the assessment of robustness and adaptability, ensuring that the final data collection framework can function as a reliable transferability tool for other mussel farming contexts across the Adriatic Sea.

PP7

The pilot action is implemented in a controlled experimental environment at Aquarium Pula, which provides the necessary infrastructure for the operation of recirculating aquaculture systems (RAS) and experimental trials on marine species.

The use of a controlled system allows precise regulation of environmental parameters such as temperature, salinity and water quality, enabling the assessment of species response under different conditions.

This approach is particularly relevant in the context of climate change, where increasing variability in natural environments affects spawning success, larval survival and recruitment of marine species.

The selected site allows the validation of data collection protocols under controlled conditions, complementing field-based pilot actions and ensuring that the developed methodologies are applicable both in experimental and real-world aquaculture contexts.

PP9

1) The pilot area comprises the subtidal zone within Zadar County. This area is generally not characterized by extensive, homogeneous habitats; rather, it consists of a mosaic of similar habitat types. For this reason, no large-scale commercial bivalve harvesting activity has developed. Conversely, habitat fragmentation has enabled the persistence of numerous species. The research will focus on habitats in the upper infralittoral zone, as these areas may be subject to considerable pressure from bivalve extraction—possibly more for subsistence purposes than for commercial exploitation. Consequently, despite the limited economic activity, bivalve populations and their habitats may nonetheless be degraded.

   The resulting dataset will contribute to refining current knowledge on the spatial distribution and conservation status of economically important bivalve populations in the Adriatic Sea. By providing structured and comparable ecological indicators, the study will support improved understanding of marine "natural capital" of conservation value, as well as the identification of potential source populations and priority areas for active restoration of both species and habitats.

   The selection of sampling stations for bivalve collection will be one of the outcomes of the preliminary research to be conducted by an external expert. Following the identification of target species and their potential habitats, test areas will be designated for each habitat type in order to evaluate the proposed hypotheses.

2) The pilot project will be carried out at the Shellfish Centre currently under construction or, alternatively, at the site where the RAS equipment for bivalve rearing is presently installed.

PP10

1) Project activities are planned across the entire area of Kaštela Bay, including the Kaštela towns section of the bay, the area around Trogir and coast of island Čiovo, and the mouth of the Jadro River.

Fishers from across the bay will be included in survey research. Biological sampling for further analysis will be conducted in areas with the highest concentration of shellfish.

2) Planned research will be conducted at marine fish and shellfish farms in Split-Dalmatia County.
   Research will be carried out directly around installed farming cages. Reference sites will consist of ecologically similar areas located near mariculture facilities but outside their direct influence.

PP13

Mali Ston bay is a 28 km long mildly eutrophic coastal ecosystem on the south of Croatia. It has ideal conditions for bivalve farming due to the inflow of freshwater and nutrients through numerous underwater springs and the river Neretva, which has a delta close to the mouth of the bay. It was declared a special marine reserve in 1986 for the purpose of oyster farming and is also a Natura 2000 site. It is often divided into three main production zones which are roughly equivalent to an interior, middle and outer part of the bay, each with differing environmental conditions. In fact, the very heterogeneous nature of the bay's environmental characteristics require the utilization of several sampling stations when performing any serious monitoring activity, the exact number depending on the observed parameter.

Bivalve farming in Mali Ston Bay is a vital branch of the local economy, a unique gastronomic tourist attraction, and a centuries-old tradition of this region. The most famous shellfish cultivated here is the European flat oyster, *Ostrea edulis*, which is designated with the European Protected Designation of Origin (PDO) under the name "Malostonska kamenica" (Mali Ston Oyster) the only shellfish in Europe to hold this status. Furthermore, more than 85% of the total Mediterranean aquaculture production of this species takes place in Mali Ston Bay. In addition to the oyster, however, the bay also accounts for the largest production of the Mediterranean mussel, *Mytilus galloprovincialis*, in Croatia.

This pilot site has been selected due to its economic, environmental and cultural importance for Dubrovnik neretva county i.e. numerous ecosystem services, but also its protection status and extensive historic study. The need to study the pilot area more extensively has been highlighted by mass mortalities of farmed bivalves recorded during 2024., as was the case in various parts of the Mediterranean Sea in previous years. In order for the county to engage in knowledge-based management of the aquaculture industry in the area, it was realised that dedicated monitoring activities were essential. The BRAVE

project will allow experts to perform much-needed "one-off" studies and monitoring activities, which will inform short-term management decisions and the content of future monitoring programs.

Pilot Actions: Execution & Tech Stack

Following the description of the testing locations, the next section outlines how pilot actions are implemented in practice, including operational procedures, technological tools and data structures used for the collection and processing of information.

PP4

Operations: The pilot action focuses on the collection of farm-level static data, operator-based monitoring of environmental and biological conditions, identification of unwanted effects affecting production, and documentation of mitigation actions and their perceived effectiveness. This activity supports the validation of the data collection standard defined under Deliverable D.1.1.7.

Technology:

- Standardised spreadsheet-based data collection tools for mussel farm operators;
- Multiparametric probes for environmental monitoring (e.g. temperature, oxygen, salinity), where available;
- Harmonised data formats for Smart Digital Platform integration.

Data profiles:

- Environmental context data (operator-based and sensor-based);
- Biological and production indicators;
- Mitigation-related information;
- Metadata supporting interoperability and transferability.

PP7

Operations: The pilot action focuses on the experimental assessment of controlled reproduction and early life-stage development of selected marine species under recirculating aquaculture systems.

The activity includes:

- preparation and testing of the recirculation system
- cultivation of algae for feeding purposes using a bioreactor
- conditioning and spawning of target species
- monitoring of larval development and survival under controlled conditions

The objective is to evaluate the feasibility of controlled reproduction as a tool to mitigate environmental constraints affecting natural populations.

Technology: The pilot relies on:

- recirculating aquaculture systems (RAS)
- algal bioreactor for feed production
- monitoring equipment for environmental parameters (temperature, salinity, water quality)
- laboratory analysis and microscopic observation for larval stages

The technological setup prioritises controlled, replicable and scalable experimental conditions.

Data Profiles: Collected data include:

- biological parameters (larval survival, growth, reproduction success)
- environmental conditions within the system (temperature, salinity, water quality)
- experimental performance indicators

The data are structured to support feasibility assessment and integration into the BRAVE Smart Digital Platform, enabling comparison with other pilot actions and supporting the development of sustainable aquaculture procedures.

PP9

First Pilot action:

Operations: The research will primarily address bivalve species occurrence and population condition. Population status will be evaluated through analysis of size–frequency distributions, enabling assessment of demographic structure, and potential pressure. In habitats characterized by higher population aggregation, quantitative estimates of population density (number of specimens or biomass per square meter) will also be conducted using standardized sampling methodologies.

Data will be collected in four phases.

1) Economically important bivalve species in the Adriatic Sea will be identified, and existing (published) data on these species and their distribution in Zadar County will be reviewed.
2) Local fishery knowledge will be gathered, along with available data on the extraction of specific bivalve species in Croatia and Zadar County.
3) Georeferenced data collection on habitats and “fishing grounds” where the presence of the target bivalve species is assumed or expected.

4) Bivalve sampling will be conducted at selected locations to determine the presence of the target species and to assess the structure of their populations.

Technology: Data will be collected through the analysis of local fishery knowledge, field observations, measurements of organism size (length and weight), number of specimens or biomass per square meter and statistical processing of field data.

Data Profiles: Metadata on target bivalve species (presence, density) and sensitive habitats, Field data derived from conducted observations and measurements – number of species, species abundance, size structure (length and weight), and population density (number of individuals or biomass per square meter).

Second Pilot action:

Operations: Experiments will be conducted in circular tanks in three replicates for each experimental group. The pilot activity will be carried out in four steps:

1) Start up the recirculating system and technically test its operation.
2) Phytoplankton cultivation in a reactor and concentration of algae for scallop feeding.
3) Scallops conditioning and spawning induction.
4) Optional: If spawning is successful, larval rearing will be undertaken until metamorphosis and the formation of spat.

Technology: Environmental data will be collected using equipment for continuous monitoring of temperature, dissolved oxygen, and pH, as well as by water sampling to determine ammonia and nitrite concentrations. The results of scallop conditioning will be assessed through biometric measurements and calculation of the condition index. Optionally, the success of spawning will be evaluated by sampling the rearing medium to estimate the relative quantity of fertilized eggs or the number of larvae during their development. The number of eggs/larvae will be determined by counting under a microscope.

Data Profiles: The measurement units will be standardized through AAC coordination for their entry into the Smart Digital Platform.

PP10

First Pilot action:

Operations: Project activities will begin with the collection of existing information on the condition of the marine environment and shellfish populations in Kaštela Bay. Special attention will be given to describing trends in anthropogenic impacts on the bay resulting from industrial and municipal pollution. All available information on historical exploitation levels and biological descriptions of shellfish populations will also be reviewed.

Technology: The main part of data collection will consist of surveys conducted among professional and other shellfish harvesters. Key survey topics will include:

- Methods of shellfish harvesting
- Catch composition
- Long-term trends and changes in catches
- Social and economic aspects of fisheries
- Additional limited field sampling is planned to better describe the composition of shellfish communities in Kaštela Bay and their population structure.

Data Profiles: All collected information will serve to assess the status of shellfish populations and provide recommendations for future management.

Second Pilot action:

Operations: The impact of mariculture on the marine environment is typically monitored through changes in seawater chemistry and the accumulation of uneaten feed and fish waste on marine sediments. Fish population studies were previously conducted using various fishing gears to collect biological samples—most commonly using gillnets.

The objective of this project is to develop and promote the use of non-invasive research methods that do not damage marine organisms or habitats.

Additionally, the research will seek to determine:

1. The presence of escapees from aquaculture facilities in the natural marine environment
2. The occurrence of invasive and non-native species

Technology: Species detection will be carried out through seawater analysis using e-DNA barcoding, while quantification will be conducted via visual census performed by trained scientific divers.

Data Profiles: Data collected at mariculture sites will be compared with data from reference stations to assess changes resulting from mariculture activities. Based on the obtained results, recommendations will be provided for incorporating fish population assessments into future monitoring programs of mariculture impacts on the marine environment.

PP13

Operations: The pilot action will rely on external expertise for monitoring of environmental and production parameters at several sampling points within the bay, based on spatiotemporal distribution of sampling points presented in one of the deliverables of the ARGOS project.

Technology: Dedicated monitoring equipment will be procured (data loggers) that will allow continuous measurement of temperature and salinity at multiple stations and depths for the duration of the activity. These will be complemented by hand-held probes for pH and dissolved oxygen taken at the same sites on a monthly schedule. Sea water samples will be taken seasonally for laboratory analysis of nutrients (spectrophotometry) and chlorophyll a (UPLC).

Data Profiles: Assessment of production quantities / standing stocks will be performed with questionnaires developed for the project and cross referenced national statistics based on reported production quantities. Condition index of bivalves, calculated as dry weight (g) / dry shell weight (g) × 100 will be analysed on subsamples of mussels and oysters taken from the bay seasonally.

Data Checklist

In order to ensure consistency across pilot actions and facilitate data integration within the BRAVE Smart Digital Platform, a common set of minimum data requirements has been defined. The following section presents the data checklist applicable to the different protocols.

Defined set of minimum data requirements:

PP4

Minimum data requirements are organised into the following macro-themes:

1. Farm and production context
   - farm identification and location
   - farm typology
   - production unit (optional identifier)
2. Environmental conditions
   - water stratification
   - visual turbidity
   - freshwater inputs
   - occurrence of extreme events
   - environmental parameters from multiparametric probes (where available)
3. Biological and production indicators
   - general mussel condition
   - growth performance
   - mortality level
   - biofouling level
   - presence of invasive or competing species
4. Mitigation measures
   - type of mitigation action
   - timing of intervention
   - perceived effectiveness

Optional data include photo documentation and operator notes.

The checklist can be refined and validated in collaboration with AAC members and stakeholders to ensure that the datasets effectively support decision-making processes and platform functionalities.

PP7

The data checklist focuses on the minimum dataset required to assess controlled reproduction and experimental performance in recirculation systems. Minimum data requirements include:

1. Experimental setup
   - system configuration (RAS characteristics)
   - species under study
   - experimental conditions
2. Environmental parameters
   - Temperature
   - Salinity
   - water quality indicators (e.g. oxygen, pH if available)
3. Biological indicators
   - larval survival rates
   - growth indicators
   - reproductive success (e.g. spawning, fertilisation)
4. Experimental performance
   - system stability
   - observed constraints and limitations

Optional data include photo documentation and laboratory observations.

The dataset will be refined in collaboration with AAC members to ensure compatibility with the Smart Digital Platform.

PP9

First Pilot action:

The data requirements are based on standardized methodologies used to assess the status of bivalve populations in natural habitats and will be adapted for integration into the Smart Digital Platform.

Georeferenced delineation of the marine area of Zadar County. Identification of economically important bivalve species. Identification of sensitive marine habitats where the presence of target bivalve species is expected. Assessment of representative sampling locations to verify their presence, abundance, and population structure. Sampling of bivalves and in situ and laboratory measurements (abundance, length, weight).

Data requirements are organized as follows:

1. Georeferenced delineation of the marine area of Zadar County.
2. Identification of economically important bivalve species.
3. Identification of sensitive marine habitats where the presence of target bivalve species is expected.
4. Assessment of representative sampling locations to verify their presence, abundance, and population structure.
5. Sampling of bivalves and in situ and laboratory measurements (abundance, length, weight).

Optional data include photo documentation and operator notes.

The checklist can be refined and validated in collaboration with AAC members to ensure that the datasets effectively support decision-making processes and platform functionalities.

Second Pilot action:

Assessment of the biological parameters of the experiment (length, weight, condition index) will be performed on at least ten scallops per replicate. The number of fertilized eggs will be estimated for each individual that releases eggs following induction.

Abiotic environmental parameters in recirculating aquaculture systems will be measured using standard methods, including continuous monitoring of temperature, salinity, pH, and dissolved oxygen with digital instruments. Ammonium (NH_4^+) concentrations will be determined from collected samples by subsequent laboratory analysis.

The data requirements are based on standardized methodologies and will be adapted for integration into the Smart Digital Platform.

Optional data include photo documentation and operator notes. The checklist can be refined and validated in collaboration with AAC members to ensure that the datasets effectively support decision-making processes and platform functionalities

PP10

First Pilot action:

Survey questionnaires will include the collection of the following data:

- Fisherman data:
  - Age
  - Gender
  - Education level

    - Primary occupation
- Fishing data:
    - Type of fishing activity
    - Fishing gear used
    - Exploitation area
    - Annual work dynamics
- Catch data:
    - Qualitative and quantitative composition of catches
    - Special focus on invasive and newly appearing species
    - Long-term changes in catches:
    - Changes in quantity and composition
    - Changes in fishing season
    - Occurrence of new species
- Socio-economic aspects:
    - Value of catch
    - Share of fishing in total income
    - Importance of fisheries for the fisher and the local community

Limited field research will be conducted to describe shellfish population density in the study area and the biological characteristics of collected specimens. This will include:

- Species identification
- Qualitative and quantitative composition
- Shell dimensions
- Condition index
- Gonadosomatic index
- Sex
- Gonad maturity stage
- Shell age (if possible)

Based on the collected data, the current status of shellfish populations in the bay will be described, and recommendations for future exploitation and conservation measures will be provided. Special attention will be given to defining critical areas for harvesting and protection.

Second Pilot action:

Second Pilot action:

E-DNA barcoding will be used for qualitative descriptions of fish communities around farming cages, with special attention given to:

- Invasive species
- Non-native species
- Escapees from cages

Visual census will provide quantitative data, including:

- Species abundance within a defined area
- Abundance of individual specimens
- Fish size
- Information on benthic habitats and benthic flora and fauna
- Type of marine sediment

PP13

Standardized monitoring equipment and procedures will be used to collect abiotic environmental data - depending on the parameter, data loggers (temperature, salinity), hand-held probes (pH, dissolved oxygen) and or sample collection with subsequent laboratory analysis will be used (NH4+, NO2-, NO3-, PO4-, chlorophyll a). This will be the same environmental data described in D.1.1.3.

Conditions of oysters and mussels will be calculated from samples from several farms within the pilot area. For bivalve condition index calculations, dry tissue weight and dry shell weight will be used, according to standard protocols.

A more detailed estimation of standing stocks (biomass of farmed bivalves) and production quantities of farmed bivalves will attempt to be obtained directly from farmers through questionnaires, but will also be cross-checked with official production numbers. Depending on the quality of obtained data, a decision will be made to integrate one or both datasets within the Smart digital Platform.

Data Standardisation

The collected data will be standardized and integrated into the Smart Digital Platform, facilitating a harmonized and comprehensive approach to analyzing pilot project results and supporting the evaluation of outcomes across the transboundary level. This approach will enhance comparability, ensure consistency, and provide a robust basis for evidence-based decision-making.

Considering the pilot character of the activity and the heterogeneity of data sources derived from different protocols, variations in the level of detail and completeness of the standardised datasets among partners are expected. Accordingly, the standardisation framework establishes a common reference structure while retaining sufficient flexibility to accommodate differences in implementation.

Harmonised Data Categories

The assessment of economically important bivalve mollusc species across different areas (PP9,PP10) of the Adriatic Sea generates a *georeferenced dataset on species composition and population parameters (including abundance, density, and size–frequency distribution)***,** as well as their ecological significance for habitat restoration in accordance with Regulation (EU) 2024/1991, providing a basis for evaluating their contribution to the recovery of habitats where these species have been irreversibly reduced due to various anthropogenic and ecological pressures. Data will be collected through a combination of *literature review, analysis of catch records, incorporation of local ecological knowledge, and targeted field sampling.*

Datasets will be collected using various methods, including *literature review, analysis of catch records, incorporation of local ecological knowledge, and targeted field sampling.* The processing of the collected data will result in *structured output datasets.*

Recirculating aquaculture systems (RAS) constitute a key strategy to mitigate the impact of adverse environmental conditions on marine species. Comprehensive management of the entire production cycle within RAS enhances the capacity for evidence-based interventions, supporting species recovery, promoting reproductive success, and contributing to the sustainable conservation and management of aquatic biodiversity in line with EU environmental objectives. Two pilot projects (PP7, PP9) focus on the development of rearing technologies in recirculating aquaculture systems (RAS) for various shellfish and crab species. By enabling *precise control over abiotic factors (temperature, salinity, pH, dissolved*

oxygen, ammonium (NH_4^+), and key biotic factors, including (*stock density, organism size, condition index, daily feeding ration, and feed composition*), these systems allow the circumvention of seasonal and temporal limitations that constrain natural growth and reproduction. (Optional) In cases of successful spawning, detailed *descriptive and quantitative measurements* will be performed to assess *embryonic and larval development, including developmental stage progression, abundance, density, and survival.* Through collaboration within the AAC, the data structure will be harmonized to ensure consistent collection at the project level and integration into the Smart Digital Platform.

The development of marine aquaculture encompasses economic, social, ecological, spatial, and technological dimensions. Farming activities influence both cultured organisms and their environment, with fish farming potentially contributing to eutrophication and shellfish aquaculture relying on the productivity of the surrounding ecosystem. Cultured species are sensitive to a range of abiotic and biotic stressors, and the introduction of non-indigenous species further complicates these interactions. Collecting data from individual farming sites is therefore essential for understanding ecosystem-level dynamics and supporting evidence-based, sustainable aquaculture management in the Adriatic Sea. Three pilot projects (PP4, PP10, PP13) will systematically investigate the interactions between cultured organisms and the surrounding abiotic conditions and biotic communities. These studies aim to elucidate how environmental factors and ecological interactions influence growth, survival, and overall ecosystem dynamics within aquaculture sites.

Data on the *presence of invasive species, non-native species, and escapees from cages* will be collected from *georeferenced farming sites* in Split-Dalmatia County using visual censuses and eDNA barcoding. These observations will be complemented by *assessments of species abundance within defined areas, abundance of individual specimens, and fish size,* alongside *information on benthic habitats, benthic flora and fauna, and marine sediment type.*

Testing the operator- and sensor-based data collection protocol (PP4) across multiple mussel farming sites. *Spatially georeferenced data on farm location, aquaculture technology, abiotic parameters (e.g., water stratification, turbidity, weather conditions, temperature, dissolved oxygen), and biotic indicators (e.g., condition, growth, fouling, presence of invasive species, mortality)* will be collected to generate comprehensive datasets that inform farming practices and support the development of mitigation strategies addressing a range of anthropogenic and environmental stressors. A similar data collection protocol will be implemented and tested under contrasting environmental conditions in the semi-enclosed Mali Ston Bay



(PP13). This pilot addresses the impacts of climate change. It focuses on *assessing the biomass and condition of farmed bivalves* at multiple locations across the bay over a one-year period. *Abiotic environmental data will be collected using data loggers (temperature, salinity), hand-held probes (pH, dissolved oxygen), and water sampling followed by laboratory analyses (NH_4^+, NO_2^-, NO_3^-, PO_4^{3-}, chlorophyll)*. These data will be combined with biological indicators, including the *bivalves condition index.*

Production data will be obtained directly from farmers *through questionnaires and cross-checked with official production records.* The integrated dataset will be used to assess the effects of environmental conditions on bivalve fitness. The analysis will consider both extreme events and seasonal variability. Particular attention will be given to the cumulative and synergistic effects of multiple stressors, which may reduce the resilience of bivalves to unfavorable conditions. The established and validated dataset protocols will provide a reliable tool for transferring methodologies to other mussel aquaculture contexts across the Adriatic Sea. All data will be collected using standardized methods and formatted for integration into the Smart Digital Platform, producing a comprehensive dataset on interactions applicable to larger-scale aquaculture sites across the Adriatic Sea.

Conclusions

The Common data collection standard for the evaluation of unwanted effects of target fish and mollusc species production in the project area with focus on mitigation addresses biological production systems in the Adriatic Sea that are currently exploited but are not directly regulated by European legislation and/or relevant international agreements (e.g., GFCM, ICAT). The natural production potential of marine organisms in the Adriatic Sea is utilized not only through capture fisheries, but also through the collection of spat originating from the natural spawning of shellfish. This practice relies on the maintenance of favourable ecological conditions and the good status of natural populations of the exploited species.

The exploitation of marine species has important implications for biodiversity; therefore, both productivity and ecological aspects must be taken into account in the assessment of these activities. In parallel, food production through aquaculture systems based on the full control of the production cycle is continuously increasing. These activities generate anthropogenic marine habitats, within which it is necessary to establish and apply acceptability standards based on systematic data collection and monitoring. Such standards are essential for assessing environmental suitability, supporting sustainable management practices, and ensuring the long-term viability of marine biological production systems in the Adriatic Sea.

In recent years, the Adriatic marine environment has been increasingly exposed to the impacts of climate change, which complicates the interpretation of conclusions drawn from data collected in previous periods. In response to these challenges, it is necessary to further develop and refine the Data Collection Protocols to enable a more accurate assessment of the vulnerabilities and strengths of exploited species and their habitats, with particular emphasis on activities aimed at addressing and mitigating the identified risks.

The collected data will be standardized and integrated into the Smart Digital Platform, facilitating a harmonized and comprehensive approach to analyzing pilot project results and supporting the evaluation of outcomes across the transboundary level. This approach will enhance comparability, ensure consistency, and provide a robust basis for evidence-based decision-making.

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ANNEXES

- ANNEX 1 – Data collection Tables – standard 5