

Technical update report

subject: TECHNICAL UPDATE ON *DELIVERABLE* N. 1 – MONITORING PROTOCOLS FOR MACROCATEGORIES

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activities: Technical report: monitoring protocol for macrocategories and examples of sampling plans on target species for evaluating the biodiversity threats driven by alien species, with relative action plan, inside BEST project - *Addressing joint Agro- and Aqua-Biodiversity pressures Enhancing SuSTainable Rural Development.*

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Premise

The purpose of this technical report is to define multiple synthetic monitoring plans divided by macrocategories, describing both their generic and specific goals and their application. For each macrocategory the main monitoring techniques are defined, through the description of methods, parameters, timing and estimation of costs (materials and human resources). The 3rd section contains an example of a species-specific management plan, necessary for the following steps of the Project, and in the Appendix some field forms are given, relatively to some of the monitoring techniques described in the present document.

Section 1: Introduction

Protection of biodiversity requires a constant effort from a technological and scientific point of view, in respect to the European directives and guidelines. Many factors represent a threat to this resource, including the so-called IAS (Invasive Alien Species), which constitute one of the main global causes of biodiversity loss, with negative effects on ecosystems, health and economy (estimating a loss of 12 billion euros each year in UE only).

On February 14th, 2018, the D.Lgs. 230/2017 has been adopted in Italy, in order to incorporate the UE Regulation No 1143/2014, concerning introduction and diffusion of IAS. This decree gives the Regions, the Autonomous Provinces and the National Parks a primary role in monitoring and managing these issues, through specific action plans and restoration of damaged ecosystems.

Monitoring represents a key tool to reach these goals, by individuating the main parameters necessary to determine the status of a species, such as presence/absence, distribution, abundance, and future trends. Data must be collected using standardized methodologies, in order to be repeatable and reproducible through time. Nevertheless, given the extreme biodiversity of IAS, no single monitoring protocol can be produced to examine all the variables, hence the necessity to produce different specific goals and to individuate different macrocategories.

Section 2: Definition of monitoring actions

1.1 Individuation of macrocategories

As aforementioned, categorizing the species into broader macrocategories allows to detect periodical ecological patterns and to define common action plans directed to multiple species. Two criteria have been considered for the production of these categories: taxonomy and ecology.

Taxonomy is a widely used criterion in conservation biology and faunal management plans, including the production of monitoring protocols. The main advantage is the ease of identification the target species, derived from a common ancestor and thus sharing some mutual physiological and behavioural features. Nevertheless, dividing the species according to this criterion represent two main risks. Firstly, some phylogenetically distant species which shares some common ecological features may not be grouped together, while they could be targeted with analogous protocols (such as net fishing for fishes and crustaceans); similarly, some closely related species could have faced different ecological pressures and evolved very dissimilar habits, for which the same monitoring protocols cannot be applied.

On the contrary, ecological features may represent valid criteria to identify some common patterns among all the target species and to adopt some generic monitoring protocols. It is obvious that an overlap between ecological and taxonomical is inevitable, but the latter has not been used as a priority criterion.

Here follows the proposed macrocategories:

- *category #1*: TERRESTRIAL PLANTS
- *category #2*: FRESHWATER AQUATIC PLANTS
- *category #3*: MARINE PLANTS
- *category #4*: TERRESTRIAL ANIMALS
 - *subcategory #4.1*: LOW-MOTILITY ANIMALS
 - *subcategory #4.2*: HIGH-MOTILITY ANIMALS
- *category #5*: FRESHWATER ANIMALS
- *category #6*: MARINE ANIMALS

1.2 Monitoring actions

The previous ecological macrocategories are hereby listed, together with a brief description of the main characteristics and the related monitoring actions.

1.2.1 Category #1: TERRESTRIAL PLANTS

Italy hosts a great plant diversity, thanks to its peculiar climatic, geomorphological, and biogeographical conditions which makes it a biodiversity hotspot, together with the Mediterranean basin. Some specific examples are represented by Tyrrhenian islands, Ligurian and Maritime alps, which hosts many endemic species as well. Direct anthropic factors represent a great threat to this rich natural resource, and introduction of invasive alien species contributes heavily to this negative picture.

On the mainland, terrestrial plants are represented by those species which complete their lifecycle on drylands, such as agricultural fields, pastures, forests, woodlands, including areas close to rivers, or urban areas, and include trees, shrubs, herbaceous plants, climbing species, and generically all those species which requires a good amount of solar radiation and soil and which are not completely or partially submerged.

In this category, IAS are mostly distributed among deeply stressed habitats (characterized by intense or less intense anthropic activities), as well as woodland and riparian habitats. A major part of the species has been detected in urban areas, followed by industrial areas and communication pathways (mainly roads), where these species have higher capacities of adaptation in terms of growth and diffusion, when compared to native species.

2.2.1.1 BRAUN-BLANQUET PHYTOSOCIOLOGICAL METHOD

Description: the phytosociological method (Braun-Blanquet, 1928), allows to recognize the types of vegetation based on their floristic, structural, ecological and dynamic characteristics. It consists in carrying out, in a chosen area of homogeneous botanical characteristics, based on physiographic and landscape criteria. A floristic survey, which is the list of all the entities present within this chosen area, is made. This is realized by drawing up a floristic list for each height range completed with the relative abundance of each species. Subsequently (synthetic phase) the various surveys are compared and the syntaxonomic processing is performed, leading to the definition of the botanical typologies through the floristic, ecological, and statistical comparison of the surveys carried out. In other cases, the collected data can be further processed (e.g. through cluster analysis or other statistical methods).

Variables to be detected: minimum area (varies according to the type of vegetation to be monitored), environmental type of reference, identification of the species, relative coverage for the different layers.

Time scale: seasonal, depending on the fluctuations of plant species throughout the year.

Detection frequency: since the method has a seasonal time scale, the maximum resolution applicable is four times a year, corresponding to the four seasons of the normal vegetative cycle.

Staff required: the use of one plant technician for each analysis station is sufficient, although the use of two technicians is recommended. It is necessary to provide for the use of at least one hour for each sampling station, in addition to the time required for returning the data relating to the synthetic phase.

Equipment:

- field equipment:
 - GPS positioner

- digital/paper support, pencil
- laboratory equipment:
 - stereoscopic microscope

Costs: medium-low. The costs, once the laboratory equipment useful for the microscopic recognition of the samples has been purchased, are related to the staff involved and the execution of each sampling campaign.

Goals: the goal of monitoring is the characterization of the entirely set of plant species that make up the population of a certain study area, both in qualitative terms (floristic lists) and in its three-dimensional structure, with consequent definition of the type of habitat and/or of the phytosociological reference association. About the study of allochthonous species, the method can be used both to obtain qualitative information, on the presence/absence of certain target species from the reference areas (through the preparation of floristic lists), and, above all, quantitative information, regarding the degree of entry of certain alien species into the populations examined, with an estimate of the relative coverage and evaluation of the effects on the phytosociological association reference (e.g. the presence of the same alien species in a given area can have different values on the basis of the relative percentages of coverage and/or any alteration of the syntaxonomy of the found population).

2.2.1.2 BOTANICAL TRANSECT

Description: the method of botanical transect is used to study in detail a certain vegetal association or to evaluate floristic and structural variations between different types along an ecological gradient. Is performed following a predetermined linear path ("transect") through the vegetated environment in characterization predicate, and describing the different species encountered in the surroundings. The inspection allows to detect the species present and to assign to each species an estimated value of abundance. Repeating the measurements over time can provide information on the dynamics of vegetation.

Variables to be detected: reference environmental typology, identification of species, estimates of percentage coverage relative to the area covered by the detection transect.

Time scale: seasonal, depending on the fluctuations of plant species throughout the year.

Detection frequency: since the method has a seasonal time scale, the maximum resolution applicable to the same is four times a year, corresponding to the four seasons of the normal vegetative cycle.

Staff required: is required only one technician for each test station. Is needed at least one hour for each sampling station, in addition to the time needed for the restitution of summary phase data (more rapid than the previous Braun-Blanquet method, since only the floristic list of the beaten transect and the estimation of the relative percentages of coverage are provided, without proceeding to the phyto-sociological characterization of the stand).

Equipment:

- Field equipment:
 - GPS positioner

- digital/paper support, pencil
- laboratory equipment:
 - stereoscopic microscope

Costs: low. the costs, once the laboratory equipment used for the microscopic recognition of the samples collected has been purchased, shall relate only to the personnel involved and to the execution of each sampling campaign. The costs are lower than those budgeted for the previous Braun-Blanquet method, since only the floristic list of the chosen transect and the estimated percentage of coverage are provided, without carrying out the phyto-morphological characterisation of the stand.

Goals: the goal of the monitoring is the characterization of the set of plant species that constitutes the population of a given study area, especially in qualitative (floristic lists) and semi-quantitative terms (estimates of percentages of coverage). Unlike the Braun-Blanquet method, the reference phyto-sociological association is not defined, to the advantage of the method's rapidity. As part of the study of allochthonous species, the method can be used to quickly obtain qualitative information about the presence/absence of certain target species from the target ranges (by drawing up the lists of flowers), in addition to having an indicative estimate on their rate of ingression (particularly useful in case of incipient colonization and/ or recrudescence post-containment interventions).

2.2.1.3 REMOTE SENSING

Description: the remote sensing method is used to study the overall distribution of a given plant combination or to assess the actual presence and spread of a plant species on the detected territory. It is performed by using an aircraft (usually a remote-controlled drone) equipped with multispectral sensors, capable of detecting the spectral signature of a particular object (in this case, a certain plant species), which is flown over the vegetated environment in predicate characterization, thus allowing both the detection of the spectral signature of the different species during the flight and the acquisition of images useful for subsequent processing. An essential prerequisite is the acquisition of the spectral signature for each species, which should be previously characterised by appropriate sampling and laboratory analysis. The area inspection shall make it possible to detect, during the flight phase, the presence and extent of all species of interest. Repeating the measurements over time can provide information on the dynamics of vegetation. This information should then be processed using appropriate software (e.g., GIS).

Variables to be detected: spectral signature of the species, atmospheric conditions for monitoring, assessment of the accuracy of remote sensing.

Time scale: seasonal, depending on the fluctuations of plant species throughout the year.

Detection frequency: since the method has a seasonal time scale, the maximum resolution applicable to the same is four times a year, corresponding to the four seasons of the normal vegetative cycle.

Staff required: only one technician is needed, supported by staff with remote control aircraft pilot licence. Prevision should be made for the use of sampling and spectral signature time of the species of interest, drone

preparation and overflight of the study area, in addition to the time required for the restitution of data related to the analysis phase using the computer.

Equipment:

- field equipment:
 - identification guides
 - software for data entry and analysis
- remote sensing equipment:
 - remote sensing drones (camera, multispectral sensors)

Costs: medium to high. The costs, once purchased or rented the equipment necessary for remote sensing, will be related both to the personnel involved and the execution of sampling, both to the application of the software for the subsequent identification of the distribution areas (in particular, the spectral signature characterisation phase for the target species, which is necessarily time and resource-consuming). The higher costs will therefore be attributable to the equipment needed to carry out the methodology.

Goals: the goal of the monitoring is the multispectral characterization of the distribution of target plant species which form the stand of a given study area, especially in quantitative terms (estimates of percentages of coverage by one or more target species following processing with appropriate software). About the study of alien species, the method is particularly useful, despite the greater economic effort required, in the quantification of the areas occupied by plant species with rapid diffusion and strong colonizing capacity (e.g. invasive succulent plants capable of colonizing open land or occupied by garrigue/low Mediterranean maquis), and/or capable of rapidly forming single-specific stands in the enlarged areas (e.g. invasive species of tall stems, such as *Ailanthus altissima* and *Robinia pseudoacacia*, in disturbed tree and shrub stands). This method can be used to monitor the progress of any containment interventions and assess their ex-post effectiveness. The method can also be used to quickly obtain qualitative information about the presence/absence of certain target species (by detecting appropriately defined species-specific spectral signatures) in reference areas with access difficulties for a detector (e.g., environmental contexts characterised by particular acclivity, situations of gullies, ravines or blades, small island contexts), in addition to having an indicative estimate of the ingression rate of the same. In addition, in this case, the method is particularly useful in case of incipient colonization and/or assessment of any resurgence of post-containment treatments.

2.2.1.4 PREDICTIVE MODELS

Description: predictive models are based on algorithms related to the interpretation of population dynamics data of the species under investigation, whose regularities and trends are identified. The methodology consists in using presence and distribution data (both from previously collected data or new ones) of the surveyed target species, and, using statistical and computer models, predicting its future distribution. A first phase of data collection should be envisaged (search for ad hoc bibliographical references, distribution data, etc.). An integration of the database with more recent data (by carrying out, if necessary, further specific sampling in the area to be monitored, for which the most appropriate methodology can be used among those set out

above), and, finally, a phase of statistical analysis useful to predict how the species being studied will spread, eventually transposing the results obtained into a digital distribution model (e.g. through GIS software).

Variables to be detected: data on the population ecology of the species in question (distribution, ecology, etc.), available from existing bibliographic information or processed ad hoc through specific monitoring campaigns.

Time scale: the data series under examination must cover significant time intervals for the elaboration of predictive hypotheses sufficiently robust, therefore the time scale for this type of analysis is necessarily very high (e.g. for plant species, intervals of years or decades depending on the growth and expansion dynamics of the different species).

Detection frequency: any sampling to support the construction of the model must be carried out in accordance with the method chosen, for which reference is made to the previous descriptions.

Staff required: only one technician is required, who knows how to use predictive models and cartographic processing. Prevision should be made for the use of sampling time (if recent data are missing) and for the collection of bibliographic data, as well as for the statistical processing of data and their transposition into GIS software.

Equipment:

- work materials:
 - identification guides (for additional sampling)
 - dedicated software for the insertion and statistical and distributive analysis of data

Costs: not *a priori* measurable. The costs consist mainly in the hours/man to be used by trained personnel in the search for previous bibliographical data, in the costs of any additional sampling, and in the necessary resources for the statistical processing of the data. A sufficiently complete starting database allows the elaboration of relatively robust predictive models with limited use of resources, while an inadequate data set requires the implementation of information by ad hoc sampling, resulting in higher costs.

Goals: the goal of the monitoring is predicting and monitoring the distribution of certain plant species within a study area. As part of the study of alien species, the method may be used to obtain qualitative information on potential areas which could be reached and/or colonised in the future by the concerned species, so as to have an indicative estimate on the rate of ingression of the same (particularly useful in case of incipient colonization).

2.2.1.5 CITIZEN SCIENCE PROJECTS

Description: citizen Science projects, of which there are many variants, are generally defined as that complex of activities linked to specific scientific research in which simple citizens participate: the definition therefore brings together a series of actions that are also very different from each other, united by the fact that the participation of citizenship in activities related to science. The contribution of the population in the active surveillance on the territory can be a crucial resource in the signalling of target species, being able to allow both the early identification as well as a constant and capillary monitoring on the territory. In the case of

citizen science environmental monitoring activities, they must invariably provide for a first phase of active training of participants in the morphological recognition of the target species and, where appropriate, the use of simple tools necessary to detect and report the presence and distribution of the target species (e.g. field binoculars, GPS tools, basic computer skills to allow the entry of data collected in free access online databases). At a later stage, the reports will be fed into a free access database, where experienced personnel will validate them.

Variables to be detected: citizen Science environmental monitoring projects are surveyed by the target species, often in terms of presence/absence (in particular for plant species).

Time scale: seasonal, depending on the fluctuations of plant species throughout the year.

Detection frequency: since the method has a seasonal time scale, the maximum resolution applicable to the same is four times a year, corresponding to the four seasons of the normal vegetative cycle. The period during which the survey sessions are carried out is determined by the project timing (two days, one month, year-round, etc.).

Staff required: only one technician is required, who can validate the photographs of the species reported by the project participants.

Equipment:

- materials for work:
 - *ad hoc* digital platforms
 - informative support for campaigns

Costs: low. The necessary resources are those relating to the training of the citizen-scientist involved in the project and, secondly, in the analysis of the data provided by the citizenship. Effective and good quality training is essential to reduce the main problems involved in using such initiatives, consisting mainly of errors or difficulties in identifying the species committed by the citizen-scientist, resulting in false positives/negatives.

Goals: the goal of the monitoring technique is to succeed, through the involvement of the citizens, in identifying and mapping the distribution of specific plant species, especially in qualitative terms. As part of the study of alien species, the method can be used to obtain rapidly and widely qualitative information about potential areas occupied by the species-target, as well as to have an indicative estimate on the rate of ingression of the same (particularly useful in case of incipient colonization). The method is particularly effective for the monitoring of large or easily identifiable plant alien species, also for the possibility of supporting each sighting with photographic material, also of detail, which can be subsequently validated by technical experts. A further benefit of the application of the method is related to the dissemination of knowledge about the problem of alien species in the population involved in the project. In particular at training events, during which the correct data about the typology, the distribution and the dangerousness of the allochthonous species present in the territory are supplied, with consequent progressive sensitization of the active citizenship towards the problem.

1.2.2 Category #2: FRESHWATER AQUATIC PLANTS

Wetlands are threatened by many anthropic factors at different scales, from climate change to habitat degradation and destruction (reclamation, urbanization, etc.), including IAS introduction, for which aquatic ecosystems are particularly vulnerable, like rivers and lakes. Concerning the plants, water represents an ideal vector for fruits, seeds, and other parts of the plant, promoting their diffusion.

The impacts caused by alien plant species are many, from the genetical to the ecosystem scale, and may alter the nutrient cycles. Temperature rises can contribute to the settling and diffusion of these species, which may lead to the local extinction of native plants.

This category of plants includes the hydrophytes, meaning those species with great water requirements and which have adapted in floating or being submerged. Other adaptations are represented by leaves with a thin cuticle, wide stomates, well developed aeriferous parenchyma and eventual aerial roots. Many aquatic plants can reproduce easily through fragmentation, which contributes to their diffusion and invasiveness.

2.2.2.1 PHYTOSOCIOLOGICAL BRAUN-BLANQUET METHOD

Description: the phytosociological method (Braun-Blanquet, 1928) is described in the previous macrocategory, but declined in the definition of associations concerning the main hydrophytes of fresh water. There are two monitoring phases: the first, more analytical, consisting in the relief and estimation of the relative abundance of the species; the second, synthetic, in the definition of the vegetation association. In case the humid environment to be investigated is a natural/artificial reservoir, it may be necessary to use a boat.

Variables to be detected: minimum range (varying depending on the type of vegetation to be monitored), environmental type of reference, identification of species and relative coverage.

Time scale: seasonal, depending on the fluctuations of plant species throughout the year.

Detection frequency: since the method has a seasonal time scale, the maximum resolution applicable to the same is four times a year, corresponding to the four seasons of the normal vegetative cycle.

Staff required: it is required only one technician for each test station, although two technicians are recommended (necessary if the boat is to be used). Provision should be made for the use of at least one hour for each sampling station, in addition to the time required for the return of summary phase data.

Equipment:

- field equipment:
 - GPS positioner
 - digital/paper support, pencil
 - boat (adapted to the environmental context in investigation)
 - sampling material (jars/bags, knives, etc.)
- laboratory equipment:
 - stereoscopic microscope

Costs: medium to low. The costs, once the boat has been chartered and the laboratory equipment used for the microscopic recognition of the samples taken has been purchased, is related to the personnel involved and the execution of each sampling campaign.

Goals: the goal of the monitoring is the characterisation of the set of plant species that constitutes the population of a given study area, both in qualitative terms (floristic lists) and in quantitative terms, with a consequent definition of the habitat typology and/or of the phyto-morphological reference association. As part of the study of allochthonous species in fresh water, the method can be used both to obtain qualitative information on the presence/absence of certain target species from the reference areas (through the drafting of floristic lists), and, above all, quantitative information on the degree of ingression of certain alien species within the populations examined.

2.2.2.2 VEGETATION TRANSECT

Description: the vegetation transect method is used to study in detail a certain plant association or to evaluate floristic and structural variations between different types along an ecological gradient. In the case of aquatic plant associations, it is applied starting from the shore and proceeding towards the centre of the reservoir, ending when no vegetation is detected at all four sampling points on the four sides of the sampling boat or when the maximum depth of the reservoir has been reached. The inspection shall allow the detection of the species present and the allocation of an abundance value (Kohler method) for each species identified. This technique gives the possibility of obtaining immediate information on the spatial structure of the populations under investigation and on the exploitation of the resources of the species that compose them. Repeating the measurements over time can provide information on the dynamics of vegetation.

Variables to be detected: environmental typology of reference, transparency of water, errors associated with the recognition of species, lentic water.

Time scale: seasonal, depending on the fluctuations of plant species throughout the year.

Detection frequency: since the method has a seasonal time scale, the maximum resolution applicable to the same is four times a year, corresponding to the four seasons of the normal vegetative cycle. The latitude and altitude of the study area should also be taken into account.

Staff required: only one technician is needed for each test station, although two technicians are recommended (necessary if the boat is to be used). Provision should be made for the use of at least one hour for each sampling station, in addition to the time required for the return of summary phase data.

Equipment:

- field equipment:
 - GPS positioner
 - digital/paper support, pencil
 - boat (adapted to the environmental context in investigation)
 - sampling material (cans/bags, knives, etc.)

- laboratory equipment:
 - stereoscopic microscope

Costs: medium to low. The costs, once the boat has been chartered and the laboratory equipment has been purchased, are related to the personnel involved and the execution of each sampling campaign.

Goals: the goal of the monitoring is the characterisation of the set of plant species that constitutes the population of a given study area, especially in qualitative (floristic lists) and semi-quantitative terms (estimates of percentages of coverage). Unlike the Braun-Blanquet method, the reference phyto-sociological association is not defined, to the advantage of the method. As part of the study of allochthonous species, the method can be used to quickly obtain qualitative information about the presence/absence of certain target species from the target ranges (by drawing up the lists of flowers), in addition to having an indicative estimate on the rate of ingression of the same (particularly useful in case of incipient colonization and/ or recrudescence post-containment interventions).

2.2.2.3 REMOTE SENSING

Description: the remote sensing method is used to study the overall distribution of a given plant combination or to assess the actual presence and spread of a plant species on the detected territory. It is performed by using an aircraft (usually a remote-controlled drone) equipped with multispectral sensors, capable of detecting the spectral signature of a particular object (a certain plant species), which is flown over the vegetated environment characterization, thus allowing both the detection of the spectral signature of the different species contacted during the flight of the area in question and the acquisition of images useful for subsequent processing. An essential prerequisite is the acquisition of the spectral signature for each species to be surveyed, which should be previously characterised by appropriate sampling and laboratory analysis. The area inspection shall make it possible to detect, during the flight phase, the presence and extent of all species of interest. Repeating the measurements over time can provide information on the dynamics of vegetation. This information should then be processed using appropriate software (e.g. GIS).

Variables to be detected: spectral signature of the species to be monitored, atmospheric conditions for monitoring, assessment of the accuracy of remote sensing.

Time scale: seasonal, depending on the fluctuations of plant species throughout the year.

Detection frequency: since the method has a seasonal time scale, the maximum resolution applicable to the same is four times a year, corresponding to the four seasons of the normal vegetative cycle.

Staff required: only one technician is needed, accompanied by staff with remote control aircraft pilot licence. Provision should be made for the use of sampling and spectral signature time of the species of interest, drone preparation and overflight of the study area, in addition to the time required for the restitution of data related to the analysis phase using the computer.

Equipment:

- field equipment:

- identification guides
- software for data entry and analysis
- remote sensing equipment:
 - remote sensing drones (camera, multispectral sensors)

Costs: medium to high. The costs, once purchased or rented the equipment necessary for remote sensing, will be related both to the personnel involved and the execution of sampling, both to the application of the software for the subsequent identification of the distribution areas (in particular, the spectral signature characterisation phase for the target species, which is necessarily time and resource-consuming). The higher costs will therefore be attributable to the equipment needed to carry out the methodology.

Goals: the goal of the monitoring is the multispectral characterisation of the distribution of characteristic plant species which form the stand of a given study area, especially in quantitative terms (estimates of percentages of coverage by one or more target species following processing with appropriate software). In the context of the study of alien species, the method is particularly useful, despite the greater economic effort required, in the quantification of the areas occupied by plant species with rapid diffusion and strong colonizing capacity (e.g. invasive succulent plants capable of colonizing open land or occupied by garrigue/low Mediterranean maquis, and/or capable of rapidly forming single-specific stands in the enlarged areas (e.g. invasive species of tall stems, such as *Ailanthus altissima* and *Robinia pseudoacacia*, in disturbed tree and shrub stands). This method can be used to monitor the progress of any containment interventions and assess their ex-post effectiveness. The method can also be used to quickly obtain qualitative information about the presence/absence of certain target species (by detecting appropriately defined spectral signatures to be species-specific) in reference areas with access difficulties for a detector (e.g. environmental contexts characterised by particular acclivity, situations of gullies, ravines or blades, small island contexts), in addition to having an indicative estimate of the ingression rate of the same. Also in this case, the method is particularly useful in case of incipient colonization and/or assessment of any resurgence of post-containment.

2.2.2.4 ANALYSIS OF ENVIRONMENTAL DNA

Description: the method consists of the collection of DNA fragments from organisms in the environment in which they live (biological fluids or tissue fragments), from which laboratory analysis (using DNA amplification and sequencing techniques), data of presence/absence and relative abundance of harvested species can be obtained. The aquatic environment lends itself optimally to this type of approach, thanks to the rapid dispersion and fragments in the water body and the ease of sampling.

Variables to be detected: genetic material related to the species to be analysed.

Time scale: seasonal, depending on the fluctuations of plant species throughout the year.

Detection frequency: since the method has a seasonal time scale, the maximum resolution applicable to the same is four times a year, corresponding to the four seasons of the normal vegetative cycle.

Staff required: for the field phase, it is sufficient only one technician to take care of the collection of samples and the correct conservation. The analytical phase must provide for the preparation of the sample, the extraction,

amplification by PCR and sequencing of the obtained amplicons, in addition to the alignment of the sequences on a specific basis and the phylogenetic interpretation of the results obtained.

Equipment:

- field equipment:
 - material for the storage of samples
 - sampling material (cans/bags, etc.)
 - boat (adapted to the environmental context in investigation)
- analytical phase:
 - structures and equipment for extraction, amplification, purification and sequencing of environmental DNA samples
 - software for the insertion and analysis of phylogenetic data

Costs: high. The costs relate mainly to the analytical phase, which includes the costs necessary for the processing of environmental DNA samples and their sequencing, usually in specialised laboratories.

Goals: the goal of the monitoring is the environmental and specific characterization of freshwater plant organisms, especially in qualitative terms (checklist of plant species) and semi-quantitative (successive estimates of relative abundance, which however require the use of additional molecular techniques, resulting in increased costs). As part of the allochthonous species study, the method can be used to quickly obtain qualitative information about the presence/absence of certain target species in the wetland under study.

1.2.3 Category #3: MARINE MACROPHYTES

The Mediterranean basin constitutes one of the world biodiversity hotspots, hosting between 10.000 and 12.000 plant and animal marine species (10% of all known marine species globally), thus requiring strict protection strategies for our and future generations.

Climate change, together with habitat degradation, is causing relevant changes in flora and fauna of the Mediterranean basin, where the introduction of more alien species has been documented, leading to an alteration of the ecological and trophic patterns. The main causes of introduction are represented by anthropic factors, such as the opening of the Suez Canal, maritime transport (ballast waters or fouling) and aquaculture.

Marine macrophytes live in aquatic, salty environment, and can be divided into marine plants and seaweeds, both unicellular and pluricellular. Algae do not have differentiated tissues, and their main colour can be helpful in their identification, mainly distributed from the surface to a depth of about 15-30 metres.

2.2.3.1 DIRECT COLLECTION METHOD

Description: the method consists in obtaining a complete checklist of the species present for the study of macrophytic biodiversity, characteristic of each marine area. The methodology provides for a first phase of direct collection of samples (through herbage and integral scratching) along the coast using a boat, paying particular attention to the integrity of the thallus structure and traveling through various transects for the collection, going from the shore towards the open sea (keeping taking into account the bionomic planes and the morphology of the seabed). A second phase of subsequent taxonomic study of the macrophytes, including also the small epiphytic species, using the optical microscope and the stereoscopic microscope. This methodology is often combined with the one of coverage (discussed in the next sub-chapter) to define macrophytic associations.

Variables to be detected: reference environmental typology and species identification.

Time scale: seasonal, depending on the fluctuations of plant species throughout the year.

Detection frequency: since the method has a seasonal time scale, the maximum resolution applicable to the same is four times a year, corresponding to the four seasons of the normal vegetative cycle.

Staff required: at least two technicians are required, of which an expert that will take care of the aquatic macrophytes during the surveys, in addition to the support staff assigned to the logistics of the means. In case of underwater detection transect/square detection transect, it is necessary to provide for the use of Underwater Scientific Operators (USO). At least two hours should be used for each sampling station, in addition to the time needed for morphological analysis of any samples collected by appropriate laboratory equipment.

Equipment:

- field equipment:
 - boat (adapted to the environmental context in investigation)
 - underwater equipment (also for photo documentation)

- material necessary for the collection of macrophytes (jars, knives, etc.)
- laboratory equipment:
 - optical microscope and stereoscopic microscope

Costs: medium to low. The costs, once the laboratory equipment used for the microscopic recognition of the samples collected and those related to the sampling effort have been purchased, shall relate to the personnel involved and to the execution of each sampling campaign.

Goals: the goal of the monitoring is the characterization of the greatest number of macrophytic species that make up the marine vegetal associations of the single marine-coastal areas, both in qualitative terms (floristic lists) that of three-dimensional structure of the same. As part of the study of allochthonous species, the method can be used to obtain qualitative information about the presence/absence of certain target species from the reference areas (through the drafting of floristic lists).

2.2.3.2 HEDGING METHOD

Description: the method consists of detecting the relative abundance of the individual species contacted on a portion of substrate and can be expressed as a surface measurement (m^2) but more commonly as a percentage of coverage. The methodology consists of a survey that is carried out within a quite large elementary stand called "Minimum Qualitative Area" (MQA), so that the surface of the survey can be increased to reach the minimum extent that allows you to have a complete set of data to validly describe communities. When the number of species inventoried increases as the sampling area does, the area can be defined as "minimum stand area", which is the minimum substrate area where the maximum number of species of the test stand is found. This method, complementary to the purely qualitative approach of direct harvesting, makes it possible to define the phytosociological associations that are established between macrophytes in marine-coastal environments.

Variables to be detected: reference environmental typology, identification of species, Minimum Qualitative Area.

Time scale: seasonal, depending on the fluctuations of plant species throughout the year.

Detection frequency: since the method has a seasonal time scale, the maximum resolution applicable to the same is four times a year, corresponding to the four seasons of the normal vegetative cycle.

Staff required: it is necessary to employ at least two technicians, of which an expert that will take care of the aquatic macrophytes during the surveys, in addition to the support staff assigned to the logistics of the means. In case of underwater detection transect/square detection transect, it is necessary to provide for the use of Underwater Scientific Operators (USO). At least two hours should be used for each sampling station, in addition to the time needed for morphological analysis of any samples collected by appropriate laboratory equipment.

Equipment:

- field equipment:
 - boat (adapted to the environmental context in investigation)

- underwater equipment (also for photo documentation)
- material necessary for the collection of macrophytes (jars, knives, etc.)
- laboratory equipment:
 - optical microscope and stereoscopic microscope

Costs: medium to low. The costs, once the laboratory equipment used for the microscopic recognition of the samples collected and those related to the sampling effort have been purchased, shall relate to the personnel involved and to the execution of each sampling campaign.

Goals: the goal of the monitoring is the morphological recognition and the estimation of the relative abundances of the macrophytic species that make up the marine vegetal associations of the single marine areas-coastal areas, in quantitative terms and in terms of the area occupied by macrophytic populations. As part of the study of alien species, the method can be used both to obtain qualitative information on the presence/absence of certain target species from the reference areas (through the drafting of floristic lists) and quantitative information on the degree of ingression of certain alien species within the populations examined, with an estimate of their coverage and an assessment of the effects on the reference phytosociological association.

2.2.3.3 VISUAL CENSUS

Description: the method consists of non-destructive sampling for the study of marine biodiversity, or even for subsequent applications of biological indices (e.g. CARLIT) that do not require an exhaustive list of flora. The monitoring technique makes use of the descriptive observation of the macrophytic diversity of the middle and infra-coastal belt, without the need for the collection of samples, even from boat. It can take place along a transect, where an observation is made along a defined path, or a square, of a well-defined reference area. The methodology also includes a good knowledge of the study area and the biological community under consideration, and it is mainly used by experienced operators. The possible collection of samples of difficult recognition will be followed by a phase of morphological recognition in the laboratory.

Variables to be detected: degree of transparency of water, identification of species of macrophytes present.

Time scale: seasonal, depending on the fluctuations of plant species throughout the year.

Detection frequency: since the method has a seasonal time scale, the maximum resolution applicable to the method is four times a year, corresponding to the four seasons of the normal growing cycle, which may be reduced to spring and autumn.

Staff required: it is necessary to employ at least two technicians, including an expert that will take care of the aquatic macrophytes during the surveys, in addition to the support staff assigned to the logistics of the means. In case of underwater detection transect/square detection transect, it is necessary to provide for the use of Underwater Scientific Operators (USO). At least one hour should be used for each sampling station, in addition to the time needed for morphological analysis of any samples collected by appropriate laboratory equipment.

Equipment:

- field equipment:
 - underwater equipment (also for photo documentation)
 - material necessary for the collection of macrophyte samples (jars, knives, GPS, etc.)
 - boat (adapted to the environmental context in investigation)
- laboratory Equipment:
 - optical microscope and stereoscopic microscope

Costs: medium to low. The costs, once the laboratory equipment used for the microscopic recognition of the samples collected and those related to the sampling effort have been purchased, shall relate solely to the personnel involved and to the execution of each sampling campaign.

Goals: the goal of the monitoring is the qualitative characterization (floristic lists) of the greatest number of species of macrophytes that make up the marine vegetable associations of the marine-coastal areas in the survey (Marine macrophytes are no longer present from the disphotic planes). As part of the study of alien species, the method can be used to obtain qualitative information about the presence/absence of certain target species in coastal environments with medium-low coastal bottom (through the drafting of floristic lists).

2.2.3.4 REMOTE SENSING

Description: the remote sensing method is used to study the overall distribution of a given plant association or to assess the actual presence and spread of a plant species on the seabed along the coast (intertidal zone). Is performed by using a drone, or even a remote-piloted submarine vehicle equipped with multispectral sensors, capable of detecting the spectral signature of an object (in our case of a certain plant species). The medium travels through the vegetated environment in predicate of characterization, thus allowing the detection of the spectral signature of the different species contacted during the immersion and the acquisition of the images useful for the successive elaborations. An essential prerequisite is the acquisition of the spectral signature for each species to be surveyed, which should be previously characterised by appropriate sampling and laboratory analysis. The inspection shall make it possible to detect the extent and presence of all species of interest. Repeating the measurements over time can provide information on the dynamics of vegetation. This information should then be processed using appropriate software (e.g. GIS).

Variables to be detected: spectral signature of the species to be monitored, atmospheric conditions for monitoring, assessment of the accuracy of remote sensing.

Time scale: seasonal, depending on the fluctuations of plant species throughout the year.

Detection frequency: since the method has a seasonal time scale, the maximum resolution applicable to the same is four times a year, corresponding to the four seasons of the normal vegetative cycle.

Staff required: it is sufficient to employ only one technician accompanied by staff in possession of the pilot licence for remotely controlled aircraft. It is necessary to provide for the use of sampling and spectral signature time for the species of interest, preparation of the medium and full survey of the study area, in addition to the time required for the return of data related to the analysis phase through the use of the computer.

Equipment:

- field equipment:
 - identification guides
 - computer for data entry and analysis
 - boat
 - marine macrophyte sampling material (knife cans/bags)
- remote sensing equipment:
 - drone/ROV
 - remote sensing equipment (camera, camera, multispectral sensors)

Costs: medium to high. The costs, once purchased or rented the equipment necessary for remote sensing, will be related both to the personnel involved and the execution of sampling, both to the application of the software for the subsequent identification of the distribution areas (in particular, the spectral signature characterisation phase for the target species, which is necessarily time and resource-consuming). The higher costs will therefore be attributable to the equipment needed to carry out the methodology.

Goals: the goal of the monitoring is the multispectral characterisation of the distribution of characteristic plant species which form the stand of a given study area, especially in quantitative terms (estimates of percentages of coverage by one or more target species following processing with appropriate software). In the context of the study of alien species, the method is particularly useful, despite the greater economic effort required, in the quantification of the areas occupied by rapidly spreading plant species with a strong colonizing capacity and/or capable of rapidly forming monospecific populations in the introduced areas (in particular the algal species).

1.2.4 Category #4: TERRESTRIAL ANIMALS

Italy, located in the centre of the Mediterranean basin, hosts a great biodiversity and a high degree of endemic species, thanks to the extreme diversity of geological, topographical and climatic features. 50'000 terrestrial species are estimated to live here, constituted for a 90% by invertebrates (mostly insects).

This richness is compromised by anthropic factors, and many species and ecosystems are facing a serious threat that may lead to their local extinction. According to the "Italian Red lists", 9% of reptiles, 36% of amphibians and 27% of breeding birds are threatened, and IAS are one of the main actors in this picture. Specific action plans are required, together with monitoring protocols based on scientific data.

The high diversity in terms of ecological adaptations of these species lead to the necessity of dividing this group in two subcategories: low-dispersion animals (represented by rodents *Rattus rattus* and *R. norvegicus*) and high-dispersion animals (represented by those species which are characterized by fast dispersion habits, like insects and birds).

2.2.4.1 TRAPPING FOR LOW-DISPERSION SPECIES

Description: the capture of specimens as a direct detection method is one of the most widely used monitoring techniques for micro-mammals. Live traps, of various models depending on the needs, catch the animals and the target species, often associated with Capture-Marking-Recapture protocols in order to avoid counting the same individuals several times and introduce an overestimation of the population. Among the numerous models, we can mention, for the micromammals, the Sherman, Longworth, Trip-Trap and Pitfall traps, while for the number it is recommended to use at least 7x7 traps for each grid, and at least two replicas (grids/ transects) for each present environmental typology.

Variables to be detected: number of individuals caught, species, biometric data, environmental reference type.

Time scale: annual, during the breeding peaks of the animals, avoiding any hibernation seasons when present.

Detection frequency: variable, from one to two sessions per year, depending on the species, which may have one or more reproductive peaks (e.g. rodents).

Staff required: we recommend the use of teams of two people for each site or group of sites nearby, necessary for the positioning, inspection and possible capture-marking of individuals. Five to six consecutive sampling days are required for each session.

Equipment:

- field material
- live traps, with baits
- individual marking material (ear tags, dyes, etc.)

Costs: average. Once the cost of the purchase of traps has been amortized, the following campaigns will have only the cost of the personnel involved and the basic preparation of the campaign.

Goals: the goal of the monitoring is the characterization of a given area of investigation, both in terms of quality (list of species present in the territory) and of abundance of populations, divided by environmental typologies. As

part of the study of alien species, this method can be applied both to monitor the presence/absence of certain target species from specific areas (such as islands or particularly sensitive areas) and to determine the absolute and relative abundance of these species within that study area, in order to quantify the degree of ingression of the species and the load it exerts on the environment. This methodology applies to those organisms with more limited dispersion capabilities (compared to other organisms like flying insects or birds), for which the trapping in a relatively restricted study area allows to have a reliable estimate on their current distribution.

2.2.4.2 INDIRECT DETECTION

Description: the presence of terrestrial species can also be assessed through indirect detection techniques, while taking into account the limitations of the methodology, exploiting the traces that the animals leave when they pass. Among the most used techniques stands out the research and analysis of birds of prey, capable of providing information on the community of micro-mammals of a territory, or the use of specific traps, called presence. In the latter case, they are tools that allow to recognize the presence of the animal starting from the traces that leave, like parts of hair (hair tubes), bite marks (Chew cards) or fingerprints (track plates).

Variables to be detected: quantification of signs of presence, variables according to the techniques used; annotation of species.

Time scale: annual, during the breeding peaks of the animals, avoiding any hibernation/hibernation seasons when present.

Detection frequency: variable, from one to two sessions per year, depending on the species, which may have one or more reproductive peaks (e.g., rodents).

Staff required: one operator is sufficient for the positioning of traps and periodic inspection (not daily) as well as for subsequent laboratory testing. Time should be allowed for any analysis of the data (such as hair samples) at the laboratory stage.

Equipment:

- field material
- presence traps
- laboratory equipment
- stereoscopic microscope (for possible laboratory analysis of hair, bones, etc.)

Costs: average. Necessary the cost of the purchase of the traps, of the personnel involved for the preparation and the basic development of the campaign, for considering the laboratory equipment for subsequent analyses.

Goals: the goal of the monitoring is the characterisation of the composition of the species community in a given study area, especially in qualitative (presence/absence) and semi-quantitative terms (estimates of abundance possibly based on frequency classes). In the case of the alien species study, this methodology may be used to assess the presence of a specific target species in a new area of interest for which no information is available. Compared to live trapping, indirect analysis of the presence of a species has the advantage of

requiring less investment in time and human resources, being the occasional collection of data over time (trapping campaigns, for comparison, in vivo need more consecutive days of sampling and more operators). As with the previous method, this protocol applies to those species which are characterized by a low vagility, having these dispersion capacities more limited than other organisms (for example flying insects, birds, etc.).

2.2.4.3 PHOTO TRAPPING

Description: photo-trapping (also called camera-trapping) is a survey and monitoring tool widely used in wildlife. In recent decades. It consists in the placement, within the area of interest, of automatic cameras and camcorders, which are able to activate, through a photocell, the passage of an animal and to record a photo or a video of it. This methodology therefore allows us to collect data at predetermined points in a standardized manner, possibly also collecting a series of metadata associated with photos or videos such as the time, date or temperature of the moment of the shot. The combination of this information makes photo trapping an excellent tool for the study of relative or absolute subscriptions, depending on the needs and the applied indices.

Variables to be detected: passage of animals taken from photos/camcorders; identification of individuals where possible.

Time scale: annual, preferably during the breeding peaks of the animals, avoiding any hibernation/hibernation seasons when present.

Detection frequency: an annual session is sufficient, provided is prolonged over time that is in the order of weeks/months. Periodic inspections are required to ensure the status of the equipment and to collect data.

Staff required: a single operator is sufficient to position photos/camcorders and data recovery. The presence of one or more additional operators shall be assessed when the sampling effort intensifies. The greatest effort is to be understood in the hours spent analysing the photographic/video samples after the collection of memory cards in the field.

Equipment:

- field material
- cameras/video cameras
- material for data analysis
- computer for image/video analysis

Costs: medium-high. Is necessary to purchase photographic equipment and any repairs/ replacements.

Goals: the goal of the monitoring is the characterisation of the composition of the species community in a given study area, both in qualitative terms (presence/absence) and in semi-quantitative terms (estimates of relative abundance). Where is possible, through photographic/video analysis, the recognition of individuals, an extensive photo-trapping campaign can also return quantitative data, through the production of estimates of absolute abundance in a given area. In the case of the alien species study, this methodology may be applied to assess the presence of target species in newly surveyed areas, and to assess the abundance of

one or more populations in an area where the presence is already established but no data on the extent of these are available. As with previous methods, this protocol applies to low-vagility species and has lower human resources sampling effort as an advantage over them, requiring the phototraps to be periodically revised but distributed over relatively long periods (weeks/months). The main disadvantage is the economic burden, due to the investment in the purchase of video/photographic equipment and their maintenance.

2.2.4.4 BREEDING BIRD SURVEY

Description: this census and monitoring technique is based on the method called Breeding Bird Survey, specific for avifauna. This investigation is applied through the creation of linear transects placed inside squares of 1 km² randomly generated in space. The surveyors shall carry out censuses in the early hours of dawn during the reproductive period of the spring season, through two transects 1 km long each within each square. The birds are divided into categories of distance from the transect, and eventually information is collected about the habitat travelled during the inspection.

Variables to be detected: identification of the species; distance from the transect; habitat concerned.

Time scale: annual, depending on the reproductive peak of the species (from April to June in the case of the ornithopod).

Detection frequency: two mornings per square each year, one for each of the two transects inside.

Staff required: the use of a transect operator is sufficient. Evaluate the presence of multiple operators in the case of an extended number of squares, to cover several points simultaneously by concentrating them in the reproductive period. In addition to the time spent in the field for each sampling station, provision should be made for the time required to return the summary phase data.

Equipment:

- field material
- notebook
- binoculars

Costs: low. The expenditure is intended for the staff involved and the purchase of any binoculars for monitoring.

Goals: the goal of the monitoring is the characterisation of the bird community in a given study area, both in qualitative terms (presence/absence) and in quantitative terms (estimates of abundance). In the case of the alien species study, this methodology may be applied in the early identification of certain target species, in areas where their presence has not yet been established, and estimates of their abundance in a given habitat/environment/area of interest. Unlike the previous techniques, this monitoring protocol applies to species with high vagility that is characterized by a high dispersion capacity. In this particular case, this methodology, which is specific to birds, has the advantage of requiring a low investment from the point of view of staff and equipment, since an operator with binoculars is needed for each site. However, is advisable to provide for the presence of multiple operators where the sampling effort is substantial, in order to concentrate the censuses during the reproductive period and avoid over-sampling.

2.2.4.5 DISTANCE SAMPLING

Description: the distance sampling method is based on the use of linear transects or point surveys in which the abundance of organisms surveyed is estimated from the distance detected by the observer. The transect can be travelled by foot or by vehicle, including boats, travelling at constant speed over the areas to be investigated. In the case of point relief, the observer shall carry out his observations within a fixed and constant period for each station. This method finds its application in the estimation of abundance of populations whose number would be unrealistically estimated through sample areas by the default extension.

Variables to be detected: identification of the species; distance from the transect.

Time scale: annual, depending on the activity peaks of the species.

Detection frequency: one session per linear transect per year

Staff required: at least two operators in the case of use of vehicles, one of which is a member of the driving and one to the census are sufficient. Evaluate the presence of multiple operators in the case of an extended number of squares to cover several points simultaneously by concentrating them in the reproductive period. In addition to the time spent in the field for each sampling station, provision should be made for the time required to return the summary phase data.

Equipment:

- field material
- notebook
- binoculars
- means of transport (motor vehicle/boat, etc.).

Costs: variable. From low in the case of transects walkable up to medium-high in the case of rental of motor vehicles/boats.

Goals: the goal of the monitoring is the characterisation of the bird community of a given study area, mainly with the aim of obtaining quantitative data (estimates of abundance). In the case of the study of alien species, this methodology can be applied in the early identification of some target species, in areas where their presence has not yet been established, but especially in the estimates of abundance of populations already settled. From the point of view of the necessary resources, in case the transect is linear such methodology has the advantage of requiring a low investment from the point of view of staff and equipment, being necessary an operator equipped with binoculars for each transect. The conditions vary if the transect is to be carried out over considerable distances or under special conditions requiring the use of motor vehicles or boats, if any, in the case of censuses in wetlands. Compared to the method of transects inside the squares, the present technique is easier to implement in the case of territories with complex topography, where roads or water routes are available. The basic assumptions is that the target species move often and preferably in group.

2.2.4.6 CENSUSES THROUGH CITIZEN SCIENCE PROJECTS

Description: these projects involve citizenship through actions of citizen science, already defined in the previous sections. The contribution of the population to active surveillance on the territory may be a crucial resource in the fight against invasive species, in particular against particularly recognisable and easy-to-contact animal species. Many projects have used this strategy (CSMON-LIFE, Life ASAP, SINAnet ISPRA), which initially provides for the production of information material aimed at facilitating the identification of target species by the citizens, and consequently the production of specific useful applications to collect these reports and put them into a common database, where they can be validated by experienced personnel.

Variables to be detected: identification of the species; photographs (by citizens); checks and validation of information (by experienced staff).

Time scale: since monitoring is entrusted to the citizenship, the whole course of the year is potentially valid as a data collection period. The period of effectiveness of the same is, however, variable from species to species.

Detection frequency: variable, information is collected at different times by citizens.

Staff required: only one expert technician is needed to analyse and evaluate the data collected by the citizen.

Equipment:

- materials for work:
- ad hoc IT platforms
- informative support for campaigns

Costs: low. The necessary resources are those relating to the training of the citizen-scientist involved in the project and, secondly, in the analysis of the data provided by the citizenship. Effective and good quality training is essential to reduce the main problems involved in using such initiatives, consisting mainly of errors or difficulties in identifying the species committed by the citizen-scientist, resulting in false positives/negatives.

Goals: the goal of the monitoring technique is to succeed, through the involvement of the citizens, in identifying and mapping the distribution of target species, especially in qualitative terms. As part of the study of alien species, the method may be used to obtain rapidly and widely qualitative information about potential target species areas. The method is particularly effective for the monitoring of easily recognizable and contactable alien species, also for the possibility of supporting each sighting by the citizen-scientist with photographic material, which can subsequently be validated by the experienced technician. A further benefit of the application of the method is related to the dissemination of knowledge about the problem of alien species in the population involved in the project. In particular at training events, during which the correct data about the typology, the distribution and the dangerousness of the allochthonous species present in the territory are supplied, with consequent progressive sensitization of the active citizenship in the face of the problem.

2.2.4.7 CENSUS OF ROOST SITES

Description: roosts are gathering sites where large groups of birds meet at the end of their daytime activities. A typical phenomenon of gregarious species, this behaviour allows to set up monitoring sessions based on the census

of the species of interest in a few specific places, concentrating the activity of operators and obtaining reliable estimates.

Variables to be detected: number of individuals; identification of species.

Time scale: annual, depending on the activity peaks of the species (the roost is a phenomenon mainly linked to the non-reproductive season, in the case of the avifauna in the autumn and winter season).

Detection frequency: once a year. The surveys are carried out at dusk, when the animals return to the dorms and the visibility allows carrying out the census.

Staff required: multiple operators. The number varies depending on the size of roost sites, which must be surveyed simultaneously to avoid over-sampling. In addition to the time spent in the field for each sampling station, provision should be made for the time required to return the summary phase data.

Equipment:

- field material
- notebook
- binoculars

Costs: medium-low. Resources should be channelled into the personnel involved in monitoring actions.

Goals: the goal of the monitoring is the quantification of target species populations in a given study area, with the aim of obtaining quantitative data with estimates of high level of reliability. In the case of the study of allochthonous species with gregarious roost formation habits, this technique allows to quantify the extent of the test population with a low approximation, concentrating these species in few specific points. The main advantage of this protocol is the relatively simple sampling and the obtaining of very reliable estimates, especially in the case of birds of considerable size, such as waders. The main disadvantage, on the contrary, is the reduced applicability of the method, this being reserved to the species with the aforementioned ecological-behavioural characteristics.

2.2.4.8 SURVEY OF NESTING SITES

Description: many species have gregarious habits also during the nesting period, forming real reproductive colonies where large numbers of individuals are concentrated. Similarly to roost sites, monitoring censuses can be carried out directly at those nesting sites.

Variables to be detected: number of individuals; identification of species.

Time scale: annual, depending on the reproductive peaks of the species (spring for the avifauna).

Detection frequency: once a year per site.

Staff required: one or more operators, the number varies depending on the size of the reproductive colonies, which must be recorded simultaneously to avoid over-sampling. In addition to the time spent in the field for each sampling station, provision should be made for the time required to return the summary phase data.

Equipment:

- field material
- notebook
- binoculars

Costs: medium-low. Resources should be channelled into the personnel involved in monitoring actions.

Goals: the goal of the monitoring is the quantification of target species populations in a given study area, with the aim of obtaining quantitative data with estimates of high level of reliability. In the case of the study of alien species with gregarious habits characterized by the formation of reproductive colonies, this technique allows to quantify the extent of the test population with little approximation, concentrating these species in few specific points. The main advantage of this protocol is the relative simplicity of sampling and the obtaining of very reliable estimates. The main disadvantage, on the contrary, is the reduced applicability of the method, this being reserved to the species with the aforementioned ecological-behavioural characteristics.

2.2.4.9 SPECIES DISTRIBUTION PATTERNS

Description: identified with the Anglo-Saxon term Species Distribution Models (SDM), such models are based on the crossing of spatial, geographical, physical, and ecological information to be able to build the distribution of species through proper ecological maps. Based on the use of software based on Geographic Information Systems (GIS), these models allow to predict any future distributions of the species at a time of incipient colonization, providing a means of prevention against potential invasive species on the territory that require accurate monitoring.

Variables to be detected: presence and/or abundance data of the species in the area concerned; geospatial data of the physical, climatic or ecological matrices that may influence the distribution of the species.

Time scale: not influent, the monitoring technique is based on analysis of data already available.

Detection frequency: not influent, the monitoring technique is based on analysis of data already available.

Staff required: a single operator is sufficient, whose use will be channelled into the collection of data from specific literature and/or databases and from the elaboration of the models in question.

Equipment:

- material for data analysis
- dedicated software for data processing and analysis (mainly geospatial, GIS)

Costs: medium to low. Resources should be channelled into the personnel involved in data analysis and the possible purchase of dedicated electronic equipment and/or software.

Goals: the goal of the monitoring is the construction of predictive models of species distribution, through the construction of maps of suitability for the presence of the species. In the case of the allochthonous species study, this methodology may be used to assess the presence of target species in areas for which no or no data are available. The advantage of this technique is to be able to build maps of potential presence of the species,

in order to organize targeted monitoring expeditions and optimize the resources available. The disadvantage, in the present case, is the necessary presence of historical data in this regard, which takes into account, in addition to the distribution of the populations analysed, also the geographical, physical and ecological characteristics related to these data.

2.2.4.10 TRAPPING FOR HIGH VAGILITY SPECIES

Description: the trapping for flying fauna is mainly used in entomological monitoring, for calculating estimates of abundance. There are many types of traps, which are based on the attraction of insects through various stimuli. We mainly distinguish those that use visual stimuli, such as light traps for nocturnal or crepuscular insects, or others based on chemical stimuli, called chemotropic traps, which exploit pheromonal substances. Trapping for monitoring purposes is generally applied to follow flight curves during peaks where there is the highest frequency of reproductive events.

Variables to be detected: number of individuals caught, identification of species.

Time scale: seasonal, depending on the reproductive peaks of the species.

Detection frequency: one or more times a year, in the case of species with more than one reproduction cycle in a year (bi-multivoltine); prolonged in time if necessary to analyse species whose biological cycle is not known.

Staff required: sufficient an operator to set traps and collect individuals periodically. In addition to the time spent in the field for each sampling station, provision should be made for the timing necessary for the counting and recognition of individuals in the laboratory.

Equipment:

- field material
- traps (of various types, such as bright or chemiotropic traps)
- laboratory equipment
- stereoscopic microscope

Costs: average. Resources should be channelled into the personnel involved in the analysis as well as the purchase of traps and microscopes necessary for the collection and analysis of data.

Goals: the goal of the monitoring is the characterization of the entomological community typical of a given area of study, both in qualitative terms (presence/absence) and in quantitative terms (estimates of abundance). In the case of the allochthonous species study, this methodology may be applied to assess the presence of a species in a given area for which no information is yet available or, where appropriate, to assess the extent of populations already settled through estimates of abundance. The advantage of this technique is that is possible to set up monitoring campaigns with relatively low economic costs, mainly related to the installation of traps in the study area. Possibly the cost can increase in case the difficult identification of the species makes it necessary the use of laboratory instrumentation for a proper analysis.

2.2.4.11 ACOUSTIC ANALYSIS

Description: bioacoustics analyses may be used to identify plant individuals infested with pathogenic insects, as in the case of Red Weevil. The sound produced by the movement and foraging of the larvae that feed on the wood can in fact be detected thanks to specific recording devices, especially during the first stages of colonization of the plant, when the concentration of larvae on the plant is still relatively low.

Variables to be detected: acoustic patterns (detected by the instrument) species-specific.

Time scale: seasonal, depending on the activity peaks of the species.

Detection frequency: one or more times a year, in the case of multivoltine species such as the red weevil, aimed at monitoring the health status of potentially infested plant essences.

Staff required: an operator using the equipment is sufficient during the monitoring phase.

Equipment:

- field material
- piezo-electric microphone for the detection of the species.

Costs: average. The resources shall be channelled into the personnel involved in the analysis and in the purchase of the acoustic devices necessary for sampling.

Goals: the goal of the monitoring is the detection of certain species during the infestation phases, both in qualitative terms (presence/absence) and in semi-quantitative terms (indirectly exploiting the abundance of insects through an estimation of infested tree essences). In the study of alien species, this methodology can be used to analyse the ingress of target species in areas not yet colonized or to make an estimate of abundance in areas where the presence is already established. The advantage is the ease of application, managing to act in advance and to identify the species in an early stage of infestation. From the point of view of the necessary resources, the technique does not involve invasive inspection methods for the plant and, having the necessary equipment, the costs are amortised with the extensive analysis of large quantities of plant individuals analysed.

2.2.4.12 OLFACTORY ANALYSIS

Description: dogs can be used as biosensors in identifying insect infestations. Dogs specially trained for the purpose can detect the chemical traces released by the secretions of infected trees, as in the case of palms, or insects/eggs during the infestation. This technique is suitable for prevention strategies in the control of plant material in ports, stations, and more generally quarantine repositories for biosecurity controls.

Variables to be detected: activity of the animal in the identification of the species.

Time scale: seasonal, depending on the activity peaks of the species.

Detection frequency: one or more times a year, in the case of multivoltine species such as the red awl, aimed at monitoring the health status of potentially infested plant essences.

Staff required: it is sufficient to employ a specialist operator during the monitoring phase, with the presence of a trained detection dog.

Equipment:

- specially trained detection dog.

Costs: medium-low. Resources should be channelled into the personnel involved in the training of animals for the purpose.

Goals: the goal of the monitoring is the detection of certain species during the infestation phases, both in qualitative terms (presence/absence) and in semi-quantitative terms (indirectly exploiting the abundance of insects through an estimation of infested tree essences). In the study of alien species, this methodology can be used to analyse the ingression of target species in areas not yet colonized or to make an estimate of abundance in areas where the presence is already established. The advantage is the ease of application, managing to act in advance and to identify the species in an early stage of infestation. From the point of view of the necessary resources, the technique does not involve invasive inspection methods for the plant and expensive, and having the necessary equipment, the costs are amortised with the extensive analysis of large quantities of plant individuals analysed.

2.2.4.13 THERMAL ANALYSIS

Description: infrared cameras can be used to detect temperature increases in infested trees, as in the case of the palm weevil. The larvae that nourish inside the trunk generate in fact fermentative processes that involve an increase of the temperature inside the tissues and detectable also from the crown of the tree.

Variables to be detected: abnormal variations in the temperature of the infested plant.

Time scale: seasonal, depending on the activity peaks of the species.

Detection frequency: one or more times a year, in the case of multivoltine species such as the red weevil, aimed at monitoring the health status of potentially infested plant essences.

Staff required: an operator using the equipment is sufficient during the monitoring phase.

Equipment:

- field material
- infrared cameras

Costs: average. Resources should be channelled into the personnel involved in the analysis and in the purchase of the equipment necessary for sampling.

Goals: the goal of the monitoring is the detection of certain species during the infestation phases, both in qualitative terms (presence/absence) and in semi-quantitative terms (indirectly exploiting the abundance of insects through an estimation of infested tree essences). In the study of alien species, this methodology can be used to analyse the ingression of target species in areas not yet colonized or to make an estimate of abundance in areas where the presence is already established. The advantage is the ease of application, managing to

act in advance and to identify the species in an early stage of infestation. From the point of view of the necessary resources, the technique does not involve invasive inspection methods for the plant and expensive, and having the necessary equipment, the costs are amortised with the extensive analysis of large quantities of plant individuals analysed.

2.2.4.14 ENVIRONMENTAL DNA ANALYSIS

Description: the method consists of the collection of DNA fragments from organisms in the environment in which they live (biological fluids or tissue fragments), from which laboratory analysis, using DNA amplification and sequencing techniques, data of presence/absence and relative abundance of harvested species can be obtained. Soil can be subjected to this type of approach, thanks also to the ease of sampling. The permanence of these fragments, combined with the current techniques of amplification and sequencing of DNA, allows to obtain a checklist of the animal and plant species that populate or have populated the environment in question.

Variables to be detected: DNA sequences belonging to the species of interest.

Time scale: annual, depending on the activity peaks of the species.

Detection frequency: once a year, necessary to monitor the state of presence and/or abundance, preferably in conjunction with the peaks of activity of the species.

Staff required: for the field phase, it is sufficient to use only one technician to take care of the collection of samples and the correct conservation. The analytical phase must provide for the preparation of the sample, the extraction, amplification by PCR and sequencing of the obtained amplicons, in addition to the alignment of the sequences on a specific basis and the phylogenetic interpretation of the results obtained.

Equipment:

- field phase:
 - material for the storage of samples
 - sampling material (cans/bags, etc.)
- analytical phase:
 - structures and equipment for extraction, amplification, purification and sequencing of environmental DNA samples
 - software for the insertion and analysis of phylogenetic data

Costs: high. The costs relate mainly to the analytical phase, which includes the necessary costs for the processing of environmental DNA samples and their sequencing, usually in specialised laboratories.

Goals: the goal of the monitoring is the detection of one or more species of a predefined study area, especially in qualitative (checklist of animal species) and semi-quantitative terms (successive estimates of relative abundance, which however require the use of additional molecular techniques, resulting in increased costs). As part of the study of alien species, this method is applied in the early identification of a target species in the early stages of colonization, when classic monitoring protocols would give unsatisfactory results to less

intensive and relatively extensive campaigns. Predominantly, this technique is used for analysis of the presence/absence of a species in a certain matrix, through the identification of specific DNA sequences. However, it is possible, through the use of standardised protocols, to apply this method for quantitative analysis, by calculating the concentrations of DNA detected (in this case mainly in cases where presence is already established and abundance data are required). The main advantage of this technique is the possibility to analyse the presence of a species in the very early stages of colonization, going to act promptly with protocols of eradication/ containment ad hoc. At the same time, the main disadvantage is the application costs, both for the field part and for the analysis part.

1.2.5 Category #5: FRESHWATER ANIMALS

Lakes, rivers and wetlands are among the most biodiverse environments, thanks to the high concentration of resources and microhabitats, composed by different matrices like the surface, the water column, the bottom, riffle-pool areas, temporary puddles, etc. Globally, freshwater environments represent 0,01% of all the water and 0,08% of land, yet hosting over 100.000 living species (about 6% of all known species).

Nowadays, we are unfortunately facing a constant degradation of these environments, both for direct and indirect causes driven by human activities. Italy has a higher number of IAS, compared to other European countries, especially among fishes (1 out of 2 species are exotic, caused mainly by voluntary introduction for fishing activities and aquaculture).

Studying and monitoring freshwater cenosis is therefore fundamental, in order to get a better picture for the understanding of the perturbed ecosystem and the undertake of specific conservation measures.

2.2.5.1 ELECTRO FISHING

Description: electric fishing is one of the most used monitoring techniques to investigate the fishing community of a freshwater body. The varied composition of aquatic ecosystems involves the use of diverse sampling tools and modes, and it is not possible to define a single monitoring protocol valid for all environments. In principle, however, there are two main sampling procedures: the first is valid for wadable watercourses (maximum depth 0,7 - 1 m also depending on the current speed) and the second for non-wadable watercourses, lakes and artificial reservoirs. Both procedures involve non-lethal sampling and release of individuals to the same capture sites.

Variables to be detected: species identification, sex (where possible), size, weight.

Time scale: annual, depending on the activity peaks of the species.

Detection frequency: once a year.

Staff required: 3-4 people needed, with an average of 4-6 hours per site, depending on safety reasons related to the riparian and riverbed conditions. In addition, provision should be made for the timing of the return of summary phase data.

Equipment:

- Field material
- Electroshunner
- Rubber underarm boots or other insulating material
- Personal protective equipment (helmet, life jacket, insulating gloves, first aid kit, etc.)
- Net with insulating handle
- Plastic containers for the transport and storage of captured specimens
- Electronic digital scale
- Ichthyometer
- Boat (in the case of sampling in lentic or lotic large waters)

Costs: high. The costs relate to the large number of operators involved, adequately trained and certified, as well as to the equipment required for sampling, subject to periodic safety reviews.

Goals: the goal of the monitoring is the characterization of the fishing community of a given survey area, both in terms of quality (list of species present in the territory) and of abundance and structure of populations. As part of the allochthonous species study, this method can be applied both to monitor the presence/absence of certain target species from areas, and to determine the absolute and relative abundance of these species within that study area, to quantify the degree of ingression of the species and the load it exerts on this environment. This methodology also allows calculating the structure of the population, through the subdivision of the individuals caught in size classes, in order to obtain information related to the population dynamics of the species in question. Electrofishing is one of the most used monitoring techniques in the field of ichthyofauna, thanks to the high catch rate and the speed of execution, which takes place within a few hours. The main disadvantage is provided by the economic factor, since both the number of operators and the equipment required are expected to be relatively high costs for the implementation of the campaign (taking into account, however, that monitoring protocols in the aquatic environment are on average more expensive than in the terrestrial environment, regardless of the techniques used).

2.2.5.2 USE OF FISHING NETS

Description: the use of nets for the capture and monitoring of aquatic fauna implies the presence of equipment and techniques now consolidated over the years. Over time, countless types of nets and fishing techniques have been perfected, but their comprehensive description is beyond the scope of this report. However, two major macro-categories can be identified:

- *gillnets: nets whose operation is based on their arrangement in such a way as to form a vertical barrier and allow the prey to remain entangled (usually through the use of stoppers and sinkers for the maintenance of the position). There are many types of mail networks, with different operating techniques. They are in any case divided into two types: fixed (anchored to the bottom) and deriving (free to move with the current).*
- *pots: nets consisting of a rigid metal or plastic mesh, with a typical funnel structure that allows the animal to enter and not be able to exit. This type of net is used not only for fish species but also for other categories of organisms, including crustaceans.*

Variables to be detected: species identification, sex (where possible), size, weight.

Time scale: annual, depending on the activity peaks of the species.

Detection frequency: once a year.

Staff required: employ at least three people, with an average of 4-6 hours. In addition, provision should be made for the timing of the return of summary phase data.

Equipment:

- field material

- nets
- rubber underarm boots (in the case of wadable river sampling)
- boat (if applicable in the case of sampling in lentic or lotic large waters)
- plastic containers for the transport and storage of captured specimens
- digital electronic scale
- ichthyometer

Costs: high. The costs relate to the high number of operators involved and the equipment required for sampling, including the boat.

Goals: the goal of the monitoring is the characterization of the fishing community of a given survey area, both in terms of quality (list of species present in the territory) and of abundance and structure of populations. As part of the allochthonous species study, this method can be applied both to monitor the presence/absence of certain target species from areas, and to determine the absolute and relative abundance of these species within that study area, to quantify the degree of ingression of the species and the load it exerts on this environment. This methodology also allows calculating the structure of the population, through the subdivision of the individuals caught in size classes, in order to obtain information related to the population dynamics of the species in question. Net fishing is widely used in the area of ichthyofauna monitoring. The use of conventional nets is used where the use of the electrostunner is impractical due to the conformation of the bed, the current, the depth, etc. From the economic point of view, the purchase of networks represents the largest expenditure, which can be amortised over time. From the point of view of human resources, the number of operators may be slightly lower than electro fishing, although the time required for sampling is higher.

2.2.5.3 MONITORING WITH MACROINVERTEBRATES SAMPLING NETS

Description: the monitoring protocol involves the use of a net with a fine mesh (500 µm), similar to the one used for the calculation of the Extended Biotic Index (EBI), with which to sieve the body of water concerned. Where possible, sampling between vegetation and sediment should be carried out at different depths: surface, medium and close to the bottom. In order to compare measurements, it is necessary to define a unit of time for sampling, such as an hour or a fraction thereof. Monitoring should be repeated over the years in order to assess the consistency and numerical development of populations, and therefore sites should be selected where no anthropogenic interventions are foreseen which could significantly change their structure.

Variables to be detected: identification of species, counting of individuals.

Time scale: seasonal, depending on the activity peaks of the species.

Detection frequency: one or more times during the year (up to one sampling per season), depending on the population dynamics of the species to be monitored.

Staff required: the presence of a single operator is sufficient. However, the presence of a second person is recommended in stations with difficult access or security problems. In addition, provision should be made for the timing of the return of summary phase data.

Equipment:

- field material
- sampling net
- chest waders
- buckets for the transport of the captured specimens.
- field analysis equipment (trays, lenses, pliers, spoons, alcohol, etc.).
- laboratory equipment
- stereoscopic/biological microscope

Costs: medium-low. Costs relate to the number of operators involved and the equipment required for sampling.

Goals: the goal of the monitoring is the characterization of the invertebrate community of a given survey area, both in qualitative terms (list of species present in the territory) and quantitative (estimates of abundance). As part of the allochthonous species study, this method can be applied both to monitor the presence/absence of certain target species from areas, and to determine the absolute and relative abundance of these species within that study area, to quantify the degree of ingression of the species and the load it exerts on this environment. This method is used for the analysis of aquatic macro-invertebrate communities, with a relatively limited investment in human resources and equipment. The use of the microscope in the laboratory is indicated in the case of the presence of species whose dimensions require magnifying instruments for correct identification.

2.2.5.4 CITIZEN SCIENCE PROJECTS

Description: these projects involve citizenship through actions of citizen science, already defined in the previous sections. The contribution of the population to active surveillance on the territory may be a crucial resource in the fight against invasive species, in particular against highly recognisable and easy-to-contact animal species. Many projects have used this strategy (CSMON-LIFE, Life ASAP, SINAnet ISPRA), which initially provides for the production of information material aimed at facilitating the identification of target species by the citizens, and consequently the production of specific applications useful to collect such reports and put them in a common database, where they will be validated by experienced staff.

Variables to be detected: identification of the species; photographs (by citizens); checks and validation of information (by experienced staff).

Time scale: since monitoring is entrusted to the citizenship, the whole course of the year is potentially valid as a data collection period.

Detection frequency: variable, information is collected at different times by citizens.

Staff required: only one expert technician is needed to analyse and evaluate the data collected by the citizen.

Equipment:

- materials for work:
 - ad hoc informatics platforms
 - informative support for campaigns

Costs: low. The necessary resources are those relating to the training of the citizen-scientist involved in the project and, secondly, in the analysis of the data provided by the citizenship. Effective and good quality training is essential to reduce the main problems involved in using such initiatives, consisting mainly of errors or difficulties in identifying the species committed by the citizen-scientist, resulting in false positives/negatives.

Goals: the goal of the monitoring technique is to succeed, through the involvement of the citizens, in identifying and mapping the distribution of target species, especially in qualitative terms. The method is particularly effective for the monitoring of easily recognizable and contactable alien species, also for the possibility of supporting each sighting by the citizen-scientist with photographic material, which can subsequently be validated by the experienced technician. A further benefit of the application of the method is related to the dissemination of knowledge about the problem of alien species in the population involved in the project. In particular at training events, during which the correct data about the typology, the distribution and the dangerousness of the allochthonous species present in the territory are supplied, with consequent progressive sensitization of the active citizenship in the face of the problem.

2.2.5.5 ENVIRONMENTAL DNA ANALYSIS

Description: the method consists of the collection of DNA fragments from organisms in the environment in which they live (biological fluids or tissue fragments), from which laboratory analysis, using DNA amplification and sequencing techniques, data of presence/absence and relative abundance of harvested species can be obtained. The aquatic environment appears to be optimal to this type of approach, thanks to the rapid dispersion and fragments in the water body and the ease of sampling. The permanence of these fragments, combined with the techniques of amplification and sequencing of DNA, allows obtaining a checklist of animal and plant species. In addition to the production of presence/absence data, it is also possible to derive relative abundance data from the samples, thanks to the percentage of obtained sequences attributed to the various species.

Variables to be detected: DNA traces belonging to the species of interest.

Time scale: annual, depending on the activity peaks of the species.

Detection frequency: once a year, necessary to monitor the presence and/or abundance status, preferably in conjunction with the activity peaks of the species.

Staff required: for the field phase, is sufficient to use only one technician to take care of the collection of samples and the correct conservation. The analytical phase must provide for the preparation of the sample, the extraction, amplification by PCR and sequencing of the obtained amplicons, in addition to the alignment of the sequences on a specific basis and the phylogenetic interpretation of the results obtained.

Equipment:

- field phase:
 - chest waders (in the case of wadable river sampling)
 - boat (if applicable in the case of sampling in lentic or lotic large waters)
 - material for the storage of samples
 - sampling material (cans/bags, etc.)
- analytical phase:
 - structures and equipment for extraction, amplification, purification and sequencing of environmental DNA samples
 - software for the insertion and analysis of phylogenetic data

Costs: high. The costs relate mainly to the analytical phase, which includes the costs necessary for the processing of environmental DNA samples and their sequencing, usually in specialised laboratories.

Goals: The goal of the monitoring is the detection of one or more species of a predefined study area, especially in qualitative (checklist of animal species) and semi-quantitative terms (successive estimates of relative abundance, which however require the use of additional molecular techniques, resulting in increased costs). As part of the study of alien species, this method is applied in the early identification of a target species in the early stages of colonization, when classic monitoring protocols would give unsatisfactory results to less intensive and relatively extensive campaigns. Predominantly, this technique is used for analysis of the presence/absence of a species in a certain matrix, through the identification of specific DNA sequences. However, it is possible, through the use of standardised protocols, to apply this method for quantitative analysis, by calculating the concentrations of DNA detected (in this case mainly in cases where presence is already established and abundance data are required). The main advantage of this technique is the possibility to analyse the presence of a species in the very early stages of colonization, going to act promptly with protocols of eradication/ containment ad hoc. At the same time, the main disadvantage is the application costs, both for the field part and for the analysis part.

2.2.5.6 VISUAL CENSUS

Description: the Visual Census is a sampling technique that takes place in immersion and that allows to record the aquatic flora and fauna simply by observing the species and recording the sightings. It is usually carried out in immersion with the help of a self-contained breathing apparatus. Applied mainly in the marine environment, cases of application are also known in the aquatic environment, where the chemical-physical conditions of the basin allow it.

Variables to be detected: number of individuals; area occupied by colonies (variable according to species and their biology).

Time scale: annual, depending on the activity peaks of the species.

Frequency of detection: once a year, better if in conjunction with the peaks of activity of the species.

Staff required: it is necessary to employ at least two Scientific Divers, in addition to the support staff assigned to the logistics of the vehicles. At least two hours should be used for each sampling station, in addition to the time needed for morphological analysis of any samples collected by appropriate laboratory equipment.

Equipment:

- Field material
- diving equipment for diving
- boat
- equipment for the delimitation of transects (ropes, weights, etc.)
- any signalling buoys for site identification in subsequent samples
- underwater data collection material (underwater notebook)

Costs: high. The costs relate to the personnel involved, the rental of the boat and the diving equipment necessary for data collection. In addition, provision should be made for the timing of the return of those summary phase data.

Goals: the goal of monitoring is the characterisation of the community of a given study area, both in qualitative terms (presence/absence) and in quantitative terms (estimates of abundance). In the case of the allochthonous species study, this methodology may be applied in the early identification of certain target species, in areas where their presence has not yet been established, and in the estimation of their abundance in a given area of interest. This protocol applies to both highly dispersive species, such as nektonic species, and sessile and/or benthic species. The main advantage that makes the Visual Census in a freshwater environment preferable to other traditional methods (fishing, poisons, and electro stunners) is given above all by the high environmental compatibility, factor that makes it an ideal protocol in protected areas. The disadvantage compared to other techniques is the high cost caused by the presence of the boat and specialized operators for scuba diving.

2.2.5.7 FAUNAL TRANSECT SURVEY

Description: observation and/or listening of all species along a predefined path, which may also provide for the estimation of the distance of each observation. Among the various types of wildlife monitoring we can mention, the Visual Encounter Survey (VES). It is mainly applied in the field of herpetology, which is based on the counting of individuals along transects or in known areas, including in wetlands where there are sites with difficult access to the water body but from which there is also good visibility of the banks.

Variables to be detected: number of individuals and of any spawning.

Time scale: annual, depending on the activity peaks of the species.

Detection frequency: once a year, better if in conjunction with the peaks of activity of the species.

Staff required: a single operator is sufficient, while considering a more intensive recruitment depending on the extent of the sampling, to be concentrated in the most active moments of the species of interest. In addition, provision should be made for the timing of the return of summary phase data.

Equipment:

- field material
- binoculars
- waders in the case of wadable waterbodies

Costs: low. The costs relate to the personnel involved, and to the field equipment (binoculars and waders, if any).

Goals: the goal of the monitoring is the characterisation of the community of a given area of study, both in qualitative terms (presence/absence) and in quantitative terms (mainly, through estimates of abundance). In the case of the allochthonous species study, this methodology may be applied in the early identification of certain target species, in areas where their presence has not yet been established, and in the estimation of their abundance in a given area of interest. This protocol is very versatile, as it does not require expensive equipment or large human resources, making it suitable for collecting data at relatively low cost. The constraint of this methodology is the applicability of the method, valid for those species whose identification is possible without diving or catching in the aquatic environment (e.g. aquatic reptiles, such as *Trachemys* sp.).

1.2.6 Category #6: MARINE ANIMALS

The Mediterranean basin is a biodiversity hotspot, with more than 17.000 species, of which one fifth is endemic. At the same time, its marine ecoregions show high levels of degradation, because of the threats they are exposed to such as IAS. Specifically, most of the introductions have been caused by Suez Canal opening, ballast waters of cargo ships, aquaculture and aquarium-related transports.

To our days, about 1.000 species have been introduced in the Mediterranean Sea, with an estimate of a 50% stabilizing. These species can substitute the native species, causing a loss of biodiversity, alteration of the habitats, trophic chains and ecosystem processes, together with economical and sanitary impacts.

Marine biodiversity protection is therefore a main goal for Italy, achievable through the adoption of standardized monitoring protocols and data collection, in order to transpose European obligations and guidelines.

2.2.6.1 USE OF UNDERWATER CAMERAS AND SONAR

Description: SONAR (Sound Navigation And Ranging) is a technique that uses the propagation of sound waves to identify and investigate objects below the surface of the water. Widely used for underwater and fisheries surveys, examples of the use of this technology for monitoring benthic communities are also known in the literature, in order to reconstruct detailed seabed maps describing the distribution and abundance of species of interest, identifying potential distribution areas on the appropriate seabed tracts. This methodology can be supported by video analysis via underwater cameras, in order to increase the level of detail and possibly predict the sampling of sediments in targeted areas.

Variables to be detected: analysis of sonar devices; analysis of the videos performed.

Time scale: annual.

Detection frequency: once during the year.

Staff required: 3-4 operators are required, responsible for driving the boat, using the equipment, and collecting any sediment samples. In addition, provision should be made for the timing of analysis of the data provided by the instrumentation, sediment analysis and return of the summary phase data.

Equipment:

- field material
- boat (appropriate to survey basin)
- SONAR equipment adapted to the type of information required
- cameras
- software for data processing
- laboratory equipment
- sediment analysis microscope (if designed)

Costs: high. The costs relate to the personnel involved, necessary also in the possible phase of analysis of the sediments, to the chartering of the boat and the necessary equipment.

Goals: the goal of the monitoring is the characterisation of the community of a given area of study, both in qualitative terms (presence/absence) and in quantitative terms (mainly, through estimates of abundance). In the case of the study of alien species, this methodology can be applied in the early identification of certain target species, in areas where their presence has not yet been established, but especially in estimates of abundance of marine communities. This technology is in fact mainly applied in the analysis of the extent of populations of benthic organisms, from algal prairies (e.g. *Caulerpa* sp.) to marine animal communities such as sponges, corals, etc. This technique allows a fine mapping of the biotic communities of the study area, returning valuable details for future management plans. The main disadvantage is mainly due to the economic factor, requiring such a monitoring protocol of very specific equipment and staff and high cost.

2.2.6.2 VISUAL CENSUS

Description: the Visual Census is a sampling technique that may take place in immersion and that allows to record the aquatic flora and fauna simply by observing the species and recording the sightings. In immersion, it is usually carried out with the help of a self-contained breathing apparatus.

Variables to be detected: number of individuals; area occupied by colonies (variable according to species and their biology).

Time scale: annual, depending on the activity peaks of the species.

Frequency of detection: once a year, better if in conjunction with the peaks of activity of the species.

Staff required: at least two operators, responsible for monitoring and guiding the boat. Evaluate the presence of multiple operators linked to the extent of sampling and safety reasons.

Equipment:

- Field material
- diving equipment
- boat
- equipment for the delimitation of transects (peaks, dead bodies, etc.)
- any signalling buoys for site identification in subsequent samples
- underwater data collection material (underwater notebook)

Costs: medium to high. The costs relate to the personnel involved, the rental of the boat and the diving equipment necessary for data collection.

Goals: the goal of monitoring is the characterisation of the community of a given study area, both in qualitative terms (presence/absence) and in quantitative terms (estimates of abundance). In the case of the allochthonous species study, this methodology may be applied in the early identification of certain target species, in areas where their presence has not yet been established, and in the estimation of their abundance in a given area of interest. This protocol applies to both highly dispersive species, such as nectonic species, and sessile and/or benthic species. The main advantage that makes the Visual Census in marine environment preferable to other traditional methods (sonar, use of fishing nets, etc.) is given above all by the high environmental

compatibility, factor that makes it an ideal protocol in protected areas. The economic factor remains, however, a limiting factor, as although the costs are lower than other monitoring techniques with specific equipment (see sonar, previous chapter) the presence of specialized operators and the use of any boats make it a technique not always applicable.

2.2.6.3 USE OF FISHING NETS

Description: the use of nets for the capture and monitoring of aquatic fauna implies the presence of equipment and techniques now consolidated over the years. Over time, countless types of nets and fishing techniques have been perfected, the comprehensive description of which falls outside the scope of this paper. However, two major macro-categories can be identified:

- *gillnets*: nets whose operation is based on their arrangement in such a way as to form a vertical barrier and allow the prey to remain entangled (usually through the use of stoppers and sinkers for the maintenance of the position). There are many types of mail networks, with different operating techniques. They are in any case divided into two types: fixed (anchored to the bottom) and deriving (free to move with the current).
- *pots*: nets consisting of a rigid metal or plastic mesh, with a typical funnel structure that allows the animal to enter and not be able to exit. This type of net is used not only for fish species but also for other categories of organisms, including crustaceans.

Variables to be detected: species identification, sex (where possible), size, weight.

Time scale: annual, depending on the activity peaks of the species.

Detection frequency: once in a year, with an average of 4-6 hours per site.

Staff required: at least 3 persons are required, with an average of 4-6 hours. In addition, provision should be made for the timing of the return of summary phase data.

Equipment:

- field material
- nets
- chest waders (in the case of wadable river sampling)
- boat (if applicable in the case of sampling in lentic or lotic large waters)
- plastic containers for the transport and storage of captured specimens.
- digital electronic scale
- ichthyometer

Costs: high. The costs relate to the high number of operators involved and the equipment required for sampling, including the boat.

Goals: the goal of the monitoring is the characterization of the fishing community of a given survey area, both in terms of quality (list of species present in the territory) and of abundance and structure of populations. As part of

the allochthonous species study, this method can be applied both to monitor the presence/absence of certain target species from areas, and to determine the absolute and relative abundance of these species within that study area, to quantify the degree of ingression of the species and the load it exerts on this environment. This methodology also allows calculating the structure of the population, through the subdivision of the individuals caught in size classes, in order to obtain information on the population dynamics of the alien species under study. Net fishing is widely used in the area of ichthyofauna monitoring. In the case of the marine environment, the use of conventional nets is economically more sustainable than other techniques with specific equipment. The purchase of networks is in fact the largest expenditure, which can however be amortised over time. From the point of view of human resources, the number of operators is comparable to that of other techniques previously analysed.

2.2.6.4 CITIZEN SCIENCE PROJECTS

Description: these projects involve citizenship through actions of citizen science, already defined in the previous sections. The contribution of the population to active surveillance on the territory may be a crucial resource in the fight against invasive species, in particular against specific recognisable and easy-to-contact animal species. Many projects have used this strategy (CSMON-LIFE, Life ASAP, SINAnet ISPRA), which initially provides for the production of information material aimed at facilitating the identification of target species by the citizens, and consequently the production of specific applications useful to collect such reports and put them in a common database, where they will be validated by experienced staff.

Variables to be detected: identification of the species; photographs (by citizens); checks and validation of information (by experienced staff).

Time scale: since monitoring is entrusted to the citizenship, the whole course of the year is potentially valid as a data collection period. The period of effectiveness of the same is, however, variable from species to species. Frequency of detection: variable, information is collected at different times by citizens.

Staff required: only one expert technician is needed to analyse and evaluate the data collected by the citizen.

Equipment:

- materials for work:
 - *ad hoc* IT platforms
 - informative support for campaigns

Costs: low. the necessary resources are those relating to the training of the citizen-scientist involved in the project and, secondly, in the analysis of the data provided by the citizenship. Effective and good quality training is essential to reduce the main problems involved in using such initiatives, consisting mainly of errors or difficulties in identifying the species committed by the citizen-scientist, resulting in false positives/negatives.

Goals: the goal of the monitoring technique is to succeed, through the involvement of the citizens, in identifying and mapping the distribution of target species, especially in qualitative terms. The method is particularly effective for the monitoring of easily recognizable and contactable alien species, also for the possibility of supporting each sighting by the citizen-scientist with photographic material, which can subsequently be validated by the experienced technician. A further benefit of the application of the method is related to the

dissemination of knowledge about the problem of alien species in the population involved in the project. In particular at training events, during which the correct data about the typology, the distribution and the dangerousness of the allochthonous species present in the territory are supplied, with consequent progressive sensitization of the active citizenship in the face of the problem.

1.3 Application in the Apulian Region

This section aims to illustrate the main application examples of the monitoring techniques described in the previous chapters, focusing on the Apulian territory and thus extending its application potentials.

2.3.1 OVERVIEW OF THE PROPOSED MONITORING TECHNIQUES

This chapter relies on previously applied monitoring protocols in the Apulian territory.

For the terrestrial plants, the most relevant IAS which have been subject of monitoring protocols are *Ailanthus altissima*, *Robinia pseudoacacia*, *Acacia saligna*, *Yucca gloriosa*, *Carpobrotus edulis*, *Paspalum distichum*, *Senecio inaequidens*. For *Ailanthus altissima* and *Robinia pseudoacacia*, through Life Project *Alta Murgia* (2013-2019), Braun-Blanquet phytosociological technique and the botanical transect have been adopted. Similarly, for LIFE *Zone Umide Sipontine* (2010-2016), Braun-Blanquet technique has been adopted to monitor *Carpobrotus edulis* and *Paspalum distichum*. Other species like *Yucca gloriosa* and *Acacia saligna* have been monitored through LIFE *Diomedee* (2018-still ongoing).

Freshwater aquatic plants monitoring protocols have focused mainly on presence/absence data (Beccarisi *et al.*, 2007), through plant survey.

Concerning marine macrophytes, the LIFE citizen science project *CSMON-LIFE (Citizen Science MONitoring)*, 2016-2019) has adopted specific campaigns, with the partnership of the Region, dedicated to giving the citizens the tool to detect IAS on the territory, such as *Codium fragile* and *Caulerpa racemosa*.

Terrestrial animals with low-motility habits, such as micromammals, have been targeted by projects like *LIFE Diomedee*, concerning the eradication of *Rattus rattus* and *R. norvegicus* on the Tremiti islands. A different situation is represented by those species characterized by high capabilities of dispersion, as for the sacred ibis (*Threskiornis aethiopicus*), for which a specific National Management Plan (2020) has been adopted. Other citizen science projects like *LIFE+ ASAP (Alien Species Awareness Program)*, 2016-2020) and *CSMON-LIFE (Citizen Science MONitoring)*, 2016-2019) have focused on other species like the monk parakeet (*Myopsitta monachus*) and the rose-ringed parakeet (*Psittacula krameri*). Among insects, trapping methods have been widely used, like for the spotted wing drosophila (*Drosophila suzukii*, Antonacci *et al.*, 2017), or the red palm weevil (*Rhynchophorus ferrugineus*), on behalf of the Department of Agriculture.

In freshwater environments, electrofishing is one of the most widely used monitoring techniques. In this respect, Bianco and de Filippo (2011), monitored the populations of *Cyprinus carpio*, *Carassius auratus*, *Ameiurus melas*, *Lepomis gibbosus* and other species inside Ofanto River Regional Park. Net fishing has been used for invertebrate species like *Procambarus clarkii*, together with other citizen science projects (*LIFE+ ASAP* 2016-2020) and a National Management Plan (2021).

Marine environments have been investigated through netfishing and underwater visual census techniques, involving fishermen (Azzurro *et al.*, 2016) to detect easily recognizable species, like *Lagocephalus sceleratus*. Visual census techniques may be used to monitor benthonic species like *Arcuatula senhousia*, *Clythia hummelincki*, *Paraleucilla magna* (Gravili *et al.*, 2008; Longo *et al.*, 2007; Mastrototaro *et al.*, 2014). Also, the utilization of sonar instruments to map

benthonic cenosis has been investigated, as for Matarrese *et al.* (2014), identifying species like *Arcuatula senhousia* inside the Gulf of Taranto.

2.3.2 APPLICATION IN THE APULIAN TERRITORY

While the previous chapter tries to depict a general picture of the main monitoring techniques used in the Apulian territory, this chapter wants to extend this picture, individuating those environments, target species and monitoring protocols which could be applied for the first time in this Region.

Puglia Region has the most extended coastal development (865km) in Italy, with very different environments that can host a great variety of species, including IAS. Concerning marine macrophytes, visual census techniques and citizen science monitoring campaigns could be easily applied.

On those rocky coastal environments difficult to access, and where exotic species may find a suitable habitat, remote sensing protocols may be used, in order to map the presence and the extension of the plant communities.

On the contrary, for those easy-to-access environments like sandy beaches, where exotic species like *Yucca gloriosa*, and *Carpobrotus edulis* can rapidly spread, Braun-Blanquet surveys represent a very suitable monitoring technique, as confirmed by the experience of Life Project Diomedee.

For freshwater aquatic plants, botanic transects can represent the best way to characterize the composition of the plant communities from a qualitative point of view. Species belonging to the genus *Azolla* and *Lemna* can be easily individuated and monitored with these relatively low-budget protocols.

More than half of Apulian territory is plain, characterized by human activities such as agriculture and urbanization. These areas are more susceptible of being invaded by tree species, easily detectable through botanic transects and through citizen science projects.

Particular attention must be directed to those high value territories represented by Protected Areas (Natura 2000 network, Natural Reserves and Parks), where the concentration of native and eventually endemic species is higher, thus representing a greater risk for local biodiversity (see Life Projects *Alta Murgia*, *Diomedee* and *Zone Umide Sipontine*). Through qualitative and quantitative monitoring techniques (like Braun-Blanquet or botanic transects), IAS can be detected and quantified, and through data collection it will be possible to set up specific predictive models.

Relatively to terrestrial animals, trapping techniques can be used for micromammals, but eradication protocols must be applied only in specific restricted areas where the ingression of new individuals can be easily monitored. In other context, trapping can be an efficient method to quantify the dimension of the local populations and to monitor population dynamics trends through time.

For high-motility animals, like birds, in the Apulian region we can identify some species characterized by gregarious habits (during nesting season or through roost sites), like *Threskiornis aethiopicus*, *Myopsitta monachus* and *Psittacula kramera*, for which s can be applied.

Flying insects can be monitored as well through trapping protocols, as for *Halyomorpha halys*, *Aleurocanthus spiniferus*, both considered agricultural pests, and *Rynchophorus ferrugineus*. For the latter, many monitoring protocols have been developed, based on chemicals, thermal or acoustic signals.

In additions, some monitoring protocols dedicated to the early identification of expanding species have been proposed, based mainly on environmental DNA analyses. The most recent amplification and sequencing techniques allows to accurately identify the presence of some target species with a relatively low sampling effort, making it suitable for analysing new areas, as for the Argentine ant (*Linepithema humile*), still not widely distributed in the Region.

In the freshwater environment, electrofishing represents one of the most common monitoring techniques, thanks to its broad applicability (like, for Apulian region, for *Micropterus salmoides*, *Perca fluviatilis* or *Gambusia holbrooki*. Also, Visual Census can be applied for some fish species. Netfishing can be applied both for vertebrates or invertebrates, as for *Trachemys scripta* spp. or *Procambarus clarkii*. For the latter, the macroinvertebrates sampling net can be used as well, together with other species like *Potamopyrgus antipodarum* (for which environmental DNA analyses have been already conducted from Goldberg *et al.* (2013).

Marine IAS can be targeted through citizen science projects and through the collaboration with fishermen who may directly contact these species (like *Callinectes sapidus*, *Percnon gibbesi*, *Fistularia commersonii*, etc.). Specific campaigns based on netfishing or visual census monitoring protocols can be applied as well, both for vertebrates and invertebrates.

Section 3: Exemplary management plan

The present section describes an example of how a management plan could be structured, on the base of which the future specific management plans will be produced, relying on the previously described macrocategories. The sections of the document are illustrated, with a brief description of them.

1. PREMISE

This chapter will provide a general overview of the species, introducing the main goals of the monitoring plan and explaining the reasons behind the choice of that species. It will be an abstract of the report, detailed in the following chapters.

Specifically, the chapter will provide a general introduction on the characteristics of the invasive species with a focus on the Apulian territory, with the most recent data about the following features:

- velocity of diffusion
- tendency to dominance
- level of threat towards local biodiversity
- extension at the regional level

2. DESCRIPTION OF THE SPECIES

The species will be described taxonomically, morphologically and according to its ecological habits. There will be a focus on its reproductive characteristics, life cycle and its typical habitats and species it interacts with.

Specifically, the chapter describes the main morphological and ecological features of the species, with a focus on the modalities of spread and reproduction, highlighting the most important aspects (included at a local level) about the invasiveness of the species.

3. DISTRIBUTION AND PATHWAYS OF INTRODUCTION

The chapter will focus on the distribution of the species, starting from its original territory till its introduction around the globe and in Italy, with a special focus in Puglia Region (depending on the available data). Subsequently, the main drivers behind its current distribution will be analysed in detail, describing the main pathways of introduction and dispersal movement of the species.

The chapter provides in detail the information about the national and international distribution of the species; the focus on the local scale is partially based on data previously collected during the same project (dedicated online databases, websites, scientific literature and documented sightings), integrating the data collected through the specific survey designed for the local managers of Protected Areas, in order to evaluate the risks related to the local IAS (up to five species for each area were identified), the most vulnerable habitats and other eventual ongoing projects.

4. IMPACTS

This section will be focused on the description of the impacts caused by the species, analysing the effects on the ecosystem through the interaction with biotic and abiotic factors, and individuating the eventual economic and sanitary impacts as well.

5. REGULATORY ASPECTS

Legislative and regulatory aspects are fundamental for the managers of the action plans, in order to respect European, national and/or local guidelines and set up campaigns based on standardized methodologies.

6. MONITORING PLAN

This chapter collects the information required to correctly apply the monitoring protocols, aiming at collecting information on the current distribution and abundance of the species.

The chapter is composed of the following sections:

- **Bibliographic research:** The main bibliographic tools are provided. Through bibliographic research, monitoring campaigns and previously documented sightings, all the data were collected and merged together, in order to provide a robust starting point from which we intend to set up the monitoring plan.
- **Description of the monitoring plan:** the chapter describes the planned monitoring actions for each target species, evaluating the different techniques and declining them according to the different ecological condition of the target area.

For each phase of the monitoring plan the main data output to provide are described, in order to produce homogeneous final reports. Specifically, the following outputs are required:

- Mapping of the target species, the data will be provided through GIS softwares;
- Sampling design: number of monitoring sites, technical details and materials required, as well as the temporal information to correctly carry out the operation: each plan has a temporal diagram with the description of the different steps;
- Synthetic data format; the information provided by the technician are described, in order to produce a standardized report, including some exemplary survey cards
- **Eventual risks related to the monitoring operations:** both for the environment and for the technicians
- **Evaluation of the monitoring results:** the variables to evaluate the results of the monitoring plan are provided (e.g. in case of a target IAS, the reduction of the species in the intervention area). The chapter describes the measurement modalities of the parameters, and the sequence of required actions are described through a flux diagram.
- **Temporal structure of the monitoring plan,** which synthetize the temporal features according to which the monitoring plan must be conducted.

7. MANAGEMENT PLAN (ERADICATION/CONTAINMENT)

Through bibliographical surveys, monitoring campaigns and documented sightings, this chapter aims to collect all the proper information necessary to produce specific monitoring protocols. For each control strategy the general goals (eradication or containment) and the specific goals are analysed, depending on the different methodologies. The chapter will individuate the most efficient methodology to reach the previously described goals, defining the applicability.

Specifically, the chapter describes:

- **Management plan goals.** The main data on the target species, including its distribution at the regional level, are described, in order to individuate a feasible management objective (e.g. eradication/containment from a specific area).
- **Description of the management plan.** The proposed management actions are described in detail, evaluating each action according to the different ecological variables.

For each phase of the monitoring plan the main data output to provide are described, in order to produce homogeneous final reports. The monitoring plan describes in detail all the proposed steps, like:

- Details on the machinery or other tools to be used to reach the proposed goals
- Management of the collected IAS during the interventions
- **Eventual risks related to the monitoring operations:** both for the environment and for the technicians
- **Temporal structure of the monitoring plan,** which synthetize the temporal features according to which the monitoring plan must be conducted.
- **Staff required.** The section provides information about the required skills for the employed staff, for each step.
- **Evaluation of the monitoring results and future actions:** The future actions to undertake depending on the monitoring and management phases are described, aiming at evaluating the effectiveness of the interventions, measured as reduction of the target species, absence of recolonization, etc. The chapter describes, through flux diagrams, the sequence of required actions and the temporal patterns which must be respected.

8. PREVENTION AND COMMUNICATION

Scientifically effective prevention and communication actions and suggestions will be described in this chapter, in order to cooperate with the population in containing the diffusion of the species on the territory. The chapter is based on the conclusions of the monitoring and management plans, as well as on the already existing strategies from other projects, aiming at reaching the main goal of this project.

As well as the previous chapter, these actions contains information on the temporal range, required resources and cost estimates. A focus on the role of the institutions and the stakeholders in managing the distribution of the species is provided, aiming at explaining the necessity of controlling the target species and describing its impacts on the environment, economy and health. In this sense we underline how the behaviour of the citizenship may lead to the spreading of a new IAS on the territory, often unconsciously, and how proper communication campaigns may prevent these risks.

The plan details the different communication steps that must be adopted, like:

- Pre-monitoring plan phase
- Pre-management plan phase
- Post-management plan phase

9. CONCLUSIONS

This last chapter will provide the proper conclusions on the efficacy of the main monitoring techniques, analysing the points of strength and weakness of the proposed methodologies, compared to the initial goals proposed during the creation of the monitoring plan.

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<http://gamberialieni.divulgando.eu/>

<http://pcwd.info/wp-content/uploads/2015/12/Monk-Parakeets.pdf>

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Appendix 1: exemplary survey cards

As an integration to the present document, some exemplary survey cards are given, referring to the macrocategories of Section 2. Specifically, we provide

- Plant survey card: valid for Braun-Blanquet method, for phytosociological surveys and valid for terrestrial plants and freshwater aquatic plants, as defined in par. 2.2.1.1 and 2.2.2.1 of the present document.
- Freshwater aquatic plants card: valid for freshwater aquatic plants, as defined in par. 2.2.2.2 of the present document.
- Marine macrophytes card: valid for marine macrophytes surveys, as defined in par. 2.2.3.1
- Micromammals card: valid for the monitoring of small mammals communities through trapping as defined in par. 2.2.4.1
- Bird survey card: valid for the monitoring of avian communities, both for visual and acoustic surveys, as defined in par. 2.2.4.4. e 2.2.4.5
- Faunal survey card: valid for direct and indirect monitoring of vertebrates and freshwater animals, when applicable, as defined in par. 2.2.4.2 e 2.2.5.7
- Freshwaters fish survey card: valid for the monitoring of fish communities in freshwater bodies, both through electrofishing or through nets, as defined in par. 2.2.5.1 e 2.2.5.2
- Marine animals card: valid for the analysis of vertebrates and invertebrates communities, as defined in par. 2.2.6.

VEGETATION SURVEY CARD

Site Code..... Operators Date

Locality

Exposition

N	NE	E	SE
S	SW	W	NW

Coordinates.....

Altitude..... Steepness (°) geological substrate

vegetational composition.....

vegetational series

management

survey method. surface mqtotal coverage..... %

STRUCTURAL ANALYSIS

Layer n.	Height	Coverage %	Medium height m	Notes
7	> 25 m			
6	12 - 25 m			
5	5 - 12 m			
4	2 - 5 m			
3	0,5 - 2 m			
2	25 - 50 cm			
1	0 - 25 cm			

COVERAGE

- 5 = continuous (> 75 %)
- 4 = interrupted (50 - 75 %)
- 3 = sparse (25 - 50 %)
- 2 = low (5 - 25 %)
- 1 = sporadic (1 - 5 %)
- + = almost no coverage (< 1 %)

BRAUN-BLANQUET SCALE:

- 5 = coverage > 75 %
- 4 = coverage 50 - 75 %
- 3 = coverage 25 - 50 %
- 2 = abundant, but with coverage < 25 %
- 1 = well represented, but with cov. < 5 %
- + = present, but with very low coverage

PHENOLOGICAL SYMBOLS (MARCELLO)

- 000 = absence of the phenomenon
- +00 = beginning of the phenomenon
- ++0 = progression of the phenomenon
- +++ = apex of the phenomenon
- 0++ = decline of the phenomenon
- 00+ = ending of the phenomenon
- 000 = ended of the phenomenon

FORM

- W = woods
- L = lianas
- AL = woody shrubs
- E = epiphytes
- H = herbs
- M = bryophytes and lichens

Num.	Floristic composition	Layer	F	Cov. Class	Cov. %	Phenolog.	Notes
1		7					
		6					
		5					
		4					
		3					
		2					
		1					
2		7					
		6					
		5					
		4					
		3					
		2					
		1					
3		6					
		5					
		4					
		3					
		2					
		1					
4		6					
		5					
		4					
		3					
		2					
		1					
5		6					
		5					
		4					
		3					
		2					
		1					
6		6					
		5					
		4					
		3					
		2					
		1					
7		6					
		5					
		4					
		3					
		2					
		1					
8		5					
		4					
		3					
		2					
		1					

Num.	Floristic composition	Layer	F	Cov. Class	Cov. %	Phenolog.	Notes
9		5					
		4					
		3					
		2					
		1					
10		5					
		4					
		3					
		2					
		1					
11		5					
		4					
		3					
		2					
		1					
12		5					
		4					
		3					
		2					
		1					
13		5					
		4					
		3					
		2					
		1					
14		5					
		4					
		3					
		2					
		1					
15		5					
		4					
		3					
		2					
		1					
16		5					
		4					
		3					
		2					
		1					
17		5					
		4					
		3					
		2					
		1					
18		4					
		3					
		2					
		1					

Num.	Floristic composition	Layer	F	Cov. Class	Cov. %	Phenolog.	Notes
19		7					
		6					
		5					
		4					
		3					
		2					
		1					
20		7					
		6					
		5					
		4					
		3					
		2					
		1					
21		6					
		5					
		4					
		3					
		2					
		1					
22		6					
		5					
		4					
		3					
		2					
		1					
23		6					
		5					
		4					
		3					
		2					
		1					
24		6					
		5					
		4					
		3					
		2					
		1					
25		6					
		5					
		4					
		3					
		2					
		1					
26		5					
		4					
		3					
		2					
		1					

Num.	Floristic composition	Layer	F	Cov. Class	Cov. %	Phenolog.	Notes
27		5					
		4					
		3					
		2					
		1					
28		5					
		4					
		3					
		2					
		1					
29		5					
		4					
		3					
		2					
		1					
30		5					
		4					
		3					
		2					
		1					
31		5					
		4					
		3					
		2					
		1					
32		5					
		4					
		3					
		2					
		1					
33		5					
		4					
		3					
		2					
		1					
34		5					
		4					
		3					
		2					
		1					
35		5					
		4					
		3					
		2					
		1					
36		4					
		3					
		2					
		1					

Num.	Floristic composition	Layer	F	Cov. Class	Cov. %	Phenolog.	Notes
37		7					
		6					
		5					
		4					
		3					
		2					
		1					
38		7					
		6					
		5					
		4					
		3					
		2					
		1					
39		6					
		5					
		4					
		3					
		2					
		1					
40		6					
		5					
		4					
		3					
		2					
		1					
41		6					
		5					
		4					
		3					
		2					
		1					
42		6					
		5					
		4					
		3					
		2					
		1					
43		6					
		5					
		4					
		3					
		2					
		1					
44		5					
		4					
		3					
		2					
		1					

Num.	Floristic composition	Layer	F	Cov. Class	Cov. %	Phenolog.	Notes
45		5					
		4					
		3					
		2					
		1					
46		5					
		4					
		3					
		2					
		1					
47		5					
		4					
		3					
		2					
		1					
48		5					
		4					
		3					
		2					
		1					
49		5					
		4					
		3					
		2					
		1					
50		5					
		4					
		3					
		2					
		1					
51		5					
		4					
		3					
		2					
		1					
52		5					
		4					
		3					
		2					
		1					
53		5					
		4					
		3					
		2					
		1					
54		4					
		3					
		2					
		1					

MARINE MACROPHYTES SURVEY CARD

Site code Operators Date

Region City I.G.M.

Locality

Orientation of the
Coast

N	NE	E	SE
S	SW	W	NW

Morphology of the coast.....

Steepness of the coast (°) Exposition.....

Degree of artificialization.....

Type fo substrate.....

Sampled area (m²) Coordinates

	Taxon	Percentage of coverage
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		
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14		

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29		
30		
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32		
33		
34		
35		
36		
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41		
42		
43		
44		
45		
46		
47		
48		
49		
50		

10. Structure of the riverbed substrate (% of the different types)	
Stable and varied	
Slightly moveable	
Easily moveable	
Evenly compact	
Compact for artificialization	

11. Granulometry (% abundance of granulometric classes)	
Rock	
Stones	
Pebbles	
Gravel	
Sand	
Silt	
Artificial substrate	

12. upper-aquatic zone (if presente)		
Left bank		Right bank
	Surface % compared with medium water level riverbed	
	Linear extension % (compared with the length of the station)	
	Maximum transverse width (cm)	
	Mean transverse width (cm)	
	Plant coverage %	

Left bank	Types of plant coverages in the upper-aquatic zone	Right bank
	Dominant species	

13. Other plant communities in the river corridor		
Left bank	Riverbed communities (other cenosis in the medium water level riverbed)	Right bank
	Coverage % of the riverbed communities in the under-aquatic zones of the riverbed	

Left bank		Right bank
	Dominant species **	
	Coverage % of exotic species	
	Exotic species	

Left bank	External formations of the riverbed considerar 10-100 m of width dependind on the dimensions of the waterbody	Right bank
	Woody riparian cenosis	
	Herbaceous riparian cenosis	
	Sparce or absent plant coverage	
	Non riparian cenosis	
	Herbaceous coverage (even not hygrophilous ones) in primary grasslands	
	Anthropic plant communities (including farmlands)	
	Absence of plant coverage for anthropic reasons, and artificial banks	
	Mean considered width (m)	

Left bank		Right bank
	Dominant communities **	
	Dominant species **	
	Coverage % of exotic species	
	Exotic species	

** list the dominant species from the proximal to the distal areas from the river

14. physicochemical parameters	
Conductivity (S/cm)	
Temperature (°C)	
Dissolved oxygen (mg/L)	
Dissolved oxygen (% sat)	
pH	

15. Erosive phenomena		
Left bank	(1) Absent or very reduced; (2) Limited; (3) Widespread; (4) very evident	Right bank

16. Hydromorphological integrity		
upstream dams		Yes/no and estimated distance
downstream dams		Yes/no and estimated distance
Presenza di <i>hydropneumatics</i>		Yes/no
Transverse section integrity		(1) Total; (2) slight artificialization; (3) compromised; (4) residual
Longitudinal section integrity		(1) Total; (2) slight artificialization; (3) compromised; (4) residual

17. Artificial intervention in the station and surroundings	
Right bank	
Left bank	
Riverbed	

18. Land Use around the riverbed

(extention % for 500 m; for big rivers: 1 km)

Left bank		Right bank
	natural areas	
	Seminatural areas	
	Extensive agriculture	
	intensive agriculture	
	Urbanized areas, industries roads and infrastructures	

19. Disturbing anthropic activities along the river corridor

(to evaluate intensity: 1 smooth, 2 medium, 3 high)

(considering up to 100 m of width, depending on the dimension of the river)

Left bank		Right bank
	water plants management	
	Human/animal trampling	
	Mowing	
	Weeding	
	Soil transportation	
	Presence of litter	
	Fires	

20. Periphyton

Diffusion		Consistence	
Absent		Visible (bidimensional)	
Circumscribed		Smooth (tridimensional)	
Widespread		Thick	
Total		Very thick	

Vertical bryophytes cenosis – facultative

Left bank		Left bank
	Linear extension of the cenosis	
	Maximum transverse width (cm)	
	Mean transverse width (cm)	

Left bank	Composition % of the cenosis	Left bank
	Acrocarpous mosses	
	Pleurocarpous mosses	
	Thallose liverworts	
	Leafy liverworts	
	Other	

Notes:

***intended as the % of the detected community

DRAWING OF THE SAMPLED AREA	
100 m	
90 m	
80 m	
70 m	
60 m	
50 m	
40 m	
30 m	
20 m	
10 m	
0 m	

MICROMAMMALS SURVEY CARD

N. site		Operator		Observers	
Region		Province		City	
Locality			GPS Coordinates		
Date			Time		

Land Use (Corine Land Cover)								
Altitude (m a.s.l.)			Steepness (°)			Survey method		
Sky					Wind			
Sunny	Cloudy 1/4	Cloudy 1/2	Cloudy 3/4	Absent	Weak		Medium	
Covered	Weak rain	Strong rain	Fog	High			Very high	

	Species	Tra	Capture/Recapture	Mark	Sex	Weight	Notes
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							
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17							
18							
19							
20							
21							
22							
23							
24							
25							

BIRD SYRVEY CARD

Site		Operator		Observers	
Regione		Province		City	
Locality			GPS Coordinate		
Date			Time		

Has the site been visited on previous years?			Yes	No	The hearing point is in the same position?			Yes	No
Exposition	N	NE	E	SE	S	SW	W	NW	360°
Altitude (m a.s.l.)			Steepness (°)			Survey method			
Sky					Wind				
Sunny	Cloudy 1/4	Sunny	Cloudy 1/4	Absent	Weak		Medium		
Covered	Weak rain	Intense rain	Fog	High			Very high		

Species		Within 100m	Over 100m
1			
2			
3			
4			
5			
6			
7			
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15			
16			
17			

CODICI: **C:** maschio in canto; **M:** maschio non in canto; **F:** femmina; **j:** giovane; **V:** volo alto di trasferimento; **R:** attività riproduttiva (nido, imbeccata, ecc.)

FAUNAL SURVEY CARD

Number site. Operators Date.....

Region City I.G.M.....

Locality Time.....

Forecast

Sunny	Cloudy 1/4	Cloudy 1/2	Cloudy 3/4	Covered
Weak rain	Intense rain	Fog	Weak wind	Strong wind

Land USE(CORINE *LandCover*)

Plant management.....

Survey method transect coordinates (GPS track).....

MACROFAUNISTIC ANALYSIS

n.	Taxon	n° counted individuals	Tracks	Notes
1				
2				
3				
4				
5				
6				
7				
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16				
17				
18				
19				
20				

FRESHWATER FISHES SURVEY CARD

RIVER				SITE (City and Province)				DATE	
SITE (name and number)				SITE COORDINATES				FORECAST	
								T (air)	
HEAD OPERATOR				TEAM (N. operatorS)					
				ELETTRO.	GUADIN.	SECCHI	SUPPORT.	Cloudness	
MODEL OF ELECTROSTUNNER				Width of active riverbed		Width of wet riverbed		Rain	
				Width of qual. transect..		Length of qual. transect.		Wind	
AN. (forme e ø)		CAT. (type, length and ø)							
				O ₂	pH	T (water)	Conduct.	Vis.	
ELETTROST. PARAMETERS				Trait	Starting time	Ending times	N. individuals	NOTES	
V		Kw peak	Freq. (Hz)	1					
				2					
				New species					
MESOHABITAT							Dams/islands	NOTES	
Ponds	Smooth	Streams	Rocks	rapids	Jumps	Falls			
DEPTH					SHADE	IDROCARBS FOAMS.	VEHICLES IN RIERBED		
≤20	21-40	41-60	61-80	>80			PAST	PRESENT	
SUBSTRATE									
ROCK	MGL>40cm	MAC _{20-40cm}	MES _{6-20cm}	MIC _{2-6cm}	GRA _{0,2-2cm}	SAND _{6μ-2mm}	SILT<6μ	ARTIFICIAL	M.emerg
FLOW TYPES									
Fall	Slide	Broken waves	Intact waves	Chaotic flow	Ridged	bubbles	Smooth	Still	Dry
VEGETATION					ORGANIC MATTER			fine deposit	
Filam. Alg..	Felt. Alg.	Sub. Macr.	Emerg-Macr	Roots	Xylal	CPOM	FPOM		

RIVER				SITE (NAME AND CODE)				DATE	N. SHEET
NOTES									
ID	SP./SEG./PASS.	L	W	ID	SP./SEG./PASS.	L	W	ID/NOTES	
1				25					
2				26					
3				27					
4				28					
5				29					
6				30					
7				31					
8				32					
9				33					
10				34					
11				35					
12				36					
13				37					
14				38					
15				39					
16				40					
17				41					
18				42					
19				43					
20				44					
21				45					
22				46					
23				47					
24				48					
25				50					

MARINE FAUNA SURVEY CARD

REGION	CITY	SITE				DATE	TIME
TRAP (name and code)		TRAP COORDINATES (from starting point)				FORECAST	
						T (air)	
OPERATORE/I (nome, cognome)							
						Cloudiness	
PHYSICO-CHEMICAL PARAMETERS	O ₂	pH	T (water)	Salinity	Conduct.	Rain	
						Wind	
INDIVIDUI CATTURATI							
ID	Species	Weight	Dimensions	Sex	Notes		
1							
2							
3							
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