Assessing and enhancing ecosystem services provided by diadromous fish in a climate change context

Deliverable title: Manual on Biological Data Collection

Deliverable reference: WP 6 – Action 6.1

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This project is co-financed by the Interreg Atlantic Area Programme through the European Regional Development Fund.

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Subsidy contract n°	EAPA_18/2018
Programme priority	4. Enhancing biodiversity and the natural and cultural assets
Start date of project	1st February 2019
End date of the project	31st January 2022
Work package n°	WP6
Indicator	Technical and scientific publications
WP Leader	IFI – partner n°7
Partners involved	INRAE – partner n°1 AZTI – partner n°2 EHEC-USC – partner n°3 MARE-UÉ – partner n°5 CMVNV – partner N°6 IFI – partner n°7 Cefas – partner n°9 MNHN – partner n°10
Submission date (month)	February 2020

Dissemination level		
PU	Public	x
PP	Restricted to other programme participants (including the Programme Authorities)	
RE	Restricted to a group specified by the consortium (including the Programme Authorities)	
CO	Confidential, only for members of the consortium (including the Programme Authorities)	

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WP 6.1 Deliverable - Executive Summary

(*The other languages are provided in Appendix 3*)

1. DiadES aims to **assess and enhance ecosystem services provided by diadromous fish** in the Atlantic Area (AA) and, in parallel, the conservation status of these species, by explicitly considering in their management expected impacts of climate change on their distribution. DiadES adopts an innovative ecosystem services approach that converts fish abundances into monetary units. DiadES will see collaborating researchers in the field of natural sciences and environmental economists helped by a strong network of diadromous fish-related managers for the entire AA. Nine case studies will be the focus of specific and joint studies. **More about the project at www.diades.eu.**

2. DiadES WP 6 "Biological and ecosystem services data collection and case studies" is on **field data acquisition**. It has a duration of 30 months, running from early 2019 to the end of July 2021. This crucial WP will involve all beneficiary partners and is led by Inland Fisheries Ireland (IFI).

3. One of the first tasks in this WP was to **agree on joint methodologies and best practices** in partners' biological studies under task 6.1 "Definition of joint methodologies for biological data collection".

4. The present report "Manual on biological data collection" represents the converging of partners in regard to target species, agreed protocols, sharing of resources. This is the deliverable expected from task 6.1 to **harmonise and standardise** the available monitoring data and ecosystem services valuation across the AA. **Sections were organised by main methodologies** and was presented as such below.

5. **A contact list** was added to the report to easily identify the most knowledgeable DiadES researchers for each methodological section.

6. A large part of partners' activities was agreed to be on **data-poor/data-deficient species** such as shads, lampreys, flounder and thin-lipped mullet (*Chelon ramada*).

7. The environmental DNA (eDNA) study builds on the fact that organisms often release their DNA to the environment by way of dislodged mucus, tissues, and discharged eggs or milt during reproduction, including DNA released by carcasses of dead individuals. If present, the released eDNA can be filtered from water samples, detected using sophisticated molecular analysis, thus providing an indication of the species' presence. The genetic analysis will be undertaken for partners **in one of two central facilities**: by AZTI (for shad species and for sea lamprey *Petromyzon marinus*) and by Cefas (for smelt *Osmerus eperlanus* and for thin-lipped mullet). **Protocols for water sample collection, filtering and storage were provided by both institutes for comparison**. This collegial approach of using discrete

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facilities will create a standardisation and help to eliminate issues of error and of contamination as much as possible.

8. Fish absorb chemical elements into their tissues and bony structures, the proportions of different elements being linked to the proportion of these elements in the aquatic environments they have inhabited. Microchemistry of otoliths (ear bone structures) and of scales provides an opportunity to examine certain key elements and develop a 'signature' for the waters in which the fish was captured and the fish's (bony) signature at a particular life stage. The proposed microchemistry studies in DiadES will mainly examine:
    a. The two shad species in an attempt to link adult fish captured at sea to the catchments of natal origin. This will expand knowledge on the migrations and movements of the species once they have left fresh water;
    b. Migratory movements of thin-lipped mullet within estuarine waters and the use of zones of specific salinity by the species.

    Protocols issued for shad samples were fully detailed with key requirements. Some interesting guidelines were also proposed for mullet samples too. This study will be led by INRAE and all analysis will be undertaken by a single laboratory team within INRAE, thereby providing a cost-effective and streamlined consistent quality of output, eliminating any issues of error.

9. The collection of scales for the microchemistry study above will also be used to examine hybridisation of the two shad species – allis (*Alosa alosa*) and twaite (*Alosa fallax*) – within DiadES along with additional tissue samples. **This is a value-added opportunity within the project**. This study will be led by INRAE and all analysis will be undertaken by a single laboratory team in INRAE, thereby eliminating any issues of error. **Key bibliographic references for protocols were listed.**

10. A series of fish migration/movement studies will be undertaken during DiadES using a variety of technologies. **Six individual DiadES partner proposals were detailed.** In conjunction with recent historical data sets, these studies will combine to provide knowledge on individual species and contribute to the distribution maps that DiadES will generate for the Interactive Web Atlas. The technologies will include:
    a. Floy tagging of juvenile out-migrating sea lamprey;
    b. Acoustic tagging of a range of species – migratory trout, smelt, shads, thin-lipped mullet;
    c. Pop-up satellite tagging of sturgeon.

11. By-catch of target DiadES species presents itself at commercial fishing ports and through investigative and monitoring fish surveys. The DiadES partners will liaise with relevant public authorities, port-based personnel and individual commercial fishermen in order to obtain by-catch data on relevant species. **Five individual DiadES partner proposals were detailed.** The team will endeavour to obtain bodies for biometric data as well as information on location of capture (within ICES grid areas) and date of capture. This may contribute significantly to knowledge of marine movements of the species and hence the distribution maps the project will generate.

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12. Partners undertake national monitoring programmes e.g. for Water Framework Directive fish in transitional waters, or have access to this data within their own country. **Six individual DiadES partner proposals were detailed.** This material will contribute to the overall distribution mapping, to more detailed life-stage mapping associated to specific habitat types and also to provision of biometric data.

13. The partners have agreed on a database protocol that will provide consistency in regard to labelling of samples, provision of biometric/environmental data relating to samples etc. This is essential in order to provide a fool-proof tracking system, necessary when several partners are sending different and disparate materials to one or more partners acting as focal points for a specific task, e.g. the INRAE laboratory servicing the microchemistry. **All information for application of this tool to other contexts was made available.**

14. Scientific outputs under WP6 from individual-partner and from shared-partner studies will be published in peer-review journals **and uploaded in the DiadES website**.

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1. Introduction to DiadES

The status of diadromous fish is in decline across their Atlantic distribution, with adverse socio-economic impacts on local populations, e.g. loss of jobs in the fishery sector, an end to European sturgeon (*Acipenser sturio*) caviar production, decreases in recreational fishing licensing and local tourism numbers, and disappearance of cultural practices linked to these species. Despite many management measures, the situation remains critical. Diadromous fish management has been commonly organized at the local scale, each river basin being a management unit, and often single-species focused. The shortcomings of this approach are apparent in recent findings, which clearly demonstrate that river basins share and exchange numbers of fish, not being isolated populations. Conjointly, climate change is an additional threat to biodiversity and related ecosystem services. Diadromous fish are vulnerable to climate change and are strongly suspected to change their geographical distribution towards the North to the detriment of southern European Union Member States. Shifts in distribution could expose diadromous fish management to an even more complex problem, which requires revisions in temporal and spatial scales through a transnational approach. In accordance with the EU Biodiversity Strategy, marine Natura 2000 biogeographic seminar, and Priority 2 of the Atlantic Strategy, DiadES will address the need to improve the management of diadromous fish species in the Atlantic Area (AA) through the explicit consideration of long-term and large-scale actions related to climate change.

DiadES aims to assess and enhance ecosystem services provided by diadromous fish in the Atlantic Area (AA) and, in parallel, the conservation status of these species, by explicitly considering in their management the expected impacts of climate change on their distribution. Diadromous fish (shads, lampreys, eel, salmon, trout and mullet) are moving between fresh and marine waters. Through their journey diadromous fish provide ecosystem services (e.g. income, food, recreation) to local communities but few quantitative estimates exist. These services could be threatened by climate change due to spatial reallocation of fish and related benefits. Building on previous EU-funded projects and monitoring programmes, DiadES will positively impact diadromous fish management in the face of global climate change by:

- Fostering the necessary level of co-operation among Member States (MS) and actors involved in diadromous fish management to enable sound decision-making;
- Improving awareness and knowledge among policy makers and other key stakeholders of the services provided by these species and the need to set common management measures targeting both anthropogenic pressures and climate change;
- Favouring a joint promotion of ecosystem services related to diadromous fish in the AA to the wider public because they influence decision-making;
- Ensuring a sustainable ecosystem-services provision by these species, combining exploitation and conservation, in support of AA economics and quality of life.

DiadES adopts an innovative ecosystem services approach that converts fish abundances into monetary units, thus circumventing the difficulty of orientating public decisions on diadromous fish management with only observed numbers of fish or stock estimates from models. DiadES will develop common methodologies for this first-ever comprehensive assessment of ecosystem services related to diadromous fish, which are unanimously recognised as having high ecological and economic value in Europe. However, there have been few quantitative studies (i.e. a handful of case studies that focused on a short list of ecosystem services). Here, maps will be produced at the AA scale for the provisioning, regulating and cultural services. The climate change dimension, and its consequences for diadromous fish management in the AA and its periphery, will be given particular attention in DiadES compared to previous projects that focussed on a single management unit (e.g. Minho River in POCTEP MIGRA-MINO-MINHO) or on one species (e.g. the European eel in SUDOE SUDOANG). DiadES will propose a global approach, not limited to one particular diadromous fish species in one management unit,

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but covering many emblematic species at the AA scale. DiadES will rely on the co-operative intensity between researchers in the field of natural sciences and environmental economists and gather, in a first attempt, a strong network of diadromous fish-related managers for the entire AA.

Populations of diadromous fish are not isolated; they share individuals between river basins, crossing administrative boundaries. The provision of ecosystem services by diadromous fish in one location may require management measures in other locations. Likewise, climate change is altering the Atlantic distribution of these species, forcing their distribution from marine areas of river basins to other locations farther north. Consequently, a transnational approach is required to account for and facilitate these spatial changes through better-adapted management plans, and ease the adaption of territories in the face of climate change. As a consequence, DiadES will bring together ecologists, economists and key stakeholders from the 5 MS of the Programme area. Joint methodology definitions and applications are planned within the DiadES Work Plan with case studies identified by each partner. Stakeholders will meet regularly during the project to share knowledge, experiences and opinions regarding long-term and large-scale issues in the sustainable conservation and exploitation of diadromous fish at multi-lingual discussion panels. Although scientists and environmental managers already collect data on diadromous fish, no initiative centralises the data to maximise the EU's investment in monitoring. Large-scale networking and upgrading activities proposed in DiadES will serve to harmonise and standardise the available monitoring data and ecosystem services valuation across the area.

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2. Introduction to WP 6 and to the survey methods

WP 6 is titled "Biological and ecosystem services data collection and case studies". This work package has a duration of 30 months, running from early 2019 to the end of July 2021. The WP will involve all beneficiary partners and is led by Inland Fisheries Ireland (IFI). Task 6.1 and 6.2 are more focused on biological data and task 6.3 on economic data. All beneficiary partners will carry out field data acquisition, biological and/or economic, in this WP under IFI's co-ordination. **This report is the deliverable of Task 6.1.**

The three actions proposed in WP 6:

1. Definition of joint methodologies for biological data collection
    - Output: Manual on biological data collection

2. Collection of biological data in field case studies
    - Output: Diadromous fish database in the Atlantic Area case studies and scientific publications

3. Case study description and ecosystem services data collection
    - Output: case study descriptions and a joint database

Under this task, partners involved in ecological studies will first agree on joint methodologies and best practices in their biological studies in a guidelines manual to assure data usefulness, quality and comparability. Nine case studies are proposed for investigation: the Mondego River (Portugal MARE-UE), Minho River (Portugal/Spain, CMVNC), Ulla River (Spain, Galicia; EHEC-USC), Gipuzkoa rivers (Spain, Basque Country; AZTI), Gironde-Garonne-Dordogne (France, Nouvelle-Aquitaine; INRAE), Normand-Breton Gulf and Loire River (France, Brittany, MNHN), river Taff, Frome and Tamar (UK; Cefas) and Waterford Harbour (Ireland, IFI). Early on, a description of the socio-economic context and conservation status of major diadromous fish populations will be prepared for each case study in specific reports based on a list of indicators required by WPs 4 and 5. Data will then be collected for 2 years (sampling seasons) and compiled into the project's database for permanent use before transfer to WPs 4 and 5 for, respectively, ecosystem services valuation and the construction of species distribution models. Data collected locally will contribute to provide results at the AA scale. Project partners will regularly communicate their findings in open-access scientific publications and scientific conferences. Most publications will present collaborative works and as such will be co-authored by several DiadES partners.

The present document provides detail on the range of species and of sampling methods to be deployed in DiadES to collect information that will feed into the project Atlas and also to WP 4 and WP5. The techniques include traditional direct sampling procedures as well as indirect 'sampling' via access to fish by-catch. However, more technological strategies such as telemetry and newer tools such as environmental DNA will also be used to generate information on species in the case studies within the Atlantic Area.

The various data collection procedures are detailed below in individual chapters, outlining the relevant partners and target species, the rationale behind the procedure, what data will be collected and how this can be used within the various DiadES work packages.

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3. Environmental DNA or eDNA

3.1 Introduction to eDNA and its proposed use by partners in DiadES

Many aquatic species of conservation concern exist at low densities or are inherently difficult to detect or to monitor using conventional sampling strategies. The introduction of environmental DNA or eDNA surveying has transformed the ability to detect the presence of these species. Environmental DNA or eDNA detection is rendered possible because living organisms have the potential to leave behind in a habitat evidence of their presence. In aquatic systems, organisms may inhabit specific niche locations or may pass through certain habitats on a migration route. The organisms may leave behind evidence of their bodily presence in the form of bodily wastes, such as faeces, or sloughed off mucus or skin cells, or products of spawning activity such as eggs or sperm. All of these contain genetic content of the organism. Therefore, if a testing of a water sample could reveal the presence of discrete or unique 'markers' for a species, then there is a very high likelihood that the species was/had been recently present. Spawning events of fish present a major potential to collect eDNA evidence of presence/absence of a species as the activity tends to be confined to specific habitat types and the level of physical activity of the adult fish leads to a concentrated release of tissue and gametes in a short time period and confined area.

Both Cefas and AZTI are using eDNA procedures as a tool for examining specific fish species ecology and distribution issues. Both have facilities to actively collect material, process the samples by way of filtration and extraction and, most significantly, facilities to undertake genetic analysis of the filtrates collected and to interpret the results.

INRAE: In parallel with the DiadES project, INRAE is involved in a discrete French project called MOMIE - Migratory movement of European sturgeon *Acipenser sturio*: habitat at sea and spawners return in fresh water (INRAE/OFB). This project will perform eDNA sampling in the Dordogne and Garonne Rivers to assess if natural reproduction of European sturgeon has occurred and which fish species are present in the area of interest (metabarcoding planned in autumn 2019 and 2020). Results from MOMIE could be potentially shared with DiadES. Protocols are not detailed as this action is not in the DiadES work plan.

AZTI: AZTI has an extensive network of national and international collaborators from which to gain experience and is a members of the Cost Action DNAqua-Net, an international network whose main aim is to nucleate a group of researchers across disciplines with the task of identifying gold standard genomic tools and novel ecogenomic indices for routine application of biodiversity assessments of European water bodies. Finally, AZTI is a member of the ICES Working Group on the Application of Genomics for Fisheries and Aquaculture and Chair of the ICES Working Group on Integrated Morphological and Molecular Taxonomy, a group it has been chairing during the period 2017-2019. AZTI is working in the development of different eDNA-based techniques in the aquatic environment. It is developing standardized eDNA and bulk sample-based qPCR and metabarcoding protocols for deriving management-relevant information for DNA-based port and ballast water surveys (Rey et al. 2019).
In addition, AZTI has analysed the effectiveness of eDNA metabarcoding for the assessment of biodiversity in the ocean (Fraija-Fernández et al. 2019).Currently in the EDAMAME project framework, AZTI is working on:

- Exploring a wider range of applications of eDNA beyond community taxonomic characterization such as biomass or number of individual quantification, genetic diversity quantification and regional connectivity assessment;
- Developing standardized sampling procedures, laboratory protocols and bioinformatic pipelines for deriving relevant management information from eDNA data;
- Developing indicators from eDNA data and benchmarking their performance and cost-effectiveness against those derived from currently used measurements;

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- Proposing strategies for integrating eDNA-based indicators into regular monitoring programs.

In the framework of another project (EDAMAME), AZTI is collecting samples to extract the DNA from the filters for analysis and presence/absence for European shads and sea lamprey (*Petromyzon marinus*) in the rivers of Gipuzkoa. The results of this work will be relevant to and will inform the DiadES project as well.

EHEC-USC: The EHEC-USC team is providing AZTI with material to detect diadromous species, mainly European shads and sea lamprey, in the rivers of Galicia using eDNA. Twaite shad fin clip samples have been forwarded to develop the shad primers. However, it has not been possible to develop primers specific to each shad species (Allis and Twaite) and therefore detection is simply at the genus level. EHEC-USC plan to collect and filter water samples and to extract the DNA from the filters, and send the DNA extraction to AZTI for analysis and presence/absence results for European shads and sea lamprey. This is planned along the Ulla River, in known areas of presence of twaite shad and sea lamprey during their time of presence, to serve as control. Further collecting of water samples is proposed:

- Along the entire potential habitat of these species in the Ulla catchment, with the hope of knowing the effective occupation of that habitat;
- In other rivers neighbouring the Ulla, in which the presence of the two species of shad and of the sea lamprey are suspected.

IFI: IFI has also been involved in eDNA studies, primarily on sea lamprey, working with National University of Ireland Dublin and the laboratory of Dr. Jens Carlsson (Gustavson et al. 2015; Bracken et al. 2019). The UCD laboratory has developed and published primers for sea lamprey and for shads. A major interest for IFI within DiadES is to explore the value or potential of eDNA in the fully-marine environment as a tool to detect evidence of its key species, allis and twaite shad and the thin-lipped mullet. Within DiadES, IFI has the capacity to collect and filter water samples for eDNA analysis but does not have the facilities to undertake any genetic analysis. IFI is particularly interested to use eDNA for several purposes:

- To detect presence/absence and habitat use by thin-lipped mullet in the case-study area and in estuaries of the south, where this species appears to be expanding its range, possibly in the context of climate change;
- To detect evidence of adult shads in the marine environment, in order to increase understanding of the movement and source of Allis shad taken off the Irish coasts.

Cefas: The Cefas team has played a key role in the development of eDNA survey techniques for freshwater fishes, designing and field-testing protocols for the detection of low populations of non-native fishes in ponds (Davison et al. 2016; 2018; 2019) and rivers in the UK (Davison and Copp, unpublished data). In the marine environment, Cefas has developed methods for the detection of an invasive ctenophore in coastal waters (Créach et al. in prep.). In addition to these research and development activities, Cefas plays an active role in advising agencies on eDNA monitoring, by participating in groups such as the UK eDNA Working Group which aim to bridge the gap between scientific projects and routine monitoring. Current Cefas projects include the use of eDNA to assess sea lamprey and smelt (*Osmerus eperlanus*) passage past barriers, and to detect marine pelagic fishes. Cefas is currently looking at the distribution of data-poor diadromous fishes in rivers (shads, lampreys, smelt) and the presence of invasive diadromous pink salmon (*Oncorhynchus gorbuscha*), In terms of marine work, Cefas is trying to detect data-poor diadromous fishes in the south-west marine areas.

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	Irstea	AZTI	EHEC-USC	IFI	CEFAS
	Gironde/Garonne/Dordogne system	**Gipuzkoan rivers**	**Ulla catchment**	**Waterford harbour and the three sisters' rivers**	**Tamar, Frome and Taff rivers**
eDNA studies	poss: Link with MOMIE project	CS	CS	CS	CS (Tamar, Frome & other SW rivers)
Focus species	European sturgeon	Analysis for detection of shad and lamprey in Gipuzkoan rivers and for other DiadES partners	Shad, sea lamprey	Shads, sea lamprey, thin-lipped mullet	smelt, lamprey species and shad spp.

Table 3.1: DiadES partner data collection for environmental DNA or eDNA. 'CS' = sampling in own case study. 'poss' = possible additional sampling that may be available or links to other projects.

3.2 Protocol issues – overview of requirements

Both the Cefas and AZTI laboratories have their own, discrete, protocols for eDNA analysis. The differences relate primarily to technical aspects of field sample collection and filtering, as opposed to any differences in treatment or analysis of the actual genetic material. Thus the spatial distribution of sampling locations in the open sea may be opportunistic, as opposed to being structured. Likewise, the volume of water sampled and the depth or depths of sampling may vary. The pore size of filters may also vary, with one laboratory using a 0.2 µm filter and the other using a 0.4 µm filter. The larger pore size enables a larger volume of water to be filtered with ease and hence a potentially larger volume of eDNA to be collected. The lead partners for eDNA work in DiadES, AZTI and Cefas concur that the differences between the two laboratories in regard to the technical aspects of sample collection, filtering and extraction do not create any differnces in outcomes of genetic analysis of samples. In this manual, details of the Cefas protocols are presented.

This manual presents protocols to cover (a) field sampling strategy, (b) sample collection and initial processing and (c) laboratory-based genetic procedures and data analysis.

3.2.1 Field sampling strategy – logistics of station network

Experience teaches us all that it is very easy to collect samples and that these can stockpile very quickly. In the case of eDNA sampling, evidence for any target aquatic species COULD be present in any number of aquatic locations – to the extent of comparing the task with "looking for a needle in a haystack…" For this reason it is important to have a plan or sampling strategy and to implement this in the field situation. What do I want to find out? Where do I want to find this out? What resources do I have – time, money, field logistics, follow-up laboratory logistics?

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These issues were discussed by the DiadES team at a meeting in Norwich in November 2019 specifically targeted at sampling and the development of a manual of methods for DiadES. In the context of eDNA and its use in the project, three field scenarios were identified:

1. Point sampling in more confined riverine locations, focusing sampling on periods of maximum likelihood of encountering the target species;
2. Sampling programme in inshore or estuarine waters at a time when species may be aggregating in advance of diadromous migration into fresh water;
3. Sampling in the open sea for indications of marine migrations etc.

Point sampling: this approach may require single sampling locations, only. It is relevant if looking for presence/absence of a species or series of species. It has an elevated likelihood of 'success' due to the more physically-enclosed sampling locations and unidirectional flow (if sampling in river locations). This sampling should be based on knowledge of a species, its likelihood of presence and the timing of species diadromous migration or movement. It can also be used to track the success or otherwise of a species in its upstream migration, for example in regard to overcoming physical barriers to passage. The sampling strategy must also be designed according to the species under detection, taking into account the available resources (which limits number of sampling locations). Given the more shallow-water nature of the sampling areas – in upper reaches of estuaries or in non-tidal, lower sections of rivers – sampling at a range of depths may not be necessary.

In Cefas' study to determine the incidence of non-native pink salmon incursions of GB rivers, as well as to assess the presence of diadromous fishes (shads, lampreys, smelt) for which data is scarce, water samples were collected at three locations per river: near the tidal limit, and at distances of ≈ 10 km and ≈ 20 km upstream of the first site. Often a weir or partial obstruction was located near the tidal limit, and this was chosen as a sample location due to the suspected likely concentration of diadromous fish eDNA at these points (due to fish delaying migration in pools below the weirs).

Sampling in more open-water scenarios: this approach is proposed for periods and locations when species may be aggregating off-shore prior to migration into estuaries and later to freshwater. As with point-source sampling, knowledge of the species' biology is relevant in helping to focus on timing and locations to optimise any positive results. The approach envisages a species beginning to converge from the open sea and to aggregate prior to moving into a defined estuarine space. The sampling strategy should create a GIS-based grid and sample within a range of squares within the grid. The grid network may be proportional to the survey area for which 5×5 or 10×10 km grids may be suitable. The aim would be to create a grid that provided sufficient degree of coverage as well as allowing for the constraints of laboratory analysis. The network generated could be sampled on a periodic basis, as the species began to aggregate and then as it moved into an estuary from the sea.

Sampling in the open sea: This is the ultimate "needle in a haystack" scenario. Interest in this sampling, for the IFI, comes from the fact that there is no established body of knowledge as to where the shads, in particular, go to at sea. Samples of marine-caught shad have been taken and landed as bycatch from a range of locations around the Irish coast, from both inshore areas and from locations remote from shore. The natal origin of these fish is not known. In the case of allis shad, these are unlikely to be of Irish origin as there are no known spawning locations for this species in Irish waters. Apart from natal origin, it would be important if any light can be shone on the possible migration routes of shads at sea.

In an open-sea context, a 10×10 km grid might be realistic. This uses the grid size as per the EU Habitats Directive. Another option is to use the ICES grid of 'boxes' delineated, as used by Cefas for reporting by-catch (see Section 7.2 below). This is an international grid and scales down from large 'box' areas with further divisions

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and sub-divisions within each large box. A simple process of GPS-recording sampling locations could be used on-board with these locations subsequently being mapped onto the ICES layer.

Even with a purpose-planned survey, the project should select key or target zones in advance on the basis of potential for sampling success, sampling in proportion to capacity to process, filter and extract samples from the water.

It is likely that the majority of any marine sampling for eDNA will be opportunistic, availing of access to a large craft that is undertaking other sampling, such as fish sampling or marine benthos sampling. The AZTI has collected water samples in the Biscay Gulf during 2017, 2018 and 2019 within the framework of the BIOMAN campaign (Assessment of anchovy stocks in the Biscay Gulf). Similarly, Cefas' DiadES team has previously made use of such 'opportunistic' marine sampling as part of a project to trial the detection of pelagic marine fishes, and therefore utilised the survey grid covered for that project by the Cefas research vessel, the "Endeavour", on a single survey in the Celtic Sea and Bristol Channel in October 2018 and October 2019. Samples of 2 L of water were collected at two water depths: from the surface using the ship's flow-through system, and additionally from at 20 m depth using a rosette sampler, where stratification was observed (stratification is assessed by measurements of deep chlorophyll maximum during this survey). Samples were collected into 2 L polycarbonate bottles, and filtered at the earliest possible opportunity using Sterivex filter cartridges of 0.22 µm pore size (EMD Millipore). Water was forced through the cartridge using a peristaltic pump; however the small pore size can create problems with clogging of the filters. Samples were frozen at sea. On return to the laboratory, extraction from the filters was undertaken using the Mu-DNA method of Sellers et al. (2018), and metabarcoding analysis (using Next Generation Sequencing and universal fish primers) undertaken at Salford University according to their protocols as part of a PhD project.

The ambitions in regard to sampling must be tempered with a degree of practicality, particularly when sampling in the open sea, where care must be taken in regard to –

- The hazards of sampling in the open sea;
- Necessity for weather awareness at all times;
- Necessity for all appropriate Health and Safety measures;
- Access to freezer facilities or to on-site filtering facilities for the collected materials;
- The potential extent of 'dispersal' or 'decay' of eDNA material with respect to the sampling site.

3.2.2 Collection of field samples, filtration and extraction

With regard to requirements at actual sampling stations, the following are relevant and should be recorded for all sampling:

- The depth regime of water in the sampling area (no. samples/depth of water column);
- Degree of replication at each sampling point;
- Volume of water to be collected and filtered for each 'sample'.

Given the sensitivity of the eDNA genetic analysis it is imperative to have as sterile or uncontaminated an environment around the sampling effort as is possible. This is difficult in a non-laboratory situation and particularly in an open-air, open-sea situation were the sampling craft may be a fishing boat that is also being used simultaneously as a fish sampling platform.

Replicate sampling is proposed with four samples to be taken at each station – three as 'active' samples from the water and one as a 'blank'.

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The Cefas river sampling protocol involves collection of 1 L water samples, which are collected from the riverbank using a 500 mL polypropylene sampling cup attached to a 183 cm polypropylene sampling pole (Camlab Ltd, Cambridge). River water is collected from the top 30 cm of water, with care taken to avoid contact with the bottom sediment. The water is immediately filtered using a sterile 50 mL syringe to push the water through a 0.47 µm pore-size Naturemetrics cartridge. Each cartridge is sealed within an individual plastic bag and placed in a portable –20^{0}C freezer. To avoid contamination among samples, new sampling cups are used for each river system. Between sites within river systems, the poles and cups are cleaned using Microsol 3+ sterilising solution (Anachem Ltd, Luton, UK) and rinsed with deionised water. After every five rivers, a 'blank' is created using de-ionised water (from a bottle brought from the laboratory), which is filtered, handled and analysed in an identical manner to the samples, to test for contamination.

In deeper water locations, sampling is undertaken using any standard sampling device that can be activated to collect at any set depth in the water column. The sampling device is flushed with water in its descent through the water column and is activated at the required depth. It is then drawn to the surface and contents poured directly into a pre-sterilised sample container. The process is repeated for the further two replicate samples. One approach to optimise sterility, proposed to the IFI team by Dr Jens Carsson of NUI Dublin, is to use a set of 4×2 L, un-opened bottled water containers (still water as opposed to sparkling water) purchased at a retail outlet. Each of the three 'replicate' bottles can be opened and emptied immediately in advance of filling with the sample for analysis. The fourth bottle can be opened and then closed and retained as a 'blank' or 'control' sample.

Each sample bottle should be labelled in such a manner as to ensure its content's 'traceability' through any subsequent sequence of storage, filtration, extraction etc.

Ideally, each sample would be filtered as soon after collection as possible. Where filtration facilities are not immediately available, sample bottles should be stored in dark conditions, to prevent any photosynthetic activity or any degradation due to solar exposure. Samples should also be stored in as cool conditions as possible, in cooler boxes on ice pads or refrigerators. It is recommended to filter water as quickly as possible, within 24 hours, but if this is not possible, refrigeration (3–5 days) is a better option than freezing (Hinlo et al. 2017).

On-site filtration, like sample collection, exposes the sample to potential contamination and all precautions should be taken to reduce and eliminate such possibilities. Filtration in a laboratory situation does allow for reduced risk of contamination. The use of filter cartridges, in which the water is pushed through the cartridge by hand using a syringe and filtered within that cartridge, provides a means of reducing contamination risk. Such filter cartridges also have the advantage of being easier to transport from the field, and reduced handling (and contamination risk) at the later laboratory stages (Spens et al. 2017). Pore size is an issue and different laboratories opt for particular pore sizes dependant on the nature of the investigation and on resources. The larger pore size of 0.47 microns permits a faster filtration rate than the finer 0.22 micron sheet. The faster filtration permits the filtering of a larger volume of water and it is considered that the quantity of candidate eDNA material retained on the 0.22 or 0.47 micron sheets is adequate for general analysis presence/absence purposes (Turner et al. 2014; Spens et al. 2017).

Filter cartridges, or filter papers, are best stored either frozen (as Cefas has done in all studies to date, as it enables long-term storage), or in a preservative solution such as ethanol or Longmire's solution. The immediate addition of Longmire's solution to a filter enables it to be stored at room temperature, for at least two weeks without significant degradation (Spens et al. 2017). For longer-term storage, freezing may be the better method to reduce degradation (Williams et al. 2016), although filters have been successfully preserved in Longmires for 150 days (Wegleitner et al. 2015).

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The next stage in the process is extraction of the DNA from the filters. There are many methods for doing this, with probably the simplest approach being to use the commercially available extraction kits (e.g. the Qiagen PowerWater Sterivex Kit, which facilitates extraction directly from Sterivex filter cartridges). Alternatively, to reduce costs many laboratories have developed other methods; one that has shown a DNA yield comparable to commercial kits is the Mu-DNA method (Sellers et al. 2018).

After extraction, by whatever method, one is left with a DNA sample of typically 100 µL, which should be stored frozen (and is robust enough to be stored for long periods and to be easily shipped). This volume is enough to run multiple analyses; enabling splitting of samples for different purposes (e.g. PCR testing for smelt, mullet and shad; or a metabarcoding screening followed by targeting a particular species).

3.2.3 Protocol for genetic analysis

Laboratory analysis of eDNA samples works on the principle of using DNA primers (small sections of DNA that can be targeted for amplification), and can follow two different approaches:

- Metabarcoding – using universal primers that enable many species to be detected simultaneously. Various primers can be used which are optimised for a particular taxonomic group, such as fishes or arthropods. The inventories produced by metabarcoding can be used to monitor fish communities in both freshwater and marine environments. Number of reads for each species can theoretically be used as a proxy for fish abundance, with some studies showing calibration with fish abundance (e.g. Evans et al. 2015; Thomsen et al. 2016).
- Single-species targeting – these use species-specific primers to amplify only the DNA of the target species, using polymerase chain reaction (PCR). Two different platforms are commonly used: 1) conventional (a.k.a. traditional or end-point) PCR, which requires viewing of the PCR amplification products under ultraviolet light on an electrophoresis gel; and 2) quantitative (a.k.a. real-time) PCR in which a fluorescent probe is activated in the presence of target DNA and measured by the instrument, providing a measurement of eDNA quantity. Quantitative PCRs are often considered more sensitive (e.g. Wilcox et al. 2013; Tréguier et al. 2014), but other studies have shown little difference in sensitivity, including extensive laboratory testing by Cefas using topmouth gudgeon (*Pseudorasbora parva*) primers (Davison et al. 2019).

A current topic of discussion in the eDNA field is the question of whether single-species targeted PCRs provide a better method of detecting a rare species of interest than metabarcoding. The consensus is that (currently) single-species targeting is the most effective method, as metabarcoding is currently constrained by issues such as including primer biases (i.e. inter-species variation in primer efficiency) and mis-assignment of taxa due to incomplete databases. Also, single-species primers can be more easily tested and fine-tuned in the lab to optimise their performance. Recent direct comparisons have shown single-species methods to more reliably detect target species (Bylemans et al. 2019; Wood et al. 2019).

The DiadES team, overall, needs to look at its set of target species and see what 'markers' are available and what 'markers' require to be developed. While only some DiadES partners are planning on direct eDNA sampling and investigations, the outcomes from any eDNA analysis, for metabarcoding or specific markers, may provide valuable information for the overall DiadES Atlas.

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3.2.4 Cefas protocol for genetic analysis

Laboratory detection protocol – single species PCRs

Filter cartridges are stored frozen (–20^{0}C) in the laboratory, before DNA extraction using a QiaGen PowerWater Sterivex kit to manufacturer's protocol. This provides 100 µL of DNA extract, which is then frozen until analysis.

Cefas PCR analysis is conducted using either conventional or quantitative PCR methods, depending on the project. Both require the design of species-specific primers, and laboratory validation for sensitivity and specificity, unless suitable primers can be found in the literature. For example, Cefas has designed conventional PCRs for smelt, but used published qPCR primers for sea lamprey (Gustavson et al. 2015) and pink salmon (Andersen et al. 2018). Primer design can be undertaken using a number of software tools, with Cefas typically using NCBI 'Primer-BLAST' to design primers and conduct *in silico* specificity tests against sequences of related species.

For both conventional and quantitative PCRs, samples are diluted 1:10 in deionised water to dilute potential eDNA inhibitors, as recommended by McKee et al. (2015). PCRs are then run using reagent mixes and PCR programmes as described in Davison et al. (2019), or using PCR programmes described in the literature if using published primer pairs. So for example the qPCR method for pink salmon detection, 2 µL of DNA sample was added to a reaction mixture 1 µL of assay mix (18 µM forward and reverse primers and 5 µM probe) for the targeted species (Applied Biosystems™), 10 µL of TaqMan® Genotyping Master Mix (Applied Biosystems™) and 7 µL of de-ionised water. The PCR thermocycling programme used was that described by Andersen et al. (2018) for use with their designed primers for pink salmon: 2 min at 50°C, 10 min at 95°C, followed by 50 cycles of 15 s denaturation at 95°C and 60 s annealing-extension at 60°C.

The number of PCR replicates varies, but general opinion is that when detecting rare DNA, it is best to collect more replicates. For example, when sampling ponds with a low density of great crested newts (*Triturus cristatus*), Rees et al. (2014) sometimes detected the species in 1 of 12 samples only. Typically, Cefas' PCR analysis includes a minimum of five PCR replicates on each sample, with more replicates when time and resources allow; for the pink salmon project, five replicates were run on all samples, with nine replicates run on samples for some key rivers.

Several checks exist to confirm that non-detection is due to absence of the species' DNA, rather than failure of the methods used. To test for inhibition of the samples at the PCR stage, tests should be conducted in which the eDNA signal of a small quantity of DNA is spiked into DNA extract and deionised water, in a direct-comparison PCR run. To demonstrate that the filtration and extraction stages have been effective at capturing macro-organism DNA, this can be done either by adding an easily-recognisable artificial DNA marker before filtration, or (as in the Cefas pink salmon method) testing some samples with generic fish primers (in any environmental samples which would be expected to contain fish DNA).

The AZTI team in DiadES has reviewed the Cefas protocols for genetic analysis, in the light of AZTI methods, and is of the view that the two protocols and procedures are essentially similar and that the Methods described here cover the processes of both the Cefas and AZTI laboratories.

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This project is co-financed by the Interreg Atlantic Area Programme through the European Regional Development Fund.

4. Microchemistry of otoliths and scales

4.1 Introduction to microchemistry of otoliths and scales and identified tasks in DiadES

Based on otolith growth patterns and chemical composition, techniques of microchemistry analysis have been used to assess either the natal sites for a range of fish species – thus linking an adult fish found in one location with its location of origin - or to obtain detailed individual chronologies of habitat use. This is a potentially exciting prospect within DiadES given, in an Irish context for example, the capture of Allis shad at a range of locations around the Irish coast but with no known spawning locations for the species in Irish waters. Where are these adult fish coming from – from the Gironde or Loire, or from the south coast of England? A collaborative approach within DiadES among the partners, using their existing collections of material and additional material to be collected within WP 6 may yield much valuable information. The interdependence of populations of Allis shad along the Atlantic coast is still a question to address to understand how the exchange of spawners that do not perform homing is driving population dynamics. Similarly, the observation of allis shad at sea along the Irish coast far from any river where a population is reported raises the question about the origin of shads in the marine environment. These two topics can be investigated using chemical tracers within otoliths (and possibly scales) to retrieve the natal origin of fish.

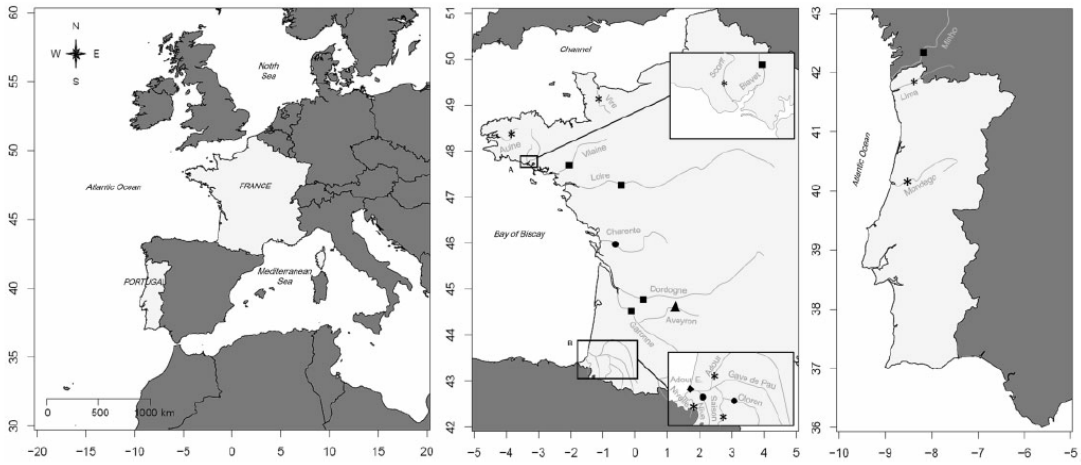

Figure 4.1.1. Rivers considered in the studies of Martin et al. (2015) and Randon et al. (2018) in France and Portugal. Symbols represent the kind of samples for each river. Filled circle: water samples; Filled diamond: adult samples; Asterisk: water and adult samples; Filled triangle: water and juvenile samples; Filled square: water, adult and juvenile samples (Map from Randon et al. 2018).

Three previous studies addressed this topic (Martin et al. 2015; Randon et al. 2018 and Nachón et al. 2019), headed by Françoise Daverat, a member of the INRAE team leading the DiadES project. Two of the studies (Martin et al. 2015; Randon et al. 2018) examined the metapopulation functioning of Allis shad in Portuguese and French rivers (Figure 4.1.1). Results showed a metapopulation dynamic with several rivers acting as sources and other as sinks (Figure 4.1.2). However, the lack of precision and homogeneity in abundance data resulted in large data gaps, which calls for a better standardization in the acquisition of abundance data. Nevertheless, this method should provide an overview of the metapopulation dynamics of other anadromous species with management concerns.

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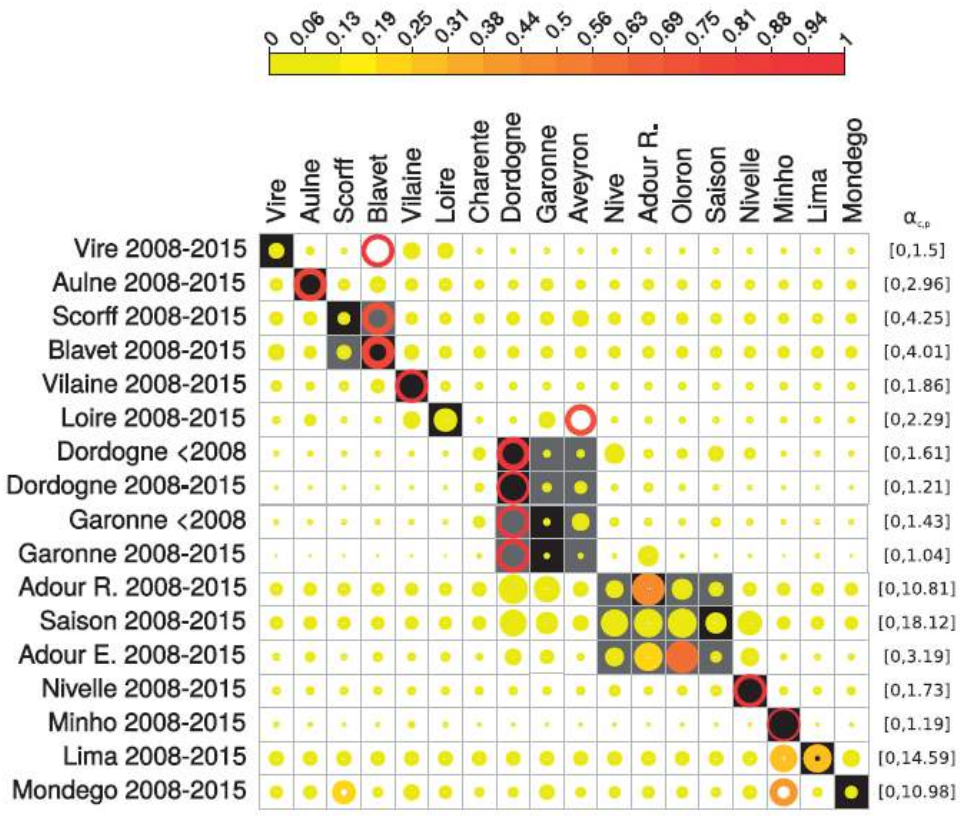

Figure 4.1.2. Probabilities of natal origins for each period and capture river. Rivers where adults were sampled are presented in row and natal rivers in columns. The horizontal bar corresponds to the probabilities. Circles are the 95% credibility intervals. Solid circles indicate large credibility intervals whereas empty circle correspond to short credibility intervals. Dark and grey cells represent respectively a homing at river scale and a homing at watershed scale. Concentration parameters are indicated for each period and capture river.

The most recent study (Nachón et al. 2019) analysed the dispersal capacities and connectivity of both shad species along the Bay of Biscay during the 1980´s (Figure 4.1.3). These results showed that the most abundant southern populations were dominant, suggesting that population-specific composition was related to population relative abundance. The dispersal in the marine environment was plastic; alternatively shads were found large distances away from their natal rivers, while others remained in the vicinity of their natal river plume.

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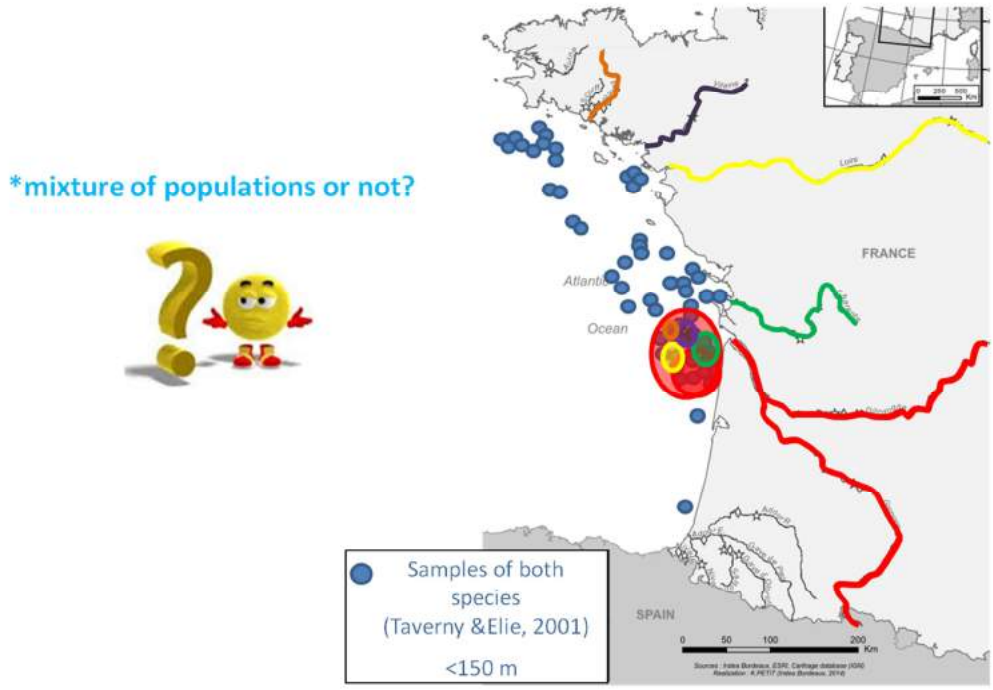

Figure 4.1.3. Illustration of population connectivity at sea suspected for Allis shad and Twaite shad in its North-Western Atlantic distribution range. Circles represent individuals from different population schooling at the Gironde entrance, close to the shores. This is one of the hypotheses of how shads may distribute at sea. Blue dots represent captures of individuals with otolith sampling in the 80's from Taverny and Elie (2001). Scheme elaborated by David J. Nachón.

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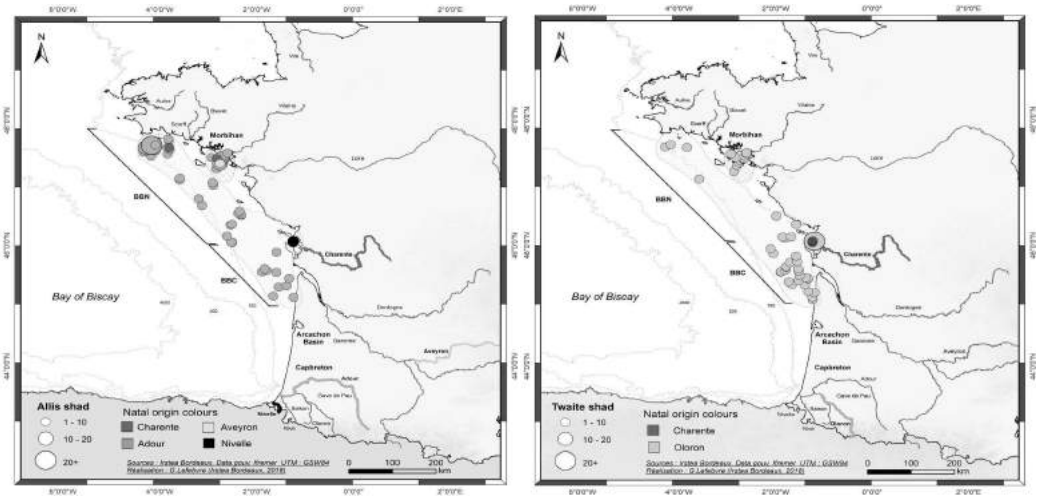

Fig 4.1.4. Most probable natal river assignations for Allis shad and Twaite shad subadults. The map shows all potential sources and those that have contributed individuals to mixtures of subadult Allis shad and Twaite shad are color-coded for the identification of the natal origin of individuals.

The second question to address using microchemistry in DiadES is the diversity of *Chelon ramada* habitat use patterns and migratory dynamics. *Chelon ramada* is a catadromous fish, as it reproduces in coastal waters and spends its growth phase in a wide range of habitats from coastal waters to tidal freshwater habitats. Mullet otolith microchemistry allows an examination of the diversity of habitat use patterns among individuals found in a single river basin. Previous work in the Gironde (SW France) provided evidence that this species displays different colonisation tactics and data collected in Portuguese rivers also highlight the existence of different patterns of habitat use.

The different behaviours of habitat occupation may have a consequence for the whole population, as it conditions growth, maturation and other demographic features. However, additional data is needed to assess the implications in terms of ecosystem services and fisheries management.

There is a degree of experience among the partners in use of microchemistry to explore the origins of populations and species, as well as the habitat use patterns. INRAE has substantial experience of investigations on Allis shad, *Alosa alosa* via microchemistry (Martin et al 2015) and more recently through shared project work with EHEC-USC. The EHEC-USC has abundant collection material (both scales and otoliths of both adults and juveniles) of twaite shad from the Ulla River. All this material comes from a doctoral thesis on the otolith microchemistry and population dynamics of the twaite shad populations of the Ulla and Minho rivers (Nachón 2017). Within the framework of the DiadES project EHEC-USC will collect individuals (and consequently scales, otoliths and samples for genetics) of both shad species from the marine environment adjacent to the region of Galicia. IFI has been active in using this technology in respect of sea bass, *Dicentrarchus labrax*, (Ryan et al. 2016) and on sea trout (Ryan et al. 2019).

It is planned that all analysis for microchemistry within DiadES would be centralised, using the INRAE facility to process all samples from all partners participating in this facet of the project. This provides scope to rationalise, to standardise and to streamline the analysis of material and the interpretation of outcomes. In such a scenario, there would be a series of 'collector' partners feeding material into the 'active' partner leading the analysis.

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	Irstea	AZTI	EHEC-USC	MARE-UÉ	CMVNC	IFI	Cefas	MNHN
	Gironde/Garonne/Dordogne system		**Ulla catchment**	**Mondego catchment**	**Minho catchment**	**Waterford harbour and the three sisters' rivers**	**Tamar, Frome and Taff rivers**	**Loire catchment**
otolith (and scale) microchemistry	CS (existing samples and data)	CS	CS (existing samples and data)	CS	CS	CS	CS (Tamar & other SW rivers and adjecent marine areas)	CS (existing samples and data)
Species	*Alosa alosa; Chelon ramada*	*Alosa alosa* scales & otoliths	*Alosa fallax (Alosa* spp. from marine areas*)*	*Chelon ramada; Alosa alosa*	*Alosa spp. and Chelon ramada* existing samples and data	*Chelon ramada; Alosa alosa*	*Alosa alosa*	

Table 4.1: DiadES partner data collection for otolith and scale microchemistry. 'CS' = sampling in own case study. 'poss' = possible additional sampling that may be available or links to other projects.

4.2 Protocol issues for shad samples – overview of requirements

This manual presents protocols to cover:

a. Collecting, storing and forwarding of water samples to the overseeing laboratory (for linking water chemistry to otolith chemistry);
b. Biometric data collection, scale collection and storage (plastic forceps etc.);
c. Harvesting and preserving otolith material for forwarding to overseeing laboratory;
d. Use of scale material as otolith surrogate and comparison of microchemistry of paired otolith and scale samples;
e. Treatment of otoliths for microchemistry analysis via LA-ICPMS;
f. Treatment of water samples submitted to overseeing laboratory in conjunction with otolith material for microchemistry analysis;
g. Data collection and analysis of outcomes for the ICPMS for otoliths (or scales, if intercalibration is successful).

Protocol a: Collecting, storing and forwarding of water samples to the overseeing laboratory (for linking water chemistry to otolith chemistry); For European shads, allis shad *Alosa alosa* (Linnaeus, 1758) and twaite shad *Alosa fallax* (Lacépède, 1803)

For rivers inhabited by shad populations, establishing the baseline of a rivers' microchemistry is the main task. In order to do this, it is essential to sample and analyse water samples from the spawning areas of the two species. It is also advisable to have and analyse otoliths of juveniles of both species captured in the riverine environment to check the chemistry with the one detected in water samples.

It is necessary to investigate the spatial variability in water chemistry in the main spawning rivers throughout the native range of the shads (Martin et al. 2015; Randon et al. 2018; Nachón et al. 2019). This is especially

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important in those rivers that flow through a variety of geological formations (Martin et al. 2013; 2015). These rivers must correspond to the rivers where the fish samples are obtained (Martin et al. 2015; Randon et al. 2018; Nachón et al. 2019). Water samples from each river should be collected in the main known spawning areas of shads and during the spawning season (Martin et al. 2015).

At each location, 100 ml of river water (Figure 4.2.1) must be collected for Sr:Ca, Ba:Ca, and 87Sr:86Sr analyses (Martin et al. 2013; 2015; Nachón 2017). Water samples must be passed through 0.45 µm Nalgene polytetrafluoroethylene filters (http://www.vgdusa.com/Nalgene-Syringe- Filters-PTFE-25mm.htm) with syringes into acid-washed, low-density polyethylene bottles and acidified (2%) using concentrated, ultrapure HNO3 (J.T. Baker, Ultrex II; http://www.avantormaterials.com/). Samples must be kept on ice in the field and refrigerated upon return to the laboratory (Martin et al. 2013; 2015; Nachón 2017).

Figure 4.2.1. Sampling water sample with a telescopic cylindrical bar (photographs taken by Nachón in 2017 and 2013 respectively).

Supplementary information

Procedure for taking and treating water samples for dissolved trace element analysis (Sr, Ca, Ba, 87Sr/86Sr, ...)

- Put on a pair of gloves. Rinse the sample bottle (250 mL wide neck bottle) thoroughly by filling it with the water from the river to be sampled and emptying it (5 to 6 times).

- At the end of the rinse, refill the sample bottle and take a syringe. Fill the syringe by pumping water into the sample bottle and then add a filter at the end. Filter the volume of the syringe into a 125 mL vial previously numbered (number or name of the sampled site). Rinse the bottle with this volume by shaking and emptying the bottle (flushing the bottle).

- Remove the filter, refill the syringe, replace the filter and filter the volume into the vial (do not empty). Repeat the operation as many times as necessary to fill the vial up to the top of the wide part of the vial (do not forget to remove the filter at the end of the syringe before filling it and replacing it immediately to filter the new volume of water to be filtered).

- Add 1 dose of nitric acid (1 small 2 mL vial per sample) and close the vial tightly. Shake to mix and store in a pocket away from light in the fridge. Fill in the field form.

At this point it should be noted that in order to establish a correct relationship between the microchemistry of the water and that of the otoliths, it is essential to obtain otoliths from the juveniles caught in the spawning areas. Furthermore, it would be interesting pooling otoliths from several years to account for the range of element values likely to be found in the adult cohorts. However, if it is not possible, due to the scarcity of the populations or the difficulty of catching the juveniles, the relationship between the microchemistry of the water and the

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microchemistry of the otoliths could be estimated, through previous work (Martin et al. 2015; Randon et al. 2018; Nachón et al. 2019).

Protocol b: Biometric data collection, scale collection and storage

For European shads, allis shad *Alosa alosa* (Linnaeus, 1758) and twaite shad *Alosa fallax* (Lacépède, 1803)

The following biometrical data is required for all samples of shads, both juvenile and adult, to permit species-specific identity:

- gill raker count (Figure 4.2.2, see also Chapter 5 on hybridization)
- fork length (LF) and total length (LT)
- wet weight
- sex – only for adults (all males are spermatic and release sperm as they enter the river and females may or may not release eggs after a brief massage, but usually have the more swollen urogenital papilla or see the gonads if dissection of individuals is performed; Figure 4.2.3)
- age

In addition, data related to the catch (date and location of catch, GPS coordinates if any and how they were captured) are needed as a minimum.

NOTE: The number of gill rakers is the most commonly used character for distinguishing the populations of shads of different river basins (Aprahamian 1982; Sabatié 1993). In addition, it is the main diagnostic character for the determination of the specific identity of the specimens of the genus *Alosa* and the detection of possible hybrid individuals (Sabatié 1993; Sabatié et al. 2000). The thresholds for the identification of specific identity differ between juveniles and adults. Consult the work of Nachón et al. (2016) and of Taillebois et al. (2019) to find these values. The first left gill arch must be removed (Figure 4.2.2) and the gills must be cleaned with plenty of water and preserved in 70º ethyl alcohol. Counting should be performed using a binocular stereomicroscope, and should include the small, not extended or fully developed, gill rakers present at each end of the gill arch, as proposed by Sabatié (1993), and King and Roche (2008).

Figure 4.2.2. Extraction of the first left gill arch, first left gill arch (from Nachón 2017) and counting of the gill rakers in the field (photograph taken by Nachón in 2017).

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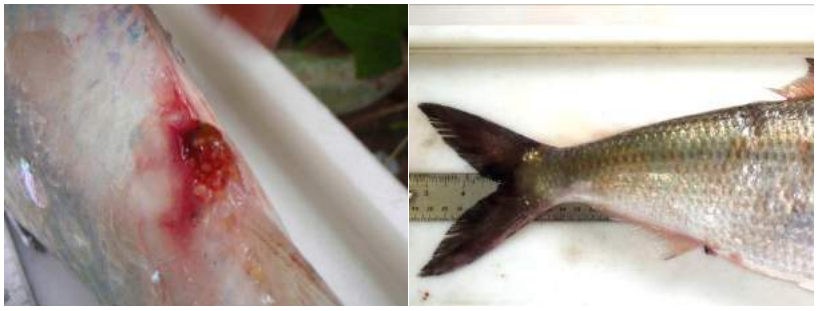

Figure 4.2.3. Eggs in the urogenital papilla and (b) detail of how it protrudes from the rest of the body both by size (thickening) and by coloration (deep red) (Nachón 2017).

Protocol c: Harvesting and preserving otolith material for forwarding to overseeing laboratory

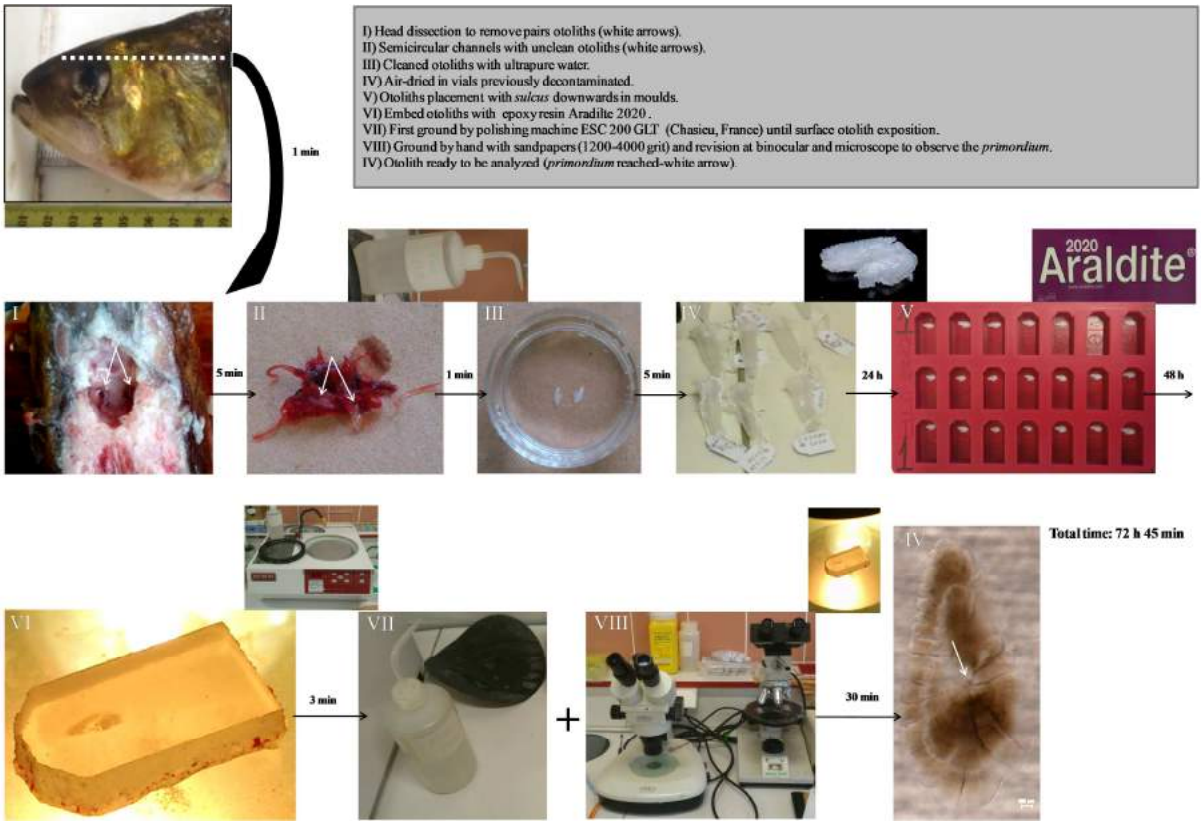

Figure 4.2.4. Schematic diagram of the otolith preparation protocol (extraction, cleaning and polishing) in which is included the total preparation time of each otolith estimated for an experienced person. Modified and reformed from Tabouret (2009), in Nachón (2017).

The analysis of the chemical composition of the otolith requires a series of precautions to be taken during preparation in order to avoid possible contamination that could alter otolith elemental composition (Proctor and Thresher 1998). Consequently, several decontamination and preparation steps must be followed. Figure 4.2.4 shows a schematic diagram of the protocol developed by Tabouret (2009) followed in this report.

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- The head of each shad must be thawed, with warm water, to facilitate the cutting and extraction of the otoliths. A longitudinal incision is made, with the help of a scalpel, just above the optical capsules, which allows the cephalic cavity to be sectioned and its contents exposed to view (see Figure 4.2.5).
- The principal concern is to cut in the optimal position, just above the upper limit of the eyes, as close as possible to that limit.
- Then, the brain mass is removed and this exposes the inner ear and the cavities where the otoliths are, and the otoliths should be visible.
- For those not experienced in otolith extraction, one option is to perform the extraction when the head is still somewhat frozen. At this stage the otoliths remain in the cavities because they have a layer of ice around them that prevents them from moving from their cavities. If the head is still frozen, when the inner ear is pulled, it breaks, and the otoliths are visible inside the cavities.

If the head is thawed, normally when pulling on the inner ear, the otoliths come stuck to the inner ear but this option is risky for non-experts, because otoliths can be lost in the extraction process

The otoliths must be removed with the help of ceramic or plastic forceps, previously decontaminated with 10% ultrapure HNO3 (Ultrex, J. T. Baker©), instead of metallic forceps, to avoid the transfer of ions between the surface of the same and the sagitta as proposed by PATTERSON et al. (1999).

Note: If the tweezers are new, decontamination with nitric acid is not necessary. Decontamination with nitric acid is only indicated if the tweezers were used for other purposes with a possible risk of contamination towards the otoliths. If the tweezers are decontaminated with nitric acid, it is necessary to dry them very well, because if not, this acid can be transposed to the otoliths and dissolve them, rendering them useless for later analysis. Drying should always take place under a laminar flow hood. The sagitta are then subjected to a series of washes with MiliQ water, to remove the tissues adhering to their surface, and are left to dry for 24 hours in a laminar flow hood inside open eppendorf vials that have been previously decontaminated with 10% ultra-pure HNO3 (Ultrex, J. T. Baker©). After this time the eppendorfs are closed and the otoliths remain stored until analysis.

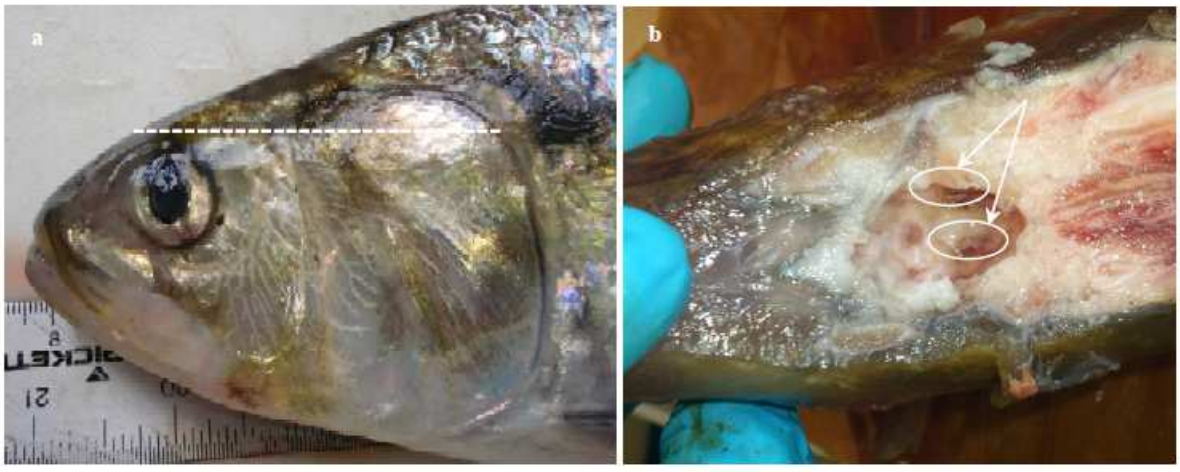

Figure 4.2.5. (a) Cutting area of the cephalic region to access, after extraction of the brain mass, (b) on a par with sagitta (white arrows) of *A. fallax* (photograph taken from Nachón 2017).

Note: Eppendorfs should only be decontaminated if they were previously used for other purposes that could be a source susceptible to otolith contamination. If they are new, it would not be necessary to decontaminate them and just rinse them a little with milliQ water several times and dry them very well. As with tweezers, if the

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eppendorfs are decontaminated with nitric acid, it is necessary to wait until they are completely dry, in order to avoid that remains or drops of acid remain inside them - the nitric acid attacks the otoliths and makes them useless for further analysis. Drying should always take place under a laminar flow hood.

Protocol d: Use of scale material as otolith surrogate and comparison of microchemistry of paired otolith and scale samples

It is absolutely essential that the scales are from the same specimens from which the otoliths were extracted, in order to be able to compare the chemical composition for the same individual between the two growth structures. For analysis, stored and dried scales (stored in paper envelopes) of adult shads returning to the river or caught in the sea, with unregenerated nuclei, should be selected. The scales will be analyzed by the same analytical processes, machines and in the same laboratory as the otoliths, in order to guarantee the comparison results. If the results are satisfactory, a detailed protocol for sample preparation and analysis will be developed. This would allow use of a non-lethal method, unlike otoliths, which is particularly beneficial when dealing with threatened or endangered populations.

Protocol e: Treatment of otoliths for microchemistry analysis via LA-ICPMS

For European shads, allis shad *Alosa alosa* (Linnaeus, 1758) and twaite shad *Alosa fallax* (Lacépède, 1803)

Otolith preparation will be carried out according to Martin et al. (2015). One sagitta per fish must be embedded in epoxy resin (Araldite 2020, Escil) with the primordial surface downwards. Resin blocks will be polished with ultrapure water and sandpaper (1200–4000 grit) until the primordium is reached. Finally, otoliths will be rinsed with ultrapure water and then air-dried before being stored in individually labelled plastic vials.

The **otoliths will be analysed** according to the protocol drawn up by Martin et al. (2015). To analyse 87Sr:86Sr, Sr:Ca and Ba:Ca ratios, two C-shaped ablation trajectories of 60 µm thickness will be performed 40 µm away from the core (Figure 4.2.6). A High Resolution (Thermo Scientific, USA) inductively coupled plasma quadrupole mass spectrometer (HR-ICP-MS) will be used to analyze Sr:Ca and Ba:Ca ratios (Figure 4.2.7). The HR-ICP-MS is coupled to a UV high-repetition-rate femtosecond laser ablation (fs-LA) system (Nexeya SA, Canéjan, France). A Nu-Plasma multicollector inductively-coupled-plasma mass-spectrometer (MC-ICP-MS, Nu Instruments, UK) coupled to a UV high-repetition-rate femtosecond laser ablation (fs-LA) system (Nexeya SA, Canéjan, France) will be used to analyze 87Sr:86Sr (Nachón et al. 2019). Analytical process can be found in Martin et al. (2015).

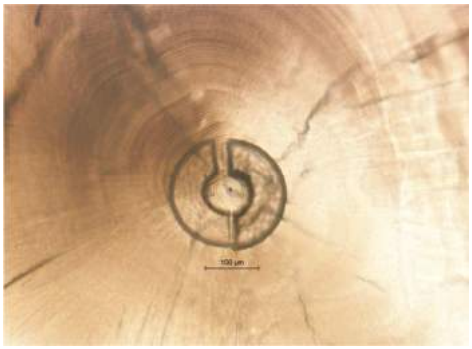

Figure 4.2.6. Picture of adult Allis shad otolith showing the two areas ablated by the laser prior to ICP-MS (left semicorona) and MC-ICP-MS analysis (rigth semicorona). Semicoronas of 60 µm thick (difference between the inner 40 µm and outer radius 100 µm) are centered on the primordium and correspond to the juvenile freshwater period of growth only (from Martin et al. 2015).

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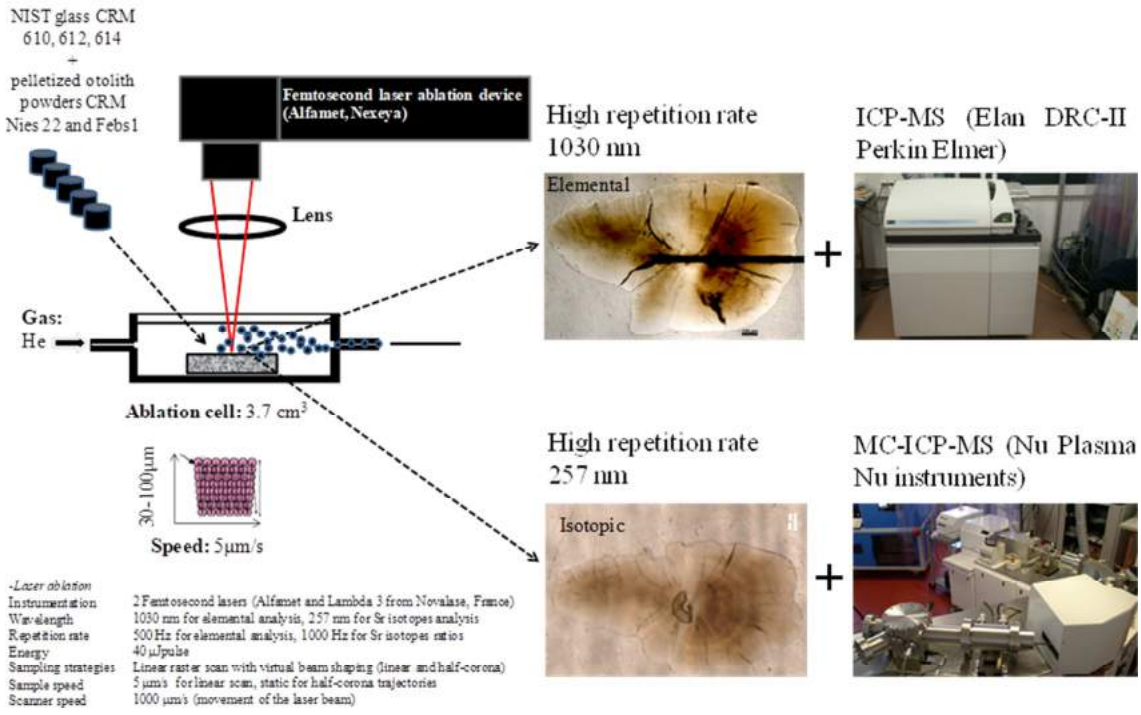

Figure 4.2.7. Ablation strategies carried out for each type of analysis, note that (from Nachón 2017). Note that for elemental analysis, a continuous transect is marked in the photo, but the trajectories to be carried out are in the form of a semicorona for both elemental and isotopic analysis, as in figure 4.2.6 above.

Protocol f: Treatment of water samples submitted to overseeing laboratory in conjunction with otolith material for microchemistry analysis

Water sample preparation

Water samples were diluted 10-fold with 2% HNO3 to measure elemental concentrations.

For 87Sr:86Sr measurements in river water, the appropriate volume of each sample (7–50 ml) was evaporated to dryness to ultimately get 4 µg of Sr and redissolved in 3M HNO3 after Martin et al. (2013). Then, Sr was separated from the rest of the matrix using columns containing Sr resin (Eichrom Technologies) and sequential elutions with ultrapure water and 3M ultrapure HNO3 (Prohaska et al. 2002). After Sr separation, the sample was then diluted in 2% HNO3 (final Sr concentration was 200 µg/l) for Sr isotope analysis. This protocol comes from Martin (2013) and Nachón (2017).

Water sample analysis

Water samples will be analysed using solution-based ultrasensitive inductively coupled plasma mass spectrometer (ICP-MS, Bruker Aurora Elite; www.bruker.com) to measure Ca, Sr, and Ba concentrations following the protocol of Martin et al. (2013). The general performance of the procedure will be checked every 10 samples using the certified reference freshwater SLRS-5 (NRCC; www.nrc-cnrc.gc.ca). External precision (percentage relative standard deviation, RSD) for the laboratory standards (n = 10) will be 3% for Ca and Ba and 4% for Sr. Sr isotope analysis will be performed using the Nu-Plasma MCICP- MS and following Martin et al. (2013). Accuracy and precision in that study was monitored with a standard reference material (SRM 987;

www.nist.gov/srm). The mean ± SD value of 87Sr:86Sr in SRM 987 (n = 54) run throughout the analyses was 0.71034 ± 0.00003, which compares favourably with the accepted value of 0.71034 ± 0.00026.

Analysis outcomes

The raw data obtained will be analysed following the processes of analysis of data detailed in previous publications (Martin et al. 2015; Randon et al. 2018; Nachón et al. 2019), which have already proved to give satisfactory results. The analysis aims to examine:

- Differences in the composition of the water between rivers;
- Correspondence between the microchemistry of the water and the microchemistry of the otoliths;
- Calculation and assignment of each of the individuals analysed to the populations studied by means of a Bayesian analysis.

4.3 Proposed studies of thin-lipped mullet in DiadES – otolith and scale studies

Within the scope of DiadES, otolith microchemistry analysis of Sr:Ca and Ba:Ca ratios will be used to study habitat use patterns and migratory dynamic of the catadromous thin-lipped mullet, *Chelon ramada*. In order to address possible behaviour-type categories within the species the following requirements are identified:

a. Microchemistry of the salinity gradient (for linking water chemistry to otolith chemistry);
b. Biometric data collection, scale collection and storage;
c. Otolith collection and analysis.

a) Microchemistry of the salinity gradient

As a first step, as described for European shads, water samples from the study area must be obtained to establish the baseline of the 'salinity gradient' microchemistry along the 'river-estuary-sea' transect in order to link water and otolith chemistry. Water samples should be obtained along the salinity gradient (following **protocol a** above) and analysed for Sr:Ca and Ba:Ca. It is also advisable to analyse otoliths of mullets captured in different environments, such as marine, estuary and river, to check the chemistry with the one detected in water samples.

b) Biometric data collection, scale collection and storage

Mullet sampling must be followed by biometric data collection and scale collection and storage. Scale reading will be used to infer mullet's approximate age and obtain an age-length relationship that will be used to define the specific size range of mullet considered for otolith analysis. The standardization will be very important since in mullet otoliths the core and the edge are not always in the same plane and even with the best possible cutting plane, we must take into consideration the trade-offs between the time scale and data loss.

c) Otolith collection and analysis

In order to avoid possible contamination that could alter otolith elemental composition (Proctor and Thresher 1998), the decontamination and preparation steps described in **protocol C** for shads (above) must be followed (Figure 4.3.1).

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Figure 4.3.1: Extraction of sagittal otoliths from thin-lipped mullet, *Chelon ramada* (photographs taken by Esmeralda Pereira in 2019).

Sagittal otolith extraction and LA-ICPMS analysis will follow that described by Daverat et al (2011) where otoliths were ground in the frontal plane to expose the core, polished using 1 micron diamond paste and stored in clean plastic sealable vials awaiting analysis. Alternatively, otoliths may be embedded in epoxy resin and a transverse section will be obtained with a diamond saw.

A High Resolution (Thermo Scientific, USA) inductively coupled plasma quadrupole mass spectrometer (HR-ICP-MS) will be used to analyze Sr:Ca and Ba:Ca ratios along the longest growth axis i.e. from the core to the edge of each otolith.

Each transect of Sr:Ca, Ba:Ca will be analysed to retrieve the movements of each fish across the salinity gradient. This analysis will be performed using all the available knowledge of the river basin i.e.: Sr:Ca and Ba:Ca in freshwater, type of estuary, features of the estuary, tide, turbidity etc. Transects will be classified into behaviour-type categories.

4.4 Data management - labelling – QA for consistency and reliability of labelling

Sample traceability and sample sharing across the project will be ensured by the construction of a joint database using Collec-Science. See section 9 on database management and use for more details. Editable label templates will be used to ensure consistency and reliability of labelling across the project. Labels will contain a QRCODE to ease stock management.

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5. Shad hybridization dynamics, dispersive capacities and homing behaviour of hybrid individuals

5.1 Introduction and state of the art

In the course of detailed discussion among partners, hybridisation of shads was identified as a key issue for future management of this species complex (e.g. legal status, conservation status, values…). This topic had not been previously planned for investigation within DIADES. Allis and twaite shad form a complex of sister species that keep hybridizing over timescales. Hybrids are fertile. Hybridization might be a process where each species gains resilience. Using recently developed genomics markers, and with a small financial contribution, this issue could be collectively addressed within DiadES.

A recent study succeeded, for the first time, in reliably detecting third-generation shad hybrids (Taillebois et al. 2019). The primary source of genetic material for this study was collected as part of monitoring programs on *Alosa alosa* and *Alosa fallax* populations in French rivers of the Atlantic coast by fisheries departments, local migratory fish associations, and planning councils. Some genetic material was also retrieved from a previous research project (Martin et al. 2015). A total of 634 sexually mature *Alosa* spp. individuals were sampled from 12 river localities across the French Atlantic coast, from the Vire River on the English Channel (Normandy region) through to the Nivelle River on the south Bay of Biscay and from one locality in the marine environment in the middle of the Bay of Biscay facing the Charente's river mouth and named hereafter Ocean (Figure 5.1.1).

The conclusions show that interspecific interactions and gene flows should not be overlooked when considering the management of these two species. Overall, contemporary and historical introgression revealed by nuclear and mitochondrial markers strongly suggests that a transfer of genes occurred from *A. fallax* toward *A. alosa* genome at least four generations ago. Moreover, the outcomes of introgression greatly depend on the catchments where local processes are thought to occur. Undoubtedly, interspecific interaction and gene flow should not be overlooked when considering the management of these species.

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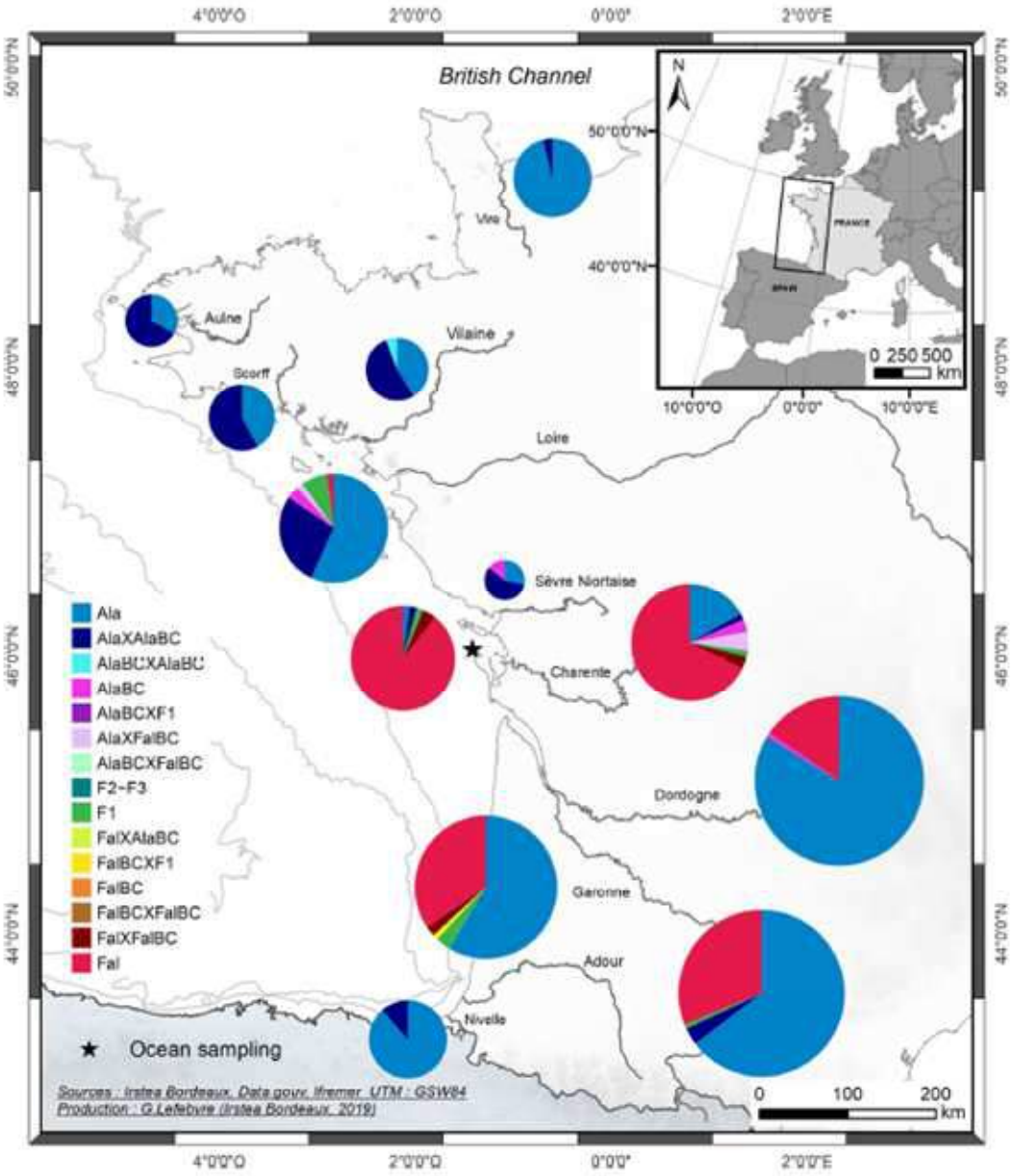

Figure 5.1.1. Sampling map of *Alosa alosa*, *Alosa fallax*, and hybrid specimens across 11 rivers and one oceanic sampling site along the French Atlantic coast. For each sampled site, the proportions of the different purebred and hybrid classes as retrieved using the SNP nuclear markers and New Hybrids are graphically represented (from Taillebois et al. 2019).

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5.2 Sample collection, storage and analysis of material

	Irstea	AZTI	EHEC-USC	MARE-UÉ	CMVNC	IFI	Cefas
	Gironde/ Garonne/ Dordogne system	**Gipuzkoan rivers**	**Ulla catchment**	**Mondego catchment**	**Minho catchment**	**Waterford harbour and the three sisters' rivers**	**Tamar, Frome and Taff rivers**
genetics of shads	CS (existing samples and data)	Fin clips available	Poss	CS	CS	CS	CS (Tamar)

Table 5.1: DiadES partner data collection for study on genetics of shad hybridisation. 'CS' = sampling in own case study. 'poss' = possible additional samples that may be available or links to other projects.

Genetic samples provided by partners (Table 5.1) should preferably be collected from the same individuals from whom the otoliths were extracted as in Martin et al. (2015). By doing this, the microchemistry of the otolith can be coupled with genetic analyses to study the population connectivity and dispersion over ecological and evolutionary time scales as in Martin et al. (2015). A further step is that this information can relate hybridization status to dispersal capabilities.

The origin of samples (fin clips or scales) can be diverse, ranging from tissue samples collected on live fish that were released after fin clipping or scale removal, through collection of fin samples from frozen fish, to tissue samples collected on fish carcasses (Taillebois et al. 2019). All tissue samples (fin clip, muscle or even scales) must be placed into vials containing molecular grade 95% ethanol (Martin et al. 2015; Taillebois et al. 2019). Scales could be stored in labelled paper envelopes (Figure 5.2.1). Samples stored in eppendorf bottles containing 95% alcohol must be properly labelled and stored in suitably labelled boxes (Figure 5.2.1). These boxes can be stored in the refrigerator to improve or ensure optimal preservation of the samples until shipment and subsequent analysis. Total genomic DNA will be extracted using Invitrogen™ PureLink™ Genomic DNA Mini Kit following the manufacturer's instructions and visualized on 1.5% agarose gels.

Biometric and specific identity information is essential for the study of hybridization. The information is the same as that indicated in the previous section on the microchemistry of otoliths (Section 4.2, Protocol b).

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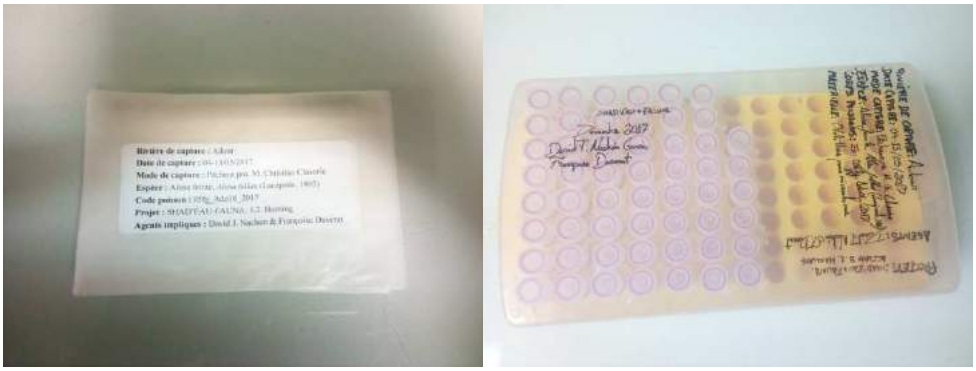

Figure 5.2.1. Examples of correct labeling and storage of scale samples and soft tissue samples respectively (photographs taken by Nachón, in 2017).

All genetic samples will be analysed following Taillebois et al. (2019). Genetic analysis will be performed at the INRAE platform – laboratory of CESTAS.

5.3 Potential outcomes

The study range can be broadened from the prior French study to include samples from the five countries of the Atlantic Area. In DiadES, contrasted situations exist that will allow characterizing the identity and genetic integrity of European shads. In Ireland and England, twaite shad is predominant and allis shad is rare. The dynamics of hybridization can be studied in that particular case study. In Galicia (Northern Spain), in contrast, there is a monospecific population of twaite shad. DiadES can also look at the genetic purity of this population. Further examples and potential candidate study cases are needed. The set of all this information will extend and improve our genetic understanding and knowledge about the two species and will give a greater insight into their joint conservation.

5.4 Data management - labelling – QA for consistency and reliability of labelling

Sample traceability and sample sharing across the project will be ensured by the construction of a joint database using Collec-Science. See section 9 on database management and use for more details. Editable label templates will be used to ensure consistency and reliability of labelling across the project. Labels will contain a QRCODE to ease stock management.

5.5 References

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This project is co-financed by the Interreg Atlantic Area Programme through the European Regional Development Fund.

6. Tracking of fish – marking studies and telemetry

6.1 Introduction

The tracking of fish using electronic tags is a powerful tool at the disposal of fisheries biologists and managers to support the conservation and management of fish stocks. The capacity to 'mark' individual fish in order to follow their movements, in space and in time, has led to a major expansion of knowledge and understanding of fish movements and migrations and the impacts of environmental and anthropogenic factors on both individuals and populations. As technology has developed so has the degree of sophistication of the types of 'marking devices' one can use. Over the last 30 years, the use of radio telemetry to study the migratory behaviour of fish in freshwater ecosystems and acoustic telemetry for research in transitional and marine environments has developed rapidly. More recently, archival or data storage tag (DST) have been developed to support the management of marine fisheries. DSTs record data such as depth, temperature and light but are fishery-dependant and the tags need to be recovered to download the data. Although DST technological innovation continues to this day with the miniaturisation of the tags and inclusion of novel sensors, it was recognised that a fishery-independent method of data collection from free ranging fish was required. This has led to the development of popup satellite archival tags (PSAT or PAT). These tags are large versions of archival tags that are combined with an Argos transmitter. A recognized advantage of PSATs is their ability to collect data on temperature, depth, and light levels and then transmit those data directly through the Argos satellite system following their programmed release and emergence at the water's surface.

Within DiadES a range of different 'marking' systems will be deployed, from numbered floy tags to radio and acoustic tags that activate listening stations and to satellite-linked pop-up tags. The suite of target fish species ranges from the large European sturgeon to the relatively small out-migrating immature river- and sea lamprey and the estuarine smelt.

All partners undertaking marking and tagging studies in DiadES will operate under the guidance of the EU directive of 2010 on the protection of animals used for scientific purposes (DIRECTIVE 2010/63/EU). DiadES partners will have received appropriate training and licencing for undertaking the work identified here and will have appropriate authorisations under their own implementing national legislation.

	Irstea	EHEC_USC	MARE-UÉ	CMVNC	IFI	CEFAS	MNHN	MNHN
	Gironde/ Garonne/ Dordogne system		**Mondego catchment**	**Minho catchment**	**Waterford harbour and the three sisters' rivers**	**Tamar, Frome and Taff rivers**	**Loire catchment**	**Normand-Breton Bay/Gulf**
Tagging - floy tags etc		CS			**CS (shad)**			
Telemetry	CS		CS	CS	CS	CS (Tamar, Frome, Taff- previous work + some new work on smelt)	CS (existing samples and data)	CS (existing samples and data)
Species	*A. sturio*	*Petromyzon marinus*	*Salmo trutta*	*Anguilla anguilla*	*Alosa fallax; Chelon ramada*	*A. anguilla/S. salar/S. trutta/Osmerus eperlanus*	*A. alosa/P. marinus/A. anguilla/S. salar*	

Table 6.1. Marking and telemetry studies in DiadES. 'CS' = sampling in own case study.

This project is co-financed by the Interreg Atlantic Area Programme through the European Regional Development Fund.

6.2 Individual DiadES partner proposals

INRAE – Marine movement of juvenile sturgeon *Acipenser sturio*

The objective of tagging *Acipenser sturio* in DiadES is to assess the individual trajectory at sea of juveniles stocked at young stages in the Gironde-Garonne-Dordogne watershed.

In 2019, 5 PSATs (Pop-up Satellite Archival Tags, Wildlife Computer, MiniPAT, length = 124 mm, weight = 60 g) were purchased to follow *A. sturio* movement at sea. *A. sturio* individuals above 7 kg will be tagged in 2020. Fish will be captured during trawling campaigns occurring in the Gironde estuary (France). They will be anesthetized and externally tagged using monofilament line. Two sites of anchoring were chosen: one line will be passed through one scute above the dorsal fin and one line will be passed through the base of the dorsal fin; both of them being linked to the tag.

The PSATs will be programmed to pop-up about 6/8 months after tagging. Fish of the size chosen are expected to leave the estuary to feed and grow at sea. Some individual fish may travel regularly between the sea and the estuarine area.

In addition to data recovered through the ARGOS system (CLS France), a specific receiver and corresponding antenna (Wildlife Computer) were purchased to try to recover the PSATs once they will have popped up to get access to more detailed data.

In parallel with DiadES, a French project called MOMIE (Migratory movement of European sturgeon *A. sturio*: habitat at sea and spawners return in freshwater; INRAE/OFB) will add 5 PSATs to supplement the experiment (tagging planned in 2020/2021).

EHEC-USC – Studies on the marine phase duration and freshwater dispersal of sea lamprey

The EHEC-USC will carry out a mark-recapture programme on sea lamprey to determine the duration of the marine phase and the dispersal capacities of the species (both at sea and in the river when they return for their spawning migration).

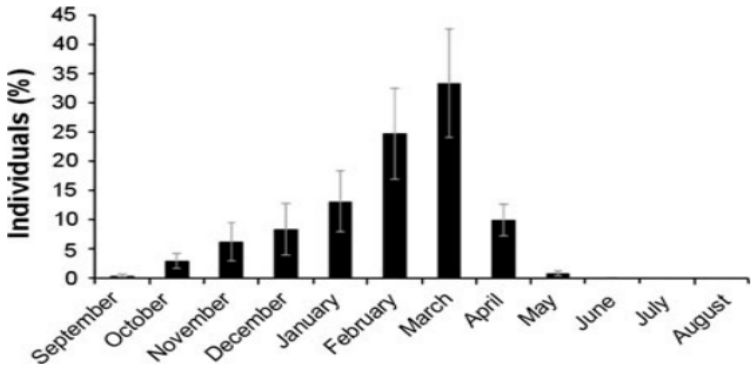

Figure 6.2.1. Monthly distribution of the percentage (mean ± standard error) of post-metamorphic lampreys captured in the locality R1 during their downstream migration (1997–2010), from Silva et al. (2013).

This project is co-financed by the Interreg Atlantic Area Programme through the European Regional Development Fund.

The mark and recapture work will coincide with the timing of the downstream migration of the post-metamorphic lampreys. Downstream migration of post-metamorphics occurs between October and May with a peak in March (Silva et al. 2013a and Figure 6.2.1)). During their downstream migration, post-metamorphic lampreys are commonly taken in a fixed trap located about 40 km from the mouth of the Ulla River (R1 in Figure 6.2.2). Post-metamorphic lampreys will be tagged at this location with individually numbered T-bar anchor tags (Figure 6.2.3) (FF-94, Floy Tag, see Silva et al. 2013a, b, for more details). The periodicity of capture of the post-metamorphic lampreys in the fixed trap is very variable and depends on annual hydrology, abundance of lampreys, etc. Therefore, the sampling rate will be adjusted to these variables. The EHEC-USC team will be in permanent contact with the technicians in charge of the management of the trap in order to go to mark the lampreys as soon as they fall into the trap. The post-metamorphic lampreys will be released several kilometres downstream to assist them in their downstream migration (a few km above E1, Figure 6.2.2). In case of difficulties with this work, the team already has previous data and information, contained in two articles (Silva et al. 2013a, b), which will be made available as needed.

Figure 6.2.2. Location of River Ulla (R) and estuary (E) sampling sites (from Silva et al. 2014), and detail of trap facilities at location R1.

Figure 6.2.3. T-type mark (Floy tag) and injector (left image) and post-metamorphic lamprey tagged with a T-type mark (right image) (pictures from Silva, 2014).

University of Évora / MARE – Trout habitat use, migratory patterns and behaviour

This telemetry study on trout (*Salmo trutta* L.) will be conducted in the river Mondego and take advantage of the array of fixed acoustic receivers (#8 VEMCO VR2) already deployed throughout the study area within the scope of the European Tracking Network (Figure 6.2.4).

This project is co-financed by the Interreg Atlantic Area Programme through the European Regional Development Fund.

It is planned to tag 20-30 trout, with most of the work focused on its anadromous form (sea trout) but also including resident brown trout specimens, to compare migratory behaviour and habitat use between both ecotypes.

Trout will be captured with the help of recreational anglers and electrofishing campaigns, and tagged with CART - combined acoustic/radio (LOTEK MM-RC-11-28 69 Khz) - transmitters, to allow detection by the fixed acoustic stations in estuarine and deep freshwater locations and by manual radiotracking in shallow freshwater areas. Selected tags will allow the monitoring of tagged trout for at least a 10-12 month period.

Data from fixed acoustic receiver stations will be downloaded every 2 months. Data will be analysed with common statistical and geospatial programs (e.g., EXCEL; R-package; ArcGIS) as well as specific movement analyses packages.

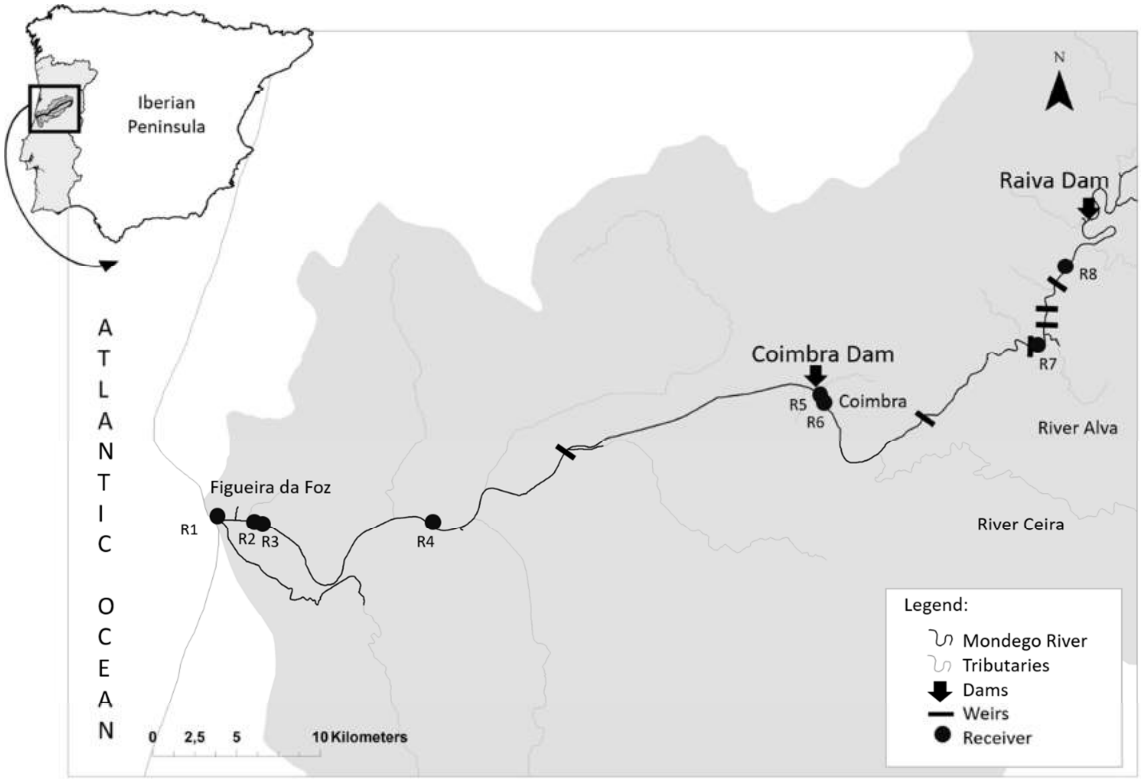

Figure 6.2.4: Location of the acoustic receivers in Mondego River.

CMVNC

CMVNC propose to undertake a joint sampling effort with EHEC-USC in regard to mark-recapture of sea lamprey juveniles in the River Minho. Post-metamorphic lampreys could be caught by two methods - with traps in tributaries or as by-catch in the glass eel fishery.

Since 2007, a mark-recapture programme of eels in one monitoring area of River Minho using PIT-Tag's has been running annually. About 3800 eels were tagged. Growth rates estimation and age validation are the main goals. Outcomes of this study will also be relevant to DiadES.

This project is co-financed by the Interreg Atlantic Area Programme through the European Regional Development Fund.

IFI – Habitat use by adult Twaite shad and thin-lipped mullet

IFI has been using acoustic telemetry since 2012 in the Waterford Harbour case study to examine spawning movements and behaviour of adult twaite shad, as well as other species. The studies have used VEMCO (Nova Scotia, Canada) acoustic technology, namely V9-2L transmitters (270-476 days battery life) and VR2W receivers. The acoustic receivers are strategically deployed at fixed locations to provide a monitoring network throughout the Barrow-Nore-Suir estuaries/Waterford Harbour (Figure 6.2.5). Data are downloaded from receivers twice-yearly. The network is currently supplemented by Irish Marine Institute (MI) receivers, deployed in collaboration with IFI to monitor movements of tagged bass.

Data organisation and examination will be undertaken using VEMCO's own VUE (VEMCO User Environment) software. Further manipulation, analysis and visualisation of the data will be undertaken using R (R Core Team, 2019) and associated packages, for instance VTrack (Campbell et al. 2012).

Since 2017, IFI telemetry research in Waterford Harbour has expanded to include tracking of thick-lipped mullet, thin- lipped mullet and European eel. Numbers tagged and tracked to date comprise 49 twaite shad, 7 thin-lipped mullet, 8 thick-lipped mullet and 40 eels. Considerable insight has been gained regarding the movements of adult shad to and from the spawning grounds during the spawning periods (Rooney and King 2015). The DiadES study will endeavour to examine adult use of the lower Waterford Harbour area and fish movements to and from associated marine inshore environs. The mullet study will examine the niche habitat use of the case study site by thin-lipped mullet and, specifically, usage of the warm-water outfall from the thermal power station at the confluence of the Suir and Barrow rivers.

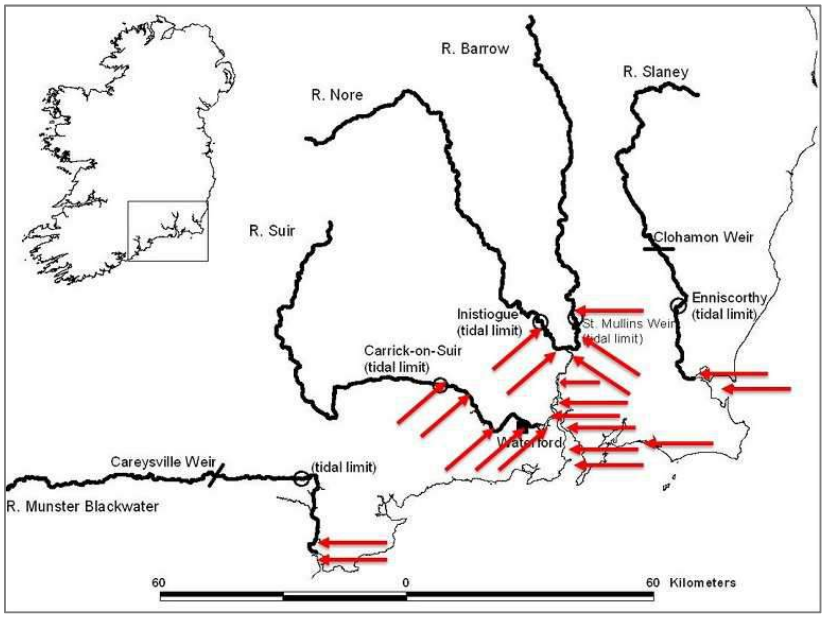

Figure 6.2.5. Locations of acoustic receivers deployed by IFI during the period 2012 - 2018 to monitor fish movements in the SE of Ireland. Marine Institute receivers are deployed in Wexford Harbour, Youghal Harbour and Bannow Bay

IFI also availed of the annual influx of anglers to the River Barrow during the shad spawning migration in May 2019 and tagged 30 shad with numbered floy tags that also contained a PIT tag. It is hoped that some recaptures will be made in 2020.

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Cefas – Studies on the Atlantic salmon (*Salmo salar*, sea trout (*Salmo trutta*), European eel (*Anguilla anguilla*) and smelt (*Osmerus eperlanus*)

Cefas has extensive experience in telemetry from working on Defra salmon and sea trout and silver eel tracking programmes and SMOLTRACK I and II projects implemented in the case study rivers Tamar, Frome and Taff, with outcomes being used under DiadES. Inter-coelomic implantation of Vemco coded acoustic transmitters was undertaken on the salmonid smolts and fish were tracked via acoustic receivers (Figures 6.2.6 to 6.2.8). In the case of eel, stomach implantation of acoustic transmitters into adult fish was undertaken and fish movements were tracked via acoustic receivers. Tag insertion of salmon smolts in SMOLTTRACK II was via stomach implantation.

Regarding future tracking projects under DiadES, Cefas is planning to undertake an acoustic tracking study in the River Tamar in 2020 to examine the spawning behaviour of European smelt in relation to Gunnislake Weir, considered to be a barrier to migration. The smelt will be caught using fyke nets in the freshwater section of the river. Smelt will be stomach tagged with Vemco V5 180 kHz coded acoustic transmitters and released back into the lower estuary. Their subsequent spawning migration will be monitored using a network of 180 kHz acoustic receivers above and below Gunnislake Weir. An array of existing acoustic receivers, operated in the estuary as part of the SAMARCH Project, will monitor the subsequent post-spawning migration as they return to sea.

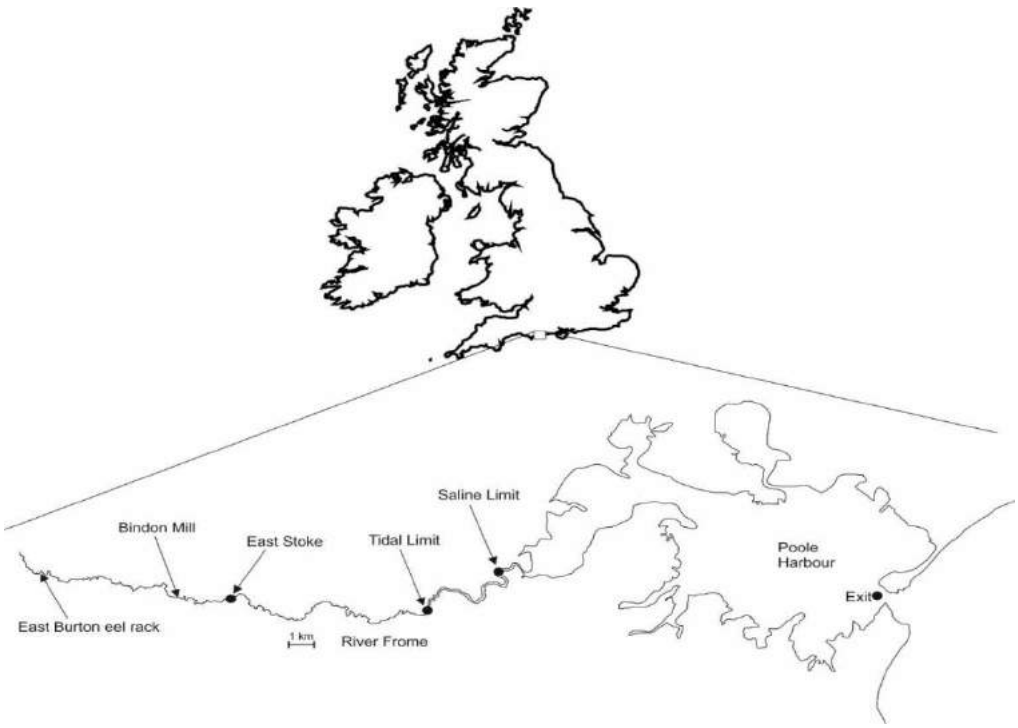

Figure 6.2.6. Position of Cefas receivers in the River Frome.

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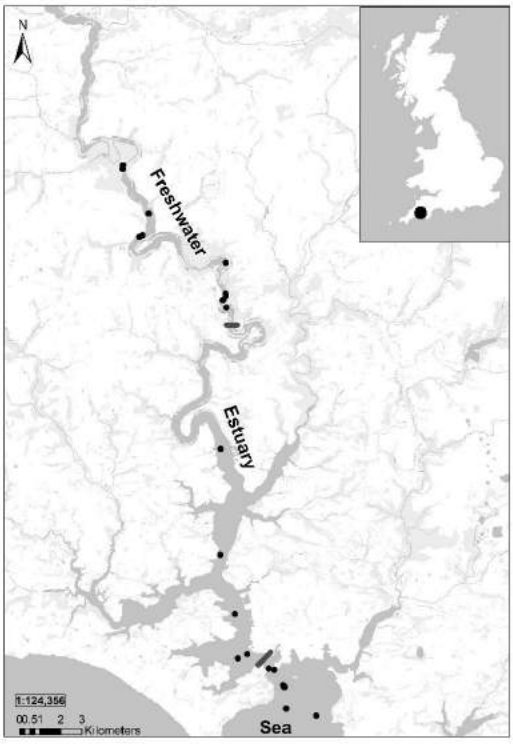

Figure 6.2.7. Receivers position in the River Tamar (black dotes), where black lines indicate boundaries between migration zones.

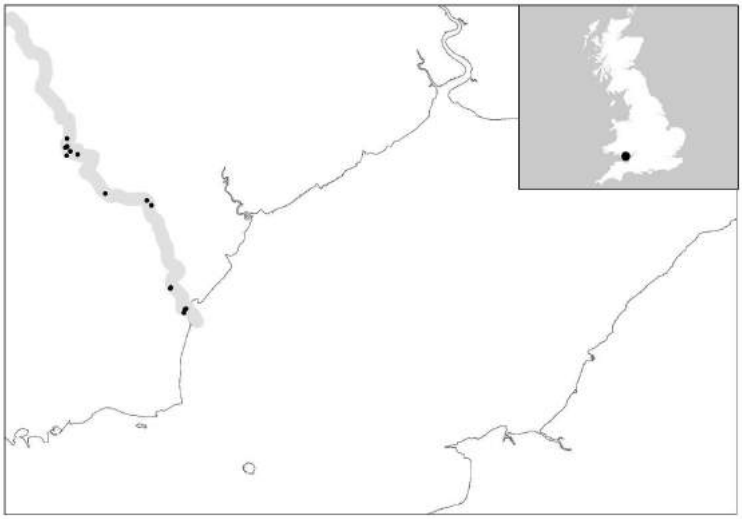

Figure 6.2.8. Cefas receiver positions in the River Taff.

MNHN – Diadromous fish movements in the River Loire

Between 2010 and 2012, a major telemetry study, focused on diadromous species, was undertaken in the Loire River (Figure 6.2.9 and Table 6.2.1). Fish have been tagged with Thelma LP9 and MP13 tags and tracked in the Loire River during their migration. The fish have been recorded with Vemco VR2W hydrophones (69 kHz) as in Figure 6.2.10.

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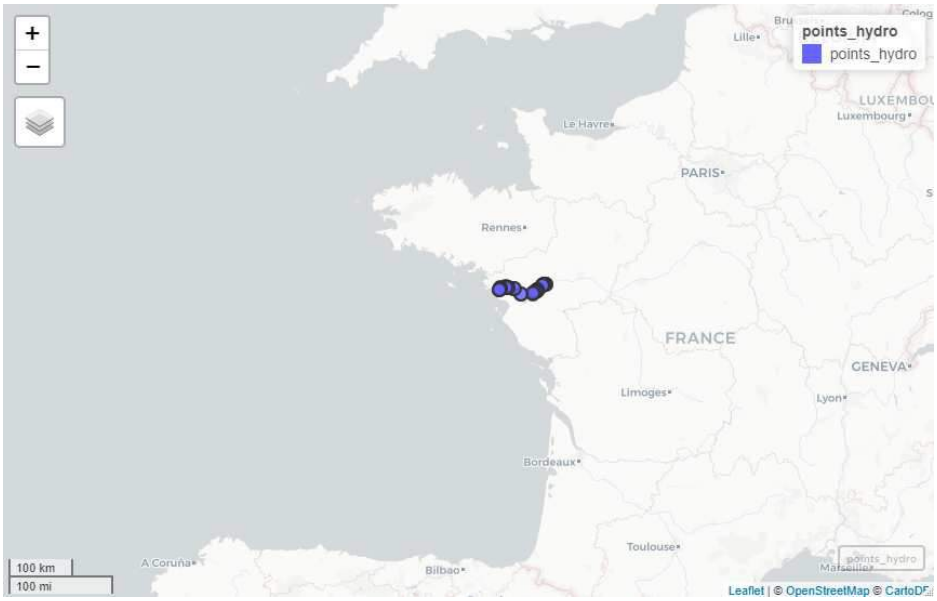

Figure 6.2.9: Location of River Loire and survey area

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Table 6.2.1: Number of tagged fish by species and by year in the River Loire study.

Species	Year	No. fish
Alosa alosa	2011	56
Alosa alosa	2012	26
Alosa fallax	2011	2
Alosa fallax	2012	2
Anguilla anguilla	2010	38
Anguilla anguilla	2011	51
Anguilla anguilla	2012	2
Chelon ramada	2011	7
Petromyzon marinus	2011	48
Petromyzon marinus	2012	64
Salmo salar	2012	3
Total no. fish		**299**

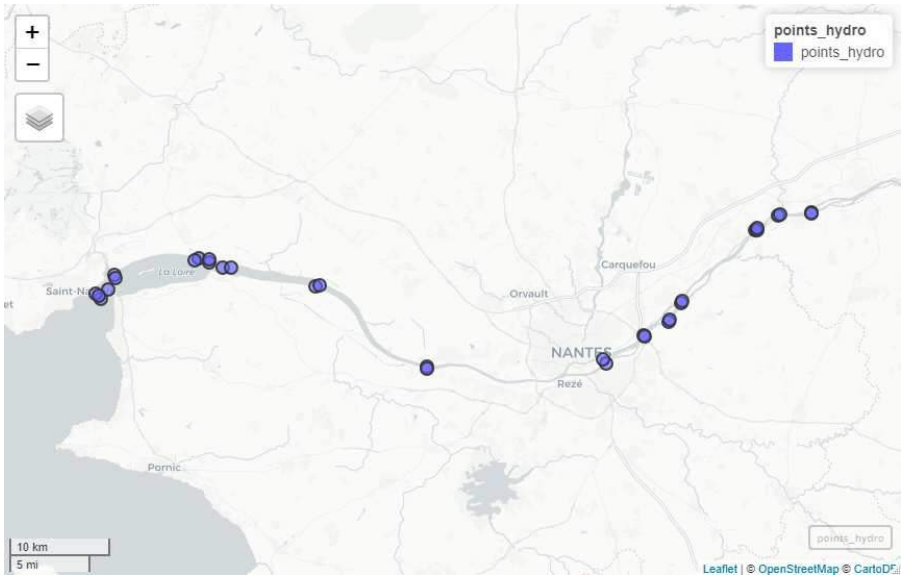

Figure 6.2.10: Sites on River Loire where hydrophones were installed

The data analysis and modelling will be made with the R software, more specifically using Tidyverse syntax and packages under the DiadES project. See also Appendix 2 for an additional study led by MNHN on characterising the food web and diet variability of shads using stable isotopes. This study was not discussed during the Norwich meeting and was as such included as an Appendix to this report.

6.3 References

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This project is co-financed by the Interreg Atlantic Area Programme through the European Regional Development Fund.

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This project is co-financed by the Interreg Atlantic Area Programme through the European Regional Development Fund.

7. By-catch data collection

7.1 Introduction

Some of the target species in the DiadES project are actively fished commercially in some of the partner Member States. Thus sea lamprey and shads are commercially fished in some parts of Portugal, Spain and France whereas there is no commercial or gastronomic interest in these species in Ireland or the UK.

Commercial sea fishing using ground or pelagic netting operations cannot be particularly selective in regard to what is collected in nets. A degree of selectivity is possible using hydroacoustic techniques to identify presence or absence of shoals but it is not possible to plan for what may be captured. In many cases, a range of non-target species are collected. These are of major potential value to a range of scientific studies – those looking at impacts of climate change and ocean current change on movements and migrations of fish; those with specific interest in particular species.

There is scope in DiadES to develop and expand existing networks and to develop new ones involving the participating DiadES teams, relevant associate partners and commercial fishermen in ports within the case study areas. Likewise, the DiadES teams in each country may be able to obtain material from studies by other state or university agencies.

The by-catch material can provide information on the species caught, when it was caught and where it was caught (GPS location; latitude – longitude etc.). If DiadES teams can obtain the body (-ies) then additional information can be harvested in regard to length, weight, gonad condition. Access to otoliths and scales can provide information on natal waters and on age and spawning history.

	Irstea	AZTI	EHEC-USC	CMVNC	IFI	Cefas
	French coastal areas	**Spanish coastal areas**	**Galician coast**	**Minho catchment**	**Irish coastal areas**	**UK coastal areas**
by-catch data	CS (Compiling existing by-catch data)	CS (Compiling existing by-catch data)	CS (new and existing data)	CS	CS	CS (Compiling existig by-catch data)

Table 7.1: Bycatch and case study locations. 'CS' for the samplings in own case study. Partners are, in general, endeavouring to capture data on national by-catch from all coastal waters.

It is recommended that raw by-catch data should not be included in the database for DiadES examination due to their sensitivity, but data summaries could potentially be used as long as specific vessels and/or individuals have not been identified.

7.2 Individual DiadES partner proposals

INRAE

ObsMer (for "Observations en Mer") is the French observation-at -sea program implementing the Data Collection Framework (EC, 2008). This collection is mandatory for all EU Members States. ObsMer aims to gather

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information to minimize bycatch and assess the incidental catches of endangered species, mainly cetaceans and turtles but also migratory fish. Onboard scientific observers randomly sample bycatch from French commercial catches since 2003. Precise information on ship characteristics (e.g. homeport, length, and engine power), fishing activity (time, latitude, longitude, gear, fishing effort, and targeted species assemblage) and catch composition (landings and discards of fish and commercial invertebrates) are collected for each fishing operation by scientific observers. For each fishing operation, a subsample of the catch (including both the part to be landed and the part to be discarded) is sorted, identified and measured. The data are compiled in Ifremer's centralised fisheries database 'Harmonie'. As fishermen target particular fish species in locations where they know the fish to be, fishing activity is non-randomly distributed in space and time.

In this context, in 2020, INRAE will make contact with DPMA (the French Directorate for Sea Fisheries and Aquaculture) and Ifremer with a view to having access to ObsMer data on diadromous fishes along the Atlantic Area, and discuss the issue of having these data displayed on the Interactive Web Atlas. INRAE will also make contact with Pôle OFB-INRAE (more particularly Laurent Beaulaton and Anthony Acou) as this group actively works to improve knowledge on diadromous fish at sea. As some work on diadromous fishes at sea has already been done at the French scale (Trancart et al 2014; Sarraj 2018), the task here will be more on maximizing the existing resources with what is intended to be done under DiadES.

In parallel with DiadES, a French project called MOMIE (Migratory movement of European sturgeon *A. sturio*: habitat at sea and spawners return in freshwater; INRAE/OFB) will assess *Acipenser sturio* habitat use at sea through the analysis of bycatch (data obtained within the French Action Plan in favor of the species restoration). Protocols are not detailed as this action is not in the DiadES work plan.

AZTI

AZTI will collect by-catch data from the Spanish commercial fleet, particularly data relating to the administrative area in which AZTI operates.

EHEC-USC

EHEC-USC has already compiled information on by-catches of the European shads (allis shad and twaite shad) in the coastal region of Galicia (NW of the Iberian Peninsula) prior to the DiadES project (Figures 7.2.1 and 7.2.2). This information has already been published and can be consulted in detail in Nachón et al. (2016).

Within the context of the DiadES project, the aim is to extend the information on by-catch to other diadromous species such as sea lamprey and to update the information for the European shads. All the information compiled comes from a web platform of the administration of the Galician region (https://www.pescadegalicia.gal/), in which the declarations of the official catches of the Galician

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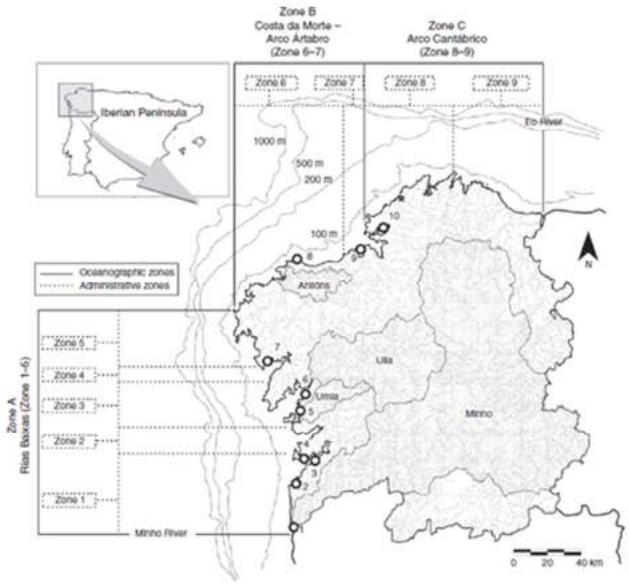

Figure 7.2.1. Location of Galician fish markets where shads were landed (white dots), administrative and ecogeographical zones for artisanal fishing (1, AGuarda; 2, Baiona; 3, Vigo; 4, Cangas; 5, Cambados; 6, Carril; 7, Muros; 8, Malpica; 9, Corunha; 10, Ferrol) and the basins of the rivers where sampling campaigns were undertaken (from Nachón et al. 2016).

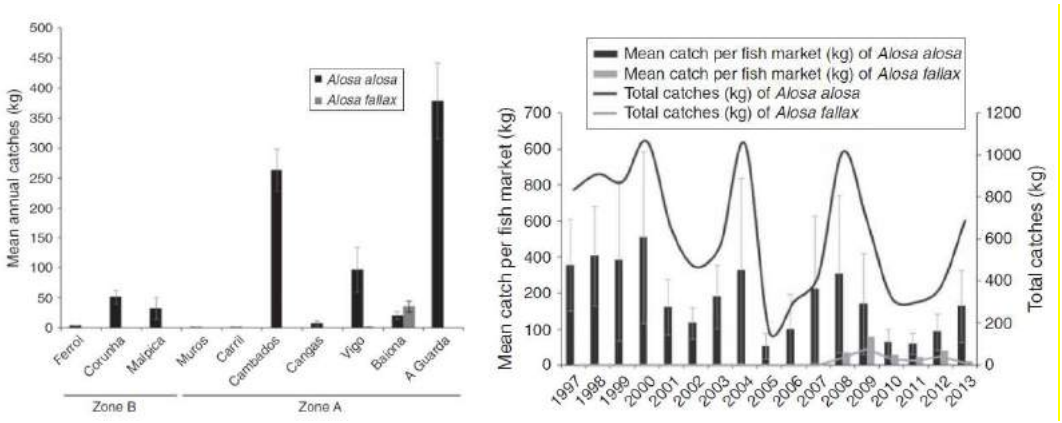

Figure 7.2.2. Distribution of mean (± s.e.m.) annual catches of *Alosa alosa* and *A. fallax* in the Galician markets between 1997 and 2013 (left graph) and mean (± s.e.m.) catch per fish market and total catches of *Alosa alosa* and *A. fallax* for all fish markets throughout the study period (from Nachón et al. 2016).

Fishing fleet are collected (see Nachón et al. 2016 for more information). However, the information only concerns the volume of landings, throughout the year, and in total and marketing value for each year, but there is no information about the precise location of the catches or on their biometric characteristics. Due to legislative rules on fishing in Galicia, it is more or less known in which coastal administrative region of Galicia the catches were made (see Nachón et al. 2016 for more details). Within the context of the DiadES project, an attempt will be made to collect such information only for the European shads, as there is interest in collecting otolith and scale samples for joint studies of otolith microchemistry and genetics of European shad populations. However, there is no guarantee that such information will be obtained.

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IFI

Through its role in EU reporting on Habitats Directive fish species, IFI has been collecting information on shads for a number of years. Research surveys in transitional waters and angler-linked activity have enabled IFI to collect much valuable information in fresh and transitional waters. IFI has developed informal linkages with three sister state agencies engaged closely with fish in the marine environment – the Marine Institute (MI), Sea Fisheries Protection Authority (SFPA) and the Sea Fisheries Board – Bord Iascaigh Mhara (BIM). These three bodies have provided IFI with data and samples of shad from the marine environment, coming from focussed pelagic sampling voyages, from gear sampling trials or as commercial fishing by-catches. On-board observer staff and port-based staff have been retaining material for IFI on an opportunistic basis for several years and this has built into a valuable resource. This network will continue to be used during the DiadES project. The SFPA is an associate partner of the project. A series of regional inshore fisheries fora (RIFF s) have been established by BIM and the Southeast RIFF, whose geographical area includes the IFI case study area, is also an associate partner of DiadES.

By-catch data, as described above, is opportunistic both in availability of accurate supporting data, e.g. location, depth of capture etc., and also in regard to whether the bodies of relevant fish species are available. Thus some scientific pelagic sampling surveys will process material on-board with no retention of carcasses - these can yield data on presence and, in some cases, fork or total length but may not have any other biometric data to offer. In other cases, bodies of fish may be provided that, due to the trawling and landing processes may be devoid of scale material.

Prior to the restriction of commercial wild salmon harvesting to discrete waters or single-stock fisheries, commercial salmon netsmen were a valued source of by-catch material in inshore waters and In transitional waters. The IFI's network of regionally-based staff worked proactively with the commercial salmon netsmen, who passed by-catch material on to the IFI personnel for forwarding for scientific data collection (King and Roche 2008).

Cefas

UK by-catch data reported here come from Cefas' off-shore sampling programme. Selection of fishing vessels for direct observation by Cefas observers is done by stratified random selection (by region, fishing methods and occasionally by vessel size) over the full list of all commercial fishing vessels, which are updated quarterly. Vessels specializing in fishing methods, fishing in foreign ports, unsafe for observers or smaller than 7m are excluded from the sampling framework. Each observer collects information for each sampled haul, specifically: gear type and mesh size, tow duration, shot and haul position, species catch composition and quantity of the landings and discards in the catch. In cases where it is not possible to process all the samples, the measured volume is estimated relative to the total catch to get a raising factor and estimate the total catch. Specifically, during each trip numbers at length are raised to the haul and then to the whole trip, which can result in rare species such as some diadromous fishes being underrepresented.

By-catch data of diadromous fishes and lampreys are reported per date, ICES rectangle and division with latitude and longitude provided, and consist of species name, catch method, estimated number of fish caught per haul, number of sampled fish and length for measured individuals. Sampling gears include beam trawls, demersal seine, dredge, gillnets, midwater trawls, otter trawls and trammel nets. Data have been recorded since 1998 onwards and include records of *A. alosa, A. fallax, P. marinus, L. fluviatilis, S. salar, S. trutta, O. eperlanus, C. ramada, P. flesus* and *A. anguilla*.

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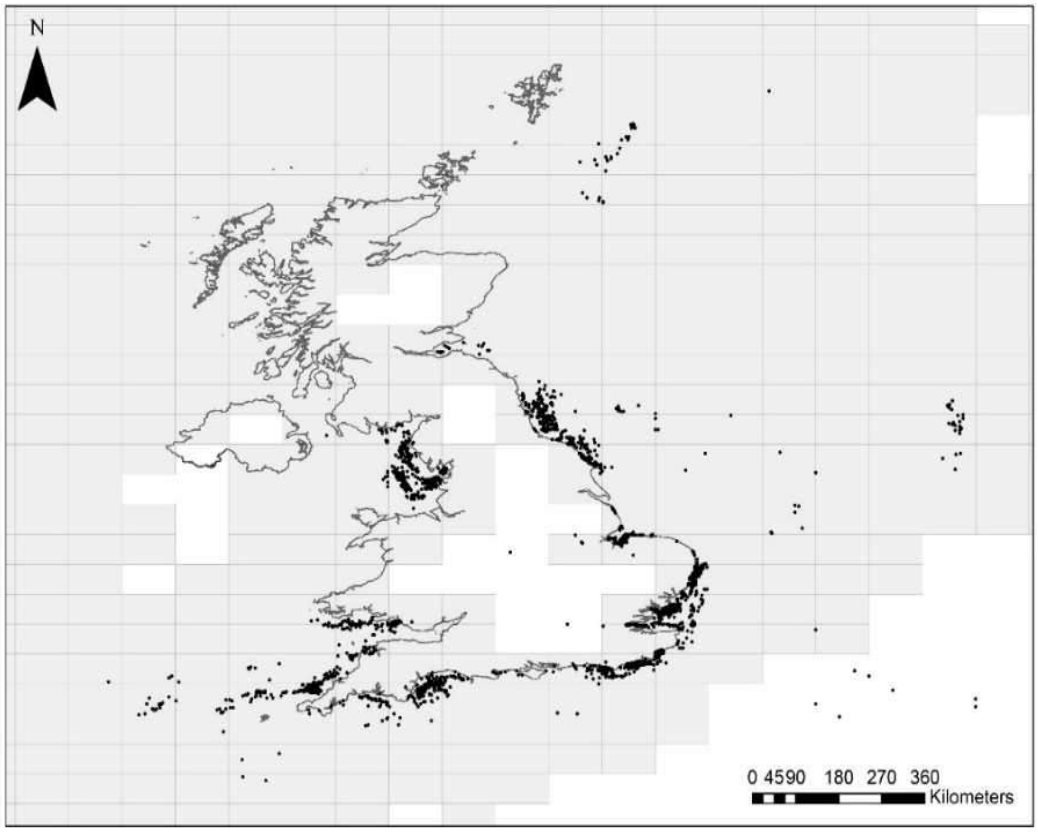

Figure 7.2.3. Approximate by-catch locations (black points) of diadromous fish species around the UK, where ICES rectangles are presented as grey polygons.

This Cefas database of bycatch contains a potentially major source of information to DiadES in regard to target fish species, dates and locations of capture and of gears used for sampling. Information in the Cefas database in regard to individual skippers and boats would remain confidential to Cefas (Figure 7.2.3).

7.3 References

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This project is co-financed by the Interreg Atlantic Area Programme through the European Regional Development Fund.

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This project is co-financed by the Interreg Atlantic Area Programme through the European Regional Development Fund.

8. Field Sampling Programmes

8.1 Introduction

Within the Water Framework Directive (WFD), EU Member States have worked together within geographic zones to develop sampling methods that provided consistency of reporting, when intercalibrated. The North-East Atlantic grouping of member states developed a sampling protocol for transitional waters described as a 'multi-method' approach, involving a suite of sampling strategies including beach seining, fyke netting and trawling (Coates et al 2007). The suite was considered pertinent in view of the wide range of habitat types, the size of many transitional waters and the diversity of fish species, life stages and body forms that might be encountered.

	AZTI	EHEC - USC	MARE-UÉ	CMVNC	IFI	Cefas
	Gipuzkoan rivers	**Ulla catchment**	**Mondego catchment**	**River Minho**	**Waterford Harbour and the three sisters' rivers**	**Tamar, Frome and Taff rivers**
baseline fish surveys	WFD trawling sampling	CS (existing data)	CS	CS	CS	CS (Compliling existing data)

Table 8.1. National or Regional Authority data collection linked to DiadES partners and case study locations. 'CS' = sampling in own case study. 'poss' = possible additional or subsidiary sampling that may be worked on.

The extent of national monitoring, for WFD or for other national programmes, may vary from one member state to another. Similarly, it is likely that different sampling strategies and technologies may be employed - for different species or for basic monitoring and data collection. As with data harvesting from fisheries by-catch (see Section 7 above) there may be scope to explore data of value to DiadES that is collected by national or regional authorities

8.2 Individual DiadES partner proposals

AZTI

AZTI carries out the sampling in transitional waters for the Basque Water Agency (URA). This includes the estuaries of Gipuzkoa. Each estuary is sampled every three years in early autumn. Demersal fauna is sampled by trawling. Data from all of these surveys would be available to DiadES for building the atlas etc. The data can

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be used to indicate presence, only, and not abundance of species recorded in the trawling surveys in the Basque transitional waters.

The dates of the URA samplings are:

- 2003-2006-2009-2012-2015-2018, Deba, Oria

- 2004-2007-2010-2013-2016-2019 Urola, Urumea, Oiartzun, Bidasoa

In addition to this, there are some surveys of the Provincial Council of Gipuzkoa and there are also some additional data from individual transitional waters: Deba (1996), Urola (1996), Oria (1996), Urumea (1995), Oiartzun (1995, 2001), and Bidasoa (1995, 2001).

EHEC-USC

EHEC-USC has current information for certain species in the upper zone of the Ulla River estuary. Thus, data on relative temporal abundance and biometric data on *A. fallax* juveniles are available (Nachón 2017). In addition, there are qualitative data (presence) of the accompanying species captured during the monitoring surveys of the *A. fallax* juveniles. There are also time series of biometric data for adult lampreys, caught by professional fisheries in the estuary during their upstream migration (Silva 2014). In addition, the EHEC-USC also has temporal relative abundance data and biometric data of post-metamorphic lampreys in the lower part of the estuary, during their downstream migration (Silva et al. 2013 a). Finally, the EHEC-USC has access to a long series of freshwater data, from 1997 to the present, on migratory species caught in the facilities of a fish trap located in the middle of the available habitat of the Ulla River.

MARE-UÉ

University of Évora / MARE has been studying diadromous species in Portugal for 20 years, mainly in the river Mondego. Besides the data collected for the Mondego Case Study in DiadES, several data sets and sample collections within other projects (e.g. AN@DROMOS.PT, ref: MAR-01.03.02-FEAMP-0002) will provide useful information to fulfil some of DiadES goals, namely:

• Trout Recreational Fisheries (angler surveys);

• Monitoring of allis shad landings (sample collection at Figueira da Foz fish market) – Samples collected include biometrics, tissue sample and the first brachial arch, scales and otoliths in a subsample of the fish;

• Allis shad and sea lamprey commercial fisheries monitoring (inquiries to commercial fishermen);

• Electric fishing for sea lamprey larvae.

This data will contribute to improve the existing knowledge about trout (both its anadromous and resident ecotypes), sea lamprey and allis shad, and the samples collected will enable the study of population dynamics of *A. alosa* in Mondego River basin and the adjacent coastline, especially regarding hybridization with *A. fallax.*

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CMVNC

In the River Minho CMVNC uses seine nets in sampling for juvenile life stages of *Alosa* spp. and mullets. In addition, fyke-nets and electric fishing for eels and trammel nets (used by commercial fishermen) for adult life stages of *Alosa* spp, lamprey species and mullet will provide additional data.

IFI

IFI has been using a multi-method approach to examine estuarine fish communities since 2004 (Harrison and Kelly 2013; Connor et al. 2019). In DiadES, IFI has been able to avail of a suite of surveys undertaken in the Irish case study – Waterford Harbour and the estuaries of the 3 Sisters rivers – as well as in the adjoining estuaries of the Munster Blackwater and Slaney during 2019. These have included:

- Juvenile bass surveys in August 2019 (beach seining) – bycatch includes shad, smelt and thin-lipped mullet;
- WFD multi-method survey in October 2019 (beach seine; fykes; beam trawl);
- Trawling survey for bass life stages in September 2019 (commercial fishing trawl operated from commercial fishing boat) – bycatch includes shad, smelt, mullet species.

These surveys also permitted collection of water samples for eDNA investigation both in the estuaries and in marine locations as the trawler travelled from one waterbody to the next along the coast.

Gear specifications:

Beach seine: The Seine net measures 30m x 3m, with a 10mm mesh size. Seine nets are deployed by boat in an arc shape from the shore and slowly drawn to shore.

Fyke nets: Fyke nets (15m in length with a 0.5m diameter front hoop, joined by an 8m leader with a 10mm square mesh). These nets are used to sample benthic fish in the littoral areas. The nets are commonly fished as a set of 3, tied end-to-end and set to fish parallel to the line of flow. The ends of the nets are weighted to ensure stability and the gang is fished overnight. This procedure is effective in sampling for fish species of vermiform body form such as eel and sea- and river lamprey.

Beam Trawl: The beam trawl measures 1.5m x 0.5m, with a 10mm mesh bag, decreasing to 5mm mesh in the cod end. The trawl is attached to a 20m tow rope and towed by a boat. Trawls are conducted along transects of 100m in length.

Trawling survey: The survey method used was first developed by Cefas (UK) in consultation with local skippers (Picket et al. 2002) primarily to sample juvenile bass populations. The trawl is based on Cefas specifications with the exception of mesh size: 80 mm diagonal stretched mesh was used throughout instead of the 70 mm used by Cefas, in order to comply with minimum mesh sizes required with Irish trawls. The cod-end is fitted with a 20 mm mesh 'Herring Brailer' net. Two 1.0m steel trawl doors (60 kg) are attached to the bridles during towing to keep the net open.

IFI will be in a position during 2020 and 2021 to link in to these studies and to harvest information of relevance to DiadES.

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Cefas

Field sampling data for England come from the National Fish Populations Database (NFPD), which consists of fisheries monitoring data from rivers, lakes and transitional and coastal waters undertaken by the regulatory authority, the Environment Agency (EA) and by third parties. Freshwater, transitional and coastal data on diadromous fishes and lampreys in Wales are collected under different sampling programmes by the Welsh regulatory authority, the Natural Resources Wales (NRW). Robust quantitative estimates of European eel and Atlantic salmon are available in freshwater habitats to support the stock assessment. The majority of other diadromous species are recorded as a part of Water Framework Directive (WFD) surveys and/or specific studies, and mainly consist of qualitative data (presence/absence).

England's' diadromous freshwater survey data span from 1975 until now, with multiple methods of sampling, including electrofishing, fyke netting, rod and line fishing, fixed and portable trap fishing, intake screen sampling, dip netting, gill netting, trawl netting and seine netting. Welsh freshwater data on diadromous fishes are available from 2001 till 2018, with sampling methods similar to those in England. Both data sets consist of sampling location, river, national grid reference (NGR), date, fished area (m^2), sampling method, species name, number of runs, total numbers caught and estimated densities where applicable (e.g. for *S. salar*, *A. anguilla*). More than 40 % of records are based on depletion methods, with multiple runs enabling robust estimates of fish densities. The remaining data represent qualitative information from single catches. Length data can be provided for measured individuals upon request.

With regard to estuarine and costal habitats, only qualitative data are available for both England and Wales (presence/absence data), with a range of methods utilised for sampling, including beam trawl netting (1.5-2.4 m), otter trawl netting, fyke netting, gill netting, seine netting, kick netting, trammel netting and intake screen sampling. Data for England are available since 1981 until 2018, while Welsh data span from 1997 to 2018. Both data sets consist of sampling location, national grid reference (NGR), date, sampling method, species name and observed total numbers per survey and species. Length data can be provided for measured individuals upon request.

All these data are public and can be fed into the DiadES database.

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This project is co-financed by the Interreg Atlantic Area Programme through the European Regional Development Fund.

9. Database development and use

9.1 Background and key requirements

One of the required outputs from DiadES WP6 is provision of a database to centralise storage of supporting data from samples collected during various biological investigations undertaken over the course of the project. This will facilitate the share of sampling data across the partnership and enable efficient sample management. Facilitating access to all sampling data will be important to allow for sample selection and subsequent analysis and will be particularly relevant for joint studies including microchemistry analyses on metapopulation dynamics and variability in habitat use, and hybridization.

It is envisaged that all publications from DiadES studies will be in open-access journals which necessitates sample data being available for other workers to review. Centralising sample data storage from the beginning of the project by all partners will allow these data to be extracted as required. Providing data to support post-project studies on diadromous species by other researchers is a requirement under the project brief.

Database development is a subject area that could absorb significant time and resources within a project and has the potential to become a project in its own right. Limited resources are available within DiadES to develop or implement a new database – this resource issue limits the choices for the partnership to existing database platforms. All partners recognise that a database is essential for the efficient and safe movement of data and samples between partners and between work packages.

Partners agreed that managing samples (water, and tissue samples including fish bodies, scales, otoliths and other tissues) across the DiadES project, with multiple partners and several different species from different water bodies, presents a considerable logistical challenge. To ensure traceability, optimise sample management and facilitate comprehensive analysis a sample management database is required. Mobilising historical and new samples, some of which will require exchange between partner laboratories to facilitate sample processing and analysis, requires a robust 'track and trace' database system. Applying a consistent coding and sample tagging system linked to a central database means that partner agencies can exchange samples efficiently.

Partners involved in WP6 agreed that the primary requirement will be to collect summary data on work done under the DiadES project. The focus would be on the joint studies on metapopulation dynamics, variability in habitat use and hybridisation (sections 4 and 5 of the present report) to ensure database is done in time and later on other studies could be added if feasible (as described in the next paragraph). The database would present only metadata, not the raw data to avoid IPR issues in the beginning and would be able to visualise collected data on a GIS dashboard. The question at stake will be what happens with the DiadES samples.

Nonetheless, for the Interactive Atlas under WP7, the main outputs will be maps of species distribution and ESs' trajectories (WP5 and WP4 key outcomes). For that reason, it would be highly desirable that the results of all the fieldwork delivered under WP6 could also be linked and interface with the outcomes of other key elements - the database should be able to facilitate all partners. Data relating to fish tagging data, by-catch surveys, eDNA and potentially the various indicators required for WP4 economic valuation should be considered for inclusion. The latter inclusions should be considered following a mid-term review of all existing or potential datasets and progress on joint case studies, and of data currently available to, the different work packages.

Key database requirements are:

a) All partners should have read/write access;

b) Sample and supporting data should be easily searched and retrieved by authorised project personnel;
c) Additionality, the basic database structure should be futureproofed to allow for future diadromous species projects to benefit from processed DiadES datasets.

9.2 Collec-Science – the DiadEs platform

The main contact person on this issue is Éric Quinton, database manager at INRAE, UR EABX (e-mail: eric.quinton@inrae.fr).

One tool already available is Collec-Science used **for traceability of field/lab samples** (Plumejeaud-Perreau et al. 2019).

More particularly, Collec-Science main characteristics are:

- Keep track with the 'genealogy' of samples (derived samples and sub-samples);
- Keep track with sample storage locations and conditions, sample loans etc.;
- Labels with QRCODE. Editable label templates;
- Possibility to add metadata (taxon, weather conditions during sampling etc.);
- GPS position of sample's containers and sampling sites;
- Fully parameterizable/configurable.

The software can be freely downloaded at: https://www.collec-science.org/en/presentation_en/ and a web demo is available at https://collec-science.irstea.fr with the Login: collec-science and password: Collec-Science (with upper cases). This software is currently in use in 10 research institutes in France. Collec-Science could be used in any context as it is free and downloadable.

A module allows you to import samples from an external database via a CSV file with fields related to samples and containers such as universal unique identifiers (UIDs), type, status, creation, location and much more. Other fields to better describe samples will be added based on WP6 partners inputs: taxon, biometry, life phase, maturity, details on sampling protocols…

Security will be ensured with different levels of authorization ranging from consultation to administration of the collection and members having access to this collection. Collec-Science could be interfaced with other existing tools such as Otolithe for otolith and scale readings (https://github.com/Irstea/otolithe) or Filo-Science for scientific surveys (demo: https://filo-science.irstea.fr, login: filo, mdp: Filo-Science) depending on DiadES partners' needs.

9.3 Collec-Science first steps for partners

The process of interagency sample transfer will start as early as March 2020. As such, here are described the first steps required from partners to activate the process of importing data in Collec-Science. Eric Quinton, INRAE database manager, will take care of creating accounts for all people involved in WP6.1 and field/lab data collection. He will also provide into the platform basic information describing partner's institute. All the information will be provided by LP to Eric Quinton. This will allow initializing the system. Then, partners with samples ready to be sent to other partners or to external companies should send an Excel file to Eric Quinton. This Excel file should be basic in a table format. Side notes or special characters (e.g. ?, 'NA'…) should be removed from the table. For numerical values, only one value should be provided by cells. Some fields in the table will be mandatory: initial sample ID and sampling localization coordinates in WGS84 numeric format. Also, the metadata agreed among partners during the Norwich meeting should be given in distinct fields: species name, basic biometric values such as fish length, degree of maturity, life stage, and any details on the sampling protocols.

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9.4 References

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Appendix 1: DiadES WP 6.1 list of people involved in biological studies.

Partner No	Partner name	Personnel	Contributing to sections of report
1	INRAE	Marie-Laure Acolas	Section 6 on tracking of fish
		Françoise Daverat	Sections 4 and 5 on microchemistry and hybridization
		Christine Gazeau	Section 4 on microchemistry
		Géraldine Lassalle	Sections 7 and 9 on by-catch data collection and database management and use
		Patrick Lambert	Sections 7 and 9 on by-catch data collection and database management and use
		Romaric Le Barh	Section 6 on tracking of fish
		Éric Quinton	Section 9 on database management and use
2	AZTI	Estibaliz Díaz	Section 3. Environmental DNA or eDNA, 7. By-catch data collection and 8. Field Sampling Programmes
		Naiara Rodriguez-Ezpeleta	Section 3. Environmental DNA or eDNA
	EKOLUR	Iker Azpiroz	Section 4. Microchemistry of otoliths and scales
	Diputación de Gipuzkoa	Aitor Lekuona	Section 4. Microchemistry of otoliths and scales
3	EHEC-USC	David J. Nachón	Sections 3, 4, 5, 6, 7, 8
		Fernando Cobo	Sections 3, 4, 5, 6, 7, 8
		Rufino Vieira	Sections 3, 4, 5, 6, 7, 8
		Sandra Barca	Section 3
		María del Carmen Cobo	Sections 3, 6
5	MARE-UÉ	Pedro R. Almeida	Section 4 on microchemistry, section 5 on hybridization, section 6 on tracking of fish and section 8 on Field Sampling Programmes
		Catarina Mateus	Section 4 on microchemistry, section 5 on hybridization, section 6 on tracking of fish and section 8 on Field Sampling Programmes
		Carlos Alexandre	Section 6 on tracking of fish and section 8 on Field Sampling Programmes
		Ana Filipa Belo	Section 4 on microchemistry,

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			section 5 on hybridization and section 8 on Field Sampling Programmes
		Esmeralda Pereira	Section 4 on microchemistry
6	CMVNC - Aquamuseu	Carlos Antunes	Section 4 and 5 on microchemistry and hybridization; Section 6 on tracking of fish; Section 8 on field sampling programmes
		Eduardo Martins	Section 4,5,6 on field work
		Ester Dias (CIIMAR)	Section 4 and 5 on microchemistry
7	IFI	James King	Overall draft compilation and IFI inserts
		William Roche	Overall draft compilation and IFI inserts
		Sean Rooney	Section 6 on tracking of fish
		Diarmuid Ryan	Section 8 on Field Sampling Programmes
9	Cefas	Andy Moore	Section 6 on tracking of fish
		Gordon Copp	Whole report QA
		Phil Davison	Section 3 on eDNA
		Tea Bašić	Section 7 on By-catch data collection and Section 8 on Field Sampling Programmes
10	MNHN	Feunteun Eric	Whole report QA
		Thomas Trancart	Section 6 tracking of fish
		Anne Lizé	Section 8 field sampling programme on Mont Saint Michel's bay and Loire

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Appendix 2: MNHN study on shad diet variability

A.1 Introduction

Life-history traits of diadromous fish are thought to vary according to biogeographical trends and other environmental factors. Food availability depends upon natural environmental and anthropogenic factors that control the quality of the food web, the diet, and associated life-history traits as growth rate, size and age at maturity, and fecundity. Therefore, understanding the characteristics of the food web and the trophic position of anadromous species on their upstream spawning migration provides indices on the growth habitats that they use and associated prey. Analysing digestive tract contents provides information on the immediate diet, while isotope ratios of muscles ($\delta^{13}C$ and $\delta^{15}N$) indicate the average composition of the diet during the past days, weeks or even months according to the growth rate and metabolism of the fish.

In order to understand the growth habitats of fish in the months preceding the migration, a comparative study of two contrasted ecosystems will be performed in the Loire estuary and in the Bay of Mont Saint Michel according the protocol proposed by Acou et al. (2013).

A.2 Methods

Most fish were collected along the coasts of the Bay of Biscay and the English Channel, thanks to cooperation with professional fishermen, environmental companies and managers. In 2012, a total of 267 shads (166 *Alosa alosa* and 101 *A. fallax*) were collected (Acou et al. 2013) (Table 1).

Tableau 1: Captures for *Alosa alosa* and *A. fallax*

	A. alosa	*A. fallax*	***Total***
Sea	***40***	***57***	***97***
Arcachon Bay	2		***2***
Eastern Channel		5	***5***
Corsica		2	***2***
Celtic Sea	1		***1***
North Sea		11	***11***
Pertuis Charentais	1	14	***15***
South Brittany	36	20	***56***
South Bay of Biscay		5	***5***
Rivers	***126***	***44***	***170***
Adour	13	16	***29***
Dordogne		15	***15***
Loire	40	8	***48***
Sélune	5		***5***
Vilaine	40	5	***45***
Vire	28		***28***
Total	**166**	**101**	***267***

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Tableau 2: Captures of *Alosa alosa* and *A. fallax* by life history stages.

	Alosa alosa				*Alosa fallax*		
	juv.	**male**	**female**	**post-repro**	**juv.**	**male**	**female**
Sea	***33***	***4***	***3***	***0***	***34***	***8***	***13***
Arcachon Bay	1		1				
Eastern Channel					5		
Corsica					2		
Celtic Sea	1						
North Sea					9	1	1
Pertuis Charentais			1		8	2	4
South Brittany	31	4	1		10	5	3
South Bay of Biscay							5
Rivers	***1***	***35***	***74***	***16***	***2***	***3***	***39***
Adour		7	6				16
Dordogne						2	13
Loire	1	8	31		1		7
Sélune				5			
Vilaine		6	23	11	1	1	3
Vire		14	14				
Total	***34***	***39***	***77***	***16***	***36***	***11***	***52***

A.3 Biological parameters

A number of life-history traits were measured on each fish: age was estimated from scales, total length and weight, body condition, age at first maturity. Significant differences of age, condition and size were recorded among species and catchments, with significantly lower conditions for *A. alosa* of the Dordogne River (Réveillac et al. 2013, in Acou et al. 2013).

A.4 Diet and position in the food web

The isotopic signatures $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) varied according to the location, sex and size for both species (Réveillac et al. op. cit.). The trophic level ($\delta^{15}N$) and carbon sources ($\delta^{13}C$) increased from the south of the Bay of Biscay to the north of the study area. Twaite shads had a higher trophic level than Allis shads whatever their localisation. The $\delta^{13}C$ signatures were more depleted (negative) in Twaite shads indicating a more estuarine signature than in Allis shads (Réveillac et al. op. cit.).

The analysis of $\delta^{13}C$ and $\delta^{15}N$ of sources in the Bay of Biscay indicated that *A. fallax* mainly depended on fish prey, while *A. alosa* mainly consumed mesozooplankton.

A.5 Proposal of collaboration to DiadES partners

The proposition is to test the variability of diets, position in the estuarine / coastal food web of both species of shads along their distribution range from North to South of Europe. The understanding of the variability of life-history traits and trophic position provides clues on the possible responses of shads to global change, including warming and human-induced environmental pressures.

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In order to test this, the MNHN plans to collect in May-June:

- 10 shads of each species of shads caught in an estuary or a river on the onset of their spawning migration. They will be measured to the nearest mm and weighed to the nearest g.

- On each fish,
    - a portion of c.a. 20 g of muscles will be collected and preserved in pure ethanol in an individual labelled container (1 per fish). The muscle sample will be taken between the mouth and the anus on the back of either side;
    - 10 scales will be extracted from the back under the dorsal fin. They will be dried on absorbent clean paper and placed in an envelope with the same label.

- In the estuary or coastal areas close to the outlet of the sampling site, samples of mesozooplankton collected with a plankton net (0.5 mm mesh) collected from the banks or a boat. A simple oblique tow of 5-10 mn is necessary.

- Samples of POM: 10-12 litres collected from surface water and filtered on separate pre-combusted glass fibre filters (GF/F, 47 mm diameter, 0.7 µm mesh). The filter will be dried at 60 °C for 12 h.

All material, containers and labels can be prepared and provided by the MNHN.

The samples well be sent to the MNHN in Dinard that will analyse the $\delta^{13}C$ and $\delta^{15}N$ ratios, following classical procedures (i.e. Ghinter et al. 2020).

A.6 References

Acou, A., Lasne, E., Réveillac, E., Robinet, T., and Feunteun, E. (2013) Programme de connaissance Natura2000 en mer : les habitats marins des espèces amphihalines. Evaluation de la cohérence du réseau Natura2000 en mer pour la grande alose (*Alosa alosa*), l'alose feinte (*A. fallax* sp.), la lamproie marine (*Petromyzon marinus*) et la lamproie fluviatile (*Lampetra fluviatilis*). Rapport scientifique préliminaire du Muséum National d'Histoire Naturelle, Stations marines de Dinard et Concarneau. 154 pages + annexes.

Ghinter, L., Dupuy, C., Miller, M., Carpentier, A., Lefrançois, C., Acou, A., Aoyama, J., Kuroki, M., Liénart, C., Watanabe, S., Tsukamoto, K., Otake, T., and Feunteun, E. (2020) Microbial functional structure and stable isotopic variation of leptocephali across three current zones in the western South Pacific. *Progress in Ocenanography*. https://doi.org/10.1016/j.pocean.2020.102264

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Appendix 3: DiadES WP 6.1 Executive Summary in the languages of the Atlantic Area (French, Spanish and Portuguese)

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Livrable pour le WP6.1 – Résumé exécutif dans les quatre langues du Programme

1. DaidES a pour but **d'évaluer et d'améliorer les services écosystémiques fournis par les poissons migrateurs amphihalins (PMAs)** dans l'Espace Atlantique (EA) et, en parallèle, le statut de conservation de ces espèces, en considérant explicitement dans leur gestion les effets attendus du changement climatique sur leurs distributions. DiadES adopte une approche innovante basée sur les services écosystémique qui convertit les abondances de poissons en unités monétaires. DiadES verra collaborer des chercheurs en sciences de l'environnement et des économistes de l'environnement aidés par un réseau solide de gestionnaires impliqués dans la gestion des PMAs sur l'ensemble de l'EA. Neuf cas d'étude seront le théâtre d'études spécifiques et conjointes. Plus d'informations sur le projet à **www.diades.eu.**

2. Le groupe de travail (GT) 6 de DiadES s'intitule "Collection de données biologiques et relatives aux services écosystémiques et cas d'étude". Ce GT **porte sur l'acquisition de données de terrain**. Il a une durée de 30 mois, de février 2019 à juillet 2021. Ce GT crucial impliquera tous les partenaires bénéficiaires et est coordonné par Inland Fisheries Ireland (IFI).

3. Une des premières tâches de ce GT a été de s'accorder sur **les méthodologies conjointes et les bonnes pratiques** dans les études biologiques menées par les partenaires dans la tâche 6.1 « Définition de méthodologies conjointes pour la collecte de données biologiques ».

4. Le présent rapport "Manuel sur la collecte des données biologiques" représente les décisions conjointes des partenaires sur les espèces d'intérêt, les protocoles, et le partage de ressources. Il s'agit du livrable attendu pour la tâche 6.1 afin d'**harmoniser et de standardiser** les données de suivi disponibles et l'évaluation des services écosystémiques sur l'ensemble de l'EA. **Les sections de ce rapport ont été organisées par grandes méthodologies** et il en est ainsi dans le reste du résumé exécutif.

5. **Une liste de contact** a été ajoutée au rapport afin de faciliter l'identification des personnes compétentes au sein du partenariat DiadES pour chaque section du rapport.

6. Une grande partie des activités des partenaires a été validée comme portant sur les espèces dites **pauvres ou déficientes en données comme les aloses, les lamproies, le flet et le mulet porc**.

7. L'étude de l'ADN environnemental (ou ADNe) repose sur le fait que les organismes laissent des traces de leur présence derrière eux, sous la forme de tissues, d'écailles, de semences suite à la reproduction, ou encore de carcasses d'animaux morts. Le contenu génétique, si présent, peut être recherché dans des échantillons d'eau et fournit une indication de présence de l'espèce. L'analyse génétique est une procédure sophistiquée qui sera mise en place dans DiadES : par l'AZTI (pour les aloses et la lamproie marine) et par le Cefas (pour l'éperlan et le mulet porc). **Les protocoles pour la collecte des échantillons d'eau, la filtration et le stockage ont été fournis par chacun des deux instituts pour comparaison**. Cette approche collégiale sera propice à la standardisation et aidera à réduire les sources d'erreur et de contamination.

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8. Les poissons absorbent les éléments chimiques dans leurs tissus et leurs structures osseuses, les proportions des différents éléments étant liées à leurs proportions dans l'environnement immédiat. La microchimie des otolithes (concrétions minérales de l'oreille interne) et des écailles fournit une opportunité pour examiner certains éléments clés et pour développer une signature de l'eau dans laquelle le poisson a été capturé et du poisson à un certain stade de vie. Les études de microchimie proposées dans DiadES examineront :
    a. Les deux espèces d'aloses et essayeront de lier les individus capturés en mer à un bassin d'origine natal. Cela contribuera à accroitre les connaissances sur les migrations et les mouvements des espèces après avoir quitté les eaux douces ;
    b. Les mouvements migratoires du mulet porc dans les eaux estuariennes et l'utilisation de zones de salinité spécifiques par cette espèce.

    Les protocoles pour les échantillons d'aloses ont été fournis en détails avec les principales exigences. Des recommandations pertinentes ont été aussi proposées pour les échantillons de mulet porc. Cette étude sera conduit par l'INRAE et toutes les analyses seront réalisées par un seul laboratoire de l'INRAE, fournissant ainsi un bon niveau de « rentabilité » et une continuité dans la qualité des sorties, éliminant des sources d'erreur majeures.

9. La collection d'écailles mentionnée dans le point précèdent sur la microchimie sera aussi examinée sous l'angle de l'hybridation entre les deux espèces d'alose - la grande alose et l'alose feinte - dans le cadre de DiadES ainsi que des échantillons additionnels de tissus mous. **Cette étude a émergé après le démarrage de DiadES et constitue de ce fait une « valeur ajoutée »**. Cette étude sera menée par l'INRAE et MARE-UÉ, et toutes les analyses seront réalisées par une seule plateforme de génétique, éliminant des sources d'erreur majeures. **Les références bibliographiques clés pour les protocoles ont été listées.**

10. Une série d'études sur les migrations/mouvements des poissons sera réalisée dans DiadES, avec une diversité de technologies. **Six propositions d'étude individuelle par six partenaires ont été détaillées.** En association avec les bases de données historiques récentes, ces données contribueront à améliorer les connaissances sur les espèces en général et les cartes de distribution que DiadES doit générer pour le web atlas interactif. Les technologies incluent :
    a. Le marquage par étiquettes pour les juvéniles dévalant de lamproie marine ;
    b. Des marques acoustiques pour une gamme d'espèces - la truite de mer, l'éperlan, les aloses, et le mulet porc ;
    c. Des marques pop-up pour l'esturgeon européen.

11. Les mentions de capture accidentelle des espèces cibles dans DiadES se retrouvent dans les déclarations des ports de pêche commerciaux et dans les campagnes de suivi de la faune piscicole. Les partenaires du projet DiadES vont prendre contact avec les autorités locales compétentes, le personnel des ports de pêche et des pêcheurs professionnels afin d'obtenir des informations sur les PMAs. **Cinq études ont été proposées/détaillées par les partenaires.** Les partenaires s'efforceront aussi d'obtenir des individus pour recueillir des données biométriques ainsi que les localisations et dates de capture (selon les grilles CIEM). Cela contribuera significativement à l'amélioration des connaissances sur les déplacements de ces espèces et des cartes de distribution que le projet doit générer.

12. Les partenaires contribuent à des programmes de suivis nationaux, e.g. la Directive Cadre sur l'Eau avec l'échantillonnage des communautés piscicoles dans les masses d'eau de transition, ou ont accès à ces

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données dans leur pays. **Six propositions de la part des partenaires DiadES ont été détaillées.** Ce matériel contribuera à l'effort de cartographie général mené dans DiadES, à des cartographies plus détaillées par stades de vie associés à des habitats spécifiques et à la provision de nouvelles données biométriques.

13. Les partenaires se sont mis d'accord sur un protocole de base de données qui fournira de la cohérence du point de vue de l'étiquetage des échantillons, de la fourniture de données biométriques/environnementales associées aux échantillons etc. Cela est essentiel afin de fournir un système de traçage efficace, particulièrement utile lorsque que plusieurs partenaires envoient du matériel varié à un ou plusieurs partenaires dans le projet assurant des tâches précises collectives, e.g. INRAE s'occupant de la microchimie pour l'ensemble du projet. **Toutes les informations pour l'application de cet outil de base de données à d'autres contextes ont été mises à disposition.**

14. Les sorties scientifiques dans le cadre du GT 6 seront des études individuelles ou conjointes publiées dans des revues soumises à la révision par les paires et **mise à disposition sur le site web de DiadES.**

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Entregável do WP 6.1 - Resumo Executivo nos quatro idiomas do Programa

1. O projeto DiadES tem como objetivo **avaliar e promover os serviços de ecossistema fornecidos pelos peixes diádromos** na Área Atlântica (AA) e, paralelamente, o estatuto de conservação dos mesmos, tendo em conta, na sua gestão, os impactos esperados das mudanças climáticas na sua distribuição. O projeto adota uma abordagem inovadora aos serviços dos ecossistemas, que converte a abundância destas espécies em unidades monetárias. Para o efeito, o DiadES estabelece colaborações entre investigadores da área das ciências naturais e da economia do ambiente, apoiadas por uma forte rede de gestores das espécies diádromos de todo o AA. Foram selecionados nove casos de estudo que serão o foco de trabalhos específicos e conjuntos. **Mais informação sobre o projeto poderá ser consultada em www.diades.eu.**

2. O WP 6 do DiadES intitula-se "Recolha de dados biológicos e de serviços do ecossistema – Casos de estudo". O programa de trabalhos (WP) é direcionado à **aquisição de dados de campo** e tem uma duração de 30 meses, com início em 2019 até o final de julho de 2021. Este WP é essencial e envolve todos os parceiros beneficiários e é liderado pelo Inland Fisheries Ireland (IFI).

3. Uma das primeiras tarefas deste WP consistiu em entrar em **acordo na definição de metodologias conjuntas e boas práticas** nas recolhas de dados biológicos entre os parceiros, no âmbito da tarefa 6.1 "Definição de metodologias conjuntas para recolha de dados biológicos".

4. O presente relatório "Manual de recolha de dados biológicos" representa a convergência dos parceiros em relação às espécies-alvo, protocolos acordados e partilha de recursos. Este é o resultado esperado da tarefa 6.1 para **harmonizar e padronizar** os dados de monitorização disponíveis e a avaliação dos serviços do ecossistema em todo o AA. **As seções foram organizadas por metodologias principais** e apresentadas tal como configura abaixo.

5. A **lista de contactos** adicionada ao relatório facilita a identificação dos investigadores da equipa DiadES que reúnem mais conhecimento sobre cada uma das secções metodológicas.

6. Foi acordado que grande parte das atividades dos parceiros incidiria sobre **espécies com informação insuficiente**, como o sável e savelha, a lampreia, a solha-das-pedras e o muge.

7. O estudo direcionado ao DNA ambiental ou eDNA baseia-se no fato de que os organismos deixam vestígios da sua presença no ambiente, sob a forma de tecidos, escamas, gâmetas resultantes da atividade de desova ou carcaças de indivíduos mortos. O conteúdo genético, se presente, pode ser recolhido a partir de amostras de água e fornecer uma indicação de presença da espécie no local amostrado. A análise genética é um processo sofisticado e será realizada por dois dos parceiros **em uma de duas instalações centrais**: pelo AZTI (para sável/savelha e para lampreia-marinha) e pelo Cefas (para o eperlano e o muge). **Os protocolos para recolha, filtração e armazenamento de amostras de água foram fornecidos pelos dois institutos para comparação.** A centralização das análises por espécie/instituição promove a padronização das mesmas e reduzirá potenciais problemas de erro e contaminação.

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8. Os peixes incorporam vários elementos químicos nos seus tecidos e estruturas ósseas. Estes são encontrados no organismo em proporções relacionadas com as existentes no meio ambiente que os rodeia. A análise microquímica de otólitos (estruturas ósseas do ouvido interno) e escamas permite examinar certos elementos-chave e desenvolver uma 'assinatura' para as águas nas quais o peixe foi capturado e uma assinatura (óssea) do peixe num estágio específico da vida. Os estudos de microquímica propostos no DiadES incidirão principalmente sobre:
    a. As duas espécies do género *Alosa*, com enfoque na identificação da bacia hidrográfica de origem dos adultos capturados no mar, de modo a ampliar o conhecimento existente sobre as migrações e movimentos das espécies após a migração trófica dos juvenis;
    b. Os movimentos migratórios do muge na zona estuarina e a respetiva utilização de zonas com salinidade especifica.

    Para as amostras de *Alosa* sp. foram incluídos protocolos detalhados, contendo os principais requisitos das análises pretendidas. Da mesma forma, foram propostas algumas diretrizes para as amostras de muge. Este estudo será conduzido pelo INRAE e todas as análises serão realizadas por uma única equipa de laboratório no INRAE, garantindo assim que a preparação e análise das amostras é realizada de forma consistente, económica e otimizada, eliminando eventuais causas de erro.

9. A coleção de escamas recolhidas para o estudo de microquímica referido acima, será ainda utilizada, juntamente com amostras de tecido adicionais, para estudar a hibridação entre as duas espécies do género *Alosa* - sável e savelha - no âmbito do DiadES. **Esta é uma oportunidade valiosa dentro do projeto.** Esta componente do projeto será conduzida pelo INRAE e pelo MARE-UÉ, sendo que todas as análises serão realizadas por uma única plataforma genética para eliminar potenciais fontes de erro. **As referências bibliográficas chave dos protocolos foram listadas.**

10. A migração/movimento de espécies alvo serão estudados no âmbito do DiadES, com recurso a diferentes tecnologias. **Foram apresentadas em detalhe seis propostas individuais por parte dos parceiros.** Em conjunto com dados históricos recolhidos, estes resultados contribuirão para o conhecimento destas espécies e para a construção dos mapas de distribuição que o DiadES irá gerar para o Atlas interativo da Web. As tecnologias referidas irão incluir:
    a. Marcação de juvenis de lampreia-marinha com floy-tags durante a migração para jusante;
    b. Marcação de diversas espécies com transmissores acústicos - truta-marisca, eperlano, sável/savelha e muge;
    c. Marcação de esturjão com transmissores pop-up com transmissão via satélite.

11. Informação sobre o bycacth de espécies-alvo do DiadES pode ser obtida em portos de pesca comercial e/ou através do estudo e monitorização das populações. Os parceiros do DiadES irão contactar as autoridades públicas relevantes, entidades de gestão portuária e os pescadores comerciais, para obter dados de capturas acessórias de espécies relevantes. **Foram apresentadas em detalhe propostas individuais de 5 parceiros do DiadES**. Procurar-se-á recolher espécimes para obtenção de dados biométricos, bem como informação sobre a data e local da captura (dentro das áreas do ICES) dos mesmos. A informação recolhida pode contribuir significativamente para o conhecimento dos movimentos marinhos das espécies e, por conseguinte, para a construção dos mapas de distribuição a gerar no âmbito do projeto.

12. Os parceiros do DiadES integram programas nacionais de monitorização, e.g. Diretiva-Quadro da Água em águas de transição, ou têm acesso a esta tipologia de dados relativos ao seu país de origem. **Foram**

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apresentadas em detalhe seis propostas individuais pelos parceiros do DiadES. Esta informação contribuirá para o mapeamento geral da distribuição, para um mapeamento mais detalhado da utilização de tipos específicos de habitat por vários estágios de vida e também para a obtenção de dados biométricos.

13. Os parceiros acordaram a utilização de um protocolo de base de dados que irá fornecer consistência em relação à etiquetagem de amostras, fornecimento de dados biométricos/ambientais relativos a cada amostra, entre outros. Esta abordagem é essencial para promover um sistema de rastreio das amostras, necessário quando existe a partilha de dados e material biológico entre um ou vários parceiros distintos que funcionam como pontos focais de uma determinada tarefa, como por exemplo, o laboratório do INRAE face à análise microquímica. **Toda a informação sobre a utilização desta ferramenta noutros contextos foi disponibilizada.**

14. Os outputs científicos produzidos no âmbito do WP6 por parceiros individuais ou por um conjunto de parceiros do projeto serão publicados em revistas científicas, com revisão por pares, **e disponibilizados na página de internet do DiadES.**

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WP 6.1 Entregable - Resumen Ejecutivo en los cuatro idiomas del Programa

1. DiadES tiene por objeto **evaluar y mejorar los servicios ecosistémicos que prestan los peces diádromos** en la Zona Atlántica (AA) y, paralelamente, el estado de conservación de esas especies, considerando explícitamente en su gestión los efectos previstos del cambio climático sobre su distribución. DiadES adopta un enfoque innovador de servicios ecosistémicos que convierte las abundancias de peces en unidades monetarias. DiadES contará con la colaboración de investigadores en el campo de las ciencias naturales y economistas medioambientales, apoyados por una sólida red de gestores de peces diádromos de todo el AA. Nueve serán el centro de estudios específicos y conjuntos. **Más información sobre el proyecto en www.diades.eu.**

2. El WP 6 de DiadES se denomina "Recopilación de datos y casos de estudio sobre servicios biológicos y de los ecosistemas". Este paquete de trabajo trata sobre la **adquisición de datos de campo**. Tiene una duración de 30 meses, desde principios de 2019 hasta finales de julio de 2021. En este programa de trabajo crucial participarán todos los socios beneficiarios y está dirigido por Inland Fisheries Ireland (IFI).

3. Una de las primeras tareas de este WP fue **acordar entre los socios metodologías conjuntas y prácticas óptimas** en los estudios biológicos en el marco de la tarea 6.1 "Definición de metodologías conjuntas para la recopilación de datos biológicos".

4. El presente informe "Manual de recopilación de datos biológicos" representa la convergencia de los socios en lo que respecta a las especies objetivo, los protocolos acordados y el intercambio de recursos. Este es el resultado esperado de la tarea 6.1 de **armonizar y estandarizar** los datos de muestreo disponibles y la valoración de los servicios ecosistémicos en todo el AA. **Las secciones se han organizado por metodologías principales** y se presentan como tales a continuación.

5. Se ha añadido al informe **una lista de contactos** para identificar fácilmente a los investigadores de DiadES con más conocimientos para cada sección metodológica.

6. Se ha acordado que una gran parte de las actividades de los socios se centrarán en las **especies con pocos datos/ datos insuficientes**, como la **alosa, la lampreas, la platija y la lisa.**

7. El estudio del ADN ambiental o eDNA se basa en el hecho de que los organismos dejan rastros de su presencia tras ellos, en forma de tejidos, escamas desprendidas, huevos o esperma de la actividad de desove y cadáveres de individuos muertos. El contenido genético, si está presente, puede ser recogido de muestras de agua indicando presencia de la especie. El análisis genético es un proceso sofisticado y se llevará a cabo para el resto de los socios **en una de las dos instalaciones centrales**: por AZTI (para la alosa y para la lamprea marina) y por Cefas (para el eperlano y para a lisa.). **Ambos institutos proporcionaron protocolos de recogida, filtrado y almacenamiento de muestras de agua para su comparación**. Este enfoque colegiado de utilizar instalaciones discretas creará una estandarización y ayudará a eliminar los problemas de error y de contaminación en la medida de lo posible.

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8. Los peces absorben elementos químicos en sus tejidos y estructuras óseas, y las proporciones de los diferentes elementos están vinculadas a su proporción en el entorno inmediato. La microquímica de los otolitos (estructuras óseas de los oídos) y de las escamas ofrece la oportunidad de examinar ciertos elementos clave y desarrollar una "firma" para las aguas en las que se capturó el pez y la firma (ósea) del pez en una etapa de vida determinada. Los estudios de microquímica propuestos en DiadES examinarán principalmente:
    a. Las dos diferentes especies de alosas e intentarán vincular los peces adultos capturados en el mar con sus cuencas natales. Esto ampliará el conocimiento sobre las migraciones y movimientos de la especie una vez que han dejado el agua dulce;
    b. Los movimientos migratorios de las lisas dentro de las aguas de los estuarios y el uso de zonas de salinidad específica por parte de la especie.

    Los protocolos emitidos para las muestras de alosas se detallan de manera completa con los requisitos clave. También se proponen algunas directrices interesantes para las muestras de lisa. Este estudio será dirigido por el INRAE y todos los análisis serán realizados por un solo equipo de laboratorio dentro del INRAE, con lo que se obtendrá un resultado de calidad constante, rentable y racionalizado, eliminando cualquier posible de error.

9. La colección de material en escala para el estudio de microquímica mencionado anteriormente también se utilizará para examinar la hibridación de las dos especies de alosas -sábalo y saboga - dentro de DiadES junto con muestras de tejido adicionales. **Esta es una oportunidad de valor añadido dentro del proyecto**. Este estudio será dirigido por el INRAE y MARE-UÉ, y todos los análisis se llevarán a cabo por una única plataforma genética, eliminando así cualquier posible de error. **Se enumeran las referencias bibliográficas clave de los protocolos.**

10. En DiadES se llevarán a cabo una serie de estudios sobre la migración/movimiento de los peces, utilizando diversas tecnologías. Se detallan **seis propuestas individuales de socios de DiadES.** Junto con los conjuntos de datos históricos recientes, éstos se combinarán para proporcionar conocimientos sobre las especies individuales y contribuir a los mapas de distribución que DiadES generará para el Atlas Web Interactivo. Las tecnologías incluirán:
    a. Marcaje de juveniles de lamprea marina en migración;
    b. Marcado acústico de una serie de especies: reo, eperlano, alosa, lisa;
    c. Marcado de esturiones por satélite.

11. La captura incidental de las especies objetivo de DiadES se presenta en los puertos de pesca comercial y mediante estudios de investigación y muestreo de los peces. Los socios de DiadES se pondrán en contacto con las autoridades públicas competentes, el personal de los puertos y los pescadores comerciales individuales para obtener datos sobre las capturas incidentales de las especies pertinentes. Se detallan **cinco propuestas individuales de socios de DiadES.** El equipo intentará obtener datos biométricos, así como información sobre la ubicación de la captura (dentro de las zonas de la cuadrícula del CIEM) y la fecha de captura. Esto puede contribuir significativamente al conocimiento de los movimientos marinos de la especie y, por lo tanto, a los mapas de distribución que el proyecto generará.

12. Los socios toman parte en programas nacionales de muestreo, por ejemplo, seguimiento de peces de la Directiva Marco del Agua en aguas de transición, o tienen acceso a estos datos dentro de su propio país. Se detallaron **seis propuestas individuales de socios de DiadES.** Este material contribuirá a la cartografía de la distribución general, a la cartografía más detallada de las etapas de la vida asociadas a tipos de

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hábitats específicos y también al suministro de datos biométricos.

13. Los socios han acordado un protocolo de base de datos que proporcionará coherencia en lo que respecta al etiquetado de las muestras y el suministro de datos biométricos/ambientales asociados. Esto es esencial para proporcionar un sistema de rastreo a prueba de errores, necesario cuando varios socios envían materiales diferentes y dispares a uno o más socios que actúan como puntos focales para una tarea específica, por ejemplo, el laboratorio del INRAE que presta servicios de análisis microquímicos. **Se facilita toda la información para la aplicación de esta herramienta a otros contextos.**

14. Los resultados científicos del WP6 de los estudios de los socios individuales y de los estudios compartidos por los socios se publicarán en revistas de revisión por pares **y se subirán al sitio web de DiadES**.

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