Natural England Commissioned Report NECR271

Regional monitoring plan for inshore fish communities in the Southwest of England

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Foreword

Natural England commission a range of reports from external contractors to provide evidence and advice to assist us in delivering our duties. The views in this report are those of the authors and do not necessarily represent those of Natural England.

Background

As there is limited knowledge of the biodiversity, variability and condition of inshore fish communities around England, there are repercussions for the efficacy of potential management measures, on estimations of Natural Capital values and on their Ecosystem Service value and contribution to societal goods and benefits.

Natural England commissioned a single piece of work with the ultimate aim of producing a detailed and fully costed monitoring plan for the monitoring of inshore fish populations in the SW of England.

This aim has resulted in the production of 3 linked reports;

1. NECR 269 (Franco, A., Nunn, A., Smyth, K., Hänfling, B. and Mazik, K. (2020a)). A review of methods for the monitoring of inshore fish biodiversity.

2. NECR 270 (Franco, A., Barnard, S. and Smyth, K. (2020b)). An assessment of the viability of fish monitoring techniques for use in a pilot approach in SW England.

3. NECR 271 (this report), (Franco, A., Hänfling, B., Young, M. and Elliott, M. (2020c)). Regional monitoring plan for inshore fish communities in the Southwest of England.

It is intended that the outputs of these linked report be used to underpin a trial of inshore fish monitoring in English inshore water, with the eventual aims of seeking to integrate inshore fish monitoring into the wider UK marine biodiversity monitoring programme

This report should be cited as: NECR 271. Franco, A., Hänfling, B., Young, M. and Elliott, M. (2020c). Regional monitoring plan for inshore fish communities in the Southwest of England

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Further information
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Regional monitoring plan for inshore fish communities in the Southwest of England

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Executive Summary

As there is limited knowledge of the biodiversity, variability and condition of inshore fish communities around England, there are repercussions for the efficacy of potential management measures, on estimations of Natural Capital values and on their Ecosystem Service value and contribution to societal goods and benefits. This study was designed to develop a regional pilot monitoring programme for inshore fish communities in the southwest (SW) of England, and it constitutes of three elements:

- The Natural England Commissioned Report: NECR 269, (Franco et al., 2020a), consists of a review of the methods that can be used to monitor inshore fish assemblages, and covering 'traditional' sampling (nets and traps), observation techniques (including visual detection, acoustic detection and others), and DNA-based methods. Based on available best practice, each method is described in the report (including technique specifications and standardisation, targeted fish components, operational constraints, logistic requirements or restrictions, licencing aspects, etc.), with the key characteristics of each technique being summarised in an Excel spreadsheet ('Fish method review table').
- The Natural England Commissioned Report (NECR 270), (Franco et al., 2020b), identifies a selection of those techniques that are potentially viable for use in the inshore areas of the SW of England. This assessment is based on the relevant characteristics of the SW study area (e.g. habitat and environmental characteristics, overall fish resource, uses of the area), and the selection of monitoring method takes into account their practicality, suitability and cost-effectiveness for use in monitoring fish communities.
- This report, which builds on the above documents and on discussions with key stakeholders (government agencies, scientific associations, academia, and consultancies) to propose a detailed, costed regional pilot monitoring programme for inshore fish communities in the SW of England.

This regional monitoring plan relies on characterising the inshore fish communities in the study area thereby both assessing the communities, indicating current and future changes and determining the value of management measures. Therefore, this plan aims to provide a clear rationale based on standardised methods to allow comparability with existing and future monitoring data.

The monitoring plan proposed here has been devised according to a modular approach, encompassing: (i) a broad-scale monitoring that allows the basic characterisation of the inshore fish assemblages by targeting key habitats and associated fish assemblage components in the study region; (ii) the use of environmental DNA (e-DNA) monitoring to provide a wider species coverage for integration and cross-validation; (iii) the use of additional targeted (reactive or strategic) monitoring to address specific interests and questions as they arise (e.g. to assess the effect of a pressure or a management measure on the fish assemblage).

The broad-scale monitoring is based on the combined use of multiple core monitoring techniques to characterise the main fish assemblage components associated with inshore aquatic habitats in the region. The methods include fyke netting, seine netting, beam trawling, otter trawling and SCUBA diving, and they have been selected based on their ability to provide both qualitative and quantitative data on the fish assemblages that are assessed at the macro-habitat scale. A key element of this monitoring plan module is the stratification of the monitoring strategy. This proposes firstly a separation of inshore coastal

waters (CW) and transitional waters (TW) for planning purposes, to account for the different environmental drivers (specifically salinity) that affect the fish assemblage structure, functioning and dynamics, and the existence of an established national monitoring programme to assess fish in transitional waters in compliance with the requirements of the Water Framework Directive (under the responsibility of the Environment Agency in England). Secondly, the monitoring design is stratified by habitat types, these being broadly defined based on the combination of water depth and type of substratum (namely sediment or rock), with the method most suited to characterise the associated fish assemblage being selected. Where relevant, the sampling design also accounts for the presence of three-dimensional habitat structures (seagrass, saltmarsh, mäerl beds, kelp, and biogenic reefs), and the hydrodynamic energy (e.g. wave exposure) of shallow water habitats, as these factors may affect the variability of fish assemblages within the broad scale habitat. The plan for core monitoring of inshore fish assemblages provides a description of the selected monitoring techniques, standard operating procedures, criteria for replication of the sampling at the habitat level (stations) and at the station level (sample replicates), and for temporal targeting, which result in a detailed allocation of sampling effort within the pilot design for the SW of England.

The use of e-DNA monitoring is proposed to integrate the data obtained from the broad scale monitoring with additional data on fish biodiversity that may allow to identify possible gaps in the above monitoring strategy, as for example poorly sampled species (e.g. rare species, more cryptic species) that may be under-represented by the core monitoring. As such, the e-DNA monitoring also allows for cross-validation of the level to which the proposed core methods are reflective of the inshore fish community as a whole, and therefore the identification of possible additional monitoring priorities and needs that will guide the development of targeted monitoring plans in the future. To maximise cost effectiveness, the monitoring strategy for e-DNA assessment is intended to take advantage of the extensive sampling effort undertaken under the broad scale monitoring for sample collection.

The inclusion of additional targeted (reactive or strategic) monitoring in the proposed plan allows to address the assessment of specific components of interest, as conditions change (e.g. new species colonising the region with climate change) or as different questions arise (e.g. where concerns arise in relation to the possible impact of an activity, a pressure, or a management action), and where identified gaps of knowledge cannot be addressed with the proposed core or e-DNA monitoring design. This component relies on monitoring strategies and designs specifically tailored to answer a question, and therefore the details of these (and the costs) are to be defined on an *ad-hoc* basis. This component also looks at possible integration of the core and e-DNA monitoring by opportunistically using data obtained from other existing or planned monitoring programmes in the study area (e.g. monitoring of artisanal and novel fisheries in the SW), by assessing the resulting potential for data integration and enhancement.

The cost for the detailed monitoring components are provided. The types of data obtained (qualitative and quantitative) from each method are also assessed, along with how these data may be integrated within and between years to provide meaningful information, in light for example of the ability to assess the fish community health, identify population trends in key species or groups.

The strength and weaknesses of the proposed monitoring plan are discussed, including for example the identification of fish assemblage components that are less well covered by the proposed methods, or limitations in the type of data that can be obtained. Recommendations for further improvement of the plan follow to address areas of uncertainty in the specific methods and protocols described in this report, as also informed by consultation with the stakeholders. The report also provides an initial (but not exhaustive) identification of the possible partners that might contribute to the delivery of the monitoring plan, by supplying expertise, equipment, staff support, delivering monitoring components, etc.

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1. Aim and scope

Detailed knowledge of the biodiversity, variability and condition of inshore fish communities around England is limited. This impacts on the confidence that can be attributed to the efficacy of potential management measures, on estimations of Natural Capital values and on their Ecosystem Service value and contribution to societal goods and benefits. As a result, Natural England has commissioned the University of Hull a study that aims at developing a regional pilot monitoring programme for inshore fish communities in the southwest regional sea area (SW) of England.

This regional monitoring plan aims at collecting a robust dataset to characterise the inshore fish communities in the study area. This is the first step for the assessment of these communities, and of changes that may indicate deleterious impacts or the effectiveness of management measures. Therefore, key to this plan is the provision of a clear rationale and the standardisation of the methods for their replicability in the future and in other regions, and to allow comparability with existing monitoring data.

Inshore fish communities are hereby defined to include species that live predominantly within 6 nautical miles (nmi) of the coast, that are dependent on such shallow inshore waters for part of their lifecycle. The latter species include for example fish using estuarine and shallow coastal waters as nursery grounds or as pathways for spawning migrations between marine and fresh waters (i.e. marine migrants and diadromous species, respectively; Franco *et al*., 2008; Potter *et al*., 2015).

The SW inshore waters relevant to this study include estuarine and marine waters (within 6 nmi of the coast) along the coast of Cornwall, Devon, Somerset, and Gloucestershire, from Start Point (south Devon) and into the Severn estuary (Somerset and Gloucestershire) (Figure 1). This corresponds to the English inshore component of the UK marine region no. 4 (Western English Channel and Celtic Sea), one of the eleven bio-regions into which UK seas have been divided based on physical and biological features, and aligning with EU Water Framework Directive (WFD) water bodies and with the regions and sub-regions of the EU Marine Strategy Framework Directive (Defra, 2010).

In this monitoring plan, the SW inshore region has been subdivided into smaller geographical units, namely four sub-regions (mainly based on relative homogeneity of general environmental characteristics) and nine zones (mainly associated to key ports of reference) (Figure 1 and Table 1). This allows the subdivision of the regional monitoring plan into smaller and more manageable spatial units, although it is emphasised that continuity and coherence between areas and sub-regions within the region are maintained through the standardisation of the design criteria.

Fish assemblages include different taxonomical, morphological and functional groups, representing different niches and inhabiting different habitats. Therefore monitoring fish communities may require the use of different, though complementary, methods. These have been reviewed in (Franco *et al*., 2020a), and their viability for potential use in the SW inshore waters has been preliminary assessed in (Franco *et al*., 2020b).The monitoring plan proposed in this report builds on that information.

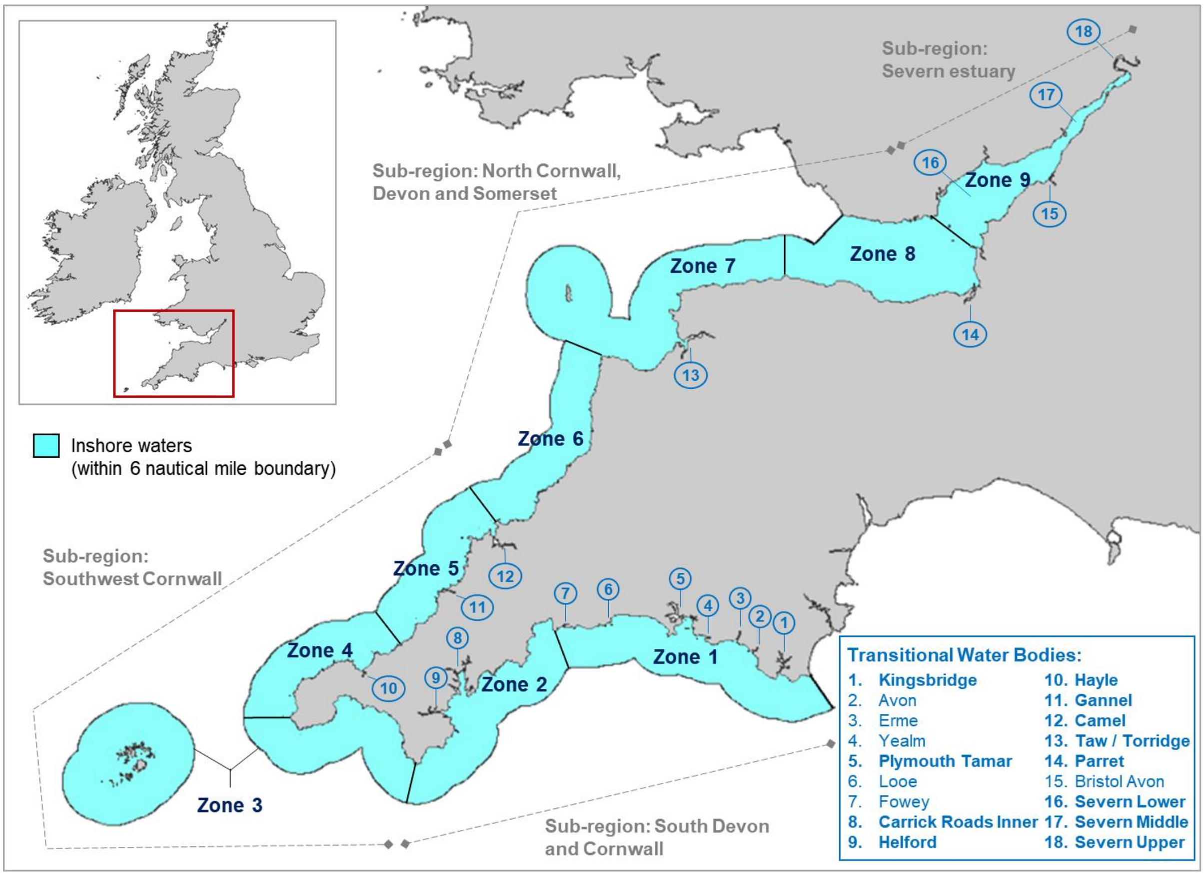

Figure 1. Study region, including coastal waters within 6 nautical miles and transitional water bodies in the SW of England. The main spatial units (sub-regions and zones) used for designing the monitoring plan are indicated, as well as the transitional water bodies included in the present monitoring plan (in bold; see Section 3.2.2.1 for details on TW body selection).

Table 1. Geographical scope of the study area and associated sub-units (sub-region and zone). Indicative topographic locations delimiting each inshore spatial unit are given, as well as MPAs (MCZs and SACs) and main transitional water systems (TW) included in each zone (* indicates TW already included in existing WFD fish monitoring undertaken by the Environment Agency; see Section 3.2.1 for further details).

Region	Sub-region	Zone (port of reference)	MPAs	TW
SW inshore waters [Start Point (South Devon) to Severn Estuary (Somerset and Gloucestershire)]	**South Devon & Cornwall** [Start Point (South Devon) to Lizard Point (South Cornwall)]	**(1) Plymouth** [Start Point (South Devon) to Fowey (South Devon)]	Skerries Bank & Surrounds MCZ Start Point to Plymouth Sound SAC Plymouth Sound and Estuaries SAC Whitsand and Looe Bay MCZ	Kingsbridge, Avon, Erme, Yealm, Tamar, Looe, Fowey

Table continued…

Region	Sub-region	Zone (port of reference)	MPAs	TW
SW inshore waters [Start Point (South Devon) to Severn Estuary (Somerset and Gloucestershire)]	**South Devon & Cornwall** [Start Point (South Devon) to Lizard Point (South Cornwall)]	**(2) Falmouth** [Fowey (South Devon) to Lizard Point (South Cornwall)]	Fal and Helford SAC The Manacles MCZ Lizard Point SAC	Carrick Roads Inner*, Helford
	SW Cornwall [Lizard Point (South Cornwall) to Camel estuary (West Cornwall)]	**(3) Newlyn/ Penzance** [Lizard Point (South Cornwall) to Land's End (SW Cornwall)]	Lizard Point SAC Mounts Bay MCZ (rock giant goby) Runnel Stone (Land's End) MCZ	
		(4) St. Ives [Land's End (SW Cornwall) to south of Portreath (West Cornwall)]	Lands End and Cape Bank SAC	Hayle
		(5) Newquay /Padstow [south of Portreath (West Cornwall) to Camel estuary (West Cornwall)]	Newquay and the Gannel MCZ (rock giant goby) Padstow Bay and Surrounds MCZ	Gannel, Camel*
	North Cornwall, Devon and Somerset [Camel estuary (West Cornwall) to Lilstock (Somerset)]	**(6) Bude** [Camel estuary (West Cornwall) to Hartland Point (North Cornwall)]	Padstow Bay and Surrounds MCZ Hartland Point to Tintagel MCZ	Camel*
		(7) Ilfracombe/ Bideford [Hartland Point (North Cornwall) to Foreland Point (North Devon)]	Lundy SAC Bideford to Foreland Point MCZ Morte Platform MCZ	Taw Torridge*

Table continued…

Region	Sub-region	Zone (port of reference)	MPAs	TW
SW inshore waters [Start Point (South Devon) to Severn Estuary (Somerset and Gloucestershire)]	**North Cornwall, Devon and Somerset** [Camel estuary (West Cornwall) to Lilstock (Somerset)]	**(8) Bridgewater** [Foreland Point (North Devon) to Hinkley Point (Somerset)]		Parret
	Severn Estuary [upstream of Hinkley Point (Somerset and Gloucestershire)]	**(9) Bridgewater/ Bristol** (lower estuary), **Sharpness** (middle and upper estuary) [upstream of Hinkley Point (Somerset and Gloucestershire)]	Severn Estuary SAC	Severn Lower*, Severn Middle*. Severn upper*, Bristol Avon (+ Usk and Wye, Wales)

Consultation with relevant stakeholders was undertaken throughout the project and a stakeholder event was held in Plymouth on the 9th April 2019 to discuss the pilot monitoring plan (the strategy, methods, their applicability, etc), its costs and the possible partnerships to implement it. Participants from government agencies (Natural England, Environment Agency (EA), Marine Management Organisation (MMO), Devon and Severn IFCA), scientific associations (Marine Biological Association, MBA), academia (University of Hull, University of Plymouth), and consultancies (Colclough & Coates Aquatic Consultants, Applied Genomics) were involved and the comments and suggestions raised during the meeting (Appendix 1) have been integrated in the monitoring plan as provided in this report[1].

The aim is that the proposed regional monitoring programme is trialled by government agencies to test costs and practicalities before seeking funding to expand nationally (T. Russell, Natural England, pers. comm.) The costs associated with the proposed monitoring have been calculated under the assumption that all monitoring requirements are outsourced, to allow the estimate of the possible budget that would be required in a worst case scenario. The cost for each monitoring component has been calculated as an average cost between a minimum and maximum to account for the possible variability in costing staff, equipment etc. depending on the supplier. Discussions with individual stakeholders/potential partners who could provide specific expertise, equipment etc. (as summarised in Section 6) have also been taken into account. Overlap with existing monitoring programmes has also been highlighted, as in some of these cases (e.g. where the same methodology is used) the integration between these and the current plan may generate savings.

[1] Where reference to a specific comment is made in the report, this is indicated as pers. comm. from the person making the suggestion or providing the information.

2. Monitoring strategy and general rationale

The monitoring strategy for the inshore waters has been devised according to a modular approach (Figure 2). The logic of this approach is briefly explained below, with detailed information given in the following sections. This is a general approach that provides a strategy for designing the monitoring of inshore fish communities around the UK, with the adaptation of the monitoring plan to the specific coastal region depending on the environmental context within the region.

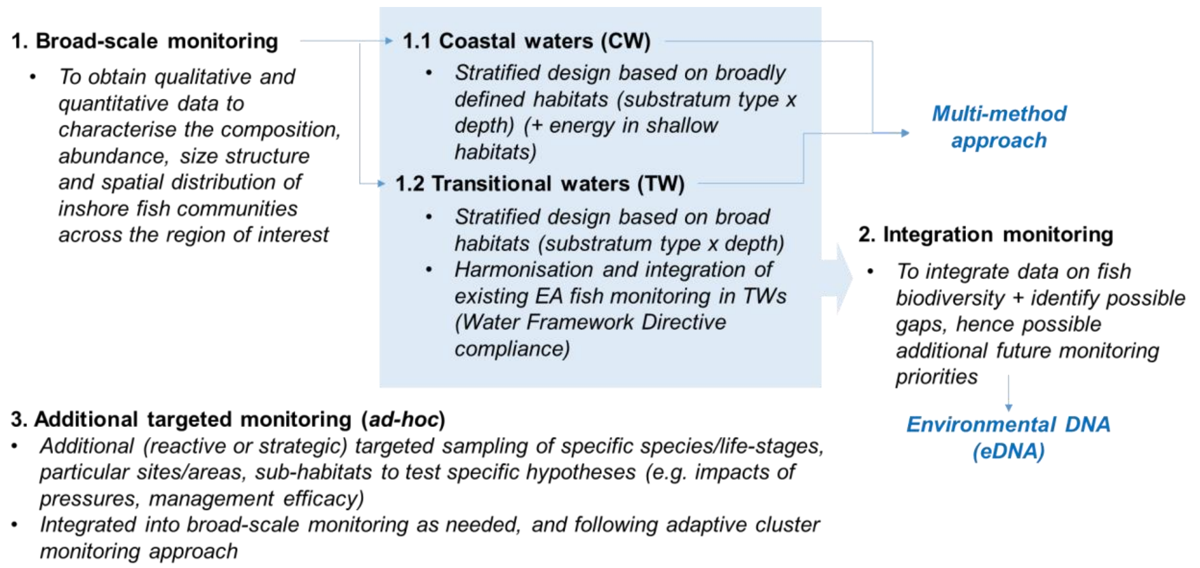

Figure 2. The modular approach to the design of the inshore fish monitoring.

2.1 Broad scale monitoring

The monitoring plan proposed in this document is designed to address the aim of characterising the inshore fish communities in the SW region via the collection of a robust dataset. With this aim in mind, the first (core) module of the monitoring aims to obtain qualitative and quantitative data to characterise the composition, abundance, size structure and spatial distribution of inshore fish communities across the region of interest.

Key elements of the design of this core component of the inshore fish monitoring are given in the sub-sections below, with further details about methods, protocols and costs provided in Section 3.

2.1.1 Multi-method approach

The multi-method approach is considered to provide a more comprehensive picture of the full species complement occurring in inshore waters, also in light of the different habitats (e.g. benthic or pelagic, different substrata) and the associated fish assemblage components (e.g. groups of species, morphological types, life stages) that are targeted by the different methods (Franco *et al*., 2020a).

The main criteria used for method selection for this core monitoring are:

- Each method provides both quantitative and qualitative data that allow to characterise the composition, abundance, size structure and spatial distribution of fish communities.
- Each method allows the collection of data at the macro-habitat scale.
- Methods providing data as representative as possible of the entire inshore fish community are prioritised over methods that are very specific (e.g. targeting a particular species).
- The combination of methods allows the collection of data on fish communities covering the broad scale fish habitats occurring in inshore waters (see Section 2.1.2 for details on these habitats).

As a result, the fish sampling methods selected for the core monitoring component of inshore fish assemblages include fyke netting, seine netting, beam trawling, otter trawling and SCUBA diving.

Other key methods were taken into account, but were considered not viable for use in the regional monitoring of inshore fish communities, also after consultation with stakeholders (Appendix 1). For example, acoustic techniques (e.g. echosounders) were not considered sufficiently developed or cost effective to contribute to the core monitoring of inshore fish assemblages, mainly due to issues associated with species identification and the need of using other methods (e.g. otter trawling) for ground truthing (Appendix 1; see also Franco *et al*., 2020a; Franco *et al*., 2020b).

Other methods as for example baited video techniques were considered not suitable for monitoring fish assemblages at the regional scale due to limitations in the spatial scale at which the data are collected and possible data artefacts (e.g. differential species attraction to the bait station) that may affect the assessment of the structure and functioning of the fish assemblage as a whole (Franco *et al*., 2020a). Similarly, mobile camera methods (e.g. towed video) were not selected for the core fish monitoring due to biases in abundance estimates due to behavioural effects on fish and species detection issues, also compared to diving underwater visual census (Franco *et al*., 2020a).

A final agreement on the selected methods and their application in different habitat contexts within the study region was reached through stakeholder consultation, also taking into account cost-effectiveness (e.g. considering the type and quality of data obtained vs. the cost of collecting these data) and possible negative implications of using a method (e.g. impact on habitats, sensitive species) (Appendix 1). This led for example to the exclusion of otter trawling and gill netting from fish monitoring in estuaries, and also informed the definition of sampling protocols (e.g. recommending increased frequency of emptying fyke nets where high fish mortality rates can be expected; see Section 3.3.1 for details).

An overview of the methods selected for the core monitoring of inshore fish assemblages and their ability to represent different inshore habitats and fish assemblage components (in terms of species and life stages) in coastal and transitional waters is given in Figure 3, with further explanation in the text below (further details on method specifications, standardised protocols and costs are provided in Section 3).

- **Beach seine netting (SEN)** is selected for the sampling of fish assemblages on sedimentary shallow habitats (up to 2 m depth), both in coastal and transitional waters. Deployed in these conditions, the net has the potential to catch both demersal and small shoaling pelagic species using these marginal habitats (e.g. flounder, plaice, smelt, herring), including both young and adult fish, thus providing information on these nursery habitats.

	Coastal waters (_CW)		Transitional waters (_TW)	
Depth category	**Sediment**	**Rock**	**Sediment**	**Rock**
Intertidal			FYK	*
Subt. - V. Shallow (0-2 m)	*SEN (+ Push Net)*	DIV	SEN	
Subt. – Shallow (2-30 m)	BT2		BT1.5	DIV
Subt. - Moderate depth (30-50 m)	*OTT (Midwater)*			
Subt. - Deep (>50 m)				
Selected key habitats	*SEN seagrass**	*DIV mäerl, kelp, biog. reef*	*SEN* FYK *seagrass* & saltmarsh**	DIV *mäerl, kelp, biog. reef* *

Figure 3. Multi-method approach for the monitoring of inshore fish communities in coastal and transitional water habitats. Methods: SEN, seine net; BT, beam trawl, OTT, otter trawl; DIV, SCUBA diving visual census, FYK, fyke nets. Asterisks indicate where the sampling is undertaken at the margin of or on substrata adjacent to the specific habitat.

Seine netting is complemented by the use of a **Riley push net** in coastal sandy beaches to effectively integrate the evidence on the use of these very shallow habitats by juvenile demersal fish (e.g. flatfish, small gobies), particularly where wave exposure may pose limits to the use of seine nets. For the purpose of this plan, push netting is included as part of the SEN_CW monitoring component.

- **Beam trawling (BT)** is selected for the sampling of inshore shallow water sedimentary habitats (<30 m) to complement the characterisation of fish assemblages using these habitats as nursery and feeding grounds. The fine meshed scientific beam trawls target efficiently benthic/demersal species using these marginal habitats at both juvenile and sub-adult stage, but bottom trawling has been also shown to take representative samples of pelagic populations, especially at the qualitative level, and to a large extent also at quantitative level (Elliott *et al.*, 1990). The use of this method is restricted to the presence of open sedimentary habitats with no obstructions or sensitive structuring features (e.g. seagrass vegetation, biogenic reefs).
- **SCUBA diving underwater visual census (DIV)** is selected to monitor fish in shallow water habitats (<30 m depth) that cannot be surveyed with seine netting and beam trawling (due to practical limitations of these methods or their possible impact on sensitive features; see Franco *et al.*, 2020a, 2020b). This monitoring component targets mainly nectobenthic fish species of small to medium size (also including a juvenile component), and associated with rocky substrata (e.g. wrasses, blennies, gobies, sparids, gadoids) in the infralittoral zone. Habitats assessed with this method also include kelp forests and other structured habitats (mäerl beds, biogenic reefs e.g. *Sabellaria*). This monitoring covers such habitats mainly in coastal waters, with a minor components represented in transitional waters (and specifically in outer estuaries).

- Midwater **otter trawling (OTT)** has been selected to integrate the monitoring of fish fauna by targeting more pelagic components of the fish assemblage and deeper areas of inshore coastal waters[2] (which in the SW region are mostly around 50 m depth). This survey component assesses the fish communities farther from the shore, mainly targeting pelagic species (e.g. sardine, anchovy, sprat and herring), using inshore waters as feeding grounds, thus complementing the data obtained with the other core methods. However it is acknowledged that this is probably a lower monitoring priority in the inshore coastal environment, compared to the other components, considering that the main provision of resources (e.g. as habitat or food) supporting diverse and productive fish communities in inshore areas is mainly associated with the bentho-demersal habitats in shallow coastal waters (e.g. functioning as nursery and feeding grounds). In addition, the fish pelagic component of deeper water assemblages (up to 20m depth) is better represented and covered by offshore monitoring (e.g. the PELTIC survey programme targeting small pelagic fish in the coastal waters of the western Channel and Celtic Sea, with a combination of acoustic surveys, pelagic trawling and plankton sampling).
- **Fyke netting (FYK)** is selected to monitor fish using intertidal habitats in transitional waters, and particularly sheltered intertidal areas (e.g. mudflats and saltmarsh) in the upper/middle reaches of estuaries. The method targets a wide variety of fish species that swim close to the shore, including marine and estuarine fish using these intertidal habitats (and adjacent shallow subtidal habitats) as nursery of feeding grounds (e.g. flatfish (particularly flounder), grey mullets, sand gobies), as spawning grounds (e.g. smelt) or as pathways for migration (e.g. eels, shads).

2.1.2 Stratification by habitat

Fish assemblages are influenced by the habitat characteristics of inshore waters (e.g. depth, salinity, seabed characteristics), due to the tolerance ranges and ecological needs of the different species (and life stages), and their interaction with the environment and other biota (Elliott and Hemingway, 2002). Habitat characteristics also influence the viability of some monitoring methods, whereby some sampling methods are not suitable for example for use on rocky substrata or within vegetation (Franco *et al*., 2020a, 2020b).

Due to their general shallower depth and higher productivity compared to offshore areas, the association of fish fauna with seabed habitats is particularly important in inshore coastal and transitional waters. This is reflected for example by the predominant benthic/demersal habits of fishes occurring in estuaries, and their dependence on the benthic/epibenthic resources for feeding (Elliott and Dewailly, 1995; Elliott and Hemingway, 2002; Franco *et al*., 2008).

In addition, estuarine and nearshore coastal habitats, particularly at shallower depths, are considered essential for the survival of early life stages of many temperate species, playing an important role as nursery grounds (Blaber *et al*., 1995; Elliott and Hemingway, 2002; Beck *et al*., 2003; McLachlan and Brown, 2006; Litvin *et al*., 2018). They provide optimal conditions for the concentration, survival and growth of juvenile stages of estuarine and marine fishes, including abundant food resources and protection from larger predators as granted by sheltered and shallow water conditions (Beck *et al*., 2003; Litvin *et al*., 2018). Similar conditions are also granted by specific structured habitats occurring in estuarine and

[2] Following discussions with stakeholders, it was agreed not to include otter trawling in the monitoring of transitional waters due to the poor cost-effectiveness of this method when applied in estuarine environments (i.e. it provides a small contribution to the information on estuarine fish assemblages for a substantial cost).

inshore coastal areas, as for example saltmarshes, seagrass meadows, kelp forests and biogenic reefs (Mathieson *et al*., 2000; Beck *et al*., 2003; Litvin *et al*., 2018).

To account for these factors, the monitoring plan is stratified by habitat as follows:

- A first level of the habitat stratification is according to salinity. Specifically, coastal waters (in fully marine conditions) are differentiated from transitional waters (estuaries, characterised by brackish conditions and a salinity gradient from marine to freshwater conditions). This distinction also considers that an existing monitoring programme is in place at the national scale and that applies to fish in transitional waters in compliance with the requirements of the Water Framework Directive (under the responsibility of the Environment Agency). Therefore, separate plans have been developed for coastal waters (CW) and transitional waters (TW), although there is some overlapping in the outer estuaries and adjacent coastal approaches to reflect the environmental continuum and the connectivity between these systems.
- A further level of habitat stratification broadly defines different habitat types based on the combination of water depth and type of substratum. Water depth is used to distinguish shallow water habitats, indicatively <30m in depth (littoral and infralittoral), from deeper water habitats which in the SW study area have depth mostly comprised between 30m and 50m, and always <100m (mostly circalittoral). The type of substratum is broadly distinguished into sedimentary and rocky substratum, with the possible addition of three-dimensional structures as submerged vegetation such as seagrass, saltmarsh and mäerl beds on littoral (the former) or infralittoral (both) sedimentary habitats, kelp on infralittoral rock, or biogenic reef features (e.g. *Sabellaria* reefs). Hydrodynamic energy (e.g. wave exposure) is also considered to characterise shallow water habitats (e.g. sandy beaches), where this may affect the fish assemblage or the ability to monitor them with a specific method. The stratification by habitat type is relevant to the choice of the most appropriate method for monitoring fish assemblages of a given habitat. An example of the habitat stratification as applied to the inshore coastal waters in the SW region is shown in Figure 4.

Further details on the monitoring design applied to the SW region (including distribution of the monitoring effort within habitat strata, methodological protocols, etc.) are given in Sections 3, separately for coastal waters (Section 3.1) and transitional waters (Section 3.2).

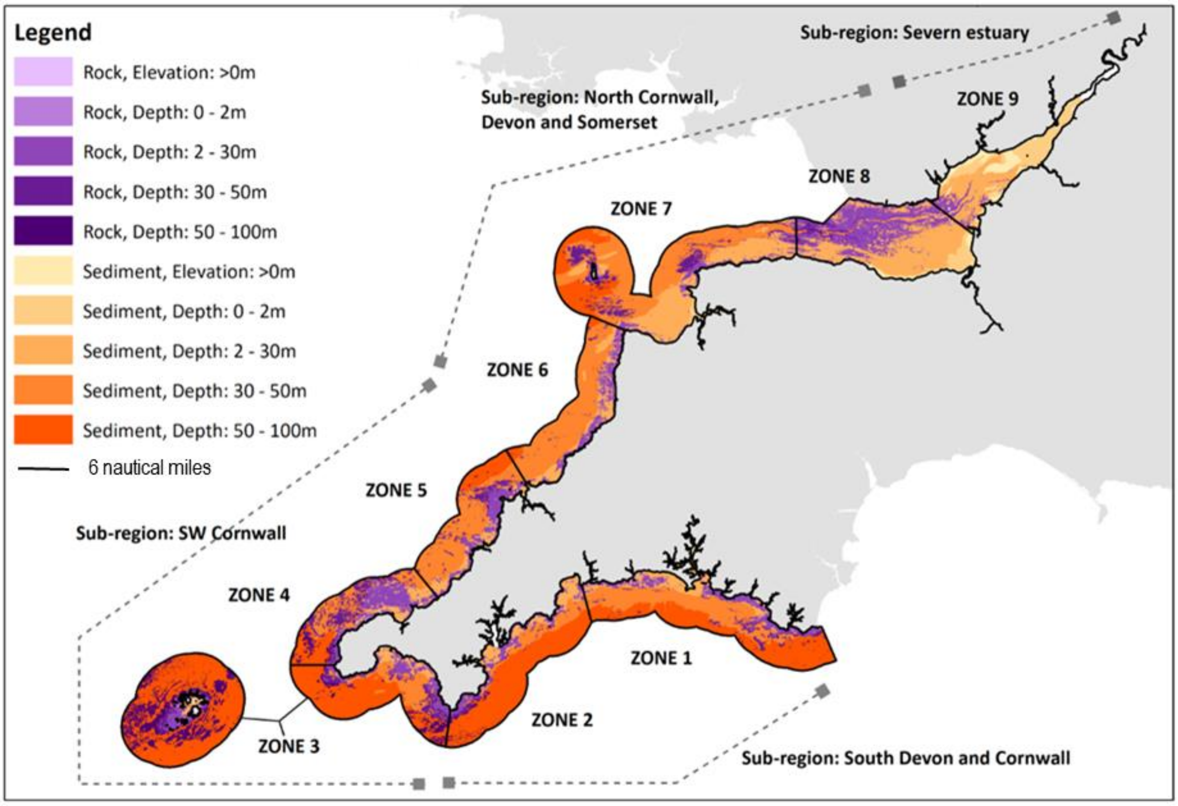

Figure 4. Main broad scale habitats (combining substratum and depth levels) distribution in the inshore zones identified for the inshore waters (within 6 nautical miles off the coast) of the SW region.

2.1.3 Temporal aspects

Fish populations in inshore coastal and transitional waters from temperate climates are subject to high levels of natural variability throughout the year reflecting the seasonality of biological processes regulating population dynamics, as for example spawning, recruitment, migrations, mortality (McErlean *et al*., 1973; Potter *et al*., 1986; Axenrot *et al*., 2004).

These changes often reflect changes in the shallow water environment (e.g. temperature) that influence the availability and distribution of the resources (habitats, food, etc), and may affect the occurrence, abundance, body size and life stages in local fish populations. Even the turbidity of the waters, which will reflect the erosion-deposition cycles in estuarine and nearshore areas, has been suggested as enhancing the refuge potential of an area (Blaber and Blaber, 1980). Therefore, the timing and frequency of the fish monitoring during an annual cycle are also important elements of the sampling design, as the above mentioned variability may significantly influence the composition, diversity and abundance of fish communities sampled throughout the year.

To account for these factors, the following elements have been considered to define the timing and frequency of the inshore fish monitoring in the SW region:

- The diversity and abundance of fish communities in shallow inshore coastal and estuarine habitats is often enhanced during the late summer – autumn, when the primary and secondary productivity of inshore water habitats is enhanced, with consequent increased food availability. In particular, the late summer-early autumn represents the post-spawning period for most temperate fish species (see for example Ellis *et al*., 2012). High numbers of juvenile fish recruit and temporary settle into shallow inshore habitats during this period, when the nursery function of these habitats is enhanced. In the late summer-autumn period (July to December), inshore habitats also function as main feeding grounds for various marine species (e.g. cod, seabass; Pawson *et al*., 2007), before the populations decrease (due to increased mortality, and migration towards deeper waters for some species) following the decrease in temperature and local resources in the winter.

- Migrations of marine species into estuaries also occur in winter (e.g. Elliott *et al*., 1990) and have been explained by the movement of species into colder waters thus giving reduced metabolism at a time of reduced food availability.
- Where inshore environments are used as migratory routes, as in the case of estuarine systems for migratory (anadromous and catadromous) fish species, the timing of such migrations, their direction and the life stages involved may differ with species, as outlined in Table 2.
- The timing in the year may affect the habitat structure (e.g. vegetative period of seagrasses and kelp) and other environmental characteristics that may influence the habitat use by fish and the effectiveness of specific monitoring techniques (e.g. increased water visibility in the summer for visual census).
- The timing in the year may also affect the logistics of the monitoring (e.g. foot access to sandy beaches in certain areas of the SW of England may be challenging during the holiday season (August), and this issue is expected to be less relevant in September or on rainy days when influx to the beach is reduced; Benjamin Ciotti, University of Plymouth, pers. comm.)
- The timing and frequency of the sampling may vary between sampling methods also in consideration of the fish assemblage components they target and the main role of the habitats that are sampled with those methods.
- Cost effectiveness and the timing and frequency of existing broad scale fish monitoring programmes in the study area have also been considered. For example: seasonality requirements of WFD fish monitoring in transitional waters identify spring (May-June) and autumn (September-October) as key monitoring periods (see Section 3.2.1 for details); the international bottom trawl surveys in the SW of England including the Irish Sea, Bristol Channel and Celtic Sea Beam Trawl Survey (ISBCBTS) are undertaken in September and the South West Ecosystem beam trawl survey (SWECOS, Western Channel and Celtic Sea) in March (MMO *et al*., 2016); the English Integrated Pelagic Survey (PELTIC) targeting small pelagic fish in the coastal waters of the western Channel and Celtic Sea include pelagic trawling undertaken in the 4th Quarter (October – December) (MMO *et al*., 2016).

As a result of the above considerations, a survey calendar is proposed, as outlined in Table 3.

In general, one survey per year is considered sufficient to characterise fish assemblages using inshore coastal waters, with surveys generally targeting the summer-autumn months to capture the highest diversity and the key functionality of these habitats as nursery and feeding grounds for fish (with some variability in the timing to accommodate logistic and other constraints as mentioned above).

In transitional waters, the monitoring frequency should be preferably twice a year, in the spring (April-June) and autumn (September-October). If needed, the frequency can be reduced to once a year, targeting the autumn period, when higher fish abundances and diversity are expected following summer recruitment for most resident and marine species using estuaries as nurseries, and migratory runs for some diadromous species (Table 2). However, the above frequency reduction should only be applied to all methods but fyke nets. This latter method is most suitable to target fish migrations (that often occur at night-time and in marginal areas – e.g. lampreys, eel), and therefore fyke netting in both spring and autumn is a minimum requirement to allow a sufficient representation of the migratory components of estuarine fish assemblages.

Table 2. Fish migration of main diadromous species in estuaries in the spring and autumn seasons (u/s, upstream migration; d/s, downstream migration; N indicates migration occurring mainly at night-time; spawning migrations of adults netting are in bold – these are more likely to be effectively targeted by fyke netting).

Species	Spring	Autumn
Eel	u/s (N)	**d/s (N)**
Salmon	**u/s** & d/s	**(u/s)**
Sea trout	d/s	**u/s**
Twaite shad	**u/s**	
Allis shad	**u/s**	
River lamprey	d/s	**u/s** (N)
Sea lamprey	**u/s (N)**	d/s **(+ u/s)**
Smelt	**(u/s)**	**u/s**

Table 3. Proposed monitoring calendar for inshore fish communities in coastal and transitional waters. Grey cells indicated possible survey time, with asterisk indicating recommended time.

Method	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Coastal waters												
SEN (incl. Push net)									*			
BT2									*			
DIV							*	*				
OTT									*	*		
Transitional waters												
SEN					(possibly)				*	*		
BT1.5					(possibly)				*	*		
FYK					*	*			*	*		

2.2 Integration with e-DNA monitoring

The second module of the monitoring is designed to integrate the data obtained from the broad scale monitoring with additional data on fish biodiversity that may allow to identify possible gaps in the above monitoring strategy, as for example poorly sampled species (e.g. rare species, more cryptic species) that may be under-represented by the core monitoring.

A key characteristic of the e-DNA method is its non-selectivity, while also having the advantage of being non-destructive. In fact, the method relies in the identification of species occurrence based on DNA traces (e.g. scales, skin, and body fluids) that are detected from water (or sediment) samples. The identification is based on comparison with reference barcodes for the species, and, for fish, the database for most regional fish fauna is almost complete and several ongoing projects focus on completing the remaining gaps (Franco *et al*., 2020a), thus ensuring a broad species coverage.

Therefore, data from the e-DNA module can be used to cross-validate the level to which the proposed core methods are reflective of the inshore fish community as a whole, and this would allow to establish possible additional monitoring priorities and needs, hence to guide the development of targeted monitoring plans in the future.

However, e-DNA techniques have also the disadvantage that it is not possible to categorically state that any species identified are within the study area given the potential transport of biological material by the prevailing hydrographic patterns. For example, material taken on one coastal area could emanate from that area, the adjoining catchments or the adjoining sea area. Hence it can indicate the structural aspects of the community, i.e. what could be in an area, but little about the functioning of the system. Therefore, e-DNA results provide information at a coarse spatial scale, and hydrodynamic factors (e.g. direction and speed of prevailing currents) need to be taken into account to identify the source location and potential transport routes.

Currently, this method only allows a qualitative assessment of biodiversity (species identification), but quantitative information may be obtained in the future as the DNA-based techniques develop further (Franco *et al*., 2020a). The low relative cost of DNA analysis methods and the easy sample collection also lend it to long-term environmental monitoring, hence playing a role in cost-effectively understanding temporal dynamic of the inshore fish community in key target locations. This may also help to understand how well this temporal variability is being catered for with traditional (core) monitoring methods.

To maximise cost effectiveness, the monitoring strategy for e-DNA assessment is intended to take advantage of the extensive sampling effort undertaken under the broad scale monitoring for sample collection. Further details on sampling effort, methodological protocols and costs are given in Section 4.

2.3 Additional targeted/integrative monitoring

The questions posed in relation to inshore fish fauna in a region may be varied and diverse, reflecting multiple interests at variable scales. They may range from life stage, species specific research questions (e.g. growth, migration, spawning, nursery use of habitats), to community scale assessments of stock health, to localised regional or water-body health assessments (such as for water body classification or the implementation of EU Directives), the response to pressures or management measures (where these are implemented), etc.

It is unlikely that any single monitoring plan can be expected to answer all the questions or provide all relevant data unless it is very detailed and comprehensive and thus perhaps costly. The recent study by Waugh *et al* (2019) shows that standardised sampling techniques will be necessary to show the trends in fish species richness between and within estuaries even if they do not use all available methods or cover all possible habitats; again this emphasises the need for clarity in defining the questions to be answered. For example to answer the question ‘what is the total fish community structure in an area’ will require a different sampling from ‘what is the effect of a particular type of stressor on the fish community’.

This additional module consists of targeted (reactive or strategic) monitoring that allows to address the assessment of specific components of interest, by answering specific questions. This is likely to provide spatial, temporal or method integration (also including data and analytical integration) of the previous modules (particularly the core broad-scale monitoring). The monitoring strategy and design for this module is to be identified as specific and local needs, interests and questions arise. As such, this monitoring component cannot be detailed or general costs identified in advance and it needs to be done on an *ad-hoc* basis. Further details on this are given in Section 5 of this report.

3. Broad scale monitoring design, methods and costs

3.1 Coastal waters (CW)

3.1.1 Detailed core monitoring design for the SW of England (CW)

The proposed detailed spatial design of the fish monitoring in inshore coastal waters in the SW of England is shown in Figure 5 (temporal aspects of the design have been addressed in Section 2.1.3), with further information on the design criteria given below.

3.1.1.1 Sampling effort distribution across methods and habitats

The proposed design takes into account the use of multiple methods in different habitat strata, as per general broad scale monitoring strategy as outlined in Section 2.1. In addition, the distribution of the sampling effort between habitat strata within zones and for different core methods takes into account the following factors and criteria:

- A minimum of 2 sampling stations is identified for each habitat stratum (and method) within a zone, to ensure that samples from representative habitats are geographically distributed throughout the whole region, with a minimum level of spatial replication within a zone.
- The number of monitoring stations allocated to each habitat stratum varies with the availability and relative extent of the stratum within a zone (in relation to the other habitats) and across zones (Appendix 2, Table A2.1).
- The value of the habitat to fish is also taken into account. This results in;
    - a proportionally higher sampling effort (in relation to the extent of the habitat) being allocated to shallower habitats (sampled with seine net, push net, beam trawl and diving surveys) compared to deeper habitats (otter trawl) in consideration of their added value as fish nursery grounds;
    - an increased number of stations allocated to a given method (hence broad scale habitat) within a zone to ensure that specific structured habitats of particular interest (e.g. due to elevated national importance of the species they support) (where occurring) are represented by the monitoring where occurring. These habitats include in particular: seagrass beds, mäerl beds, kelp, or biogenic reef features (e.g. *Sabellaria* reefs).

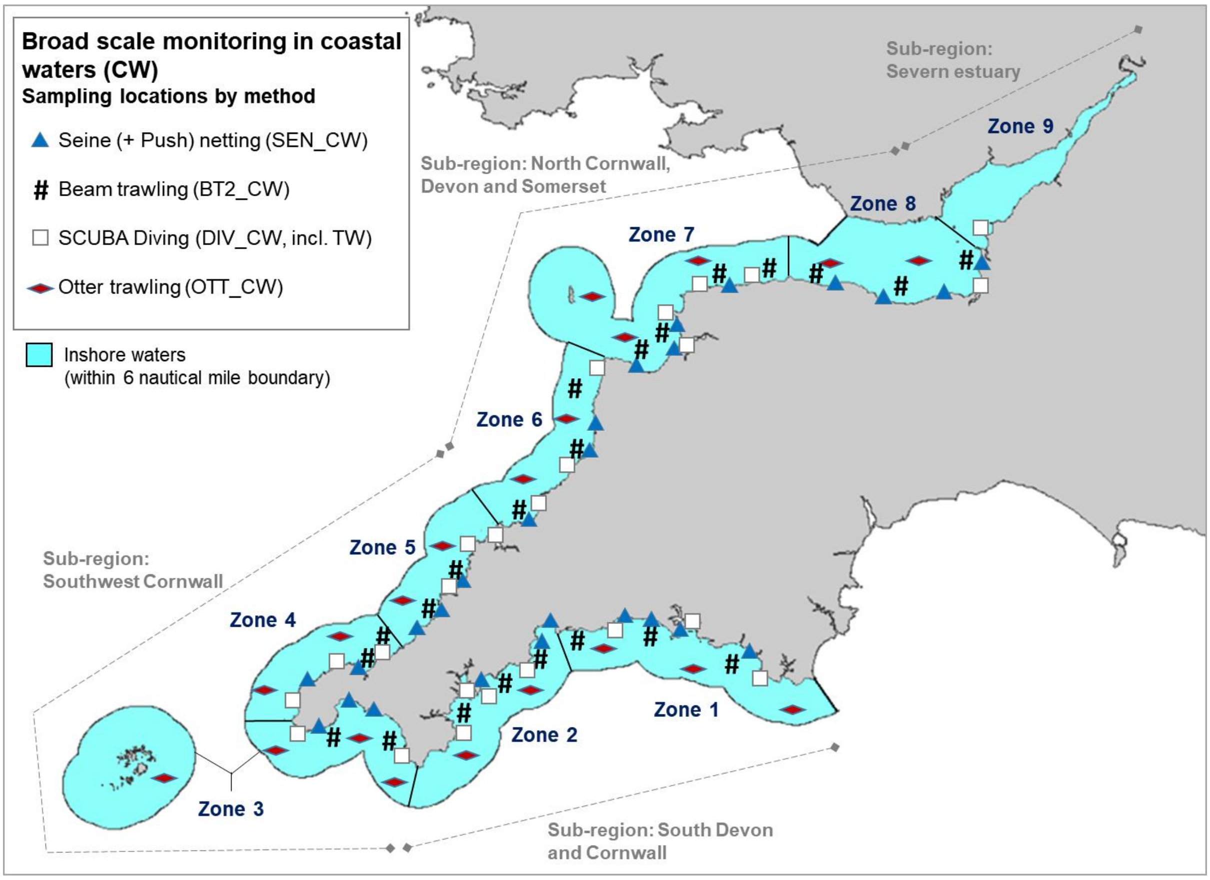

Figure 5. Distribution of sampling stations (indicative target locations) for the proposed monitoring of fish communities in inshore coastal waters of the SW region. Symbols indicate the different sampling methods targeting fish assemblage components according to the proposed habitat stratification (as in Figure 4).

Additional factors that may affect the ability to locate suitable sampling stations in a habitat stratum within a zone (e.g. degree of fragmentation and patchiness of the habitat in relation to sampling area associated with a given method, visibility and local hydrodynamic conditions, site accessibility issues) have also been considered (as informed by stakeholder consultation) to define the sampling design in Figure 5.

As a result, a total of **93 stations** has been identified in the SW inshore coastal waters and distributed throughout Zones 1-8[3] (Figure 5; Appendix 2, Table A2.2):

- **26 stations** located on both sheltered (low energy) and exposed beaches (high energy), to be sampled with seine net and push net (SEN_CW). Where both habitats are not available in one zone, the two stations should be located in the one habitat that is available. These stations should also include at least one location near to seagrass habitat, where this is available within a Zone.
- **22 stations** located on open sedimentary habitats at depth <30 m, to be sampled with beam trawl (BT2_CW). Care should be taken to avoid trawling on sensitive or unsuitable habitats (rock substratum, kelp, seagrass, mäerl, biogenic reefs).
- **18 coastal stations**, distributed in each of Zones 1-7 to represent open rock habitats at depth <30 m, as well as specific habitats of interest (kelp, mäerl, biogenic reefs) where these are available within a zone. These are to be surveyed by using underwater visual

[3] Zone 9 is not included here as it is transitional waters (Severn estuary).

census (DIV_CW). Strong tides and visibility issues in the marine approaches to the Severn estuary (Zone 8) prevent the application of diving surveys in this zone. One additional station has been added for each medium-sized transitional water body occurring in a zone (adding to max. **6 TW stations** in the region) to cover possible survey requirements for rocky habitats in outer estuaries (see Section 3.2.3).

- **21 stations** located on open water habitats at depth >30 m, to be sampled with otter trawl (OTT_CW).

3.1.1.2. Sample replication at station level

As for the sampling effort within a station, a minimum of 3 replicate samples is to be collected per station (this is the minimum sample replication for any inferential analysis). The level of sample replication influences the precision of mean estimates of main community descriptors (e.g. number of species, abundance), and this is related to the confidence in such estimates (Andrew and Mapstone, 1987). There is no information on this in relation to fish monitoring with the sampling methods considered here. However, Franco *et al*. (2015) found that the use of 3 replicate samples produces mean estimates with acceptable levels of precision for most community descriptors in benthic monitoring studies at offshore wind farm sites. This paper also emphasises that knowing the degree of change that is desired to be detected is the main determinant in deciding the level of replicability; for example the detection of a 20% change in the fish fauna characteristics between sampling sites or occasions will require more replicates than a large change. Furthermore, where the same habitat is to be sampled with different methods (as in the case of seine netting and push netting on sandy beaches), the sample replication also takes into account the different habitat area sampled with the two methods, such that a similar area is cumulatively explored at a sampling site (resulting in 3 seine net replicates and 5 push net replicates; see details on sampling methods and protocols in Section 3.1.2).

3.1.2 Core monitoring methods, SOPs and survey requirements (CW)

Details on the method specifications, protocols and survey requirements pertaining the different monitoring components for the broad scale (core) monitoring of inshore fish communities in coastal waters are given in this section. Additional information on protocols for fish sample handling and data collection, and required licencing is given in Sections 3.3 and 3.4, respectively.

3.1.2.1 Beach seine net and push net (SEN_CW)

Beach seine netting is used for the sampling of fish assemblages on sedimentary shallow habitats (up to 2 m depth), as for example sandy beaches and the adjacent shallow subtidal area. Discussion with stakeholders has highlighted that seine netting might be prevented in certain conditions of high wave energy (e.g. as common along the north coast of Cornwall) and is preferably used in sheltered beaches. Therefore it is recommended that seine netting surveys are coupled with the use of a Riley push net. Sampling experience with both methods has shown that the push net can be applied in a wider range of wave exposure (albeit still limited by very high wave energy), and therefore this method can be used where seine netting is not possible (Benjamin Ciotti, University of Plymouth, pers. comm.) However, where seine netting can be undertaken, it is recommended that both methods are used, in order to provide an assessment of the comparability of the catches and therefore of the data (inter-calibration), as this is not currently available. The use of the seine net that is also used in transitional waters will ensure continuity and consistency of the beach sampling in the region.

Both methods are used on open seabed. However, where seagrass vegetation occurs in these conditions, seine netting can also be undertaken to allow representation of the fish fauna associated with this habitat. While the net can be modified for use on seagrass beds trampling on vegetation might be an issue for habitat damage, and therefore it is recommended that, where seagrass occurs, the sampling is undertaken in the adjacent open habitat in the vicinity of vegetation. When sampling adjacent to seagrass beds, Natural England's advice on a site-specific acceptable buffer should be sort.

Gear specification and operational standards for seine netting are derived and adapted from those for seine netting for WFD fish monitoring in estuaries (EA, 2011a). The beach seine used for this purpose is 43 m long and 4 m deep, with knotless mesh size of 14 mm on the wings and 6.5 mm on the central panel (where the fish are gathered when hauling the net) (EA, 2011a). This method is also consistent with the method being currently used by the University of Plymouth for fish sampling on sandy beaches along the coast of SW of England (Benjamin Ciotti, University of Plymouth, pers. comm.) Three replicate samples are to be collected per station, taking care to avoid overlapping of the swept areas. The encircling net is set in a semicircle (D shape) from the shore by surveyors wading in the shallow waters (no leader ropes used). The size of the area enclosed by the net should be estimated to allow standardisation of abundance data as density estimates (it is estimated that the area swept with a net of the above size specifications could be as big as 400-500 m^2 per haul).

Push netting is to be undertaken using a Riley push net, a hand-held 1.5 m beam trawl (with a single spiked tickler chain and 10 mm mesh) that is towed manually in water between 0.5 and 1.5 m deep. This method has been used to sample flatfish in inshore nursery grounds on the west of Scotland (Ciotti *et al*., 2010, 2013a, 2013b, 2013c; Fox *et al*., 2014), and is currently being used on sandy beaches in the SW of England (Benjamin Ciotti, University of Plymouth, pers. comm.) At each site the net is towed at walking pace for 5 min (equivalent to a swept area of approximately 200-300 m^2 per tow), with five replicate samples to be collected. The distance covered by each tow is to be recorded using hand-held Garmin GPS units to allow data standardisation by swept area.

Sampling with both methods is to be undertaken in slack tide conditions, with low slack tide being preferable if possible (when tidal migrants are concentrated at lower levels on the beach and the net will also sample those species that do not migrate intertidally) (Wilding *et al*., 2001; EA, 2011b; Benjamin Ciotti, University of Plymouth, pers. comm.)

Survey operations require 6 surveyors (two teams of 3 persons, with two surveyors in water and one for shore support in each team) to undertake both seine netting and push netting simultaneously at one site, also considering the restricted tidal window available for the sampling. All surveyors should be preferably trained for fish identification, handling and measuring (including juvenile stages), with a minimum of two surveyors being a senior level and the rest at junior level.

3.1.2.2 Beam trawl (BT2_CW)

Beam trawling is used to target fish assemblages on open sedimentary substrata, with no obstructions. Trawling is to be avoided on rocky habitats or where sensitive features occur (e.g. seagrass, mäerl, biogenic reefs).

Gear specification and operational standards have been derived and adapted from those adopted by for the young fish surveys undertaken between 1981 and 2015 on the east coast of England (Rogers *et al*., 2000). A 2 m beam trawl is used, with fine mesh net with cod end liner of 4 mm knotless mesh, light chain footrope and 3 tickler chains.

Three samples are to be collected per station, with three separate tows undertaken along a direction parallel to the shore, and representing the main depth strata <5m, 5-15m depth, 15-30m depth where possible. Each sample is collected by towing the beam trawl at low speed (approximately 1 knot) for 500 m (corresponding to a sampled area of 1,000 m^2 for a gear of the above size specifications) or for approximately 15 minutes. To allow standardisation of abundance data as density estimates, the size of the area towed should be estimated by multiplying the beam trawl width (2 m) by the tow length (this can be estimated by using an odometer attached to the beam trawl shoe; Rogers *et al*., 2000). Overlapping of the towed areas should be avoided.

A small inshore vessel (e.g. inshore trawler or RV, 10-12 m long) is needed for this monitoring component. In addition to the vessel skipper and crew, survey operations require two surveyors. All surveyors should be preferably trained for fish identification, handling and measuring (including juvenile stages), with a minimum of one surveyor being a senior level and the other at junior level.

3.1.2.3 SCUBA diving (DIV_CW)

Underwater visual census is undertaken to assess fish assemblages associated shallow water habitats (<30 m depth) in the infralittoral zone, including open rock substratum, kelp forest, mäerl beds and biogenic reefs (e.g. *Sabellaria*). Where similar habitats occur in outer estuaries, and tidal and visibility conditions allow to undertake visual surveys, these are also included in this monitoring component (see also Section 3.2.3.4).

Standardised protocols for underwater visual census of fish have been developed for kelp habitats on the west coast of the U.S. (PISCO[4], Partnership for Interdisciplinary Studies of Coastal Oceans, a long-term, large scale monitoring programme led by California and Oregon universities), and for rocky habitats in Mediterranean MPAs (Prato *et al*., 2017). Protocols are currently being developed for fish monitoring in European/ North Atlantic waters (e.g. underwater visual census of fish in kelp habitats being tested by Pierre Thiriet and colleagues), this is still an ongoing effort and a definite methodology is not currently available (Benjamin Ciotti a Keiron Fraser, University of Plymouth, pers. comm.)

Given the above, the specification and operational standards for the SCUBA diving fish surveys in this document provisionally refer to those of the PISCO protocol, as this has been considered as a suitable starting point. However, it is recommended that further effort is made to develop and trial a standardised protocol that can be applied to rocky habitats around the UK (see Section 7). The Approved Code of Practice (ACOP) for Scientific and Archaeological diving issued by the Health and Safety Executive should also be followed to comply with Diving at Work Regulations (1997) (HSE, 2014).

In the PISCO method the survey at each station is stratified by depth, using the following strata: 5 m, 10 m and 15 m (and the addition of a 20 m stratum where available). Three replicate strip transects are to be surveyed at each depth level within a station, with the search area at each transect measuring 30 m x 2 m x 2 m. Each transect is to be marked (e.g. using a fixed rope or natural features, and two divers move along the transect in bounds, by collecting fish count and size data within sequential windows of 2-3 m (using habitat markers for spatial reference), and one meter either side of the transect. A first snapshot is taken after a scan at the beginning of each bound to record mobile exposed fish, and a second search is undertaken while diving the bound to record unexposed fishes (e.g. sedentary, solitary, cryptic species). Overlapping of the transect areas should be avoided. It

[4] http://www.piscoweb.org

is estimated that the size of the area covered by the survey would be as large as 60 m^2 per transect, 540 m^2 per station.

A team of four surveyors (buddy system) is to be deployed at each transect, including two divers (buddy system), one supervisor (in contact with the diving team for the whole duration of the survey) and one surface standby (the latter may not be required in shallower waters, where the dive buddy can act as in-water back up; Trudy Russell, Natural England, pers. comm.) The first diver should be an experienced scientific diver with diving certification as stated in the ACOP and fish identification skills, and would be tasked with recording the data in the field. The second diver acts as a buddy, providing support to the first diver, recording data about the transect (e.g. GPS line), and also carries a video camera for validation and integration of the data recorded by the first diver (comparison between fish records and video should be done on site at the end of each transect). Diving certification as stated in the ACOP and basic scientific diving experience would be required for the buddy diver.

It has been estimated that approximately 10 minutes are required to survey a 30 m-long transect (PISCO). Considering that each diver can do 2 dives per day, the length or which is determined by depth (e.g. each dive can be over 2 hours at depth ≤10 m, 1 hour for 15 m depth and 45 minutes for 20m depth; Trudy Russell, Natural England, pers. comm.), a maximum of three dive pairs would be required for surveying two stations in one day (1 pair for the transects at 5 and 10 m, one pairs at 15 m and 1 additional pair at 20 m where required), in addition to the supervisor and the surface standby. All surveyors should have appropriate training for fish identification and size measuring on diving surveys (including juvenile stages), with a minimum of four surveyors (3 divers and the supervisor) being at senior level and the others at junior level.

A large diving RIB or a commercial diving hard-boat is needed for survey support, the former being preferred as it would allow more flexibility of movement of the survey team between sites and zones in the region should the local conditions in one area/site not be suitable for the survey, thus reducing costs and the risk of downtime.

3.1.2.4 Otter trawl (OTT_CW)

Otter trawling is undertaken to assess fish assemblages inhabiting deeper areas of inshore waters in the region. In particular, the otter trawl is to be towed on midwater to target demersal/pelagic fish that are less well represented by the other monitoring components (e.g. sardine, anchovy, sprat and herring, and larger cod or seabass).

Gear specification and operational standards have been derived and adapted from those for otter trawls to be used in estuaries and coastal surveys (e.g. Clean Seas Environment Monitoring Programme (CSEMP); EA, 2013a). A 6 fathom [11 m] otter trawl is used, with 2 x 70 fathom [128 m] warps, 2 x 9 ft [2.7 m] combination bridles, and 2 x 3 ft [0.9 m] otter boards.

A minimum of 3 x 30-minute tows are to be undertaken at each station, by towing the trawl at a speed of around 5 knots or more when undertaking midwater trawls (EA, 2013a). Tow duration may be reduced to 15 minutes to reduce damage to fish caught (e.g. where samples are also collected for stomach content analysis), or the length of a tow may be altered if the site is too limited to allow full tow (EA, 2011b). Overlapping of the towed areas should be avoided. A survey vessel (CSV) that is equipped with a winch for gear deployment and retrieval is required due to the weight and size of the trawl.

In addition to the vessel skipper and crew, survey operations require three surveyors. All surveyors should be preferably trained for fish identification, handling and measuring, with a minimum of one surveyor being a senior level and the others at junior level.

As a minimum, one survey should be undertaken at each station per year. To better represent fish use of inshore waters, sampling should be undertaken in the late summer-autumn (July to December), when inshore habitat function as main feeding grounds for various marine species (e.g. cod, seabass; Pawson *et al*., 2007).

3.1.3 Costs of core monitoring for the SW of England (CW)

3.1.3.1. Unit survey cost and assumptions

Unit costs (i.e. per day of survey) have been estimated for the different survey components and are provided below. All of these estimates cover survey operations from planning to data collection (including sample laboratory analysis, where needed). A mean cost is provided for each component, as well a range of variability taking into account uncertainty of generic unit costs allocation. In particular, the minimum cost assumes the use of a local survey team, at most competitive rates and with minimum travel and subsistence (T&S; e.g. no additional hotel costs), whereas the maximum cost assumes a survey team from outside the region is used, hence with additional costs (e.g. T&S, staff mobilisation/demobilisation) and at higher rates. Downtime, if incurred, would be charged on a pro-rata basis, and therefore costs for this have not been included here.

SEN_CW = £5,100 per day of survey.
The estimate above represents a mean unit cost for the combined seine netting and push netting in coastal stations, with the possible range of variability being £2,100 to £8,200 per day.
This cost includes survey staff, T&S (mainly consisting of car/van hire, including fuel, and subsistence costs), and equipment hire and consumables. It assumes that a team of 6 undertakes the survey of 2 sites in a day by accessing the survey sites on foot from the beach. This also includes the push netting, that can be undertaken in parallel (for inter-calibration) or in replacement of seine netting (where the latter is not possible due to sea energy conditions). The cost for laboratory analysis of samples is also included, whereas costs for sample transport to the analysing laboratory (if the analysing laboratory differs from the survey supplier) are excluded.

BT2_CW = £4,300 per day of survey.
The estimate above represents a mean unit cost, with the possible range of variability being £2,400 to £6,200 per day.
This cost includes survey staff, vessel hire, T&S (mainly consisting of car/van hire, including fuel, and subsistence costs), and equipment hire and consumables. It assumes that a team of 2 surveyors (1 senior and 1 junior) undertakes the survey of maximum 3 sites in a day and the survey site access and gear deployment is undertaken using an inshore coastal survey vessel (CSV) equipped with necessary winches. The cost for laboratory analysis of samples is also included, excluding costs for sample transport to the laboratory (if the analysing laboratory differs from the survey supplier).

DIV_CW = £5,600 per day of survey.
The estimated above represents a mean unit cost, with the possible range of variability being £2,600 to £8,700 per day. Estimates of staff rates, vessel rates and charges from different sources have been considered, including NE dive team costs as provided on consultation

(Trudy Russell, Natural England, pers. comm.) The latter mostly account for costs on the lower part of the range.

This cost includes survey staff, vessel hire, T&S (mainly consisting of car/van hire, including fuel, and subsistence costs), and equipment hire and consumables. It assumes that a team of 8 (4 senior and 4 junior) undertake the survey of 2 sites in 1 day and the survey site access and team deployment is undertaken using a large diving RIB or a commercial diving hardboat. No samples are taken, hence no costs for laboratory analysis are included.

OTT_CW = £4,300 per day of survey.
The estimated above represents a mean unit cost, with the possible range of variability being £2,300 to £6,300 per day taking account on uncertainty of generic unit costs allocation. This unit cost includes survey staff, vessel hire, T&S (mainly consisting of car/van hire, including fuel, and subsistence costs), and equipment hire and consumables. It assumes that a team of 3 surveyors (1 senior and 2 junior) undertakes the survey of 2 sites in a day and the survey site access and gear deployment is undertaken using an inshore coastal vessel (CSV) equipped with necessary winches. No samples are taken, hence no costs for laboratory analysis are included.

3.1.3.2 Total survey cost for CW inshore fish core monitoring

Considering the above unit costs, the sampling effort identified for the different zones and survey components (Appendix 2, Table A2.2) and the minimum sampling frequency required, the total mean cost for the fish monitoring of inshore fish communities in the coastal areas of the SW region is estimated at around **£217,100 per year**, with a 53% uncertainty (i.e. ranging £103,000 – £331,200) (see Appendix 2, Table A2.2). This figure includes both field work costs and the costs for the sample analysis. Additional costs for data analysis and reporting are provided separately in Sections 3.4 and 3.5.

This total cost is thus divided between different survey components (mean cost in bold):

SEN_CW = £66,800 per year.
The cost for the seine netting programme (also including push netting) in coastal waters of the SW region ranges between £26,800 and £106,800. This assumes a total of 26 sampling stations in the region, and includes both field work costs and the costs for the sample analysis. The variability of the programme cost (60%) accounts for the uncertainty of generic unit costs allocation.

BT2_CW = £37,700 per year.
The cost for the beam trawling programme in coastal waters of the SW region ranges between £21,100 and £54,300. This assumes a total of 22 sampling stations in the region, and includes both field work costs and the costs for the sample analysis. The variability of the programme cost (44%) accounts for the uncertainty of generic unit costs allocation.

DIV_CW = £67,700 per year.
The cost for the diving programme in coastal (and transitional) waters of the SW region ranges between £31,300 and £104,200. This assumes a total of 24 sampling stations (18 coastal + 6 in outer estuaries) in the region, and includes field work costs (no additional sample analysis is required). The variability of the programme cost (54%) accounts for the uncertainty of generic unit costs allocation, although a higher degree of uncertainty is nominally associated to the costs for this component, considering that it is based on a tentative definition of the sampling design and protocol, and these need further development.

OTT_CW = £44,900 per year.
The cost for the otter trawling programme in coastal waters of the SW region ranges between £23,800 and £65,900. It assumes a total of 21 otter trawling stations in the region, and includes field work costs (no additional sample analysis is required). The variability of the programme cost (47%) accounts for the uncertainty of generic unit costs allocation.

3.2 Transitional waters (TW)

A national monitoring programme targeting fish communities in transitional waters (TWs, mainly estuaries) is currently in place as undertaken by the Environment Agency. This monitoring is aimed at assessing the ecological status of transitional waters using fish fauna as indicators, in compliance with the requirements of the Water Framework Directive (WFD) (see Section 3.2.1 for details on this programme). However, the current WFD TW fish monitoring has some limitations in its ability to provide a comprehensive characterisation of the fish assemblages using estuarine environments (see Section 3.2.1).

Therefore, the monitoring plan designed here for TW fish aims at providing data that are consistent with the WFD fish monitoring undertaken so far (for comparability, continuity and compliance, should this still be required in the future), but also aims at providing more comprehensive data on the fish assemblages in these environments by addressing the limitations above, where possible.

3.2.1 Current national monitoring programme for fish in TWs

The WFD TW fish monitoring in England and Wales is based on a multi-gear approach to ensure that data on a representative sample of the habitats and fishes present are obtained (Coates *et al*., 2007; EA, 2011a, 2011b, 2013a, 2013b; WFD-UKTAG, 2014). This combines sampling with fyke nets in intertidal habitats (mainly in the upper parts of an estuary, in sheltered conditions), seine net in marginal (intertidal, shallow subtidal) sedimentary habitats along the estuarine gradient, a small (1.5 m) beam trawl in shallower open sedimentary habitats, and otter trawl in deeper areas (e.g. estuarine channel) (EA, 2011b). Occasionally, additional methods (albeit not considered compliant with the WFD requirements) have been included in the sampling programme (e.g. trammel netting in the outer and middle Severn, or larger (2.4 m) beam trawl used in deeper water where otter trawling is not possible; Table 4) to integrate the estuarine habitat monitoring.

The main requirements of the fish monitoring to provide suitable data for the WFD TW assessment are summarised below:

- Gear specifications and survey protocols should follow EA operational instructions (EA, 2011a, 2011b, 2013a, 2013b).
- Sampling can be bi-annual, in the spring (May – June) and autumn (September – October), or annual (September – October) (WFD-UKTAG, 2014).
- Sampling effort may vary depending on the size of the waterbody[5], but minimum requirement for England and Wales is 3 samples for otter trawling and 6 samples from other methods (WFD-UKTAG, 2014), although actual sampling effort has been adapted to local conditions and has changed over time (Table 4).

[5] A waterbody generally corresponds to the whole estuary, except for bigger estuaries that may be divided into multiple waterbodies. In the SW region, the latter is only valid for the Severn, which is divided into upper, middle and lower Severn waterbodies.

Table 4. Number of sampling stations in TW bodies sampled in SW of England for WFD monitoring between 1997 and 2016. Data from: EA database on TraC Fish Counts for all Species for all Estuaries and all years (downloaded 30/01/2019).

TW waterbody	Survey method	1997	1998	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
Camel	Seine netting										5	5	4	4	4	4	4	5	4	4
	Beam Trawl Netting 1.5m											2					4	2	2	2
	Otter trawl netting										1	1	1	1	1	1	1	1	1	1
	Camel Total										**6**	**8**	**5**	**5**	**5**	**5**	**9**	**8**	**7**	**7**
Carrick Roads Inner	Seine netting					5	5		4	4	4	5	4	4	4	4	4	4	4	4
	Beam Trawl Netting 1.5m					1	2										4	3	3	3
	Beam Trawl Netting 2.4m					1					1	1	1	1	1	1				
	Otter trawl netting					2	1										1	1	1	1
	Carrick Roads Inner Total					**9**	**8**		**4**	**4**	**5**	**6**	**5**	**5**	**5**	**5**	**9**	**8**	**8**	**8**
Plymouth Tamar	Fyke netting																		1	
	Seine netting																		8	
	Beam Trawl Netting 2.4m					1	1													
	Otter trawl netting						1													
	Plymouth Tamar Total					**1**	**2**												**9**	
Severn Lower	Beam Trawl Netting 2.4m												2							
	Otter trawl netting					1	2	1			3	3	2	3	3	3	3	3	3	1
	Severn Lower Total					**1**	**2**	**1**			**3**	**3**	**4**	**3**	**3**	**3**	**3**	**3**	**3**	**1**
Severn Middle	Fyke netting	1	1	1																
	Seine netting								1	1		2	1	1	1	1	1	1	1	
	Beam Trawl Netting 1.5m	1	1	1	1	1	1	2	1	1		1								
	Trammel netting	2	2	2	2	2	2	3				2								
	Severn Middle Total	**4**	**4**	**4**	**3**	**3**	**3**	**5**	**2**	**2**		**5**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	
Severn Upper	Fyke netting				1	1					1	4	4	4	4	4	4	4	4	1
	Seine netting									1		1	1	1	1	1				
	Severn Upper Total				**1**	**1**				**1**	**1**	**5**	**5**	**5**	**5**	**5**	**4**	**4**	**4**	**1**
Taw / Torridge	Seine netting										6	5	5	5	5	5	6	4	4	4
	Beam Trawl Netting 1.5m																2	2	2	2
	Beam Trawl Netting 2.4m										2									
	Otter trawl netting																1	1	1	1
	Taw / Torridge Total										**8**	**5**	**5**	**5**	**5**	**5**	**9**	**7**	**7**	**7**

- Sampling protocols (EA, 2011b) require the following level of sampling effort at each site/station:
  - a minimum of two hauls with seine netting (at low slack tide);
  - one fyke net deployment per station, using two pairs of nets over a full 12 hour tidal cycle;
  - 200 m tow length for 1.5 m beam trawl (tow length may be altered if the site is too limited to allow a full tow);
  - One tow of 30 minutes against flooding tide for larger beam trawl and otter trawl (this can be reduced to 15 minutes over rough ground to reduce damage to fish).

However, there are some limitations in the way the WFD monitoring of fish in TWs has been implemented in the SW region:

- Similarly, in recent years monitoring has been reduced to one season only (autumn) (Rob Hillman, EA, pers. comm.); this has the potential to reduce the ability of representing all fish species using estuaries in different seasons (e.g. migratory species; see also point below).
- Not all main habitat and species are adequately represented by the sampling methods used. For example, fish assemblages associated with rocky substrata are not represented. Also, migratory species known to occur in some areas (e.g. shad in the Taw Torridge) are not sampled with the current monitoring programme (Rob Hillman, EA, pers. comm.)

3.2.2 Detailed proposed core monitoring design for the SW of England (TW)

The detailed spatial design of the fish monitoring in transitional waters in the SW of England is graphically outlined in Figure 6 (temporal aspects of the design have been addressed in Section 2.1.3), with further information on the design criteria given below.

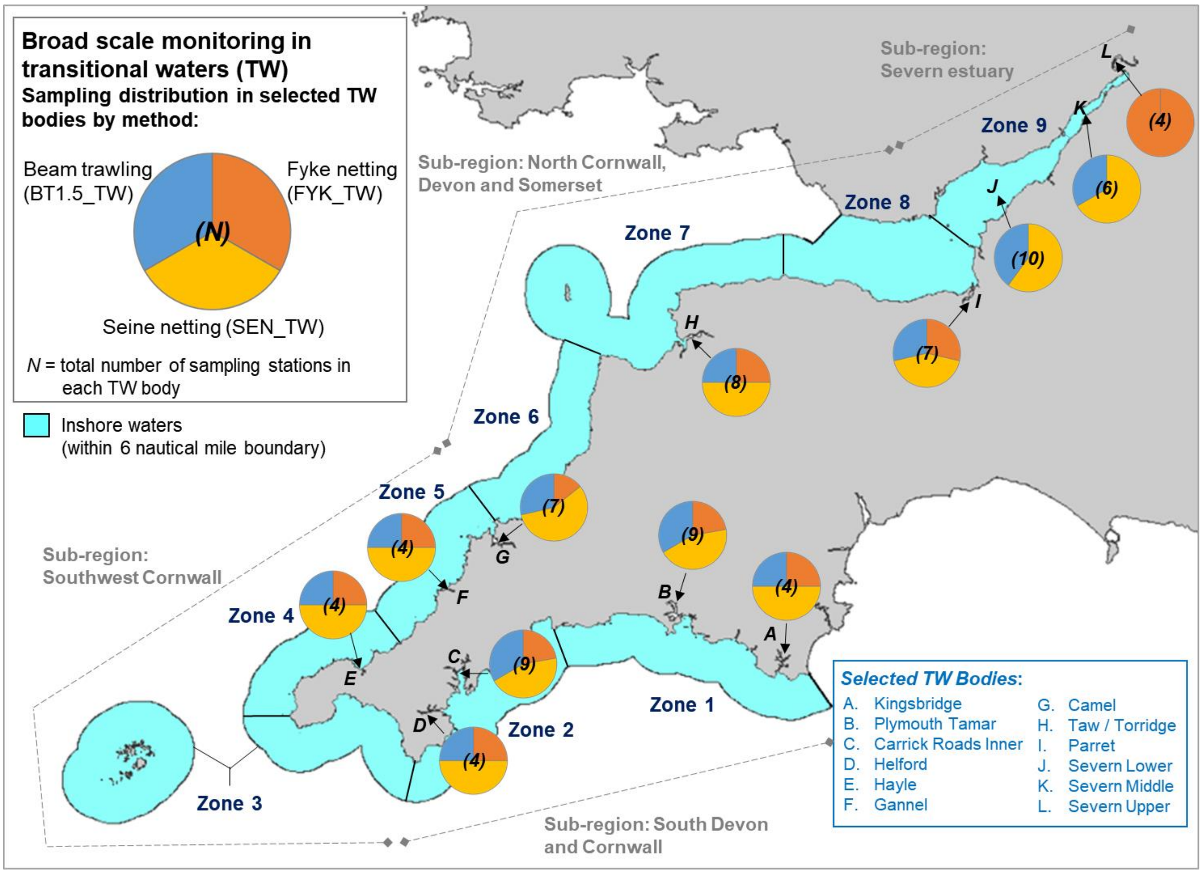

Figure 6. Distribution of the number of sampling stations by method in each of the selected transitional water bodies in the SW region.

3.2.2.1 Selection of TW bodies

Despite the fact that 18 TW bodies exist in the SW of England (within the study region, sub-regions and zones as previously defined; Table 1), only a third of these are currently monitored by the EA for WFD purposes (Table 5). It is unlikely that all the TW bodies in the region can be monitored, mainly due to likely budget limitations. Instead, it is suggested that the selection of the TW bodies for fish monitoring is undertaken based on size and habitat criteria.

The size of an estuary is often correlated with the availability and diversity of habitats in it (Elliott and Hemingway, 2002; Franco *et al*., 2008b). By influencing the diversity and availability of ecological niches for fish to occupy, this may affect the fish assemblage composition, abundance and distribution. For example, Waugh *et al* (2019) and Pasquaud *et al*. (2015) indicate the influence of estuary size on the fish species richness. The habitat availability and diversity in relation to the estuary size also affects the attractiveness of the system to different species or functional groups (Amorim *et al*., 2018).

Table 5. Transitional water bodies present in the SW of England, categorised according to size[6] and location within sub-regions and zones (see Table 1 for definition of these geographical areas of interest). TWs in bold are included in current WFD fish monitoring. TWs underlined represent additional water bodies considered in the monitoring plan provided in this document.

<table>
<tr><th rowspan="2">Region</th><th rowspan="2">Sub-region</th><th rowspan="2">Zone</th><th colspan="3">Estuary size</th></tr>
<tr><th>Small
(<1,000 ha)</th><th>Medium
(1,000 - 10,000 ha)</th><th>Large
(>10,000 ha)</th></tr>
<tr><td rowspan="9">SW</td><td rowspan="2">South Devon & Cornwall</td><td>1</td><td><u>Kingsbridge</u>, Fowey, Yealm, Avon, Erme, Looe</td><td><u>Plymouth Tamar</u></td><td>-</td></tr>
<tr><td>2</td><td><u>Helford</u></td><td>Carrick Roads Inner</td><td>-</td></tr>
<tr><td rowspan="3">SW Cornwall</td><td>3</td><td>-</td><td>-</td><td>-</td></tr>
<tr><td>4</td><td><u>Hayle</u></td><td>-</td><td>-</td></tr>
<tr><td>5</td><td><u>Gannel</u></td><td rowspan="2">Camel</td><td>-</td></tr>
<tr><td rowspan="3">North Cornwall, Devon and Somerset</td><td>6</td><td>-</td><td>-</td></tr>
<tr><td>7</td><td>-</td><td>Taw / Torridge</td><td>-</td></tr>
<tr><td>8</td><td>-</td><td><u>Parret</u></td><td>-</td></tr>
<tr><td>Severn Estuary</td><td>9</td><td>Severn Upper, Bristol Avon</td><td>Severn Middle</td><td>Severn Lower</td></tr>
</table>

The current sampling effort in TWs in the SW of England appears to concentrate on medium sized estuaries, whereas small estuaries that are more frequently occurring in the region are under-represented. In order to have a better representation on inshore fish assemblages occurring in TWs of the region overall, the list of TWs currently monitored by the EA was integrated with additional estuaries for the purpose of this monitoring plan to ensure that at least one water body for each available size category within a zone is sampled (Table 5).

When the selection based on estuary size could not include all waterbodies in a zone, a habitat availability criterion was used for the choice of the waterbody, by favouring estuaries with higher habitat diversity (number of habitats) (Appendix 2).

As a result, a total of **12 TW bodies** are considered in the inshore fish monitoring plan for the SW region, representing waterbodies of different size (5 small, 6 medium, 1 large) and distribution within the region (Table 5, Figure 6).

3.2.2.2 Sampling effort distribution across methods and habitats within an estuary

The design takes into account the use of multiple methods in different habitat strata, as per general broad scale monitoring strategy as outlined in Section 2.1, and to reflect the multi-method approach and requirements of the existing WFD TW fish monitoring. The multi-method approach better represents the diversity of fish assemblages at the estuarine scale (i.e. along the whole estuarine gradient), reflecting different habitats (providing different suitable conditions for use by different species) and different uses of the estuary by fish fauna (e.g. nursery and feeding grounds, migration pathways).

[6] Size data obtained from: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/623572/wfd_water_body_summary_table.xlsx

For this, standardised methods as applied in the WFD fish monitoring programme have been selected, including fyke nets in intertidal habitats (mainly in the upper estuary), seine netting in marginal sedimentary shallow habitats along the estuarine gradient, and beam trawling in shallower open sedimentary habitats (Figure 3). Following discussions with stakeholders, it was agreed not to include otter trawling in the monitoring, due to the poor cost-effectiveness of this method when applied in estuarine environments (i.e. it provides a small contribution to the information on estuarine fish assemblages for a substantial cost). In turn, the sampling effort of the other methods has been intensified and extended to also cover key habitats occurring in transitional waters (e.g. by deploying fyke nets in the vicinity of saltmarsh, seagrass and rocky substrata). A SCUBA diving component has also been added to estuarine monitoring to target fish assemblages associated with sublittoral rocky habitats in the outer estuary.

The spatial distribution of the sampling effort between different methods within an estuary takes into consideration the size of the estuary, and the availability and distribution of different habitats that may influence the composition of the associated fish assemblage and the applicability of different sampling methods.

As a general rule, the number of sites to be sampled in an estuary has been identified as:

- 3 to 4 in small sized TW bodies (<1,000 ha area)
- 4 to 7 in medium sized TW bodies (1,000 -10,000 ha area)
- 7 to 10 in larger sized TW bodies (>10,000ha area)

The distribution of sampling effort between different methods should reflect the range of habitats within the TW body, and their distribution along the estuarine (salinity) and exposure gradients.

Based on the above criteria and the characteristics for the specific TW bodies selected for the SW of England, the total sampling effort in the SW transitional waters includes **76 stations**, including 17 fyke netting stations, 36 seine netting and 22 beam trawling (Figure 6, Appendix 2, Table A2.4). Where possible, two methods (e.g. seine net and beam trawl) should be applied at the same site (EA, 2011b).

3.2.2.3 Sample replication at station level

As for the sampling in coastal waters, a minimum of 3 replicate samples collected per station is preferable to allow any inferential analysis and precision in the estimate of community descriptors. However, EA guidance (EA, 2011b) and the examination of the actual sample replication applied in the current WFD TW fish monitoring (EA, 2019) show that the level of sample replication at the station level is generally reduced to 2 replicates (1 in some cases) per method per station. This is mainly ascribed to logistical reasons as sampling in transitional water sites is most often restricted to low water slack tide conditions, which limits the ability to collect more than two replicates at a site (Adam Waugh, EA, pers. comm.). In addition, spatial limitations (e.g. narrow estuarine channel, small habitat patch) may also influence the ability of collecting more than two replicates at certain estuarine sites. In consideration of these factors, the minimum number or replicate samples to be collected at a site (with a given method) is identified as 2, although 3 replicates should be collected where logistics and safety conditions allow it.

3.2.3 Core monitoring methods, SOPs and survey requirements (TW)

Details on the method specifications, protocols and survey requirements pertaining the different monitoring components for the broad scale (core) monitoring of inshore fish communities in transitional waters are given in this section. Additional information on protocols for fish sample handling and data collection, and required licencing is given in Sections 3.3 and 3.4, respectively.

3.2.3.1 Fyke nets (FYK_TW)

These are to be used on intertidal areas of the estuary, in sheltered conditions, particularly in the upper estuary. The method mainly applies to sedimentary habitats (e.g. estuarine mudflats), but, where possible, they should deployed within or in the vicinity of saltmarshes, in areas adjacent to rocky shores (also including kelp) or in the vicinity of intertidal seagrass beds, to allow integration of the fish catches from these habitats.

Net specifications and deployment should follow EA operational instructions for compliance with WFD, with two double-ended Dutch 'D' type fyke nets (2 x 5.3 m long fyke nets, with a D shaped opening, 100 cm height, 32' leader, 14 mm mesh, fitted with otter guards and tags) being deployed at each site at low water, and for a cumulative period of 24h. Where possible, fyke nets should be deployed in the lower shore, possibly below to the low water mark, to maximise fishing time, and reduce air exposure of the catch. It is standard procedure for the nets to be emptied at a 12h frequency (on low tides) as per EA requirements (EA, 2011b, 2013b). However, a high mortality has been recorded on some occasions (e.g. in the Tamar; Rob Hillman, EA, pers. comm.) Where this may be the case, the fyke nets should be emptied with higher frequency over the 24 h period, particularly where the nets cannot be located at low shore level (e.g. due to safety issues) or where there are reasons to expect high fish mortality (e.g. high abundance of crabs, high water temperatures and low oxygenation). The nets should be secured to the seabed with stakes and anchors (see EA protocol, 2013b for details), and should not be deployed in areas or during periods of strong currents to avoid the net to be washed out. Sampling sites are normally accessed on foot from the shore, but a small boat (e.g. 6 m RIB) can also be used for support where access from shore is difficult or unsafe.

Fish identification and sample data collection is undertaken on site, with the survey team being composed of three surveyors. All surveyors should be preferably trained for fish identification, handling and measuring (including juvenile stages), with a minimum of one surveyors being a senior level and the rest at junior level.

At least one fyke net at each site should be equipped with a data logger to obtain a continuous recording of water pressure (as a minimum) throughout the deployment. This allows calculation of the time the nets have been submerged and fishing within a sampling cycle and therefore standardisation of abundance as catch per unit effort (CPUE; no. individuals per net per 24h fishing time). The use of data loggers also allows the collection of additional supporting environmental data such as water temperature and salinity (or conductivity).

3.2.3.2 Beach seine net (SEN_TW)

This method is to be used on intertidal and shallow subtidal sedimentary substrata in the estuary. The net has the potential to catch both demersal and small shoaling pelagic species using these marginal habitats (e.g. flounder, plaice, gobies, smelt, herring), including both young and adult fish, thus providing information on fish using these nursery habitats.

Gear specification, operational standards and deployment protocol for seine netting are derived follow the EA operational instructions for compliance with WFD (EA, 2011a, 2011b). The beach seine used for this purpose is 43 m long and 4 m deep, with knotless mesh size of 14 mm on the wings and 6.5 mm on the central panel (where the fish are gathered when hauling the net) (EA, 2011a). These specifications are also consistent with those required for seine netting in coastal waters as outlined in Section 3.1.2.1 of this document.

The encircling net is set from the shore in a semicircle (D shape), with the aid of a small vessel, and it is hauled on the shore (where possible) or on the boat for the catch collection. Additional 10 m leader ropes can be used to increase area coverage where the channel is wider than 40 m (EA, 2011a).

Two replicate samples at least (3 where possible) are to be collected per station, with three seine hauls to be undertaken at each site. Overlapping of the swept areas should be avoided. Sampling is to be undertaken in slack tide conditions, with low slack tide being preferable if possible (when tidal migrants are concentrated at lower levels on the shore and the net will also sample those species that do not migrate intertidally) (Wilding *et al*., 2001; EA, 2011b; Benjamin Ciotti, University of Plymouth, pers. comm.)

A RIB can be used for the net deployment, although a hardboat (e.g. aluminium skiff) with a net tray in the bow (from where the net is paid out) is preferable for higher stability (less snags) on deploying and recovering the net (Rob Hillman, EA, pers. comm.)

Survey operations require a team of 4 surveyors to undertake the seine netting at one site. All surveyors should be preferably trained for fish identification, handling and measuring (including juvenile stages), with a minimum of two surveyors being a senior level and the rest at junior level.

3.2.3.3 Beam trawl (BT1.5_TW)

This method is to be used in shallow and intertidal habitats, to target small demersal fish. Gear specification, operational standards and deployment protocol for beam trawling are derived follow the EA operational instructions for compliance with WFD (EA, 2011b, 2013a). A small beam trawl is used for this purpose, with the following specifications: beam 1.5 m wide and 0.45 m high; mesh size 20 mm, 5 mm codend. This gear is towed manually from a small boat (e.g. a 6 m RIB), at low speed (3 knots), with a required tow length of 200 m, although this may be reduced depending on local conditions (EA, 2011b). The beam trawl should be towed against the current, preferably around low water to maximise the catch, since fish tire more quickly swimming against the tide (EA, 2013a).

A minimum of two replicate tows per station is required (3 replicates where possible). Overlapping of the towed areas should be avoided.

In addition to the skipper, survey operations require two surveyors. All surveyors should be preferably trained for fish identification, handling and measuring (including juvenile stages), with a minimum of one surveyor being a senior level and the other at junior level.

3.2.3.4 SCUBA diving (DIV_TW)

This monitoring component has been included to allow the assessment of fish assemblages in shallow water habitats (<30 m depth) in the infralittoral zone of estuarine environments, including open rock substratum, kelp forest, mäerl beds and biogenic reefs (e.g. *Sabellaria*).

It is recognised that this habitat component only represents a small portion of the estuarine habitat (Appendix 2), and that reduced visibility might restrict the applicability of this method in TWs. As such, this technique is most likely to be applied in some medium sized estuaries (e.g. Carrick, Tamar), and particularly in the outer estuary, where environmental conditions are closer to those of coastal waters. In this regard, underwater visual census in the outer estuary (where possible) is seen as an extension of the coastal surveys, with monitoring protocol and costs being integrated as part of that coastal monitoring component (DIV_CW) as specified in Section 3.1.

3.2.4 Cost of core monitoring for the SW of England (TW)

3.2.4.1 Unit survey cost and assumptions

Unit costs (i.e. per day of survey) have been estimated for the different survey components and are provided below. The existing WFD fish monitoring undertaken by the EA in some of the selected TWs has been used to inform on survey requirements and operations for the purpose of this costing.

All of the estimates below cover survey operations from planning to data collection (including sample laboratory analysis, where needed). A mean cost is provided for each component, as well a range of variability taking into account uncertainty of generic unit costs allocation. In particular, the minimum cost assumes the use of a local survey team, at most competitive rates and with minimum travel and subsistence (T&S; e.g. no additional hotel costs), whereas the maximum cost assumes a survey team from outside the region is used, hence with additional costs (e.g. T&S, staff mob/demob) and at higher rates. Downtime, if incurred, would be charged on a pro-rata basis, and therefore costs for this have not been included here.

FYK_TW = £3,400 per day of survey
The estimate above represents a mean unit cost for fyke netting in estuaries, with the possible range of variability being £1,270 to £5,520 per day.
This cost includes survey staff, T&S (mainly consisting of car/van hire, including fuel, and subsistence costs), and equipment hire and consumables. It assumes that separate surveys are undertaken within each estuary, with a minimum of 1 station and a maximum of 2 stations (depending on relative location) being surveyed in one day by a team of 3 surveyors, and the survey sites are accessed on foot from the shore/bank (hence no costs for a boat are included). The cost for laboratory analysis of samples is also included, whereas costs for sample transport to the analysing laboratory (if the analysing laboratory differs from the survey supplier) are excluded. The cost accounts for the fact that two seasonal surveys are required in a year.

SEN_TW + BT1.5_TW = £4,325 per day of survey
The estimate above represents a mean unit cost for seine netting and/or beam trawling in estuaries, with the possible range of variability being £2,020 to £6,630 per day. The costs for seine netting and beam trawling have been combined as a unit as both methods need a small vessel (e.g. a 6 m RIB or similar small hard boat), and the survey operations can be combined in the same day.
This cost includes survey staff, T&S (mainly consisting of car/van hire, including fuel, and subsistence costs), and equipment hire and consumables. It assumes that separate surveys are undertaken within each estuary, with a minimum of 2 stations being surveyed in one day by a team of 4 surveyors. The cost for laboratory analysis of seine samples is also included, whereas costs for sample transport to the analysing laboratory (if the analysing laboratory differs from the survey supplier) are excluded.

3.2.4.2 Subtotal survey cost for TW inshore fish core monitoring

Considering the above unit costs, the sampling effort identified for the selected TW bodies (Appendix 2, Table A2.4) and the minimum sampling frequency required, the total mean cost for the fish monitoring in the 12 selected TW bodies in the SW region is estimated at **£169,785 per year**, with a 57% uncertainty (i.e. ranging £72,380 – 267,190) (see Appendix 2, Table A2.4). This figure includes both field work costs and the costs for the sample analysis, and it is also inclusive of the WFD monitoring component currently covered by the EA. Additional costs for data analysis and reporting are provided separately in Section 3.4.

This total cost is thus divided between different survey components (mean cost in bold):

FYK_TW = £74,640per year
The cost for the fyke netting programme in the 12 selected estuaries of the SW region ranges between £27,940 and £121,330. This assumes a total of 17 sampling stations in the region (min. 0 - max. 4 per estuary) that are samples twice in a year (spring and autumn). It includes both field work costs and the costs for the sample analysis. The variability of the programme cost (63%) accounts for the uncertainty of generic unit costs allocation.

SEN & BT1.5_TW = £95,150 per year
The cost for the seine netting and beam trawling programme in the 12 selected estuaries of the SW region ranges between £44,440 and £145,860. This assumes a total of 41 sampling stations in the region (with 19 of these being sampled with SEN only, 4 with BT1.5 only and 18 with both gear), and includes both field work costs and the costs for the sample analysis. The variability of the programme cost (53%) accounts for the uncertainty of generic unit costs allocation.

The breakdown of the total survey cost by individual estuary is given in Appendix 2 (Table A2.4).

3.3 Licencing requirements (CW & TW)

Appropriate consents are to be obtained from relevant authorities to undertake the monitoring surveys. For example, a permit from IFCA is required to deploy survey equipment for scientific investigation purposes, also considering local byelaws. An MMO marine licence is also required for the deposition/removal of objects from the seabed (such as nets) undertaken from a vessel (e.g. for beam trawling and otter trawling). Where the research activities are likely to affect UK or European protected species (as listed in https://www.gov.uk/government/publications/protected-marine-species), a Marine wildlife licence is required. An authorisation/licence by the EA is also needed to use fishing instruments other than rod and line (S27A authorisation) to take freshwater fish (as relevant particularly for sampling in estuaries).

3.4 Fish sample handling and data requirements (CW & TW)

Where the monitoring is carried out by fish sampling, the net is to be hauled on the shore (seine and push net) or on board (beam trawl and otter trawl), or emptied on site (fyke nets) and the live catch quickly transferred into a container filled with water (taken on the site, and frequently topped up to ensure constant water temperature and oxygenation are maintained) where the fish are held until sample processing is completed. If the sample is being processed on board of the vessel, this can be equipped with floating tanks secured to the sides for holding the catch (Rob Hillman, EA, pers. comm.) Where the sample can be processed on site, fish should be promptly released alive after processing. Care should be taken in collecting the fish from the net to reduce damage and avoid them slipping through the net (e.g. pipefish; Rob Hillman, EA, pers. comm.) Where samples are to be taken for identification and/or measuring in the lab (e.g. juveniles and post-larvae occurring in seine net, push net and beam trawl samples), appropriate methods for humane killing (e.g. overdose of anaesthetic) should be employed according to [Schedule 1 of the Animals (Scientific Procedures) Act 1986](). Subsampling (volumetric method) may be used where large catches are recorded, provided the subsample is representative of the species diversity and size range for the species in the catch (the subsampling criterion (% volume) is to be recorded). Field staff should be trained for fish identification, handling and measuring (including juvenile stages), and the laboratory analysing the samples should have appropriate level of certification to ensure QC in fish identification (e.g. NMBAQC).

The following data should be recorded for each sample (i.e. sample catch from netting and trawling surveys, or observation records from each transect for underwater visual census).

- **Fish data:**
  - Fish should be identified to species level where possible, and the species present in a sample recorded;
  - Species abundance should be recorded as the number of individuals counted for each species in the sample;
  - Individual body size (total length for marine species, fork length for freshwater species[7]) should be estimated to the nearest centimetre for all fish observed along a transect in diving surveys, and it should be measured to the nearest millimetre in up to 50 individuals for each fish species in each sample for the netting and trawling surveys.
- **Sampling data:**
  - Date and time of the sampling and sampler names;
  - Sampling site location, as GPS position at approximately mid-site location (seine netting, fyke netting), at the start and end of the tow (push netting, beam trawling, otter trawling) or of the transect line (diving survey);
  - Method used and replicate number;
  - Sampling unit effort should be recorded for data standardisation, including: haul/tow length (or tow duration and towing speed) to estimate the area swept by the net with each haul for seine netting, push netting, beam trawling and otter trawling; length, width and height of the transect explored by the diver; length of time fished for fyke nets (this is obtained from the water pressure data recorded by the data logger attached to the nets);
  - Tow depth for otter trawling.
- **Supporting data:**
  - Tidal conditions, sea state, prevailing weather conditions;
  - Water depth (m), water temperature (°C) and dissolved oxygen (% saturation);
  - In TW surveys, salinity (PSU);
  - In diving surveys, water visibility (m), and supplementary notes on habitat characteristics and variability along the transect (using natural marks for reference) and, where possible, information on the fish-habitat association (e.g. vertical habitat position of the fish, behaviour etc.) that can provide additional information on how fish use of the habitat.

[7] The latter is relevant for samples collected in estuaries, particularly in the upper reaches

It is recommended that standardised forms are used to record the data collected in the field (e.g. using DCF/FAO specific codes identifying fish species) and that the data are entered into a common shared database. There is variety of record sheets and databases that are currently used in different monitoring contexts and with different purposes, e.g.: the PISCO data sheets to record fish data from underwater visual census; the EA National Fish Populations Database (NFPD) to record fish data from WFD monitoring in transitional waters; the DATRAS online database of trawl surveys hosted by ICES and used for fishery-related assessments; the Marine Recorder database used by nature conservation bodies for marine benthic sample data. Although these tools may partially meet the inshore fish data recording requirements for individual monitoring components, it is recommended that a coordinated, integrated and fit-for-purpose system for recording and storing data from inshore fish is developed to ensure standardisation and QA of data records within and between the monitoring components making up this plan and throughout regions.

3.4.1 Cost

While fish sample handling and data collection is part of the field survey components costed in Section 3.2.4, and additional cost is envisaged for the building and inputing of data in the database from both the coastal and transitional water monitoring in the region.

This is estimated at around **£7,000 (± 20%)**, cumulatively for the TW and CW core monitoring components. Considering that a database (the EA NFPD) already exists to record fish data from WFD monitoring in transitional waters, it is suggested that a concerted effort with the EA is made to adapt that database to accommodate the additional data requirements as outlined above, and to ensure that data are entered consistently (e.g. sampling effort data are not consistently recorded in the current NFPD). Where possible the database structure should be harmonised between CW and TW (e.g. using similar fields and species codes). Considering the above, a split of £2500 and £4500 between TW and CW respectively is anticipated for this cost component.

3.5 Data analysis and reporting (CW & TW)

Qualitative (species presence) and quantitative fish data (species abundance and individual body length) are obtained from each of the core methods included in the broad scale monitoring of inshore fish assemblages. These are used to characterise the inshore fish communities based on univariate and multivariate descriptors:

- The number of species (S) is derived from species presence data as a measure of species richness in the sample community, with rarefaction methods combining data from multiple samples (species-accumulation curve) being used to estimate the total species complement (species richness) for the habitat (S_{max}) (Southwood and Henderson, 2000; Waugh *et al*., 2019);
- The fish species abundance (A) gives an indication of the size of the populations of each species in a community (and cumulatively of all species in the sampled community) and is derived from fish count data and standardised as density or catch per unit effort (CPUE) according to sampling effort; where abundance is standardised over a unit area (as density, e.g. individuals per m^2), sample density data may be extrapolated to estimate the overall species and community abundance in the region, based on the extent of the specific habitat the sample density refers to and under the assumption that fish density is maintained across the habitat in the region;

- Community indices are derived from the species presence and abundance to provide additional information on the distribution of individuals among the species occurring in the community, including indices of dominance (e.g. abundance ratio, A/S, , or biomass ratio (B/A) where biomass has been determined – the latter will indicate the dominance of small and juvenile species), evenness and diversity (e.g. Shannon-Weiner function) (Southwood and Henderson, 2000; Elliott and Hemingway, 2002);
- When the identity of the species is also taken into consideration, in addition to their number and abundance, a multivariate assessment of the fish community composition (based on species presence/absence data only) and structure (also including species abundance information) is undertaken, with similarity coefficients (e.g. Bray-Curtis) being used to identify patterns of variability in the community distribution (Clarke and Warwick, 2001);
- Species body size distribution (from length frequency histograms) provides evidence of the demographic structure of the sampled population, and it can be used to derive biomass estimates where weight-length regressions are available for a species (Elliott and Hemingway, 2002).
- In addition to the taxonomic approach, functional guilds can also be used to characterise the community by grouping species according to the way they use the habitat, spatially (e.g. demersal, pelagic) or temporally (e.g. resident, migrants (nursery), stragglers), and its resources (e.g. feeding guilds including benthivores, piscivores, detritivores, etc.) (Franco *et al*., 2008a; Potter *et al*., 2015).

The species diversity and composition, species abundance, as well as the functional guild composition allow to assess the health of the fish community, whereby a taxonomically and functionally diverse and abundant community is generally indicative of good health, reflecting the availability and use of multiple ecological niches in the habitat.

The identity of the species in the community also contributes to qualify fish community health, and specifically the presence and/or absence of species of particular relevance because of their conservation status and protection under existing international and national legislation, including EU Habitat Directive 92/43/EEC, OSPAR list of threatened species, the IUCN Red List, UK BAP priority species list, Wildlife and Countryside Act 1981, etc. (e.g. salmonids, lampreys, smelt, seahorses, rays; see JNCC Conservation Designations Spreadsheet for a detailed list of fish species designations found in the UK), also due to their sensitivity to disturbance (e.g. changes in hydromorphology or water quality) and/or anthropogenic exploitation (e.g. river and sea lamprey, Allis shad and Twaite shad, salmonids, smelt, eel) (Coates *et al*., 2007). Such an approach is used for example to assess the ecological health of fish communities in TWs for WFD assessment purposes, with the metrics used to calculate the Transitional Fish Classification Index (TFCI) being outlined in Table 6. The community health is assessed by comparison to reference conditions that are defined for each individual metric according to water body type and sampling approach (effort and method) (for details see WFD-UKTAG, 2014). Such methods have been intercalibrated and quality-controlled across areas in Europe (Elliott and Hemingway, 2000; Franco *et al*., 2012; Perez-Domingues *et al*., 2012; Alvarez *et al*., 2013).

Table 6. Metrics that comprise the Transitional Fish Classification Index (TFCI) for the WFD assessment of fish fauna in TWs (from WFD-UKTAG, 2014).

Community characteristic	No.	Metric
Species diversity and composition	1	Species composition
	2	Presence of indicator species
Species abundance	3	Species relative abundance
	4	Number of taxa that make up 90% of the abundance
Nursery function	5	Number of estuarine resident taxa
	6	Number of estuarine-dependent marine taxa
	7	Functional guild composition
Trophic integrity	8	Number of benthic invertebrate feeding taxa
	9	Number of piscivorous taxa
	10	Feeding guild composition

Univariate analysis (e.g. analysis of variance (ANOVA)) and multivariate analysis (e.g. cluster analysis, multidimensional scaling (MDS), principal component analysis (PCA), correspondence analysis (CA)) are applied to the above data to identify spatial patterns of variability in the distribution of the fish community (Clarke and Warwick, 2001; Zuur *et al.*, 2007). Population demographics of key species or groups of interest are also analysed based on length frequency distributions and the derived variables (e.g. mean body size and range, % individuals at juvenile stage, % individuals above minimum landing size for species of commercial interest).

Where the monitoring is repeated over the years, time series can be analysed to also assess possible temporal trends in the fish community characteristics and in the structure of populations of interest. These spatial and temporal patterns can be related to the variability in the habitat characteristics (depth, salinity, etc.) using correlative or regression analysis (e.g. BIOENV, distance-based linear models, general linear models, mixed models; Clarke and Warwick, 2001; Zuur *et al*., 2007), and interpreted in the light of evidence on the presence, distribution and variability of other potential influential factors (e.g. local pressures, effects of management measures). Furthermore, the multivariate analysis of patterns in the fish community data may also allow to identify species that are mostly responsible for the observed changes (e.g. gradient analysis as PCA or CA, SIMPER analysis; Clarke and Warwick, 2001; Zuur *et al*., 2007).

Where the patterns observed in the data suggest such effect may be occurring, further targeted investigations may be planned (see Section 5) with the choice of the most appropriate monitoring method and design being dependent on the specific question being asked (e.g. to detect an impact in a certain area). Where given species may be identified as the main responsible for the community change (e.g. species most sensitive to a pressure), these can be used as proxy species for more targeted monitoring (Section 5). Statistical tools as for example Threshold Indicator Taxa ANalysis (TITAN; Baker and King, 2010) may be used to detect changes in taxa distributions along observed gradients over space or time, and identify indicator species that may be used as proxies for these changes (such an approach has been recently applied by JNCC to identify indicator species/groups for changes in sublittoral rock communities along a gradient of anthropogenic resuspension associated with fishing activities; Franco *et al*., 2018).

The analysis of qualitative and quantitative data (and the associated community descriptors) as outlined above, is relatively straightforward when applied to data obtained from individual methods, hence characterising fish communities associated with a particular habitat in the region, provided there is standardisation of the sampling design and the methods across surveys (Waugh *et al*., 2019). The sample replication within a station, and of stations within a habitat in a zone, sub-region or region also allow community characteristics to be estimated and their variability at these different scales assessed.

The integration of data obtained with the different methods is also possible for both qualitative and quantitative data, although the methods used and the distribution of sampling effort between them need to be taken into account in the analysis.

Qualitative data (species lists) may be combined to obtain an integrative species inventory for the region (or for sub-areas of interest within it, e.g. sub-regions, zones). The resulting inventory would reflect the diversity and availability of the different habitats within an area, but they may also be affected by the variable selectivity of the different methods used towards certain fish components (morphological groups, life stages, demersal/pelagic, etc.) and the effort distribution between them. Where these factors are standardised, e.g. by applying the same sampling approach (methods and effort distribution) in the area over multiple years of monitoring, the species lists can be compared to assess patterns of change in the overall richness and composition of the inshore fish assemblages in the area (e.g. appearance of new species or loss of species from the region due to climate change, species disappearance from an area due to impacts on localised populations, reappearance of species previously lost from the area following successful management of impacting pressures). Similarly, changes in the functional composition of fish assemblages can be assessed, provided the standardisation of sampling approach is maintained. Where spatial comparisons are made (e.g. between different regions, or areas within a region), the differences in sampling methods and effort must be taken into consideration as possible factors affecting the observed differences.

Quantitative data, and particularly species abundance obtained from different methods are dependent on the nature and amount of sampling effort, which is standardised in different ways depending on the method (e.g. as sampling area in seine netting, beam trawling, and diving surveys; number of nets and fishing time in fyke netting) or estimated using proxies (e.g. count of all individuals seen in a given time period from visual surveys). The integration of these data is only possible as cumulative species count data. These would be meaningless in absolute terms, as they would combine fish counts obtained with different methods and on different effort units, but they may be used to assess relative differences in the overall assemblage diversity, relative abundance of species or functional groups, and taxonomical of functional structure over space (e.g. between areas) and time (e.g. between years). However, these spatial or temporal comparisons of the integrated dataset are only possible if the sampling approach is fully standardised across the spatial or temporal units compared (i.e. the compared areas or years must have the same methods used, same number of sampling stations per method (habitat), same replication within station, same effort unit for each replicate, same timing of the surveys), so that the effect of these factors on the fish assemblage structure is controlled for.

3.5.1 Cost

Based on the data and analyses outlined above, a cost of around **£12,000 (±20%)** is anticipated for the data analysis and reporting of both the coastal and transitional water monitoring in the region (£6,000 for CW, £6,000 for TW)..

4. e-DNA monitoring

4.1 e-DNA monitoring strategy

To maximise cost effectiveness, the monitoring strategy for e-DNA assessment is intended to take advantage of the extensive sampling effort undertaken under the broad scale monitoring (as described in Section 3 of this document) for sample collection.

e-DNA is dispersed in the aquatic environment as DNA traces are released by the fish moving in the coastal and transitional water areas in the region, and are also transported by moving masses of water (with tides, currents, etc). As such, the proposed monitoring design for the e-DNA component includes a subset of the sampling stations that are included under the core monitoring (Section 3), as detailed in Table 7.

Sampling stations selected for the e-DNA monitoring should aim at covering the broad-scale habitat variability in the region (e.g. distribution along the estuarine gradient, in coastal habitats with variable substratum and at different depth) to maximise the possible fish diversity recorded by of the sampling. For the same reason, areas acting as a possible sink of particulate material (e.g. managed realignments in estuaries) could be favoured. Seasonal variability should also be considered where the broad-scale monitoring allows it (e.g. fyke net stations in estuaries being monitored in both spring and autumn). This will provide a dataset with substantial spatial coverage, including some temporal variability, particularly if the monitoring is continued over the years.

Table 7. Sampling effort in the SW of England to measure e-DNA for integrating the monitoring of inshore fish communities in the region.

Effort for e-DNA monitoring		**proposed**	**min**	**max**
TW	number of estuaries	12	12	12
	stations/estuary	4	3	6
	Total stations TW	48	36	72
CW	number of zones	8	8	8
	stations/zone	5	4	8
	Total stations CW	40	32	64
TW+CW	**Total stations**	**88**	**68**	**136**

4.2 e-DNA monitoring methods

4.2.1 Sample collection and filtering

Best practice for water sample collection for e-DNA analysis in the marine environment requires manual collection of water samples before any survey operation start and gear are deployed. A water sampling bottle (e.g. Niskin bottle or Ruttner sampler) is used to obtain three replicate water samples at each station (the samples can be collected at different depths along the vertical profile in the water column). The sample is to be emptied in 2 L plastic water bottles. The outside of bottles are to be cleaned with 10% bleach after filling and they are stored upright and kept refrigerated (e.g. in a cool box or a fridge where available) prior to filtration. To avoid contamination, gloves are to be used when handling samples and sample bottles, and all equipment used to sample and store samples for e-DNA analysis are to be sterilised by washing in 10% bleach. The water sampler is also sterilised between sampling locations using 10% bleach followed by 10% microsol (detergent) and rinsed with purified water. Where possible, a clean dedicated area on the boat should be identified where samples are handled and stored.

Water samples will be filtered within 24 hours from collection. Filtration can be undertaken in a laboratory, provided the samples are delivered within the required timeframe. If this is not possible, filtration can be undertaken in the field with the appropriate filtration equipment (Laramie *et al*., 2015). All samples are filtered through a 0.45 micron cellulose nitrate filter using Nalgene filtration units in combination with a vacuum pump. All filtration equipment is sterilized in 10% bleach solution for 10 minutes after each filtration. Filtration blanks are run before the first filtration and then approximately after each sixth sample, in order to test for possible contamination at the filtration stage. After filtration the samples is frozen immediately at -20°C and can be stored for at least 4 months before molecular analysis (Li *et al*., 2018).

In alternative to the manual collection of replicate water samples for e-DNA analysis, a large volume e-DNA sampler may be used. Such a technique has been recently piloted in the SW of England for the monitoring of inshore fish communities (Mynott S., 2020. Natural England Commissioned Reports, NECR287. This consists of a sampler that is deployed on the seabed, thus remotely collecting bottom water where a higher concentration of e-DNA is likely to be present compared to the water column (e.g. due to precipitation; Sebastian Mynott, Applied Genomic, pers. comm.) The water is filtered *in situ* by the sampler (a 1µm filter is used for coastal waters, but a 4µm pre-filter can be fitted in areas with a high concentration organic matter, e.g. in estuaries, to avoid filter clogging and reduction in filtration efficiency). In addition, the sampler is battery operated, self-contained, and programmable, and it allows the filtration of large volumes of water over time, thus likely increasing the chances of obtaining more diverse e-DNA in the sample (it has been estimated that around 50L of water are filtered in a day in open coastal areas, with; Sebastian Mynott, Applied Genomic, pers. comm.) The filter is encapsulated (thus reducing issues of contamination on handling the sample) and on retrieval it is treated with a non-toxic DNA preservative for storage and subsequent analysis. Compared with the manual sampling and filtration of the water, this technique has the advantage of allowing the collection of e-DNA samples from a larger volume of water (increasing with the duration of the deployment, although it is noted that the half-life time for e-DNA in coastal inshore waters being estimated as of 21.5 hours; Sebastian Mynott, Applied Genomic, pers. comm.) In addition, issues that may arise on maintaining a clean working area (to avoid sample contamination) on a boat and by staff also undertaking other survey operations are reduced. As the two methods may entail significantly different costs (section below) and benefits (as mentioned above), both methods have been costed in Section 4.3).

4.2.2 Molecular sample analysis

The molecular sample analysis includes e-DNA extraction, amplification, high throughput sequencing, bioinformatics and data analysis. Best practice (e.g. as applied at the University of Hull) includes e-DNA extraction being carried out using the method of Sellers *et al*. (2018). Library preparation is carried out using a two-step PCR amplification approach as described in Li *et al*. (2018) using the mitochondrial 12S primers described in Miya *et al*. (2015). Bioinformatics analysis is carried out using the pipeline metaBEAT as described in Hänfling *et al*. (2016).

It is proposed that the molecular sample analysis of all samples collected in the region is undertaken centrally by a single laboratory, thus ensuring consistency and standardisation of the analysis and QA/QC procedures. The centralisation of the sample analysis also increases cost-effectiveness, considering that sequencing is normally undertaken on sample batches (e.g. a 200 sample batch per flow cell is currently being used at the University of Hull for lake fish monitoring; Bernd Hänfling, University of Hull, pers. comm.), and therefore undertaking the analysis of samples of (or close to) the maximum batch size allows to optimise sequencing costs.

4.3 Data analysis and reporting

Currently, the e-DNA approach only allows to obtain qualitative data on the species occurring in an area (species presence), and studies have shown that it is an effective approach for producing an inventory of fish in estuarine and marine systems (Thomsen *et al*., 2012; Myia *et al*., 2015; Evans *et al*., 2016). The species identification relies on the barcode matching of the e-DNA samples with an existing reference database (e.g. GenBank), which for most regional fish faunas is almost complete (a number of ongoing projects focus on completing the remaining gaps; Franco *et al*., 2020a), thus maximising detection ability of the species occurring in an area. Where compared with traditional sampling methods (including netting), e-DNA has already been recognized as a more sensitive method for fish detection (i.e. providing more comprehensive species lists) (e.g. Hänfling *et al*., 2016). In turn, the e-DNA approach cannot provide quantitative data on inshore fish populations and assemblages (e.g. abundance, size/age structure) as yet, although it cannot be excluded that further development of DNA-based techniques could allow quantitative information to be obtained in the future (Franco *et al*., 2020a).

The species presence data obtained from e-DNA monitoring allow to determine species richness (S) and the assemblage composition of the fish fauna in the region, with possible univariate (e.g. ANOVA) or multivariate analysis (e.g. cluster analysis, MDS, PCA) being applied to identify spatial patterns of variability or temporal trends (where monitoring is repeated over the years, with standardised methodology and design) in the distribution of the fish community (Clarke and Warwick, 2001; Zuur *et al*., 2007). However, the spatial resolution at which the distribution of species occurrence within the region may be assessed depends on the ability to identify the spatial scale to which e-DNA samples refer. This takes into consideration that DNA particles may be transported away from the source, and therefore their detection at a given site and the ability to discriminate between different sites will depend on the site distribution in relation to local transport patterns (e.g. prevailing current direction), while also taking into consideration the rate of DNA degradation (Franco *et al*., 2020a).

Given the higher sensitivity of the e-DNA approach to detect fish species compared to traditional sampling methods (Franco *et al*., 2020a), this technique is likely to provide a more comprehensive assessment of the fish diversity in the regional inshore waters, albeit only as at the qualitative level. As such these data can be used to integrate the species inventories obtained from the broad scale (core) monitoring, with the analysis of spatial patterns and temporal in changes in the integrated species inventory being undertaken in a similar fashion as described in Section 3.5 (for qualitative data).

The e-DNA monitoring results can also allow to identify species or groups that are poorly represented in the data obtained with the core methods. This cross-validation may be used to identify gaps and limitations in the ability of the core methods to fully reflect the inshore fish community as a whole, thus guiding further development and improvement of the monitoring strategy and design in the future to address these gaps (e.g. by intensifying sampling effort of core methods in areas or habitats in the region where species gaps are more substantial, or by integrating the monitoring with additional alternative methods that may provide more targeted data on specific components; see Section 5).

4.4 Cost of e-DNA monitoring

It is estimated that the e-DNA analysis of water samples collected in the SW region following the design in Table 7 can be undertaken at a total cost of around:

- **£19,000 per year** with manual sampling and filtering with a 15% uncertainty to account sampling variability between minimum and maximum effort;
- **£31,000 per year** with remote sampling and filtering with a 40% uncertainty to account sampling variability between minimum and maximum effort.

In both cases, these estimates assume that the sample collection in the field (manually or by deploying the water sampler) is done by the survey teams (manually or by deploying the water sampler) undertaking the broad scale monitoring at no additional costs (hence with saving in terms of field survey staff and vessel).

Where manual sampling and filtering is undertaken, the cost also includes the required filtration equipment and consumables, with four vacuum filtration kits included to cover possible sample collection by multiple teams across the region, with filtration being undertaken in the field.

The costs above also assume that the analysis of the filtered samples is undertaken at a centralised laboratory, and therefore they include equipment use and consumables for the molecular sample analysis (sequencing), and the associated labour (laboratory work, bioinformatics and data analysis). Costs for sample transport to the analysing laboratory have not been included.

An additional cost of **£3,300** (± 15%) should be included for the analysis and report writing of the e-DNA data collected for the region over an annual cycle.

5. Additional targeted/integrative monitoring

5.1 Additional reactive or strategic monitoring

Specific additional (reactive or strategic) targeted monitoring needs are likely to be identified as conditions change (e.g. new species colonising the region with climate change) or as different questions arise (e.g. where concerns arise in relation to the possible impact of an activity or a pressure, or to show the results and efficacy of a management action). There are ten types of monitoring (Elliott, 2011) each with their own approaches, methods and means of analysing the data produced and each designed to answer different questions. The broad scale monitoring plan proposed here cannot answer all of these specific questions, but it has the potential to be integrated with additional *ad-hoc* monitoring following an adaptive cluster monitoring approach. According to this approach, the existing sampling effort is increased adaptively in areas of higher interest (Thompson, 2012). These areas may be identified based on the results of the initial broad scale monitoring (e.g. data indicating the presence of an area of extremely high or low diversity), or based on other factors, as for example the identification of an area of concern, due to high intensity pressure. As a result, increasing the sampling effort in and around the area of interest, and/or in time (e.g. increased frequency) may be added to the existing monitoring plan. The specifics of the adapted design and strategy will depend on the particular conditions to be assessed and the associated testing of hypotheses (also considering spatial and temporal scale, footprint, etc.) and therefore will have to be defined on an *ad-hoc* basis. As mentioned above, the amount of sampling will also relate to the degree of change expected to be detected. Therefore costs for these monitoring components cannot be identified in advance.

Increasing the sampling effort can be in the number of stations and samples collected in the area or over time using the monitoring techniques applied in the core (broad scale) monitoring outlined in this document. This will allow the collection of additional qualitative and quantitative data, as and where needed, to which the broad scale monitoring can provide context for spatial and temporal comparisons. At the same time, these additional data will integrate the existing broad scale monitoring providing further information for the characterisation of the inshore fish communities in the region.

However, the sampling effort may also be intensified with the addition of specific targeted monitoring programmes employing different monitoring techniques that are better suited to answering a specific question. For example, tagging studies may be undertaken where the interest is on the migration movements of a certain species in an area, or more localised surveys (e.g. baited remote underwater video) may be used to assess the local effect on the distribution and behaviour of fish (e.g. Griffin *et al*., 2016; Roberts *et al*., 2016). Feeding, stomach contents and stable isotope studies will be needed to determine whether quality objectives related to predators and prey are met.

Citizen science programmes, including the records of recreational fishing clubs, may also provide valuable knowledge on inshore fish communities in the region. Although this type of monitoring cannot constitute core sampling, it has the advantage of potentially extending the monitoring over spatial/ temporal scales, making good use of participative voluntary work. This type of integration may rely on small scale, specific programmes (e.g. kick sampling in areas of estuaries, as done in the Thames, making use of Lottery and Thames Partnership Funding; Steve Colclough, IFM, pers. comm.), or on larger coordinated survey programmes (similarly to Seasearch diving surveys, but targeting fish). A key factor for these programmes to be successful in integrating the core regional monitoring proposed in this plan is the use of clear and standardised protocols (to be developed for fish diving surveys) and appropriate training.

5.2 Existing monitoring

Targeted monitoring already existing (or planned) in the region can also contribute to the integration of the core monitoring plan by providing a more diverse assessment of specific components of the inshore fish assemblage in the area. Discussions with stakeholders have been initiated during this project to collate information about these existing monitoring initiatives in the SW of England. Further collation and integration will be required in due course should this pilot be taken forward. For example a large tagging project aimed at monitoring movement patterns of sea bass within and in close proximity of three estuaries is being undertaken at Plymouth University and Devon and Severn IFCA (Thomas Stamp, Plymouth University, pers. comm.).

Of particular note is also the commercial fish potting activity in the SW. This fishery uses specially designed lightweight baited pots and targets five species of wrasse for live collection, to be used as cleaner fish to control sea lice populations in the salmon farming industry (e.g. in Scotland). This sector is rapidly expanding in the SW of England to meet demand, and it could provide some integrative information on inshore fish populations associated with rocky habitats, albeit this is limited specifically to wrasse species. The wrasse potting fishery is currently managed via the Devon & Severn (D&S) IFCA potting permit byelaw (introduced in 2017), and a fully documented fishery has been implemented by D&S IFCA (in 2017 and continued in 2018), with two primary sources of data collected: i) landings data, recorded by the fishers and ii) on-board observer surveys, undertaken by IFCA Environment Officers (Table 8).

Due to the nature of this fishery (fishers in this sector often work single-handed and need to keep fish handling and processing time to a minimum), the fishers recording requirements are kept to a minimum to avoid disruption (D&S IFCA, 2018; Libby West, D&S IFCA, pers. comm.) These are limited to wrasse landings (total numbers of wrasse retained per day), with only ballan and cuckoo wrasse being identified, while the other wrasse species are grouped. Additional information on the fishery (type and quantity of fishing gear employed and the areas fished) is also recorded in fisher logbooks, but with no requirement to link this to the catches (e.g. fishers are not required to report how many fish were caught in each area (grid cell) fished) (D&S IFCA, 2018). More detailed qualitative and quantitative data are collected by on-board observer surveys (Table 8), with the identification of all wrasse species, the quantitative assessment of the catch (as CPUE) and additional characterisation (sex, individual body size, and spawning status). Although these surveys may provide data on wrasse population structure and size that may integrate the monitoring information (at least for the areas where the fishery occurs), no records are taken on by-catch species or abundance (due to time restrictions for collecting the data during on-board observations; D&S IFCA, 2018; Libby West, D&S IFCA, pers. comm.), and therefore the contribution of these data to integrated fish species inventories or community-wide characterisation is very limited (to wrasse species only). It is noted that by-catch was qualitatively recorded in very early on-board observation surveys in 2017, and this showed a low by-catch of species, due to the selectivity of the pots, and with rockling, sea scorpions, and blennies being most common, and other species including conger, gobies, weever fish, and juvenile gadoids (D&S IFCA, 2017).

Table 8. Difference between landing data and on-board observer surveys documenting wrasse potting fishery in the SW of England (from: Devon & Severn IFCA, 2018).

	Landings data (from fishers)	**On-board observer surveys**
Data from every day of fishing effort	✓	
Fishing effort (no. pots per day) recorded	✓	✓
Daily total number of fish caught recorded	✓	✓
Daily total number of fish returned recorded		✓
Total number of fish caught per string		✓
Spatial LPUE/CPUE		✓
Species-level data recording		✓
Sizes of fish (kept and returned) recorded		✓
Spawning state of fish recorded		✓
Approximate location of fishing effort recorded (1 km^2 grid)	✓	✓
Precise location of fishing effort recorded		✓

The marine recreational fishery including all forms of non-commercial fishing from shore and boats may also be a source of integrative information on the fish species occurring in the region. Estimates for this sector give the number of sea anglers in England at around 900,000 and almost 4 million days of sea angling recorded over 2012 (Sea Angling 2012 project; Armstrong *et al*., 2013). There are obligations under the EU Data Collection Framework (DCF) and the EU Council Regulation 1224/2009 to report on recreational catches of certain species (e.g. seabass and cod), and to meet these requirements projects have been undertaken by Cefas in the past years to collect catch data from recreational sea anglers (e.g. Sea Angling 2012 and 2017 projects, the latter also providing a bespoke online catch diary tool for sea anglers to record fishing trips, catches and expenditure). Data collected from these surveys mostly regard the species as outlined in Figure 7. Most of these species are likely to be captured already by the broad scale (core) monitoring in the region, hence the ability of recreational fishery data to integrate the species inventory obtained for the region is likely to be limited. Furthermore, the use of recreational fishery data at the regional or smaller spatial scales needs to be evaluated on a case-specific basis depending on the data availability and on the distribution of the anglers contributing to these surveys; for example, Armstrong *et al*. (2013) reported that the data collected in 2012 could support estimates of catch for England as a whole, but were not sufficient for estimates at smaller spatial scales.

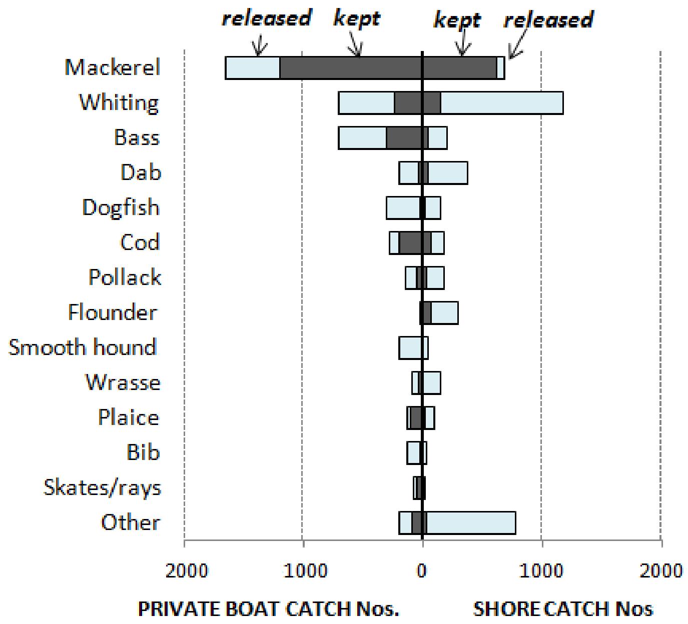

Figure 7. Annual number of fish (as thousands fish) caught from private and rented boats and from the shore in England in 2012. From Armstrong *et al*. (2013).

5.3 Data analysis

The choice of the analysis of the qualitative (species presence, assemblage richness and composition) or quantitative data (fish species abundance, fish assemblage structure, body size, etc.) obtained from individual targeted monitoring depends on the specific monitoring aim, questions and design. For example, a distance gradient analysis may be used to detect near field and far field effects on the fish community characteristics along a spatial distance gradient from a point source (Ellis and Schneider, 1997) or TITAN may be used to identify threshold values along a selected gradient (e.g. a pressure gradient) where community changes occur (Baker and King, 2010). A Before-After-Control-Impact (BACI) and 'beyond BACI' analysis may be used to detect the presence of an impact on the fish assemblage characteristics (Underwood, 1994).

These targeted/integrative elements of fish monitoring/recording in SW inshore waters also contribute to increase the overall understanding of the inshore fish communities in the region, although, given the difference in methods and approaches, and therefore the limitations in direct data comparability, they are likely to only provide a qualitative integration of the broad scale characterisation described in Sections 3 and 4 (i.e. integration of species inventory for the region, or at smaller spatial scale where the data allow it). As described in Section 3.5, the integrated species inventory for the region (or for sub-areas of interest within it, e.g. sub-regions, zones) may be analysed to detect patterns of change in the overall richness and taxonomic or functional composition of the inshore fish assemblages in the area. This is provided that factors associated with the sampling approach (methods and effort distribution) are controlled for as these may affect the results of the analysis (see Section 3.5 for details).

As the data analysis required for the specific targeted monitoring and for the consequent data and analytical integration into the regional inshore fish community characterisation depend on *ad-hoc* choices of the additional monitoring to be undertaken, costs for this cannot be identified in advance.

6. Discussion

6.1 Strengths and weaknesses

Most governance measures related to environmental quality and the detection of change due to anthropogenic pressures rely on the ability to determine the status of a biological element affected by natural features or by the pressures and the changes in that element in time and space. It requires those changes to be assessed in the context on environmental variables and the methods and data are required to be quality controlled, robust and defendable. This approach is to be followed whether the assessment is for condition monitoring (e.g. for natural conservation status or WFD or MSFD implementation), for compliance monitoring (where a developer is required to test against licence conditions), for operational monitoring (e.g. for Environmental Impact Assessment), or investigative or diagnostic monitoring (where applied research questions require new information) (Elliott, 2011).

The multimethod approach proposed in this monitoring plan and the associated stratification by habitat ensures that most fish species and groups likely to occur in inshore waters are assessed both qualitatively and quantitatively. Individual methodologies may have some limitations in capturing the full species complement of a habitat (e.g. diving is likely to under-detect smaller species/individuals (e.g. juveniles), cryptic species and larger shy predators due to avoidance behaviour), and rare species may also be under-assessed. However, the addition of e-DNA monitoring and possibly additional targeted monitoring may partly mitigate this weakness, albeit providing qualitative integration of the overall data.

It is notable that most of the methods described here relate to producing structural data, i.e. the nature of the fish community at one place or time, and so these require to be supplemented with further analyses to give ecological functioning data, i.e. that information relating to rate processes. While fish species identities can be taken further to link to the ecological guilds present, this requires the basic information on the breeding, feeding, life-cycle and habitat preferences of the species. Furthermore, the information generated by sampling of the fishes requires environmental data to put it into context, for example, this includes physico-chemical information (temperature, salinity, dissolved oxygen and turbidity), hydrographic patterns (current speed, erosion-deposition patterns, tidal regimes, density related currents, etc.) and habitat and physiographic structure (e.g. presence of seagrass beds, substratum type, impediments to migration, etc.).

As shown here, all the methods are selective to some extent, hence the need for a suite of methods. Pelagic species are less well covered by the core monitoring component compared to demersal species. Small pelagic fish (as small species or juveniles) are adequately sampled by seine netting when undertaken on near shore shallow open beach habitat (Southwood and Henderson, 2000) and midwater otter trawling in deeper waters also assess pelagic species (most likely as adults). All the other core methods target mostly benthic and demersal fish. This reflects a higher interest in these components (as also agreed with stakeholders), to reflect the fact that the main resources (e.g. as habitat or food) provided by inshore habitats in support of diverse and productive fish communities are mainly associated benthic/detrital compartment (Day *et al*. 1981). Similarly, most anthropogenic stressors affect the bed either by changing the ecohydrology (e.g. see Wolanski and Elliott, 2015) or where the bed receives polluting materials. Furthermore, while the pelagic species are more likely to move with the water mass and even be more ephemeral, demersal and benthic species may be more sedentary and so reflect the prevailing conditions in an area. Of the dominant food chains in estuaries, more are related to benthic prey and bed detritus (Elliott and Hemingway, 2002). In addition, pelagic species are better covered in offshore monitoring (e.g. PELTIC survey programme in the western Channel and Celtic Sea). Should the interest on pelagic fish in inshore habitats in the region increase in the future, additional targeted monitoring (e.g. by increasing the effort for pelagic trawling in both deeper and shallower areas) may be included in the plan.

The more intensive effort to represent shallow inshore habitats in the monitoring, and the use of relative finer meshed nets (e.g. seine, push net, coastal beam trawl) ensures that the key nursery function of inshore shallow habitat is represented, by allowing the capturing of earlier life stages (mainly juveniles). However, any assessment of the fish community in an area is required to consider that the influences on the ecological element studied may be outside the area being studied. For example, the presence of juveniles in an area may depend on breeding patterns away from the area where they were taken, the water currents required to deliver the juveniles into an area and the predator populations in the source area or receiving area. This is especially the case with diadromous species whose nearshore or estuarine populations will depend on factors in the breeding areas (in the catchment for anadromous species or at sea for catadromous species) and in the growing areas (at sea or in the catchment, respectively).

The seasonality of the surveys also ensures that inshore fish communities are sampled when their diversity and abundance is likely to be at their peak, during periods of high productivity (late summer – autumn), when inshore habitats are used by fish at different stages reflecting the multiple roles (as nursery, feeding grounds, etc) that these habitats have in supporting fish populations. However, this may give an elevated impression of the carrying capacity of an area, in sampling the peak populations. The representation of the multiple functioning of inshore habitats for different species is also captured by increasing the sampling frequency for fyke netting to spring and autumn monitoring, to capture the variable timing of use of transitional waters as pathways of migration for different diadromous species. This also allows to compensate for limitations identified in the current TW monitoring and hence to give a more accurate indication of the carrying capacity of an area. Furthermore, repeated sampling in an area will allow the population dynamics (through size-frequency data), recruitment patterns, and growth and mortality rates to be determined. If biomass per species is measured and/or length-weight regressions computed then the production (yield) and productivity (production:biomass ratios) can be determined.

There are limitations in the integration of the qualitative and quantitative data from different monitoring methods, due to different sampling units, strategies and effort distribution, as described in Sections 3.5, 4.3 and 5.3. However, such integration is not impossible, and the analysis of the integrated dataset may allow to detect spatial or temporal trends in the regional inshore fish assemblages (their health, distribution, etc), provided the variability in monitoring methods and effort is taken into consideration and controlled for in the analysis.

Once the samples have been obtained by field sampling then the quality of the biological data also depends on the sample treatment (in the field or in the laboratory). Taxonomic accuracy is determined by good laboratory practice and field/laboratory staff training. This involves having skilled taxonomists, up to date literature, standardised methods and checking against reference or voucher specimens. Intercalibration exercises can ensure the accuracy of field and laboratory methods and are especially important for the application of assessment schemes across borders and where data from different laboratories or field teams are combined and collated. For example, the determination of community structure and ecological quality status, e.g. for the Water Framework Directive, in one area carried out by one field team or laboratory, has to be consistent with those data and assessments from other areas, laboratories and dates.

6.2 Recommendations for future development

The recommendations below arise from the identification of areas of uncertainty in the specific methods and protocols described in this report, following discussions with the stakeholders:

- Development of diving survey protocol for underwater visual census of fish on rocky habitat (also including kelp and other structured habitats such as mäerl and biogenic reefs): discussion with stakeholders highlighted that there is no standardised operation protocol for this monitoring component to be applied in European (North Atlantic) habitats, and that existing protocols from other regions need to be adapted and trialled before being validated. There is an increasing trend for biological field methods to be included in the ISO/CEN framework such that methods used for the implementation of EU Directives are to the CEN Standard; as such these are listed in the Annexes for the Directives. Similarly, while AQC/QA has long been required for physico-chemical analyses and benthic invertebrate analyses (such as through the UK NMBAQC Scheme), it is recommended that a dedicated working group is formed to take this forward and develop the standardised methodology to be used in this plan. As such this survey module will need to be revised (also including costs) once the method has been defined and accepted.
- Estuarine surveys: the rationale behind the multi-method approach devised for fish in TWs is now well-established and designed to ensure that the sampling methodology (and the combination of methods) represents the specific and local conditions of habitat variability/heterogeneity in TWs (WFD-UKTAG, 2014). Although this ensures that the fish assemblage in the studied water body is effectively represented by the samples collected, the variability in the methods (e.g. seine or fyke nets of different size, design, different protocols for net deployment) poses significant limitations in terms of standardisation and data comparability. To ensure the latter, the current WFD fish monitoring programme for TW has concentrated on a set of standardised methods. Similarly, this pilot monitoring plan for TW fish in the SW has been based on methods that are consistent with the existing WFD fish monitoring to ensure a required degree of standardisation, hence data comparability and cost-effectiveness (in the use of existing data and possibility of integration with existing monitoring programmes). However, it has been highlighted how in certain conditions (e.g. small estuaries) some of these methods (e.g. large seine net) are difficult to apply (Steve Colclough, IFM, pers. comm.) There is a variety of alternative valid methods that have been applied on similar cases (e.g. kick sampling, smaller fyke nets or block netting in saltmarsh creeks), depending on the characteristics of the specific habitat being sampled and the local conditions. At this stage it is difficult to identify a set of approaches that satisfy both the requirement for standardisation and generalisation, and for effectiveness in sampling the fish assemblage under specific local conditions. It is recommended that further discussion addressing this aspect are undertaken, to possibly identify a set of alternative methods that can be applied in a standardised and coherent way to integrate the proposed core monitoring (e.g. as additional integrative monitoring using specifically designed methods where the application of core methodologies as proposed here is difficult).
- Staff training: the training of staff undertaking the monitoring and their understanding of the criteria/rationale for the surveys are key elements of standardisation and quality assurance of the programme. Therefore, a system of support and training that is

consistent over time (e.g. with changes in survey teams) will be needed. Furthermore, the exchange of field staff between areas will increase both the training and the comparability of analyses.

7. Potential partnerships

During the project, discussions were undertaken with stakeholders. Partners that could possibly contribute to the delivery of the monitoring plan (by supplying expertise, equipment, staff support, delivering monitoring components, etc.) have been identified, as outlined below. In some cases (e.g. provision of staff, equipment from government agencies, integration of the monitoring into existing plans) this may also lead to cost savings.

It is emphasised that the list of potential partners given below is influenced by the limited scope of the investigations undertaken in the time available to this project, and it is not intended to be in any way exhaustive.

Natural England

- Marine Monitoring generic advice – survey design, interaction with protected sites, features and species etc. Also to co-ordinate with and utilise the experience of NE survey staff in field and analytical elements. (contact Mike Young)
- Advice on inshore fisheries (contact Duncan Vaughan)
- Advice relating to diving surveys (contact Trudy Russell)

Environment Agency (contacts: Rob Hillman (regional), Adam Waugh (national)):

- Provision of large boats (CSV) to support otter trawling surveys in coastal waters, as well as local support for monitoring implementation and consents in TWs
- There may be some flexibility to already incorporate additional effort (e.g. fyke netting) in autumn fish surveys in estuaries that are being currently monitored by the EA (Carrick Roads, Camel, Dart), provided this incurred a small amount of additional effort.
- Through knowledge and monitoring experience of estuaries in the region (e.g. Tamar, Hayle, Gannel), the EA may help with the selection of potential sampling locations.

University of Plymouth (contacts: Benjamin Ciotti and Keiron Fraser):

- Fleet of coastal research vessels, equipment for fish sampling using multiple gears and a state of the art scuba facility which is equipped and staffed for scientific diving operations.
- SEN_CW/TW - capacity, experience and equipment to undertake the seine and push net sampling for the regional monitoring plan (they are currently undertaking Southwest Inshore Fish Surveys (in their third year) using these methods; see Appendix 3).
- BT2_CW, BT1.5_TW and FYK_TW: also ability to undertake shallow water beam trawling and fyke netting.
- DIV_CW: state of the art scuba facility which is equipped and staffed for scientific diving operations that has the capability conduct the diving surveys.
- Experienced staff who would be able to contribute to discussions on diving protocol development.
- Knowledge and monitoring experience of coastal habitats in the SW (sandy beaches, rocky habitats) (Appendix 3), with help with the selection of potential sampling locations.

Marine Biological Association (MBA) UK (contact: Rachel Brittain)

- Research vessels, including:
  - RV MBA Sepia: a custom built 15.45 m category 2 Maritime and Coastguard Agency (MCA) coded workboat, capable of operating from shallow river and estuarine environments, up to 60 miles offshore. It has the capacity of undertaking trawling surveys, and to deploy and recover divers.
  - RV MBA Laminaria: a 5.8 m rigid inflatable boat (RIB) coded by the Maritime and Coastguard Agency (MCA) for use in Category 6 waters (estuarine and inshore waters, up to 3 miles from safe heaven. The vessel is powered by a single 115 hp Suzuki engine allowing rapid travel to and from survey sites and its deep 'V' hull design provides stability as well as manoeuvrability. It can accommodate max. 6 persons on board (incl. 2 crew) It has the capacity to undertake seine netting and beam trawling in estuaries.
- Gear for trawling surveys (BT2_CW, BT1.5_TW, OTT_CW).
- Experienced staff who would be able to contribute to discussions on diving protocol development.
- Knowledge and monitoring experience of coastal and estuarine habitats in the SW (e.g. off the coast of Plymouth, within Plymouth Sound and estuaries) (Appendix 3), with help with the selection of potential sampling locations.

Applied Genomics Ltd (contact: Sebastian Mynott)

- Capacity, experience and equipment to undertake the e-DNA monitoring for the regional monitoring plan (using large volume e-DNA sampler)

University of Hull (contact: Anita Franco (IECS), Bernd Hänfling (eDNA), Magnus Johnson (diving))

- Highly qualified IECS laboratory for taxonomical analysis (NMBAQC, with joint 1st place and full marks received for fish ring test, and 1st place on marine invertebrate ring test achieved in 2018) can provide sample analysis from any of the fish surveys, and training for fish ID (training given to EA staff in the identification of estuarine and marine fish, including juvenile stages)
- SEN_CW/TW, BT2_CW, BT1.5_TW, FYK_TW: Capacity, experience and equipment of IECS staff to undertake fish monitoring in estuarine and coastal marine waters (experience of similar surveys from around the UK)
- DIV_CW: Experienced staff who would be able to contribute to discussions on diving protocol development.
- Capacity, experience and equipment to undertake centralised e-DNA metabarcoding analysis of samples (with used of e-DNA metabarcoding approach developed by UHULL and currently being used by monitoring programmes of the DEFRA agencies)

IFM, Colclough & Coates Aquatic Consultants:

- Expertise and experience to contribute to multi-method approach, particularly in estuaries.
- Capacity, expertise and experience to provide staff training on juvenile fish ID skills
- Contribution to citizen science programmes (together with ZSL and some wildlife trusts)

References

Alvarez, M., Franco, A., Pérez-Domínguez, R. and Elliott, M. (2013). Sensitivity analysis to explore responsiveness and dynamic range of multi-metric fish-based indices for assessing the ecological status of estuaries and lagoons. Hydrobiologia, 704(1), 347-362.

Amorim E., Ramos S., Elliott M., Franco A. and Bordalo A.A. (2017). Habitat loss and gain: influence on habitat attractiveness for estuarine fish communities. Estuarine Coastal and Shelf Science, 197, 244-257.

Andrew, N.L. and Mapstone, B.D. (1987). Sampling and the description of spatial pattern in marine ecology. Oceanography and Marine Biology, 25, 39-90.

Armstrong, M., Brown, A., Hargreaves, J., Hyder, K., Pilgrim-Morrison, S., Munday, M., Proctor, S., Roberts, A. and Williamson, K. (2016). Sea Angling 2012 – a survey of recreational sea angling activity and economic value in England. Defra contract MF1221 and the Marine Management Organisation. November 2013.http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&Completed=2&ProjectID=17675

Axenrot, T. and Hansson, S. (2004). Seasonal dynamics in pelagic fish abundance in a Baltic Sea coastal area. Estuarine, Coastal and Shelf Science, 60, 541-547.

Baker, M. E. and King, R. S. (2010). A new method for detecting and interpreting biodiversity and ecological community thresholds. Methods in Ecology and Evolution, 1, 25-37.

Beck, M. W., Heck, J. K. L., Able, K., Childers, D., Eggleston, D., Gillanders, B. M.,Weinstein, M. P. (2003). The Role of Nearshore Ecosystems as Fish and Shellfish Nurseries. Issues in Ecology, 11, 1-12.

Blaber, S.J.M. and Blaber, T.G. (1980). Factors affecting the distribution of juvenile estuarine and inshore fish. J. Fish Biol., 17, 143-162.

Blaber, S.J.M, Brewer, D.T. and Salini, J.P. (1995). Fish communities and the nursery role of the shallow inshore waters of a tropical bay in the Gulf of Carpentaria, Australia. Estuarine Coastal and Shelf Studies, 40, 177-193.

Ciotti, B.J., Targett, T.E., Nash, R.D.M., Batty, R.S., Burrows, M.T. and Geffen, A.J. (2010). Development, validation and field application of an RNA-based growth index in juvenile plaice Pleuronectes platessa. J. Fish Biol., 77: 2181-2209.

Ciotti, B.J., Targett, T.E. and Burrows, M.T. (2013a). Decline in growth rates of juvenile European plaice (Pleuronectes platessa) during summer at nursery beaches along the west coast of Scotland. Can. J. Aquat. Sci., 70: 720-734.

Ciotti, B.J., Targett, T.E. and Burrows, M.T. (2013b). Spatial variation in growth rate of early juvenile European plaice Pleuronectes platessa. Mar. Ecol. Prog. Ser., 475: 213-232.

Ciotti, B.J., Targett, T.E., Nash, R.D.M. and Burrows, M.T. (2013c). Small-scale spatial and temporal heterogeneity in growth and condition of juvenile fish on sandy beaches. J. Exp. Mar. Biol. Ecol., 448: 346-359.

Clarke, K. R. and Warwick, R. M. (2001). Change in marine communities: an approach to statistical analysis and interpretation. Second edition. PRIMER-E, Plymouth.

Coates, S., Waugh, A., Anwar, A. and Robson, M. (2007). Efficacy of a multi-metric fish index as an analysis tool for the transitional fish component of the Water Framework Directive. Marine Pollution Bulletin, 55, 225-240.

Defra (2010). Charting Progress 2. Feeder Report: Healthy and Biologically Diverse Seas. Available at: http://chartingprogress.defra.gov.uk/ocean-processes-feeder-report

Devon & Severn IFCA (2017) Management of the "live" wrasse pot fishery. Revised report for all D&S IFCA stakeholders. Version 3, August 2017. https://secure.toolkitfiles.co.uk/clients/15340/sitedata/4F/Byelaw_development_reports/Wrasse/Final-Wrasse-v-3-new-cover-Aug16th-2017.pdf

Devon & Severn IFCA (2018). Live Wrasse Fishery in Devon and Severn IFCA District. Research Report November 2018. https://www.devonandsevernifca.gov.uk/content/download/2878/21936/version/1/file/Live+Wrasse+Fishery+Data+Analysis+November+2018.pdf

Elliott, M. (2011). Marine science and management means tackling exogenic unmanaged pressures and endogenic managed pressures - a numbered guide. Mar Pollut Bull, 62(4), 651-655.

Elliott, M. and Dewailly, F. (1995). The structure and components of European estuarine fish assemblages. Neth. J. Aquat. Ecol., 29, 397-417.

Elliott, M., O'Reilly, M. G. and Taylor, C.J.L. (1990). The Forth estuary: a nursery and overwintering area for North Sea fishes. Hydrobiologia, 195, 89-103.

Elliott, M. and Hemingway, K.L. (Eds.) (2002). Fishes in Estuaries. Blackwell Science.

Ellis, J. I. and Schneider, D.C. (1997). Evaluation of a gradient sampling design for environmental impact assessment. Environmental Monitoring and Assessment, 48(2), 157-172.

Environment Agency (2011a). Seine netting for monitoring fish. Operational instruction 145_07. Issued 23/03/2011.

Environment Agency (2011b). Data requirements for WFD transitional fish surveillance monitoring. Operational instruction 328_07. Issued 22/12/2011.

Environment Agency (2013a). Trawling for fish (otter, beam and oyster dredge). Operational instruction 011_07. Issued 30/07/2013.

Environment Agency (2013b). Fyke netting for monitoring fish. Operational instruction 25_07. Issued 06/08/2013.

Environment Agency (2019). TraC Fish Counts for all Species for all Estuaries and all years. Available at https://data.gov.uk/dataset/trac-fish-counts-for-all-species-for-all-estuaries-and-all-years.

Evans, N.T., Olds, B.P., Renshaw, M.A., Turner, C.R., Li, Y., Jerde, C. L., Mahon, A.R., Pfrender, M.E., Lamberti, G. A. and Lodge, D.M. (2016). Quantification of mesocosm fish and amphibian species diversity via environmental DNA metabarcoding. Molecular Ecology Resources, 16, 29–41.

Fox, C.J., Targett, T.E., Ciotti, B.J., de Kroon, K., Hortsmeyer, L. and Burrows, M.T. (2014) Size variation of 0-group plaice: Are earlier influences on growth potential a contributing factor? Journal of Sea Reasearch, 88: 59-66.

Franco, A., Elliott, M., Franzoi, P. and Torricelli, P. (2008a). Life strategies of fishes in European estuaries: the functional guild approach. Marine Ecology Progress Series, 354, 219-228.

Franco, A., Franzoi, P. and Torricelli, P. (2008b). Structure and functioning of Mediterranean lagoon fish assemblages: a key for the identification of water body types. Estuarine, Coastal and Shelf Science, 79(3), 549-558.

Franco, A., Pérez-Ruzafa, A., Drouineau, H., Franzoi, P., Koutrakis, E.T., Lepage, M., Verdiell-Cubedo, D., Bouchoucha, M., Lopez-Capel, A., Riccato, F., Sapounidis, A., Marcos, C., Olica-Paterna, F.J., Torralva-Forero, M. and Torricelli, P. (2012). Assessment of fish assemblages in coastal lagoon habitats: Effect of sampling method. Estuarine, Coastal and Shelf Science, 112, 115-125.

Franco, A., Quintino, V. and Elliott, M. (2015). Benthic monitoring and sampling design and effort to detect spatial changes: a case study using data from offshore wind farm sites. Ecological Indicators, 57, 298-304.

Franco, A., Mazik, K. and Thomson, S. (2018). Sublittoral rock indicator: Spatial correlation between environmental conditions and biological data (Phase 2). JNCC Report No., May 2018, Peterborough.

Franco, A., Nunn, A., Smyth, K., Hänfling, B. and Mazik, K. (2020a). Monitoring methods for assessing inshore fish communities. Natural England Commissioned Reports, NECR 269. ISBN 978-1-78354-594-0

Franco, A., Barnard, S. and Smyth, K. (2020b). Viability of fish monitoring techniques in inshore areas of the Southwest of England. Natural England Commissioned Reports, NECR 270. ISBN 978-1-78354-595-7

Franco, A., Hänfling, B., Young, M. and Elliott, M. (2020c). Regional monitoring plan for inshore fish communities in the Southwest of England. Natural England Commissioned, Reports

Hänfling, B., Lawson Handley, L., Read, D.S., Hahn, C., Li, J., Nichols, P., Blackman, R.C., Oliver, A. and Winfield, I.J. (2016). Environmental DNA metabarcoding of lake fish communities reflects long-term data from established survey methods. Molecular Ecology, 25, 3101-3119.

Li, J., Lawson Handley, L.-J., Read, D.S. and Hänfling, B. (2018). The effect of filtration method on the efficiency of environmental DNA capture and quantification via metabarcoding. Molecular Ecology Resources, 18,1102-1114.

Litvin, S.Y., Weinstein, M.P., Sheaves, M. and Nagelkerken, I. (2018). What Makes Nearshore Habitats Nurseries for Nekton? An Emerging View of the Nursery Role Hypothesis. Estuaries and Coasts, 41, 1539–1550.

Laramie, M.B., Pilliod, D.S., Goldberg, C.S. and Strickler, K.M. (2015). Environmental DNA sampling protocol - Filtering water to capture DNA from aquatic organisms: U.S. Geological Survey Techniques and Methods, book 2, chap. A13, 15 p., http://dx.doi.org/10.3133/tm2A13.

Marine Management Organisation (MMO England), Agri-Food and Biosciences Institute (AFBI Northern Ireland), Marine Scotland / Marine Laboratory (MS, Scotland), Centre for Environment, Fisheries and Aquaculture Science (Cefas, England), Environment Agency (EA), Natural Resources Wales (NRW), and Welsh Government (2016). United Kingdom

Work Plan for data collection in the fisheries and aquaculture sectors, 2017-2019. Version 1. York, 31 October 2016.

https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/605378/Data_collection_framework_annual_work_plan_2017_to_2019_UK.pdf [last accessed 07.05.2019]

Mathieson, S., Cattrijsse, A., Costa, M.J., Drake, P., Elliott, M., Gardner, J. and Marchand, J. (2000). Fish assemblages of European tidal marshes: a functional guilds-based comparison. Marine Ecology Progress Series, 204, 225-242.

McLachlan, A. and Brown A. C. (2006). The Ecology of Sandy Shores (second ed.). Academic Press, Amsterdam, 373 pp

McErlean, A. J., O'Connor, S. G., Mihursky, J. A. and Gibson, C. I. (1973). Abundance, diversity and seasonal patterns of estuarine fish populations. Estuarine and Coastal Marine Science, 1, 19-36.

Miya, M., Sato, Y., Fukunaga, T., Sado, T., Poulsen, J.Y., Sato, K., Minamoto, T., Yamamoto, S., Yamanaka, H., Araki, H., Kondoh, M. and Iwasaki, W. (2015). MiFish, a set of universal PCR primers for metabarcoding environmental DNA from fishes: detection of more than 230 subtropical marine species. Royal Society Open Science, 2, 150088.

Mynott S., 2020. Pilot study to validate an environmental DNA sampler for monitoring inshore fish communities. Natural England Commissioned Reports, NECR287.

Pawson, M.G., Pickett, G.D., Leballeur, J., Brown, M. and Fritsch, M. (2007). Migrations, fishery interactions, and management units of sea bass (*Dicentrarchus labrax*) in Northwest Europe. ICES Journal of Marine Science, 64, 332–345.

Pérez-Domínguez, R., Maci, S., Courrat, A., Lepage, M., Borja, A., Uriarte, A., Neto, J.M., Cabral, H., St.Raykov, V., Franco, A., Alvarez, M.C. and Elliott, M. (2012). Current developments on fish-based indices to assess ecological-quality status of estuaries and lagoons. Ecological Indicators, 23, 34-45.

Potter, I. C., Claridge, P. N. and Warwick, R. M. (1986). Consistency of seasonal changes in an estuarine fish assemblage. Marine Ecology Progress Series, 32, 217-228.

Potter, I. C., Tweedley, J. R., Elliott, M. and Whitfield, A. K. (2015). The ways in which fish use estuaries: a refinement and expansion of the guild approach. Fish and Fisheries, 16(2), 230-239.

Prato, G., Thiriet, P., Di Franco, A. and Francour, P. (2017). Enhancing fish Underwater Visual Census to move forward assessment of fish assemblages: An application in three Mediterranean Marine Protected Areas. PLoS ONE, 12(6), e0178511. https://doi.org/10.1371/journal.pone.0178511

Rogers, S.I., Millner, R.S. and Mead, T.A. (2000). Demersal fish populations of the Humber estuary and adjoining coasts. In Jones N.V. and Elliott, M. (Eds.) Coastal Zone Topics: Process, Ecology and Management. 4. The Humber Estuary and adjoining Yorkshire and Lincolnshire Coasts. ECSA, pp.97-106.

Rotherham, D., Underwood, A.J., Chapman, M.G. and Gray, C.A. (2007). A strategy for developing scientific sampling tools for fishery-independent surveys of estuarine fish in New South Wales, Australia. ICES Journal of Marine Science, 64(8), 1512-1516.

Sellers, G.S., Di Muri, C., Gómez, A. and Hänfling, B. (2018). Mu-DNA: a modular universal DNA extraction method adaptable for a wide range of sample types. Metabarcoding and Metagenomics, 2, e24556.

Southwood, T.R.E. and Henderson, P.A. (2000). Ecological methods. Third edition. Blackwell Science, Oxford, UK, 575 pp.

Thompson, S.K. (2012). Sampling. Third Edition. John Wiley & Sons

Thomsen, P.F., Kielgast, J., Iversen, L.L., Møller, P.R., Rasmussen, M. and Willerslev, E. (2012). Detection of a diverse marine fish fauna using environmental DNA from seawater samples. Plos ONE, 7(8), 4173. https://doi.org/10.1371/journal.pone.0041732.

Underwood, A.J. (1994). On Beyond BACI: Sampling Designs that Might Reliably Detect Environmental Disturbances. Ecological Applications, 4(1), 4-15.

Waugh A., Elliott M. and Franco A. (2019). Debunking paradigms in estuarine fish species richness. Marine Ecology Progress Series, 613, 125-138. DOI: 10.3354/meps12883

WFD-UKTAG (2014). UKTAG Transitional Water Assessment Method Fish Fauna. Transitional Fish Classification Index. July 2014. ISBN: 978-1-906934-32-3 https://www.wfduk.org/sites/default/files/Media/Characterisation%20of%20the%20water%20environment/Biological%20Method%20Statements/TW%20Fish%20UKTAG%20Method%20Statement.pdf

Wilding, T.A., Gibson, R.N. and Sayer, M.D.J. (2001). Procedural Guideline No. 4-3 Sampling benthic and demersal fish populations on sediments. In J. B. Jon Davies (senior editor), Martin Bradley, David Connor, Janet Khan, Eleanor Murray, William Sanderson, Caroline Turnbull and Malcolm Vincent (Ed.), Marine Monitoring Handbook March 2001: JNCC. http://jncc.defra.gov.uk/PDF/MMH-mmh_0601.pdf

Wolanski, E. and Elliott M. (2015). Estuarine Ecohydrology: an introduction. Elsevier, Amsterdam, 322 pp. ISBN 978-0-444-63398-9.

Zuur, A.F., Ieno, E.N. and Smith, G.M. (2007). Analysing Ecological Data. USA: Springer.

Appendix 1. Stakeholder workshop, Plymouth, 9th April 2019

Table A1.1. Participants

Delegate name	Role/organisation
Anita Franco (AF)	Institute of Estuarine and Coastal Studies (IECS), University of Hull
Steve Barnard (SB)	Institute of Estuarine and Coastal Studies (IECS), University of Hull
Rachel Brittain (RB)	Research Vessel Manager; MBA
Benjamin Ciotti (BC)	University of Plymouth
Steve Colclough (SC)	Independent consultant; IFM, and Colclough & Coates Aquatic Consultants
Paul Elsmere (PE)	Technical Advisor (Marine), Monitoring Programmes & Technical Services, and Environment Monitoring Service; Environment Agency
James Highfield (JH)	Natural England
Rob Hillman (RH)	Fisheries Research Specialist (TW fish monitoring); Environment Agency
Sebastian Mynott (SM)	Chief Operations Officer & Principal Molecular Ecologist; Applied Genomics
Trudy Russell (TR)	Marine Ecology Specialist, Marine Habitats & Species Team; Natural England
Thomas Stamp (TS)	University of Plymouth
Libby West (LW)	Senior Environment Officer; Devon and Severn IFCA
Amy Willcocks (AW)	Local Marine Planner; MMO

Apologies: Colin Trundle (Cornwall IFCA)

Table A1.2. Comments and replies (including how the comments have been integrated and addressed in the plan).

By	Comment	Reply (AF, IECS)
LW	Include saltmarsh within the TW habitats	Included as part of fyke netting
LW	Fyke netting in the estuaries would need dispensation, maybe some PR issues.	Noted
LW	Hinkley Point sampling is an example of 'stand-alone' programme that could be incorporated?	Component of targeted monitoring
SC	IFM would be happy to engage.	
LW	How much of the monitoring programmes is being picked up by MMO planning process? Can this programme (and others) feed into development of marine plans?	
BC	Really exciting project. Currently involved in regional monitoring by beach (sandy) seining – would it be good to integrate to cross reference against wider sampling methods across wider areas?	
BC	Would like to see some element of method development. E.g. French colleagues are developing a standardised method for diving surveys of kelp habitats in European waters. Nothing has been published as yet.	Need for working group on this (particular for diving protocols) emphasised
SC	Echoed need for development of method. WFD methods might be difficult to apply to small estuaries. Smaller mobile methods may need picked up by 'citizen science' approaches.	Component of targeted monitoring
SC	Southern IFCA developed programme for southern beaches and began to build a database, but then discovered 20 year data-set based on a push-netting that had been/was undertaken independently.	
SC	Some marine habitats are under estimated in terms of their importance for fisheries.	
RH	Intertidal fyke netting in TW: High mortality (100% in some cases) seen in Tamar studies and therefore need to be sensitive if migratory species present. Proposed balance between fykes and seines in estuaries is a little heavy in the fykes. Environment Agency may have better information for seine netting in TW than for fyke netting (seines produce greater diversity and enable most of fish to be returned alive. Also fykes might not catch some migrant species such as shad which tend to swim high in the water column	Static nets as FYK are important to monitor migratory species. A way to mitigate the issue of high mortality rate would be to empty the nets more frequently than every 12h. Balance of effort between fykes and seines has been modified in favour of seine netting.
RH	Specific methods need to be defined. For example, seine nets can be fished in different ways – to ropes or without	Use of standardised protocols for this

	ropes adding 'length' to the top line or nets being shot and held in a current or flow before being closed off.	(with the need of indicating when these are modified on implementation (e.g. to accommodate logistic constraints) in order to allow evaluation of data comparability. Where alternative methods are to be applied, this can be included on a case specific basis as additional targeted monitoring
SC	Also timing and veracity of sampling team may have a method that should be used at high slack, but the team may be "running late" and fish for falling tide instead.	
BC	Is aware of convergence of methodologies in US (involving multiple states of multiple countries) bagged seines versus unbagged; black versus white mesh.	
PE	Smaller estuaries might require use of smaller seine nets	
TR	Can methodologies have defined standard protocols or procedures? Money for monitoring if or when it becomes available, may be provided in a relatively short time frame and to be implemented at short notice and therefore having closely defined program ahead of time would be very valuable.	
AF	Importance of developing a common shared database for inshore fish data (similar to Marine Recorder)	Included in plan
SC (and others)	There are juvenile fish ID skills and hence the need for standardised training (4 day IFM course?) (SC provides a 2 day cut-down version of this) and continuous support. Also lab certification for fish ID (e.g. NMBAQC)	
	Seine netting and sampling of juvenile fish: Survey staff would need training if schedule one species [under Animal (Scientific Procedures) Act 1986] are involved (i.e. for juveniles retained for subsequent laboratory ID).	Use of standardised (e.g. WFD) protocols for this, with appropriate licences obtained (e.g. from EA) Home Office licence mentioned.
LW	Even if a sample is "bycatch", if it is measured as part of a recoding programme then it is deemed as having been "taken".	
BC	Sampling/monitoring is a grey area in terms of Home Office licencing requirements. The type of sampling we are talking about doesn't need a Home Office project licence for. A more general institute licence can be used.	
RH	May be problems if deploying seine nets in TW, single hand may retain 3,000 juvenile fish, in warm shallow waters, potential high mortality. Problems exacerbated if multiple or replicate sampling is proposed. Need to have very good set of protocols for processing samples quickly and effectively, more important still if public observers are present.	
SC	IFM (together with ZSL and some of the wildlife trusts) may well contribute via citizen science programmes.	Component of targeted monitoring
LW	Core sampling should be agency-based, not citizen science.	
SC	But citizen science provides a good resource.	

SC	Thames-based citizen science approaches (e.g. kick sampling for juvenile stages of fish species) is based on external funding, but (TR) suggested that this proposed a regional programme should be developed with the aim of being implemented by government agencies under existing or anticipated funding schemes.	
RH	Otter trawling probably most expensive of WTD methods but provides limited information. Also otter trawling tends to be an outer boundary of estuaries, effectively more a coastal sampling method.	It was agreed at the meeting to drop otter trawling from the TW monitoring. It has been kept in CW monitoring, but mostly as midwater sampling to integrate information on pelagic fish.
SC	Otter trawling for TW developed primarily for the Thames, but very specific for Thames conditions (developed for bass), so may not be suitable for transfer to other TW.	
SC	Otter trawl used in WFD has been developed from Cefas sea bass otter trawl specifications. But it can be easily adapted to midwater trawls	
RH	Otter trawling in TW: OK on relatively clean rias of south coast (easy), but in some TWs in the north coast (e.g. Carrick Roads) the net fills with red seaweed with need to be cleaned out and sorted through for sampled fish (very much time consuming, or a small return in terms of data obtained)	
RB	What about pelagic fish? Otter trawls, but in deeper water may only pick up target species on deployment and on retrieval, not on main trawl. Acoustic techniques? Can a high resolution multi-beam echo sounding be used for pelagic species? But would need otter trawling for ground-truthing, and there are not enough studies	It was recognised that acoustic techniques need further development. Otter trawling (in midwater, for pelagic fish) have been included in the plan for CW. This component is better covered by offshore surveys (e.g. PELTIC) and only aims at complete coverage of inshore areas, but with smaller sampling effort (higher value attributed to shallow water habitats)
BC	PELTIC surveys undertaken by Cefas (up to 20 depth) Video footage of net retrieval for commercial fisheries?	
LW	Commercial catches can be cross referenced to iVMS data but RB noted that cod-end mesh sizes for commercial gear may well be too large to effectively sample juvenile age groups. BC highlighted that on board observer / CCTV monitoring systems may be used to record catch (not just landings), but these may be very expensive.	Commercial catches can integrate but not replace core monitoring data (the target is different)
KF	Diving approach appears to be the right choice (e.g. compared to cameras). Tend to have very limited visibility for transect dives. Also fixed cameras e.g. the PlutoCam (off Plymouth sound)	Diving as core monitoring approach, but with recommendation

	indicate that they may represent an artificially narrow range of species and also species hierarchies came into play (e.g. due to differential bait attraction)	that a dedicated working group is required to take the method/protocol development further for application in European waters. Alternative methods (cameras, potting) may be included as additional targeted/local methods where diving is not possible
TR/RB	Approach requires more closely defined protocols.	
RB	Use of high definition acoustic camera techniques (can resolve down to a single fish) being developed off freshwater acoustic monitoring methods Acoustic techniques - there are not enough studies, and still requires ground truthing (trawling)	
LW	Some work done on using baited traps (pots), e.g. used for wrasse (but also with good catches of other fishes, e.g. cod). Could be used instead of diving, if no other option is possible.	
LW/SC	Use of gill nets in estuaries is controversial (mortality etc) – possible issue.	It was agreed at the meeting to drop gill netting from the plan. This limits the coverage of sublittoral rocky habitats in TWs, but this is a small part of habitats in estuaries anyway (and risk of gill netting is too high). This habitat can be sampled with diving where visibility allows it.t
RH	Issues for use of gill nets, especially if being used over a long time period (12 or 24 hours) intended to be used on outer edge of TW.	
AF	Increased frequency of checking and emptying nets could reduce mortality, but it might still be an issue	
RH	Issues in gill net bycatch (especially seabirds), net effectively needs to be actively managed (in bycatch, for example birds being released).	
TR	Gill netting may present key concerns, to the point where it should be dropped and alternative methods used (diving could be fine in medium sized estuaries, e.g. Carrick, Tamar, and is preferred method)	
RH/SC	Upper estuaries tend to be under represented [by current TW monitoring]	Fyke netting intensified in estuaries (part. upper estuaries) compared to WFD monitoring By preferentially deploying fyke nets in the vicinity of saltmarshes, seagrass, rocky habitat, they would allow to integrate fish assemblages from these habitats H&S aspects taken into account in protocol and costings
SC	There are variants of standard gear that can be used in different conditions. E.g. small 5-hoop fyke nets set with wings as an open Y shape and set in main saltmarsh drainage channels. On a flood tide, juveniles will move into and over, saltmarsh in top of water column. On ebb, fish move down following main drainage creeks (therefore easily caught by Y shaped nets/leaders, where leaders block the channels and catch majority of fish as they fall back on the dropping tide). BC highlighted the possible issue of very high mortality when nets are set on falling tide	
LW	For health and safety, D&S IFCA require a minimum of 3 people on-site if fyke netting.	
KF	Dive surveys on north coast may be more expensive than on the south coast simply related to the provision of ports and harbours etc. for boat use.	Aspects addressed in the calculation of cost ranges and protocols

BC	Seine netting & push netting in coastal waters need 5-6 people working at the same time (2 teams of 3) Need of consistent survey teams	
	Combined seine netting and beam trawling in estuaries – 3-4 stations per day seems too optimistic	
SM	eDNA 25 hour deployment for 50L of water filtered. Battery operated, self-contained, 1µm filter contained within a "capsule" that once removed, can be flooded with preservative and stored. Minimum pump deployment time (which is programmable) can be as little as about 6 hours. For poor water quality areas, can fit in 4µm pre-filter.	
SM	eDNA is about £350 per sample. 25 hour deployment, done on an open coast site where idea was to 'average out' the variability related to local currents. The 6 hour deployment was a more specific inshore site where specific tidal states/currents being targeted.	
TR	Need to have reporting costs in as an identifiable cost.	Included as separate cost
SC	Offers expertise in training and QA (TW)	
BC/AF	Potential for developing research/studentships to trial methods, develop targeted studies in areas needing more research + training students	

Appendix 2. Habitat availability and sampling effort

Habitat availability and sampling effort and cost distribution in the SW region:

Coastal waters (CW):

Table A2.1. Broad scale habitat availability and distribution (% area) in CW of the study region, overall (Total SW) and divided into zones (Zone 1 to 8, see Table 1 in the main body of text for geographic references to these zones). Areas calculations have been derived from UKSeaMap 2018 and should be taken as indicative (there are gaps in the map coverage, particularly in marginal shallow inshore areas resulting in a likely underestimate of these habitats).

Depth category	Substratum	Zone 1	Zone 2	Zone 3	Zone 4	Zone 5	Zone 6	Zone 7	Zone 8	Total SW
Littoral and sublittoral to 30 m	Sediment	12%	13%	4%	12%	11%	17%	30%	50%	24%
	Rock	11%	10%	8%	25%	16%	15%	6%	44%	14%
30-50 m	Sediment	33%	14%	8%	25%	51%	62%	41%	2%	25%
	Rock	4%	3%	5%	19%	8%	1%	8%	4%	6%
>50 m	Sediment	39%	59%	60%	15%	14%	4%	14%	-	27%
	Rock	<1%	2%	16%	3%	<1%	<1%	<1%	-	4%

Table A2.2. Distribution of sampling effort (number of stations) across methods applied in the coastal waters (CW) of the SW of England: SEN, beach seine net, also including the use of a push net (with effort distributed between low energy and high energy beaches and shallow waters with seagrass); BT2, 2 m beam trawl; DIV, SCUBA diving (with effort distributed between shallow open rock habitat, kelp, mäerl, biogenic reef habitats in CW, and also including diving surveys in rock habitats located in outer estuaries (TW)); OTT, otter trawl. The sampling effort is given per zone per year, and cumulatively for the SW region (see Table 1 in the main body of text for geographic references to the zones). The estimated mean annual cost for each survey component and overall for the region is also given (this includes all costs for data collection, including sample analysis, but excludes possible downtime and costs for data analysis and reporting; the latter are given separately in the main body of text of this report). The min-max range in cost is also provided as a result of the uncertainty in cost estimates.

Survey effort (no. stations)		**Coastal waters (CW)**												
Habitat:	Substratum:	Sediment (beach) (incl. seagrass)				Sediment	Rock and other structured habitats						(Sediment/rock)	**Inshore Fish Coastal Waters TOTAL**
	Depth:	<2m				<30m	<20m						>30m	
	Method:	**SEN_CW (+ PUSH net)**				**BT2_CW**	**DIV_CW (incl. TW)**						**OTT_CW**	
Sub-region	**Zone**	**Total**	Low energy	High energy	Seagrass	**Total**	**Total**	Rock (CW)	Kelp	Mäerl	Biog reef	Rock (TW)	**Total**	
SOUTH	Zone 1	4	2	1	1	3	3	1	1	-	-	1	3	13
	Zone 2	3	1	1	1	3	4	1	1	1	-	1	2	12
SW CORNWALL	Zone 3	3	1	1	1	2	2	2	-	-	-		4	11
	Zone 4	2	1	1	-	2	3	3	-	-	-		2	9
	Zone 5	3	2	1	-	2	3	1	1	-	-	1	2	10
NORTH CORNWALL/ DEVON/ SOMERSET	Zone 6	3	2	1	-	3	3	2	-	-	1		2	11
	Zone 7	4	2	1	1	4	4	1	1	-	1	1	4	16
	Zone 8	4	2	2	-	3	1					1	2	10
SEVERN	Zone 9 (TW)	-	-	-	-	-	1	-	-	-	-	1	-	1
Total SW (sum, no. sites)		**26**	13	9	4	**22**	**24**	11	4	1	2	6	**21**	**93**
no. samples per site		3 (+ 5)				3	max. 9						3	
Frequency (per year)		1				1	1						1	
Total mean estimated cost (range)		**£66,790 (£26,780 - £106,800)**				**£37,710 (£21,120 - £54,300)**	**£67,720 (£31,270 - £104,170)**						**£44,890 (£23,840 - £65,940)**	**£217,100 (£103,010 - £331,200)**

Transitional waters (TW):

Table A2.3. Main habitat distribution (% area) in TWs of the study region, and within its sub-regions and zones (see Table 1 in the main body of text for geographic references). TW in bold are consistently included in recent WFD TraC fish monitoring undertaken by the EA. Habitats and areas data have been obtained from https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/623572/wfd_water_body_summary_table.xlsx

				Biology: higher sensitivity habitats								Biology: lower sensitivity habitats					
				Source: Natural England marine evidence database								Source: Natural England marine evidence database					
Sub-region	**Zone**	**TW body name**	**Total area (ha)**	**Clam, cockle and oyster beds (ha)**	**Intertidal seagrass (ha)**	**Maerl (ha)**	**Mussel beds, including blue and horse mussel (ha)**	**Polychaete reef (ha)**	**Saltmarsh (ha)**	**Subtidal kelp beds (ha)**	**Subtidal seagrass (ha)**	**Cobbles, gravel and shingle (ha)**	**Intertidal soft sediment (ha)**	**Rocky shore (ha)**	**Subtidal boulder fields (ha)**	**Subtidal rocky reef (ha)**	**Subtidal soft sediments (ha)**
South Devon & Cornwall	1	Looe	48	-	-	-	-	-	12%	3%	-	<1%	68%	16%	-	3%	-
		Erme	136	-	-	-	-	-	21%	-	-	1%	51%	11%	-	-	-
		Avon	183	-	-	-	-	-	8%	-	-	<1%	71%	9%	-	-	-
		Yealm	203	-	-	-	-	-	2%	7%	8%	-	70%	15%	-	-	6%
		Fowey	265	-	-	-	-	-	5%	-	2%	-	61%	9%	-	6%	10%
		Kingsbridge	520	-	4%	-	-	-	<1%	<1%	-	<1%	75%	12%	-	2%	4%
		Plymouth Tamar	3021	-	1%	-	<1%	-	9%	1%	<1%	<1%	57%	2%	-	2%	30%
	2	Helford	762	-	<1%	3%	1%	-	<1%	17%	3%	4%	29%	6%	-	17%	14%
		Carrick Roads Inne	**1261**	<1%	<1%	**-**	<1%	**-**	**5%**	<1%	<1%	**-**	**48%**	**1%**	**-**	**5%**	**38%**
SW Cornwall	4	Hayle	187	-	-	-	-	-	7%	-	-	-	47%	3%	-	-	3%
	5	Gannel	108	-	-	-	<1%	-	14%	<1%	-	-	59%	6%	<1%	<1%	-
		Camel	**1087**	**-**	**-**	**-**	**-**	**-**	**5%**	**1%**	**-**	<1%	**35%**	**8%**	<1%	**3%**	**30%**
North Cornwall,	7	**Taw / Torridge**	**1459**	**-**	**-**	**-**	<1%	**-**	**16%**	**-**	**-**	**-**	**64%**	**7%**	**-**	**2%**	**12%**
Devon and Somerset	8	Parrett	7085	-	-	-	-	1%	3%	-	-	<1%	81%	2%	-	-	11%
Severn Estuary	9	Bristol Avon	202	-	-	-	-	-	24%	-	-	-	85%	3%	-	-	3%
		Severn Upper	**836**	**-**	**-**	**-**	**-**	**-**	**6%**	**-**	**-**	**-**	**40%**	**-**	**-**	<1%	**3%**
		Severn Middle	**6216**	**-**	**-**	**-**	**-**	**-**	**6%**	**-**	**-**	<1%	**71%**	**6%**	**-**	<1%	**-**
		Severn Lower	**46598**	**-**	<1%	**-**	<1%	**1%**	**1%**	**-**	**-**	<1%	**35%**	**5%**	**-**	**19%**	**33%**

Table A2.4. Distribution of sampling effort (number of stations) across methods applied in the transitional waters (TW) of the SW of England: SEN, beach seine net; BT1.5, 1.5 m beam trawl; FYK, Fyke nets. The sampling effort is given per estuary per year, and cumulatively for the SW region (see Table 1 in the main body of text for geographic references to the zones). The estimated mean annual cost for each survey component and overall for the region is also given (this includes all costs for data collection, including sample analysis, but excludes possible downtime and costs for data analysis and reporting; the latter are given separately in the main body of text of this report). The min-max range in cost is also provided as a result of the uncertainty in cost estimates.

Survey effort (no. stations)			**Transitional waters (TW)**				
	Habitat:	Substratum:	Sediment (+ rock and key habitats nearby)		Sediment		
		Depth:	<0m - 1m	<2m	<30m		
		Method:	**FYK_TW**	**SEN_TW**	**BT1.5_TW**	**TOTAL number of sites (*)**	**Total mean estimated cost**
Sub-region	**Zone**	**TW body (Size)**	**Total**	**Total**	**Total**		
SOUTH	Zone 1	Kingsbridge (S)	1	2	1	4	£11,110
		Plymouth Tamar (M)	2	4	3	9	£19,760
	Zone 2	Carrick Roads Inner (M)	2	(4)	(3)	9	£19,760
		Helford (S)	1	2	1	4	£11,110
SW CORNWALL	Zone 3	-				-	
	Zone 4	Hayle (S)	1	2	1	4	£11,110
	Zone 5	Gannel (S)	1	2	1	4	£11,110
		Camel (M)	1	(4)	(2)	7	£15,435
N CORNWALL/ DEVON/ SOMERSET	Zone 6	-				-	
	Zone 7	Taw / Torridge (M)	2	(4)	(2)	8	£15,435
	Zone 8	Parrett (M)	2	3	2	7	£15,435
SEVERN	Zone 9	Severn Lower (L)	-	6	4	10	£17,300
		Severn Middle (M)	-	(1) + 3	2	6	£8,650
		Severn Upper (S)	(4)	-	-	4	£13,570
		Total SW (sum, no. sites)	**17**	**36**	**22**	**76**	
		no. replicates per site	2	2	2		
		Frequency (per year)	2	1 (poss. 2)	1 (poss. 2)		
		Total mean estimated cost (range)	**£74,635 (£27,940 - £121,330)**	**£95,150 (£44,440 - £145,860)**		**£169,785 (£72,380 - £267,190)**	

(*) SEN and BT1.5 sampling should be combined in the same site in at least 1-2 stations per water body

Appendix 3. Existing fish monitoring in SW inshore waters

Below is a table summarising the information on existing fish monitoring/studies in the SW of England (recently undertaken, ongoing or planned), as filled by the stakeholders who were consulted during this project.

Who	What	Why	Where	When	Cost	Reporting
Who does (has done, or will do) the monitoring	*What method(s) are used*	*Aim(s) of the monitoring*	*Location and spatial scale (in the SW)*	*Duration, seasonality, frequency, etc.*	*Information on costs of the monitoring if available (even if indicative)*	*Has this monitoring been reported? If so, where?*
Marine Biological Association	Otter trawl	Monitor inshore fish communities off the coast of Plymouth	• Whitsand Bay (Cornwall) • ICES station L4 (10 miles south of Rame Head) • Bigbury Bay.	Twice monthly at each site	Funded by NERC (Funding will be discontinued in 2020)	Contributes to the Western Channel Observatory (WCO) – NERC National Capability. In discussions to contribute data to Marine Strategy Framework Directive group. Work contributes to MBA time-series data. Findings disseminated in various MBA publications.
Marine Biological Association	Beam trawl	Annual survey – combined with MRes student field course	• Cargreen (River Tamar) • Kingsmill Lake (River Tamar)	Annual – October. 4 days	Standard charter rate (£1500 per day)	Contributes to MBA time series data and students use data as part of their MRes course.

Who	What	Why	Where	When	Cost	Reporting
			• West Mud (River Tamar) • Cawsand Bay (outside of seagrass area)			
Marine Biological Association	Seine net and push net	Annual BioBlitz survey – running for 10 years (Citizen science). See https://www.mba.ac.uk/citizen-science#b18	• River Yealm (Newton Ferriers and Noss Mayo) • Various locations in SW on an ad-hoc basis	Annual - July	Funded by Heritage Lottery Fund	Data contributed to National Biodiversity Network
Ben Ciotti, Anna Persson (PhD student)	Riley push net, beam trawl	Understand drivers of spatiotemporal variation in abundance and growth of juvenile fishes in inshore areas.	20 sandy beaches throughout SW UK coasts between Weston-Super-Mare and Salcombe	Monthly. Currently in third year of sampling.		For PhD thesis and scientific publication.

Note: Although not included in the table above as information was limited, further dataset potentially identified include;

- Cefas inshore sampling inshore sampling (using a beach seine) along the south coast.
- PhD at Univ. Plymouth with the Devon and Severn IFCA on how European bass (Dicentrarchus labrax) move and use estuaries. A large tagging project, which involved implanting acoustic transmitter tags within 150 fish across 3 estuaries. To monitor movement patterns, both within and in close proximity to the estuaries, a series of acoustic receivers are used.
- Fyke netting surveys in saltmarsh habitat to look at juvenile fish habitat use (Univ. Plymouth, Thomas Stamp)
- Live-wrasse potting in the SW (D&S IFCA, Libby West – more information requested)
- Hinkley point sampling, standalone programme (D&S IFCA, Libby West – more information needed)
- 20 year dataset on push netting on south coast
- Annual fish sampling programme at one site near the estuary mouth of the Camel estuary (University of Bristol).
- Shad and smelt monitoring planned in the upper Tamar estuary for 2019 to assess spawning distribution.