Atlantic Cod (*Gadus morhua*) Stock identification In ICES area 6a

Proposal by Edward Farrell, EDF Scientific Ltd and Peter Wright, Marine Scotland Science

Introduction

ICES currently recognises and provides advice for sixteen cod stocks and conducts assessments for all, except that in Division 6.b Rockall (Table 1). The stock delineations are aligned with ICES statistical areas, divisions and subdivisions for the purposes of data collection, assessment and management. However within and between these stocks there are known and recognised issues of mixing and substructure. Therefore these stocks, in at least some cases, do not represent single biological populations.

Whilst delineation by management area may be more convenient for management and regulation purposes, accurately assessing the status, biomass and sustainable exploitation rates of mixed 'stocks' is inherently difficult if not impossible as they do not correspond to biological units. Fisheries dependent and independent data may be confounded in such mixed 'stock' scenarios, which may mask changes in the abundance of individual populations and lead to biased estimates of population abundance and consequently overexploitation of smaller populations (Hintzen et al., 2015). Such overexploitation can also have genetic effects that threaten the long-term sustainability of a fisheries resource (Pinsky & Palumbi, 2014) and reduce recovery rates despite management actions (Walsh et al., 2006).

Table 1. The sixteen cod stocks recognised by ICES

Stock Key Label	Stock Key Description	Eco Region	EG
cod.21.1	NAFO Subarea 1, inshore (West Greenland cod)	Arctic Ocean Ecoregion, Greenland Sea Ecoregion	NWWG
cod.21.1a-e	NAFO divisions 1.A-E, offshore (West Greenland)	Arctic Ocean Ecoregion, Greenland Sea Ecoregion	NWWG
cod.2127.1f14	ICES Subarea 14 and NAFO Division 1.F (East Greenland, South Greenland)	Arctic Ocean Ecoregion, Greenland Sea Ecoregion, Iceland Sea Ecoregion, Norwegian Sea Ecoregion, Oceanic Northeast Atlantic Ecoregion	NWWG
cod.27.1-2	subareas 1 and 2 (Northeast Arctic)	Arctic Ocean Ecoregion, Barents Sea Ecoregion, Norwegian Sea Ecoregion	AFWG
cod.27.1-2coast	subareas 1 and 2 (Norwegian coastal waters cod)	Arctic Ocean Ecoregion, Barents Sea Ecoregion, Norwegian Sea Ecoregion	AFWG
cod.27.21	Subdivision 21 (Kattegat)	Greater North Sea Ecoregion	WGBFAS
cod.27.22-24	subdivisions 22–24, western Baltic stock (western Baltic Sea)	Baltic Sea Ecoregion	WGBFAS
cod.27.24-32	subdivisions 24–32, eastern Baltic stock (eastern Baltic Sea)	Baltic Sea Ecoregion	WGBFAS
cod.27.47d20	Subarea 4, Division 7.d, and Subdivision 20 (North Sea, eastern English Channel, Skagerrak)	Greater North Sea Ecoregion	WGNSSK
cod.27.5a	Division 5.a (Iceland grounds)	Greenland Sea Ecoregion, Iceland Sea Ecoregion	NWWG
cod.27.5b1	Subdivision 5.b.1 (Faroe Plateau)	Faroes Ecoregion	NWWG
cod.27.5b2	Subdivision 5.b.2 (Faroe Bank)	Faroes Ecoregion	NWWG
cod.27.6a	Division 6.a (West of Scotland)	Celtic Seas Ecoregion	WGCSE
cod.27.6b	Division 6.b (Rockall)	Celtic Seas Ecoregion, Oceanic Northeast Atlantic Ecoregion	WGCSE
cod.27.7a	Division 7.a (Irish Sea)	Celtic Seas Ecoregion	WGCSE
cod.27.7e-k	Divisions 7.e-k (eastern English Channel and southern Celtic Seas)	Celtic Seas Ecoregion, Greater North Sea Ecoregion, Oceanic Northeast Atlantic Ecoregion	WGCSE

Cod stocks around Ireland and Britain

The primary focus of the following proposal is the Cod in ICES Division 6a, however due to unknown levels of mixing with cod from the adjoining stocks it is necessary to also consider the North Sea, Irish Sea and Celtic Sea stocks. Since 2003 ICES has advised zero catch in Division 6a. Zero catch advice was also given for the North Sea and Irish Sea stocks during the same period, however the North Sea catch advice has increased significantly in recent years and a small catch has been advised for the Irish Sea in 2018.

Numerous studies have applied a range of methods, including conventional and electronic tagging, otolith elemental analysis and molecular genetics, to investigate the population structure of cod around Ireland and Britain. These studies have confirmed that the population structure of cod in this area is significantly more complex than is recognised in the currently assessed stocks. The ICES Stock Annexes for *West of Scotland Cod, Cod in North Sea, eastern English Channel and Skagerrak*, *Cod in the Irish Sea* and *Cod in the eastern English Channel and southern Celtic Seas* all provide detailed overviews of the current state of knowledge regarding stock structure in these areas. The key points are extracted below.

Cod to the west of Scotland are believed to comprise of at least two subpopulations of cod that remain geographically separate throughout the year. The latitudinal boundary of these groups is between 57 and 58°30' N. The southern component is characterised by coastal groups with a tendency towards year-round residency, although there is some exchange with the Irish Sea. The northern component appears to inter-mix with cod in 4a at all stages of the life history.

Within the North Sea and neighbouring areas, several studies have indicated finer scale structuring on sub-stock scales….Recent evidence points to two populations; one inhabiting the north east North Sea (centred on the Viking Bank) and the other in shallower waters. This is supported by studies using both microsatellite DNA (Nielsen et al., 2009) and SNPs (Poulsen et al., 2011, Heath et al., 2014; WD1 by Wright et al., in WKNSEA 2015)….. There may be further structuring within the North Sea than that indicated by the genetic evidence alone. There is extensive evidence for persistent resident behaviour in many groups of cod since the 1960s associated with spawning aggregations from the eastern channel north to Shetland (ICES NSRWG 1971, Metcalfe 2006, Neat et al., 2006, Wright et al., 2006a & 2006b, Righton et al., 2007, Neat et al., 2014).

Historical tagging studies indicated spawning site fidelity but varying degrees of mixing of cod between the Irish Sea, Celtic Sea and west of Scotland/north of Ireland. Studies based on meristic characteristics, allele frequencies and microsatellite markers genetics and population structure have not provided unequivocal evidence of genetically isolated stocks in the Irish Sea and surrounding waters. A recent tagging programme run from 1997–2000, in which over 2200 cod were tagged using external and data storage tags, revealed that although there was some movement of cod between the Irish and Celtic Seas, the component of Irish Sea cod in the Celtic Sea was low. Furthermore, no cod tagged in the Celtic Sea were recovered from the Irish Sea (Connolly and Officer, 2001). More recent tagging of cod off Greencastle on the north coast of Ireland (Ó Cuaig and Officer, 2007), and limited tagging on UK Fisheries Science Partnership surveys, have demonstrated movements of cod between Division 6a and 7a. Most recaptures in 7a from cod tagged in 6a have come from the North Channel and in or near the deep basin in the western Irish Sea that is a southward extension of the North Channel. Extensive tagging off the West of Scotland produced no recaptures from the Irish Sea.

The Irish Sea front, running from SE Ireland (Carnsore point) to the Welsh Coast, appears to act as boundary between the Irish Sea and Celtic Sea stock. Juveniles found close to the SE Irish Coast (south of 7a) are considered part of the Celtic Sea stock. Some migrations and mixing are known to occur in

this cod stock. Both conventional and DST tagging information for 7g (where the majority of landings are made) shows that distribution remained fairly constrained within 7g.

Cod in Division 6a

Of particular relevance to the current proposal is the population structure of cod in ICES Division 6a. Holmes et al. (2014) suggested a revision to the current stock delineations based on genetic, tagging, and otolith microchemistry studies together with density distributions of species based on research vessel survey data. The revised 6a area included division of 6a into three subpopulation areas; Clyde, southwest and the Minch (Figure 1). This is similar to the findings of Heath et al. (2014), who used single-nucleotide polymorphism (SNP) data to delineate the geographic limits of three population units of cod around Ireland and Britain. The cod from the Clyde had a greater affinity to a western population encompassing the Celtic and Irish Sea, rather than cod from further north in 6a (Figure 2).

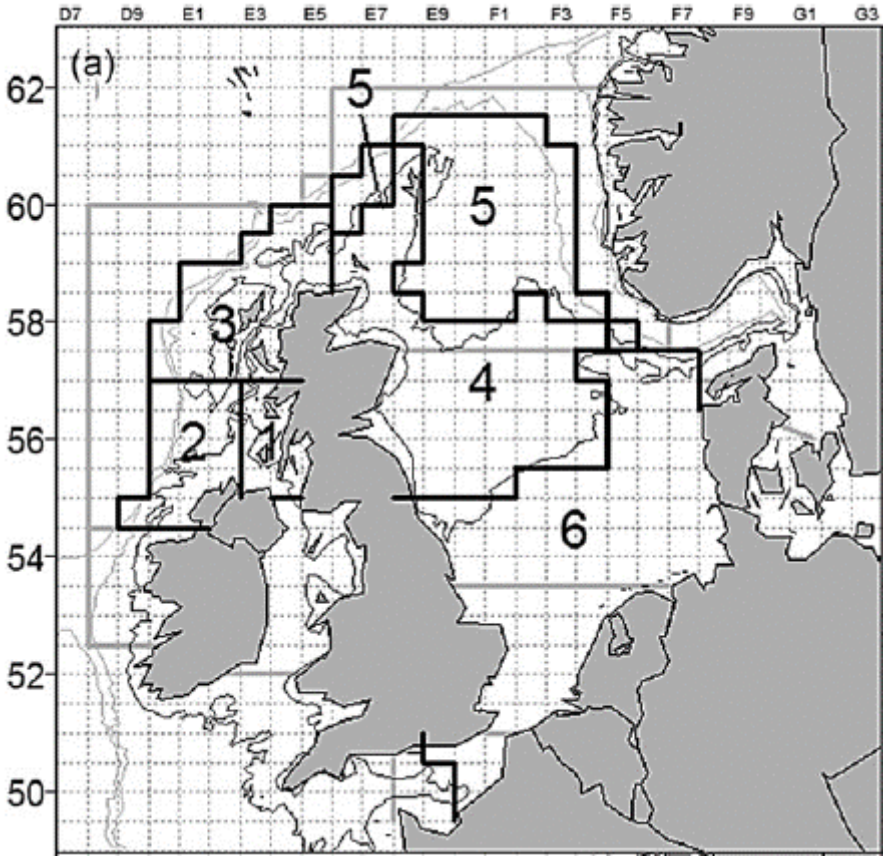

Figure 1. Putative stock or subpopulation areas for cod. 1, Clyde; 2, Southwest (SW); 3, Minch; 4, Northwest North Sea (NWNS); 5, Viking; 6, SNS. From Holmes et al., 2014.

More recently Doyle et al. (2016), also using SNP markers, uncovered finer scale population structure between resident inshore and offshore migratory cod populations around Shetland (4a) and westwards into 6a (Figure 3). Genetic and maturity evidence from this study was consistent with a reproductively isolated 'Viking' cod population, the distributional limits of which extend west to the continental shelf and not the 4° longitude as used in the stock assessment. The inshore cod to the west of Scotland (6aN) may be genetically distinct from other groups sampled (Figure 3).

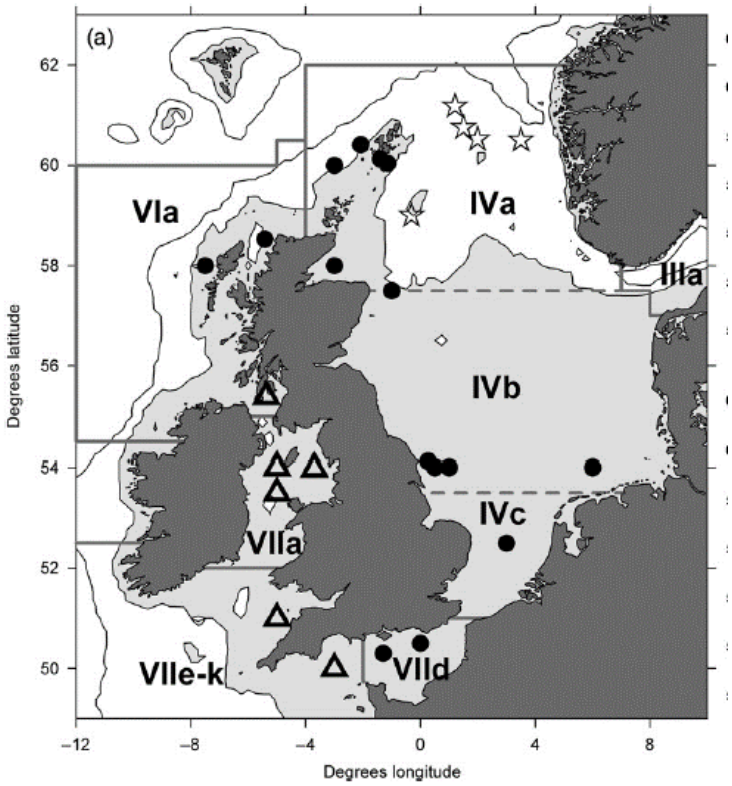

Figure 2. Cod population units based on SNP's and model grid cell properties. (a) Stars, samples of the "Viking" unit; filled circles, "Dogger" unit; open triangles, "Celtic" unit. From Heath et al. 2014.

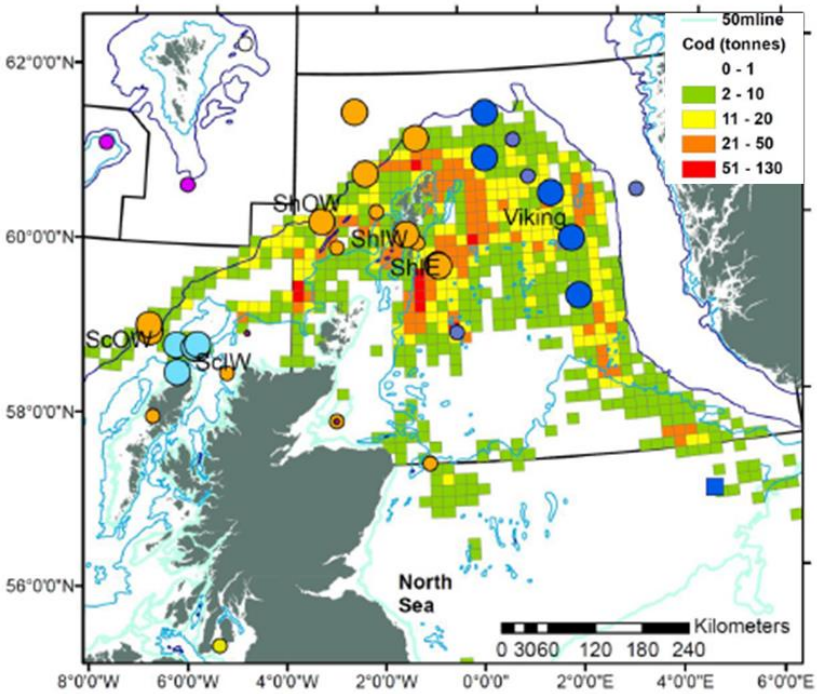

Figure 3. Location of population groups from SNP evidence. Large circles refer to results from the current study, small circles refer to Heath et al. (2014) samples and square refers to Poulsen et al. (2011). Dark blue = Viking, orange = shallow water deme, light blue = new structuring indicated in ScIW by this study. Population samples are overlaid on estimated landings per 1/16th ICES rectangle in 2011 to show approximate distribution of major fishery. From Doyle et al., 2016.

The knowledge of population structure in 6aS is based more on tagging data than on genetics studies. Neat et al. (2014) demonstrated connectivity between the Clyde area and the Irish Sea, based on analyses of data storage tag data (Figure 4). Further tagging studies are being conducted by the Irish Marine Institute and Northern Ireland's AFBI and between 2016 and 2018 over 2500 cod have been tagged in the Irish Sea and Celtic Sea areas. Up to January 2018 there have been 56 recaptures, which indicate some mixing between the Irish Sea and the Clyde and between the Irish Sea and the Celtic Sea (Figure 5).

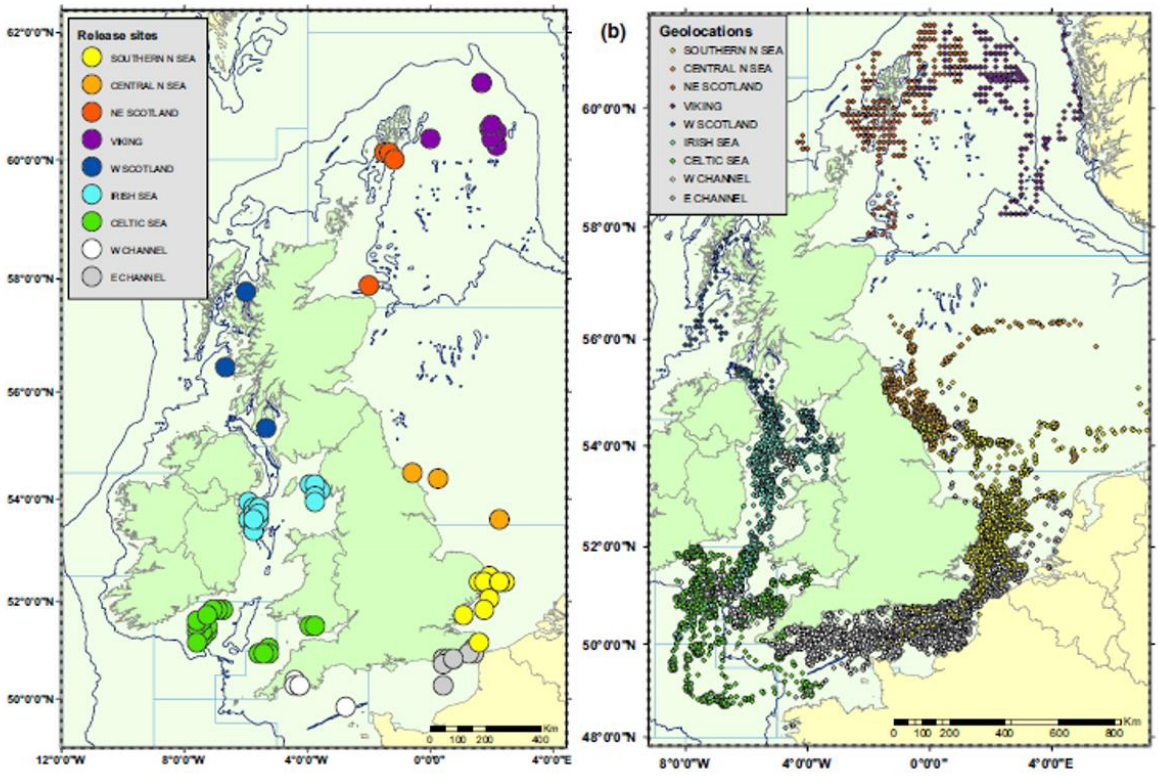

Figure 4. (A) Release sites of cod tagged with data storage tags. (B) Estimated positions of individual cod during their time at liberty during the spawning period (1 January to 30 April). From Neat *et al*., 2014.

Ó Cuaig & Officer (2007) also reported on the tagging of over 11,500 cod between 2003 and 2004. The recapture positions of 1,265 cod are displayed in Figure 6. The majority of recaptures were to the east of the Cape grounds and in the Clyde and Irish Sea areas, further illustrating the connectivity of these areas. The furthest north a cod was recaptured was west of Stanton bank, while the furthest south were two cod recaptured off the southwest of Ireland. Results appear to agree with the Celtic Seas group of Heath et al (2014).

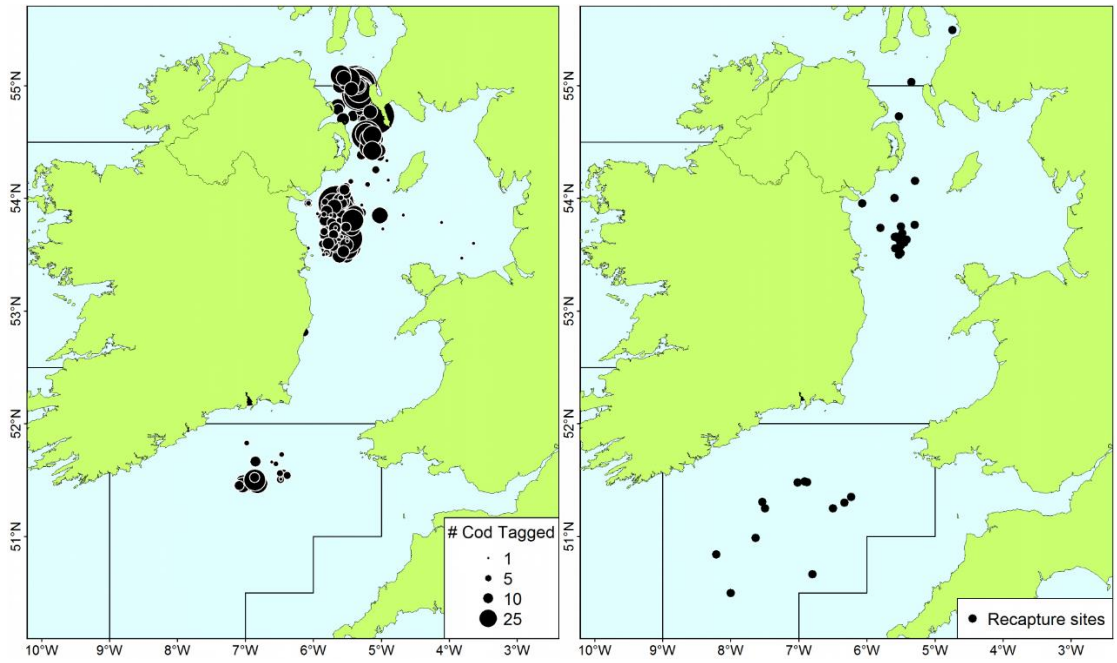

Figure 5. The release sites of tagged cod in the Irish Sea and neighbouring areas (N=2622, Jan 2018) and the recapture sites (N=52, Jan 2018).

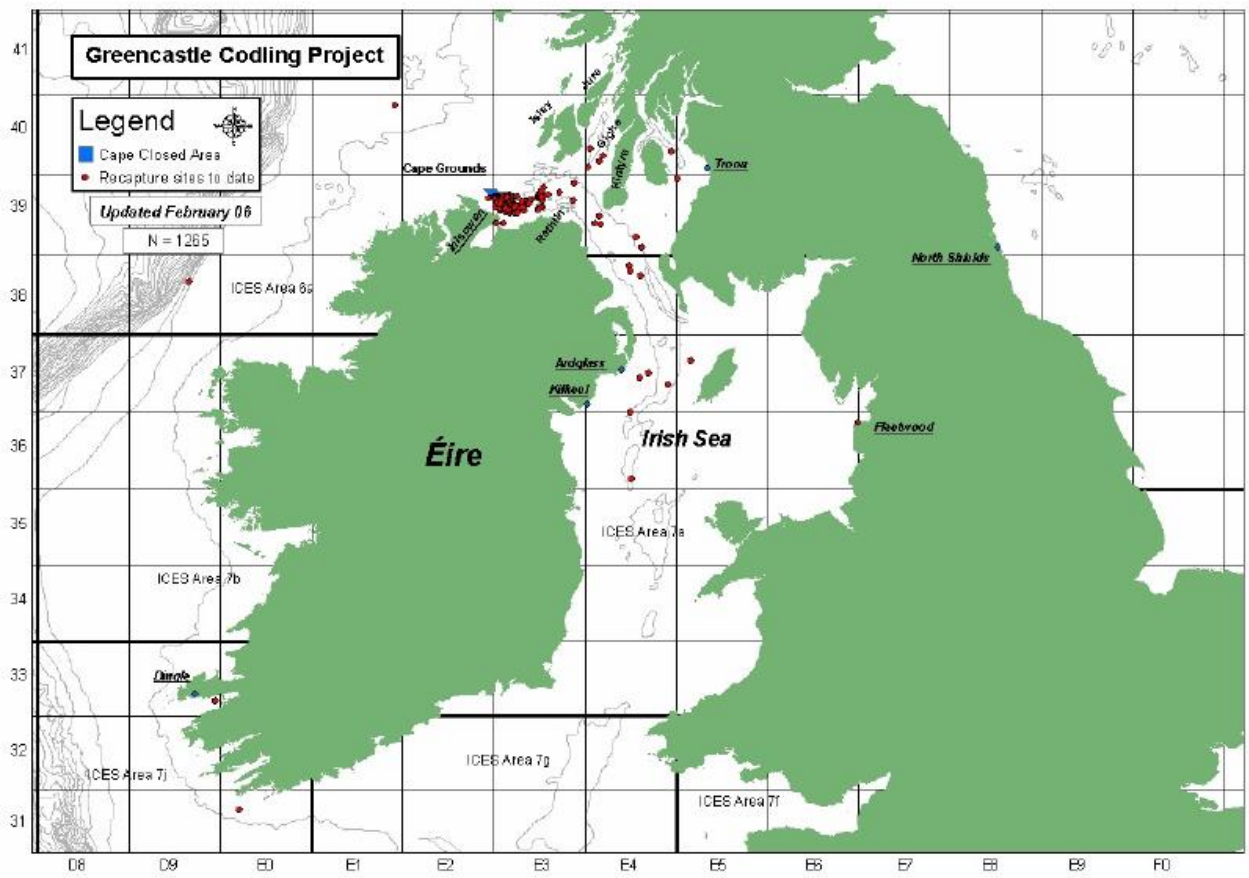

Figure 6. Recapture sites of tagged cod released on the Cape grounds

Key Points

- The 6a management/assessment area likely comprises multiple populations
- The 6a area does not contain these populations as they mix with other areas
- Other populations from other areas also mix into 6a at certain times
- The delineation between 6aN population and 6aS population is not defined
- The level of mixing between 6aN population and 6aS population is not known

Key Questions **(to be developed in consultation with industry partners and assessment scientists)**

- Are there multiple biological populations within 6a?
- Can they be discriminated genetically?
- Are they different from adjoining areas?
- Do the stock boundaries between 4a and 6a and between 7a and 6a reflect population boundaries?

Proposed Project

In order to answer the questions above it is proposed to divide the project into stages so that the progress of each stage can be monitored and only when one stage is successfully completed will the project progress to the next stage, thus minimising the risk to potential funding bodies and ensuring that resources are not utilised unproductively. The project will be divided into the following stages;

1. **Stage 1 – Literature review and Sampling programme**

   Given the extensive history of research on cod stock identification it is necessary to undertake an extensive review of all pre-existing studies. This will help to identify the best sampling strategy and also assess what resources are already available both in terms of samples and genetic resources. There has been extensive development of genetic markers in previous studies, therefore there may already be informative markers available which would reduce the time and cost involved in developing project specific markers. Archived samples may also be available that would reduce the time required to collect the multiple years of samples required for assessing the temporal stability of any population structure identified. It should be noted however that if sufficient existing informative genetic markers are not identified it may be necessary to develop these de novo. This would entail additional time and cost and could be estimated in the region of €10,000. Provision should be made for this outside of the budget in table 3.

   In order to develop a robust genetic baseline for the spawning populations it is necessary to collect genetic samples of muscle tissue from spawning fish on the spawning grounds. Each sample should be collected according to predefined protocols and should consist of muscle tissue samples collected from 100 fish per putative spawning population of interest. At least one sample (n=100 fish) should be collected per spawning population however if spawning occurs over a prolonged period or a large geographic area it may be necessary to collect multiple samples per population to ensure coverage of intra-population variability. In order to confirm the temporal stability of any population structure identified it is necessary to collect samples over at least two annual spawning periods. The sampling programme will have to be developed in collaboration with the Irish and Scottish industries and also the Marine Institute and Marine Scotland. Samples should be collected during the 2019 spawning season

from the areas indicated in Figure 7. Sampling will be coordinated as part of stage 1 and sampling consumables supplied to samplers.

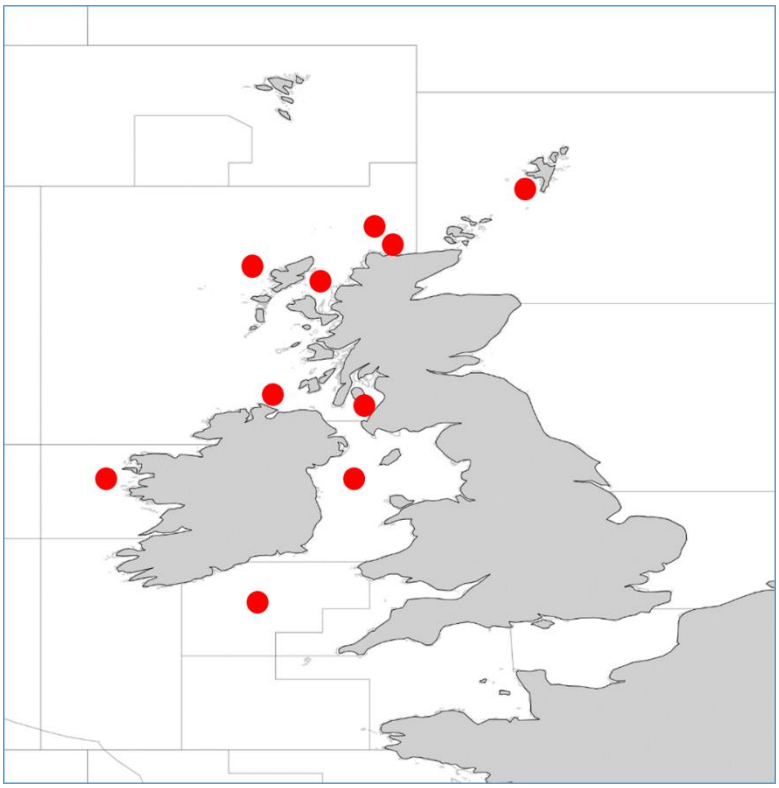

Figure 7. Proposed sampling locations for baseline genetic study.

2. **Stage 2 – Development of genetic baseline**

The approach followed and costs for the development of a genetic baseline for the 6a cod stocks depends on the results of stage 1. The availability of existing genetic markers will reduce costs and time significantly. The number of samples will also have a significant impact on the costs involved. A number of potential existing samples have been identified, though the viability of some of the older samples is to be confirmed (Table 2). Once the marker panel has been finalised in Stage 1 and the 2019 samples have been collected then it will be possible to proceed with processing the samples as per the high throughput methods developed during the 6a/7bc herring stock identification project and currently being employed in the horse mackerel stock identification project (see Farrell et al., 2016; Farrell & Carlsson, 2018).

The number of samples to analyse and the priority areas will have to be defined in consultation with the industry. For the purposes of estimating an overall cost the horse mackerel project is used as an example. The costing was based on the collection, processing and analysis of 2,112 horse mackerel samples at 80 microsatellites markers. Given the existing cod samples (Table 2) and the proposed collection of c. 1000 tissue samples in 2019, this is seen as a good indication of costs. Table 3 details the costs for stage 1 and 2.

Table 2. Cod genetic samples potentially available for use in the proposal

Area	Date	N	Type	Source
Celtic Sea	March 2018	100	Fin and muscle in EtOH	MI Tagging
Irish Sea South	February 2018	100	Fin and muscle in EtOH	MI Tagging
Irish Sea North	March 2018	50	Fin in EtOH	AFBI Tagging
6aN	2014	388	Gill in EtOH	Doyle et al in prep
North Sea	2014	505	Gill in EtOH	Doyle et al in prep
Papa Bank	2003	47	DNA extract-80	Heath et al. 2014
Outer Hebrides	1998	44	DNA extract-80	Heath et al. 2014
North Minch	2003	50	DNA extract-80	Heath et al. 2014
Clyde	2003	77	DNA extract-80	Heath et al. 2014
Moray Firth	2003	49	DNA extract-80	Heath et al. 2014
Long Hole	2009	17	DNA extract-80	Heath et al. 2014
West of Shetland	2003	119	DNA extract-80	Heath et al. 2014
West of Shetland	2002	48	DNA extract-80	Heath et al. 2014
East of Shetland	2002	42	DNA extract-80	Heath et al. 2014
Viking Bank	2002	44	DNA extract-80	Heath et al. 2014
Viking Bank	2003	36	DNA extract-80	Heath et al. 2014
Viking Bank	2007	49	DNA extract-80	Heath et al. 2014

Table 4. Costing for Stage1 & 2

Item	Description	Timing	Cost €
Stage 1	Literature and marker review and 2019 Sampling programme coordination	Jan-April 2018	10,000
Stage 2	DNA Extraction, sample processing, laboratory preparation, sequencing, data analyses, reporting and all associated costs	May-Nov 2018	63,000
Subtotal ex VAT			**73,000**
VAT @ 23%			16,790
Total			89,790

3. **Stage 3 – 2020 genetic baseline and mixed sample analysis**

Following successful completion of stage 1 and stage 2 it will be necessary to collect and screen an additional year of baseline spawning samples in 2020. The number and type of molecular markers used will be determined by the results of Stage 2. Areas of potential mixing, identified through existing knowledge and the literature review, should also be sampled in order to more accurately delineate the populations. This will enable the population boundaries to be compared to the existing stock boundaries. Provision should also be made in Stage 3 to further refine the marker panel and develop a rapid onboard method of stock identification, which may be used in areas of mixing.

At this stage it is not possible to accurately cost Stage 3.

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