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A microsatellite baseline for genetic stock identification of European Atlantic salmon (*Salmo salar* L.).

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Running title: Stock identification of European Atlantic salmon

Abstract

Atlantic salmon (*Salmo salar* L.) populations from different river origins mix in the North Atlantic during the marine life stage. To facilitate marine stock identification, we developed a genetic baseline covering the European component of the species' range excluding the Baltic Sea, from the Russian River Megra in the north-east, the Icelandic Ellidaar in the west, and the Spanish Ulla in the south, spanning 3737 km North to South and 2717 km East to West. The baseline encompasses data for 14 microsatellites for 26,822 individual fish from 13 countries, 282 rivers and 467 sampling sites. A hierarchy of regional genetic assignment units was defined using a combination of distance-based and Bayesian clustering. At the top level three assignment units were identified comprising Northern, Southern, and Icelandic regions. A second assignment level was also defined, comprising eighteen and twenty-nine regional units for accurate individual assignment and mixed stock estimates respectively. The baseline provides the most comprehensive geographical coverage for an Atlantic salmon genetic data-set, and a unique resource for the conservation and management of the species in Europe. It is freely available to researchers to facilitate identification of the natal origin of European salmon.

Key words: Atlantic salmon, genetic stock identification, individual assignment, marine ecology, microsatellites

Introduction

Homing of Atlantic salmon to natal rivers, in combination with factors such as founder effects, isolation, selection and genetic drift, and broad scale phylogeographic processes, has resulted significant population structuring at a hierarchy of levels from intra-river to inter-continental (King et al., 2001) and locally adapted populations (Garcia de Leaniz et al., 2007) including variations in marine migratory patterns among populations from different parts of the species range (Webb et al., 2007). However, the full extent of differences in

migratory patterns among populations and how this may be changing in response to shifting environmental conditions remains to be resolved (Jonsson et al., 2016).

Advancing understanding of population and stock-specific migration, distribution and feeding patterns, and their implications for marine mortality rates, and the impact of climate change, is hampered by a lack of information relating to the marine-phase of the lifecycle (Crozier et al., 2004). This situation makes it difficult to appropriately target actions to mitigate anthropogenic influences on different stock components e.g. the impacts of mixed-stock fisheries and bycatches. Thus a tool that allows the accurate identification of genetically distinct populations and regional entities (MacKenzie et al., 2011) and discrimination of the stock origins of fish in mixed feeding aggregations or during migratory phases would be invaluable in species' and North Atlantic marine ecosystem management.

DNA profiling methods for identifying the region or river/tributary of origin of salmonids have advanced over recent decades and are widely applied to Pacific salmon (*Oncorhynchus* spp.) stock management (e.g. Shaklee et al., 1999; Beacham et al., 2004; Beacham et al., 2006; Shedd et al., 2016). Their application to Atlantic salmon stock management has provided valuable insights into stock mixing at several spatial scales, including intercontinental (e.g. North American and European stocks in the West Greenland fishery: Gauthier-Ouellet et al., 2009), regional (e.g. stock composition in Canadian gill-net fisheries: Bradbury et al., 2016) and river level (e.g. population structuring in the River Teno/Tana: Vähä et al., 2016). However, overall, its use has been more limited due to the lack of useful genetic baselines for many parts of the species range.

Genetic baselines are available for the western side of the Atlantic (e.g. Bradbury et al., 2015; Sheehan et al., 2010), including a recently developed fine scale range-wide North American microsatellite baseline (Bradbury et al., 2016), that facilitate within-region

identification of fish originating from Western Atlantic populations at high geographic resolution. In contrast, only partial baselines have been developed for the eastern side of the Atlantic (e.g. Griffiths et al., 2010; Verspoor et al., 2012; Ensing et al., 2013; Gilbey et al., 2016a; Vähä et al., 2016) and no high-resolution baseline exists for the species' non-Baltic, eastern Atlantic range. Such a baseline would allow a DNA-based approach to the genetic stock identification (GSI) of marine samples from the Eastern Atlantic and, in conjunction with ecological studies, would help to provide a more detailed understanding of variations in the North Atlantic migration and distribution patterns of different European Atlantic salmon stocks. Such insight could improve understanding of the factors conditioning marine mortality, and facilitate the implementation of more effective management programmes (Crozier et al., 2004).

Genetic stock identification (GSI) has been carried out using various genetic markers, with early work successfully using allozymes (Koljonen and McKinnell, 1996) and mitochondrial DNA (Moriya et al., 2007) for salmonid species in some contexts, including for Atlantic salmon. However, higher levels of resolution and more widespread application has been subsequently achieved using microsatellite loci and they became the genetic marker most widely used in studies of Atlantic salmon stock differentiation. Even though, more recently, attention has turned to Single Nucleotide Polymorphisms (SNPs), the existing large body of microsatellite data available remains a unique and powerful resource that can be exploited for GSI in Atlantic salmon. However, it also has limitations (reviewed in Moran et al., 2006) related to laboratories using different sets of markers, variations in allele-calling with different size markers or allele-size bins, different screening platforms; differences in chemistry, differences in the fluorophore markers across loci and whether the forward or reverse primer is labelled as well as differences in primer sizes. All of these can result in

inconsistent allele-size designations across data sets generated by different laboratories. Nevertheless, evidence from large-scale standardisation projects for salmonid species such as *Oncorhynchus mykiss* (Stephenson et al., 2009) and *O. tshawytscha* (Seeb et al., 2007), as well as Atlantic salmon (e.g. Ellis et al., 2011), indicate these issues can be addressed and comprehensive, large scale integrated genetic baselines constructed (Moran et al., 2006).

Described here is a trans-European GSI baseline for Atlantic salmon (excluding Baltic salmon stocks which do not migrate to the North Atlantic) constructed by linking existing national and international microsatellite screening programmes. Baltic salmon populations are excluded from the baseline, as they do not migrate outside the Baltic Sea (Karlsson and Karlstrom, 1994; Torniainen et al., 2013). Data was integrated for a common set of 14 microsatellite loci for a geographically representative set of rivers spanning the species' Eastern Atlantic European range from the Russian River Megra in the north-east (66.151 N, 41.484 W), to the Icelandic Ellidaar in the west (64.117 N, 21.833 E) and the Spanish Ulla in the south (42.639 N, 8.761 E). Baseline samples encompassed rivers responsible for about ≈85% of wild-salmon production in the study region (based on rod-catch data derived from numerous sources). Existing and new data supplied by partners in a multi-laboratory trans-European consortium were calibrated (Ellis et al., 2011), subjected to stringent quality control and integrated to produce the new baseline. A hierarchical assignment unit approach was used and the baseline resolved into genetically distinctive regional assignment units. Assignment power and accuracy to these units were assessed, using both simulations and test samples, the latter constructed by removing fish from the dataset, to establish the utility of the baseline for regional assignment of marine-phase European origin salmon in the North Atlantic.

Methods

Baseline samples

Samples were collected from 32,888 Atlantic salmon from 551 sites representing 325 rivers in 13 countries across Europe (Denmark, England, Finland [two rivers with outlets in Norway], France, Iceland, Ireland, Northern Ireland, Norway, Russia, Scotland, Spain, Sweden and Wales) (Fig. 1, Table 1, Supplementary data S1 & S2), including the Baltic River Torne to act as a genetic out-group. Sampled sites spanned the species' entire eastern Atlantic range and spanned 3737 km from North to South and 2717 km from East to West.

Samples were collected from 1994 to 2010, with the majority collected in 2008–2009. Mainly juvenile fish were sampled, mostly parr and fry, but in some cases tissues from smolts or mature salmon returning to fresh water to spawn were sampled. Numbers sampled at a site ranged from 11 to 300 with a mean of 58, and rivers were characterised by 1 to 12 sites, depending largely on river size, with a mean number of sample sites per river of 1.7. Full details of sites are given in the Supplementary material (S1 & S2).

Genotyping

Microsatellite data were obtained from DNA extracted from tissue samples (typically fin clips or scales) screened by a consortium of 11 laboratories located across Europe (Table 1) for 14 of the 15 loci identified by a consortium of researchers and described by Olafsson *et al*. (2010). *SsaD486* (King et al., 2005) was excluded from the analysis due to its lack of variation over much of the European range. The panel of 14 loci used here were *SsaF43* (Sanchez et al., 1996), *Ssa14*, *Ssa289* (McConnell et al., 1995), *Ssa171*, *Ssa197*, *Ssa202* (O'Reilly et al., 1996) *SSsp1605*, *SSsp2201*, *SSsp2210*, *SSsp2216*, *SSspG7* (Paterson et al., 2004), *SsaD144*, *SsaD157* (King et al., 2005) and *SSsp3016* (unpublished, GenBank number AY37820).

PCR conditions, thermocyclers and multiplexes varied across laboratories, as did genotyping platforms, size standards and other chemistry employed. Genotyping details and standardisation of genotype assignments among laboratories appear in Ellis et al. (2011). In summary, two 96-well 'control plates' were prepared (Matis, Iceland) containing template DNA extracted from samples representing the widest coverage of the range of *S. salar* as was practicable and which covered sites from both the Eastern and Western Atlantic. These were subsampled and typed by each laboratory. Genotypes were submitted by each member of the consortium to a single depository (Exeter University) where conversion algorithms and standardised nomenclature were applied. For each locus, lists of allele counts and sizes for each laboratory were aligned and cross-referenced for the sample genotypes in the control plates. Standard allele scores were designated for each locus and size differences between allele lists from each laboratory were determined, which allowed laboratory specific standardisation rules to be defined. It should be noted that using this approach not every possible allele was screened, but the approach did allow the individual microsatellite bin ladders to be defined at each location. It cannot be ruled out therefore that rare alleles or alleles affected by regional indels may be have been missed using such an approach, although the coherence of the reference baseline produced (see below) suggests this is unlikely to have been a major influencing factor.

Based on the standardisation rules, all data generated for baseline sites were converted to the standard size ranges and stored in a single bespoke database for further analysis (see Ellis et al., 2011 for full details). Sib-ship analysis among individuals in each sample was investigated using the pedigree-likelihood approach implemented within the program COLONY (Jones and Wang, 2010) and used to exclude all but one fish from each full-sib family in each sample prior to inclusion in the database. Fish with less than 10 loci

genotyped were removed from further analysis due to concerns with DNA and genotype quality. Sites with more than half of the loci out of Hardy-Weinberg equilibrium (examined in GENEPOP 4.2.2; Rousset, 2008) (potentially not representative of a single population), those that had less than 70% of fish scored at all loci (potentially poor quality DNA and genotypes), and those consisting of less than 30 individuals after quality control checks listed above (potential failure to provide accurate estimates of allele frequencies), were also removed. We estimated descriptive statistics with GenAlEx 6 (Peakall and Smouse, 2006).

Assignment units

Assignment units were defined in an iterative way similar to that employed by Gilbey et al. (2016a). Units were first defined by a combination of distance-based and Bayesian clustering. Individual assignment accuracies using these units were then examined and units where accuracies did not meet a predefined threshold were combined with units that saw reciprocal misassignments, until all units had accuracies at or above the threshold level.

The distance-based approach was based on a neighbour-joining tree (Saitou and Nei, 1987) constructed using Nei's genetic distance D_A (Nei et al., 1983) calculated in POPTREE2 (Takezaki et al., 2010) and visualised in MEGA7 (Kumar et al., 2016). The clustering approach was carried out in STRUCTURE (Pritchard et al., 2000), using a burn-in of 100,000 and a run phase of 300,000 iterations during each application. Three replicates for each cluster number (K) were run with values of K from 1 to 10. K = 10 emerged as an upper limit after monitoring of the results of the runs while they were underway. In each case stable estimates of true K at the level under analysis had been identified by this point (see results). Prior site information was incorporated into the analysis using the LOCPRIOR option. The smallest K capturing the major structure in the dataset was defined by the ΔK method of Evanno et al. (2005), which was calculated using STRUCTURE HARVESTER (Earl and

vonHoldt, 2012). Replicate membership coefficients were combined with CLUMPP (Jakobsson and Rosenberg, 2007) using the Full Search method.

The Bayesian clustering was carried out using a hierarchical approach, starting with the full dataset. Evanno et al. (2005) showed that STRUCTURE tends to capture the major structure in a reference dataset but that more fine scale structure may become evident if a hierarchical analysis is performed. In the current analysis, at each hierarchical level a STRUCTURE analysis was performed and the minimum best *K* identified. The data were then split up into the cluster units and further STRUCTURE analysis performed on each one independently. This was repeated at each hierarchical split until either single-river structuring was observed or geographical coherence of the clusters was lost.

Once both the distance-based and clustering analysis had been performed, the degree to which the assignment units identified by each technique corresponded was examined. Where the same units were identified these were incorporated into the initial assignment unit panel. Where the two approaches had identified different units the smallest unit from either approach was incorporated into the initial assignment unit panel, for example in a situation where one technique had identified a single unit and another had identified sub-units the sub-units were added to the initial assignment panel. In this way, the smallest units identified by one or both technique were incorporated into the initial assignment unit test panel.

Once the initial assignment unit panel had been identified, individual assignment accuracy was calculated for each of these units (see below). If the assessed accuracy to a unit was at or above 80% the unit was retained in the panel. If accuracy was below this level the unit was combined with other units to which reciprocal misassignments were occurring. Accuracies were tested again and the process repeated until all units in the panel had

individual assignment accuracies at or above the 80% level. Nei's genetic distance D_A (Nei et al., 1983) was again calculated for all pairwise final assignment combinations using the POPULATIONS 1.2.3 software package (Langella, 1999).

Assignment analysis

Individual assignment

Individual assignment accuracy was calculated using maximum likelihood-based mixture analyses carried out using ONCOR (Kalinowski et al., 2007) with mixture proportions estimated using the EM algorithm and genotype probabilities calculated by the method of Rannala and Mountain (1997). In order to estimate unbiased assignment accuracies using fish not represented in the baseline, assignment tests were based on fish randomly removed from the reference baseline and combined into a mixture file. A randomly selected 10% of fish were removed from each of the three top level assignment units identified (see results) resulting in a total of 2682 fish in the mixture file. For each fish the most likely assignment unit of origin and associated assignment probability was calculated. Fish with assignment probabilities below 0.8 were classified as unassigned and excluded from the analysis. Accuracy to the assignment units was then calculated with the remaining fish. Using such a cut-off meant that fish whose origin was difficult to determine (low probability) were removed from the analysis and so potential accuracy could be increased (Gilbey et al., 2016a; Bekkevold et al., 2015). However, the application of cut-off scores also increased the proportion of unassigned fish (Gilbey et al., 2016a) and can thus influence apparent stock proportions if calculated from the individual assignments. As such, this should not be performed for this purpose and so, in order to estimate accurate stock proportions a Mixed Stock Analysis (MSA) approach was utilised (see below).

100% simulations

Simulated fishery mixtures were analysed in ONCOR and comprised sets of 100% simulated samples of fish from each assignment unit. Genotypic frequencies for each locus in each unit were re-sampled following Anderson *et al*. (2008). The 100% simulations were based on 1000 simulations of 200 fish per hierarchical assignment unit and the same simulated reference sample sizes as in the actual dataset.

Mixed stock analysis

Mixed stock proportions were calculated for each assignment unit. The same set of 2682 randomly selected fish used for the individual assignments was used and mixture proportions estimated in ONCOR using conditional maximum likelihood (Millar, 1987) with confidence intervals calculated based on 1000 bootstraps.

Equal proportions

Mixed stock proportions were calculated for each assignment unit using simulated fishery mixtures with equal proportions of fish at each assignment unit in ONCOR. One hundred fish were simulated for each unit and confidence intervals of the estimates calculated using 1000 bootstraps.

Baseline coverage analysis – River removal

A baseline rarely covers all possible source populations completely, and so some fish in real fishery mixtures may be from populations not included in the baseline. Hence, simulation analysis may overestimate the success rates of assignments of fish in an actual fishery due to being based only on samples from sites and rivers contained in the baseline (Waples et al., 2008). This issue was addressed using a further test panel and associated test baseline. A random 10% of the rivers in each assignment unit were removed from the baseline and used as test mixtures that were then assigned back to the reconstructed baseline. All assignment units comprising more than one river had at least one river randomly removed (see

Supplementary material S1 for details of sites and rivers removed). Fish in these 'unrepresented' mixture panels were thus from sites and rivers not included in the reconstructed baseline. In this way, we tested the capability of the baseline to reflect the regional signal of each assignment unit and to assign fish from sites and rivers not included in the baseline but from the assignment unit. This procedure was repeated at both assignment unit levels, again using ONCOR, with confidence intervals calculated based on 1000 bootstraps.

Results

Baseline QC

From a total of 551 sites sampled, 84 sites were removed, leaving 467 sites containing 26,822 fish representing 282 rivers in the final baseline (Table 1). From those removed, 17 sites were not in H-W proportions, 51 had <70% of fish screened at all loci, and 15 had <30 individuals representing the site after correction for full-siblings and individual fish for which <10 loci could be reliably genotyped. A further site (a sample of adult rod-caught fish from the Norwegian River Flekkeelva in 2007) was removed due to extreme outlier behaviour in the STRUCTURE analysis (data not shown). Full site details are contained in Fig. 1, Table 1 and Supplementary data S1 & S2. Across sites most loci were highly variable, with allele numbers ranging from 10 for *Ssa14* to 46 for *SsaD157* (mean 29.9). Additional descriptive and diversity estimates for each locus and site are presented in Supplementary material S3.

Definition of initial assignment regions

A neighbour-joining tree of Nei's D_A is summarised in Fig. 2 with an expanded version detailed in Supplementary data S4 and full site level D_A matrix in Supplementary data S5. A plot of ΔK, and a map showing the geographic positioning of the clusters at each hierarchical

STRUCTURE level are shown in Fig. 3. Assignment units as defined by POPTREE and STRUCTURE are compared in Supplementary data S6.

Both distance-based N-J tree and Bayesian STRUCTURE approaches identified three large regional groupings of sites covering the Northern, Southern and Icelandic regions and these will henceforth be referred to as the Level 1 assignment units. There was in general a good agreement between the two population structuring techniques at the lowest level units identified. Indeed, of the 26 and 22 units defined by the NJ Tree and Bayesian clustering methods, respectively, 17 units were identical (Supplementary data S6). Using the lowest level divisions produced from each technique resulted in a total of 29 units identified for the initial Level 2 assignment accuracy testing (column 1 in Table 2, Supplementary data S6). The assignment units at both initial levels are mapped in Fig. 1, with D_A matrixes detailed in Supplementary data S8.

Assignment analysis

Initial assignment accuracy

Using the 2682 fish removed from the baseline, individual assignments were performed at Level 1 and at the initially defined Level 2 assignment units. At Level 1 the assignment accuracy of all fish to the Northern, Southern and Icelandic unit respectively was 90.8%, 92.7% and 99.5% respectively. Using a probability cut-off score ≥ 0.8 this increased to 94.2%, 95.5% and 100% with 86.8%, 90.2% and 99.5% of fish in the mixture being assigned.

Assignment accuracy of fish with probability scores ≥ 0.8 to the Level 2 units was ≥ 80% in 19 of the 29 units (Table 2; for full breakdown of assignments at each Level 2 iterative level see Supplementary data S7). After combining assignment units based on reciprocal misassignments, 21 assignment units remained with recalculated accuracies ≥

355 80%. A final round of assignment unit combination resulted in 18 assignment units for which
356 assignment accuracies were all ≥ 80% (Table 2, Supplementary data S7).

357 *100% simulations*

358 The 100% simulations for each assignment unit showed robust estimates of stock
359 proportions at both assignment levels (Fig. 4). At Level 1, the mean estimates matched the
360 actual proportions extremely well with a maximum difference of just 0.3% between the
361 actual and estimated values and all upper CI at 100%. The initial Level 2 assignment units
362 again showed relatively accurate estimates with an average difference between the
363 estimated and actual mean proportions of 4.5%. The West and Central Scotland level,
364 however, showed a difference of 17.6% between estimated and actual proportions. At the
365 first round of assignment unit combinations accuracies were seen to improve, as expected,
366 with average and maximum differences between the estimated and actual mean
367 proportions of 4.5% and 9.0%. These levels reduced to 1.9% and 8.0% respectively at the
368 final Level 2 assignment unit combination round.

369 *Mixed stock analysis*

370 The results of the MSA using the 2682 fish removed from the baseline and used as a fishery
371 mixture are shown in Fig. 5A. For all assignment units, within both assignment levels, apart
372 from a single unit in Level 2, South France/Spain, where the upper CI was 0.19 below the
373 actual value, the estimated proportions of fish in the unit mixtures matched actual
374 proportions (i.e. were within the CI bands). The estimates were also very precise with
375 average CI bands of just 2.2 and a maximum of 4.7. Considering the high accuracy of the
376 mixed stock estimates at this initial assignment unit composition, no further assignment unit
377 amalgamations were deemed necessary for mixed stock analysis.

378 *Equal proportions*

As with the previous analysis the equal proportion simulation showed excellent agreement between the actual and estimated proportions in the mixture (Fig. 5B). At Level 1 there was an average difference between actual and estimated of just 0.06% and a maximum of 0.09% (Southern unit) and at Level 2 these two differences only rise to a mean difference of 0.4 and a maximum of 1.1% (North Ireland unit).

Baseline coverage analysis – River removal

The most demanding test of assignment capabilities of the baseline was the "river removal" test in which entire river systems were removed from the baseline and their fish assigned to region of origin using the remainder of the rivers in the reference baseline. However, even here relatively high levels of assignment accuracy were obtained (Fig. 5C). Average differences between actual and estimated mixture proportions were 1.9% with a maximum of 2.3% (Southern unit) at Level 1 and 1.3% and 2.9% (Central Scotland/North England) respectively at Level 2. At no time were significant proportions assigned to any of the six single-river assignment units which were not represented in the mixture file (lower CI at zero in these units).

Discussion

The study, encompassing the largest analysis of Atlantic salmon population structure in the Eastern Atlantic, for the first time, provides a genetic framework to exploit the power of microsatellite variation to assign Atlantic salmon from this part of the species' range to smaller scale regional stock groups. As such, the reported genetic baseline provides a powerful resource that can be used to increase understanding of the biology of European Atlantic salmon stocks in the North Atlantic marine environment. Enhanced understanding of stock-specific marine migration, distribution, feeding patterns, exploitation and mortality

403 rates, will help to provide guidance towards a more efficient management of Atlantic
404 salmon in a changing environment (Crozier et al., 2004).

405 Distance-based and Bayesian cluster based analyses both reveal hierarchical
406 structuring of river populations of European and Icelandic salmon into regional groups. At
407 the highest level, this structure encompasses large-scale geographical discontinuities
408 between northern (Scandinavia-Russia), Icelandic, and southern regions (Britain-Ireland-
409 France-Denmark-Spain). Such differences have been identified in previous analyses of
410 Atlantic salmon population structure. For example, King et al. (2001) showed with
411 microsatellites an unambiguous separation of Iceland, Norway and Scotland-Ireland-Spain
412 (their Fig. 3), and Verspoor et al. (2005) identified an Icelandic group together with a
413 southern British Isles-Northern France group using allozymes, although a more complex
414 pattern was apparent in their analysis among the more central range groups.

415 At the next highest level, two assignment units shared the largest average degree of
416 distinctiveness from other units, the two also being on opposite extremes of the neighbour-
417 joining tree (Fig. 2). The Baltic unit had a mean D_A of 0.236 to other units (Supplementary
418 data S8), a level of differentiation to other European rivers seen in previous studies (Bourret
419 et al., 2013) and consistent with the restricted migration of Baltic stocks (Karlsson and
420 Karlstrom, 1994) and their long history of geographical isolation (Bourret et al., 2013). A
421 second assignment unit, the English Chalk streams, also shared a similarly high mean D_A of
422 0.236. Griffiths et al. (2010) and Ikediashi et al. (2018) also reported these rivers in Southern
423 England to be highly differentiated from others in the southern part of the European range.
424 However, it is unexpected in the context of the entire European and Icelandic range, that
425 the degree of differentiation matches that of the Baltic.

Within Iceland the salmon populations segregate into Northern and Western Icelandic units as was also reported by Olafsson et al. (2014) which is thought may reflect the patterns of recolonisation after the Last Glacial Maximum.

Initially the Northern Level 2 unit subdivided into eleven geographically coherent genetic clusters that matched well with previously reported structure in this region. Bourret et al. (2013), using SNP markers, found separation of northern Norway and Russian rivers from the Norwegian and Swedish Atlantic coast rivers, and Kjærner-Semb et al. (2016) found separation of northern and southern Norwegian groupings. Within the northern Norway-Russian complex, Ozerov et al. (2017) also found the same North Kola, Northern Norway and Russia-White sea units as reported here. However, their use of 33 microsatellites and a more comprehensive geographical coverage allowed them to define structure at further hierarchical levels within these groups unresolved in the present study using only 14 microsatellites and more limited population coverage.

The population structuring of rivers from across the part of the range covered by the Level 1 Southern unit into an initial sixteen Level 2 units accords well with that reported by Griffiths et al. (2010) based on 12 microsatellites, 11 of which form part of in the panel used in the present study. Their study encompassed fish from 57 rivers across the Southern region but excluded rivers from the East coast of Scotland and Northern Ireland an showed similar geographic patterns of genetic structure (their Fig. 2). Similar assignment units in France and Northern Spain appeared in both analyses and also broadly reflected allozyme-based regional differentiation (Verspoor et al., 2005). However, some differences were seen with some of the units between the two methods used to resolve assignment units. Griffiths et al. (2010) identified groupings stretching across both Scotland and Ireland (see their Fig. 2) and similar groups were identified here using the STRUCTURE based approach (Fig. 3). In

contrast, using the distance-based approach the various Scottish and Irish units were clearly separated (Fig. 2) to which generally good assignments of fish could be made. Nevertheless, some reciprocal misassignment was still evident (Supplementary data S7) suggesting a degree of homology between the units. Further, finer-scale investigation is perhaps required to disentangle completely the complex patterns of population grouping within these regions.

Accurate assignments to the initial Level 2 units was not possible at the individual level but was achieved for mixed stock fishery estimates. Acceptable levels of individual assignments could be made to some defined units using the initial split but some areas proved problematic at this scale particularly for Britain and Ireland. This difference reflects the differing power of the two IA and MSA techniques (Manel et al., 2005) and suggests that, when using the baseline for a particular purpose, the required levels of both accuracy and resolution should be defined *a priori.* In turn, this will depend on the specific questions being examined and the tools being utilised.

Overall, the two levels of genetic structure are geographically consistent and in basic agreement with major regional phylogeographic groups previously reported using a variety of markers, suggesting the higher level regional structuring is geographically and temporally robust. In contrast, differentiation between regional units identified at the finer geographic scales may in part be conditioned by human activities, such as the transport and escape of fish from aquaculture facilities, stocking, habitat alteration, fisheries-induced evolution, and indirect genetic changes from disease and ecological disturbances. Such genetic structuring, if defined by such contemporary influences, may not have temporal stability and such lower level units thus will need to be monitored to determine if they are stable. Encouragingly, in a previous assessment of temporal stability on assignment of Atlantic salmon in the species'

474 southern European range (Griffiths et al., 2010), test samples collected 20 years before the
475 baseline samples still showed predominant allocation back to region of origin. This finding
476 suggests, at least at the larger scale, regional level units are likely to be temporarily stable.
477 However, this should not be assumed to always be the case and a program of resampling
478 should be incorporated if the baseline is exploited in the future.

479 For the Level 1 and the final Level 2 regional units, all tests of power suggest high
480 accuracies can be achieved with both individual assignments and mixed stock analysis.
481 Accuracies are improved by use of a probability cut-off of 0.8 for individual assignments,
482 which may be useful in some contexts. However, this will reduce the proportion of fish
483 assigned. Thus in application, the best cut-off will depend on the question address and will
484 need to be decided by each individual user. This will also apply to the assignment units used;
485 if reduced accuracies to some of the combined units are acceptable these may also be used
486 in specific circumstances.

487 The assignment tests carried out indicate that the described baseline can be
488 exploited to help investigate patterns of ocean utilisation and associated differences in
489 marine mortality operating at the regional stock level. However, important quantitative
490 variation linked to how individual population components use the ocean, which may affect
491 mortality rates, also exists at the level of individual rivers within regions and among river
492 tributaries (Barson et al., 2015). Evaluation of river-specific problems, likely to exist in some
493 contexts, will require assignments at the individual river level, for which the current baseline
494 appears to have limited usefulness. Nevertheless, even if river-level identification is
495 problematic, identification of region of origin may allow finer scale analysis using higher
496 resolution region-specific baselines.

Resolution of intra-regional population contributions in mixed oceanic samples, including within-river contribution assessments, would be facilitated by further increases in the coverage and resolution of the baseline. Higher resolution is already being achieved in selected areas covered by the baseline reported here (Gilbey et al., 2016a; Ozerov et al., 2017; Vähä et al., 2016). Ideally, future work will likely increase baseline coverage to include most of the estimated 2000 rivers in the North-East Atlantic Commission area. However, this will involve diminishing returns given that the rivers currently in the baseline represent an estimated ≈85% of the non-Baltic European adult salmon production. Nevertheless, genetic characterisation of as many populations as possible will be important for biodiversity inventory and assessment. Considerable value could also be added by combining the European baseline reported here with North American information to provide a trans-ocean baseline and thus enable oceanic scale investigations. This has already started using a reduced set of microsatellite markers and shows promise in the ability to assign fish from the entire species' range (Gilbey et al., 2016b).

Supplementary data

Supplementary material is available at the ICESJMS online version of the manuscript.

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710 Table 1. Sample baseline coverage pre- and post-genotype quality control (see text for
711 details).

Country	Pre-QC			Post-QC		
	Rivers	Sites	Fish	Rivers	Sites	Fish
Denmark[1]	3	6	253	2	4	189
England[2,3]	24	38	1652	23	35	1498
Finland[4]	2	5	395	2	5	393
France[2,3,5,6]	13	16	759	9	9	450
Iceland[7]	17	25	2352	16	22	1986
Ireland[8]	29	45	2345	29	40	2053
Northern Ireland[9]	9	20	1469	7	18	1302
Norway[4,10,11]	90	109	7749	81	99	7008
Russia[4,10, 12]	33	36	2506	30	33	2350
Scotland[3]	87	230	11625	69	185	8884
Spain[6]	7	7	342	4	4	190
Sweden[1,4]	4	4	180	4	4	172
Wales[2]	7	10	375	6	9	347
Total	325	551	32002	282	467	26822

712 Institutions contributing data: [1] Danish Institute for Fisheries Research, Denmark; [2] University of Exeter,
713 England; [3] Marine Scotland Science, Scotland; [4] University of Turku, Finland; [5] Geneindex, France; [6] University
714 of Oviedo, Spain; [7] Marine and Freshwater Research Institute, Iceland; [8] University College Cork, Ireland; [9]
715 Queen's University Belfast & Agri-Food and Biosciences Institute Northern Ireland, Northern Ireland; [10]
716 Institute of Marine Research, Norway; [11] Norwegian Institute for Nature Research, Norway, [12] Knipovich Polar
717 Research Institute of Marine Fisheries & Oceanography, Russia.
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721 Table 2. Individual assignment accuracy using fish removed from the reference baseline. Initial assignment units in first column defined by
722 distance and STRUCTURE based analysis. Remaining assignments represent amalgamations of units where assignment accuracy is <80%.
723 Assignment accuracy was calculated using only fish with individual assignment probabilities ≥ 0.8. Values in bold represent accuracy of at least
724 80% to assignment units. Sample sizes represent baseline/mixture size.
725

Assignment unit	Sample size	Assigned %	Correct %	Assignment unit	Assigned %	Correct %	Assignment unit	Assigned %	Correct %
White Sea	758/86	68.6	**90.2**	White Sea	70.9	**90.3**	White Sea	72.1	**90.3**
Kola	1561/160	50	**82.1**	Kola	51.9	**82.1**	Kola	53.1	**82.1**
Kola (Tuloma Basin)	287/39	61.5	**100**	Kola (Tuloma Basin)	66.7	**96**	Kola (Tuloma Basin)	66.7	**96**
Finnmark	1109/107	54.2	**84.7**	Finnmark	59.3	**82.9**	Finnmark	59.3	**82.9**
Teno/Tana	271/28	42.9	10						
Mid Norway	3195/369	54.5	**84.1**	Mid & SW Norway	68.3	**84.4**	Mid & SW Norway	69.2	**84.4**
South West Norway	816/95	42.1	73.8						
South Norway	693/83	32.5	**81.25**	South Norway	45.8	**82.4**	South Norway	47.0	**82.4**
Enningdalselva	86/8	87.5	**100**	Enningdalselva	87.5	**100**	Enningdalselva	87.5	**100**
Sweden	108/12	33.3	**100**	Sweden	41.7	**100**	Sweden	41.7	**100**
Baltic	47/5	60	**100**	Baltic	80.0	**100**	Baltic	80.0	**100**
Denmark	176/13	61.5	**100**	Denmark	76.9	**100**	Denmark	76.9	**100**
Central Scotland/North England	1711/200	32	73.5	Scotland/North East England	66.3	**80.4**	Scotland/NE England/Irish Sea	76.4	**87.2**
North East Scotland	2183/233	42.5	56.5						
Kyle/Ness	814/99	42.4	78.7						
North & West Scotland	2005/255	35.7	72						
Water of Luce	225/20	30	40						
West Central Scotland	242/28	46.4	**83.3**						
Irish Sea	1992/214	39.7	77.3	Irish Sea	52.3	76.3			
Leven	324/41	75.6	**100**	Leven	82.9	**96.9**	Leven	85.4	**96.9**
English Chalk	134/9	88.9	**100**	English Chalk	88.9	**100**	English Chalk	100	**100**
North France	283/35	45.7	78.9	North France	51.4	78.9	France/Spain	68.0	**91.7**
South France/Spain	282/40	70	**100**	South France/Spain	72.5	**100**			
North Ireland	1519/161	50.3	**87.0**	North Ireland	59.6	**85.9**	Ireland	64.0	**87.0**
South West Ireland	341/35	54.3	**85.7**	South West Ireland	52.2	77.4			
South Ireland	572/57	29.8	58.8						
Bann	619/51	66.7	**93.9**	Bann	66.7	**93.9**	Bann	66.7	**93.9**
North Iceland	976/110	95.5	**96.3**	North Iceland	95.5	**96.3**	North Iceland	95.5	**96.3**
West Iceland	811/89	91.0	**98.7**	West Iceland	92.1	**98.7**	West Iceland	93.3	**98.7**

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Figure 1. Map of sampling region. Points represent sample sites and/or river mouths. Full site information is contained in Supplementary data S1 and an expanded map with all rivers identified is in Supplementary data S2. Regions noted are all those referred to in the text. The Level one assignment units (see text) are delineated by the dashed line and the initial Level 2 units by coloured points.

Figure 2. Neighbour-joining phylogenetic tree of sample sites based on D_A with major clusters coloured and named. Expanded tree with all sites identified is detailed in Supplementary data S4.

Figure 3. Hierarchical STRUCTURE based clustering analysis of sites. Each cluster analysis is described using three components. Firstly the results of the STRUCTURE analysis are shown with vertical bars representing individual sites and colours relating to cluster membership of that site. A plot of the ΔK values (Earl and vonHoldt, 2012) associated with the analysis is also shown defining the K identified in that cluster analyses. Finally a map is shown detailing the geographic location of the clusters identified. Cluster names in italics refer to clusters for which further hierarchical analysis was performed. Cluster names in regular text refer to final cluster assignment groups.

Figure 4. Proportion estimates from independent 100% simulation studies of the genetic baseline at Level 1 and all stages of the iterative formation of the Level 2 assignment unit levels. Points represent mean estimates with bars showing 95% confidence intervals.

750 Figure 5. A) Mixed stock fishery estimates using fish removed from the baseline and used as
751 fishery mixtures. B) Mixed stock fishery estimates using simulated equal proportions of fish
752 from each assignment unit in the mixture. C) Mixed stock fishery estimates using entire
753 rivers removed from the baseline and used as fishery mixtures. Dark bars represent actual
754 proportions in the mixture files and grey bars ONCOR estimates. Bars represent mean
755 estimates with 95% confidence intervals around these estimates. NOTE change of Y-axis
756 scale for the Level 1 and 2 assignment levels.

757

758 Fig. 1.

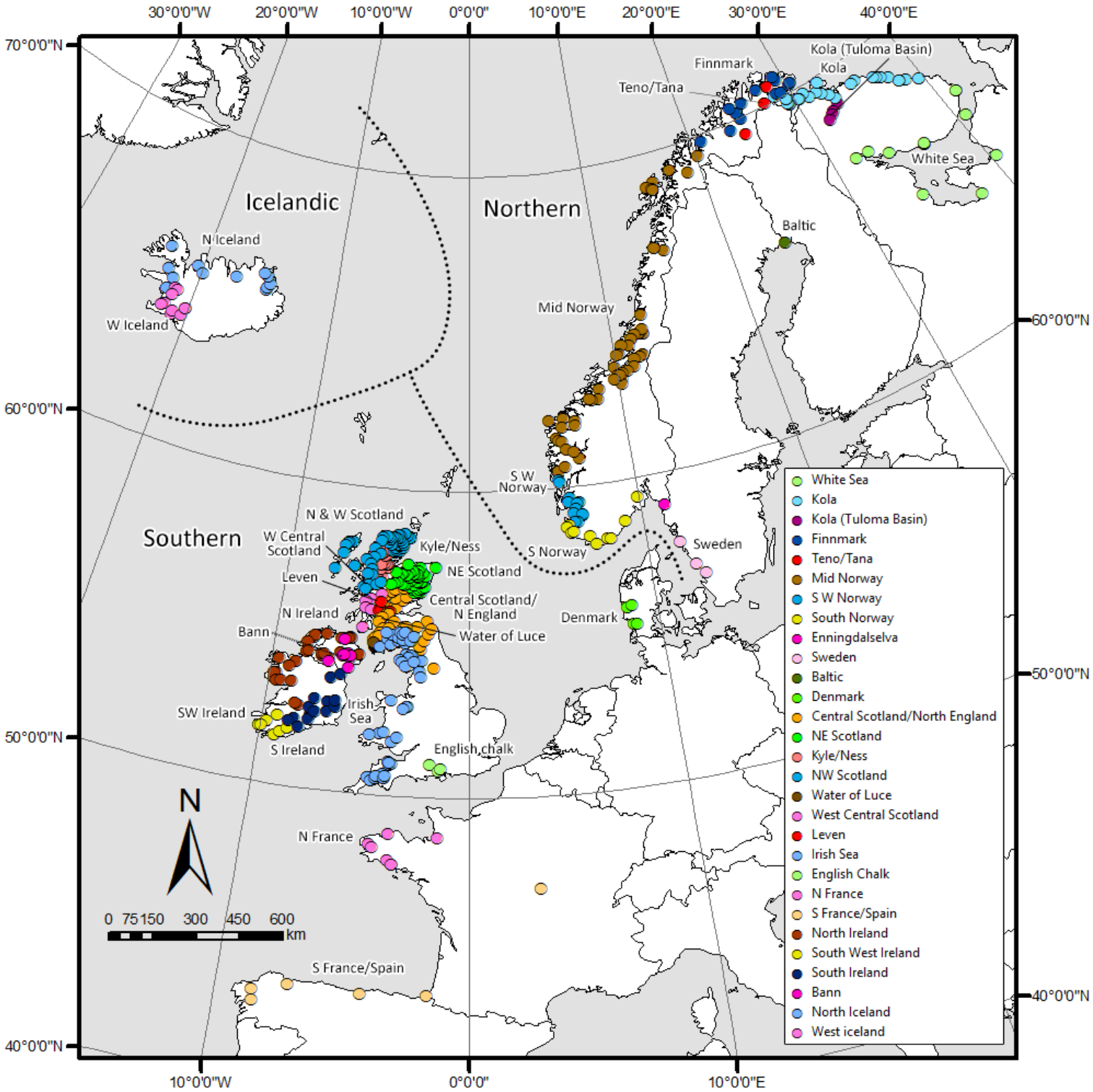


Fig. 2.

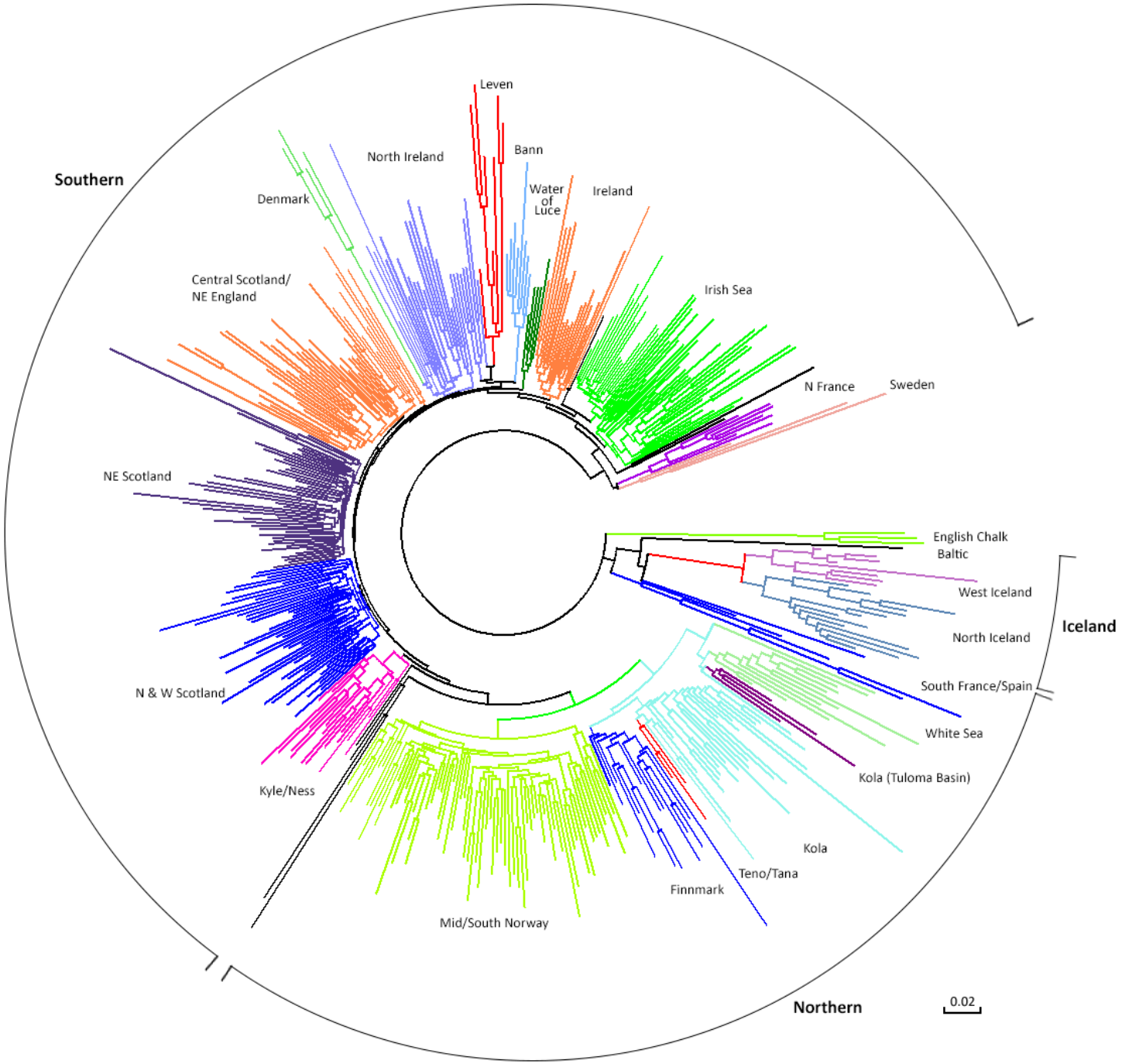
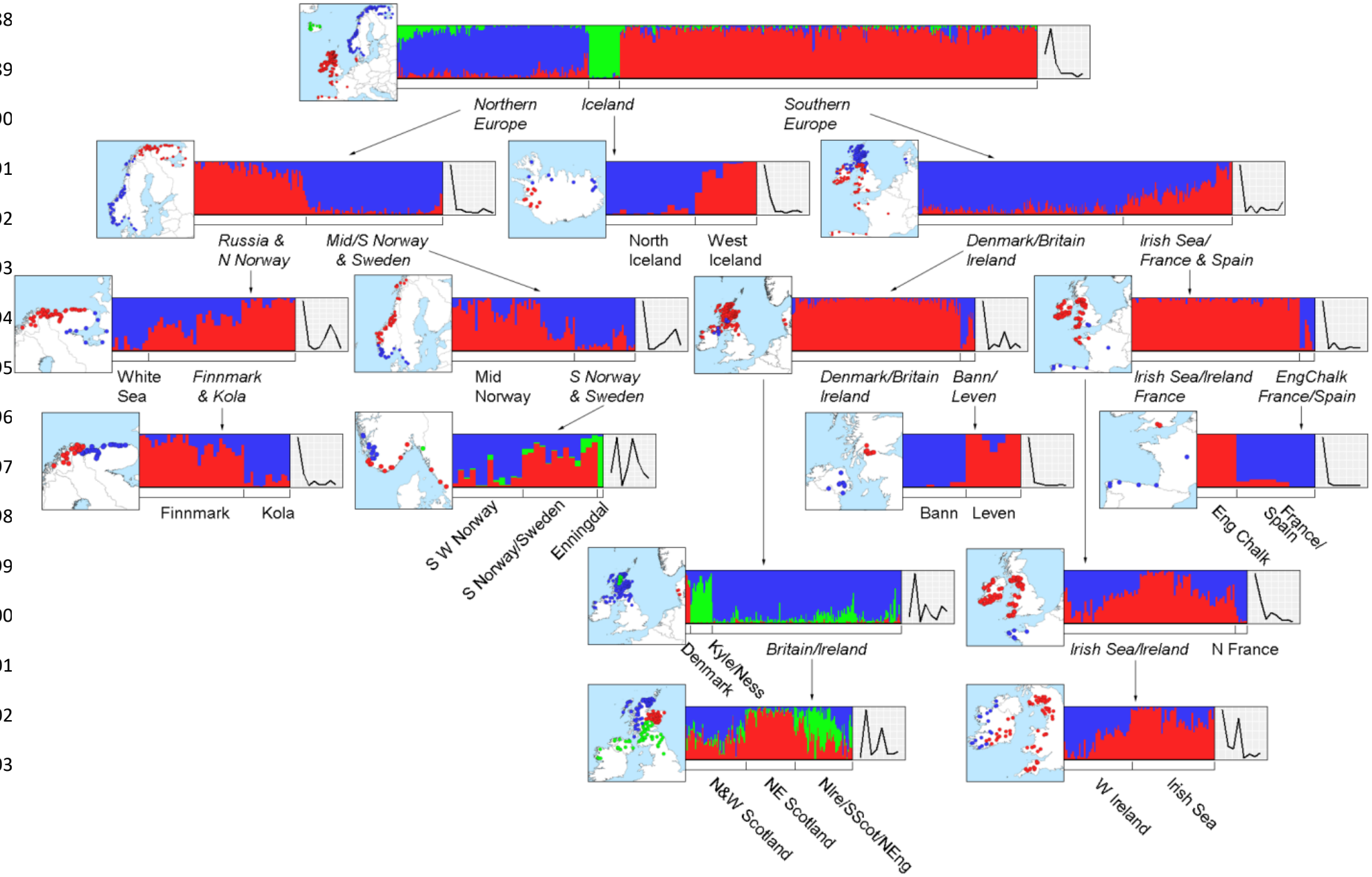
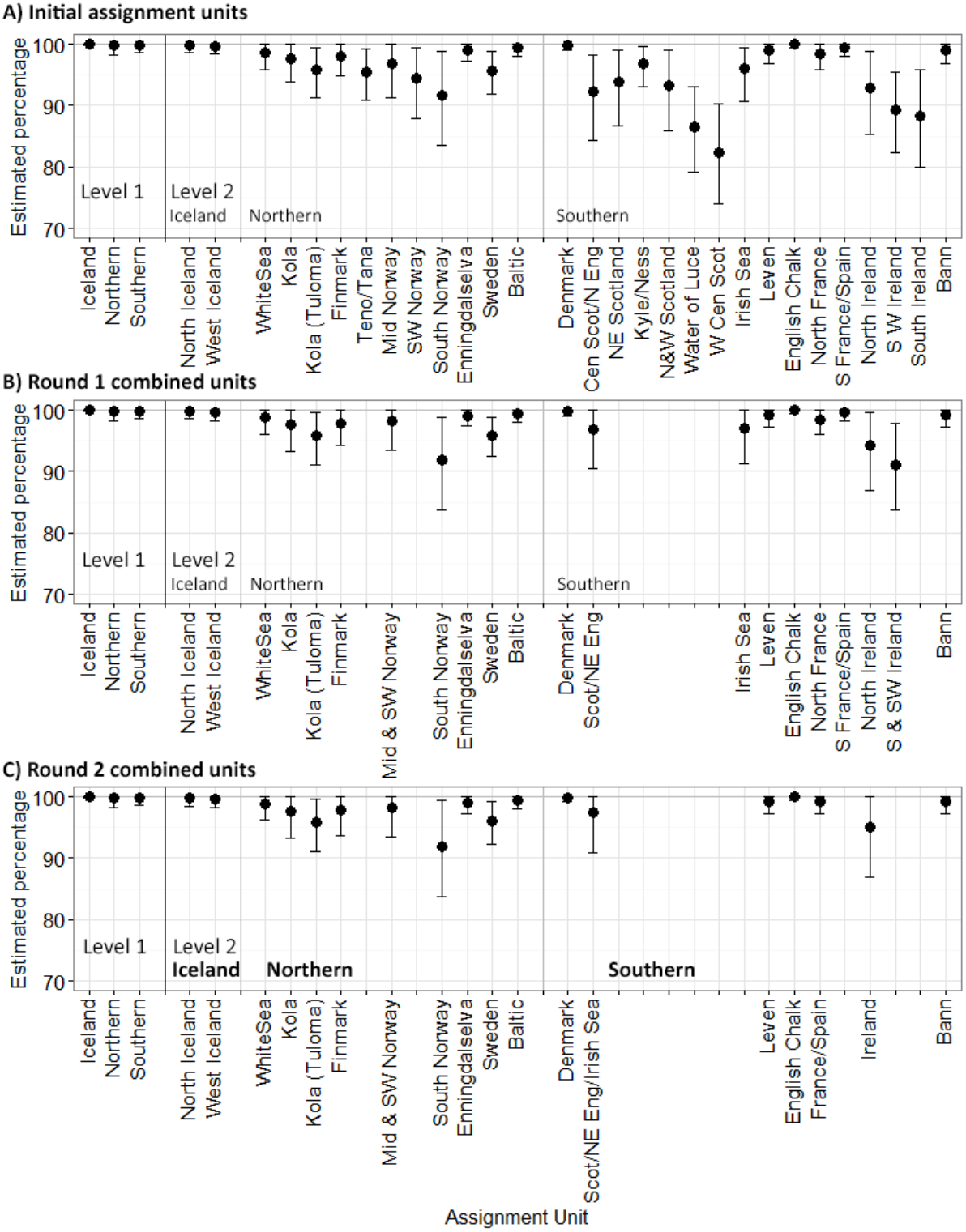


Fig. 3.



804 Fig. 4



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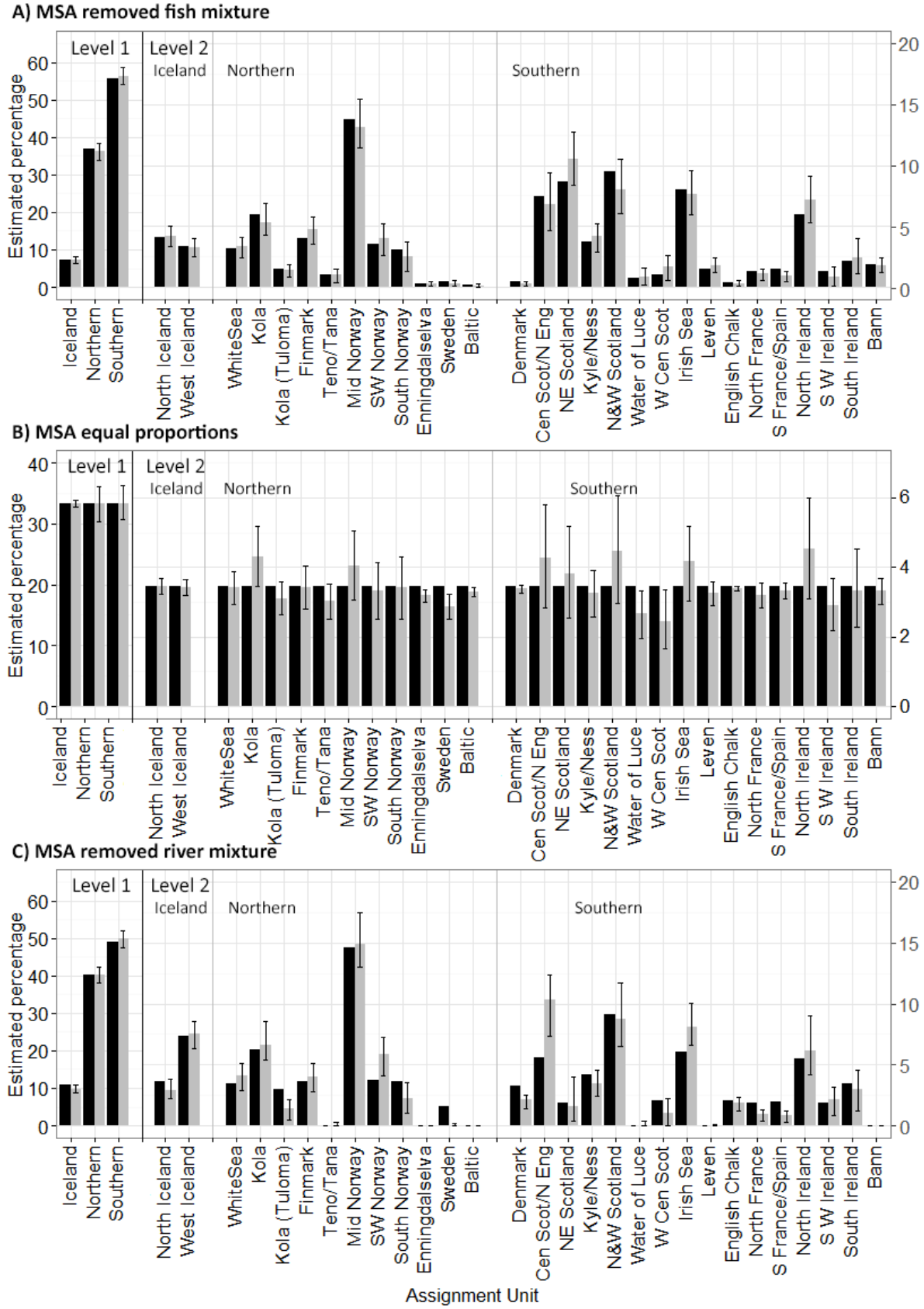
807 Fig. 5



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