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E. Cauwelier, J. Gilbey, and S.J. Middlemas



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# **GENETIC ASSIGNMENT OF MARINE-CAUGHT ADULT SALMON AT ARMADALE TO REGION OF ORIGIN**

E.Cauwelier, J. Gilbey, and S.J. Middlemas

Marine Scotland Science, Freshwater Fisheries Laboratory, Faskally, Pitlochry,  
PH16 5LB, UK.

## **Executive Summary**

The Scottish Government aims to generate 100% of its electricity from renewable resources, including tidal and wave power, by 2020. These devices have the potential to influence the migratory behaviour and survival of salmon. Little is known about migratory behaviour of returning adults and a better understanding of the migratory routes of fish in the Scottish coastal area will be of benefit in the ongoing development of this new industry. Historical tagging studies have shown that there was considerable scatter in the recaptures of salmon tagged in various locations around Scotland, with fish being found far from their natal rivers. More recently, satellite tags have enabled at-sea locations of salmon on their return migrations to be collected in Scottish waters. However, a thorough understanding of the tagging information was constrained by a lack of knowledge of where the salmon were headed before their tags detached. Here, genetic assignment techniques are used in order to investigate the origins of salmon satellite-tagged at Armadale on the north coast of Scotland. Of all the tagged fish with a location error of less than 25 km, 53 out of 75 (70%) fish could be assigned accurately to river or region of origin. A single fish was found to be of Norwegian origin and, within Scotland, the majority of fish assigned to the North/West (46%) and East Coast (44%) assignment regions. The marine distribution of tagged fish assigned to different parts of Scotland showed no obvious pattern. As such, results of this analysis confirm those seen with conventional tagging investigations and show that homing fish show considerable spatial mixing around the Scottish coast before finally returning to their natal rivers.

It is thus also clear that the deployment of renewable energy devices may not only affect local salmon populations, but also more distant ones.

## Introduction

The Scottish Government aims to generate 100% of its electricity from renewable resources, including from wind, tidal and wave power, by 2020 (Anon, 2011) and part of this development will be the installation of offshore power generating devices. However, these devices, along with the disturbance created during installation, could potentially have effects on migratory behaviour and survival of salmon through collisions and interactions with the electromagnetic fields that may be generated (Gill, 2005; Malcolm *et al.*, 2010; Slabbekoorn *et al.*, 2010; Gill *et al.*, 2012). A better understanding of the migratory routes of fish in the Scottish coastal area will thus be of benefit in the ongoing development of this new industry.

Atlantic salmon (*Salmo salar* L.) in Scotland are anadromous, spawning in freshwater, and migrating to the marine environment in search of feeding opportunities. Adult fish then return to their natal river to spawn, although little is known about the routes they take during their return migration. What little is known about the period of migration between encountering the UK coastline and entering natal rivers, for example, is based on the recovery and reporting of conventional tags at coastal netting stations (Shearer, 1992), constraining knowledge to the nearshore area. Malcolm *et al.* (2010) reviewed the migratory routes of returning salmon along the Scottish coast, based on such tag- recapture studies. They observed that there was considerable scatter in the recaptures of salmon tagged in various locations around Scotland with, for example, fish tagged on the west coast subsequently appearing on the east (Menzies, 1937; 1938a,b). For those fish tagged on the north coast subsequent recaptures were made from throughout Scotland (Calderwood, 1920; Menzies, 1937).

Recent advances in telemetry have enabled at-sea locations of salmon on their return migrations, provided by pop-up satellite transmitters, to be collected in Scottish waters (Godfrey *et al.*, 2014; 2015). While these data provide unique insight

into the use of Scottish coastal waters by salmon, they were constrained by a lack of knowledge of where the salmon were headed before their tags detached. For example, it is unclear whether all salmon locations to the east of the tagging site on the north coast were fish heading to rivers in the east or whether, as previous work would suggest (reviewed in Malcolm *et al.*, 2010), there was more mixing and the lack of any obvious spatial pattern with respect to final destination.

Here, we use genetic stock identification techniques (Hess *et al.*, 2011, 2014; Larson *et al.*, 2014) in order to investigate the origins of the satellite-tagged salmon reported in Godfrey *et al.* (2014; 2015). Salmon are assigned using a baseline of single nucleotide morphisms (SNPs) developed for Scotland and the northeast of England (Gilbey *et al.*, submitted). Where possible, genetic assignments were combined with geographical location information to examine the hypothesis that there is no obvious patterns of migratory routes displayed by fish of different origin.

## **Methods**

### **Genetic samples**

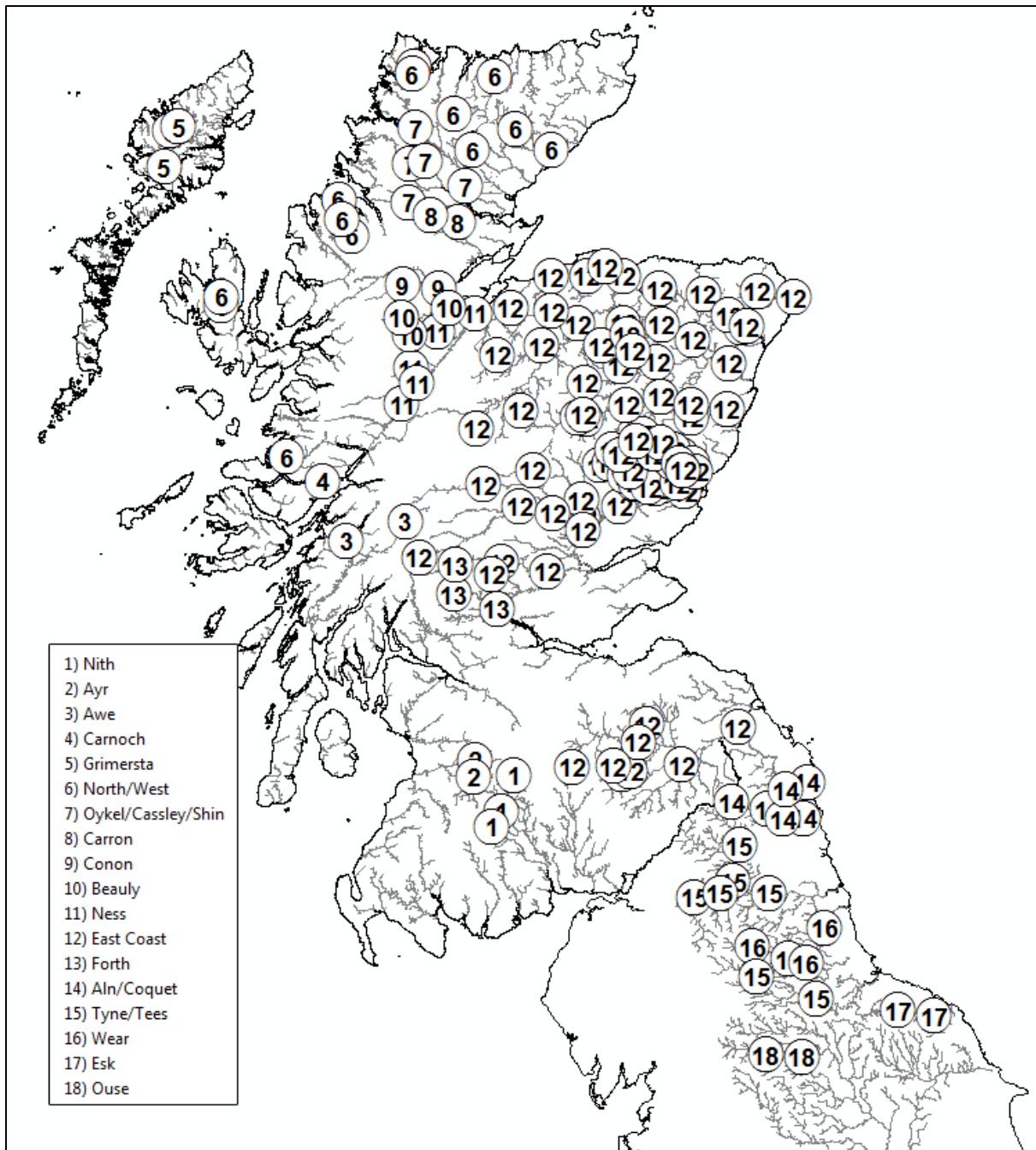
Adult salmon were caught at Armadale (Lat. 58.556; Long. -4.093), on the north coast of Scotland from May till July in two consecutive years (2013 and 2014) using bag nets. One hundred and thirty two fish larger than 70 cm and in good condition were fitted with satellite tags (Godfrey *et al.*, 2014) and a fin clip was taken from each tagged fish and stored in ethanol until analysis. The tags were programmed to release from the fish at periods from 1-10 (2013) and 1-20 (2014) days after tagging. The location of tag release was recorded, as well as subsequent recaptures of any fish post-tag release. Only tagged fish with a tag release location error of less than 25 km (Godfrey *et al.*, 2014, 2015) were used in the analysis.

## **Assignment regions**

Eighteen assignment regions were used in the analysis, based on those found by Gilbey *et al.* (submitted). Some of these regions consisted of a single river, while others comprised a number of adjacent rivers (Fig. 1). In addition, genetic information from three rivers in Ireland and four rivers in Norway were obtained (Bourret *et al.*, 2013) and added to the baseline, as returning adults of non-Scottish origin could potentially be caught off the Scottish coast (Malcolm *et al.*, 2010).

## **SNP genotyping**

Genomic DNA was extracted from the fin clips of the fish using the DNEasy blood and tissue purification kit (Qiagen), following the manufacturer's protocol. Baseline sample SNP genotyping was carried out at the centre for integrative genetics (CIGENE), Norway. Fish were genotyped at 5,568 SNP loci using a modified version of a custom-designed Illumina® iSelect SNP-array (Bourret *et al.*, 2013). A panel of 288 SNPs was identified for regional assignment from those available (Gilbey *et al.*, submitted). Adults were subsequently screened at this panel using the Fluidigm EP1 SNP genotyping platform (Fluidigm Europe b.v., Amsterdam, the Netherlands), again based on the manufacturer's protocols and work flow. SNP genotypes were determined using the Fluidigm SNP genotyping analysis software (Fluidigm Europe b.v., Amsterdam, the Netherlands) with all genotypes being triple checked. SNPs for which less than 60% of the fish could be reliably genotyped were removed from the analysis. Once those SNPs were removed, individuals that were genotyped for less than 70% of the SNPs were also removed.



**Figure 1** Map of Scotland and north England showing baseline samples representing assignment regions identified and defined by Gilbey *et al.* (submitted).

### Assignment of Armadale fish to baseline

Individual assignment of each adult to the baseline was carried out both using a Monte-Carlo resampling approach (Rannala & Mountain, 1997) implemented in the programme GENECLASS (Piry *et al.*, 2004) and a Bayesian assignment approach



as implemented in ONCOR (Kalinowski *et al.*, 2007). Individual assignments were made to site and scores combined to assignment region to obtain an overall score of assignment to region. The assignment results of each individual fish were compared between the two approaches. An assignment score likelihood (GENECLASS) cut off of 80 or assignment probability (ONCOR) cut off of 0.80 was applied, with fish having scores below this threshold being defined as unassigned. This has previously been shown to provide a balance between the numbers of fish assigned and the accuracy of the assignments (Gilbey *et al.*, submitted).

### **Tagged fish**

The geographical location of tag release/recovery of the tagged fish was mapped and combined with the individual assignments to examine the distribution of fish along the coast.

## **Results**

### **Genetic samples**

In total, 135 were tagged over the two years of the study. Three individuals had a SNP scoring percentage less than 70% and were removed from subsequent analyses, giving a total of 132 tagged fish, 50 from 2013 and 82 from 2014. Of these, geographical location was available for 103 fish, 75 of which had a location error less than 25 km, 34 from 2013 and 41 from 2014.

### **Individual assignment**

Using the two cut-off thresholds resulted in 61 (81%) of the 75 fish with acceptable locations being assigned using GENECLASS and 63 (83%) using ONCOR. When both sets of assignment were combined, and after applying the cut off to both sets of assignments, 53 fish (70%) were considered to be assigned (Table 1). Of these, three fish (4%) assigned to different regions when the two different programmes, ONCOR and GENECLASS, were compared. In all three cases, ONCOR assigned

the fish to the North/West region, whilst GENECLASS assigned two fish to the East Coast region and the third to the Beaulieu.

### **At-sea location**

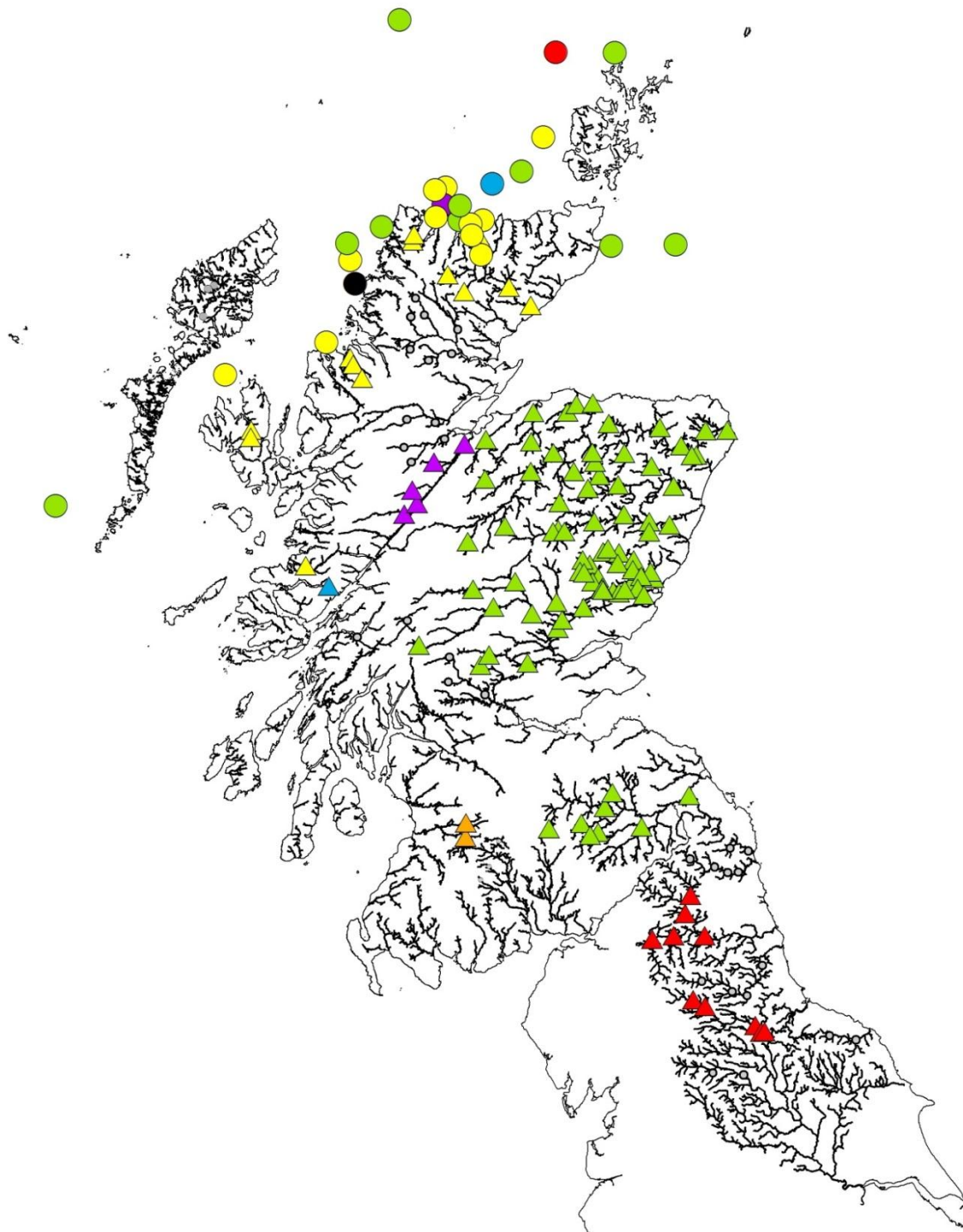
In 2013, out of the 34 tagged fish that had a tag release location error < 25 km, 25 were assigned with likelihood/probability scores  $\geq 80/0.8$  for both GENECLASS and ONCOR (Table 1, Fig. 2). In 2014, 43 fish had a tag release location error < 25 km and 29 of these fish were assigned to their genetic region of origin with likelihood/probability scores  $\geq 80/0.8$  (Table 1, Fig. 3).

In 2013, tagged fish covered six regions, dominated by the North/West and East Coast regions, whilst in 2014, only three regions were represented in the tagged fish, mostly assigning to the East Coast region and the North/West. In the latter year, three fish that entered the rivers Spey, Deveron and Dee assigned as originating from the East, which included those rivers.

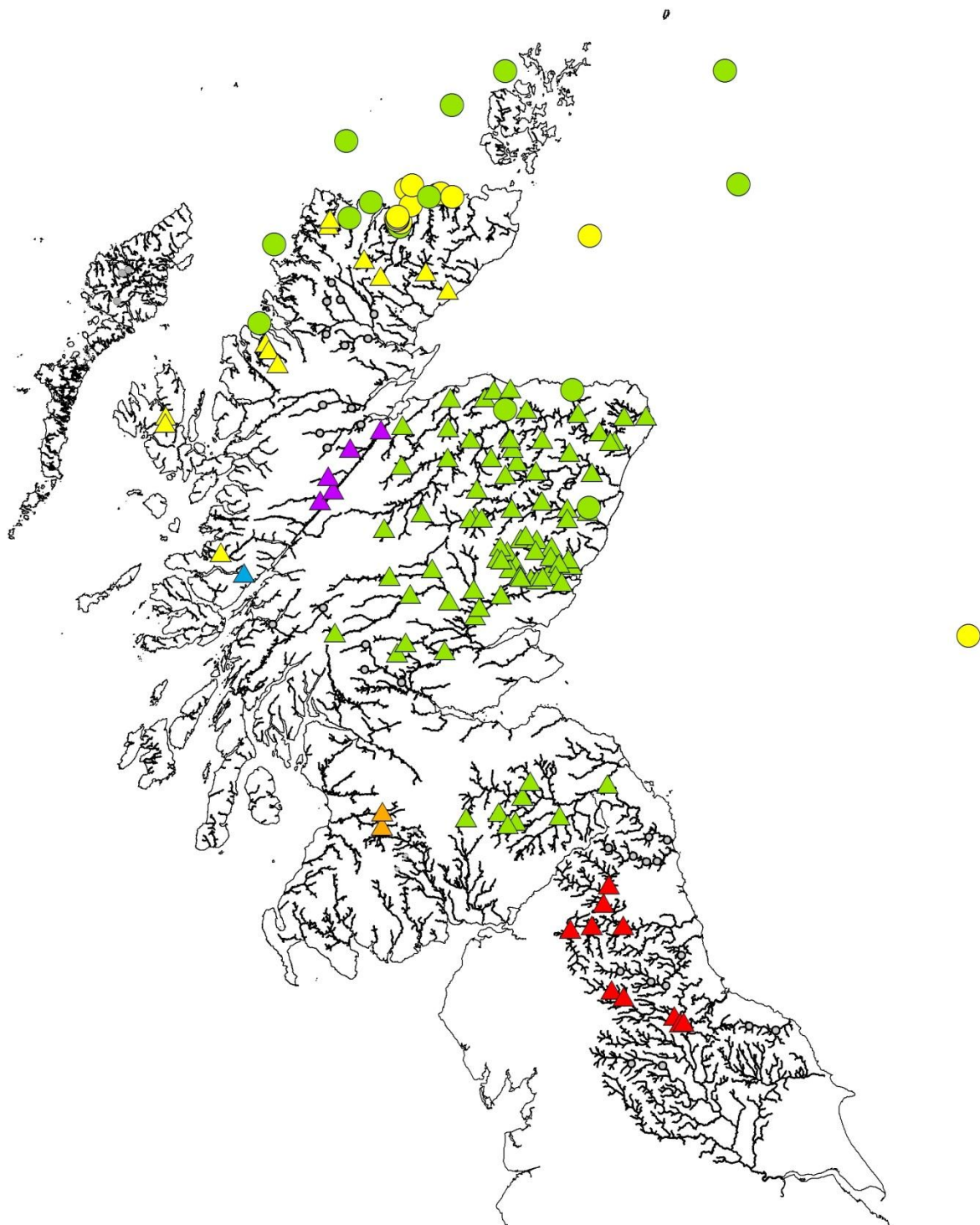
Five fish (two in 2013 and three in 2014) that were genetically assigned were recaptured following tag release (Table 2). For three of these fish (L5159, N5739 and N5732), the satellite tag had been released in the river in which they were subsequently recaptured, two of which at spawning time. They were all correctly assigned to the region they were found, North/West and East Coast, respectively. The other two fish, which both assigned to the East Coast region, were subsequently caught at sea (Table 3).

**Table 1** Number (and percentage) of the satellite tagged fish with acceptable location error assigned to the various assignment regions (cut-off score of 80% for both ONCOR and GENECLASS applied and differently assigned fish have been removed). Fish have been divided into year of capture.

Assignment region	2013	2014
Ireland		
Norway	1 (4.0)	
Awe		
Aln/Coquet		
Ayr		1 (3.4)
Beaully		
Carnoch	1 (4.0)	
Carron		
Conon		
East Coast	10 (40.0)	14 (48.3)
Esk		
Forth		
Grimersta		
Ness	1 (4.0)	
Nith		
North/West	11 (44.0)	14 (48.3)
Ouse		
Oykel/Cassley/Shin		
Tyne/Tees	1 (4.0)	
Wear		
Total number assigned	25	29



**Figure 2** Map of Scotland showing baseline samples (triangles) representing assignment regions: Ayr (orange), Carnoch (blue), North/West (yellow), Ness (purple), East Coast (green) and Tyne (red). The other baseline sites, representing region where the tagged fish did not assigned to, are represented by small grey circles. Big circles represent the tag location of the adult fish from 2013 and colour-coded to the region they assigned to. Black circles represent fish assigned to Norway.



**Figure 3** Map of Scotland showing baseline samples (triangles) representing assignment regions: Ayr (orange), Carnoch (blue), North/West (yellow), Ness (purple), East Coast (green) and Tyne (red). The other baseline sites, representing region where the tagged fish did not assigned to, are represented by small grey circles. Circles represent the tag location of the adult fish from 2014 and colour-coded to the region they assigned to.

**Table 2** Details of recaptured fish after tag release, including fish ID, assignment region, tag release location and subsequent recapture location.

Fish ID	Genetically assigned region	Tag release location	Subsequent recapture location
L5159	East Coast	40km East of Wick	Fish caught as brood stock in River Errochty, Tay
L5173	East Coast	20km west of Hoy	Caught in NE England drift nets
N5713	East Coast	8km W Westray	Fish subsequently re-caught at original capture site (Armadale nets) approx 3 weeks later
N5739	North/West	River Naver	Fish angled in river Naver approx 3 weeks later
N5732	East Coast	River Spey	Fish angled in the river Spey approx 3 months later

## Discussion

Across all years, 70.1% of those fish which produced accurate satellite tag locations were considered to be assigned when a cut-off was applied to both individual assignment techniques. A single fish (1%) was of Norwegian origin and, within Scotland, the majority of fish were assigning to the North/West (46%) and East Coast (44%) regions. The at-sea pop-up locations of the fish assigned to the various regions show considerable mixing. The accuracy of the technique was illustrated by the fact that all three fish subsequently re-caught in rivers were assigned to the correct regions, as were three fish where tags deployed within rivers.

Most fish assigned to the two largest geographic regions, the East Coast and the North/West. This is perhaps not surprising, as those regions cover a significant proportion of the Scottish coast and associated rivers. The East coast covers the rivers from the Nairn to the Tay and the river Tweed, which includes some of the largest rivers in Scotland and which have a combined share of the rod catch of salmon of ~72-74% of the Scottish total (based on 2013-2014 rod catch returns; Marine Scotland Science, 2014). Similarly, the North/West region currently stretches from the Moidart in Argyll to the Helmsdale near the Dornoch Firth and thus contains a significant number of smaller salmon rivers that have a combined rod catch of ~10-14% of the Scottish total. Even though it is currently not possible to accurately and

reliably assign fish to individual rivers within each of these regions, this study gives an insight into marine utilisation of returning adults from different parts of Scotland.

The at-sea distribution of adults assigned to the different regions is consistent with previous physical tagging results, with fish from the different assignment regions showing considerable spatial mixing. This would again suggest that fish of different origin migrate around the north coast of Scotland and, once the coastal environment is reached, home towards their natal river, a process that can take a few weeks and may involve large distances in areas far from their natal rivers and not on direct routes to these rivers. Malcolm *et al.* (2010) reviewed and summarised the migratory routes of adult Atlantic salmon, based on physical tag-recapture studies, which also showed that adult fish returning on the north coast appear to move both to the east and west before eventually finding their natal rivers (Menzies, 1937).

The findings of this study, together with previous observations, show that the deployment of renewable energy devices and/or other offshore installations have the potential to affect both local and more distant salmon populations. Furthermore, individual fish may move past the same point on a coastline more than once and hence, potentially, encounter the same installation multiple times.

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