Scottish Government
Riaghaltas na h-Alba
gov.scot

Application of acoustic tagging, satellite tracking and genetics to assess the mixed stock nature of coastal net fisheries

Scottish Marine and Freshwater Science Vol 9 No 5

J D Armstrong, N R Gauld, J Gilbey and D J Morris

marinescotland

Application of acoustic tagging, satellite tracking and genetics to assess the mixed stock nature of coastal net fisheries

Scottish Marine and Freshwater Science Vol 9 No 5

J D Armstrong, N R Gauld, J Gilbey and D J Morris

Published by Marine Scotland Science
ISSN: 2043-7722
DOI: 10.7489/12094-1

Marine Scotland is the directorate of the Scottish Government responsible for the integrated management of Scotland's seas. Marine Scotland Science (formerly Fisheries Research Services) provides expert scientific and technical advice on marine and fisheries issues. Scottish Marine and Freshwater Science is a series of reports that publishes results of research and monitoring carried out by Marine Scotland Science. It also publishes the results of marine and freshwater scientific work that has been carried out for Marine Scotland under external commission. These reports are not subject to formal external peer-review.

This report presents the results of marine and freshwater scientific work carried out by Marine Scotland Science.

Application of acoustic tagging, satellite tracking and genetics to assess the mixed stock nature of coastal net fisheries

John D. Armstrong, Niall R. Gauld, John Gilbey & David J. Morris

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

Executive summary

An array of 78 acoustic receivers was distributed among 47 rivers predominantly on the east and north coasts of Scotland. A further 8 receivers were moored on the north and east coasts near to shore. A total of 81 salmon captured in the Armadale coastal net fishery between 7 July and 25 August 2017 were tagged externally with small acoustic transmitters and released. Genetic samples were taken from each fish. Of the tagged fish, 44 (54%) were detected in rivers, one on the east coast (River Spey) and the others on the north coast (Rivers Naver, Borgie, Strathy, Halladale, Kinloch and Polla). The results are consistent with the main impact of the fishery being in the order of 100km, with occasional salmon captured from further away. Genetic analysis (in the absence of confirmation by tracking) would have suggested that the fishery was substantially more mixed stock with 30% of the catch being from the east coast and Hebrides. In fact, many of the fish assigned by genetics to east coast stocks appeared to return to north coast rivers. Possible explanations for this discrepancy are discussed. A range of patterns of movement of salmon among different rivers was observed. Tagged fish were detected near to shore on the north coast in the marine receiver array.

Results of these 2017 experiments were compared with data collected in 2013 and 2014 using satellite tagging and associated genetics. In that case there was evidence of greater interception of east coast salmon at Armadale and good correspondence between predictions of home river from genetics and observations from satellite tagging, albeit based of a small sample size.

Each salmon sampled in 2017 was assessed for presence of red vent syndrome (RVS) whereby the vent area of the fish was swollen and reddened. The incidence of RVS was 82%, higher than recorded previously. No difference could be detected in the subsequent survival of fish with and without RVS, but the power to detect any effect was very low because there were so few fish without the syndrome.

1. Introduction

The tagging/netting studies detailed in Downie et al. (2018) provide an impression, from historic investigations of certain Scottish rivers and netting stations, of the geographic range of river stocks that comprise a coastal net fishery. However, the extent of that assessment is inevitably limited both in the detail that it provides and the geographical coverage. This is largely due to limitations in the technology available at the time that required tagged animals to be recaptured. Other scientific methods are now available that may extend our understanding, including satellite tracking, acoustic tracking and genetic analysis. This report considers results from these approaches by re-analysing data from a satellite tracking experiment (Godfrey et al. 2014) and obtaining new information using acoustic tracking. In both cases, the tracking data were coupled with genetic assessment of the home regions of the sampled fish.

The study site was the Armadale netting station on the Scottish north coast. Previous work (Downie et al. 2018) indicates that salmon intercepted at this station are likely to be from river stocks on the north, east and west coasts of Scotland. Similarly, tracking fish at this site using satellite transmitters (Godfrey et al. 2014) found evidence of interception of spring-running salmon from a wide area.

Genetic stock identification (GSI) may have potential for identifying the stock origins of all coastal net fisheries without any need for physical tagging (Gilbey et al. 2016a). For example, GSI of salmon captured in the NE English coast net fisheries indicated that although most fish were from local rivers, a significant number were from different areas around the Scottish coast (Gilbey et al. 2016b). Salmon are believed generally home to their native river, although a proportion of the population strays (Dolloff et al. 1994; Keefer et al. 2014; Salmenkova 2017). Such homing to natal rivers, in combination with factors such as founder effects, isolation, selection, genetic drift, and broad scale phylogeographic processes, has resulted in significant population structuring at a hierarchy of levels from intra-river to inter-continental (King et al. 2001). Furthermore, local adaption of populations has evolved (Garcia de Leaniz et al. 2007) including variations in marine migratory patterns amongst populations from different parts of the species range (Webb et al. 2007). Genetic differences have thus developed between stocks among rivers, areas within rivers, and/or between regional groups of rivers in Scotland (Gilbey et al. 2016a; Cauwelier et al. 2018). By analysing the genotypes of salmon caught in the fishery it may be possible to determine, with some degree of precision and certainty, the rivers and regions that are being affected by capture of homing salmon in that fishery. Such an approach has been used to manage salmonid and other fisheries (e.g. Waples et al. 1990; Griffiths et al, 2010; Araujo et al. 2014; Casey et al. 2016) and depends among other factors on the robustness of the baseline of genetic variation among rivers and regions. The baseline developed by Gilbey et al. (2016a) is skewed, with a focus towards the large east coast rivers. The west coast coverage is at a lower level and as such is sub-optimal. Here, in some cases single rivers act as regional representatives and in other areas there is no coverage at all. The Scottish genetic assignment baseline is based on Single Nucleotide Polymorphic (SNP) markers and although in some cases single rivers can be identified, in the majority of situations adjacent rivers have been combined to act as regional assignment units to which assignments are made. There are 18 assignment units which include large regional

east coast and north/west assignment units comprising a number of rivers (Gilbey et al. 2016a).

Acoustic tracking has potential to assess directly the destination rivers of fish intercepted by nets. It may also provide greater insight into the detail of homing behaviour and hence likely vulnerability of fish to interception by nets. Salmon have been recorded migrating close to shore as they return home (Hawkins et al. 1979). Some salmon captured and tagged in rivers have then returned to sea, suggesting that they explore lower ends of a number of rivers as they search for their home river, or use large rivers as over-summering refuges (Priede et al.1988; Stewart et al. 2009). It is not known whether this behaviour of searching lower rivers is widespread nor over what geographic distances salmon swim close to shore. However, it is likely that salmon are particularly vulnerable to coastal nets when in this near-shore coastal return stage of the life cycle. To explore their behaviour, salmon captured at the northern netting station at Armadale were tagged with miniature acoustic transmitters. A network of acoustic receivers was deployed within the lower ends of many of the rivers on the northern and eastern coasts of Scotland, with additional more sparse coverage in west Scotland, with the aim of following movements of the tagged salmon among lower rivers. Furthermore, near-shore coastal receivers, one array to the west of the tagging location and several on the east coast, were set up to determine whether salmon were indeed swimming near to shore on sections of their migration.

The opportunity was also taken to assess whether the incidence of “red vent syndrome” (RVS), a symptom of infection by the parasite *Anisakis simplex* whereby the vent is swollen and reddened, was associated with subsequent mortality. Captured fish were visually examined for RVS and then it was determined whether the occurrence of RVS influenced chance of surviving to return to a river as indicated by acoustic detection.

The main aims of this part of the project were to:

1) describe the range of river stocks that contributed to the catches at the Armadale coastal net station;
2) identify patterns of movements of salmon among rivers during the homing migration;
3) compare between results from acoustic tracking and genetics approaches;
4) determine whether incidence of RVS affected subsequent detection.

2. Materials and Methods

2.1 2017 Acoustic Tagging and Genetics Experiments

2.1.1 Receiver Deployment

With the help of local Fisheries Boards and Trusts, an array of acoustic receivers (TBR 700, Thelma Biotel, Trondheim, Norway; VR2/VR2W/VR2AR, Vemco, Bedford, Nova Scotia, Canada) was set up around the east and north coasts of Scotland and in a few rivers on the west coast (Fig. 1). In total, 78 acoustic receivers were

deployed in 47 rivers and eight marine deployments were positioned around the coastline (Fig. 1).

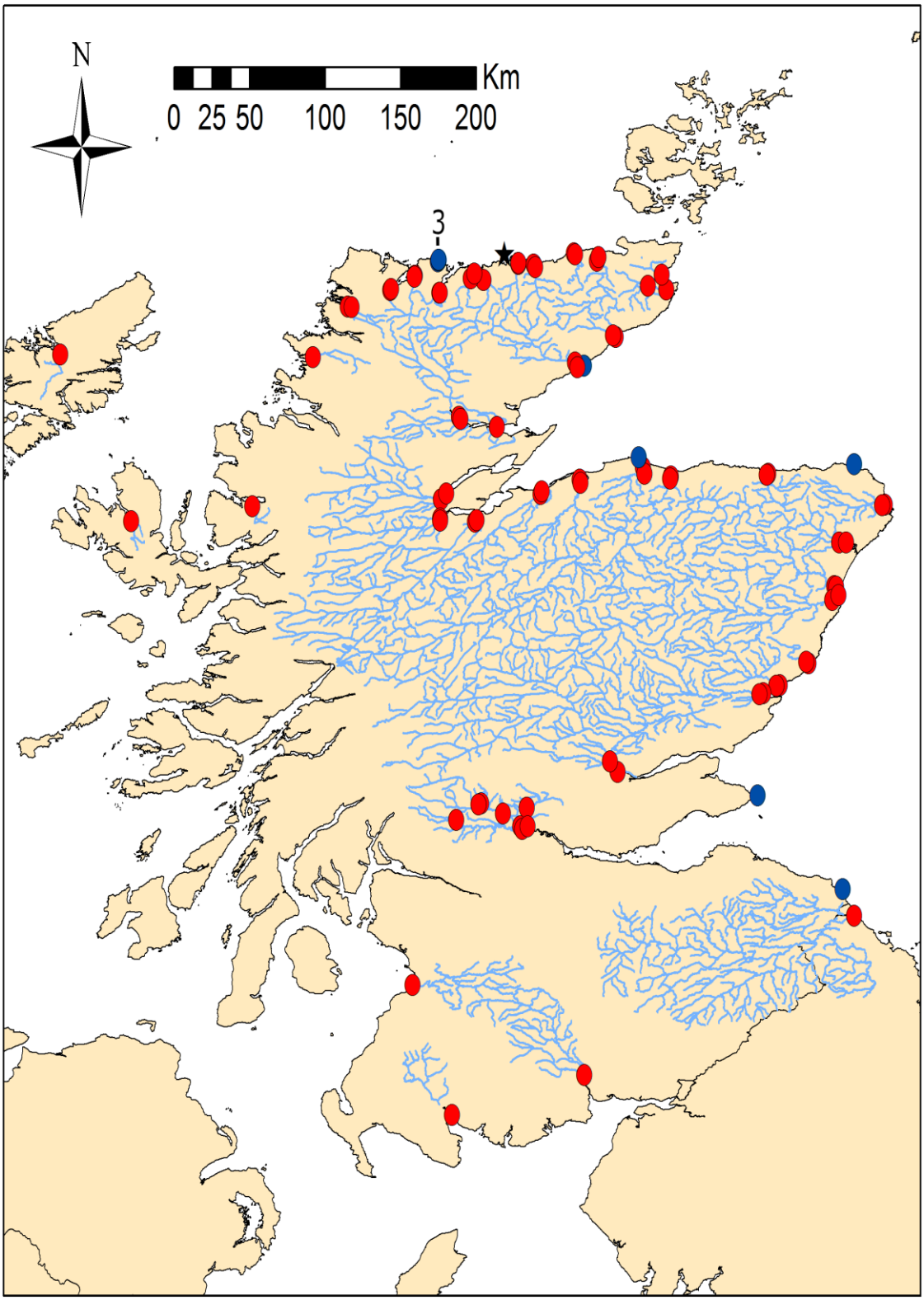

Figure 1: *A map of the receiver locations around Scotland. The black star denotes the release point, the red points represent receivers deployed in rivers and the dark blue points represent coastal receiver deployments. The light blue lines represent river catchments covered by the receiver array*.

2.1.2 River receivers

Pairs of receivers were deployed in lower reaches of each of the major east and north coast rivers. Single receivers were positioned in certain west coast rivers. Where possible, the lower receiver was positioned near the head of tide and the upper receiver was 1000-2000m further upstream. Locations were selected by local river managers to ensure safe access reasonable security from theft and vandalism and suitability for detecting salmon. Deployment locations are listed in Appendix 1.

2.1.3 Marine receivers

In addition to the river array, eight VR2AR acoustic receivers with integrated acoustic releases (Vemco, Bedford, Nova Scotia, Canada) were deployed in release canisters at seven locations around the Scottish coastline (Fig. 1). Deployments on the east coast were 400-600m from shore. The deployment to the west of the netting location was a line of three receivers at 400m intervals from shore and termed the “northern array”. Each release canister comprised a stainless steel cylinder containing a length of rope (5 mm diameter, Dyneema) with three flotation buoys (275 mm diameter). The release canisters were anchored by two 50 kg ship links and held 2m off the sea bed by a mooring rope. Deployment off the sea bed resulted in the equipment being less sensitive to shifting due to surface wave action and also less susceptible to damage from passing boats. For retrieval of a moored receiver, a portable acoustic receiver with transponding hydrophone (VR100, Vemco, Bedford, Nova Scotia, Canada) was used to trigger the acoustic release, causing the rope encased in the release canister to uncoil and the canister to raise to the sea surface. The entire mooring could then be retrieved without leaving hardware on the sea bed.

2.1.4 Tag design

Acoustic tags were modified so that they could easily be attached externally to salmon. Each acoustic tag (LP7.3 & DT-LP7, Thelma Biotel, Trondheim, Norway) was attached to a conventional “floy” tag (Floy FT-4 Lock-on, Floy Tag, Seattle, Washington, USA) just below the lock-on fastening using epoxy resin and the two tag types were secured together with 8 mm of adhesive-lined heat shrink tubing (Fig. 2). The tags were cured for 12 hours to allow the epoxy to set before use. Tags were made up using either 12.5 cm - or 20 cm- long floy tags with 20 cm tags being reserved for larger multi sea winter (MSW) fish. This choice of length prevented the trailing tags from damaging the dorsal fins of salmon. Choice of small light acoustic tags (rather than larger longer-lasting tags) was to minimise the possibility of abrasion from the trailing transmitter, and to use the smallest practical payload, thus minimising potential for impacts of the tags on fish performance.

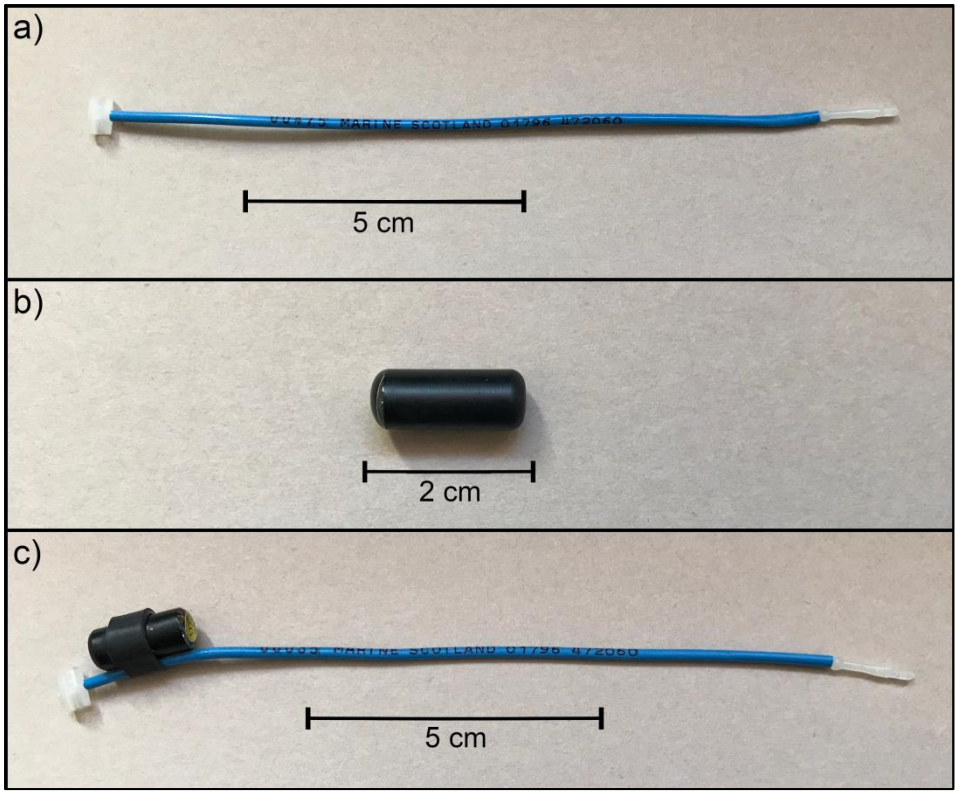

Figure 2: *Components of the external acoustic tag attachment including a) the floy tag, b) the acoustic tag and c) the fully integrated tags.*

2.1.5 Fish capture and tagging

During the period of 7 July – 25 August 2017, Atlantic salmon were caught in a bag net (Mills, 1989) at Armadale (Lat. 58.556; Long. -4.093) on the north coast of mainland Scotland. During the 50 days of the study period 56 tides were fished, whilst a further 44 tides remained unfished due to poor sea conditions.

Fish were removed from the bag net and immediately transferred into a holding tank (90 x 60 x 60 cm) of fresh sea water situated on the fishing vessel. Prior to tagging, a fish was placed in an sedation tank (80 x 60 x 40 cm) filled with anaesthetic solution (MS222, 80 mg.l^{-1}) until it no longer responded to external stimuli. It was then placed on a plastic lined v-shaped foam board with the dorsal surface facing upward. The fork length of the fish was then measured, 3-4 scales were collected from the area adjacent to the dorsal fin (for subsequent determination of sea age) and approximately 0.5 mm^2 of tissue for genetic analysis was collected by clipping the upper lobe of the caudal fin. The vent of the fish was also assessed for presence and level of the red vent symptom of *A. simplex* infection using a photo guide based on previous samples of salmon (e.g. Noguera et al. 2009). The levels of infection were categorised on a four point scale; 0 – no infection, 1 – mild infection, 2 – moderate infection and 3 –severe infection.

Both the tag and the tagging needle (157 mm long, 2 mm diameter, surgical steel, solid tipped cutting point) were sterilised with ethanol and then rinsed with sterile

saline. Before attachment the floy tag was fed into the hollow cavity in the end of the needle. The needle was then carefully pushed through the dorsal area of the fish approximately 10 mm below the dorsal fin, taking care not to hit and damage any pterygiophores. Once through the dorsal area, the needle was fed completely through the incision and the floy tag was removed from the hollow region of the needle. The two ends of the floy tag were then fastened together, locking the looped tag in place. The tag was positioned so that the acoustic emitter trailed behind the dorsal fin and then the fish was placed back into the holding tank. Once the fish was able to right itself and responded to external stimuli it was released via a landing net back into the sea, away from the capture net. Procedures were conducted under Home Office project licence number PPL 70/8928 by trained Marine Scotland Science staff with Home Office personal licences. During the study, 83 fish were tagged over 17 days, with 81 in total being released (Table 1).

Date	Number of fish tagged	Number of fish released	Average fork length (mm) [± SE; min-max]
07/07/2017	7 †	5	585 [± 11.5; 565 - 620]
08/07/2017	5	5	581 [± 7.8; 560 - 605]
09/07/2017	9	9	630 [± 33.1; 530 - 825]
10/07/2017	7	7	581 [± 15.3; 530 - 640]
12/07/2017	8	8	570 [± 10; 520 - 610]
14/07/2017	1	1	610
18/07/2017	1	1	530
21/07/2017	1	1	590
22/07/2017	1	1	592
23/07/2017	2	2	582 [± 12; 570 - 594]
27/07/2017	4	4	572 [± 24.6; 530 - 620]
28/07/2017	7	7	553 [± 14.8; 505 - 605]
30/07/2017	4	4	540 [± 7.1; 530 - 560]
31/07/2017	2	2	535 [± 25; 510 - 560]
01/08/2017	17	17	579 [± 14.4; 490 - 750]
02/08/2017	4	4	537 [± 11.1; 510 - 560]
13/08/2017	3	3	572 [± 21.7; 550 - 615]
Total	83	81	577 [± 53.9; 490 - 825]

† Two fish did not recover from anaesthesia during recovery after the procedure.

Table 1: *Dates and numbers of fish tagged and released during the study period along with the average fork lengths.*

In total 111 fish were caught in the bag net of which 81 (mean fork length 577, range 490-825 mm; Table 1) were tagged and released. A further 20 fish caught in the net were dead or moribund and had evidence of damage consistent with attack by seals. A further 8 salmon were euthanized because they were seriously damaged from the net or jellyfish stings. Finally, two fish were tagged but died as they did not recover from anaesthesia.

2.1.6 2017 Genetic analysis

Mixed stock fishery analysis was used to examine the origins of the sampled fish (Utter and Ryman, 1993, Begg et al. 1999, Bradbury et al. 2016). Assignments were performed using 288 Single Nucleotide Polymorphic (SNP) genetic markers as detailed in Gilbey et al. (2016a) using the genetic baseline as detailed in Fig. 3 which comprised 3,787 fish from 147 sites covering 27 rivers and consisting of 18 assignment units. All assignments were performed in ONCOR (Kalinowski et al. 2007) using the conditional maximum likelihood model of Rannala and Mountain (1997). Individual assignments were performed using a probability exclusion cut-off of 0.8 (see Gilbey et al. 2016a) and mixture proportions calculated using Mixed Stock Analysis (MSA).

It can be seen from Figure 3 that although the east coast of Scotland and NE of England has good reference baseline coverage, the baseline representation from the north and west of Scotland was relatively poor. The geographically large North/West assignment unit (No. 6 in yellow) has 13 sites and 354 fish (9.4% of total baseline fish). It should also be noted that the southern region of the west coast also has little coverage with large areas having no baseline representation at all.

2.2 2013/14 Satellite Tagging and Genetics

Data were considered from an investigation of salmon captured previously at Armadale and fitted with satellite transmitters (Godfrey et al. 2014). In that study, adult salmon were caught at Armadale from May till July in two consecutive years (2013 and 2014) using bag nets. A total of 132 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 genetic 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) were used in the analysis (Cauwelier et al. 2016).

Genomic DNA was extracted from the fin clips and each sample screened at a panel of 288 SNP genetic markers (Gilbey et al. 2016a) on the Fluidigm EP1 SNP genotyping platform (Fluidigm Europe b.v., Amsterdam, the Netherlands). SNP genotypes were determined using the Fluidigm SNP genotyping analysis software (Fluidigm Europe b.v., Amsterdam, the Netherlands) with all genotypes being triple checked.

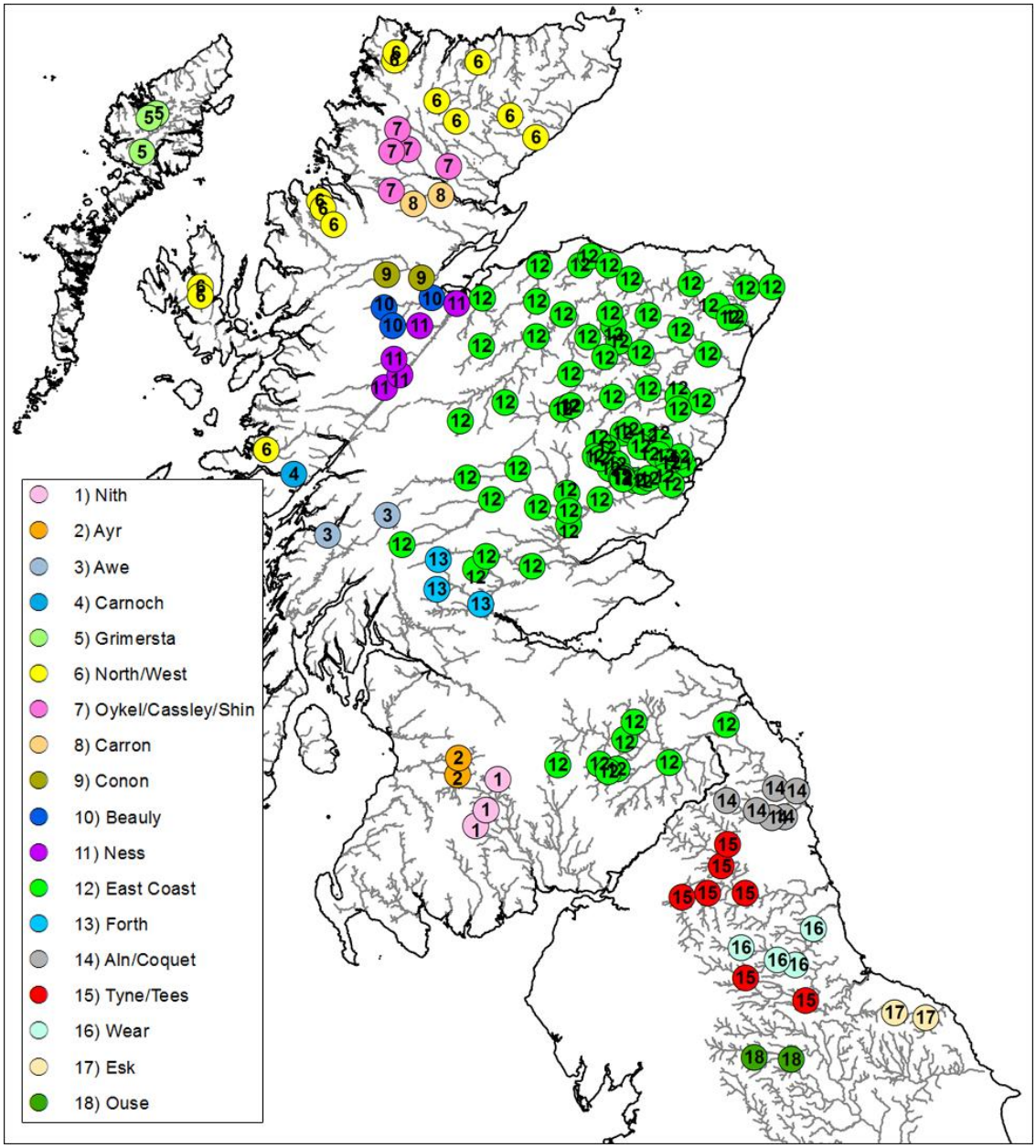

Figure 3: *Assignment units used for determining origin of tagged fish.*

Individual assignment of each fish to the genetic 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 an appropriate balance between the numbers of fish assigned and the accuracy of the assignments (Gilbey et al. 2016a).

3. Results

3.1 Salmon Tagged in July 2017

3.1.1 Acoustic Tagging

Of 81 fish tagged, 44 (54%) were detected in rivers. A single fish was recorded on the east coast that entered the River Spey 11 days after having been tagged. No fish were detected on the west coast. However, an account was received from an angler who had reportedly captured and released a tagged salmon in the River Balgy, but had not recorded the tag number. Efforts subsequently to detect the fish using an acoustic receiver were unsuccessful. All other records of salmon in rivers (43) were from the north coast. The majority of these fish were from the Naver (26, 60%) and Borgie (11, 25%). Receivers in the Hope detected two fish and single salmon were recorded in each of the Rivers Strathy, Halladale, Kinloch and Polla.

Of the 43 salmon recorded in northern rivers, 12 fish registered on the lower receiver only (Borgie: 5, Naver: 4, Kinloch: 1, Polla:1, Strathy:1). There is ambiguity over whether these fish moved upstream and were not detected on the upper receiver or departed to sea. Of these salmon, genetic assignments were North/West: 10 fish, East: 1 fish, Hebrides: 1 fish. Subsequent analyses are based on an assumption that these fish remained within the river and were not detected.

In total, seven fish were detected on the North Coast Marine Array and subsequent records of these fish were in the Naver (three fish), Kinloch (one) and Hope (one), two fish were not recorded again. There was no record of tagged fish on the east coast marine receivers.

The majority of fish movements were apparently directed immediately towards a final river, with 80% of detected fish entering only a single river. However, a proportion of the tracked fish (20%) entered multiple rivers before apparently committing to a single river catchment (Fig. 4). There did not appear to be a single general pattern to describe movements among river catchments, as, for example, six fish initially travelled west of their final river and three fish went further east initially. The time for tagged fish to enter their final river varied widely. Multiple-river-entrants took over 8 days longer on average than single-river-entrants to enter their final river (23.04 [range: 0.36- 75.86] and 14.72 [range: 0.36-86.67] days respectively).

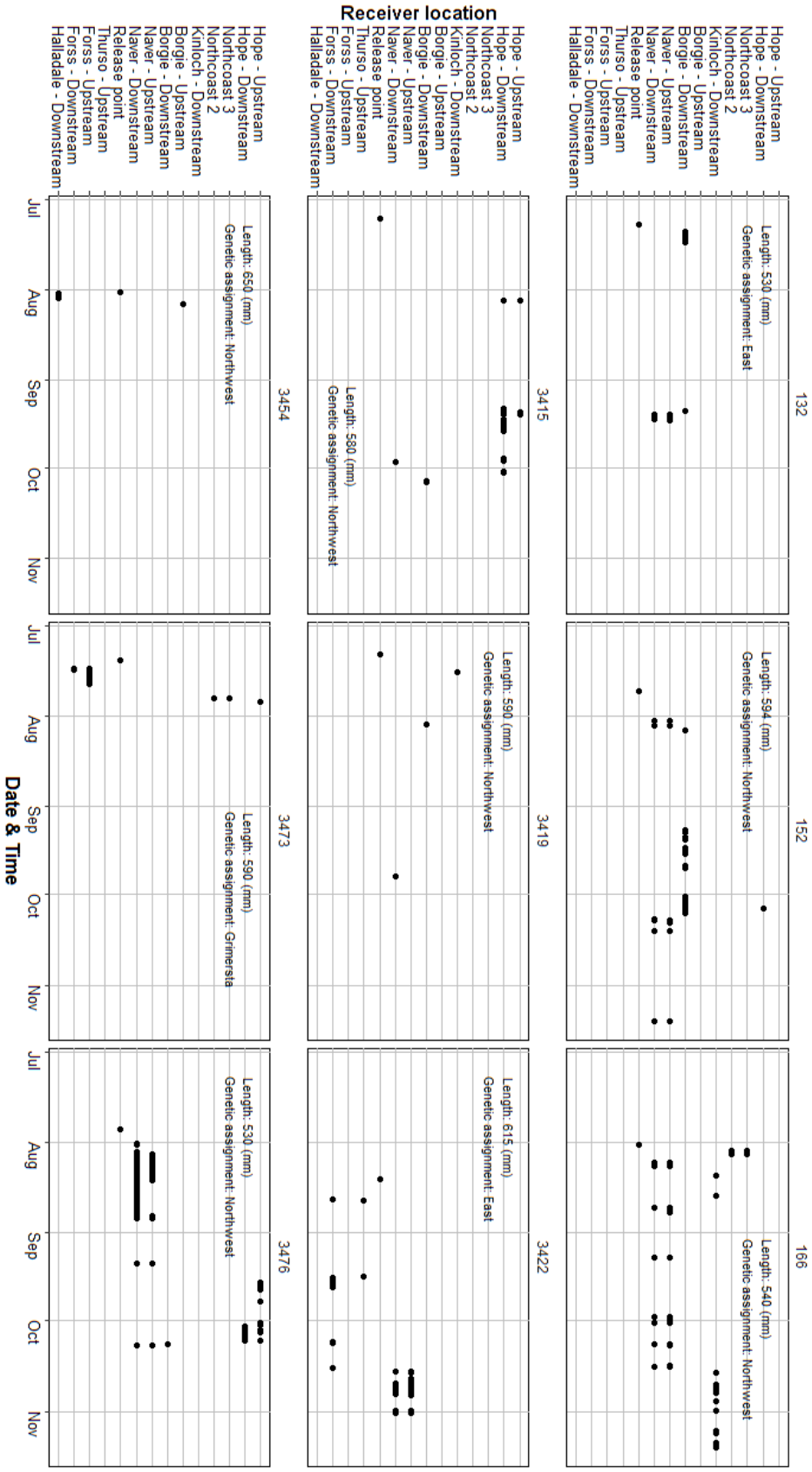

Figure 4: *Tracks of salmon that entered multiple rivers.*

3.1.2 Genetic Assignment of Acoustically-Tagged Salmon

The assessment of region/river of origin using individual assignment suggested that 57 salmon were from the North/West region, which includes Naver, Borgie, Halladale, Kinloch, Hope and Polla. A further 16 salmon of the sample were believed most likely to have originated from the east coast, 5 fish were assigned to the Hebrides and one fish appeared to be from the Nith (Table 2). Mixed Stock Fishery proportions suggested that ~68% of the fish were of North/West origin, ~22% of east coast, and ~8% of Hebridean origins.

Assignment unit	Number[1]	Percentage[2]
North/West	57	67.6
East Coast	16	22.2
Hebrides	5	7.9
Nith	1	0.1
Ness	1	0.1
Total	77	

Table 2: *Summary of numbers of fish assigning to each assignment unit. [1] numbers based on individual assignments, [2] proportions based on mixed stock analysis*

Actual river chosen (from acoustic tagging) was compared with that predicted from genetics (Table 3; Fig. 5). All salmon assigned by genetics to the North/West region returned to rivers in that region. However, nine of ten salmon assigned genetically to the east coast entered north coast rivers and only one was detected on the east (Spey). Of the six salmon assigned to Hebrides, only one was detected and it was in the North/West region (Naver).

		Return region (acoustic detection)		
Assignment region (genetics)		North/West	East	Hebrides
	North/West	34	0	0
	East	9	1	0
	Hebrides	1	0	0

Table 3: *Comparison of regions salmon were assigned to, from genetic analysis, with those they returned to, from acoustic tracking.*

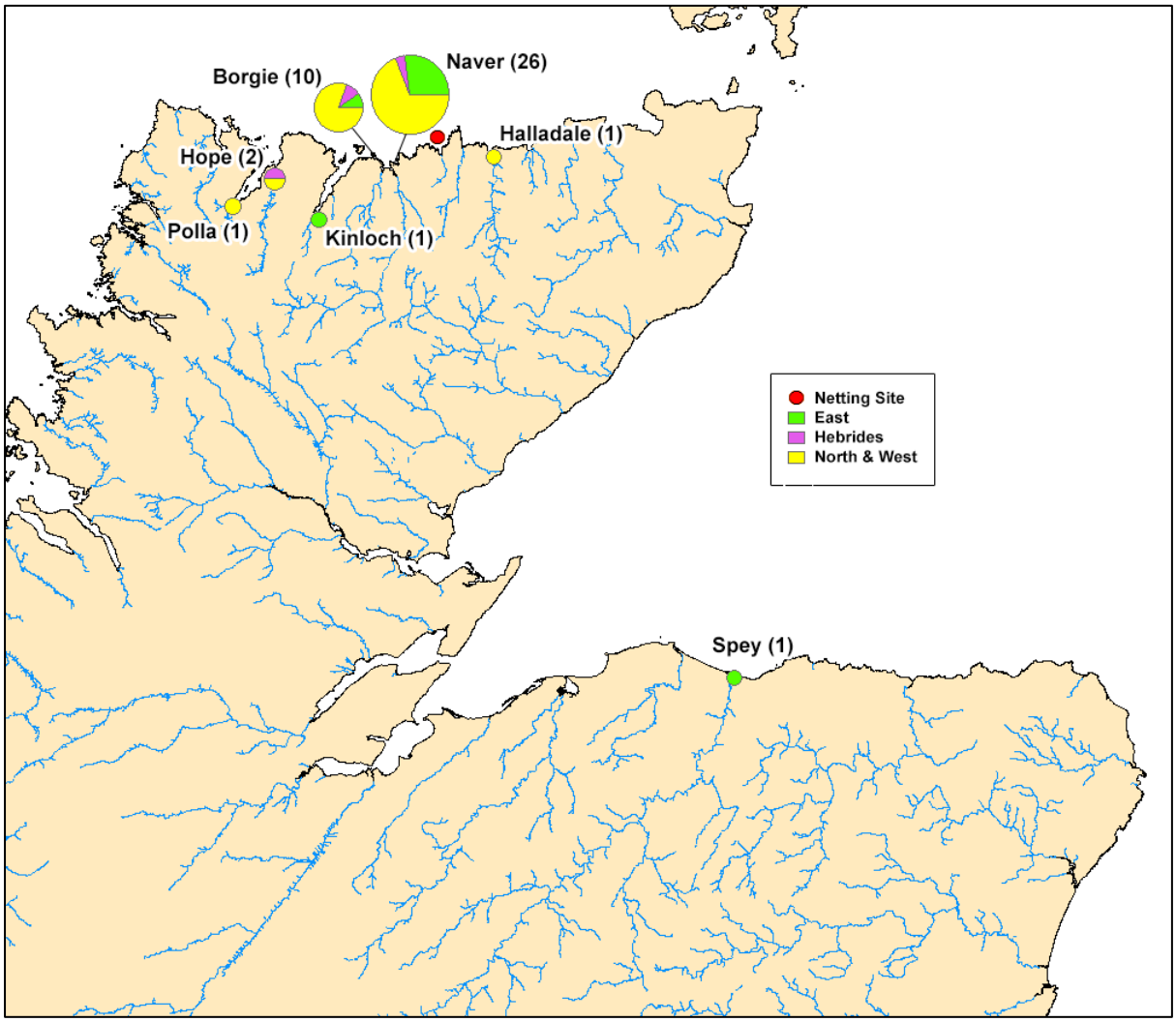

Figure 5: *Map showing acoustic tag detections of fish tagged at the Armadale netting station in 2017. Netting station shown in red, with fish genetically assigned as originating from the East as green, from the North and West as yellow, and from the Hebrides in purple. Sizes of charts are proportional to the numbers of fish detected at each site with these numbers also shown after each river name.*

3.2 Red Vent Syndrome

The majority of fish displayed signs of RVS (82%). This corresponds with previous reports that a high proportion of wild salmon returning to Scottish waters have *A. simplex* infection (Noguera et. al. 2008). There was no significant difference in the proportions of fish subsequently detected by receivers, between those displaying red vent syndrome and those with no obvious symptoms (p=0.210, binomial proportion test, R base package) (Fig. 6). However, the sample was small and imbalanced and hence the power to detect any effect was low.

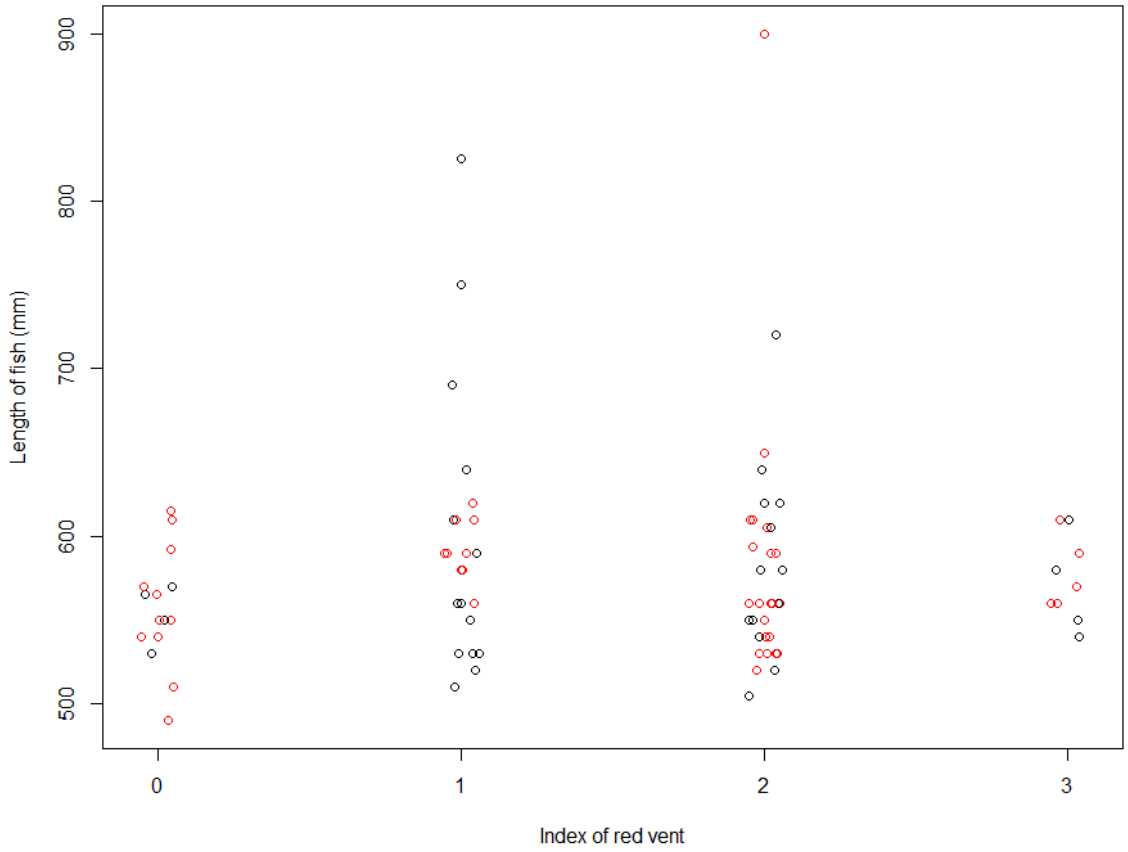

Figure 6: *Index of severity of RVS compared to length of fish. Red circles indicate whether the fish was subsequently detected on a receiver and black circles indicate no subsequent detection.*

3.3 Salmon Tagged in 2013/2014

3.3.1 Genetic Assignments

In 2013, tagged fish covered six regions, dominated by the North/West and East Coast regions, whereas in 2014 three regions were represented in the tagged fish, mostly assigning to the East Coast region and the North/West (Table 4).

Assignment region	2013	2014
Norway	1 (4%)	
Ayr		1 (3.4%)
Carnoch	1 (4%)	
East Coast	10 (40%)	14 (48.3%)
Ness	1 (4%)	
North/West	11 (44%)	14 (48.3%)
Tyne/Tees	1 (4%)	
Total number assigned	25	29

Table 4: *Number (and percentage) of the satellite-tagged fish with acceptable location error and assignment scores assigned to the various regions. Fish have been divided into year of capture.*

3.3.2. Genetic assignment of satellite-tagged salmon in rivers

All those salmon assigned to the North/West region that were detected in rivers (n = 8) were located within that region (Fig. 7). Of those salmon assigned to the east region, one was in the Naver (North/West region) after 3.2 days and three were in east coast rivers (Spey, Deveron and Dee) (Fig. 7). A further fish also assigned to the East region was captured by electrofishing on spawning grounds on the River Tay.

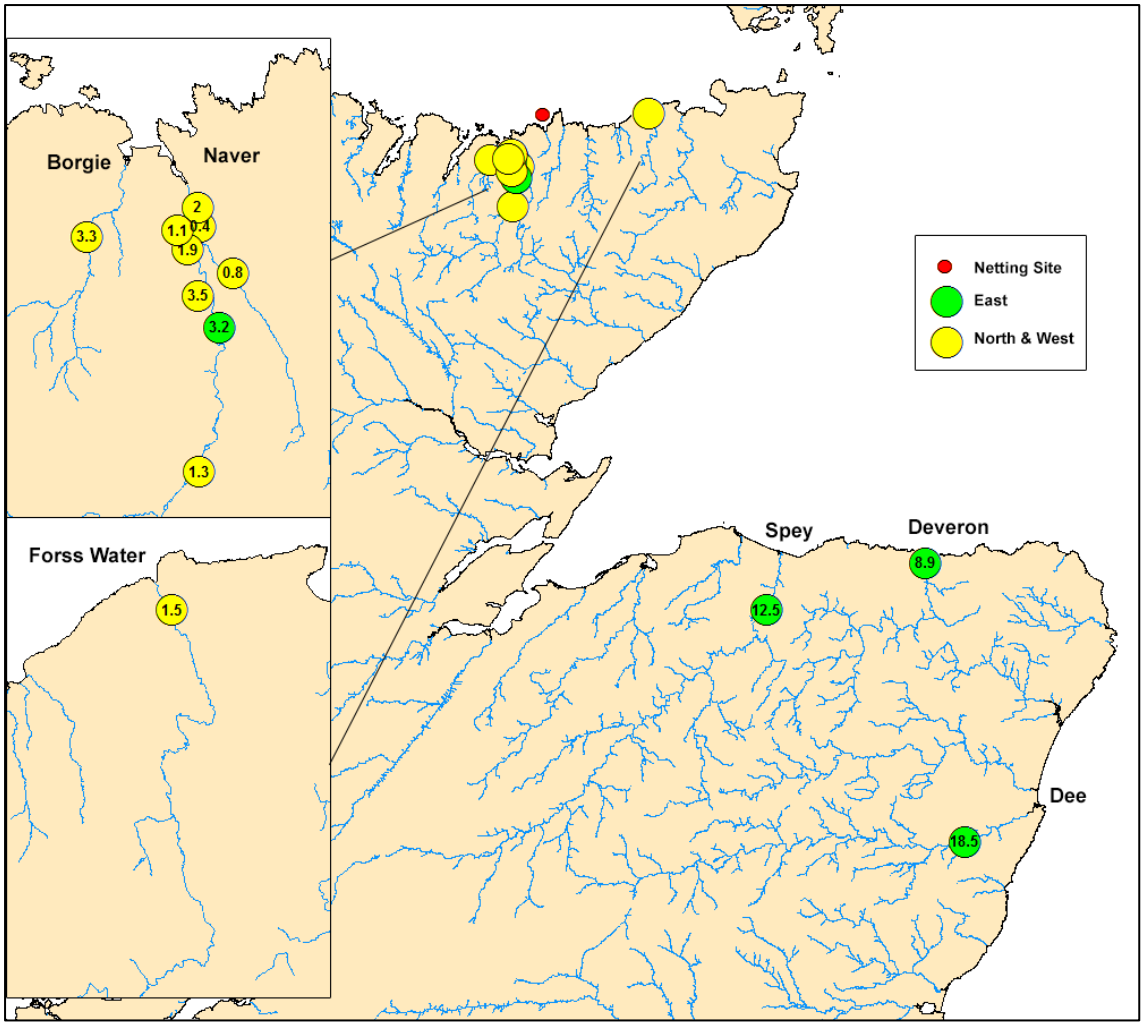

Figure 7: *Map showing satellite pop-off locations within rivers from 2013/4 satellite tagging of fish at the Armadale netting station. Netting station shown in red, with fish genetically assigned as originating from the East as green and from the North and West as yellow. Numbers refer to the number of days after tagging the fish/tag popped-off and was detected in the river.*

3.4 Summary Comparison of Acoustic Tagging and Genetics Assignment Distributions

Impressions of the distributions of salmon captured at Armadale using acoustic tracking and genetics assignment methods are summarised in Table 5.

Region	Method/year		
	Genetics 2013/14	Genetics 2017	Acoustics 2017
North/West	46	73	98
East	44	21	2
Other	10	6	0

Table 5: *Comparison between years and methods of estimates of stock composition by region (percentage).*

The types of data are rather different because acoustic tracking is limited to the area covered by receivers, whereas genetics can encompass the wider area covered by the genetic baseline. Hence, a further comparison, is made (Table 6) showing the proportional representation of East and North/West locations, where geographic coverage was good for both methods.

Region	Method/year		
	Genetics 2013/14	Genetics 2017	Acoustics 2017
North/West	51	78	98
East	49	22	2

Table 6: *Comparison between years and methods of the percentage contribution of East and North/West regions to the catch of salmon at Armadale*

Clearly, the impression provided from Table 6 is that genetics suggests that Armadale represents a much more mixed stock fishery than the impression from the results obtained by acoustic tracking. Also it is notable that the contribution of salmon assigned to the east coast was higher in 2013/14 than 2017, potentially reflecting differences in year, or season or size of salmon, or some other factor(s).

4. Discussion

4.1 The Acoustic Tagging Method

To our knowledge, this is the first time that acoustic tagging has been used to examine the spatial extent of impacts of coastal netting. The method was shown to be a powerful tool as the return (river detection) rate of tags exceeded 50%. By comparison, using simple external tags and returns from angling would have been expected to result in recaptures of only 5%, assuming a 10% exploitation rate. Furthermore, simple tagging would be likely to be even less efficient if not all recaptured fish were reported, and likely would be subject to unknown bias due to variation in exploitation and reporting rates among rivers. Radio tagging, using local tracking stations and airplane surveys rather than satellites, has been applied previously to determine the stock composition of the Usan fishery near the South Esk (Orpwood et al. 2017). Acoustic tagging has the advantage that fish may be detected in both fresh- and seawater, whereas radio tracking is not a feasible method in the marine environment.

The acoustic tagging method is not likely to provide full coverage because salmon may use small rivers in which there were no receivers. Similarly, there is the possibility that salmon swim past receivers without being recorded, for example in noisy river conditions. Hence there may be errors of fish being undetected either entering of subsequently leaving rivers. Such errors were minimised by specifying locations for deploying receivers where range of detection would be maximised. However, there may have been instances where salmon appeared to choose a river but had returned to sea and not subsequently been detected, resulting in miss-assignment. Analyses assumed that this did not happen. This assumption would not be expected to affect the main findings because most of the salmon for which

there was clear ambiguity (that is not being detected on the upper receiver), were assigned to the North West (rather than East) region.

Far fewer salmon were captured at Armadale than anticipated based on past catches. The low catch probably reflected to some extent the generally low and declining numbers of salmon in Scotland. There is clearly substantial year-to-year variation in catches associated with fishing effort, due to weather and a multitude of potential impacting factors, such as local numbers of seals. However, it is probably no longer possible sensibly to use simple tag-recapture methods that were viable when stocks were more healthy (Downie et al. 2018). The acoustic tracking method developed here provides an alternative viable approach. From an ethical standpoint, the acoustic tagging method should be preferred because it reduces the number of animals required to obtain data. As will be discussed later, acoustic tagging also provided insights into the detailed behaviour of salmon during their return migration. Such information not only informs on the spatial impact of the netting station where the study animals were captured, but also the likelihood that other netting stations on the migration route would intercept the fish.

4.2 The Range of Rivers Affected by Interceptory Coastal Netting at Armadale

Based on tracks of acoustically-tagged fish, the majority of salmon captured at Armadale (>90%) went to rivers on the northern coast over a range in the order of 100km, consistent with the predictions from historic netting recapture data (Downie et al. 2018). A single fish went to the River Spey, again consistent with wide geographic impact, albeit at a relatively low level, on distant rivers.

4.3 Comparison between Results from Tracking (satellite and acoustic) and Genetics

Although results of acoustic tracking were broadly similar to mixed-stock levels determined in earlier tagging studies, the genetic composition of samples of salmon captured at Armadale both in 2013/14 and 2017 give a rather different impression. For the early running predominantly multi-sea-winter salmon in 2013/14 only 46% of fish in the sample were assigned to the North/West region and for the predominantly later running grilse in 2017 the equivalent estimate was ~68%. A plausible explanation for this discrepancy between the results of the methods is that genetics measures the proportion of the population heading for different regions whereas tagging measures the proportion that survives. Those fish from nearby regions may be subject to lower predation mortality because they have less distance to travel and are exposed for a shorter period of time. If this argument applies, then a comparison of the ratios of fish from different regions in tagging and genetics studies could be used to estimate levels of coastal predation. Because individual genetic assignments could be related to tracks of salmon it was possible to explore this issue further.

Of the total of 14 grilse in 2017 assigned with confidence to the east coast, only one was subsequently recorded in an east coast river. A total of nine were detected, six entered and probably stayed within northern rivers, and two entered and possibly left northern rivers (in the sense that they were not registered in the upper receiver). Hence the evidence is that straying of east coast fish into northern rivers, rather than

differential mortality, accounted for much of the discrepancy in impressions of mixed stock levels between genetic assignments and tagging. The assignment method is subject to errors, for example, an intrinsic component of the methodology is a threshold of 80% certainty that a fish arises from a river or region, and hence some apparent straying may be miss-assignment. However, this factor would not explain the high level of straying, particularly as several of the fish involved had very high assignment scores to the east coast. It is conceivable that fish may stray in response to the capture and tagging process, moving into a nearby river as a consequence. However, this is not consistent with several of these fish being detected in different rivers or on the north coast array before arriving at their final northern river destination. Therefore, they appeared to have been moving around the northern coast before choosing and staying in a particular river. It is therefore feasible that a proportion of the fish caught at Armadale originated from rivers remote from the North West region, but were already straying before being tagged. These fish may have been particularly vulnerable to capture. They would likely represent a small fraction of the total numbers of fish destined for the east coast rivers but could form a sizable fraction of those obtained at the netting station.

In contrast to the 2017 acoustic tracking study, with one exception which was soon after tagging, satellite tags on salmon assigned to east coast rivers popped off in east coast rivers. Although the sample size was small, this contrast might indicate that earlier running salmon do not stray to the same extent as later running fish, or some other factor may explain the difference.

4.4 Patterns of Movement of Salmon among Rivers during the Homing Migration

The majority of tagged fish that were subsequently detected (~80%) showed directed movement into the river that they were assumed to have selected to spawn in. The remaining 20% of fish entered multiple rivers before selecting a final river. There was no consistent pattern to these movements. Fish that entered multiple rivers took over a week longer on average to enter what is presumed to be their destination river compared to fish that entered a single river. The similarity in data ranges between multiple- and single-river-entrants suggests that a portion of the single-river-entrants either remained within the coastal zone for an extended period (up to 86 days) before committing to a river catchment or moved to locations outside the range of the acoustic receiver array before returning to their destination river. Due to the movement between catchments and subsequent delay before entering their destination river it is anticipated that multiple-river-entrants potentially suffer higher predation risk as well as being subject to higher risks associated with human activities, such as coastal netting.

4.5 Red Vent Syndrome

Unfortunately the sample of fish obtained was too small to test effectively the influence of RVS on the subsequent survival of salmon. However, RVS, which is usually associated with *A. simplex* infection was found in the majority of fish. The incidence of RVS in returning salmon appears to have increased substantially in recent decades across the North Atlantic region (Beck et al. 2008; Norguera et al. 2008; Larrat et al. 2013). In 2008 a survey of RVS conducted at the Armadale

netting station stated that the prevalence of the syndrome was 14% in the sample of returning salmon examined (Pert et al. 2009). In the 2017 sample the prevalence was 82% and hence much higher than the previous estimate. An additional survey, conducted in 2008/ 2009 that counted anisakid parasite numbers in salmon tissues from 3 sites around Scotland (Armadale, Spey, Montrose), found that while 100% of salmon had anisakid infection, only a minority of fish displayed RVS and these were all from Montrose in a 2008 sample (Wootten et al. 2010). The reason for the substantial increase in the expression of RVS in returning salmon at Armadale is unclear. RVS is considered to be caused by the progression of anisakid infection rather than reflecting parasite burden per se (Larrat et al. 2013). However, this hypothesis has yet to be experimentally confirmed. In additional to time from infection, other potential influencing factors such as temperature or immunological status have also yet to be examined.

The life cycle of *A. simplex* is complex and passes through a number of hosts. Initially *A. simplex* eggs are eaten by marine crustaceans, which are subsequently eaten by fish or squid. The parasite migrates through the tissues of these hosts which are then eaten by a higher predator, such as a marine mammal or seabird. Eggs are produced in these hosts and are then released back into the water to complete the life cycle (Wootten et al. 2010). Therefore it is an integral and essential part of the parasites life cycle for the fish host to be eaten.

Parasite induced changes to hosts are well documented from numerous host parasite systems, including fish, and often facilitate transmission to subsequent hosts (Barber and Rushbrook 2008). There is currently a lack of data regarding the influence of Anasakis/ RVS on the salmonid host that may impact on its ability to successfully migrate back to its river of origin. The reddened vent may reduce camouflage and hence increase vulnerability of salmon to predation. However, the role of the red vent as an attractant for potential predator hosts has also not been investigated. Therefore it cannot be discounted that *A. simplex* induces behavioural/ physiological changes that impact on the numbers or migratory behaviours of salmon returning to spawn.

5. Synthesis and conclusions

Genetics has promise for assessing the mixed stock nature of fisheries, and indeed is widely applied. However, careful consideration is required regarding the handling of inherent errors associated with probabilistic nature of the method and possibility of inherent bias. Such bias may relate to sufficiency of the reference baseline sampling. Furthermore, there may be bias in sampling towards fish that are straying. This issue was exemplified by comparisons of rivers used by tagged fish and those predicted from genetics.

Acoustic tagging provides an alternative to traditional methods of simple mark-recapture that were useful when salmon were more abundant and the coastal netting industry was more active and widespread. The approach depends on an extensive network of receivers, which is logistically complex to deploy. However, compared with standard tagging, the data return is high relative to the number of fish tagged. Satellite tagging may reduce the need for an array of receivers, but the tags are

suitable for use only on large salmon, are very expensive and provide only single point locations.

The impression from contemporary data on the mixed stock nature of the Armadale fishery from acoustically-tagged salmon is broadly similar to that derived from historic data using standard tags (Downie et al. 2018). However, differences in genetic samples suggest that the fishery is more mixed stock for early than later returning grilse. The combination of genetics and acoustic tracking provides a promising approach for assessing mixed stock status of a fishery, combining genetics at a population level to determine spatial and temporal variations and tracking to refine and ground-truth estimates. However, this is contingent on sufficiently large samples of salmon being available. It is notable that the numbers of fish displaying red vent syndrome at Armadale was substantially higher than an earlier assessment conducted at the site. The acoustic tagging method appears to be a useful tool for testing effects of RVS on marine survival of salmon if sufficiently large samples of salmon with and without the syndrome can be obtained.

Sufficient salmon were captured to determine that there was a mixture of fish searching among rivers and those exhibiting movement to a nearby home river. It was evident that some fish do move close to the coast as they search for home and in this respect the behaviour of salmon in the rocky seascape of the northern coast may be similar to that in east coast sandy areas (Hawkins et al., 1979). Further work with a larger sample of salmon heading to home rivers remote from the capture location is required to determine the spatial range across which such near-shore swimming occurs.

Acknowledgements

Thanks to many colleagues within Marine Scotland Science and Policy, the Salmon Net Fishing Association of Scotland, and Fisheries Management Scotland, without whom this project would not have been possible. Thanks also to Christopher Somers, University of Regina, Canada for his advice on the tag type used for externally tagging fish with acoustic transmitters.

References

Araujo, H.A., Candy, J.R., Beacham, T.D., White, B. and Wallace, C., 2014. Advantages and challenges of genetic stock identification in fish stocks with low genetic resolution. Transactions of the American Fisheries Society, 143(2), pp.479-488.

Barber, I. and Rushbrook, B.J. 2008. Parasites and fish behaviour. In: Magnhagen, C., Braithwaite, V.A., Forsgren, E. and Kapoor, B.G. (eds). Fish Behaviour. Science publishers, Enfield, pp. 525-561.

Beck, M., Evans, R., Feist, S.W., Stebbing, P., Longshaw, M. and Harris, E. 2008. *Anisakis simplex* sensu lato associated with red vent syndrome in wild adult Atlantic salmon *Salmo salar* in England and Wales. Diseases of Aquatic Organisms, 82, pp. 61-65.

Begg, G.A., Friedland, K.D., Pearce, J.B. 1999. Stock identification and its role in stock assessment and fisheries management: an overview. Fisheries Research 43,1-8.

Bradbury, I.R., Hamilton, L.C., Chaput, G., Robertson, M.J., Goraguer, H., Walsh, A., Morris, V., Reddin, D., Dempson, J.B., Sheehan, T.F., King, T., Bernatchez, L. 2016. Genetic mixed stock analysis of an interceptory Atlantic salmon fishery in the Northwest Atlantic. Fisheries Research 174, 234-244.

Casey, J., Jardim, E. and Martinsohn, J., 2016. The role of genetics in fisheries management under the EU common fisheries policy. Journal of Fish Biology, 89(6), pp.2755-2767.

Cauwelier, E., Gilbey, J., and Middlemas, S. 2016. Genetic assignment of marine-caught adult salmon at Armadale to region of origin. Scottish Marine and Freshwater Science Report. 6 No 16. Marine Scotland Science. Aberdeen, Scotland, U.K.

Cauwelier, E., Verspoor, E., Coulson, M. W., Armstrong, A., Knox, D., Stradmeyer, L., Webster, L. M. I. & Gilbey, J. 2018. Ice sheets and genetics: Insights into the phylogeography of Scottish Atlantic salmon, *Salmo salar* L. Journal of Biogeography 45, 51-63.

Dolloff, C.A., Flebbe, P.A. and Thorpe, J.E., 1994. Salmonid flexibility: responses to environmental extremes. Transactions of the American Fisheries Society, 123(4), pp.606-612.

Downie, H., Hanson, N., Smith, G.W., Middlemas, S.J., Anderson, J., Tulett, D. and Anderson, H. 2018. Using historic tag data to infer the geographic range of salmon river stocks likely to be taken by a coastal fishery – Scottish Marine and Freshwater Science Vol 9 No 6 (DOI: 10.7489/12095-1).

Garcia de Leaniz, C., Fleming, I.A., Einum, S., Verspoor, E., Jordan, W.C., Consuegra, S., Aubin-Horth, N., Lajus, D., Letcher, B.H., Youngson, A.F. and Webb, J.H., 2007. A critical review of adaptive genetic variation in Atlantic salmon: implications for conservation. Biological Reviews, 82(2), pp.173-211.

Gilbey, J., Cauwelier, E., Coulson, M.W., Stradmeyer, L., Sampayo, J.N., Armstrong, A., Verspoor, E., Corrigan, L., Shelley, J. and Middlemas, S., 2016a. Accuracy of assignment of Atlantic Salmon (*Salmo salar* L.) to rivers and regions in Scotland and Northeast England based on single nucleotide polymorphism (SNP) markers. PloS One, 11(10), p.e0164327.

Gilbey, J., Cauwelier, E., Stradmeyer, L., Sampayo, J., Middlemas, S., Corrigan, L. & Shelly, J. 2016b. Assignment of fish from the north east English net fishery to origin using SNP genetic markers. p. 45. Faskally, Pitlochry, Scotland. U.K.: Marine Scotland Science and the Environment Agency.

Godfrey, J.D., Stewart, D.C., Middlemas, S.J. and Armstrong, J.D., 2014. Depth use and migratory behaviour of homing Atlantic salmon (*Salmo salar*) in Scottish coastal waters. ICES Journal of Marine Science, 72(2), pp.568-575.

Griffiths, A.M., Machado-Schiaffino, G., Dillane, E., Coughlan, J., Horreo, J.L., Bowkett, A.E., Minting, P., Toms, S., Roche, W., Gargan, P. and McGinnity, P., 2010. Genetic stock identification of Atlantic salmon (*Salmo salar*) populations in the southern part of the European range. BMC genetics, 11(1), p.31.

Hawkins, A.D., Urquhart, G.G. and Shearer, W.M., 1979. The coastal movements of returning Atlantic salmon, *Salmo salar* (L.). Scottish Fisheries Research, 15 pp 1-13.

Kalinowski, S.T., Manlove, K.R., and Taper, M.L. 2007. ONCOR: a computer program for genetic stock identification. Department of Ecology, 310 Lewis Hall, Montana State University. Available from http://www.montana.edu/kalinowski/Software/ONCOR.htm

Keefer, M.L. and Caudill, C.C., 2014. Homing and straying by anadromous salmonids: a review of mechanisms and rates. Reviews in Fish Biology and Fisheries, 24(1), pp.333-368.

King, T.L., Kalinowski, S.T., Schill, W.B., Spidle, A.P. and Lubinski, B.A., 2001. Population structure of Atlantic salmon (*Salmo salar* L.): a range-wide perspective from microsatellite DNA variation. Molecular Ecology, 10(4), pp.807-821.

Larrat, S., Bouchard, F., Seguin, G. and Lair, S. 2013. Relationship between red vent syndrome and anisakid larvae burden in wild Atlantic salmon (*Salmo salar*). Journal of Wildlife Diseases. 49(2). Pp 229-234.

Mills, D. 1989. Ecology and Management of Atlantic salmon. Chapman and Hall, London. 351 pp.

Noguera, P., Collins, C., Bruno, D., Pert, C., Turnbull, A., McIntosh, A., Lester, K., Bricknell, I., Wallace, S. and Cook, P., 2009. Red vent syndrome in wild Atlantic salmon *Salmo salar* in Scotland is associated with *Anisakis simplex* sensu stricto (Nematoda: Anisakidae). Diseases of Aquatic Organisms, 87(3), pp.199-215.

Orpwood, J.E., Mackay, F., Smith, G.W., Stewart, D.C., Henry, J.I., Anderson, J.M., Morgan, T., Millar, C.P., Malcolm, I.A., Cauwelier, E., Counter, S.-L., Gilbey, J., Sampayo, J., Stradmeyer, L., Simpson, I., Downie, H.K., Wyndham, M., Middlemas, S.J., MacLean, J.C. & Armstrong, J.D. (2017). Spring salmon on the River South Esk, Scotland. Scottish Marine and Freshwater Science Vol 7 No 10

Pert, C.C, Noguera, P.A. and Bruno, D.W. 2009. Scottish red vent syndrome survey 2008. Marine Scotland Science internal report, No. 07/09. 6pp.

Piry, S., Alapetite, A., Cornuet, J.M., Paetkau, D., Baudouin, L. and Estoup, A., 2004. GENECLASS2: a software for genetic assignment and first-generation migrant detection. Journal of Heredity, 95(6), pp.536-539.

Priede, I.G., Solbé, J.D.L., Nott, J.E., O'Grady, K.T. and Cragg-Hine, D., 1988. Behaviour of adult Atlantic salmon, *Salmo salar* L., in the estuary of the River Ribble in relation to variations in dissolved oxygen and tidal flow. Journal of Fish Biology, 33(sA), pp.133-139.

Rannala, B. and Mountain, J.L., 1997. Detecting immigration by using multilocus genotypes. Proceedings of the National Academy of Sciences, 94(17), pp.9197-9201.

Salmenkova, E.A., 2017. Mechanisms of homing in salmonids. Biology Bulletin Reviews, 7(4), pp.287-298.

Stewart, D. C., Middlemas, S. J., Mackay, S., & Armstrong, J. D. (2009). Over-summering behaviour of Atlantic salmon *Salmo salar* returning to rivers in the Cromarty Firth, north–east Scotland. Journal of fish biology, *74*(6), 1347-1352.

Utter, F. & Ryman, N. 1993. Genetic Markers and Mixed Stock Fisheries. Fisheries. 18, 11-21.

Waples, R.S., Winans, G.A., Utter, F.M. and Mahnken, C., 1990. Genetic approaches to the management of Pacific salmon. Fisheries, 15(5), pp.19-25.

Webb, J., Verspoor, E., Aubin-Horth, N., Romakkaniemi, A., and Amiro, P. 2007. The Atlantic Salmon. In The Atlantic Salmon: Genetics, Conservation and Management, pp. 17–45. Ed. by E. Verspoor, L. Stradmeyer, and J. L. Nielsen. Blackwell Publishing, Oxford, U.K.

Wootten, R., Yoon, G.-H. and Bron, J.E. 2010. A survey of anisakid nematodes in Scottish wild Atlantic salmon. FSAS project S14008. Final report. pp.25.

Appendix 1: Receiver deployment information including location of deployment

River/Site	Deployment type	River Position	Grid Reference
Allan	River	Upstream	NN7902305419
Allan	River	Downstream	NS7899096491
Ayr	River	-	NS3366522252
Balgy	River	Upstream	NG8517053278
Beauly	River	Downstream	NH5198544446
Beauly	River	Upstream	NH5200443357
Bervie	River	Downstream	NO8335372709
Bervie	River	Upstream	NO8263673354
Borgie	River	Upstream	NC6685958768
Borgie	River	Downstream	NC6810461095
Conon	River	Upstream	NH5262353669
Conon	River	Downstream	NH5460456339
Crail	Marine	-	NO6439309730
Cree	River	-	NX241641564657
Dee	River	Upstream	NJ9209102963
Dee	River	Downstream	NJ9436405168
Deveron	River	Downstream	NJ6949163349
Deveron	River	Upstream	NJ6897662819
Don	River	Upstream	NJ9282109445
Don	River	Downstream	NJ9352009099
Dunbeath	River	Downstream	ND166294
Dunbeath	River	Upstream	ND157302
Eas Ghobain	River	-	NN6120707372
Evelix	River	-	NH7366187558
Eyemouth	Marine		NT9564664684
Findhorn	River	Downstream	NJ0238061456
Findhorn	River	Upstream	NJ0256160026
Forss	River	Downstream	ND030697
Forss	River	Upstream	ND033690
Forth	River	Upstream	NS7701695319
Forth	River	Upstream	NN5281000305
Fraserburgh	Marine	-	NK0001467842
Garb Uisige	River	-	NN6223807898
Grimersta	River	-	NB2183729999
Halladale	River	Downstream	NC8891064803
Halladale	River	Upstream	NC8940463504
Helmsdale	River	Upstream	ND0207617400
Helmsdale	River	Downstream	ND0283315156
Helmsdale mouth	Marine		ND0511215960
Hope	River	Downstream	NC4743060653

Hope	River	Upstream	NC4724260152
Inver	River	-	NC1021423497
Kinloch	River	Upstream	NC5570852301
Kinloch	River	Downstream	NC5576452401
Kyle of Sutherland	River	Upper	NH6036992710
Kyle of Sutherland	River	Lower	NH6088991792
Laxford	River	Downstream	NC2352146988
Laxford	River	Upstream	NC2486646696
Lossie	River	Downstream	NJ2499066870
Lossie	River	Upstream	NJ2540063835
Lossiemouth	Marine	-	NJ2353371741
Nairn	River	Upstream	NH8815555010
Nairn	River	Downstream	NH8865056150
Naver	River	Upstream	NC7121357775
Naver	River	Downstream	NC7115857957
Ness	River	Upstream	NH6438342483
Ness	River	Downstream	NH6497343126
Nith	River	-	NX9733177170
North Esk	River	Downstream	NO7296162237
North Esk	River	Upstream	NO7165062121
Northcoast 1	Marine	-	NC5573267419
Northcoast 2	Marine	-	NC5591767810
Northcoast 3	Marine	-	NC5604468139
Polla	River	Upstream	NC3864754138
Polla	River	Downstream	NC3893954757
Snizort	River	-	NG4147248690
South Esk	River	Downstream	NO6680158416
South Esk	River	Upstream	NO6543657889
Spey	River	Upstream	NJ3479562313
Spey	River	Downstream	NJ3448561486
Strathy	River	Upstream	NC8360665154
Strathy	River	Downstream	NC8360165509
Tay	River	Downstream	NO1299621626
Tay	River	Upstream	NO1019726711
Teith	River	Downstream	NS7634496582
Teith	River	Upstream	NN7013302767
Thurso	River	Upstream	ND109660
Thurso	River	Downstream	ND114677
Tweed	River	Downstream	NT9994952164
Ugie	River	Downstream	NK1094448327
Ugie	River	Upstream	NK1004648246
Wester	River	-	ND3313458801
Wick	River	Downstream	ND3461451736
Wick	River	Upstream	ND2830053590

Ythan	River	Upstream	NJ9468330343
Ythan	River	Downstream	NJ9712030248