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1. BACKGROUND

Infectious salmon anaemia (ISA) is a viral disease caused by the ISA virus (ISAV) (OIE 2009). Clinical disease affects farmed Atlantic salmon (*Salmo salar*) and the virus can be carried by other salmonid species such as brown and sea trout (*Salmo trutta*) (OIE 2009). It was first reported from Norway in farmed Atlantic salmon in 1984 (Thorud and Djupvik 1988; Lyngstad et al. 2008) and has caused economic losses in several major salmon producing regions of the World (OIE 2009). There was a previous outbreak of ISA in farmed Atlantic salmon in Scotland in 1998-99 (Anon 2000; Stagg 2003) which was successfully eradicated.

In January 2009 ISA was confirmed at an Atlantic salmon farm in South West Shetland (Anon. 2009a) in management area 3(a) (Anon. 2009b) (Figure 1). An epidemiological investigation was quickly implemented to investigate potential routes of virus transmission. One aspect of this research involved screening wild marine and freshwater fish for ISAV.

There is evidence that ISAV can be carried by wild salmonids (Nylund and Jakobsen 1995; Raynard et al. 2001) however no signs of ISA disease have been reported. There have also been reports of the virus in marine fish although no clinical ISA disease has been observed. These reports relate to species that were artificially challenged such as Atlantic cod (*Gadus morhua*) (Grove et al. 2007) and herring (*Clupea harengus*) (Nylund et al. 2002), both in Norway, or obtained from cages, saithe (*Pollachius virens*) and cod, containing clinically infected farmed Atlantic salmon (Kibenge et al. 2004) from Canada. As such, it was important to investigate the possibilities for wild fish to act as reservoirs and vectors of ISAV.

This was achieved by implementing two discreet field trips in South West Shetland in the spring of 2009, the first for wild marine and the second for wild freshwater fish. Due to the presence of clinical ISA in farmed Atlantic salmon within the management area the wild marine survey was undertaken first in February 2009 and the freshwater investigation was implemented later in the spring.

2. TARGET SPECIES

The species and numbers sampled are to an extent determined by what is present in the catch. However, a priority species list based on previous knowledge of ISAV in wild fish and published literature was produced (Table 1).

Wild Marine Fish

Previously ISAV, but not clinical disease, has been reported in Atlantic cod, herring and saithe (Grove et al. 2007; Nylund et al. 2002; Kibenge et al. 2004). As a result these were included in the list along with other gadoid species (related to cod) and sprat (*Sprattus sprattus*) (related to herring). Previous surveys undertaken by Marine Laboratory researchers revealed that common dab (*Limanda limanda*), plaice (*Pleuronectes platessa*)

and gurnards (various species) were abundant in this area of Shetland. Additionally they had previously been caught close to salmon cages (Wallace et al. 2008) so these were also included (Table 1).

Wild Fish Found in Freshwater

As ISA is a disease of salmonids these were primarily targeted for the freshwater investigation. The most abundant wild salmonid species in Shetland is brown/sea trout. Other species include Atlantic salmon, which are present in some freshwaters, 3-spined-stickleback (*Gasterosteus aculeatus*), European eel (*Anguilla anguilla*) and flounder (*Platichthys flesus*) which are often found in the lower reaches of rivers (Table 1).

TABLE 1

List of target species

Wild marine fish	**Wild fish found in freshwater**
Atlantic cod (*Gadus morhua*)	Brown & sea trout (*Salmo trutta*)
Pollock (*Pollachius pollachius*)	Atlantic salmon (*Salmo salar*)
Saithe (*Pollachius virens*)	3-spined-stickleback (*Gasterosteus aculeatus*)
Haddock (*Melanogrammus aeglefinus*)	European eel (*Anguilla anguilla*)
Whiting (*Merlangius merlangus*)	Flounder (*Platichthys flesus*)
Poor cod (*Trisopterus minutus*)	
Norway pout (*Trisopterus esmarki*)	
Herring (*Clupea harengus*)	
Sprat (*Sprattus sprattus*)	
Gurnard (various species)	
Common dab (*Limanda limanda*)	
Plaice (*Pleuronectes platessa*)	
Total: 12	**Total: 5**

3. TARGET TISSUES

The ISA virus can be present in a variety of organs (Snow et al. 2003) so from each fish 4 different tissue types were collected. These were: gill; kidney; heart and brain with each type being kept separate for individual screening. Gill and kidney are reported as being important target tissues for ISAV surveillance (OIE 2009) and as a result these were initially screened. Depending on the results of this testing a decision was made whether or not to test the heart and brain samples.

4. TESTING PROCEDURE

Quantitative Real-time polymerase chain reaction (qPCR) was chosen as the diagnostic testing procedure (Snow et al. 2009). This was the most appropriate test as it is sensitive and very specific (Nérette et al. 2008) and hence affords the best chance of detecting ISAV carrier populations. In addition it facilitates the rapid testing of large numbers of samples.

5. NUMBERS AND POOLING

In order to detect infection at assumed low prevalence in the wild populations large numbers of fish from each species were screened. Grouping fish for the purpose of sampling (pooling)

was necessary to facilitate laboratory testing of these large numbers. As such, the wild marine fish were pooled in 5's and as there were less wild fish caught from freshwaters these were sampled and tested individually.

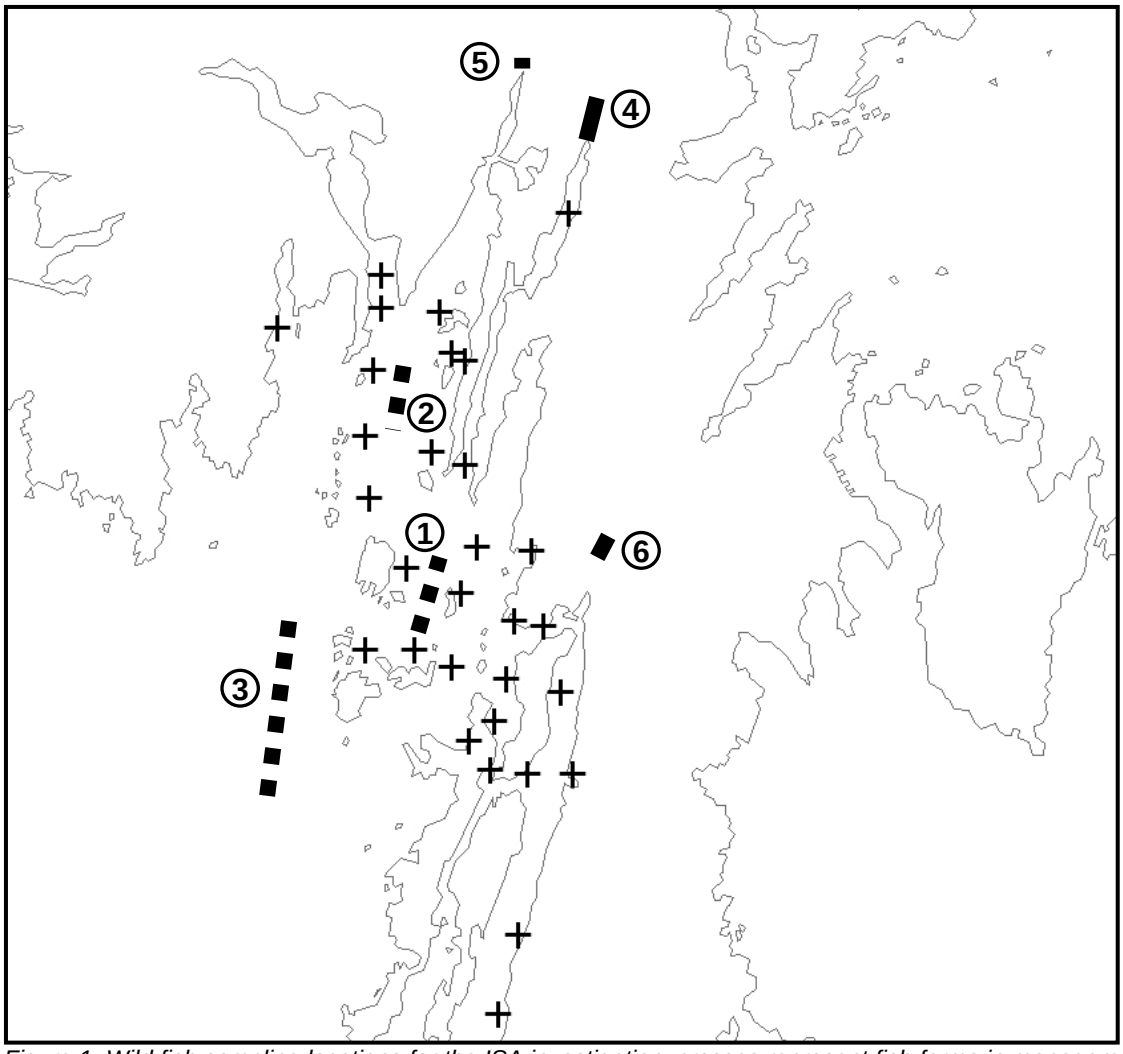

Figure 1. Wild fish sampling locations for the ISA investigation: crosses represent fish farms in management area 3(a); dotted lines are numbered marine tows; solid lines are numbered electrofishing sites. The circled numbers refer to the tow and electrofishing locations (Tables 2 – 7).

6. FIELD TRIPS AND RESULTS

Wild Marine Fish

The aim of this exercise was to catch wild marine fish from the waters close to Atlantic salmon farms in the infected management area. Demersal trawls were undertaken in the area from the 8th to 11th February 2009. Tow locations were chosen in consultation with the vessel skipper to be close to the infected farms but also taking into consideration the dynamics of each location. Three locations were confirmed: 1; 2 & 3 (Figure 1 & Appendix 1) two being close to fish farms and one being situated further out into deeper waters. Tows were undertaken each morning and the catch was iced up and transported to an on-shore laboratory for sampling.

The fish were sorted into species and a decision on what species and numbers to sample was made based on the catch and with reference to the priority species list (Table 1). For

each species the fish were grouped in 5's (with a small number of exceptions depending on the catch) and samples of the target organs were aseptically removed. These were placed in transport media containing 1.0 mL of RNALater solution (Ambion plc.) and kept cool at 4^0C for transport to the permanent laboratory in Aberdeen for screening. To maintain good bio security all waste fish and consumables were sent for incineration.

Samples of gill and kidney were screened in February and March 2009. The heart and brain samples were not tested. The catch breakdown and testing results for the wild marine fish are presented (Tables 2; 3 & 4).

TABLE 2

Tow location 1

Species	**Number sampled**	**ISAV test result**
Long rough dab (*Hippoglossoides platessoides*)	150	-ve
Saithe	30	-ve
Whiting	35	-ve
Common dab	100	-ve
Plaice	55	-ve
Short-horn sculpin (*Myoxocephalus scorpius*)	70	-ve
Atlantic cod	4	-ve
Flounder	2	-ve
Total	**446**	

TABLE 3

Tow location 2

Species	**Number sampled**	**ISAV test result**
Long rough dab	50	-ve
Saithe	10	-ve
Whiting	150	-ve
Common dab	150	-ve
Norway pout	150	-ve
Herring	30	-ve
Total	**540**	

TABLE 4

Tow location 3

Species	**Number sampled**	**ISAV test result**
Haddock	30	-ve
Saithe	30	-ve
Whiting	110	-ve
Atlantic cod	25	-ve
Lesser Argentine (*Argentina sphyraena*)	15	-ve
Total	**210**	

Wild Fish from Freshwater

The aim was to catch wild fish from freshwater catchments draining into the infected management area. Electrofishing was implemented as the primary catching method with gill netting as a back up procedure if insufficient fish numbers were caught. At the time of the wild marine fish investigation, in February 2009, the freshwater temperatures were low so it was decided to implement this sampling later in the spring, late March, when the water temperatures would be higher. From previous personal experience these higher temperatures make the fish more active thus increasing the chances of catching them. Additionally there would be a greater chance of catching migratory sea trout returning to freshwaters at this time of year.

The area was closely scrutinised using geographic and satellite maps and a list of potential electrofishing/netting locations was constructed. The final locations were chosen from this list to give the maximum likelihood of catching good numbers of fish and so were essentially the larger rivers and burns 4; 5 & 6 (Figure 1 & Appendix 1). They also gave an increased chance of catching sea trout which move between fresh and marine waters and Atlantic salmon were present in one of the rivers. All three rivers drained into the management area and the outflows were hydrographically separated by several km's giving a wide distribution (Figure 1).

Electrofishing was implemented from 23rd to 25th of March 2009 at the 3 locations. Atlantic salmon parr and European eel were very abundant in location 4, brown trout were present at a high density in location 6 and in general, fish were less abundant in location 5. A proportion of all the fish caught at each location were immediately released to avoid over exploitation of the stocks. The numbers of fish retained for sampling are illustrated in tables 5, 6 & 7. The fish were sorted into species and sampled individually rather than in pools. As before the target organs were aseptically removed from each fish and stored in media at 4^{0}C for transport to the permanent laboratory. All waste fish and consumables were incinerated.

Gill and kidney tissue samples were screened in April 2009 and the heart and brain samples were not screened. The catch breakdown and testing results for the fish caught from freshwater are presented (Tables 5; 6 & 7).

At location 4 additional samples for a variety of disease agents were taken from 30 of the Atlantic salmon for a fish health inspectorate (FHI) disease survey. When completed, the results of this testing will be reported separately by FHI.

TABLE 5

Electrofishing location 4 on Stromfirth burn (lower)

Species	Number sampled	ISAV test result
Atlantic salmon parr	64	-ve
Brown trout	3	-ve
Sea trout	7	-ve
European eel	25	-ve
3-spined-stickleback	2	-ve
Flounder	1	-ve
Total	**102**	

TABLE 6

Electrofishing location 5 on burn of Weisdale (lower)

Species	Number sampled	ISAV test result
Brown/sea trout	22	-ve
European eel	8	-ve
Total	**30**	

TABLE 7

Electrofishing location 6 on Scalloway burn (upper)

Species	Number sampled	ISAV test result
Brown/sea trout	82	-ve
European eel	1	-ve
3-spined-stickleback	1	-ve
Total	**84**	

7. SUMMARY

Marine Waters

For the epidemiological investigation a total of 1196 wild marine fish were sampled. From these fish, all the samples of gill and kidney were screened for ISAV and were found to be negative. The samples of heart and brain were not tested in view of the negative results from the first two tissue types. Almost all the target species were caught and overall a reasonable number of fish from a variety of species were screened. This is believed to be a good representation of the populations that were present in the area at this time and as such is an appropriate sub sample for disease screening purposes.

Freshwaters

A total of 216 wild fish from freshwaters were sampled. All the samples of gill and kidney were found to be negative for ISAV. As before, the samples of heart and brain were not screened. All the target species were caught in varying numbers depending on the location and are a good representation of the species and numbers in each area.

8. CONCLUSIONS

This work has provided important information to the ISA epidemiological investigation. Good numbers of fish of a diverse range of species from a variety of locations were screened to assess any possible interactions between farmed and wild fish. All the samples were screened using a sensitive and specific testing procedure and found to be negative for ISAV and as a result, in this instance, no wild reservoir for ISAV was identified. This may illustrate wild fish to be of a relatively low risk in the spread of ISAV between marine fish farms.

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APPENDIX 1

Location details for the wild fish sampling in Shetland for the ISA investigation

Papa – Hildasay (1)	**North Havra – Fora Ness (2)**	**Scalloway Deeps (3)**
Shot: 60,07,88 N; 01,20,25 W Haul: 60,09,00 N; 01,19,63 W	Shot: 60,10,43 N; 01,20,98 W Haul: 60,11,27 N; 01,20,76 W	Shot: 60,08,30 N; 01,23,76 W Haul: 60,05,78 N; 01,24,33 W
30 FTM average	30 FTM average	50 – 55 FTM
30 mins	30 mins	60 mins
Soft ground	Soft ground	N/A
Stromfirth burn (4)	**Burn of Weisdale (5)**	**Scalloway burn (6)**
From: HU 407 507 To: HU 408 513	From: HU 394 525 To: HU 394 527	From: HU 411 405 To: HU 412 410
Description: Commencing at the first riffle above the deep section of lower burn (above loch of Strom) and terminating at the start of the deep section 50m above road bridge.	Description: Commencing above the old road bridge (behind the church yard) and terminating approximately 200 metres up river.	Description: Commencing at the B9074 road bridge and terminating just below the loch Asta out flow.