Managing Interactions Aquaculture Project

River and Fisheries Trusts of Scotland

Sea Trout Post Smolt Monitoring Project Regional Report 2011

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Summary

The River and Fisheries Trusts of Scotland (RAFTS) Sweep Netting Monitoring Project, which forms part of the wider Managing Interactions Aquaculture project funded by Marine Scotland and coordinated and delivered by RAFTS and partner fishery trusts and district salmon fishery boards, was undertaken in 2011 to examine the sea trout post smolt populations and the potential interactions with sea lice for the West Coast of Scotland. This report presents details, analysis and findings from the twenty eight monitoring sites, which are summarised below.

The focus of this project was in two main areas;

1) Describe the current status of the post smolt sea trout populations surveyed; and

2) Present the number and stages of development of sea lice that were found on post smolt sea trout at each monitoring location.

In examining the current status of the post smolts the lengths of the sea trout were examined and found to be predominately under 260mm. In comparison, the weights of the post smolts exhibited variation across the monitoring sites. The majority of the monitoring sites had post smolts described as of "good condition" with the exception of the two monitoring sites in Wester Ross. Predation pressure was provisionally explored with only two sites indicating levels of predation that might require further exploration both these sites are located in Lochaber.

Two species of sea lice were examined, *L. salmonis* and *C. elongates.* The analysis focused on the sea lice loadings and examined the comparisons of these loadings across the monitoring sites. The *L. salmonis* loading pressure on the sea trout post smolts was further examined using two critical threshold levels which were the epizootic threshold (Costello, 2009) and the *L. salmonis* mobile threshold (Wells *et al* 2006).

The results indicated that five of the monitoring sites in 2011 experienced extensive heavy infestations (epizootic). To explore the impact of these heavy infestations further the Wells *et al*, 2006 threshold was explored to determine if the infection levels at the monitoring sites resulted in a detrimental impact. The implemented critical threshold level indicated that at one site >40% of the sampled post smolts were experiencing critically detrimental infestation level and five further sites experienced <10% of the sampled post smolts were experiencing critically detrimental infestation levels.

Further discussion is presented on the monitoring site and the comparisons to the fish farm activities within the study area this includes the distance to the nearest active fish farm, site biomass and year of production. Finally a comparison of wild sea lice counts to the published fish farm sea lice counts is also documented.

The report concludes on the lessons learnt from this first year of study and discusses the implications for the development and future direction of this project.

Managing Interactions Aquaculture Project
Sea Trout Post Smolt Monitoring Programme
Regional Report 2011

1. Project Background

In 2011, the Rivers and Fisheries Trusts of Scotland (RAFTS) and its member fishery trusts and partner district salmon fishery boards on the west coast of Scotland began a programme of work funded by the Scottish Government associated with the interactions between aquaculture and wild fish populations. The Managing Interactions Project is designed to support the better coordination and management of wild fisheries and stocks with the aquaculture industry. Underpinning this programme of work were the wild fish priorities of protecting sensitive and high value fresh water sites, improving practice and management at existing aquaculture sites and finally informing decisions on the location and biomass production at aquaculture sites both current and proposed. To achieve these strategic objectives three projects were identified as key priorities and work streams within the overall Project.

These were:

- Strategic programme of post smolt sweep netting and analysis;
- Programme of genetic sampling and analysis; and
- Locational guidance and zones of sensitivity analysis.

The three Managing Interaction projects are overseen by a Steering Group, chaired by RAFTS, which includes representatives from a range of west coast fishery trusts and boards, Marine Scotland Science and Marine Scotland Policy.

The participating fishery trusts and boards are:

- Argyll Fisheries Trust
- Argyll District Salmon Fishery Board
- Lochaber Fisheries Trust
- Wester Ross Fisheries Trust
- Wester Ross District Salmon Fishery Board
- Skye Fisheries Trust
- Skye District Salmon Fisheries Board
- West Sutherland Fisheries Trust
- Outer Hebrides Fisheries Trust
- Western Isles Salmon Fisheries Board

This paper will discuss further the cooperative sea trout post smolt monitoring programme which was organised to monitor wild sea trout populations and sea lice levels on the west coast of Scotland. Further details on the other two Managing Interactions projects will be made available on the RAFTS website (www.rafts.org.uk) and be reported separately.

In 1999 the Tripartite Working Group (TWG) had set up Area Management Agreements (AMA) which had been developed between local industry and wild fisheries interests throughout the west coast and the Western Isles. The AMAs were designed to encourage aquaculture and wild fisheries interests to work collaboratively on a number of objectives. These objectives included:

- single year class management and synchronised production / fallowing cycles within AMA zones;
- synchronised lice treatments zero ovigerous salmon lice particularly during the critical wild smolt migration period (Feb - June);
- the preparation of containment and contingency plans to minimise escapes impacts;
- ensure adherence to industry Codes of Practice;
- regular monitoring and information exchange between AMA partners;
- adherence to disease control mechanisms in wild fisheries; and
- finally a number of other local specific management aspects.

Under the TWG support to the AMAs a network of sweep netting sites were set up to monitor the wild fish populations and to support the local Area Management Groups (AMG). The results of these sweep netting activities were reported individually and locally to respective AMGs. The TWG project ended in March 2011 and the monitoring project was continued under the Managing Interactions project. Despite the cessation of the TWG, many of the AMAs remain active and are now run at the local level. Figure 1 indicates the distribution of currently signed AMAs on the West Coast of Scotland and the Western Isles.

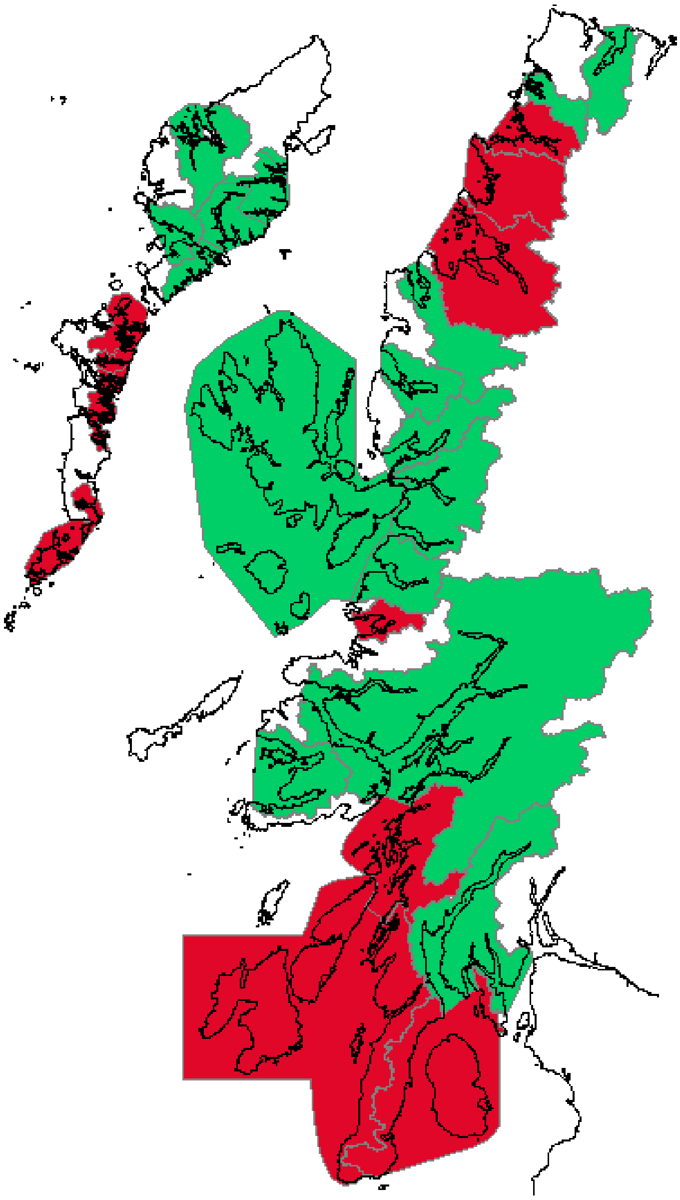

Figure 1: Map of Area Management Group Regions. Signed and active AMAs indicated by green shading with grey boundaries and Un-signed areas are shown as red with grey boundaries.

1.1 Strategic programme of post smolt sweep netting and analysis 2011

In early 2011 a complete and rigorous assessment of previous monitoring sites sampled under the TWG project along with a suite of potential monitoring sites were considered for inclusion in this project. The initial site assessments involved Trusts, Boards and Marine Scotland Science. The final network of sites identified includes twenty eight core sites throughout the West Coast of Scotland which aimed to give extended coverage of sites across a range of distances from fish farms. The project also aimed to focus sampling efforts on the sea trout smolt run as previous studies have shown that post smolts are potentially the most vulnerable stage to sea lice infection (Finstad *et al*.,2000).

2. Methods and Site Information

2.1 Sweeping Survey Techniques and Data Analysis

All chosen monitoring sites were surveyed in accordance with the Scottish Fisheries Co-Ordination Centre (SFCC) sampling protocol, “Sea Trout Netting and Sea Lice Sampling: A Standard Sweep Netting Protocol for Management, 2009”. This ensured that the project complied with current recommended standards. The data gathering was conducted by participating fisheries trusts during the months of May, June and July 2011

Sea Trout were captured during the hours of daylight using a sweep net which was deployed from the shoreline. Trust teams using the sweep nets would either employ hand hauling techniques or deploy the net from a boat. The sweep nets used were fifty metres in length and had a standard stretched mesh size of 20 mm. All sea trout caught within the sweep were removed and anaesthetised. Under anaesthesia the length (±1mm) and weight (±1g) were recorded and where possible, a scale sample was also taken. The Sea Trout were examined for the presence of sea lice, which if found to be present were counted and staged. Sea Lice counts were classified according to the two species under investigation *Lepeophtheirus salmonis* (Krøyer 1837) and/or *Caligus elongatus* (Nordmann 1832). *L. salmonis* was further staged by one of three gender and life-stages which were copepodid/chalimi, pre-adult/adult and ovigerous females as per the SFCC Protocol. Additional information was also collected on any other parasites present or any predator damage to the fish.

The focus of the subsequent analysis at the monitoring sites described is on the post smolt sea trout populations and included weights, lengths, condition indices and predator damage. Further to the population analysis there will be analysis on the sea lice loadings with comparisons between the monitoring sites.

Four assessment methods were implemented to analyse and describe the sea lice distribution on the sea trout post smolt populations at the monitoring sites. These were:

- Prevalence: The percentage of fish in the sample infected by sea lice.
- Abundance: The mean number of sea lice per fish in the whole sample.
- Intensity: The mean number of sea lice per infected fish
- Abundance Median: The middle value when ranked numerically of sea lice within the population of fish.

Prevalence is an indication of the percentage of infected sea trout versus uninfected sea trout. To obtain a more comprehensive view of the distribution of sea lice amongst the sea trout sampled, abundance and intensity analysis was explored. Abundance gives an indication of the overall number of lice within the population whilst intensity provides a more accurate indication of the level of infestation on infected fish.

As highlighted by Hazon *et al* 2006, parasite infestations of hosts generally do not show a normal distribution of variation among individual hosts. Typically, parasite populations show “over-dispersion”, or “aggregation” on certain individual hosts (i.e. many or most hosts are parasite-free, but a small number of hosts carry exceptionally heavy infestations). From a statistical viewpoint, it is inappropriate to calculate the arithmetic mean and error terms of infestation intensities if the data are not normally distributed. All lice data in the present study has therefore been log transformed prior to the calculation of the normal mean and error terms. A log transformation usually will stabilize the variance and render the error terms normal. However, calculated means and error terms were subsequently back transformed in order to allow the data to be displayed in a meaningful way. It should be noted however that the back-transformed mean will always be lower than the arithmetic mean. Ensuring that the distribution variation is normalised and appropriately accounted for is crucial to determine if the populations being monitored are experiencing lice loads that could be reported as having a detrimental impact. Analysing such lice loads appropriately can support the local management strategies and policies.

Finally a full range of site environmental factors was recorded at each site. On every visit to the monitoring site, water temperature, air temperature and salinity profiles were recorded. The collection of these environmental factors is important as it has been shown previously that temperature and salinity influence sea lice population dynamics (Butterworth *et al*, 2006).

In accordance with the SFCC protocol, the project Steering Group agreed that for each site a target of >30 fish should be included in each sample and that this sample should be collected from a minimum of two survey dates at each site. Additional survey dates and greater number of fish would further improve and enhance the sample size available for analysis and the robustness of the analysis subsequently possible.

The sampling data from all the Trusts was compiled by the project coordinators in a structured Access Database (2010) in preparation for analysis. Analyses of the data involved descriptive statistics and graphs which were prepared in Excel (2010).

2.2 Site Information

The final network of sites identified includes twenty eight core sites throughout the West Coast of Scotland aimed at achieving good coverage of sites across a range of varying distances from active fish farms (Figure 2). The twenty eight sites were identified across six fisheries trusts on the west coast (Table 1). Each individual Trust was responsible for completion of the sweep netting surveys of the sites within their own area.

Table 1: Monitoring Site Details.

Map Site ID (Figure 2)	Sweep Netting Site	Fisheries Trust	Number of Site Visits	Number of Sea Trout Caught 260mm Threshold	Current Distance to Active Fish Farm (Km)
1	Carradale	Argyll	2	36	9
2	Southend	Argyll	1	0	44
3	Machrihanish	Argyll	1	0	31
4	Loch Fyne	Argyll	2	60	24
5	West Riddon	Argyll	2	33	3
6	Dunstaffnage	Argyll	2	41	4
7	West Tarbert	Argyll	1	0	22
8	Laggan Bay	Argyll	1	0	62
9	Kinlocheil	Lochaber	8	100	20
10	Camas na Gaul	Lochaber	6	83	6
11	Sunart	Lochaber	9	8	10
12	Tong	Outer Hebrides	4	71	40
13	Ardroil	Outer Hebrides	4	11	23
14	Borve	Outer Hebrides	4	181	10
15	Eishken	Outer Hebrides	3	41	3
16	Kyles	Outer Hebrides	3	55	23
17	Malacleit	Outer Hebrides	3	26	25
18	Slapin	Skye	3	27	4
19	Harport	Skye	3	29	2
20	Kyle of Durness	West Sutherland	2	59	22
21	Polla	West Sutherland	3	33	7
22	Laxford	West Sutherland	2	41	4
23	Kinloch	West Sutherland	1	0	35
24	Kannaird	Wester Ross	2	28	1.5
25	Boor Bay	Wester Ross	3	31	8
26	Flowerdale	Wester Ross	2	36	26
27	Carron	Wester Ross	3	0	10
28	Gruinard Bay	Wester Ross	1	0	14

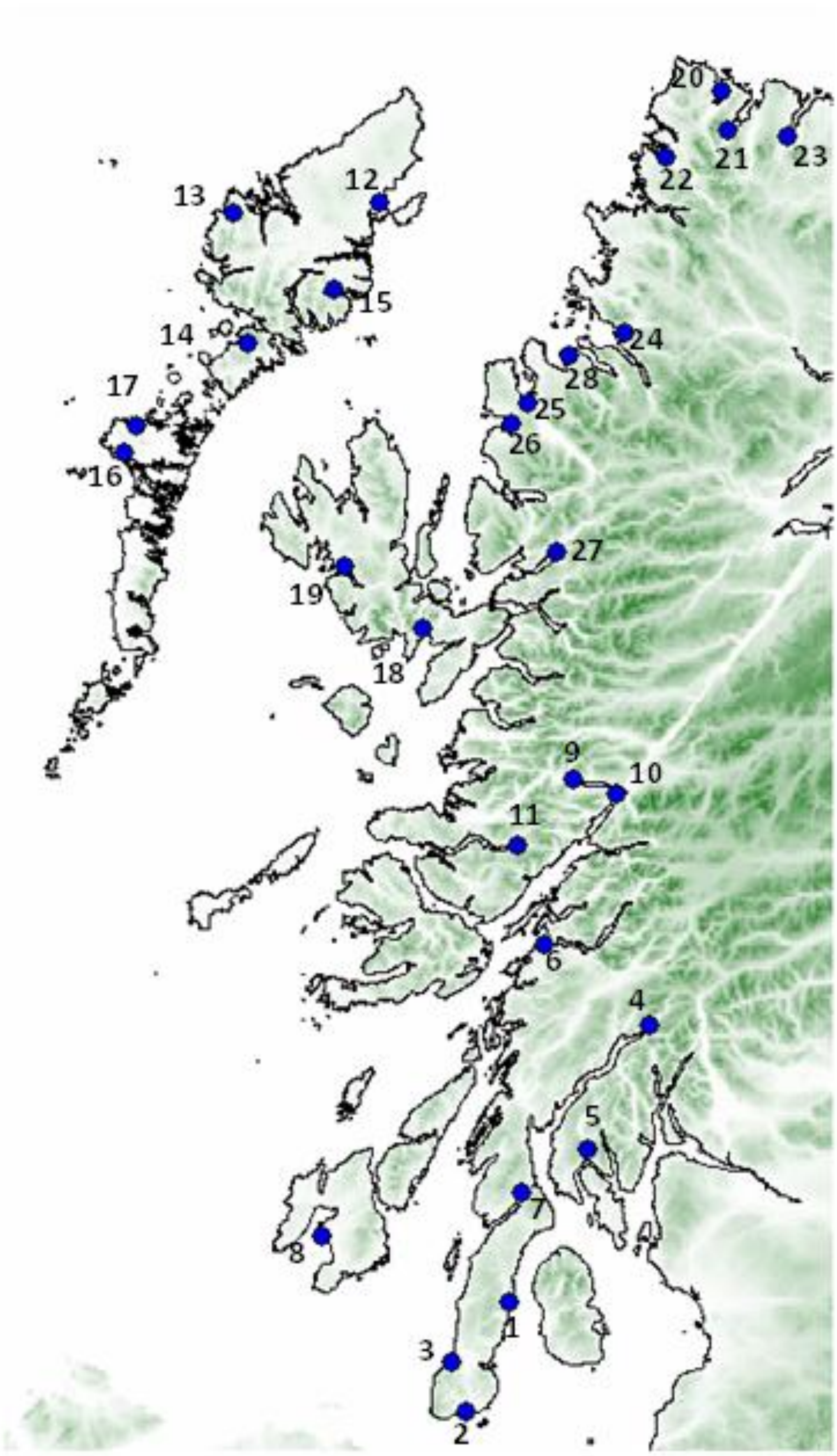

Figure 2: Geographical spread of monitoring sweeping sites sampled in 2011 (Blue dot indicates monitoring site please see Table 1 for full site details).

3. Sweep Netting Analysis Results

3.1. Sea Trout Analysis

The total number of post smolts caught at each site varied. The variation arose due to a number of factors such as weather conditions which led to unsafe sampling conditions and the simple absence of fish from both new sweep netting sites included to provide sample points at a range of distances from active fish farms and established sites where previous surveys had been successful in fish capture. Some sites did not produce the numbers of fish noted in previous years despite significant effort from the Trust teams. In addition five of the new monitoring sites were not successful although in some of these sites the minimum planned sampling effort was not delivered by the surveying trusts. An assessment and review of sites in the current study which were unsuccessful and did not provide the desired sample numbers will be required in advance of any future sweep netting activities.

Under the SFCC protocol the recommended minimum sample size for statistical analysis is currently advised as thirty fish. As can be seen from Table 1 fifteen of the initial twenty eight sites achieved this minimum sample size, four sites fell just below the minimum sample size and finally nine sites either produced very few fish or no fish at all well below the minimum sample size. However this report does present results for all sites that recorded data even if they fell below this minimum sample size and aims to report all observation of the samples taken over the study period.

3.1.1 Length, Weight and Condition Factor

Across the monitoring sites as anticipated the sea trout were predominately under 260mm (Figure 3). Unlike the sea trout post smolt length, the weight of the post smolts shows a much greater variation across the monitoring sites (Figure 4). It must be noted that weight data was not collected at all sites due to factors including severe weather conditions which created problems sampling weights under the environmental conditions being experienced. To explore the sea trout post smolt condition factor, Fultons condition factor (Ricker, 1975) was employed. This factor assumes a relationship between the weight of a fish and its length, which calculates and allows for the description of the individual fish condition. The formula for Fultons Condition Factor is:

$$K = \frac{W}{L^3}$$

K = Fulton Condition Factor
W = Weight
L = Total Length
Finally a scaling factor is implemented to bring the factor close to 1.

For monitoring sites that had available length and weight data the condition factor was calculated for all fish at each monitoring site and is summarised in Figure 5. As a general rule if a fish has a condition factor of 1 or above it would be considered healthy and of the fourteen sites with available data only two fall below the 1 factor level, Kinnaird and Flowerdale in Wester Ross (See Appendix 3 for further details).

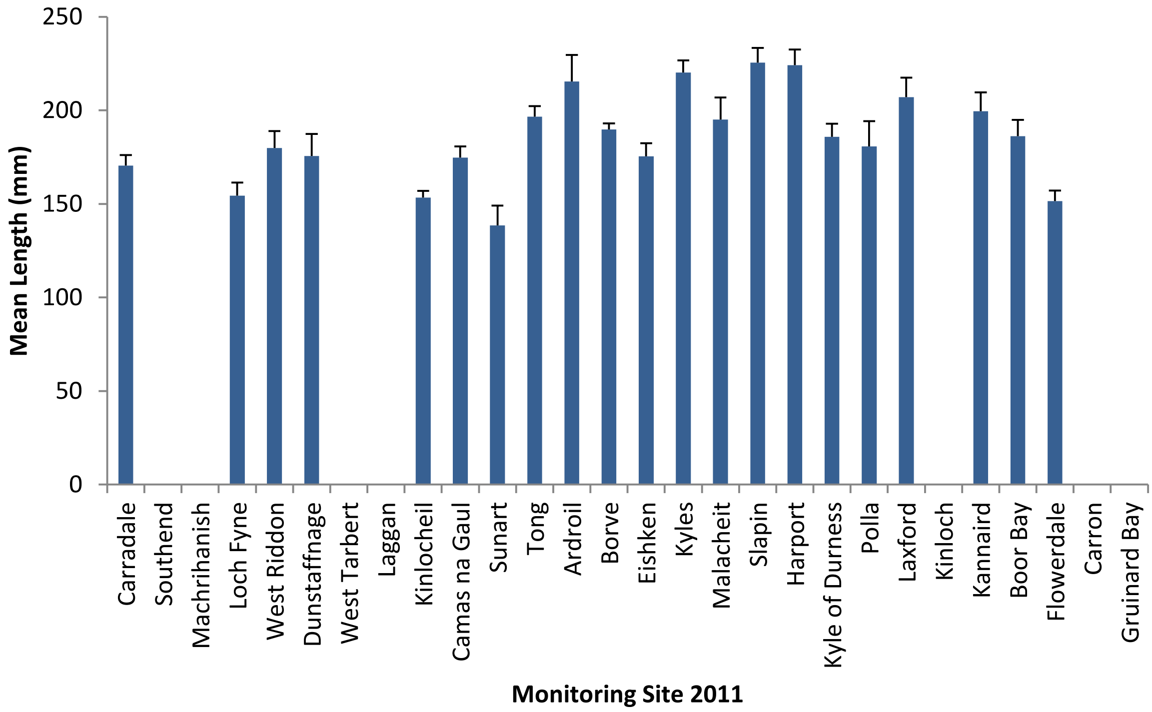

Figure 3: The mean sea trout lengths (mm) at each monitoring site.

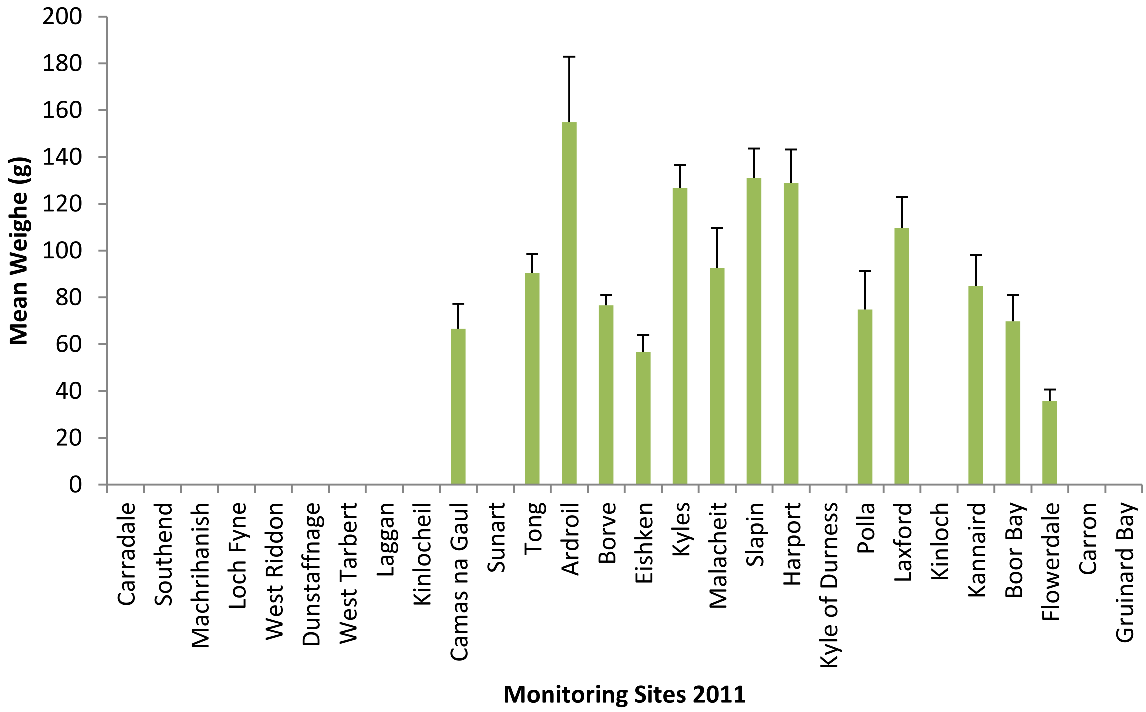

Figure 4: The mean sea trout weights (g) at each monitoring site. * Weight data was collected at only the sites indicated, please see Appendix three for full details.

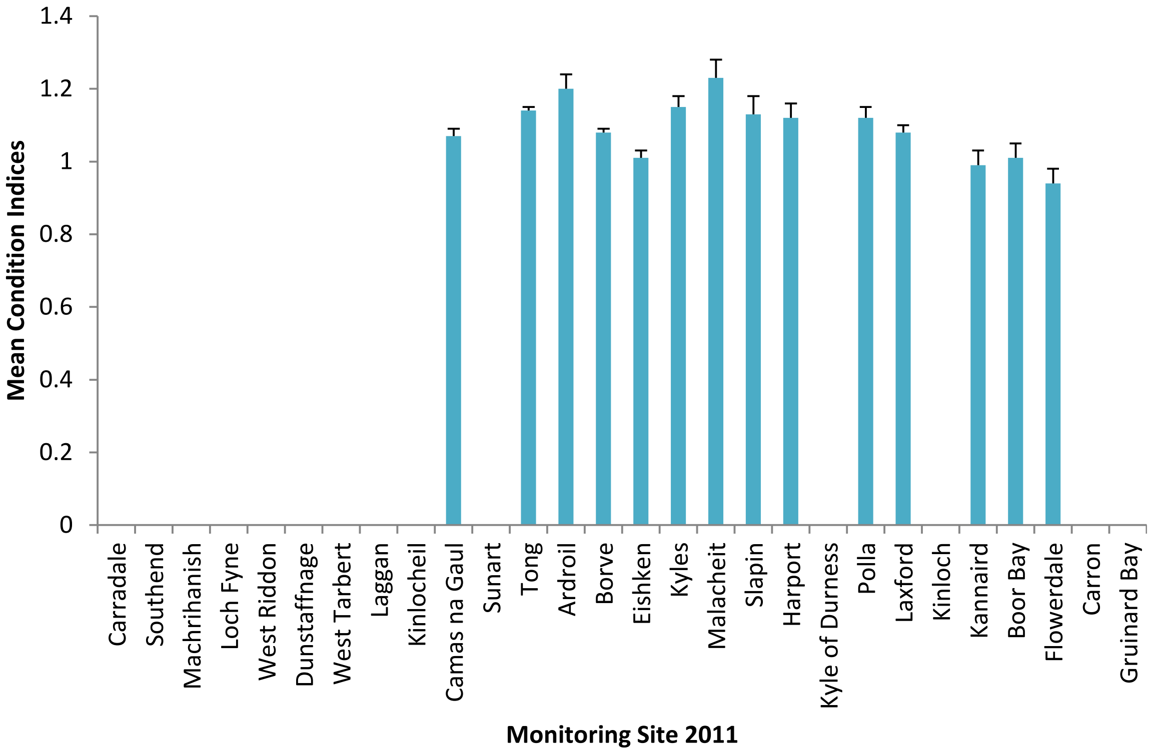

Figure 5: The mean sea trout Condition Indices at each monitoring site. * Weight data was collected at only the sites indicated, please see Appendix three for full details.

3.1.2 Predation Pressure

As with all ecosystem interactions the prey/predator relationships for sea trout is a natural process, however as identified the sea trout populations on the West coast are under pressure and declining (AST, 2011). It is important to understand the dynamics of the predation occurring. One of the dynamics relating to sea lice loadings and predation is particularly important to consider for example at sites were lice loads may be at elevated levels and weakening the fish, it may therefore be increasing a fish population's susceptibility to predation. Sea trout can encounter a range of predators throughout their life cycle. These include predators ranging from birds such as the Osprey or Heron, to mammals such as mink or otters and to marine mammals such as common and grey seals. Predation pressures are difficult to quantify and currently out with the scope of this study. It has been shown that predation by marine mammals may have a role in stock declines, but this impact is not well understood (Middlemas, *et al* 2003; Butler *et al*, 2006; Butler *et al*, 2011).

The scope of the study here is limited to examining whether predation could be identified as occurring or not occurring. There are no conclusions drawn on the detrimental level of impact on the sea trout populations under study may be experiencing due to predation. Whilst examining the sea trout for physical damage, if observed it was categorised to the likely predator species and the percentage level of damage/scale loss was also recorded by the Fisheries Biologist. Predation was observed at fifteen sites across the West Coast and the Western Isles (Figure 6).

From the predation recorded the majority were from seals and birds with a few recorded as due to otter damage. Two monitoring sites recorded indicative high levels of physical damage from predation which are Kinlocheil and Camas na Gaul both in Lochaber. It is recommended that further studies into predation pressure on the sea trout post smolts should be explored to further understand the pressure dynamics being experienced at these sites.

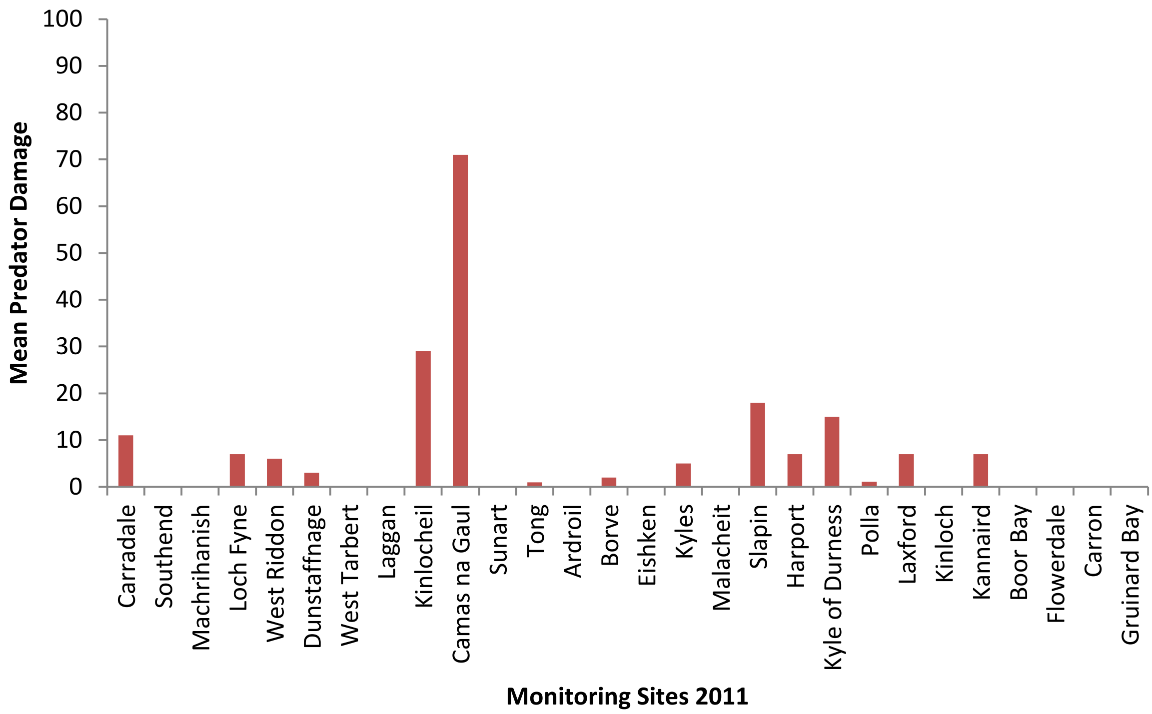

Figure 6: The percentage sea trout predator damage recorded at each monitoring site.

3.2 Sea Lice Analysis

3.2.1 *L. salmonis* Copepodid and Chalimi life Stages

The *L. salmonis* immature life stages under examination here are the Copepodid and Chalimi stages. These initial stages include the four stages of immature sea lice which attached to the sea trout by a frontal filament around which they feed on the fish mucus and skin. These immature stages are the smallest and are often extremely hard to discern on the fish host and as a result they are often under estimated in counts (Tully, 1989).

It can be extremely hard to determine significant levels for each of the sites with no information on background levels of sea lice data available. From the data collected in 2011 and considering the individual sites compared to the regional mean of 2.33 for abundance, a mean regional intensity of 8.36 and a regional mean prevalence of 31 it can been seen that the majority of sites reported and recorded levels of Copepodid/Chalimi presence below the regional mean for abundance, intensity and prevalence (Figure 7 and Figure 8). However there are three sites which could be classed as experiencing elevated levels of Copepodid/Chalimi presence when considering the regional means for abundance, intensity and prevalence these are Camas na Gaul (Lochaber), Kinnaird (Wester Ross) and Laxford (West Sutherland). To ensure that the regional means are not being representing by any particularly high outliners the median which is less influenced by outliers was explored. As can be seen from Figure 9 again Camas na Gaul (Lochaber), Kinnaird (Wester Ross) and Laxford (West Sutherland) are indicated as experiencing elevated levels.

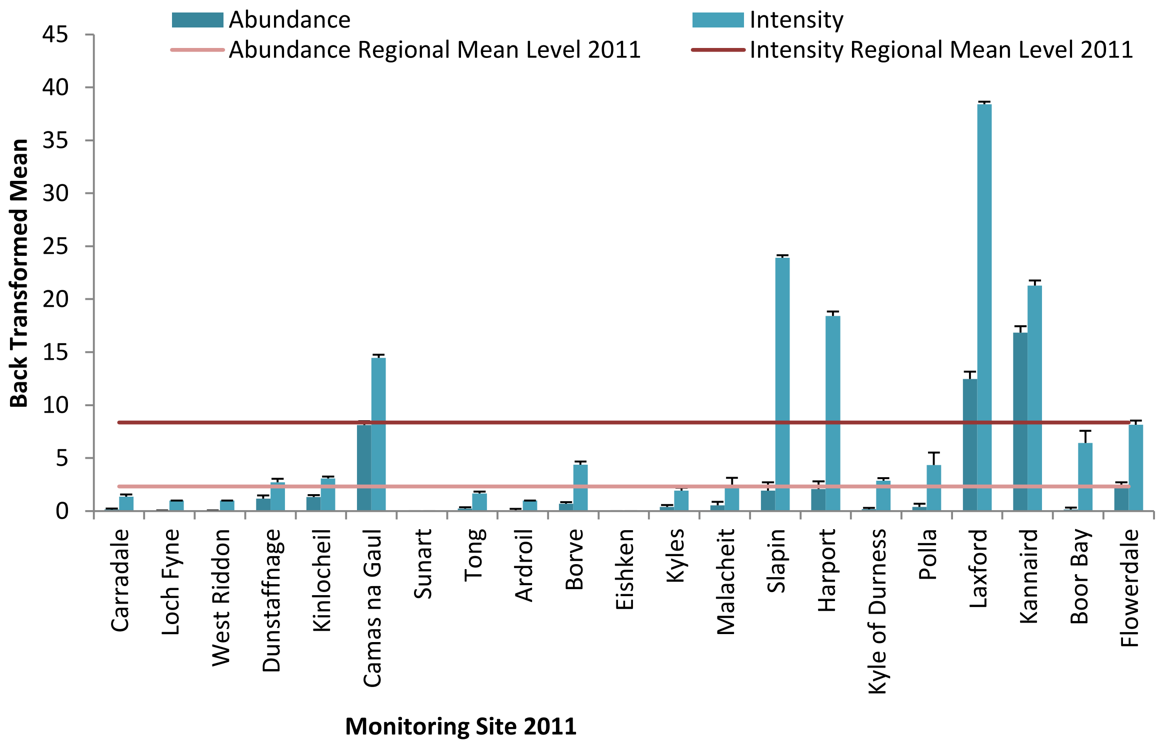

Figure 7: Back Transformed means in 2011 for Abundance and Intensity for Copepodid/ Chalimi at each monitoring site (including 95% confidence intervals).

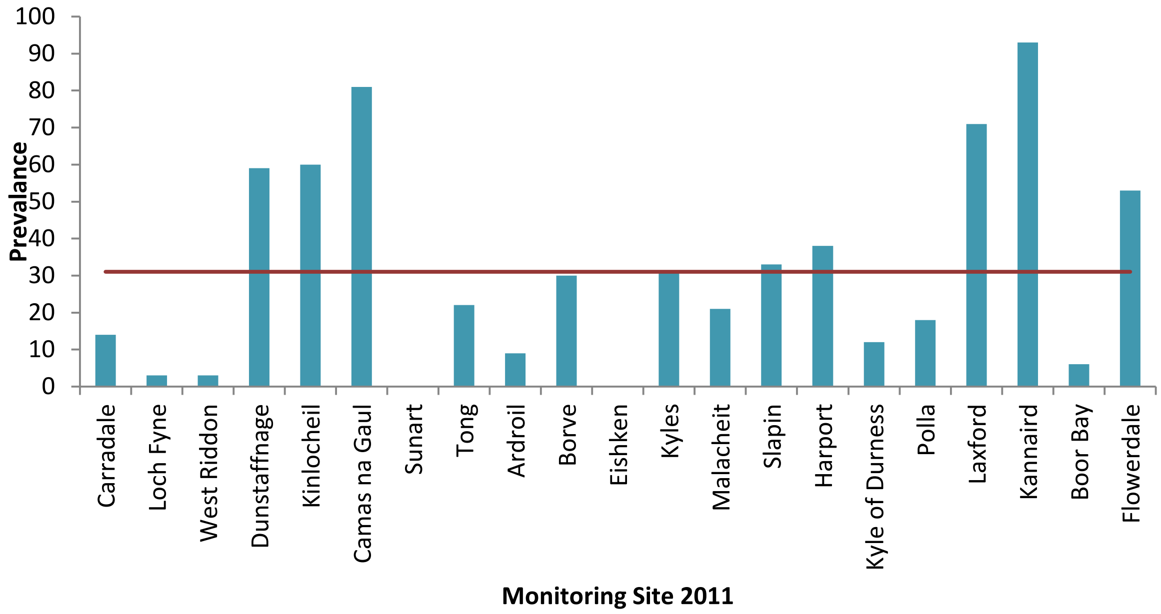

Figure 8: Prevalence results of Chalimi/Copepodid stages at each monitoring site. The prevalence regional mean level for 2011 is indicated on the graph as a red solid line.

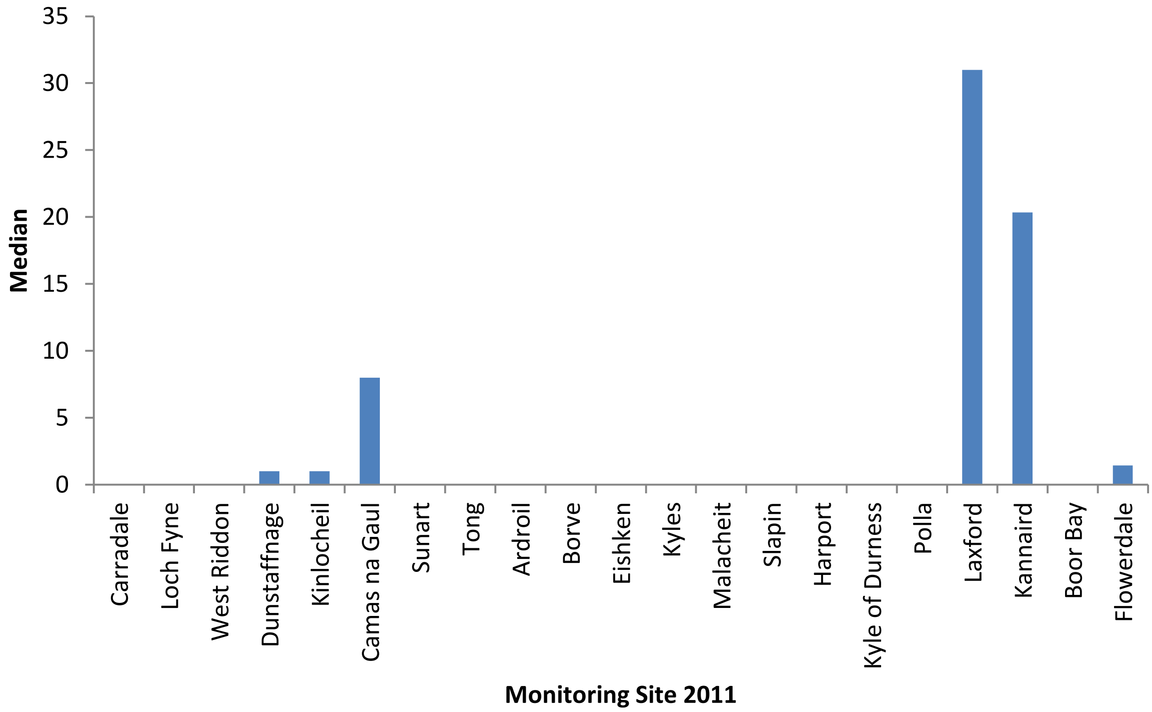

Figure 9: Median results of Chalimi/Copepodid stages at each monitoring site. The median regional level for 2011 is 0.

3.2.2 *L. salmonis* Mobile life Stages

The *L. salmonis* stages under examination here are commonly referred to as the mobile life stages, which includes the two pre-adult stages of the male and female. The adult life stage here includes the adult male and female (without eggs strings). These life stages are easier to identify as they are larger and move freely to feed over the fish mucus and skin.

From the data collected in 2011 and considering the individual sites compared to the regional mean of 1.17 for abundance, a mean regional intensity of 3.41 and a mean region prevalence of 37 it can been seen that the majority of sites reported and recorded levels of preadult and adult presence below the regional mean for abundance, intensity and prevalence (Figure 10 and Figure 11). However there are five sites which could be classed as experiencing elevated levels of preadult and adult presence when considering the regional mean for abundance, intensity and prevalence. These are Dunstaffnage (Argyll), Kyles (Outer Hebrides), Malacleit (Outer Hebrides), Camas na Gaul (Lochaber) and Laxford (West Sutherland). There is a potential for the regional means to be representing particularly high outliners, therefore the median which is less influenced by outliers was explored to confirm the indicative elevated levels. As can be seen from Figure 12 four of the five sites Dunstaffnage (Argyll), Kyles (Outer Hebrides), Malacleit (Outer Hebrides) and Camas na Gaul (Lochaber) are indicated as experiencing elevated levels. However the fifth site Laxford (West Sutherland) is below the regional median and therefore less likely to be experiencing elevated mobile life stages.

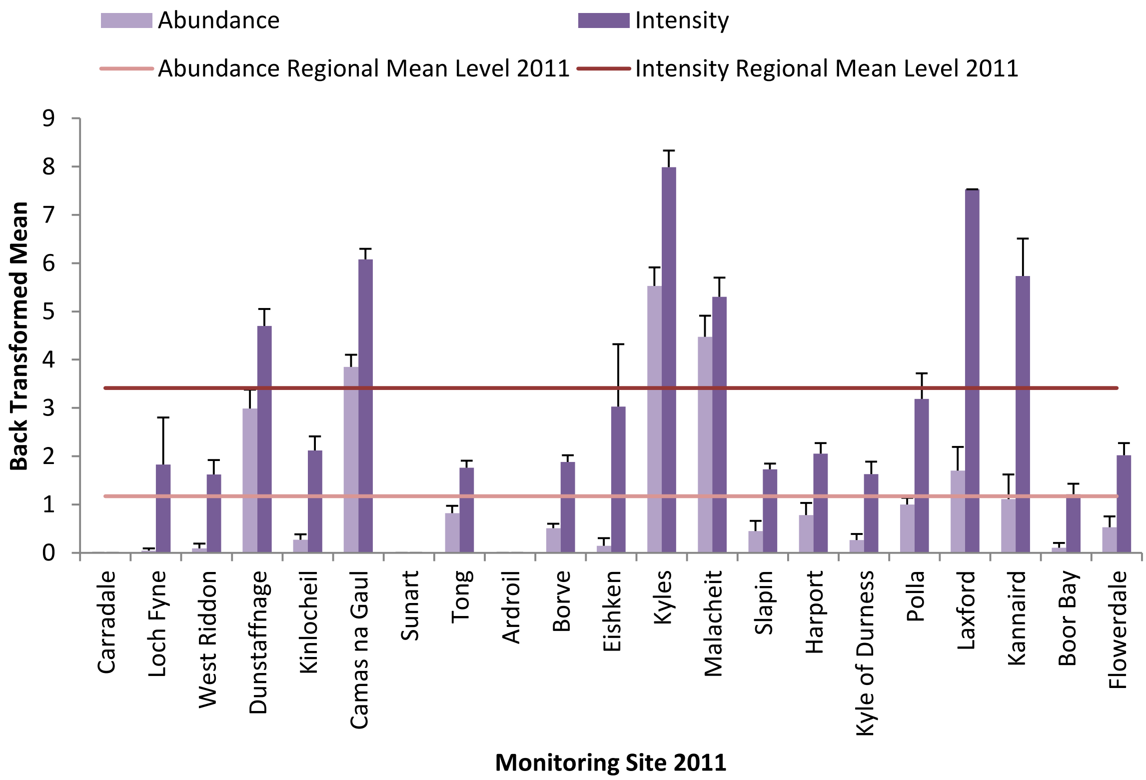

Figure 10: Back Transformed means in 2011 for Abundance and Intensity results for Preadult/Adult at each monitoring site (including 95% confidence intervals).

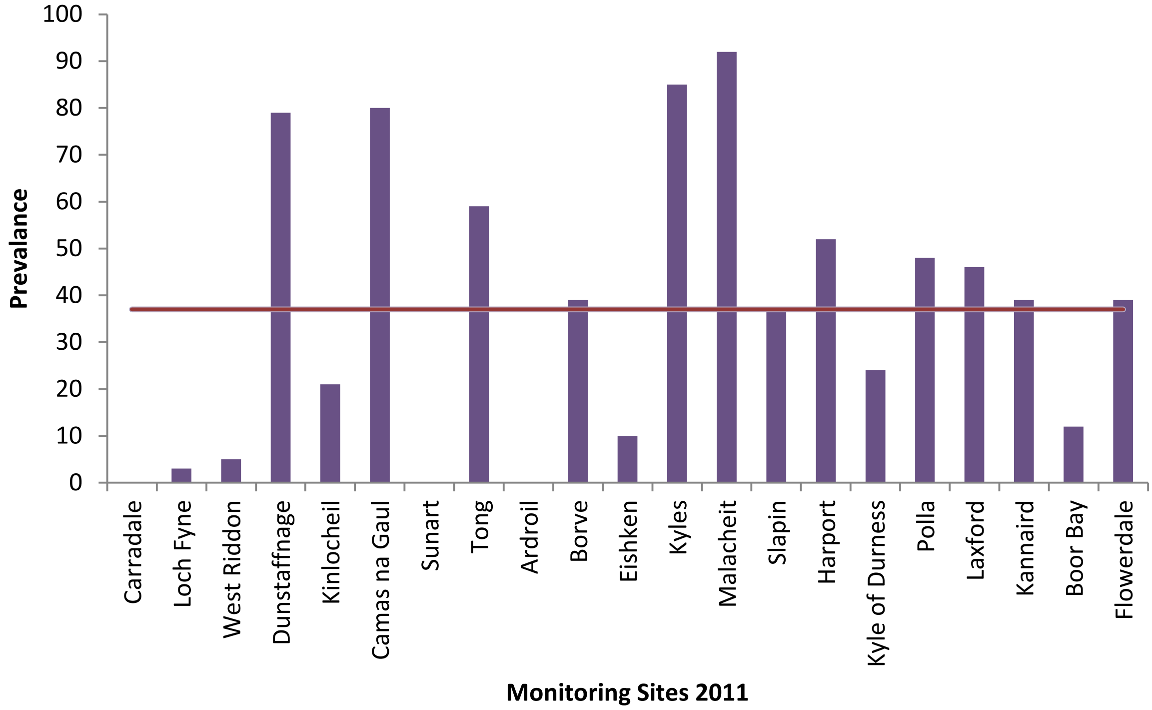

Figure 11: Prevalence results for Preadult/Adult *L. salmonis* stages at each monitoring site. The prevalence regional mean level for 2011 is indicated on the graph as a red solid line.

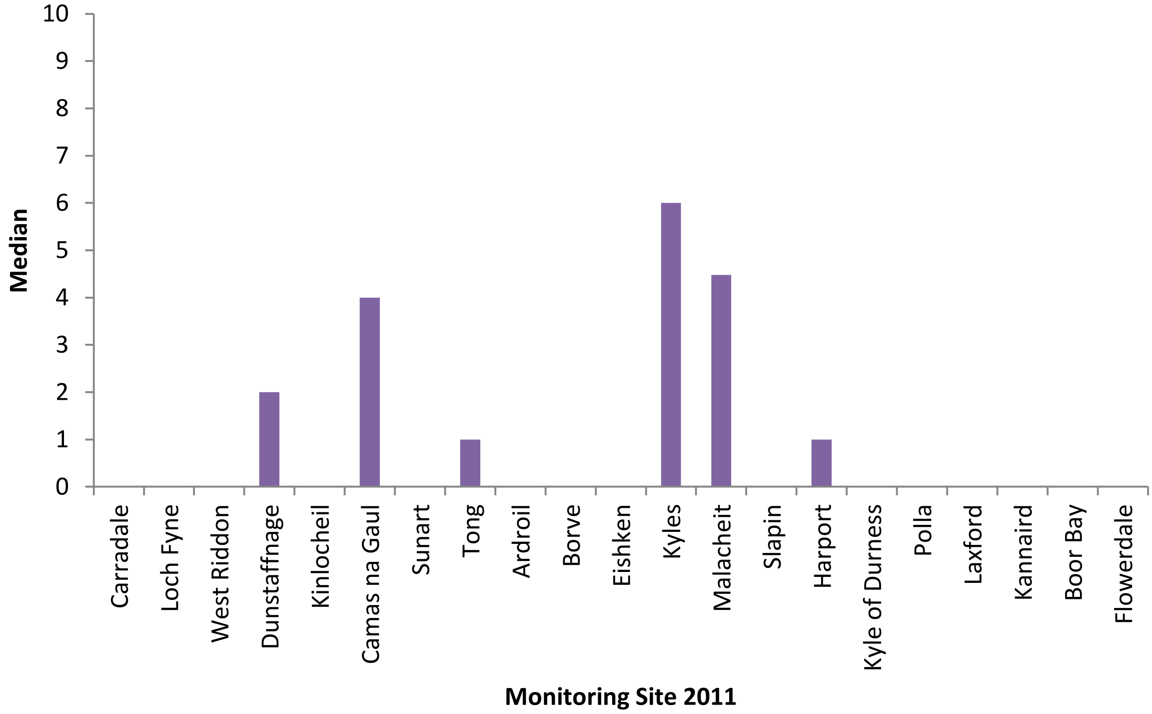

Figure 12: Median results for Preadult/Adult L. salmonis stages at each monitoring site. The median regional level for 2011 is 0.

3.2.3 *L. salmonis* Ovigerous Female life Stage.

The final *L. salmonis* life stage examined on the post smolt sea trout was the Ovigerous female. Ovigerous females are easily identified by two visible egg strings which can average carry a total of a 1000 eggs.

From the data collected in 2011 and considering the individual sites compared to the regional mean of 0.21 for abundance, a mean regional intensity of 1.65 and a regional mean prevalence of 15 it can been seen that the majority of sites reported and recorded levels of ovigerious female presence below the regional mean for abundance, intensity and prevalence (Figures 13 and 14). Only three sites could be classed as experiencing elevated levels of ovigerious female presence when considering the regional mean for abundance, intensity and prevalence these are Kyles (Outer Hebrides), Malacleit (Outer Hebrides) and Polla (West Sutherland). There is a potential for the regional means to be representing particularly high outliners, therefore the median which is less influenced by outliers was explored to confirm the indicative elevated levels. As can be seen from Figure 15 two of the three sites Kyles (Outer Hebrides) and Malacleit (Outer Hebrides) are indicated as experiencing elevated levels. However the third site Polla (West Sutherland) is below the regional median and therfore unlikely to be experiencing elevated mobile life stages.

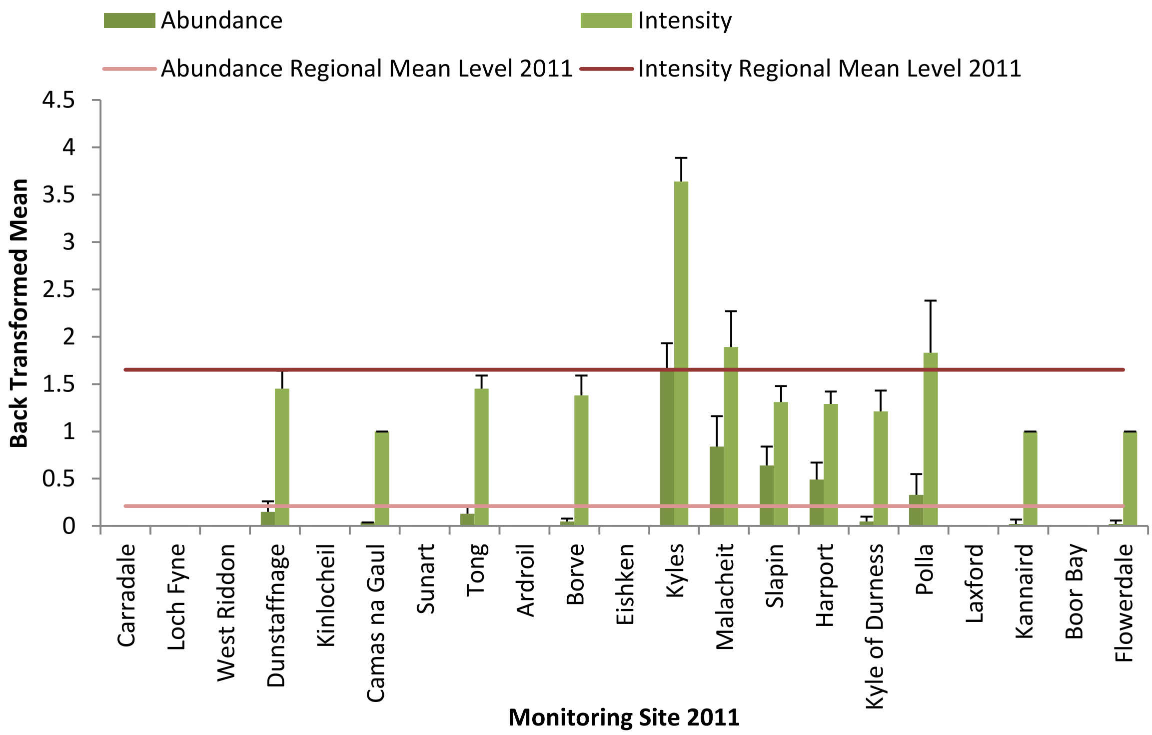

Figure 13: Back Transformed means in 2011 for Abundance and Intensity results *for L. salmonis* ovigerous females at each monitoring site (including 95% confidence intervals).

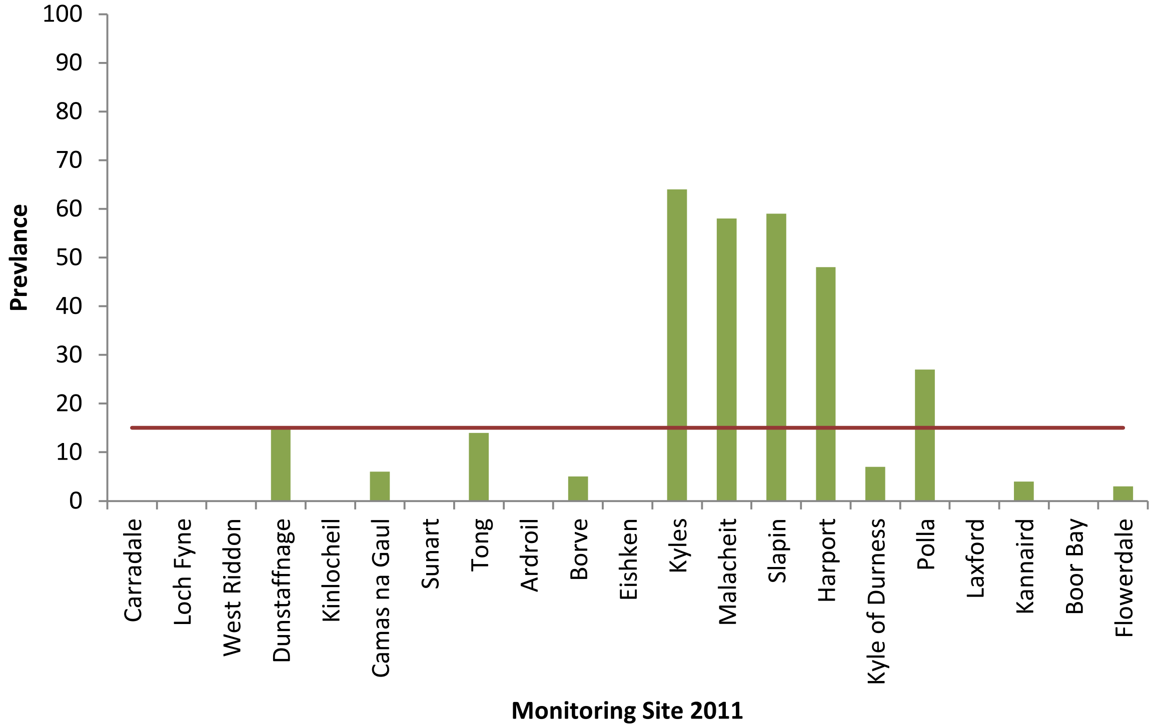

Figure 14: Prevalence results for *L. salmonis* ovigerous females stage at each monitoring site. The prevalence regional mean level for 2011 is indicated on the graph as a red solid line.

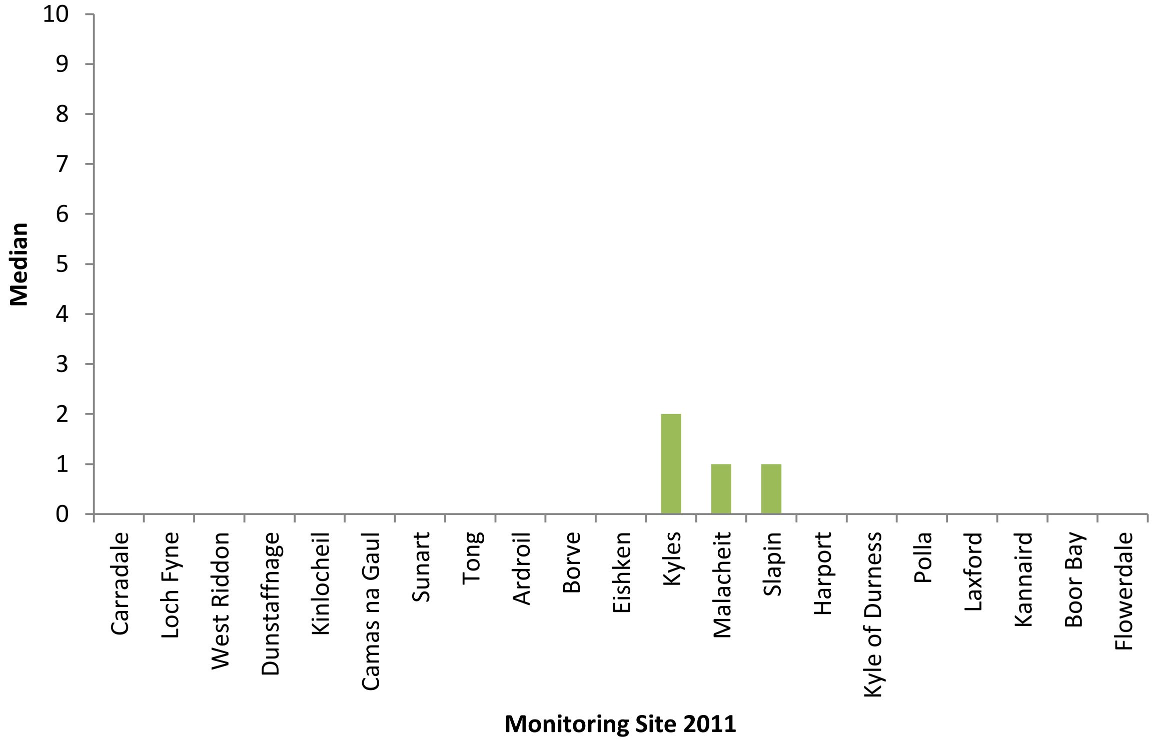

Figure 15: Median results for L. salmonis ovigerous females stage at each monitoring site. The median regional level for 2011 is 0.

3.2.4 *L. salmonis* all life Stages.

A final examination of the total counts of the all the *L. salmonis* life Stages was under taken. Overall the majority of the monitoring sites sampled experienced low levels of *L. salmonis* presence when considering the regional mean for abundance 3.81, regional mean for intensity of 7.75 and a regional mean prevalence of 50 in 2011 (Figures 16 and 17). However there are four sites which indicate elevated presence levels in comparison to the regional means. These are Kyles (Outer Hebrides), Camas na Gaul (Lochaber), Kinnaird (Wester Ross) and Laxford (West Sutherland). There is a potential for the regional means to be representing particularly high outliners, therefore the median which is less influenced by outliers was explored to confirm the indicative elevated levels. As can be seen from Figure 18 all four sites Kyles (Outer Hebrides), Camas na Gaul (Lochaber), Kinnaird (Wester Ross) and Laxford (West Sutherland) are indicated as experiencing elevated levels. Further exploration of these results and their potential detrimental impacts can be found in section 4.

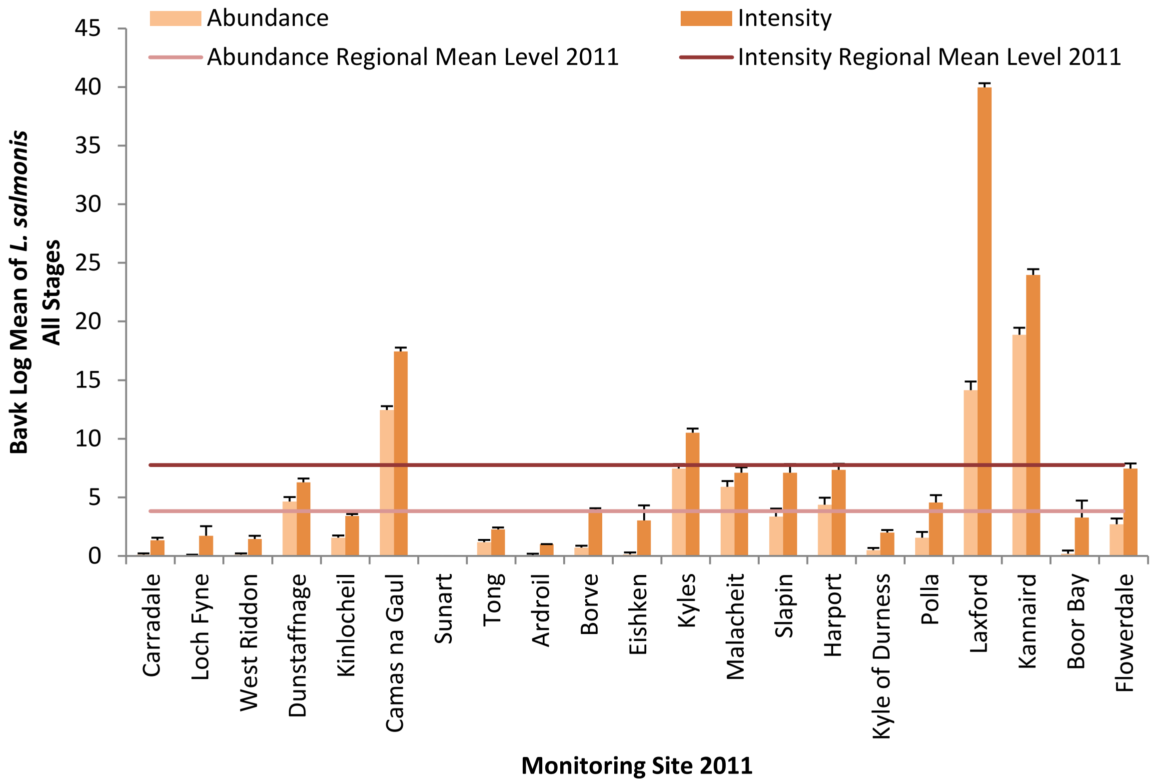

Figure 16: Back Transformed means in 2011 for Abundance and Intensity results for all *L. salmonis* stages at each monitoring site (including 95% confidence intervals).

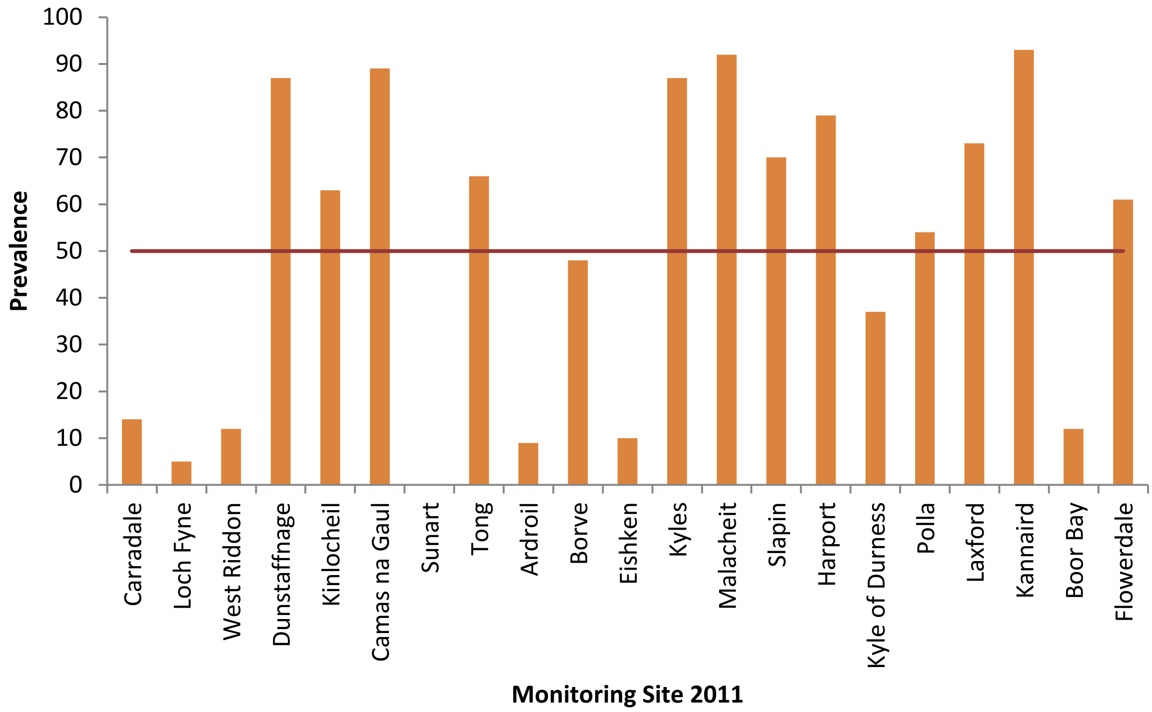

Figure 17: Prevalence results for all *L. salmonis* stages presence at each monitoring site. The prevalence regional mean level for 2011 is indicated on the graph as a red solid line.

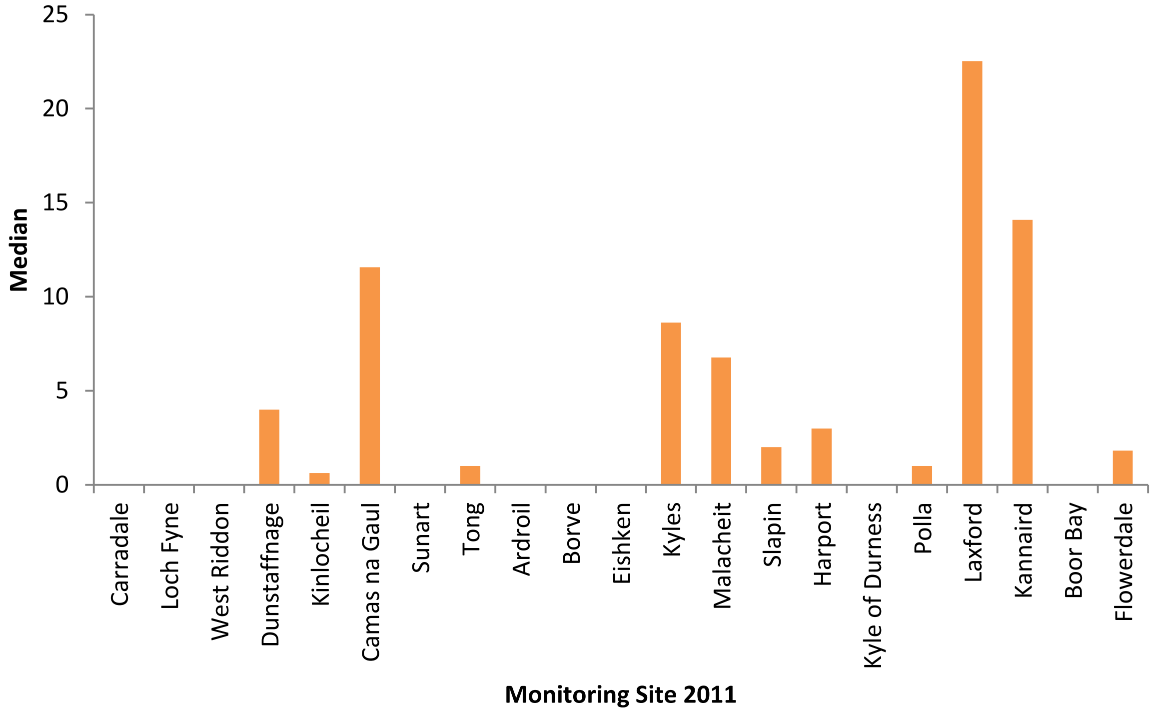

Figure 18: Median results for all *L. salmonis* stages presence at each monitoring site. The median regional level for 2011 is 1.

3.1.5 *C. Elongatus* all life Stages

Caligus elongatus is much smaller, lighter in colouration and a host generalist (Wootten *et al*., 1982) that has been recorded on over eighty host species (Kabata, 1979). The *C. elongatus* life cycle has less stages then *L. salmonis* as it moults directly from chalimus IV to the adult stages (Piasecki,1996). Whilst currently of lesser concern in Scotland than the sea louse *L. salmonis*, *C. elongatus* is present and does have the potential to become a problem which should not be underestimated. Bergh *et al*., 2001 reported high intensity *C. elongatus* infestations, and consequentially severe head lesions, were reported for juvenile farmed halibut *Hippoglossus hippoglossu*. As a host generalist there are possibilities in Scotland that if presence levels become elevated, farmed and wild fish could experience detrimental problems from *C. elongatus.*

From the data collected throughout the monitoring sites *C. elongatus* was only identified as being present in Skye, West Sutherland and the Outer Hebrides. It can be extremely hard to determine significant levels for each of the sites with no information on background levels of sea lice data available. From the data collected in 2011 and considering the individual sites compared to the regional mean of 0.48 for abundance, a mean regional intensity of 5.59 and a regional prevalence mean of 10. Where this species was identified as present, overall it was at extremely low presence levels. Only one site in West Sutherland Laxford demonstrates elevated presence levels in comparison to the regional means for abundance, intensity and prevalence (Figures 16 and 17). There is a potential for the regional means to be representing particularly high outliners, therefore the median which is less influenced by outliers was explored to confirm the indicative elevated levels. As can be seen from Figure 18 Laxford (West Sutherland) is indicated as experiencing elevated levels.

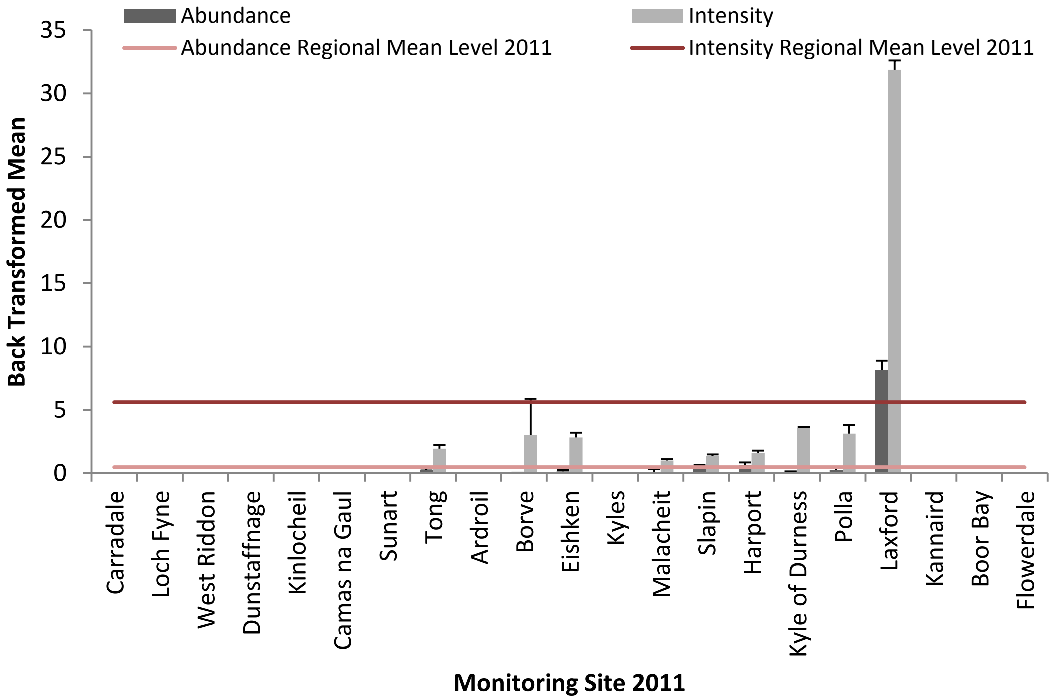

Figure 16: Back Transformed means in 2011 for Abundance and Intensity results for all *C. elongatus* stages at each monitoring site (including 95% confidence intervals).

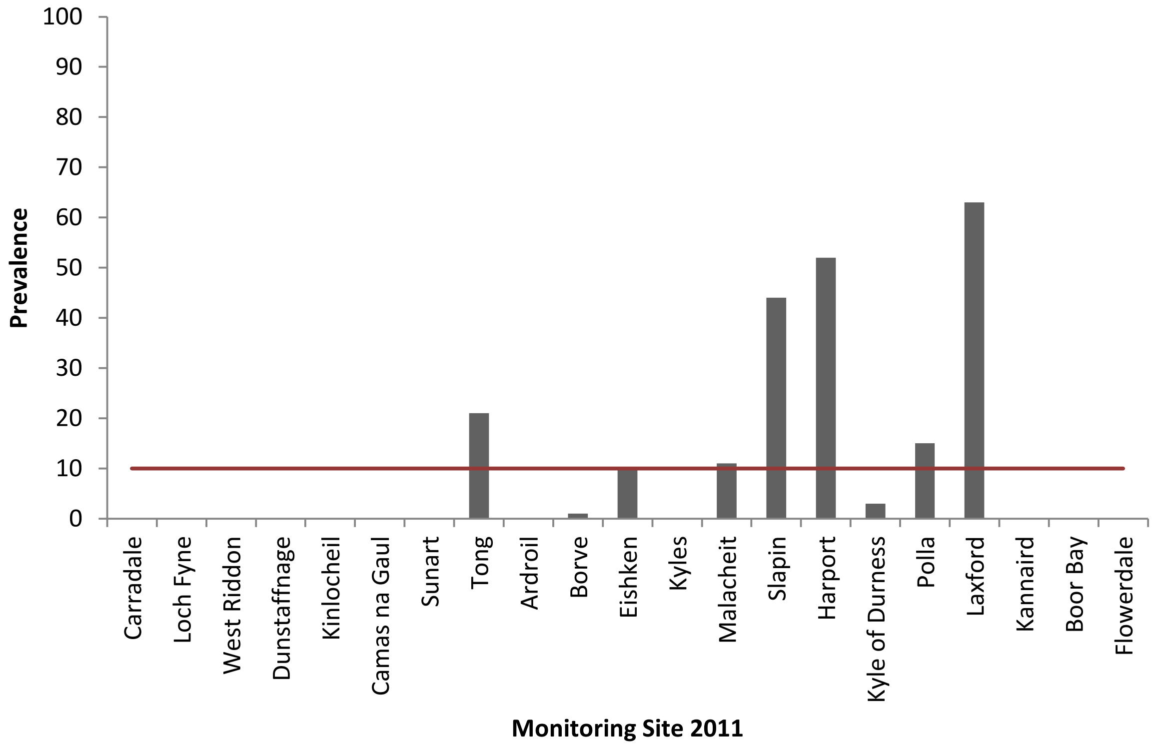

Figure 17: Prevalence results for Total *C. elongatus* presence at each monitoring site. The prevalence regional mean level for 2011 is indicated on the graph as a red solid line.

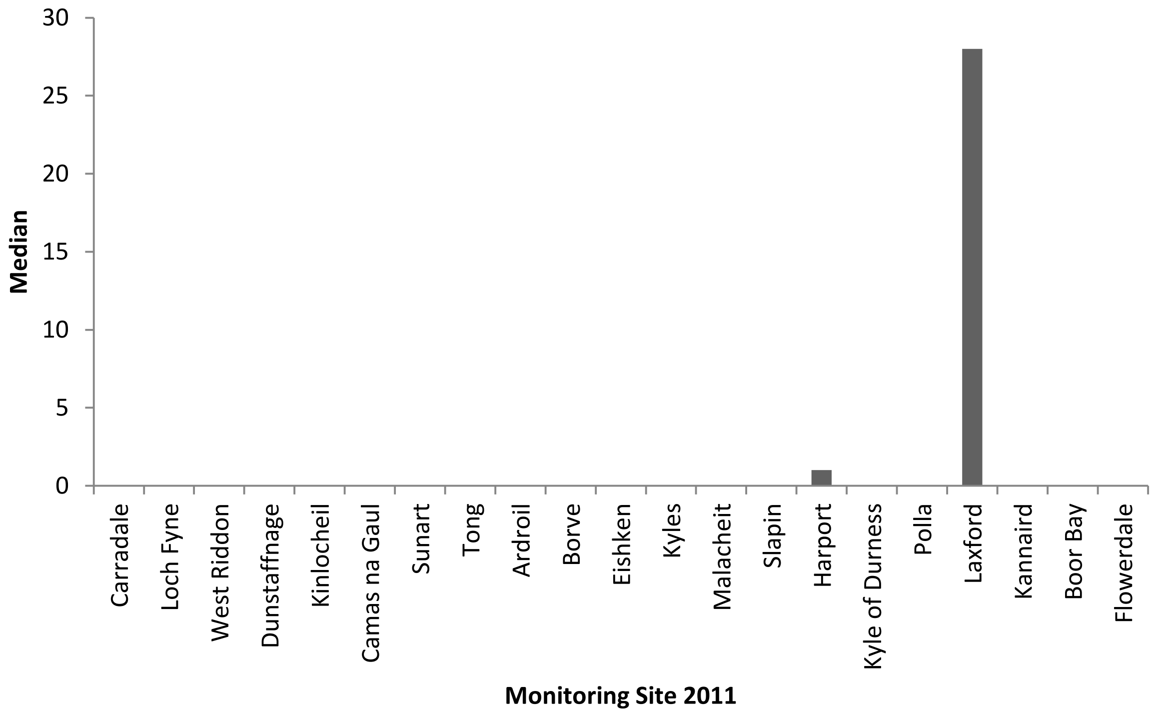

Figure 18: Median results for Total *C. elongatus* presence at each monitoring site. The median regional level for 2011 is 0.

4. Discussion

Overall when considering the results of the post smolt sea trout populations the lengths and weights are in line with the predicted results and from these, the mean condition factors across the populations are encouraging and indicative of fish in good condition. The majority of the sites showed low levels of damage from predation but some identified sites recorded levels of predator damage which may merit further work to attempt to quantify any detrimental impact caused by predators. Particularly as one of these sites Camas na Gaul (Lochaber) as discussed in section 4.1 are also indicating elevated lice loadings that may be having an impact on the dynamics of the prey/predator relationship in these areas.

To fully understand the implications of the sea lice presence at the monitoring sites and whether or not detrimental impacts were being experienced further analyses were performed based on the results of previous studies.

4.1 Exploring the pressures from Sea Lice on wild sea trout post smolt populations.

A number of factors need to be considered when analysing the results collected at the monitoring sites. Sweep netting studies may over- or under-estimate the levels of lice on wild fish. It is sometimes impossible to sample those fish which have succumbed to heavy infestation loads and therefore such fish will not be sampled potentially leading to an underestimate of the true lice levels. Equally, it is possible that those fish with no lice, or small levels of lice are better able to evade the net than fish with higher lice levels, potentially leading to overestimates. Therefore presenting a true reflection of infestation levels on the sea trout population as a whole is problematic and leads to an inherent difficulty in drawing meaningful conclusions on threshold levels and their impact on sea trout populations (Middlemas *et al*., 2010). As long as these inherent difficulties are presented and considered it is possible to draw conclusions that can be attributed to the population and inform local management strategies and policies.

To further explore the sea lice infestation pressure on wild sea trout populations data from each monitoring site was examined to determine if the levels of observed sea lice infection could be classed as an epizootic. Sea lice epizootics are characterised by unusually high infestations that are maybe fatal and although currently rare in Scotland they have previously been reported (Butler, 2002). Epizootics recorded on sea trout in Europe and Pacific salmon in British Columbia tend to have over 60% prevalence and more than 5 lice per fish (Costello, 2009 and Beamish *et al*, 2009).

Based on the results of calculating threshold levels for an epizootic occurring there are five sites that have experienced sea lice levels that could potentially be categorised as epizootics (Figure 19). This, however, is not the final picture as this is only indicates that these sea trout populations are experiencing heavy, large infestations and further analysis is required to determine if these high observed levels are having a detrimental impact. To examine these high levels in more depth a tolerance threshold level was explored.

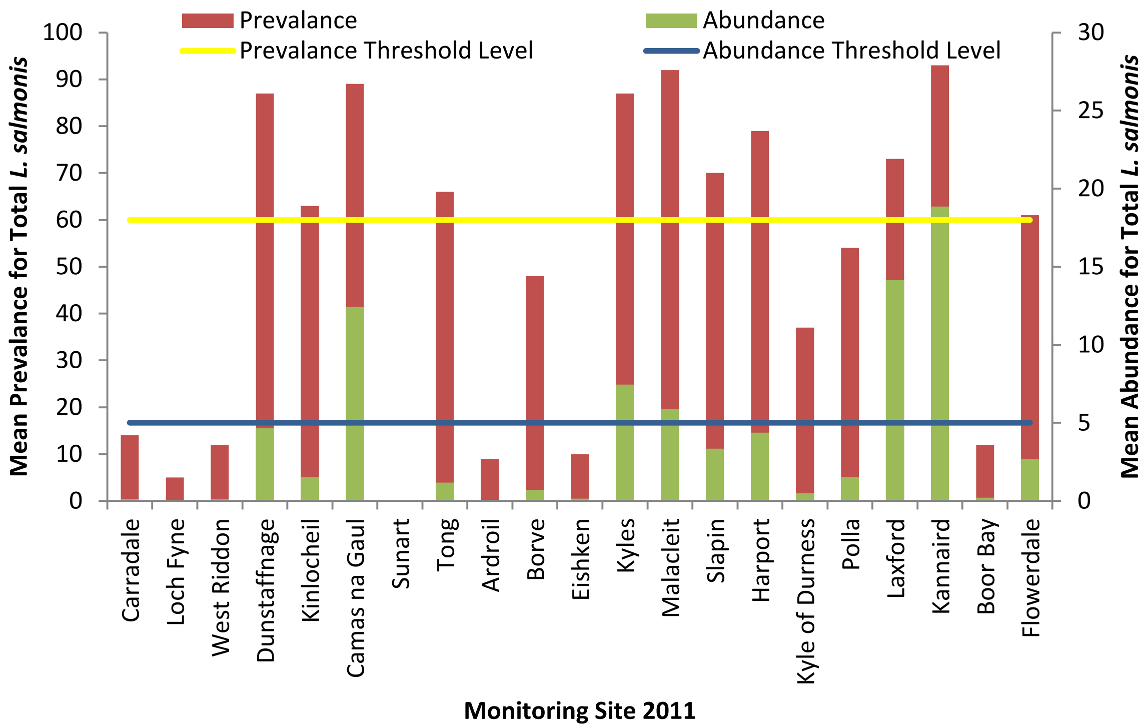

Figure 19: Prevalence and Abundance results for all *L. salmonis* stages at each monitoring site in 2011. The Costello 2009 threshold levels for identifying epizootics are highlighted on the graph by a solid yellow line for the prevalence threshold and a solid blue line for the abundance threshold.

The threshold level for impact to be explored is from Wells *et al*. (2006) where this study found that abrupt changes in a range of physiological parameters occurred at thirteen mobile lice per fish (weight range 19-70g). This level could be detrimental to the fish host. It was suggested within this study that a management strategy should be applied if the populations are experiencing more than 13 mobile lice per fish. The lice figures used in this analysis were all mobile stages and the proportion of chalimi converted into the expected number of mobile lice. To calculate the likely survival rate of chalimi to adult stages Bjørn and Finstad 1997 recommended survival rate of 0.63 was implemented. As not all weight data was available for all sites as was employed under the previous study by Middlemas *et al* 2010, only those fish below 198mm (the equivalent of 70g) were considered in this analysis. It was also deemed appropriate only to consider monitoring sites that have sample sizes of thirty fish or greater.

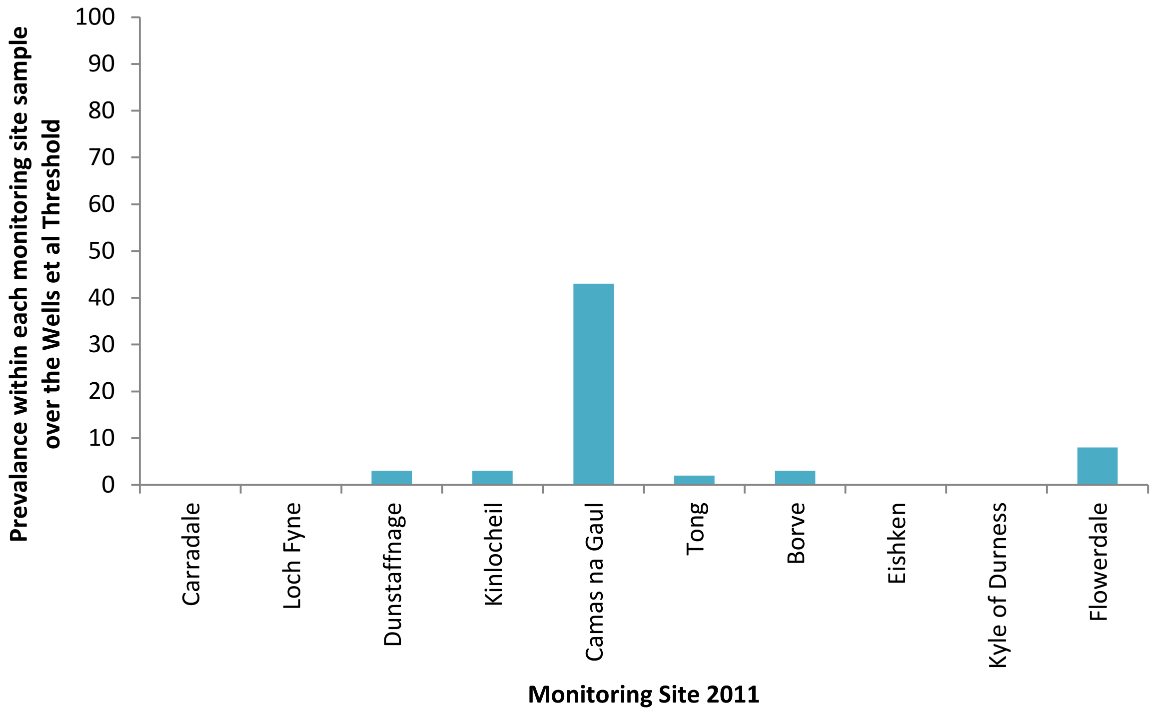

Figure 20: Percentage of fish within each monitoring site sample which has been identified over the Wells et al, 2006 threshold.

Within each of the monitoring samples the percentage of individual fish in each sample that appeared over the threshold and therefore more likely to be carrying a detrimental sea lice burden were identified for each monitoring site (Figure 20). One monitoring site Camas na Gaul which has 43% of the sample carrying detrimental lice loads. In comparison all other sites with a valid sample size have experienced less than 10% of the sample recorded as carrying detrimental loads.

There is currently no guidance on the acceptable proportion of fish exceeding the Wells *et al* 2006 threshold. Interestingly, Hazon *et al* 2006 recommend in the EU project "Sustainable Management of Interactions between Aquaculture and Wild Salmonid":

> "*that a level of 10% or fewer of wild sea trout in any given population in Ireland bearing total infestations of ≥13 lice • fish-1 should be adopted as indicative of a satisfactory or acceptable lice loading. Within any given sea trout stock, frequencies of heavily-infested juvenile sea trout (i.e. those ≥13 lice • fish-1) >10% should perhaps be considered a cause for concern*."

Being able to adopt such an acceptable or unacceptable proportion of lice loadings in Scotland would aid the local management strategies and policies greatly. To achieve this would require the collation and evaluation of sea trout captured at 50km and greater from active fish farms in Scotland and this is one of the aims of the managing interactions monitoring work as it goes forward into 2012.

In conclusion when considering the epizootic threshold (Costello, 2009) and the *L. salmonis* mobile threshold (Wells et al 2006), it is possible to identify the post sea trout populations in the study areas that are under pressure from detrimental sea lice loadings and where management strategies are required to support the reduction of sea lice burdens on the post smolts. However it should be noted that the detrimental impact from sea lice has concentrated solely on one species *L. salmonis* in this study. At a number of the monitoring sites in 2011 *C. elongates* was identified as also present and although not seen as such a serious problem species as *L. salmonis* the relationship and the likely additive effect of the two species occurring together merits further exploration in the future.

4.2 Managing Interactions

4.2.1 Monitoring Site comparisons to nearest active Fish Farm.

Previous monitoring data collected under the TWG project was analysed by Marine Scotland Science (Middlemas *et al*, in peer review) which explored the levels of sea lice in relation to distance to fish farm covering the period of 2003 to 2009. Whilst only a preliminary analysis of the 2011 data could be carried out as part of this study, further exploration of this factor remains a priority going forward into 2012.

Data was obtained from Scottish Environment Protection Agency on the nearest farms to the monitoring site. Data acquired included year of production and mean biomass at fish farm site for the period of May to July 2011. As can be seen from Figure 21 the majority of the active fish farm sites were in the second year of production. It was anticipated that the data collected in 2011 would allow for further analyses of the distance aspect of sea lice interactions between wild fish and farmed fish. This year monitoring sites between 10km to 20km and 25km to 40km have not been as successful as in previous years. As mentioned previously this was is due to factors including severe weather conditions which created unsafe sampling conditions and the simple absence of fish at survey times. In respect of some the new sites the minimum planned sampling effort was not implemented by trusts and, therefore, it is not clear whether these sites are unsuitable for further sampling effort or should be retained. Whilst a decreasing infestation pattern can be observed as distance is increased from the active fish farm it is not statistically robust to draw any conclusions at this time (Figure 22).

In comparison when considering the fish farm site biomass levels it has previously been reported that with increasing biomass levels it can create a situation were greater infestation levels on wild fish are experienced. Again this pattern of increasing infestation levels with greater biomass on site can be observed for 2011, however it is not statistically robust to draw any conclusions at this time (Figure 23).

Finally when considering the interactions of the farmed with the wild fish it is not only the nearest farm but the accumulation of active fish farms that needs to be considered when trying to objectively address management and policy practices to help manage the interactions. A distance band analysis was carried out in Idrisi from the monitoring site to indicate how many active fish farms were present (Appendix 7). A further complicating factor when considering the accumulation of fish farms in an area is the presence of non-active licenses and a number of these do fall within the distance bands from the monitoring sites. There is currently no clear framework on notification of when any of these inactive sites may become active again and this is a highly complicating factor for wild fisheries when trying to manage the interactions. This is a factor that is currently under consideration in the Scottish Government Consultation Bill and hopefully from this clarification will be achieved that will aid the appropriate management and policy guidance on this factor in the future. The data gathered in the 2011 surveys reported here is available to Marine Scotland Science for use in further and on going analysis of sweep netting results that may be undertaken.

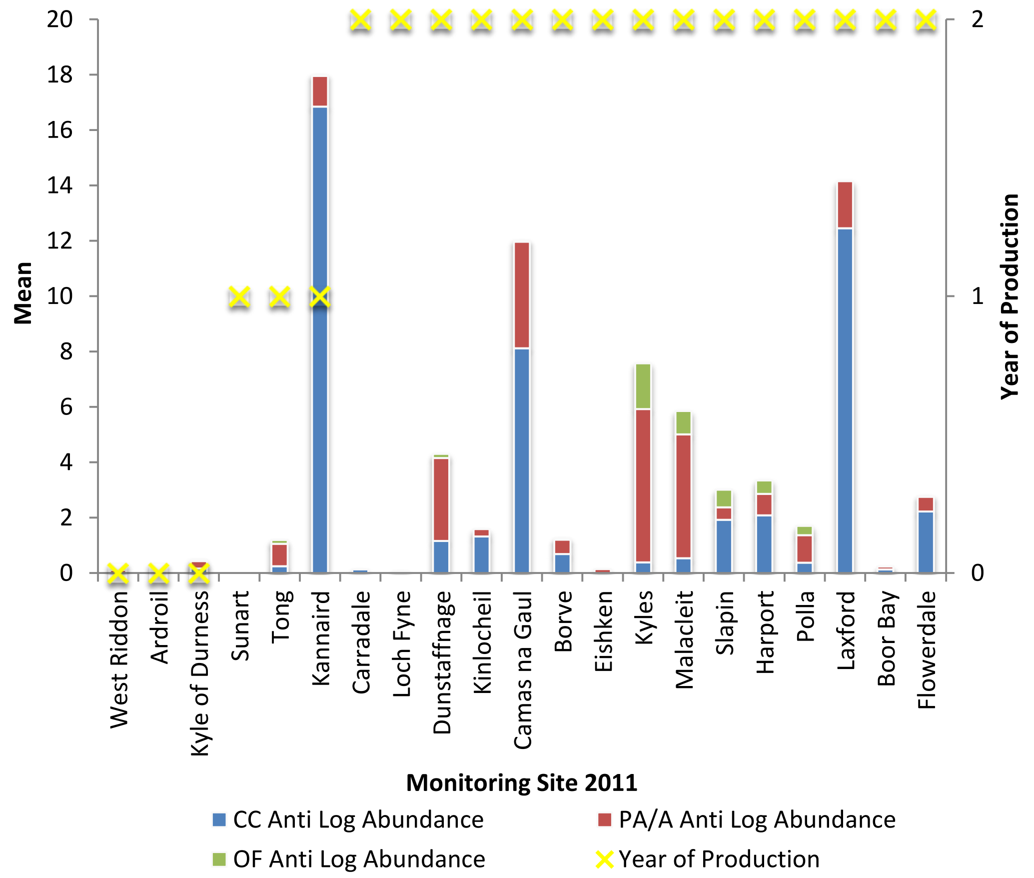

Figure 21. Year of production in 2011 of the nearest active fish farm to the monitoring site (yellow cross) and the mean abundance of *L. salmonis* staged per SFCC protocol.

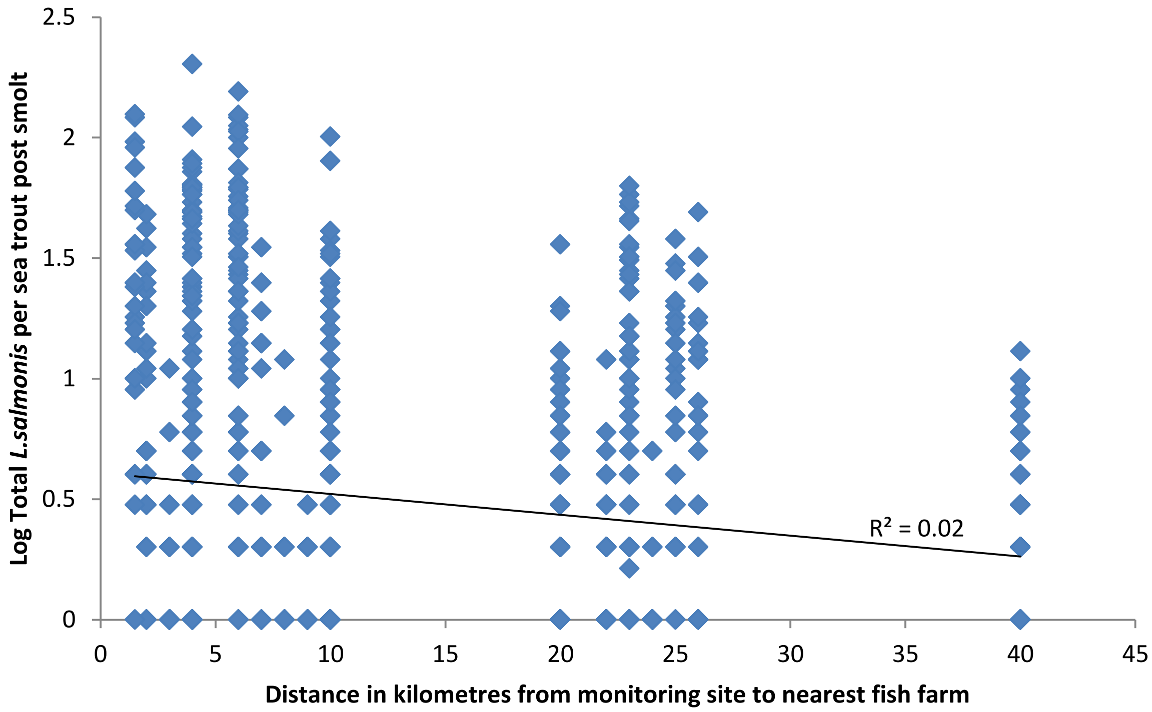

Figure 22 *L. salmonis* Log total per individual fish host for each monitoring site compared to the distance in km to the nearest active fish farm 2011.

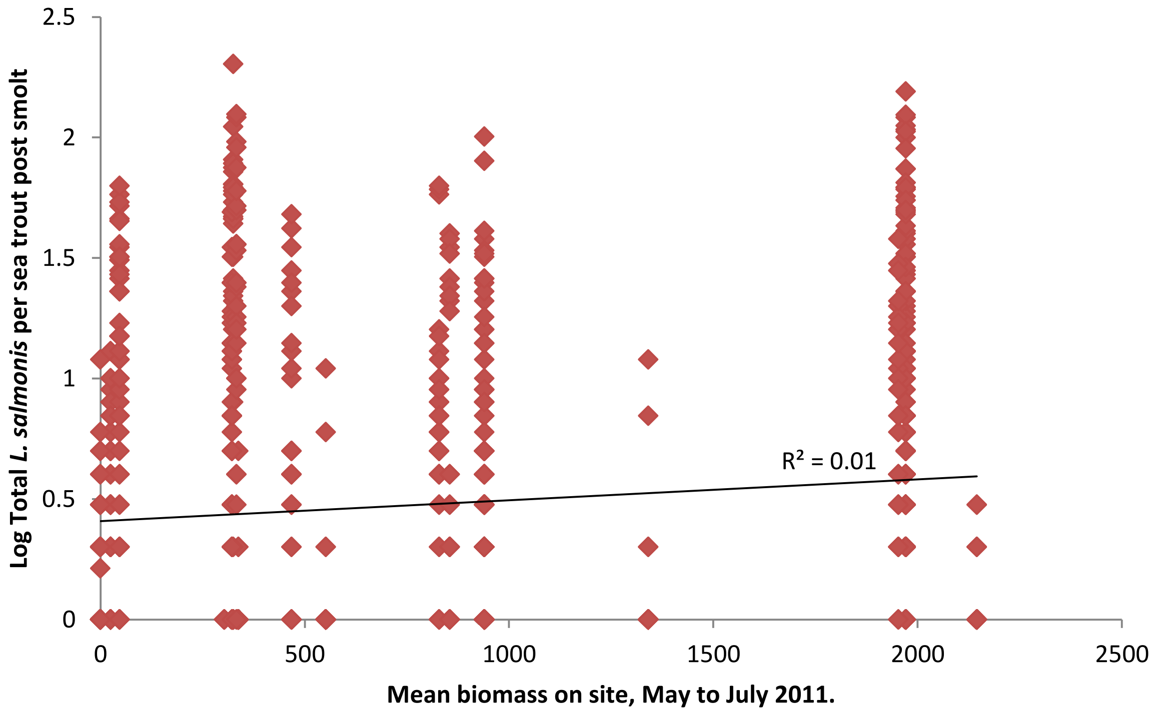

Figure 23. *L. salmonis* total per individual fish host for each monitoring site compared to the mean biomass on the nearest active fish farm for the period of May to July 2011.

4.2.2 Monitoring Site Sea Lice Counts in comparison to Farmed Fish sea lice counts.

In 2010 the Scottish Salmon Producers Organisation (SSPO) developed a dedicated health management system which is specifically designed to assist its members to improve lice management across Scotland. The information gathered and analysed in this system is published in reports on their website for six management regions across Scotland[1].

The six management regions are Orkney, West Shetland, East Shetland, North Mainland, South Mainland and the Western Isles. The monitoring sites within the Managing Interactions project fall into the North Mainland (encompassing the coastline (and associated islands) from Loch Eriboll in the north to Rubh' Arisaig, near Loch nan Ceall on the west coast), South Mainland (encompassing the coastline (and associated islands) from Rubh' Arisaig, near Loch nan Ceall on the west coast, to Irvine, towards south west Scotland.) and the Western Isles (encompasses all islands in the Western Isles including Harris, Lewis, North and South Uist, Benbecula, Barra and the associated smaller islands).

The interactions between farmed and wild fish in relation to sea lice is a contentious issue in Scotland and elsewhere which are not yet fully researched or understood (Harvey, 2009). Nonetheless the most realistic approach within the current understanding of the wild and farmed fish interactions should be a precautionary approach as highlighted by Revie *et al* 2009. It had been anticipated that the published wild fish lice counts could be examined alongside the published farm lice counts. However, the highly aggregated form, covering large geographical areas, in which the SSPO published their results did not allow this comparative evaluation to be undertaken. Nevertheless it is possible to report on the regional lice count information published by SSPO.

The SSPO reports indicate that in the period of May 2011 the Western Isles and the South Mainland lice numbers across these two regions, on average, remained below the suggested lice treatment threshold set out in the National Treatment Strategy for the Control of Sea Lice on Scottish Salmon Farms (NTS) and the Code of Good Practice (CoGP). However, the North Mainland, lice numbers across this region were, on average, 32% above the suggested treatment threshold set out in the NTS and CoGP (Figure 24A). In June 2011 the SSPO reports indicate that again the Western Isles and the South Mainland, lice numbers across these two regions, on average, remained below the suggested lice treatment threshold set out in the NTS and CoGP and the North Mainland had lice numbers across this region, on average, which were 138% above the suggested lice treatment threshold set out in the NTS and CoGP (Figure 24B). Finally in July 2011 the SSPO reports indicated that the Western Isles and the South Mainland, lice numbers across these two regions, on

[1] (http://www.scottishsalmon.co.uk/science/sea_lice/regional_reports(1).aspx)

average, remained below the suggested lice treatment threshold set out in the NTS and CoGP and for the North Mainland during July, lice numbers were, on average, 149% above the suggested lice treatment threshold set out in the NTS and CoGP (Figure 24C).

The principle objective of comparing the results of sea lice counts on wild fish with the counts on the farmed fish was to assess their interrelationships. This will enable appropriate management strategies and policies to be utilised to protect vulnerable wild fish stocks. Unfortunately due to the published data on farmed sea lice counts being produced in an aggregated regional form, it has not yet been possible to make these comparisons. Potentially this is probably the most challenging issue between wild and farmed sectors regarding the publication of fish farm sea lice data and is currently under discussion in the Aquaculture and Fisheries consultation bill. It is recommended that further work, at a local level, on this potential interrelationship is needed to understand the relationships. In order to fully explore the potential interrelationships, and the sea lice pressure dynamics being experienced on farmed and wild fish, extended local data sharing protocols are required.

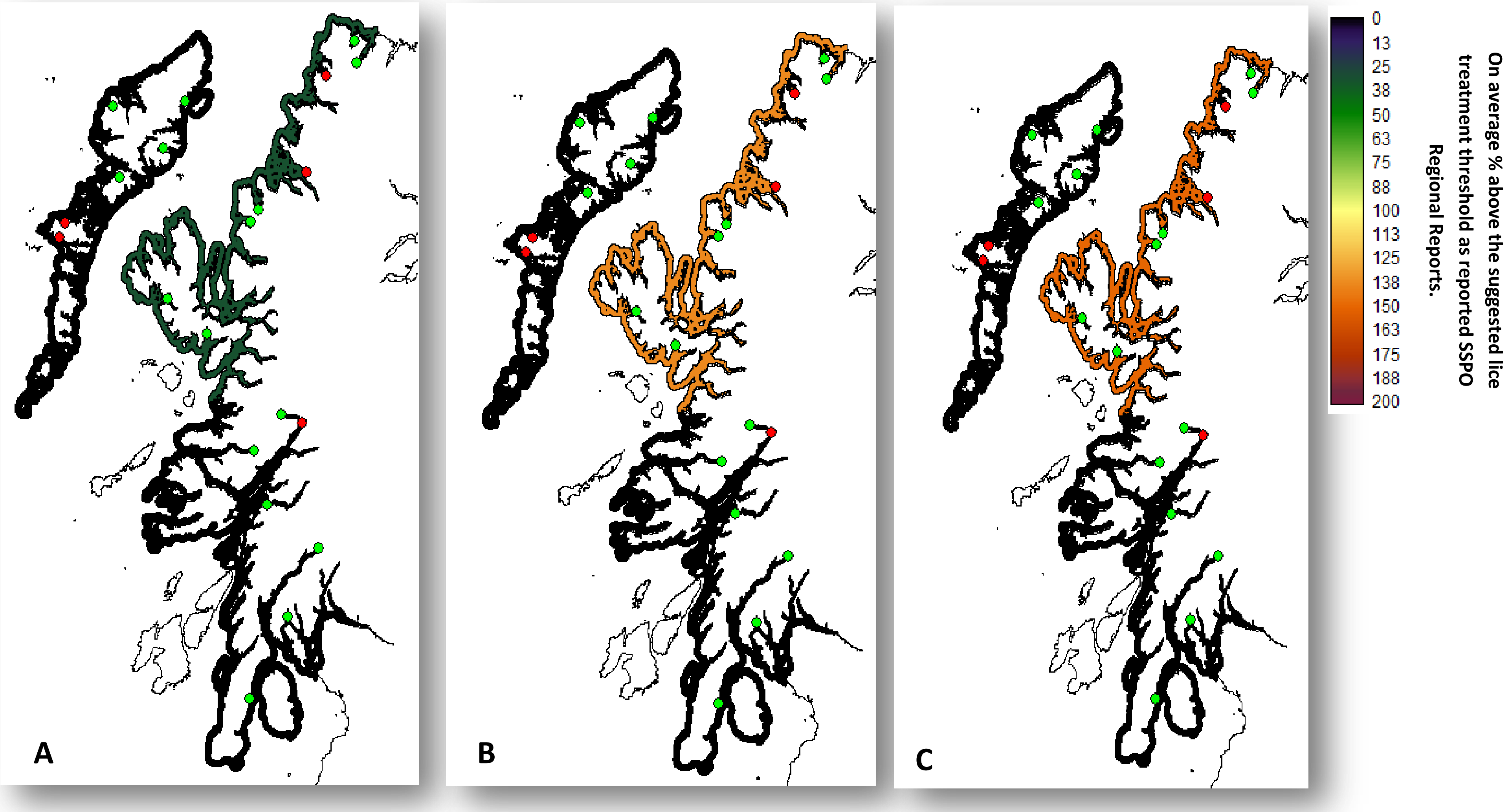

Figure 24: Map layers representing the reported farm sea lice levels in relation to the CoGP and NTS threshold levels. 0 indicates for that period on average the region is below the threshold level. The green dots indicate wild monitoring sites which did not exceed one of predetermined explored determinant threshold levels. In comparison the red dots indicate wild monitoring sites that did exceed one or more of the predetermined explored determinant threshold levels.

5. Conclusions

This first year of the sweep netting monitoring programme coordinated by RAFTS as part of the Managing Interactions Aquaculture Project has seen a number of refinements being made to the protocol, data collection and analyses at a regional level. There has also been a number of important lessons learnt that have indicated where further refinements are needed. This was in relation to the identified monitoring sites, the sea lice counting protocol and development of the current RAFTS structured Access database for collation and analysis of data to be developed in the future to create an online management support tool.

Future sampling should be undertaken at a further refined network of sweep netting sites to ensure the inclusion of sample locations across a full range of distances from active fish farms. To deliver such a sample network, and in particular one which includes sites of greater distance from aquaculture production sites, locations may need to be sought from outside the current study area; potentially from the lower Clyde, Ayrshire or Solway areas.

Some of the current monitoring sites were not successful in yielding desired fish numbers and a reassessment of sites to be retained or discarded will be made in early 2012 which will support the refinement and extension of the sampling network across the West Coast. Although it should be noted there will always be an inherent unpredictability of sampling fish in these environments and it may be that in any given sample year some sites do not provide the desired sample sizes and fish numbers aimed for.

In a number of sites a full set of environmental information was not recorded at survey. This, alongside a review of the method of lice counts to be used (underwater or above water) should be improved and concluded in future sampling protocols.

The provision of sample records from trusts to RAFTS for coordination may be enhanced and made more administratively efficient were an online data base available for use by trusts. RAFTS is currently exploring the viability of such a system for its current staff and whether the operating system being considered could host such a database.

For 2011 the results indicated that five monitoring sites experienced extensive heavy infestations (epizootic). The management threshold level for infestation levels (Wells *et al*, 2006) was used to determine if the infection levels resulted in detrimental impact effects. The implemented critical threshold level indicates that potentially one of the monitoring sites had elevated levels of sea lice presence within the fish population above the critical detrimental impact threshold level.

This study was able to explore the comparisons of the monitoring data to nearest active fish farm year of production and biomass. However it was unable to explore the reported high levels of sea lice counts at the monitoring sites in 2011 and their potential link to the high sea lice levels reported as being above the trigger threshold treatment levels of farms. This was due to the current regional nature of the data released by the SSPO. Being able to properly draw conclusions on what is occurring between farmed fish, wild fish and sea lice within a local area is of paramount importance in ensuring that the appropriate management strategies and policies are employed for the health and wellbeing of the wild fish and for the sustainable development of farmed fish within a defined area.

It is recognised that there are concerns around confidentiality aspects within the Scottish aquaculture industry regarding the reporting of sea lice counts from farms and the way this data is handled. Previously data sharing has taken place where it was possible to resolve conflicts to accommodate the perceived concerns. One solution included having Regional Development Officers working on the Tripartite Working Group Project. It should be recognised that there are potential benefits for all parties and it is hoped that new arrangements can be put in place and be implemented to aid the principal objective of evaluating the interrelationships between farmed fish, wild fish and the problematic parasitic sea lice species on the West Coast of Scotland.

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7. Appendix

Appendix 1

Scottish Government Managing Interactions:
North West Coast Aquaculture Project.

Sweep Netting Project

Counts carried out by............................ No of personnel.................................... Method of Counting Sea Lice.........................

Weather Conditions............................... Water Temperature (deg C)............................. Air Temperature (deg C).............................

Salinity (PSU)..

Site Code			Date		Time		Catchment			
Fish	Length (mm)	Weight (g)	Copepodid/Chalimus	Preadult/Adult	Ovigerous Females	Caligus	Dorsal Fin Damage	Predator Damage	Black Spots	Any Additional Notes/Scale REadings

Appendix 2

Table A1: Site Environmental Conditions over sample period

Sweep Netting Site	Mean Water Temperature	Mean Air Temperature	Mean Salinity
Carradale	*	*	*
Southend	*	*	*
Machrihanish	*	*	*
Loch Fyne	*	*	*
West Riddon	*	*	*
Dunstaffnage	*	*	*
West Tarbert	*	*	*
Laggan Bay	*	*	*
Loch Eil	11.60	10.80	28.80
Camas na Gaul	13.20	11.80	35.20
Sunart	14.50	11.80	33.20
Tong	10.95	13.23	35.00
Ardroil	12.85	14.03	19.50
Borve	13.78	14.63	17.75
Eishken	13.67	13.80	35.00
Kyles	15.13	14.13	23.30
Malacheit	16.03	15.63	35.00
Loch Slapin	11.86	12.50	31.10
Loch Harport	10.50	11.57	26.62
Kyle of Durness	13.35	14.35	11.50
Polla	11.88	14.50	2.50
Laxford	9.50	13.70	5.50
Kinloch	*	*	*
Kannaird	13.00	10.00	27.00
Boor Bay	12.25	13.50	24.50
Flowerdale	11.50	14.00	16.00
Carron	*	14.50	*
Gruinard Bay	*	*	*

* No Data

Appendix 3

Table A2: Sea Trout Post Smolt (Threshold 260mm) Analysis

Sweep Netting Site	Mean length (± s.d.) (mm)	Mean Weight (± s.d.) (g)	Mean Condition Factor (± s.d.)
Carradale	170.39 (±17.39)	*	*
Southend	*	*	*
Machrihanish	*	*	*
Loch Fyne	99.35 (±75.97)	*	*
West Riddon	32.20 (± 46.15)	*	*
Dunstaffnage	71.19 (± 83.15)	*	*
West Tarbert	*	*	*
Laggan Bay	*	*	*
Loch Eil	153.40 (± 18.29)	*	*
Camas na Gaul	174.69 (±27.95)	66.63 (± 30.90)	1.07 (± 0.06)
Sunart	138.50 (± 15.30)	*	*
Tong	196.52 (±24.65)	90.35 (±35.17)	1.14 (±0.06)
Ardroil	215.45 (±24.06)	154.80 (± 32.06)	1.20 (± 0.70)
Borve	189.78 (± 22.83)	76.56 (± 29.96)	1.08 (±0.09)
Eishken	175.37 (± 23.03)	56.66 (± 23.50)	1.01 (± 0.08)
Kyles	220.22 (±24.38)	126.62 (±37.35)	1.15 (± 0.11)
Malacheit	195.08 (± 30.35)	92.43 (±45.12)	1.23 (± 0.13)
Loch Slapin	225.41 (± 20.96)	130.96 (±33.45)	1.13 (± 0.13)
Loch Harport	220.03 (± 23.30)	128.83 (±39.66)	1.12 (± 0.11)
Kyle of Durness	185.76 (± 27.71)	*	*
Polla	180.72 (± 39.31)	74.88 (±47.90)	1.12 (± 0.11)
Laxford	207.07 (± 34.09)	109.72 (±40.52)	1.08 (± 0.07)
Kinloch	*	*	*
Kannaird	199.43 (± 27.64)	84.96 (±35.46)	0.99 (± 0.12)
Boor Bay	185.61 (± 25.49)	69.55 (±34.22)	1.02 (± 0.13)
Flowerdale	151.39 (± 17.77)	35.67 (±15.11)	0.97 (± 0.11)
Carron	*	*	*
Gruinard Bay	*	*	*

* No Data

Appendix 4

Table A3: Prevalence, Abundance, Intensity and Median analysis for Copepodid/ Chalimi at each monitoring site.

Monitoring Site	Prevalence	Abundance (± s.d.)	Intensity (± s.d.)	Median
Carradale	14	0.13 (± 0.36)	1.35(± 0.25)	0
Southend	*	*	*	*
Machrihanish	*	*	*	*
Loch Fyne	3	0.02 (± 0.14)	1(± 0)	0
West Riddon	3	0.02(± 0.13)	1(± 0)	0
Dunstaffnage	59	1.16(± 1.33)	2.70(±1.04)	1
West Tarbert	*	*	*	*
Laggan Bay	*	*	*	*
Kinlocheil	60	1.32(± 1.31)	3.08(± 0.84)	1
Camas na Gaul	81	8.12(± 3.05)	14.45(± 2.14)	8
Sunart	0	0(± 0)	0(± 0)	0
Tong	22	0.24(± 0.55)	1.65(± 0.42)	0
Ardroil	9	0.06(± 0.23)	1(± 0)	0
Borve	30	0.69(± 1.64)	4.38(± 0.31)	0
Eishken	0	0(± 0)	0(± 0)	0
Kyles	29	0.39(± 0.78)	1.91(± 0.28)	0
Malacheit	31	0.54(± 1.14)	2.47(± 1.07)	0
Loch Slapin	33	1.92(± 3.74)	23.92(± 0.24)	0
Loch Harport	38	2.08(± 3.51)	18.41(± 0.80)	0
Kyle of Durness	12	0.17(± 0.57)	2.87(± 0.32)	0
Polla	18	0.37(± 1.19)	4.33(± 1.68)	0
Laxford	71	12.45(± 4.81)	38.41(± 0.78)	31
Kinloch	*	*	*	*
Kannaird	93	16.85(± 2.54)	21.28(± 1.73)	20.35
Boor Bay	6	0.13(± 0.65)	6.42(± 0.75)	0
Flowerdale	53	2.22(± 2.46)	8.15(± 1.10)	1.45
Carron	*	*	*	*
Gruinard Bay	*	*	*	*

* No Data

Appendix 5

Table A4: Prevalence, Abundance, Intensity and Median analysis for Preadult/Adult at each monitoring site.

Monitoring Site	Prevalence	Abundance (± s.d.)	Intensity (± s.d.)	Median
Carradale	0	0(± 0)	0(± 0)	0
Southend	*	*	*	*
Machrihanish	*	*	*	*
Loch Fyne	3	0.04(± 0.23)	1.83(± 0.63)	0
West Riddon	5	0.09(± 0.33)	1.62(± 0.26)	0
Dunstaffnage	79	2.99(± 1.84)	4.70(± 1.37)	2
West Tarbert	*	*	*	*
Laggan Bay	*	*	*	*
Kinlocheil	21	0.27(± 0.71)	2.12(± 0.80)	0
Camas na Gaul	80	3.85(± 1.78)	6.08(± 1.31)	4
Sunart	0	0(± 0)	0(± 0)	0
Tong	59	0.82(± 0.85)	1.76(± 0.59)	1
Ardroil	0	0(± 0)	0(± 0)	0
Borve	39	0.51(± 0.87)	1.88(± 0.76)	0
Eishken	10	0.14(±0.61)	3.03(± 1.33)	0
Kyles	85	5.53(± 2.41)	7.99(± 1.78)	6
Malacheit	92	4.47(± 1.60)	5.30(± 1.34)	4.48
Loch Slapin	37	0.45(± 0.67)	1.73(± 0.26)	0
Loch Harport	52	0.78(± 0.88)	2.05(± 0.47)	1
Kyle of Durness	24	0.26(± 0.59)	1.63(± 0.55)	0
Polla	48	1(± 1.56)	3.19(± 1.40)	0
Laxford	46	1.70(± 2.69)	7.53(± 0.63)	0
Kinloch	*	*	*	*
Kannaird	39	1.11(± 2.06)	5.73(± 1.66)	0
Boor Bay	12	0.10(± 0.31)	1.21(± 0.22)	0
Flowerdale	39	0.53(± 0.83)	2.02(± 0.53)	0
Carron	*	*	*	*
Gruinard Bay	*	*	*	*

*No Data

Appendix 6

Table A5: Prevalence, Abundance, Intensity and Median analysis for Ovigerous Females at each monitoring site.

Monitoring Site	Prevalence	Abundance (± s.d.)	Intensity (± s.d.)	Median
Carradale	0	0(± 0)	0(± 0)	0
Southend	*	*	*	*
Machrihanish	*	*	*	*
Loch Fyne	0	0(± 0)	0(± 0)	0
West Riddon	0	0(± 0)	0(± 0)	0
Dunstaffnage	15	0.15(± 0.40)	1.45(± 0.25)	0
West Tarbert	*	*	*	*
Laggan Bay	*	*	*	*
Kinlocheil	0	0(± 0)	0(± 0)	0
Camas na Gaul	6	0.04(± 0)	1(± 0)	0
Sunart	0	0(± 0)	0(± 0)	0
Tong	14	0.13(± 0.38)	1.45(± 0.24)	0
Ardroil	0	0(± 0)	0(±0)	0
Borve	5	0.05(± 0.23)	1.38(± 0.36)	0
Eishken	0	0(± 0)	0(± 0)	0
Kyles	64	1.66(± 1.50)	3.64(± 0.95)	2
Malacheit	58	0.84(± 1.05)	1.89(± 0.89)	1
Loch Slapin	59	0.64(± 0.62)	1.31(± 0.37)	1
Loch Harport	48	0.49(± 0.57)	1.29(± 0.26)	0
Kyle of Durness	7	0.05(± 0.23)	1.21(± 0.22)	0
Polla	27	0.33(± 0.78)	1.83(± 0.96)	0
Laxford	0	0(± 0)	0(± 0)	0
Kinloch	*	*	*	*
Kannaird	4	0.02(± 0.14)	1(± 0)	0
Boor Bay	0	0(± 0)	0(± 0)	0
Flowerdale	3	0.02(± 0.12)	1(± 0)	0
Carron	*	*	*	*
Gruinard Bay	*	*	*	*

* No Data

Appendix 7

Table A6: Prevalence, Abundance, Intensity and Median analysis for Total *L. salmonis* at each monitoring site.

Monitoring Site	Prevalence	Abundance (± s.d.)	Intensity (± s.d.)	Median
Carradale	14	0.12(±0.36)	1.35(±0.25)	0
Southend	*	*	*	*
Machrihanish	*	*	*	*
Loch Fyne	5	0.05(±0.28)	1.71(±0.70)	0
West Riddon	2	0.11(±0.36)	1.45(±0.26)	0
Dunstaffnage	87	4.65(±1.80)	6.29(±1.31)	5
West Tarbert	*	*	*	*
Laggan Bay	*	*	*	*
Kinlocheil	63	1.55(±1.46)	3.41(±0.97)	1
Camas na Gaul	89	12.44(±2.77)	17.44(±2.33)	16
Sunart	0	0(±0)	0(±0)	0
Tong	66	1.18(±1.06)	2.26(±0.75)	1
Ardroil	9	0.06(±0.23)	1(±0)	0
Borve	48	0.71(±1.88)	3.83(±1.73)	0
Eishken	10	0.14(±0.61)	3.03(±1.33)	0
Kyles	87	7.44(±2.59)	10.52(±1.86)	9
Malacheit	92	5.89(±1.89)	7.10(±1.54)	7
Loch Slapin	70	3.35(±3.12)	7.09(±2.44)	2
Loch Harport	79	4.38(±2.52)	7.35(±1.77)	3
Kyle of Durness	37	0.50(±0.83)	1.99(±0.61)	0
Polla	54	1.55(±2.22)	4.56(±1.93)	1
Laxford	73	14.13(±5.14)	39.96(±1.33)	34
Kinloch	*	*	*	*
Kannaird	93	18.85(±2.69)	23.98(±1.82)	23
Boor Bay	12	0.20(±0.76)	3.28(±1.47)	0
Flowerdale	61	2.69(±2.50)	7.46(±1.39)	2
Carron	*	*	*	*
Gruinard Bay	*	*	*	*

* No Data

Appendix 8

Table A7: Prevalence, Abundance, Intensity and Median analysis for *C. elongatus* at each monitoring site.

Monitoring Site	Prevalence	Abundance (± s.d.)	Intensity (± s.d.)	Median
Carradale	0	0(± 0)	0(± 0)	0
Southend	*	*	*	*
Machrihanish	*	*	*	*
Loch Fyne	0	0(± 0)	0(± 0)	0
West Riddon	0	0(± 0)	0(± 0)	0
Dunstaffnage	0	0(± 0)	0(± 0)	0
West Tarbert	*	*	*	*
Laggan Bay	*	*	*	*
Kinlocheil	0	0(± 0)	0(± 0)	0
Camas na Gaul	0	0(± 0)	0(± 0)	0
Sunart	0	0(± 0)	0(± 0)	0
Tong	21	0.25(±0.65)	1.93(±0.68)	0
Ardroil	0	0(±0)	0(±0)	0
Borve	1	0.01(±0.18)	3(±1.66)	0
Eishken	10	0.14(±0.51)	2.83(±0.39)	0
Kyles	0	0(± 0)	0(± 0)	0
Malacheit	11	0.08(±0.25)	1(± 0)	0
Loch Slapin	44	0.46(±0.58)	1.34(±0.28)	0
Loch Harport	52	0.64(±0.70)	1.60(±0.36)	1
Kyle of Durness	1.69	0.05(±0.33)	3.58(±0.82)	0
Polla	15	0.24(±0.75)	3.12(±0.82)	0
Laxford	63	8.16(±4.99)	31.87(±1.01)	28
Kinloch	*	*	*	*
Kannaird	0	0(± 0)	0(± 0)	0
Boor Bay	0	0(± 0)	0(± 0)	0
Flowerdale	0	0(± 0)	0(± 0)	0
Carron	*	*	*	*
Gruinard Bay	*	*	*	*

* No Data

Appendix 9

Table A8: Percentage of individual sea trout (198mm) within each sample at the individual monitoring sites over the threshold levels.

Site Name	% of Sea trout over the Wells et al Threshold	Sample Size
Carradale	0	34
Southend	*	*
Machrihanish	*	*
Loch Fyne	0	52
West Riddon	0	24
Dunstaffnage	3	31
West Tarbert	*	*
Laggan	*	*
Kinlocheil	3	99
Camas na Gaul	43	69
Sunart	0	8
Tong	2	41
Ardroil	0	3
Borve	3	131
Eishken	0	35
Kyles	0	9
Malacheit	29	14
Loch Slapin	67	3
Loch Harport	20	5
Kyle of Durness	0	43
Polla	9	22
Laxford	18	11
Kinloch	*	*
Kannaird	33	12
Boor Bay	0	24
Flowerdale	8	36
Carron	*	*
Gruinard Bay	*	*

*= No Data

Appendix 10

Table A9: Information on active fish farms in km distance bands to the monitoring site.

Monitoring Site	Number of Fish farm Sites up to 5km from monitoring Site Active in 2010/11	Number of Fish farm Sites up to 10km from monitoring Site Active 2010/11	Number of Fish farm Sites up to 20km from monitoring Site Active 2010/11
Carradale	0	1	1
Southend	0	0	0
Machrihanish	0	0	0
Loch Fyne	0	0	0
West Riddon	1	1	3
Dunstaffnage	1	6	9
West Tarbert	0	0	0
Laggan Bay	0	0	0
Kinlocheil	0	0	1
Camas na Gaul	0	1	2
Sunart	0	1	3
Tong	0	0	0
Ardroil	0	0	0
Borve	0	1	2
Eishken	2	3	5
Kyles	0	0	0
Malacleit	0	0	0
Slapin	1	1	1
Harport	1	1	1
Kyle of Durness	0	0	0
Polla	0	2	2
Laxford	3	3	4
Kinloch	0	0	0
Kannaird	1	1	4
Boor Bay	0	1	1
Flowerdale	0	0	0
Carron	0	1	4
Gruinard Bay	0	0	4

Managing Interactions Report on Genetic Tool Development for distinguishing farmed vs. wild fish in Scotland

Mark Coulson

Rivers and Fisheries Trusts of Scotland

Steering Group Meeting

22nd June, 2012

Executive Summary

The purpose of this report is to present the results of the development and application of genetic tools to support the identification of wild and aquaculture origin fish from west coast catchments in Scotland.

Four main objectives have been outlined and will be discussed in turn. These are as follows:

1. Development of a cost effective and Scotland specific tool to allow wild and aquaculture strains to be identified from tissue sample analysis.
2. Develop an annual sampling network across the west coast that allows these catchments to be sampled systematically to assess the extent or presence of genetic materials of aquaculture origin.
3. Support fishery trusts in the gathering of samples from an agreed network across west coast catchments.
4. Provide resources for samples gathered to be analysed and results reported for prospective application in policy and management practice.

The genetic markers developed in Norway and implemented here provide strong resolution between Scottish fish and Norwegian farmed strains. This offers a powerful tool for the identification of fish as either a wild Scottish fish or a direct escapee from a Norwegian aquaculture strain. Furthermore, these markers offer the possibility of being used in identifying samples where aquaculture origin genetic material is still present after initial introgression in the past. To this end, further sampling of both wild and a robust farmed strain baseline in Scotland, is needed. Additionally, the particular panel of genetic markers can be revised as more baseline data is accumulated.

Introduction

The Atlantic salmon genome consists of approximately 7 billion DNA base pairs, which is about 2x the size of the human genome. Differences that occur among these base pairs allow for the identification of different individuals as well as populations or 'stocks'. Indeed such differences have been used in fisheries management for the identification of region/river of origin of individual fish as well as the reconstruction of parent-offspring relationships in supportive breeding programmes. These differences evolve either as random processes among groups of individuals inhabiting different environments or as a result of direct selective processes acting upon characteristics that differentially affect individual survival and reproduction.

One area where such differences are apparent is in the distinction between wild and aquaculture fish. Selective processes are involved in the domestication process that may differ from those in the wild (either intentionally or unintentionally). Furthermore, genetic drift (random differences) occurs not only as a result of domestication, but also within different cohorts or even family groups of individual aquaculture strains.

Single nucleotide polymorphisms (SNPs) are a class of genetic markers that differ by a single base change at a given location in the genome. Recently, a set of 60 SNPs has been identified that distinguish between Norwegian wild versus Norwegian farmed strains (Karlsson *et al*. 2011) with high accuracy. These SNPs differ in the frequencies of the genetic variants rather than being diagnostic for 'farmed' or 'wild' origins. Therefore across all 60 SNPs, a probability is associated with any individual as coming from either of these sources. Karlsson *et al*. (2011) demonstrated, with individuals of known source, a high accuracy of these markers to correctly identify individuals of either purebred wild or purebred farmed origin.

The prevalence of Norwegian farmed strains in the Scottish aquaculture industry allows for the development and application of these markers in the Scottish context. Given the ability of these markers to distinguish Norwegian farmed strains from wild Norwegian fish, such markers would confidently be expected to distinguish more readily between Norwegian farmed and wild Scottish fish, as greater genetic differentiation occurs between these regions (Gilbey *et al*. in preparation).

The purpose of this study was two-fold. Firstly, to confirm as expected, that the set of farm-wild markers developed by Karlsson *et al*. (2011) would allow for differentiation between Norwegian strains of farmed fish versus wild Scottish fish (see Objective 1 below). The second aim was to screen a number of fish from the west coast of Scotland to distinguish between wild and farmed fish as assess the possibility of further distinguishing fish with mixed ancestry (i.e. introgression) (Objective 4 below).

Summary of Methods

DNA was extracted and quantified for all samples prior to SNP processing. Samples that met quality and quantity controls were subsequently sent to CIGENE (Norway) where they were assayed for either a V2 Illumina panel of 5,500 SNPs (see Objective 1) or the reduced set of 60 farm-wild SNPs (Karlsson *et al*. 2011) (see Objective 4).

The raw data was subsequently returned to RAFTS staff for analysis and interpretation. SNPs common to both the V2 and farm-wild panels were extracted from the database and included in the analysis for all individuals. As further quality control, individuals that failed at more than 10% of the SNPs were excluded for analysis. Analysis consisted of two levels: 1) a population (or sample) level analysis and 2) an individual level analysis. For the sample level, principal component analysis (PCA) was conducted on the raw genotype frequencies and represented as sample means. This was used to define the number of distinct groups identified by the SNP panel. Subsequently, individual-level analysis was conducted using the program Bayesian Analysis of Population Structure (BAPS) (Corander *et al*. 2008). This program was used to assign individual fish to one of the groups as identified by the PCA analysis. Additionally, this procedure estimates whether individuals are purely from a single group or represent admixed individuals. For each individual, a probability is assigned that it comes solely from a single group or the probability that the individual is admixed.

Objectives and Results

1. Development of a cost effective and Scotland specific tool to allow wild and aquaculture strains to be identified from tissue sample analysis.

To date, a set of 60 SNP markers has been developed by Karlsson *et al*. (2011) in Norway [Norwegian Institute for Nature Research (NINA) & Centre for Integrative Genomics (CIGENE)] to distinguish between wild and farmed Norwegian salmon. The aim of the current project was to build upon and develop these markers in a Scottish context. This involved the confirmation that such markers would be largely applicable in Scotland followed by subsequent sample screening to distinguish between fish of wild and farmed origin. Given the extent of Norwegian strains of salmon used by the Scottish aquaculture industry, the first step was to verify, as expected, that such markers distinguish Scottish versus Norwegian fish.

Given the ongoing development of SNP markers as part of FASMOP and various Marine Scotland Science (MSS) internal projects, a baseline of Scottish samples had been screened for a larger panel of 5,500 SNPs on a V2 Illumina Array at CIGENE. This larger panel includes a subset of the 60 farm-wild SNPs identified by Karlsson *et al*. (2011). However, a noticeable gap in the geographical coverage of Scotland for these markers was identified along the west coast. To this end, the first phase of this project involved the processing of 2-3 sites from each of 4 west coast Scottish rivers (Snizort, Carnoch, Moidart, Ghriomarstaidh and the Gruinard) for this SNP panel. These sites are identified in Figure 1 along with the wider geographical coverage mentioned above. This allowed for a more comprehensive baseline of the variability and applicability of these markers for distinguishing among wild versus farmed fish.

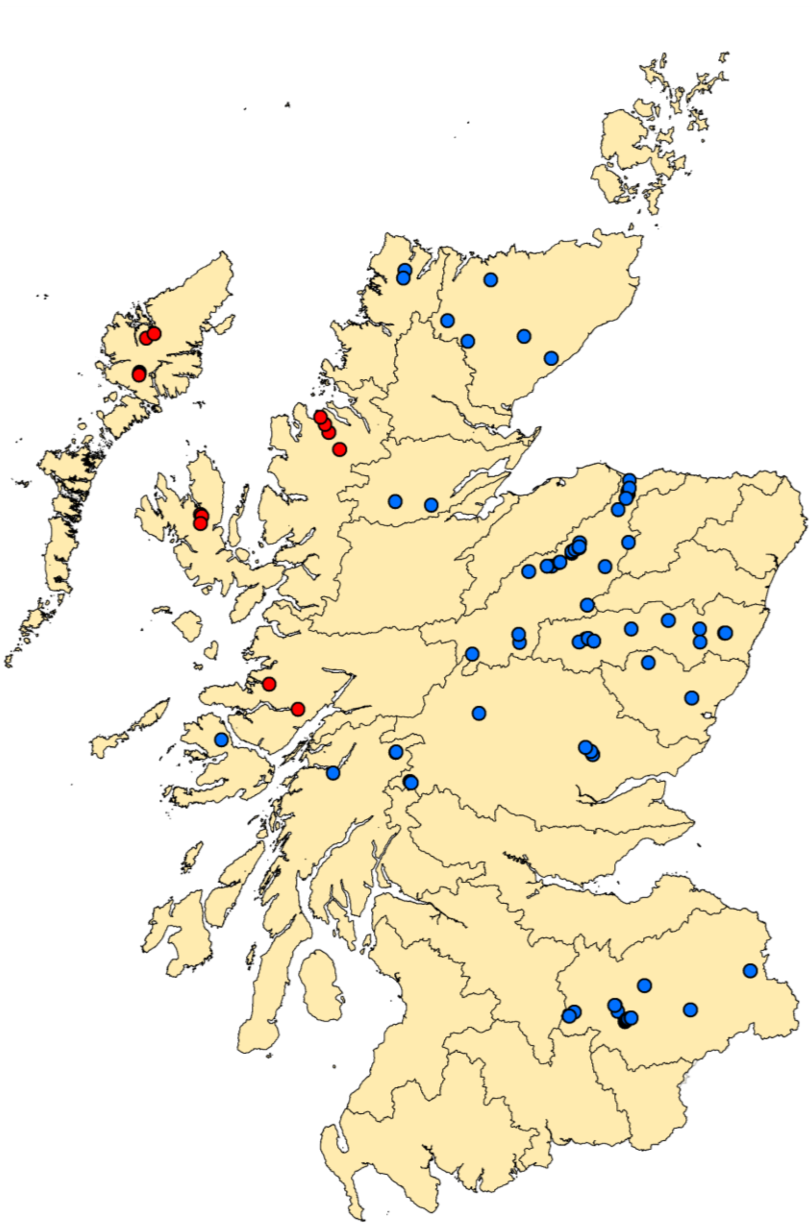

Figure 1. Location of sites screened for the V2 Illumina SNP panel (5,500 genetic markers). Sites in blue represent those screened as part of FASMOP and/or internal MSS projects. Sites in red are those screened as part of the Managing Interactions project to extend the geographical coverage of this panel.

Data from the sites illustrated in Figure 1 were combined with data from three Norwegian rivers (Gaula, Laerdalselva, & Numedalslagen), which were screened with the 5,500 SNP panel. These sites are used to represent a wild Norwegian baseline. Additionally, the genetic profiles from 756 individuals representing 12 samples of Norwegian farmed fish [Aqua Gen, SalmoBreed and Marine Harvest (Mowi strain)] were provided by Sten Karlsson (NINA, Trondheim, Norway) for comparison against the Scottish baseline. Two samples from Scottish fish farms were also included, and thought to be predominantly of Norwegian origin (referred to as Scottish 'Norwegian' farm in Figure 2). A total of 49 of the 60 farm-wild SNPs identified by Karlsson *et al*. (2011) were in common across these samples.

A principal component analysis was conducted on the samples and results are displayed in Figure 2. Each point represents the sample site mean and points further apart from one another are more genetically distinct, while those closer together are more genetically similar. As can be see, three groups are present: Norwegian farmed strains (including the two samples taken from Scottish fish farms), the three Norwegian rivers representing wild Norwegian fish, and the Scottish samples. These results confirm expectations that the markers are able to distinguish Scottish fish from Norwegian fish of either farmed or wild ancestry. The greater spread of points within the Norwegian farmed fish compared to the Scottish fish should be noted. Farmed strains are notably variable in their genetic make-up and for this reason it is necessary to have as complete a baseline of farmed strains as possible. Given only Norwegian strains are present in the plot, it is uncertain at present how well these markers will distinguish wild Scottish fish from strains of domestic origin. Therefore, it is possible that the SNP panel, as currently exists, may need revisiting in the event of the provision of aquaculture strains of local origin. In the meantime, these markers can be used to address the identification of wild Scottish versus Norwegian farmed fish.

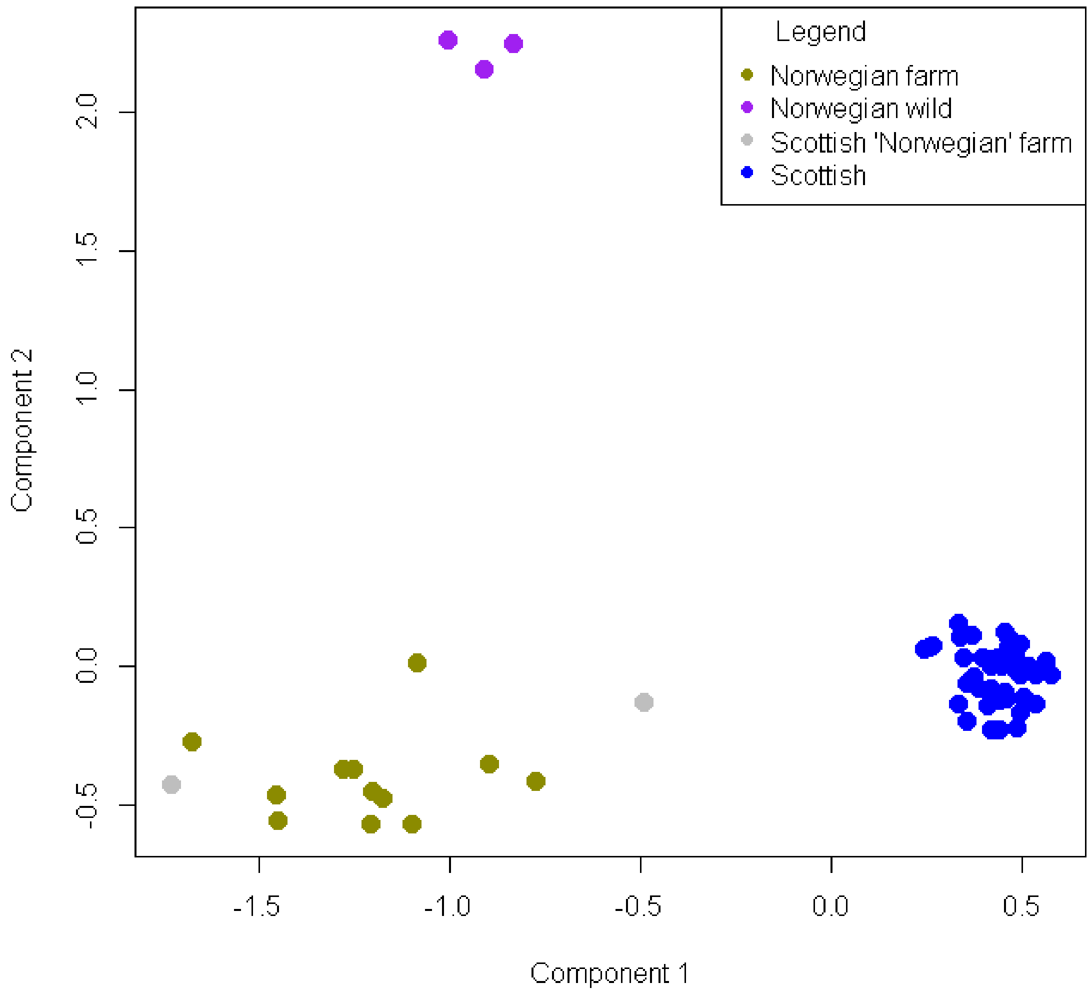

Figure 2. Principal component analysis based on 49 farm-wild SNPs from Karlsson *et al*. (2011). Points represent sample means.

2. Develop an annual sampling network across the west coast that allows these catchments to be sampled systematically to assess the extent or presence of genetic materials of aquaculture origin.

Each of the participating fisheries trusts was allocated a total of 100 samples for subsequent screening with the developed 60 farm-wild SNPs. The locations of these samples are shown in Figure 3. Additionally a number of locations have been screened with these SNPs as part of the **F**ocusing **A**tlantic **S**almon **M**anagement **O**n **P**opulations (FASMOP) project, thereby extending the geographical coverage.

The locations surveyed aimed to initiate a robust, pan-west coast sampling network, with trusts focusing on areas of particular concern or interest. In addition to samples collected in areas near fish farming operations, sites located further away were also targeted to represent a wide range of the genetic diversity present. The choice of sites was agreed upon by the Trusts and RAFTS staff and in some cases, prior results (e.g. samples of direct escapes). A summary of all samples screened (including those processed as part of FASMOP) is presented in Table 1.

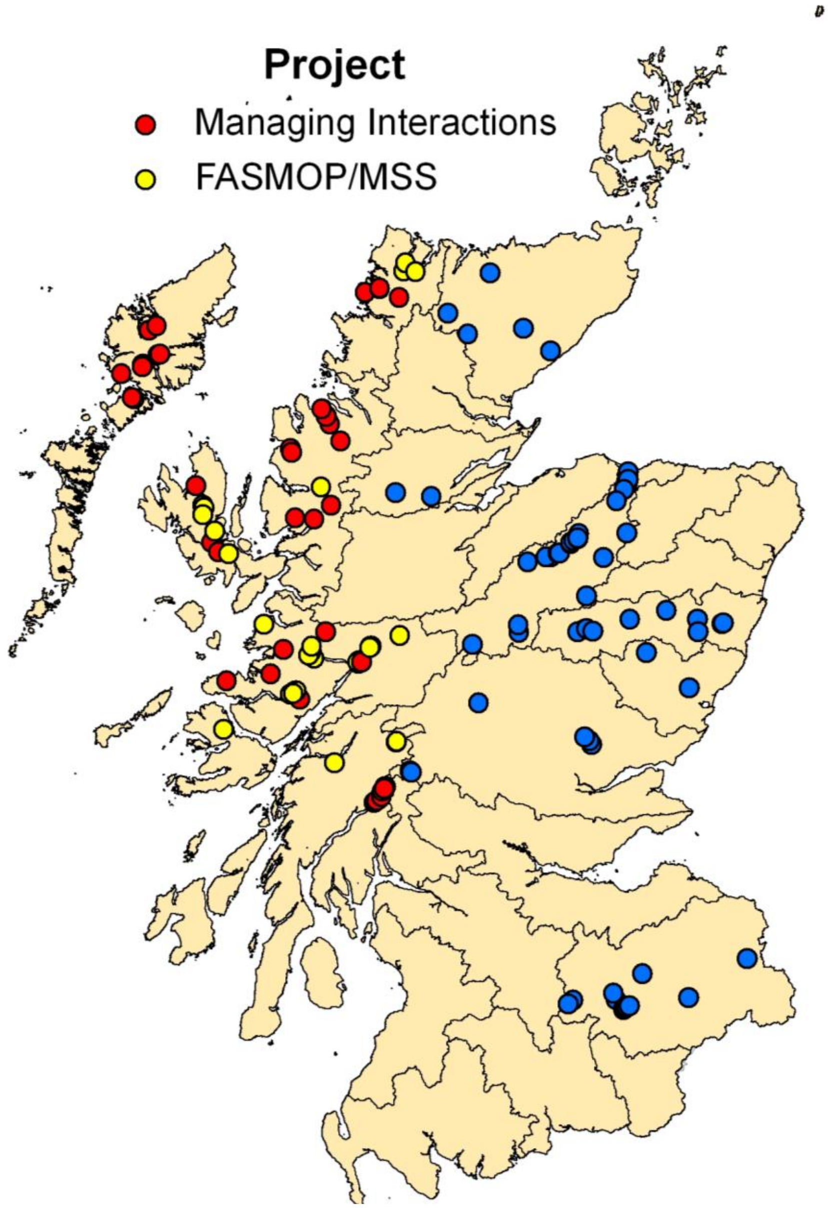

Figure 3. Map of locations sampled screened for farm vs. wild SNPs. West coast samples are coded by the project with which they were screened. Locations in blue represent east coast sampling sites used as a 'wild Scottish' baseline (see text for details).

Table 1. West coast of Scotland sampling locations analysed for farm-wild SNPs.

Trust	River	Site	Year of Collection	Number of Samples
Argyll	Aros River	Loch Frisa	2010	32
	River Awe	Lower Awe	2010	32
		Upper Orchy	2010	32
	River Fyne	various locations	2011	100
Lochaber	Achateny Water	Achateny Burn	2006	15
	River Ailort	mainstem	2011	30
	Carnoch River	various locations	2010	32
	River Lochy	Lower Lochy	2008	29
		Lundy	2011	31
		Pean	2011	24
		Roy	2008	43
		Upper Lochy	2005	34
		Loch Lochy (farm escapes)	2010	35
	River Moidart	mainstem	2006	32
	River Morar	Loch an Nostarie	2005	21
	River Shiel	River Callop	2010	30
		River Finnan	2008	12
			2010	22
		River Shlatach	2005	52
			2008	24
			2010	8
	Strontian River	various locations	2008	23
			2010	8
Outer Hebrides	Kintaravay	mainstem	2011	33
	Ghriomarstaidh	Langadale River	2009	22
		Langavat – Grimersta	2005	21
		Langavat – March Burn	2005	21
	Laxadale	mainstem	2011	34
	Loch Leosaid	River Leosaid	2011	33

Trust	River	Site	Year of Collection	Number of Samples
Skye	River Drynoch	mainstem	2011	33
	River Hinnisdal	mainstem	2011	31
	River Sligachan	mainstem	2010	19
			2011	35
	River Snizort	Lower Snizort	2010	40
		Upper Snizort	2010	32
	Varagill River	mid river	2010	20
Wester Ross	Balgy River	smolt trap	2006	59
			2007	20
	River Carron	River Lair	2011	33
		Tullich burn (escapes?)	2011	7
	Gruinard River	Lower river	2008	21
		Mid river	2005	20
		Upper river	2007	23
	River Kerry	Mid river	2011	33
	River Kishorn	Lower river	2011	27
	River Torridon	Mainstem	2007	45
West Sutherland	River Dionard	Mainstem	2006	19
		Rhigolter Burn	2006	19
	River Laxford	Allt Horn	2011	32
		Bad na Baighe	2011	34
	Allt a Mhuilinn	Bhadaidh Daraich	2010	34
	River Polla	Allt Coire an Uinnseinn	2008	32

3. Support fishery trusts in the gathering of samples from an agreed network across west coast catchments.

The above sampling network (Table 1) was supported by a £2,000 payment to each of the participating trusts and was a combination of newly acquired samples on the part of the trust and/or existing samples being stored at the Marine Scotland Freshwater Laboratory, on behalf of the trusts. All trusts have invoiced and been paid for their sampling contributions.

4. Provision of resources for samples gathered to be analysed, and results reported for prospective application in policy and management practice.

All sites in Figure 3 plus the Norwegian wild and farm baselines were analysed for a reduced set of 35 farm-wild SNPs in common. A principal component analysis of all sites is shown in Figure 4. As before, there is strong separation between the Norwegian wild, Norwegian farm and Scottish wild baselines. The sites in blue represent east coast Scottish samples, while those in red represent west coast Scottish samples. Three west coast samples fall well within the Norwegian farm group: Shlatach (2008), Carron (Tullich) and Loch Lochy. Two of these sites (Loch Lochy and Tullich) were known or suspected to be direct escapes and previous work (FASMOP) suggested there may be a farm effect among the temporal replicates from the Shlatach (2005 vs. 2008).

A farm sample had also been provided by Loch Duart Ltd, which represents a Scottish aquaculture strain. This sample grouped most closely with the Scottish wild samples and could not be distinguished from them based on group or individual-level analyses. However it may be possible, given this sample was screened for the V2 5,500 SNP chip, to determine if other SNPs (apart from those used here) may be useful in distinguishing local strains from wild Scottish fish. These efforts would be greatly improved if other local strains could be acquired and incorporated into the analysis.

A number of other west coast Scottish samples (labelled in Figure 4) appear to fall between the large Scottish group and the Norwegian farmed strains. This would suggest that these fish represent admixed individuals, a mixture of pure farmed and pure wild individuals, or some combination of both. To look at this issue further, the results of the individual-level analysis are presented in Table 2. Given the purpose of the individual level analysis is to determine if fish captured within the west coast aquaculture zone are wild, farmed or potentially admixed, only the east coast Scottish samples were used a a 'wild' Scottish baseline. The reason for this is that if there has been a long history of introgression from aquaculture into west coast fish, then they may not actually represent truly wild Scottish fish. Therefore, the east coast, which is further removed from aquaculture was kept as the wild baseline and all west coast samples were treated as test cases.

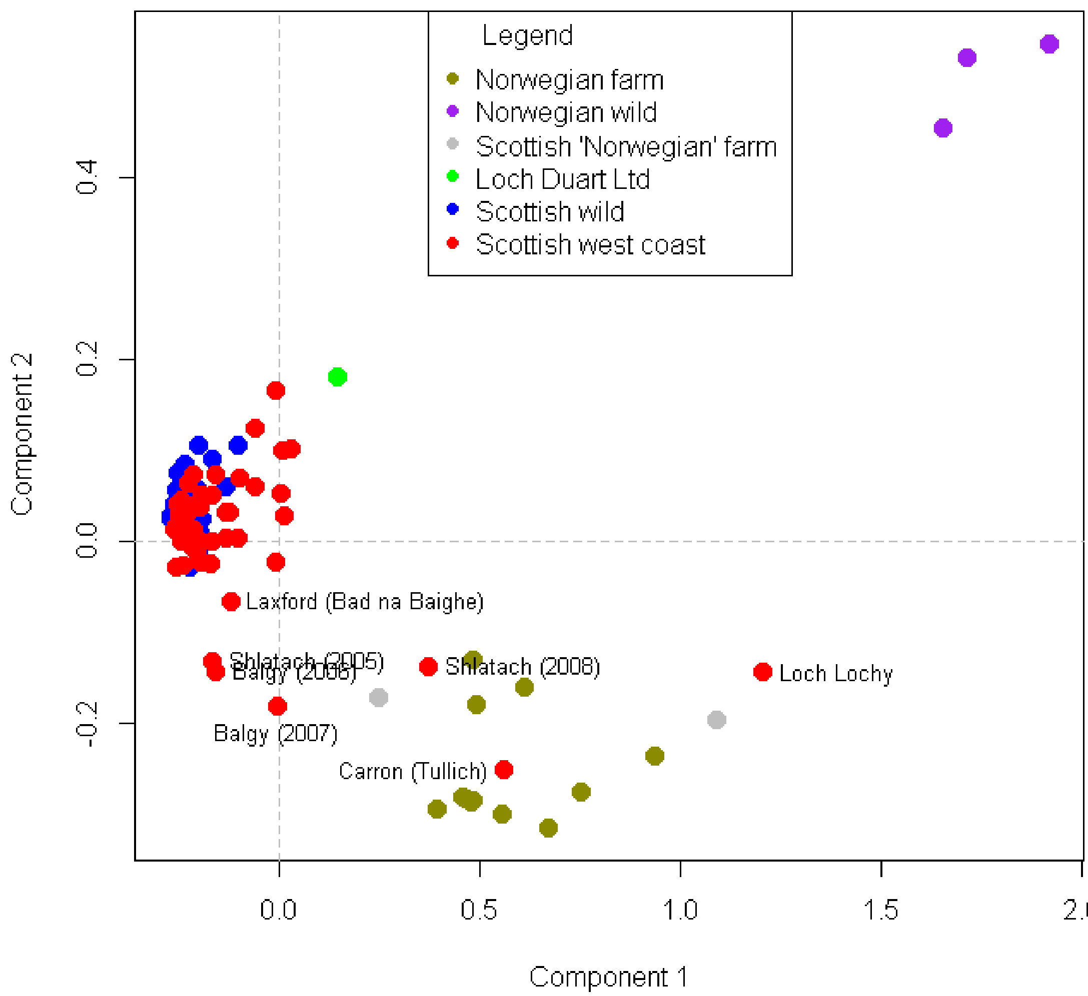

Figure 4. Principal component analysis based on 35 farm-wild SNPs including all west coast samples screened (Managing Interactions & FASMOP).

For the individual-level analysis, each west coast fish was either assigned to a single cluster (‘farmed’ or ‘wild’) or to more than one cluster. This latter scenario represents potentially admixed individuals and is tabulated below.

Table 2. Sample sites with the number of individuals classified as wild (‘Scottish’), farmed (‘Norwegian’) or admixed

Trust	River	Site	Scottish	Farmed	admixed
Argyll	Aros River	Loch Frisa (farm escapes)	0	32	0
	River Awe	Lower Awe	32	0	0
		Upper Orchy	31	0	1
	River Fyne	various locations	97	0	3
Lochaber	Achateny Water	Achateny Burn	13	0	2
	River Ailort	mainstem	30	0	1
	Carnoch River	various locations	32	0	0
	River Lochy	Lower Lochy	29	0	0
		Lundy	29	0	0
		Pean	20	0	3
		Roy	43	0	0
		Upper Lochy	33	0	0
		Loch Lochy (farm escapes)	0	34	0
	River Moidart	mainstem	30	0	2
	River Morar	Loch an Nostarie	18	0	1
	River Shiel	River Callop	29	0	1
		River Finnan 2008	21	0	0
		River Finnan 2010	9	0	2
		River Shlatach 2005	32	0	15
		River Shlatach 2008	0	19	5
		River Shlatach 2010	7	1	0

	Strontian River	2008	23	0	0
		2010	6	0	0
Outer Hebrides	Kintaravay	mainstem	32	0	1
	Ghriomarstaidh	Langadale River	22	0	0
		Langavat – Grimersta	18	0	3
		Langavat – March Burn	21	0	0
	Laxadale	mainstem	34	0	0
	Loch Leosaid	River Leosaid	33	0	0
Skye	River Drynoch	mainstem	32	0	1
	River Hinnisdal	mainstem	31	0	0
	River Sligachan	2010	17	0	1
		2011	35	0	0
	River Snizort	Lower Snizort	39	0	0
		Upper Snizort	32	0	0
	Varagill River	mid river	19	0	1
Wester Ross	Balgy River	2006	41	0	16
		2007	9	0	11
	River Carron	River Lair	30	0	2
		Tullich burn (escapes?)	0	7	0
	Gruinard River	Lower river	21	0	0
		Mid river	20	0	0
		Upper river	20	0	1
	River Kerry	Mid river	33	0	0
	River Kishorn	Lower river	25	0	2
	River Torridon	Mainstem	41	0	1
West Sutherland	River Dionard	Mainstem	19	0	0
		Rhigolter Burn	19	0	0

	River Laxford	Allt Horn	32	0	0
		Bad na Baighe	33	0	0
	Allt a Mhuilinn	Bhadaidh Daraich	34	0	0
	River Polla	Allt Coire an Uinnseinn	28	0	4

As can be seen from the table above, cases of known escapes (Aros River, Loch Lochy, Tullich burn) were all confidently assigned as being of pure farmed origin. Most other sites were of pure Scottish origin with a number of sites containing between 1 and 16 individuals that were classified as 'admixed'. Generally, these were most prevalent in sites suspected of being farmed influenced (e.g., Shlatach, Balgy). Only five individuals from the east coast of Scotland were classified as 'admixed' (two from the Dee and three from the northeast).

Given that the dataset does not contain individuals that are known to be admixed, this scenario was simulated using the farmed and Scottish baseline data. The program Hybridlab (Nielsen *et al*. 2006) was used to create 200 F1 hybrids between a pool of the Norwegian farmed strains and a pool of the east coast Scottish fish. These individuals were subsequently classified according to the program BAPS (as above). Of 200 simulated F1 hybrids, 73 (36.5%) were assigned as admixed, 80 (40%) as pure farmed and 46 (23%) as wild.

Discussion and recommendations

These results show the ability of this panel of farm-wild SNPs to reliably distinguish direct escapes of Norwegian origin from wild Scottish fish. However, the grouping of the Loch Duart farm strain with the Scottish wild samples based on the individual-level assignment clearly demonstrates that these markers may not work for all domesticated strains. Given the current panel was developed specifically to distinguish Norwegian farm strains from Norwegian wild fish, the make-up of the panel may need to be revised if local sources of aquaculture strains become available for SNP screening. Given that most farmed fish in Scotland are of Norwegian origin, however, allows for this tool to be widely (but not universally) applied in Scotland.

The results of the individual-level assignment demonstrate several points. Scottish fish were largely classified as wild and the three examples of known direct escapes were each confirmed by the assignment results. Several fish from a number of sites were classified as ‘admixed’ and all but five of these were west coast fish. Given that this type of analysis assigns a probability that an individual is ‘pure’ or ‘admixed’, does not directly allow for an assessment of how accurate such a classification may be. The ability to classify individuals as either ‘wild’, ‘farmed’ or ‘admixed’ will be affected by the extent to which each of the pure baselines are represented. A wide and robust baseline has been, and continues to be, developed for Scottish ‘wild’ fish, however such a baseline is still sparse for aquaculture strains being used in Scotland. Indeed, if such a baseline can be improved, this would aid in strengthening the robustness of this type of analysis.

Furthermore, without individuals known to be introgressed, a preliminary simulation analysis was included as a proxy for the ability to correctly identify individuals as admixed. These results suggested that while ~80% of the fish were identified as either farmed or ‘admixed’, there were about equal numbers of each for a group of 200 F1 hybrids. Only ~20% of these hybrids were classified as pure Scottish (i.e. having no farm influence). This suggests, that while this SNP panel can possibly identify admixed individuals, there is still room for improvement in the accuracy of classification. Part of the reason for this may be the fact that only 35 SNPs (out of a possible 60) were used for the current analysis. Given that the data was a combination of several SNP chips (V2 Illumina vs. farm-wild panel) not all 60 SNPs were in common across all individuals. As future samples would be processed at the full 60 SNPs, this would allow for farmed and wild baselines as well as new test samples to be screened for almost twice the number of SNPs, which will likely improve resolution and increase the accuracy around these assignments.

Future work should therefore focus on at least three main recommendations:

- Widen the Scottish baseline samples to be screened. This would obviously include greater sampling along the west coast of Scotland in areas of interest and of primary concern. Additionally, areas removed from aquaculture, and possibly including east coast sites, should be screened for the full 60 SNP panel.

- Increased coverage of the various farmed strains utilized by the Scottish aquaculture industry. This is also particularly relevant for local strains. This coverage would ideally be repeated at regular time intervals and would aim to capture the diversity of the different strains being utilized. Furthermore, this coverage would allow for the specific make-up of the SNPs involved to be revisited to improve resolution in the Scottish context.

- Further analysis of simulated individuals of varying degrees of introgression (e.g. F2, backcrosses, etc.) to determine the depth with which assignment to farmed ancestry will be possible.

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Managing Interactions Aquaculture Project

Steering Group Meeting 22/06/12

Paper 1: MIASG Monitoring Sites 2012

Purpose

This paper summarises the current post smolt sweep netting activiites being undertake by fishery trusts in Spring 2012 in advance of confiermed funding for this work for any continuation of the Managing Interactions Aquaculture project, proposes minor revisions to the methodology in terms of fish sizes to be included in the sample and deadlie for sample collection and introduces the need to further review the final network of sites to be surveyed this year subject to funding confirmation.

1. Introduction

Funding for this proposed area of work is still uncertain and not guaranteed. However, in advance of the funding decision it is important to set out the work currently being undertaken by trusts im 2012, consider the value of refining the sampling methodology based on 2011 experience and to assess what might be delivered in 2012.

2. Current Activities

In advance of any funding decision by Marine Scotland five trusts are currently undertaking sweep netting monitoring actvities on the west coast. A total of twenty two sites from last year (see **Table 1**) and two new proposed sites (see **Table 2**) are being surveyed. These twenty four sites cover a range of distances from active fish farms from 1.5km to 43km (see **Figure 1**). This work is being taken forward by trusts without guarantee of funding due to the requirement to complete this field work over the period May-July. It is proposed that a review of these twenty four sites is undertaken and the development of new network of sites (up to a maximum of thirty monitioring) is considered at the steering group meeting. The discussions held on new sites should also consider the inclusion of locations in other trust areas outside the aquaculture production area e.g. in the Solway. However, the network may require to be limited by the finance ultimately available to support this sampling effort.

3. Methodology Refinements

In 2011 each site was to be surveyed on at least two separate occasions to collect a total sample of at least thirty fish. It is proposed that these aspects of the methodology are maintained.

Whilst the number of thirty fish in the sample is still appropriate one refinement is proposed in relation to fish size and to allow thecollection of a sample which can be fully incorporated into later analysis. Presently the SFCC protocol used advises a cut off size of 260mm for the identification of post smolts. However, evidence was provided by three trusts in 2011 which indcated that it would be possible and desirable to include fish over the 260mm size threshold in the anaylsis as these were considered in these cases to still be post smolts. To recognise this it is proposed that fish >260mm shoudl be eligible for inclusion in the post smolt sample but that their status as post smolts should be verified and confirmed by the trust from the reading of scale samples. This revision would allow larger fish to be included in the sample returns and later analysis where they are demonstrated to be post smolts and assist the collection of the necessary 30 fish minimum sample size at each site.

In addition, evidence was also supplied by two trusts that the movement of post smolts in certain rivers happens later than the mid July end point for sampling which was agred in 2011. In light of this request it is proposed that an extension on the sampling period is incorporated in to this project to allow surveys to be undertaken to the end of July 2012.

4. Project 2012 Deliverables

It has been proposed that a network of thirty monitoring sites are targeted over the 2012 study period although this proposal may need to be revised in the light of funding constraints.

The data collected by the trusts will be collated and anyalsed by the project coordinators. The project coordinators will produce a full regional report in the same format as the 2011 regional report and all data collected in 2012 will be made available to MSS for further detailed and strategic anaylsis such as consideration with previous historic time series data of lice counts.

5. Recommendations

The Steering Group is requested to:

i. Review and approve the completion of surveys at the 24 currently active survey sites (subject to funding confirmation);
ii. Consider the inclusion of additional survey sites and their location (subject to necessary funding availability);
iii. Review and approve the revisions to sample methodology and protcol re maximum fish size and scale age verificaion and end point of the survey period; and
iv. Review and approve the project 2012 deliverables of a single regional report of format as per 2011 and with all data available to MSS for strategic analysis within, for example, longer time series data sets.

Donna Claire Hunter

08 June 2012

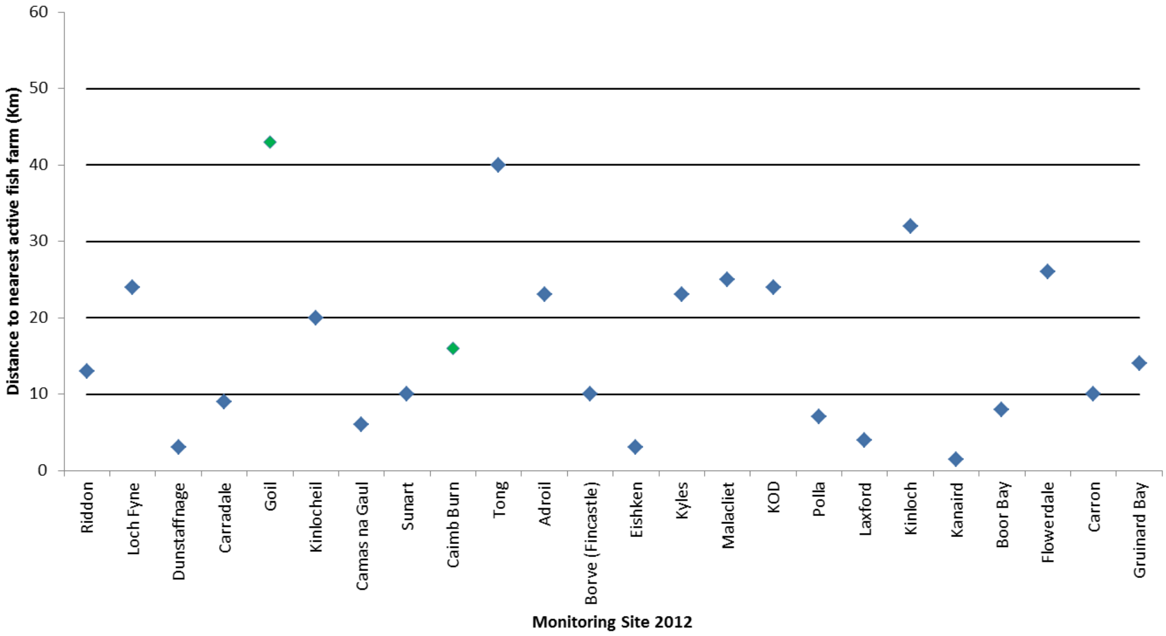
Table 1: Monitoring sites that were undertaken in 2011 and are currently being monitored in 2012

Region	Core Sites	RAFTS Distance Verification to nearest active fish farm (Km)	Within an FMA	Location Reference (Easting's and Northings) Supplied by Trusts	National Grid Square
Argyll	Carradale	9	Yes/ M-47	180344 637209	NR83
Argyll	Loch Fyne	24	Yes/ M-42	218415 712259	NN11
Argyll	West Riddon	3	Yes/ M-45	201273 678457	NS07
Argyll	Dunstaffnage	4	Yes/ M-36	189988 733924	NM83/ NM93
Lochaber	Kinlocheil	20	Yes/ M-33	NM978790 = 197800 779000	NM97
Lochaber	Camas na Gaul	6	Yes/ M-33	NN095751 = 209500 775100	NN07
Lochaber	Sunart	10	Yes/ M-34	NM827608 = 182700 760800	NM86
Outer Hebrides	Tong	40	No	144950 935075	NB43
Outer Hebrides	Ardroil	23	No	105270 932575	NB03
Outer Hebrides	Borve (Fincastle)	10	Yes/ W-7	109180 897220	NG09
Outer Hebrides	Eishken	3	Yes/W-5	132780 911850	NB31
Outer Hebrides	Kyles	23	No	75725 867155	NF76
Outer Hebrides (with site 17)	Malacleit	25	No	79260 874525	NF77
West Sutherland	KOD	22	No	238080 965094	NC36
West Sutherland	Polla	7	Yes/M-1	239330 954667	NC35
West Sutherland	Laxford	4	Yes/M-3	222634 947668	NC24
West Sutherland	Kinloch	35	No	255494 953128	NC55
Wester Ross	Kinnaird	1.5	Yes/M-11	211728 899917	NH19/NC10
Wester Ross	Boor Bay	8	Yes/M -15	185000 881000	NG88
Wester Ross	Flowerdale	26	Yes/M-16	180850 875100	NG87
Wester Ross	Carron	10	Yes/M-20	193199 840702	NG94
Wester Ross	Gruinard Bay	14	Yes/M-4	196500 893500	NG99

Table 2: New proposed monitoring sites for 2012 which are currently being monitored

Region	New Site	RAFTS Verification of Distances (Km)	Within an FMA	Location Reference (Eastings and Northings) Supplied by Trusts	National Grid Square
Lochaber	Caimb Burn	16	Yes/M-29	165000 789000	
Argyll	Loch Goil	43	No	219500 701000	

Figure 1: Twenty four current monitoring sites (Blue dots indicate sites carried on from 2011 sampling and green dots are proposed new sites for 2012) and their distance to the nearest active aquaculture in 2012



Managing Interactions Aquaculture Project

Locational Guidance Tool Development

Paper 4: MIASG Proposed Approach to Consider Distance in Locational Tool

Developing an Approach to Include Distance in Sensitivity / Risk Matrix

1. Purpose and Introduction:

This is a discussion paper to propose a system to incorporate "distance" in both the sensitivity / risk matrix of the aquaculture and river/fisheries sections of the locational guidance tool.

It tries to recognise that the relationship between an aquaculture site (a static point at varying distances from a range of river mouths with each distance presenting a different risk) to river(s) is different from a river (a static point at varying distances of range of aquaculture sites with each distance presenting a different risk with this risk influenced also by the biomass / production at each aquaculture site). Given this difference a different system of recognising the distance of a farm to river(s) and the distance of a river from farm(s) is required in each of the aquaculture and river/ fisheries assessment matrices.

2. Distance of Aquaculture Production Site from River(s)

Each farm unit has a fixed location. That location will have a calculable distance to a number of river mouths. The distance from each river will change the risk of sea lice from that farm affecting any given river (accepting that smolt migration needs to be considered separately and in addition to try and generate some basis for interaction between smolt and farm where general migration routes can be generated or assumed). The risk from any farm is also related to the volume of production at the site as large production sites are likely to generate greater lice numbers due simply to the larger numbers of fish held.

To match distance of farm from rivers risk is proposed to vary on the bands below:

- 0-5km = High risk
- 5-15km = Medium Risk
- 15 -25km = Low Risk

For farms >25km from the nearest river it is assumed there is no risk. This end point is required as without it all farms will present a risk to all rivers. However, smolt migration routes (where these are known or can be assumed) will be required in addition to identify situations where a farm distant from a river mouth may still have an influence due to proximity during smolt migration.

To consider the production of the site and relate this to distance the following tonnage bands are proposed for consideration:

- <1000 tonnes = Low Risk
- 1000 – 2000 tonnes = Medium Risk
- >2000 tonnes = High Risk

These bandings would combine in **Table 1** below to generate a risk assessment for each farm which considers both the distance of any farm to any river and also the production of fish from at that site.

Table 1: Combined Aquaculture Risk Matrix Considering Distance from River and Farm Production

Distance (km) and Production (tonnes)	<1000	1000 - 2000	>2000
0-5	High x Low = Medium	High x Medium = High	High x High = High
5-15	Medium x Low = Low	Medium x Medium = Medium	Medium x High = High
15-25	Low x Low = Low	Low x Medium = Low	Low x High = Medium

To give balance to the combined assessment any risk derived from a low risk factor multiplied by medium risk is scored low risk. Any risk from a high risk multiplied by medium is scored high. If either a uniformly conservative or uniformly precautionary approach is adopted the table would change to those below (**Tables 1A** (conservative) and **Table 1B** (precautionary). The approach here should be discussed as either option has consequences for the output derived.

Table 1A: Combined Aquaculture Risk Matrix Considering Distance from River and Farm Production (Conservative)

Distance (km) and Production (tonnes)	<1000	1000 - 2000	>2000
0-5	High x Low = Medium	High x Medium = Medium	High x High = High
5-15	Medium x Low = Low	Medium x Medium = Medium	Medium x High = Medium
15-25	Low x Low = Low	Low x Medium = Low	Low x High = Medium

Table 1B: Combined Aquaculture Risk Matrix Considering Distance from River and Farm Production (Precautionary)

Distance (km) and Production (tonnes)	<1000	1000 - 2000	>2000
0-5	High x Low = Medium	High x Medium = High	High x High = High
5-15	Medium x Low = Medium	Medium x Medium = Medium	Medium x High = HIgh
15-25	Low x Low = Low	Low x Medium = Medium	Low x High = Medium

This risk assessment approach can be replicated for each river within each distance band and an accumulated risk assessment derived for each site.

E.g. 1 A farm of 1500 tonnes production may have x3 rivers between 0-5km, a further 5 rivers within 5-15km and 10 within 15-25km and this would generate 18 risk scores to accumulate.

E.g.2 A farm elsewhere of the same 1500 tonnes production may have x1 river between 0-5km, a further 2 rivers within 5-15km and 5 within 15-25km and this would generate 8 risk scores to accumulate.

3. Distance of River from Aquaculture Production Site(s)

Each river mouth has a fixed location. That location will have a calculable distance to any fish farm. The distance of each river from each farm will change the risk of sea lice from that farm affecting any given river. The risk from any farm is also related to the volume of production at the site as large production sites are likely to generate greater lice numbers due simply to the larger numbers of fish held but this factor, it is proposed, is better handled in the aquaculture assessment set out in **Section 2** (above).

There are at least two main ways to consider the relationship between river distance and farm. Most simply it is just a distance relationship where risk decreases with distance from farm (see **Table 2**). Alternatively the assessment might be based on a combination of distance and "total score" generated from the rest of the river assessment matrix (see **Table 2A**).

To match distance of river to each farm risk is proposed to vary on the bands below:

- 0-5km = High risk
- 5-15km = Medium Risk
- 15 -25km = Low Risk

For rivers >25km from the nearest farm it is assumed there is no risk. This end point is required as without it all rivers will collect a risk score from all farms. However, smolt migration routes (where these are known or can be assumed) will be required in addition to identify situations where a farm distant from a river mouth may still have an influence due to proximity during smolt migration.

To consider the overall assessment score of the river and relate this to distance bands a general grouping could be generated as below. Note what constitutes a low, medium or high total assessment score is not currently determined as the assessment matrix and weightings within it are not finalised.

- Low total assessment score = Low Priority
- Medium total assessment score = Medium Priority
- High total assessment score = High Priority

Table 2: River Risk Matrix Based Upon Distance of River from Farm

Distance (km)	0-5	5-15	15-25
All rivers	High	Medium	Low

Table 2A: Combined River Risk Matrix Considering Distance from Farm and Overall River Assessment Score

Distance (km) and River Assessment Score	Low Score	Medium Score	High Score
0-5	High x Low = Medium	High x Medium = High	High x High = High
5-15	Medium x Low = Low	Medium x Medium = Medium	Medium x High = High
15-25	Low x Low = Low	Low x Medium = Low	Low x High = Medium

In the same way summarised in **Section 2 Table 2A** could be revised based on a conservative or precautionary approach to risk and generate matrices similar to **Tables 1A** and **1B**. The preferred approach to be followed requires discussion amongst the project partners.

In respect of the two approaches presented in **Table 2** and **Table 2A** the simple distance relationship of **Table 2** is preferred. This will calculate a cumulative risk to rivers from the aquaculture unit which is based on the fixed variable of distance from farm to river mouth. The **Table 2A** approach uses the priority score of the river which is itself an modelled and derived output and open to change determined by the rules of the river prioritisation and the extent of data and information behind the prioritisation score. As such it is considered to be less applicable for use in the generation of the aquaculture risk score.

4. Discussion Points and Recommendations:

Distance of farm location from rivers and distance of river from farm locations are important features of the relationship between wild fish and fisheries and aquaculture. The extent of production on any farm is also an important part of this relationship and interaction. The paper above presents options for discussion as to how these issues can be pragmatically considered within the locational guidance part of the Managing Interactions Project.

Suggested discussion points are:

a) Are the distance bands and tonnage levels reasonable to generically separate risk and levels of influence?
b) In **Tables 1, 1A** and **1B** is there a preference as to how risk should be considered which affords reasonable protection and is seen externally to be fair and equitable? Do any of the options presented provide this?
c) Does this sort of approach allow the identification of accumulated risk from a farm linked to many rivers at different distances and a river linked to many farms at different distances?

Having discussed the issues above the Steering Group is recommended to:

a) Approve the use and integration of these systems into the river and aquaculture prioritisation and risk assessment matrices to support prioritisation and risk assessments for later review within the overall protocol development.
b) Approve the use of the simple distance relationship between farms and rivers where all rivers are considered equally (**Table 2**) as opposed to the use of overall assessment scores in combination with distance (**Table 2A**) to provide a risk score.

Callum Sinclair, Diane Kennedy and Donna-Claire Hunter
RAFTS
08 June 2012

4. Programme Costs

Project activities are summarised in **Table 1.** These have been used to develop project milestones and a proposed grant payment schedule summarised in **Table 2** and recognising the indicative project costs and activities set out in **Table 3.** A maximum grant for this work from Marine Scotland of £100k is assumed.

A total spend in 2012 of **£113 000** is proposed which would:

- Support the delivery of sweep netting activities proposed and to be undertaken by summer 2012;
- Secure the necessary staff resource (from existing MIA staff employed by RAFTS) to take forward the Locational Guidance work and co-ordinate the post smolt sweep netting activities until end March 2013;
- Maintain the long term sequence of sweep netting and the technical development of the Locational Guidance in 2012/13 and allow further review of priorities and funding opportunities for future years.

Although a 3-year programme of work and funding support is desirable a single year award is requested at this stage following guidance from Marine Scotland. Single year funding will allow work to progress and be safeguarded in 2012 whilst future arrangements are considered.

Table 2: Summary of activities, milestones and proposed grant payment schedule

Activity	Milestone	When	Proposed Payment Schedule
1.Co-ordinating staff resource	Contracted August 2012 - March 2013	June 2012	1. August 2012: £40k (for salary and payments to trusts for field work completed)
2. Sweep Netting Network Refinement and Site Selection	Sites confirmed and applied	June 2012	
Field Surveys, Data Submission and Payments to Trusts	Field work completion Data submission Payments to trusts	May-July 2012 July - Aug 2012 Sept - Oct 2012	
Access Database Development	Database developed and functional	July 2012	2. October 2012: £50k (for salary and payments to trusts for field work completed)
Regional Analysis and Reporting	Final regional report of surveys	Dec 2012	
Data Available to MSS	Sweep netting information to MSS	Dec 2012	
3. Locational Guidance GIS development, data collation and operating rules and systems	GIS map outputs from river prioritisation to provide coastal water priorities	Aug 2012 -March 2013	3. March 2013: £10k (for salary after receipt of sweep netting report and satisfactory progress on locational guidance)
	Data collated and collected from range of sources	Aug 2012 - March 2013	
	Preparation of "operating rules / manual / guidance)	March 2013	
Partner and interested party consultation and engagement	Meetings with trusts and boards	Aug 2012 - March 2013	
	Meetings with interested parties e.g. SNH, MSS, SEPA, Crown Estate, Aquaculture in	Aug 2012 - March 2013	