Using fatty acid markers to distinguish between effects of salmon (*Salmo salar*) and halibut (*Hippoglossus hippoglossus*) farming on mackerel (*Scomber scombrus*) and whiting (*Merlangius marlangus*)

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Abstract

Presence of coastal aquaculture activities in marine landscapes is growing with impacts on the wild fish that share these habitats. However, it is difficult to disentangle subsequent ecological interactions between these activities and marine fish communities. We evaluated the impact of both salmon and halibut farms on mackerel (*Scomber scombrus*) and whiting (*Merlangius merlangus*) sampled near sea cages using condition indices and fatty acid (FA) biomarkers. Results of the stomach content analysis indicated that mackerel and whiting consumed waste feed which was also reflected in their modified FA profiles. Both mackerel and whiting had elevated levels of FAs that are of vegetable oils origin. The use of vegetable oils as replacement for marine oils is a lot more common in salmon farming than halibut farming. Additionally,

the overall effects of the two fish farms were more pronounced in whiting than in mackerel sampled near the sea cages. By allowing discrimination between source of trophic interactions, this method could lead to more informed decisions in managing different farming activities.

KEYWORDS

Fish farming, halibut farming, salmon farming, wild fish populations, fatty acid biomarkers, linear discriminant analysis

1. INTRODUCTION

As aquaculture production increases, there is a trend for diversifying the range of species produced, for example cold water marine production of salmonids (principally *Salmo salar*) is being joined by production of high value marine species such as halibut (*Hippoglossus hippoglossus*) and cod (*Gadus morhua*). Different production systems and species have differential impact on the environment. Because of the need for increased aquaculture production and diversification to remain environmentally sustainable (Diana et al., 2013), we require tools for distinguishing the impacts of different production systems on the ecosystem.

Fish production in mesh cages allows the release of organic by-products in the form of particulate matter originating from uneaten food and faeces, dissolved metabolic waste including ammonia and urea excreted from the gills and organic matter resulting from scraping of biofouling on cages in the surrounding environment (reviewed by Holmer, 2010; Uglem, Karlsen, Sánchez-Jerez & Saether, 2014; Price, Black, Hargrave & Morris, 2015). Nutrient emission from fish farms can have a range of ecological impacts on the surrounding aquatic environment such as local

47 eutrophication, impacts on benthic fauna and local wild fish populations (see Mente,
48 Pierce, Santos & Neofitou, 2006; Holmer, 2010; Uglem et al., 2014). Gaining
49 knowledge on how the environment is affected by aquaculture activities is important for
50 the long term sustainability of the sector (Diana et al., 2013).

51 Biochemical tracers such as lipids are often used in food web ecology (see
52 reviews by Dalsgaard, St. John, Kattner, Müller-Navarra & Hagen, 2003; Bergé &
53 Barnathan, 2005; Kelly & Scheibling, 2012; Parrish, 2013; White et al. 2019). The main
54 reasoning behind the use of FAs as biomarkers is that groups of primary producers
55 possess unique FAs or ratios of FAs and that this can be conservatively transferred
56 through the aquatic food web (see reviews by Dalsgaard et al., 2003; Bergé &
57 Barnathan, 2005; Kelly & Scheibling, 2012; Parrish, 2013). A number of studies have
58 used terrestrial FA biomarkers to assess whether coastal fish farming influences wild
59 marine fish in the vicinities of the sea cages (reviewed by Fernandez-Jover et al.,
60 2011ab; see also Arechavala-Lopez, Sæther, Marhuenda-Egea, Sanchez-Jerez &
61 Uglem, 2011, 2015; Izquierdo-Gómez et al., 2015).

62 The farming of species such as Atlantic salmon, Atlantic halibut and cod require
63 a sufficient dietary supply of FAs such as 22:6n-3, 20:5n-3 and 20:4n-6 for optimal
64 growth and health status. The farming industry relies on capture fisheries for the supply
65 of fish oil. However, as the capture fisheries is stagnating the farming industry has
66 explored alternative sources such as vegetable oils (e.g. soybean, rapeseed, linseed,
67 palm oils) (Tacon & Metian, 2008). However, vegetable oils are rich in 18:2n-6 and
68 18:3n-3 but lack n-3 PUFAs (20:5n-3, 22:6n-3) (Turchini, Torstensen & Ng, 2009).
69 Similar to cultured fish, wild fish incorporate these FAs into their tissues as a result
70 from feeding on waste feed from fish farms. Therefore, influence of fish farming on

wild fish populations can be detected using FAs such as 18:2n-6 and low ratio of n-3/n-6 (reviewed by Fernandez-Jover et al., 2011b).

As the marine aquaculture sector is rapidly increasing and diversifying it is important to evaluate the impacts of various fish farming activities on the wild fish populations. Knowledge of how wild fish are affected by different forms of aquaculture can guide the site selection of fish farms, management of fish farming activities and wild fish stocks, and conservation of wild fish. The aim of this study was to evaluate the impacts of a halibut and a salmon farm on diet, condition and total lipid and FA profiles of mackerel and whiting sampled near the sea cages. Moreover, comparison between the farmed species and the two impacted fish species was assessed in order to determine how the source of effects (salmon vs. halibut aquaculture) can be distinguished in two distinct target species (mackerel and whiting).

2. MATERIALS AND METHODS

2.1 Sampling sites

The project was approved by the University of Stirling, Institute of Aquaculture ethics committee (in April 2013), and that fish were sacrificed in accordance with Schedule 1 of the UK Animals (Scientific Procedures) Act 1986.

Sampling sites were selected to evaluate the impacts of salmon and halibut farming on wild fish populations around sea cages. Farm and reference sites were selected for each farming activity. All sampling sites (Figure 1) were located on the West Coast of Scotland and selected based on the cooperation of fish farmers and the accessibility to the selected sites.

94 The halibut farm was located in Loch Melfort (Figure 1; 56.2475 N, 5.5145 W)
95 which is a fjordic type small sea-loch that extends about six km in length, maximum
96 depth of 73 metres and a fresh/tidal flow per thousand of 10.2 (Edwards & Sharples,
97 1986). The halibut farm was almost adjacent to the shore in water depth of 14-23
98 metres. The farm was accessed from the shore by a jetty. The farm consisted of six
99 circular cages each having a diameter of 22.3 metres and 7-8 metres depth. The farm
100 produced Atlantic halibut with maximum consented biomass of 250 tonnes/year.

101 The salmon farm was located in Loch Leven (Figure 1; 56.6880 N, 5.1375 W), a
102 sea loch of 13.4 km in length, a maximum depth of 62 metres. The fresh/tidal flow ratio
103 per thousand is 40.5 (Edwards & Sharples, 1986). The selected farm is about 120
104 metres off the shore at an average depth of 25 metres. The farm was accessed from the
105 shore by a boat. The farm comprises of twelve 24 metres^2 steel pens and produces
106 Atlantic salmon (*Salmo salar* L.) with maximum consented biomass of 1450
107 tonnes/year.

108 Loch Melfort and Loch Leven are both relatively small lochs. The catchment
109 area for Loch Leven is larger than for Loch Melfort which indicates a larger freshwater
110 input in Loch Leven. The flushing time (the time it takes for all or some of the water in
111 the loch to be replaced by the tidal currents (Gillibrand, 2001)) in Loch Leven is three
112 days whereas that of Loch Melfort is nine days. The flushing time difference between
113 the two lochs indicates that resident times for phytoplankton and nutrients is higher for
114 Loch Melfort than for Loch Leven.

115 Details on farm management, locations and abbreviations used throughout the
116 studies are given in Table 1. Halibut farming has a limited production as compared to
117 salmon production in Scotland. The maximum allowed biomass for the chosen salmon
118 farm is almost six times more than the halibut farm production (Table 1). The halibut

farm is located in a very sheltered bay whereas the salmon farm is located in a well flushed area indicating that nutrients from the salmon farm will be more dispersed than those of the halibut farm. The halibut farm was towards the end of the production cycle (36-56 months) whereas the salmon farm was in the beginning of the production cycle (18 months) indicating differences in the diets fed to the cultured fish. At the halibut farm the feeding frequency was manual whereas at the salmon farm feeding was automated which may indicate more waste feed at halibut farm (Table 1). However, halibut farming often has a tarpaulin at the bottom of the cage which allows the halibut to consume settled feed and therefore less artificial feed would be lost (Gillibrand, Gubbins, Greathead & Davies, 2002).

2.2 Fish sampling at farm sites

Wild fish were sampled by using baited rod and line fishing gear. Fish collection using rod and line selects for feeding fish. Mackerel were caught using three hook feather rig (Shakespeare Mackerel Rig; SP 3240; "J" hooks size 1/0) placed on a monofilament main line (0.25 mm) on a conventional spinning reel and a 3 metres rod. Whiting were caught using three hook rig (Shakespeare SP 3280; "J" hooks size 2). The rig encompassed a 100 g lead at the end of the main line. The rig was placed on a monofilament main line (0.25 mm) on a conventional spinning reel and a 3 metres rod.

2.3 Fish sampling at reference sites

Three reference sites were chosen for each sampled species (mackerel and whiting) (Figure 1). Reference sites were chosen based on distance from farm and accessibility. Majority of the fish were sampled by local fisherman using rod and line. Whiting caught at a third reference site were bigger in size compared to those caught

near the two farms and thus were not included in the study. Fish sampling at the salmon farm took place in July/August 2014.

2.4 Fish processing

All fish were immediately placed on ice and transported to the Institute of Aquaculture, University of Stirling where they were kept at -20°C until processing. At the time of processing fish were defrosted and individual mass (g) and length (cm) were recorded. Individual fish were dissected. Following dissection fish livers were weighed.

Stomachs (from the oesophagus to the pyloric sphincter) were removed and stored in 70% ethanol. Stomachs of mackerel and whiting were analysed between 10-12 weeks. Stomach contents were emptied, and prey items were categorized into pellets, invertebrates, fish and unknown. Frequency of occurrence (FO) was calculated using the formula:

$FO = J_i / P \times 100$

where J_i is the number of fish containing prey i and P is the number of fish with food in their stomachs (Hyslop 1980). Fulton's condition index (FCI) was calculated using the formula: $FCI = W / L^3 \times 100$

where W = mass (g), L = length (cm). The hepatosomatic index (HSI) was calculated with the formula:

HSI= Liver mass (g) / Total mass (g) ×100.

2.5 Lipid extraction and fatty acid methyl esters (FAMEs)

Samples of the muscle (flesh) and liver tissues were taken from individual mackerel and whiting. Commercial feed pellets were also collected from the halibut and salmon farms.

Total lipids were extracted from feed pellets, muscle and liver tissues of fish according to the method of Folch, Lees & Sloane-Stanley (1957). In brief, total lipids were extracted from samples (~ 0.5 g) by homogenising in 20 volumes of chloroform:methanol (2:1, v/v) using Ultra-Turrax tissue disrupter (Fisher Scientific, Loughborough, UK) in a fume cupboard. Samples were left on ice for one hour followed by addition of 5 ml of 0.88% (w/v) potassium chloride (KCl) to remove non-lipid impurities. Samples were centrifuged at 400 × g (1500 rpm Jouan C 412 bench centrifuge) for 5 minutes and the top layer (aqueous) was removed by aspiration. The percentage of lipids was determined gravimetrically after evaporation of solvent under stream of oxygen-free nitrogen (OFN) and overnight desiccation under vacuum. Lipids were re-dissolved in chloroform:methanol (2:1, v/v) containing 0.01% butylated hydroxytoluene (BHT) at a concentration of 10 mg/ml and stored under nitrogen at -20°C prior to FA analysis. All lipid extractions were done in duplicate. Percent lipid was calculated as follows:

$$\% \text{ Lipid} = \text{Mass Lipid (g)} / \text{Mass Sample (g)} \times 100$$

FA methyl esters (FAME) were prepared from total lipids by acid-catalysed transesterification according to the method of Christie (1982) and extracted and purified as described by Tocher and Harvie (1988). Total lipids (100 µl) and 17:0 free FA standard (heptadecaenoic acid) at 10% of the total lipid (100 µl) were mixed and the solvent evaporated under nitrogen evaporator. Toluene (1 ml) was added to dissolve

neutral lipids followed by addition of 2 ml methylating reagent (1% (v/v) solution of sulphuric acid in methanol). After mixing, the tubes were incubated overnight (16 hours) in a hot block at 50°C. Following incubation, tubes were cooled to room temperature and 2 ml of 2% (w/v) $KHCO_3$ and 5 ml of iso-hexane:diethyl ether (1:1, v/v) + 0.01% (w/v) BHT were added, mixed and centrifuged at 400 x g for 2 minutes. The upper organic layer was transferred to another test tube and additional 5 ml of isohexane:diethyl ether (1:1, v/v) (no BHT) was added and same procedure repeated. The solvent was evaporated under nitrogen evaporator and FAMEs re-dissolved in 100 µl of iso-hexane.

FAMEs were purified by thin layer chromatography (TLC) plates (20 × 20 cm). FAMEs were loaded on the plates using Hamilton syringe (100 µl). Plates were chromatographed in iso-hexane:diethyl ether:acetic acid (90:10:1, v/v/v). To visualise the FAMEs the margins from the edges of the plates were sprayed with 1% (w/v) iodine in chloroform. FAMEs were eluted from the silica with 10 ml of iso-hexane:diethyl ether (1:1, v/v) + 0.01% (w/v) BHT followed by centrifugation. FAMEs were stored under nitrogen at -20°C until further analysis.

FAMEs were separated and quantified by gas-liquid chromatography using a Fisons GC-8160 (Thermo Scientific, Milan, Italy) equipped with a 30 m × 0.32 mm i.d. × 0.25 µm ZB-wax column (Phenomenex, Cheshire, UK), on-column injector and a flame ionization detector. Hydrogen was used as a carrier gas with initial oven thermal gradient 50°C to 150°C at 40°C/min to a final temperature of 230°C at 2°C/min. Individual FAME were identified by comparison of their retention times with known standards (heptadecanoic acid (17:0) (internal standard); marinol oil (reference standard); SupelcoTM 37-FAME mix (Sigma-Aldrich Ltd., Poole, UK)) and by reference to published data (Ackman, 1980; Tocher & Harvie, 1988). Data were

collected and processed using Chromcard for Windows (version 2.01; Thermoquest Italia S.p.A., Milan, Italy). Individual FA concentrations were expressed as percentages of the total content. All samples were analysed in duplicates to ensure precision of the method.

Of the 33 identified fatty acids (FAs), 15 fatty acids were selected for statistical analysis based on the abundance and/or importance (14:0, 16:0, 18:0; 16:1n-7; 18:1n-7; 20:1n-9; 22:1n-11, 20:4n-6, 18:4n-3, 20:5n-3, 22:5n-3, and 22:6n-3) and potential aquaculture biomarkers (18:2n-6, 18:3n-3 and 18:1n-9) (Iverson, 2009).

2.6 Statistical Analysis

All analysis were conducted and figures (including maps) plotted using the statistical software R (R Development Core Team 2019) run in RStudio (version 3.6.2, RStudio Team 2019) with libraries rgdal (Bivand, Keitt & Rowlingson, 2016), ggplot2 (Wickham, 2009), rgeos (Bivand & Rundel, 2016), and maptools (Bivand & Lewin-Koh, 2016) and Global Administrative Areas (GADM) database. Confidence intervals for frequency of occurrence were estimated using the function binconf in library Hmisc (Harrell, 2016). The package lsmeans (Lenth, 2016) was used for contrasts between groups. The package plyr was also used for data arrangement (Wickham, 2011). LDA was performed using the package MASS (Venables & Ripley, 2002) with function lda. Packages ggplot2 (Wickham, 2009) and cowplot (Wilke, 2015) were used to plot the data.

Prior to applying any statistical models to the data graphical exploratory tools were used as suggested by Zuur, Elena & Elphick (2010). Boxplots were used to detect outliers or observations that are too far off from most of the observations. Both boxplots and a quantile-quantile (Q-Q) plots were used to get a general impression of the

242 homogeneity and data distribution. Boxplots for length, weight, condition indices, lipid
243 and fatty acids are provided as supplementary information. Linear regressions were
244 used to check for differences in the length and weight of each species between farm and
245 control sites, as this is a potential confounding variable.

246 In order to determine the dietary composition of the wild fish frequency of
247 occurrence of each group of items (fish, fish pellets, invertebrates and unidentified) was
248 calculated and plotted for both mackerel and whiting.

249 In order to detect whether there was any impact of the farming on condition
250 indices and fatty acids, one way analysis of variance (ANOVA) models were applied
251 with single degree contrasts used to evaluate differences between farm and control and
252 the two farms. First, one-way ANOVAs were fitted separately to mackerel and whiting
253 to evaluate differences in length, mass, total lipid and selected individual fatty acid
254 contents of the wild fish, between sites (farms and controls). Single degree of freedom
255 contrasts were then used to detect differences between the combined farm and control
256 sites; and then between the two farms (excluding control sites). This followed the
257 procedure in Mangiafico (2015).

258 LDA was used to distinguish between mackerel and whiting sampled at the
259 different locations. Linear discriminant analysis (LDA) is a multivariate technique that
260 calculates the combination of FAs that produce the maximum multivariate distance
261 among groups by creating uncorrelated linear equations of the original FAs (Budge et
262 al. 2006). The main assumptions for LDA include that observations are independent,
263 the covariance matrices are homogeneous and the data are multivariate normal (Budge
264 et al. 2006). Budge et al. (2006) notes that these assumptions are rarely met with FA
265 data and one should be aware of the limitations and potential effects on the
266 interpretation of the results.

3. RESULTS

3.1 Stomach contents

Stomach content analysis is presented in Figure 2. Of the mackerel caught near both fish farms 7% had empty stomachs and of reference sites 16% had empty stomachs. Fish (clupeids) was the main item found in most of the stomachs of mackerel sampled near the two fish farms and reference sites (Figure 2A). About 10% of the mackerel sampled near the sea cages had consumed waste pellets and none were found in fish from reference sites. Because of longer transport time and cooling failure, from mackerel collected at Reference Mackerel 3 was difficult to identify because digestion was at its final stages.

Of the whiting caught near both fish farms 17% had empty stomachs and of reference sites 40% had empty stomachs. Invertebrates were the main item found in most of the stomachs of whiting sampled near the sea cages and reference sites (Figure 2B). Of the whiting caught near the sea cages 31% had consumed waste pellets and none were found in whiting caught at reference sites.

3.2 Length, mass and condition

Descriptive statistics for length, mass and condition indices are presented in Table 2. Total length of mackerel sampled near both farms was significantly different than those sampled away from cages. Total length of mackerel sampled near the halibut farm were statistically significant as compared to those sampled near the salmon farm (Table 2). The mass of mackerel near the farms was statistically different than the mass of mackerel sampled away from the cages. The mass of mackerel sampled near the halibut farm was significantly different than the mass of mackerel sampled near the

salmon farm (Table 2). The FCI of mackerel sampled near the sea cages was significantly different than the FCI of mackerel sampled at the reference sites and no statistical differences were found in the FCI of mackerel sampled at the two farms (Table 2). The HSI for mackerel sampled near the farms was significantly different than the HSI for mackerel sampled away from the cages. The HSI for mackerel sampled at the halibut farm was significantly different than the HSI for mackerel sampled at the salmon farm (Table 2).

The total length of whiting sampled near the fish farms was statistically different than the total length of whiting sampled away from the cages. The total length of whiting sampled at the halibut farm was significantly different than the total length of whiting sampled at the salmon farm (Table 3). The mass of whiting sampled near the fish farms was significantly different than the mass of whiting sampled away from the cages. The mass of whiting sampled at the halibut farm was significantly different than the mass of whiting sampled at the salmon farm. No statistical differences were detected in the FCI of whiting sampled near and away sea cages and between both farms. The HSI of whiting sampled near the farms was statistically different than the HSI of whiting sampled away from the cages (Table 3). No statistical differences were found in HSI of whiting sampled near the halibut and salmon farms.

3.3 Lipid and fatty acid composition

The lipid and FA analysis of the diets fed to farmed fish in both farms can be found in Table 4. Lipid content and levels of selected FAs for mackerel and whiting sampled near the two fish farms and at reference sites can be found in Tables 5 and 6, respectively.

3.4 Commercial diet composition

315 The proportion of total lipid in commercial fish feeds used in the halibut and
316 salmon farms in 2014 was about 25.6% (Table 4). The diet at the salmon farm was rich
317 in terrestrially based oils such as 18:2n-6, 18:3n-3 whereas the diet at the halibut was
318 rich in marine oils such as 22:6n-3 (Table 4). The halibut diet was also rich in 20:1n-9
319 and 22:1n-11 (Table 4).

320 **3.5 Lipid and fatty acid composition of wild fish**

321 Total lipids of muscle tissues of mackerel sampled near sea cages did not
322 statistically differ from the total lipids in mackerel sampled from reference sites (Table
323 5). No statistical differences were found in the lipid proportions of mackerel sampled
324 near the halibut and salmon farms (Table 5).

325 Fatty acids that differed between mackerel sampled near and away from fish
326 farms included: 14:0, 16:0, 18:0, Total Saturated FAs, 16:1n-7, 18:1n-9, 18:1n-7,
327 20:1n-9, 22:1n-11, Total Monosaturated FAs, 20:4n-6, Total n-6 PUFAs, 18:3n3,
328 18:4n-3, 20:5n-3, 22:5n-3, 22:6n-3, Total n-3 PUFAs, Total PUFAs, n-3/n-6 (Table 5).

329 Fatty acids that differed between mackerel sampled near a halibut and a salmon
330 farm included: 20:4n-6, 20:5n-3 (Table 5).

331 Total lipids of muscle tissues of whiting sampled near sea cages were similar to
332 total lipids of muscle tissues sampled at reference whiting sites (Table 6). Total lipids of
333 whiting sampled near the halibut farm were similar to those of whiting sampled near the
334 salmon farm (Table 6).

335 Fatty acids that were found statistically different between the muscle tissue of
336 whiting sampled near and away from sea cages were: 14:0, 16:0, 18:1n-7, 20:1n-9,
337 22:1n-11, Total Monosaturated FAs, 18:2n-6, 20:4n-6, Total PUFAs, 18:3n-3, 18:4n-3
338 20:5n-3, 22:5n-3, 22:6n-3, n-3 PUFAs, Total PUFAs, n-3/n-6 (Table 6).

Fatty acids found statistically different between the muscle tissue of whiting sampled near the halibut farm and the salmon farm were: 14:0, 16:0, 20:1n-9, 22:1n-11, 20:5n-3, 22:5n-3, 22:6n-3 (Table 6).

3.6 Linear Discriminant Analysis

Results of LDA for mackerel and whiting sampled near and away from sea cages can be found in Figures 3 and 4. The coefficients of the LDA functions for the fatty acids for mackerel and whiting can be found in Tables 7 and 8, respectively.

For mackerel, the linear discriminant function plot showed partial separation between control and farm sites (Figure 3, LD1 axis LD2 partially discriminates the two farms. The FAs that contributed to the most separation between mackerel sampled near and away from sea cages were: 18:3n-3, 18:1n-7, 14:0, and 18:0. The FAs 18:3n-3, 18:0, 14:0, 18:1n-7, and 20:5n-3 contributed to the separation between mackerel sampled near sea cages of the salmon and halibut farms (see also Tables 7). Linear discriminant function correctly assigned 52.2% of all samples to their origin (Melfort Farm (50%), Leven Farm (77%), Reference Mackerel 1 (24%), Reference Mackerel 2 (65%) and Reference Mackerel 3 (47%)). The reference sites were not separated well indicating dietary similarities.

For whiting, the linear discriminant function plot separated the whiting sampled near the sea cages and those caught away from cages more clearly than for mackerel (Figure 4). LD1 separated farm from reference sites, LD2 separated the two reference sites and LD3 separated the salmon and the halibut farms. The FAs that contributed most to the discrimination between whiting sampled near and away from sea cages were: 22:5n-3, 16:1n-7, 22:1n-11 and 18:2n-6. The FAs 18:4n-3, 20:1n-9, 14:0 and 18:3n-3 contribute to the discrimination between the two reference sites of whiting (see also Table 8). It is also worth noting that within the whiting sampled at Reference 1 site

364 there appears to be two distinct groups (Figure 4A). The FAs 14:0, 18:3n-3, and 16:1n-
365 7 contributed to the separation between whiting sampled near the halibut and salmon
366 farm (Table 4B). Linear discriminant analysis correctly assigned overall 90.4% of all
367 samples (Melfort Farm (89.5%), Leven Farm (76.5%), Reference Whiting 1 (95%) and
368 Reference Whiting 2 (100%)).

369 ## 4. DISCUSSION

370 Both the salmon and halibut farming had an impact on the mackerel and whiting
371 as both species consumed waste feed detected in their stomach and fatty acid profiles.
372 The LDA was able to distinguish between fish sampled near the salmon farming and
373 those sampled near the halibut farming. The overall impacts of both the halibut farm
374 and the salmon farm appear to be more evident in whiting than in mackerel.

375

376 ### 4.1 Impacts of fish farming on wild mackerel and whiting

377 As it has been noted by various studies (see reviews by Sanchez-Jerez et al.,
378 2011; Uglem et al., 2014) sea cages have a large attractive effect which could be
379 because of habitat provision, food availability and/or chemical attraction to the farmed
380 fish. Food availability has been suggested as the strongest attractant of wild fish to fish
381 farms (e.g. Uglem et al., 2014). This has also been termed the "birdfeeder effect"
382 (Eveleigh et al., 2007). The present study provides evidence that both farming activities
383 increased the presence of mackerel and whiting possibly as a response to the presence
384 of food resources.

385 Some of the feed from both types of fish farming is lost to the environment.
386 More of this waste feed is expected to be lost through salmon cages than the halibut
387 farming. The reason for this is that halibut is a sedentary species and the presence of
388 tarpaulin would allow some of these waste pellets to be consumed by the halibut

389 (Davies & Slaski, 2003). Some of the feed will also be indigested by both the halibut
390 and the salmon. The average feed conversion ratios for halibut are 1.3 and for salmon
391 about 1.1-1.2 (Davies & Slaski, 2003). The rest of the feed is converted in fish biomass
392 and some is excreted as dissolved nutrients that become available for microbial and
393 primary production (Davies & Slaski, 2003).

394 Although the halibut farm was much smaller in scale as compared to the salmon
395 farm both farms appear to impact mackerel and whiting sampled near the sea cages.
396 Both mackerel and whiting sampled near both farming activities were found with
397 aquaculture pellets and other food items in their stomachs. Mackerel sampled near both
398 fish farming activities were overall longer and heavier than mackerel sampled away
399 from the farms, potentially this is a confounding variable that may be driving some of
400 the differences between farm and control sites. Similarly, whiting sampled near the
401 farms were bigger and heavier than those sampled away from the farms. The whiting
402 sampled at the salmon farm were bigger than whiting sampled from all other sites.

403 Both species sampled near the salmon farm were heavier and longer which
404 could be because of the presence of the farm, loch effect and/or age-related differences.
405 The salmon farm is located in Loch Leven which has a higher flushing rate than Loch
406 Melfort indicating potential higher nutrients availability in Loch Melfort. Thus, the wild
407 fish in Loch Leven might benefit more from the additional nutrients released from the
408 salmon farm.

409 The abundance of prey reduces foraging times of an animal which results in
410 improved biological condition (Oro, Genovart, Tavecchia, Fowler & Martínez-Abraín,
411 2013). Some differences in condition indices were noted for mackerel and whiting
412 sampled near and away from the sea cages. However, these indices were not highly

413 reliable to indicate whether the differences were because of the presence of the farms or
414 the loch effect.

415 Results for mackerel differed from whiting. There was both a lower proportion
416 of fish with pellets in the stomach contents, and also a less clear separation between
417 farm and control sites in terms of fatty acid composition (compare Figures 3 and 4).
418 This is likely due to the more mobile behaviour of the mackerel leading to a weaker
419 association between the farm and the fish, with the mackerel visiting the farms for
420 shorter periods and relying less on direct feeding on pellet waste than for the whiting.

421 Mackerel is a species that needs to continuously swim (lack of swimbladder)
422 which raises the energy requirements of the fish (Juell, Holm, Hemre, & Lie, 1998)
423 whereas whiting is a benthopelagic species. A higher portion of the whiting sampled
424 near both farming activities were found with artificial pellets than mackerel sampled
425 near the farms suggesting a strong dependence on the farm by these fish. Other gadoids
426 such as saithe have been found with pellets in their stomachs when caught near cages
427 (Carss, 1990; Skog, Hylland, Torstensen & Berntssen, 2003). Fernandez-Jover et al.
428 (2011a) reported 6-96% of the diet of cod and saithe near fish farms in Norway was
429 composed of waste feed. In contrast, Mente et al. (2008) studied the diets of demersal
430 fish including whiting at four sea lochs that support fish farms on the West Coast of
431 Scotland and did not find any pellets in the diet of whiting. The diet of whiting
432 consisted mainly of *Malacostracan crustacea* (e.g. shrimp) and teleost fish (e.g.
433 clupeids and gadoids) (Mente et al., 2008). Dietary difference between lochs were noted
434 but dietary differences related to the presence of fish farming were less consistent with
435 differences found for individual lochs (Mente et al., 2008). Mente et al. (2008) did not
436 find clear causal relationship between fish farming development and impacts on diet
437 composition. Moreover, Mente et al. (2008) noted lack of clear aquaculture influence

on the diets of the sampled fish might be related to the sampling methodology which was using bottom trawlers within 50 m from the nearest sea cages. In the present research, sampling took place at the sea cages using rod and line which selects for feeding fish. The presence of waste pellets in whiting sampled next to the cages indicates direct effect of the halibut and salmon farms. Although this may indicate a local-only effect as Mente et al. (2008) pointed out there may be a wider-scale ecological impact of fish farming on marine fish populations.

Although, the weight, length, FCI and HSI were not strong indicators for fish farming influence on the wild fish the FA analysis was better in detecting the impact of farming activities on wild fish. Both mackerel and whiting sampled near both farms had modified FA profiles as compared to those sampled away from the cages. LDA indicated clear separation between fish sampled near the salmon and halibut farms. The difference between fish sampled near the salmon and halibut farms is related to the differences in the aquaculture feeds at both farms. The salmon diet contained higher levels of the FA 18:2n-6, 18:3n-3, 18:1n-9, and lower n-3/n-6 ratios as compared to the halibut diet. The FA 18:2n-6 appears to be a clear causal contributor towards the separation between farm and reference sites. The main contributing FA for the separation between mackerel and whiting sampled near the halibut and salmon farms appears to be 18:3n-3.

The impact of both fish farming activities was stronger in whiting than in mackerel. The LDA was able to classify 90.4% of whiting sampled near and away from the sea cages. The classification was much higher than that for mackerel (52.2%) indicating a stronger influence of both the halibut and the salmon farms on whiting than on mackerel.

462 The LDA was also able to classify 89.5% of the whiting sampled near the
463 halibut farm and 76.5% of the whiting sampled near the salmon farm. In mackerel, the
464 LDA correctly differentiated 50% of the mackerel sampled near the halibut farm and
465 77% of the mackerel sampled near the salmon farm. Similar to the LDA results of
466 mackerel, the FA 18:3n-3 appears to be a strong signal for the salmon farm. Fernandez-
467 Jover et al. (2011a) also used LDA to distinguish between cod and saithe sampled near
468 and away from sea cages in Norway. The LDA classified 88.5% and 96.7% of the cod
469 muscle and liver, respectively and 85.7% and 96.7% of the saithe muscle and liver,
470 respectively (Fernandez-Jover et al., 2011a).

471 As indicated by the stomach content and fatty acid results the presence of
472 various farming activities can have an impact on the wild fishes with stronger impacts
473 on more residential species such as whiting. There is limited information on the ecology
474 of whiting in both lochs but it is expected to be similar to other gadoids. In general,
475 gadoids spend their first year in various Lochs on the West Coast of Scotland and could
476 remain inshore for about 2 to 4 years before joining the offshore populations (Hawkins
477 et al. 1985). During the winter months the food availability is scarce in the loch
478 resulting in poor condition and growth of the juvenile gadoid populations (Hawkins et
479 al. 1985). Thus the presence of additional feed resources from the farms could be of
480 benefit for the juvenile gadoid populations. However, it is not clear from this study how
481 changes in their fatty acid profiles would impact the growth and reproduction.

482 **4.2 Study limitations**

483 The study design needs to have lochs without aquaculture activities; however this
484 is very difficult to accomplish as there are almost no lochs without aquaculture
485 activities on the West Coast of Scotland.

Both the stomach content and the fatty acid analysis were useful tools for detecting the impacts of the halibut and the salmon farms on migratory and a residential species. However, fatty acids give a better indication of long-term influence of marine farming on the wild fish and other organisms (White et al. 2019).

FA analysis was useful in distinguishing between salmon and halibut farming. The use of individual FAs as biomarkers (e.g. 18:2n-6 and 18:3n-3) of terrestrial origin should be taken with caution as some of these FAs are also present in low levels in the marine environment (Fernandez-Jover et al., 2011b). Fish oil and fish meal containing high levels of n-3 PUFAs (20:5n-3 and 22:6n-3) are limited and expensive and therefore there has been increasing research efforts to find alternative replacements such as using plant-based ingredients (Tacon & Metian, 2008). Other potential alternatives for terrestrial based feeds for fish meal and fish oil include microalgae (Sprague, Dick & Tocher, 2016) or genetically modified oilseed crop plants that can synthesize n-3 PUFAs (Betancor et al., 2015). Changes in FA profiles of wild fish feeding waste feed will be minimal as ingredients in the fish feed change towards ingredient that are similar to the natural feed of fish. Thus, to monitor the sustainable growth of marine aquaculture alternative techniques such as stable isotope analysis or a combination of new techniques is needed to detect the environmental impacts.

The univariate and multivariate techniques were useful approximation to fit to the data. However, the LDA was a more powerful approach in detecting the differences between fish sampled at the various locations. Although some statistical differences were noted using the univariate approach caution should be taken as not of all these differences were noted using LDA.

It is also important to note that although there may be some statistical significance in some of the variables it may not have any ecological relevance (Wilding & Hughes

2010). Any anthropogenic activity will have a localised impact with potential broader impacts (Wilding & Nickell 2007). Thus, it would be of high importance to take a pluralistic approach into detecting broader scale impacts of various farming activities.

5. CONCLUSIONS

Both the salmon and halibut farms provided additional food resources for mackerel and whiting. There is potential for both species to stay longer near this readily available food resource which could have an impact on migration and reproduction. The FA analysis indicated that the feed ingredients of the salmon farm could be detected more easily than those used for the halibut farm. Other methods or a combination of methods would be needed to detect the impact of fish farming on wild fish populations.

As marine aquaculture expands there will be further interactions with the capture fisheries sector and it is of high importance that these two sectors are managed in a sustainable manner. Long-term regional additive effects between both sectors would be of importance to be evaluated. This could be done using various ecosystem-based modelling approaches, spatial planning, stock enhancement and cooperative management of the sectors.

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DATA AVAILABILITY

The data that support the findings of this study are openly available in DataSTORRE (Stirling University Online Repository for Research Data) at http://hdl.handle.net/11667/135, reference number 11667/135.

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