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ARTICLE

Spatiotemporal Variations in Trace Element Compositions in Pollock Populations under the Influence of Coastal Norwegian Salmon Farms

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Abstract

Pollock *Pollachius virens* (also known as Saithe) modify their feeding habits when including in their diet uneaten feed pellets from salmon aquaculture sea cages. To determine the influence of salmon farms on Pollock, multivariate and univariate analyses were conducted on the trace element signatures from muscle and liver tissues. Sample fish were caught in the vicinity of salmon farms and in control areas (>3-km distance from the farms) on the coast of Hitra Island (western Norway) over two consecutive years (2012 and 2013). The hepatosomatic index was calculated as a proxy of fish body condition and was higher in Pollock captured near the salmon farms in both years. Variations in specific trace element profiles revealed the influence of farming on the Pollock assemblages (i.e., arsenic, manganese, and copper in muscle; vanadium and manganese in liver). Differences in element composition between sampling years were notable and may, in addition to influence from salmon feed, reflect temporal variation in Pollock migrations or natural food availability. Multivariate analyses of each sampling year showed significant differences in trace element composition of both tissue types among the Pollock groups. Therefore, trace element assessment is a potential tool for determining the influence of aquaculture on Pollock populations, although other natural sources of variation must be taken into account when considering future aquaculture and fishery management strategies.

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Trace element levels in fish tissues are related to their concentrations in the environment and to different physiological processes, including uptake, elimination, and bioaccumulation (Langston and Spence 1995; Rainbow 1997). Trace elements have been examined in marine habitats around the world to increase our understanding of human impacts on marine ecosystems, as the environmental concentrations derived from natural origins are also affected by anthropogenic sources (Gillanders and Kingsford 2000; Demétrio et al. 2012). Several trace elements are essential for metabolic activities in organisms; however, elements like silver (Ag), arsenic (As), chromium (Cr), mercury (Hg), lead (Pb), and tin (Sn) could become harmful at high concentrations and have adverse effects even at low concentrations (Soto-Jiménez 2011). Trace elements are also interesting because of their potential toxicity to humans due to accumulation through the trophic food web in different fish tissues (Fisher and Reinfelder 1995; Ni et al. 2000; Wang 2002).

Marine coastal aquaculture is growing exponentially, affecting other coastal users who share the space and resources and also having negative effects on the environment (FAO 2014). This expansion of the fish farming industry along the coastline may lead to both potential synergies and unwanted interactions between aquaculture and fisheries (Uglem et al. 2014). For this reason, tools that detect aquaculture–fishery interactions are of particular importance (Cataudella et al. 2005).

In addition to release of nutrients and modification of the benthic communities located beneath the cages (Mazzola et al. 2000; Nickell et al. 2003; Hargrave et al. 2008; Holmer 2010), one well-known consequence of salmon aquaculture is the aggregation of wild fish in the vicinity of the farms, which then feed on the nonconsumed pellets from the fish cages (Dempster et al. 2009). The Pollock *Pollachius virens* (also known as Saithe), which is commercially important in the North Atlantic, is the most common species attracted to fish farms in Norway (Dempster et al. 2009). Pollock spawn during winter on shallow, offshore banks, whereupon the eggs drift with the currents and the juveniles settle in nearshore waters (Nedreaas 1987). Juvenile Pollock stay in nearshore waters during their first 2–4 years before they typically migrate to offshore waters; thereafter, they seasonally move between spawning and feeding areas (Clay et al. 1989; Armannsson et al. 2007; Olsen et al. 2010; Homrum et al. 2012). The population structure of Pollock is still unclear, but four genetic clusters have been identified throughout the species' distributional range (Saha et al. 2015). However, the Pollock is a highly migratory species (e.g., Armannsson et al. 2007; Homrum et al. 2013), and it is reasonable to assume that there is substantial gene flow among population units (Olsen et al. 2010). Salmon farming may thus interfere with their natural migration pattern since the farms constitute artificial feeding grounds with a continuous supply of uneaten salmon feed (Otterå and Skilbrei 2014).

A dietary switch from natural prey to salmon feed may also induce differences in the behavior, metabolism, and morphology of Pollock, including variation in the size and weight of their organs (Skog et al. 2003; Dempster et al. 2011). Previous studies have detected compositional side effects in the fatty acid (Fernandez-Jover et al. 2011a) and trace element profiles of Pollock (Arechavala-Lopez et al. 2015) due to this trophic supplement, which may lead to physiological alterations in the wild fish targeted by artisanal fisheries (Otterå et al. 2009; reviewed by Uglem et al. 2014).

These potential impacts of salmon farms have generated a succession of conflicts in local Norwegian fisheries (Uglem et al. 2014; Ertör and Ortega-Cerdà 2015). However, few studies have examined how salmon farms influence the trace element concentrations in the tissues of wild Pollock (Bustnes et al. 2011; Arechavala-Lopez et al. 2015). In a previous study (Arechavala-Lopez et al. 2015), we assessed spatial variations in profiles of total lipids, fatty acids, and trace elements in the liver and muscle of wild Pollock under the influence of salmon farms. However, no studies have focused on the temporal variability of trace element composition in wild Pollock that aggregate around fish farms or explored how aquaculture could be affecting different tissues. For that reason, the aim of this study was to analyze the possible influence of salmon farms on changes in trace element concentrations in Pollock tissues over two consecutive years. The specific objectives of this study were (1) to assess the variations in body condition and trace element composition in two tissue types (liver and muscle) from Pollock sampled in two different areas (close to salmon farms [farm associated] or distant from the influence of farming [control]); and (2) to determine the temporal persistence and variability of trace element composition in the muscle and liver tissues from the two fish groups.

METHODS

Study location and fish sampling.— This study was conducted in September 2012 and 2013 on the Norwegian coast of Hitra Island (63.603658°, 8.645661°), where salmon farming coexists with local Pollock fisheries (Figure 1). Pollock were captured by using nets and hooks, and the catches were adapted to the opportunity to fish near farms. The sampling scheme was developed to test the hypothesis that Pollock caught near farms are directly influenced by aquaculture activity (i.e., feed surplus). Consequently, sampling locations were selected based on distance to a farm; Pollock that were captured less than 0.5 km from a farm were considered to be farm associated

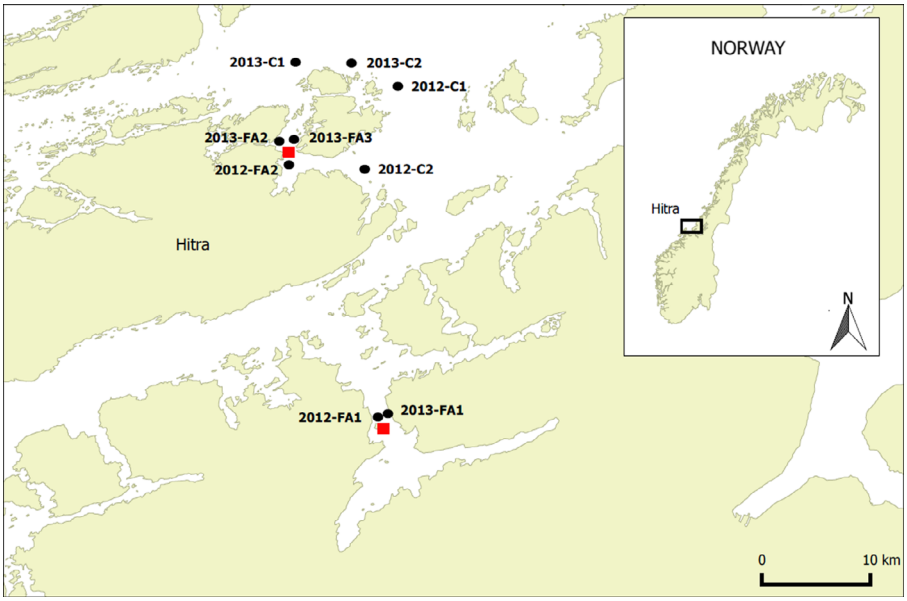

FIGURE 1. Study area map showing two different salmon farming areas (represented by red squares) around Hitra Island, Norway. Farm-associated (FA) and control (C) Pollock sampling areas and years (2012, 2013) are shown with black circles.

(FA-Pollock), while Pollock that were captured over 3 km from farms were regarded as controls (C-Pollock; Figure 1). In 2012, a total of 32 Pollock were captured in two control locations ($n = 8$; 2012-C1, 2012-C2) and two farm locations ($n = 8$; 2012-FA1, 2012-FA2; Figure 1; Supplement S1 available separately online). This fish sampling (2012) corresponds to that previously carried out by Arechavala-Lopez et al. (2015). To assess temporal variations and patterns, a total of 60 Pollock were caught in 2013 from two control locations ($n = 12$; 2013-C1, 2013-C2) and three farm locations ($n = 12$; 2013-FA1, 2013-FA2, 2013-FA3; Figure 1; Supplement S1). Each Pollock was placed on ice, and its stomach was removed. In gadoid species, lipids are stored primarily in the liver, making liver weight a measure of spawner quality (Marshall et al. 1999). The hepatosomatic index (HSI) was calculated and applied as an index of Pollock body condition: $\text{HSI} = 100 \times \text{LW}/W$, where LW is liver weight and W is empty body weight (i.e., without stomach contents).

For trace element analysis, tissue samples (10 g of liver and 10 g of muscle per fish) were collected, rinsed in distilled water (Milli-Q), and stored at −80°C in acid-washed tubes. Samples were freeze dried for 1 h and weighed to estimate dry mass. Trace element concentrations in tissues were determined according to Türkmen and Ciminli (2007). A 1-g sample of either tissue was extracted and then homogenized using a mixture of nitric acid and hydrogen peroxide (4:1, weight/weight). The samples were digested in a high-pressure microwave system, where concentrations of specific trace elements were identified and quantified through inductively coupled plasma mass spectrometry (Agilent 7700x mass spectrometer), and peak areas were compared with calibration curves. The equipment provides the possibility of directly introducing samples with a high dissolved solids content, such as seawater, without the need for prior dilution. This is achieved due to the high matrix introduction device, which dilutes the aerosol by means of argon gas before reaching the interface, thus avoiding clogging problems. Blank samples were prepared in the same manner and were used for blank corrections and to calculate the limits of detection. Spiked samples were also analyzed every 10 samples to assess instrument drift. The samples were randomized within every tissue. This analytical technique allows the simultaneous determination of trace element concentrations of up to 1 mg/kg (1 ppm [1×10^6]), which is the best option for environmental studies that involve fish farms (Dean et al. 2007).

In total, 30 trace elements were determined: Ag, As, barium (Ba), beryllium (Be), bismuth (Bi), cadmium (Cd), cobalt (Co), copper (Cu), Cr, iron (Fe), gallium (Ga), Hg, indium (In), lithium (Li), manganese (Mn), molybdenum (Mo), nickel (Ni), Pb, antimony (Sb), selenium (Se), strontium (Sr), thallium (Tl), vanadium (V), and zinc (Zn).

Statistical analyses.— To assess the influence of salmon farms on wild Pollock populations, multivariate (permutational multivariate ANOVA [PERMANOVA]) and univariate (ANCOVA) analyses were performed. The HSI and the trace element composition of muscle and liver tissues were compared between the two fish groups (fixed factor; FA-Pollock and C-Pollock) captured during the 2

years (fixed factor and orthogonal with fish group) at different locations (random factor nested within the interaction of fish group × year). The mean weight of the captured Pollock was compared among the groups, sampling localities, and years by using a Monte Carlo test to rule out a type I error in further analyses (Anderson and Robinson 2003). For the univariate analysis of changes in trace element concentrations, an ANCOVA was used, taking into account the weight of individuals as a covariate. The analysis was performed considering two orthogonal factors: year and fish group. Covariance coefficients were analyzed with heteroscedasticity-corrected covariance matrices using the rstatix package in RStudio.

During the multivariate analysis, PERMANOVAs were performed with the Bray–Curtis dissimilarity coefficient on fourth-root-transformed concentrations to give greater weight to the less abundant elements, with fish weight considered as a covariate (Anderson 2017). In fact, these types of elements are often present at high concentrations that can influence false differences in the analysis (Kunzendorf et al. 1986; Rago and Breland 2011). Fourth-root, square-root, and $\log(x+1)$ transformations were performed on different element concentrations when required to homogenize the variance among the samples during the univariate analysis. Similarity percentage (SIMPER) analysis was used to test for significant differences between the fish groups (FA-Pollock and C-Pollock) according to the contribution of specific trace elements. Principal coordinates analysis (PCO) was run to explore and visualize similarities and dissimilarities in the trace element composition between the fish groups according to sampling locality and year. The statistical analysis was performed using PRIMER 6 and PERMANOVA+ software (Plymouth Routines in Multivariate Ecological Research, Auckland, New Zealand).

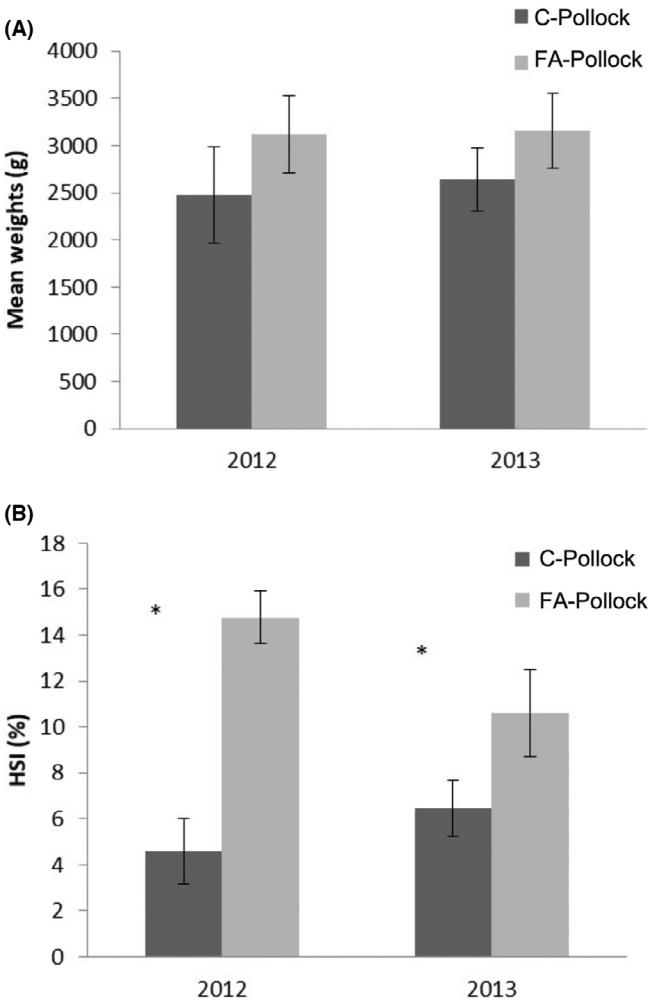

FIGURE 2. Bar plot of **(A)** mean weight (±SD) and **(B)** mean hepatosomatic index (HSI; ±SD) for control Pollock (C-Pollock; $N = 16$ in 2012; $N = 24$ in 2013; dark gray) and farm-associated Pollock (FA-Pollock; $N = 16$ in 2012; $N = 36$ in 2013; light gray) that were sampled during 2012 and 2013. Asterisks indicate significant differences ($P < 0.05$).

RESULTS

Fish Weight and Hepatosomatic Index

The mean weights of FA-Pollock captured in 2012 (mean ± SD = 3,116 ± 844 g) and 2013 (3,157 ± 812 g) were higher than those of C-Pollock captured in 2012 (2,476 ± 1,047 g) and 2013 (2,642 ± 682 g), although no significant differences were detected (fish group × year: Monte Carlo P-value, $P_{[MC]} = 0.843$; Figure 2A; Supplement S2). Significant differences were, however, detected at the location level (location: $P_{[MC]} = 0.012$), indicating high spatial heterogeneity at this scale (Supplement S2). The HSI values obtained for FA-Pollock for both sampling years were over 10%, while the HSI values for C-Pollock were below 7% (Figure 2B; Supplement S2). The statistical analysis indicated significant differences between fish groups in 2012 ($P_{[MC]} = 0.026$) and 2013 ($P_{[MC]} = 0.012$), thereby revealing consistent differences between the groups at the temporal scale (Figure 2B; Supplement S2).

Trace Element Concentration: Univariate Analysis

On the whole, the trace element concentrations detected in muscle tissues were generally higher in C-Pollock than in FA-Pollock (Supplements S2–S4). Trace element concentrations in muscle tissues were higher in C-Pollock for As, Zn, and Fe (Supplements S2, S3). In the liver samples, the trace element concentrations were also generally higher for C-Pollock than for FA-Pollock (Supplements S2, S4). In this case, the highest concentrations were detected for Fe, Zn, As, and Cu.

TABLE 1. Analysis of covariance results for trace element (TE) concentrations in Pollock, including *P*-values (*$P < 0.05$, **$P < 0.01$) for each factor (fish weight, year, fish group, and fish group × year).

	Muscle				Liver			
TE	Fish weight	Year	Fish group	Fish group × year	Fish weight	Year	Fish group	Fish group × year
Ag	0.144	0.00038**	0.593	0.679	0.533	0.002**	0.261	0.186
As	3.68×10^{-1}	6.02×10^{-21}**	9.09×10^{-7}**	9.00×10^{-3}**	2.34×10^{-1}	5.39×10^{-9}**	4.10×10^{-11}**	3.00×10^{-3}**
Ba	8.28×10^{-1}	8.26×10^{-13}**	1.21×10^{-1}	4.40×10^{-1}	0.401	0.067	0.213	0.9
Be	0.460	0.079	0.033*	0.387	0.579	0.032*	0.583	0.894
Bi	6.05×10^{-1}	3.79×10^{-5}**	1.87×10^{-1}	6.40×10^{-2}	0.033*	0.014*	0.482	0.095
Cd	0.786	0.680	0.986	0.521	1.73×10^{-1}	5.35×10^{-6}**	1.03×10^{-6}**	4.54×10^{-5}**
Co	9.53×10^{-1}	1.07×10^{-27}**	8.49×10^{-1}	8.50×10^{-1}	8.96×10^{-1}	1.11×10^{-7}**	1.53×10^{-5}**	1.10×10^{-4}**
Cu	5.10×10^{-1}	2.55×10^{-19}**	2.40×10^{-2}*	1.62×10^{-1}	2.88×10^{-1}	1.31×10^{-7}**	9.35×10^{-1}	7.10×10^{-2}
Cr	5.06×10^{-1}	8.73×10^{-5}**	9.72×10^{-1}	5.45×10^{-1}	1.80×10^{-1}	3.92×10^{-25}**	7.73×10^{-1}	5.57×10^{-1}
Fe	7.91×10^{-1}	3.50×10^{-7}**	9.01×10^{-1}	6.70×10^{-2}	9.80×10^{-2}	6.00×10^{-6}**	9.24×10^{-6}**	2.00×10^{-3}**
Ga	7.49×10^{-1}	6.03×10^{-17}**	1.75×10^{-1}	6.59×10^{-1}	0.438	0.177	0.221	0.852
Hg	1.61×10^{-1}	7.80×10^{-19}**	7.03×10^{-9}*	2.13×10^{-9}**	0.660	0.106	0.203	0.044*
In	4.75×10^{-1}	1.06×10^{-8}**	1.44×10^{-1}	7.30×10^{-2}	5.90×10^{-2}	4.61×10^{-5}**	7.40×10^{-2}	4.00×10^{-3}**
Li	7.20×10^{-1}	1.05×10^{-6}**	2.00×10^{-3}*	6.49×10^{-5}*	0.889	0.607	0.135	0.029*
Mn	6.50×10^{-1}	1.13×10^{-26}**	2.76×10^{-5}**	1.00×10^{-3}**	7.61×10^{-1}	1.31×10^{-6}**	1.00×10^{-2}*	1.00×10^{-3}**
Mo	7.00×10^{-1}	1.51×10^{-10}**	8.84×10^{-1}	7.08×10^{-1}	8.02×10^{-1}	3.88×10^{-11}**	1.91×10^{-7}**	6.83×10^{-8}**
Ni	0.435	0.002**	0.413	0.313	0.425	0.264	0.638	0.344
Pb	0.844	0.001**	0.512	0.205	3.96×10^{-1}	6.92×10^{-10}**	4.04×10^{-1}	3.64×10^{-1}
Sb	8.14×10^{-1}	6.58×10^{-11}**	3.80×10^{-2}*	3.60×10^{-2}*	0.376	0.087	0.058	0.063
Se	9.91×10^{-1}	9.94×10^{-40}**	2.72×10^{-5}**	1.03×10^{-4}**	4.95×10^{-1}	1.70×10^{-4}**	2.68×10^{-18}**	1.19×10^{-6}**
Sr	5.50×10^{-1}	1.66×10^{-8}**	3.80×10^{-2}*	3.59×10^{-1}	0.371	0.383	0.003**	0.353
Tl	0.782	0.00063**	0.416	0.199	2.9×10^{-2}*	3.38×10^{-10}**	2.99×10^{-1}	4.40×10^{-2}*
V	5.61×10^{-1}	1.11×10^{-5}**	8.33×10^{-1}	3.60×10^{-1}	0.002**	0.0003**	0.021*	0.0004**
Zn	4.36×10^{-1}	1.36×10^{-17}**	7.51×10^{-1}	3.46×10^{-1}	6.55×10^{-1}	2.81×10^{-15}**	1.90×10^{-2}**	4.40×10^{-4}**

Significant interactions (fish group × year) were found in muscle for six elements (As, Hg, Li, Mn, Sb, and Se; $P < 0.05$; Table 1). The posteriori pairwise comparison test for Mn indicated that the concentrations were significantly higher in FA-Pollock compared to C-Pollock during both 2012 and 2013 (2012: $P = 0.016$; 2013: $P = 0.018$). The concentrations of Hg and As were significantly higher in C-Pollock during 2012 and 2013 (Hg and As: $P < 0.01$). Furthermore, the concentrations of Li and Se were significantly higher in C-Pollock during 2012, and the concentration of Sb was significantly higher in C-Pollock during 2013 (Table 1; Supplement S3).

For the liver samples, the ANCOVA results indicated significant differences in the fish group × year interaction ($P < 0.05$; Table 1) for 13 elements (As, Cd, Co, Fe, Hg, In, Li, Mn, Mo, Se, Tl, V, and Zn). The posteriori pairwise comparison test for Co, Fe, Hg, In, Li, and Zn indicated statistical differences between fish groups in 2012 and in 2013 for Mn (Table 1; Supplement S4). The concentrations of As, Cd, Mo, and Se were significantly higher in C-Pollock during both years ($P < 0.05$). Furthermore, the concentration of V was significantly higher in C-Pollock during 2012 and was significantly higher in FA-Pollock during 2013. Concentrations of Ti and V in 2012 showed significant differences between fish groups, despite the covariable having a significant effect.

Trace Element Composition: Multivariate Analysis

The signatures of trace elements in the muscle samples were significantly different between fish groups and years (Table 2), with a significant effect of fish weight. The SIMPER analysis showed that As, Cr, and Zn contributed most to the dissimilarities between the fish groups (As: 8.56%; Cr: 6.32%; Zn: 6.17%), and As, Ag, and Cr differed most between sampling years (As: 7.73%; Ag: 6.79%; Cr: 6.22%; Table 3). Axis 1 of the PCO explained 65.2% of the total variation in muscle samples, basically discriminating between years in both cases (Figure 3A).

For the liver samples, the PERMANOVA results indicated significant differences between fish groups and years (Table 2). The SIMPER analysis identified that the dissimilarity was mainly related to variation in the Fe concentration (93%). The elements Zn, As, and Cu made the greatest contributions to the dissimilarity between fish groups (Zn: 42.48%; As: 28.77%; Cu: 19.41%) and sampling years (Zn: 48.95%; As: 23.37%; Cu: 20.08%; Table 3). Axis 1 of the PCO explained 79% of this variation, with a large difference between sampling years and less discrimination between treatments (Figure 3B).

DISCUSSION

Variations in trace element composition and HSI were detected in wild Pollock captured near Norwegian salmon farms compared to Pollock that were captured further away. These variations support the hypothesis that farming activity can induce diet changes in farm-associated wild fish (Sepúlveda and Oliva 2005). However, variation in trace element profiles between sampling localities and years indicates that both salmon feed and natural prey

TABLE 2. Permutational multivariate ANOVA results for differences in mean trace element concentrations in Pollock, with fish weight used as a covariate. Pairwise tests were used to compare differences within the levels of each factor (Lo = location; SS = sum of squares; MS = mean sum of squares; pseudo-F = F-value by permutation; unique perms = unique permutations; $P_{[MC]}$ = Monte Carlo P-value, where $*P_{[MC]} < 0.05$, $**P_{[MC]} < 0.01$). Muscle concentration data were fourth-root transformed.

Factor	df	SS	MS	Pseudo-F	$P_{(MC)}$	Unique perms
			Muscle			
Fish weight	1	325.95	325.95	2.5356	0.037*	999
Fish group	1	773.06	773.06	3.5678	0.02*	998
Year	1	18,853	18,853	88.96	0.001**	999
Fish group × year	1	319.94	319.94	1.5028	0.205	999
Lo (fish group × year)	5	1,103	220.61	2.369	0.001**	999
Residual	83	7,636	93.122			
Total	91	29,011				
			Liver			
Fish weight	1	220.19	220.19	2.745	0.025*	999
Fish group	1	1,130.5	1,130.5	8.084	0.003**	999
Year	1	6,647.5	6,647.5	48.409	0.001**	999
Fish group × year	1	235.19	235.19	1.7145	0.183	998
Lo (fish group × year)	5	696.09	139.22	2.2178	0.001**	999
Residual	76	4,708	62.773			
Total	84	13,637				

TABLE 3. Results of the similarity percentage analysis, showing the dissimilarities between the two fish groups (control [C-Pollock] and farm associated [FA-Pollock]) and two sampling years (2012 and 2013), according to permutational multivariate ANOVA (dissimilarity = average dissimilarity; contribution = contribution of each element to the total dissimilarity; cumulative = average cumulative contribution to the total dissimilarity; TE = trace element). Muscle concentration data were fourth-root transformed.

Statistic	TE	C-Pollock versus FA-Pollock	2012 versus 2013
		Muscle	
Dissimilarity (%)		23.25	32.41
Contribution (%)	As	8.56	7.73
	Cr	6.32	6.22
	Zn	6.17	6.00
	Hg	6.09	4.87
	Pb	6.01	5.02
	Fe	5.57	4.21
	Ag	5.36	6.79
	Ni	5.33	5.1
	Ga	5.12	5.7
	Ba	5.02	3.9
	Sr	4.39	3.31
	Se	3.86	4.32
	Li	3.70	3.49
	Cd	3.60	3.01
	Bi	3.29	
	Mn	3.17	3.24
	V	3.11	3.01
	Cu	3.10	
	Sb	2.71	3.23
Cumulative (%)		90.48	79.15
		Liver	
Dissimilarity (%)		32.42	37.55
Contribution (%)	Zn	42.48	48.95
	As	28.77	23.37
	Cu	19.41	20.08
Cumulative (%)		90.66	92.40

may influence the accumulation of trace elements (Sanchez-Jerez et al. 2002). In addition, changes in migration patterns, natural spatial distribution, or residence time around fish farms may influence the accumulation of trace elements (Arechavala-Lopez et al. 2016). For instance, Uglem et al. (2009) found that tagged Pollock stayed close to the cages at salmon farms for a prolonged period (days to months), which indicates that the high availability of waste feed originating from salmon farms may influence the accumulation of trace elements.

In the northern and northeastern portions of the North Sea, wild Pollock are important predators of sand lances *Ammodytes* spp., clupeids (e.g., Atlantic Herring *Clupea harengus*), Norway Pout *Trisopterus esmarkii*, and Haddock *Melanogrammus aeglefinus* (Daan 1989; Hislop et al. 1997; Floeter et al. 2005). More specifically, the trophic level of Pollock reaches 4.3 ± 0.4 under normal conditions (Yang 1982). The incorporation of salmon farms into marine coastal ecosystems causes an increase in species such as Pollock under the cages, whose feeding habits are affected due to the fish feed derived from these salmon farms (Dempster et al. 2002). This formulated fish feed may affect fish quality in addition to the organoleptic characteristics of fillets from farm-associated wild fish (Carss 1990; Skog et al. 2003; Otterå et al. 2009; Dempster et al. 2011; Uglem et al. 2020). It has also been shown that if Pollock stay near fish farms for several months, physiological changes and modifications of the metabolic profile can occur due to a diet switch from wild prey to uneaten feed pellets (Dempster et al. 2009; Bustnes et al. 2010; Fernandez-Jover et al. 2011a, 2011b; Maruhenda-Egea et al. 2015). Moreover, the inclusion of vegetable ingredients in the salmon feed could considerably reduce the Pollock's trophic level. Nevertheless, the reduced difference in trace element signatures between groups in 2013 compared with 2012 has two potential explanations: (1) the FA-Pollock fed under the salmon farms for short periods in 2013 or (2) the C-Pollock were previously feeding in the farming areas. In addition to changes in trophic behavior influenced by fish farming, there are many other abiotic and biotic factors that may influence the trace element composition of wild fish (Bustnes et al. 2010). For instance, water chemistry, temperature, and salinity may interact with the effects of fish farming, generating differences that are not easily explainable because of the high variability of environmental and physical conditions characterizing the Norwegian fjords. Thus, combinations of natural and anthropogenic processes are likely to influence the trace element profiles of wild fish populations inhabiting the fjords. Information on fish metabolic profiles and levels from these northern coastal waters is quite limited, but there are likely to be significant sources of many elements derived from anthropogenic activities.

The variations in specific trace element concentrations in Pollock tissue (e.g., As, Mn, and Cu in muscle; V and Mn in liver) could be used as indicators of salmon farming influence on wild Pollock. For instance, high concentrations of Mn and V in FA-Pollock livers may be indicative of Pollock individuals feeding in the areas surrounding salmon farms. A minimal supplementation of Mn in dry feed is necessary to meet the Mn requirement

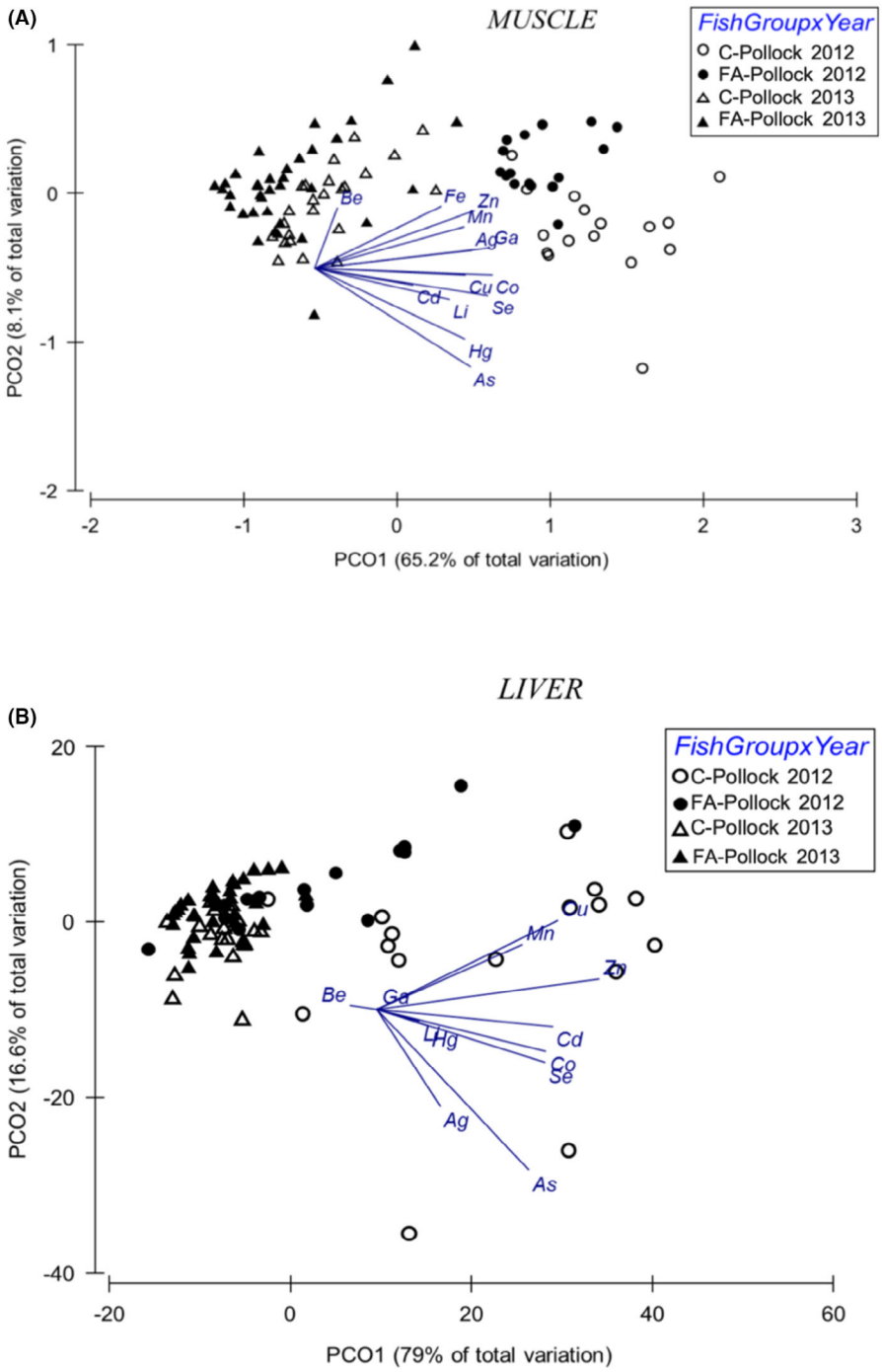

FIGURE 3. Principal coordinates analysis (PCO) of the trace elements in **(A)** muscle and **(B)** liver (fourth-root transformed) sampled from control Pollock (C-Pollock; $N = 16$ in 2012; $N = 24$ in 2013) and farm-associated Pollock (FA-Pollock; $N = 16$ in 2012; $N = 36$ in 2013). Only vectors with a correlation greater than 0.2 are plotted.

of Atlantic Salmon *Salmo salar* so as to prevent some negative effects of Mn deficiency (e.g., dwarfism, low body weight; Howe et al. 2004; Prabhu et al. 2019). In fact, the use of enriched diets with certain essential elements, including Cu, Fe, Zn, and Mn, might lead to higher concentrations of these elements in FA-Pollock tissues. Additionally, As, Cd, Pb, and Hg can also appear in the environment and potentially in animal feeds as result of

natural causes as well as industrial and agricultural activities (Bampidis et al. 2013); however, the practice of reducing or eliminating elements from commercial salmon feeds (Lorentzen and Maage 1999) might be reflected by lower concentrations of Hg, As, Cd, Fe, and Se in the tissues of FA-Pollock.

Moreover, although Pollock muscle may have greater implications with regard to human consumption, the results obtained suggest that the liver could be a better aquaculture indicator. The functional state of the liver and its implications for physiological processes may affect Pollock health and, consequently, the quality of fish fillets. However, such variations in trace element profiles between the fish groups studied (FA-Pollock and C-Pollock) were not detected in our previous study (Arechavala-Lopez et al. 2015). In that study, we reported a higher concentration of Hg in muscle samples from C-Pollock compared to FA-Pollock in 2012. Mercury is a naturally occurring element released into the environment from a variety of sources, including human activities, and can appear as methylmercury when converted by bacteria. This organic compound is deposited in water and sediment, and it bioaccumulates and biomagnifies up the aquatic food chain (EFSA 2004). It is therefore possible that wild Pollock captured near farms might also have fed on other wild fish that were aggregated around the farms (Uglem et al. 2009), transferring any nutritional deficits through the food chain (Hamre et al. 2008; Nordgreen et al. 2013). This predation may also contribute to lower levels of some elements and, consequently, a lower trophic level for wild FA-Pollock compared to noninfluenced C-Pollock individuals (Arechavala-Lopez et al. 2015). Le Croizier et al. (2016) found significant differences in terms of metal concentrations in the livers of fish from different trophic groups, indicating an effect of anthropogenic input whereby the consumption of benthic prey led to a greater accumulation of several trace metals, including Cd, Ni, and Pb. In any case, it is noteworthy that aquaculture does not seem to negatively affect the concentration of heavy metals that could impact the health of consumers.

Additionally, Skog et al. (2003) found that wild Pollock associated with a single fjord-based farm in Norway had a higher somatic condition than control fish that were taken from within the same fjord. This phenomenon has been demonstrated for other species (Fernandez-Jover et al. 2007, 2011b), and it could be a simple and quick proxy for assessing the possible influence of salmon farming on wild fish populations.

In conclusion, our results indicate that salmon grow-out cages appear to have a clear effect on Pollock body condition, as reflected by the HSI, due to changes in trophic behavior. These changes appear to potentially have an effect on the trace element composition of Pollock tissues, such as muscle and liver, in short-term studies. Monitoring these changes may be of interest because they can provide environmental information regarding the influence of salmon farming on wild fish, especially in terms of changes in multivariate trace element composition. For example, the period of residence of Pollock around fish farms could be affecting the trace element composition. However, the environmental variability of marine systems and the natural changes in fish distribution, migration, and prey availability could affect the concentrations of elements stored in fish tissues, as well as changes or fluctuations in the composition of aquaculture feed. Studies on larger spatial and temporal scales are needed to draw firmer conclusions regarding the effect of salmon farming on changes in the trace element composition in wild fish populations. In short, the analysis of trace element concentrations in fish samples associated with aquaculture facilities can be a good indicator of the interrelations between aquaculture and wild fish populations in coastal environments, providing information on food safety in relation to heavy metal concentrations.

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SUPPORTING INFORMATION

Additional supplemental material may be found online in the Supporting Information section at the end of the article.