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NOTE

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Abstract

Saithe *Pollachius virens* are attracted to uneaten salmon feed underneath cages at open-cage salmon farms in Norway. The aggregated Saithe have modified their feeding habits as they have switched from wild prey to uneaten food pellets, which could lead to physiological and biochemical changes in the Saithe. Variations in profiles of total lipids, fatty acids, and trace elements in Saithe liver and muscle were measured to evaluate the influence of fish feed from salmon farms on wild Saithe populations. Farm-aggregated Saithe had higher fat content in liver tissues than did individuals captured more than 25 km away from farms, but no clear differences were found in muscle tissues. High proportions of fatty acids of terrestrial origin, such as oleic, linoleic, and linolenic acids, in liver and muscle tissues of farm-aggregated Saithe reflected the presence of wild Saithe at farms. Accordingly, low proportions of arachidonic, eicosapentaenoic, and docosahexaenoic acids in Saithe tissues mirrored the feeding activity at farms. Variations in specific trace element signatures among fish groups also revealed the farming influence on wild Saithe. High levels of Fe, As, Se, Zn, and B in liver, but also As, B, Li, Hg, and Sr in muscle of Saithe captured away from farms indicated the absence of feeding at farms.

Many fish species are attracted to natural or artificial floating objects, often referred to as "fish aggregation devices" (FADs) (Dempster and Taquet 2004). Marine fish farms may serve as FADs by providing uneaten fish feed and structural habitat and by attracting small prey species (Sanchez-Jerez et al. 2011). Since fishing is not allowed near farms, these effects of fish farming may create conflicts with local fisheries because wild fish stocks become less available for

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exploitation. Moreover, a diet shift from wild prey to a diet consisting partly of artificial fish feed also may affect fish quality and fish fillet organoleptic characteristics (Carss 1990; Skog et al. 2003; Otterå et al. 2009; Dempster et al. 2011). Saithe *Pollachius virens* is an important commercial fish species in Norway, and they typically occur in pelagic schools along the coast during part of their migration and range extensively through a wide number of fjords (Bjordal and Skar 1992; Bjordal and Johnstone 1993). In addition, Saithe is one of the most abundant wild fish species found around Norwegian salmon farms (Dempster et al. 2009).

Wild Saithe reside near fish farm facilities for several months (Bjordal and Skar 1992; Bjordal and Johnstone 1993; Uglem et al. 2009; Dempster et al. 2009, 2010), a sufficient period to cause physiological changes and modification of metabolic profiles due to a diet switch from wild prey to uneaten feed pellets (Skog et al. 2003; Dempster et al. 2009; Otterå et al. 2009; Bustnes et al. 2010; Fernandez-Jover et al. 2011a, 2011b). Significant differences in body condition, relative liver size, lipid content, and fatty acid (FA) composition in both muscle and liver of Saithe have been reported in previous studies for farm-aggregated and unaggregated fish. Saithe fillets from a fjord without salmon farms tasted better than those collected in a fjord where farms were present (Skog et al. 2003). Besides providing nutrient inputs, fish farms may be supplying additional sources of other trace elements, since fish diets are enriched with various essential elements, including copper (Cu), iron (Fe), zinc (Zn), manganese (Mn), cobalt (Co), and chrome (Cr) among others (CIESM 2007). In addition, Cu is still used as an antifouling treatment (copper-based algaecides) for the net-pens and related equipment (e.g., Solberg et al. 2002; Brook and Mahnken 2003; Braithwaite and McEvoy 2005; Braithwaite et al. 2007). Bustnes et al. (2011) found that mercury (Hg) concentrations in the livers of farm-aggregated Saithe were higher than in unaggregated fish, but not at critically elevated levels of public health concern, suggesting that the distribution of Hg and other elements in Saithe and Atlantic Cod *Gadus morhua* in Norwegian coastal waters may be influenced by a combination of habitat use, diet, geochemical conditions, and water chemistry rather than farming activity alone. The overall knowledge about the presence and origin of essential and nonessential trace elements in Saithe populations is still sparse.

To address conflicts between fish farming and fisheries, a quantitative tool to determine retrospectively whether Saithe have been eating feed pellets would be useful. In some cases the fish might have been feeding actively on pellets over prolonged periods even though pellets are not found in stomach samples. The evacuation time for food consumed in gadoids usually varies (Andersen 2001), and stomach analyses would thus only reveal recent feeding on artificial fish feed. Enlarged livers can be used as an indication of active feeding by fish on salmon pellets (Dempster et al. 2009, 2011); however, it is also possible that Saithe will develop enlarged livers due to feeding on natural prey with high fat content, like Atlantic Herring *Clupea harengus* or Capelin *Mallotus villosus*. Thus, the FA and trace element (TE) profiles of commercial fish feed, which differ from those in natural food, might be used to compare their biochemical variation with those in tissues to characterize the dietary prehistory of Saithe. For instance, FA profiles in liver, muscle, and eggs vary between farm-aggregated and unaggregated gadoids (Skog et al. 2003; Fernandez-Jover et al. 2011b; Uglem et al. 2012). In particular, the FAs from vegetable oils vary between the two groups, since vegetable fats are used as a substitution for marine fat in artificial fish feed (e.g., Bell et al. 2001, 2003). In the current study we examined whether TEs in addition to FAs and lipid content could be used to distinguish between farm-aggregated and unaggregated Saithe. The specific objectives of the study were to (1) compare the composition of total lipids, FAs, and TEs between Saithe captured both at salmon farms and in areas having no farming activity, and (2) determine the reliability of using these compounds as indicators to detect the influence of salmon farming on wild Saithe assemblages.

METHODS

Fish sampling and preparation.—A total of 32 Saithe were captured between September 19 and 21, 2012, in the vicinity of Hitra Island, Norway (63.603658°N, 8.645661°E), with bottom nets and hooks or jigging (automatic jigging–juksa machines) around salmon farms and in control areas located more than 25 km away (Figure 1). Altogether, two groups of Saithe (16 individuals in each group) were designated as farm-aggregated fish (henceforth, F-Saithe) and unaggregated fish (henceforth, U-Saithe). Muscle and liver tissue samples (approximately 6 g) were collected from captured fish and stored at −80°C for further analyses of total lipids, FAs, and TEs.

Total lipids and FA analysis.—Extraction and determination of lipids and FA composition of the total lipid fraction in muscle and liver was determined in each sampled individual, after tissue homogenization, by fat extraction following the method of Folch et al. (1957) using a mixture of chloroform and methanol (1:1 proportion for the first extraction and 2:1 proportion for the second). Fatty acid methyl esters (FAMEs) samples were analyzed according to the method of Stoffel et al. (1959) by HPLC. Individual methyl esters were identified by comparison with known standards. The lipid content was expressed as percentage of ash-free dry matter, and individual FA concentrations were expressed as percentages of the total FA composition.

Trace elements analysis.—Approximately 1 g each of muscle and liver from each individual Saithe was subjected to wet mineralization following homogenization using a mixture of nitric acid and hydrogen peroxide (4:1, w/w) to extract TEs from the sample matrix through a vessel microwave digestion system. A total of 26 minor and 4 major elements were

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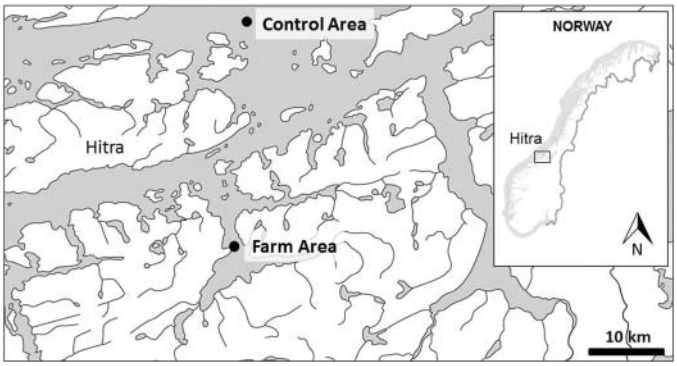


FIGURE 1. The study area around Hitra Island, Norway. Black circles represent the salmon farming and control areas where wild Saithe individuals were sampled.

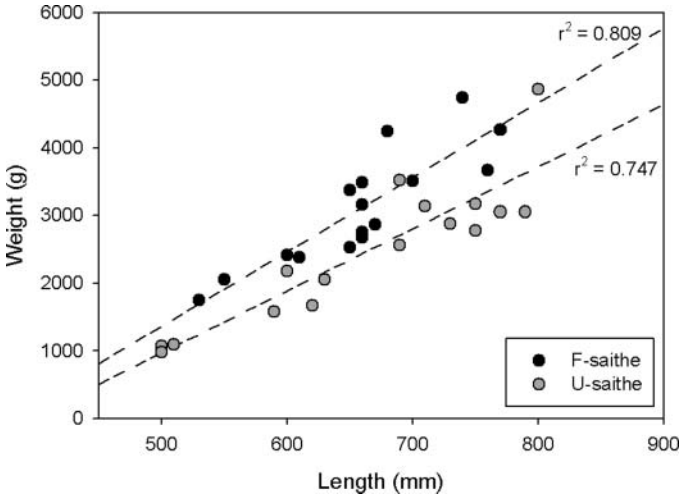


FIGURE 2. Scatterplot and linear regressions depicting the body length (cm) and weight (g) of unaggregated (U-Saithe) and farm-aggregated (F-Saithe) Saithe sampled. Data specific to U-Saithe individuals ($n = 16$) are represented by gray circles, while F-Saithe individuals ($n = 16$) are represented by black circles.

analyzed through inductively coupled plasma mass spectrometry (ICP-MS).

Each sample was analyzed in triplicate. The ICP-MS method is the routine choice for determination of trace elements in environmental studies involving fish farms and allows simultaneous determination of most elements within the periodic table with limits of detection below one part per billion (ppb; i.e., 1×10^9) (e.g., Campana et al. 1994; Dean et al. 2007). Nevertheless, minor and major elements were expressed in parts per billion and parts per million, respectively. Trace elements were quantified on the basis of peak areas and comparison with a calibrated curve obtained using the corresponding standards.

Statistical analysis.—Analysis of variance and linear regressions were applied to examine the effect of body length (L) and weight (W) between and within F-Saithe and U-Saithe groups. Differences between both fish groups in total lipids proportions and specific FAs and TEs on muscle and liver samples were also analyzed through ANOVA. Fourth-root transformations were performed on FAs and TEs to homogenize the variance among samples. Principal component analyses (PCAs) were used as the ordination method of Saithe assemblages with the elements that presented significant differences between groups. Moreover, cross-validation discriminant analysis (DA) was applied as a method of classifying Saithe individuals within groups according to the FA and TE profiles. Statistical analyses were performed with IBM-SPSS Statistics-20 and PRIMER-6 software packages.

RESULTS

Sampled Fish and Total Lipids

U-Saithe individuals presented larger mean body length and lower mean body weight ($L = 664.4 \pm 25.7$ mm, mean $\pm$ SE; $W = 2{,}475.6 \pm 261.8$ g) than F-Saithe ($L = 659.4 \pm 16.6$ mm, $W = 3{,}115.8 \pm 211.1$ g). However, there were no significant differences in total lengths and weights among the fish groups (ANOVA: total L: $P = 0.871$; total W: $P = 0.067$) (Figure 2). Livers from F-Saithe contained significantly higher proportions of total lipids than did those from U-Saithe (ANOVA: $P = 0.001$) (Figure 3). However, there were no differences in lipid content in muscle between both Saithe groups (ANOVA: $P = 0.365$) (Figure 3).

Fatty Acid Profiles

The FA profiles of liver and muscle differed between U-Saithe and F-Saithe (Table 1). Palmitic acid (PA, 16:0), oleic acid (OA, 18:1[n-9]), eicosapentaenoic acid (EPA, 20:5[n-3]), and docosahexaenoic acid (DHA, 22:6[n-3]) were the most abundant FAs in both tissues. Liver samples from U-Saithe had significantly higher proportions of PA, palmitoleic acid (PAL, 16:1), vaccenic acid (18:1[n-7]), nervonic acid (NEA, 24:1), arachidonic acid (ARA, 20:4[n-6]), EPA, and DHA (Table 1). However, liver samples from F-Saithe had significantly higher proportions of OA, erucic acid (22:1[n-9]), linoleic acid (LA, 18:2[n-6]), and linolenic acid (LNA, 18:3[n-3]) (Table 1). Similarly, muscle samples from F-Saithe presented higher proportions of OA, LA, LNA, EPA, and docosapentanoic acid (DPA, 22:5[n-3]) (Table 1). The percentage of total saturated FAs in liver and muscle samples was significantly higher in U-Saithe (liver: $27.06 \pm 0.78\%$, mean $\pm$ SE; muscle: $40.16 \pm 1.05\%$) compared with F-Saithe (liver: $21.47 \pm 0.73\%$; muscle: $36.16 \pm 0.76\%$) (Table 1). In contrast, proportions of total unsaturated FAs were significantly higher in livers and muscles from F-Saithe (liver: $78.95 \pm 0.72\%$; muscle: $71.96 \pm 0.47\%$) compared with U-Saithe (liver: $71.60 \pm 1.05\%$; muscle: $69.72 \pm 0.85\%$) (Table 1). Similarly, the proportion of total monounsaturated FAs (MUFAs) in liver and

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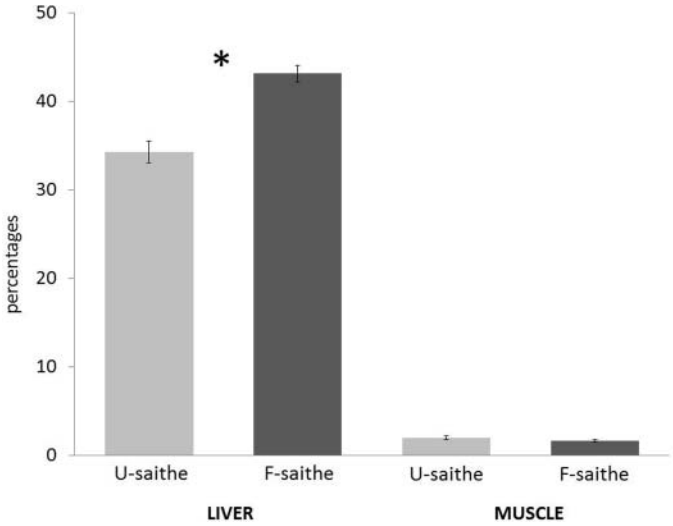


FIGURE 3. Proportion of total lipids in liver and muscle of unaggregated (U-Saithe) and farm-aggregated (F-Saithe) Saithe sampled. Error bars represent ± SE. Significant differences between groups determined by ANOVA are shown with an asterisk ($P < 0.05$). Data specific to U-Saithe individuals ($n = 16$) are represented by gray bars, while F-Saithe individuals ($n = 16$) are represented by black bars.

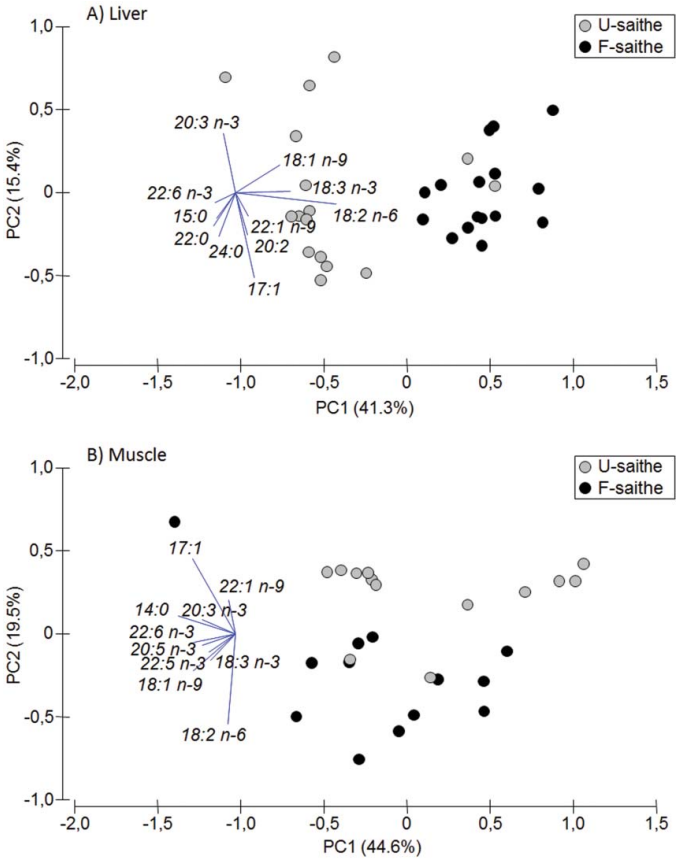


FIGURE 4. Principal component analysis of fatty acids in (**A**) liver and (**B**) muscle samples (fourth-root transformed) from unaggregated (U-Saithe) and farm-aggregated (F-Saithe) Saithe. Data specific to U-Saithe individuals ($n = 16$) are represented by gray circles, while F-Saithe individuals ($n = 16$) are represented by black circles. Only vectors with a correlation > 0.15 are plotted.

muscle of F-Saithe (liver: 47.54 ± 0.54%; muscle: 19.40 ± 0.57%) was significantly higher than in U-Saithe (liver: 42.87 ± 1.07%; muscle: 16.05 ± 0.41%) (Table 1). Conversely, proportions of total long-chain polyunsaturated FAs (PUFAs) in Saithe liver and muscle were significantly higher in U-Saithe (liver: 25.24 ± 1.34%; muscle: 52.21 ± 0.97%) compared with F-Saithe (liver: 17.92 ± 0.82%; muscle: 47.01 ± 0.99%) (Table 1). Altogether, proportions of the n-3: n-6 ratio were significantly higher in both tissues from U-Saithe samples (liver: 1.54 ± 0.06%; muscle: 1.23 ± 0.01%) than from F-Saithe samples (liver: 1.03 ± 0.04%; muscle: 1.18 ± 0.01%) (Table 1). A combination of two principal components (PCs) explained 56.7% of the total variation of FA profiles in liver samples (PC1: 41.3%, PC2: 15.4%) (Figure 4a). Variations in OA, LA, LNA, and DHA (the latter with negative correlation) among liver samples are explained by PC1, while PC2 contained the variations of the heptadecenoic acid (HA, 17:1), eicosadienoic acid (20:2), and lignoceric acid (24:0) (Figure 4a). The 64.1% of total variation in muscle samples were explained by two PCs (PC1: 44.6%; PC2: 19.5%) (Figure 4b). Principal component 1 mainly comprised the variations in DHA, EPA, and DPA, while variations in HA, OA, LA, and LNA are contained by PC2 (Figure 4b). Discriminant analysis with selected FAs in liver and muscle samples (those with significantly different proportions among groups; see Table 1) showed that 62.5% and 76.9%, respectively, were correctly classified. About 69% of U-Saithe liver and muscle samples were correctly classified from selected FA profiles, whereas higher percentages were correctly classified in F-Saithe liver (93.7%) and muscle (84.6%) (Table 2).

Composition of Trace Elements

Variations of TEs between U-Saithe and F-Saithe were found in both sampled tissues (Table 3). Regarding liver samples, a total of 15 minor elements, lithium (Li), boron (B), vanadium (V), Fe, Co, nickel (Ni), Zn, arsenic (As), selenium (Se), strontium (Sr), molybdenum (Mo), cadmium (Cd), antimony (Sb), and Hg and three major elements potassium (K), magnesium (Mg), and sodium (Na) were detected in significantly higher concentrations (ANOVA: $P < 0.05$) in U-Saithe individuals (Table 3). However, gallium (Ga), indium (In), and barium (Ba) were detected in significantly higher concentrations in F-Saithe (Table 3). Within the aforementioned minor elements, Fe (U-Saithe: 144.93 ± 31.15 ppb; F-Saithe: 39.28 ± 7.02 ppb), Zn (U-Saithe: 40.99 ± 3.81 ppb; F-Saithe: 26.80 ± 3.26 ppb), As (U-Saithe: 19.48 ± 3.20 ppb; F-Saithe: 4.92 ± 0.54 ppb), Se (U-Saithe: 2.43 ± 0.26 ppb; F-Saithe: 0.52 ± 0.06 ppb), and Sr (U-Saithe: 0.92 ± 0.14 ppb; F-Saithe: 0.42 ± 0.08 ppb) presented the highest concentrations in liver samples for both fish groups (Table 3). Regarding TE analysis

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TABLE 1. Proportions of total FAs in liver and muscle of unaggregated (U-Saithe) and farm-aggregated (F-Saithe) fish groups. Data are expressed as mean ± SE; ND = not detected. Significance level (ANOVA, fourth-root transformed data): *$P < 0.05$, **$P < 0.01$.

	Liver			Muscle		
FA	U-Saithe	F-Saithe	*P*-value	U-Saithe	F-Saithe	*P*-value
14:0	3.02 ± 0.28	2.44 ± 0.14	0.079	0.98 ± 0.22	0.91 ± 0.31	0.768
15:0	0.32 ± 0.03	0.19 ± 0.02	0.025*	0.01 ± 0.01	ND	0.154
16:0	12.33 ± 0.59	10.39 ± 0.28	0.006**	4.93 ± 0.49	6.55 ± 0.63	0.049*
17:0	0.18 ± 0.02	0.18 ± 0.01	0.928	0.03 ± 0.02	0.02 ± 0.01	0.347
18:0	3.87 ± 0.22	3.91 ± 0.14	0.778	2.28 ± 0.32	3.16 ± 0.48	0.109
20:0	0.17 ± 0.01	0.23 ± 0.02	0.025*	0.01 ± 0.01	0.02 ± 0.01	0.081
22:0	0.19 ± 0.11	0.04± 0.01	0.148	0.01 ± 0.01	ND	0.327
24:0	0.04 ± 0.01	0.02 ± 0.01	0.039*	0.01 ± 0.01	0.01 ± 0.01	0.848
Total saturated FAs	27.06 ± 0.78	21.47 ± 0.73	0.001**	40.16 ± 1.05	36.16 ± 0.76	0.005**
14:1	0.01 ± 0.01	0.01 ± 0.01	0.314	0.02 ± 0.01	0.01 ± 0.01	0.040*
15:1	0.01 ± 0.01	ND	0.075	0.01 ± 0.01	0.01 ± 0.01	0.480
16:1	5.21 ± 0.23	3.81 ± 0.18	0.001**	0.40 ± 0.06	0.48 ± 0.09	0.642
17:1	0.23 ± 0.04	0.32 ± 0.04	0.169	0.18 ± 0.04	0.29 ± 0.13	0.330
18:1(n-7)	5.07 ± 0.30	4.30 ± 0.14	0.050*	1.30 ± 0.11	1.51 ± 0.15	0.272
18:1(n-9)	15.11 ± 1.64	25.53 ± 1.58	0.001**	1.81 ± 0.26	4.43 ± 0.51	0.001**
20:1	6.56 ± 0.59	5.07 ± 0.42	0.068	0.43 ± 0.07	0.47 ± 0.07	0.652
22:1(n-9)	0.35 ± 0.07	0.59 ± 0.06	0.004**	0.031 ± 0.01	0.02 ± 0.01	0.192
24:1	0.62 ± 0.04	0.40 ± 0.03	0.001**	0.16 ± 0.03	0.17 ± 0.02	0.724
Total MUFAs	42.87 ± 1.07	47.54 ± 0.54	0.001**	16.05 ± 0.41	19.40 ± 0.57	0.001**
18:2(n-6)	1.764 ± 0.62	8.32 ± 0.67	0.001**	0.36 ± 0.14	1.47 ± 0.19	0.005**
18:3(n-3)	0.94 ± 0.17	3.18 ± 0.29	0.001**	0.08 ± 0.02	0.31± 0.05	0.001**
20:2	1.25 ± 0.12	1.45 ± 0.06	0.204	0.07 ± 0.01	0.14 ± 0.03	0.101
20:3(n-3)	0.06 ± 0.02	0.03 ± 0.01	0.499	0.09 ± 0.02	0.10 ± 0.03	0.592
20:4(n-6)	0.70 ± 0.13	0.36 ± 0.02	0.001**	0.63 ± 0.10	0.72 ± 0.07	0.304
20:5(n-3)	7.12 ± 0.45	5.18 ± 0.22	0.001**	2.95 ± 0.27	4.13 ± 0.44	0.038*
22:2	0.39 ± 0.02	0.44 ± 0.02	0.062	0.09 ± 0.01	0.14 ± 0.02	0.017*
22:4(n-6)	0.32 ± 0.02	0.26 ± 0.01	0.030*	0.05 ± 0.01	0.07 ± 0.01	0.385
22:5(n-3)	0.78 ± 0.03	0.85 ± 0.05	0.375	0.32 ± 0.05	0.61 ± 0.07	0.003**
22:6(n-3)	8.66 ± 0.45	6.39 ± 0.30	0.001**	9.63 ± 1.01	11.69 ± 1.09	0.148
Total LC-PUFAs	25.24 ± 1.34	17.92 ± 0.82	0.001**	52.21 ± 0.97	47.01 ± 0.99	0.001**
Total PUFAs	28.72 ± 1.11	31.42 ± 0.52	0.032*	53.67 ± 0.94	52.56 ± 0.78	0.382
Total unsaturated FAs	71.60 ± 1.05	78.95 ± 0.72	0.001**	69.72 ± 0.85	71.96 ± 0.47	0.029*
n-3:n-6 ratio	1.54 ± 0.06	1.03 ± 0.04	0.001**	1.23 ± 0.01	1.18 ± 0.01	0.005**

in muscle samples, a total of eight minor elements: Li, B, Cu, As, Se, In, Sb, and Hg, and two major elements, Mg and Na, showed significantly higher concentrations in U-Saithe individuals, while two minor elements, Mn and Ni, were detected in higher concentrations (ANOVA: $P < 0.05$) in F-Saithe (Table 3). Within these minor elements, As (U-Saithe: 22.73 ± 6.84 ppb; F-Saithe: 7.18 ± 0.08 ppb), Se (U-Saithe: 1.49 ± 0.06 ppb; F-Saithe: 0.98 ± 0.03 ppb), Cu (U-Saithe: 1.14 ± 0.12 ppb; F-Saithe: 0.85 ± 0.05 ppb), B (U-Saithe: 1.02 ± 0.21 ppb; F-Saithe: 0.31 ± 0.11 ppb), Mn (U-Saithe: 0.46 ± 0.05 ppb; F-Saithe: 0.79 ± 0.08 ppb), and Hg (U-Saithe: 0.71 ± 0.13 ppb; F-Saithe: 0.16 ± 0.03 ppb) presented the highest concentrations in muscle samples for both fish groups (Table 3). A combination of two PCs explained 70% of the total variation of TE profiles in liver samples (PC1: 62%, PC2: 8%) (Figure 5a); PC1 mainly represented the variations in Fe, As, Se, Zn, and B concentrations among samples, while variations in Cu, Zn, Cd, and Fe are explained by PC2

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TABLE 2. Classification (in percent) through cross-validation DA of unaggregated (U-Saithe) and farm-aggregated (F-Saithe) fish groups according to selected liver and muscle FAs ($P < 0.01$).

Tissue	Group	U-Saithe	F-Saithe	*n*
Liver	U-Saithe	68.7	31.3	16
	F-Saithe	6.3	93.7	16
Muscle	U-Saithe	69.2	30.8	16
	F-Saithe	15.4	84.6	16

(Figure 5a). Of the total variation among muscle samples, 52.9% was explained by two PCs (PC1: 37.1%, PC2: 15.8%) (Figure 5b). Variations in B, As, Li, aluminum (Al), and Cr among muscle samples are represented by PC1, while PC2 mainly contained the variations in Al, Cr, As, Ga, and Hg (Figure 5b). Discriminant analysis with selected TEs in liver and muscle samples (those with significantly different proportions among groups; see Table 3) showed that 51.6% and 65.6%, respectively, were correctly classified (Table 4). A total of 93.3% and 93.7% of U-Saithe were correctly classified from selected TE profiles in liver and muscle, respectively, and similar percentages were obtained from selected TEs in F-Saithe liver (93.7%) and muscle (87.5%) (Table 4).

TABLE 3. Trace elements in liver and muscle of unaggregated (U-Saithe) and farm-aggregated (F-Saithe) fish groups. Data are expressed as mean ± SE; ND = not detected. Significance level (ANOVA, fourth-root transformed data): $*P < 0.05$, $**P < 0.01$.

	Liver			Muscle		
TE	U-Saithe	F-Saithe	*P*-value	U-Saithe	F-Saithe	*P*-value
Minor elements						
Li	0.025 ± 0.0125	ND	0.006**	0.087 ± 0.028	0.012 ± 0.006	0.001**
Be	0.001 ± 0.001	0.001 ± 0.001	0.106	ND	0.001 ± 0.001	0.154
B	0.164 ± 0.086	ND	0.001**	1.022 ± 0.212	0.312 ± 0.107	0.001**
Al	2.359 ± 0.264	2.317 ± 0.283	0.888	1.216 ± 0.274	0.695 ± 0.178	0.313
V	0.193 ± 0.067	0.063 ± 0.017	0.032*	0.013 ± 0.008	0.009 ± 0.003	0.751
Cr	0.132 ± 0.0175	0.127 ± 0.013	0.960	0.117 ± 0.076	0.162 ± 0.138	0.825
Mn	1.589 ± 0.235	1.417 ± 0.244	0.498	0.457 ± 0.048	0.789 ± 0.084	0.001**
Fe	144.928 ± 31.510	39.281 ± 7.017	0.001**	11.744 ± 2.606	7.726 ± 1.177	0.065
Co	0.105 ± 0.0172	0.025 ± 0.004	0.001**	0.004 ± 0.001	0.004 ± 0.001	0.959
Ni	0.127 ± 0.018	0.074 ± 0.013	0.007**	0.041 ± 0.019	0.061 ± 0.061	0.049*
Cu	13.916 ± 1.881	11.867 ± 2.091	0.371	1.139 ± 0.123	0.845 ± 0.050	0.022*
Zn	40.995 ± 3.805	26.797 ± 3.263	0.003**	14.997 ± 0.558	16.063 ± 0.981	0.300
Ga	0.066 ± 0.017	0.119 ± 0.018	0.004**	0.039 ± 0.014	0.029 ± 0.010	0.945
As	19.480 ± 3.201	4.919 ± 0.544	0.001**	22.727 ± 6.836	7.179 ± 0.924	0.001**
Se	2.434 ± 0.259	0.521 ± 0.059	0.001**	1.497 ± 0.056	0.976 ± 0.034	0.001**
Sr	0.919 ± 0.143	0.417 ± 0.084	0.001**	2.525 ± 0.383	1.746 ± 0.258	0.072
Mo	0.384 ± 0.059	0.109 ± 0.013	0.001**	0.011 ± 0.002	0.010 ± 0.004	0.362
Ag	0.123 ± 0.014	0.120 ± 0.013	0.432	0.069 ± 0.009	0.075 ± 0.014	0.347
Cd	0.675 ± 0.164	0.111 ± 0.028	0.006**	0.006 ± 0.001	0.011 ± 0.006	0.822
In	0.001 ± 0.001	0.008 ± 0.003	0.006**	0.007 ± 0.001	0.004 ± 0.002	0.001**
Sb	0.003 ± 0.001	0.001 ± 0.001	0.021*	0.013 ± 0.003	0.007 ± 0.003	0.049*
Ba	0.201 ± 0.052	0.366 ± 0.056	0.003**	0.293 ± 0.054	0.273 ± 0.042	0.983
Hg	0.041 ± 0.031	ND	0.011*	0.713 ± 0.132	0.164 ± 0.035	0.001**
Tl	0.002 ± 0.001	0.005 ± 0.002	0.095	0.007 ± 0.004	0.003 ± 0.001	0.181
Pb	0.284 ± 0.103	0.209 ± 0.072	0.556	0.158 ± 0.039	0.128 ± 0.027	0.237
Bi	0.004 ± 0.001	0.032 ± 0.024	0.033*	0.059 ± 0.026	0.025 ± 0.017	0.059
Major elements						
Ca	35.71 ± 5.76	27.01 ± 8.84	0.470	287.72 ± 29.56	283.35 ± 25.06	0.941
K	1,304.64 ± 130.54	838.29 ± 78.14	0.002**	10,884.82 ± 349.69	10,208.60 ± 145.15	0.062
Mg	113.29 ± 15.33	43.90 ± 4.39	0.001**	931.65 ± 29.25	846.36 ± 16.20	0.006**
Na	1,049.93 ± 144.10	427.14 ± 52.87	0.001**	2,454.52 ± 351.46	1,608.25 ± 196.89	0.020*

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TABLE 4. Classification (in percent) through cross-validation DA of unaggregated (U-Saithe) and farm-aggregated (F-Saithe) fish groups according to selected liver and muscle TEs ($P < 0.01$).

Tissue	Group	U-Saithe	F-Saithe	*n*
Liver	U-Saithe	93.3	6.7	15
	F-Saithe	6.3	93.7	16
Muscle	U-Saithe	93.7	6.3	16
	F-Saithe	12.5	87.5	16

DISCUSSION

Variations in profiles of total lipids, FAs, and TEs in Saithe liver and muscle were to a large extent associated with presence at fish farms. Saithe can be found in far higher concentrations immediately beside and beneath salmon cages than just 25–200 m distance away from the nearest cage, most likely because they feed on waste fish food (Cromey et al. 2002; Tuya et al. 2006; Dempster et al. 2010). Hence, wild Saithe that normally feed on crustaceans and fish (e.g., Du Buit 1991; Carruthers et al. 2005) substitute , feed pellets for these natural items, when they aggregate at farms. This change in diet affects the chemical composition of the fish in a way that is similar to that seen in cultured fish species (Skog et al. 2003; Fernandez-Jover et al. 2011a).

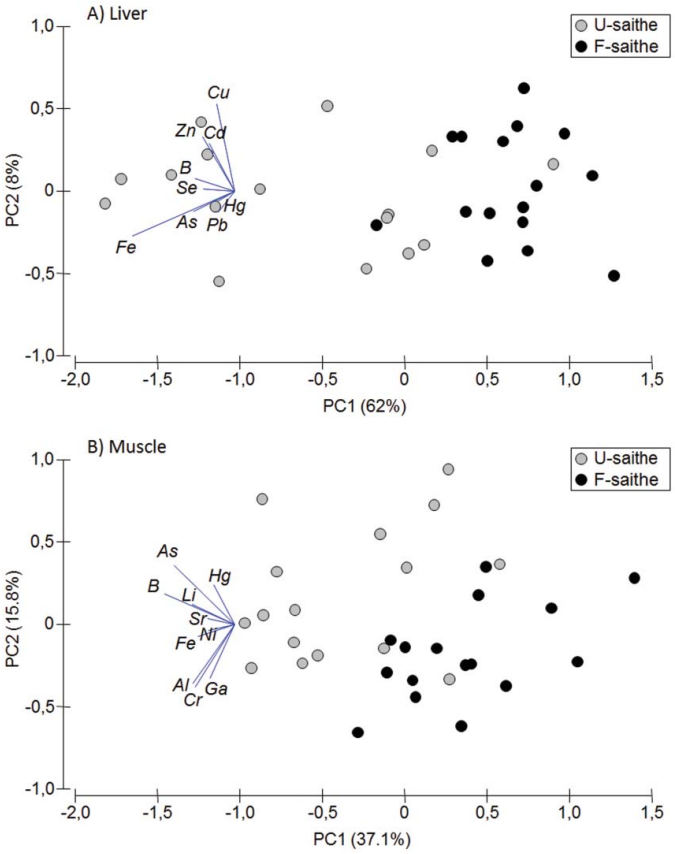


FIGURE 5. Principal component analysis of minor elements in (**A**) liver and (**B**) muscle samples (square-root transformed) from unaggregated (U-Saithe) and farm-aggregated (F-Saithe) Saithe. Data specific to U-Saithe individuals ($n = 16$) are represented by gray circles, while F-Saithe individuals ($n = 16$) are represented by black circles. Only vectors with a correlation > 0.15 are plotted.

After fish switch to a diet of salmon pellets, it is reasonable to assume that the high lipid content of the salmon feed will result in a higher fat content of the fish (Lopparelli et al. 2004). In our study, higher fat content in liver tissues was detected in F-Saithe than in U-Saithe, which were captured >25 km away from farms. Previous studies on Saithe, but also on other farm-aggregated species, revealed higher lipid levels and condition indices (i.e., Fulton's condition index, hepatosomatic index) in farm-aggregated fish compared with nonaggregated individuals (Skog et al. 2003; Fernandez-Jover et al. 2007, 2011b; Arechavala-Lopez et al. 2011; Izquierdo-Gomez et al. 2015). The incorporation and storage of FAs in fish tissues strongly depends on the FA profile of the diet (Sargent et al. 2002). The current practice of substituting fish oils with other vegetable lipid sources in farmed marine fish diets leads to notable changes in lipid composition and FA profiles in fish tissues (Fernandez-Jover et al. 2011a). Wild Saithe feeding around Norwegian salmon farms had liver and muscle FA profiles similar to the feed pellets used at the farm (Skog et al. 2003; Fernandez-Jover et al. 2011b). Our study on Saithe confirms that the presence of high proportions of FAs of terrestrial origin, such as OA, LA, and LNA, in liver and muscle tissues indicates that these fish have been feeding at farms. Consequently, the feeding habits of Saithe at farms are also reflected through a lower n3:n6 ratio or low proportions of ARA, EPA, and DHA. Therefore, variation in dietary FA profiles or specific FAs can be used to detect the occurrence of wild Saithe feeding at fish farms.

This study confirmed that the influence of fish farms on wild Saithe populations is also reflected by variation in TE signatures in muscle and liver tissues. Wild U-Saithe could be placed in a higher trophic level as they feed on a wide variety of prey items compared with F-Saithe that usually feed heavily on pellets (Fernandez-Jover et al. 2011a). The higher levels of TEs found in U-Saithe liver and muscle tissues compared with F-Saithe might be a result of the accumulation of TEs from natural prey (e.g., As, Se, Zn, Hg, Fe). Accordingly, F-Saithe could be placed in a lower trophic level, and consequently the accumulation of TEs in their tissues would be lower due to them directly feeding on pellets or consuming the aquaculture-related deposition of elements in the vicinity of the farms (Solberg et al. 2002; Bustnes et al. 2011). Contrary to expectations, the used of enriched diets (with essential elements such as Cu, Fe, Zn, and Mn among others) and antifouling treatments (i.e., Cu-based algaecides) at farms were not reflected in TE profiles of farm-aggregated Saithe tissues. However, the lower Fe levels in liver samples from F-Saithe might also be explained by the current practice of reducing or eliminating

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the Fe supplementation of commercial salmon feeds (Lorentzen and Maage 1999), since farmed Atlantic Salmon *Salmo salar* have a limited capacity to regulate Fe absorption and thus can develop winter ulcers (Salte et al. 1994). Our results show that concentrations of specific TEs (e.g., Fe, As, Se, Zn, and B in liver, and As, B, Li, Hg, and Sr in muscle) might be used to differentiate F-Saithe and U-Saithe, but that differences between groups may change over time, perhaps due to changes in TEs in natural prey or waste pellets.

The results of the present study suggest that variation in specific metabolic elements may be used to detect whether specific fish have inhabited the areas surrounding fish farms for a certain period of time. However, how long these farm-related characteristics persist in the fish's body, which will influence the accuracy of detection, remains unknown. The FA and TE content in both natural prey and waste feed may vary in time and with location, and it is thus important to correlate variations in FAs and TEs with potential recent changes on fish feed formulation when using such substances for examining fish farm influence on wild fish. Bustnes et al. (2011) suggested that the presence of specific essential elements in Norwegian coastal waters may be influenced by a combination of habitat use, diet, geochemical conditions, and water chemistry rather than by fish farming activity alone. Moreover, the possibility of the existence of several ecologically different Saithe assemblages within a metapopulation, having different feeding activities or movement patterns, indicate that the prediction of origin and potential effects due to variations in FAs and TEs might be more complex than previously assumed. Nevertheless, the ready availability of waste feed at farms provides a trophic subsidy in coastal waters (Fernandez-Jover et al. 2011b), altering the metabolite composition of wild fish populations. Whether metabolic variations lead to potential effects, such as affected physiological performance and reduced fish fitness, reproductive potential, spawning success, or flesh quality, on wild Saithe populations requires further research.

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