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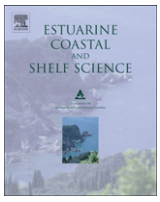

Research highlights

► Waste feed from coastal fish farms modify the diet of wild cod and saithe. ► Pelleted food modifies the Fatty Acid profiles of their muscle and liver. ► Cod's liver condition was significantly increased. ► These effects seem to occur globally on farm-aggregated fish that feed on lost pellets. ► Linear discriminant analysis is a useful tool to detect fish farm effects on FAs profiles.

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Waste feed from coastal fish farms: A trophic subsidy with compositional side-effects for wild gadoids

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ABSTRACT

Aquaculture of carnivorous fish species in sea-cages typically uses artificial feeds, with a proportion of these feeds lost to the surrounding environment. This lost resource may provide a trophic subsidy to wild fish in the vicinity of fish farms, yet the physiological consequences of the consumption of waste feed by wild fish remain unclear. In two regions in Norway with intensive aquaculture, we tested whether wild saithe (*Pollachius virens*) and Atlantic cod (*Gadus morhua*) associated with fish farms (F_{assoc}), where waste feed is readily available, had modified diets, condition and fatty acid (FA) compositions in their muscle and liver tissues compared to fish unassociated (UA) with farms. Stomach content analyses revealed that both cod and saithe consumed waste feed in the vicinity of farms (6–96% of their diet was composed of food pellets). This translated into elevated body and liver condition compared to fish caught distant from farms for cod at both locations and elevated body condition for saithe at one of the locations. As a consequence of a modified diet, we detected significantly increased concentrations of terrestrial-derived fatty acids (FAs) such as linoleic (18:2ω6) and oleic (18:1ω9) acids and decreased concentrations of DHA (22:6ω3) in the muscle and/or liver of F_{assoc} cod and saithe when compared with UA fish. In addition, the ω3:ω6 ratio clearly differed between F_{assoc} and UA fish. Linear discriminant analysis (LDA) correctly classified 97% of fish into F_{assoc} or UA origin for both cod and saithe based on the FA composition of liver tissues, and 89% of cod and 86% of saithe into F_{assoc} or UA origin based on the FA composition of muscle. Thus, LDA appears a useful tool for detecting the influence of fish farms on the FA composition of wild fish. Ready availability of waste feed with high protein and fat content provides a clear trophic subsidy to wild fish in coastal waters, yet whether the accompanying side-effect of altered fatty acid compositions affects physiological performance or reproductive potential requires further research.

1. Introduction

Anthropogenic changes to natural habitats have precipitated shifts in the abundance of many vertebrate populations (e.g. Gill et al., 1996; Wang et al., 2001). When changes have resulted in increased abundance of food for wild animals, super-abundance has sometimes resulted (Garrott et al., 1993). In terrestrial habitats, waste dumps in particular have directly increased populations of birds (Ramos et al., 2009) and bears (Eberhardt and Knight, 1996), which utilize these areas as feeding habitats. Increases in the populations of such species have been attributed to their gregarious nature and their flexible, opportunistic feeding behaviors, which makes them highly adapted to utilising new feed resources. In the marine environment, coastal sea-cage fish farms may represent an analogous scenario; an increased abundance of food is constantly available in their vicinity due to the loss of waste feed and wild fish aggregate in their vicinity in great biomass (Dempster et al., 2002, 2009; Fernandez-Jover et al., 2008) to feed upon this resource (Tuya

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et al., 2006; Fernandez-Jover et al., 2008). Wild fish may benefit from this feed resource (Fernandez-Jover et al., 2007; Sanchez-Jerez et al., 2008; Dempster et al., in press), which could act as a trophic subsidy. However, whether they are exposed to physiological changes as a result of consuming large amounts of waste feed has not been thoroughly explored.

In coastal Norway, 1142 concessions for salmonid farming were active in 2008 and used 1.2 million tons of fish food to produce 827 000 t of fish (Kjønhaug, 2009; Norwegian Fisheries Directorate, 2009). The amount of food that goes uneaten by salmon during production and falls through the sea-cages as waste varies, but estimates of up to 5% (Otterå et al., 2009) indicate that >50 000 tons of waste feed is directly available to wild fish in the vicinity of farms each year. Wild carnivorous gadoid fish, such as Atlantic cod (*Gadus morhua*), saithe (*Pollachius virens*) and haddock (*Melanogrammus aeglefinus*), are the main fish present around Norwegian fish farms (Dempster et al., in press, 2010b). Aggregations sizes of gadoids have been estimated to be over 10 tons in the summer months within the typically less than 1 ha of sea surface area that salmon farms occupy (Dempster et al., 2009).

These wild gadoids consume large amounts of waste feed in the vicinity of farms, which results in a significant shift away from their natural diets (Dempster et al., in press). Marine fish, especially carnivores, have a natural diet rich in polyunsaturated ω3 fatty acids (PUFA), and as a consequence, ω3 PUFAs occur in higher concentrations in marine fish muscle (Ackman, 1967). Fish meal and fish oil are added into commercial fish feeds to supply the requirements of reared carnivorous fish. However, as the demand for and the cost of these fish feed components is high, the fish feed industry has developed feeds that contain substantial amounts of vegetable-derived oils and meals of terrestrial origin that consist mainly of ω6 PUFAs rather than ω3 PUFAs. This fundamentally changes the fatty acid profile of feeds. Sunflower, soya bean, palm or rapeseed oils are used extensively in fish feed production and result in high concentrations of oleic acid (18:1ω9) and linoleic acid (18:2ω6), reducing the concentration of ω3 PUFAs (Pickova and Mørkøre, 2007; Turchini et al., 2009). If wild fish feed extensively on lost waste pellets from coastal fish farms, their total fat content and fatty acid composition may change in the same way as occurs with reared fish (e.g. Bell et al., 2006; Fernandez-Jover et al., 2007; Jobling et al., 2008). A previous study carried out at a single farm showed that wild saithe that were captured nearby had a different muscle fatty acid composition than saithe caught distant from the farm (Skog et al., 2003).

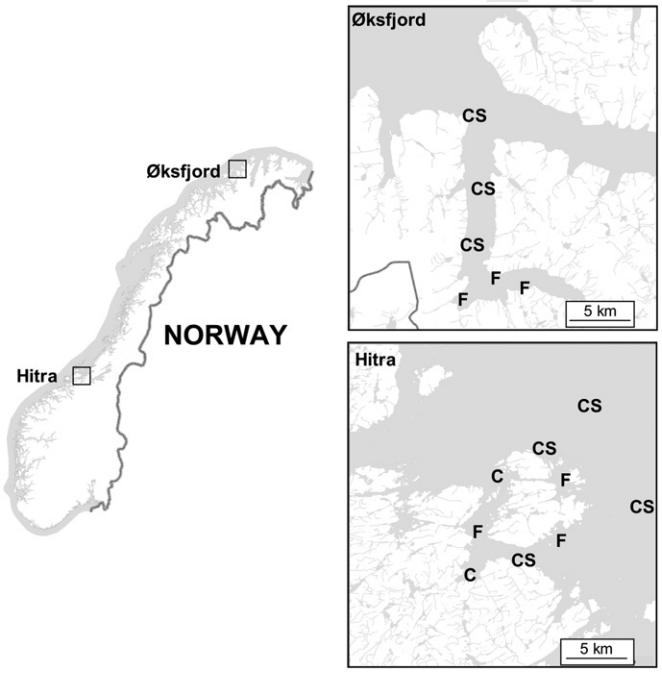

Fig. 1. Map of the Hitra and Øksfjord salmon farming areas in Norway showing the sampling locations for farm-associated (F) saithe *Pollachius virens* and Atlantic cod *Gadus morhua* and farm-unassociated sampling locations for saithe (S) and cod (C).

As a first step towards determining whether the modified diets available to wild fish around Atlantic salmon (*Salmo salar*) farms have physiological or ecological consequences for wild fish, we tested whether the diets of cod (*Gadus morhua*) and saithe (*Pollachius virens*) differ when they are aggregated around fish farms compared to natural control locations. Further, we tested whether differences we detected in diets translated into differences in traditional measures of condition, including Fulton's condition index and the hepato-somatic index, and fatty acid concentrations in body tissues. In doing so, we tested the fatty acid compositions of muscle and liver tissue, liver being the main fat storage organ in gadoid fish (Dos Santos et al., 1993).

2. Materials and methods

2.1. Study locations and fish sampled

Saithe and cod were sampled from two salmon farming areas (Fig. 1): Hitra within the South-Trondelag region (63°N; 94 farms; 82,000 t) and Øksfjord within the Troms region (70°N; 123 farms; 72,000 t; Norwegian Fisheries Directorate, 2009). Farm-associated (hereafter F_{assoc}) fish were defined as those captured within 5 m of sea-cages containing Atlantic salmon. Both cod and saithe were sampled from within 5 m of the sea-cages at 3 farms at both Hitra and Øksfjord. These were the same farms used to assess aggregation sizes by Dempster et al. (2009). Farm-unassociated fish (hereafter UA) were defined as those captured 4–20 km distant from the nearest salmon farms (Fig. 1) to limit the possibility of sampling fish at non-farm locations that had interacted recently with a farm. Depending on the species and farming area, UA fish were sampled from 3 to 6 locations. The 4 km minimum limit was based on telemetry-derived observations of the predominant movements of wild cod and wild saithe (Uglem et al., 2008, 2009, 2010) in the vicinity of fish farms. UA areas were of similar depth and bottom habitat as those of the salmon farms. All fishes were captured between June–August 2007 with standardized hook and line fishing gear. Due to the low number of fish obtained at some UA and F_{assoc} sampling locations, samples were pooled for diet, condition and fatty acid analyses at the level of the farming area (i.e. Hitra F_{assoc}, Hitra UA, Øksfjord F_{assoc}, and Øksfjord UA; Table 1).

In addition to the fish samples, dry food pellets were directly collected from the feed bags at each of the studied farms in Hitra and Øksfjord. 4 pellet types were collected at Hitra and 3 at Øksfjord. Pellets from the various farms were pooled for Hitra and Øksfjord for later analysis of their FA profiles.

2.2. Diet and condition indices

Upon capture, fish were immediately placed on ice before transfer to the laboratory where they were measured (fork length: FL) and weighed. Livers were then dissected and weighed and stomach contents from the foregut were dissected. We calculated two condition indices: Fulton's Condition Index (FCI = [100 × W]/L^3, where W = weight in g (after withdrawing stomach content weight) and L = length in cm), and the Hepato-Somatic Index (HSI = 100 × [liver weight/total weight]). FCI is widely used to

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Table 1
Size, total weight (g), fork length (cm), mean stomach content (g) and proportion of occurrence (percentage of fish containing each prey item) of Atlantic cod (*Gadus morhua*) and saithe (*Pollachius virens*) captured adjacent to farms (F_{assoc}) or unassociated with farms (UA). Mann–Whitney test were apply to detect significant differences for weight, length and mean stomach content between F_{assoc} and UA fish within each locality. Significance level: *0.05, **0.01. Q8

	G. morhua				*P. virens*			
Location	Hitra		Øksfjord		Hitra		Øksfjord	
Treatment	UA	F_{assoc}	UA	F_{assoc}	UA	F_{assoc}	UA	F_{assoc}
No. fish	25	24	32	27	24	25	28	30
Fork length (cm)	38.2 ± 1.8	45.1 ± 4.6	61.5 ± 3.1	74.5 ± 4.5	30.0 ± 1.3	33.0 ± 4.1	47.2 ± 1.7	51.0 ± 1.2
Total weight (g)	540 ± 107	970 ± 538	2359 ± 467	4035 ± 726	310 ± 45	390 ± 31	1098 ± 98**	1360 ± 9*
Mean stomach content (g)	6.3 ± 3.3	19.3 ± 13.6	32.1 ± 8.4	56.1 ± 17.8	0.45 ± 0.12	58.2 ± 55.9**	4.8 ± 2.9	15.5 ± 3.2*
% Invertebrates	90.1	10.1	73.0	31.1	100	3.1	21.1	30.4
% Fish	9.9	79.8	25.5	35.4	0	1	36.8	11.8
% Food pellets	0	6.3	0	24.9	0	95.9	0	44.3
% Other	0	3.8	1.5	8.7	0	0.4	42.1	13.6

compare growth conditions of fish and HSI, which represents the ratio of liver weight to body weight, provides an indication on status of energy reserve in an animal, especially in fish that use liver as the main storage organ.

Stomach contents from the foregut were examined and prey species were identified to the highest possible taxonomic separation. Prey categories were later reduced to 11 for saithe (pellets, Brachyura, Osteichthyes, Polychaeta, Caridea, zooplankton, Phaeophyceae, Bivalvia (principally *Mytilus edulis*), Ophiuridae, Hydroida (principally *Ectopleura larynx*), and unidentified benthic matter) and 13 for cod (pellets, Brachyura, Osteichthyes, Polychaeta, Caridea, Phaeophyceae, Bivalvia (*Mytilus edulis*), Holothuria, Ophiuridae, Echinoidea, Octopoda, Amphipoda and unidentified benthic matter). Prey items within each category were weighed.

2.3. Fatty-acid analyses

Samples of the anterior-dorsal white muscle and the homogenized liver (approximately 6 g each) were obtained from individual fish, packed in aluminum foil, frozen at −20 °C and analyzed within one week. After individual tissue homogenisation, the FA composition of the total lipid fraction was determined by fat extraction following the method of Folch et al. (1957), with a mixture of chloroform and methanol (1:1 proportion for the first extraction and 2:1 proportion for the second extraction). Fatty acid methyl ester samples were prepared and analyzed according to Stoffel et al. (1959) by gas–liquid chromatography using a SP™ 2560 flexible fused silica capillary column in a Hewlett–Packard 5890 gas chromatograph. Individual methyl esters were identified by comparison with known standards purchased from the Sigma Chemical Company (St. Louis, MO, USA). Individual FA concentrations were expressed as percentages of the total FA composition.

2.4. Statistical analyses

To test for significant differences between UA and F_{assoc} cod or saithe in FCI, HSI or fatty acid profiles within each farming locality (Hitra or Øksfjord), we used non-parametric Mann–Whitney tests. The significance level was adjusted for multiple comparisons associated with pairwise tests to $\alpha = 0.01$ to reduce the probability of making Type I errors (Bonferroni procedure; Rice, 1989). A principal component analysis (PCA) was used to explain the variance in the FA data. Due to a high number of variables (i.e. FAs), the PCA was used to transform the original variables into new, uncorrelated variables called principal components, which were plotted to obtain a more informative, two-dimensional picture than the raw FA table values. To test the suitability of FA profiles for classifying the fish depending on their origin (F_{assoc} or UA), a linear discriminant analysis (LDA) was applied for both tissue types. This analysis searches for the linear combination of variables which best separates the different groups of samples and gives a final output in which every sample is classified using the calculated discriminant model (Duda et al., 2001). All of the fish used for FA analysis were used to run the LDA. Distinction of each individual fish to F_{assoc} or UA origin was done by the leaving-one-out method, which classifies each case while leaving it out of the model calculations.

3. Results

3.1. Diet of F_{assoc} and UA Atlantic cod and saithe

In total, 57 UA and 51 F_{assoc} cod and 52 UA and 55 F_{assoc} saithe were captured for analyses (Table 1). There were no significant differences between the fork lengths of UA and F_{assoc} cod or saithe at both localities. No differences in mean weights were detected between UA and F_{assoc} cod or saithe, apart from in Øksfjord where F_{assoc} saithe (1360 ± 9 g) were significantly heavier than UA saithe (1098 ± 98 g). Comparisons of diets, condition and fatty acid compositions between UA and F_{assoc} cod or UA and F_{assoc} saithe at each locality where thus made based on fish of broadly similar length and weight.

Diets of cod and saithe at UA locations were mainly composed of invertebrates, fish and other items (mainly crustaceans; Table 1). These differed from diets of F_{assoc} fish at both Hitra and Øksfjord mainly due to the presence of food pellets, which ranged between 6.3% and 24.9% of the stomach content for cod and 44.3%–95.9% for saithe.

3.2. Condition of F_{assoc} and UA fish

FCI differed significantly between UA and F_{assoc} cod at Hitra (UA: 0.92 ± 0.02 vs. FA: 1.09 ± 0.04) but not Øksfjord (UA: 0.97 ± 0.39 vs. FA: 1.00 ± 0.02; Fig. 2). Significant differences between HSIs were detected between UA and F_{assoc} cod at both localities, with livers of F_{assoc} fish consistently larger as a proportion of total body weight than their UA counterparts. At Hitra, average HSIs were over 2 times greater for F_{assoc} (2.5 ± 0.4) than UA cod (1.1 ± 0.2). Similarly, at Øksfjord, mean HSI values were over 3 times greater for F_{assoc} (10.0 ± 2.1) than UA (2.9 ± 0.3) cod.

For saithe, we did not detect significantly increased levels of FCI and HSI for F_{assoc} compared to UA fish, with the only exception of significantly higher FCIs for F_{assoc} saithe at Hitra compared to UA fish (Fig. 2). UA saithe had average FCIs of 0.97 ± 0.07 and 1.00 ± 0.36 at Hitra and Øksfjord, respectively. F_{assoc} fish values ranged between 1.13 ± 0.04 at Hitra and 1.09 ± 0.03 at Øksfjord. No significant differences were found among F_{assoc} and UA fish at any

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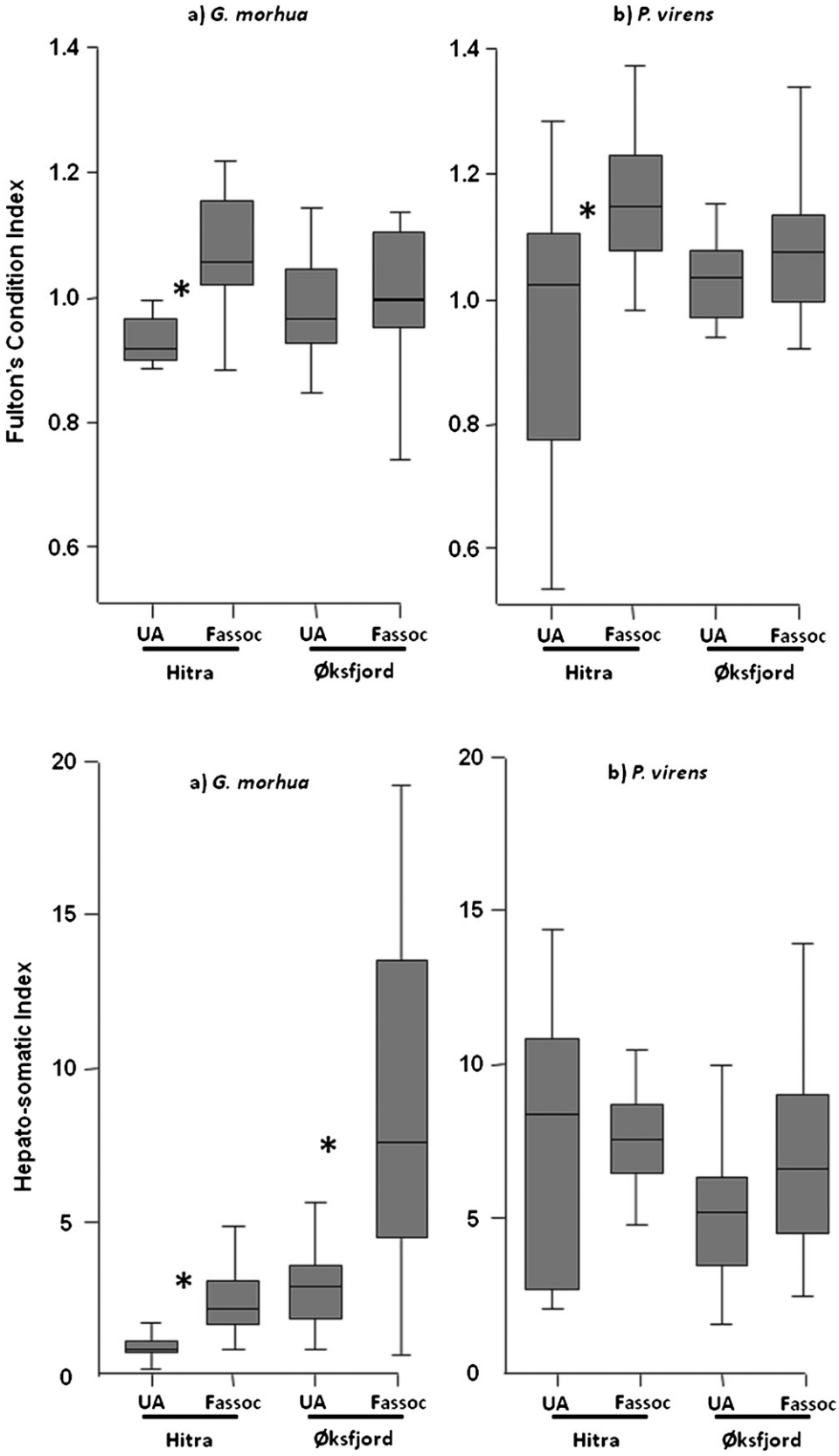

Fig. 2. Box plots of the Fulton's Condition Index (FCI) and Hepato-somatic Index (HSI) of farm-associated (F_{assoc}) and unassociated (UA) cod (*Gadus morhua*) and saithe (*Pollachius virens*).

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Table 2
Fatty acid composition (% of total fatty acids) of the food pellets used at Øksfjord and Hitra. Numbers are mean ± standard deviation of 3 types of food pellets used at Øksfjord and 4 types used at Hitra. Only fatty acids with a concentration higher than 1% are shown.

Fatty acids	Øksfjord	Hitra
C16:0	17.1 ± 1.90	11.4 ± 0.30
C18:0	2.74 ± 0.07	2.80 ± 0.23
Total saturated	27.9 ± 2.45	20.6 ± 0.45
C16:1ω7	5.65 ± 0.87	3.48 ± 0.23
C18:1ω7	2.65 ± 0.23	2.72 ± 0.04
C18:1ω9	16.1 ± 5.92	30.8 ± 1.33
C20:1ω9	5.23 ± 1.31	5.05 ± 0.94
Totalω9	22.6 ± 4.57	37.2 ± 0.29
Total monounsaturated	31.1 ± 4.00	43.5 ± 0.18
C18:2ω6	6.37 ± 2.01	12.0 ± 0.55
Total ω6 PUFA	8.16 ± 1.58	12.9 ± 0.60
C18:3ω3	2.07 ± 1.05	5.13 ± 0.25
C18:4ω3	2.36 ± 0.46	1.51 ± 0.17
C20:5ω3	12.7 ± 2.43	6.97 ± 0.28
C22:5ω3	1.16 ± 0.10	0.66 ± 0.05
C22:6ω3	14.4 ± 1.02	8.55 ± 0.38
ω3 PUFA	32.3 ± 2.97	23.0 ± 0.56
Total polyunsaturated	41.0 ± 1.56	35.9 ± 0.34
ω3/ω6	4.17 ± 1.12	1.79 ± 0.12
ω3 HUFA	28.5 ± 3.56	16.4 ± 0.64

locality for HSI. At Hitra, mean HSI values were similar for UA (7.9 ± 1.5) and F_{assoc} (7.5 ± 0.5) saithe. At Øksfjord, UA saithe had a lower mean HSI value (5.5 ± 0.57) than F_{assoc} fish (7.0 ± 0.7), but due to high variability in HSI values among individuals, no significant difference was detected.

3.3. Fatty-acid compositions of waste feed

Food pellets collected at both Øksfjord and Hitra had broadly similar FA compositions. Pellets contained high amounts of ω3 (23.0–32.8%), ω9 (22.6–37.1%) and ω6 fatty acids (8.2–13.0%; Table 2). High concentrations of the palmitic (16:0, 11.4–17.1%), oleic (18:1ω9, 30.8–16.1%), linoleic (18:2ω6, 6.4–12.0%) EPA (20:5ω3, 7.0–12.7%) and DHA (22:6ω3, 8.6–14.4%) fatty acids were also detected. Øksfjord pellets contained high levels of EPA and DHA.

3.4. Fatty acid compositions of F_{assoc} and UA fish

When FAs were individually compared, several fatty acids in both muscle and liver differed between F_{assoc} and UA cod and saithe at both localities (Table 3). For cod, linoleic (18:2ω6) acids were significantly increased in F_{assoc} fish compared to UA fish in both muscle and liver tissues. Significantly higher levels of eicosadienoic (20:2ω6), linolenic (18:3ω3) and docosapentaenoic (22:5ω3) acids were also detected in the muscle of F_{assoc} compared to UA cod. Even greater differences between the F_{assoc} and UA groups were found in the cod liver tissue. In addition to linoleic acid, differences were detected for oleic acid (18:1ω9) and total monounsaturated acids due to higher levels in F_{assoc} cod. In contrast, significantly lower levels of DHA (22:6ω3), total ω3, ω3/ω6 ratio and total Highly Unsaturated ω3 acids (HUFA) were detected in the livers of F_{assoc} cod compared to UA cod.

Principal components analysis indicated that PC1 and PC2 explained 24.6% and 19.3%, respectively, of the total variability for cod muscle and 24.2% and 13.7% for cod liver (Fig. 3). Linoleic and linolenic acid were the factors that showed the highest positive correlation with PC1 regarding cod muscle, DHA and 20:4ω6 showed the highest negative correlations. DHA also showed a marked negative correlation with PC2. This was translated in the differentiation of some F_{assoc} fish along PC1 and PC2, but many F_{assoc} and UA fish remained overlapped in the plot. Differentiation was clearer regarding cod liver; F_{assoc} fish showed a tendency to order along PC1 with linoleic, linolenic and oleic acids with the highest negative correlation loads, with DHA presenting the highest positive correlation. According to the FA profile of cod muscle, linear discriminant analysis (LDA) correctly assigned 88.5% of the individuals to their F_{assoc} or UA origin (69 out of 78). LDA was even more

Table 3
Fatty acid composition (% of total fatty acids) of farm-associated (F_{assoc}) and farm-unassociated (UA) cod (*Gadus morhua*). Data are expressed as mean ± standard deviation. Mann–Whitney test were apply to detect significant differences for weight, length and mean stomach content between F_{assoc} and UA fish within each locality. Significance level: **0.01, ***0.001.

	Muscle				Liver			
	Hitra		Øksfjord		Hitra		Øksfjord	
	UA	F_{assoc}	UA	F_{assoc}	UA	F_{assoc}	UA	F_{assoc}
n	20	19	22	17	5	5	10	10
C16:0	17.14 ± 0.24	17.45 ± 0.37	17.35 ± 0.16	16.79 ± 0.22	0.39 ± 0.86	0.53 ± 0.65	0.44 ± 0.45	0.34 ± 0.44
C18:0	3.62 ± 0.08	3.71 ± 0.08	4.18 ± 0.23	4.32 ± 0.21	4.62 ± 0.3	4.73 ± 0.45	3.54 ± 0.33	3.42 ± 0.26
Total saturated	22.53 ± 0.31	23.05 ± 0.38	23.55 ± 0.37	23.23 ± 0.43	25.82 ± 0.93	22.59 ± 1.75	23.95 ± 0.77	21.08 ± 0.8
C16:1ω7	1.08 ± 0.05	1.08 ± 0.06	1.06 ± 0.04	1.22 ± 0.07	3.52 ± 0.77	5.76 ± 0.71	5.52 ± 0.42	5.16 ± 0.37
C18:1ω9	5.75 ± 0.22	6.31 ± 0.29	5.88 ± 0.26	8.48 ± 0.41***	8.33 ± 0.89	19.89 ± 3.06**	12.18 ± 0.65	20.99 ± 1.48***
C18:1ω7	2.36 ± 0.09	2.76 ± 0.23	2.31 ± 0.07	2.54 ± 0.1	4.19 ± 0.15	6.74 ± 1.85**	3.69 ± 0.22	4.12 ± 0.33
C20:1ω9	0.89 ± 0.1	1.31 ± 0.19	1.09 ± 0.11	1.25 ± 0.08	3.9 ± 1.29	4.93 ± 0.93	7.6 ± 0.89	4.76 ± 0.34
Totalω9	7.91 ± 0.32	8.79 ± 0.4	7.99 ± 0.33	10.7 ± 0.43***	14.81 ± 1.93	25.58 ± 3.43	21.25 ± 1	26.93 ± 1.1***
Total monounsaturated	11.65 ± 0.42	12.92 ± 0.46	11.63 ± 0.34	14.74 ± 0.48***	22.75 ± 2.63	38.62 ± 2.72**	30.71 ± 1.1	36.53 ± 1.54***
C18:2ω6	0.8 ± 0.05	1.31 ± 0.11***	0.7 ± 0.02	2.24 ± 0.28***	0.98 ± 0.09	4.64 ± 1.17**	2.09 ± 0.31	5.57 ± 0.84**
C20:4ω6	4.5 ± 0.29	3.8 ± 0.35	3.26 ± 0.3	2.81 ± 0.2	4.29 ± 0.69	2.19 ± 0.93	0.83 ± 0.16	1.06 ± 0.22
Total ω6 PUFA	6.65 ± 0.35	6.81 ± 0.49	5.59 ± 0.29	7.02 ± 0.27***	7.79 ± 0.8	10.61 ± 1.35	4.34 ± 0.37	8.33 ± 0.67***
C18:3ω3	0.39 ± 0.02	0.52 ± 0.03**	0.26 ± 0.01	0.59 ± 0.08***	0.92 ± 0.1	3.09 ± 0.21***	1.42 ± 0.11	1.91 ± 0.26
C18:4ω3	0.44 ± 0.04	0.38 ± 0.06	0.46 ± 0.06	0.37 ± 0.06	0.82 ± 0.33	1.13 ± 0.47	2.53 ± 0.46	1.31 ± 0.15**
C20:5ω3	14.34 ± 0.43	15.27 ± 0.83	15.36 ± 0.63	14.95 ± 0.67	11.04 ± 0.78	10.04 ± 1.34	12.44 ± 0.81	11.34 ± 0.56
C22:5ω3	1.1 ± 0.03	1.58 ± 0.16**	0.85 ± 0.02	1.28 ± 0.06***	1.53 ± 0.22	1.43 ± 0.23	1.14 ± 0.05	1.54 ± 0.09***
C22:6ω3	42.71 ± 1.16	39.25 ± 1.2	42.15 ± 0.82	37.71 ± 0.76***	28.98 ± 2.36	11.82 ± 2.8**	23.21 ± 1.26	17.43 ± 1.87**
Total ω3 PUFA	59.16 ± 0.82	57.19 ± 0.62	59.2 ± 0.74	54.99 ± 0.77***	43.61 ± 2.27	28.17 ± 2.7**	40.98 ± 1.5	34.04 ± 1.53**
Total PUFA	65.81 ± 0.68	64.01 ± 0.66	64.79 ± 0.64	62.02 ± 0.67**	51.18 ± 5.2	38.78 ± 1.59**	45.32 ± 1.44	42.37 ± 1.18
ω3/ω6	9.49 ± 0.61	9.31 ± 0.73	11.36 ± 0.73	8.09 ± 0.43***	5.76 ± 0.42	2.95 ± 0.63**	10.05 ± 0.89	4.59 ± 0.75***

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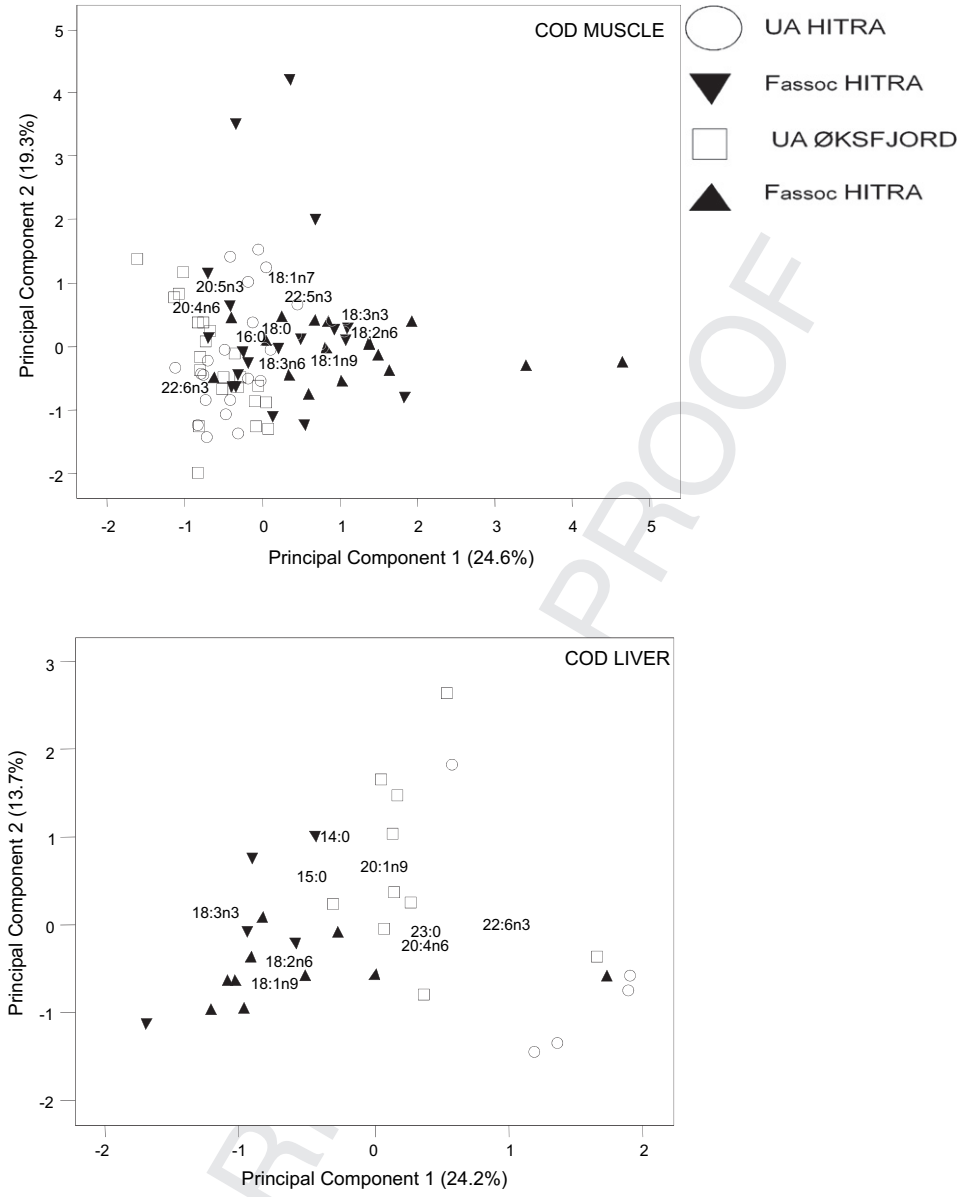

Fig. 3. Principal components analysis of the fatty acid profile of muscle and liver samples of farm-associated (filled symbols) or unassociated (empty symbols) Atlantic cod (*Gadus morhua*).

accurate in assigning individuals to F_{assoc} or UA origin based on the liver samples, correctly classifying 96.7% of individuals (29 out of 30).

Similar results were detected for saithe: FAs in F_{assoc} saithe were significantly modified away from those of UA fish. Higher levels of linoleic acid (18:2ω6), and a decreased ω3/ω6 ratio were detected in farm-associated saithe at both localities in both tissues (Table 4). Moreover, for saithe muscle, the total amount of ω6 FAs was significantly higher in F_{assoc} saithe compared to UA saithe.

Principal components analysis indicated that PC1 and PC2 explained 22.8% and 15.6%, respectively, of the total variability for saithe muscle and 24.0% and 18.6% for saithe liver (Fig. 4). Linolenic (18:3ω3), linoleic (18:2ω6) oleic (18:1ω9) and 22:5ω3 acids showed the highest correlation with PC1, while DHA presented a high negative correlation. Even though some overlapping between F_{assoc} and UA profiles existed, separation was greater than for cod muscle. For saithe liver, F_{assoc} profiles showed greater variability among individuals than those of UA fish. The highest positive correlations were reached by palmitic (16:0) and 24:1ω9, while the highest negative correlations occurred for linoleic (18:2ω6) and 22:5ω3 FAs. When LDA was applied to the total FA profile of saithe muscle, the analysis correctly assigned 85.7% of the individuals according to their F_{assoc} or UA origin (66 out of 77). For saithe liver, LDA correctly classified 96.7% of individuals to their F_{assoc} or UA origin (29 out of 30).

4. Discussion

Waste feed from coastal fish farms are shown to modify the diet of wild cod and saithe in their vicinity, which increases their condition relative to fish captured several km away from farms. In effect, the ready availability of waste feed, which is known to have a high protein and fat content (Hardy and Barrows, 2002; Fernandez-Jover et al., 2007), provides a trophic subsidy to these wild fish. Waste feed contained high levels of FAs of terrestrial origin and its consumption by wild fish led to detectable changes to the FA profiles of their muscle and liver tissues. Modification of the

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diet, condition and FA profiles of wild fish associated with sea-cage aquaculture appears to be a general effect for wild fish that feed heavily on the waste food pellets (Skog et al., 2003; Fernandez-Jover et al., 2007, 2009).

4.1. FAs as biomarkers of waste feed consumption by wild fish

Large aggregations of wild cod and saithe occur around fish farms (Dempster et al., 2009) and are resident for periods of weeks to months (Uglem et al., 2008, 2009). Aggregation by wild fish at fish farms is principally due to the increased presence of food (Tuya et al., 2006; Dempster et al., 2010b), and our results demonstrate that persistent consumption of the waste feed by wild fish alters the FA profile of their tissues. This change in cod and saithe diet directly influenced the FA profile of muscle and liver, which is the main fat storage organ of gadoids (Dos Santos et al., 1993). Specifically, increases in concentrations of linoleic (18:2ω6) and oleic (18:1ω9) acids and the ω3/ω6 ratio were detected, and decreases in concentrations of DHA (22:6ω3). Linoleic and oleic acid in particular were present in high concentrations in the formulated pellets. These FAs (linoleic, oleic and DHA) stand out as candidates for use as biomarkers of the influence of fish farms on wild fish.

Liver was the most sensitive tissue to FA modification and enabled correct classification into UA or F_{assoc} origin for 97% of cod and saithe individuals. Muscle was also a good indicator, enabling correct classification into UA or F_{assoc} origin for 89% of cod and 86% of saithe. The difference in classification accuracy between the two tissue types may be explained by differences in the time required for FA turnover in muscle and liver. Liver tissue, especially of gadoids, is rich in neutral lipids, which are more rapidly mobilized than the abundant polar lipids present in gadoid muscles (Dos Santos et al., 1993; Jobling, 2001; Sargent et al., 2002; Tocher, 2003; Jobling et al., 2008). Therefore, FA mobilization in the liver is a more rapid and dynamic process and is more likely to provide a snapshot of a recent diet than muscle tissue. In contrast, the lipid content of the muscle of gadoids is very low (~0.5%), with phospholipids being the major class (Dos Santos et al., 1993; Jobling et al., 2008). This indicates the structural role of fatty acids in this tissue, which generally presents a more conservative profile than the liver. This could be noted from the PCA plots (Figs. 3 and 4); the origin of the fish was clearly differentiated by analyzing cod liver, with DHA as the main differentiating FA. For saithe liver, the PCA plots showed a more conservative pattern, as shown by the lower plot dispersion of F_{assoc} saithe liver. In contrast, UA fish showed a more dispersed pattern, which may have been due to individual variability in natural diets.

Therefore, as turnover of the FA content in muscle tissues is more conservative than liver, changes in the muscle fatty acid profile may be a more informative tool than changes in the liver to investigate the influence of waste feed on wild fish over long time scales. However, since the liver composition of gadoids is greatly modified, and HSI values of F_{assoc} cod were 2–3 times those of UA cod, liver tissues could be useful in addressing potential impacts of fish farm diets on gadoid energy stores. Previous analyses of the FA profiles of farmed and wild cod livers using gas chromatography found clear differentiation (Standal et al., 2008) indicating that this procedure could also be applied to determine the influence of sea-cage aquaculture on the local ichthyofauna.

Tracking of dietary components through the food web cannot be entirely achieved using other methods such as examination of stomach contents, which are modified by digestion, or stable isotopes, which are useful in estimating the trophic level of a predator but cannot determine the species composition of the diet (Hobson, 1993; Gilmore et al., 1995; Koch et al., 1995). FAs could, therefore, be used as biomarkers in the study of the structure and dynamics of fish food webs around fish farms, as alternatives to direct or indirect methods that provide information of the most recent meal and may not be representative of the longer term diet (see Dalsgaard et al., 2003 for a review on FA trophic markers). Their qualitative use has inferred trophic levels and spatial and temporal differences in diets both within and among species (Kakela et al., 1993; Smith et al., 1996, 1997; Iverson et al., 1997a, b).

Table 4
Fatty acid composition (% of total fatty acids) of farm-associated (F_{assoc}) and farm-unassociated (UA) saithe (*Pollachius virens*). Data are expressed as mean ± standard deviation. Mann–Whitney significance level: ** 0.01, *** 0.001.

	Muscle				Liver			
	Hitra		Øksfjord		Hitra		Øksfjord	
	UA	F_{assoc}	UA	F_{assoc}	UA	F_{assoc}	UA	F_{assoc}
n	20	20	18	20	5	5	10	10
C16:0	17.18 ± 0.25	16.6 ± 0.54	15.65 ± 0.44	16.03 ± 0.51	0.58 ± 0.52	0.53 ± 0.63	0.48 ± 0.37	0.38 ± 0.75
C18:0	4.13 ± 0.1	4.09 ± 0.15	4.07 ± 0.16	4.21 ± 0.21	4.48 ± 0.23	3.56 ± 0.42	4.02 ± 0.13	4.13 ± 0.18
Total saturated	24.08 ± 0.36	24.03 ± 0.68	22.49 ± 0.61	23.37 ± 0.3	28.17 ± 0.76	25.9 ± 1.08	25.57 ± 0.62	23.83 ± 0.99
C16:1ω7	1.32 ± 0.09	1.81 ± 0.17	1.88 ± 0.16	2.14 ± 0.25	6.19 ± 0.39	5.26 ± 0.25	5.97 ± 0.34	5.75 ± 0.26
C18:1ω9	5.78 ± 0.32	8.24 ± 0.84**	7.31 ± 0.63	10.01 ± 1.05	11.03 ± 1.74	8.39 ± 2.06	16.24 ± 1.8	19.67 ± 1.24
C18:1ω7	2.41 ± 0.23	1.91 ± 0.14	2.3 ± 0.08	2.75 ± 0.13**	3.52 ± 0.26	2.28 ± 0.23	3.81 ± 0.12	4.2 ± 0.29
C20:1ω9	2.83 ± 0.43	3.29 ± 0.39	2.82 ± 0.35	2.34 ± 0.26	9.59 ± 2.11	16.19 ± 1.9	7.67 ± 1.05	6.85 ± 0.8
Total ω9	9.66 ± 0.42	12.51 ± 0.71***	11.15 ± 0.82	10.5 ± 0.53	22.07 ± 2.3	28.77 ± 2.14	25.2 ± 1.68	27.57 ± 0.73
Total monosaturated	13.62 ± 0.61	16.45 ± 0.99	15.51 ± 0.98	14.54 ± 0.62	32.06 ± 2.58	36.45 ± 1.88	35.28 ± 1.72	37.82 ± 0.79
C18:2ω6	0.95 ± 0.05	3.48 ± 0.55***	1.14 ± 0.2	2.91 ± 0.49**	1.61 ± 0.1	2.8 ± 0.58**	2.31 ± 0.7	4.79 ± 0.79**
C20:4ω6	1.34 ± 0.07	1.24 ± 0.07	1.13 ± 0.05	1.53 ± 0.09***	0.49 ± 0.05	0.27 ± 0.06	0.42 ± 0.03	0.59 ± 0.06
Total ω6 PUFA	3.95 ± 0.15	6.45 ± 0.51***	3.27 ± 0.24	5.5 ± 0.49***	3.34 ± 0.12	4.38 ± 0.72	3.77 ± 0.67	6.58 ± 0.87**
C18:3ω3	0.62 ± 0.05	1.06 ± 0.1***	0.87 ± 0.24	0.89 ± 0.11	1.68 ± 0.11	2.17 ± 0.19	1.63 ± 0.23	1.96 ± 0.16
C18:4ω3	0.81 ± 0.1	0.93 ± 0.11	0.83 ± 0.05	0.58 ± 0.05**	3.02 ± 0.32	5.17 ± 0.5	2.26 ± 0.18	1.89 ± 0.1
C20:3ω3	0.14 ± 0.02	0.11 ± 0.01	0.31 ± 0.18	0.1 ± 0.01	0.3 ± 0.02	0.3 ± 0.04	0.24 ± 0.01	0.19 ± 0.02
C20:5ω3	13.22 ± 0.29	13.54 ± 0.37	12.95 ± 0.58	12.51 ± 0.64	12.03 ± 1.01	10.54 ± 0.23	12.09 ± 0.76	11.69 ± 0.52
C22:5ω3	0.92 ± 0.05	1.24 ± 0.09***	0.9 ± 0.02	1.16 ± 0.1	0.77 ± 0.03	0.74 ± 0.05	0.76 ± 0.02	1.12 ± 0.08**
C22:6ω3	42.58 ± 0.86	36.15 ± 0.85***	42.81 ± 0.95	38.01 ± 2.18	18.59 ± 2.23	14.3 ± 0.39	18.34 ± 1.64	14.87 ± 1.18
Total ω3 PUFA	58.33 ± 0.66	53.05 ± 0.9***	58.71 ± 1.09	56.57 ± 1.12	36.41 ± 3.27	33.24 ± 0.73	35.36 ± 2.06	31.75 ± 1.11
Total PUFA	62.29 ± 0.62	59.5 ± 0.49**	61.99 ± 0.94	62.08 ± 0.67	39.75 ± 3.21	37.63 ± 0.97	39.13 ± 1.6	38.33 ± 1.25
ω3/ω6	15.22 ± 0.7	9.7 ± 1.08***	19.5 ± 1.28	12.78 ± 1.56***	11.03 ± 1.38	8.21 ± 0.97**	11.07 ± 1.25	5.98 ± 1**

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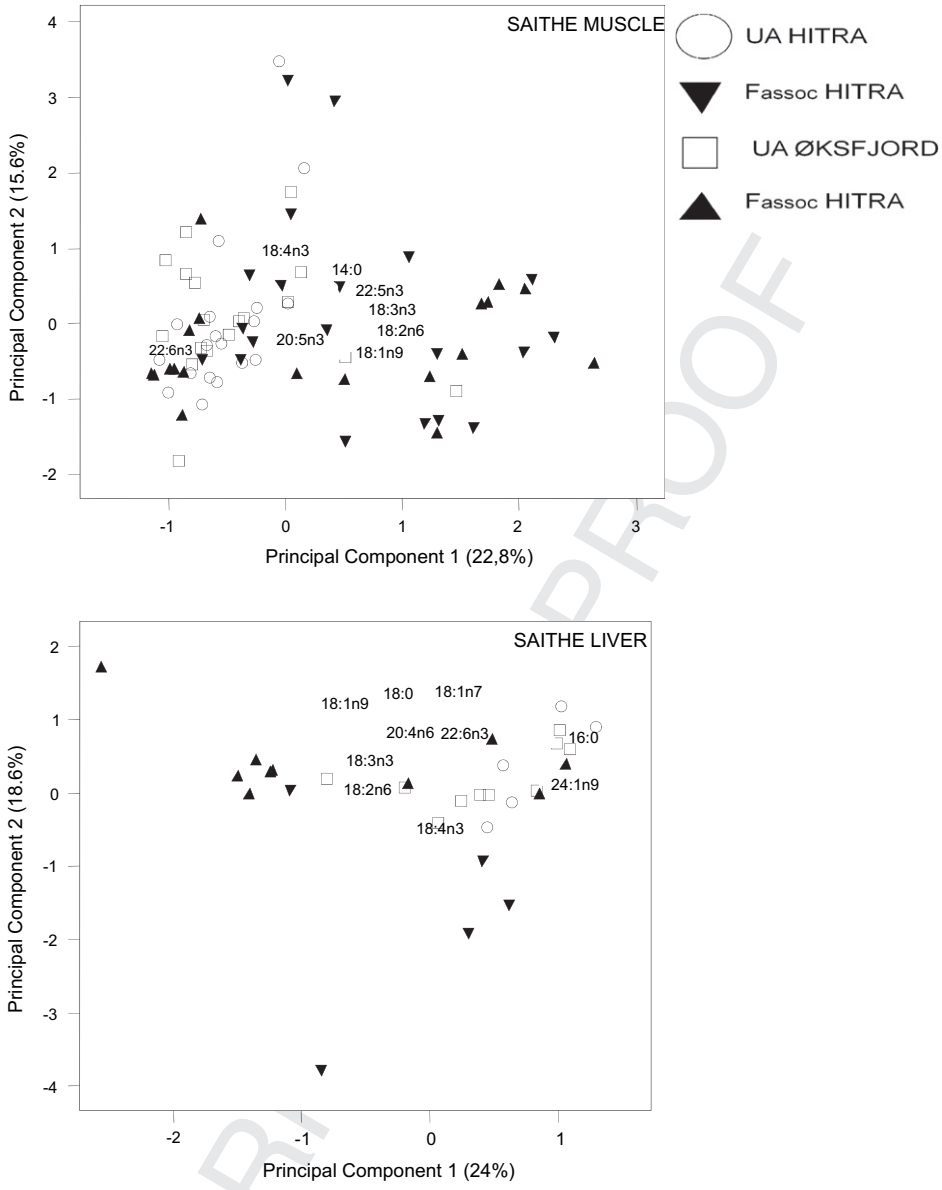

Fig. 4. Principal components analysis of the fatty acid profile of muscle and liver samples of farm-associated (filled symbols) or unassociated (empty symbols) saithe (*Pollachius virens*).

Further, the effect of the terrestrial-originated FAs on the entire food web (Fernandez-Jover et al., 2009; Olsen et al., 2009) requires further study as marine organisms are not typically exposed to such high levels of these FAs.

4.2. Waste feed as a trophic subsidy with compositional side-effects for wild fish

Traditional indicators of fish condition (FCI, HSI) indicate that cod and saithe around fish farms in our two study regions directly benefited from consuming the readily available waste feed. Similarly, in an extensive study comparing the diets of farm-associated and wild fish, Dempster et al. (in press) detected significantly elevated FCIs and HSIs for both saithe and cod associated with fish farms in three intensive fish farming regions in Norway. Waste feed thus provides a trophic subsidy to wild fish in coastal waters. Increased FCIs and HSIs translate to increased energy stores in wild fish, which is strongly correlated with the amount of energy fish are able to invest in spawning (Marshall et al., 1999), which may ultimately translate to spawning success (Izquierdo et al., 2001).

Cod and saithe are among the most abundant species aggregated around Norwegian fish farms (Dempster et al., 2009). They are attracted and concentrated from surrounding habitats and a high proportion of fish that aggregate are of adult size (Dempster et al., 2009). In addition, saithe have been shown to repeatedly visit and reside for months at multiple farms in regions with intensive aquaculture (Uglem et al., 2009), effectively using farms as an interconnected network of preferred feeding habitats. Therefore, the effects of waste feeds as a resource subsidy may be enhanced in areas with higher farming density.

Whether the side-effect of altered fatty acid composition of tissues that accompany increased fish condition has consequences for the physiological performance or spawning success of farm-associated wild fish remains unknown. Significantly increased levels of linoleic acid (18:2ω6) for both species and oleic acid for cod (18:1ω9) were found in the liver of F_{assoc} fish relative to UA fish. Oleic acid, in particular, is used by gadoids as an energy source

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(Jobling et al., 2008). However, if negative physiological or ecological effects override the likely positive effects on fish condition provided by the trophic subsidy, FAs from terrestrial origins may represent a new source of pollution for wild fish. Unfortunately, limited knowledge exists concerning the physiological effects of FA modification in wild fish over short or long temporal scales.

Marine fish require certain essential FAs, and their dietary requirements cannot be entirely fulfilled with oils from terrestrial origins. Alteration of the FA compositions of diets for cultivated fish is an active research field that aims at clarifying the limits of substitution of terrestrial-derived ω6 FAs for marine-derived ω3 FAs (e.g. Pickova and Mørkøre, 2007). Specifically, DHA (which was found significantly decreased in saithe muscle at Hitra and cod liver at both localities) is a key FA in neural tissues (brain or eyes) in marine fish since it can form up to 72% of its phosphoglycerides composition (Sargent et al., 1999, 2002; Tocher, 2003). We also found decreased levels of total ω3 PUFAs in cod liver and the ratio ω3/ω6 in cod liver and saithe muscle and liver. Polyunsaturated FAs are of particular importance for the formation of gametes. They are selectively transferred to the eggs and deficiencies in the amounts of these FA lead to reduced growth, egg quality, fecundity and larval survival (Sargent et al., 1999, 2002; Turchini et al., 2009).

However, evidence from studies related to the spawning of farmed cod within sea-cages suggests that the modified diets around fish farms and their alteration of wild fish composition is unlikely to change the ability of cod and saithe to spawn. Farmed cod fed farm diets their entire lives within sea-cages both mature and spawn viable eggs which hatch and contribute to the larval pool in fjord systems (Jørstad et al., 2008). These larvae have been documented to survive to young-of-the-year stage in the wild and contribute to the recruit pool in fjord populations of cod (van der Meeren and Jørstad, 2009). Thus, it is possible that the trophic subsidy provided to wild fish in the vicinity of farms could translate to enhanced spawning success. Research to document the relative value of natural diets compared to farm modified diets to spawning success and timing must be clarified to determine the ecological role of waste feed as a trophic subsidy.

Up to 170 species of wild fish have been documented to associate with fish farms as adults or juveniles worldwide (Sanchez-Jerez et al., in press). Wild fish populations at aquaculture sites are subject to several anthropogenic impacts, including fishing (Akyol and Ertosluk, 2010) or aquaculture-originated contaminants (DeBruyn et al., 2006; Bustnes et al., in press). If the alteration of FA profiles of farm-associated wild fish diminishes their performance, they may be subject to additional synergistic effects with the other anthropogenic impacts. Further research into the potential effects on wild fish caused by aggregation at fish farms, modified dietary intake and altered fatty acid compositions should target the mechanisms driving the changes we have observed.

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