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Response of tissue lipids to diet variation in Atlantic salmon (*Salmo salar*): Implications for estimating diets with fatty acid analysis

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ABSTRACT

The influence of feeding duration and tissue type on fatty acid (FA) deposition in Atlantic salmon (*Salmo salar*) tissues was investigated to establish the appropriate foundation for quantitative estimates of diet in wild fish. Fish were adapted to seawater and were fed one of four formulated diets based on krill or herring oil for 22 weeks. After 12 weeks of feeding, proportions of most FA in both belly flap and muscle tissues of salmon had changed substantially to resemble dietary FA proportions, showing little variation after an additional 10 weeks of feeding. However, tissues showed a differential response to diets, depending on the proportions of dietary FA. When present in large proportions in the diet, FA, including 18:2n-6 and monounsaturated FA, seemed to be selectively catabolized; when present in low proportions, selective retention in tissues occurred. Proportions of the polyunsaturated FA, 20:5n-3 and 22:6n-3, in tissues also varied with diet but not in a predictable manner related to dietary FA proportions. Further, the response to a change in the FA profile of diet was not equal for both tissues with belly flap tissue developing a FA signature that was more similar to the diet than muscle tissue. These results suggest that fish may represent a more complex study species than marine mammals for quantitative estimation of diet using FA.

1. Introduction

Understanding predator–prey relationships is essential in the implementation of ecosystem-based approaches to fisheries management and relies on accurate estimates of predator diets. Traditional methods of diet estimation involving the examination of stomach contents and hard parts in fish and mammals have some well-known limitations (Cortés, 1997; Hyslop, 1980) and this has led to the development of alternative techniques, including the use of biomarker-based approaches (e.g., Gannes et al., 1997; Iverson et al., 2004). Analysis of fatty acids (FA) in predator tissues has become a popular choice to investigate diet. The use of dietary FA is appropriate because there is little alteration in FA structure as they are incorporated into animal tissues (Tocher, 2003), making them useful as biomarkers (Dalsgaard et al., 2003; Iverson, 2009; Kirsch et al., 1998).

FA are most commonly used in a qualitative manner to examine general differences in predator diets on a variety of spatial or temporal scales, without identifying specific prey items. This type of analysis has been applied to a range of predators at all trophic levels including, for example, zooplankton (Auel and Hagen, 2005; Graeve et al., 1994), fish (Budge et al., 2002; Petursdottir et al., 2008) and marine mammals (Cooper et al., 2009; Thiemann et al., 2007). Recently, Iverson et al. (2004) have described a more complex method that attempts to estimate the proportions of individual prey species in a predator's diet. This method, coined 'Quantitative FA Signature Analysis' (QFASA; Iverson, 2009; Iverson et al., 2004), uses predator and prey FA proportions and prey weights relative to fat proportions to estimate the proportions of prey species in the diet of a predator. A distance minimizing technique is used to compare the predator's FA profile with combinations of prey FA profiles that have been modified to compensate for lipid metabolism in the predator; full details of the mathematical procedure are given in Iverson et al. (2004). QFASA has been applied to a number of different predators in a variety of environments, including seals, seabirds, and polar bears (Beck et al., 2007; Iverson et al., 2007; Meynier et al., 2010; Thiemann et al., 2008; Wang et al., 2010); however, its application in fish has not yet been investigated.

An important step in QFASA is the modification of the predator's FA profile before analysis to compensate for any changes in FA proportions brought about by metabolism (Iverson, 2009; Iverson et al., 2004). While FA structures generally experience little change during deposition or mobilization in fish (Tocher, 2003), their proportions

Abbreviations: FA, fatty acid; QFASA, quantitative fatty acid signature analysis; HO, herring oil; KO, krill oil; FAME, fatty acid methyl ester; MANOVA, multivariate analysis of variance; ANOVA, analysis of variance; ANOSIM, analysis of similarity; MDS, multidimensional scaling; TAG, triacylglycerol; PL, phospholipid.

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are usually modified. For instance, both catabolism and *de novo* synthesis of FA may be responsible for variations in tissue signatures; minor modifications in dietary FA structure through elongation and desaturation can also alter the ingested FA profile. These subtle changes result in a predator FA profile that is different than that of its prey (reviewed in Budge et al., 2006), and such variation must be accounted for. This is normally done through controlled feeding studies, where a predator is fed a single diet until its tissue FA profile resembles that of its diet as closely as possible (e.g., Iverson et al., 2004; Nordstrom et al., 2008; Thiemann et al., 2008; Wang et al., 2010). Predator metabolism can then be taken into account by calculating a "calibration coefficient" for each FA; this is the ratio of the proportion of the FA in the predator relative to the proportion in its diet. Determining the necessary duration of such feeding studies is critical.

Aquaculture literature offers some insight into this problem. For instance, several studies (e.g., Bell et al., 2003; Jobling, 2003; Torstensen et al., 2004) suggest that a 'wash-out' period for durations ranging from 14 to 25 weeks is necessary to establish a new dietary FA signal in salmon tissues. However, these studies conducted with formulated feeds invariably involve an initial feeding of diets based on a vegetable oil with FA profiles bearing little resemblance to typical marine diets. The period required to accomplish such a large change in signature is likely to be far greater than that necessary when switching from one marine diet to another marine diet of relatively similar FA composition. Further, there is evidence to suggest that gene expression involved in lipid metabolism is influenced by the FA composition of diet, particularly when vegetable oil-based diets are fed (Jordal et al., 2005; Stubhaug et al., 2005); conclusions drawn from aquaculture data may not apply in ecological studies. Thus, new feeding studies are necessary using diets with FA profiles typical of marine sources to determine the period of time required for a fish tissue to resemble its new diet after a switch in dietary sources.

QFASA requires that FA be sampled from a tissue that functions as a fat storage depot so that the FA signature is more likely to reflect diet. This usually means that tissues consisting primarily of triacylglycerols (TAG) are targeted. All studies of QFASA to date have been conducted with animals that have such tissues, i.e., adipose or blubber that has a primary function of fat storage (e.g., Beck et al., 2007; Iverson et al., 2004; Thiemann et al., 2008; Wang et al., 2010). Most fish species do not have such dedicated tissues and store excess fat in their muscles, viscera or liver. For instance, lean fish such as Atlantic haddock (*Melanogrammus aeglefinus*) and Atlantic cod (*Gadus morhua*) primarily store dietary fat in the liver (Nanton et al., 2003). In salmonids, the muscle and belly flap are the major fat storage sites (Jobling et al., 2002; Nanton et al., 2007) so are the most appropriate tissues to sample for QFASA.

The purpose of this study was to perform the necessary preliminary work prior to applying QFASA to Atlantic salmon, highlighting the differences between its new application in fish with its more typical use with marine mammals and birds. Specifically, the objectives were: 1) to determine the period of time required for the FA profile of belly flap and muscle tissues to closely resemble that of a new diet; and 2) to compare the dietary FA signatures in belly flap and muscle tissues.

2. Materials and methods

2.1. Experimental animals and diets

Farmed juvenile Atlantic salmon (n = 260), originally collected from the Saint John River in NB, Canada, were held in freshwater aquaria (1300 L) and gradually acclimated to seawater by mixing freshwater and seawater to achieve the full salinity (31 ppt) in about two weeks prior to the start of this experiment. Each fish was identified by inserting a Passive Integrated Transponder (PIT) tag near a dorsal fin. The salmon were raised under a photoperiod of 12 L:12D and were housed in 16 tanks (110 L each) which were arranged randomly in two layers in a "U" configuration, with four tanks per diet. The tanks operated on a filtered flow-through system where temperature followed natural variation and ranged from 7 to 11 °C.

For 12 months prior to the beginning of the experiment, fish were fed a commercial salmonid feed produced by Corey Feeds Ltd. (Fredericton, Canada) with a lipid proportion of 24% dry weight (DW). At week 0, diets were switched to one of four formulated feeds based on either herring oil (HO) or krill oil (KO) and designed to contain 14% lipid wet weight (WW) or 16% DW. Fully refined KO was supplied by Aker BioMarine (Oslo, Norway) and was composed entirely of triacylglycerols (TAG). All other components of the diets were identical (Table 1), with nutrient concentrations following recommendations of the National Research Council (1993). Two of the four diets were based on only one oil source (100% HO or KO); the other two were mixtures of the oils at ratios of 30:70 and 70:30 HO:KO to establish a gradient of HO in the diets. These four diets were mixed using a Hobart mixer (Model H600T, Rapids Machinery Co., Troy, OH) and steam-pelleted into 5 mm pellets using a pellet mill (California Pellet Mill Co., San Francisco, CA). The pellets were then dried in a forced-air drier at 80 °C for 90 min, and stored at -40 °C in airtight containers until fed.

All fish were fed twice per day until apparent satiation and were sampled at weeks 0, 12 and 22. The fish were euthanized using an overdose of the anesthetic MS-222 and lengths (snout to tail tip) and weights were recorded. Condition factor ($K = 100 \times W/L^3$) was also calculated. Whole fish were immediately frozen at −40 °C for < one week before analysis.

2.2. Lipid extraction, transesterification and FA analysis

The entire belly flap and lateral and dorsal muscular tissues of each salmon were rapidly homogenized in a blender, weighed into 1.5 g portions and immediately immersed in 2:1 chloroform:methanol. Samples of each diet were crushed using a glass mortar and pestle and percent moisture (WW) was determined. Percent lipid was determined gravimetrically on a WW basis. Lipids were then extracted using chloroform

Table 1
Formulations of Atlantic salmon experimental diets. KO — formulated diet with krill oil as the lipid source; HO — formulated diet with herring oil as the lipid source; 30:70 HO:KO — formulated diet incorporating a 30:70 mixture of herring oil:krill oil; 70:30 HO:KO — formulated diet incorporating a 70:30 mixture of herring oil:krill oil.

Ingredient	KO (g/kg)	30:70 HO:KO (g/kg)	70:30 HO:KO (g/kg)	HO (g/kg)
Herring meal	420	420	420	420
Corn gluten meal	80	80	80	80
Soybean meal	120	120	120	120
Wheat middlings	102	102	102	102
Dried whey	70	70	70	70
Pre-gelatinized starch	40	40	40	40
Vitamin premix[a]	17	17	17	17
Mineral premix[b]	10	10	10	10
Herring oil	0	42	98	140
Krill oil	140	98	42	0
Choline chloride	1	1	1	1

[a] Vitamin added to supply the following (per kg diet): vitamin A (retinol acetate), 8000 IU; vitamin D_3 (cholecalciferol), 4500 IU; vitamin E (all-race-α-tocopheryl acetate), 300 IU; vitamin K_3 (menadione sodium bisulfite), 40 mg; vitamin B_1 (thiamin HCl), 50 mg; vitamin B_2 (riboflavin), 70 mg; D-calcium pantothenate, 200 mg; biotin, 1.5 mg; folic acid, 20 mg; vitamin B_{12}, 0.15 mg; niacin, 300 mg; pyridoxine HCl, 20 mg; vitamin C (ascorbic acid, stay C), 300 mg; inositol, 400 mg; butylated hydroxytoluene (BHT) 15 mg; butylated hydroxyanisole (BHA), 15 mg.

[b] Minerals added to supply the following (per kg diet): manganese ($MnSO_4 \cdot H_2O$, 32.5% Mn), 40 mg; iron ($FeSO_4 \cdot H_2O \cdot 7H_20$, 20.1% Fe), 50 mg; copper ($CuSO_4 \cdot 7H_2O$, 25.4% Cu), 10 mg; zinc ($ZnSO_4 \cdot 7H_2O$, 22.7% Zn), 75 mg; cobalt ($CoCl_2 \cdot 6H_2O$, 24.8% Co), 5 mg; selenium (Na_2SeO_3, 45.7% Se), 1 mg; fluoride (NaF, 45.3% F), 4 mg.

and methanol, following a modified Folch method (Folch et al., 1957). Lipids were transesterified to yield FA methyl esters (FAME), using an acidic methanol solution (see Budge et al., 2006 for details). FAME were analyzed using a Perkin-Elmer Autosystem II capillary gas chromatograph (GC), with a flame ionization detector (FID). A flexible fused silica column (30×0.25 mm ID) coated with 50% cyanopropyl polysiloxane (0.25 μm film thickness; J&W DB-23, Agilent Technologies, Folsom, California) was used for separation and helium was employed as the carrier gas. Operating conditions and temperature ramps are described in Budge et al. (2006). Co-elution of pairs of specific isomers, 18:1n-11 and 18:1n-9, and 20:1n-11 and 20:1n-9, occurred as the GC column aged over time. For consistency, each pair was considered a single FA and was reported as the sum of both isomers. FA were quantified as percent by weight of total FA identified. The entire suite of FA in a sample, reported in this manner as percent of total FA, was referred to as the FA composition or profile.

2.3. Statistical analyses

Multivariate analysis of variance (MANOVA) and Tukey post-hoc tests were used to examine variation in length, weight and lipid percentage in tissues at weeks 0, 12 and 22, using the SPSS program for Windows (Version 11.0.1, SPSS, Inc.). We considered each tank, rather than individual fish, to be the experimental unit, yielding n = 4 for each diet treatment. Before analysis, FA<0.1% were removed from the data set and the remaining 52 FA were renormalized to 100% and transformed using an arcsine square root function. Pearson correlations were calculated to assess temporal variation in FA profiles of fish tissues using the 15 most abundant FA, shown in Figs. 2–4, representing FA present at proportions >0.5%. Analysis of variance (ANOVA) was used to determine temporal variation in individual FA using an adjusted α of 0.003 (0.05/15). Analysis of similarity (ANOSIM) and non-metric multi-dimensional scaling (MDS) with Bray–Curtis similarity matrices were used to examine variation in FA signatures with tissue.

3. Results

3.1. Diet composition

FA compositions of the experimental diets varied substantially (Table 2, Fig. 1). For instance, proportions of 18:1n-11 + 9 and 18:2n-6 were much greater in the initial diet than in the formulated diets (18:1n-11 + 9 at ~30% in initial vs. ~8–15% in the formulated diets; 18:2n-6 at ~14% in initial vs. ~5% in the formulated diets) while long chain monounsaturates (20:1n-11 + 9, 22:1n-11) dominated the profiles of the HO diet. There was also a trend of increasing 20:5n-3 and 22:6n-3 as the proportion of HO increased in the diet, reflecting the greater amounts of these FA in HO. The KO diet contained higher proportions of saturates (~37% vs. ~19% in HO) and intermediate proportions of the long chain monounsaturates 20:1n-11 +9 and 22:1n-11 compared to the other diets. These variations in FA composition resulted in distinct groupings in the MDS plot (Fig. 1).

3.2. Growth and tissue lipid composition

Lengths and weights increased significantly for all fish over the course of the 22 week study for all diets (MANOVA; $p<0.001$; Table 3) with body weight increasing to ~4–5× that of initial weight. There was also a significant increase (MANOVA; $p<0.05$) in percent lipid of belly flap tissue for all groups and salmon fed the KO diet showed the greatest increase in belly flap% lipid (from ~11% initial to 17% final). Salmon fed diets incorporating large portions of HO showed smaller increases (from ~11% initial to 13 and 15% for fish fed HO and 70:30 HO:KO, respectively). Percent lipid in muscle showed a slight but significant increase ($p<0.001$) over the course of the experiment. After the initial sampling, condition factor was constant for all dietary treatments and coincided with the increase in percent lipid in the tissues.

Table 2
Proportions of major FA (weight% of total FA identified; mean ± SD; n = 3) of diets fed to Atlantic salmon. Initial — commercial diet; KO — formulated diet with krill oil as the lipid source; HO — formulated diet with herring oil as the lipid source; 30:70 HO:KO — formulated diet incorporating a 30:70 mixture of herring oil:krill oil; 70:30 HO:KO — formulated diet incorporating a 70:30 mixture of herring oil:krill oil.

	Initial	KO	30:70 HO:KO	70:30 HO:KO	HO
Saturated					
14:0	2.9 ± 0.0	15.6 ± 0.1	12.5 ± 0.0	7.8 ± 0.0	5.2 ± 0.0
16:0	17.4 ± 0.0	19.2 ± 0.0	17.1 ± 0.0	14.0 ± 0.0	12.3 ± 0.0
18:0	4.2 ± 0.0	1.7 ± 0.0	1.6 ± 0.0	1.3 ± 0.0	1.2 ± 0.0
Subtotal	24.5 ± 0.0	36.6 ± 0.1	31.2 ± 0.1	23.2 ± 0.0	18.7 ± 0.0
Monounsaturated					
16:1n-7	7.0 ± 0.0	10.0 ± 0.0	8.8 ± 0.0	7.1 ± 0.0	6.2 ± 0.0
18:1n-11 + 9	29.4 ± 0.1	14.5 ± 0.0	12.5 ± 0.0	9.6 ± 0.0	7.9 ± 0.0
18:1n-7	2.5 ± 0.0	5.9 ± 0.0	4.8 ± 0.0	3.3 ± 0.0	2.4 ± 0.0
20:1n-11 + 9	3.0 ± 0.0	4.0 ± 0.1	6.8 ± 0.0	10.8 ± 0.0	13.3 ± 0.0
20:1n-7	0.2 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.6 ± 0.0	0.7 ± 0.0
22:1n-11	1.8 ± 0.0	4.5 ± 0.1	9.6 ± 0.1	16.9 ± 0.0	21.2 ± 0.1
22:1n-9	0.2 ± 0.0	0.6 ± 0.0	0.9 ± 0.0	1.2 ± 0.0	1.5 ± 0.0
Subtotal	44.1 ± 0.0	39.8 ± 0.1	44.0 ± 0.1	49.6 ± 0.1	53.2 ± 0.2
Polyunsaturated					
16:2n-4	0.4 ± 0.0	0.7 ± 0.0	0.6 ± 0.0	0.5 ± 0.0	0.5 ± 0.0
16:4n-1	0.6 ± 0.0	0.9 ± 0.0	0.8 ± 0.0	0.6 ± 0.0	0.5 ± 0.0
18:2n-6	13.7 ± 0.0	4.9 ± 0.0	4.8 ± 0.0	4.7 ± 0.0	4.4 ± 0.0
18:3n-3	1.2 ± 0.0	0.8 ± 0.0	0.9 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
18:4n-3	0.7 ± 0.0	1.9 ± 0.0	1.8 ± 0.0	1.6 ± 0.0	1.5 ± 0.0
20:4n-6	0.5 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
20:5n-3	5.0 ± 0.0	4.3 ± 0.0	4.9 ± 0.0	5.7 ± 0.0	6.1 ± 0.1
22:5n-3	1.1 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.6 ± 0.0	0.7 ± 0.0
22:6n-3	3.6 ± 0.0	3.9 ± 0.0	4.7 ± 0.0	6.1 ± 0.1	6.9 ± 0.1
Subtotal	26.2 ± 0.0	17.8 ± 0.0	18.9 ± 0.1	20.9 ± 0.1	21.6 ± 0.2
Total	94.9 ± 0.1	94.2 ± 0.1	94.1 ± 0.1	93.8 ± 0.1	93.6 ± 0.0

3.3. Temporal variation in FA composition

FA profiles of tissues were compared by sampling weeks and, in all cases, correlations were greatest between weeks 12 and 22 (Table 4), indicating that tissue FA compositions were similar to that of the new

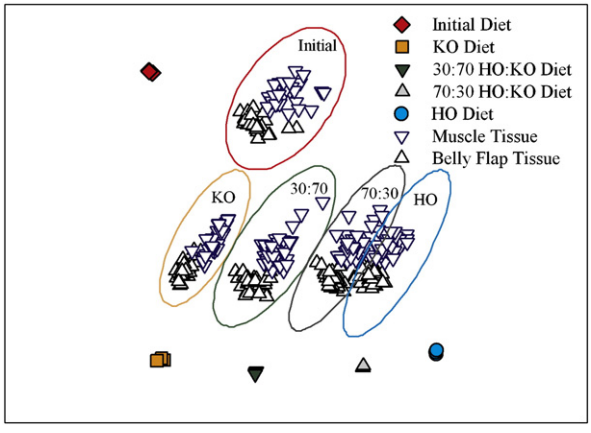

Fig. 1. MDS plot of FA in diets, belly flap tissue (BF) and muscle tissue (M) of Atlantic salmon. Ellipses surround tissues of fish fed the diet indicated within the ellipse. Fish fed the initial diet were sampled at week 0; all other fish were sampled at week 22. Initial — commercial diet; KO — formulated diet with krill oil as the lipid source; HO — formulated diet with herring oil as the lipid source; 30:70 HO:KO — formulated diet incorporating a 30:70 mixture of herring oil:krill oil; 70:30 HO:KO — formulated diet incorporating a 70:30 mixture of herring oil:krill oil.

Table 3
Condition factor (K), length, weight and percent lipid (mean ± SD; n = 4) of Atlantic salmon at each sampling event. Lipid was determined gravimetrically as a percentage of wet weight. Initial — commercial diet; KO — formulated diet with krill oil as the lipid source; HO — formulated diet with herring oil as the lipid source; 30:70 HO:KO — formulated diet incorporating a 30:70 mixture of herring oil:krill oil; 70:30 HO:KO — formulated diet incorporating a 70:30 mixture of herring oil:krill oil.

Week	Diet	K	Length (cm)	Weight (g)	Lipid content	
					Belly flap (% WW)	Muscle (% WW)
0	–	0.9 ± 0.2	20 ± 1	76 ± 17	10.6 ± 5.3	2.6 ± 0.7
12	KO	1.3 ± 0.3	25 ± 2	196 ± 39	14.3 ± 5.4	4.0 ± 1.5
22	KO	1.2 ± 0.4	30 ± 3	312 ± 97	17.3 ± 5.3	3.7 ± 1.0
12	30:70 HO:KO	1.3 ± 0.4	26 ± 2	218 ± 59	15.5 ± 7.1	3.6 ± 0.7
22	30:70 HO:KO	1.2 ± 0.3	31 ± 2	355 ± 79	17.1 ± 6.8	3.8 ± 1.1
12	70:30 HO:KO	1.2 ± 0.2	27 ± 1	237 ± 32	18.9 ± 6.6	3.2 ± 0.9
22	70:30 HO:KO	1.2 ± 0.3	31 ± 3	353 ± 96	14.8 ± 4.7	3.3 ± 0.7
12	HO	1.3 ± 0.2	26 ± 2	218 ± 38	16.2 ± 5.2	3.1 ± 0.6
22	HO	1.2 ± 0.3	30 ± 3	334 ± 71	13.0 ± 3.2	3.4 ± 0.9

diet fed within 12 weeks of the diet switch, and differed little after that point. This temporal variation in FA composition within a tissue and dietary treatment was also obvious in the bar graphs (Figs. 2 and 3). In general, FA proportions varied according to the diet fed, with patterns in results for fish fed KO and HO diets representative of all four diets (Figs. 2 and 3). Proportions of most FA did not change significantly after week 12 in both tissues, reaching a value intermediate between proportions in the initial and formulated diets (Figs. 2 and 3). However, proportions of several FA, such as 20:5n-3 and 22:6n-3, reached plateaus at week 12 that did not mirror the change in dietary FA proportion. Finally, a few FA (e.g., 18:1n-11 + 9 and 18:2n-6) were present in very different proportions in the initial and prepared diets, and did not reach constant proportions in the fish tissues by the end of the experiment (ANOVA: $p<0.003$).

3.4. Variation with tissue type

ANOSIM showed a significant variation in FA composition of belly flap and muscle tissue within a diet treatment and sampling period (e.g., Fig. 4; full data not shown). However, while FA signatures indicated that belly flaps and muscles were discrete, individual FA proportions varied subtly between these tissues, with obvious differences in only a few FA (e.g., 14:0, 16:1n-7, 18:1n-11 + 9, 20:5n-3 and 22:6n-3). The MDS plot of diets and tissues also showed that belly flap and muscle from fish fed the same diet were in distinct groups yet also in close proximity to each other (Fig. 1). Certainly, diet had a greater impact than tissue on the final FA proportions in fish. On the basis of individual FA, belly flaps had FA proportions more similar to the diet fed (Fig. 4). The proximity of belly flap tissue of salmon to the diets fed in the MDS plot (Fig. 1) also indicated that belly flaps had a FA distribution more similar to the diet fed than did the muscle tissue.

Table 4
Correlations between FA profiles at different sampling weeks in fish tissue of Atlantic salmon. FA used in correlations are shown in Figs. 2 and 3. Pearson correlation coefficients (r) demonstrate similarity in FA profiles of weeks compared. KO — formulated diet with krill oil as the lipid source; HO — formulated diet with herring oil as the lipid source; 30:70 HO:KO — formulated diet incorporating a 30:70 mixture of herring oil:krill oil; 70:30 HO:KO — formulated diet incorporating a 70:30 mixture of herring oil:krill oil.

Diet	Weeks compared	Correlation coefficient	
		Tissue	
		Belly flap	Muscle
KO	0, 12	0.90	0.90
	0, 22	0.84	0.86
	12, 22	0.99	0.99
30:70 HO:KO	0, 12	0.89	0.90
	0, 22	0.86	0.86
	12, 22	0.99	0.99
70:30 HO:KO	0, 12	0.83	0.83
	0, 22	0.77	0.78
	12, 22	0.99	0.99
HO	0, 12	0.76	0.76
	0, 22	0.65	0.70
	12, 22	0.99	0.99

4. Discussion

4.1. Variation in growth rates and lipid composition with diet

Although all fish increased in length and weight over the course of the experiment, there was notable variation among groups. For instance, salmon fed KO had significantly lower (MANOVA, $p<0.005$) weight at week 12 than all other groups and had significantly greater (MANOVA, $p<0.001$) muscle percent lipid. Also, fish fed diets high in HO (both 70:30 HO:KO and HO) showed an unusual drop in belly flap percent lipid from week 12 to week 22. These results are atypical for dietary treatments that are equivalent in all respects except the FA composition of the oil. It appears that dietary FA composition had a subtle effect on growth and lipid deposition, in addition to FA composition.

4.2. Temporal variation

Over the course of the 22 week study, proportions of most FA approached, but did not achieve proportions found in the formulated diets (Figs. 2 and 3). Likewise, initial proportions of FA did not match that of the initial diet, despite feeding for ~1 year prior to the start of the experiment. Similar results have been reported with other species, including pinnipeds, birds and fish (e.g., Arts et al., 2010; Iverson et al., 2004; Lane et al., 2006; Wang et al., 2010), further supporting the conclusion that a predator's tissues do not acquire a FA composition that is identical to that of its diet due to metabolism of dietary lipids. However, after a sufficiently long period of time on a constant diet, one expects a steady state to be reached, where the tissue FA signature is no longer changing to resemble the diet. Most FA seemed to reach, or approach, this steady state (Figs. 2 and 3; Table 4). Of the entire profile, only 18:1n-11 + 9 and 18:2n-6 often did not approach a clear plateau. These FA were present in much larger proportions in the initial diet than in any formulated diet and were at proportions of ~24–26% and ~9–10%, respectively, in the fish tissues at week 0. It seems that it is more difficult to replace such large proportions of a tissue FA with a lower dietary proportion of a FA, suggesting that longer feeding periods may be necessary for FA signatures to stabilize when differences in dietary FA profiles are relatively large. A similar problem has been reported with salmon fed terrestrial oil-based diets, followed by a finishing diet based on marine fish oil (Bell et al., 2003; Jobling et al., 2002), where the terrestrial oil-based diets were relatively high in 18:1n-9 and 18:2n-6, while the finishing diet based on marine fish oil contained ~4–8× less of those FA on a percent basis. As a result, even after 14–20 weeks of feeding the finishing diet, proportions of both FA remained high in the salmon tissues, relative to the marine oil-based diet.

To explain the patterns of lipid deposition, competing hypotheses of either dilution or turnover can be considered. Jobling (2003) has described turnover as a change in a predator's FA composition after the addition of a new diet, due to mobilization and oxidation of existing FA in the tissue and deposition of FA from the new diet. Dilution, however, occurs as the signal of the original FA in tissues becomes diluted as greater amounts of FA obtained from the new diet are deposited (Jobling, 2003). Dilution does not assume that the original FA are removed from the tissue. Instead, the original FA contribute proportionally less to the overall FA profile as the animal grows and deposits

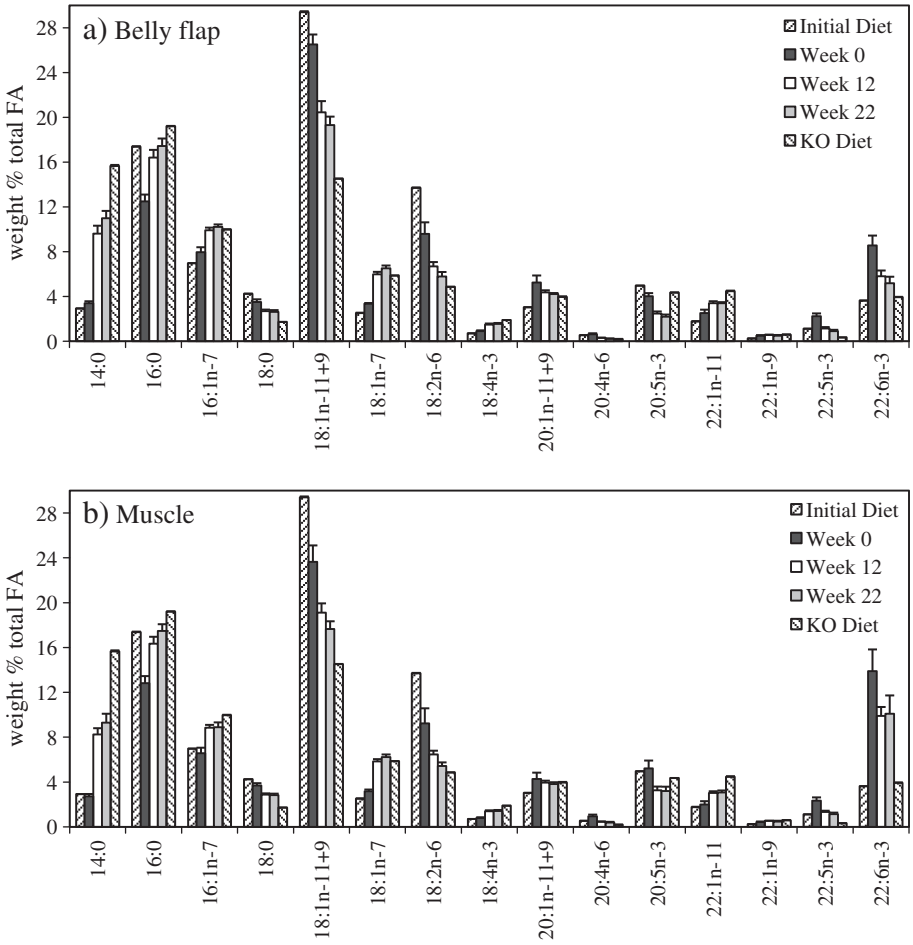

Fig. 2. FA proportions (mean ± SD) in a) belly flap and b) muscle tissues of Atlantic salmon fed the krill oil-based (KO) diet at weeks 0, 12 and 22. FA proportions in the initial commercial diet and KO diets are also shown for comparison.

more FA from the new diet. There is evidence to support both theories in the current study.

With dilution, an increase in the proportions of dietary FA would be relatively easy to record in a fish tissue as it would represent an addition to amounts already present. A major decrease in the proportion of a dietary FA, such as 18:2n-6, would, however, require substantial growth of the fish and deposition in the tissue of a large amount of fat with a lower proportion of 18:2n-6 to literally dilute the original signal. In our data, the failure of 18:1n-11 + 9 and 18:2n-6 to reach a clear plateau in proportions by week 22 supports the dilution theory; the experiments were not of a sufficient duration to allow the fish to deposit enough lipid with lower proportions of those two FA to fully modify the tissue FA composition. Such dilution models have been shown to generally apply in a number of different cultured fish species, including Atlantic salmon (Jobling, 2003; Lane et al., 2006; Robin et al., 2003).

Alternatively, the differential response of fish tissue to the diets also reinforces the importance of lipid metabolism in modifying tissue FA composition. For instance, at week 0, in the belly flaps of fish fed the initial diet, 18:2n-6 was ~4% less than its proportion in that diet. In contrast, the same FA was ~2% greater than its proportion in the KO diet by week 22 (Fig. 2a). Since 18:2n-6 is only found at ~5% in the KO diet, it is unlikely that the results simply reflect a failure to reach a steady state between diet and tissue FA and that proportions of 18:2n-6 would continue to drop to ~1%, to mirror the difference found between the initial diet and tissue, if the experiment had continued. Instead, selective oxidation of dietary lipids is almost certainly occurring, particularly when the dietary FA are present in large proportions. FA present in relatively larger proportions in fish diets are more readily catabolized than those present in smaller proportions. A number of studies have reported such results for high proportions of 18:1n-9, 18:2n-6, 18:3n-3 and 22:1n-11 in fish diets (Bell et al., 2003; Olsen and Henderson, 1997; Stubhaug et al., 2007). In this study, at 14% in the initial diet, 18:2n-6 was among the FA present in greatest proportions and it was likely experiencing substantial oxidation before deposition in tissues. In the KO diet, other FA, such as 14:0 and 16:1n-7, were major FA, leaving 18:2n-6 less important as a substrate for oxidation and allowing more to be deposited. While this logic does not apply to all major FA, similar patterns can be found in 14:0, 16:0 and 16:1n-7 in the KO diet (Fig. 2), and 20:1n-11 + 9 and 22:1n-11 in the HO diet (Fig. 3). *De novo* synthesis of FA or chain elongation and desaturation of dietary precursors may also modify the FA profile, although such processes are unlikely to occur to a large extent in fish fed such high fat diets, rich in polyunsaturated FA (Likimani and Wilson, 1982; Tocher, 2003; Turchini et al., 2009). In the wild, where fish may undergo regular periods of fasting associated with spawning, selective mobilization and oxidation of major FA from tissues for energy and reproduction may also influence the FA patterns acquired by tissue. Certainly, a combination of both dilution and turnover had occurred to yield the FA profile found in the fish tissues. This mixture of processes in fish separates them from other species, such as seals, polar bears and birds, whose diets have been estimated by QFASA (Iverson et al., 2004; Thiemann et al., 2008; Wang et al., 2010); in those animals, growth ceases when maturity is reached, so modification of the FA profile through dilution is not possible and only turnover due to lipid metabolism can occur.

Regardless of the mechanisms responsible for tissue FA composition, two FA showed unexpected patterns; 20:5n-3 and 22:6n-3

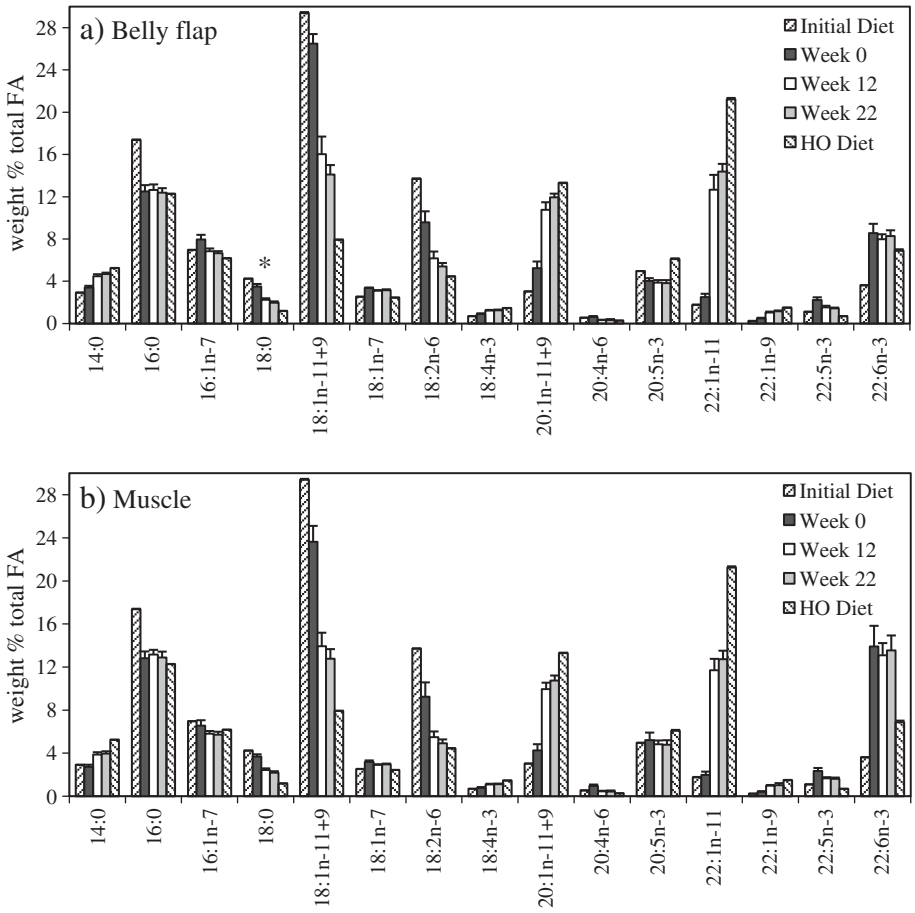

Fig. 3. FA proportions (mean ± SD) in a) belly flap and b) muscle tissues of Atlantic salmon fed the herring oil-based (HO) diet at weeks 0, 12 and 22. FA proportions in the initial commercial diet and HO diet are also shown for comparison.

reached a steady state by week 12, but the change in proportion was independent of dietary FA composition. For instance, in belly flap and muscle tissue of fish fed the KO diet (Fig. 2a and b), from week 0 to 22, the decrease in tissue proportions of 20:5n-3 and 22:6n-3 of ~2 and ~4%, respectively, was much greater than the subtle difference in proportions of those FA in the initial and KO diet. Similarly, in fish fed the HO diet (Fig. 3a and b), 22:6n-3 did not respond to the change in diet, despite very different proportions of that FA in the initial and HO diets. Similar results have been found for 22:6n-3 in Atlantic salmon fed vegetable oil-based diets (Bell et al., 2003; Torstensen et al., 2004). Both 20:5n-3 and 22:6n-3 are essential FA in fish, with 20:5n-3 a required precursor for vital eicosanoids and 22:6n-3 important in neural tissues (reviewed in Tocher, 2003). Thus, their proportions may be more strictly controlled by physiological processes and less influenced by diet. Tocher (2003) also suggested that 22:6n-3 may be selectively retained in tissues, while saturated and monounsaturated FA are catabolised for energy. In contrast, a similar study in which juvenile salmon were fed fish oil-based diets, rather than the typical vegetable oil-based feeds used in aquaculture studies, found conflicting results (Arts et al., 2010). That work reported increases in 20:5n-3 and 22:6n-3 in muscle tissue that followed the increases in dietary concentrations of those FA. Controlled feeding studies with pinnipeds and birds have also failed to show the unusual patterns in 20:5n-3 and 22:6n-3 observed here (Iverson et al., 2004, 2007; Nordstrom et al., 2008; Wang et al., 2010). Because 20:5n-3 and 22:6n-3 can only be acquired from diet, they have been considered important in examining trophic relationships (e.g., Iverson et al., 2004) and have been included in all QFASA applications in marine mammals and birds (i.e., Beck et al., 2007; Meynier et al., 2010; Thiemann et al., 2008; Wang et al., 2010). However, they were clearly not following the expected deposition patterns in this study and should only be used with caution when investigating diets in Atlantic salmon.

All results (Table 4; Figs. 1, 2 and 3) suggest that FA proportions in both tissue types resembled their novel diets within 22 weeks, with only slight changes occurring in most FA in the final 10 weeks of the study. The period required for tissue FA profiles to reflect a dietary change will, however, be influenced by the amount of lipid in the diet, with diets with higher percent lipid leading to more rapid reflection of dietary FA in tissues. An obvious concern, therefore, was the applicability of our results to wild salmon consuming natural diets with lower percent lipid. Our formulated diets were typical of those available for use in aquaculture with lipid proportions of ~14% WW and a moisture content of ~9%. On a DW basis, this represents ~16% lipid. Diets of wild adult salmon are usually composed of shrimp, sandlance and herring (Shearer, 1992). Although seasonal variation occurs, one would expect lipid proportions of ~3, 6 and 8% WW, respectively, in those fish species (Budge et al., 2002). However, when converted to a DW basis, assuming 80% moisture in these fish, percent lipid increases to ~15, 30 and 40%, respectively. Thus, the percent lipid DW of the experimental diets was actually similar or less than what might be encountered in the wild, indicating that wild fish tissue lipids likely reflect the FA composition of diets consumed within a 12–22 week period.

4.3. Variation in tissue FA

Belly flaps and muscles of the salmon were readily distinguishable at all sampling periods by comparison of FA profiles (e.g., Figs. 1 and 4), agreeing with results from a number of other studies (e.g., Jobling et al., 2002; Nanton et al., 2001; Torstensen et al., 2000).

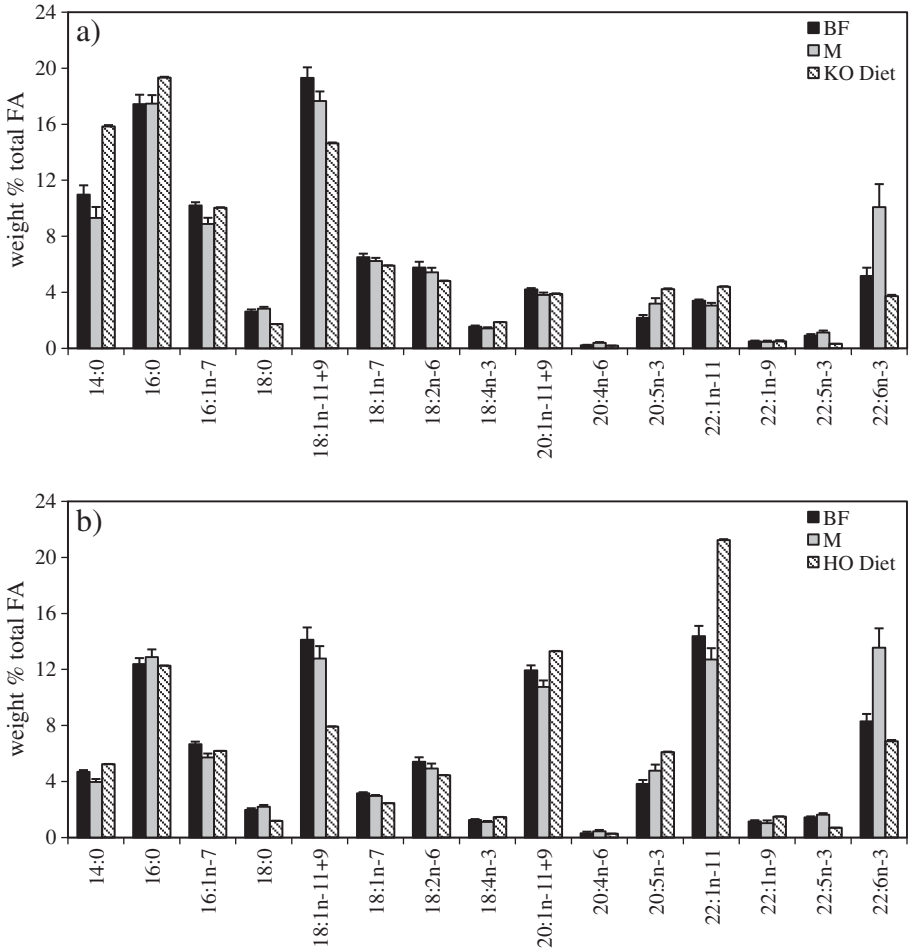

Fig. 4. FA proportions (mean ± SD) in belly flap tissue, muscle tissue and diets at week 22 in Atlantic salmon fed a) krill oil-based (KO) diet and b) herring oil-based (HO) diet. Differences in FA profiles between tissues of fish fed a single diet were significant (ANOSIM; $R = 0.90$, $p < 0.05$, and $R = 0.93$, $p < 0.03$, for the KO and HO diets, respectively).

These tissues have differing cellular compositions and physiological roles so different biochemical compositions are expected. The belly flap is the major lipid depot in Atlantic salmon, containing predominantly adipocytes, or fat cells (Nanton et al., 2007). Numbers of adipocytes decrease sharply from the belly flap to the muscle tissue in salmon (Zhou et al., 1996). Greater amounts of adipocytes in the belly flaps result in greater amounts of total lipid and higher proportions of TAG (> 94%) in that tissue than in the muscles (< 75%), while higher proportions of phospholipids (PL) are found in the muscles (~20%) than the belly flaps (2–3%; Nanton et al., 2007). TAG are primarily used for energy storage, while PL serve as essential components of structural membranes. As such, TAG have been found to be less selective in the dietary FA that they contain, while PL seem to preferentially incorporate certain FA (Trushenski et al., 2008). Thus, one would anticipate the greater similarity between dietary FA and FA in TAG-rich belly flaps than FA in PL-rich muscle tissue observed here.

In applications for diet estimation with QFASA, a key aspect is to identify and sample the tissue that most readily reflects dietary FA. In mammals and marine birds, discreet fat depots, high in TAG, have been sampled; in pinnipeds, blubber has been used, while subcutaneous adipose tissue was sampled in birds and polar bears (Iverson, 2009). In Atlantic salmon, the belly flap is the analogous tissue and is clearly a better choice than muscle. However, there is an interesting trade-off involved. Assuming that dilution does have an influence on FA tissue composition, the more lipid that is present in a tissue, the longer the time that will be required for a new dietary signal to develop. The belly flap does have a high proportion of TAG but it also contains a large amount of lipid in general, relative to other tissues, indicating that although TAG are likely to be more easily replaced, a large amount of dilution must take place in this tissue. Thus, a better choice may be to sample a tissue with a lower amount of TAG so dilution is relatively faster and the tissue's FA will more rapidly respond to a change in diet. A particularly useful strategy in this respect may be to examine the FA composition of only isolated TAG (i.e., Benedito-Palos et al., 2010; Trushenski et al., 2008). FA in TAG from muscle may have a profile more representative of diet than that of FA from total lipids, where PL FA would be making a substantial contribution to the signal. This may also be a useful approach in fish without prominent belly flaps, such as cod or other gadoids.

4.4. Recommendations for application of QFASA

Calculation of accurate calibration coefficients is a critical requirement for application of QFASA. We have demonstrated that after 12 weeks of feeding diets with new FA compositions, fish tissues begin to strongly reflect the FA profiles of the new diet; 22 weeks was sufficient to reach a steady state for most FA in salmon belly flap and muscle tissue and is, therefore, sufficient duration for feeding studies in Atlantic salmon to establish calibration coefficients for QFASA applications. On a DW basis, percent lipid was similar in the experimental diets and prey of wild salmon, suggesting that tissues of wild salmon likely reflect diet over the past 12–22 week period. Belly flaps more closely resembled the dietary FA composition and therefore better reflect diet than muscle tissue. Based on our results, we recommend the sampling of this tissue which is high in

adipocytes, and likely TAG; however, future work with salmonids should consider analyzing FA within muscle TAG that may more rapidly respond to a change in dietary FA profile. Ontogentic and reproductive effects on tissue FA profiles were not investigated and are difficult to predict based on the current study. Until further study has been completed, it would be prudent to limit QFASA analyses to juvenile salmon.

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