Estimating diets of Atlantic salmon (*Salmo salar*) using fatty acid signature analyses; validation with controlled feeding studies

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Abstract: Diets incorporating homogeneous binary mixtures of herring or krill oil were fed to Atlantic salmon (*Salmo salar*) for 22 weeks, and belly flap and muscle tissues were then analyzed for fatty acid (FA) composition. Quantitative fatty acid signature analysis (QFASA) was able to estimate proportions of major dietary components within ~10% of actual values, but the accuracy of the estimates depended on the FA set and calibration coefficients (CC) used in the modelling. FAs present at low levels had little influence on estimates, despite having only dietary sources; the FA set used in the modelling must incorporate the major FAs in tissues to ensure accurate estimates of diet. CC, which reflect modifications that consumers make to dietary FAs, were similar in the two tissues but varied with diet. When CC were applied to correct for fish metabolism, QFASA tended to overestimate the dietary component that had been fed to determine the CC. Diet estimates were most accurate when CC that had been developed from feeding the krill oil-based diet were applied. This first application of QFASA to fish therefore establishes a set of FAs and CC to begin to investigate diets of salmonids.

Résumé : Des saumons atlantiques (*Salmo salar*) ont reçu des régimes comprenant des mélanges binaires homogènes de hareng ou d'huile de krill pendant 22 semaines, après quoi les filets abdominaux et les tissues musculaires ont été analysés pour en déterminer la composition d'acides gras (FA). L'analyse quantitative de la signature des acides gras (QFASA) a permis d'estimer les proportions des principales composantes du régime avec une exactitude de ± ~10 % par rapport aux valeurs réelles, bien que cette exactitude dépende de l'ensemble de FA et des coefficients d'étalonnage (CC) utilisés dans la modélisation. Les FAs présents en faibles concentrations avaient peu d'incidence sur les estimations, même s'ils étaient exclusivement de sources alimentaires. Pour obtenir des estimations exactes du régime alimentaire, l'ensemble de FA utilisé dans la modélisation doit comprendre les principaux FAs présents dans les tissus. Les CC, qui reflètent les modifications apportées par les consommateurs aux FAs présents dans le régime, étaient semblables pour les deux types de tissus, mais variaient selon le régime. Quand des CC étaient appliqués pour tenir compte du métabolisme des poissons, la QFASA avait tendance à surestimer la composante du régime donnée aux poissons pour déterminer les CC. Les estimations les plus exactes du régime alimentaire ont été obtenues dans les cas où des CC établis à partir du régime à base d'huile de krill ont été appliqués. Cette première application de la QFASA aux poissons établit donc un ensemble de FAs et des CC permettant d'étudier les régimes alimentaires des salmonidés.

[Traduit par la Rédaction]

Introduction

Accurate estimates of predator diets are essential in understanding predator–prey relationships and have commonly involved examination of stomach contents and hard parts in fish and mammals. These techniques have some well-known limitations in fish (Hyslop 1980; Cortés 1997) that have led to the development of alternative methods, including the use of biomarker-based approaches (e.g., Petursdottir et al. 2008; McMahon et al. 2010; Piché et al. 2010). Analyses of fatty acid (FA) profiles of consumer tissues are being increasingly used to investigate predator diets. FAs are useful as biomarkers because they are transferred conservatively through food webs, with little modification of structure (Dalsgaard et al. 2003; Iverson 2009).

FA profiles may be used in a qualitative manner to investigate temporal or spatial variation in predators foraging at a variety of trophic levels, without identifying specific prey types (e.g., Wang et al. 2009; Czesny et al. 2011). Alternatively, a more complex method has been proposed that attempts to estimate proportions of prey species in predator diets by comparing FA profiles of prey and predator tissues. This model, quantitative fatty acid signature analysis (QFASA; Iverson et al. 2004), uses a distance minimizing technique to compare the FA profiles of the predator with combinations of FA signatures of possible prey, taking into

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account modifications in FA proportions due to lipid metabolism in the predator. This technique has been applied to a number of different predators in a variety of ecosystems, including seals, seabirds, and polar bears (Thiemann et al. 2008; Meynier et al. 2010; Wang et al. 2010), but its application in fish has not yet been investigated.

FAs are typically deposited in consumer tissues in different proportions compared with diet because of a number of biochemical processes, including selective mobilization, catabolism through β-oxidation, and de novo synthesis; compensating for these changes in proportions is a critical aspect of QFASA (Iverson et al. 2004; Williams and Buck 2010). These differences are normally assessed with controlled feeding studies where the consumer is fed a known diet for a sufficiently long period of time so that its tissue FA profile reaches a constant state relative to the FA profile of the diet. "Calibration coefficients" (CC) can then be determined for each FA by calculating the ratio of the amount of the FA in the consumer tissue relative to that of its diet or prey (CC = percent FA in consumer/percent FA in prey). Before comparing a predator's FA signature with potential prey signatures using QFASA, each FA in the consumer tissue is divided by the appropriate CC to modify its levels and take into account the effects of consumer lipid metabolism. All previous applications of QFASA have focused on marine mammals and birds and have assumed that FA metabolism, and therefore CC, are specific to the consumer, such that the consumer consistently modifies dietary FA in the same manner, regardless of their proportions (Iverson et al. 2004; Nordstrom et al. 2008; Wang et al. 2010). However, a number of aquaculture studies have shown that FA present in large proportions in fish diets are more readily catabolized than those present in smaller proportions (Olsen and Henderson 1997; Bell et al. 2003; Stubhaug et al. 2007). Thus, in fish, CC may vary with the FA composition of the prey species, and the impact of that variation on QFASA diet estimates is unknown.

The accuracy of diet estimates produced by QFASA may also be influenced by the FA set used in the modelling and the consumer tissue sampled (Iverson et al. 2004; Nordstrom et al. 2008; Meynier et al. 2010). Different FAs can provide varying degrees of information about diet. For instance, a number of FAs are derived solely from the diet, such as those with n-6 or n-3 double bonds, while others arise from biosynthesis. Similarly, certain tissues may better reflect dietary FA profiles. Iverson et al. (2004) suggested sampling metabolically active fat storage depots for QFASA applications; blubber, subcutaneous fat, and synsacral fat have been used in studies with seals (Tucker et al. 2009), polar bears (Thiemann et al. 2008), and birds (Wang et al. 2010). In salmonids, the belly flap and muscle are the major fat storage sites (Tocher 2003; Nanton et al. 2007) and so are the most appropriate tissues to sample.

The purpose of this study was to investigate the application of QFASA, originally developed to estimate diets in marine mammals with large fat depots, to fatty fish (Atlantic salmon, *Salmo salar*). Salmon were selected because they deposit fat in both belly flap and muscle tissues and can serve as a model species for other fatty fish. In this study, salmon were fed diets with differing FA compositions for 22 weeks to investigate the variation in CC with FA profiles of the diet. Known mixtures of those diets were also fed to salmon, and QFASA was used to estimate proportions of diets consumed. The specific objectives were to evaluate the influence of the following parameters on the accuracy of QFASA estimates: (*i*) tissue; (*ii*) FA set; and (*iii*) CC set.

Materials and methods

Fish and experimental diets

Tank-reared juvenile Atlantic salmon (n = 161) were adapted to seawater by gradual exposure to brine 2 weeks prior to the start of the experiment. The fish were housed in 16 tanks (110 L each) operating on a filtered flow-through system maintained at a temperature of approximately 9 °C under a photoperiod of 12 h light : 12 h dark. The tanks were arranged in two levels in a "U" configuration, with four tanks per diet. To control for possible tank effects, no tanks with the same diet treatment were adjacent to each other.

For 12 months prior to the beginning of the experiment, fish were fed a commercial salmonid feed produced by Corey Feeds Ltd. (Fredericton, New Brunswick, Canada), consisting of >46% protein, >24% lipid, and <2% fibre on a dry mass basis and referred to as the initial diet. 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 mass or 16% dry mass. All other components of the diets were identical (Table 1), with nutrient levels 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 HO:KO and 70:30 HO:KO, arbitrarily chosen to establish a gradient of HO in the diets. These four diets were mixed using a Hobart mixer (Model H600T, Rapids Machinery Co., Troy, Ohio) and steam-pelleted into 5.0 mm pellets using a pellet mill (California Pellet Mill Co., San Francisco, California). The pellets were then dried in a forced-air drier at 80 °C for 90 min and stored frozen in airtight containers until use.

All fish were fed twice per day until apparent satiation. Fish were sampled at weeks 0 and 22 to ensure that sufficient turnover had occurred in fish tissue lipids (Budge et al. 2011). The fish were euthanized using an overdose of the anaesthetic tricaine methanesulfonate (MS-222). Whole fish were immediately frozen at –40 °C for less than 1 week before analysis.

Lipid extraction, transesterification, and FA analysis

Belly flap and muscle tissue (lateral and dorsal muscles combined) of each salmon were homogenized separately in a blender. Samples of each pelleted diet were crushed using a glass mortar and pestle, and moisture content was determined. Lipids were extracted following a modified Folch method (Folch et al. 1957) using chloroform and methanol. FA methyl esters (FAME) were synthesized by transesterification 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. A flexible fused silica column (30 mm × 0.25 mm internal diameter) coated with 50% cyanopropyl polysiloxane (0.25 μm film thickness; J&W DB-23,

Table 1. Formulations of Atlantic salmon experimental diets.

Ingredient	KO (g·kg^{-1})	30:70 HO:KO (g·kg^{-1})	70:30 HO:KO (g·kg^{-1})	HO (g·kg^{-1})
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
Pregelatinized starch	40	40	40	40
Vitamin premix[a]	17	17	17	17
Mineral premix[a]	10	10	10	10
HO	**0**	**42**	**98**	**140**
KO	**140**	**98**	**42**	**0**
Choline chloride	1	1	1	1

Note: Herring oil (HO) and krill oil (KO) are noted in bold, as they were the only ingredients that occurred in different amounts in each diet.

[a]See Budge et al. (2011) for details of vitamin and mineral premixes.

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). FAME were identified by comparison of retention times with those of mixed FAME standards (Nu-Chek Prep, Elysian, Minnesota). 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. All FA with mean values <0.10% were removed from the data set. Several other FAs, such as *i*-16:0 and 22:4n-6, that were not consistently identified were also removed, leaving 46 FAs in total.

FA sets and CC calculation

Four FA sets were used: (*i*) a full FA set of all FAs identified at levels >0.1% of total, including 46 FAs and comprising 100% of total FAs in both tissues; (*ii*) a dietary set including 21 FAs that can only be acquired from diet (i.e., FAs that cannot be synthesized by the fish), comprising ~39% and 43% of total FAs in belly flap and muscle tissues, respectively; (*iii*) an extended dietary set, consisting of the dietary set plus 8 FAs that may have both a dietary and biosynthetic origin, comprising ~97% of total FAs in both tissues; and (*iv*) a reduced set consisting of 14 FAs present in at least one diet at proportions >1% of total FAs, comprising approximately 92% of total FAs in both tissues. FAs in the full set are reported in Tables 2 and 3; FAs in the other three sets are indicated within the tables.

Three CC sets were derived from tissues of salmon fed the initial, HO, and KO diets. They were calculated separately for each tissue and diet type by dividing the percentage of an individual FA in the salmon tissue by the percentage of the same FA in the diet (Iverson et al. 2004). The mean of those three created a fourth CC set (Iverson et al. 2004). A fifth calibration scenario was also considered when tissues were modelled without application of CC (i.e., none). A nomenclature incorporating subscripts for diet and, as necessary, tissue (i.e., $CC_{diet,tissue}$) was adopted to specify the conditions under which a particular CC was derived.

QFASA modelling

The ability of the model to distinguish diets without considering the effects of predator metabolism was first evaluated by challenging the model with calculated diet mixtures, referred to as "pseudopredators" in Iverson et al. (2004). Test mixtures were created to mimic the proportions of diets actually fed by summing individual FA percentages derived from 70% of each FA in the HO diet and 30% of each FA in the KO diet. A 30:70 HO:KO mixture was also calculated. CC were not applied because diets had not been consumed, so there was no need to correct for effects of predator metabolism. Eight trials were conducted using the four FA sets and the two mixtures, with only initial, HO, and KO diets entered in the model as possible diet items (Table 4). The Kulback–Liebler (KL) distance was used to assess the similarity of the actual and estimated FA profiles. This distance is defined as

$$\mathrm{KL} = \sum_j (y_j - \hat{y}_j) \log (\hat{y}_j / y_j)$$

where y_j and $\hat{y}_j$ represent the proportions of the *j*th FA in the consumer and estimated diet, respectively. The combination of diet types generating a FA composition with the smallest KL distance was considered to be the estimated diet. Full details of the QFASA modelling procedures can be found in Iverson et al. (2004).

FA signatures of tissues of salmon fed the 70:30 and 30:70 HO:KO diets were then modelled to investigate the effects of different modelling scenarios on the accuracy of QFASA diet estimates. Belly flap and muscle tissues were modelled using only three FA sets (full, extended, and reduced; see Results section for explanation), and all CC sets with initial, HO, and KO diets as possible diet items with CC applied to the appropriate tissues (percent FA in consumer/CC = modified consumer FA profile for comparison with the three diet items).

Statistical analysis

Analysis of similarity (ANOSIM) of Bray–Curtis matrices was used to evaluate similarity of FA profiles among diets and tissues (Primer 6, Version 6.1.6, PRIMER-E, Ivybridge, United Kingdom, http://www.primer-e.com/). FA data were transformed using an arcsine square root function. FA most responsible for differences were identified using the similarity percentages routine (SIMPER).

Table 2. Fish mass (mean (standard deviation, SD)), tissue lipid percentage (% wet mass; mean (SD)), and fatty acid (FA) composition (% total FA; mean (SD)) of belly flaps of Atlantic salmon reared on five diets: Initial — commercial feed; HO — formulated diet with herring oil as the lipid source; KO — formulated diet with krill oil as the lipid source; 70:30 — formulated diet incorporating a 70:30 ratio of HO to KO; 30:70 — formulated diet incorporating a 30:70 ratio of HO to KO.

	Diet				
	Initial (n = 29)	HO (n = 36)	KO (n = 28)	70:30 (n = 34)	30:70 (n = 34)
Mass (g)	76 (17)	334 (71)	312 (97)	353 (96)	355 (79)
Tissue lipid (%)	10.6 (5.3)	13.0 (3.2)	17.3 (5.3)	14.8 (4.7)	17.1 (6.8)
Saturates					
14:0[e,r]	3.44 (0.17)	4.73 (0.13)	11.07 (0.67)	6.23 (0.25)	8.98 (0.43)
i-15:0	0.10 (0.01)	0.18 (0.01)	0.24 (0.01)	0.19 (0.01)	0.21 (0.01)
ai-15:0	0.04 (0.00)	0.07 (0.01)	0.10 (0.01)	0.08 (0.00)	0.09 (0.01)
15:0	0.20 (0.01)	0.34 (0.01)	0.38 (0.02)	0.35 (0.01)	0.36 (0.01)
16:0[e,r]	12.67 (0.61)	12.49 (0.43)	17.60 (0.67)	14.09 (0.32)	16.1 (0.38)
i-17:0	0.03 (0.00)	0.05 (0.00)	0.03 (0.00)	0.05 (0.00)	0.04 (0.00)
ai-17:0	0.08 (0.01)	0.05 (0.01)	0.06 (0.00)	0.06 (0.01)	0.06 (0.01)
17:0[e]	0.19 (0.01)	0.18 (0.04)	0.11 (0.01)	0.30 (0.02)	0.24 (0.18)
18:0[e,r]	3.55 (0.23)	1.99 (0.12)	2.65 (0.15)	2.14 (0.12)	2.51 (0.14)
20:0					
0.11 (0.01)	0.14 (0.01)	0.11 (0.01)	0.14 (0.01)	0.12 (0.01)	
Monounsaturates					
14:1n-5					
0.19 (0.01)	0.09 (0.01)	0.20 (0.01)	0.11 (0.01)	0.16 (0.01)	
16:1n-11	0.24 (0.01)	0.36 (0.02)	0.47 (0.02)	0.38 (0.01)	0.43 (0.02)
16:1n-9	0.39 (0.02)	0.23 (0.02)	0.26 (0.02)	0.24 (0.02)	0.25 (0.02)
16:1n-7[e,r]	8.07 (0.44)	6.72 (0.18)	10.30 (0.22)	7.47 (0.18)	9.02 (0.18)
16:1n-5	0.13 (0.01)	0.25 (0.01)	0.37 (0.02)	0.27 (0.01)	0.32 (0.01)
Me-16:1a[a]	0.09 (0.00)	0.12 (0.02)	0.18 (0.01)	0.10 (0.00)	0.14 (0.03)
Me-16:1b[a]	0.11 (0.01)	0.12 (0.03)	0.07 (0.01)	0.14 (0.01)	0.09 (0.02)
17:1	0.21 (0.01)	0.25 (0.03)	0.29 (0.01)	0.24 (0.01)	0.28 (0.03)
18:1n-11+9[e,r]	26.87 (0.91)	14.24 (0.89)	19.48 (0.76)	15.47 (0.90)	18.12 (0.99)
18:1n-7[e,r]	3.40 (0.06)	3.19 (0.08)	6.57 (0.25)	3.90 (0.06)	5.41 (0.13)
18:1n-5	0.27 (0.01)	0.45 (0.01)	0.30 (0.01)	0.41 (0.01)	0.34 (0.01)
20:1n-11+9[d,e,r]	5.31 (0.63)	12.04 (0.36)	4.25 (0.09)	10.03 (0.35)	6.76 (0.20)
20:1n-7[d,e]	0.40 (0.05)	0.67 (0.03)	0.52 (0.02)	0.59 (0.02)	0.57 (0.03)
22:1n-11[d,e,r]	2.54 (0.31)	14.5 (0.74)	3.42 (0.09)	11.62 (0.64)	7.00 (0.35)
22:1n-9[d,e,r]	0.49 (0.06)	1.16 (0.09)	0.50 (0.06)	1.07 (0.05)	0.71 (0.10)
22:1n-7[d,e]	0.07 (0.01)	0.23 (0.01)	0.13 (0.02)	0.20 (0.02)	0.15 (0.02)
24:1	0.35 (0.04)	0.66 (0.06)	0.37 (0.04)	0.55 (0.04)	0.47 (0.06)
Polyunsaturates					
16:2n-4[d,e]	0.47 (0.03)	0.45 (0.02)	0.64 (0.02)	0.47 (0.02)	0.56 (0.02)
16:3n-4[d,e]	0.37 (0.03)	0.25 (0.02)	0.16 (0.02)	0.23 (0.02)	0.20 (0.02)
16:4n-1[d,e]	0.40 (0.04)	0.31 (0.02)	0.46 (0.03)	0.34 (0.02)	0.40 (0.02)
18:2Δ5,11	0.15 (0.01)	0.05 (0.01)	0.13 (0.01)	0.05 (0.01)	0.10 (0.01)
18:2n-7	0.09 (0.01)	0.05 (0.01)	0.09 (0.01)	0.06 (0.01)	0.08 (0.01)
18:2n-6[d,e,r]	9.71 (1.06)	5.45 (0.32)	5.83 (0.41)	5.71 (0.34)	5.73 (0.29)
18:2n-4[d,e]	0.20 (0.01)	0.13 (0.01)	0.15 (0.01)	0.13 (0.01)	0.14 (0.01)
18:3n-6[d,e]	0.31 (0.04)	0.18 (0.03)	0.26 (0.02)	0.21 (0.02)	0.22 (0.02)
18:3n-3[d,e]	0.81 (0.09)	0.95 (0.04)	0.76 (0.03)	0.87 (0.02)	0.82 (0.03)
18:4n-3[d,e,r]	0.91 (0.07)	1.26 (0.06)	1.56 (0.08)	1.29 (0.06)	1.40 (0.10)
18:4n-1[d,e]	0.31 (0.03)	0.31 (0.05)	0.45 (0.04)	0.26 (0.02)	0.38 (0.05)
20:4n-6[d,e]	0.63 (0.08)	0.31 (0.11)	0.22 (0.03)	0.27 (0.03)	0.23 (0.02)

Table 2 (*concluded*).

	Diet				
	Initial (n = 29)	HO (n = 36)	KO (n = 28)	70:30 (n = 34)	30:70 (n = 34)
20:3n-3[d,e]	0.07 (0.01)	0.11 (0.01)	0.07 (0.01)	0.25 (0.08)	0.10 (0.02)
20:4n-3[d,e]	0.48 (0.02)	0.60 (0.04)	0.56 (0.05)	0.57 (0.04)	0.59 (0.06)
20:5n-3[d,e,r]	4.08 (0.27)	3.86 (0.28)	2.20 (0.20)	3.44 (0.31)	2.66 (0.20)
21:5n-3[d,e]	0.36 (0.02)	0.28 (0.02)	0.20 (0.01)	0.27 (0.01)	0.23 (0.01)
22:5n-6[d,e]	0.18 (0.02)	0.14 (0.02)	0.08 (0.01)	0.10 (0.01)	0.09 (0.01)
22:5n-3[e,r]	2.26 (0.25)	1.45 (0.07)	0.91 (0.11)	1.32 (0.11)	1.08 (0.09)
22:6n-3[d,e,r]	8.66 (0.90)	8.36 (0.54)	5.22 (0.59)	7.70 (0.50)	6.04 (0.39)
Full	100.00 (0.00)	100.00 (0.00)	100.00 (0.00)	100.00 (0.00)	100.00 (0.00)
Dietary	36.79 (1.11)	51.57 (1.05)	27.64 (0.98)	45.63 (0.88)	35.00 (0.85)
Extended	97.23 (0.06)	96.55 (0.11)	96.35 (0.11)	96.57 (0.08)	96.46 (0.13)
Reduced	91.95 (0.20)	91.44 (0.15)	91.58 (0.18)	91.49 (0.18)	91.52 (0.23)

Note: All fish were fed the initial diet for 12 months prior to the beginning of the experiment; 29 of these were collected at week 0 as representative of fish fed the initial diet. The remainder were then switched to one of the four formulated diets and fed for a further 22 weeks. Superscript letters represent the following: a, methyl branch was present at an unknown position; d, dietary FA set consisting of FAs that fish can only acquire from diet; e, extended FA set consisting of the dietary set plus eight FAs that may have both a dietary and biosynthetic origin; r, reduced FA set consisting of 14 FAs present in at least one diet at proportions >1%.

The influence of tissue, FA set, and CC set on model performance was assessed by comparing the sums of the absolute differences between the actual and estimated proportions for each diet in each modelling scenario following the equation

$$\text{sum of differences} = (|\text{actual}_{\text{initial}} - \text{estimated}_{\text{initial}}| + |\text{actual}_{\text{HO}} - \text{estimated}_{\text{HO}}| + |\text{actual}_{\text{KO}} - \text{estimated}_{\text{KO}}|)$$

The sums were log-transformed and compared using a three-factor repeated-measures analysis of variance (ANOVA) (SPSS for Windows, Version 11.0.1, SPSS Inc., Chicago, Illinois) to determine effects of tissue, FA set, and CC set on diet estimates. Pairwise Bonferroni post hoc tests, with appropriate correction of the α level for multiple comparisons, were performed to identify the combination of factors yielding the minimum sum of differences. The optimal combination of tissue, FA set, and CC set for use in modelling were those that generated significantly lower sums of differences.

Results

FA composition of diet and fish tissues

Using the full FA set, ANOSIM indicated significantly different FA profiles of the three pure diet types (initial, HO, and KO; $R = 1$, $p < 0.004$). Of the major FAs present at amounts >1% (reduced FA set), the initial diet had greatest levels of 18:0, 18:1n-11+9, and 18:2n-6, while HO had highest levels of the monounsaturates 20:1n-11+9, 22:1n-11, and 22:1n-9, as well as 22:6n-3 (Fig. 1). FA levels of KO were typically intermediate between those of the initial diet and HO, except for levels of 14:0, 16:0, 16:1n-7, and 18:1n-7, which were greatest in KO. Similarly, FA profiles (full FA set) of both tissues from fish fed the different diets were significantly different (ANOSIM: $R = 1$; $p < 0.001$) after 22 weeks of feeding, with SIMPER indicating that the same FA that distinguished among the diets were responsible for the differences (Tables 2 and 3).

CC variation

Patterns of CC were broadly similar in belly flap (denoted with subscript BF) and muscle (subscript M) tissues of fish fed the same diet (Figs. 2*a* and 2*b*), and despite different dietary FA levels, CC_{HO} and CC_{KO} calculated for most FAs within a tissue followed similar trends. For instance, very similar values of $CC_{HO,BF}$ and $CC_{KO,BF}$ were calculated for monounsaturated 20:1n-11+9, 22:1n-11, and 22:1n-9,and PUFA 18:2n-6, 20:5n-3, and 22:6n-3 (Fig. 2*a*). However, within a tissue, CC_I (initial diet) for the same FA were quite different than CC_{HO} and CC_{KO} (Fig. 2*a*).

Modelling

Calculated diet mixtures

In the calculated diet mixtures, all four FA sets correctly estimated a proportion of zero for the initial diet but did not perform equally well for HO and KO content (Table 4). The dietary set consistently overestimated the HO proportions in both diet mixtures, predicting 83% and 47% in the 0:70:30 and 0:30:70 mixtures, respectively. Modelling using the full, extended, and reduced FA sets gave the exact proportions expected. The poor performance of the dietary set led to its exclusion from further modelling.

Tissue and FA set

There was a significant effect of tissue for both diets (70:30: $F_{[1,33]} = 15$, $p < 0.001$; 30:70: $F_{[1,33]} = 12$, $p = 0.001$), but the effect was not consistent across CC sets. For instance, for the full FA set, sums of differences were lower for the 70:30 mixture when based on FA profiles of belly flaps using CC_{none}, CC_{HO}, and CC_{KO}; values were higher when CC_{mean} and CC_I were applied (Figs. 3*a* and 3*c*). FA set (excluding dietary) had a significant effect on the sums of differences (70:30: $F_{[2,66]} = 1100$, $p < 0.001$; 30:70: $F_{[2,66]} = 760$, $p < 0.001$; Fig. 3), and within a tissue, lowest sums of differences were generally achieved with the reduced FA set. There were also significant interactions of tissue, FA set, and CC. Because modelling results were broadly similar

Table 3. Tissue lipid percentage (% wet mass; mean (standard deviation, SD) and fatty acid (FA) composition (% total FA; mean (SD)) of muscle tissue of Atlantic salmon reared on five diets: Initial — commercial feed; HO — formulated diet with herring oil as the lipid source; KO — formulated diet with krill oil as the lipid source; 70:30 — formulated diet incorporating a 70:30 ratio of HO to KO; 30:70 — formulated diet incorporating a 30:70 ratio of HO to KO.

	Diet				
	Initial (n = 29)	HO (n = 36)	KO (n = 28)	70:30 (n = 34)	30:70 (n = 34)
Tissue lipid (%)	2.6 (0.7)	3.4 (0.9)	3.7 (1.0)	3.3 (0.7)	3.8 (1.1)
Saturates					
14:0[e,r]	2.77 (0.21)	4.00 (0.21)	9.41 (0.79)	5.36 (0.41)	7.71 (0.58)
i-15:0	0.08 (0.01)	0.15 (0.01)	0.20 (0.02)	0.17 (0.01)	0.19 (0.01)
ai-15:0	0.03 (0.00)	0.06 (0.01)	0.08 (0.01)	0.06 (0.00)	0.07 (0.01)
15:0	0.18 (0.01)	0.31 (0.01)	0.34 (0.02)	0.32 (0.02)	0.32 (0.02)
16:0[e,r]	13.02 (0.64)	13.01 (0.55)	17.67 (0.61)	14.57 (0.41)	16.36 (0.42)
i-17:0	0.03 (0.01)	0.06 (0.01)	0.03 (0.00)	0.06 (0.01)	0.04 (0.00)
ai-17:0	0.08 (0.01)	0.04 (0.01)	0.05 (0.00)	0.05 (0.00)	0.05 (0.00)
17:0[e]	0.18 (0.02)	0.18 (0.04)	0.11 (0.01)	0.32 (0.02)	0.26 (0.22)
18:0[e,r]	3.75 (0.21)	2.21 (0.13)	2.86 (0.14)	2.35 (0.16)	2.67 (0.17)
20:0	0.10 (0.01)	0.13 (0.01)	0.10 (0.01)	0.14 (0.01)	0.11 (0.01)
Monounsaturates					
14:1n-5	0.15 (0.02)	0.07 (0.01)	0.16 (0.01)	0.09 (0.01)	0.13 (0.01)
16:1n-11	0.21 (0.02)	0.34 (0.02)	0.43 (0.03)	0.36 (0.02)	0.40 (0.02)
16:1n-9	0.36 (0.02)	0.22 (0.02)	0.25 (0.01)	0.23 (0.02)	0.24 (0.02)
16:1n-7[e,r]	6.67 (0.50)	5.77 (0.28)	8.97 (0.44)	6.43 (0.38)	7.83 (0.44)
16:1n-5	0.12 (0.01)	0.23 (0.01)	0.33 (0.03)	0.25 (0.01)	0.30 (0.02)
Me-16:1a[a]	0.08 (0.01)	0.11 (0.02)	0.17 (0.01)	0.09 (0.00)	0.13 (0.04)
Me-16:1b[a]	0.11 (0.01)	0.11 (0.02)	0.06 (0.02)	0.14 (0.01)	0.09 (0.01)
17:1	0.18 (0.02)	0.22 (0.03)	0.27 (0.02)	0.22 (0.01)	0.25 (0.03)
18:1n-11+9[e,r]	24.01 (1.49)	12.89 (0.90)	17.85 (0.69)	13.81 (0.82)	16.27 (0.91)
18:1n-7[e,r]	3.23 (0.15)	3.00 (0.06)	6.29 (0.23)	3.67 (0.09)	5.10 (0.21)
18:1n-5	0.23 (0.01)	0.43 (0.02)	0.28 (0.01)	0.38 (0.01)	0.33 (0.01)
20:1n-11+9[d,e,r]	4.33 (0.58)	10.85 (0.47)	3.85 (0.17)	9.06 (0.52)	6.10 (0.31)
20:1n-7[d,e]	0.34 (0.05)	0.61 (0.04)	0.48 (0.03)	0.55 (0.03)	0.52 (0.04)
22:1n-11[d,e,r]	2.02 (0.30)	12.84 (0.80)	3.09 (0.19)	10.41 (0.78)	6.35 (0.42)
22:1n-9[d,e,r]	0.42 (0.06)	1.04 (0.19)	0.47 (0.08)	0.99 (0.06)	0.66 (0.07)
22:1n-7[d,e]	0.06 (0.01)	0.20 (0.02)	0.11 (0.01)	0.17 (0.04)	0.14 (0.01)
24:1	0.37 (0.05)	0.66 (0.05)	0.40 (0.05)	0.59 (0.06)	0.53 (0.07)
Polyunsaturates					
16:2n-4[d,e]	0.37 (0.03)	0.39 (0.02)	0.55 (0.03)	0.40 (0.03)	0.48 (0.03)
16:3n-4[d,e]	0.29 (0.04)	0.21 (0.02)	0.13 (0.02)	0.20 (0.01)	0.17 (0.02)
16:4n-1[d,e]	0.31 (0.05)	0.28 (0.03)	0.39 (0.03)	0.31 (0.03)	0.36 (0.02)
18:2Δ5,11	0.13 (0.01)	0.05 (0.01)	0.13 (0.01)	0.05 (0.00)	0.09 (0.01)
18:2n-7	0.08 (0.01)	0.05 (0.01)	0.08 (0.01)	0.06 (0.00)	0.07 (0.01)
18:2n-6[d,e,r]	9.39 (1.36)	4.97 (0.36)	5.48 (0.33)	5.17 (0.35)	5.24 (0.31)
18:2n-4[d,e]	0.19 (0.01)	0.12 (0.01)	0.14 (0.01)	0.12 (0.01)	0.13 (0.01)
18:3n-6[d,e]	0.29 (0.04)	0.16 (0.03)	0.24 (0.02)	0.19 (0.02)	0.20 (0.02)
18:3n-3[d,e]	0.80 (0.10)	0.88 (0.04)	0.73 (0.02)	0.82 (0.03)	0.78 (0.05)
18:4n-3[d,e,r]	0.79 (0.07)	1.12 (0.07)	1.43 (0.08)	1.16 (0.07)	1.28 (0.10)
18:4n-1[d,e]	0.36 (0.07)	0.28 (0.05)	0.43 (0.04)	0.24 (0.02)	0.36 (0.06)
20:4n-6[d,e]	0.96 (0.14)	0.45 (0.10)	0.39 (0.06)	0.42 (0.05)	0.39 (0.06)
20:3n-3[d,e]	0.07 (0.01)	0.11 (0.01)	0.07 (0.01)	0.22 (0.03)	0.11 (0.05)
20:4n-3[d,e]	0.46 (0.03)	0.60 (0.05)	0.60 (0.06)	0.57 (0.04)	0.61 (0.06)
20:5n-3[d,e,r]	5.30 (0.71)	4.82 (0.43)	3.23 (0.39)	4.48 (0.56)	3.74 (0.50)
21:5n-3[d,e]	0.31 (0.02)	0.26 (0.02)	0.20 (0.01)	0.25 (0.01)	0.22 (0.01)
22:5n-6[d,e]	0.24 (0.03)	0.19 (0.02)	0.12 (0.01)	0.14 (0.01)	0.14 (0.02)
22:5n-3[e,r]	2.38 (0.29)	1.64 (0.12)	1.15 (0.14)	1.47 (0.11)	1.29 (0.14)
22:6n-3[d,e,r]	14.12 (1.96)	13.68 (1.40)	10.2 (1.66)	12.89 (1.81)	11.2 (1.84)

Table 3 (*concluded*).

	Diet				
	Initial (n = 29)	HO (n = 36)	KO (n = 28)	70:30 (n = 34)	30:70 (n = 34)
Sums					
Full	100.00 (0.00)	100.00 (0.00)	100.00 (0.00)	100.00 (0.00)	100.00 (0.00)
Dietary	41.46 (1.83)	54.05 (1.06)	32.34 (1.73)	48.76 (1.15)	39.16 (1.55)
Extended	97.48 (0.13)	96.76 (0.13)	96.63 (0.21)	96.74 (0.11)	96.66 (0.13)
Reduced	92.22 (0.33)	91.84 (0.23)	91.95 (0.27)	91.82 (0.19)	91.80 (0.22)

Note: All fish were fed the initial diet for 12 months prior to the beginning of the experiment; 29 of these were collected at week 0 as representative of fish fed the initial diet. The remainder were then switched to one of the four formulated diets and fed for a further 22 weeks. Superscript letters represent the following: a, methyl branch was present at an unknown position; d, dietary FA set consisting of FAs that fish can only acquire from diet; e, extended FA set consisting of the dietary set plus eight FAs that may have both a dietary and biosynthetic origin; r, reduced FA set consisting of 14 FAs present in at least one diet at proportions >1%.

Table 4. Variation in estimates of calculated diet mixtures with the fatty acid (FA) set employed in modelling (mean (SD)).

		Estimated diet proportions		
FA set	Actual diet proportions (Initial:HO:KO)	Initial	HO	KO
Full	0:70:30	0 (0)	70 (0)	30 (0)
	0:30:70	0 (0)	30 (0)	70 (0)
Dietary	0:70:30	0 (0)	83 (0)	17 (3)
	0:30:70	0 (0)	47 (0)	53 (3)
Extended	0:70:30	0 (0)	70 (0)	30 (0)
	0:30:70	0 (0)	30 (0)	70 (0)
Reduced	0:70:30	0 (0)	70 (0)	30 (0)
	0:30:70	0 (0)	30 (0)	70 (0)

Note: Calculated diet mixtures (n = 8 for each mixture) were created using only the FA composition of diets and tested the ability of the model to differentiate among diet items with contrasting FA signatures.

across both tissues, results for all modelling scenarios are only shown for belly flaps. Results for muscle tissue are shown only for the full FA set (Fig. 3).

CC

CC had a significant impact on the sums of differences (70:30: $F_{[4,132]} = 540$, $p < 0.001$; 30:70: $F_{[4,132]} = 360$, $p < 0.001$; Fig. 3). Use of a CC set derived from feeding a specific diet generally resulted in overestimation of that dietary component. For instance, for all FA sets, modelling of the 70:30 and 30:70 mixtures with $CC_{I,BF}$ or $CC_{I,M}$ consistently resulted in estimates that included a large contribution from the initial diet, although that dietary component was not present in the actual diet (Fig. 3). Despite the similarity in CC_{HO} and CC_{KO}, the same effect was also seen with their application, particularly with the 70:30 HO:KO diet. For both mixtures, modelling without using CC (CC_{none}) predicted the correct major component of the diet (i.e., for the 30:70 mixture, KO was the major component estimated) and produced more accurate results than using CC_I. In several cases (30:70 mixture for belly flap with all FA sets (Figs. 3*b*, 3*f*, 3*h*) and 70:30 mixture for muscle with the full FA set (Fig. 3*c*)), the sum of the differences based on the mean of the three CC (CC_{mean}) was not significantly different than that calculated with CC_{HO}. However, estimates based on CC_{mean} always included contributions at >15% of the total from the initial diet that had not been fed in 22 weeks, indicating poor accuracy. The most accurate estimates were consistently those derived from the use of CC_{KO}, with estimates for HO and KO within 10% of actual values; results were particularly accurate for the 30:70 mixtures.

Discussion

Tissue effects

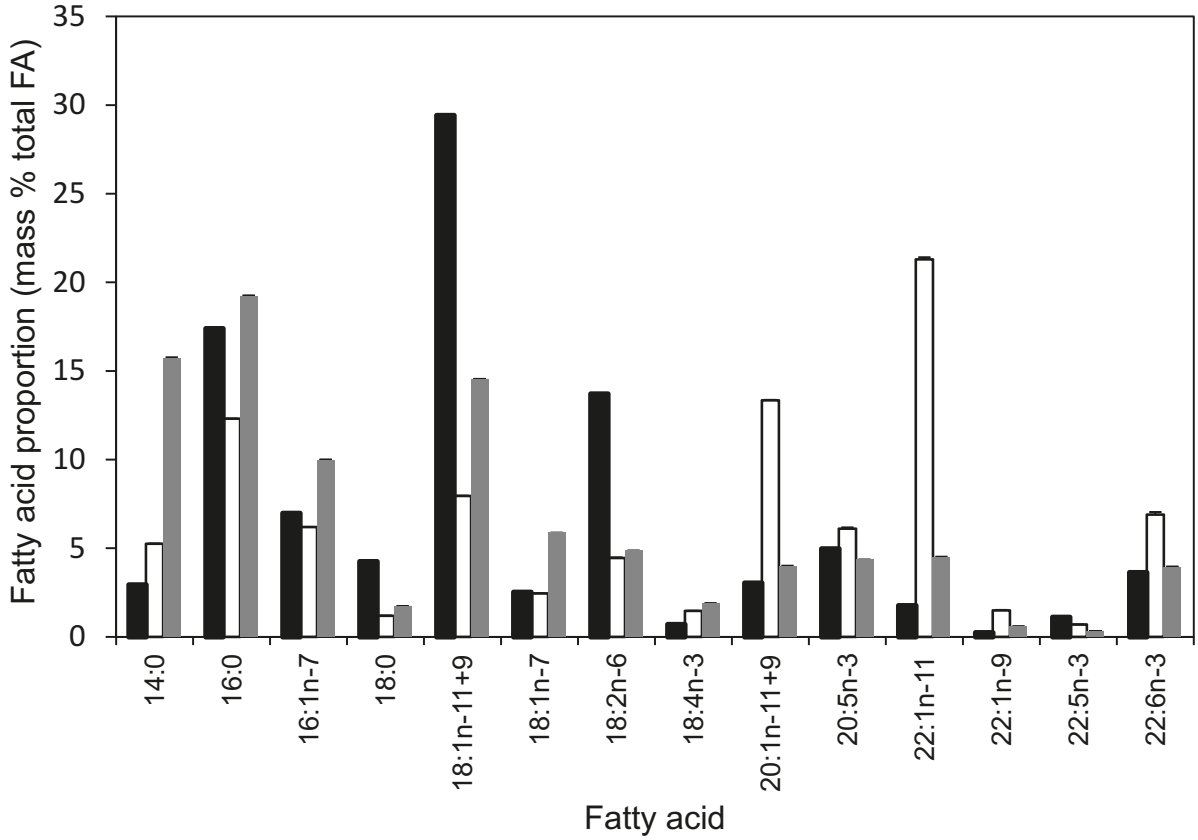
Tissue had a significant effect on the accuracy of diet estimates but the differences were subtle. Our previous work had shown that belly flap FAs had a greater similarity to dietary FAs than muscle tissue in salmon for all diets studied (Budge et al. 2011), so more accurate estimates from belly flap tissue were expected. However, the diet estimates in this study were made using tissue-specific CC, so we were compensating for any differences in the assimilation patterns of FAs into the tissues. This demonstrates that any suitably calibrated storage tissues should be appropriate for QFASA modelling in fatty fish similar to salmon.

The most appropriate tissue to analyze may become a more important consideration when QFASA is applied to lean fish, such as Atlantic cod (*Gadus morhua*) or haddock (*Melanogrammus aeglefinus*). In such fish, there are much lower levels of lipids within muscle tissue compared with the liver, their major lipid storage site (Nanton et al. 2003), with muscles having higher amounts of phospholipids (PL) and lower amounts of triacylglycerols (TAG). TAG stored within tissues have been found to more closely resemble the FA composition of dietary lipids than do structural PL (Castledine and Buckley 1980; dos Santos et al. 1993), which tend to remain relatively unchanged. Thus, in lean fish, a tissue high in PL and low in TAG, such as muscle, may not readily reflect dietary FA composition and would require controlled feeding studies of much longer duration to establish CC. Certainly, if CC calculated for muscle tissue in salmon were applied to muscle tissue of lean fish, inaccurate diet estimates would result because of differences in the lipid class composition. Liver tissue would be a more appropriate choice to model in such fish, but calculation of new CC for liver tissue in lean species would likely be necessary; the extent of variation of CC among fish species and tissue is unknown.

Influence of FA set

The KL distance measure, used to compare FA signatures, was considered to be superior to other measures, such as squared error or relative squared error, because it gave more weight to FAs present in small proportions (Iverson et al.

Fig. 1. Proportion of fatty acids (FAs) (% total FA; mean ± standard deviation (SD), $n = 3$ for each diet) present at mean levels >1%, representing the reduced FA set, in diets fed to determine calibration coefficients in Atlantic salmon. Black, white, and grey bars represent initial, herring oil (HO), and krill oil (KO) diets, respectively.



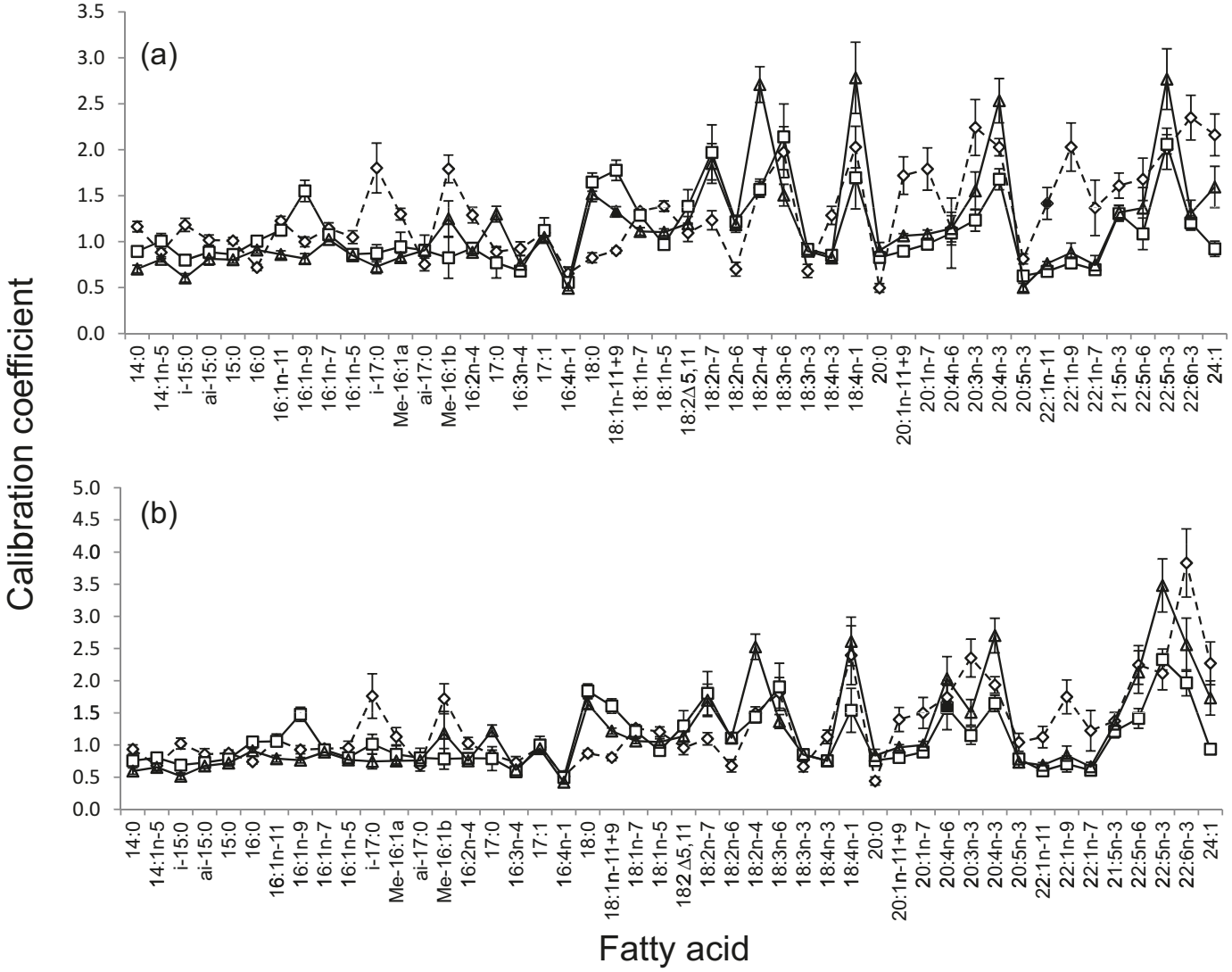
2004); despite this, the measure remains heavily influenced by FAs present in large proportions. This can be clearly seen in the results of the modelling of the calculated diet mixtures with the dietary FA set. FAs in this set only represented ~39%–43% of total FAs identified, while those in the full, extended, or reduced sets all comprised >90% of total tissue FAs. Within the dietary set, 15 of 21 tissue FAs were present at <1% of total and contributed little to the distance measure. The proportions of the remaining FAs in the dietary set (18:2n-6, 18:4n-3, 20:1n-11+9, 20:5n-3, 22:1n-11, 22:6n-3) did not vary substantially in all three diets. When additional FAs (14:0, 16:0, 16:1n-7, 17:0, 18:0, 18:1n-11+9, 18:1n-7, and 22:5n-3) were added to create the extended FA set, accuracy of diet estimates improved. In fact, the much greater influence of FAs present in large proportions on the distance measure was obvious when the calculated diets were modelled using the reduced FA set. This result was equivalent to that obtained using the full or extended FA set, although only 14 FAs present in at least one diet at levels > 1% were used.

Several recent studies employing QFASA with seals (Nordstrom et al. 2008) and eiders (Wang et al. 2010) have optimized the FA set used in the modelling so that the most accurate result was achieved with consumers fed known diets. In these studies, it was assumed that uncertainty in CC was responsible for deviations in diet estimates from actual values, so FAs were removed from the modelling sets according to decreasing CC variability. However, FAs present in small amounts in both consumer and diet items would be more likely to generate CC with high variability, since a small change (i.e., 0.01%) in an FA present at a low level (<0.1%) in either consumer or diet item would result in a large change in CC. Thus, FAs with CC with high variability are also more likely to be FAs present in small amounts and therefore contributing little to the KL distance and the diet estimate. A better strategy may be to remove FAs according to concentration. If only FAs present in large proportions must be retained for accurate estimates, determining the minimum number of FAs required would be a useful exercise. Certainly, the modelling would be simplified with a reduced number of FA variables. It would also serve to streamline data processing; extracting data from chromatograms generated by GC can be time-consuming, and the process would be more efficient if only data from a few FAs present at large levels were required. However, an obvious caveat is that this study involves a captive feeding experiment where FA signatures were different among diets but showed great consistency within diets and consumers, making for optimal modelling conditions. It may not be possible to use such a reduced FA set if proportions of those major FAs are similar among diet items. Careful assessment of the FA compositions of the diet items, as well as modelling of calculated diet mixtures, would be necessary when using a reduced FA set to ensure that diet FA proportions were sufficiently different to allow diets to be distinguished based on their signatures.

Variation in CC

Wang et al. (2010) demonstrated that CC calculated for spectacled (*Somateria fischeri*) and Steller's (*Polysticta stel-*

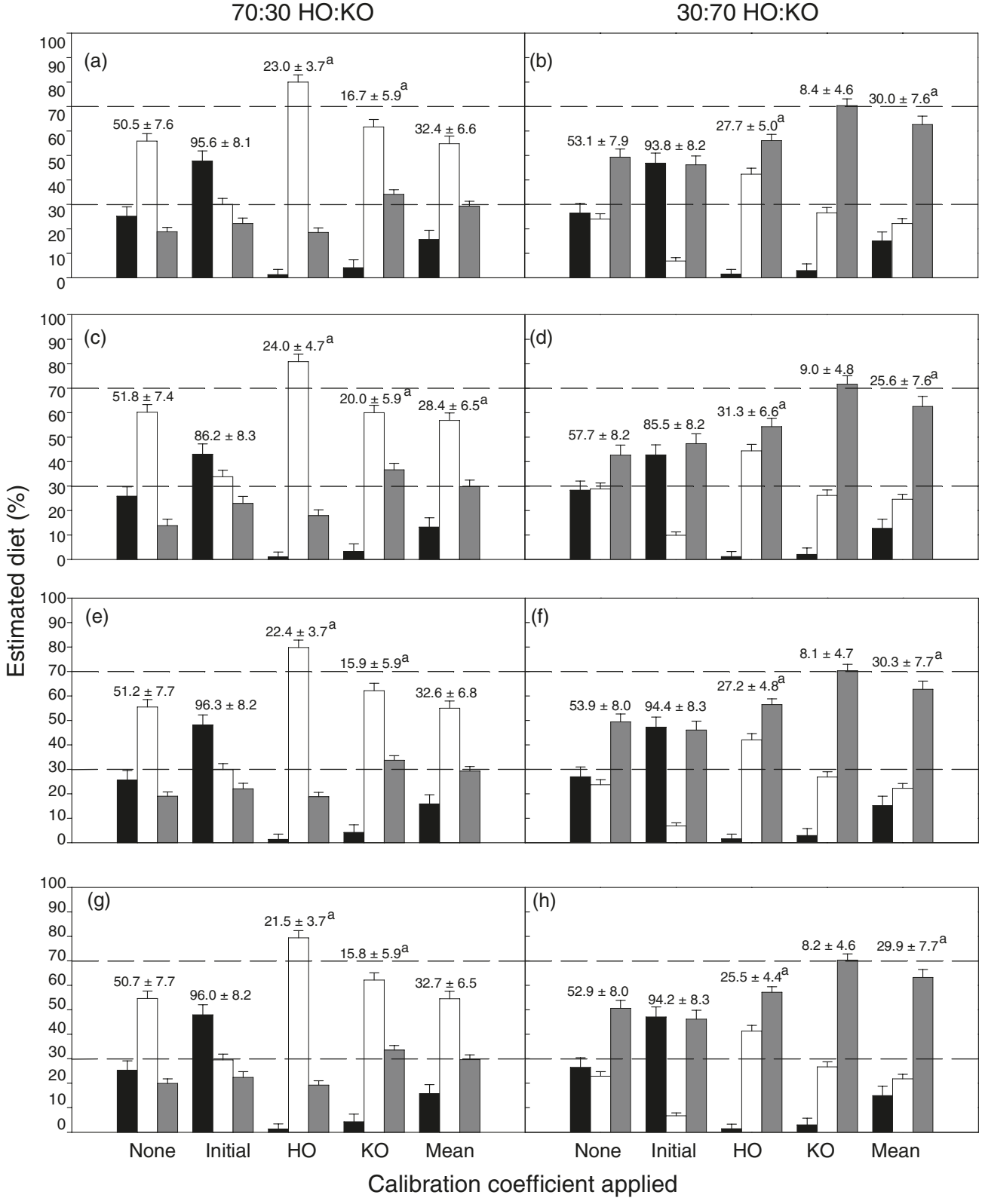
Fig. 2. Calibration coefficients (mean ± standard deviation (SD)) for each fatty acid calculated for Atlantic salmon tissues after feeding a constant diet for 22 weeks: (*a*) belly flap; and (*b*) muscle. Diamonds, squares, and triangles represent initial, HO, and KO diets, respectively.



leri) eiders fed diets with a terrestrial FA signature high in 18:2n-6 and relatively low in 20:1 and 22:1 isomers were quite different for those specific FAs than CC for murres (*Uria aalge*) fed a more typical marine diet with low levels of 18:2n-6 and elevated 20:1 and 22:1 proportions. This suggests that FAs may be metabolized differently according to dietary proportions, and substantial evidence exists for this effect in fish (Bell et al. 2003; Torstensen et al. 2004; Stubhaug et al. 2007). Thus, we expected that FAs present at high levels in a diet would be metabolized differently than when present at lower levels in another diet, resulting in different CC for those FAs depending on diet consumed. For instance, 22:1n-11 was about four times more abundant in the HO than the KO diet, and we anticipated that a larger amount of dietary 22:1n-11 would therefore be oxidized for energy in fish fed the HO diet compared with KO, leaving a relatively smaller amount available for deposition. Similar effects were also anticipated for other major FAs present in contrasting amounts in the HO and KO diets (e.g., 14:0, 18:1n-11+9, and 20:1n-11+9). Instead, the CC derived for those FAs from fish fed HO and KO diets were similar and did not seem to be influenced by dietary level. Considered alone, this supports the hypothesis of consumer-specific CC, where a predator consistently modifies levels of dietary FAs regardless of composition of diet. Indeed, the CC calculated here had little similarity to those derived for other species, including seals (Iverson et al. 2004; Nordstrom et al. 2008), seabirds (Wang et al. 2010), and mink (*Mustela vison*; Thiemann et al. 2008), offering further support for consumer-specific CC. However, while most CC_{HO} and CC_{KO} did follow similar trends, very obvious diet-specific CC were found for several FAs in both tissues, including 18:1n-11+9, 20:4n-3, 22:5n-3, and 24:1. In the muscle, $CC_{HO,M}$ and $CC_{KO,M}$ for 22:6n-3 also differed. Further, CC_I within a tissue showed clear deviation from CC_{HO} and CC_{KO} for most FAs, indicating that CC were not consumer-specific and therefore not equivalent for all diets. The interpretation of this data is complicated by several FAs, such as 20:1n-11+9 and 22:6n-3, that have very similar levels in the initial and KO diets but very different CC_I and CC_{KO}.

The variation of CC with diet is likely a product of differential fish lipid metabolism. Based on Wang et al. (2010), we

Fig. 3. Estimated percentages of individual diets fed to Atlantic salmon: (*a* and *b*) belly flap tissue modelled using the full FA set; (*c* and *d*) muscle tissue modelled using the full FA set; (*e* and *f*) belly flap tissue modelled using the extended FA set; and (*g* and *h*) belly flap tissue modelled using the reduced FA set. Proportions of HO and KO in the diet are indicated above the panels. For each panel, results were estimated without applying CC or with the CC indicated. Sums of the differences from the actual amount fed (mean ± standard deviation (SD); $n = 34$) for each estimated proportion are given above each result. Means with the same superscript within a panel indicate that sums of the differences were not significantly different. Black, white, and grey bars represent proportions of initial, HO, and KO diets, respectively. The horizontal dashed lines mark 30% and 70%, representing the actual percentages of HO and KO diets.



had proposed the simple hypothesis that FA amounts above some minimum dietary level might be catabolized for energy, resulting in CC that varied with diet. However, it is clear that the relationship between tissue and dietary FA level is more complex. Studies with amino acids have shown that oxidation of essential amino acids varies with the concentrations of other dietary amino acids (Roberts et al. 2001; Elango et al. 2008), and similar processes may occur with FAs, where the levels of one FA in the diet influences the manner in which another FA is deposited in tissue. For instance, if a FA is present at a very large level in the diet, such as 18:1n-9 or 18:2n-6 in the initial diet, that FA may be preferentially catabolized, sparing another FA. Both the initial and KO diets had similar levels of 20:1n-11+9 and 22:1n-11, but the initial diet had an approximately twofold higher concentration of 18:2n-6, and the corresponding CC for those FAs did not agree. It may be that 18:2n-6 was preferentially oxidized for energy in the fish fed the initial diet, resulting in less catabolism and greater deposition of 20:1n-11+9 and 22:1n-11, leading to larger CC derived from the initial diet than from the KO diet. In contrast, in the KO diet, with much lower proportions of 18:2n-6, there may have been less of a sparing effect with more 20:1n-11+9 oxidized, giving lower $CC_{KO,BF}$ and $CC_{KO,M}$. Differential catabolism with varying dietary FA levels has been demonstrated in Atlantic salmon for a number of FAs, including monounsaturated 18:1n-9 and 22:1n-11 and polyunsaturated 18:2n-6 and 18:3n-3 (e.g., Torstensen et al. 2004; Stubhaug et al. 2007), and Bell et al. (2003) has suggested that increased metabolism of FAs occurred in fish muscle as dietary concentrations increased. In fish, diet-specific CC may be necessary to compensate for this differential catabolism.

Other explanations for the variation in CC with diet that are related to experimental design can be eliminated. For instance, the salmon fed the initial diets were reared in fresh water in a single large tank before the diet switch and transfer to seawater, and there is some evidence indicating that salinity may influence the FA composition of fish tissues (Leray et al. 1984; Li and Yamada 1992; Tocher et al. 2000). However, we have demonstrated in previous work (Penney 2010) that FA signatures of tissues of salmon fed identical diets based on KO were not influenced by salinity after a 22-week feeding study. Similarly, the duration of the feeding study could influence the CC. The initial diet had been fed to salmon for approximately 1 year before beginning the experiment. After the diet switch, HO and KO diets were fed for a relatively shorter period of only 22 weeks, and it is possible that the salmon were not fed the new diets for a sufficiently long period of time for their tissues to fully resemble the FA signature of the new diets. However, the majority of FAs did, in fact, reach a constant level after 22 weeks of feeding (Budge et al. 2011). Levels of most FAs in fish tissue sampled after only 8 weeks of feeding were very similar to those at 22 weeks, demonstrating that the differences in CC found in the current study between the initial and HO–KO diets could not be due to insufficient duration of the experiment.

Last, CC derived here might also be influenced by the non-lipid components of the diet. All ingredients, except the oil, were identical in the HO and KO diets, but differences in composition between HO–KO and the initial diets were likely much greater. The proximate composition of all three diets was similar based on data available from the manufacturer of the initial commercial feed. However, detailed carbohydrate and amino acid analyses were not conducted, and the initial diet contained a number of ingredients that were not included in the HO or KO diets, including poultry feather meal, yeast, lysine, and gums. Carbohydrate content can influence lipid metabolism (e.g., Lin et al. 1977; Wilson 1994; Coyle et al. 1997), and proteins may have a similar effect. Thus, differences in the specific components in the fish diets may contribute to the variation in FA metabolism observed here; however, without knowledge of the exact composition of all diets, it is difficult to speculate on potential causes of the dietary differences. A combination of both differences in dietary components and FA composition of oils likely led to differential oxidation of FAs and the variation in CC with diet.

Influence of CC variation on diet estimates

Application of a CC derived from feeding a specific diet tended to overestimate that dietary component and therefore influenced the proportional composition of the other components. This tendency likely arises because the application of a set of CC changes the consumer's tissue FA profile so that it more closely resembles the diet that was fed to determine CC. For example, application of $CC_{I,BF}$ for 18:2n-6, which is ~60% that of $CC_{HO,BF}$ or $CC_{KO,BF}$ (0.70 vs. 1.22 and 1.18, respectively), to the belly flap of salmon fed the 70:30 HO:KO diet with 18:2n-6 at ~5.7% would modify that value to make it larger and more similar to the level of 13.7% found in the initial diet. However, application of either $CC_{HO,BF}$ or $CC_{KO,BF}$ would make the 18:2n-6 level smaller and more similar to that of those diets (~4.5% and 4.9%, respectively). Overall, this problem occurred least with $CC_{KO,BF}$ and $CC_{KO,M}$, leading to the conclusion that the most accurate results were achieved using those CC. However, these results are almost certainly specific to this situation. The superior performance of the CC_{KO} likely arose because of the particular FA composition of the diets and salmon tissues; estimates from tissues of salmon fed a diet with a very different FA composition may not be as accurate when CC_{KO} are applied.

Applications to wild populations

Iverson et al. (2004) describe four key requirements in the application of QFASA: (*i*) FA profiles of potential prey; (*ii*) CC to account for consumer metabolism; (*iii*) sampling of an appropriate consumer tissue that reflects dietary FA; and (*iv*) a statistical model to compare FA profiles of consumer and prey. To apply to wild salmon, the same statistical model as employed here and by Iverson et al. (2004) can be used. The current work also suggests that the FA profile of either muscle or belly flap would be representative of diet. The first two requirements, however, are more difficult to address in wild fish. In this controlled study with homogenous diets, we know the precise FA profiles of the two prey items. In wild fish, the FA profile of all likely prey items must be determined with sufficient sampling effort so that an estimate of the individual variation in FA composition can be made. Temporal and spatial variation in prey FA signatures must also be assessed, as well as variation with size and reproductive status (e.g., Iverson et al. 2002; Piché et al. 2010). The most accurate results achieved in this study were within 10%

of the actual values; we would expect larger errors in diet estimates of wild fish simply because of the variety in prey species that could be consumed and the potential variation in their FA profiles.

We have also addressed the second requirement of development of CC to account for consumer metabolism. However, the salmon in this study were fed diets with higher lipid content (on average 14% wet mass) than those normally consumed by fish in the wild, which may influence derived CC. Typical diet items of large adult marine salmon include Atlantic herring (*Clupea harengus*), sandlance (*Ammodytes dubius*), and shrimp (*Pandalus borealis*; Shearer 1992), which have lipid contents of 3%, 6%, and 8% wet mass, respectively (Budge et al. 2002; Iverson et al. 2002). High lipid diets may suppress de novo synthesis or increase β-oxidation of FAs in tissues of captive fish (Turchini et al. 2009), making the CC calculated here inaccurate. Lin et al. (1977) did report suppressed de novo synthesis of FAs in the liver of coho salmon (*Oncorhynchus kisutch)* as dietary lipid content increased from 2.5% to 10% wet mass but did not find a change in lipogenic enzymes in mesenteric adipose. Similar effects were also reported in channel catfish (*Ictalurus punctatus*; Likimani and Wilson 1982). These results suggest that dietary fat content has little effect on FA metabolism, including both synthesis and oxidation, in adipose tissue of fish and that the CC derived here for belly flaps, consisting of adipose tissue, will remain accurate in salmon consuming lower fat diets. There is less evidence for such a conclusion in muscle tissue, and future studies must address this possibility. It is also clear that hepatic lipid metabolism is influenced by dietary lipid level (Lin et al. 1977; Likimani and Wilson 1982). The liver is the main FA storage tissue in lean fish such as cod and haddock, so with those consumers, caution should be taken in extrapolating data from captive feeding studies that fed high fat diets to wild fish consuming lower fat diets. Certainly, further studies using lower fat diets in other fish species are required to fully assess the influence of dietary fat on consumer lipid metabolism.

Water temperature is known to influence fish metabolism, with lower temperatures suppressing general metabolic rates (Johnston and Dunn 1987) and likely lipid metabolism as well. Specific effects on hepatic de novo synthesis of PL have also been noted in carp (*Cyprinus carpio*; Farkas et al. 1980). Water temperature in this study was ~9 °C and likely represents an upper limit that fish would experience in the North Atlantic Ocean. Lower temperatures would likely result in a decrease in rates of de novo synthesis, elongation, desaturation, and oxidation of FAs, potentially affecting tissue lipid profiles. However, feeding rate is often decreased with cold temperatures (Johnston and Dunn 1987) so that dietary lipid intake and deposition would also be decreased. Combined with a lower rate of metabolism, this may result in no net difference in FA profile of tissue with changing temperatures. There are few reports investigating the influence of temperature on the FA composition of fatty tissues; however, a recent study (Elsdon 2010) showed that temperature did not have an effect on the FA composition of muscle tissue lipid at relatively high temperatures of 16 and 23 °C in black bream (*Acanthopagrus butcheri*). Thus, temperature may have minimal influence on FA composition.

It is also well-known that growth and reproductive stage may influence FA profiles of tissues. Aquaculture literature (reviewed in Tocher 2010) has shown that essential FA requirements are different for larvae than for juveniles and subadults of many freshwater, marine, and diadromous fish species, indicating that lipid metabolism and therefore CC would also vary. However, there seems to be little evidence to support differences in FA metabolism between subadults and adults of fish species. A more important consideration may be reproductive state. For instance, the proportions of 20:5n-3 and 22:6n-3 in fish eggs seems to be somewhat fixed and independent of diet, pointing to changes in fish lipid metabolism in gravid females during egg development (Sargent et al. 2002). Such effects on tissue FA profiles are difficult to predict, and application of QFASA to gravid or recently spawned females should be avoided until further study is completed.

This work represents the first investigation of the application of QFASA to marine fish. Here, we have shown that QFASA was able to estimate the proportions of the dietary components within ~10% of actual values but that the accuracy of estimates depended on both the CC and FA set employed in the modelling. Small but significant differences were found in the accuracy of estimates generated from FA profiles of belly flap or muscle tissue. Evaluation of a number of FA sets indicated that FAs present at low levels had little influence on estimates, despite having only dietary sources; the FA group used in modelling must incorporate the major dietary FAs to ensure accurate estimates of diet. CC were similar in the two tissues but varied with diet. When modelling diets, application of a CC set derived from feeding a specific diet item resulted in an overestimate of that diet component. This problem occurred least, and diet estimates were most accurate, when CC_{KO} were applied. Much of this work will be broadly applicable to consumers in general and establishes a basis for further investigation of QFASA in marine fish.

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