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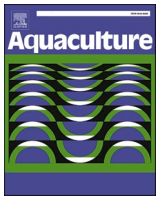

Verifying the relationship between $\delta^{13}C$ isotope profile variables and individual feed conversion ratio in large rainbow trout (*Oncorhynchus mykiss*)

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ABSTRACT

The study aimed to verify the relationship found between individual indicator traits, derived from an isotope ratio of $^{13}C/^{12}C$ in various tissues (muscle, liver, and adipose), and individual feed efficiency. A 50-day experiment was performed with 46 rainbow trout (*Oncorhynchus mykiss*) kept in individual tanks after a dietary switch, using an experimental feed based on maize gluten with an enhanced level of ^{13}C, during the grow-out phase. Individual growth, feed intake, and isotope profiles (muscle, liver, fin, and adipose tissue) were recorded. Using a Lasso-regression model, feed efficiency was found to be positively associated with visceral adipose tissue synthesis, meaning that feed efficiency was worsened when visceral adipose synthesis increased. The results indicated that deposition efficiency through lipid metabolism ($\delta^{13}C$ in adipose tissue) was the most important determinant of feed efficiency of rainbow trout at this life stage. The results suggest that phenotyping of lipid deposition efficiency is possible through the use of feed ingredients with a natural abundance of stable isotopes.

1. Introduction

Feed efficiency is among the most economically important traits in animal production (Gjedrem, 2005). Improving feed efficiency, by genetic selection on growth or other means, will reduce the production costs and reduce the environmental footprint per unit produced (Besson et al., 2016; de Verdal et al., 2011). Feed efficiency can be assessed as the feed efficiency ratio (FER), i.e., growth per unit of feed consumed, or the feed conversion ratio (FCR), i.e., amount of feed consumed per unit of growth. However, genetic improvement of efficiency implies large-scale recording of growth and feed intake at an individual level. Recording growth is relatively straightforward while recording individual feed intake is difficult in aquatic species. Farmed fish are typically kept in large sea cages, ponds, or tanks and fed communally by dispersing feed into the water, making it practically impossible to record individual feed intake under commercial conditions.

In the past, two experimental non-invasive methods have been used to record individual feed intake within a group of fish, either based on X-radiography or video recording. The first method uses radio-opaque ballotini glass beads for which the number of ingested beads is subsequently detected by x-raying, allowing to predict feed intake (Jobling et al., 2001). Single meal prediction is highly accurate (McCarthy et al., 1993), but the method requires repeated anesthesia and handling of the fish, increasing the stress and exposing the fish to injuries and diseases. Furthermore, rainbow trout (*Oncorhynchus mykiss*) have been shown to have substantial daily variation in feed intake (Bolliet et al., 2000; Boujard and Leatherland, 1992; McCarthy et al., 1992; Thodesen et al., 1999), and 3–6 measurements are thus needed to obtain a satisfactory estimate of the individual feed intake (Kause et al., 2006). Potentially even more measurements may be needed if the aim is to assess feed intake over a longer period, and the intense handling of fish limits the use of this method in a commercial setting. The second method is video recording, manually feeding of one and one pellet with retrospective video identification of individual fish and number of pellets eaten (de Verdal et al., 2017, 2018a, 2018b). However, even if the individual feed intake can be recorded over a longer period without disturbing the fish,

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the method is labor-intensive, and the time required to analyze the video is extensive. The method also requires external tagging and tracking of the individual fish. This limits the group size to 10–20 individuals (de Verdal et al., 2018a), which is far from commercial conditions, where thousands of fish are kept in the same unit.

Direct selective breeding for improved feed efficiency implies recording individual feed intake. Consequently, selective breeding for improved feed efficiency has had to rely on indirect selection through increased growth rate (Gjedrem and Baranski, 2010; Thodesen et al., 1999, 2001), which is expected to reduce the maintenance requirement per unit produced, e.g., by reducing time to slaughter. At a family group level, additive genetic correlations ranging from 0.60–0.82 have been estimated between growth and feed efficiency in Atlantic salmon (Dvergedal et al., 2019b; Kolstad et al., 2004). The size of these estimates points to a substantial fraction of the additive genetic variation in feed efficiency being due to other factors than growth, with ample room for improvement.

One alternative to the former approaches is to assess feed efficiency by stable isotope recording (Dvergedal et al., 2019a), with the objective of establishing indicator phenotypes more closely related to feed efficiency than growth alone (Dvergedal et al., 2019b). The results indicated individual variation in metabolic efficiency, with efficient fish allocating a larger fraction of the ingested nutrients from feed to growth and less to maintenance of existing body tissue (Dvergedal et al., 2019a, 2019b). In a family-tank experiment, as much as 79% of the between-tank variance in the feed conversion ratio (using leave-one-out cross-validation) was explained by relative growth, isotope-based indicator traits, and sampling day (Dvergedal et al., 2019b). In comparison, 62% of the variance was explained by growth and sampling day alone. The ratio of tissue turnover, estimated by the change in isotope fractions to body growth after a dietary switch, was used as an individual indicator of feed conversion (IFCR). For these indicator traits (IFCR or its inverse, efficiency, IFER), the estimated genetic correlation to the feed conversion ratio (on a group level) approached unity. The estimated heritabilities of the indicator traits were low (0.06–0.11), but still substantial genetic gain can be achieved by genomic selection using a large reference population (Hayes et al., 2009). In summary, the study showed that individual indicator traits have the potential to assess individual feed efficiency in salmonids for use in selective breeding.

In this study, we aimed to verify the relationship between the individual indicator traits and individual FCR by carrying out an experiment with 46 rainbow trout (*O. mykiss*) kept in individual tanks and recorded for growth, feed intake, and isotope profiles. We hypothesized that a universal phenotypic relationship existed between the indicator traits and FCR at the individual level. The experiment was carried out with large-sized rainbow trout since the production in Norway is carried out in saltwater (average slaughter weight 3–4 kg).

2. Material and methods

The experiment was carried out at the Center for Sustainable Aquaculture at the Norwegian University of Life Sciences (NMBU), Aas, Norway, following the laws and regulations for experiments on live animals in the EU (Directive 2010/637EU) and Norway (FOR-2015-06-18-761).

2.1. Fish maintenance

The experiment included 46 rainbow trout of both sexes from the breeding company AquaGen AS (Trondheim, Norway) and was conducted in freshwater. From the eyed egg-stage until the start of the experiment, the fish were reared in a single tank. At an average weight of 1.23 ± 0.20 kg, the fish were randomly allocated to individual tanks (one fish per tank). The circular tanks (height = 70 cm and diameter = 78 cm), each with a 300 l capacity, were supplied with recirculated freshwater from the recirculating aquaculture system (RAS), at a flow rate of 7–8 l min^{-1}, and the fish were kept under a 24 h light regime, with an average temperature of 13.9 °C. The tanks were controlled by OxyGuard water quality monitoring and control systems for aquaculture (OxyGuard International AS, Denmark), and the water quality (<0.05, 0.03, and 8.15 mg l^{-1} for ammonium (NH_4^+), nitrite (NO_2–), and nitrate (NO_3-), respectively) was within legal legislation. Dissolved oxygen was measured daily and kept above 8 mg l^{-1} in the outlet water. All the fish were healthy throughout the experiment.

2.2. Dietary treatment and feeding

A diet with a natural inclusion level of the stable isotope ^{13}C was fed during the experimental period of 50-days. The formulation and analyzed chemical composition of the diet is presented in Table 1. The enrichment of ^{13}C to alter the ratio between $^{13}C/^{12}C$ was mainly obtained by using a high inclusion of maize gluten in the diet $\delta^{13}C$ isotope level ($\delta^{13}C = -16.5$, Table 1) relative to the baseline diet ($\delta^{13}C = -24.2$, data not shown), meaning that the protein fraction was enriched with ^{13}C. Maize is a C4 plant with a higher level of ^{13}C than C3 plants (e.g., wheat and soybean) due to differences in photosynthetic pathways (Gannes et al., 1998; Staddon, 2004; typically, −14.35 vs. -28.79). Thus, the inclusion of maize gluten in the diet creates a contrast in $\delta^{13}C$ enabling to trace nutrient allocation to various tissues. The experimental diet was formulated to meet the nutritional requirements of rainbow trout and was produced at the BioMar AS pilot plant (Tech Center, Brande, Denmark). The fish were fed twice daily (07:00 and 15:00) for a period of 1 h by automatic belt feeders. The feeding level equaled 1–3% of the estimated body weight and was adjusted for uneaten feed. Registrations of uneaten feed and calculations of feed intake were performed according to Helland et al. (1996). The daily feed intake per tank was calculated by first collecting the waste feed on a wedge wire screen (Shomorin et al., 2019) and correcting the total waste feed for leasing losses. As explained by Shomorin et al. (2019), the wedge wire is placed at an inclined position in the outlet water column of the tank. The design of the screen ensures efficient drainage so that uneaten feed that is trapped on the screen is exposed minimally to water. Then, the difference between total fed feed and total uneaten feed was calculated as g dry matter intake, after drying the uneaten feed at 105 °C overnight.

Table 1
Formulation and analyzed content of the experimental diet for 1–2 kg rainbow trout.

Formulation and content	
Formulation, g kg^{-1}	
Fish meal	201.0
Maize gluten	503.0
Wheat	89.0
Fish oil	85.0
Alga meal	120.0
Premix and other	12.9
Analyzed content, g kg^{-1}	
Dry matter	955.7 ± 0.1
Crude protein	505.7 ± 7.6
Lipid	165.1 ± 4.6
Starch	111.0 ± 0.6
Ash	46.0 ± 0.0
Gross energy, MJ kg^{-1}	23.9 ± 0.0
Analyzed content, ‰	
$\delta^{13}C$	−16.5 ± 0.12

The analysis was a mean of duplicates with standard deviation; Fish Meal SA Super Prime, Köster, FF Skagen, Peru; Maize gluten, Norsildmel, Ukraine; Wheat, Hedegaard, Denmark; SA Omega fish oil, ED & F, Peru; Alga Prime, BioMar UK, Brazil; Premix and other, owned by BioMar AS, used under license for this study, and not publicly available.

2.3. Tissue sampling

At the end of the experiment, fish were anesthetized with metacaine, MS-222TM; 1 g l^{-1} water (Finquel® vet., MSD Animal Health, Intervet International B.V., Netherlands) and killed with a sharp blow to the head prior to dissection. Tissue samples from muscle, liver, adipose fin, and adipose tissue were collected, snap-frozen in liquid nitrogen, and stored at −20 °C until stable isotope analysis. Tissue sampling was standardized; muscle was sampled in front of the dorsal fin on the left side in the filet (1 × 1 cm cube), the liver was divided into four small pieces, the whole adipose fin was collected, and adipose tissue from the fat deposited around the gut from the pyloric ceca until the distal intestine was sampled.

2.4. Chemical analysis

The diet was dried and ground prior to analysis which were performed in duplicates (Table 1). The diet was analyzed for dry matter by drying to constant weight at 104 °*C*, ash by combustion at 550 °*C*, crude protein by Kjeldahl nitrogen x 6.25 according to Commission Regulation (EC) No 152/2009, and starch as described in McCleary et al. (1994). Lipid was determined after extraction with petroleum ether and acetone (70/30) on an Accelerated Solvent Extractor (ASE 200) (Dionex Corp, Sunnyvale, CA, USA), while gross energy was established with the PARR 1281 Adiabatic Bomb Calorimeter (Parr Instruments, Moline, IL, USA) according to ISO 9831.

2.5. Muscle lipid extraction

Muscle fat content varies between individual fish and may thus affect the isotope profile in muscle tissue samples. Lipids are typically depleted in ^{13}C relative to protein and carbohydrates, and variation in fat content can thus be confounded with the incorporation of ^{13}C-enriched amino acids in muscle samples. How to deal with lipids in stable isotope analyses involving ^{13}C has been discussed (Wessels and Hahn, 2010), and one solution is chemical extraction of lipids from samples (Logan et al., 2008; Post et al., 2007; Wessels and Hahn, 2010). Thus, we divided each muscle sample into two subsamples. In one subsample, stable isotopes were analyzed directly while in the other subsample (denoted MC ($\delta^{13}C$ in muscle) and MCP ($\delta^{13}C$ in muscle protein), see below), we extracted lipids from the muscle by adding 1000 μl of 10:5:4 methanol:chloroform:water to a finely ground sample (21.6–379.9 mg) and vortexing the mixture before centrifugation (Bligh and Dyer, 1959; Pinnegar and Polunin, 1999). The mixture was spun down at 5000 xg for 10 min, and after centrifugation, the supernatant was discarded. The procedure was repeated three times (repeated until the supernatant was blank). On the final run, 1000 μl of methanol was added, and the solution was centrifuged at 5000 xg for 10 min. The sample was left in the fume hood for 10 min for evaporation of the methanol. The pellet retained was dried at 60 °C overnight. The lipid content of tissues was determined by weighing before and after these treatments, and the sample was then reground before stable isotope analysis.

2.6. Stable isotope analysis

Tissue samples (muscle, liver, adipose fin, and adipose tissue) were freeze-dried and homogenized (except adipose fin), and approximately 1 mg per sample was weighed into small tin capsules (8 × 5 mm, Elemental Microanalysis, Devon, UK). Samples were analyzed for C-isotope compositions using a Nu Horizon isotope-ratio mass spectrometer (IRMS) (Nu Instruments, Wrexham, UK) coupled to a Eurovector element analyzer (EA) 3028 (Eurovector S.p. A, Redavalle, Italy) at the Institute for Energy Technology (Kjeller, Norway) and $\delta^{13}C$ was calculated as follows (Fry, 2006):

$$\delta^{13}C = \left(\frac{\frac{C_{13}}{C_{12}} Sample}{\frac{C_{13}}{C_{12}} Standard} - 1 \right) 1000,$$

where the two ratios are the proportions of ^{13}C divided by the proportion of ^{12}C, in the sample and the reference standard ((Vienna Pee Dee Belemnite for carbon, VPDB); $\delta^{13}C_{Standard}$= 0.0112372 (Craig, 1957)). Analyzed content of $\delta^{13}C$ in the diet is given in **Table 1**.

The calibration of ^{13}C was performed against international certified reference materials and internal standards, and the results of $\delta^{13}C$ analyses were plotted on a two-point calibration line calculated from the analysis of the USGS-24 standard (−16.05‰) from the United States Geological Survey and an in-house (Institute for Energy Technology) graphite standard (−31.56‰) from Spectrapure. The internal IFE Trout standard was prepared by Soxhlet extraction with CH_2Cl_2: 7% CH_3OH for approximately two hours, cleansed with 2 N HCl, and rinsed with distilled water to a neutral pH. The $\delta^{13}C$ composition of IFE trout was calibrated against the USGS-24 standard. The average $\delta^{13}C$ from six analyses of the IFE trout was −20.05‰, with a standard deviation of 0.11.

2.7. Phenotypes analyzed

The initial (*IW*) and final (*FW*) weights were recorded for each fish. From these variables, individual weight gain (*WG*) and relative weight gain (*RG*) were calculated as follows:

$$WG = FW - IW,$$

$$RG = \frac{FW - IW}{FW} \times 100.$$

For each fish, the feed intake (*FI*, g dry matter) was recorded, and relative feed intake (*rFI*) was calculated as:

$$rFI = \frac{FI}{FW} \times 100.$$

Individual feed conversion ratio (*FCR*) was calculated as:

$$FCR = \frac{FI}{WG} = \frac{rFI}{RG}.$$

From the tissue samples, the following individual variables were available: $\delta^{13}C$ in muscle (MC), $\delta^{13}C$ in muscle protein (MCP), $\delta^{13}C$ in the liver (LC), $\delta^{13}C$ in adipose fin (FC), and $\delta^{13}C$ in adipose tissue (AC).

Individual isotope-based indicator variables for the feed conversion ratio (*IFCR*) were derived as described by Dvergedal et al. (2019b). First, atom percentage (Atom %) ^{13}C was calculated for $\delta^{13}C$ in muscle (AMC) and $\delta^{13}C$ in muscle protein (AMCP) as described by Fry (2006):

$$Atom\ \%^{13}C = \left(\frac{\left(\delta^{13}C_{Sample} + 1000\right)}{\left(\delta^{13}C_{Sample} + 1000 + \left(\frac{1000}{\delta^{13}C_{Standard}}\right)\right)} \right) 100,$$

where $\delta^{13}C_{Sample}$ and $\delta^{13}C_{Standard}$ are the proportions of ^{13}C divided by the proportion of ^{12}C, in the sample and the reference standard (Vienna Pee Dee Belemnite for carbon, VPDB), respectively; $\delta^{13}C_{Standard}$= 0.0112372 (Craig, 1957) (Note that the number of $\delta^{12}C$ atoms in this study is greater than the number of $\delta^{13}C$ atoms, and atom percentage $= \frac{\delta^{13}C}{\delta^{12}C+\delta^{13}C} \approx \frac{\delta^{13}C}{\delta^{12}C} = \delta^{13}C$). Secondly, after feeding with enriched feed, the atom % ^{13}C in excess (*APE*) is proportional to the fraction of newly deposited nutrients in the tissue, resulting from both tissue growth and the replacement of previously deposited carbon, denoted as metabolism. *APE* is the *Atom % ^{13}C* in the sample adjusted for the initial isotope percentage in the sample (*IA %*). The *IA %* was assessed by using 20 randomly sampled fish at the start of the experiment. The ^{13}C average

and standard deviations in the muscle with and without fat were: 1.084 ± 0.001 and 1.085 ± 0.001, respectively. Thirdly, IFCR was defined as follows (taking *IFCR_AMC* as an example):

$$IFCR\_AMC = \frac{FW \times APE}{FW - IW} = \frac{APE}{RG},$$

where $APE = (AMC - IA\%)$, with $IA\%$ equal to 1.084 for AMC and 1.085 for AMCP. The basis of the IFCR indicator is to quantify the fraction of metabolic turnover (indicated by the APE) that is allocated to growth. For a more detailed description of the variable, see Dvergedal et al. (2019b).

A priori to the calculations, data were thoroughly inspected. One fish was excluded from the analysis as it was inferred to not eat sufficiently during the 50-days. Furthermore, missing values were assigned both to IFCR_AMC and IFCR_AMCP for one fish with negative IFCR values due to lower atom percentage ^{13}C in muscle than in IA %.

2.8. FCR and associations

The Pearson correlation coefficients between WG, RG, FI, rFI, FCR, MC, MCP, LC, FC, AC, IFCR_AMC, and IFCR_AMCP variables were estimated using SAS®, V.9.4 (SAS, Inst. Inc., Cary, NC).

Associations to FCR were estimated using a statistical learning method that has advantages compared with conventional statistical methods when there is a relatively high number of possible explanatory variables compared to the number of observations ($i = 1, \ldots, N$) and there is collinearity between variables. A shrinkage method approach such as Lasso-regression (least absolute shrinkage and selection operator) (Hastie et al., 2009) can be used to deal with these problems. The Lasso-regression formula can be written:

$$\widehat{\beta}_{lasso} = argmin_{\beta}\left\{\frac{1}{2}\sum_{i=1}^{N}\left(y_i - \beta_0 - \sum_{j=i}^{p} x_{ij}\beta_j\right)^2 + \lambda\sum_{j=1}^{p}|\beta_j|\right\}.$$

The Lasso-regression uses $\sum_{j=1}^{p}|\beta_j|$ as a penalty, and λ controls the amount of shrinkage. The Lasso-regression fits a model involving all p predictors but shrinks the coefficient estimates towards zero. Some estimates are forced to be exactly equal to zero, yielding a sparser model. Thus, the Lasso-regression performs variable selection, which is useful to exclude the least important variables from a multiple regression model.

The relationship between FCR and the nine explanatory variables (except FI and rFI, because these variables cannot be recorded in the sea) was explored by using the SAS® PROC GLMSELECT procedure with the Lasso-selection option and the LSCOEFFS sub-option. This sub-option uses Least Angle Regression (LAR) to determine the sparse model and ordinary least-squares regression to obtain the regression coefficients and associated test statistics. The LAR algorithm searches for solutions over a set of values (Hastie et al., 2009). The predicted residual error sum of squares (PRESS) was used as a stop criterion. The coefficient of determination under prediction was computed for FCR as:

$$\widehat{R}^2 = 1 - \frac{PRESS}{SS_{tot}},$$

where $PRESS = \sum (y_i - \widehat{y}_i)^2$, and $\widehat{y}_i$ is the predicted individual phenotype using regression coefficients estimated using data from all other individuals in the analysis, and SS_{tot} is the total sum of squares. The $\widehat{R}^2$ is an estimate of the fraction of variance in *FCR* explained by the model in the prediction of missing observations under leave-one-out cross-validation.

3. Results

The fish grew on average 688.9 ± 170.6 g over the 50-day period (Table 2). The coefficient of variation was smaller for FCR (9.5%) than for RG and FI (15.7% and 27.3%, respectively). The mean $\delta^{13}C$ in muscle with and without fat (MC and MCP), liver (LC), adipose fin (FC), and adipose tissue (AC) ranged from −16.8 to −24.4‰, with the largest value in the liver. Extraction of lipids from muscle did not have a large effect on the measured delta values, and therefore not on the IFCR_AMC and IFCR_AMCP. Finally, a larger coefficient of variation was found for the IFCR variables than for FCR.

Table 2
Descriptive statistics with the coefficient of variation (CV) of observed variables in an experiment with 46 rainbow trout kept in individual tanks and recorded for growth, feed intake, and isotope profiles to verify the relationship between isotope profile variables and individual FCR, by feeding a diet with an enhanced natural abundance of $\delta^{13}C$.

	N	Mean	Min	Max	SD	CV
IW, g	45	1229.27	896.00	1643.00	194.52	15.82
FW, g	45	1918.04	1203.00	2628.00	306.78	15.99
WG, g	45	688.78	307.00	991.00	170.57	24.76
RG, %	45	35.63	21.44	47.71	5.58	15.67
FI, g dry matter	45	594.39	223.44	872.01	162.33	27.31
rFI, %	45	30.73	13.84	45.97	6.21	20.21
FCR	45	0.86	0.65	1.05	0.08	9.49
MC, ‰	45	−21.04	−24.92	−19.21	0.92	−4.36
MCP, ‰	45	−20.78	−24.10	−18.84	0.91	−4.36
LC, ‰	45	−16.83	−19.56	−16.00	0.74	−4.42
FC, ‰	45	−18.73	−20.59	−17.25	0.89	−4.76
AC, ‰	45	−24.43	−26.58	−22.84	0.77	−3.14
IFCR_AMC, %	44	0.10	0.04	0.15	0.019	18.58
IFCR_AMCP, %	44	0.09	0.03	0.15	0.02	21.21

IW = Initial weight; FW = Final weight; WG = Weight gain: FW – IW; RG = Relative weight gain: ((FW – IW) / FW) x 100; FI = Feed intake: Calculated according to Helland et al. (1996); rFI = Relative feed intake: (FI/FW) x 100; FCR = Feed conversion ratio: FI / WG; MC = $\delta^{13}C$ in muscle; MCP = $\delta^{13}C$ in muscle protein; LC = $\delta^{13}C$ in liver; FC = $\delta^{13}C$ in adipose fin; AC = $\delta^{13}C$ in adipose tissue; IFCR_AMC = Indicator variable for FCR in muscle; IFCR_AMCP = Indicator variable for FCR in muscle protein.

Feed conversion ratio (FCR) had a positive Pearson correlation to FI and rFI ($r = 0.53$ and 0.71, respectively, Table 3). Moreover, FI and rFI were strongly correlated with each other ($r = 0.84$). One of the most striking results, however, was the positive correlation between FCR and growth, $r = 0.36$ for RG (fast-growth implies less feed efficient fish). Weight gain (WG) and RG were closely correlated ($r = 0.80$) and both correlated to FI ($r = 0.95$ and 0.81, respectively), and rFI ($r = 0.70$ and 0.91). The correlations between FCR and MC, MCP, and AC were all positive ($r = 0.54$, 0.47, and 0.61, respectively). Despite the positive correlation between growth and FCR, the correlation between FCR and IFCR_AMC was positive ($r = 0.32$), while being lower and non-significant to IFCR_AMCP. The correlation between whole-muscle and fat-extracted muscle $\delta^{13}C$ (MC and MCP) was high ($r = 0.88$), another indication that fat-extraction of muscle tissue had a very small effect on isotope values. Further, $\delta^{13}C$ in muscle (MC and MCP) correlated positively to $\delta^{13}C$ in adipose fat (AC; $r = 0.71$ and 0.69, respectively), and all these $\delta^{13}C$ variables correlated positively with WG, RG, FI, and rFI ($r =$ 0.44–0.90). Both MC and MCP had positive correlations to IFCR-variables ($r = 0.52$–0.83, respectively), and the IFCR-variables had a positive correlation to each other ($r = 0.76$). Note also the slightly positive correlation between $\delta^{13}C$ in MC and LC ($r = 0.34$).

When regressing all the explanatory variables (except FI and rFI) on FCR using Lasso-regression, only AC was significant $F = 18.71$ ($P < 0.0001$); Table 4. The model with the smallest PRESS value had a coefficient of determination (R^2) of 0.31, while leave-one-out cross-validation gave a coefficient of determination under prediction ($\widehat{R}^2$) of 0.24.

4. Discussion

Nitrogen and carbon isotopes are the most relevant when assessing feed efficiency; by definition, all organic compounds contain carbon,

Table 3
Pearson correlation coefficients between variables in an experiment with 46 rainbow trout kept in individual tanks and recorded for growth, feed intake, and isotope profiles to verify the relationship between isotope profile variables and individual FCR, by feeding a diet with an enhanced natural abundance of $\delta^{13}C$.

Variables	WG	RG	FI	rFI	FCR	MC	MCP	LC	FC	AC	IFCR_AMC	IFCR_AMCP
WG		**0.80**	**0.95**	**0.70**	0.25	**0.47**	**0.44**	0.25	−0.09	**0.63**	−0.17	−0.10
RG			**0.81**	**0.91**	**0.36**	**0.69**	**0.70**	0.23	0.01	**0.84**	−0.03	0.12
FI				**0.84**	**0.53**	**0.54**	**0.50**	0.24	−0.04	**0.73**	−0.08	−0.03
rFI					**0.71**	**0.73**	**0.71**	0.21	0.06	**0.90**	0.11	0.20
FCR						**0.54**	**0.47**	0.18	0.07	**0.61**	**0.32**	0.27
MC							**0.88**	**0.34**	−0.02	**0.71**	**0.75**	**0.64**
MCP								0.22	0.07	**0.69**	**0.52**	**0.83**
LC									−0.005	0.22	0.08	−0.06
FC										0.03	0.18	0.26
AC											0.19	0.25
IFCR_AMC												**0.76**

Significance levels: Bold = $P \leq 0.05$; WG = Weight gain; RG = Relative weight gain; FI = Feed intake; rFI = Relative feed intake; FCR = Feed conversion ratio, MC = $\delta^{13}C$ in muscle; MCP = $\delta^{13}C$ in muscle protein; LC = $\delta^{13}C$ in liver, FC = $\delta^{13}C$ in adipose fin; AC = $\delta^{13}C$ in adipose tissue; IFCR_AMC = Indicator variable for FCR in muscle; IFCR_AMCP = Indicator variable for FCR in muscle protein.

Table 4
Lasso-regression analysis results as obtained when regressing nine experimental variables in Table 2 (except feed intake and relative feed intake) on feed conversion ratio (FCR). In Lasso, a hybrid version of Least Angle Regression (ordinary least-squares for determination of coefficients in a second step) was used. The predicted residual error sum of squares (PRESS) criterion was used to select the final model.

Step	Source	Estimate	*F*	PRESS	Model			
					F	*P*	R^2	$\widehat{R^2}$
0	Intercept	2.27	0	0.2592	18.71	0.0001	0.31	0.24
1	AC	0.058	18.71	0.1871				

F: *F*-value; *P*: *P*-value; $\widehat{R^2}$:The coefficient of determination under prediction; AC = $\delta^{13}C$ in adipose tissue.

while nitrogen is common to all amino acids. For nitrogen, two stable variants exist; $\delta^{14}N$ and $\delta^{15}N$ (natural abundance 99.63% and 0.37%, respectively) (Lide, 2005). Likewise, carbon has two stable variants; $\delta^{12}C$ and $\delta^{13}C$ (natural abundance 98.93% and 1.07%, respectively) (Lide, 2005). Using feed with an enhanced level of certain isotopes (i.e., with altered ratios of $^{14}N/^{15}N$ and/or $^{13}C/^{12}C$) and monitoring the subsequent rate of change in isotope profile of different tissues, the relative contribution of the nutrients to protein growth can be assessed (Houlihan et al., 1995; Le Vay and Gamboa-Delgado, 2011; MacAvoy et al., 2005). After a dietary switch, say towards a diet with an enhanced natural abundance of $\delta^{13}C$, the isotopic signature of tissue samples can be used to assess the fraction of "new" protein in different tissues, using $\delta^{13}C$ (Fry, 2006) as an indicator. Changes in the isotopic composition of tissues after a change in diet occurs through two processes (Fellerhoff, 2002; Jardine et al., 2003). The first process involves the metabolic breakdown of tissues that were synthesized during feeding on the previous diet and their subsequent replacement with tissues synthesized on a new diet. Secondly, the synthesis of new tissue after a diet switch will reflect the isotopic composition of the current diet and will contribute to the overall isotopic composition of the fish. Efficient fish should be characterized by a low ratio between total synthesis (to replace degraded nutrients and synthesis of new tissue) and growth, i.e., as much as possible of the synthesis should be allocated to growth and as little as possible to replace degraded nutrients.

In a similar study on Atlantic salmon parr, Dvergedal et al. (2019b) regressed all explanatory variables simultaneously on FCR using backward elimination: The preferred model with the lowest PRESS value explained 79% of the variation in FCR. The variables retained in the model were RG, atom % ^{15}N in muscle (AMN), LC, and AC. In this study, using backward elimination, explanatory variables were able to explain 62% (R^2) of the variation in FCR (data not shown). The variables retained were RG ($F = 23.29$), FI ($F = 12.58$), MC ($F = 6.93$), and AC ($F = 16.86$). However, Pearson correlations (Table 3) showed that most of the variables had a high positive phenotypic correlation with each other. Due to the relatively high number of possible explanatory variables compared to the number of observations and collinearity between variables, we used Lasso-regression to obtain a higher resolution on which of these variables are most important for feed efficiency. The Lasso-regression analysis revealed a significant relationship between FCR and AC ($F = 18.71$ ($P < 0.0001$); Table 4), while the coefficient of determination under prediction was rather limited ($\widehat{R^2} = 0.24$), meaning that increasing the power of the experiment would likely have detected more marginal explanatory variables and increased the coefficient of determination of the prediction model. The estimated regression coefficient of AC was positive, meaning that an increased level of $\delta^{13}C$ worsened (increased) FCR. The experimental diet with an enhanced level of ^{13}C from the inclusion of maize gluten, i.e., the enhanced level of ^{13}C was largely restricted to the protein fraction of the diet. In adipose tissue, the main carbon source is lipids, but the origin of lipid carbon can be from oxidative degradation and deamination of amino acids, or carbohydrates through acetyl-CoA formed in mitochondria (Tocher, 2003). The correlation between high levels of ^{13}C in adipose tissue and FCR indicates that inefficient fish convert protein/amino acids to fat, which is expected to result in poor feed efficiency. The most expensive ingredient in fish feed is the protein source, so it is imperative that the protein source is used for muscle growth. Selecting animals that produce less visceral adipose tissue from protein would improve feed efficiency and reduce production costs, which is in accordance with previous results (Dvergedal et al., 2019b; Kause et al., 2016). Growth-efficient fish seem to utilize a low-protein turnover strategy (Carter et al., 1993; McCarthy et al., 1993), and reduced capacity for body lipid deposition is favorably associated with high protein retention efficiency (de Verdal et al., 2017).

Our results confirm that during the grow-out phase, the lipid metabolism seems to control a significant part of the variation in feed efficiency in rainbow trout, in accordance with Kause et al. (2016). At this life stage, the relative weight gain (RG) (per time unit) is expected to slow down (Davidson et al., 2014; Gjedrem and Gunnes, 1978; Santosh, 1999), and a relatively larger fraction of the feed is allocated to energy

and lipid deposition (Einen and Roem, 1997). Neely et al. (2008) showed that selection for increased growth in coho salmon (*Oncorhynchus kisutch*) resulted in improved feed efficiency with the priority of dietary lipids for energy (sparing of protein for growth), meaning that fish gained less body fat, but the relationship depended on the physiological age and feed composition. Moreover, Quinton et al. (2007) found that selection for both growth and reduced whole-body lipid percentage would accelerate the improvement in the daily gain/daily feed intake ratio, over just selection for growth alone. Further, Kause et al. (2016) have shown that, in rainbow trout of 2–3 kg body weight, selection towards low muscle lipid % and for increased growth is expected to increase the genetic response in FCR by 49% compared to selection for growth alone. These results predict that fish with genetically low body and muscle lipid percentages are more efficient by allocating the ingested protein to growth and indicate that muscle lipid percentage might be essential to consider to enhance genetic progress for feed efficiency in fish in later life stages. In this study, growth (WG and RG) correlated positively with FCR (r = 0.25 and 0.36, respectively), meaning that fast growth, on average, made fish less feed efficient. This can be explained by whole-body growth at this life stage compared to that in juvenile fish (Dvergedal et al., 2019b), being to a larger extent due to growth in the visceral adipose tissue and to a smaller extent due to muscle protein growth. Secondly, the indicator AC correlated positively with WG (r = 0.63), RG (r = 0.84), FI (r = 0.73), and rFI (r = 0.90), and thus also with FCR (r = 0.61). This confirms the relationship between increased $\delta^{13}C$ content in adipose tissue (AC) and increased FCR (adverse effect on feed efficiency).

In this study, the IFCR-variables suggested by Dvergedal et al. (2019b) showed a low phenotypic correlation to FCR (r = 0.32 for IFCR_AMC), indicating a weak relationship between metabolic efficiency and FCR during the grow-out phase in rainbow trout. As in Dvergedal et al. (2019b), the phenotypic correlation between FCR and IFCR was positive despite the sign of the phenotypic correlations between FCR and traits like growth (WG and RG) and MC were reversed compared with those in the juvenile Atlantic salmon. One of the major differences between the two data sets was the relative importance of fat deposition for feed efficiency (highly important here, but likely of limited importance in juvenile Atlantic salmon). One of the most important parameters in aquaculture is to obtain high growth. There are many factors (i.e., water temperature, photoperiod, health, genetics, adiposity, maturation, smoltification, water quality, fish size, dietary composition, and feed regime; Dessen, 2018) affecting the growth during the grow-out phase. As seen in this study, the RG had a high phenotypic correlation to the protein-to-fat synthesis in the visceral adipose tissue (r = 0.84). This high synthesis of adipose tissue in this study can be explained by fish storing more fat because they are coming closer to sexual maturation. As well as photoperiodic stimuli, the sexual maturation period requires sufficient fat and energy reserves (Kadri et al., 1996; Rowe and Thorpe, 1990; Taranger et al., 2010).

Fat extraction did not significantly affect the $\delta^{13}C$ isotope profile of the sample, and further inference was based on MC, which was estimated with a correlation to FCR of r = 0.54. This indicates that MC closely reflects body growth and maintenance in the muscle during the grow-out phase. However, body growth correlated, as mentioned, closely with carbon lipid metabolism (AC), and therefore, MC was found to have an unfavorable correlation to efficiency (FCR). The correlation between FCR and MC was more pronounced than between FCR and RG because MC is also linked to excessive turnover (maintenance) beyond what is allocated to growth. However, at this life stage, deposition efficiency through lipid metabolism is likely a more important determinant of feed efficiency, which can be measured as $\delta^{13}C$ in adipose tissue (AC). However, it remains to validate the relationship between AC and the amount of visceral fat, which would require fat measurements.

In Dvergedal et al. (2019b), LC stood out as another unique individual indicator trait for the feed conversion ratio. However, in this experiment, no association with FCR could be found, which can be explained by the fact that the liver is an organ with high metabolic activity, reaching equilibrium with the feed ($\delta^{13}C = -16.5$, Table 1) at a faster rate than muscle and adipose tissue (Table 2). Towards equilibrium, the LC stabilizes, resulting in a reduced ability to explain variation in FCR.

Phenotyping of stable isotopes at an individual level requires sampling from the muscle and adipose tissues, and this normally implies that the fish must be sacrificed prior to phenotyping. Prediction of breeding values for selection candidates thus relies on data from a separate training population (e.g., sibs). Alternatively, the phenotyping may be performed on the candidate itself if an association exists between FCR and a fin-clip to determine the isotope profiles of adipose fin samples (FC). However, no such associations were uncovered in the current experiment, meaning that carbon metabolism in FC was insignificantly correlated to all variables (Table 3). This means that selective breeding is still among un-phenotyped selection candidates. For such sib-recorded traits, genomic selection methods are substantially more efficient than classical pedigree-based methods (Vallejo et al., 2017; Ødegård et al., 2014), but individual phenotyping of the training population animals is still needed. The results suggest a potential for phenotyping feed efficiency during the grow-out phase of salmonids by feeding an experimental diet with an enhanced level of ^{13}C from the inclusion of maize gluten, and subsequently recording the level of ^{13}C in central tissues, i.e., muscle and adipose tissues. This provides an individual feed efficiency assessment that does not require individual recording of feed intake.

5. Conclusion

The study verifies that individual indicator traits based on stable isotope-derived variables can add information to individual feed efficiency in salmonids, after a dietary switch to a feed based on maize gluten with an enhanced level of ^{13}C. During the grow-out phase in rainbow trout, deposition efficiency through lipid synthesis is the most important determinant of feed efficiency, which can be assessed as a fraction of ^{13}C in adipose tissue.

Submission declaration

All authors read and approved the final manuscript. The content of the manuscript has not been published or submitted for publication elsewhere.

Authors' contributions

H.D., J.Ø., T.G., S·S., M.S., and G.K. designed the experiment. S.S and M.S. formulated and produced the diet. H.D. carried out the experiment, the sampling, and prepared samples for stable isotope analysis. H.D., J. Ø., and G.K. conducted the statistical analyses. H.D. wrote the first draft.

Declaration of Competing Interest

The authors declare they have none.

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References

Besson, M., Aubin, J., Komen, H., Poelman, M., Quillet, E., Vandeputte, M., de Boer, I.J. M., 2016. Environmental impacts of genetic improvement of growth rate and feed conversion ratio in fish farming under rearing density and nitrogen output

limitations. J. Clean. Prod. 116, 100–109. https://doi.org/10.1016/j.jclepro.2015.12.084.
Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911–917. https://doi.org/10.1139/o59-099.
Bolliet, V., Cheewasedtham, C., Houlihan, D., Gélineau, A., Boujard, T., 2000. Effect of feeding time on digestibility, growth performance and protein metabolism in the rainbow trout *Oncorhynchus mykiss*: interactions with dietary fat levels. Aquat. Living Resour. 13, 107–113. https://doi.org/10.1016/S0990-7440(00)00147-9.
Boujard, T., Leatherland, J.F., 1992. Circadian pattern of hepatosomatic index, liver glycogen and lipid content, plasma non-esterified fatty acid, glucose, T_3, T_4, growth hormone and cortisol concentrations in *Oncorhynchus mykiss* held under different photoperiod regimes and fed using demand-feeders. Fish Physiol. Biochem. 10, 111–122. https://doi.org/10.1007/BF00004522.
Carter, C., Houlihan, D., Brechin, J., McCarthy, I., 1993. The relationships between protein intake and protein accretion, synthesis, and retention efficiency for individual grass carp, *Ctenopharyngodon idella* (Valenciennes). Can. J. Zool. 71, 392–400. https://doi.org/10.1139/z93-055.
Craig, H., 1957. Isotopic standards for carbon and oxygen and correction factors for mass-spectrometric analysis of carbon dioxide. Geochim. Cosmochim. Acta 12, 133–149. https://doi.org/10.1016/0016-7037(57)90024-8.
Davidson, J., Kenney, P., Manor, M., Good, C., Weber, G., Aussanasuwannakul, A., Summerfelt, S.T., 2014. Growth performance, fillet quality, and reproductive maturity of rainbow trout (*Oncorhynchus mykiss*) cultured to 5 kilograms within freshwater recirculating systems. J. Aquacul. Res. & Develop. 5, 1. https://doi.org/10.4172/2155-9546.1000238.
de Verdal, H., Narcy, A., Bastianelli, D., Chapuis, H., Même, N., Urvoix, S., Mignon-Grasteau, S., 2011. Improving the efficiency of feed utilization in poultry by selection. 2. Genetic parameters of excretion traits and correlations with anatomy of the gastro-intestinal tract and digestive efficiency. BMC Genet. 12, 71. https://doi.org/10.1186/1471-2156-12-71.
de Verdal, H., Mekkawy, W., Lind, C.E., Vandeputte, M., Chatain, B., Benzie, J.A.H., 2017. Measuring individual feed efficiency and its correlations with performance traits in Nile tilapia, *Oreochromis niloticus*. Aquaculture 468, 489–495. https://doi.org/10.1016/j.aquaculture.2016.11.015.
de Verdal, H., Komen, H., Quillet, E., Chatain, B., Allal, F., Benzie, J.A., Vandeputte, M., 2018a. Improving feed efficiency in fish using selective breeding: a review. Rev. Aquac. 10, 833–851. https://doi.org/10.1111/raq.12202.
de Verdal, H., Vandeputte, M., Mekkawy, W., Chatain, B., Benzie, J.A.H., 2018b. Quantifying the genetic parameters of feed efficiency in juvenile Nile tilapia *Oreochromis niloticus*. BMC Genet. 19, 105. https://doi.org/10.1186/s12863-018-0691-y.
Dessen, J.-E., Norges miljø- og biovitenskapelige universitet Institutt for husdyr- og akvakulturvitenskap, 2018. Growth, feed utilization, health and biometric parameters in Atlantic salmon (*Salmo salar* L.) : influence of dietary protein-to-lipid ratio and body fat status. Department of Animal and Aquacultural Sciences, Faculty of Biosciences, Norwegian University of Life Sciences, p. 103. https://hdl.handle.net/11250/2834483.
Dvergedal, H., Ødegård, J., Mydland, L.T., Øverland, M., Hansen, J.Ø., Ånestad, R.M., Klemetsdal, G., 2019a. Stable isotope profiling for large-scale evaluation of feed efficiency in Atlantic salmon (*Salmo salar*). Aquac. Res. 50, 1153–1161. https://doi.org/10.1111/are.13990.
Dvergedal, H., Ødegård, J., Øverland, M., Mydland, L.T., Klemetsdal, G., 2019b. Selection for feed efficiency in Atlantic salmon using individual indicator traits based on stable isotope profiling. Genet. Sel. Evol. 51, 13. https://doi.org/10.1186/s12711-019-0455-9.
Einen, O., Roem, A.J., 1997. Dietary protein/energy ratios for Atlantic salmon in relation to fish size: growth, feed utilization and slaughter quality. Aquac. Nutr. 3, 115–126. https://doi.org/10.1046/j.1365-2095.1997.00084.x.
Fellerhoff, C., 2002. Feeding and growth of apple snail *Pomacea lineata* in the Pantanal wetland, Brazil-a stable isotope approach. Isot. Environ. Health Stud. 38, 227–243.
Fry, B., 2006. Isotope notation and measurement. In: Fry, B. (Ed.), Stable Isotope Ecology. Springer, New York, pp. 21–39.
Gannes, L.Z., Martínez del Rio, C., Koch, P., 1998. Natural abundance variations in stable isotopes and their potential uses in animal physiological ecology. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 119 (3), 725–737. https://doi.org/10.1016/S1095-6433(98)01016-2.
Gjedrem, T., 2005. Selection and Breeding Programs in Aquaculture. Springer, Dordrecht.
Gjedrem, T., Baranski, M., 2010. Selective Breeding in Aquaculture: An Introduction. Springer Science & Business Media, Berlin.
Gjedrem, T., Gunnes, K., 1978. Comparison of growth rate in Atlantic salmon, pink salmon, Arctic char, sea trout and rainbow trout under Norwegian farming conditions. Aquaculture 13, 135–141. https://doi.org/10.1016/0044-8486(78)90107-2.
Hastie, T., Tibshirani, R., Friedman, J., 2009. The Elements of Statistical Learning. Data Mining, Inference, and Prediction. Springer, New York.
Hayes, B.J., Bowman, P.J., Chamberlain, A.J., Goddard, M.E., 2009. Invited review: genomic selection in dairy cattle: Progress and challenges. J. Dairy Sci. 92, 433–443. https://doi.org/10.3168/jds.2008-1646.
Helland, S.J., Grisdale-Helland, B., Nerland, S., 1996. A simple method for the measurement of daily feed intake of groups of fish in tanks. Aquaculture 139 (1), 157–163. https://doi.org/10.1016/0044-8486(95)01145-5.
Houlihan, D.F., Carter, C.G., McCarthy, I.D., 1995. Chapter 8 protein synthesis in fish. In: Hochachka, P.W., Mommsen, T.P. (Eds.), Biochemistry and Molecular Biology of Fishes. Elsevier Press, Amsterdam, pp. 191–220.
Jardine, T.D., McGeachy, S.A., Paton, C.M., Savoie, M., Cunjak, R.A., 2003. Stable Isotopes in Aquatic Systems: Sample Preparation. Analysis and Interpretation, Canadian Manuscript Report of Fisheries and Aquatic Sciences, No, p. 2656.
Jobling, M., Covès, D., Damsgård, B., Kristiansen, H.R., Koskela, J., Petursdottir, T.E., Gudmundsson, O., 2001. Techniques for measuring feed intake. In: Houlihan, D., Boujard, T., Jobling, M. (Eds.), Food Intake in Fish. Wiley-Blackwell, Oxford, pp. 49–87.
Kadri, S., Mitchell, D.F., Metcalfe, N.B., Huntingford, F.A., Thrope, J.E., 1996. Differential patterns of feeding and resource accumulation in maturing and immature Atlantic salmon, *Salmo salar*. Aquaculture 142, 245–257. https://doi.org/10.1016/0044-8486(96)01258-6.
Kause, A., Tobin, D., Dobly, A., Houlihan, D., Martin, S., Mäntysaari, E.A., Ruohonen, K., 2006. Recording strategies and selection potential of feed intake measured using the X-ray method in rainbow trout. Genet. Sel. Evol. 38, 389. https://doi.org/10.1186/1297-9686-38-4-389.
Kause, A., Kiessling, A., Martin, S.A.M., Houlihan, D., Ruohonen, K., 2016. Genetic improvement of feed conversion ratio via indirect selection against lipid deposition in farmed rainbow trout (*Oncorhynchus mykiss* Walbaum). Br. J. Nutr. 116, 1656–1665. https://doi.org/10.1017/S0007114516003603.
Kolstad, K., Grisdale-Helland, B., Gjerde, B., 2004. Family differences in feed efficiency in Atlantic salmon (*Salmo salar*). Aquaculture 241, 169–177. https://doi.org/10.1016/j.aquaculture.2004.09.001.
Le Vay, L., Gamboa-Delgado, J., 2011. Naturally-occuring stable isotopes as direct measures of larval feeding efficiency, nutrient incorporation and turnover. Aquaculture 315, 95–103.
Lide, D.R., 2005. CRC Handbook of Chemistry and Physics, 85 ed. CRC Press, Boca Raton.
Logan, J.M., Jardine, T.D., Miller, T.J., Bunn, S.E., Cunjak, R.A., Lutcavage, M.E., 2008. Lipid corrections in carbon and nitrogen stable isotope analyses: comparison of chemical extraction and modeling methods. J. Anim. Ecol. 77, 838–846. https://doi.org/10.1111/j.1365-2656.2008.01394.x.
MacAvoy, S.E., Macko, S.A., Arneson, L.S., 2005. Growth versus metabolic tissue replacement in mouse tissues determined by stable carbon and nitrogen isotope analysis. Can. J. Zool. 83, 631–641.
McCarthy, I.D., Carter, C.G., Houlihan, D.F., 1992. The effect of feeding hierarchy on individual variability in daily feeding of rainbow trout, *Oncorhynchus mykiss* (Walbaum). J. Fish Biol. 41, 257–263. https://doi.org/10.1111/j.1095-8649.1992.tb02655.x.
McCarthy, I.D., Houlihan, D.F., Carter, C.G., Moutou, K., 1993. Variation in individual food consumption rates of fish and its implications for the study of fish nutrition and physiology. Proc. Nutr. Soc. 52, 427–436. https://doi.org/10.1079/PNS19930083.
McCleary, B.V., Solah, V., Gibson, T.S., 1994. Quantitative measurement of total starch in cereal flours and products. J. Cereal Sci. 20, 51–58. https://doi.org/10.1006/jcrs.1994.1044.
Neely, K.G., Myers, J.M., Hard, J.J., Shearer, K.D., 2008. Comparison of growth, feed intake, and nutrient efficiency in a selected strain of coho salmon (*Oncorhynchus kisutch*) and its source stock. Aquaculture 283, 134–140. https://doi.org/10.1016/j.aquaculture.2008.06.038.
Ødegård, J., Moen, T., Santi, N., Korsvoll, S.A., Kjøglum, S., Meuwissen, T.H.E., 2014. Genomic prediction in an admixed population of Atlantic salmon (*Salmo salar*). Front. Genet. 5, 402. https://doi.org/10.3389/fgene.2014.00402.
Pinnegar, J.K., Polunin, N.V.C., 1999. Differential fractionation of $\delta^{13}C$ and $\delta^{15}N$ among fish tissues: implications for the study of trophic interactions. Funct. Ecol. 13, 225–231. https://doi.org/10.1046/j.1365-2435.1999.00301.x.
Post, D.M., Layman, C.A., Arrington, D.A., Takimoto, G., Quattrochi, J., Montana, C.G., 2007. Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia 152, 179–189. https://doi.org/10.1007/s00442-006-0630-x.
Quinton, C.D., Kause, A., Ruohonen, K., Koskela, J., 2007. Genetic relationships of body composition and feed utilization traits in European whitefish (*Coregonus lavaretus* L.) and implications for selective breeding in fishmeal- and soybean meal-based diet environments. J. Anim. Sci. 85, 3198–3208. https://doi.org/10.2527/jas.2006-792.
Rowe, D.K., Thorpe, J.E., 1990. Suppression of maturation in male Atlantic Salmon (Salmo salar L.) Parr by reduction in feeding and growth during spring months. Aquaculture 86, 291–313. https://doi.org/10.1016/0044-8486(90)90121-3.
Santosh, P.L., 1999. Atlantic salmon, *Salmo salar* Linnaeus, 1758. http://www.fao.org/fileadmin/user_upload/affris/img/pdf/FAO_Fisheries__Aquaculture_-_AFFRIS_-_Salmo_salar_Linnaeus_1758_.pdf (accessed 15 February 2022).
Shomorin, G.O., Storebakken, T., Kraugerud, O.F., Øverland, M., Hansen, B.R., Hansen, J.Ø., 2019. Evaluation of wedge wire screen as a new tool for faeces collection in digestibility assessment in fish: the impact of nutrient leaching on apparent digestibility of nitrogen, carbon and Sulphur from fishmeal, soybean meal and rapeseed meal-based diets in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 504, 81–87. https://doi.org/10.1016/j.aquaculture.2019.01.051.
Staddon, P.L., 2004. Carbon isotopes in functional soil ecology. Trends Ecol. Evol. 19 (3) https://doi.org/10.1016/j.tree.2003.12.003.
Taranger, G.L., Carrillo, M., Schulz, R.W., Fontaine, P., Zanuy, S., Felip, A., Hansen, T., 2010. Control of puberty in farmed fish. Gen. Comp. Endocrinol. 165, 483–515. https://doi.org/10.1016/j.ygcen.2009.05.004.
Thodesen, J., Grisdale-Helland, B., Helland, S.J., Gjerde, B., 1999. Feed intake, growth and feed utilization of offspring from wild and selected Atlantic salmon (*Salmo salar*). Aquaculture 180, 237–246. https://doi.org/10.1016/S0044-8486(99)00204-5.
Thodesen, J., Gjerde, B., Grisdale-Helland, B., Storebakken, T., 2001. Genetic variation in feed intake, growth and feed utilization in Atlantic salmon (*Salmo salar*). Aquaculture 194, 273–281. https://doi.org/10.1016/S0044-8486(00)00527-5.

Tocher, D.R., 2003. Metabolism and functions of lipids and fatty acids in teleost fish. Rev. Fish. Sci. 11 (2), 107–184. https://doi.org/10.1080/713610925.
Vallejo, R.L., Leeds, T.D., Gao, G., Parsons, J.E., Martin, K.E., Evenhuis, J.P., Palti, Y., 2017. Genomic selection models double the accuracy of predicted breeding values for bacterial cold water disease resistance compared to a traditional pedigree-based model in rainbow trout aquaculture. Genet. Sel. Evol. 49, 17. https://doi.org/10.1186/s12711-017-0293-6.
Wessels, F.J., Hahn, D.A., 2010. Carbon 13 discrimination during lipid biosynthesis varies with dietary concentration of stable isotopes: implications for stable isotope analyses. Funct. Ecol. 24, 1017–1022. https://doi.org/10.1111/j.1365-2435.2010.01716.x.