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Two-way selection for muscle lipid content in pan-size rainbow trout (*Oncorhynchus mykiss*)

Edwige Quillet[a,*], Sandrine Le Guillou[a], Joël Aubin[b], Benoît Fauconneau[c]

[a]*INRA, Laboratoire de Génétique des Poissons, 78352, Jouy-en-Josas Cedex, France*
[b]*SEMII, Barrage du Drennec, 29450 Sizun, France*
[c]*INRA, Station SCRIBE, Campus de Beaulieu, 35042 Rennes Cedex, France*

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Abstract

A two-way selection for muscle lipid content was performed in rainbow trout. One-year-old pan-size individuals were mass selected using Distell Fish Fatmeter values corrected for absolute weight (fat index, FI), this being a non-destructive measurement of the muscle lipid content in live fish. Direct and correlated responses at pan-size (around 260 g) were estimated after two generations of selection. Absolute Fat values and FI values in the upward line (fat line, FL) and downward line (lean line, LL) differed from the control line and from each other. The mean realized heritability achieved for FI was 0.25. The difference in Fat values between FL and LL resulted in a significant difference in muscle lipid content (29.6% dry matter content in FL, vs. 25.6% in LL, P=0.003). The differences in Fat and FI values between FL and LL were sustained until maturation at 2 years old. At the age of 1 year, the two lines did not differ significantly in terms of weight, length or most body shape traits (height, width or cross-section shape). However, FL fish had a better condition factor than LL (1.44 vs. 1.38), and a greater belly thickness (11% relative increase), the control line displaying intermediate values. The abdominal internal fat coat was thicker in FL, and represented a larger proportion of the entire abdominal thickness than in LL. The relative visceral weight (in % of total body weight), carcass and fillet yield were not modified by selection. The frequency of precocious males was significantly higher in FL (16.5%, vs. 11% and 10.1% in control and LL fish, respectively), but there was no difference between the reproductive traits of 2-year-old LL and FL females. These results indicate that breeding based on Fatmeter values was efficient in selecting the fillet lipid content in rainbow trout, with limited correlated responses at the size where selection was applied. In particular, no adverse effects were recorded of a reduction in muscle lipid content on carcass or fillet yield under the experimental conditions applied during this study.

Keywords: Rainbow trout; *Oncorhynchus mykiss*; Mass selection; Fatmeter; Lipid content; Carcass traits; Body composition; Reproduction

* Corresponding author. Tel.: +33 1 34 65 23 93; fax: +33 1 34 65 23 90.
E-mail address: edwige.quillet@jouy.inra.fr (E. Quillet).

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1. Introduction

Alongside growth rate and slaughtering weight, quality traits (carcass and fillet yields, flesh colour, chemical composition) have, in the past 10 years, become of considerable importance to the aquaculture industry (Alsted et al., 1995; Gjedrem, 1997). In salmonids, body and fillet lipid contents are important attributes, influencing both the technical and sensory characteristics of the product. High body lipid content is usually associated with a higher viscero-somatic index, and a reduction in carcass yield. The fillet lipid content is of major importance to flesh texture and flavour (Johansson et al., 2000; Morkore et al., 2001). Many studies have concluded that the whole body lipid content can easily be modified by controlling the feeding regime (Shearer et al., 1997; Jobling et al., 1998; Johansson et al., 2000). However, increasing the lipid supply in the diet results in increased fat deposition throughout the body, in both consumable parts of the fish (i.e. trimmed fillet) and in 'waste' compartments such as the viscera and abdominal belly wall (Weatherup et al., 1997; Jobling et al., 1998). On the other hand, reducing the lipid and energy content of the diet reduces growth and increases nitrogen excretion (Steffens et al., 1999). The genetic control of body composition and fatness is an alternative means of improving body and flesh characteristics.

In salmonids, heritabilities have been estimated for a number of traits related to body composition and carcass quality, such as carcass yield, fillet yields, body shape, flesh composition and colour (Kinghorn, 1983; Gjerde and Schaeffer, 1989; Iwamoto et al., 1990; Gjedrem, 1992, 1997; Elvingson and Johansson, 1993; Elvingson and Nilsson, 1994; Rye and Gjerde, 1996; Kause et al., 2002, 2003). With the exception of some traits displaying heritabilities close to zero (percent fillet protein, ash and water), most estimates are positive and of medium to high magnitude.

As far as lipids are concerned, two tissues are usually considered, the viscera and fillet, as being the most important with respect to marketability and product quality. In salmonids, the most marked variation in viscera weight is due to differences in visceral fat deposits (Gjerde and Schaeffer, 1989; Elvingson and Johansson, 1993). According to the figures on body composition published by Hoffman et al. (1999) for hatchery lake trout (1 kg), the viscera and muscle each contribute about 30% to the whole-body total lipid content.

Viscera weight and abdominal fat deposition exhibit moderate heritability (0.22 to 0.28, Rye and Gjerde, 1996 in salmon; Gjerde and Schaeffer, 1989, Kause et al., 2002 in rainbow trout). Some studies have produced relatively low estimates of the heritability (0.2 or less) of meat fat content in coho salmon (Iwamoto et al., 1990) and Arctic charr (Elvingson and Nilsson, 1994). However, most estimates regarding Atlantic salmon and rainbow trout were fairly high. In rainbow trout, a value of 0.47 was found by both Kinghorn (1981 cited in Gjedrem, 1997, but only mentioned in Kinghorn, 1983) and by Gjerde and Schaeffer, 1989. For Atlantic salmon, the values were 0.3 (Rye and Gjerde, 1996) and 0.46 (Rye cited in Gjedrem, 1997). Thus, the prospects of achieving a rapid genetic gain in muscle fat content by the selection of salmonids appear quite hopeful.

However, limitations to including flesh components into breeding schemes arise from the difficulty of phenotyping the large numbers of animals required. Chemical analyses, as well as the NMR techniques recently developed (Toussaint et al., 2002) remain time-consuming, cannot be used in the field, and above all require that fish should be sacrificed, thus rendering the individual evaluation of breeding candidates impossible.

Attempts have been made to develop non-destructive measurements of body lipid content in fish. X-ray tomography has been developed in several species (Rye, 1991; Jopson et al., 2002; Kolstad et al., 2004), but it is difficult to apply in the field. Alternative methods, such as body electric conductivity (Novinger and Martinez Del Rio, 1999; Hancz et al., 2003), near infrared reflectance spectroscopy (Gjerde and Martens, 1987) or morphometrics (Simpson et al., 1992; Kora et al., 2000; Rikardsen and Johansen, 2003) have been tested. For example, condition factor has been proposed as an indirect indicator of lipid or energy status in salmonids (Herbinger and Friars, 1991), but some results have called its usefulness into question (Johansen and Jobling, 1998; Shearer and Swanson, 2000; Rikardsen and Johansen, 2003), especially when the muscle lipid content, rather than the whole body energy content, is targeted. An inexpensive and portable method was proposed by

Kent (1990). The transmission of micro-waves depends on the composition of tissues, and especially on the tissue water content. The Distell Fish Fatmeter is based on this principle. Calibration equations established for different fish species are used to deduce the lipid content from the water content value (Distell.com, 2001). In rainbow trout, the conditions for use and accurate measurements using the Fatmeter on whole fish were described in detail by Douirin et al. (1998). Moreover, high estimates of the heritability of Fatmeter values have been published in this species (Chevassus et al., 2002).

In this paper, we present the results of experimental two-way mass selection for muscle lipid content in pan-sized rainbow trout. Selection was performed over two generations with the aim of evaluating the efficiency of Fatmeter values as a non-destructive predictive trait for selection. Direct and correlated responses for some major productive features (growth, body shape and composition, reproductive characteristics) were examined.

2. Materials and methods

2.1. Selection process

Two-way selection for muscle lipid content was performed during two successive generations of the spring-spawning INRA experimental strain of rainbow trout (G0), at the INRA experimental fish farm (SEDI, Finistère, France). The pre-dorsal muscle lipid content was measured using a Distell Fish Fatmeter, according to the method described by Douirin et al. (1998), the probe being placed over the dorsal muscle, anterior to the dorsal fin and above the lateral line. A positive correlation between the percent lipid content of the flesh and body weight has been described (Alsted et al., 1995; Johansen and Jobling, 1998), and was also recorded in our strain (R^2=0.4). Therefore, the absolute value provided by the Fatmeter (referred to as 'Fat') had to be adjusted for the underlying allometric relationship between the two traits. The relative Fat Index, FI=Fat/log (W), where W is the body weight of the fish, was chosen as the selection operating trait which enabled the best correction for weight effect. Selection was applied to 1-year-old fish, in order to avoid the lipid mobilization towards gonad growth observed in older fish (Shearer, 1994). The few males that had matured at the age of 1 year were discarded. The fish were fed with commercial pellets (containing about 22% lipids). Selection was performed within candidate groups of more than 800 individuals (Table 1). Before each selection process, the distribution curve of FI and the thresholds for about 10% upward and/or downward selection were determined by the random sampling of 100 individuals from each of the candidate populations (G0, or fat or lean G1 lines). Some of the selected fish could not be used at time of reproduction (dead or immature individuals), so that a minimum of 65 to 120 breeders were used at each step of selection to produce the next generation, according to the following mating system: equal volumes of ova from several females were mixed, and each mixture was fertilized with a mixture

Table 1
Means, standard deviation (S.D.) and coefficient of variation (CV, %) of FI, the selected trait, selection thresholds and pressure of selection in the two first generations of selection

Generation of selection	Characteristics of FI in one year old fish	
G0	Initial number of fish: 1024 Mean (S.D.): 1.30 (0.57) CV=43.8	
	Lean line	Fat line
Selection of G1 parents	Downward threshold value: 0.56 Percent selected: 11.8%	Upward threshold value: 2.16 Percent selected: 8.7%
G1	Initial number of fish : 809 Mean (S.D.): 1.67 (0.48) CV=28.7	Initial number of fish : 866 Mean (S.D.): 2.17 (0.58) CV=26.7
Selection of G2 parents	Downward threshold value: 1.13 Percent selected: 12%	Upward threshold value: 2.50 Percent selected: 13.8%
G2	Mean (S.D.): 0.99 (0.29) CV=29.3	Mean (S.D.): 1.50 (0.44) CV=29.3

The control line was no more recorded during the selection process (G1 and G2 steps).
G2 fish were used at 2 years old as breeders of the LL and FL experimental groups used in the present study.

of milt of approximately the same number of males. The milts (equal volume from each male) were mixed more than 1 h before fertilization, so as to equalize the genetic contribution of the different males (Withler and Beacham, 1994). Details about the selection scheme and the evolution of FI values in successive generations are given in Table 1. Data show that the relative distance between fat and lean line mean values increased from G1 to G2 (it represented 26% and 41% of the between-line mean, respectively). Yet, data also indicate that the FI absolute mean and relative variability changed substantially from one generation to the other. In particular, G1 fish had mean FI values much higher than G0 or G2 values, whatever the line (fat or lean). We suspect that variations in the thermal regime the fish were exposed to during the period of time that preceded records have contributed to the year to year variation of mean Fat values (G1 fish were reared during a quite mild winter). The increasing experience of the staff may also have induced progressive changes in the husbandry, and modify performances. Whatever the reasons, the large year to year variations impaired a proper estimation of response to selection on the basis of the comparison of groups reared at different periods. Thus, a special spawning was done to perform appropriate comparison of the two selected lines and the control, all being reared in the same conditions. The experiment aimed at measuring the response after 2 generations of selection, and was therefore performed using G2 fish as parents of the experimental progeny.

2.2. Measurement of response to selection

The study was performed at the SEMII experimental farm (Finistère, France). Three experimental groups were produced. The Lean (LL) and Fat (FL) lines were generated using G2 fish as breeders. The control line (C) was sampled from the original spring-spawning strain (standing for G0). For each line (LL, FL and C), about 30 females and 40 to 50 males were used as breeders. Within each line, an equal volume of ova was used from each female, and fertilizations were performed with a mixture of milt from the different males (Day 0). Mixture of milt was prepared as described above. The experimental groups were constituted at the eyed stage, just before hatching. The experimental design involved three replicated tanks for each selected line (LL and FL), and two replicated tanks for the C line. The experiment started with 500 eyed eggs per tank.

Survival and growth were regularly monitored, and the fish were automatically fed in excess of predicted needs with commercial pellets. The response to selection was measured on 1-year-old individuals (Day 341), that had received a diet containing 45% protein and 20% lipids for their last 6 months of growth. The fish were sacrificed (lethal dose of anaesthetic) and a number of traits were recorded. *Growth* was measured by body weight (W, in g) and fork length (L, in mm). Several traits related to *body shape* were recorded: pre-dorsal fin height (H, in mm), pre-dorsal fin width (W_d, in mm), both measured using a calliper (Fig. 1). The condition factor ($K=10^5*W/L^3$) and cross-section shape ($SS=H/W_d$) were derived from these values. Finally, traits related to *body compartments* were recorded: carcass weight (W_C, in g), viscera weight (V=digestive tract with fat and spleen, in g), liver weight (liv, in g) and total (untrimmed) fillet weight (W_F). The following ratios were derived: viscero-somatic index ($VSI=100*V/W$), hepato-somatic index ($HSI=100*liv/W$), carcass yield ($CY=100*W_C/W$) and fillet yield ($FY=100*W_F/W$). Gonadic sex was recorded. Precocious maturing males were recorded, but discarded from the measurements. The abdominal wall thickness was measured using a Toshiba and Hospimedi 100 LC ultrasound scanner (7.5 MHz probe frequency). Both total belly thickness (T_T) and 'muscle' belly thickness (T_M) were measured, as shown in Fig. 1. The thickness of the internal fat coat (T_{FC}) was calculated as the difference between these values (T_T-T_M) (Fig. 1). About 100 individuals were sampled in each tank (total numbers of observations for the different traits are given in Table 2).

Traits related to the *muscle lipid content* were Fat values and FI, as previously described. In the LL and FL lines, fillets from 15 to 17 individuals per tank were randomly sampled, skinned and immediately frozen. They were sent to a private company (Cervac Midi Atlantique, 64500 Saint-Jean-de-Luz, France), for determination of the lipid content in each individual fillet, the soxhlet method being used for extraction. Unfortunately, because of a misunder-

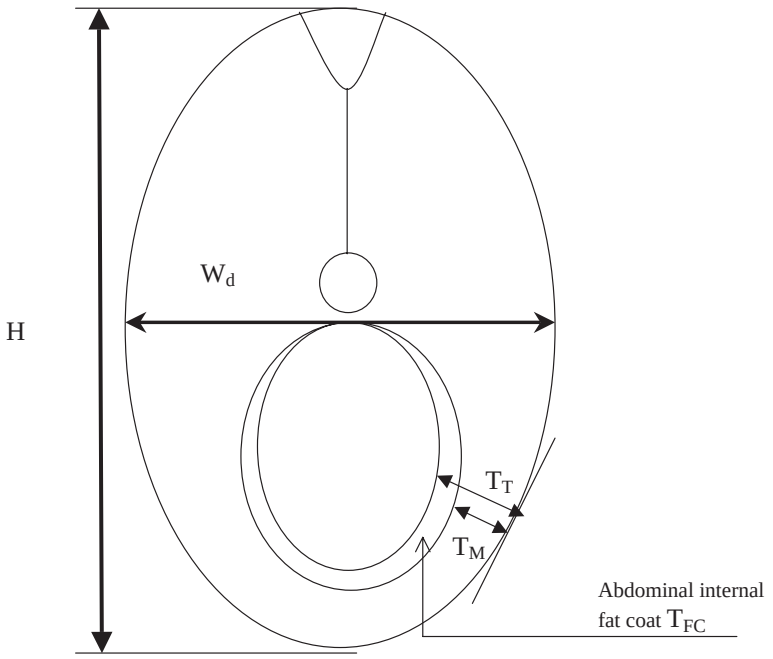

Fig. 1. Cross-section anterior to the dorsal fin showing the body shape and belly thickness traits observed in experimental groups to measure the correlated response to selection for muscle lipid content (H: pre-dorsal fin body height, W_d: pre-dorsal body width, T_T: total belly thickness, T_M: 'muscle' belly thickness, T_{FC}: thickness of abdominal internal fat coat).

standing, lipid values (Lip) were provided as a percent dry matter only, and not as a percent fresh matter.

A sample of fish from the LL and FL lines was kept for further study of their *reproduction traits*. About ninety fish per line (30 per replicate) were grouped in a single tank (tagging by adipose fin clipping). At Day 433, growth (L, W) and lipid muscle content (Fat and FI) were measured in 50 fish per line. At Day 593, males that were already recognizable were discarded in order to limit the biomass. The remaining fish were individually PIT tagged in order to further distinguish the females, and L, W, Fat and FI were measured once again. At spawning time, the last males were discarded, and 38 and 34 mature females remained in the LL and FL lines, respectively. Females were sorted every week from mid-March to mid-May (Day 692 to Day 734). Eggs were taken from each spawning female and the following traits were recorded: total body weight before spawning (W_B), L, Fat and FI, W_C, V, liv as previously described, total weight of dripped ova (W_O in g), mean weight of ova (w, in mg, estimated by weighing to 50 ova-samples). Derived traits were the gonado-somatic index (GSI=100*W_O/W_B) and the number of ova per female (N_O=W_O/w). For each female, a batch of 200 to 300 ova was fertilized with a mixture of milt from several males, and survival was recorded at the following stages: fertilization (100 °C×day), late eyed stage, and end of yolk–sac resorption. Survival was expressed as a percentage of fertilized ova.

At the ovulation peak, a 5 g sample of ova was collected from 7 and 8 females in FL and LL lines, respectively, and frozen for further chemical analysis of the lipid content (Folch et al., 1957).

2.3. Statistical analyses

Statistical analyses were performed using the SAS® GLM procedure. Angular transformation was applied to proportions (Lip, VSI, HSI, CY, FY and GSI). Analyses were performed using the mixed ANOVA model:

$$P = \mu + S + L + r(L) + e \qquad \text{(M1)}$$

Table 2
Total number of observations (n), phenotypic mean, standard deviations (S.D.) and coefficient of variation (CV, %) for observed traits at Day 341

Traits	n	Pooled mean[a]	Pooled S.D.[a]	Pooled CV[a]
Muscle lipid content				
Fat	741	3.33	0.83	24.7
FI	741	1.39	0.33	23.7
Lip (%DM)[b]	96	27.6	6.1	22.1
Growth				
Length (mm)	742	261	18.9	7.3
Weight (g)	741	256	63.3	24.8
Body shape				
K	741	1.40	0.13	9.5
H (mm)	742	65.2	6.6	10.4
W_d (mm)	742	30.6	3.3	10.7
SS	742	2.14	0.13	6.0
Belly thickness				
T_T (mm)	742	4.15	0.61	14.7
T_M (mm)	742	3.55	0.56	15.8
T_{FC} (mm)	742	0.60	0.13	22.3
Body compartments				
Viscera weight (V, g)	742	23.4	7.2	30.6
VSI (%)	741	9.1	1.3	13.9
Liver weight (liv, g)	742	2.9	0.9	31.2
HSI (%)	741	1.14	0.17	15.2
Carcass weight (W_C, g)	742	227	56	24.7
CY (%)	741	88.7	1.7	1.9
Fillet weight (W_F, g)	674	81.5	21.0	25.8
FY (%)	674	64.3	6.1	9.4

K: condition factor, H: pre-dorsal fin height, W_d: pre-dorsal fin width, SS: cross-section shape, T_T: total belly thickness, T_M: muscular belly thickness, T_{FC}: thickness of internal fat coat; V, liv, W_C and W_F: absolute weight of viscera, liver, carcass and fillet, with their corresponding ratios to total body weight (see details in the text).

[a] Mean of LL, C and FL mean values.

[b] Values available only for LL and FL lines.

where P is the individual value of the trait, μ is the overall mean, S is the fixed effect of sex (immature male or immature female), L is the fixed effect of the line (LL, FL or C), $r(L)$ is the random tank effect within the line (3 tanks for L and F lines, 2 tanks for C line), and e is the within-tank residual term. The sex-line interaction I_{S*L} was never significant and was therefore removed from the model.

However, most traits were correlated with fish size. Thus, in a second step, and in order to correct for size difference when necessary, comparisons between lines were performed using the following ANCOVA model:

$$P = \mu + S + L + r(L) + a\mathrm{Ln}(\mathrm{size}) + e \qquad \text{(M2)}$$

where P is the log-transformed trait value, μ, S, L, $r(L)$ and e are the same as described above, 'size' is either W or L (depending on traits), and a is the common allometry regression slope of the trait on size. Length was used as the covariate for body shape length traits (H, W_d), and weight was used as the covariate for belly thickness traits. Model M2 was also used for a detailed analysis of cross-section shape (W_d analysed with H as the covariate) and belly thickness traits (T_M and T_{FC} analysed with T_T as the covariate). The homogeneity of slopes was checked before ANCOVA analyses were performed. Tests for significance of line effect and differences between lines were performed using $r(L)$ as the error term.

To ensure that the use of ratios (VSI, HSI, CY and FY) provided adequate correction for size effects, ANCOVA was also performed on the V, liv, W_C and W_F values, with W as the covariate. The results were the same as those obtained using the link M1 model (not shown). Thus, the customary ratios were used.

Male maturation rates were compared using the chi-square test with a correction for continuity. ANOVA was applied using the following model for performance (L, W, Fat, FI) at Day 433, Day 593 and at spawning time and for female reproduction traits: $P=\mu+L+e$, where P is the trait (arc–sin transformed for ratios and survivals), L the fixed line effect (LL or FL) and e, the within-line residual (individual fish of unknown sex at Day 433 or females only later on).

3. Results

Overall means and standard deviations of the main traits are summarized in Table 2.

3.1. Effect of sex at Day 341

Males were significantly heavier than females (263 g vs. 250 g, $P<0.05$), and had a higher liver weight (+9%, $P<0.001$) and HIS (+4%, $P<0.001$). There was no difference between sexes for traits related to muscle composition (Fat, FI, Lip). Whatever the trait, the sex*line interaction was never significant.

3.2. Muscle lipid content at Day 341

The LL, control and FL lines differed significantly with respect to Fat values, with LL being the lowest, FL the highest and controls being intermediate (Fig. 2). The difference remained after correction for underlying differences in weight, and FI was significantly different between the three groups, with the same ranking. Mean FI values were 1.08, 1.37 and 1.72 for LL, C and FL lines, respectively. Corresponding standard deviations were 0.22 in LL line, and 0.39 in both C and FL lines. Thus, C control exhibited the highest within group variation (coefficient of variation was 28.5%, vs. 20.4 and 22.7 in LL and FL lines, respectively). Changes to FI mean values corresponded to 12% gain per generation.

Realized heritability h_r^2 was estimated using the standard formula $R=ih_r^2\sigma$, where R is the response to selection, i, the cumulative intensity of selection known from the proportion selected during the selection process, and σ, the phenotypic standard deviation of the trait. R was estimated as the difference either between values for FI in the LL and FL lines, or between the values for the C line and each of the other two. The standard deviation of C control rather than the standard deviation in G0 was used to estimate σ, because it was recorded under the same experimental conditions as the between lines response. The resulting mean h_r^2 value was 0.25 (0.22 and 0.27 in the LL and FL lines, respectively, which is quite similar).

Lip values in percent dry matter indicated that selection had actually modified the muscle composition (Fig. 2). Assuming an equal response to upwards and downwards selection, the correlated changes in Lip associated with selection on FI values corresponded to a 4% gain per generation.

3.3. Growth and body shape at Day 341

Comparisons of weight and length revealed no significant differences between the LL and FL lines (Table 3). Thus, a comparison between the two lines for other traits could have been performed with simple ANOVA. However, the control group was significantly smaller, and ANCOVA with 'size' as a covariate was required to perform overall comparisons. For LL and FL comparisons, ANCOVA and ANOVA provided consistent results (ANOVA not shown).

The relationship between length and weight was modified by selection in both lines, as shown by the differences in K values, and the calculated values of weight using the length cubed as the covariate. For a

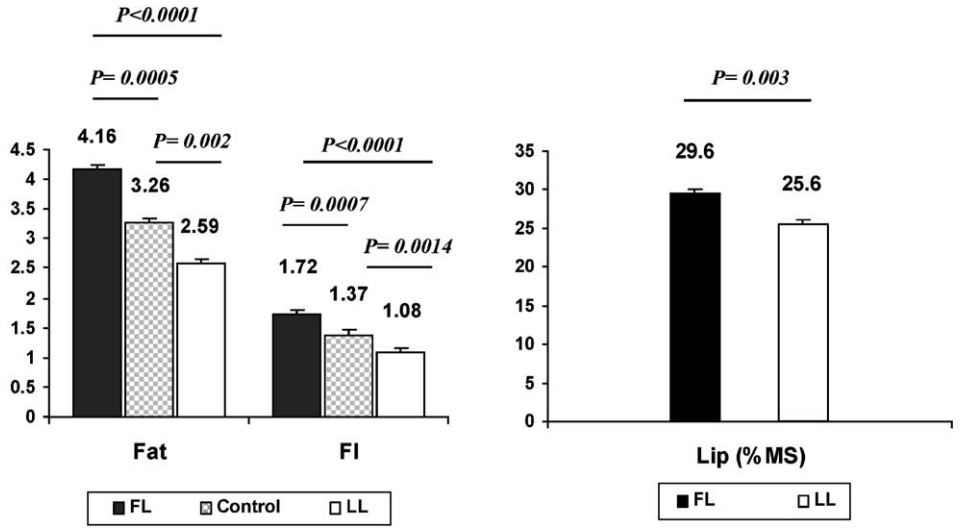

Fig. 2. Least squares means and standard errors of muscle lipid content traits at Day 341 in Fat line (FL), Lean line (LL) and control line (C) (ANOVAs performed on data combined for all three lines for Fat and FI, and for FL and LL lines for Lip). Italic figures above charts are P-values of the contrasts between Control and FL or LL line, or between FL and LL lines. R^2 of the ANOVA model is 0.42, 0.44 and 0.11 for Fat, FI and Lip, respectively. Line effect is significant at $P<0.0001$ for Fat and FI, and at $P=0.003$ for Lip.

Table 3
Growth and body shape traits of the three lines at Day 341 (Least square means and levels of significance of ANOVAs and ANCOVAs performed on data combined for all lines)

Traits	Line	Model[1]	R^2	Fat line	Control	Lean line	P-value for line effect
Growth	Length (L)	M1	0.07	263^{ab}	255^{b}	267^{a}	0.025
	Weight (W)	M1	0.05	266^{a}	237^{b}	268^{a}	0.029
Body shape	Weight	M2, L cubed	0.88	265^{a}	260^{ab}	253^{b}	0.005
	K	M1	0.05	1.44^{a}	1.40^{b}	1.38^{b}	<0.0001
	H	M1	0.05	65.7^{a}	63.2^{b}	66.8^{a}	0.004
		M2, L	0.72	65.4	65.0	65.2	0.751
	W_d	M1	0.15	31.7^{a}	28.7^{b}	31.2^{a}	0.275
		M2, L	0.70	31.6^{a}	29.4^{b}	30.4^{ab}	0.027
		M2, H	0.77	31.5^{a}	29.4^{b}	30.5^{ab}	0.065
	SS	M1	0.24	2.08^{a}	2.21^{b}	2.14^{a}	0.081

K: condition factor, H: pre-dorsal fin height, W_d: pre-dorsal fin width, SS: cross-section shape (see details in the text).
a, b, c: within a row, values with not a common superscript differ ($P<0.05$).
[1] In the case of ANCOVA (M2), the covariate is indicated. All regressions are significant ($P<0.0001$).

given length, FL fish were heavier than LL fish, while controls displayed intermediate values. Other traits (H, W_d and SS) did not differ between LL and FL lines.

3.4. Body compartments at Day 341

The data are summarized in Table 4. Belly thickness was increased in the FL line, while the LL line remained similar to the control. The increase in thickness concerned both the meaty part of the belly and the internal fat coat. However, correction for differences in total belly thickness between the LL and FL lines showed that T_M was higher in LL (ANCOVA with T_T as a covariate restricted to FL and LL lines, $P<0.02$), while T_{FC} was higher in FL (ANCOVA restricted to FL and LL lines, $P<0.03$). Thus, the relative share of the

Table 4
Body compartment traits in the three lines at Day 341 (Least square means and levels of significance of ANOVAs and ANCOVAs performed on data combined for all lines)

Traits	Line	Model[1]	R^2	Fat line	Control	Lean line	P-value for line effect
Belly thickness	T_T	M1	0.14	4.46^{a}	3.97^{b}	4.03^{b}	0.011
		M2, W	0.53	4.37^{a}	4.08^{b}	3.93^{b}	0.004
	T_M	M1	0.10	3.78^{a}	3.42^{ab}	3.47^{b}	0.020
		M2, W	0.49	3.69^{a}	3.51^{ab}	3.38^{b}	0.008
		M2, T_T	0.96	3.48^{a}	3.55^{ab}	3.56^{b}	0.067
	T_{FC}	M1	0.21	0.68^{a}	0.55^{b}	0.56^{b}	0.014
		M2, W	0.30	0.66^{a}	0.55^{ab}	0.54^{b}	0.018
		M2, T_T	0.38	0.64^{a}	0.56^{ab}	0.56^{b}	0.063
Viscera	V	M1	0.05	24.0^{a}	21.2^{b}	25.0^{a}	0.007
	VSI%	M1	0.03	9.0	8.9	9.2	0.201
Liver	liv	M1	0.04	3.05^{a}	2.78^{b}	2.99^{ab}	0.106
	HSI%	M1	0.06	1.15	1.17	1.11	0.246
Carcass	W_C	M1	0.05	236^{a}	209^{b}	239^{a}	0.021
	CY%	M1	0.03	89.0	88.5	88.9	0.418
Fillet	W_F	M1	0.06	86.5^{a}	71.5^{b}	85.5^{a}	0.005
	FY%	M1	0.01	65.0	64.0	64.1	0.443

T_T: total belly thickness, T_M: muscular belly thickness, T_{FC}: thickness of internal fat coat; V, liv, W_C and W_F: absolute weight of viscera, liver, carcass and fillet, with their corresponding ratios to total body weight (see details in the text).
a, b, c: values with not a common superscript differ ($P<0.05$).
[1] In the case of ANCOVA (M2), the covariate is indicated. All regressions are significant ($P<0.0001$).

internal fat coat was higher in FL individuals (15.4% vs. 13.9% in LL), with controls displaying intermediate values. There were no differences between lines for the other traits (visceral weight, liver weight, carcass and fillet weight), except that controls exhibited lower values because of their smaller absolute size. All differences disappeared after correction for weight differences.

3.5. Further growth and reproductive traits

Precocious males were easily detected (external body pigmentation and production of milt after abdominal pressure). Their incidence was 11%, 10.1% and 16.5% in control, LL and FL groups, respectively. This incidence differed significantly between the FL and LL groups ($P<0.005$).

Measurements performed from Day 433 to first spawning (Table 5) confirmed the results recorded on Day 341 for weight, length (no difference between LL and FL), and muscle lipid traits (significant differences), but indicated that there were no longer any differences with respect to K values. Thus, the difference in muscle lipid content between FL and LL lasted from immature pan-size stage up to the end of reproduction. However, at that time, the relative difference between the two lines was less than it was in immature fish (about 33% difference, vs. 60% at Day 341). The principal reproduction traits of 2-year-old females are summarized in Table 6. There were no significant differences between lines for any of the recorded traits.

Table 6
Principal female reproductive traits at first spawning in Fat and Lean lines (Least square means, standard errors)

Traits	Line	
	Fat Line	Lean Line
Relative fecundity		
GSI%	11.4±0.5	11.8±0.5
N_O	4952±188	4830±178
Characteristics of ova		
w	47.7±1.4	50.0±1.3
Lipid content (%FM)	10.83±0.22	10.70±0.22
Fertilization rate (%)	86.5±1.8	92.2±1.7
Yield of eyed embryos (%)	77.5±3.9	70.6±3.8
Yield of swimming larvae (%)	64.1±4.0	69.6±3.9

GSI%: gonado-somatic index, N_O: total number of ova per female, w: mean weight of individual ova (see details in the text)
Within a row, values never differ ($P<0.05$).

4. Discussion

4.1. Muscle lipid content

The value of realized heritability for FI, the selection trait, was moderate ($h^2_r=0.25$), and very similar in the LL and FL lines. Among the calculated heritabilities of lipid content traits that have been published, only few have concerned Fatmeter values. Jopson et al. (2002) found heritability of 0.55 for

Table 5
Growth and muscle lipid content of Fat and Lean lines from Day 433 to first spawning (Least square means, standard errors and level of significance of ANOVA)

Age	Day 433[1]		Day 593[1]		At spawning[1] (Day 692 to 734)	
Traits	Line					
	Fat Line	Lean Line	Fat Line	Lean Line	Fat Line	Lean Line
Length	350.8±3.1	353.3±2.9	523.0±7.0	520.9±6.7	560±5	559±4
Weight[2]	694.9±20.0	707.5±18.8	2517.2±89.0	2477.2±85.3	3156±89	3055±84
K	1.59±0.02	1.59±0.02	1.75±0.03	1.75±0.03	1.78±0.03	1.75±0.03
Fat	$4.56±0.13^a$	$3.11±0.13^b$	$7.65±0.31^a$	$5.72±0.30^b$	$5.84±0.34^a$	$4.38±0.32^b$
FI	$1.61±0.05^a$	$1.09±0.04^b$	$2.25±0.09^a$	$1.69±0.09^b$	$1.68±0.10^a$	$1.26±0.09^b$

K: condition factor, Fat: Fatmeter absolute value, FI: Fat Index=Fat/log(W) (see details in the text).
a, b: within a row and for a given date, values with different superscript differ ($P<0.003$).
[1] At Day 433: immature males+immature females; At Day 593 and at spawning time: females only.
[2] Total body weight including weight of ova (W_B) at spawning time.

Fatmeter values in chinook salmon fillet. In the trout, Chevassus et al. (2002) estimated heritabilities for Fat and FI of between 0.36 and 0.72, depending on the position of the probe on the fish and on the experiments. The estimates from that study which best matched our experimental conditions ranged from 0.36 to 0.40. Thus, a stronger response to selection might have been anticipated.

Several factors may have contributed to the results we obtained: different populations/species were used, the mean lipid content of fish at the time of measurements may have differed and affected their precision. Another reason may have been the operating conditions. Stress induces changes in the molecular uptake of cellular water, which will affect the probe energy transmission of the apparatus. Thus, the accuracy of Fat values may be reduced as fish are gradually removed from a tank to be measured. The experimental conditions were not specified by Jopson et al. (2002), but Chevassus et al. (2002) measured individuals that had been fished and killed within a very short period of time (personal communication). This could obviously not be applied during the selection process (numerous fish to measure and kept alive), though our aim was to standard the duration of exposure to manipulation stress among individuals. Further investigations are necessary to improve management of the selection process. The selection-induced difference in lipid muscle content in pan-size fish was sustained throughout the life of the fish, and based on Fatmeter values was still significant after spawning in 2-year-old females.

4.2. Correlated responses

The control line was much smaller than the two selected lines, and for most traits more variable than the two experimental lines. Such a result was unexpected. Control fish exhibited growth similar to that seen in the other groups during the first months of life (data not shown), and no experimental event was identified that could have contributed to the difference observed at later stages. Nevertheless, the control line appeared to be valid as it was intermediate between LL and FL for most other traits.

Little information has so far become available on possible genetic correlations between muscle lipid content and growth. During this study, no difference in growth was seen between the two selected lines, at least in pan-size individuals. Assuming a positive environmental correlation between Fat and weight and a non-nil genetic correlation, adjustment of phenotypic Fat values to phenotypic weight values would result in a weight difference in favour of LL fish, the magnitude of which depending on the sign and value of the genetic correlation. Thus, the lack of a correlated response for growth indicated that the genetic correlation between the two traits may be limited. At 1-year-old, both belly thickness and condition factor increased in the FL line and decreased (but to a lesser extent) in the LL line. This contrary evolution from values in the control line was consistent with published results which confirmed that the two traits are genetically correlated with muscle fat content (Gjerde and Schaeffer, 1989 in rainbow trout; Rye and Gjerde, 1996 in Atlantic salmon) and phenotypically and genetically correlated with each other (Elvingson and Johansson, 1993). However, the difference in condition factor disappeared in older fish. This feature needs to be confirmed: it either reflects an actual change in morphology as size/age increases, or may also be the result of some uncontrolled bias when sampling the fish kept for the final measurements.

In trout, the greatest variation in viscera weight is due to a difference in visceral fat deposits (Gjerde and Schaeffer, 1989; Elvingson and Johansson, 1993). In large animals (more than 2 kg), negative estimates of a genetic correlation between visceral fat deposition and meat fat content have been published (−0.33 in trout, Gjerde and Schaeffer, 1989; −0.43 in trout, Kause et al., 2002; −0.67 in salmon, Rye and Gjerde, 1996). Such correlations are in favour of a physiological balance between the two sites of deposition, and are consistent with the positive genetic correlation observed between carcass or fillet yield and meat fat content (Gjerde and Schaeffer, 1989; Rye and Gjerde, 1996; Kause et al., 2002). Thus, higher abdominal fat deposition, associated with a higher viscera weight and a reduction in carcass yield or fillet yield could be anticipated in the LL line. The positive correlations between belly thickness and carcass yield (Gjerde and Schaeffer, 1989) constituted a further reason to anticipate lower carcass yield in LL fish, but in fact this was not the case.

The small size of fish at the time of examination in the present study is one possible explanation. Fat deposition increases with size and differences may have been too small at the time of examination to be significant. Another explanation could be that the difference in fat deposition was counterbalanced by a lower weight for the digestive tract in the LL line, but this latter trait was not recorded.

The increase in internal fat coat thickness, associated with an increased muscle fat content in the FL line, was consistent with the positive genetic correlation (+0.59) estimated by Kause et al. (2002) between percent fillet fat and fillet trimming waste in the abdominal region. In the present study, fillets were not trimmed because of the small size of fish, but one could anticipate higher trimming wastes in larger sized animals from the FL line.

4.3. Reproduction traits

The incidence of precociously maturing males was significantly increased in the FL line. Early sexual maturation may be correlated with growth rate at periods long before the reproduction season, with slow-growing individuals initiating maturation at a later age (Crandell and Gall, 1993; Morita and Morita, 2002). However, the weight of fish in the FL line was never higher than those in the LL line during the first year of life (data not shown), and this could not explain the difference. More recently, several studies have described an association between early maturity, energy storage and body lipid levels at a far younger age (Silverstein et al., 1997; Shearer and Swanson, 2000). Thus, the higher maturation rate in the FL line may have been a consequence of the higher muscle lipid content, but further investigation is required to confirm this hypothesis.

Modifications of body fat deposition may interfere with reproduction success. The total body lipid content (and especially abdominal fat) may play important biological functions during sexual maturation (Aksnes et al., 1986; Washburn et al., 1990). During this study, no difference could be recorded between LL and FL with respect to female reproduction traits, despite consistently higher muscle lipid content in FL females. However, there was a very broad variation between females regarding reproductive traits (and especially survival traits), which may have hidden other differences. Further study is necessary to conclude that selection has no adverse effect on the reproduction process in either of the two lines.

5. Conclusion

The results obtained demonstrated the efficiency of indirect mass selection for muscle lipid content using the Fatmeter in pan-size rainbow trout. To our knowledge, this is the first report on a response to selection for a meat quality trait. The experimental lines developed will constitute valuable material to enable nutritional, genetic and functional analyses on lipids in trout.

In term of breeding objectives, the principal finding was that the deterioration in carcass yield, associated with an increased visceral fat deposition in the lean line, which were anticipated from data in the literature, were not recorded. If this finding were to be confirmed, it might have important practical implications, because it would mean that the muscle fat content could be monitored in either direction with limited adverse effects on other carcass quality traits. Further investigations on larger fish and over several generations of selection are required to check this point and make appropriate decisions with respect to future breeding.

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