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Changes in fatty acid composition in muscle and adipose tissue of farmed rainbow trout (*Oncorhynchus mykiss*) in relation to ration and age

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Abstract

To evaluate the importance of age and feed ration level (RL) on the composition of tissue fatty acid (FA) in rainbow trout (*Oncorhynchus mykiss*), fish were fed rations ranging from appreciable underfeeding to gross overfeeding in a longitudinal experiment lasting from start of feeding to onset of sexual maturation 2.4 years later. In order to study the effects of compensatory growth and reduced feed availability, fish were moved from high to low ration and vice versa. Changes of individual FA of total lipid (TL), triacylglycerols (TAG) and total phospholipids (PL) were studied in white and red muscle, as well as in three major adipose tissues. The effect of saltwater transfer on FA composition was also examined. A strong interdependence was found between the relative proportion of PL and TAG with changes in TL content. This was most prominent in white muscle. In parallel with this change in relative lipid class composition, a major effect was seen on FA in the TL fraction. The most marked effect of RL was an inverse relationship between 22:6 n-3 of the PUFA n-3 series and 16:1 and 18:1 of the MUFA series. This was seen in all tissues studied. It is suggested that the most important factor governing FA composition in muscle, pending changes in feed intake, is the TL content, affecting the relative level of PL and TAG. In adipose tissue, consisting mainly of TAG, more subtle changes were observed. The FA compositions of PL and TAG were not affected to any major extent by RL, except at extreme reductions. Significant changes in FA of PL and TAG were observed as an effect of saltwater transfer.

Keywords: Growth rate; Fatty acid composition; Lipids; Muscle; Rainbow trout; Ration level

1. Introduction

In farmed rainbow trout (*Oncorhynchus mykiss*), feed ration size determines growth (Storebakken & Austreng, 1987) and partly, also, fat deposition (Kiessling, Kiessling, Storebakken & Åsgård, 1991c). In a number of studies, environmental and nutritional effects on fat content and its composition have been investigated (e.g. Polvi & Ackman, 1992). However, due to the limited time span of most experiments and the use of small fish, the results are often difficult to relate to the more practical fish farm situations. Therefore, more long-term studies are needed.

During recent years, fish lipids have been focused on as being beneficial for human health (Anon., 1992). The muscle is often the main part of fish used for human consumption and salmon has been suggested to be a major human dietary source of n-3 fatty acids of nutritional and medical interest (Ackman, 1990; Burr et al., 1989). The fat component of muscle is generally presented as composition of total fat content. To understand changes in this component it is, however, important to investigate the proportional contribution of the two major lipid classes, PL and TAG. Further,

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fatty acid compositions of PL and TAG are likely to change differently with alteration in the farming protocol as a function of different roles in the metabolism of the fish, with PL being important constituents of membranes and functioning as a depot for precursor to the eicosanoid metabolism while TAG serve mainly as depots for fatty acids catabolised in the energy metabolism (Henderson & Tocher, 1987).

From earlier work (e.g. Kiessling et al., 1991c) we know that the total fat content will increase with increasing fish size. In a stable environment, growth is a direct effect of feed ration level (RL) and age (Kiessling, Kiessling, Storebakken & Åsgård, 1991a). However, alterations in the feed level (RL) will affect this relationship, with an increased RL resulting in a higher lipid deposition than expected. This becomes obvious when relating lipid content to body weight in fish given an increased feed ration with fish given the high ration continuously. The contrary, reducing the feed level, will result in a maintained high tissue fat level compared to fish of the same size but given the lower ration continuously (Kiessling et al., 1991c); i.e. lipid content is not only a factor of attained weight but also of the growth curve attaining that specific weight. From a commercial point of view, it is of great interest to elucidate whether different feeding protocols (continuous, intensive during early life or intensive feeding during later growth stages) will significantly affect the nutritional value of the fish, as reflected in the FA composition of the flesh. Further, it is reasonable to presume that such changes may be different, depending on whether total lipid or lipid class is studied in a specific tissue.

The aim of the present study was to evaluate the importance of age, body size and growth rate, altered via feed RL, from start of feeding until slaughter at 2.4 years, on lipid classes and FA composition in white (WM) and red muscle (RM) and three major adipose tissues (visceral,V, dorsal, D and abdominal wall, AW). In order to elucidate the long-term effects, of inflicted underfeeding as well as recovery growth, in freshwater (hatchery) environment as well as after transport to, and during, saltwater rearing (net pen), groups of fish were transferred from low initial RL to high RL and vice versa. As the experiment included saltwater adaptation, which is known to significantly affect lipid mobilisation (Sheridan, 1994) and partly depend on fatty acid precursors (Sargent, 1995), special attention was paid to this phase.

2. Material and methods

2.1. Rearing and feeding

The study was carried out at the Institute of Aquaculture Research, Sunndalsøra (freshwater) and Averøy (saltwater), Norway, and the muscle analyses at the Swedish University of Agricultural Sciences, Uppsala, Sweden. A detailed description of the fish, experimental design, feeding schedules, sampling, sampling times, locations of the samples and statistics are given in Kiessling et al. (1991a). A brief presentation is given below. All fish, regardless of ration level, were fed Tess Elite (T. Skretting A/S, Stavanger, Norway). The mean levels (wet weight bases) of proximate composition constituents of different batches (from 1.2 to 2.4 years) from this diet were as follows ($n=4$; g kg^{-1}): protein 451±26 (S.D.); lipid, 183±22; ash, 87±4; crude fibre, 10±2; nitrogen-free extract, 178±6; and dry matter, 909±6.

The rainbow trout were given feed ration levels (RL) as a percentage of the ration size necessary to obtain maximal growth (RL = 100, Austreng, Storebakken & Åsgård, 1987). Fish were either kept on the same level throughout the experiment (e.g. RL100/100/100 or 25/25/25) or moved from one RL to another at 0.2 year (e.g. RL100/50/50 or 25/100/100) or at 1.0 year (e.g. RL100/100/50 or 50/50/100). The later transfer between RLs took place just before saltwater transfer (at age 1.0 year). The experiment was started by counting 3000 eyed eggs into 12 hatching trays. The fry were transferred to 1 m^2 fibreglass tanks in a through-flow system, 1 week after hatching, in accordance with that described by Storebakken and Austreng (1987). Oxygen was monitored regularly and was never allowed to fall below 6 mg/l in the effluent water. After 1 year the salinity was gradually increased for 1 month before the fish were transferred to saltwater at a salinity of 3.2% and kept in net pens of 27 m^3. The fish on ration levels RL 25 and RL100/25 remained in freshwater, owing to their small size. Fish from RL = 200 were split out and kept in freshwater as control. The fish were kept in a 24-h light regime during the freshwater stages and in ambient light during the saltwater stage. Water temperature varied between 4 and 15°C, though the temperature was close to 10°C on all sampling occasions. The experiment started in May and samples were taken before (beginning of May) and after (beginning of October) each growth season. In addition, an extra sample was taken 1 month after saltwater transfer (end of June). At this first sample in salt water, only fish from RL100/100/100, RL75/75/75 and RL50/50/50 were sampled. Groups with altered RL were omitted from this sample as the main interest was to study the possible effects of the salt water transfer, avoiding possible stress reactions caused by alterations in feeding pattern.

2.2. Preparation of tissue samples

Muscle samples for analyses of fatty acid composition were taken in front of the dorsal fin and immediately dorsal to the lateral line. The red muscle was carefully dissected under a magnifying glass and 3–10 mm of the

underlying muscle (depending on fish size) was removed before sampling the white muscle. This was done to ensure that no intermediate muscle contaminated the sample. The main fat depots were sampled as follows: (1) dorsal adipose tissue, located between the epaxial muscle fillets, was dissected free of WM from the dorsal fin to the caudal part of the pectoral girdle. The dorsal fin muscle (enclosed by adipose tissue) was included in the weight of this sample. (2) The visceral adipose tissue was freed from all internal organs, except for the pyloric caeca, which was included in the sample weight. (3) The adipose tissue in the abdominal wall was taken, from the pectoral fin to the pelvic fin and dorsally to the end of the ribs vertebrae. A more detailed description, including drawings, is presented in Kiessling et al. (1991a). FA analyses from the dorsal and intestinal fat depots were performed on pure adipose tissue while abdominal wall sample contained a mixture of adipose tissue and white muscle. This sampling procedure was chosen in order to obtain anatomically comparable samples from rainbow trout of different sizes. Samples were frozen in liquid nitrogen and stored at −80°C.

Usually six of the 10 sampled fish in each group were analysed. Fish with gonads larger than 5% of total body weight were excluded except at the last sampling (2.4 years) where all 10 sampled fish were analysed to render possible a correction, by linear regression (GLM, SAS, 1985), for incipient sexual maturation.

2.3. Lipid analyses

Lipids were extracted according to Folch, Lees and Sloan-Stanley (1957), as modified by Bell, Henderson and Sargent (1985). Fatty acids were analysed by gas–liquid chromatography, after methylation, on a 30 m fused silica capillary column (Supelcowax 10) with nitrogen as carrier gas using a split ratio of either 25:1 or 50:1, for non-fatty and fatty tissues, respectively. Injection port and the flame ionisation detector were held at 220 and 240°C, respectively. The column temperature was held at 160°C for 1 min and then increased by 2°C per min to a final temp of 220°C (9 min). Lipid classes were separated by thin-layer chromatography, as described by Thomassen, Strom, Christiansen and Norum (1979) and fatty acid methyl esters were identified according to retention times of authentical standards. Separation and identification of the two major lipid classes (TAG and total PL) and their FA compositions were only carried out on samples from RL100 at 1.0, 1.1 and 2.0 years, from RL75 at 1.1 and 2.0 years, from RL50 at 1.1 and 2.0 years and RL100/25 at 1.0 year. The relative percentage of individual FA and fatty acid groups (saturates (SAFA), monounsaturates (MUFA) and polyunsaturated fatty acids (PUFA n-3, and PUFA n-6) are always expressed as area percentage of the sum of the identified FA. Values for tissue fat content used in the statistical evaluation are given in Kiessling et al. (1991c). These are in percent of tissue weight for white and red muscle, while those for visceral, dorsal and abdominal wall adipose tissue are given in percent fat of total body weight. Due to the extensive size of this material (25 identified fatty acids × two lipid classes × nine feed ratios × six sampling occasions), complete data are presented only for white muscle and dorsal adipose tissue. Fatty acid composition of TL, for red muscle, visceral and abdominal adipose tissue, is only presented as sum of fatty acids within SAFA, MUFA and PUFAn-3 and PUFAn-6, R^2 and P values of the different models. PL and TAG analyses are shown from all tissues. The complete data set from the total material is presented in Pickova (1997).

2.4. Statistical analyses

Statistical analyses were carried out using the general linear models procedure (GLM) of the Statistical Analysis System (SAS) software package (SAS, 1985). F-test was used to test for significant differences between treatment groups and t-test for paired comparisons between tissues within individual fish. The standard GLM-procedure for multiple regression and the stepwise procedure was used to test effects on fatty acid groups, considering the effects of treatment (age and ration level), body weight, tissue- and feed-lipid content, tissue TAG and PL content, sex and gonad weight, as sources of variation. These models were derived by backward stepwise procedure, which started with the full models (including all factors and possible interactions), and then non-significant ($P < 0.05$) terms were subsequently excluded. The stepwise procedure lists the variables in decreasing order of best fit, with the full R^2 given for the variable with the best fit. The successive variables are then ranged according to the degree in which they further add to the model.

3. Results

Individual weights of the analysed fish throughout the experiment ranged between 2.4 g and 5.5 kg (Kiessling et al., 1991a). No effect of sex or incipient sexual maturation (measured as gonad somatic index) was found on any of the analysed variables.

The results accounted for below are mainly concerned with changes from 1.0 to 2.4 years. The data from 0.3 year are presented in Kiessling, Johansson and Storebakken (1989). In the statistical evaluation, all data, with fish ranging from 0.3 to 2.4 years, are included (Tables 1 and 6). Observed changes in individual fatty acids (FA) and fatty acid groups of the total lipid fraction (TL) are shown in Tables 2, 3 and 4. The data shown for individual fatty acids of TL are limited to two

tissues, WM, being the tissue with lowest lipid content and the most even distribution between membrane lipids (PL) and depot lipids (TAG). Dorsal adipose tissue (D), represents a pure depot tissue dominated by TAG. Data of individual fatty acids of TL from other tissues investigated are presented in Pickova et al. (1997).

A change in fatty acid composition of total lipids was observed in all tissues and at all samplings in saltwater between grossly over-fed (RL200) and fully-fed fish (RL100). Despite no difference in either body weight or total lipid content (Kiessling et al. 1991c), fatty acids affected by RL increased or decreased as if RL200 fish had a higher feed intake compared to RL100 fish (Tables 3, 4 and Pickova, 1997).

3.1. Changes in saturates, monoenes and PUFA of total lipid

3.1.1. Saturates

The total level of saturates was in the same range in all tissues studied (Table 2, 3 and 4). In WM and D, a general trend was seen with a decreased SAFA level with age (Table 1). In RM, V and D saturates showed a slight increase with age (Tables 1 and 2). Combining all factors, a high R^2 value was found in all tissues except WM and abdominal wall (AW) (Table 1). WM-specific growth rate (SGR), i.e. the growth rate in the period preceding the sampling, seemed to have some influence on the observed variation. This factor (SGR) also influenced the dorsal lipid depot. Also, lipid content of the tissue affected the level of saturates. This was most prominent in RM and V (Table 1). Variations in saturates were well described by a combination of growth factors such as lipid content, age, SGR, body weight and given feed ration in the adipose tissues. This was, however, not the case for WM or AW. The most notable effects seen in single saturated fatty acids were in 14:0 and 18:0 which both decreased with RL, but did not change with age.

3.1.2. Monounsaturates

The general levels of the studied tissues were roughly in the same range (around 50% of all FA), but the

Table 1
Regression coefficient, R^2, for FA groups in tissue and growth factors including both fish given stable RL and fish exposed to altered RL[a]

	Lipid content		Age	SGR	Body weight	Ration level		$R2$ value	PL/TAG[b]
	Feed	Tissue				Start	End	complete model	
SAFA WM				28(40)				28(40)	
SAFA RM		24(47)	15(13)	10		11(7)		60(67)	
SAFA V		18(27)	12(16)	24		(12)	6	60(55)	
SAFA D			9(11)	39(30)	14(21)			62(62)	
SAFA AW					12	(17)	13	25(17)	
MUFA WM		67(76)						67(76)	81
MUFA RM			4	4(5)	73(72)			81(77)	
MUFA V		7(8)	70(70)			(7)	4	81(85)	
MUFA D		7	71		(68)			78(68)	
MUFA AW				4	65(65)			69(65)	75
PUFA n-3 WM		52(60)			5			57(60)	93
PUFA n-3 RM		71(76)		3	3			77(76)	58
PUFA n-3 V		9	45(51)					54(51)	
PUFA n-3 D					50(52)			50(52)	
PUFA n-3 AW		57(57)						57(57)	84
PUFA n-6 WM				16	11		15	42	
PUFA n-6 RM		3		22(22)	45(53)			70(75)	57
PUFA n-6 V		71(74)			11(13)			82(87)	
PUFA n-6 D		3		7(8)	67(68)			77(76)	
PUFA n-6 AW					79(83)		3	82(83)	
Lipid content WM	3			7(5)	74(83)			84(88)	66
Lipid content RM			2(3)	5	76(82)			83(85)	48
Lipid content V	44(45)		9(6)	12(11)	14			79(62)	
Lipid content D				5	56(60)			61(60)	
Lipid content AW	(5)				74(80)		4	78(85)	

[a] (FA-groups = aX.... + gY + u; stepwise procedure, SAS, 1985). $P < 0.05$. The values for fish given a stable RL throughout the experiment are given within brackets. Values are expressed as $R^2 \times 100$. Abbreviations: SGR, specific growth rate; SAFA, saturated fatty acids; MUFA, monounsaturated; PUFA, polyunsaturated; PL, phosholipids; TAG, triacylglycerols; WM, white muscle; RM, red; V, visceral adipose tissue; D, dorsal; AW, abdominal wall; FA, fatty acid; RL, feed ration level.

[b] The effect of this factor was only analysed for set RL levels at 1.0 and 2.0 years.

response to treatment varied in absolute numbers (Table 1). In WM, effects were seen with tissue fat content, in RM and AW with body weight, in V with age and, in D with age (both steady and altered RL) and with body weight (only fish given a steady RL, Table 1). When fish were moved to sea water, a marked increase was observed of this fatty acid group in all tissues in all fish studied (Table 1), even if this was most prominent in the groups with high ration levels. Groups remaining in fresh water (FW 1.4 years), naturally did not show this increase.

In general, the content of monounsaturates increases with age and decreases with reduced growth (Tables 1 and 2). Combining variables (SGR, body weight, lipid content, etc.) directly related to growth, in common with saturates, yielded a high R^2 (degree of explanation). This, as was also the case with saturates, was less apparent in WM and AW (Table 1). It is interesting to note that WM and AW are the only tissues investigated with a strong relationship between level of monounsaturates and the relative level of PL and TAG (Table 1).

In WM, all the individual FA of monounsaturates increased with age and decreased with decreasing ration level (RL). Fish moved between different RLs, from low to high RL showed an increased monounsaturate incorporation and fish from high to low RL showed an opposite response (Table 3). In all tissues, except WM, the same tendency is seen for 16:1 and 18:1 (increase with age, but decrease with RL) while 20:1 and 22:1 show the opposite response with an increase with lower RL and a slight decrease with age (Tables 3 and 4 and Pickova, 1997).

3.1.3. PUFA n-3

Lipid content of the analysed tissue is the dominating factor affecting PUFA n-3 levels in most tissues (WM, RM, AW). PUFA in V is, however, more related to age

Table 2
Changes in total lipid composition with age and ration level in red muscle, visceral and abdominal adipose tissue[a]

	RL	Red muscle[b]				Viscera				Abdominal wall[c]			
FA-groups		SAFA	MUFA	PUFA n3	PUFA n6	SAFA	MUFA	PUFA n3	PUFA n6	SAFA	MUFA	PUFA n3	PUFA n6
1.0 year FW	100	22.4	50.5	20.0	7.1	22.3	49.9	20.5	7.2	23.4	49.0	20.7	6.8
	75	22.4	50.5	20.2	7.0	22.2	49.1	21.2	7.5	–	–	–	–
	50	21.8	50.2	20.6	7.4	21.2	49.3	21.2	8.2	22.5	49.1	20.9	7.4
	25	21.5	50.9	20.5	7.1	22.0	51.7	18.7	7.6	–	–	–	–
	100/25	21.3	48.4	22.3	8.0	22.5	51.4	17.6	8.4	23.7	45.2	24.8	6.2
	100/50	21.6	50.1	20.8	7.5	22.3	49.3	20.7	7.7	–	–	–	–
	25/100	21.7	50.4	20.6	7.3	22.5	48.9	21.1	7.5	–	–	–	–
1.1 year SW	100	24.5	52.2	18.6	6.5	23.1	51.9	18.5	6.6	23.1	52.7	17.8	6.3
	75	24.3	48.3	20.2	7.0	23.0	51.7	18.3	6.5	23.2	52.2	18.0	6.4
	50	23.6	47.5	21.4	7.5	22.0	52.0	18.6	7.3	21.7	52.5	19.0	6.9
1.4 year SW	200	24.9	52.6	16.9	5.5	24.7	53.7	16.6	5.0	24.3	54.1	16.5	5.0
	100	23.9	52.5	18.1	5.5	23.7	53.7	17.1	5.5	23.7	53.5	17.3	5.4
	75	24.3	50.2	18.9	6.6	24.3	52.7	17.1	5.9	23.2	53.6	17.6	5.6
	50	23.9	50.2	19.2	6.7	22.9	51.8	18.3	7.0	22.1	51.5	19.7	6.7
	100/50	23.8	50.3	19.1	6.8	22.3	53.4	17.8	6.5	22.8	51.9	19.1	6.2
	100/100/50	23.6	50.4	19.6	6.4	22.5	53.9	17.4	6.1	22.2	54.4	17.4	6.0
	25/100	24.8	52.0	17.7	5.5	24.5	54.1	16.3	5.1	24.1	54.4	16.4	5.1
	50/50/100	25.1	52.4	17.2	5.3	24.5	53.5	16.8	5.1	24.1	53.7	16.9	5.3
1.4 years FW	200	23.3	50.5	19.8	6.4	24.0	51.6	17.9	6.4	22.8	49.7	21.0	6.5
	25	20.0	50.8	21.2	7.9	22.1	53.9	16.1	7.9	23.1	43.0	26.4	7.5
	100/25	19.7	50.3	22.5	7.6	21.9	54.2	16.3	7.6	22.4	45.2	24.6	7.8
2.4 years SW	200	21.8	56.3	17.2	4.7	23.2	57.2	15.2	4.5	23.0	56.5	15.8	4.7
	100	21.5	56.6	17.2	4.7	22.4	57.2	15.6	4.8	22.2	56.7	16.2	4.9
	75	22.7	55.9	16.8	4.6	21.7	58.5	15.1	4.7	22.4	57.4	15.9	4.3
	100/50	20.9	56.8	17.7	4.6	22.3	57.3	15.2	5.1	22.5	56.0	16.5	4.9
	100/100/50	23.3	53.8	17.8	5.1	19.7	59.2	15.5	5.5	22.1	56.4	16.6	4.9
	25/100	23.3	54.5	17.4	4.8	21.6	57.7	16.2	4.6	22.5	57.3	17.0	3.2
	50/50/100	24.0	54.1	17.2	4.7	22.7	56.7	15.9	4.6	23.3	56.6	15.7	4.4
S.E.%		1.0	1.0	1.0	1.0	2.0	1.0	2.0	2.0	2.0	1.0	2.0	2.0

[a] FW, freshwater; SW, saltwater.
[b] The values for 0.3 years of age are from Kiessling et al. (1989).
[c] –, not analysed. Abbreviations: See Table 1.

but, in D, body weight seems to be the dominating factor (Table 1). PUFA n-3 can be said to mirror the changes in monounsaturates, with a general decrease with time and increase with depressed growth. However, the total statistical model, consisting of the combined effect of all growth factors studied, showed a lower degree of explanation for changes in PUFA n-3 than for monounsaturates. This could possibly be ascribed to the fact that changes in PUFA n-3 are even more strongly related to the changes in the relative contents of PL and TAG than is the case with monounsaturates (Table 1) and that the relative level of PL and TAG is not included in the main model. In single fatty acids, 22:6n-3 is the FA most obviously altered by reduction in RL and age. Fish moved from a high to low RL and vice versa showed a pronounced response. This is manifested in that the RL25/100 and RL50/100 groups show the lowest values of all fish. This effect was most pronounced in tissues with the lowest proportion of adipose tissue, i.e. WM > AW > RM > D > V (Tables 3, 4 and Pickova, 1997). Further, in fish kept until 1.5 years in FW, the content of 18:4n-3 is very low in D and AW. In WM, 20:5n-3 increased with continued or induced underfeeding (high to low ration, Tables 3 and 4 and Pickova, 1997).

3.1.4. PUFA n-6

PUFA n-6 is low in all tissues throughout the study. In RM, D and AW the most prominent effect was seen

Table 3
Changes in fatty acid composition of white muscle with age and ration level. The fatty acids are given as percent of total identified fatty acids. S.E.% is the average standard error in percent of the mean[a]

Fatty acids	RL	14:0	16:0	18:0	16:1 n7	18:1[b] n5,7,9	20:1[c] n9,11	22:1[d] n9,11	18:2 n6	18:3 n3	18:4 n3	20:4 n6	20:5 n3	22:5 n3	22:6 n3
1.0 year FW	100	4.0	19.5	2.7	5.5	15.7	7.3	6.6	4.1	0.9	1.5	0.5	6.4	1.5	22.8
	75	3.9	19.3	2.7	5.1	15.6	8.7	8.5	4.0	0.8	1.4	0.4	5.6	1.3	20.7
	50	4.5	25.2	3.0	3.9	12.3	4.7	3.6	3.5	0.7	1.2	0.9	6.5	1.3	28.1
	100/25[e]	2.1	19.5	2.9	2.5	9.7	4.1	2.6	2.9	0.5	0.9	0.7	9.2	1.4	41.0
	100/50	5.1	23.7	2.0	4.5	12.9	6.1	6.0	3.9	0.9	1.2	1.0	5.5	1.1	24.8
	25/100	5.4	21.9	2.4	6.1	16.5	6.3	6.2	4.4	0.9	1.5	0.2	5.6	1.1	20.0
1.1 year SW	100	3.6	18.7	2.6	5.9	16.6	9.0	8.0	4.1	0.8	1.2	0.4	5.2	1.4	22.6
	75	3.4	17.6	2.3	5.0	13.7	8.0	7.4	3.7	0.7	1.2	0.4	5.1	1.4	20.4
	50	3.3	18.9	2.7	4.9	14.2	8.2	7.0	3.8	0.7	1.1	0.5	5.9	1.5	27.3
1.4 year SW	200	4.1	19.7	2.6	7.1	17.7	7.7	6.5	3.6	0.7	1.3	0.4	5.2	1.3	19.4
	100	4.0	18.6	2.4	6.3	17.4	8.3	7.1	3.8	0.7	1.3	0.4	5.2	1.4	20.4
	75	3.9	19.0	2.3	6.0	16.9	8.2	6.9	3.8	0.7	1.4	0.4	5.2	1.5	21.3
	50	2.7	18.3	1.9	4.1	13.3	6.1	4.8	3.7	0.7	1.4	0.5	7.3	1.5	31.0
	100/50	4.2	20.2	2.1	5.7	14.7	8.6	7.2	3.8	0.7	1.3	0.4	5.4	1.4	24.0
	100/100/50	4.2	20.4	2.3	6.5	16.8	8.0	6.5	3.6	0.6	1.2	0.4	5.0	1.3	22.6
	25/100	5.1	20.3	2.6	8.2	19.7	9.2	7.3	3.7	0.7	1.4	0.3	4.4	1.3	15.3
	50/50/100	5.0	19.1	2.3	8.7	19.7	9.2	7.6	3.9	0.7	1.5	0.3	4.5	1.2	15.6
1.4 years FW	200	3.9	18.6	2.5	5.9	16.5	7.9	6.7	4.1	0.8	1.3	0.4	6.1	1.5	21.0
	25	2.7	20.2	2.5	2.7	9.3	5.4	3.3	3.3	0.6	0.9	0.6	7.8	1.9	37.6
	100/25	2.9	18.3	2.1	3.7	11.2	7.2	6.1	3.2	0.7	1.1	0.5	7.6	1.4	31.7
2.0 years SW	200	5.2	18.6	2.4	8.8	20.2	9.9	8.5	3.4	0.7	1.6	0.2	4.2	1.3	12.5
	100	4.8	17.9	2.3	9.0	20.6	10.2	8.7	3.3	0.6	1.6	0.3	4.2	1.2	14.1
	75	4.7	20.0	2.1	8.2	19.0	9.6	7.7	3.6	0.7	1.3	0.3	4.0	1.3	17.6
	50	4.0	19.0	1.7	5.8	15.1	7.5	5.8	3.3	0.6	1.2	0.5	6.1	1.5	27.7
2.4 years SW	200	4.9	17.3	2.1	9.5	20.6	11.0	9.2	3.5	0.6	1.3	0.4	4.3	1.4	13.9
	100	5.1	16.3	1.9	9.3	20.9	11.4	9.2	3.7	0.7	1.4	0.4	4.4	1.5	13.6
	75	5.5	17.7	2.1	9.9	21.8	10.8	8.4	3.2	0.6	1.3	0.4	4.2	1.4	12.4
	100/50	4.8	18.4	1.9	7.8	17.6	9.6	7.9	3.1	0.6	1.3	0.4	5.5	1.6	19.5
	100/100/50	4.9	19.1	2.1	8.2	18.8	9.8	7.9	3.2	0.6	1.1	0.4	4.8	1.5	17.2
	25/100	5.3	19.2	2.3	8.6	19.2	10.2	8.6	3.1	0.6	1.3	0.3	4.4	1.4	15.0
	50/50/100	5.6	17.7	2.2	10.2	21.6	10.7	8.6	3.3	0.6	1.1	0.3	4.2	1.4	12.3
S.E.%		3	2	2	3	3	4	2	2	3	3	5	2	4	3

[a] FW, freshwater; SW, saltwater; RL, feedration level.
[b] The average relative distribution of n5,7,9 was 3:14:83.
[c] The average relative distribution of n9,11 was 4:96.
[d] The average relative distribution of n9,11 was 4:96.
[e] Presented instead of RL = 25, which was not analysed in this tissue at this sampling.

with changes in body weight. In V, tissue lipid content related most to PUFA n-6. In WM, a small contribution was seen both by SGR, body weight and ration level (change in RL, Table 1). The change seen in single FA of PUFA n-6 is that of 18:2n-6, decreasing with age. An opposite but, in absolute numbers, less pronounced trend was seen for 20:4n-6 (Table 3). The changes observed in 18:2n-6, however, were best explained by changes in the diet (Table 5). Considering that this FA is a dominating one in this fatty acid group, it is feasible to assume that the relation to growth observed for the PUFA n-6 group in fact was a dietary effect, since the level in the diet was inversely related to pellet size (Table 5). This led to a lower dietary intake of 18:2n-6 as the fish received a larger pellet (Tables 1, 2, 3 and Pickova, 1997).

3.2. Fish remaining in fresh water

Fish on maintenance ratio (RL25/25/25 and RL100/25/25) were never transferred to salt water (to small size at 1.0 year of age), but kept on in the fresh water environment until 1.4 years of age. In order to have fish with the same RL in both environments, RL 200 was divided into two subgroups; one group remained in freshwater while the other was transferred to seawater. All fish remaining in fresh water maintained or even increased the level of PUFA in TL of all tissues studied (Table 2). The reverse was seen for monounsaturates. However, in WM this difference was not significant. This difference was the reverse of that of the diet (Table 2). This indicates that the difference observed at saltwater transfer (decrease of PUFA and increase of

Table 4
Changes in fatty acid (in percent of identified FA) composition of dorsal adipose tissue with age and ration level[a]

Age	Fatty acids RL	14:0	16:0	18:0	16:1 n7	18:1[b] n5,7,9	20:1[c] n9,11	22:1[d] n9,11	18:2 n6	18:3 n3	18:4 n3	20:4 n6	20:5 n3	22:5 n3	22:6 n3
1.0 year FW	100	5.5	15.9	2.3	8.3	20.2	11.2	9.8	6.0	1.2	2.5	0.2	4.1	1.2	9.0
	50	5.2	14.1	2.0	6.9	18.9	12.7	11.3	6.6	1.3	2.6	0.3	4.3	1.2	9.5
	100/25[e]	5.2	15.2	2.5	6.7	19.7	12.7	11.1	6.6	1.2	2.0	0.3	3.8	1.0	9.2
1.1 year SW	100	5.2	15.3	2.2	9.0	22.2	11.9	9.6	6.6	1.2	2.1	0.2	3.7	1.2	9.1
	75	5.3	15.1	2.0	8.6	20.7	12.1	10.0	6.7	1.3	2.1	0.2	4.2	1.2	9.3
	50	5.4	14.6	2.1	7.3	19.7	13.7	11.9	7.0	1.2	2.1	0.2	4.0	1.1	9.4
1.4 year SW	200	5.1	15.9	2.2	10.0	24.2	11.1	8.8	5.3	1.0	1.8	0.2	4.1	1.3	8.5
	100	5.2	15.5	2.1	9.3	23.1	12.0	9.7	5.7	1.1	1.9	0.2	4.1	1.3	8.8
	75	5.2	15.1	2.0	9.3	22.7	12.1	9.5	6.2	1.1	2.0	0.2	4.2	1.3	8.8
	50	5.5	13.9	1.7	8.5	20.9	13.0	10.7	6.7	1.2	2.3	0.2	4.5	1.3	8.7
	100/50	5.7	14.3	1.5	9.3	20.6	12.9	10.6	6.4	1.2	2.1	0.2	4.3	1.4	8.9
	100/100/50	5.2	14.7	1.8	9.6	22.7	12.4	9.8	6.1	1.1	1.9	0.2	3.8	1.3	8.6
	25/100	5.4	15.8	1.9	10.1	23.4	11.6	9.3	5.5	1.1	1.9	0.2	3.8	1.2	8.3
	50/50/100	5.3	15.5	1.6	10.3	22.8	11.7	9.7	5.5	1.1	2.0	0.2	3.9	1.2	8.6
1.4 years FW	200	5.0	14.9	2.2	8.4	13.5	14.3	13.6	6.5	1.3	0.0	0.3	5.3	1.8	12.0
	25	5.8	13.7	2.1	7.2	9.6	16.6	15.6	7.8	1.5	0.0	0.3	5.2	1.6	12.2
	100/25	5.5	13.6	2.0	7.2	9.6	16.4	15.6	8.1	1.4	0.0	0.3	5.6	1.8	12.0
2.0 years SW	200	5.8	15.2	2.1	12.2	24.9	10.9	8.3	4.4	1.0	2.0	0.2	3.8	1.2	7.7
	100	6.2	14.8	1.9	11.8	23.6	11.2	8.4	4.8	1.0	2.2	0.2	4.0	1.2	8.0
	75	6.0	14.3	1.9	11.1	23.7	11.9	8.9	5.2	1.0	2.1	0.2	4.0	1.3	8.2
	50	6.4	12.8	1.5	10.6	21.7	12.5	9.4	6.1	1.1	2.6	0.3	4.4	1.4	8.8
2.4 years SW	200	5.8	14.5	1.9	12.5	24.2	11.4	8.8	4.5	0.9	1.7	0.2	3.7	1.4	8.0
	100	6.6	14.7	1.8	12.4	24.3	11.4	8.3	4.6	0.9	1.9	0.2	3.6	1.3	7.9
	75	6.5	15.0	1.9	12.3	24.6	11.6	8.5	4.2	0.8	1.5	0.2	3.7	1.4	7.5
	100/50	6.8	13.8	1.6	11.6	21.6	13.6	10.2	4.8	0.9	1.8	0.2	3.9	1.4	7.5
	100/100/50	6.2	13.6	1.7	11.2	22.9	13.0	9.9	5.0	0.9	1.7	0.2	3.8	1.5	7.9
	25/100	5.9	14.2	1.9	11.4	23.2	12.2	9.3	4.5	0.9	1.8	0.3	4.1	1.5	8.4
	50/50/100	6.1	14.8	1.9	12.5	23.9	11.5	8.5	4.3	0.9	1.9	0.2	3.9	1.4	8.0
S.E.%		3	2	3	2	2	2	2	3	3	3	8	3	3	1

[a] FW, freshwater, SW, saltwater; RL, feed ration level.
[b] The average relative distribution of n5,7,9 was 4:15:81.
[c] The average relative distribution of n9,11 was 4:96.
[d] The average relative distribution of n9,11 was 4:96.
[e] Presented instead of RL = 25, which was not analysed in this tissue at this sampling.

MUFA) mirrors a physiological effect rather than diet changes or TAG mobilisation. The lower content of monounsaturates observed in fish remaining in fresh water can mainly be ascribed to a decrease of 16:1 and 18:1, as both total 20:1 and 22:1 were higher in fresh water fish than fish transferred to salt water. In PUFA, all identified FAs were higher in fish remaining in fresh water than those in saltwater-transferred fish. However, 18:4n-3 constitutes the exception with a very low level in AW and a non-detectable level in the dorsal fat depot of RL 200 fish remaining in fresh water (Pickova, 1997).

3.3. Variation in total triacylglycerols and phospholipids

Table 7 shows changes in total lipid class and fatty acid groups of each class. Changes of both lipid class and fatty acid group were tested against the main factors, ration level and age (Table 6). Changes in relative level of the total lipid class were also included in the full model (Table 1). In WM, variation in lipid class was well explained by the combined factors of ration level and age. Also WM lipid content related well to changes in total content of the two lipid classes ($r^2 = 0.66$, $P < 0.05$, Table 1). In all other tissues, the relative level of PL and TAG showed a poor relationship with RL and age (Table 1). Specific growth rate (SGR) relates well to relative level of the two lipid classes in RM ($r^2 = 0.74$, $P < 0.02$). Also, in RM, in parity with that of WM, a significant but less pronounced relation was found with lipid content ($r^2 = 0.48$, $P < 0.05$, Table 1). Relative levels of PL and TAG also relate well to SGR in V ($r^2 = 0.81$, $P < 0.02$), D ($r^2 = 0.88$, $P < 0.02$) and AW ($r^2 = 0.83$, $P < 0.02$). The correlation for PL with RL and tissue fat content were negative but, with SGR, positive.

A correlation was found between levels of the two lipid classes in the different tissues (RM, V, D and AW, $r^2 = 0.85$–1.0, $P < 0.0001$, Table 1) of the same fish. The correlation between these lipid classes (PL and TAG) in WM and the other tissues was less pronounced ($r^2 = 0.58$–0.71, $P < 0.001$, Table 1).

3.3.1. Variation in fatty acid groups of triacylglycerols and phospholipids

As a general trend, PUFA n-6 in TAG was the fatty acid group best explained by RL and age (Table 6). Also, monounsaturates were well explained by these variables in TAG. Age, more often than RL, seemed to have the strongest influence on changes in level of a specific FA group. The most obvious exception was PL of RM and D. Only in WM was total lipid separated into two lipid classes at the last sampling before saltwater transfer (Tables 8 and 9), thereby allowing a comparison, before and after saltwater transfer. At least in WM, saltwater transfer has a much more profound effect on fatty acid composition of each class than RL or age. This effect is most obvious in the PL fraction.

3.3.2. Variation in fatty acids of triacylglycerols and phospholipids

Individual FA in the two major lipid classes, PL and TAG, seemed to change, as an effect of RL and age, in

Table 5
Fatty acid composition of feed (in percent of identified fatty acids) in stable feed ratios (SFR), decreased feed ratios (DFR) and increased feed ratio (IFR)

Age	% FA RL	14:0	16:0	18:0	16:1 n7	18:11[a] n7,9	20:1 n9	22:12[b] n9,11	18:2 n6	18:3 n3	20:4 n6	20:5 n3	22:5 n3	22:6 n3
1.0 year FW	SFR	5.4	13.6	2.0	5.5	12.3	13.0	17.2	7.3	1.7	0.4	8.9	0.8	10.8
	IFR (25/100), DFR (100/50)	5.4	13.6	2.0	5.5	12.3	13.0	17.2	7.3	1.7	0.4	8.9	0.8	10.8
	DFR (100/25)	4.9	14.1	1.9	5.5	13.1	11.7	15.3	8.7	1.6	0.5	8.9	0.8	11.9
1.1 year SW	SFR	5.4	12.8	1.9	6.1	12.2	14.5	19.2	6.1	1.5	0.4	8.8	0.8	9.1
1.4 year SW	SFR; IFR; DFR	5.2	13.0	2.1	5.5	10.3	12.7	17.9	7.0	1.7	0.5	10.1	0.9	12.0
1.4 years FW	SFR	5.8	12.9	1.4	7.1	12.8	15.2	19.3	4.3	1.1	0.3	9.1	0.7	8.6
	DFR	5.6	13.6	1.7	6.3	12.7	12.9	16.9	7.2	1.5	0.4	8.8	0.8	10.6
2.0 years SW	SFR (200, 25)	5.8	12.3	1.5	8.0	14.1	15.2	20.1	4.3	1.0	0.4	7.7	0.6	7.5
	SFR (75, 50)	5.7	12.5	1.5	8.2	13.6	14.4	19.3	5.0	1.1	0.4	8.1	0.7	8.2
2.4 years SW	SFR	5.8	12.1	1.7	9.1	11.8	13.3	18.0	4.8	1.2	0.5	10.1	1.0	9.4
	SFR (75)	6.0	13.1	1.5	8.9	13.1	13.9	18.4	3.3	1.0	0.4	9.9	0.8	8.6
	DFR (100/50/50)	6.0	12.3	1.7	9.1	11.6	13.1	17.6	3.7	1.1	0.5	10.6	1.0	10.4
	DFR (100/100/50)	6.0	13.1	1.5	8.9	13.1	13.9	18.4	3.3	1.0	0.4	9.9	0.8	8.6
	IFR	5.8	12.1	1.7	9.1	11.8	13.3	18.0	4.8	1.2	0.5	10.1	1.0	9.4

[a] The average relative distribution of n-9,11 was 24:76.
[b] The average relative distribution of n9,11 was 3:97.

Table 6
Correlation between FA-classes within lipid classes with ration level (RL) and age (1.0–2.0 years)[a]

	Lipid class %					
			SAFA	MUFA	PUFA n3	PUFA n6
White muscle						
	RL	49	–	14	12	13
	AGE	23	20	44	23	21
	TOT	72	20	58	36	35
Red muscle						
	RL	26	15	19	17	10
	AGE	6	–	–	–	28
	TOT	33	15	19	17	38
Visceral adipose tissue						
	RL	–	–	–	12	7
	AGE	19	–	34	21	43
	TOT	19	–	34	33	51
Dorsal adipose tissue						
	RL	22	22	15	30	–
	AGE	–	–	57	26	43
	TOT	22	22	72	57	43
Abdominal adipose tissue						
	RL	–	8	8	13	8
	AGE	18	15	49	43	–
	TOT	18	22	57	56	8

[a] Values are given as $R^2 \times 100$. The full model: (FA-class = aRL + bAge + u). Lipid classes percent included are PL and TAG. See Table 1 for abbreviations.

parity with that of the whole fatty acid group. In PUFA n-3, of PL in WM and RM, 22:6n-3 decreased with increasing RL and decreased with age. In the adipose tissues an increase of 22:6n-3 was more pronounced with a decreased RL; also the content decreased with age. 20:4n-6, on the other hand, increased slightly with age and with lowered RL. In absolute number, the changes in fatty acids observed within a lipid class were small compared with those of total lipid fraction containing a mixture of PL and TAG (Tables 2–4, 8 and 9).The FA composition of TAG did not show any pronounced differences between the tissues with age. Some trends can be recognised both, with RL and age, in occurrence of 16:1 and 18:1, 16:0 and 18:0; these vary slightly between tissues (Table 8).

4. Discussion

Deposition of fatty acids as triacylglycerols in salmonids is suggested to be a non-selective process (Thomassen & Røsjø, 1989) contrary to that of mobilisation (Kiessling & Kiessling, 1993). Excess dietary energy is mainly stored in the form of TAG deposited in muscle and adipose tissue, these being the main fat depots of salmonids (see Kiessling et al., 1991c). At low dietary lipid levels, lipid storage is influenced by de novo lipid synthesis and by lipid deposition from plasma lipoproteins (Sheridan, 1994). Mobilisation and utilisation of lipid reserves in salmonids are under hormonal and enzymatic control and they occur normally during periods of starvation. Examples of such periods in wild fish are the egg and yolk sac stage, smoltification, spawning migration and subsequent winter in fresh water (Sheridan). Farmed fish, on the other hand, are also starved pre handling and pre slaughter and at high or low water temperatures. Such periods of starvation are, however, few and limited in time. Contrary to starvation, long periods of low feed intake can often occur with farmed fish as the result of either inadequate feeding routines or stress as by high fish densities, poor water quality or high infection. If also feed intake is low, in parity with starvation, induced mobilisation of lipid reserves is less well known and no longitudinal studies exist for salmonids in general or for farmed rainbow trout in particular. It is therefore important to underline that this study does not deal with effects of starvation but rather with the effects of sub-optimal feeding regimes, ranging from underfeeding to gross over feeding, phenomena also known to occur frequently within aquaculture.

In summary observed changes in levels of different fatty acids from the five investigated tissues included in this study indicate that, in parity with starvation, underfeeding as well as gross over-feeding, induce a selective mobilisation and deposition of fatty acids in farmed rainbow trout. Sheridan (1994) suggests RM and liver to act as short-term, and WM and mesenteric fat, as long-term fat storage depots in salmon. Kiessling et al. (1991c) suggested that, in rainbow trout also, the dorsal fat depot serves as a long-term depot. Kiessling et al. also found WM of rainbow trout to function as a short-term rather than long-term fat depot. This was most pronounced during the freshwater period. Our data in this study tend to support the earlier finding (Kiessling et al.) in that WM is the tissue showing the largest changes in fatty acid composition with ration level of all tissues studied. Further, both quantitative and qualitative changes of TAG, the class representing storage lipids, also vary significantly with ration level in WM.

The fact that several fatty acids, which were observed to change with a reduction in feed ration level, were also found to differ between RL200 fish and RL100 fish is intriguing. This indicated to us that RL200 fish in fact ingested more feed than RL100 fish without an accompanying increase in body growth (Kiessling et al., 1991a). A general belief with over-feeding is that excess feed is ignored by the fish and will end up as feed waste, as over feeding is defined as a level where no addition of feed improves overall growth. The alternative would be

Table 7
Changes in lipid composition (in percent of identified FA) of phospholipids and triacylglycerols with age and ration level[a]

	RL	PL%	Phospholipids				TAG%	TRIACYLGLYCEROLS			
			SAFA	MUFA	PUFA n3	PUFA n6		SAFA	MUFA	PUFA n3	PUFA n6
White muscle											
1.0 years FW	100	20.2	33.3	14.7	50.0	2.0	79.8	26.0	51.9	16.9	5.2
	100/25	65.6	34.3	12.4	51.2	2.1	34.4	25.9	54.2	15.1	4.8
1.1 years SW	100	23.5	30.2	9.8	58.0	2.0	76.5	25.2	52.6	16.9	5.3
	75	21.4	29.7	10.0	58.0	2.3	78.6	26.4	50.1	18.0	5.5
	50	41.1	30.3	9.0	58.7	2.0	58.9	26.3	51.1	16.9	5.7
2.0 years SW	100	11.4	28.9	14.7	54.6	1.8	88.6	24.2	55.3	16.6	3.9
	75	14.9	29.5	10.7	58.2	1.6	85.1	25.0	53.5	17.1	4.4
	50	31.0	29.2	11.8	56.9	2.1	69.0	23.3	53.8	17.8	5.2
Red muscle											
1.1 years SW	100	1.7	29.7	19.4	48.1	2.8	98.3	24.5	51.3	17.3	6.9
	75	5.4	26.4	20.0	50.3	3.3	94.6	24.0	51.5	17.4	7.1
	50	3.8	27.3	18.7	50.4	3.7	95.4	24.9	49.8	17.8	7.4
2.0 years SW	100	2.0	27.7	22.5	47.0	2.8	98.0	21.8	55.9	17.3	5.1
	75	2.7	27.9	19.2	50.4	2.5	97.3	22.3	55.0	17.4	5.3
	50	3.2	26.5	17.5	53.3	2.8	96.8	21.0	53.4	19.1	6.5
Visceral adipose tissue											
1.1 years SW	100	0.1	26.5	37.6	29.7	6.2	99.9	24.4	50.4	18.5	6.7
	75	0.2	28.4	38.6	26.7	6.3	99.8	24.3	50.6	18.2	6.9
	50	0.3	27.8	43.5	22.8	5.9	99.7	23.9	49.9	18.5	7.8
2.0 years SW	100	0.4	27.6	46.1	22.2	4.2	99.6	22.6	55.3	17.3	4.8
	75	0.3	25.6	48.3	21.2	4.9	99.7	23.8	53.0	18.0	5.2
	50	0.3	27.5	44.5	22.4	5.6	99.7	22.0	51.5	19.5	7.1
Dorsal adipose tissue											
1.1 years SW	100	0.1	30.0	39.9	24.0	6.1	99.9	23.8	51.1	17.7	7.4
	75	0.3	25.8	38.1	30.3	5.8	99.7	23.6	51.0	17.9	7.4
	50	0.5	28.0	34.0	32.9	5.2	99.5	23.0	51.7	17.6	7.8
2.0 years SW	100	0.3	28.6	46.3	21.3	3.9	99.7	22.0	55.8	17.1	5.1
	75	0.3	27.0	42.4	26.6	3.9	99.7	23.4	53.0	17.9	5.7
	50	0.3	26.5	44.1	24.1	5.3	99.7	22.0	51.6	19.6	6.8
Abdominal adipose tissue											
1.1 years SW	100	0.4	30.9	18.0	48.1	3.1	99.6	23.6	52.9	17.3	6.3
	75	0.6	29.3	20.6	46.9	3.2	99.4	23.8	52.1	17.3	6.8
	50	1.3	28.6	16.7	51.6	3.1	98.7	23.1	53.0	17.0	6.9
2.0 years SW	100	1.5	28.3	29.9	38.9	2.9	98.5	22.7	55.8	16.5	5.0
	75	1.0	29.2	25.8	42.2	2.9	99.0	23.2	54.4	17.1	5.3
	50	1.2	28.9	22.9	45.0	3.1	98.8	22.1	53.3	18.3	6.3
S.E.%		16	3	5	4	4	1	2	1	2	3

[a] FW, freshwater; SW = saltwater.

that feeding at a high level would decrease feed digestibility. Further, no consistent differences were found in tissue growth measured as muscle lipid content (varied between samplings in a non consistent way (Kiessling et al., 1991c), protein content, glycogen content or metabolic activity (Kiessling, Kiessling, Storebakken & Åsgård, 1991b; Kiessling et al., 1991c, respectively), which refutes the idea of a difference in feed intake between fully and over fed fish. However, a few, but striking, differences were found. The most noteworthy was a higher level of lipid deposition in viscera, dorsal and abdominal wall of RL200 compared to RL100 fish at all samplings during the sea phase (Kiessling et al., 1991c). Based on these data we suggest that over-fed fish in fact ingested more diet than fully fed fish, but they only have the digestible and metabolic capacity to utilise this extra feed for lipid accretion in adipose tissue, possibly by a preferential use of protein for aerobic phosphorylation. Further, in WM, a low level of PUFA n-3, mainly 22:6n-3, and high level of saturates was found in RL200 fish compared to RL100 fish. In all data where lipid classes are separated, such changes indicate an increased level of TAG, i.e. lipid deposition. This indicates that WM may function as a lipid depot in parity

Table 8
Changes in fatty acid composition (in percent of identified FA) of triacylglyceroles with age and ration level[a]

	Fatty acid RL	14:0	16:0	18:0	16:1 n7	18:1 n7,9	20:1 n11	22:1 n11	18:2 n6	18:3 n3	18:4 n3	20:4 n6	20:5 n3	22:5 n3	22:6 n3
White muscle															
1.1 years SW	100	4.7	17.0	3.5	7.8	22.8	11.3	10.6	5.0	0.8	1.4	0.3	3.6	1.1	9.9
	75	5.0	17.4	4.0	7.5	19.8	11.7	11.0	5.2	0.9	1.5	0.3	3.7	1.3	10.6
	50	4.5	15.3	6.5	6.1	19.0	12.7	13.2	5.4	0.9	1.4	0.4	3.4	1.3	10.0
2.0 years SW	100	5.2	16.6	2.4	9.6	22.6	12.4	10.7	3.6	0.8	1.7	0.3	3.7	1.2	9.2
	75	5.6	16.6	2.7	10.3	22.0	11.5	9.3	4.2	0.8	1.6	0.3	3.6	1.2	9.9
	50	5.9	15.8	1.6	9.5	20.6	12.5	11.0	4.9	0.9	1.9	0.3	3.8	1.3	9.9
Red muscle															
1.1 years SW	100	5.2	15.0	4.3	8.6	21.9	11.1	9.6	6.7	1.3	2.0	0.3	3.9	1.2	8.9
	75	5.2	15.1	3.7	8.1	20.4	11.9	11.0	6.9	1.2	2.2	0.2	4.2	1.1	8.7
	50	4.8	13.5	7.4	7.0	18.5	11.7	11.1	7.0	1.2	2.1	0.1	3.8	1.1	8.7
2.0 years SW	100	5.5	14.4	1.9	10.9	23.6	12.2	9.1	4.9	1.0	2.4	0.1	3.9	1.3	8.7
	75	6.0	14.2	2.2	11.1	23.2	11.8	8.6	5.1	1.0	2.1	0.2	3.8	1.3	9.2
	50	5.7	13.0	2.3	9.9	21.0	12.8	9.4	6.2	1.1	2.5	0.3	4.2	1.5	9.7
Visceral adipose tissue															
1.1 years SW	100	6.1	15.7	2.6	9.3	20.9	11.4	8.8	6.5	1.2	2.3	0.1	4.4	1.2	9.5
	75	5.9	15.9	2.6	8.5	20.1	11.9	10.0	6.7	1.1	1.9	0.3	4.4	1.2	9.5
	50	6.4	15.1	2.4	8.0	19.1	12.4	10.2	7.5	1.3	2.4	0.3	4.3	1.2	9.2
2.0 years SW	100	5.8	14.7	2.1	11.2	23.3	11.6	9.2	4.7	1.0	2.4	0.1	4.1	1.3	8.5
	75	6.9	14.9	2.0	12.4	23.1	10.0	7.0	5.0	1.0	2.4	0.1	4.4	1.3	8.9
	50	6.9	13.2	1.8	10.9	20.7	11.4	8.2	6.9	1.3	2.8	0.2	4.8	1.4	9.2
Dorsal adipose tissue															
1.1 years SW	100	6.0	15.6	2.1	9.8	21.9	11.0	8.4	6.9	1.3	2.4	0.5	4.4	1.2	8.5
	75	5.8	15.6	2.1	9.3	21.2	11.4	9.2	7.1	1.3	2.3	0.3	4.4	1.1	8.8
	50	6.0	15.0	2.0	7.9	20.0	12.8	10.9	7.4	1.3	2.3	0.4	4.2	1.1	8.8
2.0 years SW	100	5.7	14.4	1.9	11.5	24.1	11.5	8.6	5.0	1.0	2.3	0.1	4.1	1.3	8.4
	75	6.8	14.4	2.1	12.6	23.2	10.4	6.7	5.4	1.1	2.3	0.2	4.4	1.3	8.9
	50	7.0	13.1	1.9	11.5	21.6	11.1	7.2	6.6	1.2	2.8	0.2	4.9	1.4	9.3
Abdominal adipose tissue															
1.1 years SW	100	5.3	15.7	2.5	9.2	22.7	11.9	9.7	6.2	1.1	2.0	0.1	4.1	1.3	8.8
	75	5.3	16.0	2.5	8.5	20.9	12.1	10.5	6.5	1.1	2.0	0.3	4.0	1.2	8.9
	50	5.3	15.1	2.6	7.1	19.6	13.7	12.5	6.8	1.1	2.0	0.1	4.0	1.1	8.8
2.0 years SW	100	5.9	14.8	2.0	11.8	24.2	11.4	8.3	4.8	1.0	2.3	0.2	3.9	1.2	8.1
	75	6.5	14.4	2.3	12.0	23.4	11.2	7.6	5.1	1.0	2.2	0.2	4.0	1.3	8.6
	50	6.2	13.0	2.9	10.1	21.2	12.4	9.3	6.0	1.1	2.3	0.2	4.3	1.4	9.1
S.E.%		2	2	6	3	3	3	3	3	4	4	19	3	3	3

[a] FW = freshwater, SW = saltwater, RL, feed ration level, FA, fatty acid.

with that found earlier for the freshwater period (Kiessling et al., 1991c). In a study by Johansson and Kiessling (1991), the eating quality of starved rainbow trout was evaluated. The fatty acid composition was altered towards higher content of PUFA n-3 and a decrease in monounsaturates, showing the same trends in change of fatty acids levels as in our present study for severely underfed fish (RL25 and RL100/25). This suggests that feed ration at, or close to, maintenance ration is physiologically more similar to starvation than to less extreme under-feeding as RL50 and RL75. The underlying rationale for WM to store lipids became obvious when it is realised that this tissue has the single largest potential (counted on whole tissue capacity) of all tissues in salmonids to utilise lipids for its energy metabolism (Frøyland, Madsen, Eckhoff, Lie & Berge, 1998). An alternative explanation could be that fish given RL200 could obtain that food with less effort as food was more plentiful, and social hierarchies and actual physical activities to obtain food would be less, i.e. less environmental stress has led to an increased fat deposition relative to protein deposition. It is also noteworthy that values in fish given RL 75 very often coincide with those from RL100. A third possible explanation, is a difference in selective utilisation of fatty acids between these two groups. Kiessling and Kiessling (1993) showed that mitochondria isolated from RM of rainbow trout utilised fatty acids selectively as substrates for aerobic phoshorylation. Mitochondria isolated from liver did not show any selectivity. WM contained too few mitochondria to be

Table 9
Changes in fatty acid composition (in percent of identified FA) of phospholipids with age and ration level[a]

	Fatty acid RL	14:0	16:0	18:0	16:1 n7	18:1 n7,9	20:1 n11	22:1 n11	18:2 n6	18:3 n3	18:4 n3	20:4 n6	20:5 n3	22:5 n3	22:6 n3
White muscle															
1.1 years SW	100	1.9	26.2	2.1	1.5	6.5	1.4	0.4	1.4	0.3	0.2	0.5	8.2	1.8	47.6
	75	1.9	25.7	2.1	1.7	6.7	1.0	0.6	1.7	0.4	0.3	0.6	8.6	1.9	46.9
	50	1.8	25.7	2.7	1.3	5.9	1.4	0.5	1.4	0.1	0.0	0.7	8.7	1.7	48.2
2.0 years SW	100	2.1	24.7	2.1	2.6	7.9	2.6	1.7	1.2	0.4	0.5	0.6	8.0	1.6	44.0
	75	1.8	26.1	1.6	1.7	6.6	1.3	0.5	1.1	0.2	0.3	0.5	7.0	1.6	49.1
	50	2.0	25.4	1.8	1.7	7.1	1.7	0.7	1.3	0.2	0.3	0.8	7.0	1.5	47.9
Red muscle															
1.1 years SW	100	2.9	23.2	3.5	2.9	11.0	3.1	2.3	2.1	0.4	0.6	0.7	6.4	1.8	38.9
	75	2.4	20.5	3.4	3.0	11.1	3.4	2.5	2.5	0.6	0.6	0.8	7.0	1.9	40.1
	50	2.5	21.6	3.2	2.7	10.9	4.0	1.2	2.8	0.6	0.6	0.8	6.4	1.9	40.8
2.0 years SW	100	2.9	21.2	3.6	3.7	12.5	3.8	2.5	2.0	1.0	1.1	0.9	6.7	1.9	36.3
	75	3.0	21.5	3.4	3.2	10.1	3.0	2.8	1.6	0.4	0.4	0.8	6.9	1.7	40.9
	50	2.9	20.2	3.3	2.7	10.0	2.8	1.9	1.9	0.4	0.5	0.9	6.6	1.8	44.0
Visceral adipose tissue															
1.1 years SW	100	4.1	17.7	4.7	6.1	16.5	6.4	8.6	4.6	0.7	1.2	1.6	6.6	1.4	19.8
	75	5.5	18.9	4.0	6.7	16.7	7.5	7.7	5.0	0.7	1.1	1.3	5.7	1.3	17.9
	50	4.3	19.5	4.0	6.8	20.0	8.4	8.2	4.9	0.7	1.0	1.0	4.6	1.0	15.5
2.0 years SW	100	5.0	18.2	4.3	8.7	19.6	9.0	8.7	3.3	0.7	1.4	0.9	5.1	1.1	13.8
	75	4.3	17.4	3.9	8.9	19.5	7.6	8.1	3.8	0.7	1.1	1.0	4.5	1.2	13.8
	50	6.4	17.9	3.2	8.9	17.5	7.6	7.0	4.7	0.8	1.3	1.0	4.7	1.1	14.6
Dorsal adipose tissue															
1.1 years SW	100	6.3	20.1	3.6	7.7	18.0	6.8	7.4	4.9	0.7	0.8	1.2	4.6	1.2	16.7
	75	4.6	17.3	3.9	6.4	16.8	7.4	7.5	4.6	0.7	0.9	1.2	5.0	1.2	22.4
	50	4.8	19.9	3.3	5.6	15.6	6.9	6.0	4.1	0.7	0.9	1.1	4.8	1.1	25.3
2.0 years SW	100	5.8	18.7	4.1	8.9	20.8	9.1	7.4	3.1	0.7	1.2	0.8	4.6	1.0	13.7
	75	4.0	18.6	4.3	7.7	18.1	6.7	7.6	2.8	1.5	0.7	1.1	4.8	0.9	18.8
	50	4.8	17.9	3.7	8.4	18.7	6.9	6.5	4.1	0.6	0.9	1.2	5.0	0.8	16.8
Abdominal adipose tissue															
1.1 years SW	100	3.1	24.2	3.6	3.0	9.8	2.6	2.5	2.3	0.4	0.5	0.8	7.1	1.7	38.4
	75	3.1	22.8	3.4	3.3	10.3	4.0	3.1	2.4	0.5	0.6	0.9	7.7	1.6	36.5
	50	2.5	22.5	3.6	2.5	8.6	3.3	2.3	2.3	0.5	0.5	0.9	7.8	1.4	41.4
2.0 years SW	100	3.6	21.3	3.4	5.5	14.0	5.1	5.2	2.2	0.6	0.8	0.7	6.8	1.5	29.3
	75	3.3	22.3	3.6	4.5	12.5	3.7	3.1	2.2	0.4	0.5	0.8	5.9	1.4	34.0
	50	3.4	22.1	3.4	3.8	11.1	3.6	2.6	2.2	0.4	0.5	0.9	6.2	1.3	36.6
S.E.%		6	3	4	8	5	9	12	6	17	15	8	5	5	5

[a] FW, freshwater, SW, saltwater; RL, feed ration level; FA, fatty acids.

effectively isolated by the method of Kiessling and Kiessling. If WMs use fatty acids as a major substrate for aerobic phoshorylation, as suggested by Frøyland et al. (1998), it is also likely that mitochondria in this tissue are capable of selective mobilisation. Such selective utilisation, possibly triggered by the above suggested behavioural differences, could then explain the observed differences in fatty acid composition between RL100 and RL200 fish groups.

Walton and Cowey (1982) pointed out the importance of distinguishing between fresh- and marine water species but also between fresh and sea water environments in regard to the lipid metabolism of the fish. We found a marked increase in PUFA n-3 in PL of WM, as fish were moved from fresh to seawater. This change is probably even more pronounced than seen from these data as this change was contradicted by an opposite change in the diets used during the fresh versus the sea period (Table 2). This is contrary to the natural situation where a salmonid migrating from fresh water leaves an environment low in dietary HUFA n-3 (highly unsaturated fatty acids) and migrates to an environment rich in these fatty acids. It is therefore tempting to speculate whether this increase of PUFA n-3, despite a decrease in dietary content (22.2 compared with 20.3% PUFA n-3 at 1.1 year in saltwater, Pickova, 1997), reflects an inherited adaptation to a higher need of these long chained and unsaturated fatty acids in the marine environment. Bell, McVicar, Park and Sargent (1991) found an imbalance of the n-3/n-6 ratio decrease stress

tolerance in salmonids. Also, a reduction of 20:5n-3 was observed as the fish were transferred from FW to SW, possibly indicating an increased need during very low dietary availability or need for physiological regulation triggered by a change in the outer environment. This is consistent with the role of this fatty acid as precursor in eicosanoid metabolism (Sargent, 1995).

In our fish, the PUFA n-6 remained relatively stable, resulting in an increase of this ratio with sea transfer. It is possible that the optimum relation between n-3 and n-6 in the PL fraction changes between these two environments, as PUFA n-3, especially 22:6 n-3, is known to have a major role in the maintenance of membrane structure stability in fish (Sargent, Bell, Bell, Henderson & Tocher, 1995). Bell, Tocher, Farndale, Cox, McKinney and Sargent (1997) have suggested that the desmoltification process is indicated by a reversion of lipid composition with a decrease in PUFA (fresh water type) in salmonids remaining in fresh water after the natural smoltification time. This could be concluded to be an active transfer of specific fatty acids from TAG, to the metabolically more active PL fraction.

Saturates show very stable levels both in TAG and in structural lipids (PL) (Table 7). This is most likely an effect of saturates being relatively evenly distributed between both lipid classes. They are not influenced by a relative shift in content of TAG and PL as seen for the monoenes and PUFA which are differently distributed between PL and TAG. The decrease in saturated fatty acids, observed in WM in connection with saltwater transfer and with extreme low RL, is consistent with the observation by Kiessling and Kiessling (1993) that mitochondria from the RM of rainbow trout preferentially utilise saturated fatty acids for energy metabolism.

Monoenes dominate TAG. As the fish grow, lipid content increases and more TAG is deposited in the tissue, thereby increasing the relative level of monoenes in the total lipid fraction. This explanation for the observed variation of monoenes is consistent with the results shown in Table 1, indicating that lipid content, relative level of lipid class or other growth variables known to relate to lipid deposition, have high correlation coefficients for monoenes. As expected (see above) some variations are seen between WM and adipose tissues, most likely as an effect of their different physiological functions. Also, within TAG, the change in relative level of monoenes is well explained in many tissues by RL and age. The importance of monoenes for energy metabolism was in this study indicated by the observed decrease with decreasing RL. This is in accordance with other studies where it has been shown that monoenes are used as the preferential substrate for catabolism (reviewed by Henderson & Tocher, 1987; Kiessling & Kiessling, 1993).

n-3 PUFA dominate structural lipids and, in common with monoenes, are highly affected by factors related to changes in lipid content, such as growth and ration level, and thus a shift between the two major lipid classes. Again these changes are most obvious in muscle and abdominal wall, containing higher amounts of structural lipids (Tables 1 and 6). In underfed groups, or groups moved from high to low rations, an increase in 20:5n-3 was observed in WM (Table 3). 22:6n-3 Is the fatty acid most obviously altered by reduction in RL and age, with a general increase and decrease, respectively. Again, fish moved from high to low RL showed an intermediate response, while fish moved from low to high level showed a much stronger effect compared with fish given a constant high ration. This is manifested in that the RL25/100 and RL50/100 groups show the lowest values of all fish (Table 3). We suggest that this is an effect of an over-compensation in lipid deposition in this fish, with an increased influence of storage lipids (TAG) on the fatty acid pattern of the total lipid.

n-6 PUFA is only present in relatively small amounts in most marine organisms including fish. Despite this low content, the fatty acids of this group are important in lipid metabolism, being the basis for formation of arachidonic acid, 20:4n-6. Arachidonic acid, and eicosaheptaenoic acid, 20:5n-3, are the precursors for eicosanoids and the relative levels of these two fatty acids will have a profound effect on the formation of these metabolically very active substances (Sargent, 1995). It was therefore of interest to register that the fish with the highest content of 18:2n-6 in the tissue is the fish with the lowest content in the feed (Table 5). Furthermore, including the whole material (not shown) it becomes apparent that the highest level of this fatty acid, and the total n-6 group, is found in the metabolically inactive adipose tissue (dorsal and viscera). Red muscle is intermediate in this respect. In common with the above discussed fatty acid groups, the underlying rationale for this difference could be the uneven distribution of the lipid classes between these tissues. However, this is not the case, as the majority of differences observed between the adipose and muscle tissue, in respect to PUFA n-6, are found within the phospholipid structure (Table 8). This difference in PL of muscle and adipose tissue is mainly due to a higher content of 18:2n-6 in the latter, while only small differences were seen in levels of 20:4n-6. We suggest that this difference is not an effect of a higher deposition in PL of adipose tissue, but rather an effect of a higher mobilisation from PL in muscle. This, as well as precursor fatty acids to eicosanoids, are mobilised from this fraction (Voss, Reinhart, Sankarappa & Sprecher, 1991 on rat; reviewed by Sargent, Bell, Bell, Henderson & Tocher, 1995, in fish).

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