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Fatty acid profiles of white muscle and liver in stream-maturing steelhead trout *Oncorhynchus mykiss* from early migration to kelt emigration

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The profiles of specific fatty acids (FAs) in white muscle and liver of fasting steelhead trout *Oncorhynchus mykiss* were evaluated at three periods during their prespawning migration and at kelt emigration in the Snake/Columbia River of Washington, Oregon and Idaho, to improve the understanding of energy change. Twenty-seven FAs were identified; depletion of 10 of these was positively correlated in liver and white muscle of prespawning *O. mykiss*. To observe relative changes in FA content more accurately over sampling intervals, the lipid fraction of tissues was used to normalize the quantity of individual FA to an equivalent tissue wet mass. Saturated and monounsaturated FAs were depleted between upstream migration in September and kelt emigration in June, whereas polyunsaturated FAs were more conserved. Liver was depleted of FAs more rapidly than muscle. Three FAs were detected across all sampling intervals: 16:0, 18:1 and 22:6n3, which are probably structurally important to membranes. When structurally important FAs of *O. mykiss* are depleted to provide energy, physiological performance and survival may be affected.

Key words: iteroparity; lipid; physiology; reproduction.

INTRODUCTION

Unlike semelparous Pacific salmon, *Oncorhynchus* spp., that die after one spawning cycle, steelhead trout *Oncorhynchus mykiss* (Walbaum 1792) are iteroparous and can spawn repeatedly. Although most *O. mykiss* begin downstream emigrations after spawning as kelts, many do not survive to spawn a second time (Busby *et al.*, 1996; Quinn & Myers, 2004). During spawning migrations, *O. mykiss* fast and rely on stored lipid and tissue protein for energy to support migration, gonadal maturation and spawning. In salmonids, white muscle serves as the primary energy storage tissue (Hendry *et al.*, 2000). Lipid is prioritized over protein for energy because it is easier to mobilize, contains more energy per unit mass (lipid 26·4 *v.* protein 20·1 kJ g^{-1}), and does not hinder swimming ability when depleted (Mommsen *et al.*, 1980; Hendry *et al.*, 2000; Penney & Moffitt, 2014*a*).

The constituents of lipids, fatty acids (FAs), can be separated based on the number of double bonds in their respective carbon chains into saturated (SFAs), monounsaturated (MUFAs) and polyunsaturated (PUFAs). The long chained MUFAs, specifically

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18:1n9, 20:1n9 and 22:1n9, are readily oxidized for energy in fishes (Tocher, 2003). In contrast, structurally important FAs, such as PUFAs like 22:6n3 (Sargent *et al.*, 1999), are generally conserved. Fishes utilize many FAs for metabolic energy *via* β-oxidation, which occurs in peroxisomes and mitochondria (Tocher, 2003). In most teleosts, the liver and red muscle are the primary sites of FA oxidation but white muscle is also important in salmonids (Ruyter & Thomassen, 1999). Identifying FAs that are specifically used for energy can be difficult without first separating neutral and polar lipids; however, it is possible to infer which individual FA or FA groups (SFAs, MUFAs and PUFAs) are selectively oxidized (consumed) in fasting or starving fishes.

The depletion of FAs in spawning semelparous salmonids of the genus *Oncorhynchus* has been documented in several studies (Ando *et al.*, 1985; Sasaki *et al.*, 1989; Hatano *et al.*, 1995, Phleger *et al.*, 1995; Ballantyne *et al.*, 1996, Magnoni *et al.*, 2006), and these species may lose >70% of lipid stores from the point of freshwater re-entry to spawning (Gilhousen, 1980; Brett, 1995). Less is known about FA depletion in iteroparous anadromous salmonids during reproduction (Jonsson *et al.*, 1997; Jobling *et al.*, 1998). Few studies describe FA depletion in populations that spend prolonged periods fasting in fresh water before spawning like Atlantic salmon *Salmo salar* L. 1758 and stream-maturing *O. mykiss*.

Stream-maturing Columbia/Snake River *O. mykiss* return to fresh water during the summer and autumn (July to October) to begin migrations upstream, but spawning occurs in the spring (March to June). In some Snake River populations, stream-maturing *O. mykiss* will swim >1500 km to reach their natal systems and spend 8 to 11 months fasting before spawning. After spawning Snake/Columbia River *O. mykiss* kelts begin emigrations back towards the Pacific Ocean (April to June) to recover lost energy and undergo gonadal recrudescence. Penney & Moffitt (2014*a*) reported that muscle lipid was effectively exhausted in stream-maturing Snake River *O. mykiss* kelts (lipid 0·1–0·3% by wet tissue mass) and hypothesized that low rates of iteroparity in these stocks (<2%) may be related to protein catabolism. Few studies address how extensive FA depletion affects the repeat-spawning potential of *O. mykiss* kelts. Like protein, the depletion of structurally important FA probably also degrades the performance and physiological condition of kelts.

In the present study, the proximate and FA compositions of white muscle and liver of *O. mykiss* were assessed at three periods during prespawning migration and once during kelt emigration. The objectives were to: (1) identify and describe the distribution and relative proportion of FAs across sample periods for the liver and white muscle; (2) compare paired white muscle and liver samples across sample periods to determine if changes in FA composition were correlated between the tissues; (3) evaluate the absolute change across sample periods in the equivalent wet mass of FAs. It was hypothesized that the FA composition between white muscle and liver tissues would vary due to differences in lipid storage capacity and metabolic function, and that structurally important FAs would be conserved.

MATERIALS AND METHODS

STUDY SITES

Stream-maturing Snake/Columbia River *O. mykiss* were sampled and killed at four intervals in their freshwater migration (1) late summer (September), (2) early autumn (October), (3) late

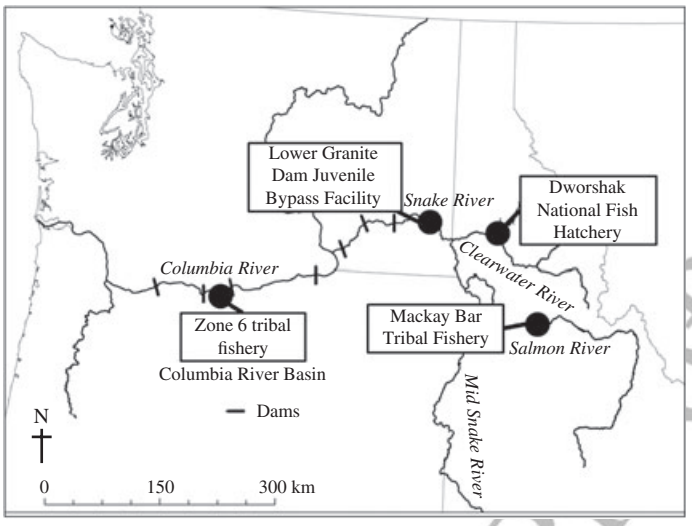

FIG. 1. Map of sample sites in the Columbia and Snake Rivers.

autumn (November) and (4) kelt emigration (June). September migrants were from mixed stocks of hatchery (adipose fin absent) and natural (adipose fin present) origin stocks captured in the zone 6 tribal gillnet fishery (45° 39′ N; 120° 57′ W) between the Dalles and John Day Dams on the Columbia River OR/WA. October migrants were from mixed hatchery and natural stocks from tribal hook and line harvests at Mackay Bar (45° 24′ N; 115° 28′ W) on the Salmon River, ID. November migrants were from known hatchery broodstock at Dworshak National Fish Hatchery (46° 30′ N; 116° 19′ W) located on the North Fork of Clearwater River, ID. Mixed stocks of unknown hatchery origin and natural-origin emigrating kelts were intercepted at the Lower Granite Dam juvenile bypass facility (46° 39′ N; 117° 26′ W), WA located on the Snake River (Fig. 1 and Table I). All kelts were sampled in spawning year 2010 (the year of spawning), whereas September, October and November migrants were sampled in spawning year 2011.

TISSUE COLLECTION AND ANALYSIS

Oncorhynchus mykiss from tribal harvests were killed by blows to the skull and samples from Dworshak National Fish Hatchery and Lower Granite Dam were killed with lethal doses (200 mg l^{-1}) of MS-222 (Finquel, Argent Laboratories; www.argent-labs.com) buffered with $NaHCO_3$. All fish were measured for fork length (L_F; 0·5 cm), examined for marks and tags, and identified as hatchery or natural origin. All September, October and November migrants were considered in good external condition using the criteria of Penney & Moffitt (2014*a*). External condition of kelts varied and was reported for individuals sampled. A muscle fillet (skin on) was removed from a location posterior to the insertion of the dorsal fin and the liver (gall bladder removed) was excised from the peritoneal cavity. Fillets and livers were weighed (0·05 g) and frozen immediately on dry ice and stored in the laboratory at −80° C until analysis.

PROXIMATE AND ENERGETIC ANALYSES

Muscle fillets were partially thawed for separation of white muscle from the skin and red muscle. White muscle and whole liver tissues were individually weighed and pureed while still partially frozen. A portion of pureed tissue was then re-weighed and dried at 105° C for 8 h to determine moisture content. Dried tissue was ground into a fine powder using a mortar and pestle for lipid and ash analysis. The ash (inorganic) component was determined by

TABLE I. Description of stream-maturing Snake/Columbia *Oncorhynchus mykiss* sampled during early upstream migration and kelt emigration including sex, external condition, sample size and fork length (L_F)

					L_F (cm)		
Sampling period	Month	Spawn year	Sex	External condition	*n*	Mean	Range (minimum–maximum)
Late summer	September	2011	F	Good	9	77·1	64·0–88·0
			M	Good	4	73·3	55·0–89·0
Early autumn	October	2011	F	Good	4	71·0	56·0–82·0
			M	Good	5	68·4	62·0–90·0
Late autumn	November	2011	F	Good	10	81·1	75·0–87·0
			M	Good	6	81·2	56·0–92·0
Kelt	June	2010	F	Good	2	55·0	53·0–57·0
				Fair	2	75·5	75·0–76·0
				Poor	0		
			M	Good	2	59·5	57·0–62·0
				Fair	3	66·0	61·0–71·0
				Poor	1	76·0	76·0

n, sample size; F, female; M, male.

incinerating a pre-weighed aliquot of dried tissue at 550° C in a muffle furnace. Crude lipid content (neutral + polar) was extracted from dried tissues using an Ankom XT15 lipid extractor (www.ankom.com). Dry protein content was calculated *via* subtraction of ash and lipid content (100 − (% dry ash + % dry lipid) = % dry protein). Energy density (lipid + protein) of tissues was determined using a Parr 6300 Calorimeter (www.parrinst.com) and expressed in kJ g^{-1}.

All white muscle and liver samples were analysed in duplicate to provide an average of % lipid, % protein, % ash and % moisture, plus energy density. The average values were then expressed as percentages of wet tissue mass using the same calculation described by Hendry *et al.* (2000). All methods were performed in accordance with standard operating procedures of the Association of Official Analytical Chemists (AOAC, 2000).

FATTY ACID ANALYSIS

The separation of unique FA was performed on samples of wet pureed liver and muscle using a two-step methylation procedure as described by Bligh & Dyer (1959), and saponification and esterification of extracted lipids followed the same procedures used by Powell *et al.* (2010). For this study, individual FAs are identified following the same designations used by Tocher (2003), which defines a FA based on its carbon chain length, number of ethylenic bonds (saturation level) and position of the ethylenic bond (*n*-series). The FA methyl esters were analysed using a Shimadzu GC17A (http://shimadzu.com/) gas chromatograph containing at 30 m capillary column. Analysis conditions were as follows: injection temperature 250° C; detector temperature 260° C; oven temperature programmed to increase from 100 to 235° C at 3·5° C per min, and held at that temperature for 35 min. The total run time was 70 min for all samples. Individual FAs were identified by retention times in accordance to the internal standards (Superlco; www.sigmaaldrich.com) and expressed as a percentage of the total amount (100%) of FAs. Each tissue sample was analysed in duplicate to provide an average value for all FAs. Tissue samples with large absolute differences between duplicate runs were censored from analysis. The total sum of % SFAs, % MUFAs and % PUFAs was determined for each individual tissue sample.

A normalized quantity of each FA was calculated as an equivalent proportion of wet mass (g) in 100 g of wet tissue to account for changes in total lipids across sample periods. For each

sample, the proportion of lipid was expressed as an equivalent mass of lipid in 100 g of wet tissue (% lipid 100 g wet tissue = g lipid mass). From the wet lipid mass, the percentage of each FAs was multiplied by the estimated wet lipid mass to determine a normalized wet mass for individual FA (% FA g wet lipid mass = g wet mass individual FA). The total wet mass sum of SFAs, MUFAs and PUFAs was then determined for each tissue sample.

STATISTICAL ANALYSIS

Because of sampling constraints associated with tribal harvest, no control of the proportions of female and male *O. mykiss* sampled was possible. Descriptive statistics and box plots were used to summarize the proximate (% moisture, % lipid, % ash and % protein) and energetic density (kJ g^{-1}) of white muscle and liver tissue, and significant differences were determined using general linear models and Tukey's HSD by tissue across sampling periods with the model: $y_i = \mu + \alpha_i + \epsilon_i$, where y_i refers to the response variables, μ is the overall mean, α_i is the sampling period (September, October, November and kelt) and ϵ_j is the random error. The relative change (%) in FA composition across sample periods was calculated for individual FA and for FA groups (SFAs, MUFAs and PUFAs). Correlations of the proportion of individual FA in white muscle *v*. liver samples of individual fish were made using Spearman correlations for three sampling periods before spawning. The FA composition in kelts was not included in correlations because of the low lipid content (<1·0%) in both the white muscle and liver tissues.

Using values of total lipid in each fish tissue, changes in the normalized wet mass of SFAs, MUFAs and PUFAs across sampling periods were analysed using MANOVA and Wilks–Lambda statistics. Analysis followed the general linear model: $y_i = \mu + \alpha_i + \epsilon_i$, where y_i refers to the response variables (sum of SFAs, sum of MUFAs and sum of PUFAs), μ is the overall mean, α_i is the sampling period (September, October, November and kelt) and ϵ_j is the random error. Significant differences detected by the MANOVA were assessed using permuted multiple comparison analysis (20 000 permutations) to identify which sampling periods and FA groups were significantly different. All data analyses were performed using SAS 9.2 (SAS Institute; www.sas.com) and tests considered significant at $\alpha = 0{\cdot}05$.

RESULTS

PROXIMATE COMPOSITION

From September to kelt emigration, the mean lipid content of white muscle and liver tissues decreased by 95·7 and 86·4% (Fig. 2). The effect of sampling period on lipid content was highly significant (ANOVA, $F_{3,44} = 54{\cdot}62$, $P < 0{\cdot}001$). The total lipids in samples of white muscle showed a progressive decrease, with significant differences detected between all sampling intervals except the October and November samples. Liver lipids were significantly different across sampling periods (ANOVA, $F_{3,44} = 11{\cdot}91$, $P < 0{\cdot}001$), driven by a rapid decline after September. White muscle and liver protein decreased by 23·5 and 6·9% from September to kelt emigration, but followed different patterns of depletion. White muscle protein followed a significant stepwise decrease similar to lipid (ANOVA, $F_{3,44} = 32{\cdot}19$, $P < 0{\cdot}001$), while the proportion of protein in liver tissues increased from September to November migration but then declined significantly by kelt emigration (Fig. 2). White muscle ash content exhibited a significant stepwise decrease across sampling periods (ANOVA, $F_{3,44} = 34{\cdot}94$, $P < 0{\cdot}001$), whereas the ash content of liver was more stable and differences across sampling periods were not significant. Changes in white muscle energy density followed trends similar to lipid and protein, exhibiting an overall decrease of 47·0 and 27·7% from September to kelt emigration. Moisture content of proximate samples followed an inverse pattern to lipid, protein and energy density in white muscle and liver

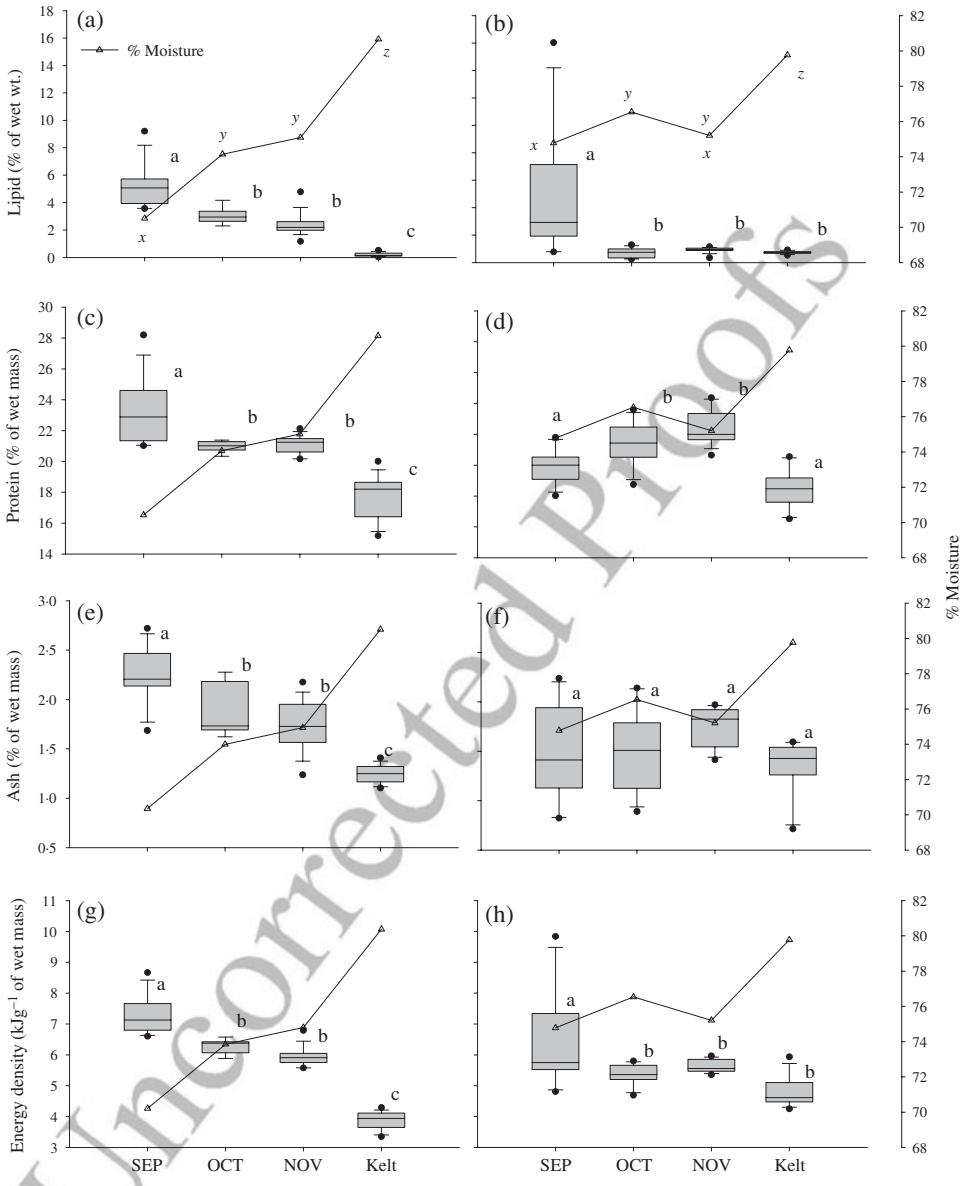

FIG. 2. Boxplots (line within box = median; ends of box = 25th and 75th percentiles; ends of whiskers = 90th and 10th percentiles; black circles = outliers) of (a), (b) lipid, (c), (d) protein and (e), (f) ash composition, and (g), (h) energy density of (a), (c), (e), (g) white muscle and (b), (d), (f), (h) liver tissue samples from Snake River *Oncorhynchus mykiss* in September (SEP), October (OCT), November (NOV) and kelt emigration (Kelt). The results of least-squared mean comparisons of lipid, protein, ash and energy density by sampling period for each tissue are marked. Those with the same lower case letters (a, b, c) were not statistically different ($\alpha = 0{\cdot}05$). The mean moisture content for samples is provided with a second *y* axis (△), and the results of least-squared mean comparisons of moisture content of muscle and liver samples from each sampling period are provided. Moisture content with the same lower case letters (*x*, *y*, *z*) were not significant different ($\alpha = 0{\cdot}05$).

TABLE II. Mean ± S.E. of *Oncorhynchus mykiss* white muscle fatty acid composition (%) between September, October, November and kelt sampling periods and total sums of saturated (SFAs), monounsaturated (MUFAs) and polyunsaturated (PUFAs) fatty acids

FAME	September ($n = 13$)	October ($n = 9$)	November ($n = 16$)	Kelt ($n = 10$)
C14:0	3·06 ± 0·12	2·75 ± 0·20	3·06 ± 0·12	0·25 ± 0·13
C14:1	0·01 ± 0·01	0·0 ± 0·0	0·0 ± 0·0	0·0 ± 0·0
C15:0	0·17 ± 0·04	0·19 ± 0·06	0·20 ± 0·04	0·0 ± 0·0
C15:1	0·00 ± 0·00	0·0 ± 0·0	0·0 ± 0·0	0·0 ± 0·0
C16:0	15·86 ± 0·30	15·82 ± 0·81	12·80 ± 0·23	17·65 ± 0·95
C16:1	3·66 ± 0·27	3·84 ± 0·34	3·10 ± 0·14	0·53 ± 0·23
C17:0	0·29 ± 0·09	0·73 ± 0·03	0·70 ± 0·06	0·0 ± 0·0
C17:1	0·08 ± 0·04	0·0 ± 0·0	0·08 ± 0·05	0·0 ± 0·0
C18:0	3·63 ± 0·11	3·79 ± 0·24	2·62 ± 0·20	4·87 ± 0·45
C18:1n9	14·27 ± 0·85	18·54 ± 1·50	13·50 ± 0·87	8·64 ± 0·94
C18:2n6	0·97 ± 0·08	0·91 ± 0·12	2·40 ± 0·62	0·0 ± 0·0
C18:3n3	0·74 ± 0·08	5·88 ± 0·57	7·32 ± 0·71	0·0 ± 0·0
C20:0	11·75 ± 0·70	0·03 ± 0·03	0·17 ± 0·13	8·34 ± 1·85
C20:1n9	1·01 ± 0·11	7·41 ± 0·59	7·18 ± 0·75	0·0 ± 0·0
C20:2n6	0·18 ± 0·05	0·83 ± 0·21	1·39 ± 0·14	0·0 ± 0·0
C20:3n3	0·29 ± 0·07	7·69 ± 0·96	9·98 ± 0·48	0·0 ± 0·0
C20:4n6	0·23 ± 0·08	1·88 ± 0·20	2·37 ± 0·11	0·70 ± 0·23
C20:5n3	6·25 ± 0·28	5·80 ± 0·13	6·71 ± 0·22	9·31 ± 0·64
C22:0	8·73 ± 0·58	0·0 ± 0·0	0·0 ± 0·0	3·90 ± 1·17
C22:1n9	1·38 ± 0·11	0·0 ± 0·0	0·08 ± 0·06	0·0 ± 0·0
C22:2n6	0·0 ± 0·0	0·0 ± 0·0	0·0 ± 0·0	0·0 ± 0·0
C22:3n6	0·01 ± 0·01	0·0 ± 0·0	0·0 ± 0·0	0·0 ± 0·0
C22:4n6	0·09 ± 0·04	0·0 ± 0·0	0·03 ± 0·02	0·0 ± 0·0
C22:5n3	2·50 ± 0·14	2·64 ± 0·16	3·26 ± 0·11	3·08 ± 0·48
C22:6n3	24·55 ± 1·45	21·17 ± 0·61	22·65 ± 0·60	42·74 ± 2·28
C24:0	0·15 ± 0·07	0·0 ± 0·0	0·0 ± 0·0	0·0 ± 0·0
C24:1	0·0 ± 0·0	0·25 ± 0·80	0·43 ± 0·08	0·0 ± 0·0
% SFAs	43·64	23·32	19·55	35·01
% MUFAs	20·41	30·04	24·38	9·17
% PUFAs	35·80	46·80	56·11	55·83

n, sample size.

tissues, with significant differences between September migrants and kelts evident in liver and white muscle samples (Fig. 2).

FATTY ACID DIVERSITY AND PATTERNS

Twenty-seven FAs were detected in white muscle and liver tissues across all sampling intervals (Tables II and III). In white muscle, 14:1, 15:1, 22:2n6, 22:3n6, 22:4n6, 24:0 and 24:1 were detected only in trace amounts (mean < 0·05%). In liver, 14:1, 15:1, 20:3n3, 22:2n6, 22:3n6 and 24:0 were detected in trace amounts in all samples.

Significant positive correlations were observed between the proportions of 10 FAs in the liver and muscle: 14:0, 16:1, 17:0, 18:3n3, 20:0, 20:1n9, 20:2n6, 20:4n6, 22:0, 22:1n9 and 24:1n9 (Fig. 3). Only one correlation was negative (20:1n9) driven by the

TABLE III. Mean ± S.E. value of liver fatty acid composition (%) between September, October, November and kelt sampling periods and total sums of saturated (SFAs), monounsaturated (MUFAs) and polyunsaturated (PUFAs) fatty acids

FAME	September ($n = 11$)	October ($n = 11$)	November ($n = 16$)	Kelt ($n = 15$)
C14:0	2·17 ± 0·17	1·22 ± 0·11	1·46 ± 0·09	1·01 ± 0·13
C14:1	0·03 ± 0·02	0·0 ± 0·0	0·0 ± 0·0	0·0 ± 0·0
C15:0	0·23 ± 0·06	0·08 ± 0·04	0·37 ± 0·04	0·16 ± 0·05
C15:1	0·0 ± 0·0	0·0 ± 0·0	0·0 ± 0·0	0·01 ± 0·01
C16:0	15·60 ± 0·94	19·29 ± 0·68	17·94 ± 0·48	19·52 ± 0·75
C16:1	3·76 ± 0·54	2·34 ± 0·25	1·67 ± 0·11	1·40 ± 0·12
C17:0	0·48 ± 0·10	0·69 ± 0·04	0·82 ± 0·02	0·30 ± 0·05
C17:1	0·20 ± 0·06	0·0 ± 0·0	0·0 ± 0·0	0·17 ± 0·05
C18:0	5·80 ± 0·70	6·99 ± 0·61	6·86 ± 0·44	6·53 ± 0·41
C18:1n9	19·08 ± 1·46	16·48 ± 0·89	13·72 ± 0·35	14·44 ± 0·42
C18:2n6	1·20 ± 0·11	0·80 ± 0·05	0·97 ± 0·06	0·90 ± 0·11
C18:3n3	0·72 ± 0·14	2·70 ± 0·40	3·57 ± 0·21	0·19 ± 0·09
C20:0	5·77 ± 1·20	0·57 ± 0·14	0·90 ± 0·09	0·0 ± 0·0
C20:1n9	1·40 ± 0·52	0·79 ± 0·42	0·0 ± 0·0	3·06 ± 0·45
C20:2n6	0·38 ± 0·06	0·37 ± 0·09	0·74 ± 0·06	0·20 ± 0·05
C20:3n3	0·03 ± 0·02	0·19 ± 0·10	0·16 ± 0·08	0·03 ± 0·02
C20:4n6	2·32 ± 0·24	2·45 ± 0·50	3·90 ± 0·15	5·42 ± 0·24
C20:5n3	7·69 ± 0·61	1·81 ± 0·42	1·53 ± 0·14	9·97 ± 0·66
C22:0	1·92 ± 0·57	0·0 ± 0·0	0·0 ± 0·0	0·60 ± 0·15
C22:1n9	2·55 ± 0·31	0·42 ± 0·16	0·0 ± 0·0	0·18 ± 0·05
C22:2n6	0·49 ± 0·49	0·0 ± 0·0	0·0 ± 0·0	0·0 ± 0·0
C22:3n6	0·11 ± 0·05	0·0 ± 0·0	0·0 ± 0·0	0·54 ± 0·18
C22:4n6	0·14 ± 0·05	0·25 ± 0·11	0·26 ± 0·08	0·0 ± 0·0
C22:5n3	4·44 ± 0·26	4·02 ± 0·26	4·35 ± 0·17	4·44 ± 0·19
C22:6n3	23·35 ± 2·04	29·80 ± 0·78	30·67 ± 0·32	30·56 ± 1·20
C24:0	0·04 ± 0·03	0·0 ± 0·0	0·0 ± 0·0	0·01 ± 0·01
C24:1	0·06 ± 0·03	8·91 ± 0·34	10·22 ± 0·16	0·24 ± 0·08
% SFAs	32·01	28·85	28·35	28·13
% MUFAs	27·07	28·93	25·61	19·50
% PUFAs	40·87	42·39	46·15	52·25

n, sample size.

absence of 20:1n9 in the white muscle of November migrants and absence in the liver for most October and November migrants (Fig. 3). White muscle and liver tissues of September migrants exhibited the highest proportions of 14:0, 16:1 20:0, 22:0 and 22:1n9 compared to October and November migrants. In contrast, 17:0, 18:3n3, 20:2n6 and 20:4n6 were highest in the white muscle and liver tissues in November migrants. In September samples, 24:1 was present in liver and absent in the white muscle.

FATTY ACID PROFILES

The proportion of SFAs in white muscle of *O. mykiss* decreased over the time spent in fresh water. In September samples, >43% of the FAs were saturated, dominated by 16:0, 20:0 and 22:0 (Table II). In samples from October and November migrants,

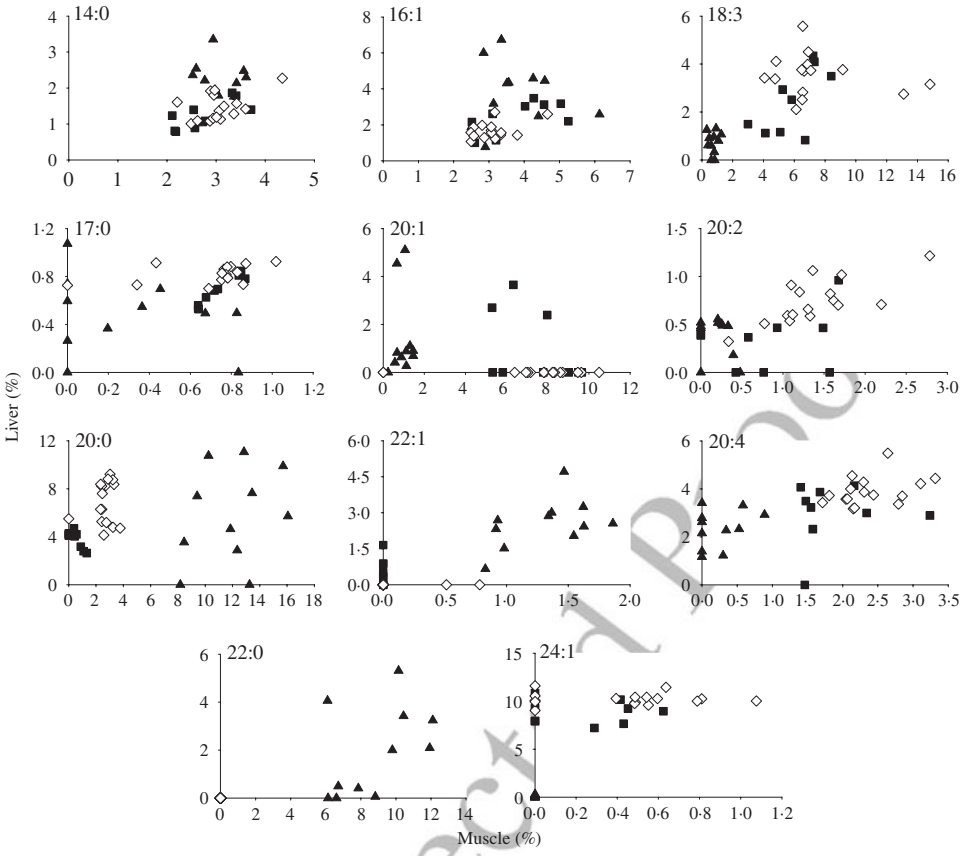

FIG. 3. Scatterplots of the proportion of specific fatty acids that had significant Spearman correlations between white muscle and liver tissues for all sampling periods (▲, September; ■, October; ◇, November) except kelts. Kelts were omitted because of the low lipid content (<1·0%) in both the white muscle and liver tissues.

SFAs comprised 23·7–19·3% of the lipids with 16:0 still dominant. In November, the majority of FAs were polyunsaturated and two were dominant: 20:3n3 (7·0–10·1%) and 22:6n3 (20·3–22·7%). Between the September upstream migration and the kelt emigration in June, the white muscle MUFA content decreased by 57·3% (Table II). The only MUFA remaining in kelts was 18:1, which comprised <9% of the total FA content. In kelts more than half the FAs in the white muscle were polyunsaturated, largely 22:6n3.

For liver tissue, SFAs comprised 33·0% of total FAs in September migrants and nearly half of these were 16:0 (16·6%), with smaller proportions of 18:0 and 20:0 (Table III). Monounsaturated FAs comprised 25·2% of FAs and were dominated by 18:1. Polyunsaturated FAs were detected across all sampling periods and constituted 42·5% of liver FAs in September dominated by 22:6n3. Saturated FAs and MUFAs decreased by 13·7 and 22·5% from September to kelt emigration, while the relative proportion of PUFAs increased by 20·4%. In kelts, the SFAs of the liver were mainly composed of 16:0. Monounsaturated FAs were dominated by 18:1 (15·0%), and PUFAs comprised more than half of the total FAs in kelt livers, dominated by 22:6n3.

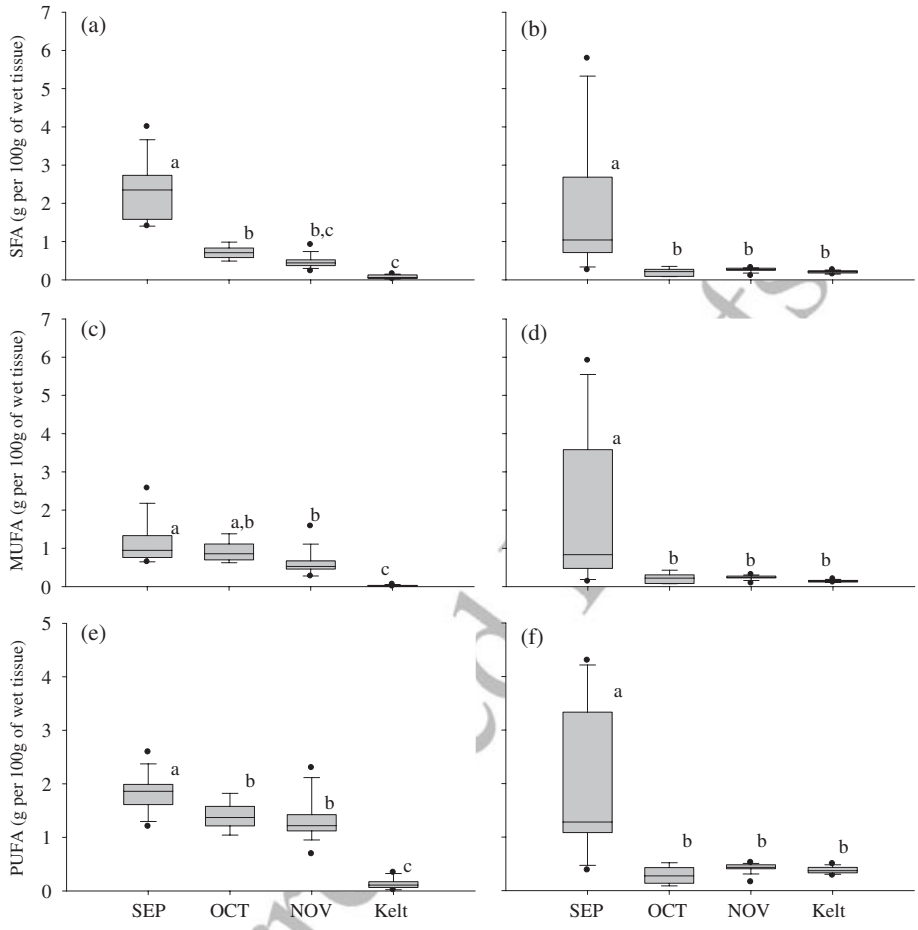

FIG. 4. Box plots (line within box = median; ends of box = 25th and 75th percentiles; ends of whiskers = 90th and 10th percentiles; black circles = outliers) of normalized composition (equivalent g) per 100 g of wet tissues, (a), (c), (e) white muscle and (b), (d), (f) liver tissue samples from Snake River *Oncorhynchus mykiss* in September (SEP), October (OCT), November (NOV) and kelt emigration (Kelt), separated by (a), (b) saturated (SFAs), (c), (d) monounsaturated (MUFAs) and (e), (f) polyunsaturated (PUFAs) fatty acids. The results of least-squared means comparisons by sampling period are shown, and those with the same lower case letters were not statistically different ($\alpha = 0{\cdot}05$).

NORMALIZED FATTY ACID PROFILES

The total lipid contents of muscle and liver tissues from *O. mykiss* sampled over time in fresh water showed significant decreases. By using these proportions to normalize the FA constituents, interpretations of changes in composition within tissues and across sampling periods are clarified. The normalized equivalent wet masses of all FA groups decreased significantly in white muscle from September migration to kelt emigration (MANOVA, Wilks' $\lambda = 0{\cdot}02$, $F_{9,102{\cdot}4} = 40{\cdot}2$, $P < 0{\cdot}001$). The sequence of changes in the groups of FA across sampling periods varied, but kelts were always significantly different from the September, October and November migrants (Fig. 4). The normalized wet masses of SFAs and PUFAs in September migrants were significantly higher

compared with samples collected at other times (ANOVA, all $P < 0{\cdot}001$). The equivalent masses of MUFAs in white muscle did not differ between September and October migrants, but September and October migrants had higher MUFAs than November migrants and kelts (ANOVA, $P < 0{\cdot}001$).

The normalized composition of FA groups in liver tissues also decreased significantly between upstream migration in September to kelt emigration (MANOVA, Wilks' $\lambda = 0{\cdot}21$, $F_{9,109{\cdot}7} = 10{\cdot}7$, $P < 0{\cdot}001$). The normalized wet masses of SFAs, MUFAs and PUFAs were significantly higher in September compared with all other sampling periods (ANOVA, all $P < 0{\cdot}01$). All SFAs, MUFAs and PUFAs declined rapidly in samples after September (Fig. 4). No significant differences in SFAs, MUFAs and PUFAs were detected in pair-wise comparisons of *O. mykiss* sampled in October, November and kelts.

DISCUSSION

This study is the first to report specific FA profiles of white muscle and liver tissues in *O. mykiss* during spawning and postspawning migrations. Stream-maturing *O. mykiss* selectively depleted SFAs and MUFAs during upstream migration and conserved PUFAs. By the time of kelt migration, most FAs, regardless of group were nearly exhausted. The majority of white muscle (>95·0%) and liver (>85·0%) lipid stores were depleted over the *c.* 8 months from the time of sampling in September to the kelt migration, and the largest decrease was observed during the period of upstream migration (Brannon *et al.*, 2004). Upstream migration can account for >75–90% of lipid use during the reproductive cycle of many anadromous salmonids (Gilhousen, 1980; Hendry & Berg, 1999; Kiessling *et al.*, 2004). Comparatively, liver lipid exhibited a more rapid decrease than that was observed in white muscle tissue. Einen *et al.* (1998) reported that liver lipid was depleted more rapidly than muscle tissue in adult *S. salar* starved for 86 days. Unlike leaner fish species that store large quantities of lipid in the liver, such as pikeperch *Sander lucioperca* (L. 1758) and cod *Gadus morhua* L. 1758, salmonids store the majority of lipid in white muscle (Dos Santos *et al.*, 1993; Uysal *et al.*, 2006).

White muscle and liver protein both exhibited overall decreases from September to kelt migration but the patterns of depletion were not consistent between the two tissues. Penney & Moffitt (2014*b*) determined that white muscle protein was conserved over lipid during the spawning migration of Snake/Columbia River *O. mykiss*. White muscle protein acts as a secondary source of energy when lipid stores are exhausted (Hendry & Berg, 1999; Hendry *et al.*, 2000). Previous studies support that liver protein generally decreases during spawning migrations, but the pattern of depletion can be variable across species (Idler & Bitners, 1960; Einen *et al.*, 1998).

Ash content comprised a small (0·5–3·0%) component of white muscle and liver tissues, but showed a gradual decrease in white muscle from September to kelt emigration. Ando *et al.* (1985) reported that the ash content of white muscle in chum salmon *Oncorhynchus keta* (Walbaum 1792) decreased during spawning migrations. The inorganic constituents and minerals of ash are generally the proximate components least affected by changes in growth (Busacker *et al.*, 1990). Love (1970) noted that the ash composition of fasting fishes generally increases due to the proportional decrease of tissue to skeletal and other bony material. Small bones sometimes present in samples

of white muscle tissue for proximate analysis could have caused variations in inorganic constituents during analysis in this study.

FATTY ACID PROFILES

The relative changes in FA composition of Snake/Columbia River *O. mykiss* in this study were similar to patterns reported for other anadromous salmonids during spawning migrations. Polyunsaturated FAs generally comprised the largest proportion of total FAs in white muscle (36–56%) and liver (42–51%) tissues. Marine fishes generally contain higher proportions of PUFAs over freshwater fishes, because marine algae are enriched in PUFAs (Heintz *et al.*, 2010). Anadromous salmonids achieve the majority of their somatic growth during marine residence and the FA composition of tissues is generally reflective of dietary source, especially PUFAs that cannot be synthesized *de novo* (Ruyter & Thomassen, 1999). From upstream migration in September to kelt emigration, the SFAs and MUFAs were selectively depleted in white muscle and liver, while PUFAs were conserved. The depletion of SFAs and MUFAs during migration and reproduction is variable within and between species of anadromous salmonids (Brett, 1995). In starved or fasting salmonids, FA oxidation appears to favour the use of short chained SFAs and MUFAs over longer chained FAs (Ando *et al.*, 1985; RingØ *et al.*, 1990; Ballantyne *et al.*, 1996; Booth *et al.*, 1999). Both SFAs and MUFAs can be catabolized for energy production *via* mitochondrial β-oxidation, but this process is more complicated for PUFAs (Tocher, 2003). Longer chained FAs are oxidized more slowly over shorter chained FAs while many PUFAs are incorporated into membrane polar lipids (Ruyter & Thomassen, 1999). Brett (1995) noted that Chinook salmon *Oncorhynchus tshawytscha* (Walbaum 1792) and coho salmon *Oncorhynchus kistutch* (Walbaum 1792) utilized shorter chained FAs first during early migration. From a physiological standpoint, oxidizing long chained PUFAs would be energetically inefficient and, more importantly, could affect membrane integrity. Another factor possibly affecting patterns in SFA and MUFA profiles during spawning migrations may be lipid allocation for gonadal maturation. In this study, sample sizes did not permit comparisons between sexes. It has been reported that 16:0 and 18:1n9 are particularly important in ovarian development (Wiegand & Idler, 1985) and 20:4n6 has been implicated as an important precursor for steroid production (Booth *et al.*, 1999). Future investigation of sex differences in FA depletion is warranted.

Although FAs were consolidated into groups of SFAs, MUFAs and PUFAs, each FA group in white muscle and liver was generally dominated by a single FA (16:0, 18:1n9 and 22:6n3). These same FAs were dominant in studies of white muscle and liver tissues in other anadromous salmonids (Ando *et al.*, 1985; Sasaki *et al.*, 1989; Hatano *et al.*, 1995; Jonsson *et al.*, 1997). Most plasma non-esterified FAs in sockeye salmon *Oncorhynchus nerka* (Walbaum 1792) (Ballantyne *et al.*, 1996) and *S. salar* (Booth *et al.*, 1999) were these same FAs. Ratnayake & Galli (2009) noted that 16:0 and 18:1n9 were among the most widely occurring SFAs and MUFAs in vegetable and animal oils. Tocher (2003) mentioned that 16:0 was among the few SFAs within animal cell membranes, but also noted that 16:0 can be rapidly oxidized to provide energy within all eukaryotic cells. Likewise, 18:1n9 has also been more commonly associated with neutral lipids in marine fish species and implicated as an important energy source during spawning migrations (Wiegand & Idler, 1985; Booth *et al.*, 1999; Huynh *et al.*, 2007).

The proportions of 10 individual FAs showed positive correlations between liver and muscle over three sampling intervals. These findings suggest that although the liver is not a primary storage tissue for lipid, it is reflective of FA consumption from other lipid depots (*e.g.* muscle, viscera and skin). Some FAs were present in one tissue type and absent another. This variation may be related to the location of specific FA oxidation in the body. Bombardier *et al.* (2010) reported that the red epaxial muscle of *S. salar* was the most efficient tissue for oxidizing lipid during migration and spawning. Only white muscle was analysed in the study. The liver is a small mass (only *c.* 1%) in salmonids, and Froyland *et al.* (2000) were uncertain if hepatic FA oxidation could regulate total body lipid composition in *S. salar*.

The extensive depletion of SFAs, MUFAs and PUFAs in stream-maturing Snake/Columbia River *O. mykiss* kelts probably limits postspawning survival. Penney & Moffitt (2014*a*) examined the histological architecture of the liver in Snake River *O. mykiss* kelts and reported that many hepatocytes were shrunk and observed cellular necroses in poor condition kelts (near death). Buelow & Moffitt (2014) found that various electrolytes in the blood plasma of migrating *O. mykiss* kelts were lower in poor condition kelts over good condition kelts indicating potential leakage through membranes as polar lipids were presumably mobilized for energy. Booth *et al.* (1999) speculated that the use of PUFAs in *S. salar* kelts may reduce the need to catabolize protein and minimize dependence on a single energy source. Considering the importance of PUFAs to membranes (Sargent *et al.*, 1999), it could be argued that the depletion of PUFAs and other FAs important to cellular membranes would be just as damaging as severe protein catabolism.

MANAGEMENT IMPLICATIONS

Somatic lipids were exhausted in emigrating *O. mykiss* in this study and in Penney & Moffitt (2014*b*). In *S. salar* kelts several studies suggest that low energy reserves limit postspawning survival (Belding, 1934; Jonsson *et al.*, 1991, 1997). Low rates of repeat spawning in *O. mykiss* and their ecological homologue *S. salar* have been associated with high energetic investments in migration and reproduction (Crespi & Teo, 2002; Fleming & Reynolds, 2004). Fleming (1998) hypothesized that the energetic trade-offs accompanying anadromy, such as increased body size and gonadal investments, probably promoted a reproductive strategy favouring juvenile over adult survival.

One management tool used to enhance iteroparity in threatened and endangered *O. mykiss* and *S. salar* populations is to capture and recondition kelts. Increasing the number of repeat-spawning individuals within a population can increase natural or hatchery production and diversify gene flow in depressed stocks (Brannon *et al.*, 2004). A key component of successful kelt reconditioning is re-establishment of feeding, but little is known about the most important nutritional sources. In *S. salar*, marine forage fishes such as Atlantic silver sides *Menidia menidia* (L. 1766), smelt *Osmerus mordax* (Mitchill 1814) and capelin *Mallotus villosus* (Müller 1776), have been successfully used to re-condition kelts (Johnston *et al.*, 1987, 1990, 1992). Hatch *et al.* (2013) re-conditioned *O. mykiss* kelts from the Yakima River, WA, using a combination of krill and specialized feeds. Marine-based feeds typically contain many of the essential FAs needed for metabolic energy and biological membranes. Although it could be presumed that any dietary source of energy is probably beneficial, additional research is needed to determine if feeds with specific FA groups could improve kelt survival.

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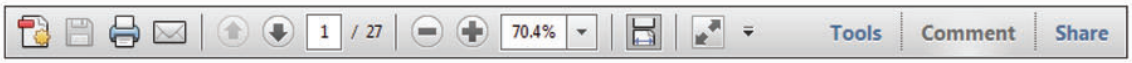

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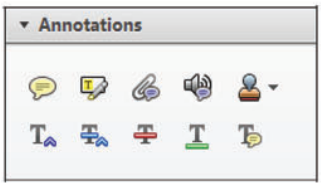

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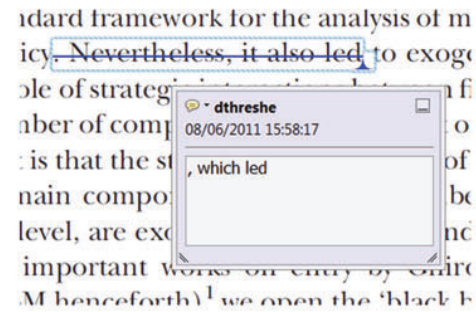

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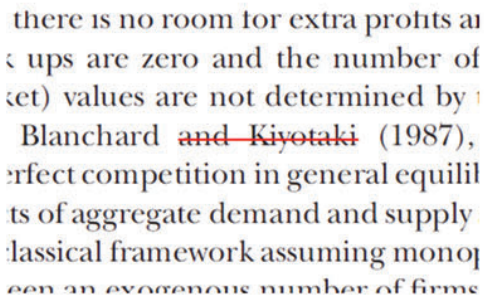

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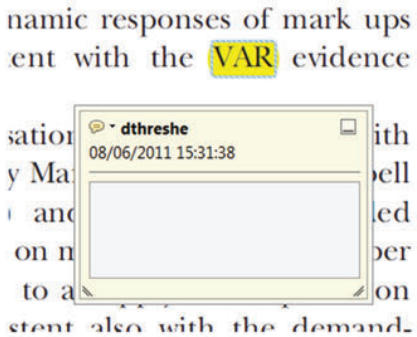
- Click on the Add note to text icon in the Annotations section.
- Type instruction on what should be changed regarding the text into the yellow box that appears.

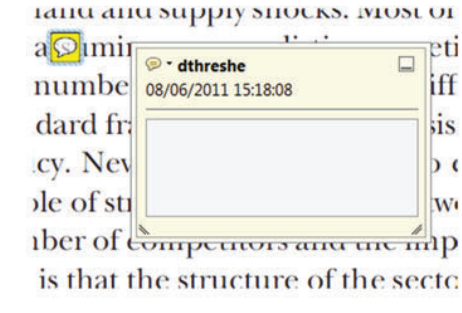


4. Add sticky note Tool – for making notes at specific points in the text.

Marks a point in the proof where a comment needs to be highlighted.

How to use it

- Click on the Add sticky note icon in the Annotations section.
- Click at the point in the proof where the comment should be inserted.
- Type the comment into the yellow box that appears.



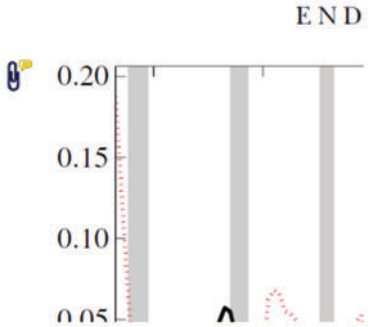
USING e-ANNOTATION TOOLS FOR ELECTRONIC PROOF CORRECTION

5. Attach File Tool – for inserting large amounts of text or replacement figures.

Inserts an icon linking to the attached file in the appropriate pace in the text.

How to use it

- Click on the Attach File icon in the Annotations section.
- Click on the proof to where you'd like the attached file to be linked.
- Select the file to be attached from your computer or network.
- Select the colour and type of icon that will appear in the proof. Click OK.

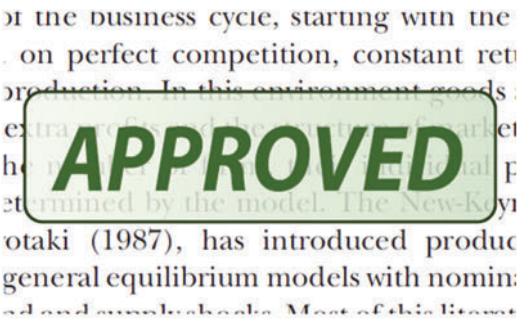


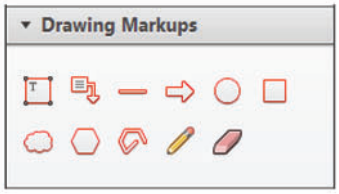
6. Add stamp Tool – for approving a proof if no corrections are required.

Inserts a selected stamp onto an appropriate place in the proof.

How to use it

- Click on the Add stamp icon in the Annotations section.
- Select the stamp you want to use. (The Approved stamp is usually available directly in the menu that appears).
- Click on the proof where you'd like the stamp to appear. (Where a proof is to be approved as it is, this would normally be on the first page).



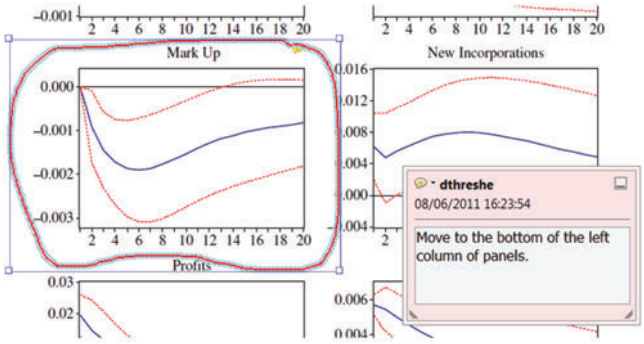


7. Drawing Markups Tools – for drawing shapes, lines and freeform annotations on proofs and commenting on these marks.

Allows shapes, lines and freeform annotations to be drawn on proofs and for comment to be made on these marks..

How to use it

- Click on one of the shapes in the Drawing Markups section.
- Click on the proof at the relevant point and draw the selected shape with the cursor.
- To add a comment to the drawn shape, move the cursor over the shape until an arrowhead appears.
- Double click on the shape and type any text in the red box that appears.



For further information on how to annotate proofs, click on the Help menu to reveal a list of further options: