

# Plasma nonesterified fatty acid profiles in male and female sockeye salmon, *Oncorhynchus nerka*, during the spawning migration

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**Abstract:** To establish if there are sex-specific differences in the utilization of specific fatty acids in salmon during migration, we monitored plasma nonesterified fatty acids (NEFA) in male and female early-run Stuart sockeye salmon, *Oncorhynchus nerka*, during their spawning migration in the Fraser River. Total plasma NEFA concentrations declined in both males and females to 60 and 40% of their respective initial levels. Palmitic (C<sub>16:0</sub>), oleic (C<sub>18:1</sub>), docosahexaenoic (C<sub>22:6n3</sub>), and eicosapentaenoic (C<sub>20:5n3</sub>) acids consistently represented between 66 and 77% of the total plasma NEFAs throughout the migration. These fatty acids are probably utilized as a source of energy to sustain swimming during the migration. A difference in monoene levels between sexes suggest that females utilized monoenes, particularly oleic acid, for yolk production. Fatty acid concentrations of the n6) series remained constant in both sexes; however, a sudden increase of C<sub>20</sub> polyunsaturate proportions of both the n3 and n6 series was observed at the time of gonadal maturation in both sexes. While plasma NEFAs are important as energy sources for migrating sockeye salmon, there is also a selective utilization of plasma NEFAs for gonadal development and reproduction that is reflected in altered NEFA profiles of male and female fish, respectively.

**Résumé :** Nous avons surveillé la concentration plasmatique d'acides gras non estérifiés (AGNE) chez des saumons rouges, *Oncorhynchus nerka*, femelles et mâles qui passent dans la rivière Fraser au début de la remonte vers la Stuart pour déterminer si l'utilisation de ces acides gras diffère selon le sexe chez le saumon en migration. Nous avons constaté, tant chez les mâles que chez les femelles, une diminution de la concentration plasmatique des AGNE totaux, les valeurs ayant baissé à 60 et 40% de ce qu'elles étaient initialement. L'acide palmitique (C<sub>16:0</sub>), l'acide oléique (C<sub>18:1</sub>), l'acide docosahexanoïque (C<sub>22:6n3</sub>) et l'acide eicosapentanoïque (C<sub>20:5n3</sub>) ont représenté 66 et 77% des AGNE plasmatiques totaux durant toute la migration. Il est probable que ces acides gras fournissent l'énergie nécessaire à la nage soutenue durant la migration. Nous avons observé une différence entre les concentrations de monoènes des deux sexes, ce qui pourrait signifier que les femelles utilisent des monoènes, et plus particulièrement l'acide oléique, pour la production du vitellus. Les concentrations d'acides gras de la série n6 sont restées constantes chez les deux sexes; toutefois, une brusque augmentation des proportions de polyinsaturés en C<sub>20</sub> des séries n3 et n6 est apparue chez les deux sexes au moment de la maturation des gonades. Les AGNE plasmatiques sont d'importantes sources d'énergie pour le saumon rouge en migration, mais certains sont aussi utilisés pour le développement gonadique et la reproduction, utilisation sélective que traduisent les changements du profil des AGNE observés chez mâles et chez les femelles.

[Traduit par la Rédaction]

## Introduction

The spawning migrations of sockeye salmon, *Oncorhynchus nerka*, typically involve sustained swimming for considerable distances, fasting, and completion of the final stages of gonadal development. The postspawning death of the fish may be a reflection of the extreme metabolic demands of this effort. Faced with these demands, fish must partition their resources

to complete their migration, produce viable gametes, and successfully spawn. In the Fraser River, early (July–August) Stuart sockeye salmon face an energetically demanding upstream migration as their timing in the river corresponds with high flows associated with the spring freshet.

Several studies of the biochemical changes in migrating Pacific salmon have been undertaken (Duncan and Tarr 1958; Idler and Tsuyuki 1958; Idler and Clemens 1959; Idler and

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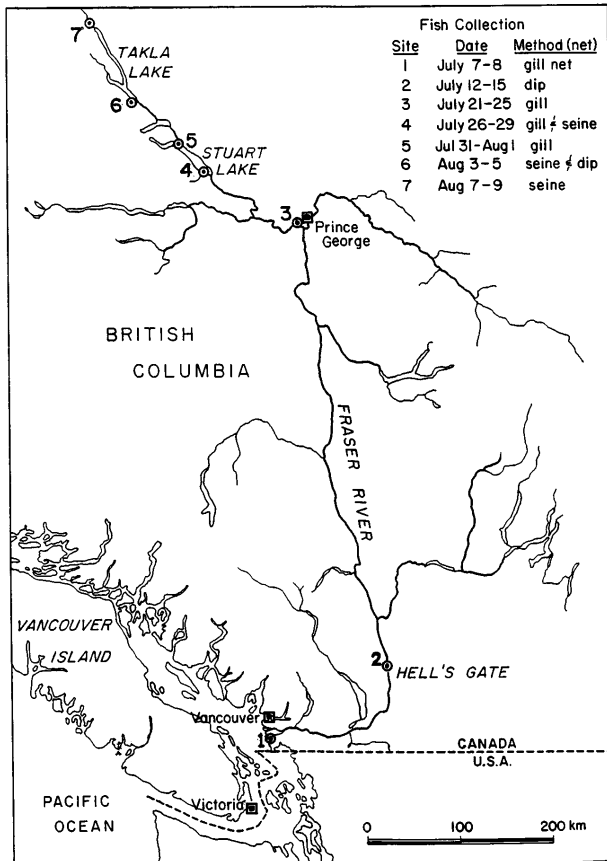
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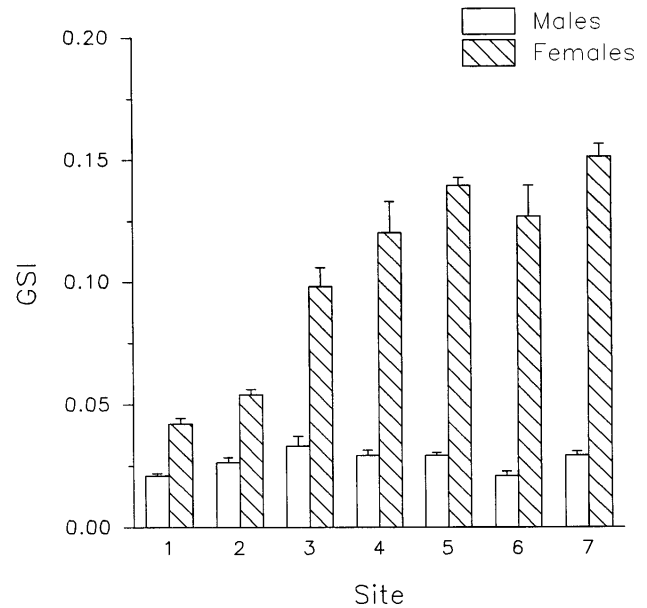
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**Fig. 1.** Map showing the sampling sites for Stuart run of sockeye salmon on the Fraser River, British Columbia.



Bitners 1958, 1959, 1960; Trams 1969; Patton et al. 1970; Gilhousen 1980; Mommsen et al. 1980; French et al. 1983; Ando et al. 1985; Hatano et al. 1989), and these provide an understanding of the changes in the major energy reserves (lipid, carbohydrate, and protein) during the course of the migration. Lipids are particularly important during this period both as energy sources for lateral red muscle, the main muscle group involved in prolonged swimming, and for gonad development. While red muscle is capable of oxidizing a variety of fatty acids (Kiessling and Kiessling 1993), specific fatty acids are needed by developing gonads. Selective utilization of fatty acids has been reported during the process of yolk deposition in eggs in female gonads (Wiegand and Idler 1985), which may represent 15–20% of the body weight at the end of the migration. In addition, specific fatty acids such as arachidonic acid are important precursors of eicosanoid messengers present in fish gonads (Stacey and Goetz 1982) and stimulate steroid production (Van Der Kraak and Chang 1990; Wade et al. 1994; Mercure and Van Der Kraak 1995). Eicosanoids such as prostaglandins are essential for ovulation in all vertebrates including fish (Murdoch et al. 1993). In addition, many fish use prostaglandins as pheromones to synchronize spawning behaviour between sexes (Stacey 1991). Thus, a detailed assessment of the changes in fatty acids during migration may provide a useful tool for monitoring metabolic demands and the importance of specific fatty acids associated with reproduction. Measurements of plasma nonesterified fatty acids

**Fig. 2.** Gonadosomatic index (GSI) of male and female sockeye salmon during the spawning migration in the Fraser River. Values are given as means  $\pm$  SE.



(NEFAs) may be the most appropriate for this purpose as they represent the most metabolically labile pool of fatty acids and have been used previously as indices of the health of fish populations (McKinley et al. 1993). Different fatty acids are likely used during the formation of eggs compared with those used in sperm formation, implying that there may be sex-related differences in the plasma of migrating salmon. Other fatty acids may be required as energy sources for locomotion. Therefore, examination of the specific fatty acids comprising the NEFA pool can provide insight into changes in lipid metabolism during the migration.

The utilization of lipid by migrating female sockeye salmon exceeds that of males because of the formation of mature ovaries (Idler and Bitners 1960). Although previous studies of the sockeye salmon migration in the Fraser River indicated that the concentrations of total plasma NEFAs declined as the migration progressed (Patton et al. 1970; French et al. 1983), only total levels of plasma NEFAs were considered and differences between the two sexes were ignored. To establish if there are sex-specific changes in plasma NEFAs associated with the differing lipid requirements of male and female fish, we sampled the early spawning migration of Stuart sockeye (July–August) in the Fraser River.

## Materials and methods

### Animals

Early Stuart sockeye (1.0–3.5 kg) were collected at seven sites on the Fraser River between 7 July and 9 August 1993 (Fig. 1). Collection occurred over a period of 2–5 days on or near the estimated dates of peak migration at each site. Fishing conditions were site specific and determined the capture method (Fig. 1). Although gill nets were used for collection at three sites (Fig. 1), the nets were tended constantly and fish were removed and sampled in less than 4 min after encounter with the net. Fish with previous encounters with gill nets were sepa-

**Table 1.** Concentrations (nmol/mL) of individual nonesterified fatty acids in the plasma of male sockeye salmon at the during the Fraser River migration.

Fatty acid	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7
14:0	84.04±6.37	108.41±14.55	49.00±7.87	63.36±8.93	46.24±3.20	38.62±7.12	50.53±6.70
14:1	4.50±1.22	23.62±3.88	3.91±1.04	10.11±3.57	5.29±0.88	10.40±2.56	4.23±0.93
16:0	518.37±40.71	613.93±70.39	425.62±50.02	606.94±53.65	399.44±37.22	296.33±61.56	381.11±41.61
16:1	118.74±13.62	161.10±33.68	59.82±12.41	86.93±14.75	79.13±11.95	49.65±8.44	55.37±11.68
18:0	81.25±7.49	95.48±14.43	45.26±6.73	52.35±6.70	47.41±5.79	29.97±4.22	27.24±2.18
18:1n9	361.97±27.82	488.31±72.75	230.95±28.49	299.87±28.87	220.54±22.16	156.29±33.04	180.62±15.07
18:2n6	83.26±8.10	120.35±13.82	31.15±4.53	38.74±3.23	37.50±6.44	31.28±6.19	28.16±4.98
18:3n3	26.16±3.00	34.04±4.24	10.18±1.59	9.84±1.31	11.60±1.54	7.74±1.34	15.59±4.13
18:4n3	33.37±4.32	67.90±7.29	16.05±2.47	22.80±2.76	19.35±1.85	10.78±2.91	25.91±9.04
20:0	0.04±0.04	nd	nd	0.21±0.14	nd	nd	nd
20:1	51.06±4.86	78.19±9.70	56.72±8.07	55.74±7.04	47.36±6.88	53.37±14.98	45.31±6.70
20:2n6	3.99±0.53	4.32±0.80	4.68±0.57	4.84±0.80	3.54±0.75	7.78±3.81	2.44±0.82
20:3n 6	0.07±0.07	nd	1.31±0.36	0.18±0.18	2.00±0.67	5.15±2.47	1.94±0.52
20:4n6	37.86±3.41	41.42±5.48	26.42±2.99	41.07±3.96	23.68±1.49	29.19±7.22	30.18±4.09
20:3n3	3.99±0.45	3.10±0.62	1.72±0.51	2.15±0.93	4.13±1.03	9.44±3.88	nd
20:4n3	13.37±1.57	12.03±2.76	12.37±3.27	8.82±2.46	8.47±2.80	12.47±4.20	14.61±2.66
20:5n3	329.25±32.20	395.95±52.35	171.41±21.63	226.25±24.47	158.50±18.06	134.94±32.80	155.15±15.57
22:0	nd	nd	nd	nd	nd	nd	0.09±0.06
22:1	36.05±4.06	43.33±5.28	39.93±6.66	41.58±5.48	30.00±8.59	34.82±11.45	25.51±5.25
22:2n6	nd	nd	nd	0.32±0.32	nd	nd	0.61±0.50
23:0	7.35±1.23	16.00±2.27	2.89±1.01	10.28±2.31	0.62±0.62	3.65±1.31	0.22±0.22
22:4n6	0.04±0.04	nd	nd	0.22±0.15	2.96±2.55	0.10±0.10	nd
22:5n6	56.09±6.73	58.92±10.89	34.71±5.16	47.10±8.09	32.85±11.67	37.67±8.09	33.69±2.43
22:5n3	14.20±2.37	3.95±3.95	24.81±5.88	7.94±4.35	22.67±7.56	8.01±3.55	5.24±3.42
22:6n3	440.30±29.45	613.35±113.70	277.85±36.12	350.03±30.28	235.95±22.08	196.91±31.95	326.79±51.40
24:0	nd	nd	nd	nd	0.15±0.15	nd	nd
24:1	10.57±2.91	25.57±6.34	10.70±3.51	5.16±2.12	4.43±1.54	6.06±2.18	0.76±0.76
Total	2315.86±178.05	3009.27±344.26	1537.45±191.89	1992.88±182.66	1443.82±109.53	1170.62±235.00	1411.29±146.34
Total saturates	691.05±53.26	833.83±95.68	522.77±63.77	733.14±67.99	493.87±44.94	368.57±73.51	459.19±48.51
Total monoenes	582.88±46.37	820.12±115.31	402.02±51.90	499.40±44.91	386.75±35.26	310.59±69.24	311.79±30.77
Total polyenes	1041.93±80.77	1355.32±179.52	612.65±78.34	760.34±73.42	563.20±42.31	491.46±95.07	640.30±80.25
n3 polyenes	860.63±68.03	1130.32±159.28	514.38±66.85	627.85±59.87	460.67±41.39	380.30±78.09	543.28±72.64
n6 polyenes	181.30±16.58	225.00±21.76	98.27±12.06	132.49±14.23	102.54±16.45	111.16±17.81	97.03±7.76

**Note:** Values are given as the mean ± SE. Number of fish sampled: site 1, 11; site 2, 8; site 3, 11; site 4, 9; site 5, 7; site 6, 8; site 7, 6. nd, not detectable.

rated from those with no previous net encounters. Only unmarked fish were used.

#### Blood samples and fish weights

Blood was sampled from the caudal vasculature of live unanesthetized fish using heparinized 10-mL Vacutainer tubes. Plasma was obtained by centrifuging at  $800 \times g$  for 10 min and the plasma samples were frozen on dry ice immediately. Total body and gonad weights were determined for each fish to calculate gonadosomatic index (GSI).

#### Plasma nonesterified fatty acid analysis

Nonesterified (free) fatty acids were methylated as previously described (Singer et al. 1990). One microlitre of carbon disulfide ( $CS_2$ ) containing methyl esters was injected into a gas chromatograph (Hewlett-Packard, HP5890A) fitted with a flame ionization detector (FID) and an automatic injector (Hewlett-Packard, 7673A). Fatty acid methyl esters (FAMES) were resolved on a DB-225 megabore fused silica column (Chromatographic Specialities Inc., Brockville, Ont.) using helium as the carrier gas. Oven temperature programming included an initial temperature of 150°C that was increased immediately after injection to 210°C at a rate of 60°C/min and held at this temperature for 30 min. FAMES from 14 to 24 carbons in length were readily resolved under these conditions. FAMES were identified by comparison of retention times with those of known standards, and the

absolute amounts were quantified with an internal standard, heptadecanoic acid (17:0), added to the plasma samples prior to methylation. All chemicals were purchased from Sigma Chemical Co. (St. Louis, Mo.). Lipid standards used were obtained from NU Chek Prep, Inc. Elysian, Minn. Additional fatty acids, not present in the standard, were added with a methylated fatty acid mixture prepared from menhaden oil extract.

#### Statistical analysis

Differences between sites and sexes were established by analysis of variance and multiple comparison based on *t* statistic comparisons ( $p < 0.05$ ), using LSMEANS (Steele and Torrie 1980). Sites were considered as treatments and individual fish as replicates (units) for each treatment. Assumptions of linearity for treatment regressions and shared slopes between treatment regressions were validated using general linear models. Assumptions of normality, independence, and homoscedasticity were verified by generating appropriate residual plots. Data transformations (log, square root, and cube root) were used when appropriate to meet the above assumptions.

#### Results

Male and female sockeye salmon at each of the seven sites on the Fraser River exhibited marked changes in gonadal development during the migration (Fig. 2). The GSI for female fish

**Table 2.** Concentrations (nmol/mL) of individual nonesterified fatty acids in the plasma of female sockeye salmon during the Fraser River migration.

Fatty acid	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7
14:0	99.51±10.49	108.24±15.42	73.88±5.07	70.38±9.20	58.06±6.12	42.25±4.82	30.35±3.07
14:1	1.90±1.24	22.50±6.42	9.88±4.86	4.24±2.03	2.81±0.75	6.84±1.48	2.67±0.69
16:0	556.52±43.87	545.93±62.31	524.47±12.25	570.60±83.42	470.16±29.22	288.90±24.65	291.88±29.11
16:1	216.25±17.12	213.86±39.75	203.53±10.36	127.65±25.84	128.62±13.92	73.00±7.54	37.30±3.71
18:0	145.01±15.90	106.46±17.62	145.06±17.51	131.79±32.11	111.50±8.10	53.96±7.32	52.85±6.49
18:1n9	597.18±62.46	577.30±63.80	609.29±33.61	539.57±72.68	478.18±35.70	192.82±21.76	220.19±23.97
18:2n6	103.77±16.64	120.64±18.56	101.10±12.31	62.38±8.66	63.37±5.22	38.35±5.14	22.69±3.93
18:3n3	30.96±12.32	34.76±5.39	35.68±7.26	19.93±5.26	19.74±3.06	6.71±1.84	10.93±2.17
18:4n3	36.96±12.32	76.97±12.24	45.47±8.46	33.52±8.63	31.70±7.57	8.21±1.54	8.12±1.43
20:0	nd	nd	nd	nd	0.11±0.11	0.06±0.06	nd
20:1	73.11±8.89	96.89±9.33	64.34±10.27	62.33±6.41	56.18±5.63	53.58±9.59	36.27±4.92
20:2n6	7.04±0.56	7.33±0.71	6.85±0.72	8.17±0.90	5.94±0.59	12.97±3.22	2.22±0.69
20:3n6	0.47±0.29	nd	0.78±0.46	0.48±0.48	1.57±0.37	6.91±2.13	1.74±0.47
20:4n6	33.39±1.27	28.45±3.38	26.80±4.21	32.46±2.66	25.88±2.23	30.91±5.44	14.69±1.64
20:3n3	5.35±0.70	2.40±0.29	4.29±1.03	3.38±0.90	3.37±0.56	12.75±3.21	1.52±0.99
20:4n3	33.06±5.66	11.54±4.95	20.37±9.72	16.76±6.65	17.63±3.62	15.10±1.98	8.60±1.91
20:5n3	423.92±28.54	400.18±50.90	315.32±10.81	291.04±37.68	263.41±20.17	187.75±18.16	151.12±14.83
22:0	nd	nd	1.72±1.72	nd	nd	nd	nd
22:1	34.45±9.46	54.40±8.60	25.30±6.43	24.03±5.33	31.38±6.45	25.00±7.84	14.09±5.12
22:2n6	nd	nd	nd	nd	nd	nd	nd
23:0	8.76±4.02	20.97±2.83	4.80±1.80	9.07±2.09	6.56±1.79	4.96±1.05	0.06±0.06
22:4n6	nd	0.05±0.05	nd	0.12±0.12	nd	nd	0.17±0.14
22:5n6	29.18±6.71	28.85±9.37	25.38±7.56	19.67±1.87	21.50±5.28	31.50±4.83	23.49±3.72
22:5n3	45.08±7.53	1.76±1.76	33.12±14.99	13.45±13.45	25.66±7.92	16.62±2.12	11.51±4.98
22:6n3	592.60±29.68	650.36±132.23	414.01±22.90	417.02±61.16	335.03±27.00	210.66±16.01	268.74±47.16
24:0	nd	nd	nd	nd	0.09±0.09	nd	nd
24:1	17.66±9.80	20.11±5.65	7.58±4.13	5.43±3.00	9.03±3.22	5.02±1.40	0.13±0.09
Total	3091.54±231.63	3129.94±398.36	2699.02±137.56	2463.48±349.93	2167.48±141.62	1324.81±115.89	1211.34±122.83
Total saturates	809.81±66.81	781.59±89.58	749.93±23.62	781.85±123.19	646.49±37.31	390.12±36.43	375.14±35.94
Total monoenes	940.56±86.51	985.06±107.98	919.91±44.38	763.25±103.94	706.20±54.98	356.25±41.71	310.66±34.58
Total polyenes	1341.17±87.42	1363.29±212.11	1029.18±74.85	918.38±130.95	814.80±58.91	578.43±48.44	525.54±65.78
n3 polyenes	1167.32±73.28	1177.97±191.67	868.26±55.28	795.10±123.29	696.54±52.00	457.80±38.52	460.54±59.10
n6 polyenes	173.85±19.93	185.32±26.84	160.91±19.99	123.29±9.44	118.26±8.03	120.64±14.33	65.01±7.37

**Note:** Values are given as the mean ± SE. Number of fish sampled: site 1, 4; site 2, 7; site 3, 4; site 4, 6; site 5, 8; site 6, 7; site 7, 9. nd, not detectable.

increased throughout the migration with the increments being greatest in the first few sites. The GSI for males was relatively constant throughout the migration.

There were two possible approaches to the analysis of plasma NEFAs. One was to examine the absolute concentrations of individual fatty acids. These data are presented for male (Table 1) and female (Table 2) fish. However, this approach may not reveal other important relationships where the proportions of specific nonesterified fatty acids change. To identify these situations, analysis of the proportions (percentages) of fatty acids has also been reported (Figs. 4–7).

The major fatty acids in the plasma of both male (Table 1) and female (Table 2) sockeye salmon during the spawning migration were 16:0, 18:1, 20:5n3, and 22:6n3. These fatty acids consistently represented 66–77% of total NEFAs in both sexes. Overall, the total plasma NEFAs of fish declined throughout the migration to 60 and 40% of the initial concentrations in males and females, respectively (Fig. 3). NEFA levels were significantly lower in males than in females at sites 1, 3, and 5.

The distributions of fatty acid classes in males and females are presented in Fig. 4. The percentage of saturated fatty acids in the plasma of males was significantly higher than that of

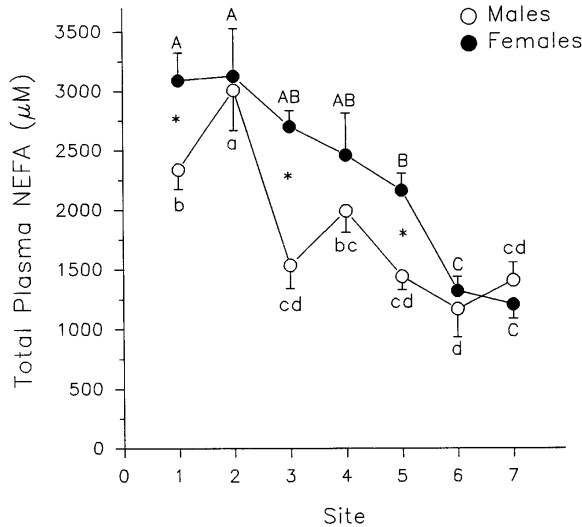
females at sites 1, 3, 4, and 5 (Fig. 4A). The percentage of monoenes in the plasma was significantly higher in females compared with males at all sites except site 6 (Fig. 4B). There were no differences in the percentages of polyenoic fatty acids between males and females at any sites (Fig. 4C).

The proportions of n3 and n6 fatty acids are presented in Fig. 5. There were no male–female differences between the percentages of n3 fatty acids at any site (Fig. 5A). The percentage of n6 fatty acids was significantly higher in males compared with females at all sites (Fig. 5B).

The percentages of the four major fatty acids in the plasma are compared in Fig. 6. The percentage of 16:0 was significantly higher in males than in females at all sites except site 7 (Fig. 6A). The percentages of 18:1 were significantly higher in females compared with males at all sites except site 6 (Fig. 6B). The percentage of 20:5n3 was significantly higher in females than in males at the last three sites (Fig. 6C). There were no significant differences in the percentage of 22:6n3 between males and females at any of the sites (Fig. 6D).

The proportions of three minor fatty acids (20:4n6, 20:3n6, 20:3n3), which are precursors of eicosanoids, are presented in Fig. 7. The proportion of arachidonic acid (20:4n6) (Fig. 7A) was significantly higher in males than in females at all sites

**Fig. 3.** Total concentrations of nonesterified fatty acids in the plasma of male and female sockeye salmon on the spawning migration in the Fraser River. Values are given as means  $\pm$  SE. Capital letters over or under means refer to statistical comparisons between sites for females and lowercase letters refer to statistical comparisons between sites for males. Means with the same letters are not significantly different. Significant differences between males and females are indicated by asterisks.



except site 6 (Fig. 7A), where a sudden increase was observed in females. Similar sudden increases at that particular site were also found with 20:3n6 (Fig. 7B) and 20:3n3 (Fig. 7C). No difference between sexes was found for these two fatty acids.

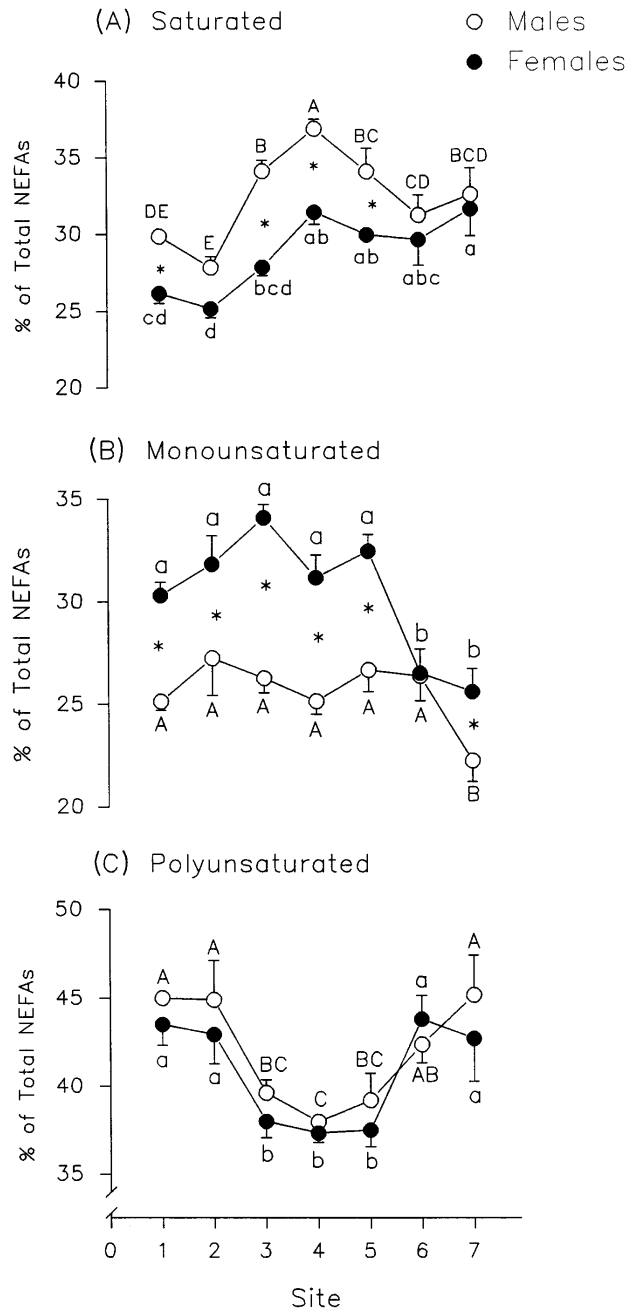
**Discussion**

The plasma NEFA profile of migrating sockeye salmon is similar in many respects to that of other fish species. The four fatty acids found in highest concentrations at all sites (16:0, 18:1, 20:5n3, and 22:6n3) are the same as those reported for temperate-zone marine fish (Ballantyne et al. 1993). The proportions of saturated, monounsaturated, and polyunsaturated fatty acids are similar to those reported for other marine or freshwater teleost fish (Ballantyne et al. 1993). Lipids are stored in several tissues in migrating salmon including liver, abdominal adipose tissue, red muscle, and white muscle (Love 1970). Mobilization of liver lipids involves increased hepatic lipase activity during spawning in both males and female pink salmon (Hatano et al. 1989). Lipids must also be mobilized from extrahepatic stores during the migration. Jezierska et al. (1982) showed tissue-specific depletion of lipids during periods of starvation. Considering this and given that the fatty acid composition of fish tissues varies (Jezierska et al. 1982), some of the changes in proportions of plasma NEFAs that we observed may be due to changing sources of fatty acids as the migration progresses. The levels and proportions of plasma NEFAs indicate utilization of fatty acids for metabolic requirements associated with swimming, fasting, gonadal growth, sexual maturation, and spawning.

**Energetics**

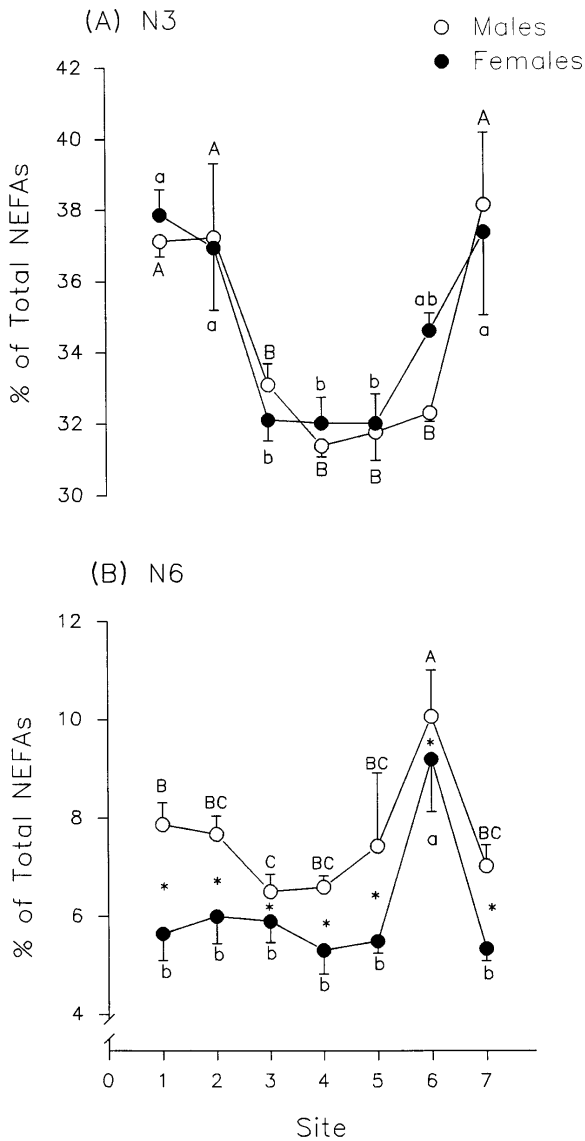
The general decline in total NEFA as the migration progressed

**Fig. 4.** Percentages of (A) saturated, (B) monoenoic, and (C) polyenoic nonesterified fatty acids in the plasma of male and female sockeye salmon on the spawning migration in the Fraser River. Values are given as means  $\pm$  SE. Capital letters over or under means refer to statistical comparisons between sites for females and lowercase letters refer to statistical comparisons between sites for males. Means with the same letters are not significantly different. Significant differences between males and females are indicated by asterisks.



suggests that in the initial stages of the migration, plasma NEFAs are important energy sources for both males and females. However, their importance may have declined as the migration progressed because of their depletion. Similar declines in

**Fig. 5.** Percentages of (A) n3 and (B) n6 nonesterified fatty acids in the plasma of male and female sockeye salmon on the spawning migration in the Fraser River. Values are given as means  $\pm$  SE. Capital letters over or under means refer to statistical comparisons between sites for females and lowercase letters refer to statistical comparisons between sites for males. Means with the same letters are not significantly different. Significant differences between males and females are indicated by asterisks.



plasma total NEFA have been reported in other migrating Pacific salmonids (Patton et al. 1970; French et al. 1983).

The changes in the major plasma NEFAs in the males during the migration may be due to the energetic costs of locomotion. In studies of sockeye salmon migrating in the Fraser River, Idler and Bitners (1958) concluded that, on the basis of measurements of gonad and total body lipid, females expended the same amount of energy as males for swimming. An increase in plasma 22:6n3 at the last site may represent the final mobilization of the longest most unsaturated fatty acid as other fatty acids are depleted.

On the basis of migration energetics studies of early Stuart sockeye conducted in 1993, site 2 (Hells Gate) represents the most difficult challenge for the migrating Fraser River fish (S.G. Hinch, Westwater Research Center, University of British Columbia, 1933 West Mall, Vancouver, B.C., unpublished data). The high water velocity at this site likely requires higher levels of red and even white muscle activity for burst swimming. Total plasma NEFAs at this site increased in males to levels comparable with that of the females.

Differences between sexes in the classes of plasma NEFAs are apparent throughout the migration. Saturated fatty acids (primarily 16:0) are higher in proportion in the plasma of males than in females. Saturated fatty acids are readily synthesized in fish and are important oxidative substrates for a variety of tissues including red muscle. However, the higher levels in males do not indicate a reduced importance in females. While the proportion of saturated fatty acids in females is lower, the absolute amounts are similar to those in males. This is likely due to the increase in other fatty acids mobilized for yolk synthesis.

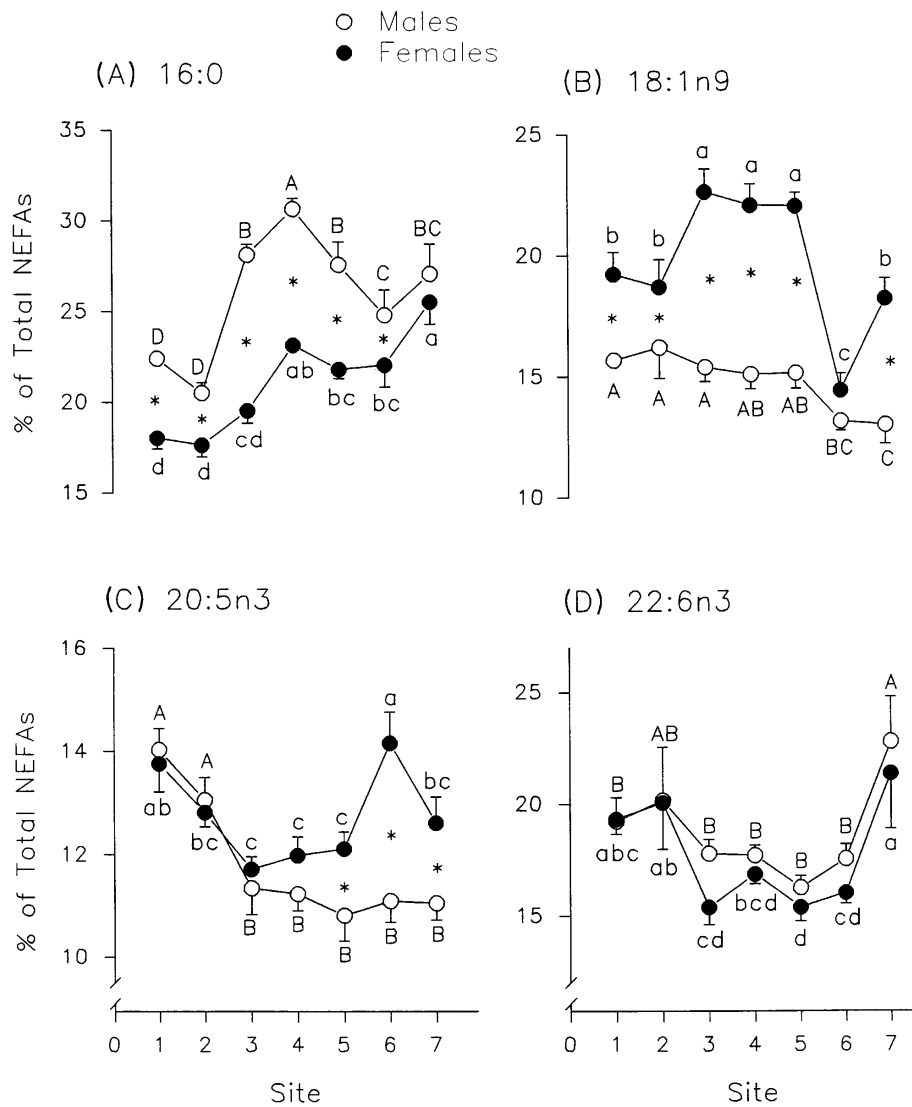
Fatty acid oxidation occurs in the mitochondria of a variety of fish tissues (see Ballantyne 1994 for review). Although liver may oxidize fatty acids to provide the energy for synthesis of proteins, carbohydrates, and other macromolecules, it is likely that red muscle is responsible for most of the fatty acid oxidation during migration, since this tissue is responsible for steady-state swimming. Several studies of mitochondria isolated from fish red muscle (Murata and Higashi 1979; Sidell et al. 1988) indicate that this tissue can oxidize a range of fatty acid chain lengths. The availability of fatty acids in the plasma NEFA fraction likely determines which fatty acids are oxidized. In migrating sockeye salmon, 16:0, 18:1, 20:5n3, and 22:6n3 would be preferred substrates because of their abundance in the plasma. Mitochondrial oxidation of fatty acids is usually considered to be complete with very few if any intermediates accumulating (Mannaerts and Veldhoven 1993). The effects of fatty acid oxidation on plasma NEFAs would be to decrease levels of all fatty acids proportionally. Therefore, deviations in the proportions of specific NEFAs in the plasma are more likely indicative of other processes.

The effects of starvation on plasma NEFAs varies in different fish species. Rainbow trout (*Oncorhynchus mykiss*) maintained constant plasma NEFA levels for 56 days of food deprivation (Black and Skinner 1986). In bass (*Dicentrarchus labrax*), food deprivation results in an initial increase in plasma NEFAs followed by a decline after 40 days (Zammit and Newsholme 1979). In spite of this interspecies variability it seems likely that the declines in NEFAs observed during the migration are due in part to the effects of starvation. The constant swimming associated with the migration may simply speed up the metabolic consequences of food deprivation, resulting in more severe declines in plasma NEFAs than would be observed if the fish were simply starving and not migrating.

### Gonad development

In females, NEFA levels were high before Hells Gate, perhaps indicating their mobilization for the production of yolk. Therefore, in females the levels of plasma lipoproteins required to bind plasma NEFAs may exceed that of males. The extra energy required to pass through site 2 may also require diversion of fatty acids from gonad development in females. This may

**Fig. 6.** Percentages of (A) palmitic acid, (B) oleic acid, (C) 20:5n3, and (D) 22:6n3 nonesterified fatty acids in the plasma of male and female sockeye salmon on the spawning migration in the Fraser River. Values are given as means  $\pm$  SE. Capital letters over or under means refer to statistical comparisons between sites for females and lowercase letters refer to statistical comparisons between sites for males. Means with the same letters are not significantly different. Significant differences between males and females are indicated by asterisks.



explain why the largest increase in gonad size in females occurs after Hells Gate (site 2).

The sex-related differences in the proportion of the four predominant plasma NEFAs (16:0, 18:1, 20:5n3, and 22:6n3) may be useful indicators of the partitioning of plasma NEFAs during the migration. Since the male gonads have apparently reached their full size early in the migration, vast amounts of plasma NEFAs are likely not required for gonadal growth.

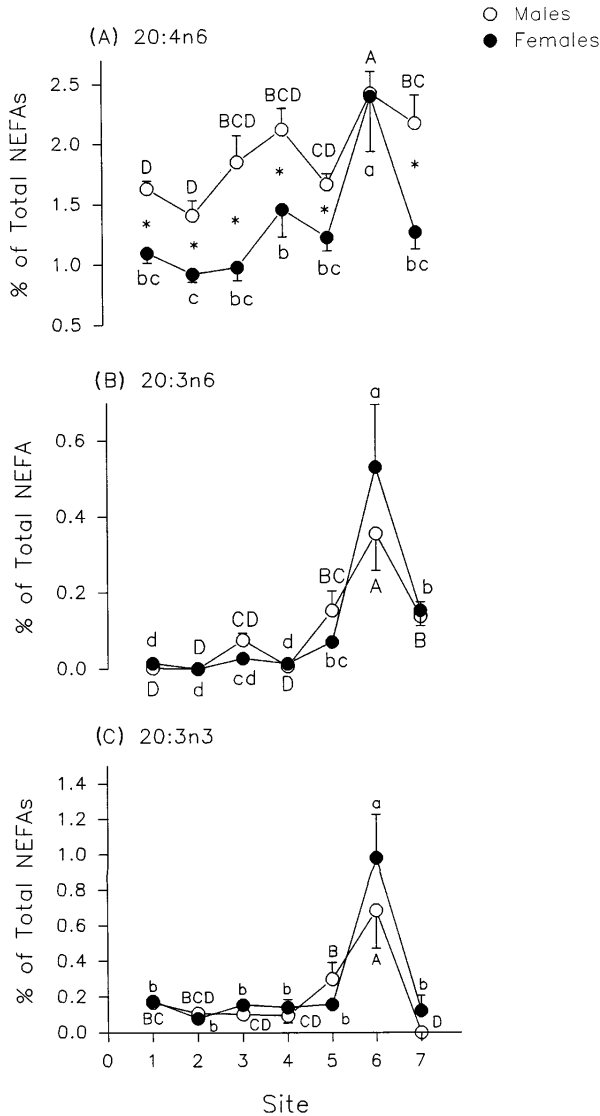
Monoenoic fatty acids (particularly 18:1) are highest in concentration and proportion in females. The importance of monoenes (18:1 and 16:1) in the neutral lipid of ovaries of Atlantic salmon, *Salmo salar*, increases with GSI while the proportion of the polyunsaturated fatty acids (20:5n and 22:6n3) declines (Wiegand and Idler 1985). The monoene 18:1 seems to be particularly important in this function with a substantial increase in this fatty acid between sites 2 and 3. These

are the sites at which ovarian growth was most apparent. High levels of 18:1 in comparison with those in males are maintained in the plasma for sites 3, 4, and 5, perhaps indicating the duration of this phase of ovarian development.

The proportions of polyunsaturated fatty acids are similar in both sexes throughout the migration. Although polyenes are important components of eggs (Wiegand and Idler 1985; Bell 1989), it appears that plasma NEFAs may not be the primary source of these fatty acids. The predominant fatty acids of fish gonads are phospholipids with a high proportion of polyunsaturated fatty acids compared with lipid reserves, which are largely neutral lipids with higher proportions of saturated fatty acids (Tocher and Sargent 1984; Henderson and Tocher 1987). Lipid classes other than plasma NEFA (e.g., phospholipid) may be involved in delivering polyenes to oocytes.

Males had substantially more n6 fatty acids, particularly

**Fig. 7.** Percentages of (A) 20:3n6, (B) 20:3n3, and (C) 20:4n6 nonesterified fatty acids in the plasma of male and female sockeye salmon on the spawning migration in the Fraser River. Values are given as means  $\pm$  SE. Capital letters over or under means refer to statistical comparisons between sites for females and lowercase letters refer to statistical comparisons between sites for males. Means with the same letters are not significantly different. Significant differences between males and females are indicated by asterisks.



20:4n6, in their plasma throughout the migration. This may imply that this class of fatty acid is important in testis function and previous studies have shown the steroidogenic effects of arachidonic acid and its metabolites in fish (Wade et al. 1994). The highest levels of arachidonic acid were found in both sexes at site 6 with the increase being most noticeable in females. Given that gonadal maturation and ovulation, which depend on eicosanoid synthesis, may occur at this site, the change in fatty acids may effect some of the physiological changes associated with spawning. Given that 20:5n3 also can be converted to eicosanoids and that a sudden mobilization of this

fatty acid in females was found in spite of its already high concentration 20:5n3 may be connected to some important physiological events associated with spawning.

**Summary**

Analyses of plasma NEFAs, the most metabolically active lipid fraction of the blood, have allowed us to infer their specific roles in supplying the energy requirements for swimming as well as the differing needs for certain fatty acids by males and females associated with the reproductive aspects of the migration of sockeye salmon in the Fraser River. The fatty acids found in highest concentrations likely serve as important energy sources whereas specific less abundant fatty acids of the n3 and n6 series may be mediators of the reproductive aspects of the migration.

**References**

Ando, S., Hatano, M., and Zama, K. 1985. A consumption of muscle lipid during spawning migration of chum salmon, *Oncorhynchus keta*. *Nippon Suisan Gakkaishi*, **51**: 1817–1824.

Ballantyne, J.S. 1994. Fish mitochondria. In *Biochemistry and molecular biology of fishes*. Vol. 3. Edited by P.W. Hochachka and T.P. Mommsen. Elsevier Science, Amsterdam. pp. 485–500.

Ballantyne, J.S., Glemet, H.C., Chamberlin, M.E., and Singer, T.D. 1993. Plasma nonesterified fatty acids of marine teleost and elasmobranch fishes. *Mar. Biol. (Berlin)*, **116**: 47–52.

Bell, M.V. 1989. Molecular species analysis of phosphoglycerides from the ripe roes of cod (*Gadus morhua*). *Lipids*, **24**: 585–588.

Black, D., and Skinner, E.R. 1986. Features of the lipid transport system of fish as demonstrated by studies on starvation in the rainbow trout. *J. Comp. Physiol. B*, **156**: 497–502.

Duncan, D.W., and Tarr, H.L.A. 1958. Biochemical studies on sockeye salmon during spawning migration. III. Changes in the protein and non-protein nitrogen fractions in muscles of migrating sockeye salmon. *Can. J. Biochem. Physiol.* **36**: 799–803.

French, C.J., Hochachka, P.W., and Mommsen, T.P. 1983. Metabolic organization of liver during spawning migration of sockeye salmon. *Am. J. Physiol.* **245**: R827–R830.

Gilhausen, P. 1980. Energy sources and energy expenditures in Fraser River sockeye salmon during their spawning migration. *Int. Pac. Salmon Fish. Comm. Bull. No. 22*.

Hatano, M.S., Mizogami, M., Sugawara, A., and Ando, S. 1989. Lipid metabolism in the liver of chum salmon during spawning migration. *Nippon Suisan Gakkaishi*, **55**: 1623–1627.

Henderson, R.J., and Tocher, D.R. 1987. The lipid composition and biochemistry of freshwater fish. *Prog. Lipid Res.* **26**: 281–347.

Idler, D.R., and Bitners, I. 1958. Biochemical studies on sockeye salmon during spawning migration. II. Cholesterol, fat, protein, and water in the flesh of standard fish. *Can. J. Biochem. Physiol.* **36**: 793–798.

Idler, D.R., and Bitners, I. 1959. Biochemical studies on sockeye salmon during spawning migration. V. Cholesterol, fat, protein and water in the body of the standard fish. *J. Fish. Res. Board Can.* **16**: 235–241.

Idler, D.R., and Bitners, I. 1960. Biochemical studies on sockeye salmon during spawning migration. IX. Fat, protein and water in the major internal organs and cholesterol in the liver and gonads of the standard fish. *J. Fish. Res. Board Can.* **17**: 113–122.

Idler, D.R., and Clemens, W.A. 1959. The energy expenditures of Fraser River sockeye salmon during the spawning migration to Chilko and Stuart Lakes. *Int. Pac. Salmon Fish. Comm. Prog. Rep. No. 6*.

Idler, D.R., and Tsuyuki, H. 1958. Biochemical studies on sockeye salmon during spawning migration. I. Physical measurements,

- plasma cholesterol and electrolyte levels. *Can. J. Biochem. Physiol.* **36**: 783–791.
- Jezierska, B., Hazel, J.R., and Gerking, S.D. 1982. Lipid mobilization during starvation in the rainbow trout, *Salmo gairdneri* Richardson, with attention to fatty acids. *J. Fish Biol.* **21**: 681–692.
- Kiessling, K.H., and Kiessling, A. 1993. Selective utilization of fatty acids in rainbow trout (*Oncorhynchus mykiss* Walbaum) red muscle mitochondria. *Can. J. Zool.* **71**: 248–251.
- Love, R.M. 1970. *The chemical biology of fishes*. Academic Press, New York.
- Mannaerts, G.P., and Veldhoven, P.P. 1993. Metabolic role of mammalian peroxisomes. *In* *Peroxisomes: biology and importance in toxicology and medicine*. Edited by G. Gibson and B. Lake. Taylor & Francis, London. pp. 18–61.
- McKinley, R.S., Singer, T.D., Ballantyne, J.S., and Power, G. 1993. Seasonal variation in plasma non-esterified fatty acids of lake sturgeon (*Acipenser fulvescens*) in the vicinity of hydro-electric facilities. *Can. J. Fish. Aquat. Sci.* **50**: 2440–2447.
- Mercure, F., and Van Der Kraak, G. 1995. Inhibition of gonadotropin-stimulated ovarian steroid production by polyunsaturated fatty acids in teleost fish. *Lipids*, **30**: 547–554.
- Mommsen, T.P., French, C.J., and Hochachka, P.W. 1980. Sites and patterns of protein and amino acid utilization during the spawning migration of salmon. *Can. J. Zool.* **58**: 1785–1799.
- Murata, H., and Higashi, T. 1979. Studies on the metabolism of fatty acid in fish. IV. Rate of fatty acid decrease based on beta-oxidation in carp dark muscle mitochondria. *Nippon Suisan Gakkaishi*, **45**: 211–217.
- Murdoch, W.J., Hansen, T.R., and McPherson, L.A. 1993. A review: role of eicosanoids in vertebrate ovulation. *Prostaglandins*, **46**: 85–115.
- Patton, S., Crozier, G.F., and Benson, A.A. 1970. Serum lipids and the death of spawning Pacific salmon. *Nature (London)*, **225**: 754–755.
- Sidell, B.D., Crockett, E.L., and Driedzic, W.R. 1988. Metabolic characteristics of muscle tissues from Antarctic fishes. *Antarct. J. U.S.* **23**: 138–140.
- Singer, T.D., Mahadevappa, V.G., and Ballantyne, J.S. 1990. Aspects of the energy metabolism of the lake sturgeon, *Acipenser fulvescens*, with special emphasis on lipid and ketone body metabolism. *Can. J. Fish. Aquat. Sci.* **47**: 873–881.
- Stacey, N. 1991. Hormonal pheromones in fish: status and prospects. *In* *Proceedings of the 4th International Symposium on the Reproductive Physiology of Fish, 7–12 July 1991, University of East Anglia, Norwich, U.K.* Edited by A.P. Scott, J.P. Sumpter, D.E. Kime, and M.S. Rolfe. Fish Symp. 91, Sheffield. pp. 177–181.
- Stacey, N.E., and Goetz, F.W. 1982. Role of prostaglandins in fish reproduction. *Can. J. Fish. Aquat. Sci.* **39**: 92–98.
- Steele, R.G.D., and Torrie, J.H. 1980. *Principles and procedures of statistics: a biometrical approach*. McGraw-Hill Inc., New York.
- Tocher, D.R., and Sargent, J.R. 1984. Analyses of lipids and fatty acids in ripe roes of some northwest marine fish. *Lipids*, **19**: 492–499.
- Trams, E.G. 1969. Hepatic insufficiency in spawning Pacific salmon. *Mar. Biol. (Berlin)*, **4**: 1–3.
- Van Der Kraak, G., and Chang, J. 1990. Arachidonic acid stimulates steroidogenesis in goldfish preovulatory follicles. *Gen. Comp. Endocrinol.* **77**: 221–228.
- Wade, M.G., Van Der Kraak, G., Gerrits, M.F., and Ballantyne, J.S. 1994. Release and steroidogenic actions of polyunsaturated fatty acids in the goldfish testis. *Biol. Reprod.* **51**: 131–139.
- Wiegand, M.D., and Idler, D.R. 1985. Ovarian neutral lipid fatty acid composition varies with state of ovarian growth in landlocked Atlantic salmon. *Can. J. Zool.* **63**: 2775–2777.
- Zammit, V.A., and Newsholme, E.A. 1979. Activities of enzymes of fat and ketone-body metabolism and effects of starvation on blood concentrations of glucose and fat fuels in teleost and elasmobranch fishes. *Biochem. J.* **184**: 313–322.