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Seasonal dynamics of fatty acid composition in female northern pike (*Esox lucius* L.)

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Abstract. Seasonal changes in the fatty acid composition of neutral and polar lipids were measured in the ovary, liver, white muscle, and adipopancreatic tissue of northern pike. The role of environmental and physiological factors underlying these changes was evaluated. From late summer (August–September) to winter (January–March), the weight percentage of n-3 polyunsaturated fatty acids (especially 22:6n3) declined significantly in the neutral lipids of all somatic tissues examined. However, large quantities of n-3 polyunsaturated fatty acids accumulated in the recrudescing ovaries during fall and the weight percentage of n-3 polyunsaturated fatty acids in ovary polar lipids also increased significantly. Additionally, the n-3 polyunsaturated fatty acid content of somatic polar lipids increased significantly during fall due to increases in the total polar lipid content of the somatic tissues. This suggests that during fall n-3 polyunsaturated fatty acid are diverted away from somatic neutral lipids and thereby conserved for use in ovary construction and for incorporation into tissue polar lipids. The percentage of n-3 polyunsaturated fatty acid in ovary neutral lipids also declined during fall and early winter, perhaps as an adaptation to conserve these fatty acids for storage in oocyte polar lipids and later incorporation into cellular membranes of the developing embryo. Reductions in the n-3 polyunsaturated fatty acids content of somatic and ovarian neutral lipids during fall were compensated for specifically by increases in the percentage of monounsaturated fatty acids rather than saturated fatty acids. This suggests that the ratio of saturated to unsaturated fatty acids in pike neutral lipid, is regulated physiologically, and hence may influence the physiological functioning of these lipids. During fall and early winter the percentage of saturated fatty acids declined significantly in the polar lipids of all tissues examined. This change was consistent with the known effects of cold acclimation on the fatty acid composition of cellular membranes. As the ovaries were recrudescing from September to January, liver polar lipids exhibited significant decreases in the percentage of total polyunsaturated fatty acids and n-3 polyunsaturated fatty acids and increases in monounsaturated fatty acids, and acquired a fatty acid composition very similar to that of ovary polar lipids. Therefore, seasonal changes in the percentage of polyunsaturated and monounsaturated fatty acids in liver polar lipids probably reflect the liver's role in vitellogenesis rather than the effects of temperature on membrane fatty acid composition. At all times of year, the fatty acid compositions of white muscle and adipopancreatic tissue neutral lipids were very similar, which may indicate a close metabolic relationship between these lipid compartments.

Key words: Essential fatty acids – Seasonal – Neutral and polar lipids – Omega 3 and 6 – Omega 6 – Freshwater fish, *Esox lucius*

Introduction

Imbalances between the dietary supply of EFA and physiological EFA requirements may occur in north-temperate fish if ovarian recrudescence and membrane lipid restructuring occur over winter as food and EFA intake decline. Ovarian recrudescence in north-temperate fish creates a large demand for EFA because mature ovaries often comprise as much as 25% of body weight and contain up to 10% of wet weight as lipids (Kaitaranta and Ackman 1981; Henderson and Tocher 1987). Furthermore, the ovarian lipids of many fish consist predominantly of PL which typically contain large amounts of n-3 EFA (Fogerty et al. 1986; Henderson and Tocher 1987; Schwalme and Mackay 1992). Additional requirements for EFAs over winter may result from incorpora-

Abbreviations: AP, adipopancreatic; BHT, butylated hydroxytoluene; CI, confidence interval; EFA, essential fatty acids; MUFA, monounsaturated fatty acids; NL, neutral lipids; PL, polar lipids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids

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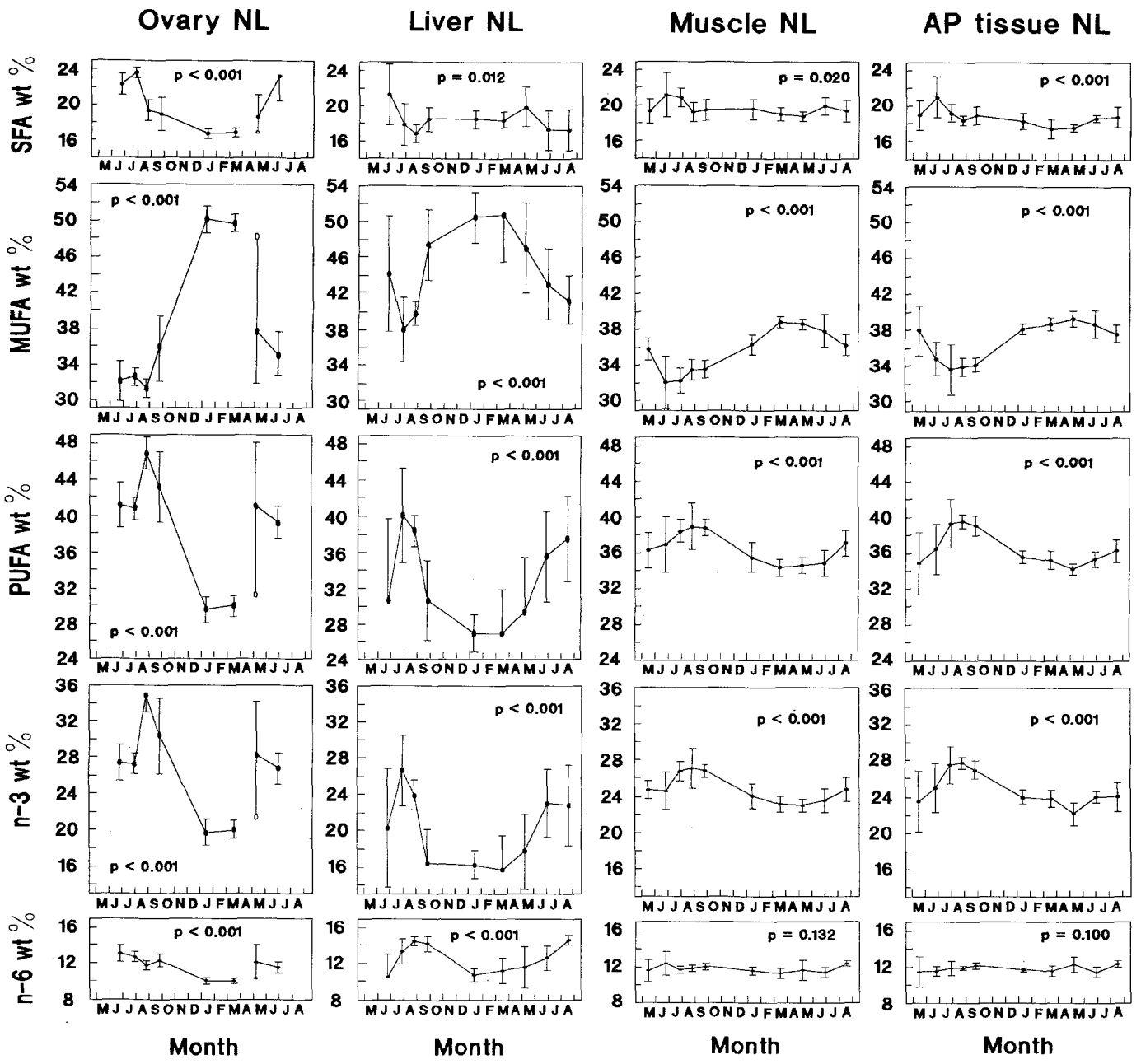

Fig. 1. Seasonal changes in the weight percentage of major fatty acid classes in neutral lipids (*NL*) of the ovary, liver, white muscle, and adipopancreatic (*AP*) tissue of female northern pike. Quantities of fatty acids (ie: wt-%) are expressed as a percentage of the total weight of all fatty acids in the indicated lipid compartment. Data are shown as the mean and 95% confidence interval (CI); *n* values are 5–8 for all means except those of May 1988. CIs are not shown if they are enclosed by the symbol. For some samples, half the CI is omitted for clarity. Variables pertaining to ovaries in Figs. 1, 2, 4 and 5 have two means in May 1988; the *open circle* shows the mean for three pike which had not spawned and still contained mature eggs, and the *solid circle* shows the mean for four pike which had spawned all their eggs. *P* values indicate the statistical significance of seasonal variation as tested using one-way ANOVA. Data from the unspawned fish caught in May 1988 were not used in the ANOVAs for ovarian fatty acids. *SFA* denotes saturated fatty acids (16:0 and 18:0 in NL; 16:0, 18:0, and 14:0 in PL), *MUFA* denotes monounsaturated fatty acids (16:1n7, 18:1n9), *PUFA* denotes polyunsaturated fatty acids (n-3 plus n-6 fatty acids), *n-3* denotes PUFA of the linolenic family (18:3n3, 20:5n3, 22:5n3, 22:6n3), and *n-6* denotes PUFAs of the linoleic family (18:2n6, 20:4n6, 22:5n6). Although not quantified, 14:0 was a minor fatty acid in NL because 14:0 plus BHT never amounted to more than 3–4 wt-% of total fatty acids in pike NL

tion of long chain EFA into cellular membranes as part of the homeoviscous adaptation phenomenon (Hazel 1989), and from increases in the PL content of somatic tissues (Schwalme and Mackay 1992) due to cold induced proliferation of cellular membranes (Egginton and Sidell 1989).

If imbalances between dietary EFA intake and physiological EFA requirements occur during winter, north-temperate fish may be expected to preferentially divert dietary EFA away from somatic NL reserves, or perhaps mobilize these fatty acids from NL reserves, so that EFA can be conserved for ovary growth and seasonal mem-

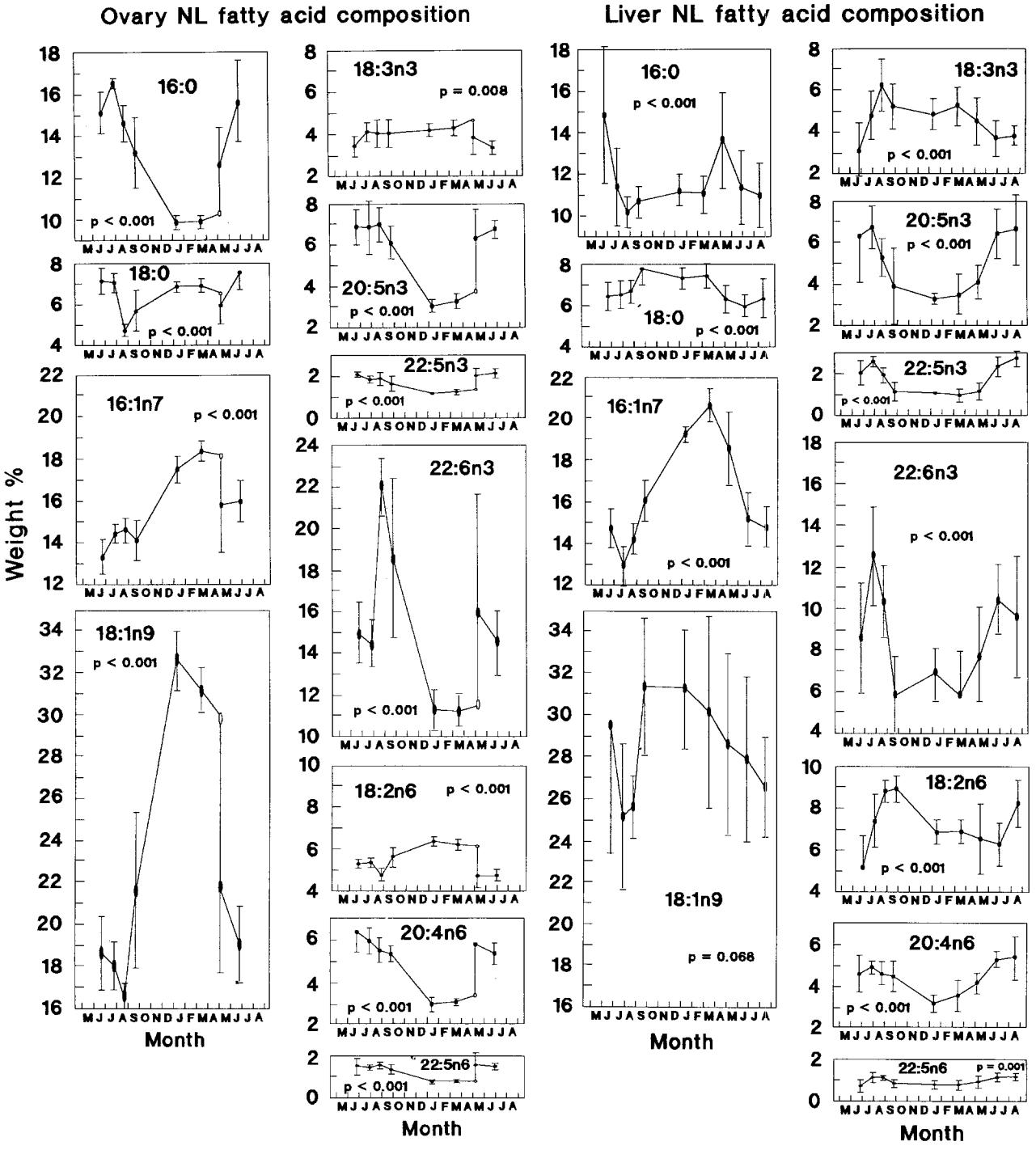

Fig. 2. Seasonal changes in the weight percentage of individual fatty acids in ovary and liver NL of female northern pike. For other details see Fig. 1

brane restructuring. Accordingly, the percentage of EFA in somatic NL would be expected to decline during fall and winter and perhaps be compensated for by increased percentages of MUFA to maintain the proper viscosity of NL reserves (Hazel 1979b).

Although a few studies have examined seasonal changes in the fatty acid composition of fish, those studies have limitations which make them unsuitable for evaluating seasonal reallocations of EFA among body compartments. These limitations include the measurement of fatty acid composition on only total tissue lipids (Jangaard et al. 1967; Henderson et al. 1984; Bergstrom 1989), on few sampling dates (Roche and Peres 1984; Dutta et al. 1985; Agren et al. 1987), and in only one or two body compartments (Hardy and Mackie 1969; Eaton et al. 1975; Yuneva et al. 1987; Henderson and Almatar 1989; Tidwell and Robinette 1990).

In the present study, seasonal changes in the fatty acid composition of NL and PL in the major lipid depots of female northern pike were measured to determine wheth-

er a reallocation of EFA from somatic NL to ovarian lipids and somatic PL occurs over winter. The study also addressed the question of the validity of extrapolating relationships between acclimation temperature and fish fatty acid composition obtained in laboratory studies to wild fish, and whether such factors as reproduction and diet modify the seasonal effects of temperature. Pike collected for this study were also the subject of an earlier report (Schwalme and Mackay 1992) in which seasonal changes in the total content of NL and PL fatty acids in pike body compartments were examined.

Materials and methods

Fish collection. Five to eight adult female pike from Lac Ste. Anne, Alberta (53°, 42′ N; 114°, 22′ W) were collected using gill nets during each of ten collection periods between May 1987 and August 1988. In total, 68 pike were sampled. Mean body weight and standard length were 944 g (SD=271 g) and 45.6 cm (SD=4.6 cm), respectively. Pike were selected for analysis so as to minimize variation in standard length between and within samples. The ten samples of pike did not differ significantly (one-way ANOVA, $P<0.05$) in either average body weight or average standard length.

Gill nets were set for 1–2 h from May to September and for 14–16 h (overnight) during winter collections in January and March. Pike were stored in a freezer (−20 °C) prior to lipid extraction from tissues which was usually completed within 6 h after removal of fish from the nets.

Lipid extraction and fatty acid analysis. The methods of lipid extraction and fatty acid analysis used in the present study were detailed in an earlier paper (Schwalme and Mackay 1992), and only a brief summary of those methods is given here. Two grams each of ovary, liver, AP tissue (Plantikow et al. 1986), and epaxial white muscle were excised from each pike. Extraction of tissue lipids with chloroform-methanol containing BHT at levels of at least 0.1% of the extract's lipid content, filtration of the extract to remove particulate matter, and removal of non-lipids by washing the extracts with 0.58% NaCl were done using the method of Folch et al. (1957). The silicic acid chromatography procedure of Rouser et al. (1967) was used to separate NL from PL. Recoveries of purified NL (tri-*O*-hexadecanoylglycerol) and PL (1,2-dieicosanoyl-*sn*-glycerol-3-phosphocholine) through silicic acid chromatography averaged 96.4±4.9% and 93.0±2.0% (mean±SD, $n=5$), respectively. Cross-contamination between the two lipid fractions was negligible. The procedure of Bannon et al. (1982) with extended heating periods, (see below) was used to saponify lipids and methylate fatty acids.

Fatty acid methyl esters were dissolved in iso-octane and quantified using a Varian model 4600 gas chromatograph equipped with a flame ionization detector and a glass column packed with Gas Chrome Q solid support (100/120 mesh) coated (10% loading) with Silar 10 C stationary phase. Other details of gas chromatography are given in Schwalme and Mackay (1992).

Fatty acids in pike lipids were identified by comparison of peak retention times with those of known standards (MaxEpa oil), by reference to published studies on the fatty acid composition of pike (Kluytmans and Zandee 1973; Kinsella et al. 1977) and other north-temperate freshwater fish (Ackman et al. 1967; Hazel 1979a, b), and by combination capillary column gas chromatography-mass spectrometry. Detector response per unit weight of fatty acid was assumed to be the same for all fatty acids measured (Ackman and Sipos 1964; Albertyn et al. 1982). A sample containing known amounts of several fatty acids was routinely chromatographed to ensure that the accuracy of fatty acid quantification did not change (due to leaks in the system or sample adsorption) during the period of test sample analysis. BHT eluted with NL during silicic acid chromatography, and with 14:0 during gas chromatography, and thereby prevented measurement of that fatty acid in pike NL. Lack of BHT in PL extracts did not lead to significant oxidation of PUFA.

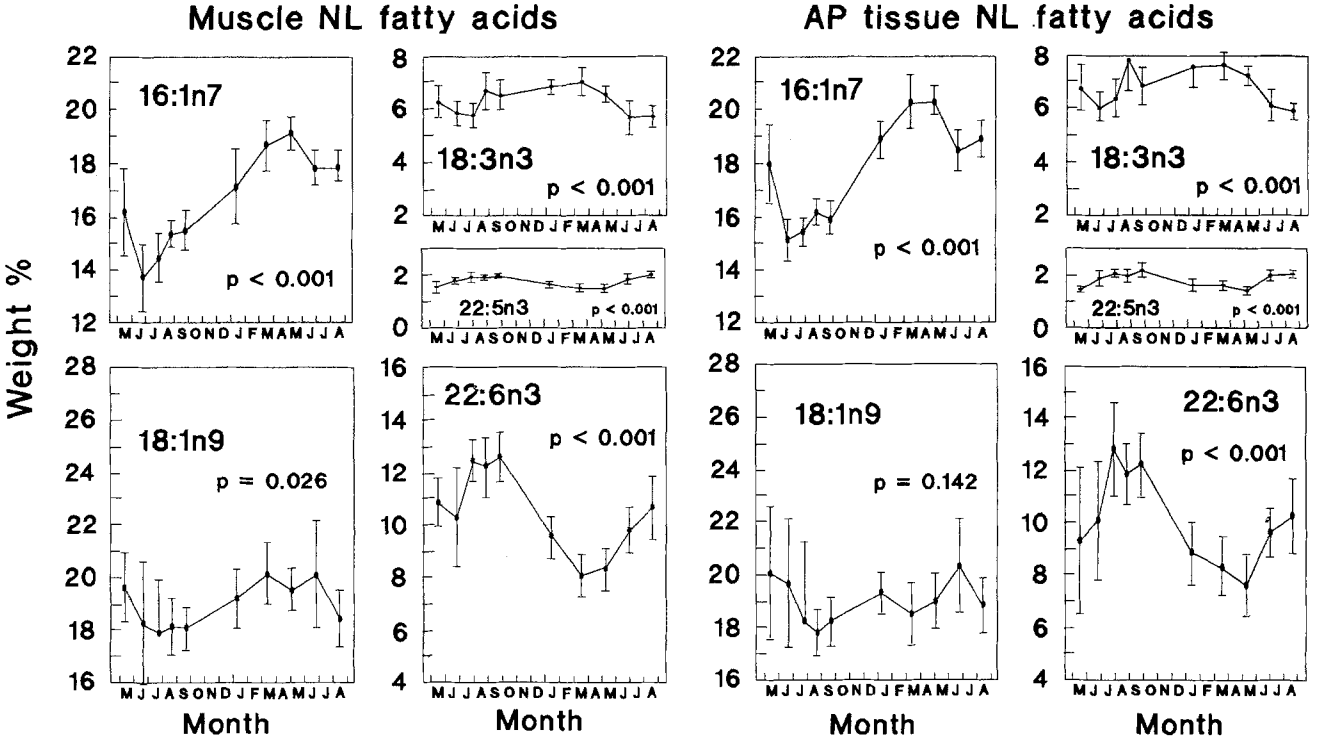

Fig. 3. Seasonal changes in the weight percentage of selected fatty acids in the NL of white muscle and adipopancreatic (*AP*) tissue of female northern pike. For other details see Fig. 1

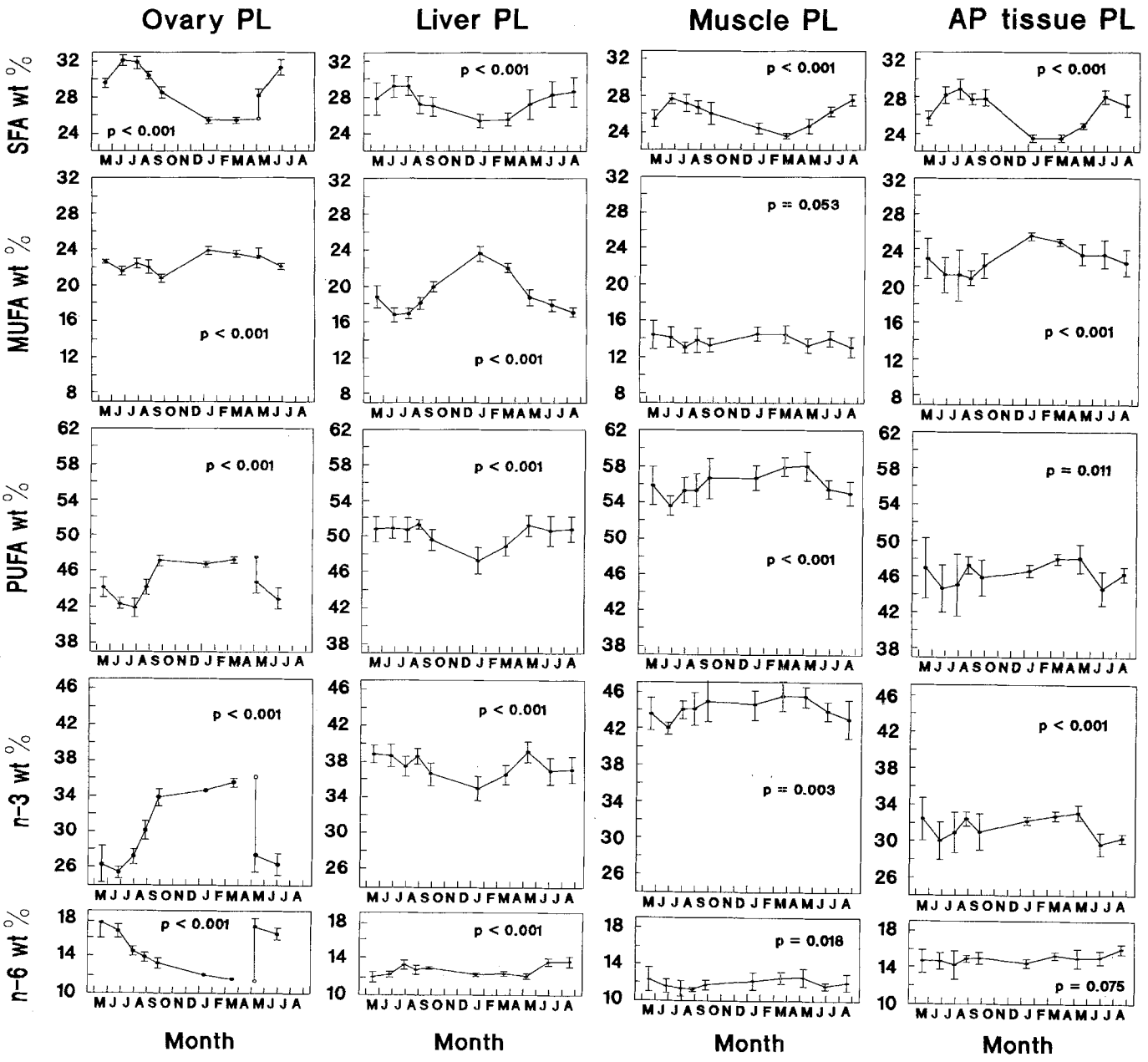

Fig. 4. Seasonal changes in the weight percentage of major fatty acid classes in tissue polar lipids (*PL*) of female northern pike. For other details see Fig. 1

Measurement of fatty acid composition in NL of liver and ovary was complicated by the presence of unidentified compounds whose peaks overlapped with those of several PUFA (18:3n3, 20:4n6, 20:5n3, 22:5n3). The unidentified compounds comprised about 10% and 5% of total peak area in chromatograms of liver and ovary NL, respectively, and were probably furan fatty acids which were first identified in fish by Glass et al. (1974). In our earlier paper (Schwalme and Mackay 1992) we gave evidence indicating that the unidentified compounds were furan fatty acids, and described the argentation chromatography procedure (Glass et al. 1977) used to quantify PUFA independently of the unidentifed compounds. The content of unidentified compounds in pike NL declined progressively during cold storage (−30 °C) and during saponification and methylation (unlike the content of identified fatty acids), and therefore is not reported. To reduce the quantity of unidentified compounds in pike lipids, and thereby provide better quantification of PUFA, the saponification and methylation periods were extended beyond those suggested by Bannon et al. (1982). Lipids dissolved in 0.5 mol · l^{-1} methanolic KOH were heated for 1 h, and subsequent to the addition of 14% boron trifluoride in methanol, heated for an additional 30 min.

Fatty acid analyses of ovary and liver NL were not done in May 1987 because the argentation chromatography procedure was not working at that time. Ovaries were not sampled in August 1988 because by that time seasonal changes in ovary fatty acid composition had been clearly described.

One-way ANOVA (at $P<0.001$) was used to determine the statistical significance of seasonal variation in the parameters measured.

Results

From late summer 1987 (August–September) to winter the NL of all four tissues underwent significant decreases

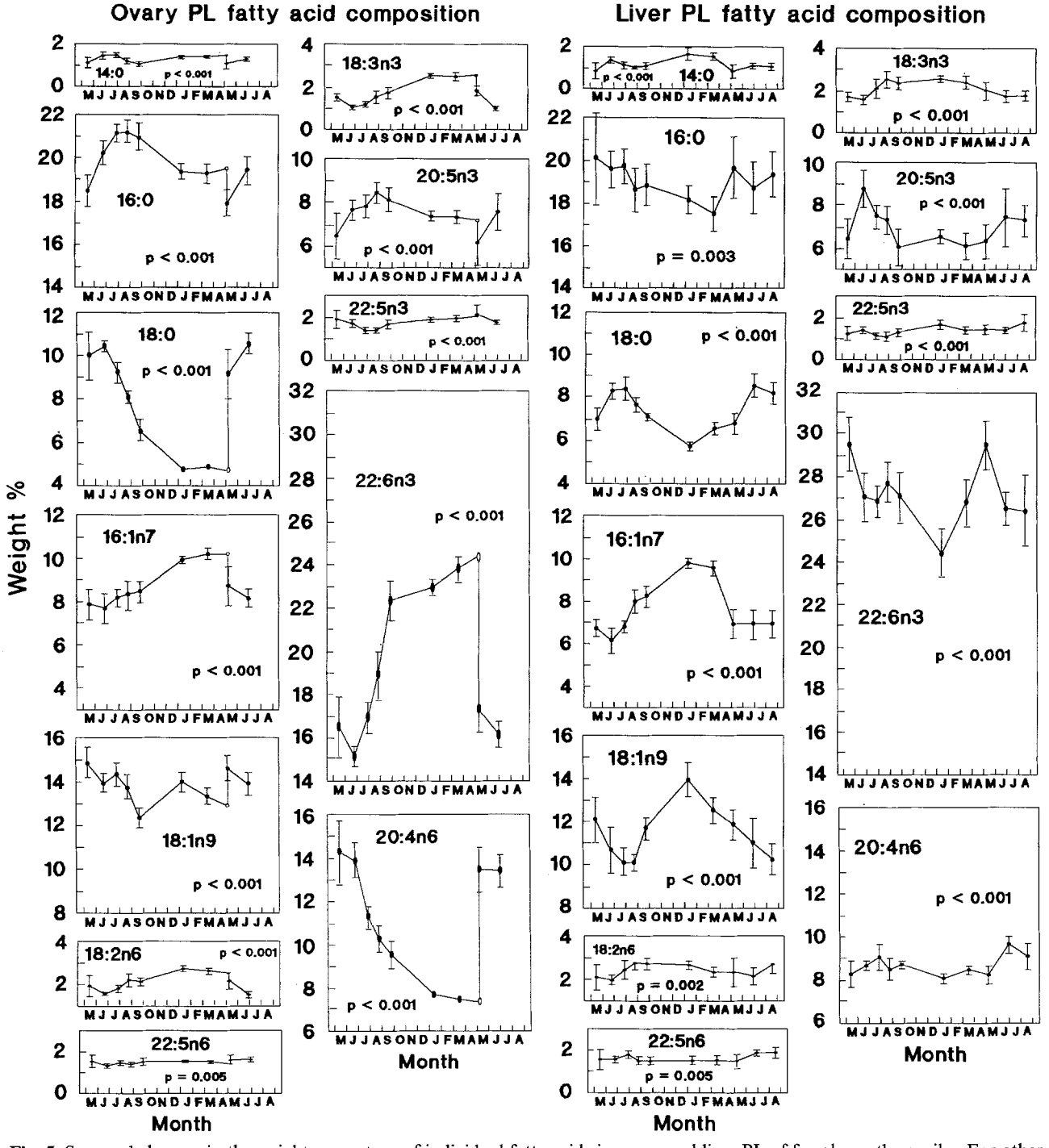

Fig. 5. Seasonal changes in the weight percentage of individual fatty acids in ovary and liver PL of female northern pike. For other details see Fig. 1

in the percentages of total PUFA and n-3 PUFA, and increases in total MUFA; those changes being of largest magnitude in ovary and liver (Fig. 1). Percentages of n-6 PUFA in ovary and liver NL also declined during late summer and fall (Fig. 1).

In the NL of all tissues examined, the dominant seasonal change among individual PUFA was a large and significant decline in the percentage of 22:6n3 from late summer to winter of 1987, followed by an increase in spring (Figs. 2, 3). Other PUFA whose abundance in NL of some or all tissues declined from summer to winter are: 20:5n3, 20:4n6, 22:5n3, and 22:5n6 (Figs. 2, 3). Percentages of short- and long-chain PUFAs in NLs changed in a reciprocal manner: 18:3n3 and 18:2n6 tended to increase during summer or fall as levels of longer chain PUFA declined (Figs. 2, 3).

Among MUFA, levels of both 16:1n7 and 18:1n9 increased greatly between late summer and winter of 1987 in NL of ovary, but only 16:1n7 showed significant ($P<0.001$) seasonal changes in NL of liver, muscle, and

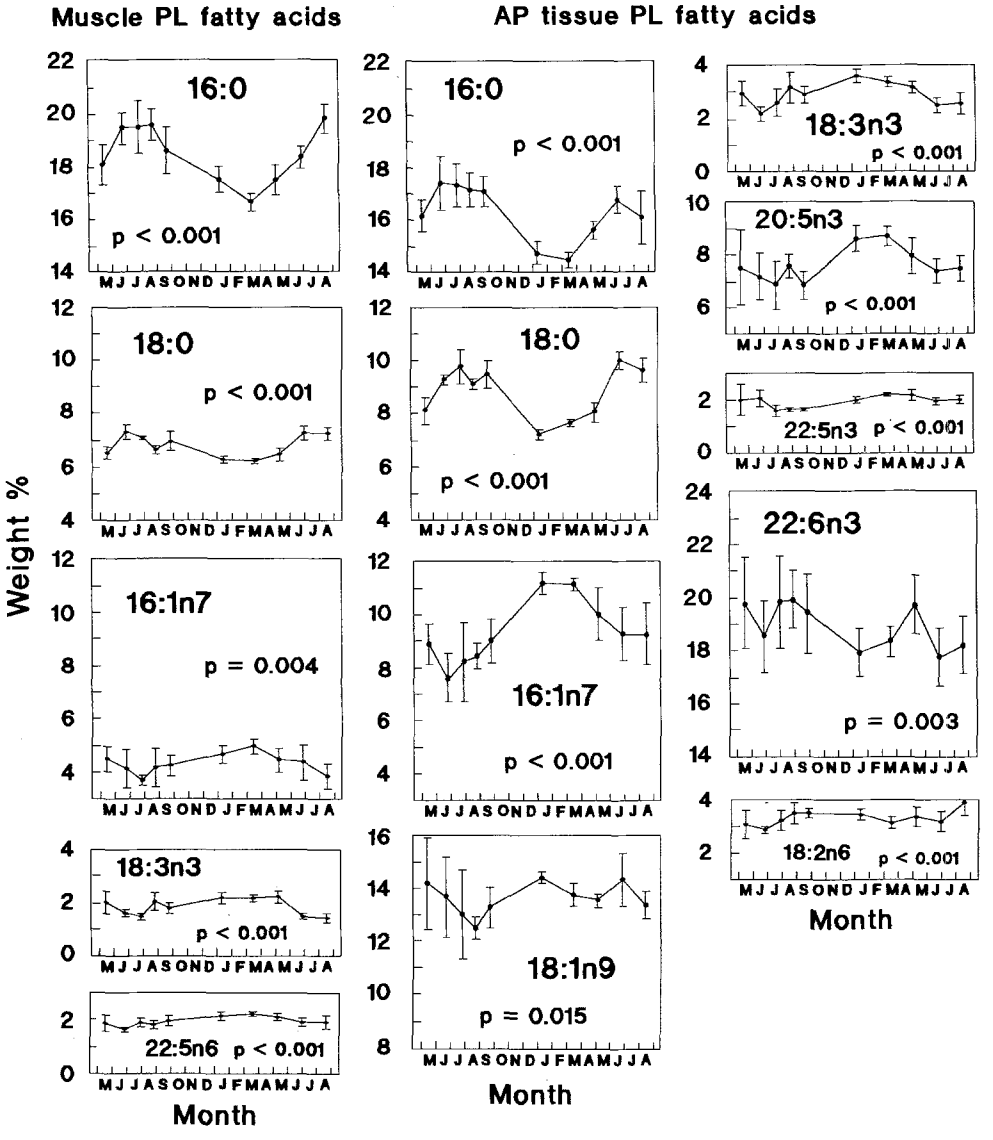

Fig. 6. Seasonal changes in the weight percentage of selected fatty acids in PLs of white muscle and adipopancreatic (*AP*) tissue of female northern pike. For other details see Fig. 1

AP tissue (Figs. 2, 3). During the spring and summer of 1988, percentages of 18:1n9 returned almost to previous levels in ovary NL, and 16:1n7 returned to previous levels in liver NL, but returned only partially in NL of other tissues (Figs. 2, 3).

The percentage of total SFA in ovary NL declined by 6.8 wt-% from July 1987 to January 1988 (Fig. 1), mainly as a result of changes in 16:0 (Fig. 2). Neutral lipids of non-ovarian tissues failed to show clearly defined changes in total SFA (Fig. 1), although liver NL did undergo small changes in the percentages of 16:0 and 18:0 (Fig. 2).

Overall, muscle and AP tissue NL had similar fatty acid compositions which followed a nearly identical pattern of seasonal variation (Figs. 1, 3). Fatty acids not reported in Fig. 3 had stable levels throughout the year which differed by no more than 0.5 wt-% between muscle and AP tissue NL. Those fatty acids, with their average levels in muscle and AP tissue NL in parentheses, are: 16:0 (13.3 wt-%), 18:0 (6.0 wt-%), 20:5n3 (6.2 wt-%), 18:2n6 (6.4 wt-%), 20:4n6 (4.3 wt-%), and 22:5n6 (1.2 wt-%).

In all tissues examined, percentages of 16:0, 18:0, and total SFA in PL declined significantly from summer to winter in 1987, and increased to previous levels during spring and summer in 1988 (Figs. 4, 5, 6). However, the timing and magnitude of changes in 16:0 and 18:0 varied considerably among tissues (Figs. 5, 6).

Percentages of total MUFA in PL increased during fall and winter and decreased during spring in liver (which showed the largest change), AP tissue, and ovary, but did not change seasonally in muscle (Fig. 4). In liver PL both 16:1n7 and 18:1n9 showed clearly defined increases during fall and winter, and decreases during spring or summer, but in PL of ovary and AP tissue only 16:1n7 underwent such changes (Figs. 5, 6). Changes in percentages of 18:1n9 in ovary and AP tissue PL were irregular or perhaps exhibited two cycles per year (Figs. 5, 6). The percentage of 16:1n7 in muscle PL increased slightly from summer to winter (Fig. 6), but that of 18:1n9 (9–10 wt-%) did not change significantly (data not shown).

From June 1987 to March 1988, the percentage of n-3 PUFA in ovarian PL increased to a greater extent (10

wt-%) than total PUFA (5 wt-%) as a result of a concurrent decrease in the proportion of n-6 PUFA (Fig. 4). Changes in percentages of total n-3 and n-6 PUFA in ovary PL resulted mainly from large increases in 22:6n3, and decreases in 20:4n6 during summer and winter of 1987 (Fig. 5). Other PUFA in ovary PL exhibited smaller seasonal changes which differed in timing and magnitude (Fig. 5).

Of the organs and tissues examined, liver was the only one in which the percentages of total PUFA and n-3 PUFA in PL declined significantly during fall and winter (Fig. 4). Large seasonal variations in 22:6n3 and smaller variations in 20:5n3 accounted for most of the change in total n-3 and total PUFA content of liver PL (Fig. 5). Percentages of 18:3n3 in liver PL increased slightly during late summer (as 22:6n3 and 20:5n3 declined), but other PUFA (22:5n3, 18:2n6, 22:5n6) in liver PL exhibited little seasonal variation (Fig. 5).

During the summer, fall, and early winter of 1987, the fatty acid compositions of ovary and liver PL gradually converged, and became nearly identical by January 1988. The similarity in ovary and liver PL fatty acid composition is clearly illustrated by levels of fatty acid classes (Fig. 4), and those of individual fatty acids, especially 22:6n3, 20:4n6, and 18:1n9 (Fig. 5).

Seasonal variations in the percentage of PUFA in PL of muscle and AP tissue were limited to small increases in total PUFA and n-3 PUFA from June 1987 to March 1988 in both tissues (Fig. 4), and to small changes in individual PUFA, notably 18:3n3, 20:5n3, and 22:6n3 in AP tissue (Fig. 6). Percentages of 22:6n3, 20:5n3, 22:5n3, 18:2n6, and 20:4n6 in muscle PL remained stable year round and averaged 32.8, 7.2, 2.2, 2.5, and 7.5 wt-%, respectively (data not shown). Average percentages of 20:4n6 and 22:5n6 in AP tissue PL were 9.9 and 1.7 wt-%, respectively (data not shown).

Discussion

Seasonal reallocations of n-3 PUFAs among body compartments

A significant decrease in the percentage of n-3 PUFA in somatic NL of pike during fall (Figs. 1, 2, 3) may be an adaption to spare these EFA for ovary construction and membrane PL synthesis, and thereby compensate for reduced dietary EFA intake. Additionally, decreases in the percentage of n-3 PUFA in NLs of maturing ovaries (Figs. 1, 2) would conserve n-3 PUFA for storage in oocyte polar lipids and later use as components of cellular membranes in the developing embryo.

Reproductively maturing Atlantic salmon also appear to conserve n-3 PUFA, since their ovary NL, like those of pike, undergo highly significant decreases in percentages of 20:5n3 and 22:6n3, and increases in 16:1n7 and 18:1n9, during maturation (Wiegand and Idler 1985). The latter authors suggested that these changes represent an adaptation to spare n-3 PUFA for storage in oocyte polar lipids, and to make MUFA available as sources of energy for the salmon embryo.

Table 1. Quantities of n-3 PUFA made available via reductions in the percentage of these fatty acids in pike NL during ovarian recrudescence from August 1987 to March 1988

	Somatic NL	Somatic plus ovarian NL
A. Content of NL fatty acids[a] ($g \cdot kg$ carcass wt^{-1})	7.4	8.0
B. Decline in wt-% of n-3 PUFA from August 1987 to March 1988	3.9	4.7
C. Grams of n-3 PUFA made available from NL (A × B/100) ($g \cdot kg$ carcass wt^{-1})	0.289	0.376
	Ovarian TL plus somatic PL	**Ovarian PL plus somatic PL**
Content of n-3 PUFA[b] ($g \cdot kg$ carcass wt^{-1}) in:		
D. March 1988	3.187	2.800
E. August 1987	0.932	0.919
F. Increase in n-3 PUFA content from August 1987 to March 1988 (D–E) ($g \cdot kg$ carcass wt^{-1})	2.255	1.881
Proportion of quantity F made available by diversion of n-3 PUFA away from NL (C/F × 100)	12.8%	20.0%

[a] The weight of pike carcass (whole body minus liver and gonad) changes little from August to March (Billard et al. 1983), and there were no significant seasonal variations in the total content of fatty acids in either somatic NL or the combined NL of somatic plus ovarian tissues (Schwalme and Mackay 1992). Therefore, the values shown are the average content of all NL fatty acids in each body compartment calculated from all pike samples collected from June 1987 to August 1988 for somatic NL, and to June 1988 for somatic plus ovarian NL (see Schwalme and Mackay 1992)

[b] Values were taken from Schwalme and Mackay (1992)

Reductions in the percentage of n-3 PUFA in pike somatic NLs during fall may have resulted from diversion of dietary n-3 PUFA away from NLs, or perhaps from mobilization of n-3 PUFA from somatic NL. We were unable to differentiate between these two mechanisms because the total n-3 PUFA content of somatic NLs varied considerably between individual pike, and thus did not exhibit statistically significant seasonal variation (Schwalme and Mackay 1992).

Admittedly, n-3 PUFA which were diverted away, or mobilized, from somatic plus ovarian NL of pike from August to March (0.38 g) account for only 20.0% of the n-3 PUFA accumulated in somatic plus ovarian PLs (Table 1). Quantities of n-3 PUFA excluded from somatic NL over winter (0.29 g) account for only 12.8% of the n-3 PUFA accumulated in ovary total lipids (Table 1). Thus, seasonal imbalances between the pike's dietary supply and physiological requirements for n-3 PUFAs appear to be of moderate severity. However, pike continue to feed throughout winter and do not significantly deplete somatic NL (at least not before March) to supply lipids for the recrudescing ovaries (Schwalme and Mackay 1992; Medford and Mackay 1978). Fish species that completely cease feeding over winter, and therefore rely solely on somatic tissues as the source of nutrients for ovary growth, may experience greater seasonal imbalances between the supply and physiological requirements for n-3 PUFA, and may possess enhanced abilities to preferentially mobilize n-3 PUFA from somatic NL.

The role of temperature in seasonal fatty acid dynamics

The most consistent seasonal change observed in pike PL was an increased degree of unsaturation during late summer and early winter as reflected in reduced percentages of SFA; changes in MUFA and PUFA varied considerably among tissues (Fig. 4). This is significant because the greatest effect of fatty acid composition on membrane physical state results from the addition of the first double bond to a saturated hydrocarbon chain (Coolbear et al. 1983). Accordingly, the percentage of SFA relative to that of unsaturated fatty acids is considered to be the primary determinant of membrane viscosity, and not the relative percentages of MUFA versus PUFA (Cossins et al. 1978; Hazel 1989). On this basis, seasonal cycles in the percentage of SFAs in pike PL appear to be attributable, at least in part, to homeoviscous adaptation.

Our results support Hazel's (1979b) proposal that the degree of unsaturation of fish NL reserves may influence the physical state (viscosity) of these reserves and the rapidity with which they can be hydrolysed to supply energy. If the degree of unsaturation of NL has no effect on the physical state or physiological functioning of these lipids, declines in the percentage of PUFA in NL during fall and winter would be unlikely to be offset specifically by increases in MUFA (Fig. 1). Therefore, increases in the percentage of MUFA in pike NL during fall and winter may be an adaptation to compensate for reductions in PUFA and thereby maintain proper NL viscosity at low temperatures.

Fatty acid composition reflects intertissue metabolic relationships

In pike, ovarian recrudescence begins in late August, and by January maturing oocytes comprise most of the ovary mass and PL comprise 70% (based on the weight of fatty acids) of ovarian lipids (Schwalme and Mackay 1992). Therefore, convergence of liver and ovary PL fatty acid composition from September to January (Figs. 4, 5) probably reflects the liver's important role in synthesizing large quantities of vitellogenin, the plasma lipoprotein which is the primary precursor of oocyte nutrients (Ng and Idler 1983; Mommsen and Walsh 1988).

Although the fatty acid composition of vitellogenin presumably resembles that of the maturing oocyte, several considerations suggest that the close similarity between the fatty acid compositions of liver and ovary PL in January cannot be explained solely by accumulation of vitellogenin within the liver. First, the PL fatty acid concentration of pike liver increased from 1.20% wet wt in July 1987 to only 1.75% wet wt in January (Schwalme and Mackay 1992). Even if this increase were due entirely to vitellogenin accumulation, vitellogenin would comprise no more than 31% of liver PL in January. To account for the very similar fatty acid compositions of liver and ovary PL in January, vitellogenin would have to represent virtually the entire content of PLs in the liver, not just 31%. Second, ovarian estrogens induce a profound proliferation of hepatocyte organelles including mitochondria, sarcoplasmic reticulum, and Golgi complex (Ng et al. 1984; Mommsen and Walsh 1988), which must surely contribute greatly to the observed increases in liver PL fatty acid concentration of pike during early winter. Therefore, we suggest that the bulk fatty acid composition of liver membrane PL comes to resemble that of ovarian PL during the period of peak vitellogenesis (January).

In pike, a functional metabolic relationship probably exists, not only between liver and ovary, but also between the NL of white muscle and AP tissue. As Figs. 1 and 3 show, the fatty acid compositions of white muscle and AP tissue NL were similar at all times of year. This suggests that AP tissue NL may serve primarily as a reserve of energy for use by white muscle which, because of its large mass, probably accounts for much of the pike's total energy consumption.

Reciprocal seasonal changes in short- versus long-chain PUFAs

During early winter, liver PL and the NL of pike ovary, liver, white muscle, and AP tissue, underwent increases in one or both of 18:2n6 and 18:3n3, while percentages of long-chain n-3 and n-6 PUFA either remained constant or more commonly declined (Figs. 2, 3, 5). Reciprocal changes in short- versus long-chain fatty acids also occurred in the muscle NL of maturing Black Sea anchovy (Yuneva et al. 1987), and may be a common feature of seasonal fatty acid cycles in fish.

In pike, reciprocal changes in 18:2n6 and 18:3n3

versus long-chain PUFA may have resulted from preferential diversion of long-chain PUFA from somatic to ovarian tissues (as apparently happens in Black Sea anchovy), and from NL to PL, or perhaps to changes in the activity of fatty acid elongase and desaturase enzymes. Declining food intake during early winter may reduce circulating insulin levels which in turn reduces the activity of elongase and delta-6 and delta-5 desaturase enzymes (Jeffcoat 1979; Brenner 1981) such that 18-carbon PUFA accumulate while longer-chain PUFA decrease in abundance.

In summary, female northern pike exhibit marked seasonal changes in the fatty acid composition of somatic and ovarian tissues which appear to be related to several environmental and physiological factors. Decreases in the percentage of n-3 PUFA in somatic NL during winter may reflect attempts to conserve limited dietary supplies of these fatty acids for use in ovary construction and incorporation into PL. Changes in the percentage of SFA in tissue PL may be attributable to seasonal temperature cycles. Seasonal changes in the percentage of PUFA and MUFA in liver PL appear to reflect the liver's role in vitellogenesis. The latter result emphasizes the need for caution when attempting to extrapolate the voluminous laboratory work on homeoviscous adaptation in fish liver (Hazel 1979a, 1989, 1990) to wild fish populations.

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References

Ackman RG, Eaton CA, Bligh EG, Lantz AW (1967) Freshwater fish oils: yields and composition of oils from reduction of sheepshead, tullibee, maria, and alewife. J Fish Res Board Can 24:1219–1227

Ackman RG, Sipos JC (1964) Application of specific response factors in the gas chromatographic analysis of methyl esters of fatty acids with flame ionization detectors. J Am Oil Chem Soc 41:377–378

Agren J, Muje P, Hanninen O, Herranen J, Pentilla I (1987) Seasonal variations of lipid fatty acids of boreal freshwater fish species. Comp Biochem Physiol 88B:905–909

Albertyn DE, Bannon CD, Craske JD, Hai NT, O'Rourke KL, Szonyi C (1982) Analysis of fatty acid methyl esters with high accuracy and reliability I. Optimization of flame-ionization detectors with respect to linearity. J Chromatogr 247:47–61

Bannon CD, Craske JD, Hai NT, Harper NL, O'Rourke KL (1982) Analysis of fatty acid methyl esters with high accuracy and reliability II. Methylation of fats and oils with boron trifluoride-methanol. J Chromatogr 247:63–69

Billard R, Mackay WC, Marcel J (1983) Progression of gametogenesis and of corporal and gonadal weight during the reproductive cycle of the pike, *Esox lucius*. In: Billard R (ed) Le brochet: gestion dans le milieu naturel et elevage. INRA Publ., Paris, pp 53–61

Bergstrom E (1989) Effect of natural and artificial diets on seasonal changes in fatty acid composition of wild and hatchery-reared Atlantic salmon (*Salmo salar* L.). Aquaculture 82:205–217

Brenner RR (1981) Nutritional and hormonal factors influencing desaturation of essential fatty acids. Prog Lipid Res 20:41–47

Coolbear KP, Berde CB, Keough KMW (1983) Gel to liquid-crystalline phase transitions of aqueous dispersion of polyunsaturated mixed-acid phosphatidylcholines. Biochemistry 22:1466–1473

Cossins AR, Christiansen JA, Prosser CL (1978) Adaptation of biological membranes to temperature: the lack of homeoviscous adaptation in the sarcoplasmic reticulum. Biochim Biophys Acta 511:442–454

Dutta H, Das H, Das A, Farkas T (1985) Role of environmental temperature in seasonal changes of fatty acid composition of hepatic lipid in an air-breathing Indian teleost, *Channa punctatis* (Bloch). Comp Biochem Physiol 81B:341–347

Eaton CA, Ackman RG, Tocher CS, Spencer KD (1975) Canadian capelin 1972–1973. Fat and moisture composition, and fatty acids of some oils and lipid extract triglycerides. J Fish Res Board Can 32:507–513

Egginton S, Sidell BD (1989) Thermal acclimation induces adaptive changes in subcellular structure of fish skeletal muscle. Am J Physiol 256:R1–R9

Fogerty AC, Evans AJ, Ford GL, Kennett BH (1986) Distribution of omega-6 and omega-3 fatty acids in lipid classes in Australian fish. Nutr Rep Int 33:777–786

Folch J, Lees M, Sloane Stanley GH (1957) A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226:497–509

Glass RL, Krick TP, Eckhardt AE (1974) New series of fatty acids in northern pike *(Esox lucius)*. Lipids 9:1004–1008

Glass RL, Krick TP, Olson DL, Thorson RL (1977) The occurrence and distribution of furan fatty acids in spawning male freshwater fish. Lipids 12:828–836

Hardy R, Mackie P (1969) Seasonal variation in some of the lipid components of sprats *(Sprattus sprattus)*. J Sci Food Agric 20:193–198

Hazel JR (1979a) Influence of thermal acclimation on membrane lipid composition of rainbow trout liver. Am J Physiol 236:R91–R101

Hazel JR (1979b) The influence of temperature adaptation on the composition of the neutral lipid fraction of rainbow trout *(Salmo gairdneri)* liver. J Exp Zool 207:33–42

Hazel JR (1989) Cold adaptation in ectotherms: regulation of membrane function and cellular metabolism. In: Wang LCH (ed) Advances in comparative and environmental physiology, vol 4. Springer, Berlin Heidelberg New York, pp 1–50

Hazel JR (1990) Adaptation to temperature: phospholipid synthesis in hepatocytes of rainbow trout. Am J Physiol 258:R1495–R1501

Henderson RJ, Almatar SM (1989) Seasonal changes in the lipid composition of herring *(Clupea harengus)* in relation to gonad maturation. J Mar Biol Assoc UK 69:323–334

Henderson RJ, Sargent JR, Hopkins CCE (1984) Changes in the content and fatty acid composition of lipid in an isolated population of the capelin *Mallotus villosus* during sexual maturation and spawning. Mar Biol 78:255–263

Henderson RJ, Tocher DR (1987) The lipid composition and biochemistry of freshwater fish. Prog Lipid Res 26:281–347

Jangaard PM, Ackman RG, Sipos JC (1967) Seasonal changes in fatty acid composition of cod liver, flesh, roe, and milt lipids. J Fish Res Board Can 24:613–627

Jeffcoat R (1979) The biosynthesis of unsaturated fatty acids and its control in mammalian liver. Essays Biochem 15:1–36

Kaitaranta JK, Ackman RG (1981) Total lipids and lipid classes of fish roe. Comp Biochem Physiol 69B:725–729

Kinsella JE, Shimp JL, Mai J, Weihrauch J (1977) Fatty acid content and composition of freshwater finfish. J Am Oil Chem Soc 54:424–429

Kluytmans JHFM, Zandee DI (1973) Lipid metabolism in the northern pike (*Esox Lucius* L.) 1. The fatty acid composition of the northern pike. Comp Biochem Physiol 44B:451–458

Medford BA, Mackay WC (1978) Protein and lipid content of gonads, liver, and muscle of northern pike *(Esox lucius)* in relation to gonad growth. J Fish Res Board Can 35:213–219

Mommsen TP, Walsh PJ (1988) Vitellogenesis and oocyte assembly. In: Hoar WS, Randall DJ (eds) Fish physiology, vol XIA. Academic Press, New York, pp 347–405

Ng TB, Woo NYS, Tam PPL, Au CYW (1984) Changes in metabolism and hepatic ultrastructure induced by estradiol and testosterone in immature female *Epinephelus akaara* (Teleostei, Serranidae). Cell Tissue Res 236:651–659

Ng TB, Idler DR (1983) Yolk formation and differentiation in teleost fishes. In: Hoar WS, Randall DJ (eds) Fish physiology, vol. IXA. Academic Press, New York, pp 373–404

Plantikow H, Letko G, Spormann H, Sokolowski A, Kemnitz P (1986) Preparation of isolated exocrine cells from adipopancreatic tissue of pike (*Esox lucius* L.) and carp (*Cyprinus carpio* L.): morphology, amylase, and lipase contents of exocrine pancreatic cells. Zool Anz 217:272–282

Roche H, Peres G (1984) Influence of acclimatization to different temperatures and of the seasonal factor on the lipid composition of liver, muscle, and intestinal tissues of the sea dace (*Dicentrarchus labrax,* Pisces). Comp Biochem Physiol 78B:755–759

Rouser G, Kritchevsky G, Simon G, Nelson GJ (1967) Quantitative analysis of brain and spinach leaf lipids employing silicic acid column chromatography and acetone for elution of glycolipids. Lipids 2:37–39

Schwalme K, Mackay WC (1992) Seasonal changes in the neutral and polar lipid fatty acid content of female northern pike (*Esox lucius* L.). Can J Zool 70:280–287

Tidwell JH, Robinette HR (1990) Changes in proximate and fatty acid composition of fillets from channel catfish during a two-year growth period. Trans Am Fish Soc 119:31–40

Wiegand MD, Idler DR (1985) Ovarian neutral lipid fatty acid composition varies with the state of ovarian growth in landlocked Atlantic salmon. Can J Zool 63:2775–2777

Yuneva TV, Shul'man GE, Chebotareva MA, Morozova AL (1987) Seasonal dynamics of fatty-acid composition of lipids in anchovy *Engraulis encrasicholus ponticus* Alexandroz. J Evol Biochem Fish 22:387–393