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Comparison of fatty acid profiles of spawning and non-spawning Pacific herring, *Clupea harengus pallasi*

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Abstract

Crude lipid and fatty acid composition from liver, intestine, roe, milt and flesh of spawning and non-spawning Pacific herring (*Clupea harengus pallasi*) were examined to determine the relative effects of spawning on the nutritional value of herring. Depletion of lipid due to spawning condition was significant ($P<0.01$) in all organ tissues and flesh of spawning herring. The lipid content ranged from an average of 1.9 to 3.4% (wet weight basis) in different organ tissues of spawning herring, to 10.5 to 16% in non-spawning fish. The fatty acid profile exhibited many differences in the relative distribution of individual fatty acids among organ tissues and between the two fish groups. Oleic acid (C18:1n-9), a major monounsaturated fatty acid (MUFA) found in all tissue lipids, decreased significantly ($P<0.01$) in spawning fish. The two monoenes, C20:1n-9 and C22:1n-11, occurred at high concentrations in the flesh but at only minor proportion in the digestive organs and gonads. Spawning herring also had significantly ($P<0.01$) higher polyunsaturated fatty acids (PUFA) content in the organ tissues, particularly in the milt and ovary, with docosahexaenoic acid (C22:6n-3, DHA) having the greatest proportion. Among the n-6 fatty acids, only C18:2n-6 and C20:4n-6 occurred at notable amounts and were present in higher proportions in spawning fish. We concluded that although relatively higher n-3 fatty acid content was found in the organ lipids of spawning herring, they are not an energy-dense prey food source due to the fact that both flesh and gonads contain a very low amount of lipid.

Keywords: Fatty acid composition; Herring; Liver; Milt; n-3 PUFA; Roe; Spawning

1. Introduction

Pacific herring (*Clupea harengus pallasi*), a pelagic fish, constitute one of the most abundant species in the North Pacific basin, inhabiting the continental shelf waters of the Pacific rim from Korea and Baja Mexico in the South to the Arctic in the North (Haegele and Schweigert, 1985). With only minor exceptions, herring stocks on both sides of the Pacific spawn during the local spring (Ware, 1985). Herring from different regions, caught in different spawning grounds show a number of common biochemical features, which reflect changes in body composition. The annual life cycle of Pacific herring comprises a period of foraging with active feeding after spawning, a maturation time that includes months of feeding and accumulation of fat for gonad development, followed by a spawning period with no feeding when gonads are discharged.

Herring spawn in late winter in British Columbia, with greatest numbers of fish occurring in March and April. Larval herring grow inshore through the summer and disappear into deeper water in the fall, thus making them unavailable for commercial fishing until full maturation at 2–3 years (Hart, 1973). Mature herring migrate inshore before spawning and are sometimes seen in September or October, immediately preceding active spawning. Unlike the summer months, Pacific herring characteristically fast in the winter, with the development of reproductive organs occurring at the expense of stored lipids (Hart et al., 1940). There are notable changes in the chemical composition of the fish tissues at each period of the life cycle as a result of feeding and sexual maturation; the most notable being the marked changes in tissue fat content and fatty acid composition.

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Pacific herring is an important prey species for many species of fish, birds and mammals (Hart, 1973; Trites and Donnelly, 2003). To understand the nutritional contribution made by different prey species consumed by marine mammals and the possible population impacts of dietary differences, important prey such as herring and walleye Pollock (*Theragra chalcogramma*) have been examined and used in several feeding studies (Kitts et al., 2004; Donnelly et al., 2003; Rosen and Trites, 2000). Most of these studies have focussed on the gross energy differences between the prey species. However, attention should also be given to the lipid composition of the fish and in particular, the variations that occur during the fish life cycle, since different lipids and associated constituent fatty acids are nutritionally important substrates for a number of important metabolic energy and maintenance processes that underlie mammalian growth and reproduction. Studies regarding fat content and fatty acid composition in other species of herring, such as the Baltic herring and Atlantic herring, have revealed notable seasonal variations that could be linked to dietary factors (Linko et al., 1985; Ratnayake and Ackman, 1979). Similar results have been found for others species such as walleye Pollock (*T. chalcogramma*) in Alaska (Kitts et al., 2004).

In this study, we examined the lipid content and fatty acid composition of Pacific herring of different maturities. Specifically, our objective was to investigate the effects of spawning on changes in the fatty acid composition of the total lipids from organ and flesh tissues. The research aim of this study was to compare the specific changes in fat content of a spawning fish (the leanest fish) compared to a feeding fish, caught at a time when it had accumulated the most lipid. We reasoned that nutritional difference, in terms of available prey fish, should not only focus on inter-species differences, but also within-species differences related to seasonality and degree of maturity.

2. Materials and methods

Fresh, whole Pacific roe herring (from a roe fishery) and frozen herring (from a food and bait fishery) which were representative prey fish for sea lions were obtained from a commercial processing plant in British Columbia. The roe herring, referred herein as "spawning herring", were obtained during the roe harvesting season in March, 2003, within 48 h of being caught. Eight to twelve fish per bag were vacuum-packed, quick-frozen and stored below −28 °C upon receiving from the fishery. The food herring, referred herein as "non-spawning herring", were collected from local fisheries. These herring were caught in September, 2002 and were immediately quick-frozen when landed (within 24 h from catch) and stored below −28 °C before being tested. After thawing, undamaged individual fish were selected and recorded for total body length (standard length), weight and maturity stage. A total of 24 herring (12 females and 12 males) from each group were tested. Roe recovered from female fish, and milt from male fish, were weighed, and the gonad index (roe weight/total fish body weight × 100) was calculated.

On the basis of collected commercial data and using the maturity scale (Hjort scale) described by Hay and Outram (1981), we estimated that our spawning fish samples were in the categories V–VI, which characterizes herring of mature and ripe condition (approaching or about spawning). In this condition, the ovaries consist mostly of ripe eggs with little ovarian tissue, and the male fish contain large milts that fully occupied the belly cavity. The spawning fish we used were estimated to be about 3–4 years old, while the non-spawning herring sample of the late summer catch were about 2–3 years of age. After the gonads were removed, liver and the remaining gastro-intestinal tract were carefully separated and sampled. The gastro-intestinal tract consisted of the stomach and intestine (not including the pyloric caeca) and included contents of both. The trunk, excluding head and tail, was separated and filleted. The fillet, excluding skin, was homogenized in a food processor. Due to the small size of the herring liver (less than 1 g in some fish), pooling of liver samples was necessary to obtain sufficient quantity for oil extraction.

Crude lipids were determined in duplicate from freshly minced samples of each tissue from individual fish which was homogenized using a modified Folch method (Folch et al., 1957). Total lipids were extracted with chloroform: methanol (2:1 v/v) containing 0.01% butylated hydroxytoluene (BHT) as the antioxidant. Fatty acid methyl esters (FAME) were prepared by transesterification with Boron trifluoride (BF3) in methanol following the method described by Kitts et al. (2004), based on the procedure described by Ackman and Eaton (1971b). After the extraction with hexane, FAMEs were analyzed by gas liquid chromatograph (GC-17A, Shimadzu Scientific Instruments Inc., Columbia, Maryland), equipped with flame ionization detector and an auto injector (AOC1400, Shimadzu Scientific Instruments Inc., Columbia, Maryland). Samples were injected onto a capillary column (30m × 0.25 mm; 0.25 μm film thickness; liquid phase: J and W DB 23), with helium as the carrier gas. Temperature programming was used according to the method described by Budge et al. (2002) with minor modification. The column temperature was initially set at 153 °C for 2 min, then increased to 174 °C at 2.3 °C/min, and then to a final temperature of 220 °C at 2 °C/min with a final hold time of 2 min. Detector and injector temperatures were both set at 250 °C. Chromatographic peaks were integrated and identified using the Shimadzu software package (version 7.2.1 SP1), which were compared to known standards supplied by Nu-Chek Prep (Elysian, MN) and a sample of well-characterized Menhaden fish oil (PUFA-3 from Matreya, Inc., Pleasant Gap, PA). Individual fatty acids are reported as weight percent of total fatty acids using mass response factors relative to C18:0. Theoretical relative response factors (Ackman, 1992) were used for calculations, with minor adjustments made after tests with standard mixtures.

Student's *t*-test was used to determine differences between the two groups of fish, spawning versus non-spawning. In all statistical tests used, $P < 0.01$ was considered statistically different.

3. Results and discussion

3.1. Total lipid content

The lipid contents of different organs of herring are presented in Table 1. A significant ($P < 0.01$) lower, but variable lipid

Table 1
Morphological parameters and lipid contents of organs in spawning and non-spawning Pacific herring

	Spawning herring	Non-spawning herring
Fish length (cm)	21.42±0.72	19.16±0.88*
Fish weight (g)	122.30±12.55	94.08±14.18*
Organ weight ratios (% body weight)		
Flesh (fillet without skin)	50.77±1.28	55.18±1.22*
Roe (gonad index)	29.95±2.32	4.25±1.16*
Milt (gonad index)	22.34±2.51	6.20±0.83*
Liver	0.56±0.19	0.92±0.22*
Gastro-intestine tract **	1.68±0.45	2.14±0.33
Fat content % (wet weight basis)		
Flesh	2.41±0.87	10.78±0.68*
Roe	2.81±0.75	–
Milt	3.40±0.62	–
Liver	1.92±0.44	10.54±0.70*
Gastro-intestine tract	2.23±0.28	16.08±0.54*

*Pairs of means corresponding to spawning and non-spawning fish were compared and those that were significantly different ($P<0.01$) are shown (*).
**Includes intestine and stomach. The stomach content was not removed.
Results represent means±S.D. ($n=12$). (−)=no sample.

content was observed in all organ tissues from spawning herring, (e.g., an average of 1.9% in liver to 3.4% in milt, on a wet weight basis). This result was in direct contrast to that obtained from the non-spawning (late summer, still feeding) herring, which had livers containing higher lipid (e.g. averaging 10.5%). An almost identical situation existed for the different flesh lipid content between spawning and non-spawning herring. In spawning herring, the viscera including liver accounted for about 3% of the total carcass weight ratio, while the liver alone accounted for 0.9%, in non-spawning herring (Table 1). Thus, fat from the liver and intestinal tract appears to only represent a minor portion of the total body fat. The main fat reserve was in the body flesh (lipid content 10.8%), with major deposits found in the abdomen. These findings for Pacific herring differ from the distribution of lipid observed in the liver of lean fish, such as cod and walleye Pollock. In our previous study of walleye Pollock, livers had a mean oil content of 54.4%, or 3.8% of the total fish weight (Kitts et al., 2004).

Decreases in whole body lipid content at the onset of spawning season are common in many species. For example, the lipid content of sockeye salmon decreases from 9.7 to 1.8% during spawning migration from the ocean to the river (Thurston and Newman, 1962). Chum salmon have a severe depletion of lipid during spawning migration that results in flesh lipid content that is only 1.2% in river fish (Huynh and Mackey, 1992). The noted decrease in fat content found in spawning herring in our study agrees with other studies that have described changes occurring as part of the fish life cycle. For example, total carcass oil content of Baltic herring (*Clupea harengus membras*) varies from 2% to 11% of wet weight, with the lowest value found in spawning fish caught in early summer (Baltic herring spawn between May and July) and the highest content present in fish caught between September and November (active feeding time) (Aro et al., 2000; Linko et al., 1985). Ackman and Eaton (1975) reported with Newfoundland herring fillets, a similar pattern of fat deposition in the summer and fall which was followed by a gradual depletion through winter.

Winter fasting and ripening of reproductive tissues at the expense of stored triglyceride in fish tissues are the main causes of decreased lipid content in spawning fish. Moreover, the mobilization of lipid stores from muscle and liver to the gonad brings forth a large fluctuation of lipid content in these tissues during gonad development. It has been estimated that about 40% of body fat deposits are transferred to the ovaries and constituent eggs in reproductive mature capelin (Henderson et al., 1984).

3.2. Fatty acid profiles

The fatty acid compositions of total lipids from different organ and flesh tissues are presented in Tables 2–5. From 50 fatty acid components originally detected, thirty fatty acids were identified and compared between spawning and non-spawning fish. The 30 fatty acids studied ranged from C14:0 to C:22:6n-3,

Table 2
Fatty acid composition (weight % of total fatty acids) in the total lipids of flesh from spawning and non-spawning herring[1]

Fatty acid	Spawning	Non-spawning
C14:0	3.56±0.74	3.99±0.48
C16:0	19.62±2.61	18.35±1.08
C17:0	1.19±0.29	0.74±0.26
C18:0	3.54±1.30	2.55±0.33
Total SFA	28.70±3.14	26.21±1.25
C16:1n-11	0.28±0.09	0.28±0.05
C16:1n-9	0.18±0.07	0.15±0.02
C16:1n-7	4.02±1.42	6.03±0.78*
C17:1	1.03±0.71	0.84±0.08
C18:1n-11	0.36±0.21	0.13±0.08
C18:1n-9	19.72±2.13	23.78±4.70
C18:1n-7	3.69±0.57	3.42±0.41
C18:1n-5	0.30±0.12	0.28±0.05
C20:1n-11	2.91±0.52	0.38±0.23*
C20:1n-9	4.20±1.01	5.20±1.31
C22:1n-11	8.23±2.45	6.37±1.77
C22:1n-9	0.35±0.15	0.30±0.17
Total MUFA	46.54±5.28	48.12±2.57
C16:2n-6	0.22±0.10	0.17±0.03
C16:2n-4	0.22±0.10	0.21±0.02
C16:3n-6	0.23±0.06	0.61±0.09*
C18:2n-6	0.97±0.33	0.99±0.22
C18:3n-6	0.20±0.10	0.19±0.06
C18:3n-3	0.25±0.11	0.41±0.08*
C18:4n-3	0.60±0.34	1.03±0.23
C20:2n-6	0.14±0.07	0.19±0.12
C20:4n-6	0.93±0.20	0.64±0.12*
C20:4n-3	0.24±0.09	0.36±0.10
C20:5n-3	7.52±1.22	8.74±0.88
C21:5n-3	0.19±0.08	0.35±0.03*
C22:5n-3	0.63±0.14	0.78±0.08*
C22:6n-3	9.98±2.04	8.58±1.55
Total PUFA	23.06±2.41	24.21±2.62
n-3 PUFA	19.73±2.50	20.59±2.25
n-6 PUFA	2.99±0.33	2.93±0.54

[1]Pairs of means corresponding to spawning and non-spawning fish were compared and those that were significantly different ($P<0.01$) are identified by (*). Total fatty acids include some components not presented due to limited amounts. Results represent means±S.D. ($n=8$).

Table 3
Fatty acid composition (weight % of total fatty acids) in the total lipids of livers from spawning and non-spawning herring[1]

Fatty acid	Spawning	Non-spawning
C14:0	0.88±0.14	1.26±0.23
C16:0	12.99±1.15	12.00±0.74
C17:0	0.94±0.29	1.90±0.64*
C18:0	5.56±0.54	2.91±0.42*
Total SFA	20.66±0.96	18.57±1.18
C16:1n-11	0.40±0.22	0.20±0.05
C16:1n-9	0.32±0.22	0.26±0.04
C16:1n-7	2.08±0.28	4.22±0.68*
C17:1	0.43±0.08	0.47±0.05
C18:1n-11	0.42±0.08	0.12±0.04*
C18:1n-9	12.84±0.57	38.14±5.48*
C18:1n-7	4.41±0.43	4.20±0.48
C18:1n-5	0.40±0.11	0.20±0.04*
C20:1n-11	0.78±0.25	0.33±0.12*
C20:1n-9	1.33±0.50	1.61±0.48
C22:1n-11	1.66±1.28	1.12±0.30
C22:1n-9	0.15±0.05	0.10±0.02
Total MUFA	25.36±1.83	51.49±4.43*
C16:2n-6	0.49±0.34	0.14±0.02
C16:2n-4	0.88±0.35	0.12±0.01*
C16:3n-6	0.27±0.08	0.29±0.08
C18:2n-6	1.14±0.15	1.14±0.16
C18:3n-6	0.15±0.04	0.14±0.04
C18:3n-3	0.20±0.09	0.51±0.11*
C18:4n-3	0.26±0.05	0.78±0.18*
C20: 2n-6	0.12±0.09	0.13±0.05
C20:4n-6	2.11±0.35	0.57±0.12*
C20:4n-3	0.21±0.10	0.60±0.13*
C20:5n-3	9.06±0.44	10.97±1.61
C21:5n-3	0.31±0.15	0.29±0.05
C22:5n-3	1.70±0.19	1.35±0.12*
C22:6n-3	32.11±0.58	11.16±2.37*
Total PUFA	49.91±0.74	28.35±4.10*
n-3 PUFA	44.28±0.38	25.76±3.83*
n-6 PUFA	4.77±0.66	2.50±0.33*

[1]Pairs of means corresponding to spawning and non-spawning fish were compared and those that were significantly different ($P<0.01$) are identified (*). Total fatty acid groups include some components not presented. Results represent means±S.D. ($n=8$).

with a selection criteria that required exceeding a minimum of 0.4% total fatty acids at least in one tissue sample. Collectively, the 30 fatty acids comprised over 95% of the total fatty acids in the total lipids. Only a few minor components of uncertain identity were omitted for calculation, including those of carbon chains exceeding C22:6n-3.

In general, the fatty acid profiles of different organ tissues in both spawning and non-spawning herring exhibited notable similarities, with high, but variable proportions of omega-3 highly unsaturated fatty acids (HUFA), predominantly C20:5n-3 (EPA) and C22:6n-3 (DHA), along with substantial proportions of monoene C18:1n-9 and saturated fatty acid C16:0. Many differences in the relative distribution of individual fatty acids were observed among organ tissues from both fish groups. Fatty acid contents in the flesh of both spawning and non-spawning herring, decreased in the order of MUFA> SFA> PUFA (Table 2); a characteristic lipid profile of most fatty fish (Kozlova and Klotimchenko, 2000). On average, 47% monounsaturates (primarily C18:1 isomers), 28% saturates (primarily C16:0), and approximately 23% polyunsaturates, of which over 85% were omega 3 fatty acids (mainly EPA and DHA), were found in the flesh of spawning herring. Organ tissues (e.g., liver, milt and roe) of spawning fish displayed a converse pattern with the PUFA prevailing and monoenes contained at lower levels. Interestingly, this pattern was not seen in the non-spawning fish, as liver and intestine contained more MUFA than PUFA.

3.2.1. Saturated fatty acids (SFA)

The most abundant SFA in herring was C16:0 (ranging from 12 to 24% in different tissues), which is noted for being a predominant source of potential metabolic energy in fish during growth and particularly during the roe formation stage in female fish (Henderson et al., 1984). The latter example explains the relatively high amount (e.g., 22%) of C16:0 present in the roe lipid of spawning herring (Table 5). Kaitaranta and Linko (1984), also reported a high proportion (23.3%) of C16:0 in Baltic herring roe that was related to a high polar lipid content. However, a decreasing trend in the amount of C16:0 in the flesh

Table 4
Fatty acid composition (weight % of total fatty acids) in the total lipids of gastrointestine and contents from spawning and non-spawning herring[1]

Fatty acid	Spawning	Non-spawning
C14:0	1.15±0.94	1.83±0.41
C16:0	24.31±1.47	18.94±1.66*
C17:0	1.02±0.42	2.04±0.20
C18:0	6.09±0.35	3.53±0.52*
Total SFA	32.57±2.72	26.96±1.60*
C16:1n-11	0.55±0.30	0.22±0.07
C16:1n-9	0.42±0.12	0.15±0.02
C16:1n-7	4.04±0.50	5.34±0.77*
C17:1	0.85±0.52	0.65±0.09
C18:1n-11	2.10±0.32	–
C18:1n-9	12.53±0.54	31.31±4.29*
C18:1n-7	5.44±0.55	4.42±0.57
C18:1n-5	0.98±0.27	0.33±0.06*
C20:1n-11	0.35±0.08	0.89±0.35*
C20:1n-9	0.81±0.19	4.39±1.41*
C22:1n-11	0.36±0.19	3.81±1.19*
C22:1n-9	0.67±0.17	0.19±0.12*
Total MUFA	31.09±1.25	52.60±2.18*
C16:2n-6	0.66±0.51	0.19±0.03
C16:2n-4	0.17±0.14	0.21±0.02
C16:3n-6	–	0.65±0.17
C18:2n-6	1.87±1.20	1.30±0.17
C18:3n-6	0.83±0.58	0.16±0.08
C18:3n-3	0.88±0.69	0.49±0.10
C18:4n-3	0.43±0.15	1.21±0.10*
C20:2n-6	0.52±0.49	0.16±0.07
C20:4n-6	1.64±0.30	0.60±0.12*
C20:4n-3	–	0.25±0.06
C20:5n-3	6.87±0.87	8.00±0.77
C21:5n-3	0.44±0.23	0.25±0.02
C22:5n-3	0.88±0.28	0.60±0.09
C22:6n-3	15.70±2.11	4.70±0.95*
Total PUFA	31.24±2.76	19.38±1.99*
n-3 PUFA	25.18±2.16	15.80±1.79*
n-6 PUFA	5.90±1.15	3.07±0.20*

[1]Pairs of means corresponding to spawning and non-spawning fish were compared and those that were significantly different ($P<0.01$) are identified (*). Total fatty acid groups include some components not presented. Results represent means±S.D. ($n=8$). (–) indicates no sample.

Table 5
Fatty acid composition (weight % of total fatty acids) in the total lipids of mature gonads from spawning herring[1]

Fatty acid	Milt	Roe
C14:0	0.26±0.05	1.91±0.08*
C16:0	11.82±1.28	22.12±2.17*
C17:0	0.30±0.10	0.42±0.10
C18:0	2.99±0.28	2.51±0.48
Total SFA	15.61±1.61	27.45±3.16*
C16:1n-11	1.23±0.35	0.43±0.12*
C16:1n-9	0.16±0.02	0.44±0.09*
C16:1n-7	1.03±0.49	4.00±0.56*
C17:1	1.08±0.53	2.12±0.45*
C18:1n-11	0.27±0.04	0.37±0.11
C18:1n-9	10.80±1.34	12.28±2.33
C18:1n-7	6.63±0.72	4.56±0.42*
C18:1n-5	0.31±0.06	0.38±0.05
C20:1n-11	0.31±0.06	0.29±0.08
C20:1n-9	0.59±0.13	0.60±0.14
C22:1n-11	0.34±0.05	0.38±0.15
C22:1n-9	0.20±0.06	0.11±0.07*
Total MUFA	24.24±2.28	26.82±1.95
C16:2n-6	0.07±0.05	0.32±0.05*
C16:2n-4	–	0.23±0.06
C16:3n-6	0.22±0.06	0.21±0.03
C18:2n-6	0.85±0.18	0.99±0.22
C18:3n-6	0.19±0.06	0.19±0.03
C18:3n-3	0.23±0.07	0.52±0.09*
C18:4n-3	0.34±0.05	0.49±0.05*
C20:2n-6	0.35±0.08	0.11±0.07*
C20:4n-6	1.35±0.17	1.23±0.22
C20:4n-3	0.52±0.08	0.43±0.09
C20:5n-3	10.84±0.78	13.72±0.94*
C21:5n-3	0.39±0.09	0.19±0.05*
C22:5n-3	3.58±0.34	1.32±0.31*
C22:6n-3	35.97±0.32	21.65±3.62*
Total PUFA	56.30±0.42	42.65±3.87*
n-3 PUFA	52.23±0.73	38.76±5.76*
n-6 PUFA	3.86±0.32	3.62±0.12

[1]Pairs of means corresponding to spawning and non-spawning fish were compared and those that were significantly different ($P<0.01$) are identified (*). Total fatty acid groups include some components not presented. Results represent means±S.D. ($n=8$). (–) indicates no sample.

total lipids was reported for Baltic herring during spawning time, which apparently corresponded to the changes in C16:0 in dietary plankton lipids (Linko et al., 1985). Interestingly, herring milt only contained half of the C16:0 found in the roe, contributing in part to the relatively lower saturated fatty acid content in the milt (15.6%) compared to the roe (27.5%).

3.2.2. Monounsaturated fatty acids (MUFA)

Monounsaturated fatty acids constituted nearly half of the total fatty acids in fish flesh (Table 2) but less than one third total fatty acids in liver, intestine, milt and roe recovered from spawning herring (Tables 3, 4 and 5). In particular, MUFA dominated in both liver and intestine lipids of the non-spawning fish due to the abundance of C18:1n-9, which occurred at more than 30% in these tissues. The proportion of C18:1n-9 was three-times higher in the liver (38.1%) and about 2.5 times greater in the intestine (31.3%) of non-spawning fish, compared to those from spawning fish. The higher level of C18:1n-9 found in non-spawning fish likely reflects a requirement for greater energy needs during the summer feeding period, when herring lipid content increases. In contrast, spawning fish may require C18:1n-9 for energy metabolism during the course of gonad development (Henderson et al., 1984), thus depleting C18:1n-9 reserves.

Our reported levels of C18:1n-9 agreed with published fatty acid data for herring. For example, Linko et al. (1985) reported a total monoene content of 25 to 40% in the flesh total lipid of Baltic herring caught from May to October off the coast of Finland, of which 13 to 26% was C18:1n-9. Pacific herring oil has also been reported to contain 29.72% C18:1n-9 in the total fatty acids (Ratnayake and Ackman, 1979). Interestingly, a much lower proportion of C18:1n-9 was reported for Atlantic herring in fish muscle and skin lipids (Ratnayake and Ackman, 1979). However, no major difference may exist between Pacific or Baltic herring and Atlantic herring (Linko et al., 1985) if the C16:1 and C18:1 acids are interchangeable (Ackman and Eaton, 1970, 1971a,b), given that Atlantic herring contain a higher proportion of C16:1. The higher proportion of C18:1n-9 in liver and intestine compared to flesh in non-spawning herring is opposite to the lower proportion of C18:1n-9 in liver and intestine compared to flesh in spawning fish. This observation suggests that this particular fatty acid is transferred directly from the diet, with the greater levels found in the digestive organs reflecting the active feeding behaviour of non-spawning fish.

Another interesting feature of the MUFA was the noticeably high levels of C20:1n-9 and C22:1n-11 acids in the flesh lipids from both spawning and non-spawning herring. These fatty acids existed between 4 and 9% in the flesh lipids (Table 2). These two MUFA have been associated with zooplankton, specifically copepod (Graeve et al., 1994), and variation in the levels could reflect varying amounts of zooplankton consumed in the diet (Budge et al., 2002). The relatively high level of these MUFAs in the lipid is characteristic of herring and likely originates from the fatty alcohols present in copepods (Ackman, 1982). According to Tocher (2003), the presence of high levels of C20:1n-9 and C22:1n-11 fatty acids in herring body oil is due to the oxidation of C20:1 and C22:1 fatty alcohols of wax esters that are predominant in the lipids of the calanoid copepods, and which constitute a large part of the herring diet. C20:1 and C22:1 occur in very high concentrations in Atlantic herring (14% and 20%, respectively) (Ratnayake and Ackman, 1979), but in very low concentration in Baltic herring flesh lipids (Linko et al., 1985). The C22:1 was reported to be the prominent MUFA, varying from 14 to 28%, in the flesh lipid of North Sea herring (Aidos et al., 2002).

Unlike the flesh, the organ tissues, including gonads of the spawning herring, contained only minor proportions of C20:1n-9 and C22:1n-11. Less than 2% was found in these tissues (Tables 3, 4 and 5). Similar low levels of these MUFA have been reported previously in capelin roe and were considered to reflect the selective oxidation of C20:1n-9 and C22:1n-11 in triacylglycerol depots. These particular MUFA are abundant in triacylglycerols found in parent fish, but exist in only small amounts in roe (Henderson et al., 1984). Similar to C18:1n-9, the C20:1n-9 and C22-1n-11 fatty acids are also consumed in

large amounts during growth and during the roe formation in female fish (Henderson et al., 1984).

In our study, we recovered significantly ($P<0.01$) lower levels of C20:1n-9 and C22:1n-11 from intestine lipid of spawning herring compared to non-spawning herring (Table 4). Taking into account that the majority of spawning fish stop feeding during spawning migration, the low percentage of these fatty acids in the intestine is expected as it reflects their reduced feeding behaviour.

3.2.3. Polyunsaturated fatty acids (PUFA)

PUFA contents of spawning herring, ranged from 23% in the flesh and 50% in the liver to 43% and 56%, respectively in the roe and the milt (Tables 2–5). A significantly ($P<0.01$) lower proportion of PUFA was found in the liver of non-spawning herring compared to spawning herring. The differences, however, were not observed in the flesh, which had fairly similar PUFA proportions in both fish groups.

The primary source of total PUFA variation found in Pacific herring was due to the difference in highly unsaturated fatty acids (HUFA), namely n-3 fatty acids EPA and DHA. The sum of both HUFAs reached approximately 17% in the flesh of both fish groups and 35% and 47%, respectively for roe and milt; a normal pattern for marine fish lipids where EPA and DHA are dominant PUFAs.

DHA occurred in greater proportion than EPA in all organ tissues of spawning herring. The highest proportion of DHA occurred in the milt (36%) while the roe contained a relatively lower proportion (22%). The high level of DHA found in the gonads is characteristic of spawning herring and agrees with values reported in the literature. The fatty acids of herring milt have been reported to contain up to 39.5% hexaenoic acids with roe containing 21.2% (Notevarp, 1961). In other studies, Tocher and Sargent (1984) reported 31.4% DHA in Atlantic herring roe and 28.6% DHA in cod roe from the total phospholipid fraction. Kaitaranta (1980), also reported average contents of 32.6% and 25.6% of DHA in the polar lipids of whitefish flesh and roe, respectively. Because DHA is an important component of membrane structural lipids (Tocher and Harvie, 1988), the relative percentage of this HUFA is expected to increase during the gonad development stage.

High polyunsaturated fatty acids (HUFA) are also the major source of metabolic energy for reproduction (Henderson et al., 1984). Although utilized for energy, DHA and EPA are relatively conserved in comparison with MUFA during the gonad development. Our data indicate that C18:1n-9 is selectively utilized for metabolic fuel use for spawning herring, thus contributing to the relatively lower level of C18:1n-9, but higher levels of DHA, found in our spawning fish samples. Moreover, a relatively higher DHA/EPA ratio (e.g. greater than 3) was obtained in both the milt and liver, in contrast to the lower ratio found in roe (1.6) and in the flesh (1.3). Similar findings on relative proportions of DHA and EPA have been reported in capelin roe (Henderson et al., 1984), and in fish roe in general (Tocher and Sargent, 1984). One explanation for this finding is the selective catabolism of C20:5n-3, relative to C22:6n-3, in fatty acid oxidation which produces energy for gonadogenesis, and the selective transfer of C22:6n-3 to the eggs (Tocher, 2003).

Our results also demonstrate a relatively low concentration of the other essential PUFA, C18:2n-6 (0.9–1.9%) and C18:3n-3 (<1%), in the organ and flesh tissues of herring, which agrees with the findings on many other marine species. Higher relative proportions of C18:2n-6 have been reported in Baltic herring lipid in May compared to August herring, which have been linked to dietary factors (Linko et al., 1985). For example, Pacific herring generally feed on pelagic species that are a poor source of these PUFA. The n-6 fatty acids were present in only negligible amounts in all herring tissue lipids, except for C18:2n-6 and C20:4n-6 (arachidonic acid), which occurred in notable levels of 0.5 to 2.1%. Fish which feed predominantly from the pelagic phytoplankton food web have been found to contain low levels of C20:4n-6 (Dunstan et al., 1988). In British Columbia, adult herring feed mostly on crustaceans such as copepods, amphipods, euphasiids, etc., with some consumption of smaller fish and marine worms (Hart, 1973). The proportions of n-6 PUFA were relatively low in all lipid fractions (<4%), except in the intestine and liver of spawning fish, where higher proportions (4–6%) were found. In fish oils, the n-6 PUFA is generally less than 5% of total fatty acids (Ratnayake et al., 1989).

Although occurring at low concentration, significantly ($P<0.01$) higher level of C20:4n-6 was observed in the lipids of flesh, liver and intestine in spawning herring compared to non-spawners (Tables 2, 3 and 4). The eggs and milt also contained notable levels of C20:4n-6 (Table 5), which was higher than that in body flesh. Bell et al. (1997) reported that C20:4n-6 levels in eggs and newly hatched larvae from eight species of marine teleosts were several folds higher than in the normal body lipids of these fish.

Similar to EPA and DHA, C20:4n-6 is involved in maintaining cell membrane structure and function and also contributed to growth promotion in fish (Cejas et al., 2004; Sargent et al., 1995). Although C20:4n-6 has similar biologically importance as EPA and DHA, it is often neglected in fish because it occurs only at very low concentration, despite possessing vital functions as a main precursor of various eicosanoids (Tocher and Sargent, 1987), that have important roles in a variety of physiological functions including osmoregulation, cardiovascular functions and the functioning of the reproductive systems (Cejas et al., 2004). In contrast, the content of C20:4n-6 is considerably high in marine mammals. For example, the n-6 PUFA/n-3 PUFA ratio in the liver of seals and dolphin is about 1 (Kakela and Hyvarinen, 1998), due mainly to the high concentration of C20:4n-6. Because fish prey source, such as herring have very low C20:4n-6 content, it stands to reason that marine mammals such as sea lions or dolphins, must obtain this fatty acid from other dietary sources, or through active biosynthesis pathways for conversion of C18:2n-6 to C20:4n-6.

In conclusion, our study shows that tissue lipid contents and fatty acid composition of Pacific herring vary with the life cycle and condition at maturity. Variation in fatty acid composition occurred not only in the flesh but also within the organ tissues of

liver, intestine and gonad. We have shown that spawning herring exhibit a marked increase in the relative concentration of PUFA in the organ lipids, particularly in the milt and ovary. A higher proportion of n-3 fatty acids was found in the gonads, with DHA representing the greatest proportion at more than twice that found in the flesh. Taken together, however, the total content of important n-3 HUFA was low, due to the fact that herring eggs and milt contain a low amount of lipid. Moreover, due to the significant lipid loss during spawning, the absolute amounts of n-3 HUFA in the flesh of spawning fish will decrease even more so, although relative proportions may be similar to those of non-spawning fish.

Former studies have reported herring to be an energy-rich food source for marine mammals compared to walleye Pollock, which has less than 2% total lipid in flesh (Rosen and Trites, 2000). Our findings that showed seasonal variation in total lipid content of Pacific herring could be contributed to lower lipid levels has lead to our conclusion that non-spawning herring are a better nutrition-dense prey food source than spawning herring for marine mammals. It is noteworthy that the n-3 fatty acids concentrations occurring in herring during the spawning season are similar to that found in a lean fish species, such as walleye Pollock. Thus caution is required in concluding the relative nutritional density of prey fish sources for marine mammals without an indication of the fish life cycle.

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