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Temperature-Dependent Fatty Acid Composition Change of Phospholipid in Steelhead Trout (*Oncorhynchus mykiss*) Tissues

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Abstract In this study, the changes of the fatty acid composition of phospholipid in different tissues (muscle, heart, brain and spleen) of steelhead trout (*Oncorhynchus mykiss*) were analyzed when the water temperature decreased gradually from 16℃ to 12℃, 8℃, 6℃, 4℃, 2℃ and 1℃. Three fish individuals each tank (average weight 70.32 g±9.12 g) were collected and used to analysis at each designed temperatures. At normal temperature (16℃), the fatty acid composition of phospholipid of muscle and heart was similar each other. The highest concentration of saturate fatty acids (SFA) was found in the phospholipid of spleen. The brain phospholipid contained higher oleic acid (18:1n9) than the phospholipid of other tissues at 16℃. When the environmental temperature decreased, the concentration of unsaturated fatty acids of phospholipids in all tissues increased, and accordingly the ratio pf the unsaturated to saturated fatty acids (U/S) and unsaturation index (UI) increased, indicating that steelhead trout can compensate temperature-dependent changes in membrane fluidity by remodeling the fatty acid composition of phospholipids. The changes in the fatty acid composition of phospholipid were tissue-specific. At the early stages of the experiment (16℃ to 8℃), the fatty acid composition of phospholipid changed remarkably in muscle, heart, and spleen. When temperature decreased to less than 8℃, an obvious response of phospholipid fatty acid was observed in all tissues. The change of phospholipid composition of steelhead trout tissues may be affected by both cold stress and starvation when the temperature decreased to 2℃, and the change of phospholipid composition of muscle was very obvious.

Key words temperature; tissue; phospholipid; fatty acid; steelhead trout *Oncorhynchus mykiss*

1 Introduction

According to the Food and Agriculture Organization of the United Nations (FAO), farming production of fish in the family Salmonidae is more than 2 million tons per year, becoming the world's third largest aquaculture fish after carp and tilapia (Pauly and Zeller, 2017). In recent years, Salmonidae mariculture has developed rapidly in China, especially the mariculture of steelhead trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*) (Han *et al*., 2016).

Phospholipid, the key structural element of cellular membranes, is sensitive to the temperature change of the environment, and is slightly influenced by diet (Hsieh *et al*., 2003; Wijekoon, 2011). Ectotherm compensates for temperature-induced change in membrane fluidity by altering the fatty acid composition of cell membrane (Wodtke, 1978; Jobling and Bendiksen, 2015; Fadhlaoui and Couture, 2016). As ambient temperature decreases, cell membrane becomes more fluid; conversely, at warmer temperatures, cell membrane become more rigid (Fokina *et al*., 2015).

Temperature can influence all physiological and behavioral parameters of poikilothermic animals (Ng *et al*., 2015). Water temperature has been described as the 'abiotic master factor' for fish (Mellery *et al*., 2015). Fish species are generally able to cope with gradual temperature change that is common in nature (*e.g*., diel variation, currents and seasonal cooling). They usually respond to such change through altering their physiological functions, attempting to maintain a relatively constant metabolism. The alteration of fatty acid composition in phospholipid associates with the low temperature acclimation of fish (Hazel, 1979; Olsen, 1999; Farkas *et al*., 2001; Snyder and

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Hennessey, 2003; Copeman *et al*., 2013). Olsen (1999) found that some fish species (such as goldfish *Carassius auratus* and catfish *Parasilurus asotus*) from cold regions have more *PUFA* (polyunsaturated fatty acid) in their phospholipid than those from warm regions. Farkas *et al*. (2001) also reported that the most obvious influence of environmental temperature on membrane lipid composition is the content of UFA (unsaturated fatty acid) in ruffe (*Acerina cernua*). The contents of 22:6n3 (DHA) and 20:5n3 (EPA) significantly increase in low temperature-acclimated freshwater fish alewives (*Alosa pseudoharengus*) (Snyder and Hennessey, 2003) and Pacific cod (*Gadus microcephalus*) (Copeman *et al*., 2013) while 16:0 (palmitic acid) in phospholipid decreases in a variety of freshwater and marine fish species at low temperatures (Hazel, 1979; Cossins and Prosser, 1982; Snyder *et al*., 2012).

Temperature plays an important role in the development, growth and survival of anadromous steelhead trout at all stages of their life cycle (Wijekoon, 2011; Schregel, 2013). Specifically, water temperature can affect the fatty acid composition of phospholipid in the tissues of the fish (Sloat *et al*., 2014). However, limited information is available for the change of fatty acid composition of phospholipid in fish tissues when the temperature gradually decreases. In the present study, phospholipid fatty acids in the muscle, spleen, heart and brain of steelhead trout were analyzed during a decreasing process of temperature. The objective of this study was to investigate the changes of key fatty acids in phospholipid of four tissues, thus guiding the selection of optimum harvesting time.

2 Materials and Methods

2.1 Experimental Design

Experiment was conducted at the Key Laboratory of Mariculture, Ocean University of China Qingdao, China, from 9 September to 27 October, 2016. Juvenile triploid steelhead trout individuals were obtained from WanZe-Feng Fishery Company, Rizhao, Shandong, China. Before the experiment, fish individuals were acclimated for two weeks in an aquarium equipped with a semi-recirculating system and maintained at 16℃ ± 0.5℃. This system is consisted of a reservoir and a supplemental aeration and a circulation pump. One hundred juvenile steelhead trout individuals (75.45 g ± 6.27 g) were randomly distributed into 4 fiberglass cylindrical tanks (277 L; 0.58 m height × 0.78 m diameter), 25 each. During the entire experiments, the fish were fed with Tianma trout feed (Tianma Company, Fujian, China), twice a day (at 8:00 and 18:00) with a daily ration of approximately 2% of wet body weight. The fatty acid composition of the feed is listed in Table 1. Uneaten feed residue and feces were collected by siphoning after 2 h of feeding. Approximately 50% of the water was exchanged daily. Water temperature was controlled using the temperature control system (ZKH-WK 2000, Zhongkehai, Qingdao, China) to an accuracy of ±0.5℃. Dissolved oxygen was $>6.0\ mg\ L^{-1}$ and the photoperiod was 12 h:12 h (L/D).

The experiment was conducted after the conditioning phase. Water temperature was reduced from 16℃ to 12℃, 8℃, 6℃, 4℃, 2℃ and 1℃ gradually at a rate of $0.5℃\ h^{-1}$ and maintained for one week at each selected temperatures for temperature acclimation. According to Hsieh *et al*. (2003) and Donaldson *et al*. (2008), fish can adapt to cold shock within one week. The fish were fed daily, but the feed intake was reduced when water temperature was below 4℃, and all fish stopped eating at 1℃. At each designated temperature, 3 fish individuals were collected each tank and euthanized with MS-222 ($70\ mg\ L^{-1}$). The muscle, spleen, brain and heart were collected from each fish and frozen with liquid nitrogen. These samples were stored at −80℃ in an ultra-low temperature freezer (New Brunswick Scientific, Edison, New Jersey, USA).

Table 1 Fatty acid composition of the diet

Saturated fatty acid content (%)		Monounsaturated fatty acid content (%)		Polyunsaturated fatty acid content (%)	
12:0	0.16	14:1n5	0.11	18:2n6	32.70
13:0	0.13	16:1n7	2.48	18:3n6	3.61
14:0	2.46	17:1n7	0.16	18:3n3	0.11
15:0	0.29	18:1n9	20.21	18:2n6	4.06
16:0	15.99	20:1n9	0.15	20:3n3	0.11
17:0	0.50	22:1n9	0.12	20:4n6	0.45
18:0	4.47	24:1n9	0.54	20:5n3	3.83
20:0	0.57	Subtotal	23.77	22:6n3	6.03
21:0	0.11			Subtotal	50.89
22:0	0.47				
24:0	0.19				
Subtotal	25.35				

Note: Values are means of four replicates. Other FAs found irregularly in trace amounts (<0.1%) include 22:2n9 and 22:5n6.

2.2 Lipid Extraction and Fatty Acid Analysis

Tissue samples of 3 fish individuals each tank, 4 tanks each temperature, were analyzed. In total, 0.1 g of tissue was homogenization with lipid extracted with chloroform/methanol (2:1) containing 0.01% butylated hydroxytoluene (BHT) as an antioxidant following the method of Folch *et al*. (1957). After separation of the chloroform and methanol layers, the chloroform layer was dried to a constant weight under a stream of nitrogen. Phospholipid was separated by one-dimensional thin-layer silica gel HSG (100 × 50 mm) (Yinlong Company, Yantai, China)

plates with N-hexane/ether/acetic acid (84:15:1) as the developing solvent according to Hazel (1979). Fatty acid methyl esters (FAMEs) were obtained through esterification with 2 mL methyl esterification reagent (hydrochloric acid-methanol) for 3 h at 90℃ as described by Fadhlaoui and Couture (2016). The upper phase was dried under nitrogen, and suspended in hexane.

FAMEs were quantified by injecting 1 μL of the sample into a gas chromatography (GC-2010 plus, Shimadzu, Kyoto, Japan) equipped with a flame ionization detector instrument (FID) (GC-2010, Shimadzu, Kyoto, Japan) and an RTX-WAX fused silica capillary column (30 m long × 0.25 mm internal diameter × 0.25 μm thickness, Phenomenex, Torrance, California, USA). The gradient temperature program was: 60℃ for 1.0 min, at a rate of 10℃ min^{-1} to 190℃, 2.0℃ min^{-1} to 260℃, and finally 260℃ for 0.6 min. FAMEs identification and quantification were performed through comparison of retention times (identification) and peak areas (quantification) with a calibration solution, 37-FAME Mix (Supelco, Bellefonte, Pennsylvania, USA).

2.3 Statistical Analysis

All data were analyzed using one-way analysis of variance (ANOVA) to determine if differences ($P<0.05$) existed among different treatments. The mean separation procedure was obtained by the Student-Newman-Keuls (SNK)'s multiple range test. The SAS statistical software version 9.4 (SAS Institute Inc., Cary, North Carolina, USA) was used for statistical analyses.

To facilitate comparisons of fatty acid composition, we used the unsaturation index (*UI*) and the unsaturated to saturated fatty acids ratio (*U*/*S*) as reported by Snyder and Hennessey (2003) and Wallaert and Babin (1994). The *UI* and *U*/*S* algorithms were as follows:

$$UI = \sum(\%\text{Monoenes} + 2\times\%\text{Dienes} + \%\text{Trienes}\cdots)/100\,,$$

$$U/S = \sum(\%UFA)/\sum(\%SFA)\,,$$

where monoenes, dienes, trienes among other are fatty acids containing 1, 2, 3, and more double bonds, respectively; % means weight percentage; *UFA* means unsaturated fatty acids; *SFA* means saturated fatty acids.

3 Results

3.1 The Fatty Acid Composition of Phospholipid in Different Tissues at 16℃

The fatty acid composition of phospholipid in different tissues of steelhead trout at normal temperature (16℃) are presented in Tables 2–5. A total of 15–20 fatty acid species of phospholipid were found in all four tissues, including 3–6 saturated fatty acids (*SFA*), 5–7 monounsaturated fatty acids (*MUFA*) and 7–8 polyunsaturated fatty acids (*PUFA*). In the four tissues examined, the highest proportions of *SFA*, *MUFA*, and *PUFA* were 16:0 (palmitic acid), 18:1n9 (oleic acid) and 22:6n3 (DHA), respectively. Moreover, the sum of palmitic acid and DHA accounted for over 50% of the phospholipid in the heart, muscle and spleen. The fatty acid composition of phospholipid in muscle and heart were similar. The brain

Table 2 Fatty acid composition (%) of muscle phospholipid of Steelhead trout acclimated to different temperatures

Fatty acid		Temperature (℃)							*P*-value	*PSE*
		16	12	8	6	4	2	1		
Saturated fatty acids content (%)	14:0	1.38^{ab}	1.47^{a}	1.23^{bc}	1.27^{bc}	1.22^{bc}	1.20^{bc}	1.15^{bc}	0.0030	0.0514
	16:0	26.89^{a}	25.79^{b}	25.02^{bc}	24.99^{bc}	24.04^{c}	22.41^{d}	22.53	<0.0001	0.2962
	18:0	10.66^{a}	9.50^{b}	9.46^{b}	8.69^{c}	8.44^{d}	7.18^{e}	7.06	<0.0001	0.1667
	∑*SFA*	38.93^{a}	36.76^{b}	35.71^{c}	34.95^{c}	33.70^{d}	30.79^{e}	30.74^{e}	<0.0001	0.3169
Monounsaturated fatty acids content (%)	16:1n7	1.66^{ab}	1.32^{b}	1.62^{ab}	1.73^{a}	1.46^{ab}	1.57^{ab}	1.53^{ab}	00.0293	0.0803
	18:1n9	7.00^{c}	7.27^{bc}	7.32^{ab}	7.49^{ab}	7.69^{a}	7.55^{ab}	7.28^{bc}	00.0007	0.1299
	20:1n9	0.60^{c}	0.75^{ab}	0.79^{a}	0.79^{a}	0.70^{b}	0.69^{b}	0.58^{c}	<0.0001	0.0227
	24:1n9	1.17	1.43	1.13	1.10	1.43	1.56	1.36	0.1253	0.1013
	∑*MUFA*	10.43^{c}	10.77^{bc}	10.87^{abc}	11.11^{ab}	11.28^{a}	11.37^{ab}	10.76^{bc}	0.0022	0.1796
Polyunsaturated fatty acids content (%)	18:2n6	5.97^{d}	6.89^{c}	7.37^{bc}	7.86^{ab}	7.83^{ab}	8.18^{ab}	8.36^{a}	<0.0001	0.2284
	18:3n3	0.66^{b}	0.77^{ab}	0.82^{a}	0.87^{a}	0.87^{a}	0.91^{a}	0.93^{a}	0.0024	0.0414
	20:2n6	1.58^{a}	1.67^{a}	1.61^{a}	1.69^{a}	1.18^{b}	0.97^{bc}	0.84^{c}	<0.0001	0.0898
	20:3n3	1.21^{e}	1.41^{de}	1.76^{cd}	2.06^{bc}	2.36^{ab}	2.67^{a}	2.41^{ab}	<0.0001	0.1231
	20:4n6	2.22	2.33	2.20	2.21	2.11	2.45	2.46	0.0539	0.0874
	20:5n3	5.86^{b}	6.00^{ab}	6.14^{ab}	6.17^{ab}	6.21^{ab}	6.45^{ab}	6.77^{a}	0.0438	0.1834
	22:2n9	2.31	2.28	2.38	2.21	2.35	2.58	2.71	0.3675	0.1657
	22:6n3	30.75^{d}	31.06^{d}	31.36^{cd}	31.41^{cd}	32.45^{bc}	33.37^{ab}	33.71^{a}	<0.0001	0.3378
	∑*PUFA*	50.55^{d}	52.40^{c}	53.64^{bc}	54.48^{b}	55.36^{b}	57.58^{a}	58.21^{a}	<0.0001	0.5001
	U/*S*	1.57^{e}	1.72^{d}	1.81^{c}	1.88^{c}	1.98^{b}	2.24^{a}	2.24^{a}	<0.0001	0.0229
	UI	2.58^{d}	2.65^{cd}	2.69^{c}	2.72^{bc}	2.78^{b}	2.88^{a}	2.91^{a}	<0.0001	0.0299

Notes: Values are means of four replicates. Significant differences in each row were indicated by superscript letters by the Student-Newman-Keuls multiple comparison ($P<0.05$). Other FAs found irregularly in trace amounts (<0.1%) include 15:0, 20:0, 21:0, 17:1n7, 18:3n6, and 22:5n6. *PSE*, pooled standard error; *SFA*, saturated fatty acids; *MUFA*, monounsaturated fatty acids; *PUFA*, polyunsaturated fatty acids; *U*/*S*, the unsaturated to saturated fatty acids ratio; *UI*, unsaturation index.

Table 3 Fatty acid composition (%) of heart phospholipid in Steelhead trout acclimated to different temperatures

Fatty acid		Temperature (℃)							*P*-value	*PSE*
		16	12	8	6	4	2	1		
Saturated fatty acids content (%)	14:0	1.24^{a}	1.16^{ab}	1.08^{b}	1.05^{b}	0.80^{c}	0.85^{c}	0.77^{c}	<0.0001	0.0354
	16:0	28.26^{a}	27.60^{ab}	25.33^{bc}	23.79^{c}	21.48^{d}	20.66^{d}	19.63^{d}	<0.0001	0.7104
	17:0	0.64^{a}	0.65^{a}	0.55^{b}	0.53^{b}	0.47^{b}	0.45^{b}	0.48^{b}	<0.0001	0.0247
	18:0	9.45^{a}	8.61^{ab}	7.53^{b}	6.64^{c}	6.06^{cd}	5.88^{cd}	5.37^{d}	<0.0001	0.3006
	20:0	0.67^{a}	0.40^{b}	0.39^{b}	0.52^{b}	0.43^{b}	0.45^{b}	0.50^{b}	0.0098	0.0491
	24:0	0.98	0.99	1.06	1.05	1.10	1.08	1.07	0.4266	0.0378
	∑*SFA*	41.25^{a}	39.40^{a}	35.94^{b}	33.59^{c}	30.35^{d}	29.37^{d}	27.81^{d}	<0.0001	0.7875
Monounsaturated fatty acids content (%)	15:1n5	1.35^{a}	1.27^{ab}	1.15^{abc}	1.04^{abc}	0.94^{bc}	0.87^{c}	1.02^{abc}	0.0062	0.0837
	16:1n7	1.04	1.23	1.06	1.03	1.02	0.98	0.99	0.2779	0.0727
	18:1n9	6.09^{d}	7.10^{c}	7.67^{bc}	8.11^{ab}	8.75^{a}	9.03^{a}	8.89^{a}	<.0001	0.2699
	24:1n9	1.45^{a}	1.26^{ab}	1.11^{b}	1.30^{ab}	1.31^{ab}	1.21^{ab}	1.30^{ab}	0.0170	0.0573
	∑*MUFA*	9.93^{b}	10.87^{ab}	10.98^{ab}	11.48^{a}	12.02^{a}	12.10^{a}	12.20^{a}	0.0006	0.3259
Polyunsaturated fatty acids content (%)	18:2n6	6.15b^{c}	5.73^{c}	6.61^{ab}	6.71^{ab}	6.77^{ab}	7.13^{a}	6.80^{ab}	0.0029	0.2114
	18:3n3	0.68b^{c}	0.64^{c}	0.75^{ab}	0.80^{a}	0.79^{a}	0.81^{a}	0.84^{a}	<0.0001	0.0248
	20:2n6	0.68	0.49	0.49	0.53	0.61	0.56	0.48	0.1760	0.0566
	20:3n3	0.73^{bc}	0.62^{c}	0.75^{bc}	0.79^{bc}	1.08^{a}	0.96^{ab}	1.23^{a}	0.0002	0.0793
	20:4n6	2.86^{c}	2.90^{c}	3.08^{bc}	3.22^{abc}	3.46^{abc}	3.58^{ab}	3.79^{a}	0.0023	0.1562
	20:5n3	3.60^{c}	4.17^{bc}	4.38^{abc}	4.89^{ab}	5.17^{a}	5.37^{a}	5.41^{a}	0.0002	0.2495
	22:2n6	1.20^{a}	0.98^{ab}	0.76^{b}	0.58^{ba}	0.82^{ab}	0.28^{c}	0.59^{bc}	0.0001	0.1061
	22:6n3	32.54^{d}	34.07^{cd}	36.16^{bc}	37.39^{bc}	38.85^{b}	39.78^{ab}	40.44^{a}	<0.0001	0.8045
	∑*PUFA*	48.44^{d}	49.59^{d}	52.98^{c}	54.91^{c}	57.56^{b}	58.46^{b}	59.58^{a}	<0.0001	0.8176
	U/S	1.42^{d}	1.53^{d}	1.78^{c}	1.98^{bc}	2.29^{b}	2.40^{b}	2.58^{a}	<0.0001	0.0473
	UI	2.55^{e}	2.66^{e}	2.82^{d}	2.94^{c}	3.07^{b}	3.13^{b}	3.19^{a}	<0.0001	0.0571

Notes: Same as those of Table 2 but other FAs found irregularly in trace amounts (< 0.1%) include 12:0, 15:0, 21:0, 17:1n7, 18:3n6, and 22:5n6.

Table 4 Fatty acid composition (%) of brain phospholipids in Steelhead trout acclimated to different temperatures

Fatty acid		Temperature (℃)							*P*-value	*PSE*
		16	12	8	6	4	2	1		
Saturated fatty acids content (%)	14:0	0.31	0.32	0.33	0.28	0.28	0.29	0.29	0.2775	0.0161
	16:0	20.13^{a}	20.07^{a}	20.12^{a}	19.07^{b}	18.26^{c}	17.32^{d}	16.11^{e}	<0.0001	0.1773
	18:0	8.49^{a}	8.56^{a}	8.12^{ab}	7.99^{b}	7.24^{c}	6.80^{d}	6.16^{e}	<0.0001	0.1293
	20:0	0.10	0.11	0.10	0.12	0.10	0.11	0.10	0.0945	0.0039
	21:0	0.17	0.18	0.18	0.19	0.19	0.22	0.16	0.1555	0.0141
	22:0	0.20	0.19	0.21	0.19	0.16	0.17	0.18	0.2902	0.0147
	24:0	0.42	0.42	0.42	0.41	0.40	0.44	0.43	0.9732	0.0301
	∑*SFA*	29.81^{a}	29.85^{a}	29.48^{a}	28.25^{b}	26.63^{c}	25.15^{d}	23.43^{e}	<0.0001	0.1888
Monounsaturated fatty acids content (%)	16:1n7	1.04	1.03	0.97	1.02	1.07	1.09	1.17	0.1092	0.1509
	17:1n7	0.41	0.40	0.44	0.37	0.39	0.39	0.38	0.3692	0.0217
	18:1n9	25.1^{d}	25.12^{d}	25.09^{d}	25.35^{d}	26.12^{c}	26.88^{b}	27.47^{a}	<0.0001	0.1188
	20:1n9	1.40^{b}	1.44^{ab}	1.46^{ab}	1.52^{ab}	1.59^{ab}	1.63^{ab}	1.73^{b}	0.0330	0.0696
	22:1n9	0.46	0.44	0.42	0.48	0.48	0.49	0.48	0.9714	0.0549
	24:1n9	6.57	6.52	6.68	6.90	6.69	6.87	6.77	0.9776	0.3325
	∑*MUFA*	34.98^{c}	34.95^{c}	35.06^{c}	35.63^{c}	36.35^{bc}	37.35^{ab}	38.00^{a}	<0.0001	0.3400
Polyunsaturated fatty acids content (%)	18:2n6	0.78	0.78	0.78	0.82	0.92	0.92	1.00	0.0615	0.0563
	20:2n6	0.27	0.28	0.29	0.29	0.30	0.32	0.31	0.2637	0.0144
	20:3n3	0.24^{c}	0.24^{c}	0.24^{c}	0.25^{c}	0.28^{bc}	0.34^{a}	0.32^{ab}	0.0001	0.0147
	20:4n6	25.1^{d}	25.12^{d}	25.09^{d}	25.35^{d}	26.12^{c}	26.88^{b}	27.47^{a}	<0.0001	0.0473
	20:5n3	4.20^{d}	4.21^{d}	4.18^{d}	4.44^{cd}	4.72^{c}	5.03^{b}	5.37^{a}	<0.0001	0.1031
	22:6n3	28.01^{d}	28.06^{d}	28.06^{d}	28.29^{cd}	28.56^{c}	29.01^{b}	29.40^{a}	<0.0001	0.1062
	∑*PUFA*	34.68^{d}	34.75^{d}	34.75^{d}	35.25^{d}	36.08^{c}	37.08^{b}	38.02^{ac}	<0.0001	0.1957
	U/S	2.34^{e}	2.34^{e}	2.37^{e}	2.51^{d}	2.72^{c}	2.96^{b}	3.24^{a}	<0.0001	0.0253
	UI	2.32^{e}	2.32^{e}	2.32^{e}	2.35^{d}	2.40^{c}	2.46^{b}	2.51^{a}	<0.0001	0.0077

Notes: Same as those of Table 2 but other FAs found irregularly in trace amounts (< 0.1%) include 12:0, 15:0, 17:0, 18:3n3, 18:3n6, and 22:5n6.

Table 5 Fatty acid composition (%) of spleen phospholipids from Steelhead trout acclimated to different temperatures

Fatty acid		Temperature (℃)							*P* value	*PSE*
		16	12	8	6	4	2	1		
Saturated fatty acids content (%)	14:0	1.27^{a}	1.31^{a}	1.25^{a}	0.89^{b}	0.83^{b}	0.71^{c}	0.80^{b}	<0.0001	0.0302
	16:0	29.71^{a}	27.44^{b}	25.25^{c}	24.92^{c}	23.46^{d}	23.58^{d}	23.44^{d}	<0.0001	0.3517
	17:0	1.02^{a}	0.86^{b}	0.77^{c}	0.58^{d}	0.56^{d}	0.51^{d}	0.53^{d}	<0.0001	0.0188
	18:0	10.55^{a}	8.23^{bc}	7.39^{bc}	7.14^{c}	8.44^{b}	7.91^{bc}	7.76^{bc}	<0.0001	0.2768
	∑*SFA*	42.54^{a}	37.84^{b}	34.66^{c}	33.53^{cd}	33.30^{cd}	32.72^{d}	32.54^{d}	<0.0001	0.3988
Monounsaturated fatty acids content (%)	16:1n7	1.25^{b}	1.54^{a}	1.4^{ab}	1.33^{ab}	1.34^{ab}	1.45^{ab}	1.41^{ab}	0.0590	0.0587
	18:1n9	12.21^{b}	13.37^{a}	14.26^{a}	13.96^{a}	13.90^{a}	13.96^{a}	13.98^{a}	0.0027	0.3115
	20:1n9	0.69^{b}	0.77^{a}	0.47^{d}	0.54^{c}	0.52^{c}	0.52^{c}	0.52^{c}	<0.0001	0.0074
	24:1n9	2.80^{a}	2.12^{b}	2.12^{b}	2.05^{b}	1.94^{b}	1.69^{b}	1.70^{b}	0.0004	0.1415
	∑*MUFA*	16.95	17.80	18.25	17.89	17.71	17.62	17.61	0.3018	0.2170
Polyunsaturated fatty acids content (%)	18:2n6	4.18^{c}	5.40^{b}	5.84^{b}	6.44^{a}	6.88^{a}	6.73^{a}	6.83^{a}	<0.0001	0.1587
	18:3n3	0.46^{d}	0.60^{c}	0.70^{b}	0.72^{b}	0.76^{b}	0.85^{a}	0.84^{a}	<0.0001	0.0175
	20:2n6	0.76^{f}	1.04^{a}	0.80^{e}	0.87^{d}	1.06^{a}	0.91^{c}	0.99^{b}	<0.0001	0.0103
	20:3n3	0.51^{f}	0.59^{e}	0.68^{d}	0.85^{c}	1.42^{a}	1.18^{b}	1.23^{b}	<0.0001	0.0248
	20:4n6	4.90^{a}	4.28^{a}	4.50^{a}	4.99^{a}	4.85^{a}	4.94^{a}	4.89^{a}	0.0836	0.1801
	20:5n3	3.17^{d}	3.37^{d}	3.6^{cd}	3.83^{bc}	4.26a^{b}	4.32^{a}	4.23^{ab}	<0.0001	0.1208
	22:6n3	26.68^{c}	28.67^{b}	30.24^{a}	30.18^{a}	29.44^{ab}	30.32^{a}	30.20^{a}	<0.0001	0.3566
	∑*PUFA*	40.67^{d}	43.95^{c}	46.35^{b}	47.88^{b}	48.66^{b}	49.25^{a}	49.20^{a}	<0.0001	0.2615
	UI	2.25^{d}	2.40^{c}	2.53^{b}	2.57^{ab}	2.57^{ab}	2.62^{a}	2.61^{a}	<0.0001	0.0150
	U/S	1.35^{d}	1.63^{c}	1.86^{b}	1.96^{a}	1.99^{a}	2.04^{a}	2.05^{a}	<0.0001	0.0234

Notes: Same as those of Table 2 but other FAs found irregularly in trace amounts (< 0.1%) include 15:0, 20:0, 21:0, 17:1n7, 18:3n6, and 22:5n6.

phospholipid content was more variable than other tissues. The levels of 18:1n9 in spleen and brain were higher than those in heart and muscle, and brain had the highest level among the four tissues.

The highest *U*/*S* was found in brain (2.32), followed by that in muscle (1.57), heart (1.42), and spleen (1.35). The highest *UI* was found in muscle (2.58), followed by that the heart (2.55), brain (2.32), and spleen (2.25).

3.2 The Fatty Acid Composition of Phospholipid at Declined Temperatures

The fatty acid composition of phospholipid from muscle, spleen, heart and brain of fish acclimated at different temperatures is presented in Tables 2–5. Two different change patterns of the fatty acid profile of phospholipid in all tissues in response to temperature decline were as follows: 1) a decline in the level of *SFA* due primarily to the loss of the concentration of 16:0 and stearate (18:0); and 2) an increase in the concentration of *UFA*. For all tissues, there was a little increase in diene content (due to an increased proportion of 18:2n6), a significant increase of monoenes (major change in 18:1n9), and a much more significant increase in polyunsaturated fatty acid (due primarily to an increased proportion of 20:4n6, 20:5n3, and 22:6n3). Consequently, with the continuous decrease in temperature, the *U*/*S* increased from 1.57 to 2.38 in the muscle, from 1.35 to 2.05 in the spleen, from 1.42 to 2.58 in the heart, and from 2.32 to 2.51 in the brain. There was also an increase in *UI* among all of the tissues examined.

To better understand the change in the fatty acid composition of phospholipid with the decrease of temperature in different tissues, six line charts for *UI*, *U*/*S*, *UFA*, *SFA*, *MUFA*, and *PUFA* are presented (Fig.1). At the early stages of the experiment when the water temperature reduced from 16℃ to 8℃, the composition of phospholipid fatty acids in the muscle, heart, and spleen changed significantly as a result of acclimating to the lower temperature. However, no alteration was found in brain phospholipid. When water temperature was reduced to 6℃, the *PUFA* and *MUFA* of phospholipids in the brain showed an obvious increase as in the muscle and heart. However, the *UI* and *U*/*S* in spleen phospholipid were relatively stable. At the end of the experiment, when the water temperature decreased from 4℃ to 1℃, the *PUFA* and *MUFA* levels in phospholipid continued to increase in the brain and heart, while *MUFA*, especially 18:1n9, in the muscle significantly decreased. No significant change of fatty acid composition in spleen phospholipid was found in this stage. In addition, only *PUFA* and *MUFA* levels in the heart phospholipid consistently increased throughout the experiment.

4 Discussion

The primary constituents of biological membrane are phospholipid and protein (Fadhlaoui and Couture, 2016). These two macromolecules associate each other through weak electrostatic and hydrophobic interactions (Yeagle, 1989; Schregel, 2013). Phospholipid in cellular membrane displays complex phase behaviors and physical properties. The difference of fatty acid composition of cell membrane can affect membrane-associated physical

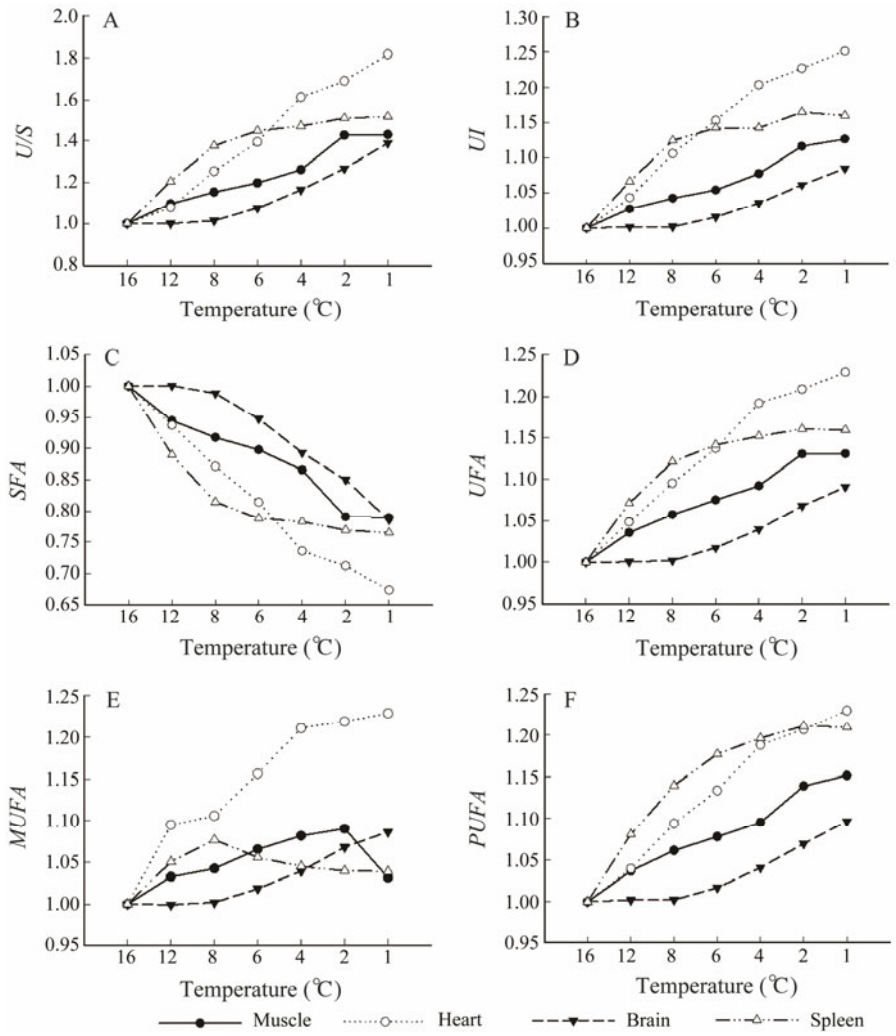

Fig.1 The tendencies of *UI*, *U*/*S*, *SFA*, *PUFA* and *MUFA* of tissue phospholipid fatty acids of Steelhead trout acclimated at different temperatures. Each value is the ratio between treatments and the normal temperature (16℃). *SFA*, saturated fatty acids; *UI*, unsaturation index; *U*/*S*, the unsaturated to saturated fatty acids ratio; *PUFA*, polyunsaturated fatty acids; *MUFA*, monounsaturated fatty acids.

attributes and biological functions, such as membrane fluidity, membrane phase behaviors, membrane thickness, membrane permeability, and membrane related enzymes (Bell *et al*., 2008; Tocher and Glencross, 2015). Thus, the difference in membrane phospholipid fatty acid composition of, for example, insect (Kostal and Simek, 1998), fish (Snyder and Hennessey, 2003) and mamma (Pettegrew *et al*., 2015), leads to the difference in the physiological and biochemical functions of animal tissues.

In the present study, 16:0 and 22:6n3 were the dominant fatty acids in phospholipid of all tissues at 16℃ (Tables 2–5). The 16:0, the main fatty acid synthesized *de novo*, is enriched in both freshwater and seawater fish species (Li *et al*., 2011). Fish membrane phospholipid preferentially store n-3 *PUFA* (such as DHA and EPA) at the *sn*-2 position (the distribution of fatty acids in phospholipid), which can easily be derived from food (Ng *et al*., 2015). Therefore, DHA is found in large quantities in all tissues. Moreover, compared with freshwater fish, marine fish have significantly higher DHA and EPA concentrations, especially in cold-water fish (Osman and Suriah, 2001; Stoknes *et al*., 2004; Snyder *et al*., 2012).

Our findings showed that the tissue patterns of phospholipid were more variable. The level of oleic acid (18:1n9) was the highest in brain phospholipid (Table 4). However, lower levels of *PUFA* and relative higher levels of *SFA* were found in spleen phospholipid (Table 5). Brain tissue has been shown to be rich in phospholipid. Bell and Tocher (1989) reported that the predominant molecular species of fatty acids in brain phospholipid was 16:0-22:6, but the level of 18:1-22:6 was higher, especially in phosphatidylethanolamine and phosphatidylcholine (two main phospholipids in brain). Moreover, the profiles of brain phospholipid fatty acids were also similar to those shown by Buda *et al*. (1994) in a study of carp (*Cyprinus carpio*). For the spleen phospholipid, Zhang *et al*. (2010) found that *PUFA* might have an adverse effect on macrophage aggregation, which in turn affected the immune system of the pompano (*Trachinotus ovatus*). In this case, the content of *SFA* in spleen was higher than those in muscle and liver.

In the current study, the levels of *U*/*S* and *UI* of phospholipid in all tissues of steelhead trout increased with the decrease of temperature, due to a decrease in the per-

centage of *SFA* (16:0 and 18:0) and a concomitant increase in both *MUFA* and *PUFA* (major 18:1n9, 20:4n6, 20:5n3 and 22:6n3). Low temperature acclimation can alter the phospholipid fatty acid composition of fish tissues, such as an increase in *PUFA* in *sn*-2 (Hazel, 1979; Wallaert and Babin, 1994; Fokina *et al*., 2015; Ng *et al*., 2015), and an increase in *MUFA* replacing *SFA* in *sn*-1 (Dey *et al*., 1993; Farkas *et al*., 2001). Compared with *SFA*, homologous *UFA* has a lower melting point and occupies a larger space in the bilayer membrane structure. Thus *UFA* accumulation can enhance the fluidity and stability of the bilayer lipid membrane (Hazel, 1979; Wijekoon, 2011; Fadhlaoui and Couture, 2016). Such adaptation in membrane lipids minimizes the stress caused by cold temperature on fish (Hazel and Williams, 1990; Farkas *et al*., 2001; Jobling and Bendiksen, 2015). Low temperatures might induce the reconstruction of poikilotherms phospholipid, which can enhance biomembrane fluidity by increasing unsaturated fatty acids concentration, in a process referred to as 'homeoviscous adaptation' (Sinensky, 1974). Our data indicated that increasing membrane *UFA* in steelhead trout ensures the existence of a liquid-crystalline phase of appropriate fluidity of biofilm to better acclimate reduction in temperature.

Although phospholipids in all tissues became more unsaturated with the declined temperature, the changes of rates or in specific tissues showed remarkable differences (Fig.1). At early stages of the experiment, the compositions of phospholipid fatty acids in the muscle, heart, and spleen significantly changed, especially in spleen. However, the change in brain phospholipid was unremarkable. The muscle and heart (blood circulation system) are sensitive to ambient environment fluctuations, and are able to make appropriate responses quickly (Ingemansson *et al*., 1993; Aho and Vornanen, 2001; Hong *et al*., 2014). Indeed, a few hours in a cold environment was sufficient to increase the expression of the *PUFA* synthase-related genes in muscle (Wijekoon, 2011). Aho and Vornanen (2001) showed that 96 h of low temperature exposure can significantly increase the basic heart rate (BHR) in rainbow trout. In addition, the spleen is essential for teleost with the functions of hematopoiesis, blood storage, and immunity (Fänge and Nilsson, 1985; Zhang *et al*., 2010). It has been well known that extreme environmental temperature change can affect the spleen functions in Atlantic salmon and other salmonids (Fänge and Nilsson, 1985). In the present study, the content of red blood cells (RBC) and platelets (PLT) in the steelhead trout were significantly increased when the temperature was decreased from 16℃ to 8℃ (unpublished data). The increase of RBC and PLT can counteract the adverse effects of low temperature. Change in spleen phospholipid occurs at early stages. One explanation for this finding is that spleen is a vital tissue for hematopoietic and immune functions.

When the temperature decreased from 6℃ to 4℃, the change rates of phospholipid fatty acids in the heart were still high, but they were not very high in the muscle and spleen (Fig.1 and Tables 2, 3, 5). In addition, the unsaturated fatty acids in the brain phospholipid significantly increased (Table 4). At this stage, phospholipids in the four tissues examined were likely to be affected by cold shock, which altered more unsaturated fatty acids. Several investigators have confirmed that the optimum growth temperature for salmon (Rainbow trout, Steelhead trout and Atlantic salmon *etc*.) ranges from 8℃ to 18℃ (Sigholt and Finstad, 1990; Biro *et al*., 2004; Wijekoon, 2011). When the ambient temperature is below a suitable temperature, fish tissues will produce a comprehensive physiological response under central nervous system (CNS) control (Donaldson *et al*., 2008).

When the temperature decreased below 4℃, fatty acid composition of spleen phospholipid became stable. In muscle phospholipid, the level of *PUFA* continued to increase, while *MUFA* (18:1n9) significantly decreased. Moreover, the phospholipids of the brain and heart became more unsaturated after the temperature decreased to 4℃, which was caused by the increases of *MUFA* and *PUFA* levels. An increase in the *UFA* content indicates a reduction in the anti-oxidant capacity of membrane phospholipid. Regardless of *MUFA* or *PUFA*, the higher proportion of UFAs in membrane phospholipid, the more susceptible to reactive oxygen species (ROS) attack, which can compromise membrane fluidity and functions (Bell *et al*., 2003; Ye *et al*., 2016). In general, oxidation of C18 *MUFA* over C20 *PUFA*, and n-6 *PUFA* over n-3 *PUFA* occurs (Crockett, 2008). When water temperature decreases below the minimum tolerable for fish, food intake reduces and even stops. Consequently, excessive ROS will be produced under cold and starvation stresses (Cengiz *et al*., 2017). For the brain and heart, the most important organs in fish, vitamin E is present in high abundance in their biomembrane, which could protect unsaturated fatty acids from ROC (Pettegrew *et al*., 2015; Cengiz *et al*., 2016). It is interesting to note that steelhead trout reduced feed intake when temperature was below 4℃, and stopped feeding at 1℃. Moreover, the condition factor (CF) of steelhead trout was significantly decreased when the water temperature was below 2℃ (CF: 1.17±0.06, 16℃; 1.09±0.04, 2℃; 1.07±0.03, 1℃). Therefore, changes in phospholipid fatty acids in the muscle and the spleen may be caused by both cold and starvation, while the brain and heart seem to be merely influenced by cold stress. Similarly, Stubhaug *et al*. (2007) found that Atlantic salmon (*S. salar*) was more likely to sacrifice *MUFA*, as opposed to *HUFA* (such as DHA and EPA), to avoid being oxidized under food deprivation. Moreover, Xu *et al*. (2015) reported that the expression of *scd 1* which plays a role in producing *MUFA* declines in muscle and increases in the brain when large yellow croaker (*Larimichthys crocea*) was under cold stress and starvation. Skuladottir *et al*. (1990) documented that the *U*/*S* and *PUFA* decrease in muscle and slightly increase in the heart when Atlantic salmon (*S. salar*) was at extremely low environmental temperature.

5 Conclusion

In the present study, the concentrations of unsaturated

fatty acids in phospholipid of four tissues of steelhead trout increased along with the decrease of environmental temperature which also caused increases of *U*/*S* and *UI*. These findings indicated that steelhead trout can compensate for temperature-induced change in membrane fluidity by remodeling the fatty acid composition of its phospholipid. Additionally, change of the fatty acid composition of phospholipid was tissue-specific. Muscle, heart and spleen respond immediately to temperature change whereas brain does so late. A comprehensive response was found in four tissues when the ambient temperature decreased to below 8℃. The compensatory restructuring of the fish phospholipid composition may be affected by both cold stress and starvation when the temperature was below 2℃, which is very obvious in muscle.

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