Effects of Constant and Diel Cyclic Temperatures on The Liver and Intestinal Phospholipid Fatty Acid Composition in Rainbow Trout *Oncorhynchus Mykiss* During Seawater Acclimation

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Abstract

Background: Rainbow trout is an important economic fish in aquaculture and a model species in environmental physiology. Despite of earlier research on the seawater adaptability of rainbow trout at different temperature regimes, the influence on liver and intestine in this species is still unknown. Two trials were conducted to investigate the effects of constant and diel cyclic temperatures on phospholipid fatty acid (PLFA) composition in the liver and intestine of rainbow trout during seawater acclimation.

Results: At the end of the growth trial 1, fish at 9 and 12.5°C showed significantly higher ratios of unsaturated to saturated (U/S) and unsaturation index (UI) than those at 16°C in liver and intestine phospholipids. After day 1 of seawater acclimation, the U/S, UI, and average chain length (ACL) of liver and intestine phospholipids in fish at 16°C significantly increased. Two weeks after seawater acclimation, liver and intestinal PLFA composition adapted to salinity change. In trial 2, significantly higher U/S, UI, and ACL were found in intestinal phospholipids at 13±2°C. On the first day after seawater acclimation, UI and ACL in liver phospholipids significantly increased in 13°C, while fish in 13±2°C showed significantly decreased U/S, UI, and ACL in the intestine. At the end of the growth trial 2, liver PLFA compositions were stable, whereas intestinal PLFA in 13 and 13±1°C showed significantly decreased U/S, UI, and ACL. A two-way analysis of variance and principal component analysis revealed significant effects of different constant temperatures, seawater acclimation, and their interaction on the liver and intestinal phospholipids, a significant effect of diel cyclic temperature on intestinal phospholipids, and the effects of seawater acclimation and its interaction with diel cyclic temperature on liver phospholipids.

Conclusion: Temperatures of 9 and 12.5°C could elevate membrane fluidity and thickness in the liver and intestine of rainbow trout in freshwater, whereas no significant effects were found with diel temperature variations. After seawater acclimation, constant and diel cyclic temperatures significantly influenced the liver and intestine's membrane fluidity and thickness. Compared with constant temperature, diel temperature variation (13±2°C) can enhance the adaptability of rainbow trout during seawater acclimation.

Background

Salmonids are one of the most important economically farmed species in the world. The global production of Atlantic salmon (*Salmo salar*) in 2018 exceeded 2.4 million tons, while that of rainbow trout (*Oncorhynchus mykiss*) reached 0.8 million tons [1]. The surge in demand for salmonids in China is mainly driven by consumers' preference for its tasty flesh and abundance of essential amino acids and polyunsaturated fatty acids (PLFAs) [2]. The discovery of the Yellow Sea Cold Water Mass opened up the possibility of offshore salmon mariculture [3, 4] and one of the pivotal stages of this project is the 'mountain-sea relay' mode, in which salmon are hatched and bred until the juvenile stage in freshwater in mountainous areas, transferred to seawater, and fattened to commercial size. Previous research has reported that the seawater tolerance of salmonids can be influenced by life stage [5], photoperiod [6], salinity stimulation [7], nutritional status [8, 9] and temperature change [10, 11].

During adaptation to environmental changes (e.g. temperature and salinity), organisms alter the physical properties of the membrane, including fluidity, thickness, permeability, and viscosity, by restructuring the phospholipid fatty acid (PLFA) composition [12, 13]. These changes will significantly influence the biological function of the membrane [14]. Biological membranes are susceptible to changes in temperature and salinity. When the temperature decreases, fish will increase the unsaturation degree to maintain sufficient fluidity, and vice versa [15, 16]. When salinity increases, some fish increase membrane fluidity by elevating the proportion of unsaturated fatty acids [17].

Rainbow trout is a cold-water fish and can tolerate relatively high fluctuations in temperature and salinity. Environmental change induces adaptive alterations in fish tissues, and the restructuring of PLFA composition is an important step in regulating membrane function [18]. For instance, a decrease in temperature can increase membrane fluidity by increasing the degree of unsaturation in fish tissues, which has been demonstrated in alewives (*Alosa pseudoharengus*) [19], yellow perch (*Perca flavescens*) [20], steelhead trout (*O. mykiss*), and Atlantic salmon [15]. Similarly, fish living in seawater will also have a higher proportion of unsaturated fatty acids in tissue phospholipids, which can be used to maintain membrane function [21, 22].

To date, studies from our laboratory demonstrated that temperature could significantly influence the PLFA composition in salmonids [15, 16]. Our data from Ge et al. [11] reported that temperatures of 12.5 and 13 ± 2 °C are preferable for rainbow trout during seawater acclimation. However, the effects of seawater acclimation at different temperature regimes on liver and intestinal phospholipids in rainbow trout remain unknown. Thus, this study aimed to acclimate rainbow trout at different temperature regimes and analyse PLFA composition changes and seawater tolerance.

Methods

Animals

Rainbow trout were purchased from Wanzefeng Fishery Company (Rizhao, China). Fish were reared in aquariums and acclimated to an experimental environment where the water temperature was maintained at optimal levels (16.0 ± 0.5 °C) by a semi-recirculating system equipped with refrigeration and aeration facilities. Throughout the experiment, rainbow trout were fed to satiation with commercial dry feed by hand (Qihao Biotechnology Company, Qingdao, China) twice daily (0800 and 1800 hrs). All fish stocking and experimental procedures were performed in accordance with the ethical approval of the Ocean University of China and National Experimental Management Regulations in China and were approved by the Animal Experimentation Committee of the Ocean University of China (Reference Number, 2018-12). All animal studies were carried out in accordance with the ARRIVE guidelines [45]. The fatty acid composition of the feed is shown in Additional file 1.

Experimental design

In trial 1, rainbow trout with a similar initial weight (94.73 ± 9.70 g) were randomly assigned to nine tanks (186 L; 0.58 m height $\times$ 0.65 m diameter), where the water temperature was maintained at 16 ± 0.5 °C by a recirculating system equipped with refrigeration facilities. Each treatment had three replications with ten fish per tank ($n = 3$; biomass, $5.09 \text{ kg} \cdot \text{m}^{-3}$). The temperature of dechlorinated freshwater was controlled by a temperature control system (ZKH-WK 2000, Zhongkehai, Qingdao, China), fluctuating within 0.5 °C. The temperature regimes were as follows: water temperature was gradually adjusted from 16 °C (T16) to 12.5 °C (T12.5) and 9 °C (T9) at a rate of 2 °C per 12 h, and then kept constant for the rest of the experiment. The fish were subjected to temperature acclimation in freshwater for 28 days. Immediately after acclimation, the salinity of all tanks was increased to 14 ppt from 0 ppt by mixing with seawater (salinity 30 ± 1 ppt) within 60 min, and then further increased from 14 ppt to 30 ppt at a rate of 2 ppt day⁻¹, according to the method described by Xiong et al. (2020). Filtered and dechlorinated seawater was drawn from the coastal waters of the Yellow Sea. The water at the desired salinity was prepared in advance and pumped into the tanks. After that, fish were stocked for 14 days.

In trial 2, juvenile rainbow trout with a similar initial weight (62.28 ± 0.41 g) were randomly allocated to 12 tanks (186 L; 0.58 m height $\times$ 0.65 m diameter). Each treatment was replicated four times, with ten fish per tank. The temperature of dechlorinated freshwater was controlled by an AI temperature controller (AI-526P, Yudian Tech, Xiamen, China) with an accuracy of 0.05 °C. The temperature regimes were conducted as follows: water temperature was gradually adjusted from 16.0 to 13.0 °C in 12 h, after which the fish were subjected to three experimental thermal regimes. These regimes are referred to as CT, VT2, and VT4. CT mimics a laboratory scenario where the temperature is constant at 13.0 °C while VT2 and VT4 mimic a natural scenario where the temperature oscillates daily by the same magnitude. In VT2, the fluctuation in temperature is 2.0 °C, changing between 12.0 and 14.0 °C, in a sinusoid manner. VT4 represents a scenario with a larger daily magnitude of fluctuation between 11.0 and 15.0 °C, also in a sinusoid manner. The fish were subjected to temperature acclimation in freshwater for six weeks. Immediately after acclimation, the salinity of all tanks was increased to 15 ppt from 0 ppt by mixing with seawater (salinity 30 ± 1 ppt), and then further increased to 30 ppt from 15 ppt at a rate of 3 ppt per day, according to the method described by Huang et al. [8]. The water at the desired salinity was prepared in advance and pumped into the tanks. After that, fish were stocked for three weeks.

Approximately 100% of the water in each tank was manually changed daily. The water was prepared to be at the desired temperature before pumping into the tanks. Water quality [dissolved oxygen (DO), pH, nitrate, and ammonia] was monitored thrice weekly using a YSI Pro Plus Multiparameter (YSI Incorporated, Yellow Springs, Ohio, USA), and salinity was measured daily using a seawater refractometer. DO content was >8.7 mg $\cdot$ L $^{-1}$, photoperiod was 12:12-h (light/dark), pH was 7.2 ± 0.7 , ammonia level was 0.04 ± 0.02 mg $\cdot$ L $^{-1}$, and nitrate was 1.17 ± 0.33 mg $\cdot$ L $^{-1}$.

Sampling procedures

Fish were fasted for 24 h prior to sampling and euthanised using MS-222 (60 mg $\cdot$ L $^{-1}$; Sigma-Aldrich, St. Louis, MO, USA). Tissue samples were collected from each treatment on the last day in freshwater (FW-28 in trial 1 and FW-42 in trial 2), and on the first (SW-1) and final (SW-14 in trial 1 and SW-21 in trial 2) days in seawater, immediately frozen in liquid nitrogen, and stored at -80 °C after dissection.

Lipid extraction and PLFA analysis

Lipid extraction and PLFA analysis were performed according to Ge et al. [11]. Briefly, liver and intestine (0.1 g) were homogenised in chloroform/methanol (2:1, v/v). The crude extract was precipitated proteins and separated into two phases. The lower phase was then dried to a constant weight under nitrogen flow to obtain lipids. Solid lipids were diluted with 20 μ L petroleum ether (60 – 90 °C) and spotted on one-dimensional thin-layer hybrid silica gel plates (100 $\times$ 100 mm, Haiyang Company, Qingdao, China) to obtain phospholipids. The phospholipids were visualised by iodine vapour and esterified at 90 °C for 90 min to obtain fatty acid methyl esters (FAMES) [16].

The FAMES were injected into a gas chromatograph (GC-2010 plus; Shimadzu, Kyoto, Japan) equipped with an RTX-WAX fused silica capillary column (30 m $\times$ 0.25 mm $\times$ 0.25 μ m, Phenomenex, Torrance, CA, USA) and a flame ionisation detector (FID; GC-2010, Shimadzu, Kyoto, Japan). The temperature setting of the column oven started from an initial temperature of 80 °C for 1 min, then serially increased as follows: 180 °C at a rate of 8 °C min $^{-1}$ for 5 min, 220 °C at a rate of 4 °C min $^{-1}$, 224 °C at a rate of 0.5 °C min $^{-1}$, 280 °C at a rate of 4 °C min $^{-1}$ for 10 min. FAMES were classified and quantified by comparing retention times and areas of the peak using the 37-FAME Mix standards (Supelco, Bellefonte, Pennsylvania, USA).

Biometric indices

The indices of PLFA composition (FA-related indices) were calculated according to the following formulae, as previously used by Wallaert et al. [46], Snyder et al. [47] and Cornelius et al. [48]:

$$UI = \sum (\%monoene + 2 \times \%dienes + 3 \times \%trienes \dots) / 100$$

$$U/S = \sum \%UFA / \sum \%SFA$$

$$ACL = \sum (14 \times \%14C + 16 \times \%16C + 17 \times \%17C \dots) / 100$$

UI: unsaturation index; monoene, dienes, trienes: the numbers of double bonds that fatty acids contain; %: weight percentage; U/S: the ratio of unsaturated fatty acids to saturated fatty acids; UFA: unsaturated fatty acids; SFA: saturated fatty acids; ACL: average chain length of phospholipid; n-3 PUFA: omega-3 series polyunsaturated fatty acids; n-6 PUFA: omega-6 series polyunsaturated fatty acids; n3/n6: the ratio of n-3 PUFA to n-6 PUFA.

Statistical analyses

Statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, NC, USA). All data sets were tested for normality and homogeneity of variances using Levene's test and then tested using one- and two-way analysis of variance (ANOVA) to determine if significance existed among different treatments, followed by the Student–Newman–Keuls multiple range test. Principal component analysis (PCA) and data visualisation were performed using GraphPad Prism 9.0.0 (GraphPad Software Inc., San Diego, CA, USA). All data are expressed as the mean $\pm$ SD. A *P* value less than 0.05 was regarded as statistically significant.

Results

Effects of constant temperature on liver phospholipid fatty acid composition (PLFA) during seawater acclimation

In trial 1, after rearing in freshwater for four weeks (FW-28), the liver PLFA composition of rainbow trout was significantly altered by different constant temperatures (Table 1). In treatments of 9 (T9) and 12.5 °C (T12.5), the proportions of 16:0 and 18:0 significantly lowered ratio of saturated fatty acid (SFA) in liver phospholipids. The proportion of monounsaturated fatty acid (MUFA) in liver phospholipids was significantly lower in treatment of 16 °C (T16) than in T12.5, but significantly higher than in treatment T9, mainly caused by 18:1n9. The proportion of polyunsaturated fatty acids (PUFAs) in the liver phospholipids of rainbow trout was negatively correlated with temperature; the proportion of PUFAs in T9 was the highest, followed by T12.5 and T16. The ratio of unsaturated to saturated fatty acid (U/S) in T9 and T12.5 was significantly higher than in T16, while the unsaturation index (UI) in T9 was significantly higher than in T16. The ratio of n-3 PUFA to n-6 PUFA (n3/n6) in T9 and T12.5 was significantly higher than in T16 (Fig. 1A–D). In addition, the average chain length (ACL) of fatty acid in T9 was significantly higher than in the other groups.

At the first day after seawater acclimation (SW-1), the liver PLFA composition in rainbow trout was significantly altered (Table 1). The proportion of SFA in liver phospholipids significantly decreased in T16, whereas those in T9 and T12.5 increased, significantly higher than in T16. The total MUFA in T12.5 significantly decreased, while those in other groups remained unchanged. The proportion of PUFAs in T9 increased to 55.75%, close to the level in T12.5. Fig. 1 shows a significantly low U/S in T9 and a significantly high ACL in T12.5, but no significant difference in UI among groups.

Two weeks after seawater acclimation, the total SFA in T9 significantly decreased and was significantly lower than in T12.5 (Table 1). The total MUFA in T16 considerably increased and was significantly higher than in other groups. Consequently, the UI in T9 and T16 increased and was significantly higher than in T12.5. These changes resulted in significantly higher UI, U/S, and ACL in T9 (Fig. 1A–D).

Effects of constant temperature on intestinal PLFA composition during seawater acclimation

In trial 1, after rearing in freshwater for four weeks, the intestinal PLFA composition in rainbow trout was altered significantly (Table 2). The total SFA in T9 and T12.5 decreased significantly, while the total PUFAs in T9 and T12.5 were significantly higher in T16. The total MUFA in T12.5 significantly decreased and was significantly lower than in T16. These changes resulted in significantly higher U/S, UI, and ACL in T9 and T12.5 than in T16 (Fig. 1E–H). The n3/n6 ratio was positively associated with temperature.

On the first day after seawater acclimation, the total SFA in T16 significantly decreased. The total MUFA in T9 and T16 significantly decreased whereas that in T12.5 increased and was significantly higher than in other groups. The total PUFA in T9 and T16 significantly increased, while that in T12.5 was relatively steady. Consequently, significant increases in n3/n6, U/S, UI, and ACL were recorded in T16.

Two weeks after seawater acclimation (SW-14), the total SFA in T16 decreased while the other groups showed no significant difference (Table 2). The total MUFA in all groups decreased significantly. Accordingly, the total PUFA in all groups increased but showed no difference. Thus, the UI in T9 was significantly higher than in other groups, whereas no difference was found in UI and ACL in liver phospholipids in rainbow trout (Fig. 1E–H).

Effects of diel cyclic temperature on liver PLFA composition during seawater acclimation

In trial 2, at the final day of growth trial (FW-42), the proportion of 16:0 in the treatment of 13 °C (CT) was significantly decreased while that of 20:4n6 in the treatment of 13 ± 1 °C (VT2) and 13 ± 2 °C (VT4) remained steady (Table 3). However, no significant change was found in total SFA, MUFA, and PUFA among the groups. Therefore, there was no difference among groups in terms of U/S, UI, and n3/n6, except for a significantly higher ACL in VT2 (Fig. 2A–D).

At SW-1, the total SFA of liver phospholipids in CT and VT2 increased significantly, while VT4 showed no significant change (Table 3). An overall decrease in total MUFA was detected in all groups, and the CT group showed significantly lower total MUFA than the VT2 and VT4 groups. Furthermore, the proportion of 22:6n3 was significantly lower in VT2 and VT4 than in CT, while total PUFA showed no difference among groups. These changes resulted in significantly higher UI in CT than in VT2. ACL and n3/n6 were significantly higher in CT than in VT2 and VT4 (Fig. 2A–D).

In trial 2, two weeks after seawater acclimation, the total SFA in all groups increased significantly and showed no difference in liver phospholipids of rainbow trout (Table 3). The total MUFA in CT increased to 7.88%, while in VT2 and VT4 it was comparatively steady. The total PUFA in VT2 and VT4 decreased significantly and showed no difference among groups. The PLFA-related parameters showed no significant difference among groups.

Effects of diel cyclic temperature on intestinal PLFA composition during seawater acclimation

In trial 2, after six weeks in freshwater, the intestinal PLFA composition of rainbow trout was significantly influenced by diel cyclic temperature (Table 4). The total SFA in VT4 was significantly lower than in CT and VT2. A significantly high proportion of 22:6n3 was recorded in VT4, resulting in a significantly high total PUFA in VT4. Correspondingly, the U/S, UI, n3/n6, and ACL in VT4 were significantly higher than in CT and VT2 (Fig. 2E–H).

At SW-1, significant decreases in the proportions of 16:0 and 18:0 were detected in CT in the intestinal phospholipids of rainbow trout (Table 4). The total MUFA in VT4 significantly increased and was significantly higher than that in CT and VT2. The proportion of 22:6n3 in CT and VT2 increased significantly, whereas that in VT4 decreased. Consequently, the total PUFA in CT was significantly higher than in VT2 and VT4 (Fig. 2E–H). These changes caused significantly higher U/S in CT than VT2 and VT4, whereas the UI and ACL in VT4 were significantly lower than in the other groups.

At SW-21, the intestinal phospholipids contained significantly lower proportions of 18:1n9 and 18:2n6 in CT than in VT2 and VT4, whereas the proportion of 22:6n3 in CT was significantly higher than in VT2 and VT4 (Table 4). However, no significant difference was observed in the total SFA, MUFA, and PUFA among the groups. The UI, n3/n6, and ACL in CT were significantly higher than in VT2 and VT4 (Fig. 2E–H).

A two-way analysis of variance

In trial 1, the two-way analysis of variance (ANOVA) indicated that both the liver and intestine in rainbow trout were significantly influenced by constant temperature, seawater acclimation, and their interaction (see Additional file 3). In trial 2, the diel cyclic temperature had no significant effect on liver PLFA composition, whereas seawater acclimation significantly influenced the total SFA, n3/n6, U/S, UI, and ACL in the liver PLFA composition. Therefore, the total SFA, n3/n6, U/S, UI, and ACL were significantly influenced by the interaction of diel cyclic temperature and seawater acclimation. In the intestinal phospholipids, all selected parameters were significantly affected by diel cyclic temperature, seawater acclimation, and their combination, except total MUFA, which was free of the effect of temperature.

Principal component analysis

In the present study, principle component analysis (PCA) was performed to reveal the tissue differences in rainbow trout at different temperature regimes and seawater acclimation stages. Based on the results of the two-way ANOVA, 16 variables (14:0, 16:0, 18:0, 16:1, 18:1n9, 24:1, 18:2n6, 20:2n6, 20:3n3, 20:4n6, 20:5n3, and 22:6n3, U/S, UI, n3/n6, and ACL) were selected for the liver of rainbow trout, while nine variables (14:0, 18:0, 14:1, 16:1, 18:1n9, 18:2n6, 18:3n3, 20:5n3, 22:6n3, U/S, UI, n3/n6, and ACL) were selected for the intestine.

In trial 1, the first two principal components (PCs) with eigenvalues of 6.68 and 3.72 were found to explain 69.34% of the overall variability. The eigenvectors of 16:1, 18:0, 18:1n9, 18:2n6, 22:6n3, U/S, UI, n3/n6, and ACL were more than 0.4, indicating a strong effect on the PCs. The score plots distinguished the liver PLFA composition of rainbow trout at different constant temperatures and seawater acclimation stages (Fig. 3A). PCA analysis of the intestinal PLFA composition yielded two PCs with eigenvalues of 6.70 and 2.44, explaining 76.06% of the dataset variance. The eigenvectors for 14:0, 14:1, 18:1n9, 18:3n3, 20:5n3, 22:6n3, U/S, UI, n3/n6, and ACL were greater than 0.4, resulting in the difference in intestinal PLFA composition of rainbow trout at different temperatures and seawater acclimation stages (Fig. 3B).

In trial 2, two of three PCs were selected for PCA on liver PLFA composition, and their eigenvalues were 5.69 and 3.54 and explained 65.88% of the total variability. The eigenvectors for 16:1, 16:0, 18:2n6, 18:1n9, 18:0, 20:5n3, 20:3n3, n3/n6 and U/S exceeded 0.4. However, individuals of liver PLFAs only clustered at FW-42 and SW-21 instead of temperature regimes (Fig. 3C). Analysis of the intestinal PLFA composition produced two PCs with eigenvalues of 6.68 and 2.13, explaining 73.45% of the accumulated variability. Eigenvectors of more than 0.4 included 16:0, 18:2n6, 18:1n9, 18:0, 20:5n3, 22:6n3, 24:1, n3/n6, U/S, UI, and ACL, contributing to the comparatively high differentiation in intestinal phospholipids of rainbow trout influenced by diel cyclic temperature and seawater acclimation (Fig. 3D).

Discussion

Effects of constant temperature on the liver and intestinal PLFA composition in rainbow trout

In order to adapt to different temperatures, fish can restructure the membrane phospholipids of tissues to maintain proper metabolism and physiological homeostasis [23-25]. In the constant temperature experiment, the two-way ANOVA indicated a significant effect of temperature on the liver and intestinal phospholipids in rainbow trout. The higher U/S and UI of the liver and intestinal phospholipids at 9 and 12.5 °C indicated an increase in membrane fluidity. Similarly, when temperature decreased from 29 to 22 °C, the liver's membrane fluidity in the European seabass (*Dicentrarchus labrax*) significantly increased [26]. Leray et al. [27] reported that rainbow trout (250 g) living at 12 °C showed a significantly higher degree of unsaturation in the intestinal phospholipids than at 17 °C. Under low-temperature conditions, regulation of the degree of unsaturation of phospholipids is essential for maintaining proper properties (fluidity, permeability, viscosity, and thickness) of cellular and organelle membranes [13, 28]. Contrarily, increased membrane fluidity of the liver and intestine helps increase the transmembrane efficiency and activity of membrane-bound enzymes, enhancing the ability to absorb and utilise nutrients [29]. This adaptation alleviates the adverse effects of low temperature, which limits the metabolic rate in fish [23, 25].

Effects of constant temperature on the liver and intestinal PLFA composition in rainbow trout during seawater acclimation

Notably, on the first day after seawater acclimation in trial 1, the UI of liver phospholipids in rainbow trout at different temperatures changed significantly and converged to a similar level; meanwhile individuals from all groups clustered at SW-1 in the PCA. The results indicated that the effect of seawater acclimation was more significant than temperature and that the tissue membranes of rainbow trout adjust for acclimation to seawater. Similarly, Borlongan et al. [30] found that milkfish (*Chanos chanos* Forsskal) contained a significantly higher degree of unsaturation of liver phospholipids in seawater than in freshwater, indicating that fish can respond to salinity change by altering membrane properties. In trial 1, two weeks after seawater acclimation, the UI and ACL in T12.5 and T16 changed

significantly and reverted to levels before seawater acclimation, demonstrating that fish had basically completed the acclimation to salinity change. However, the liver PLFA composition of rainbow trout in T9 was significantly altered after seawater acclimation, but the fluidity remained unchanged, reflecting that temperature had more influence than salinity change.

In trial 1, on the first day after seawater acclimation, the U/S, UI, n3/n6, and ACL of intestinal phospholipids significantly increased and reached a level close to T9 and T12.5, indicating a significant increase in fluidity, permeability, and thickness of the intestinal membrane. On the one hand, fish consume a large amount of energy for osmoregulation during seawater entry and can improve energy conversion efficiency by increasing membrane fluidity of the intestine to increase the efficiency of cross-membrane transportation and activities of membrane-bound enzymes [31, 32]. Brijs et al. [33] reported a dramatic increase in intestinal contractile activity in rainbow trout during seawater exposure, increasing the efficiency of absorptive processes. On the other hand, when fish are acclimated to seawater, ingested water and ions are absorbed by the intestine to maintain osmotic homeostasis [34]. Genz et al. [31] found a significant decrease in water, Na⁺, and Cl⁻ in intestinal fluids after rainbow trout were acclimated to seawater, suggesting an enhanced absorption rate of the intestinal membrane. In the present study, the intestinal PLFA composition of rainbow trout showed no significant difference at SW-14, indicating that they had adapted to salinity change.

Effects of diel cyclic temperature on the liver and intestinal PLFA composition in rainbow trout

In trial 2, the liver phospholipids in rainbow trout showed no difference in U/S and UI. The two-way ANOVA and PCA analyses demonstrated that only seawater acclimation significantly influenced the intestinal PLFA composition. This because a) the liver of rainbow trout might have adapted to the diel cyclic changes in temperature after a 6-week stocking period under different temperature variations; b) the liver of rainbow trout contains large amounts of long-chain PUFAs, suggesting better adaptability to temperature change due to higher membrane fluidity. Similarly, Eldridge et al. [35] subjected Atlantic salmon to periodic temperature for 30 days, and the results showed no significant difference in plasma cortisol, triglyceride, and histopathology.

It is noted that the U/S, UI, and ACL in intestinal phospholipids of rainbow trout in VT4 were significantly higher than at constant temperature, indicating an increase in membrane fluidity of the intestine. PCA analysis results demonstrated that intestinal phospholipids in rainbow trout were more susceptible to temperature variations than liver phospholipids. Under diel cyclic temperature variation conditions, feed intake of fish will increase, and therefore the absorption efficiency of the intestinal membrane will be enhanced [35, 36]. Thomas et al. [37] reported that coho salmon (*Oncorhynchus kisutch*) under diel temperature cycles increased its energy reserves by enhancing energy utilisation efficiency.

Effects of diel cyclic temperature on the liver and intestinal PLFA composition in rainbow trout during seawater acclimation

In trial 2, after the first day of seawater acclimation, the liver phospholipids of rainbow trout significantly increased the UI and ACL, consequently increasing the liver membrane's fluidity and thickness, indicating the liver's adaptation to seawater acclimation. Enhanced fluidity of the liver membrane is beneficial for the utilisation of nutrients and can increase the synthesis and secretion of antioxidants [38, 39]. In trial 2, the liver PLFA composition of rainbow trout at

VT4 was relatively stable, implying that diel cyclic temperatures could improve salinity tolerance to some extent. Similarly, after acclimation to periodic temperature variations for three days, the tolerance to acute heat stress in Atlantic salmon significantly improved [40]. Hokanson et al. [41] reported that rainbow trout subjected to daily temperature variations showed a higher growth rate and survival than those living at a constant temperature (15 °C). Our data showed that the liver PLFA compositions in all treatments were steady with no difference, suggesting that the adaptation to seawater had completed.

In trial 2, on the first day after seawater acclimation, PCA analysis indicated that clusters appeared at different stages of seawater acclimation, demonstrating that intestinal phospholipids were more susceptible to salinity change. Therefore, the U/S and UI of intestinal phospholipids significantly increased in CT but decreased in VT4, lowering the fluidity, permeability, and thickness of the intestinal membrane. These alterations represented adaptive changes in the intestine to counteract the salinity change in rainbow trout [42]. Decreased membrane fluidity can stimulate the activity of Na⁺-K⁺ ATPase located on the intestinal membrane and consequently contribute to osmotic homeostasis in fish [13, 43, 44]. In trial 2, the intestinal PLFA composition in all groups stabilised, implying that fish had adapted to the salinity change. Moreover, fish under diel temperature variation up to 4 °C showed a faster adaptation, proving that diel cyclic temperature can enhance the adaptability of rainbow trout to seawater acclimation.

Conclusions

In this study, different constant temperatures significantly influenced the liver and intestinal PLFA composition in rainbow trout. The fluidity and thickness of the liver and intestinal membrane significantly increased at 9 and 12.5 °C, respectively. During seawater acclimation, the liver and intestinal PLFA compositions were initially influenced by salinity change, and at 12.5 °C more resilience was observed although fish at all selected temperatures showed stable PLFA composition after two weeks. At different diel cyclic temperatures, the intestinal membrane of rainbow trout at 13 ± 2 °C had significantly increased fluidity, permeability, and thickness. During seawater acclimation, the liver's PLFA composition was more stable, while the PLFA composition of the intestine responded to salinity changes more rapidly. Therefore, different constant temperatures significantly influenced the liver and intestinal phospholipids in rainbow trout, while the appropriate diel cyclic temperature can improve the adaptability to seawater acclimation in the liver and intestine of rainbow trout.

Declarations

Ethics approval and consent to participate

Ethical approval for fish experiment was permitted by the Animal Experimentation Committee of the Ocean University of China (Reference Number, 2018-12). Fish husbandry and sample collection were in compliance with animal care protocols at the Ocean University of China and National Experimental Management Regulations in China.

Consent for publication

Not applicable.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Competing interests

All authors declare no conflict of interests.

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Authors' contributions

JG carried out the entire experiment, analysed the data and drafted the manuscript with the guidance of YZ and SD. MH helped analyse samples. YZ was responsible for the integrity of the work as a whole. QG, and YD helped edit this manuscript.

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Tables

Table 1 Liver PLFA composition of rainbow trout in different constant temperatures during seawater acclimation.

SP	FW-28			SW-1			SW-14		
Temperature	9 °C	12.5 °C	16 °C	9 °C	12.5 °C	16 °C	9 °C	12.5 °C	16 °C
Saturated fatty acid									
C14:0	1.11 ^{Ab}	1.11 ^A	0.82 ^B	1.38 ^{Aa}	0.83 ^B	0.97 ^B	1.04 ^b	1.25	1.03
C16:0	17.09 ^{Ba}	17.39 ^B	18.95 ^{Aa}	17.40 ^{Aa}	17.55 ^A	14.80 ^{Bb}	15.53 ^{Bb}	18.78 ^A	14.56 ^{Bb}
C17:0	0.44	0.42	0.41	0.37	0.35	0.60	0.57	0.52	0.49
C18:0	9.45 ^{Ab}	7.40 ^{Bb}	9.30 ^A	11.29 ^{Aa}	10.24 ^{Ba}	9.21 ^C	9.18 ^{Ab}	9.25 ^{Aa}	8.65 ^B
C20:0	0.28	0.25	0.35 ^a	0.20	0.19	0.24 ^{ab}	0.22	0.29	0.18 ^b
C22:0	0.05 ^b	0.08	0.09 ^b	0.18 ^{Aa}	0.05 ^B	0.06 ^{Bb}	0.05 ^{Bb}	0.08 ^B	0.23 ^{Aa}
C23:0	0.25 ^b	0.52 ^a	0.39 ^b	0.63 ^a	0.54 ^a	0.70 ^a	0.05 ^{Bc}	0.06 ^{Bb}	0.23 ^{Ab}
C24:0	0.33 ^{Ac}	0.41 ^{AB}	0.60 ^{Ab}	0.65 ^{Bb}	0.63 ^B	1.16 ^{Aa}	1.08 ^{Aa}	0.51 ^B	1.15 ^{Aa}
ΣSFA	29.00 ^{Bb}	27.57 ^{Cb}	30.9 ^{Aa}	32.11 ^{Aa}	30.38 ^{Ba}	27.75 ^{Cb}	27.72 ^{Bc}	30.73 ^{Aa}	26.51 ^{Bb}
Monounsaturated fatty acid									
C14:1n5	0.73	0.60 ^b	0.55 ^b	0.54	0.62 ^b	1.01 ^a	1.09	1.33 ^a	1.05 ^a
C16:1n7	1.07 ^C	2.18 ^{Aa}	1.96 ^{Bb}	1.21	1.71 ^b	1.35 ^c	1.24 ^B	1.86 ^{Bb}	3.29 ^{Aa}
C17:1n7	0.25	0.20	0.14	0.13 ^b	0.15	0.20	0.26 ^a	0.17	0.21
C18:1n9	7.23 ^C	11.55 ^{Aa}	9.18 ^{Bb}	7.33 ^B	8.51 ^{ABb}	9.79 ^{Ab}	7.68 ^B	7.48 ^{Bc}	13.95 ^{Aa}
C20:1n9	0.28	0.21	0.30	0.24	0.22	0.25	0.26	0.30	0.25
C22:1n9	0.44 ^a	0.31	0.49	0.25 ^b	0.23	0.33	0.05 ^{Bc}	0.34 ^A	0.41 ^A
C24:1n9	3.05 ^{Aa}	2.55 ^B	3.16 ^A	2.57 ^{Bb}	2.44 ^B	3.24 ^A	3.39 ^{Aab}	2.67 ^{AB}	2.41 ^B
ΣMUFA	13.04 ^C	17.59 ^{Aa}	15.78 ^{Bb}	12.26 ^B	13.87 ^{Bb}	16.18 ^{Ab}	13.98 ^B	14.16 ^{Bb}	21.57 ^{Aa}
Polyunsaturated fatty acid									
C18:2n6	4.57 ^{Ba}	5.25 ^{Aa}	4.17 ^{Cb}	3.61 ^b	3.43 ^c	4.24 ^{ab}	4.58 ^{ab}	4.56 ^b	5.52 ^a
C18:3n3	2.01 ^a	2.02 ^a	2.07	1.71 ^{Bb}	1.64 ^{Bc}	1.94 ^A	2.11 ^{Aa}	1.87 ^{Bb}	2.03 ^A
C18:3n6	0.23	0.19	0.21	0.17	0.11	0.14	0.18	0.15	0.14
C20:2n6	1.85 ^{Aa}	1.09 ^{AB}	0.82 ^B	0.95 ^{ABc}	0.87 ^B	1.44 ^A	1.52 ^b	1.35	1.51
C20:3n3	2.07 ^{Ab}	1.26 ^{Bb}	1.19 ^B	1.23 ^c	2.04 ^a	1.52	2.19 ^{Aa}	1.57 ^{Bb}	1.24 ^C
C20:3n6	1.06 ^a	1.60 ^a	1.83 ^a	0.66 ^b	0.93 ^b	0.83 ^b	0.62 ^{Bb}	1.05 ^{Ab}	0.64 ^{Bb}
C20:4n6	5.30 ^{Aa}	4.26 ^{Ba}	2.72 ^{Cb}	3.73 ^{Bc}	3.59 ^{Bb}	4.76 ^{Aa}	4.61 ^b	4.42 ^a	4.02 ^a

C20:5n3	4.36 ^b	4.14	4.39	5.40 ^{Aa}	4.61 ^B	4.63 ^B	5.87 ^{Aa}	4.71 ^B	4.98 ^B
C22:2n6	0.20 ^b	0.17 ^b	0.11 ^b	0.08 ^{Bc}	0.45 ^{Aa}	0.34 ^{Aa}	0.30 ^{Aa}	0.09 ^{Cb}	0.21 ^{Bab}
C22:6n3	36.31 ^{Ab}	34.85 ^{Cb}	35.81 ^{Ba}	38.09 ^{Aa}	38.07 ^{Aa}	36.23 ^{Ba}	36.31 ^{Ab}	35.34 ^{Ab}	31.67 ^{Bb}
∑PUFA	57.96 ^{Aa}	54.84 ^B	53.32 ^{Cb}	55.63 ^b	55.75	56.07 ^a	58.30 ^{Aa}	55.11 ^B	51.92 ^{Cb}

Note: Values are means of 3 replications. Different lowercase letters indicate significant differences ($P < 0.05$) among different treatments at the same time, and different capital letters indicate significant differences at different times at the same treatment. Data containing mean $\pm$ SD are included in Additional file 2. FW-28: end of growth trial. SW-1: one day after salinity reached 30. SW-4: four days after salinity reached 30. SW-7: seven days after salinity reached 30. SW-14: fourteen days after salinity reached 30.

Table 2 Intestinal PLFA composition of rainbow trout in different constant temperatures during seawater acclimation.

SP	FW-28			SW-1			SW-14		
Temperature	9 °C	12.5 °C	16 °C	9 °C	12.5 °C	16 °C	9 °C	12.5 °C	16 °C
Saturated fatty acid									
C14:0	2.73 ^B	2.26 ^C	3.32 ^{Aa}	2.48	1.67	2.20 ^{ab}	2.50	2.49	1.63 ^b
C16:0	22.31	22.06	23.99 ^a	22.37	21.55	23.58 ^a	21.16 ^B	23.10 ^A	21.38 ^{Bb}
C17:0	0.78 ^B	0.49 ^{Bb}	1.25 ^{Aa}	0.81 ^A	0.75 ^{ABa}	0.58 ^{Bb}	0.62	0.54 ^b	0.67 ^b
C18:0	10.27 ^{AB}	11.51 ^A	10.22 ^{Bb}	9.29 ^B	11.69 ^A	11.44 ^{Aab}	10.45	11.23	12.57 ^a
C20:0	0.29	0.32	0.45 ^a	0.25	0.22	0.31 ^{ab}	0.17	0.26	0.17 ^b
C22:0	0.18 ^A	0.10 ^{Ba}	0.11 ^B	0.14	0.16 ^a	0.18	0.11	0.05 ^b	0.16
C23:0	0.12 ^{AB}	0.07 ^B	0.13 ^A	0.13 ^A	0.04 ^B	0.11 ^A	0.12	0.07	0.11
C24:0	1.04 ^B	0.76 ^C	2.91 ^{Aa}	1.05	0.96	1.19 ^b	1.54	0.68	1.16 ^b
ΣSFA	37.71 ^B	37.59 ^B	42.38 ^{Aa}	36.53 ^B	37.04 ^B	39.60 ^{Ab}	36.66	38.42	37.84 ^c
Monounsaturated fatty acid									
C14:1n5	1.64 ^{Ba}	1.06 ^C	2.93 ^{Aa}	0.86 ^{Cb}	1.19 ^B	1.61 ^{Ab}	0.93 ^b	1.05	0.62 ^c
C16:1n7	1.86	1.51	1.51	2.40 ^A	1.26 ^B	1.39 ^B	2.13 ^A	1.72 ^{AB}	1.38 ^B
C17:1n7	0.53 ^a	0.47	0.45	0.29 ^{Bb}	0.45 ^A	0.19 ^B	0.34 ^b	0.40	0.34
C18:1n9	10.85 ^a	9.39 ^a	10.21 ^a	8.20 ^{Bb}	10.68 ^{Aa}	9.47 ^{ABab}	8.32 ^b	7.42 ^b	8.86 ^b
C20:1n9	0.23 ^b	0.22	0.35 ^a	0.21 ^b	0.17	0.19 ^b	0.35 ^a	0.23	0.27 ^{ab}
C22:1n9	0.60	0.54 ^b	0.85 ^a	0.71	0.77 ^a	0.64 ^b	0.52	0.57 ^b	0.52 ^b
C24:1n9	2.49	3.22 ^a	3.24 ^a	3.01	3.49 ^a	2.53 ^{ab}	2.39 ^A	2.55 ^{Ab}	1.63 ^{Bb}
ΣMUFA	18.19 ^{ABa}	16.42 ^{Ba}	19.54 ^{Aa}	15.67 ^{Bb}	18.02 ^{Aa}	16.01 ^{Bb}	14.98 ^b	13.93 ^b	13.62 ^c
Polyunsaturated fatty acid									
C18:2n6	3.83 ^{Bb}	5.52 ^{Aa}	4.96 ^{Ab}	5.07 ^a	4.50 ^{ab}	4.19 ^b	3.93 ^b	3.75 ^b	7.01 ^a
C18:3n3	2.75 ^{AB}	2.30 ^B	3.80 ^A	2.63	2.85	3.12	2.82	3.30	2.50
C18:3n6	0.34 ^a	0.22	0.43	0.15 ^b	0.16	0.18	0.12 ^b	0.15	0.24
C20:2n6	0.79	0.70 ^a	0.78	0.67	0.72 ^a	0.72	0.79	0.56 ^b	0.74
C20:3n3	1.01	1.11	0.97	0.75 ^B	1.36 ^A	0.82 ^B	1.17	1.05	0.70
C20:3n6	0.55	0.67	0.73 ^a	0.56 ^A	0.47 ^A	0.31 ^{Bb}	0.45	0.64	0.53 ^a
C20:4n6	2.49	2.45	2.61	3.04	2.60	2.30	2.94	2.77	2.29

C20:5n3	1.76 ^{Bb}	2.94 ^{Aa}	2.17 ^{AB}	2.90 ^{ab}	2.39 ^b	2.81	3.70 ^a	3.70 ^a	2.74
C22:2n6	0.33 ^a	0.18 ^b	0.38 ^a	0.15 ^{Bb}	0.31 ^{Aa}	0.25 ^{Aab}	0.16 ^b	0.23 ^{ab}	0.19 ^b
C22:6n3	30.24 ^{Ab}	29.90 ^A	21.25 ^{Bb}	31.89 ^{ab}	29.58	29.71 ^a	32.28 ^a	31.50	31.61 ^a
∑PUFA	44.09 ^{Ab}	45.99 ^{Aab}	38.07 ^{Bc}	47.80 ^{Aa}	44.94 ^{Bb}	44.39 ^{Bb}	48.35 ^a	47.65 ^a	48.54 ^a

Note: Same as Table 1.

Table 3 Liver PLFA composition (%) in rainbow trout at different sampling points at diel cyclic temperatures.

SP	FW-42			SW-1			SW-21		
Temp	CT	VT2	VT4	CT	VT2	VT4	CT	VT2	VT4
Saturated fatty acid									
C14:0	0.96 ^b	1.00 ^b	0.97 ^b	1.18 ^a	1.16 ^a	1.13 ^a	1.07 ^{ab}	1.19 ^a	1.11 ^a
C16:0	17.26 ^{AB}	15.98 ^{Bc}	17.90 ^{Aa}	16.95	17.40 ^b	16.50 ^b	18.41	18.77 ^a	18.26 ^a
C17:0	0.61 ^{ab}	0.53	0.71 ^a	0.44 ^{ABb}	0.57 ^A	0.26 ^{Bb}	0.74 ^a	0.61	0.67 ^a
C18:0	6.23 ^b	6.21 ^b	6.27 ^c	7.66 ^a	7.63 ^a	7.35 ^b	8.09 ^a	7.70 ^a	8.20 ^a
C20:0	0.21	0.19 ^a	0.19	0.13	0.13 ^{ab}	0.14	0.15	0.09 ^b	0.15
C22:0	0.12	0.09	0.16	0.08	0.12	0.08	0.10	0.09	0.08
C24:0	0.52 ^{Bb}	0.94 ^A	0.79 ^{ABa}	0.76 ^a	0.82	0.38 ^b	0.63 ^{Aab}	0.42 ^B	0.48 ^{ABb}
ΣSFA	25.92 ^b	24.94 ^b	27.00 ^b	27.20 ^{ab}	27.82 ^a	25.84 ^b	29.19 ^a	28.87 ^a	28.96 ^a
Monounsaturated fatty acid									
C14:1n5	0.79 ^b	0.78	0.98 ^b	0.60 ^b	0.92	0.59 ^c	1.42 ^a	1.01	1.28 ^a
C16:1n7	2.61 ^a	2.54 ^a	2.70 ^a	1.42 ^b	2.04 ^{ab}	2.14 ^{ab}	1.23 ^b	1.34 ^b	1.59 ^b
C17:1n7	0.23	0.19	0.20	0.23 ^A	0.20 ^A	0.17 ^B	0.19	0.22	0.21
C18:1n9	2.01 ^a	2.09 ^a	2.11 ^a	1.51 ^{Bb}	1.68 ^{ABb}	1.86 ^{Aa}	1.60 ^b	1.55 ^b	1.56 ^b
C20:1n9	0.20	0.17	0.15	0.18	0.16	0.20	0.18	0.13	0.17
C22:1n9	0.08	0.05	0.08	0.06	0.05	0.05	0.06	0.06	0.04
C24:1n9	2.81	2.91	2.62 ^b	2.88	2.75	2.71 ^b	3.18	3.01	3.19 ^a
ΣMUFA	8.72 ^a	8.72 ^a	8.84 ^a	6.88 ^{Bb}	7.80 ^{Ab}	7.72 ^{Ab}	7.88 ^a	7.32 ^b	8.04 ^b
Polyunsaturated fatty acid									
C18:2n6	12.89 ^a	12.86 ^a	12.92 ^a	10.22 ^{Bb}	12.00 ^{Aa}	13.23 ^{Aa}	9.18 ^b	8.89 ^b	9.58 ^b
C18:3n3	0.10	0.10	0.10 ^{ab}	0.15 ^A	0.10 ^B	0.08 ^{Bb}	0.14	0.14	0.13 ^a
C18:3n6	0.16	0.16	0.13	0.13	0.14	0.15	0.15	0.13	0.10
C20:2n6	1.26 ^{Ab}	1.48 ^{Aa}	1.12 ^B	1.18 ^a	1.21 ^{ab}	1.36	0.79 ^b	0.97 ^b	1.18
C20:3n3	0.91 ^b	0.84 ^b	0.84 ^b	1.33 ^{Aa}	0.96 ^{Bb}	0.94 ^{Bb}	1.24 ^a	1.28 ^a	1.21 ^a
C20:3n6	1.93 ^A	1.50 ^{Bab}	1.29 ^{Bb}	2.03	1.83 ^a	2.06 ^a	1.82 ^A	1.14 ^{Bb}	1.54 ^{ABb}
C20:4n6	2.92 ^{Ab}	2.78 ^{Ac}	1.85 ^{Bc}	3.37 ^b	3.53 ^b	3.06 ^b	5.19 ^a	4.93 ^a	4.63 ^a
C20:5n3	2.69 ^b	2.52	2.12 ^b	3.01 ^{ab}	2.90	2.80 ^a	3.64 ^{Aa}	2.60 ^B	2.92 ^{Ba}
C22:2n6	0.34	0.33	0.38	0.23	0.37	0.28	0.23	0.29	0.30

C22:6n3	42.16 ^b	43.78 ^a	43.4 ^a	44.29 ^{Aa}	41.33 ^{Cb}	42.47 ^{Bab}	40.54 ^{Bb}	43.45 ^{Aa}	41.39 ^{Bb}
PUFA	65.36	66.34 ^a	64.16 ^b	65.92	64.38 ^{ab}	66.44 ^a	62.93	63.81 ^b	63.00 ^b

Note: Values represent the mean of 4 replicates. Different lowercase letters indicate significant differences ($P < 0.05$) among different treatments at the same time, and different capital letters indicate significant differences at different times at the same treatment. Data containing mean $\pm$ SD are included in Additional file 2. FW-42: end of growth trial. SW-1: one day after salinity reached 30. SW-21: 21 days after salinity reached 30. SFA: saturated fatty acid, MUFA: monounsaturated fatty acid, PUFA: polyunsaturated fatty acid.

Table 4 Intestinal PLFA composition (%) in rainbow trout at different sampling points at diel cyclic temperatures.

SP	FW-42			SW-1			SW-21		
Temp	CT	VT2	VT4	CT	VT2	VT4	CT	VT2	VT4
Saturated fatty acid									
C14:0	1.27 ^b	1.14 ^b	1.02 ^c	1.28 ^b	1.20 ^b	1.25 ^b	1.50 ^a	1.72 ^a	1.67 ^a
C16:0	19.84 ^{ABa}	21.32 ^A	18.76 ^B	16.96 ^{Bb}	20.7 ^A	20.46 ^A	20.95 ^a	21.37	20.20
C17:0	0.62	0.73	0.63	0.82	0.68	1.01	0.88	1.11	0.85
C18:0	11.07 ^{Aa}	10.36 ^A	8.69 ^{Bc}	9.19 ^{Bb}	10.41 ^B	12.59 ^{Aa}	10.67 ^a	9.81	10.49 ^b
C20:0	0.27	0.38 ^a	0.33 ^a	0.26 ^{AB}	0.29 ^{Aab}	0.21 ^{Bb}	0.22	0.23 ^b	0.22 ^b
C22:0	0.11	0.14	0.13	0.15	0.10	0.14	0.11	0.19	0.12
C24:0	0.77 ^B	1.20 ^{Aa}	0.81 ^B	0.60 ^B	0.81 ^{Ab}	0.86 ^A	0.74	0.90 ^{ab}	0.77
ΣSFA	33.94 ^{Aa}	35.28 ^A	30.37 ^{Bb}	29.26 ^{Bb}	34.19 ^A	36.51 ^{Aa}	35.08 ^a	35.32	34.31 ^a
Monounsaturated fatty acid									
C14:1n5	0.55 ^c	0.74 ^c	0.69 ^b	1.00 ^b	1.00 ^b	1.41 ^a	1.37 ^a	1.28 ^a	1.28 ^a
C16:1n7	1.12 ^b	0.96 ^b	0.96 ^b	1.30 ^b	0.90 ^b	1.40 ^b	2.22 ^a	2.78 ^a	2.97 ^a
C17:1n7	0.34	0.30	0.26 ^b	0.45 ^{AB}	0.25 ^B	0.59 ^{Aa}	0.34	0.40	0.37 ^b
C18:1n9	2.07	2.22 ^b	1.87 ^b	2.08 ^B	1.89 ^{Bc}	2.57 ^{Aa}	2.25 ^B	2.63 ^{Aa}	2.70 ^{Aa}
C20:1n9	0.28	0.33	0.28	0.26	0.26	0.33	0.27	0.33	0.31
C22:1n9	0.07	0.10	0.08	0.07	0.09	0.08	0.10	0.09	0.10
C24:1n9	2.64	2.54 ^a	2.34 ^{ab}	2.18	2.06 ^b	2.69 ^a	2.36 ^A	1.95 ^{Bb}	1.73 ^{Bb}
ΣMUFA	7.07 ^b	7.20 ^b	6.46 ^b	7.32 ^{Bb}	6.46 ^{Bb}	9.07 ^{Aa}	8.90 ^a	9.47 ^a	9.46 ^a
Polyunsaturated fatty acid									
C18:2n6	10.58 ^A	9.43 ^{Ab}	7.43 ^{Bb}	11.7 ^B	7.06 ^{Cc}	13.56 ^{Aa}	11.90 ^C	13.35 ^{Ba}	14.86 ^{Aa}
C18:3n3	0.12	0.11	0.11 ^b	0.1	0.09	0.11 ^b	0.09	0.15	0.20 ^a
C18:3n6	0.16 ^b	0.16 ^b	0.15 ^b	0.26 ^{ab}	0.13 ^b	0.19 ^b	0.36 ^a	0.58 ^a	0.67 ^a
C20:2n6	0.80 ^A	0.51 ^{Bb}	0.59 ^{ABb}	0.61	0.55 ^b	0.76 ^a	0.71	0.81 ^a	0.82 ^a
C20:3n3	0.79	0.74 ^b	0.69	0.73	0.69 ^b	0.88	0.93	0.98 ^a	0.96
C20:3n6	1.32 ^{Aa}	0.89 ^B	0.91 ^B	0.89 ^b	0.84	1.00	0.91 ^b	0.90	0.96
C20:4n6	3.28 ^a	3.20	3.02	2.68 ^b	3.11	2.86	2.99 ^{ab}	2.72	2.76
C20:5n3	2.36 ^b	2.06 ^b	2.13 ^b	2.34 ^b	2.14 ^b	2.45 ^b	3.03 ^a	3.19 ^a	3.24 ^a
C22:2n6	0.37	0.42	0.43	0.40	0.46	0.42	0.38	0.51	0.49

C22:6n3	39.21 ^{Bb}	40.01 ^{Bb}	47.71 ^{Aa}	43.71 ^{Aa}	44.27 ^{Aa}	32.16 ^{Bb}	34.71 ^{Ac}	32.02 ^{Bc}	31.28 ^{Bb}
PUFA	58.99 ^{Bb}	57.52 ^{Bb}	63.17 ^{Aa}	63.42 ^{Aa}	59.34 ^{Ba}	54.42 ^{Bb}	56.02 ^c	55.21 ^c	56.23 ^b

Note: Same as Table 3.

Figures

Figure 1

Liver and intestinal PLFA compositions at different constant temperatures during seawater acclimation of rainbow trout. The ratio of unsaturation (A&E), unsaturation index (B&F), the ratio of n-3 PUFA to n-6 PUFA (C&G) and the average length of fatty acid chain (D&H). Values are the means of 3 replicates. Capital letters indicate intergroup differences at the same sampling point.

Figure 2

Liver and intestinal PLFA compositions at diel cyclic temperatures during seawater acclimation in rainbow trout. The ratio of unsaturation (A&E), unsaturation index (B&F), the ratio of n-3 PUFA to n-6 PUFA (C&G) and the average length of fatty acid chain (D&H). Values are the means of four replicates. Capital letters indicate intergroup differences at the same sampling point. Values are the means of four replicates. Capital letters indicate intergroup differences at the same sampling point.

Figure 3

Score plots of liver and intestinal PLFA composition using PCA analysis. Liver (A) and intestine (B) of rainbow trout at different constant temperatures, and liver (C) and intestine (D) of rainbow trout at diel cyclic temperature regimes, using principal component analysis.

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