Journal of Thermal Biology 83 (2019) 165–171

Contents lists available at ScienceDirect

Journal of Thermal Biology

journal homepage: www.elsevier.com/locate/jtherbio

Influence of long-term temperature stress on respiration frequency, Na^+/K^+-ATPase activity, and lipid metabolism in common carp (*Cyprinus carpio*)

Jun-long Sun[1], Liu-lan Zhao[1], Can Cui, Zong-jun Du, Zhi He, Yan Wang, Xue-wei Li**, Song Yang*

College of Animal Science and Technology, Sichuan Agricultural University, Chengdu, Sichuan, 611130, China

ARTICLE INFO

Keywords:
Eurythermic fish
Environmental temperature
Stress
Respiration
Lipid metabolic
Gene expression

ABSTRACT

Temperature is a key factor that influences fish metabolism. Therefore, it is important to understand how temperature variation affects lipid metabolism and energy consumption in fish. We determined respiration frequency, Na^+/K^+-ATPase activity, and lipid metabolism in common carp (*Cyprinus carpio*) after 18 days of temperature stress (5 °C and 30 °C, with 17 °C as the control). We found that respiratory frequency was positively correlated with water temperature ($p < 0.01$), whereas red blood cell count and mean corpuscular hemoglobin content and concentration were negatively correlated with temperature. In liver and heart, triglyceride (TG) levels were lower in both stress groups (5 °C and 30 °C) than in the control (17 °C) ($p < 0.01$), and highest in muscle from the high temperature stress group ($p < 0.01$). The non-esterified fatty acid concentration was negatively correlated with TG levels. In brain, lipoprotein lipase (LPL) activity decreased with increasing temperature and hepatic lipase (HL) activity was lower in both stress groups than in the control ($p < 0.01$). In muscle, the activity of LPL and HL was lowest in the high temperature stress group, resulting in a significant increase in TG levels. Na^+/K^+-ATPase activity in heart was lower in both stress groups than in the control ($p < 0.01$). The expression of *LPL* mRNA in heart increased with increasing temperature ($p < 0.01$), whereas *LPL* mRNA expression in brain and liver increased in both stress groups ($p < 0.01$). Our results show that temperature can significantly affect lipid metabolism in common carp, and that different tissues respond differently to changes in temperature.

1. Introduction

World average temperature, atmospheric CO_2 concentration, and tropospheric ozone concentration are increasing, leading to climate extremes (Intergovernmental Panel on Climate Change, 2014). Water temperature varies in day-and-night and seasonal patterns (Crozier and Hutchings, 2014; Harley et al., 2006) and is affected by the external climate. Water temperature is a key environmental factor that affects the survival, growth, and reproduction of fish. It also plays an important role in the productivity of farmed fish, mostly because of its effect on metabolism (Killen et al., 2016; Kordas and Harley, 2016; Angilletta et al., 2002; Gillooly et al., 2001; López-Olmeda and Sánchez-Vázquez, 2011). Most fish species lack insulation and temperature regulating mechanisms and have low metabolic rates. When environmental factors lie outside an optimal range, fish experience stress and exhibit disruption of normal physiological activity and even death (Barton, 2002; Donaldson et al., 2010; Corey et al., 2017). Within their tolerance range, fish have positive regulatory mechanisms that help them adapt to environmental changes (Aguiar et al., 2002). Changes in temperature usually lead to changes in respiratory frequency (Maricondi-Massari et al., 1998; Aguiar et al., 2002). At high temperatures, accelerated physiological rates can result in higher energy requirements (Johansen et al., 2015; Dillon et al., 2010), whereas, at low temperatures, reduced physiological rates can lower energy demands and affect metabolism. Many studies have shown that temperature fluctuations have a large impact on aquatic animals (e.g., Long et al., 2012; Zhang et al., 2017). The magnitude of the response to thermal stress in aquatic animals depends on the duration and magnitude of the temperature change.

Under environmental stress, fish can switch from sugar metabolism to lipid metabolism (Mengxiao et al., 2018). Lipid metabolism involves the synthesis and degradation of lipids in cells, including the

* Corresponding author.
** Corresponding author.
E-mail addresses: 1988sunjunlong@sina.com (J.-l. Sun), zhaoliulan2007@163.com (L.-l. Zhao), 1297316616@qq.com (C. Cui), 756662181@qq.com (Z.-j. Du), zhihe@sicau.edu.cn (Z. He), 594843662@qq.com (Y. Wang), xuewei.li@sicau.edu.cn (X.-w. Li), ysys210@hotmail.com (S. Yang).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.jtherbio.2019.05.009
Received 21 November 2018; Received in revised form 3 April 2019; Accepted 18 May 2019
Available online 21 May 2019
0306-4565/

breakdown or storage of fats for energy. Lipoprotein lipase (LPL) is a glycoprotein produced in tissues; it plays a key role in lipoprotein metabolism, mainly in tissues that require high lipid concentrations. LPL hydrolyzes triglycerides (TGs) carried by plasma low-density lipoprotein and chylomicrons, releasing non-esterified fatty acids (NEFAs) that are taken up by nearby tissues, used as energy, or stored (Goldberg, 1996). Hepatic lipase (HL) is a lipolytic enzyme that plays an important role in the clearance of plasma TGs, as well as in the metabolism of high-density lipoproteins and other lipoproteins (Annema and Tietge, 2011). Na+/K + -ATPase (NKA) is a key enzyme for osmotic regulation and maintenance of ionic equilibrium (Kong et al., 2008). NKA is also affected by environmental temperature (Guo et al., 2014).

In this study, common carp (*Cyprinus carpio*) was selected as a representative eurythermic species because of its high economic value and easy availability. Common carp is also an excellent model for studying the mechanisms of temperature acclimation because of its high tolerance of varying temperatures and high resistance to stress. The objectives of this study were: 1) to record the respiratory frequency and to measure plasma biochemical indexes of common carp under different temperature conditions; 2) to determine NKA and lipid-related enzyme activities in main tissues of common carp; and 3) to quantify the expression of the LPL-encoding gene in main tissues. The results will contribute to understanding the physiological mechanisms involved in temperature stress and adaptation in eurythermic species.

2. Materials and methods

2.1. Ethics statement

All experiments were performed according to the Guidelines for the Care and Use of Laboratory Animals in China. All experimental procedures and sample collection were approved by the Institutional Animal Care and Use Committee (IACUC) of the College of Animal Science and Technology of Sichuan Agricultural University, Sichuan, China, under permit No. DKY-B20161701.

2.2. Fish preparation and maintenance

Common carp (*Cyprinus carpio*) were obtained from Chengdu Tongwei Aquatic Engineering Technology Research Center, Tong Wei Group Company Ltd. The healthy fish with an average weight of 500 ± 50 g were marked with PIT marker and kept in a recirculating system (600-L tanks with circulating aerated water at 17 °C) to acclimate the experimental condition for two weeks with natural photoperiod. Fish were hand-fed to apparent satiation twice daily (9:00 and 17:00) with carp diet (31.3% crude protein, 11.6% crude fat, 11.7% ash). Water quality was monitored daily and maintained as follows: temperature 17.0 ± 0.5 °C, pH 7.5 ± 0.2, dissolved oxygen > 5.8 mg L^{-1} (> 80% of air saturation).

2.3. Experimental design and sample collection

Nine rectangular tanks (60 L) were used for stress experiment. Ninety fish were randomly separated into groups A, B and C (3 tanks in each group, 10 fish in each tank). For temperature stress: the temperature in group A were gradually decreased from 17 °C to 5 °C at a rate of 1 °C/h, named cold stress group (CSG). The temperature in group B were still maintained at 17 °C named control group (CG). Accordingly, the temperature in group C were gradually increased from 17 °C to 30 °C at a rate of 1 °C/h, named heat stress group (HSG). Under the experimental conditions, each tank was continuously aerated to saturate the dissolved oxygen in the water and replace 25% of the water every day. The water quality monitoring was operated and maintained same just like during acclimation period. The experiment lasted for 18 days, and fish were hand-fed twice per day to satiation at 09:00 and 17:00, and then fasted 24 h prior to sample. (Fig. 1)

Five fish in each tank were sampled at the end of the trial, blood were drawn from the fish caudal vessel using heparinized syringes. The fish were anesthetized with MS-222, after weighting fish were killed by cutting the spine, and immediately dissected for sampling liver, muscle, heart and brain. All the samples were immediately frozen in liquid nitrogen, and stored at −80 °C until use.

2.4. Measurement of respiratory frequency

The respiratory frequency from three groups were measured twice a day during the experiment (8:00 and 16:00, respectively), the operation was as follows: firstly, determin the identity of each fish by scanning the PIT marker, secondly, record the number of breaths for 1 min with five fish from each tank and each fish was repeat measured 5 times. The entire measurement process was carried out in the experimental tanks.

2.5. Plasma biochemical indexes and blood parameters

Part of blood samples were centrifuged at 2500 rpm for 10 min after stabilizing 30 min. The plasma was transferred into a new Eppendorf tube for immediate analysis. The mean erythrocyte hemoglobin (MCH) and mean erythrocyte hemoglobin concentration (MCHC) in plasma were measured by an automated biochemical analyzer by Chengdu Lilai Biotechnology Co., Ltd., Sichuan, China. Small part of blood samples were used for red blood cells (RBC) count by Neubauer hemocytometer after diluted 200-times.

2.6. Lipid metabolism parameters

The tissues used for the determination of lipid metabolism parameters and enzyme activities were homogenized on ice with 0.9% sodium chloride buffer in a glass hand-homogenizer. Then the homogenate was centrifuged (2500 g for 10 min at 4 °C). The supernatant obtained by centrifugation was immediately transferred into an Eppendorf tube and stored at −20 °C. All enzyme activity analysis were completed within 7 days. The LPL and HL activities in the liver, muscle, brain and heart were measured, using reagent kits (Product Code A067). The activity of NKA in the liver, muscle, brain and heart were measured, using reagent kits (Product Code A070-2). The TG and NEFA in the liver, muscle, brain and heart were determined by reagent kit (Product Code A110–2, A042 and A019-2, respectively). These kits were purchased at Nanjing Jiancheng Institute of Biotechnology and biochemical parameters assayed according to the manufacturer's instructions.

2.7. Quantification of LPL gene expression

Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and stored at −80 °C. RNA was quantified and quality was assessed by NanoDrop Spectrophotometer 2000c (NanoDrop Technologies, Wilmington, DE, USA) and 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). Three high quality RNA samples from each group were used for RT-qPCR (The standard for high quality RNA is the OD ratios of 260/280 and 260/230 greater than 1.8 and RNA integrity number greater than 8).

Total RNA was used to synthesize mRNA cDNA by using TianGen® FastKing RT Kit (With gDNase). The qRT-PCR primers were designed with reference to the relevant known common carp sequences by Primer Primer 5.0. The expression level of mRNA was determined using quantitative PCR (qPCR). The emission intensity was detected by Step One real-time PCR system (Applied Biosystems) under the following steps: initial denaturation step at 95 °C for 20 s, 40 thermal cycling steps consisted of 3 s at 95 °C, 30 s at 60 °C. The relative expression of gene was calculated by $2^{-\Delta\Delta Ct}$ method which normalized to the geomean of *β-actin* and *18s* RNA (Livak and Schmittgen, 2001) (The reference gene primers and *LPL* gene primers are shown in Table 1). All reactions were

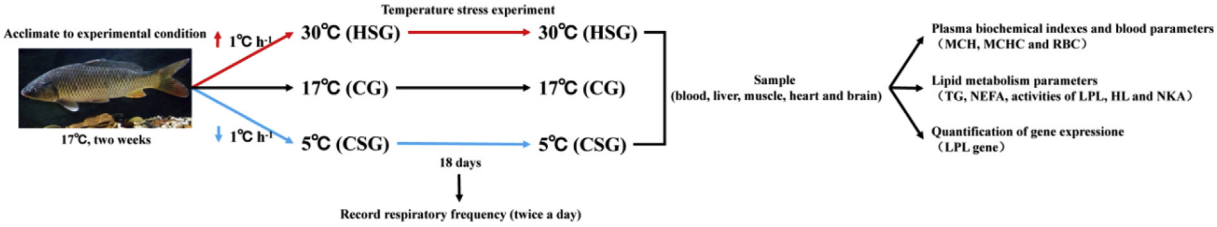

Fig. 1. Experimental design and technical route.

run in triplicate and included no template controls for each gene.

2.8. Statistical analysis

The data were input into excel file to set up database, and they were presented as mean ± SEM (Standard Error of Mean). Correlation analysis was performed using SPSS. The level of significance was determined by one-way analysis of variance (ANOVA) with SPSS, a significance level of 0.05 ($p < 0.05$) and great significance level of 0.01 ($p < 0.01$) were used in all tests.

3. Results

3.1. Respiratory and blood biochemical indexes

There were no mortalities throughout the trial in any of the experimental groups (CG, control group at 17 °C, CSG, cold stress group at 5 °C, and HSG, heat stress group at 30 °C). Significant differences were observed in the respiratory frequency among the three groups ($p < 0.01$); the respiratory frequency increased with increasing temperature (Fig. 2 A). Red blood cell count (RBC) count was significantly higher in CSG compared with CG and HSG ($p < 0.01$) (Fig. 2B). Mean corpuscular hemoglobin (MCH) content and MCH concentration showed a similar trend to that of the RBC count ($p < 0.05$) (Fig. 2 C, D). Overall, the respiratory frequency showed positive correlation with temperature ($p < 0.01$), whereas the RBC count, MCH content, and MCH concentration showed negative correlation with temperature ($p < 0.05$). The correlation coefficients are shown in Table 2.

3.2. Activity of NKA

In CSG and HSG, NKA activity in heart was 0.202 ± 0.017 U/mg protein and 0.423 ± 0.068 U/mg protein, respectively, which was significant lower than in CG (1.542 ± 0.067 U/mg protein) by 85.21% and 67.02%, respectively ($p < 0.01$). In HSG, NKA activity in muscle was 0.708 ± 0.026 U/mg protein, 46.00%.higher than in CG (0.485 ± 0.072 U/mg protein) ($p < 0.05$); there was no significant difference in NKA activity in muscle in CSG and CG ($p > 0.05$). There also were no significant differences in NKA activity in brain and liver among the three groups ($p > 0.05$) (Fig. 3).

3.3. TG and NEFA content

TG content in liver was 19.62% lower in CSG (0.418 ± 0.015 mmol/mg protein) than in CG (0.520 ± 0.004 mmol/mg protein) ($p < 0.01$). TG content in heart was 42.19% lower in HSG (0.380 ± 0.017 mmol/mg protein) than in CG (0.658 ± 0.051 mmol/mg protein) ($p < 0.01$). TG content in muscle was 12.96% higher in HSG (0.795 ± 0.009 mmol/mg protein) than in CG (0.704 ± 0.018 mmol/mg protein) ($p < 0.01$). There were no significant differences in TG content in brain among the three groups ($p > 0.05$) (Fig. 4A).

The NEFA content in brain was significantly different among the three groups. The lowest NEFA content was in HSG (652.689 ± 7.589 μmol/g protein) ($p < 0.05$) and the highest was in CSG (862.764 ± 18.537 μmol/g protein) ($p < 0.01$). In contrast, in liver, the NEFA content was higher in CSG (292.766 ± 11.017 μmol/g protein) and HSG (347.261 ± 9.262 μmol/g protein) than in CG (245.967 ± 16.031 μmol/g protein) ($p < 0.01$). There were no significant differences in NEFA content in heart and muscle among the three groups ($p > 0.05$) (Fig. 4B). The NEFAs showed negative correlation with the TGs, especially in heart and brain where the negative correlation was significant ($p < 0.05$). The correlation coefficients are shown in Table 3.

3.4. Activities of LPL and HL

There were no significant differences in LPL and HL activity in heart and liver among the three groups ($p > 0.05$). LPL activity in brain was highest in CSG (1.629 ± 0.025 U/mg protein) and lowest in HSG (0.987 ± 0.047 U/mg protein); that is, 5.91% higher ($p < 0.05$) and 35.83% lower, respectively, than in CG (1.538 ± 0.011 U/mg protein) ($p < 0.01$). HL activity in brain was 27.79% and 67.85% lower in CSG and HSG (1.912 ± 0.027 U/mg protein and 0.852 ± 0.065 U/mg protein, respectively) than in CG (2.648 ± 0.297 U/mg protein) ($p < 0.01$). LPL activity in muscle was lowest in HSG (0.409 ± 0.002 U/mg protein), which was 23.26% lower than in CG (0.533 ± 0.036 U/mg protein) ($p < 0.01$). There were no significant differences in HL activity in muscle among the three groups ($p > 0.05$) (Fig. 5).

3.5. Expression of LPL gene

LPL mRNA primers are shown in Table 1 and *LPL* expression levels in the various tissues are shown in Fig. 6. *LPL* expression in heart was

Table 1
Primer sequences used for real-time PCR analysis.

Gene	Primer name	Primer sequence	Product length (bp)	Annealing temperature (°C)	Accession number
β-actin	β-actin-F	CATCAGGGTGTCATGGTTGGT	163	61.0	M24113.1
	β-actin-R	CTCAAACATGATCTGTGTCAT			
18s	18s-F	AAACGGCTACCACATCCAA	86	59.5	AF133089.2
	18s-R	TTACAGGGCCTCGAAAGAGA			
LPL	LPL-F	AACCCGAAGACGATCTATGCT	122	59.9	FJ716101.1
	LPL-R	AAACATACCCGTAACCGTCC			

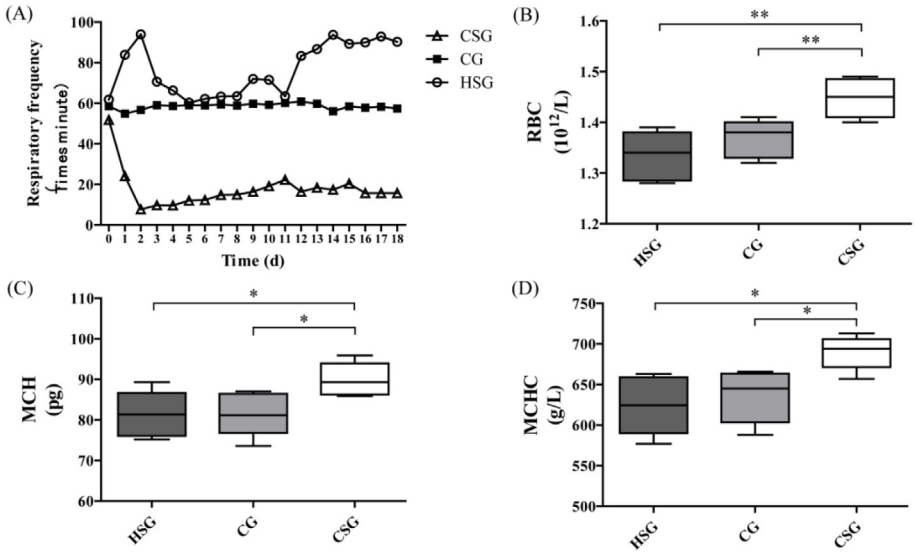

Fig. 2. Respiratory frequency, RBC, MCH and MCHC for during exposure to different temperature **Note:** (A) Respiratory frequency was measured each morning (08:00) and evening (16:00) for 18 days. For each measurement, Determined the identity of each fish by scanning the PIT marker. Five fish from each tank were randomly selected to record the number of breaths for 1 min, and each fish was repeat measured 5 times. The entire measurement process was carried out in the experimental tanks. The value is average of respiratory frequency; (B), (C) and (D) Five fish in each group were selected for determination of RBC, MCH and MCHC in plasma. Values are means of five replicates ± SEM. *, ** indicate significant differences at $p < 0.05$ and $p < 0.01$, respectively.

2.712-fold higher in HSG and 0.523-fold lower in CSG than in CG ($p < 0.01$), showing *LPL* expression in heart increased with the temperature. In contrast, *LPL* expression levels in brain and liver were 4.490-fold and 4.370-fold higher in CSG, respectively, than in CG ($p < 0.01$) and, in liver, *LPL* expression was 2.490-fold higher in HSG than in CG ($p < 0.01$). *LPL* expression levels in muscle were 0.351-fold and 0.360-fold lower in CSG and HSG, respectively, than in CG ($p < 0.01$). LPL activity was positively correlated with *LPL* expression. The correlation coefficients are shown in Table 5.

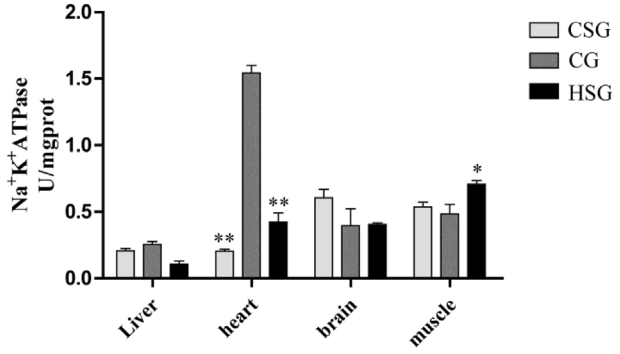

Fig. 3. The activity of Na^+/K^+-ATPase in liver, heart, brain and muscle. Note: Values are means of five replicates ± SEM. *, ** indicate significant differences from the control group (17 °C) at $p < 0.05$ and $p < 0.01$, respectively.

4. Discussion

4.1. The respiratory frequency of carp is significantly affected by temperatures

In ectothermic organisms, compensation for temperature changes is achieved through physiological processes such as temperature acclimation by adjusting respiratory frequency and metabolic rate, for example (Withers, 1992). In the present study, the respiration frequency of the common carp was found to be related to water temperature. This is consistent with the results of studies on the metabolic rate of fish (Maricondi-Massari et al., 1998; Gillooly et al., 2001; Aguiar et al., 2002), which showed that temperature changes affected the metabolic rate and changed the demand for oxygen, hence, changing respiration frequency (Weiss, 1970; Bartelme, 2006; Sukhan et al., 2010). Respiration frequency was found to increase with rises in water temperature, indicating changes in metabolic rate (Tantarpale et al., 2012). At low temperatures, fish are likely to be in a resting state with low respiration frequency (Murugaian et al., 2008), whereas, at high temperatures, fish are likely to be active with high respiration frequency. The high respiration frequency observed at high temperatures can be explained by an increase in metabolism rate and the requirement for more oxygen. However, the amount of dissolved oxygen declines as water temperature increases, resulting in insufficient levels of dissolved oxygen (Bartelme, 2006). Therefore, the reduced amount of dissolved oxygen together with the higher metabolic rate of fish in water at high temperatures lead to increased respiratory frequency to meet the increased demand for oxygen in tissues (Sukhan et al., 2010).

4.2. Changes in hematological parameters are opposite to respiratory frequency and may be a compensation mechanism

In several of the measured hematological parameters (RBC count, MCH content, and MCH concentration), large differences were observed between CG and the temperature stress groups (HSG and CSG). The trend of the changes in the three parameters was consistent among the three groups, and showed an inverse relationship with temperature. This is similar to the results of previous studies of fish under hypoxia conditions, which showed that increasing the number of RBCs

Table 2
Correlation analysis results between respiratory rate, plasma biochemical parameters and temperature.

Pearson	Temperature	RBC	MHC	MCHC	Respiratory frequency
Temperature	1	−0.765**	−0.571*	−0.671**	0.971**
RBC	−0.765**	1	0.593*	0.642**	−0.790**
MHC	−0.571*	0.593*	1	0.939**	−0.634**
MCHC	−0.671**	0.642**	0.939**	1	−0.716**
Respiratory frequency	0.971**	−0.790**	−0.634**	−0.716**	1

Note: *, ** indicate significant level at $p < 0.05$ and $p < 0.01$, respectively.

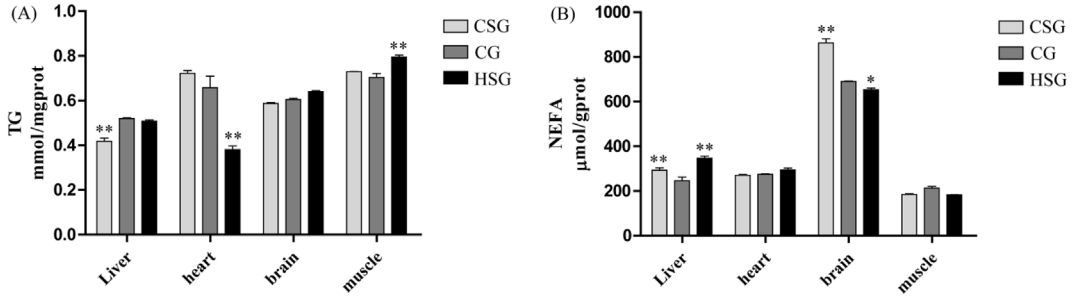

Fig. 4. The content of TG and NEFA in liver, heart, brain and muscle **Note:** Values are means of five replicates ± SEM. *, ** indicate significant differences from the control group (17 °C) at $p < 0.05$ and $p < 0.01$, respectively.

Table 3
Correlation analysis results between TG and NEFA.

Pearson		TG			
		Liver	Heart	Brain	Muscle
NEFA	Liver	−0.102			
	Heart		−0.745*		
	Brain			−0.756*	
	Muscle				−0.560

Note: * indicate significant level at $p < 0.05$.

effectively increased the transport capacity of oxygen (Murad et al., 1990; Wu et al., 2016). In this study, we found that, in CSG, the respiratory frequency of the common carp was reduced and the RBC count and MCH content and concentration were increased compared with CG, which may help to regulate the exchange of oxygen between tissues under low temperature conditions. In HSG, no significant differences were detected in the RBC count and MCH content and concentration compared with CG, indicating that low temperature stress is a more serious threat than high temperature stress in aquaculture (Qi et al., 2013).

4.3. NKA participates in energy distribution during temperature change

NKA is a key enzyme for osmotic regulation and maintenance of ionic equilibrium (Kong et al., 2008). The “channel stop” hypothesis predicts the suppression of functional ion channels that are ATP-consuming ion pumps to reduce the energy cost of maintaining an ion gradient at low temperatures (Hochachka, 1986; Lutz et al., 1985). We found that NKA activity in heart was reduced by 85.21% and 67.02% in CSG and HSG, respectively, compared with CG. This is similar to the response of turtle heart at low and high temperatures (Overgaard et al., 2005). The reduced NKA activity in heart in HSG indirectly indicates the occurrence of “channel arrest” in heart under high temperature stress. Similar results have been reported in crucian carp (Aho and Vornamen, 1997). NKA activity affects the electrical membrane

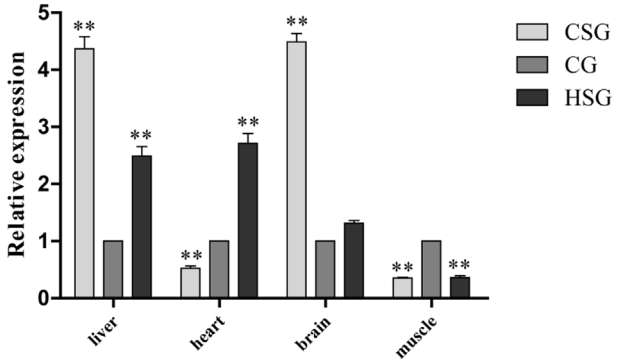

Fig. 6. Expression of *LPL* mRNA in liver, heart, brain and muscle **Note:** Relative expressions of *LPL* mRNAs were quantified by qPCR. *LPL* mRNA expression was measured in the total RNA prepared from samples subjected to 5 °C ($n = 3$), 17 °C ($n = 3$) and 30 °C ($n = 3$). The relative expressions of specific genes were calculated by the $2^{-\Delta\Delta Ct}$ method normalized to the geomean of β-actin and 18s RNA; error bars represent SEM. *, ** indicate significant differences from the control group (17 °C) at $p < 0.05$ and $p < 0.01$, respectively.

potential of cardiac cells both directly and indirectly (Lichtstein, 1995). Inhibition of NKA activity at low and high temperatures causes changes in the membrane potential of these cells. These changes may cause excitatory changes in specific cells.

4.4. Common carp need to regulate lipid metabolism to provide energy when temperature changes

High water temperatures (e.g., 30 °C) may induce a physiological response in fish so that energy is used primarily to sustain homeostasis. When fish were exposed to high temperature, the rates of enzymatic reactions increased leading to increased demand for ATP (Qi et al., 2013). Low temperature stress can also affect these processes. Stress caused by temperature changes modified the energy distribution in fish (Cheng et al., 2010; Qiang et al., 2013). Liver is considered to be the

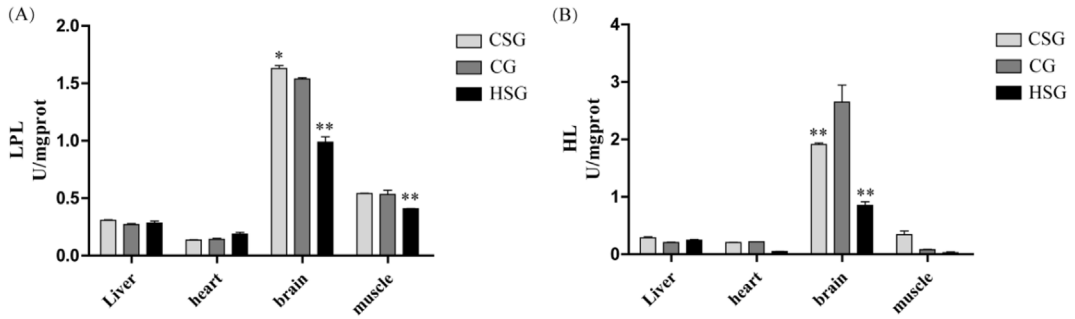

Fig. 5. The activity of LPL and HL in liver, heart, brain and muscle **Note:** Values are means of five replicates ± SEM. *, ** indicate significant differences from the control group (17 °C) at $p < 0.05$ and $p < 0.01$, respectively.

Table 4
Correlation analysis results between activity of LPL and expression of *LPL* mRNA.

Pearson		The activity of LPL			
		Liver	Heart	Brain	Muscle
Expression of *LPL* mRNA	Liver	0.621			
	Heart		0.793*		
	Brain			0.524	
	Muscle				0.394

Note: * indicate significant level at $p < 0.05$.

Table 5
Correlation analysis results between TG and the activity of LPL.

Pearson		TG			
		Liver	Heart	Brain	Muscle
The activity of LPL	Liver	−0.651			
	Heart		−0.709*		
	Brain			−0.934**	
	Muscle				−0.645

Note: *, ** indicate significant level at $p < 0.05$ and $p < 0.01$, respectively.

main organ for fat metabolism. TG levels can reflect the level of liver fat storage (Di Marco et al., 2008). We found that in CSG, liver TGs reduced significantly and NEFAs showed negative correlation with TGs, whereas there was no significant difference in LPL and HL activity in CSG compared with CG. However, the changes in TG levels differed among the tissues from both temperature stress groups. In heart, the TG level increased in HSG compared with CG, indicating the utilization of lipid increased and fat deposition reduced at the high temperature. Lipids were found to be one of the most readily available energy sources (Das et al., 2004; Wang et al., 2012; Ferreira et al., 2015). The TG level in brain reduced with decreasing temperature ($p > 0.05$) and LPL activity increased with increasing temperature ($p < 0.05$). The TG level and LPL activity showed a significant negative correlation, suggesting that under low temperature stress, lipid metabolism in brain increased. Some studies have suggested that common carp may be suitable for aquaculture in high water temperatures and may require a larger volume of fat cells to ensure adequate fat as energy at low temperatures (Ma et al., 2015). The negative correlation between TG level and LPL activity was observed in all the tissues (Table 5). LPL and HL both promote the uptake of lipoproteins (Liang et al., 2002). We found that HL activity showed significant changes in brain. Lipid metabolism in brain increased at low temperatures leading to significant increases in NEFAs. NEFAs have been reported to act via changes in the activity of the autonomic nervous system. Beta oxidation of cerebral NEFAs was shown to be a prerequisite for glucose-induced insulin secretion and insulin action (Cruciani Guglielmacci et al., 2004).

4.5. Temperature affects LPL expression and LPL activity in different tissues

Several studies have demonstrated that *LPL* is expressed in multiple organs of fish and that *LPL* expression tends to be higher in liver and lower in heart and muscle (Cheng et al., 2010). Interestingly, *LPL* mRNA expression in muscle decreased in CSG and HSG in our study, but increased in liver (Fig. 6). Furthermore, the *LPL* expression levels essentially mirrored LPL activity (Table 4). These data indicate that the changes in enzyme activity can be explained in part by the *LPL* expression levels. However, it has been reported that changes in LPL activity were not always accompanied by changes in mRNA levels (Lee et al., 1998), implying regulation of LPL is complex and multilevel. Moreover, we found significant changes in *LPL* mRNA expression and LPL enzyme activity in brain. To function, the brain requires high energy in the form of ATP, which is fueled mainly by fatty acids and glucose (Lopaschuk et al., 2010). In the brain, ketone bodies converted from fatty acids are additional fuel sources for ATP generation (Kelly et al., 2000). Liver LPL targets TGs circulating to the liver and increases the production of ketones. Ketone bodies can be used as an alternative energy source to glucose. However, we found increased *LPL* expression levels and increased LPL activity in liver in CSG and HSG compared with CG. The changes in LPL activity may compensate for the increased metabolic rate. LPL has a short half-life so its enzymatic activity could be rapidly regulated by changes in mRNA levels (Liang et al., 2002).

5. Conclusions

The respiratory frequency of the common carp showed a positive correlation with temperature. RBC count and MCH content and concentration were negatively correlated with temperature, possibly as a compensatory mechanism. NKA activity in heart was significantly decreased in the high and low temperature stress groups compared with the control, possibly reflecting a reduction in ion transportation allowing the allocation of more energy to survival. These changes may cause changes in the excitability of specific cells. Changes in the lipid metabolism indicators (LPL, HL, and TGs) indicated that the different tissues may have different energy metabolism patterns after temperature stress. Changes in LPL activity may be a compensation mechanism for changes in metabolic rate. TGs in brain were more likely to decompose after low temperature stress. The brain's energy may be derived from ATP produced in other tissues under high temperature stress. Lipid metabolism in liver increased under temperature stress, indicating that the ambient temperature significantly affected lipid metabolism in carp tissues. We strongly suggest that lipid metabolism is an important energy supplier in the physiological response to temperature stress in eurythermic fish.

Declarations

Funding

This research was supported by the Double Support Project fund of the Sichuan Agricultural University (SICAU, No. 03572406).

Availability of data and materials

All data generated and analysed during this study are included in this published article.

Author contributions

Jun-long Sun, Song Yang, and Xue-wei Li conceived and designed the experiments; Jun-long Sun, Can Cui, Zong-jun Du, Zhi He and Yan Wang performed the experiments; Jun-long Sun performed data analysis; Jun-long Sun wrote the paper; Liu-lan Zhao and Song Yang assisted with writing and proofreading.

Conflicts of interest

The authors declare no conflict interest.

Consent for publication

Not applicable.

Ethics approval and consent to participate

All experiments were performed according to the Guidelines for the Care and Use of Laboratory Animals in China. All experimental procedures and sample collection were approved by the Institutional Animal Care and Use Committee (IACUC) of the College of Animal Science and Technology of Sichuan Agricultural University, Sichuan, China, under

permit No. DKY-B20161701.

Acknowledgments

We are grateful to Ming-zhou Li at Sichuan Agriculture University (SICAU) for the conception of research, we also thank to Ji-deng Ma at SICAU for technical support, and we acknowledge the assistance of Hao Wu, Wen-qiang Lian, Qing Xiao and Jie Du with fish rearing and tissue sampling. We thank Margaret Biswas, PhD, from Liwen Bianji, Edanz Group China (www.liwenbianji.cn/ac), for editing the English text of a draft of this manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtherbio.2019.05.009.

References

Aguiar, L.H., Kalinin, A.K., Rantin, F.T., 2002. The effects of temperature on the cardiorespiratory function of the neotropical fish *Piaractus mesopotamicus*. J. Therm. Biol. 27, 299–308. https://doi.org/10.1016/S0306-4565(01)00092-4.

Aho, E., Vornamen, M., 1997. Seasonality of ATPase activities in crucian carp (*Carassius carassius* L.) heart. Fish Physiol. Biochem. 16, 355–364. https://doi.org/10.1023/A:1007727024877.

Angilletta, M.J., Niewiarowski, P.H., Navas, C.A., 2002. The evolution of thermal physiology in ectotherms. J. Therm. Biol. 27, 249–268. https://doi.org/10.1016/S0306-4565(01)00094-8.

Annema, W., Tietge, U., 2011. Role of hepatic lipase and endothelial lipase in high-density lipoprotein mediated reverse cholesterol transport. Curr. Atheroscler. Rep. 13, 257–265. https://doi.org/10.1007/s11883-011-0175-2.

Bartelme, T.D., 2006. Metabolism, Energy Use and Feeding Behaviour in Fish. http://advancedaquarist.com/2006/4/aafe ctures1.html.

Barton, B.A., 2002. Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. Integr. Comp. Biol. 42 (3), 517–525. https://doi.org/10.1093/icb/42.3.517.

Cheng, H.L., Sun, S.P., Peng, Y.X., Shi, X.Y., Xin, S., Meng, X.P., et al., 2010. Cdna sequence and tissues expression analysis of lipoprotein lipase from common carp (*Cyprinus carpio var*. jian). Mol. Biol. Rep. 37 (6), 2665–2673. https://doi.org/10.1007/s11033-009-9797-7.

Corey, E., Linnansaari, T., Cunjak, R.A., Currie, S., 2017. Physiological effects of environmentally relevant, multi-day thermal stress on wild juvenile Atlantic salmon (*Salmo salar*). Conserv. Physiol. 5, cox014. https://doi.org/10.1093/conphys/cox014.

Crozier, L.G., Hutchings, J.A., 2014. Plastic and evolutionary responses to climate change in fish. Evol. Appl. 7, 68–87. https://doi.org/10.1111/eva.12135.

Cruciani Guglielmacci, C., Hervalet ADouared, L., Sanders, N.M., Levin, B.E., Ktorza, A., Magnan, C., 2004. Beta oxidation in the brain is required for the effects of non-esterified fatty acids on glucose-induced insulin secretion in rats. Diabetologia 47 (11), 2032–2038. https://doi.org/10.1007/s00125-004-1569-2.

Das, T., Pal, A.K., Chakraborty, S.K., Manush, S.M., Chatterjee, N., Mukherjee, S.C., 2004. Thermal tolerance and oxygen consumption of Indian Major Carps acclimated to four temperatures. J. Therm. Biol. 29 (3), 157–163. https://doi.org/10.1016/j.jtherbio.2004.02.001.

Di Marco, P., Priori, A., Finoia, M.G., Massari, A., Mandich, A., Marino, G., 2008. Physiological responses of European sea bass *Dicentrarchus labrax* to different stocking densities and acute stress challenge. Aquaculture 275, 319–328. https://doi.org/10.1016/j.aquaculture.2007.12.012.

Dillon, M.E., Wang, G., Huey, R.B., 2010. Global metabolic impacts of recent climate warming. Nature 467, 704. https://doi.org/10.1038/nature09407.

Donaldson, M.R., Cooke, S.J., Patterson, D.A., Macdonald, J.S., 2010. Cold shock and fish. J. Fish Biol. 73 (7), 1491–1530. https://doi.org/10.1111/j.1095-8649.2008.02061.x.

Ferreira, N.G., Morgado, R., Santos, M.J., Soares, A.M., Loureiro, S., 2015. Biomarkers and energy reserves in the isopod Porcellionides pruinosus: the effects of long-term exposure to dimethoate. Sci. Total Environ. 502, 91–102. https://doi.org/10.1016/j.scitotenv.2014.08.062.

Gillooly, J.F., Brown, J.H., West, G.B., Savage, V.M., Charnov, E.L., 2001. Effects of size and temperature on metabolic rate. Science 293, 2248–2251. https://doi.org/10.1126/science.1061967.

Goldberg, I.J., 1996. Lipoprotein lipase and lipolysis: central roles in lipoprotein metabolism and atherogenesis. J. Lipid Res. 37, 693–707.

Guo, Q.D., Wang, Y.J., Lü, W.Q., 2014. Combined effects of temperature and salinity on the physiological osmotic induction and antioxidant responses in the juvenile Japanese Flounder (*Paralichthys olivaceus*). Acta Hydrobiol. Sin. 38, 58–67.

Harley, C.D., Randall Hughes, A., Hultgren, K.M., Miner, B.G., Sorte, C.J., Thornber, C.S., Rodriguez, L.F., Tomanek, L., Williams, S.L., 2006. The impacts of climate change in coastal marine systems. Ecol. Lett. 9, 228–241. https://doi.org/10.1111/j.1461-0248.2005.00871.x.

Hochachka, P.W., 1986. Defense strategies against hypoxia and hypothermia. Science 231, 234–241. https://doi.org/10.1126/science.2417316.

Intergovernmental Panel on Climate Change, 2014. Summary for policymakers. Climate Change 2013 – The Physical Science Basis: Working Group I Contribution to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK, pp. 1–30. https://doi.org/10.1017/CBO9781107415324.004.

Johansen, J.L., Pratchett, M.S., Messmer, V., Coker, D.J., Tobin, A.J., Hoey, A.S., 2015. Large predatory coral trout species unlikely to meet increasing energetic demands in a warming ocean. Sci. Rep. 5. https://doi.org/10.1038/srep13830.

Kelly, D.P., Lehman, J.J., Barger, P.M., Huss, J.M., Weinheimer, C.J., Kovacs, A., et al., 2000. Ppar signaling in the control of cardiac energy metabolism: lessons from genetically altered mice. Trends Cardiovasc. Med. 10 (6), 238. https://doi.org/10.1016/S0022-2828(01)90610-8.

Killen, S.S., Glazier, D.S., Rezende, E.L., Clark, T.D., Atkinson, D., Willener, A.S.T., Halsey, L.G., 2016. Ecological influences and morphological correlates of resting and maximal metabolic rates across teleost fish species. Am. Nat. 187, 592–606. https://doi.org/10.1086/685893.

Kong, X.H., Wang, G.Z., Li, S.J., 2008. Seasonal variations of ATPase activity and antioxidant defenses in gills of the mud crab Scylla serrata (*Crustacea, Decapoda*). Mar. Biol. 154, 269–276. https://doi.org/10.1007/s00227-008-0920-4.

Kordas, R.L., Harley, C.D.G., 2016. Demographic responses of coexisting species to in situ warming. Mar. Ecol. Prog. Ser. 546, 147–161. https://doi.org/10.3354/meps11620.

Lee, J.J., Smith, P.J., Fried, S.K., 1998. Mechanism of decreased lipoprotein lipase activity in adipocytes of starved rats depend on duration of starvation. J. Nutr. 128, 940–946. https://doi.org/10.1093/jn/128.6.940.

Li, Mengxiao, Wang, Xiaodan, Qi, Changle, Li, Erchao, Du, Zhenyu, Qin, Jian G., et al., 2018. Metabolic response of nile tilapia (*Oreochromis niloticus*) to acute and chronic hypoxia stress. Aquaculture 495, 187–195. https://doi.org/10.1016/j.aquaculture.2018.05.031.

Liang, X.F., Oku, H., Ogata, H.Y., 2002. The effects of feeding condition and dietary lipid level on lipoprotein lipase gene expression in liver and visceral adipose tissue of red sea bream *Pagrus major*. Comp. Biochem. Physiol. A 131, 335–342. https://doi.org/10.1016/S1095-6433(01)00481-0.

Lichtstein, D., 1995. Na^+, K^+-atpase and heart excitability. Adv. Exp. Med. Biol. 382, 23.

Livak, J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta Ct}$ method. Methods 25, 402–408. https://doi.org/10.1006/meth.2001.1262.

Long, Y., Li, L., Li, Q., He, X.Z., Cui, Z.B., 2012. Transcriptomic Characterization of temperature stress responses in larval zebrafish. PLoS One 7, 37209. https://doi.org/10.1371/journal.pone.0037209.

Lopaschuk, G.D., Ussher, J.R., Folmes, C.D., Jaswal, J.S., Stanley, W.C., 2010. Myocardial fatty acid metabolism in health and disease. Physiol. Rev. 90, 207–258. https://doi.org/10.1152/physrev.00015.2009.

López-Olmeda, J.F., Sánchez-Vázquez, F.J., 2011. Thermal biology of zebrafish (danio rerio). J. Therm. Biol. 36 (2), 91–104. https://doi.org/10.1016/j.jtherbio.2010.12.005.

Lutz, P.L., Rosenthal, M., Sick, T.J., 1985. Living without oxygen: turtle brain as a model of anaerobic metabolism. Mol. Physiol. 8, 411–425.

Ma, X.Y., Qiang, J., He, J., Gabriel, N.N., Xu, P., 2015. Changes in the physiological parameters, fatty acid metabolism, and SCD activity and expression in juvenile GIFT tilapia (*Oreochromis niloticus*) reared at three different temperatures. Fish Physiol. Biochem. 41, 937–950. https://doi.org/10.1007/s10695-015-0059-4.

Maricondi-Massari, M., Kalinin, A.L., Glass, M.L., Rantin, F.T., 1998. The effects of temperature on oxygen uptake, gill ventilation and ECG waveforms in the Nile tilapia, *Oreochromis niloticus*. J. Therm. Biol. 23, 283–290. https://doi.org/10.1016/S0306-4565(98)00019-9.

Murad, A., Houston, A.H., Samson, L., 1990. Haematological response to reduced oxygen-carrying capacity, increased temperature and hypoxia in goldfish, *Carassius auratus* L. J. Fish Biol. 36, 289–305. https://doi.org/10.1111/j.1095-8649.1990.tb05610.x.

Murugaian, P., Ramamurthy, Karmegam, N., 2008. Effect of temperature on the behavioural and physiological response of catfish, mystus gulio (*Hamilton*). J. Appl. Sci. Res. 4 (11), 1454–1457.

Overgaard, J., Wang, T., Nielsen, O.B., Gesser, H., 2005. Extracellular determinants of cardiac contractility in the cold anoxic turtle. Physiol. Biochem. Zool. 78, 976–995. https://doi.org/10.1086/432853.

Qi, Z.H., Liu, Y.F., Luo, S.W., Chen, C.X., Liu, Y., Wang, W.N., 2013. Molecular cloning, characterization and expression analysis of tumor suppressor protein p53 from orange-spotted grouper, Epinephelus coioidesin response to temperature stress. Fish Shellfish Immunol. 35, 1466–1476. https://doi.org/10.1016/j.fsi.2013.08.011.

Qiang, J., Wang, H., Kpundeh, M.D., He, J., Xu, P., 2013. Effect of water temperature, salinity, and their interaction on growth, plasma osmolality, and gill Na^+, K^+-ATPase activity in juvenile gift tilapia *Oreochromis niloticus*, (l.). J. Therm. Biol. 38 (6), 331–338. https://doi.org/10.1016/j.jtherbio.2013.04.002.

Sukhan, Z.P., Chowdhury, M.T., Hannan, M.A., 2010. Climate change and its impact on fisheries resource in Bangladesh. In: Proc. of International Conference on Environmental Aspects of Bangladesh (ICEAB10), (Japan).

Tantarpale, V.T., Rathod, S.H., Kapil, S., 2012. Temperature stress on opercula beats and respiratory rate of freshwater fish of Ghanna punctatus. International Journal of Scientific and Research Publications 2, 2250–3153.

Wang, X., Wang, L., Yao, C., Qiu, L., Zhang, H., Zhi, Z., Song, L., 2012. Alternation of immune parameters and cellular energy allocation of Chlamys farreri under ammonia N exposure and Vibrio anguillarum challenge. Fish Shellfish Immunol. 32 (5), 741–749. https://doi.org/10.1016/j.fsi.2012.01.025.

Weiss, R.F., 1970. The solubility of Nitrogen, Oxygen and Argon in water and sea water. Deep Sea Res. 17, 721–735. https://doi.org/10.1016/0011-7471(70)90037-9.

Withers, P.C., 1992. Comparative Animal Physiology. Saunders College Publishing, Orlando, pp. 1024.

Wu, Z., You, F., Wen, A., Ma, D., Zhang, P., 2016. Physiological and morphological effects of severe hypoxia, hypoxia and hyperoxia in juvenile turbot (*Scophthalmus maximus* L.). Aquacult. Res. 47, 219–227. https://doi.org/10.1111/are.12483.

Zhang, Y.Y., Loughery, J.R., Martyniuk, C.J., Kieffer, J.D., 2017. Physiological and molecular responses of juvenile shortnose sturgeon (*Acipenser brevirostrum*) to thermal stress. Comp. Biochem. Physiol., A 203, 314–321. https://doi.org/10.1016/j.cbpa.2016.10.009.