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Effects of fasting, temperature, and photoperiod on *preproghrelin* mRNA expression in Chinese perch

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Abstract Preproghrelin, a gut/brain peptide, plays an important role in the regulation of food intake and energy homeostasis in teleost and mammals. In the present study, we obtained the full-length *preproghrelin* cDNA in Chinese perch. The *preproghrelin* messenger RNA (mRNA) tissue expression showed that level was much higher in stomach and pituitary than in other tissues. The fasting study showed, after gastric emptying (3–6 h), short-term fasting (6–12 h) increased *preproghrelin* expression in the stomach. While in the pituitary, fasting reduced *preproghrelin* expression at 1, 3, 12, and 48 h, presenting state fluctuation of self-adjustment. The temperature study showed that the mRNA expression of *preproghrelin* was the highest in the brain at 26 °C and highest in the stomach at 32 °C, respectively, with different optimum temperature in these two tissues, reflecting spatiotemporal differences of regulation by central nervous system and peripheral organs. The photoperiod study showed that normal light (11 h of lightness and 13 h of darkness) led to highest *preproghrelin* expression, both in the brain and in the stomach, than continuous light or continuous dark, proving food intake is adapted to natural photoperiod or normal light in this study. These results all indicated that tissue-specific *preproghrelin* expression of Chinese perch could be significantly affected by environmental factors. Short-term fasting of 6 h after gastric emptying, 26 °C, and normal light led to higher *preproghrelin* expression, which indicated potential appetite increase in Chinese perch.

Keywords Chinese perch · *preproghrelin* · mRNA expression · Fasting · Temperature · Photoperiod

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Introduction

Food intake in vertebrates is regulated through a complex interaction of several neuroendocrine factors derived from both the central nervous system and peripheral organs (Volkoff et al. 2009). Preproghrelin, a gut/brain peptide, was originally

purified and characterized in the stomach of rats and humans, and identified as the first endogenous ligand of the growth hormone secretagogue receptor (GHSR). Central or peripheral administration of preproghrelin stimulates the release of growth hormone (GH) (Kojima et al. 1999; Wren et al. 2000), promotes food intake, and increases body weight gain in rats (Wren et al. 2000; Nakazato et al. 2001). Therefore, preproghrelin is considered the third polypeptide that regulates GH secretion in addition to growth hormone releasing hormone and somatostatin (Wang et al. 2002). In fish, preproghrelin is also considered to regulate somatic growth and energy metabolism, by stimulating GH secretion, appetite, and gastric function (Kaiya et al. 2006, 2008; Kang et al. 2011a, 2011b).

Preproghrelin has been identified from several fish species, including goldfish (Unniappan et al. 2002), Japanese eel (Kaiya et al. 2003a), Mozambique tilapia (Kaiya et al. 2003b), rainbow trout (Kaiya et al. 2003c), Nile tilapia (Parhar et al. 2003), channel catfish (Kaiya et al. 2005), sea bream (Yeung et al. 2006), common carp (Kono et al. 2008), zebrafish (Amole and Unniappan 2009), Atlantic cod (Xu and Volkoff 2009), Arctic charr (Frøiland et al. 2010), Pacific bluefin tuna (Suda et al. 2012), crucian carp (Zhou et al. 2012), grass carp (Feng et al. 2013), and David's schizothoracin (Cai et al. 2014). Gene structure and messenger RNA (mRNA) quantification during fasting and re-feeding provided evidence for preproghrelin's stimulatory role in food intake, and central and peripheral injections of preproghrelin also increased food intake in goldfish (Unniappan et al. 2002, 2004; Miura et al. 2006; Matsuda et al. 2006). Similarly, after a chronic treatment with native preproghrelin, the food intake and body weight were increased in Mozambique tilapia (Riley et al. 2005). But the aforementioned reports revealed that the function mechanisms of preproghrelin are species-specific, suggesting the necessity of further investigations on the physiological roles of preproghrelin in various fish species (Kaiya et al. 2008).

An understanding of the interactions between environmental factors and the mechanisms of appetite control is fundamental to the development of practical approaches to optimize feed intake, which will usher in a new era of research in redefining the limits of productivity (Matteri 2001). Apart from nutrient availability, temperature and photoperiod are two other important environmental factors that may affect feeding and appetite in fish. However, their effects vary among fish species.

The Chinese perch, *Siniperca chuatsi* (Basilewsky), has unique food preference (Liang et al. 2001; Liang 2008). In the wild, the fry, from the initiation of feeding, feed solely on live fry of other fish species (Chiang 1959). For many years, to reduce the high production costs of carnivorous fish by domesticating them to feed on artificial diet has been a challenge (Kubitza and Lovshin 1999), and gaining deep insight into the regulation of food intake in carnivorous fish would have great significance in solving this problem. The present study was designed to improve our understanding of the neuroendocrine regulation of food intake in this economically valuable species, by cloning the complementary DNA (cDNA) sequence of *preproghrelin*, analyzing its structure, tissue distribution, and changes in mRNA expression in various tissues of animals exposed to different short-term fasting, temperatures, and photoperiods. Our results will provide new insights into the physiological roles of preproghrelin in the regulation of the growth and food intake of Chinese perch.

Materials and methods

Animals and samples

The Chinese perch (120 ± 15 g body weight) were obtained from Jiangxia National Fine Breed of Chinese Perch Farm (Jiangxia, Hubei, China) and were acclimated to natural photoperiod (11 h of light and 13 h of dark) and temperature for 2 weeks with a circulating freshwater system before the experiments. Fourteen groups of fish (7 fasted and 7 control groups) were used to evaluate the effect of short-term fasting on *preproghrelin* mRNA expression in Chinese perch. The fish were maintained separately in 42 plastic aquariums (100 × 50 × 50 cm^3) (3 aquariums per group and 8 fish per aquarium). The fish in the control groups were fed to apparent satiation with live mud fish (*Misgurnus anguillicaudatus* Cantor) once daily at 9:00 a.m., and three fish per group were randomly sampled at 0, 1, 3, 6, 12, 24, and 48 h post-feeding. The fish in fasted groups were not fed at 9:00 a.m. and then sampled at the above indicated time points. The sampled fish were anesthetized in 0.02% tricaine-methanesulfonate (MS-

222); the stomach and brain tissues were collected and stored at −80 °C until RNA isolation.

After a 2-week temporary rearing, all fish were randomly divided into four temperature groups (8, 18, 26, 32 °C, 3 aquariums per group and 8 fish per aquarium) and three photoperiod groups (continuous light (CL), continuous dark (CD), and normal light (NL) (11 h of light and 13 h of dark), 3 aquariums per group and 8 fish per aquarium), and then all the fish were fed to satiation with live mud fish once daily at 9:00 a.m. for 2 weeks. Three fish were randomly sampled from each group from the respective aquariums at 9:00 a.m. (fish in both the control group and the fasted group were fasted for 1 day before sampled, which has been used in many similar experiments), and then anesthetized in 0.02% MS-222 before tissue collection. The stomach and brain collected were immediately frozen in liquid nitrogen and stored at −80 °C until total RNA extraction. Other tissues including the cerebellum, diencephalon, telencephalon, pituitary, eye, gill, heart, pyloric caecum, liver, intestinal tract, head kidney, body kidney, gall bladder, spleen, and muscle were collected at natural temperature (18 °C) for detection of the tissue distribution of *preproghrelin*.

Cloning of *preproghrelin* in Chinese perch and tissue distribution analysis

Total RNA was extracted using Trizol® Reagent (TaKaRa, Dalian, China) and was treated with RNase-Free DNase I Water (provided in the kit) according to the manufacturer's instructions. The concentrations and integrity of the RNA were determined by a photometer (Bio-Rad, Hercules, CA, USA) fixed at 260 and 280 nm wavelengths and RNA electrophoresis, followed by storing the total RNA at −80 °C until the next step.

In order to clone Chinese perch *preproghrelin* open reading frame (ORF), primers (Table 1) were designed based on other fish *preproghrelin* sequences deposited in the NCBI GenBank (http://www.ncbi.nlm.nih.gov/genbank). PCR reaction was performed in total volume of 25 μL reaction system containing 12.5 μL dNTP Mix (Invitrogen, Carlsbad, CA, USA), 1.25 μL each of sense primer and antisense primers (10 μM), 2.5 μL cDNA, and 7.5 μL ddH_2O, under following parameters: pre-denaturation at 94 °C for 4 min, − 30 cycles of 94 °C for 30 s, 57 °C for 30 s, 72 °C for 30 s, and a final extension at 72 °C for 10 min. The PCR products were purified from agarose gel using Universal DNA Purification Kit (Tiangen, Beijing, China), and then cloned into pMD®19-T plasmid vector (TaKaRa,). The inserts were sequenced by automated sequence analysis (TaKaRa).

Table 1 Primer sequences used for cloning Chinese perch *ghrelin*

Primer name	Primer sequence(5′-3′)	Location
GHRE-1	F:CTGTTTGCTGGTCTTCC	21–37
GHRE-2	F: TGCTGGTCTTCCTGTTGT	26–43
3'RACE-RT	CTGATCTAGAGGTACCGGAT CCTTTTTTTTTTTTTT	

Structural analysis

Multiple sequence alignments were generated using the Clustal X 2.1. The cleavage site of the signal peptide was estimated using Signal P Ver. 4.0 program (http://www.cbs.dtu.dk/services/ SignalP/). A phylogenetic tree was constructed based on the amino acid sequences by the neighbor-joining method of the Clustal W (http://www.ddbj.nig.ac.jp/search/clustalw-e.html) and MEGA 6.0 program (http://www.megasoftware.net/index.html).

Real-time quantitative PCR

After the sequence was confirmed, the tissue distribution of *preproghrelin* mRNA was studied in the brain, eye, gill, heart, stomach, pyloric caecum, liver, intestinal, head kidney, body kidney, gall bladder, spleen, and muscle. The *preproghrelin* mRNA expression after 0, 1, 3, 6, 12, 24, and 48 h of fasting, as well as post-feeding, was studied in the pituitary and stomach. The *preproghrelin* mRNA expression at 8, 18, 26, and 32 °C, and at different conditions of light, was studied in the brain and stomach

Real-time PCR was carried out on the CFX Connect Real-Time System (Bio-Rad) using SYBR Green (TaKaRa). The primers used are listed in Table 2. All real-time PCR reactions were performed in triplicate. Each PCR mixture (25 μL) contained 12.5 μL of 2 × SYBR^R Premix Ex Tap™ (TaKaRa), 0.5 μL of each primer, 2.5 μL of cDNA, and 9.5 μL of RNase-Free H_2O. The PCR amplification was performed at 95 °C for 30 s, followed by 45 cycles of 95 °C for 5 s, and 60 °C for 30 s.

Table 2 Primer sequences used for Real-time quantitative RT-PCR of Chinese perch *ghrelin*

Primer name	Primer sequence(5′-3′)	Product Location	Tm (°C)
Ghre-RT2	F: GCTTTCTCAGCCCTTCAC R: GCGCTACACCGTACTCCT	86–259	57

Statistical analysis

The β-actin gene was used as a housekeeping gene in this study. The $2^{-\Delta\Delta Ct}$ method was used to determine the relative mRNA abundance for the surveyed samples (Livak and Schmittgen 2001). Treatments that do not share a common letter are significantly different from each other as determined by one-way ANOVA ($P < 0.05$). In fasting study, fish groups with an asterisk above show significant differences between the control and the experiment group.

Results

Molecular cloning of Chinese perch *preproghrelin*

From the RACE PCR, the full-length *preproghrelin* cDNA sequence was obtained. The *preproghrelin* (GenBank Accession No. KR491948) nucleotide sequence of Chinese perch was 434 bp long, containing a 321-bp ORF and a 113-bp 3′-untranslated region (3′-UTR). The deduced preproghrelin peptide was composed of 107 amino acid residues, with an N-terminal 26 amino acid signal peptide, a 20 amino acid mature peptide (GSSFLSPSQKPQNKGKPFRV), a potential amidation-proteolytic site (Gly-Arg), and a 61 amino acid C-terminus peptide of preproghrelin (Fig. 1).

Tissue distribution of *preproghrelin* mRNA in Chinese perch

Real-time quantitative PCR (RT-qPCR) was performed to analyze *preproghrelin* mRNA expression in central nervous and peripheral tissues (Fig. 2). Results showed that expression of *preproghrelin* mRNA was detected in all the tissues tested, with the highest levels observed in the stomach, followed by the pituitary and liver, moderate in the cerebellum, diencephalon, telencephalon, pyloric caecum, intestine, and spleen, and the lowest in the eye, gill, heart, head kidney, body kidney, gall bladder, and muscle.

Expression of *preproghrelin* in response to fasting

Analysis of *preproghrelin* mRNA expression in the stomach after different periods of fasting showed that, after gastric emptying (3–6 h), short-term fasting (6–12 h) increases *preproghrelin* expression when compared to control. This increase in expression was observed until 24 h of fasting; then, *preproghrelin* levels returned to basal. Before 3 h, incompletely digested prey fish were found in the stomach and preproghrelin expression was lower than basal level. In pituitary, fasting decreased *preproghrelin* expression at 1, 3, 12, and 48 h but did not exert any change at 6 and 24 h (Fig. 3).

Expression of *preproghrelin* in fish reared at different temperatures

As shown in Fig. 4, *preproghrelin* mRNA was expressed at the highest level in the brain and the stomach at 26 and 32 °C, respectively. With the temperature increasing from 8 to 32 °C, the expression level increased all the time in the stomach, but it increased until 26 °C and decreased at 32 °C in the brain.

Expression of preproghrelin at different photoperiods

The expression levels of *preproghrelin* mRNA in the stomach showed small but statistically significant differences at the different photoperiod tested. The *preproghrelin* mRNA levels under both CD and CL conditions were significantly lower than those under the NL condition (Fig. 5). Additionally, *preproghrelin* mRNA levels under CD and CL conditions did not have statistically significant differences in the brain, both of which were significantly lower than those under the NL condition (Fig. 5).

Discussion

In the present study, we obtained the full-length *preproghrelin* cDNA and predicted mature peptide in Chinese perch. The putative mature peptide of Chinese

Fig. 1 Nucleotide and deduced amino acid sequences of the Chinese perch preproghrelin. The putative signal peptide region is underlined, the mature peptide regions are represented in *gray shadow*, and the putative cleavage site is indicated by the *frame*. The translation start codon (*ATG*) and stop codon (*asterisk*) are *bold-typed*. The putativepolyadenylation signal (*ATTAAA*) is shown by the *double lines*

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ATGTTTCTGAAGAGAAACACCTGTTTGCTGGTCTTCCTGTTGTGTTCTCTGACC      54
 M  F  L  K  R  N  T  C  L  L  V  F  L  L  C  S  L  T      18
TTGTGGTGCAGGTCGACCAGTGCCGGCTCCAGCTTTCTCAGCCCTTCACAAAAA    108
 L  W  C  R  S  T  S  A  G  S  S  F  L  S  P  S  Q  K      36
CCTCAGAACAAGGGGAAGCCTTTCAGAGTCGGCCGCCAAGTCATGGAGGAGCCT    162
 P  Q  N  K  G  K  P  F  R  V  G  R  Q  V  M  E  E  P      54
AATCAACCCACTGAGGACAACCACATCACAATAAGTGCCCCCTTTGAAATTGGC    216
 N  Q  P  T  E  D  N  H  I  T  I  S  A  P  F  E  I  G      72
ATCACTATGAGAGAAGAGGACTTTGAGGAGTACGGTGTAGCGCTGCAGGAGATC    270
 I  T  M  R  E  E  D  F  E  E  Y  G  V  A  L  Q  E  I      90
GTTCAGCGTCTGCTGGGAAACACACAGACAGCAGAGGGACGATCACAACTTTGA    324
 V  Q  R  L  L  G  N  T  Q  T  A  E  G  R  S  Q  L  *     107
AGATCATGGACAAGAATTTCAAATTTGCTGTCTCAATGCCTTCCAATTTCAACTTC  380
ATTAGATAGTGATCATTAAAATGCTGAAAGCAATTAGCCCGTAAAAAAAAAAAA    434
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perch is composed of GSSFLSPSQKPQNKGKPFRV, with the first five amino acids (GSSFL) considered the "active core" (Bednarek et al. 2000). This sequence is the same as that of rainbow trout (Kaiya et al. 2003a), Japanese eel (Kaiya et al. 2003b), Mozambique tilapia (Kaiya et al. 2003c), Nile tilapia (Parhar et al. 2003), channel catfish (Kaiya et al. 2005), sea bream (Yeung et al. 2006), Pacific bluefin tuna (Suda et al. 2012), sea bass (Terova et al. 2008), Atlantic salmon (Murashita et al. 2009), orange-spotted grouper (Chen et al. 2008), and mammals (rat and human) (Kojima et al. 1999), but different from the active core (GTSFL) in goldfish

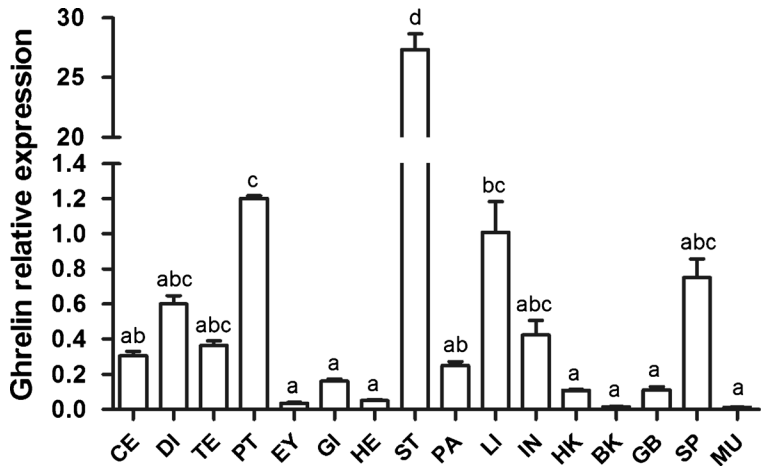

Fig. 2 Tissue distribution of *preproghrelin* mRNA in Chinese perch. The results were expressed as relative expression levels. After standardization by *β-actin* gene, *preproghrelin* mRNA levels were normalized as the average of gallbladder mRNA levels = 1. *Error bars* represent standard error of the mean ($n = 3$ fish). *CE* cerebellum, *DI* diencephalon, *TE* telencephalon, *PT* pituitary, *EY* eye, *GI* gill, *He* heart, *ST* stomach, *PA* pyloric caecum, *Li* liver, *IN* intestine, *HK* head kidney, *BK* body kidney, *GB* gall bladder, *SP* spleen, *MU* muscle. Treatments do not share a *common letter* are significantly different from each other as determined by one-way ANOVA ($P < 0.05$), like "a" and "b" are significantly different from each other, but "a" and "ab" do not have significant difference

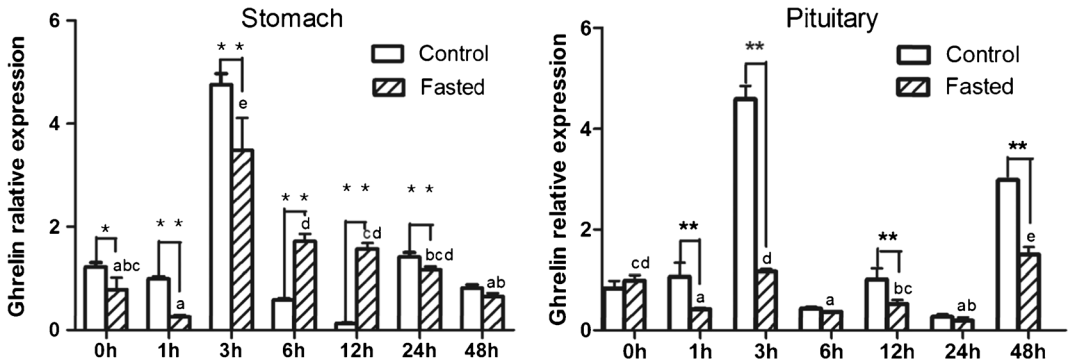

Fig. 3 The *preproghrelin* mRNA expression in the pituitary and stomach of Chinese perch during 48 h after being fed (control) or not (fasted) at time 0. Data are presented as mean ($n = 3$) mRNA copy number ± SEM normalized against *β-actin* copy numbers. Mean values with an *asterisk above* show significant differences between fish groups at a given time point ($P < 0.05$). Treatments do not share a *common letter* are significantly different from each other as determined by one-way ANOVA ($P < 0.05$), like "a" and "b" are significantly different from each other, but "a" and "ab" do not have significant difference

(Unniappan et al. 2002), zebrafish (Amole and Unniappan 2009), and grass carp (Feng et al. 2013) (Supple. File 1). Notably, in the N-terminal portion of the mature preproghrelin peptide, the first seven amino acids are highly conserved, including a serine[3] residue, which is the site of acylation, an essential modification for receptor binding and biological activity (Kojima et al. 1999). The mature preproghrelin peptides in vertebrates vary in their length—28 amino acids for rats and humans (Kojima et al. 1999), but 20 amino acids for Chinese perch, bluefin tuna (Suda et al. 2012), sea bream (Yeung et al. 2006), sea bass (Terova et al. 2008), and Nile tilapia (Parhar et al. 2003).

As in mammals, preproghrelin is primarily produced in the stomach and intestine (stomach-less) of fish, but also detected in the kidney, hypothalamus, heart, brain, and gills (Unniappan et al. 2002; Parhar et al. 2003; Kono et al. 2008). In our study, *preproghrelin* mRNA was primarily expressed in the stomach of Chinese perch, consistent with the finding in mammals (Ariyasu et al. 2001). In Chinese perch, *preproghrelin* mRNA expression level in the brain and liver was only a little lower than that in the stomach, which was similar to the results in goldfish (Unniappan et al. 2004), rainbow trout (Kaiya et al. 2003a), Japanese eel (Kaiya et al. 2003b), Mozambique tilapia (Peddu et al. 2009), hammerhead shark (Kawakoshi et al. 2007), and Atlantic cod (Xu and Volkoff 2009), indicating the important roles of stomach, brain and liver in preproghrelin secretion. Furthermore, the expression of *preproghrelin* mRNA was detected in all the tissues studied, similar to previous studies on *preproghrelin* mRNA in human tissues (Gnanapavan et al. 2002) and the tissues of several fish species (Kaiya et al. 2008).

The effect of fasting on the expression level of *preproghrelin* mRNA was studied in zebrafish, and in both the gut and brain the expression level increased significantly by fasting, but declined sharply after re-

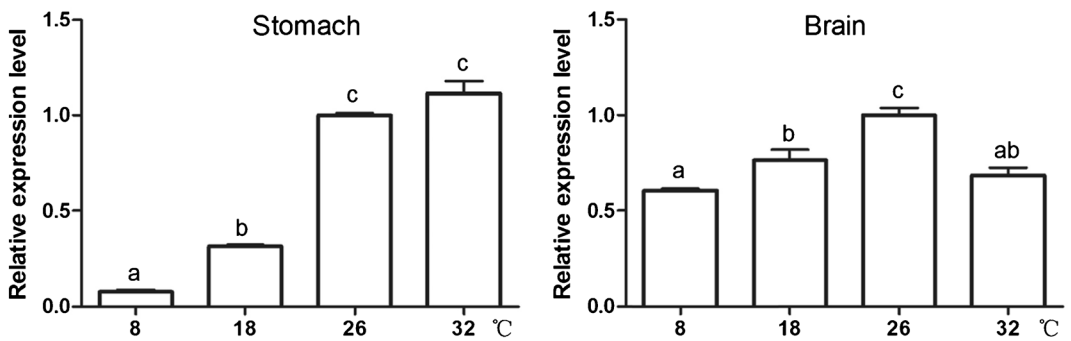

Fig. 4 Expression of *preproghrelin* mRNA in the brain and stomach of Chinese perch maintained at different temperatures. Data are presented as mean ($n = 3$) mRNA copy number ± SEM normalized against *β-actin* copy numbers. Mean values with *different letters* show significant differences between fish groups at a given tissue ($P < 0.05$)

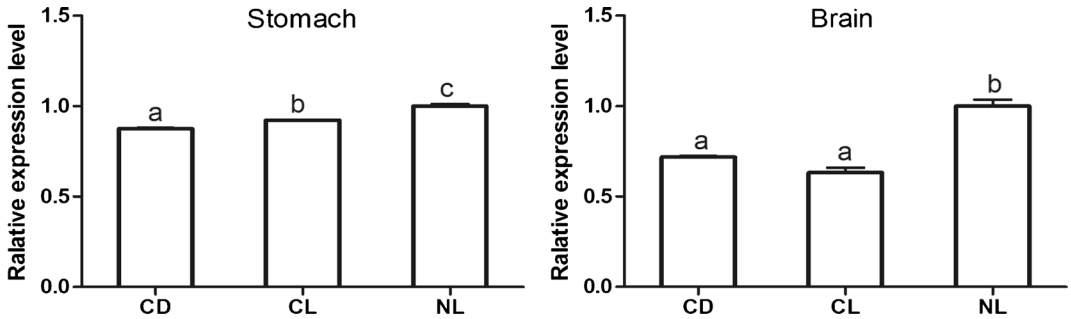

Fig. 5 Effects of different photoperiods on stomach and brain *preproghrelin* mRNA expression in Chinese perch. *CL* continuous light, *CD* continuous dark, *NL* normal light. Data are presented as mean ($n = 3$) mRNA copy number ± SEM normalized against *β-actin* copy numbers. Mean values with *different letters* show significant differences between fish groups at a given tissue ($P < 0.05$)

feeding (Amole and Unniappan 2009). In Chinese perch, the potential role of preproghrelin in the regulation of appetite and food intake was further supported by fasting-induced up-regulation of *preproghrelin* mRNA expression in the stomach from 6 to 12 h fasting. At 3 h fasting, incompletely digested prey fish were found in the stomach and *preproghrelin* expression was low. After 3 h fasting, *preproghrelin* in the stomach increased significantly. This can also be seen in goldfish (Unniappan et al. 2004) and sea bass (Terova et al. 2008), but no change is observed in rainbow trout (Jönsson et al. 2007). No effect on *preproghrelin* mRNA by fasting or re-feeding was found in Nile tilapia and Atlantic cod (Parhar et al. 2003; Xu & Volkoff 2009). These results suggest that the preproghrelin physiology varies in fish. In the present study, the *preproghrelin* mRNA expression in the stomach of Chinese perch was more sensitive (relative expression level from 0.3 to 3.5) to fasting than that in the pituitary/brain (relative expression level from 0.2 to 1.7). During the entire process of fasting, *preproghrelin* expression in the pituitary presented state fluctuation of self-adjustment with up and downregulation.

To further improve the growth rate of fish, it is necessary to increase the food intake, which is closely related with two important environmental factors: temperature and photoperiod (Volkoff et al. 2009). However, the individual effects of the two factors are very difficult to determine because fish is subjected to seasonal cycles and both parameters vary under natural conditions.

In this study, the *preproghrelin* mRNA expression was higher in both the stomach and the brain at 26 °C than at a lower temperature, but lower in the brain at 32 °C than at 26 °C, suggesting that the higher the temperature is, the more the preproghrelin expression is in the stomach and the stomach has a higher optimal temperature than the brain for preproghrelin expression. In some fish species, food consumption and growth rates tend to increase with rising temperature (Bendiksen et al. 2002; Sunuma et al. 2007). The food intake of grass carp is also regulated by water temperature (Wen et al. 1998). In the burbot, fasting decreases plasma preproghrelin-immunoreactive peptide levels at 2 °C but not at 10 °C (Nieminen et al. 2003). Picha et al. (2009) reported that blood preproghrelin of hybrid striped bass increases significantly by fasting at 14 and 24 °C. Xu and Volkoff (2009) found that during fasting, *preproghrelin* mRNA expression remained unchanged during catabolism in Atlantic cod, but plasma preproghrelin-immunoreactive peptide levels decrease in linear relation with the temperature at 20 °C. Two recent studies on Atlantic salmon generated conflicting results. In one study, when the Atlantic salmon were kept at different temperatures for 3 months, their growth and food intake are decreased at 19 °C compared to fish at 14 °C (Hevrøy et al. 2012) while in the other study, the Atlantic salmon showed no significant difference in plasma preproghrelin levels at three different temperatures (8, 12, and 18 °C) (Kullgren et al. 2013). In summary, high temperature increases appetite to some extent in most cases, but some cold water fish adapt to low temperature and have stronger appetite in a low temperature.

Additionally, both continuous dark and continuous light reduced *preproghrelin* mRNA expression compared to the normal light (Fig. 5). Previous studies showed that feeding activity is affected by photoperiod and light regimens in a number of fish species including European sea bass (Sanchez-Vazquez et al. 1998),

yellowtail (Kohbara et al. 2000), and barfin flounder (Sunuma et al. 2007). However, Atlantic cod showed no obvious differences in neither food intake nor *preproghrelin* mRNA expression under different photoperiods (24 h light, 24 h dark, 16 h light: 8 h dark) (Xu and Volkoff 2009). Above studies indicated that photoperiod may affect food intake, but this effect is species-specific and may have relationship with surroundings.

The endocrine mechanism for the regulation of feeding by environmental factors (fasting, temperature, and photoperiod) is interactive. Future studies should focus on the interaction of these three key factors as well as tissue-specific effects on *preproghrelin* mRNA expression to further understand the physiological roles of preproghrelin in Chinese perch.

Conclusion

In this study, we characterized the structure of *preproghrelin* cDNA, the deduced mature peptide and tissue expression of *preproghrelin* mRNA in Chinese perch. The widespread distribution of *preproghrelin* mRNA in tissues implicated in the regulation of metabolism (such as the stomach, brain, liver, and spleen) suggested that preproghrelin might play an important role in energy balance in Chinese perch. Interactions between environmental factors and the mechanisms of appetite control are fundamental to the development of practical approaches to optimize feed intake. Fasting-induced increase of *preproghrelin* mRNA expression in the stomach suggests an orexigenic role of preproghrelin in Chinese perch. Higher temperature increased the level of *preproghrelin* mRNA expression in the stomach, which had a higher optimal temperature (32 °C) than the brain (26 °C). Additionally, the normal light is an appropriate photoperiod for Chinese perch growth. These findings suggest that short-term fasting of 6 h after gastric emptying, 26 °C, and normal light would be appropriate for culturing Chinese perch. The overall results from this study provide some insights into *preproghrelin* mRNA expression of Chinese perch in response to fasting, temperature, and photoperiod as well as useful information for culturing this valuable fish species.

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