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The transcripts of CRF and CRF receptors under fasting stress in Dabry's sturgeon (*Acipenser dabryanus* Dumeril)

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ABSTRACT

Dabry's sturgeon (*Acipenser dabryanus* Dumeril, 1868) belongs to Sturgeon and is distributed throughout the mainstream of the upper Yangtze River. While there is little research on physiological mechanism of Dabry's sturgeon, such as feeding regulation by the CRF system. At present, CRF is thought to regulate feeding via CRF receptors (CRF-Rs) in several mammals, but relatively few studies of CRF and feeding exist in teleosts. Herein, the transcripts of CRF and CRF-Rs under fasting stress in Dabry's sturgeon (*Acipenser dabryanus* Dumeril) have been explored. A full length Dabry's sturgeon CRF cDNA of 953 bp was identified, which contained a 447 bp open reading frame (ORF). A partial CRF-R1 cDNA of 1053 bp and CRF-R2 cDNA of 906 bp corresponding to the coding sequences (CDS) was obtained. In addition, analysis of the tissue distribution of CRF and CRF-Rs mRNAs revealed they were widely distributed in the central and peripheral nervous systems. Furthermore, periprandial (preprandial and postprandial), fasting, and re-feeding experiments revealed CRF mRNA was significantly increased 1 h and 3 h after feeding and CRF and CRF-Rs transcripts were significantly decreased after 10 days fasting, and significantly increased on re-feeding on day 10. These results suggest that CRF and CRF-Rs might regulate feeding by acting as satiety factors.

1. Introduction

Feeding plays a critical role in providing nutrition and energy, which accelerates the growth and development of fish (Saper et al., 2002). Feeding is affected by various extrinsic (light, temperature, stress, and the palatability of food) and intrinsic factors (Hoskins and Volkoff, 2012; Volkoff et al., 2010). The brain and other tissues (e.g. intestine, eye, gills) are connected by a network of peptides and hormones modulate hunger and satiety signals (Matsuda, 2009; Volkoff et al., 2010). An intricate network of factor regulates feeding and includes the CRF system.

The CRF system is comprised of six related neuropeptides, CRF (Vale et al., 1981), sauvagine (SVG) (Chang et al., 1993), urotensin I (UI) (Lederis et al., 1982), and orthologs of urocortin 1, 2, and 3 (UCN1, UCN2 and UCN3) (Lewis et al., 2001; Reyes et al., 2001; Spina et al., 1996), two main G protein-coupled receptors, CRF-R1 and CRF-R2 (Chang and Hsu, 2004; Pohl et al., 2001; Vita et al., 1993) and a binding protein, CRF-BP (Doyon et al., 2005; Huising et al., 2004). CRF, a major peptide and a paralogue of UCN1, was first found in 1995 (Saffran and Schally, 1955). Additionally, many studies identifying UCN1 and UI and their role in feeding regulation exist, and in a previous study we reported another CRF-related peptide (UCN3) in the Siberian sturgeon (*Acipenser baerii*) (Zhang et al., 2016). At present, CRF has been identified in the brain or hypothalamus in many vertebrates, including mammals (Furutani et al., 1983; Patthy et al., 1985; Seasholtz et al., 1991; Shibahara et al., 1983; Thompson et al., 1987; Vale et al., 1981), birds (Vandenborne et al., 2005), amphibians (Stenzel-Poore et al., 1992), and teleosts (Ando et al., 1999; Bernier et al., 1999; Chandrasekar et al., 2007; Chen and Fernald, 2008; Doyon et al., 2003; Huising et al., 2004; Lu et al., 2004; Okawara et al., 1988; Wang et al., 2014). In animals, CRF acts when it binds to two similar, yet functionally distinct, G protein-coupled receptors (CRF-R1 and CRF-R2) (Grammatopoulos, 2012). Two primary receptors have been reported in several species of fish (Arai et al., 2001; Chen and Fernald, 2008; Huising et al., 2004; Pohl et al., 2001). However, the identification of CRF and CRF-Rs has only been reported in two fish, the

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common carp (*Cyprinus carpio*) (Huising et al., 2004) and cichlid (*Haplochromis burtoni*) (Chen and Fernald, 2008).

The function of the CRF system in feeding has been reported in some vertebrates including fish. Central or peripheral injection of CRF system-related peptides can decrease food intake in the rainbow trout and Siberian sturgeon, respectively, which suggests that CRF system peptides may have an anorexic function (Ortega et al., 2013; Zhang et al., 2016). Additionally, downregulation of the transcription of CRF during fasting stress is reported in mice (Yadawa and Chaturvedi, 2016), frogs (Prater et al., 2018), and several fish (Maruyama et al., 2006; Wang et al., 2014). Although CRF and CRF-Rs have been discovered in fish species with sequenced genomes there are relatively few studies of their function in fish (except in goldfish (*Carassius auratus*) and Ya-fish (*Schizothorax prenanti*)) (Maruyama et al., 2006; Wang et al., 2014). Moreover, as fish have different feeding habits are extremely diverse, have complex genetic backgrounds, and a broad variety of habitats, studies of the CRF system in a diversity of fish is necessary (Volff, 2005). So far only a single CRF-related peptide, UCN3 has been reported to regulate feeding in sturgeon (Zhang et al., 2016). In the present study we explore other CRF-related peptides and two major receptors to enrich knowledge about regulation of feeding by the CRF system in fish, such as the sturgeon.

Dabry's sturgeon (*Acipenser dabryanus* Dumeril, 1868) is distributed throughout the mainstream and tributaries of the upper Yangtze River (Zhuang et al., 1997). Dabry's sturgeon has an important ecological role in the river ecosystems, which makes its protection important (Chen, 2007; Zhou et al., 2014). Thus, it is necessary to study the physiological mechanisms of Dabry's sturgeon to provide a theoretical basis for species protection. However, there is little known about the physiological mechanisms of Dabry's sturgeon, in particular in relation to regulation of feeding.

To determine the change in transcripts of the CRF system under fasting stress in Dabry's sturgeon, the cDNA sequences of CRF and CRF-Rs were cloned, and the tissue distribution of these genes was assessed in Dabry's sturgeon for the first time. Furthermore, the transcriptional response of the CRF system in the brain in periprandial (preprandial and postprandial), fasting and re-feeding experiments were performed, with an aim of investigating the possible roles of CRF and CRF-Rs in appetite regulation in Dabry's sturgeon.

2. Material and methods

2.1. Animals

Dabry's sturgeon were purchased from the Fisheries Institute of Sichuan Academy of Agricultural Sciences (Yibin, China). Juvenile fish (n = 102) were acclimated in three indoor tanks (dimensions = 0.80 m*1.00 m, volume = 0.50 m^3, flow rate = 0.12 m/s) in the Aquaculture Laboratory of Sichuan Agricultural University (Chengdu, China). The tanks were supplied with constantly aerated and filtered water at 13 ± 1 °C and maintained under a natural photoperiod (12 h light: 12 h dark). Fish were fed to satiety with commercial sinking pellets (nutrient content: crude protein ≥ 40%, crude fat ≥ 12%, coarse fiber ≤ 6%, crude ash ≤ 18%, water ≤ 18% and total phosphorus ≥ 1.2%; Tongyi, Suzhou, China) in an excess of 2% body weight (BW) at 16:00 once a day. Before the experiments, Dabry's sturgeon were acclimated to the experimental circuit for more than two weeks to minimize stress.

All animal handling procedures were approved by the Animal Care and Use Committee of Sichuan Agricultural University under permit No. DKY-S20160417, and followed the guidelines for animal experiments of Sichuan Agricultural University.

2.2. Experimental design

Prior to sampling of the fish used for CRF system cDNA cloning and tissue distribution experiments, fish were deprived of food for 24 h. 6 Dabry's sturgeon (251.47 ± 12.75 g) were randomly selected from the experimental circuit and were anesthetized in 0.02% tricaine methanesulfonate (MS-222), weighed and killed by spinal section. Taking into consideration the reported role of CRF in feeding and feed regulation , whole brain and peripheral tissue, the eye, gills, esophagus, stomach, rectum, duodenum, intestine valvular, pyloric caeca, kidney, spleen, liver and pancreas were collected, washed with sterile saline, snap-frozen in liquid nitrogen, and stored at −80 °C until total RNA isolation.

For fasting stress experiments, 96 Dabry's sturgeon (239.12 ± 5.43 g) were placed in three experimental tanks. In the control tank the fish were fed (1–10 days), and in the remaining two tanks the fish were fasted (1–10 days) and re-fed (on the 10th day), respectively. In the periprandial (preprandial and postprandial) experiment, standard feeding procedures were followed for the "fed" tank and the fasted tank was deprived of food. The whole brain was sampled at 3 h (13:00, −3h) and 1 h (15:00, −1h) prior to feeding, 0 h at start feeding (16:00, 0 h), 1 h (17:00, +1h) and 3 h (19:00, +3h) after feeding. In the fasting and re-feeding experiments, the fish in the "fed" tank and "fasted" tank were provided with food or food was withheld for 10 days, and they were sampled 1 h after feeding (16.00 h) on the days the fish were sampled (3rd-, 6th- and 10th-day). 6 fish per tank were sampled at each time point. After fasting or food restriction, the metabolic rate was increased when adequate food was provided, and metabolite levels were restored to pre-starvation values. To compensate for a lack of nutrients during fasting, the "re-fed" treatment was performed. The whole brain, which regulates feeding and energy balance was sampled and preserved as described above (Stachniak et al., 2014). There were no obvious differences in feeding behavior in any of the fish stocked in the experimental tanks before the experiments

2.3. RNA extraction and cDNA synthesis

Total RNAs were isolated from Dabry's sturgeon tissue samples using a Spin Column Animal Total RNA Purification Kit (Sangon Biotech, Shanghai, China) following the manufacturer's protocol. Final RNA concentrations were determined by 1.0% agarose gel electrophoresis. The optical density reading at 260 nm using a NanoDrop 2000 spectrophotometer (Thermo Scientific, MA, USA) and qualities of RNA samples were assessed by measuring the ratio of sample absorbance at 260 and 280 nm. Only RNA samples with a ratio between 1.8 and 2.1 were used for cDNA synthesis.

1 μg total RNA was reverse-transcribed (RT) into cDNA using the PrimeScript™ RT Reagent Kit (Takara, Dalian, China). First-strand cDNA of the whole brain with 5′ or 3′ adaptors added was synthesized using SMART RACE cDNA Amplification Kit (BD Biosciences Clonetech, Palo Alto, CA, US) for the rapid amplification of cDNA ends (RACE).

2.4. Cloning CRF and its two receptors

For cloning Dabry's sturgeon CRF and its two receptors (CRF-R1 and CRF-R2), core fragment primers and 5′ and 3′ RACE primers (Table 1) were designed using Primer premier 6.0 usingwhole brain or intestine transcriptome data of Siberian sturgeon (unpublished). 5′ and 3′ RACE primers were designed to amplify the core sequences. PCR methods and procedures were as previously (Lin et al., 2014).

CRF and CRF-Rs nucleotide sequences and deduced protein sequences were analyzed by employing BLASTn and BLASTp (http://www.ncbi.nlm.nih.gov). The ORF was predicted using the Open Reading Frame Finder (http://www.ncbi.nlm.nih.gov/ gorf /gorf.html). The cleavage site of the signal peptide was predicted using the SignalP 4.1 Server (http://www.cbs.dtu.dk/services/SignalP/). Multiple alignments of amino acid sequences and phylogenetic analysis were performed using MEGA 7.0 software and themaximum likelihood method with default settings (http://www.megasoftware.net/index.

Table 1
Primer sequences used in cloning and gene expression studies.

Primer	Sepuence(5′-3′)	Applications
CRF-F	GAATCAAATGGAGAAAGACGAG	CRF-cloning
CRF-R	GCCCGAGTATCAAAATCAGAC	CRF-cloning
CRF-F1	GGCCCAGAAGGCTTTGAGCAATAA	CRF-3′ RACE outer
CRF-F2	ACGTTTCAAAGCTGGAACTGCGAACC	CRF-3′ RACE inner
CRF-R1	AGGTTCGCAGTTCCAGCTTTGAAACG	CRF-5′ RACE outer
CRF-R2	GTTGGGTTTGCCGTCACGCCTTTT	CRF-5′ RACE inner
CRFR1-F-1	AGTGCCAGGAAATACTAAACGAAGA	CRFR1-cloning (1)
CRFR1-R-1	TCAAGAAGCAGTAAAACACAGAGAC	CRFR1-cloning (1)
CRFR1-F-2	ACAAAACTTCGCGCCTCAAC	CRFR1-cloning (2)
CRFR1-R-2	AGTTGAGGCGCGAAGTTTTG	CRFR1-cloning (2)
CRFR2-F	GCAATGAGCCCTGGTGTC	CRFR2-cloning
CRFR2-R	TGGAGAGGTTGGGATGGA	CRFR2-cloning
CRF-qF	CTGGAACTGCGAACCTACATCT	CRF-qPCR
CRF-qR	GCCCGAGTATCAAAATCAGACA	CRF- qPCR
CRFR1-qF	CATGAGAGCAATGTGATCTGGTG	CRFR1- qPCR
CRFR1-qR	AGGACGATGGCAGTATGAAGGT	CRFR1- qPCR
CRFR2-qF	CTGCGGTGAGGAAAAGATGG	CRFR2- qPCR
CRFR2-qR	TGTGGATTGCTTGATGCTGTG	CRFR2- qPCR
β-actin-qF	GCCCCACCTGAGCGTAAATA	β-actin-qPCR
β-actin-qR	CCTGCTTGCTGATCCACATC	β-actin-qPCR
EF1α-qF	AAGATTGACCGTCGTTCCG	EF1α-qPCR
EF1α-qF	ATGTTCACAATGGCAGCGTC	EF1α- qPCR

html). The reliability of the phylogenetic tree was assessed using 1000 bootstrap replicates.

2.5. Quantitative real-time PCR analysis

Total RNA of the samples collected to analyse the tissue distribution of the CRF system, periprandial and in fasting, and re-feeding experiments were isolated and reverse transcribed as described above. In order to analysis the transcript expression of CRF, CRF-R1, and CRF-R2, cDNA samples were diluted 1:3 in nuclease-free water and specific primers for qPCR were designed basing on the cloning sequences (Table 1).

β-actin, 18S, EF1-α (elongation factor) and GAPDH (glyceraldehyde-3-phosphate dehydrogenase) were evaluated as reference gene candidates. β-actin and EF1-α were used as reference genes as their expression presented negligible variation between tissues and treatments (as seen by similar Ct values).

Procedures and methods have been described previously (Lin et al., 2014). For all standard curves, the primer amplification efficiencies of CRF, CRF-R1, CRF-R2, β-actin and EF1-α were from 90.4% to 115.5%, and R^2 were from 0.992 to 1.000 (Table). The relative expression analyses of the target genes were normalized using the reference genes (geometric averaging of β-actin and EF1-α Ct values) in the same sample via the $2^{-\triangle\triangle Ct}$ method.

2.6. Statistical analysis

All data were expressed as mean ± SEM. Statistical analyses were performed using SPSS (version 21.0) statistical software (SPSS Inc., Chicago, IL, USA). Student's t-tests were used for the comparison between two groups. For multiple group designs, one-way ANOVA analyses was used followed by an *LSD* post-hoc test after confirming normal distribution of the data. "LSD", least significant difference, is an extended Student's *t* test which uses a more sensitive estimate of error in the data for making statistically valid mean comparisons. $P < 0.05$ was considered to be statistically significant.

Table. 2
The primer amplification efficiencies and R^2 used in gene expression studies.

Primer	Amplification efficiencies	R^2
CRF	102.0%	0.996
CRF-R1	97.5%	0.995
CRF-R2	96.5%	0.992
β-actin	115.5%	0.999
EF1α	90.4%	1.000

3. Result

3.1. Molecular cloning of Dabry's sturgeon CRF and CRF-Rs

cDNA for the coding sequences of the CRF (GenBank accession number: MK434204), CRF-R1 (GenBank accession number: MK434206) and CRF-R2 (GenBank accession number: MK434205) of Dabry's sturgeon was cloned from whole brain by RACE. The full-length cDNA sequence of CRF was 953 base pairs (bp) and included a 42 bp 5′ untranslated region (5′UTR), a 447 bp open reading frame (ORF) that encoded a protein of 149 amino acids, and a 3′ untranslated region (3′UTR) of 464 bp that contained a putative AATAAA polyadenylation signal and a poly (A) tail. Furthermore, the putative 149 amino acid precursor protein of CRF had a 20-amino acid signal peptide, a cryptic region, and a 41-amino acid mature peptide (Fig. 1A). The partial coding sequences (CDS) of two G-protein-coupled receptors (CRF-R1 and CRF-R2) were cloned, but it was not possible to clone CRF-R3 in Dabry's sturgeon. A 1053 bp partial CDS of CRF-R1 and 906 bp partial CDS of CRF-R2 were cloned and encoded 351 amino acids (Fig. 1B). and 302 amino acids (Fig. 1C), respectively. The predicted transmembrane (TM) domains of the cloned G-protein-coupled receptors are shown in Fig. 1B and 1C.

The deduced amino acid sequences of Dabry's sturgeon CRF (Fig. 2A), CRF-R1 (Fig. 2B), and CRF-R2 (Fig. 2C) were moderately conserved compared to other species. In the phylogenetic trees, Dabry's sturgeon CRF clustered alone, which may be related to the special evolutionary status of the sturgeon in the history (Fig. 3A). CRF-R1 and CRF-R2 did not cluster together in the phylogenetic tree and CRF-R1 clustered with the Cyprinidae while CRF-R2 clustered with chicken and frog (Fig. 3B).

3.2. Tissue distribution of Dabry's sturgeon CRF and CRF-Rs

CRF mRNA expression was detected in all sampled tissues (Fig. 4A). High mRNA levels of CRF were observed in the whole brain, esophagus, stomach, duodenum, and pyloric caeca.

Within the whole brain, CRF-R1 and CRF-R2 mRNA expression was detected. The most abundant levels of CRF-R1 transcripts were detected in the stomach, pyloric caeca, and gill (Fig. 4B). CRF-R2 mRNA was most abundant in the stomach and lower levels were detected in the whole brain (Fig. 4C).

3.3. Effects of fasting and re-feeding on body weight (BW) change in Dabry's sturgeon

The change in BW of Dabry's sturgeon for 10 days fasting is shown in Fig. 5. BW showed an 8% increase in the fish that were fed for 10 days, and a 14% decrease in fish fasted for 10 days.

3.4. Effects of periprandial experiment on CRF expression in Dabry's sturgeon brain

CRF mRNA expression in the fasted and refed fish was significantly decreased 1 h after feeding ($P < 0.05$), and remarkably decreased 3 h after refeeding ($P < 0.01$). There was no obvious difference of CRF mRNA in the fasted fish at any of the time points analyzed (–3h, −1h, 0, +1h, +3h) (Fig. 6).

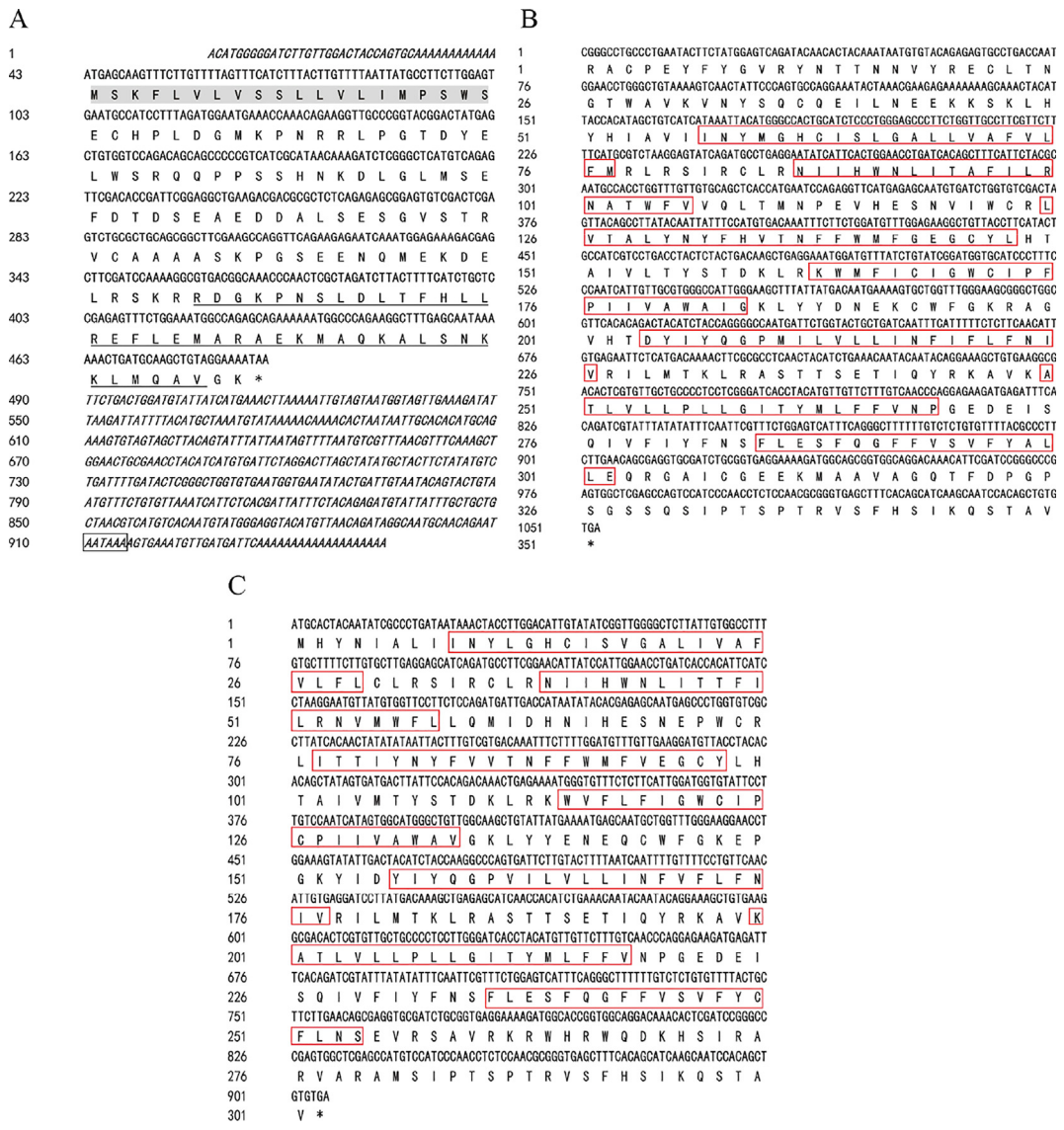

Fig. 1. cDNA sequence and deduced amino acids for A. *dabryanus* CRF (A), A. *dabryanus* CRF-R1 (B), and A. *dabryanus* CRF-R2 (C). 3′ and 5′ untranslated regions are in *italics*. Amino acids coding for the putative signal peptide is shaded. Amino acids coding for the mature peptide are in underline. The polyadenylation signal in 3′-UTR is shown in black box. The seven transmembrane domain regions (TM) for each receptor sequence is in red boxes. The stop condon is indicated by asterisk (*).

3.5. Effects of fasting and re-feeding on CRF and CRF-Rs expression in Dabry's sturgeon brain

CRF expression was decreased in the fasted fish during the experiment. On the 3rd and 6th day of fasting a significant decrease of CRF mRNA expression occurred in relation to the fed control fish ($P < 0.05$). On the 10th day, CRF mRNA expression in fasted and re-fed fish sharply decreased ($P < 0.001$). There were no significant differences in CRF mRNA levels in the fasted fish, but in fed fish up-regulation of CRF mRNA occurred in fed fish during the experiment (Fig. 7A).

CRF-R1 transcripts were significantly decreased after 3 days fasting ($P < 0.05$), but were not significantly different on day 1, 6 or 10 between fasted and re-fed fish or between re-fed and fed fish on day 10 (Fig. 7B). CRF-R2 mRNA expression was significantly decreased on the 3rd day of fasting ($P < 0.05$) and was even lower on the 10th fasting day ($P < 0.01$). Re-feeding induced a remarkable decrease in CRF-R2 mRNA expression in the re-fed fish compared to the fed fish ($P < 0.01$), and had an increased CRF-R2 mRNA expression compared to the fasted tank (Fig. 7C).

4. Discussion

In the current research, Dabry's sturgeon CRF and CRF-R sequences were obtained for the first time. Dabry's sturgeon full-length CRF cDNA was 953 bp and consisted of a 447 bp ORF (Fig. 1A). CRF cloning has also been reported in other several fish (Chandrasekar et al., 2007; Lu et al., 2004; Van Enckevort et al., 2000; Wang et al., 2014). However, two subtypes of CRF (CRF1 and CRF2) have been identified in common carp (Huising et al., 2004), white sucker (*Catostomus commersoni*) (Okawara et al., 1988), sockeye salmon *(Oncorhynchus nerka)* (Ando et al., 1999), goldfish (Bernier et al., 1999), and rainbow trout (*Oncorhynchus mykiss*) (Doyon et al., 2003). Thus, some teleosts have duplicated copies of CRF genes, although it is currently unclear if all teleost fish lineages have CRF gene duplication. In our study, a single CRF isoform was identified with Dabry's sturgeon, suggesting that Dabry's sturgeon may have lost one of the copies of the CRF gene. Furthermore, two partial cDNAs of Dabry's sturgeon CRF-R1 and CRF-R2 were cloned (Fig. 1B and 1C). In common with our results, CRF-R1 and CRF-R2 have also been cloned in other fish, like the chum salmon (*Oncorhynchus keta*) (Pohl et al., 2001), catfish (*Ameiurus nebulosus*) (Arai et al., 2001), and cichlid (Chen and Fernald, 2008). However, in

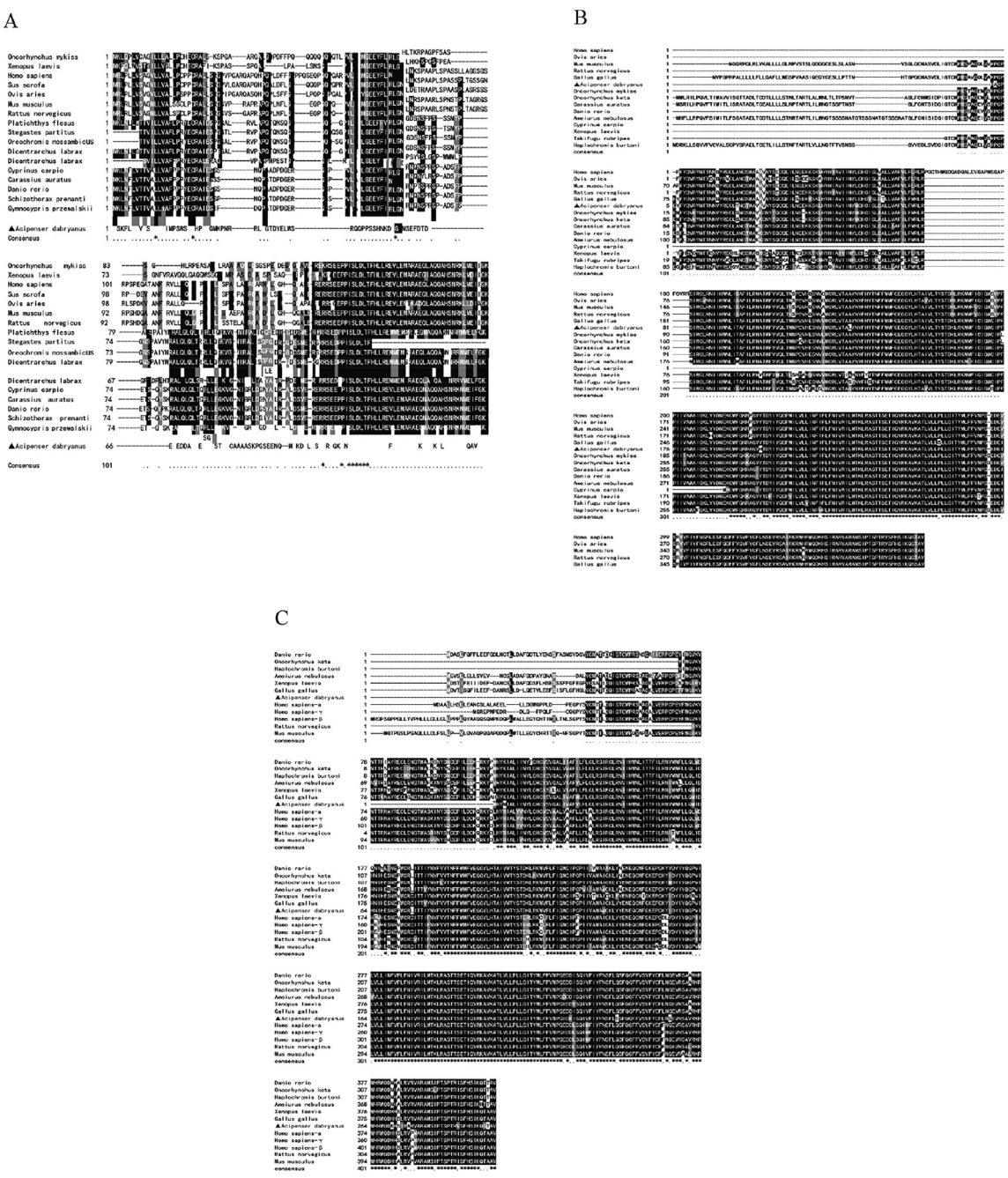

Fig. 2. Amino acid sequence alignments for A. *dabryanus* CRF (A), and A. *dabryanus* CRF-R1 (B) and CRF-R2 (C). In alignments, black shading indicates identical amino acids and white background indicates unrelated amino acids. GenBank accession numbers of CRF and CRF-Rs are as follows: A. *dabryanus* CRF, MK434204; A. *dabryanus* CRF-R1, MK434206; A. *dabryanus* CRF-R2, MK434205.

common carp (Huising et al., 2004) and fugu (*Takifugu rubripes*) (Cardoso et al., 2003) only a CRF-R1 was identified, and only in the catfish has a third form of CRF receptors (CRF-R3) been identified (Arai et al., 2001). In our studies, Darby's sturgeon CRF-R1 and CRF-R2 were successfully cloned. Multiple amino acid sequence alignments showed that Dabry's sturgeon CRF, CRF-R1, and CRF-R2 shared a high identity with other vertebrate forms (Fig. 2). Phylogenetic analysis showed that Dabry's sturgeon CRF clustered alone, and Dabry's sturgeon CRF-R1 was clustered with Cyprinidae CRF-R1 while CRF-R2 was clustered with chicken and frog CRF-R2 (Fig. 3), which may be related to the special evolutionary status of the sturgeon in the history (Alexandrou et al., 2013).

To explore whether CRF and CRF-Rs were distributed in tissues that relate to feeding regulation in Dabry's sturgeon quantitative PCR was performed. CRF was distributed widely in the brain and peripheral tissue (e.g. gut and gills) , and there was an especially high level of CRF mRNA in the entire brain of Dabry's sturgeon (Fig. 4A), which is similar to what is found in the flounder (*Platichthys flesus*) (Lu et al., 2004), zebrafish (*Danio rerio*) (Chandrasekar et al., 2007), and Ya-fish (Wang et al., 2014). The brain significantly regulates feeding behavior and energy balance (Stachniak et al., 2014). Therefore, the levels of CRF in the brain suggest that CRF might be involved in the central regulation of feeding in Dabry's sturgeon. Furthermore, via the detection of CRF transcripts in different brain regions, it was discovered that CRF was expressed abundantly in the hypothalamus of the common carp (Huising et al., 2004), goldfish (Bernier et al., 1999), rainbow trout (Doyon et al., 2003), flounder (Lu et al., 2004), and Ya-fish (Wang et al., 2014). In the present study, the whole brain was sampled to

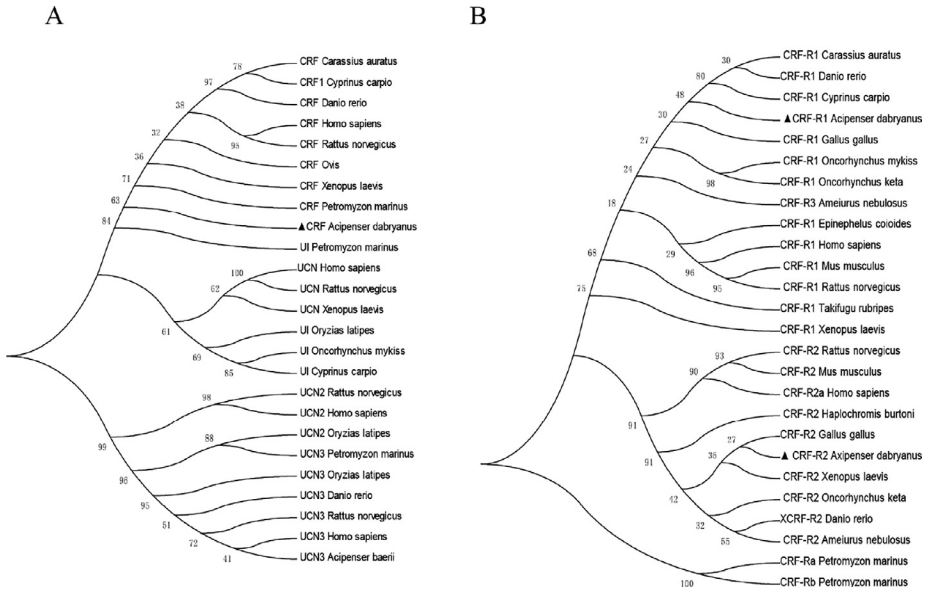

Fig. 3. Phylogenetic trees for A. *dabryanus* CRF family member pre-propeptides(A), and A. *dabryanus* CRF-R1 and CRF-R2 (B). The phylogenetic trees was inferred using the neighbor-joining, and bootstrap values (1000 replicates) are given at each node. The CRF system were used a secretin-based system as an outgroup.

assess the distribution of CRF and CRF-Rs mRNA. CRF-R1 and CRF-R2 were not only expressed in the whole brain but were also abundantly expressed in the stomach, pyloric caeca, and gill of Dabry's sturgeon (Fig. 4B and C). Similar to our results, an abundant expression of CRF-Rs in the brain has also been reported in some mammals (Vita et al., 1993) and species of fish (Arai et al., 2001; Chen and Fernald, 2008; Huising et al., 2004), suggesting that central CRF-Rs might also be closely associated with the regulation of food intake. Additionally, the distribution of CRF-Rs in the gastrointestinal tract (stomach) has been reported in several mammals (Porcher et al., 2006; Yuan et al., 2012a, 2012b) and the catfish (Arai et al., 2001), which indicates that CRF-Rs may also have peripheral actions on both gastrointestinal motility and the regulation of feeding. Because CRF has a high affinity for CRF-R1 and CRF-R2 in mammals, diverse functions of the CRF system in regulating biological functions, including locomotor activity, energy homeostasis, and even feeding behavior, have been reported (Larauche, 2012; Volkoff, 2016). The present study showing the localization in the same tissue of CRF and its receptors suggests it may bind to CRF-Rs in

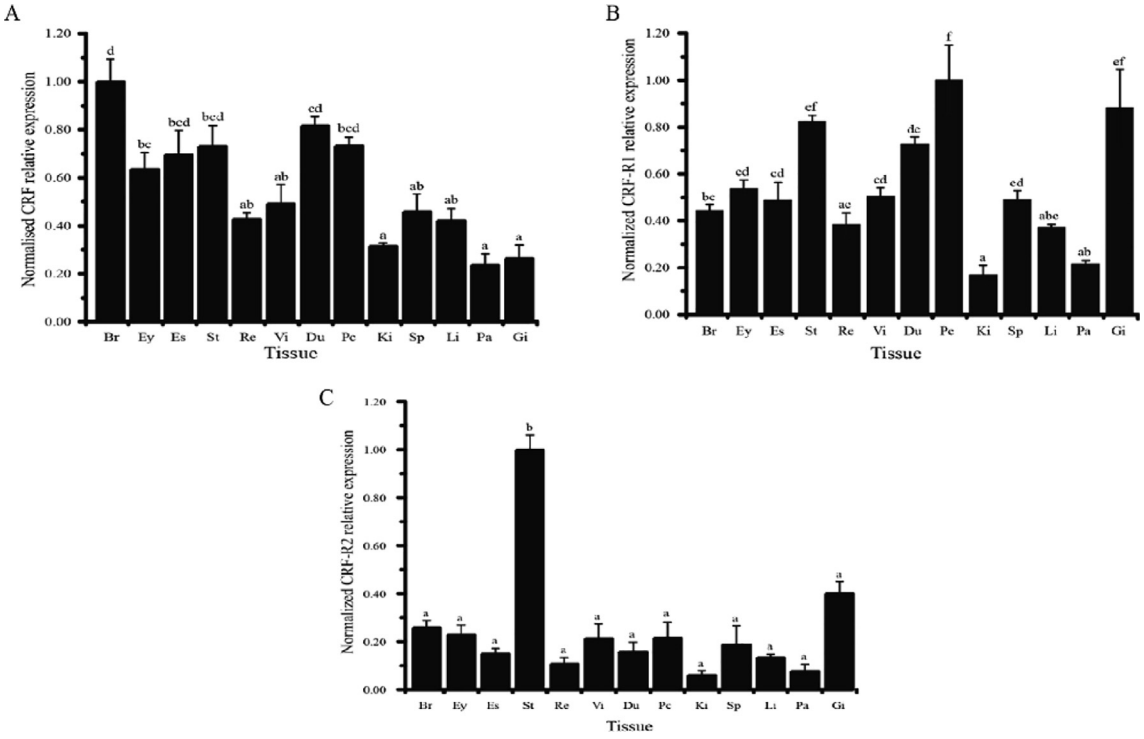

Fig. 4. Tissue distribution for CRF (A) and its two receptors, CRF-R1 (B) and CRF–R2 (C) in A. *dabryanus*. Results were expression as relative expression levels after standardization by β-actin and EF1-α, and the highest level of target gene mRNA expression was set equal to 1.0. Bars with different letters represent significant differences between unfed groups (ANOVA, $P < 0.05$). Br, whole brain; Ey, eye; Es, esophagus; St, stomach; Re, rectum; Vi, valvular intestine; Du, duodenum; Pc, pyloric caeca; Ki, trunk kidney; Sp, spleen; Li, liver; Pa, pancreas and Gi, gill. Data are means ± SEM; n = 6 per tissue.

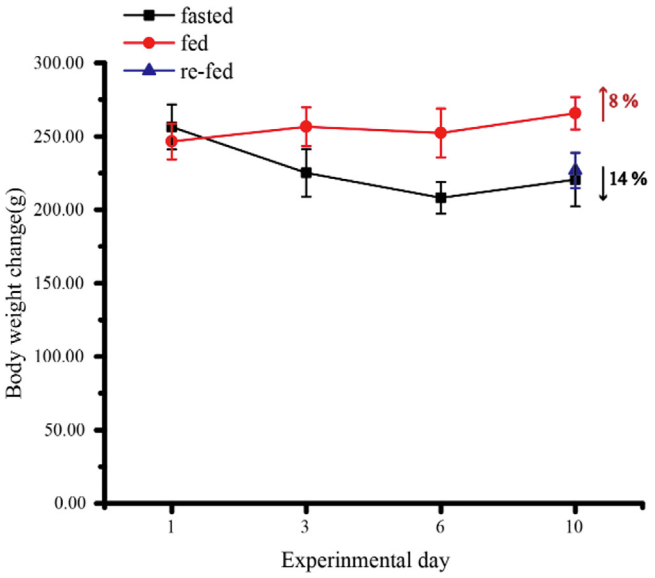

Fig. 5. Effects of fasting and re-feeding experiments on body weight (BW) change in A. *dabryanus*. BW showed an 8% increase in fed tank during 10 days, and a 14% decrease in fasted tank during 10 days. Data are means ± SEM; n = 6 per group. Black frame indicates fast. Red circle indicates fed. Blue triangle indicates re-fed.

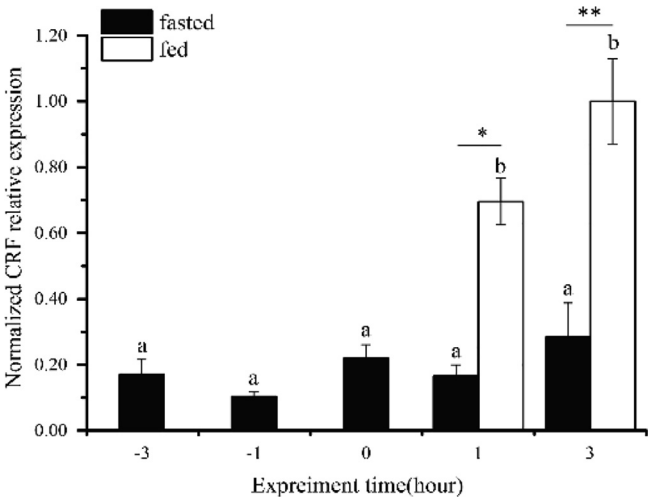

Fig. 6. *Periprandial* experiments induced changes with CRF mRNA in A. *dabryanus* of whole brain. The mRNA expression was normalized to β-actin and EF1-α, and the highest level of target gene mRNA expression was set equal to 1.0. Bars with different letters represent significant differences between unfed groups (ANOVA, $P < 0.05$). Asterisks represent significant differences between the two objects (Student's *t*-test, $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$). Data are means ± SEM; n = 6 per group.

the brain and gastrointestinal tract, which are related to the control of feeding.

To understand the transcript of CRF under fasting stress in Dabry's sturgeon, the entire brain was observed to detect the expression of CRF in periprandial, fasting, and re-feeding experiments. The results indicate that the fasting induced a low level of CRF mRNA expression during short- and long-term fasting that was reversed by re-feeding on the 10th sampling day (Figs. 6 and 7A). A 4-day or 7-day fast induces the down-regulation of CRF in mice (Yadawa and Chaturvedi, 2016) and frogs (Morimoto et al., 2011). Fasting for 7 days decreased the CRF expression in goldfish (Maruyama et al., 2006) and Ya-fish (Wang et al., 2014), and CRF expression increased after re-feeding in Ya-fish (Wang et al., 2014). This is presumably because fasting stimulates a decrease in the concentration of satiety factors, while the reverse is true for re-feeding (Rønnestad et al., 2017; Volkoff, 2016). Our previous studies have also identifiedin Dabry's sturgeon some satiety factors, such as apela (Tang et al., 2018), CCK (Yuan et al., 2014), and lpetin (Yuan et al., 2014). Ultimately, CRF may play a role in feeding regulation, and may act as a long-term satiety factor in Dabry's sturgeon. However, preprandial CRF expression was not obviously different in Ya-fish compared to that of postprandial CRF (Wang et al., 2014), which indicate that short-term stress might activate the transcription and release of CRF, resulting in the immediate secretion of CRF in the hypothalamus to increase the expression of CRF. This counteracts the decrease in CRF expression due to fasting (Chen and Fernald, 2008; Doyon et al., 2006). Therefore, short-term fasting may cause an increase or decrease in CRF expression, even having no obvious changing. In 10 days, the BW of fed Darby's sturgeon underwent an 8% increase but a 14% decrease in the fasted fish (Fig. 5). Similar results have also been reported in frogs in which the BW of fed frogs increased by 7%, and decreased by 20% in fasted frogs (Morimoto et al., 2011).

Because of the feeding suppression effects of CRF via CRF-Rs in mammals, the transcripts of CRF-R1 and CRF-R2 were also detected during fasting and re-feeding experiments (Ohata and Shibasaki, 2011; Yeh et al., 2016). Similarly in the present study, CRF-R1 and CRF-R2 mRNA in fasted fish decreased and re-feeding increased CRF-R2 expression. Overall, CRF with CRF-Rs play a potential role in the feeding regulation of Dabry's sturgeon, and the CRF-R1 expression should decrease in the early phase of fasting stress. Similar observations have been reported in the common carp (Huising et al., 2004) and cichlid (Chen and Fernald, 2008). CRF-R2 may still be downregulated until the cessation of this fasting stress, and the decreasing expression of the ligand and its receptors suggest that a common regulator or induction of expression of CRF-R2 might be implicated by its ligand, similar to the positive regulation of CRF-R2 by UCN3 (Poulin et al., 2012). Although the present study suggests that CRF, as well as CRF-Rs, could be involved in the feeding regulation in Dabry's sturgeon, their precise involvement in feeding regulation in Dabry's sturgeon needs to be further investigated.

5. Conclusion

The cDNA sequences of Dabry's sturgeon CRF and CRF-Rs have been cloned. Distributions of CRF, CRF-R1, and CRF-R2 mRNA was widespread in the whole brain and peripheral tissues. To explore the involvement of the CRF system in the endocrine regulation of feeding in Dabry's sturgeon, transcripts of CRF and CRF-Rs were detected under fasting stress. CRF expression in the whole brain was decreased significantly through periprandial and 10 days fasting and this was reversed by re-feeding. CRF-R1 and CRF-R2 expression decreased significantly during 10 fasting days, whereas re-feeding increased CRF-R2 expression. These results suggest that CRF with CRF-Rs might act as anorexigenic factors to regulate feeding through the central system in a long- fasting term.

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Conflict of interest

The authors declare that there is no conflict of interest.

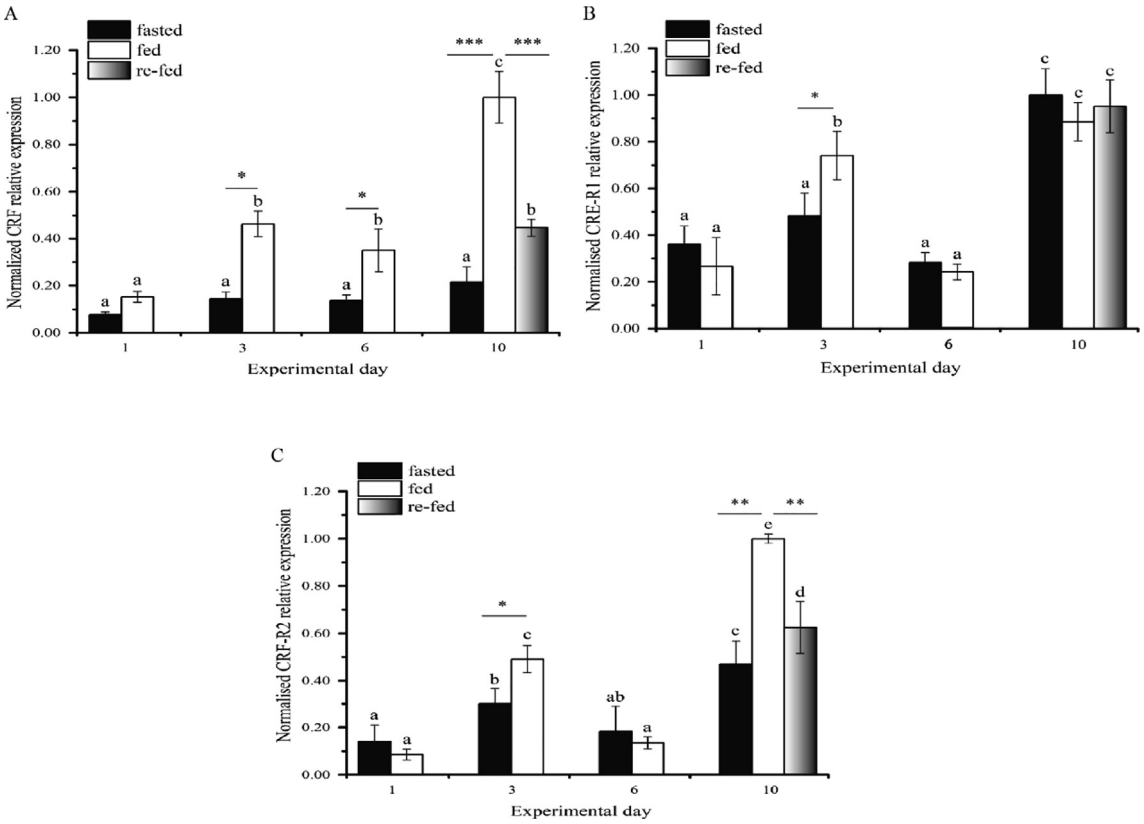

Fig. 7. Fasting and re-feeding experiments induced changes with CRF mRNA (A), CRF-R1 (B) and CRF–R2 (C) in A. *dabryanus* of whole brain. The mRNA expression was normalized to β-actin and EF1-α, and the highest level of target gene mRNA expression was set equal to 1.0. Bars with different letters represent significant differences between unfed groups (ANOVA, $P < 0.05$). Asterisks represent significant differences between the two objects (Student's *t*-test, $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$). Data are means ± SEM; n = 6 per group.

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