Modulation of plasma protein expression in bullfrog (Rana catesbeiana) tadpoles during seasonal acclimatization and thermal acclimation

Modulation of plasma protein expression in bullfrog (*Rana catesbeiana*) tadpoles during seasonal acclimatization and thermal acclimation

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Abbreviations: CBB, Coomassie Brilliant Blue; CTLD, C-type lectin-like domain; MBL, mannose-binding lectin; qPCR, quantitative polymerase chain reaction; RT, reverse transcription; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SEM, standard error of the mean; SFTPD, pulmonary surfactant-associated protein D; T3, 3,3',5-triiodothyronine; TBS, Tris-buffered saline; TH, thyroid hormone; TTR, transthyretin.

Abstract (239 words)

Biological activities in ectothermic vertebrates depend to a great extent on ambient temperature. Adapting their biological systems to annual or short-term alterations in temperature may play an important role in thermal resistance or overwintering survival. Using SDS-PAGE and western blot, we examined plasma proteins in bullfrog (*Rana catesbeiana*) tadpoles that were seasonally acclimatized (winter vs. summer) or thermally acclimated (4 °C vs. 21 °C) and identified two season-responsive proteins. The first, transthyretin (TTR), is a plasma thyroid hormone distributor protein that was abundant in summer, and the second is a protein containing C-type lectin-like domain (CTLD) that was abundant in winter and cold acclimation of 4 weeks. Sequence analysis revealed that the C-terminal carbohydrate recognition domain of this CTLD protein (termed collectin X) was highly similar to those of the collectin family members, which participate in the innate immune system; however, it lacked a collagen-like domain. Among the hepatic genes involved in the thyroid system, *ttr* and *dio3* were up-regulated, whereas *thra* and *thrb* were down-regulated, in summer acclimatization or warm acclimation. In contrast, the collectin X gene (*colectx*), as well as *colect10 and colect11* in the collectin family involved in the innate immune system, were down-regulated during warm acclimation, although *fcn2* in the ficolin family was up-regulated during summer acclimatization and warm acclimation. These findings indicate that seasonal acclimatization and thermal acclimation differentially affect some components of the thyroid and innate immune systems at protein and transcript levels.

Keywords: transthyretin, collectin, expression, thermal acclimation, amphibian tadpoles, *Rana catesbeiana*.

1. Introduction

Ectothermic vertebrates have multiple layers of excellent systems to cope with annual, middle-term, or diurnal changes in ambient temperatures, as they cannot maintain their bodies at a metabolically favorable temperature. Temperature profoundly affects their cellular metabolism, including processes involved in energy metabolism (Rogers et al., 2004), restructuring of cellular membranes (Trueman et al., 2000), and gene transcription and translation (Gracey et al., 2004; Kiss et al., 2011). As a result, their endocrine (Wright et al., 1999) and immune (Ferguson et al., 2018) systems, and development (Scott and Johnston, 2012) and locomotor performance (Wilson et al., 2000) can be accommodated by these cellular and biochemical responses.

A typical example is amphibian metamorphosis, which is obligatorily controlled by thyroid hormones (THs) (Tata, 1970). This post-embryonic transformation is highly sensitive to ambient temperature. Metamorphosis can be experimentally arrested by exposure to cold temperature even after treatment with THs, and then resumed by transferring the tadpoles to warm water (Frieden et al., 1965). In natural environments, tadpoles of some amphibian species, including the American bullfrog *Rana catesbeiana*, can overwinter without metamorphosis (Viparina and Just, 1975). The arrest of metamorphosis is beneficial for their survival in winter, because small froglets are most vulnerable to predator attacks, and it is difficult to obtain prey on land in the winter season, whereas tadpoles can remain relatively stable and safe in aqueous environments. The immune system in amphibians is also thermally sensitive, and cold temperatures have complicated effects on their survival and disease resistance. In many ectothermic vertebrates, innate defenses are known to be maintained or up-regulated during winter (Ferguson et al., 2018).

In the present study, we focused on plasma proteins in seasonally acclimatized and thermally acclimated amphibian tadpoles and detected two season-responsive proteins. One was up-regulated and the other down-regulated in summer. The up-regulated protein was transthyretin (TTR), a TH distributor protein, whereas the down-regulated protein was identified as a protein containing C-type lectin-like domain (CTLD) (Zelensky and Gready, 2005), termed collectin X, the biological role of which has not been elucidated. We next investigated their transcript levels accompanied with those of functionally related proteins. Finally, we discuss possible roles of these proteins in relation to seasonal acclimatization and thermal acclimation in tadpoles.

2. Materials and Methods

2.1. Animal care and experimental design

American bullfrog *R. catesbeiana* tadpoles at stages VI–XI (Taylor and Kollros, 1946), weighing 6–11 g,

were collected from ponds in the southern suburbs of Shizuoka, or in Ibaraki from a commercial supplier, in Japan, in summer (September, 2016 and June and August, 2017) or winter (March, 2016 and January, 2017). They were anesthetized by immersion in 0.02% 3-aminobenzoic acid ethyl ester, without acclimation to laboratory conditions. The truncus arteries of tadpoles were cut with scissors and blood was collected into heparinized microhematocrit tubes. The blood was centrifuged at 500 ×*g* for 10 min at 4 °C to separate the plasma from the blood cells. The liver was dissected, and small pieces of the tissues (each 20–40 mg) were snap-frozen in liquid nitrogen. The plasma and liver tissues were stored at -35° C and -84° C, respectively, for later use.

For the warm acclimation experiments, only tadpoles collected in winter were used. They were maintained in aerated and dechlorinated tap water at 4 °C, under natural lighting conditions and were fed boiled spinach *ad libitum* (approximately 0.5 g of a frozen block/tadpole) at 9:00 AM thrice a week. After acclimation to laboratory conditions at 4 °C for at least 1 week, 48 tadpoles were divided into 6 groups (8 individuals/10 L water per group), three each of the 4 °C and the 21 °C groups. For the 4 °C groups (control), tadpoles were maintained at 4 °C until Day 3, Day 14, or Day 28. For the 21 °C groups, tadpoles were subjected to a stepped warming regime of 1 °C/2 h to a maximum of 6 °C/day, to 21 °C, over 3 days (from Day -3 to Day 0), and then maintained at 21 °C until Day 3, Day 14, or Day 28. The mean body mass of each group was adjusted to be similar at the beginning of the experiment. Half the water volume in the aquaria (5 L) was changed thrice a week on the day after feeding. On the last days of the acclimation experiments, tadpoles were anesthetized with 3-aminobenzoic acid ethyl ester. The liver and plasma, as in the acclimatization study, were collected and stored.

All housing and experimental procedures were conducted in accordance with the guidelines for the care and use of laboratory animals of the Shizuoka University (permit #29F-8) under the international guideline "Act on Welfare and Management of Animals" (Ministry of the Environment of Japan).

2.2. Protein analyses

Plasma proteins (2 μL) were analyzed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in a 15% acrylamide gel according to the method described by Laemmli (1970) with molecular markers (bovine serum albumin, 66 kDa; ovalbumin, 45 kDa; lysozyme, 14 kDa), and visualized by staining with Coomassie Brilliant Blue (CBB) R-250.

Western blotting was performed as previously described (Yamauchi et al., 2000). After electrophoresis, the resolved proteins were transferred onto a nitrocellulose membrane (Protran, 0.45 μm, GE Healthcare, Little

Chalfont, Buckinghamshire, UK) at 1.2 mA/cm^2 for 1 h. After blocking with 10% skim milk in Tris-buffered saline (TBS, 50 mM Tris-HCl, pH, 7.6, and 140 mM NaCl) overnight at 4 °C, the membrane was then incubated for 1 h at room temperature with rabbit primary antibody directed against *R. catesbeiana* TTR (1:1,000) (Yamauchi et al., 2000) in TBS containing 1% skim milk. The antibody dilution was first optimized in our laboratory. After incubation, membranes were rinsed thrice with TBS containing 0.1% Tween 20 and then incubated with the secondary antibody (1:2,500, alkaline phosphatase-linked anti-rabbit immunoglobulin, raised in goat) (Promega, Madison, WI, USA) in 1% skim milk/TBS for 30 min at room temperature. Immunoblots were then developed using a detection kit containing 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium to detect alkaline phosphatase activity (ProtoBlot AP System, Promega). Band intensity was quantified using an image analyzer (LAS-4000, GE Healthcare Life Sciences, Chicago, IL, USA).

For protein sequencing, proteins were transferred to a polyvinylidene difluoride membrane (Thermo Fisher Scientific, Waltham, MA, USA) after SDS-PAGE, and then stained using CBB R-250. The band was cut and analyzed with a protein sequencer (Thermo Fisher Scientific, Procise 491 cLC).

2.3. Quantitative polymerase chain reaction (qPCR)

Total RNA was extracted from the liver of tadpoles using the acid guanidinium thiocyanate-phenol-chloroform method (Chomczynski and Sacchi, 1987). The quantity of specific RNA species in each sample was estimated by qPCR using THUNDERBIRD SYBR qPCR Mix (Toyobo, Osaka, Japan) and Thermal Cycler Dice (TaKaRa, Shiga, Japan) after the RNA samples had been treated with M-MLV Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA) and random hexamers for 10 min at 25 °C, for 50 min at 37 °C, and then for 15 min at 70 °C according to the manufacturer's instructions. Each PCR was run in duplicate to control for PCR variation. Detailed information of primer sets is shown in Supplementary Table 1. Primer specificity was confirmed by BLAST searches, the appearance of a single band on gel electrophoresis, and melting curve analysis. The thermocycler program included a step of 50 °C (2 min) and 95 °C (10 min), and 40 cycles of amplification at 95 °C (15 s) and 60 °C (1 min), and then a step of 95 °C (15 s). All assays gave unique dissociation curves. PCR efficiency, which was 82.9–115.9%, was determined by qPCR using reverse transcription (RT)-qPCR or RT product of total RNA as a template at different concentrations that covered 3−5 orders of magnitude. Control reactions lacking reverse transcriptase were tested for residual genomic DNA, and contamination was evaluated by non-template controls without RNA into the cDNA synthesis. All negative controls had undetermined Cq or Cq > 35. Relative quantification of transcript amounts was calculated using the

comparative Cq method (Livak and Schmittgen, 2001), and the transcript amount was set to be 1.0 in the winter or 4 °C groups. We tested three candidates as reference genes: β-actin (*actb*), ribosomal protein L8 (*rpl8*), and lactate dehydrogenase B (*ldhb*) genes. We arbitrarily used the *actb* as a reference gene, because no significant differences between two groups (winter vs. winter, or 4 °C vs. 21 °C) were detected in Cq values for the three transcripts.

2.4. Statistics

The data are presented as the mean ± standard error of the mean (SEM) ($n = 8$), unless otherwise noted. Differences between groups were analyzed using the Student's *t*-test. Differences were considered statistically significant $p < 0.05$. Statistical analyses were conducted using Microsoft Excel 2003 Data Analysis Software (SSRI, Tokyo, Japan).

3. Results and Discussion

CBB staining after SDS-PAGE of plasma proteins revealed that a 20-kDa protein was two-fold more abundant whereas a 15-kDa protein was less abundant (not clearly visible), in the winter tadpoles than in the summer tadpoles (Fig. 1A). Western blot analysis using the specific antibody against *R. catesbeiana* TTR indicated that the 15-kDa protein was TTR, and that the amount of TTR in the summer plasma was nearly twice as high as in the winter plasma. To investigate whether these seasonal changes in protein level are experimentally reproduced or not, we set up the experiments for thermal acclimation, where the winter tadpoles were acclimated to 4 °C or 21 °C for 3 days, 2 weeks, or 4 weeks, under laboratory conditions. In CBB staining, the 20-kDa protein level varied individually within a group with no significant differences between the 4 °C and 21 °C acclimation groups at 3 days, 2 weeks, or 4 weeks. In western blotting, the amount of TTR was two-fold higher in the 21 °C plasma than in the 4 °C plasma after acclimation for 4 weeks, although there was no significant difference in band intensity of TTR between the 4 °C and 21 °C acclimation groups at 3 days and 2 weeks. Therefore, at least plasma TTR level is likely to respond to habitat temperatures under natural and experimental conditions.

Protein sequencing revealed that the N-terminal 8 residues of the 20-kDa protein was NSKVRPDA. Subsequent BLAST search of this peptide against the public genomic databases (https://www.ncbi.nlm.nih.gov/) demonstrated that the 20-kDa protein belongs to the CTLD superfamily (Zelensky and Gready, 2005). Its

precursor form predicted from the cDNA (GDDO01018430 in the NCBI genome database) comprises 196 amino acid residues (Fig. 1B), starting with a signal peptide of 19 residues. The calculated molecular mass of the mature protein is 19,624 Da, which was in agreement with the molecular mass estimated from SDS-PAGE. BLAST search of the C-terminal carbohydrate recognition domain of the *R. catesbeiana* CTLD protein against public protein databases showed the highest amino acid sequence identity (54.3%) to *Nanorana parkeri* (a frog in the same superfamily Ranoidea) pulmonary surfactant-associated protein D (SFTPD)-like (XP_018413317), followed by *Xenopus laevis* mannose-binding protein A-like (XP_018080250, 48.4%), and *Xenopus tropicalis* SFTPD-like (XP_002933943, 46.1%), all of which belong to the collectin family. Therefore, we named it collectin X.

The mature collectin X contains a short N-terminal region of 24 residues, a neck region with a coiled-coil structure of 22 residues, and a C-terminal carbohydrate recognition domain of 131 residues. However, unlike most known members of the collectin family, it lacks a large part of collagen-like region, Gly-X-Y amino acid repeats (where X and Y are any amino acid), with only two repeats remaining, and conserved cysteine residues at the N-terminal region that may participate in forming inter chain disulfide bonds in the collectin family (Wallis and Drickamer, 1999). The C-terminal carbohydrate recognition domain contains the four conserved cysteine residues (at positions 96, 167, 181, and 189) that are known to form four disulfide bonds (between the residues at positions 96 and 189, and those at positions 167 and 181) in the collectin family (Drickamer, 1988). Five amino acid residues (Glu155, Asn157, Glu64, Asn177, and Asp178), that are known to interact directly with mannose, glucosamine, and glucose in the presence of Ca^{2+} in rat mannose-binding lectin (MBL) (Weis et al., 1992), are completely conserved. Collectin X, like mammalian MBLs, has the EPN motif in this domain (at positions 156-158), which is important for mannose specificity. These sequence characteristics suggest that collectin X has similar binding specificity for carbohydrates as mammalian MBLs. Collectin X may have diverged from a common ancestor of MBL/SFTPD that are involved in the innate immune system, despite the fact that it has a domain structure similar to the other soluble CTLD protein families in the CTLD protein superfamily, such as groups VII, IX and XII (Zelensky and Gready, 2005).

We next investigated the transcript levels of hepatic genes involved in the thyroid system and the innate immune system, including *ttr* and *colectx* genes (Fig. 2). The *ttr* transcript amounts were significantly higher in the summer tadpoles than in the winter tadpoles, although there were no significant differences in transcript amounts between the tadpoles in 4 °C and 21 °C groups in any acclimation period tested. A unique transcriptional change was detected in the gene for deiodinase III (*dio3*), which can inactivate the active form of

THs, 3,3',5-triiodothyronine (T3), by removing an inner ring iodine. The acclimation to 21 °C for 3 days transiently up-regulated the *dio3* transcript. This may attenuate T3 actions under a rapid shift to warm conditions. The transcript levels for TH receptors (*thra* and *thrb*) were higher in winter than in summer. The *thra* transcript was also at least twice as high in the 4 °C group as that in the 21 °C group after thermal acclimation of 2 and 4 weeks.

Transcript amounts of *colectx* were the highest among those of the tested genes that are or may be involved in the innate immune system. Although the amounts of collectin X in plasma were clearly higher in winter than in summer, its transcript levels in winter were the same as those in summer. There were also no significant differences in transcript levels of collectin 10 (*colect10*) and collectin 11 (*colect11*) between the winter and summer tadpoles. Only the ficolin 2 gene (*fcn2*) was up-regulated in summer. In the thermal acclimation study, the *colectx*, *colect10*, *and colect11* transcript levels gradually decreased at 21 °C with increasing acclimation periods, whereas the *fcn2* transcript levels increased at 21 °C after acclimation of 3 days and 2 weeks.

The amphibian thyroid system may respond to seasonal acclimatization and thermal acclimation. Amphibians have a thyroid system that is highly sensitive to temperature. TTR is a major plasma TH distributor protein with high affinity for T3 in amphibian tadpoles (Yamauchi et al., 1993). T3 binding activity at 20 °C is approximately one-tenth of that at 4 °C (Yamauchi et al., 1993). These facts suggest that a high level of the *ttr* transcript and plasma TTR in summer can compensate for the decreased TH binding activity at warm temperatures to maintain TH homeostasis in plasma. The bullfrog tadpole thyroid glands become less sensitive to thyrotropin at low temperatures or in winter (Wright et al., 1999), resulting in low levels of plasma THs. It is likely that TH receptors act as repressors of TH-inducible genes in the absence of TH in winter (Buchholz and Shi, 2018). Furthermore, higher expression of TH receptors in winter may sensitize target tissues to THs, when the thyroid glands start to secrete TH into the bloodstream. Temperature may be a critical factor that directly or indirectly controls the *thra* expression in tadpole liver.

Analyses of plasma proteins and hepatic transcripts indicate that seasonal acclimatization and thermal acclimation modulate the innate immune system of bullfrog tadpoles. Our surprising finding was that collectin X, with high sequence similarity to MBL/SFTPD in the collectin family and a lack of collagen-like repeats, was abundantly present in plasma of winter tadpoles: ~approximately 0.5 mg/mL, roughly estimated from the band intensity of SDS-PAGE. This was two orders of magnitude higher than the plasma concentrations of human collectin family members such as MBL, collectin 10, and collectin 11 (Casals et al., 2019). There are closely similar expression patterns among *colectx*, *colect10*, and *collect11* during seasonal acclimatization and thermal

acclimation. Collectin X, like collectins 10 and 11, may be involved in the innate immune system. Further studies are needed to confirm the function of this protein.

4. Conclusions

In conclusion, seasonal acclimatization and thermal acclimation strongly affected the levels of plasma proteins and/or hepatic transcripts that participate in the thyroid and innate immune systems in bullfrog tadpoles. The thyroid system was less active in winter and may become active in spring, whereas the innate immune system is more active in winter. These modulations may have beneficial effects for survival in unsuitable thermal conditions in overwintering tadpoles and to drive appropriately many biological processes in the transition from cold to warm conditions in spring.

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Declarations of interest

The authors of this present study declare no conflicts of interest.

Appendix A. Supplementary data

Supplementary material related to this article (Supplementary Table 1) can be found, in the online version, at doi: ***.

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Figure legends

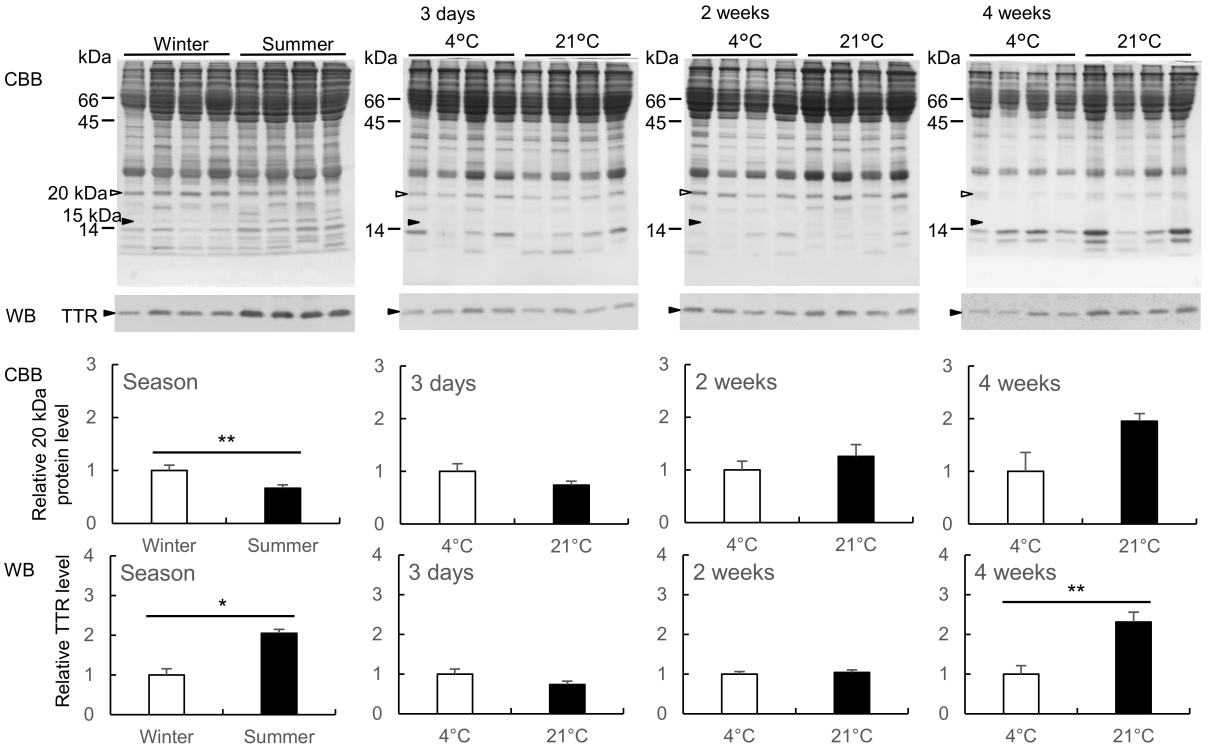
Fig. 1. Quantitative changes in plasma proteins in response to seasonal acclimatization and thermal acclimation in bullfrog tadpoles and identification of responsive proteins. (*A*), Plasma proteins were obtained from the tadpoles that were seasonally acclimatized (winter, *open*; summer, *closed*) or acclimated

to 4 °C (*open*) and 21 °C (*closed*) for 3 days, 2 weeks, or 4 weeks. Four of eight plasma/group (2 μL) were analyzed by SDS-PAGE, followed by staining with Coomassie Brilliant Blue (*CBB*) R-250 or western blotting (*WB*) using antibody against bullfrog transthyretin (*TTR*). Band intensities after CBB staining or western blotting for TTR were quantified in an image analyzer. Arrowheads, the position of TTR of 15-kDa (*closed*) and 20-kDa protein (*open*). These experiments were repeated twice using the plasma from different animals in the same groups, with similar results. Each value represents the mean ± SEM (n = 8). Asterisks denote significantly different means between two groups (*, $p < 0.05$; **, $p < 0.01$). (*B*), Nucleotide and deduced amino acid sequence of cDNA encoding 20-kDa C-type lectin-like domain (CTLD) protein. The initiation methionine is marked as +1. Eight amino acid residues that were determined by protein sequencing of the 20-kDa protein are underlined. Conserved 4 cysteine and 5 amino acid residues that are involved in direct interactions with carbohydrates in the carbohydrate recognition domain are double underlined and boxed, respectively.

Fig. 2. Transcript levels of genes involved in the thyroid and innate immune systems in the livers of seasonally acclimatized and thermally acclimated bullfrog tadpoles. Total RNA was prepared from the liver of bullfrog tadpoles (each n = 8) that were seasonally acclimatized (winter, *open*; summer, *closed*) or acclimated to 4 °C (*open*) and 21 °C (*closed*) for 3 days, 2 weeks, or 4 weeks. The RNA was analyzed by real-time polymerase chain reaction (PCR) after reverse transcription. Gene transcripts investigated were transthyretin (*ttr*), deiodinase III (*dio3*), and thyroid hormone receptor α and β (*thra* and *thrb*) in the thyroid system, and C-type lectin-like domain (CTLD) protein, termed collectin X (*colectx*), collectin 10 *(colect10*), collectin 11 (*colect11*), and ficolin (*fcn2*). The vertical axis represents the amounts of gene transcripts after normalization to the *actb* transcript, and the values are expressed relative to those of the winter or the 4 °C group that was set to 1.0. These experiments were repeated twice using the liver from different animals in the same groups, with similar results. Each value represents the mean ± SEM (n=8). Asterisks denote significantly different means between two groups (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

Fig. 1

A



B

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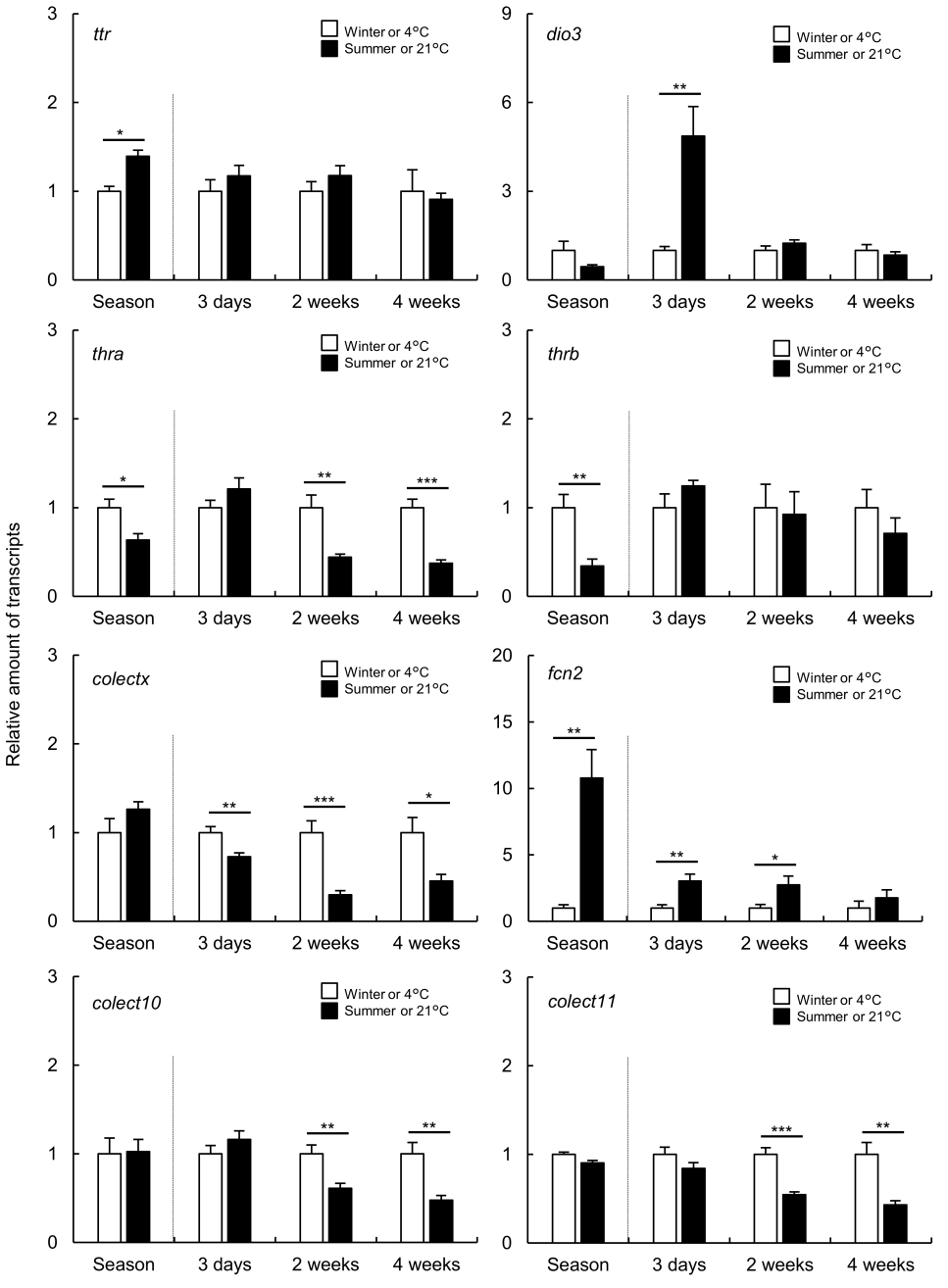
 101 tatcctgatcagctgtgttcagaatATGAAAGCTCTGCAGGTTTTCTGTGTACTTTTCCTCTATGCGGCTTTAGTGACGTCTAACTCAAAGGTACGCCCT
                          +1 M  K  A  L  Q  V  F  C  V  L  F  L  Y  A  A  L  V  T  S  N  S  K  V  R  P   25
 201 GATGCTGGAAAAGCTGGAGTGAAAGCAGAATCAGTATCAAGTTCTCTTTGCGCAGTGTGTGAAGATCTTCGAGAGCAAGTCAAAGTCTTGAATGAACAAC
      D  A  G  K  A  G  V  K  A  E  S  V  S  S  S  L  C  A  V  C  E  D  L  R  E  Q  V  K  V  L  N  E  Q  Q   59
 301 AAAAAGCACTCAAAACAGCATTTTTATTTTTCAAAGGAAGAGCTACAAGTGGTGACAAAATCTACCTCTCAGATGGTGACCAAGGCAATTTTAACGATGC
        K  A  L  K  T  A  F  L  F  F  K  G  R  A  T  S  G  D  K  I  Y  L  S  D  G  D  Q  G  N  F  N  D  A   92
 401 TAGAGAAAGGTGCACAAAGGCGGGAGGTCAAATGGCCTCTCCAAGGAATGCTGAAGAGAACGAAGCCATACTGTTCATCACCAATCAGTACGGTGTTGCT
       R  E  R  C  T  K  A  G  G  Q  M  A  S  P  R  N  A  E  E  N  E  A  I  L  F  I  T  N  Q  Y  G  V  A   125
 501 TCTTATCTTGGAATAAATGAACTTGAGGAACTTGGGACTTATAGATATCCAGGCGGTGAGGCGATTTCCTTCACCAACTGGAATGTTGGTGAACCCAATA
       S  Y  L  G  I  N  E  L  E  E  L  G  T  Y  R  Y  P  G  G  E  A  I  S  F  T  N  W  N  V  G  E  P  N  N  159
 601 ATTTTGAAGGCAGACAGGAGAATTGTGTAGAGATGCAAAATACAGGAAGATGGAATGACATTGTTTGTGCAAATACACGTCTTATCATCTGCGAGTTTTC
        F  E  G  R  Q  E  N  C  V  E  M  Q  N  T  G  R  W  N  D  I  V  C  A  N  T  R  L  I  I  C  E  F  S   192
 701 TATCACTAGATTTTAAaataattacttctttcgtcactttaatttgtaagggagttttggcttttgaggtcttaaagaagaaaacgaatgcaactatcac
        I  T  R  F  *                                                                                      196
 801 atttcagcagtttgtcagtcatcaataaaacctgccttctgattttgtagtataaaaaaaaaaatttgtcacattaaaaaaaaatgcacatactgtattt
 901 atatatatcattatgtatttccatattataagaaagcacaaggagaaatattgacaacagcgagaagtggtaaacctatgtagttcactagtctaagggg
1001 agttcagattaacaaaacaaattcttttttgcgctttattgtacattaaaatgtaacttcagcaccaaaaaaaaa 1075
```

Fig. 2


Relative amount of transcripts
ttr
dio3
thra
thrb
colectx
fcn2
colect10
colect11
Winter or 4°C
Summer or 21°C
Season
3 days
2 weeks
4 weeks