Differential expression of aquaporin genes and the influence of environmental hypertonicity on their expression in juveniles of air-breathing stinging catfish (*Heteropneustes fossilis*)

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Research Article

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Abstract

Aquaporins (AQPs) are a superfamily of transmembrane channel proteins that are responsible for the transport of water and some other molecules to and from the cell, mainly for osmoregulation under anisotonicity. We investigated here the expression patterns of different AQP isoforms and also during exposure to hypertonicity (300 mOsmol/L) for 48 h in juvenile stages of air-breathing stinging catfish (*Heteropneustes fossilis*). A total of 8 mRNA transcripts for different isoforms of AQPs and their translated proteins could be detected in the anterior and posterior regions of S1, S2, and S3 stages of juveniles of stinging catfish at variable levels. In general, more expression of mRNAs for different *aqp* genes was seen in the S2 and S3 juveniles than in the S1 juvenile. Most interestingly, exposure to hypertonicity of S2 juvenile for a period of 48 h led to increased expression of most of the *aqp* genes both at transcriptional and translational levels, except for *aqp3* in the anterior and posterior regions and *aqp1* in the anterior region, showing maximum expression at later stages of hypertonic exposure. Thus, it is evident that AQPs play crucial roles in maintaining the water and ionic balances under anisotonic conditions even at the early stages of development of stinging catfish as a biochemical adaptational strategy to survive, grow and reproduce in anisotonic environment.

Introduction

All living organisms try to maintain their internal osmolarity by involving specific channel proteins that control the concentrations of inorganic ions, some organic solutes, and water inside their cellular systems. A relatively limited number of solutes seem to serve the function of osmolytes, which include inorganic ions like K^+, Na^+, Cl^-, and also some organic osmolytes such as polyols like inositol and sorbitol, methylamines like betain and α-glycerophos, phorycholine, and certain amino acids like taurine, and also urea (Goldstein and Pearlman 1995; Häussinger 1996). The adaptive responses to osmolarity changes mainly deal with the involvement of water channels, electrolyte, and osmolyte transporters for the movement of water, ions, and osmolytes in and out of various cellular systems, thereby maintaining the constancy in cellular volume. Out of these, aquaporins (AQPs) are the essential transmembrane channel proteins involved mainly to facilitate water transport and, in some cases, small solutes across the membrane (Verkman and Mitra 2000; Takata et al. 2004). AQPs exist as homotetramers embedded in the lipid bilayer, and each monomer functions independently as a single pore channel (Gomes et al. 2009). Very little information is available on the expression of AQPs in freshwater teleosts, except for some information on euryhaline species. Cutler and Cramb (2000) reported for the first time the expression of AQPs in some marine and freshwater fishes, the expression of which is said to be regulated that allow them to survive in their respective environments with various salinity changes (Watanabe et al. 2009; Kim et al. 2014; Tipsmark et al. 2010; Lee et al. 2017). Various researchers have reported the ubiquitous presence of different isoforms of AQPs in different tissues of certain teleosts. A total of 11 AQP sub-families are reported in the genome of zebrafish (*Danio rerio*), which include mammalian isoforms AQP 0–1, 3–5, and 7–12 (Cerda` and Finn 2010).

The air-breathing stinging catfish (*Heteropneustes fossilis*) are commercially important freshwater fish species with high market, nutritional and medicinal value. They are known to be one of the richest dietary sources of iron and calcium for human welfare in Southeast Asian countries (Kohli and Goswami 1989). The stinging catfish are more resistant to environmental challenges such as high environmental ammonia, desiccation, and hypoxic stresses (for reviews, see Saha and Ratha 1998; 2007). This fish also face the problems of osmolarity changes regularly in their natural habitats during different seasons of the year, especially in summer when the ponds and lakes dry up, thereby compelling them to migrate inside the mud peat to avoid total dehydration, and also during the monsoon season when the water in the same habitat gets diluted.

The physiology of development of teleost fish is incredibly reliant on their osmoregulatory efficiency (Evans 1993; Moustakas and Copeland 2004; Cuesta-Alberto et al. 2005). The culture of salinity tolerant freshwater fish in saline water is a common practice mainly to reduce the mortality rate as a consequence of infestation with pathogenic bacteria and fungal infection in fish and ultimately to improve fish production (Hatting et al. 1975; Long et al. 1977; Kutty et al. 1980; Altinok and Grizzle 2001; Martı´nez-Palacios et al. 2004). Furthermore, salinity has significant positive effects at the initial stages of development, such as egg fertilization, incubation, reabsorption of the yolk sac, and the overall development of fish (Alderdice 1988; Boeuf and Payan 2001). At intermediary salinities, the better growth rates at juvenile stages were observed almost in all the freshwater and marine fish species examined (Boeuf and Payan 2001; Ahmmed et al. 2017).

The ability of fish larvae to survive on changing salinity depends on their capacity to function at least for a short period in an altered cellular fluid osmolarity and ionic concentrations or on the capacity of the larvae in maintaining the constancy of cellular fluid osmolarity. The latter is achieved through tight regulation of different ions and water channels to survive under the cellular osmolarity challenges (Holliday 1969). Therefore, the present study aims to investigate the expression pattern of multiple *aqp* genes and the corresponding AQP proteins in juvenile stages of stinging catfish (*H. fossilis*) and also while exposed to a hypertonic environment (300 mOsmol/L).

Materials And Methods

Antibodies and reagents

Rabbit polyclonal AQP1, AQP3, AQP4, AQP7, AQP8, AQP11, AQP12, and HRP-conjugated goat anti-rabbit and anti-mouse IgG were procured from ThermoFisher Scientific, USA. The mouse monoclonal GAPDH antibody was procured from Santacruz Biotechnology, USA. Oligonucleotide primers were obtained from GCC Biotech, India. Quantinova 2X Rotor-Gene SYBR Green PCR Master mix was obtained from Qiagen, Germany. Other chemicals were of analytical grade and obtained from local sources. Milli-Q water was used in all preparations.

Animal and Experimental set up

The hatchlings of stinging catfish (*Heteropneustes fossilis*) were bred and reared in the fish breeding facility of the Biodiversity Campus of Gauhati University, Guwahati, India, which were produced from healthy brood pairs. The hatchlings were maintained in the laboratory in a glass fish tank (2 ft x 4 ft x 1 ft). The larvae were maintained at a temperature of 27°C ± 1°C (pH 7.1 ± 0.1) with 12h: 12h photoperiods. They were fed daily ad libitum with planktons, later switched to juvenile fish feed when the hatchlings grew. The study was approved by the Institutional Animal Ethical Committee (IAEC) of Gauhati University, Guwahati, India (IAEC/Per/2019/PP-IAEC/2019-036). All experiments with fish were carried out in accordance with relevant guidelines and regulations of the Code of Ethics of the World Medical Association (Declaration of Helsinki) for animal experiments.

To compare the expression patterns of AQPs in stinging catfish at juvenile stages, 7-days old larvae (average length: 0.8–1.5 cm, S1), 14-days old (3–4 cm, S2), and 21-days old (5–6 cm, S3) juveniles were taken (Fig. 1). However, to study the effect of hypertonicity on the expression of AQPs, the experiments were conducted only with 14-days old juveniles (S2), mainly due to preferred size and growth rate. To study the effect of environmental hypertonicity, four sets of S2 juveniles were exposed separately in a set of 5 to the hypertonic environment (300 mOsmol/L NaCl) in a volume of 5 L in plastic tumblers for 6, 12, 24, and 48 h. The hypertonic NaCl solution in different tumblers was replaced every day at a fixed time with fresh media. The juveniles, after exposure to a hypertonic environment for different time intervals, were anesthetized in 1 L of 0.2% neutralized 3-aminobenzoic acid ethyl ester (MS-222) for 5 min, followed by decapitation at the head region just beneath the pectoral fin and divided the whole body into anterior and posterior regions. The body parts were dipped in liquid nitrogen and stored at − 80°C until used for further analyses. All analyses were completed within 3 weeks of preserving the tissue.

RNA extraction and cDNA synthesis

The total RNA was extracted from 50 mg of each tissue using RNAiso Plus (Takara, Japan), following the method of Rio et al. (2010), subsequently treated with DNase (Invitrogen) for the removal of DNA, if at all present. The isolated RNA was quantified in a Qubit® 3.0 Fluorometer (ThermoFisher Scientific, USA). First strand cDNA was synthesized from 400 ng of total RNA in a volume of 20 µL using High Capacity cDNA reverse transcription kit (ThermoFisher Scientific, USA) following the manufacturer's protocol. For quantitative real-time PCR studies, the cDNA samples were diluted 50 times with sterile Milli-Q water.

Quantitative real-time PCR analysis

The quantitative real-time PCR (qPCR) analysis of different *aqp* genes were performed with a Rotor-Gene Q SYBR Green PCR (Qiagen). In a 25 µl reaction volume, 12.5 µl QuantiNova™ 2x Rotor-Gene SYBR Green PCR Master Mix (Qiagen), 1.2 µl of each forward and reverse primers (10 µM), and 5 µl of cDNA template were used. The qPCR reactions were carried out at the following thermal cycling conditions: 95°C for 10 min, followed by 45 cycles of denaturation at 95°C for 15s, annealing at respective annealing temperatures for 30 s, and elongation at 72°C for 30s. The qPCR was performed in triplicate for each sample, and a negative control using no cDNA was run for each gene. Melting curve analysis was used to

confirm the amplification of only a single PCR product. Further, to re-confirm the amplification product, the sequencing of the PCR product was performed in an AB13130 Genetic Analyzer (ThermoFisher Scientific, USA) using the Sanger dideoxy method. Two reference genes (GAPDH and β- actin) were used to normalize the qPCR data. Relative mRNA expression of each gene was calculated using the modified ΔΔCT method (Livak and Schmittgen, 2001). The used primer pairs were designed, and the specificity of each primer pair was checked by using the Primer-BLAST tool (Ye et al. 2012) (Table 1).

Table 1
Sequences of the specific primer pairs of *H. fossilis* aquaporin isoforms *aqp1, 1b, 3, 4, 7, 8, 11, 12* and *β-actin* for qPCR analysis.

Aquaporin	Forward primer	Reverse Primer	Accession no
Aqp1	5' TGTCAGGACCTCCGACTGAT 3'	5' TCTACTGCAAGTCCGAGGGA 3'	MF100768
Aqp1b	5' TGGTGTCAGCCCAGGACAAG 3'	5'ACCAAACGATCGAGCAGGGT 3'	MK723989
Aqp3	5' GCCACACTTGGAGTCTTGGT 3'	5' TGGACCTTCCGAGAATGCAG 3'	MG545607
Aqp4	5' GTCACGCTCTGGTTGTGGAG 3'	5'AGGCTGGTTGAACCTTTGAGA3'	MN593356
Aqp7	5'GTAATACCAGGGCCCCGACC3'	5' GCAACGGCGGGAATTTTTGC 3'	MK689684
Aqp8	5'TCAGTAATGCGTCTGGAGCTG3'	5'CGCCGTGGTCAGAAACACAG 3'	MG545608
Aqp11	5' CAACTGGATGCGTGAACGTG 3'	5' CACCACTGCAGGATGTCTGT 3'	MK689683
Aqp12	5' ACTCAGTGCAGTTCATCGCT 3'	5' GCTAAGGTGCGCAAGGTGAT 3'	MG545609
β-actin	5' CAGCTGAGCGTGAAATCGTG 3'	5'TCCAGAGAGGATGAGGAGGC 3'	FJ409641

Western blot analysis

Tissues were homogenized in a lysis buffer consisting of 50 mM Tris-HCl buffer (pH 7.5) with 0.33 M sucrose, 1 mM EDTA, 1% TritonX, and a protease inhibitor cocktail (Complete™ MINI EDTA-free, Roche, Mannheim, Germany). The lysates were centrifuged at 10,000 x g for 10 min at 4^{o}C. The protein concentrations in supernatants were determined by the dye-binding method (Bradford, 1976). Samples were solubilized and denatured by the addition of 0.5 volume of loading buffer (220 mM TrisHCl, 22.5 M EDTA, 9% SDS, 1.8 M sucrose, 180 mM DTT, 0.125% bromophenol blue), and incubated at 37^{o}C for 30 min. The proteins were separated by SDS-polyacrylamide gel electrophoresis. After electrophoresis, the proteins were transferred to polyvinylidene difluoride (PVDF) membranes (0.2 µm; Immuno-Blot®, Bio-rad). Non-specific binding sites were blocked with 1% bovine serum albumin in 1X PBST buffer (pH 7.4) overnight at 4^{o}C in a shaker bath. The blots were incubated for 2 h with primary antibodies at 1:5000 dilution in 1X PBS containing 1% BSA and 0.1% Tween 20, followed by incubation with horse-radish peroxidase (HRP) conjugated anti-rabbit or anti-goat IgG secondary antibody (1: 5000 dilution) for 2 h with washing steps with PBST in between. The chemiluminescence was detected using Clarity™ Western

ECL Substrate (Bio-Rad) in an Image Quant LAS 500 system (GE Healthcare Life Sciences, USA). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as a protein loading control.

Statistical Analysis

The data, collected from different experiments, were statistically analyzed by One-way ANOVA test, followed by Turkey's multiple comparison test, performed in GraphPad Prism software and presented as mean ± S.E.M (n = 3–5 in each set of experiments). Levene's test was also performed to verify the homogeneity of variance of different parameters between the control and treated groups of each set of experiments.

Results

Expression of aqp genes and AQP transporter proteins in juvenile stages of stinging catfish

The relative expressions of mRNAs for different *aqp* genes (Fig. 2) and their translated AQP transporter proteins (Fig. 3) were analyzed both in the anterior and posterior parts in three juvenile stages (S1, 7-days old; S2, 14-days old; S3, 21-days old, Fig. 1) of stinging catfish. The relative changes in the levels of expression of mRNAs of different *aqp* genes and their translated proteins in the S2 and S3 stages were compared with the levels found in the S1 stage.

In general, the levels of mRNA for different *aqp* genes were found to be more expressed in S2 and S3 juveniles compared to the levels found in S1 juveniles in both the anterior and posterior parts of juveniles (Fig. 2a & b). In general, the levels of mRNAs for *aqp1, aqp1b, aqp3, aqp4, aqp7, aqp8, aqp11*, and *aqp12* were found to be significantly higher in the anterior regions of S2 and S3 juveniles than in S1 juveniles.

As shown in Fig. 2a, the expression of mRNA for *aqp1* gene was found to be 8.1-fold higher in S2 juveniles and 5.5-fold higher in S3 juveniles compared to the level found in S1 juveniles. The expression of mRNA for *aqp1b* gene was also found to be 31.1- and 23.0-fold higher in S2 and S3 juveniles, respectively, compared to the level found in the S1 juvenile. Whereas, in cases of *aqp3*, *aqp4*, and *aqp7* genes, the mRNA levels were found to increase significantly by 2.5-, 3.0- and 2.7-fold in S2 juvenile, respectively, and by 3.1-, 3.3- and 3.5-fold in S3 juvenile, respectively, compared to the levels found in S1 juvenile. In cases of *aqp8*, and *aqp11*, the fold increases of mRNA levels were again found to be much higher in S2 and S3 juveniles compared to levels seen in S1 juveniles, which were 24.5-, and 11.3-fold higher in S2 juveniles, and 37.3- and 27.5-fold higher, respectively, in S3 juvenile. In the case of *aqp12* gene, the level of mRNA expression was again higher in S2 juveniles by 6.5-fold, and by 5.4-fold in S3 juveniles compared to the level found in S1 juveniles.

Similarly, the posterior region of three juvenile stages of stinging catfish showed some variations in the expression of mRNA transcripts for different *aqp* genes (Fig. 2b). For *aqp1*, there were 7.7- and 17.4-fold more expression in S2 and S3 juveniles, respectively, compared to the level found in S1 juveniles. For *aqp1b*, the mRNA level was found to be 53.5- and 38.5-fold higher in S2 and S3 juveniles, respectively,

compared to the level found in S1 juveniles. Whereas, in the case of *aqp3* and *aqp4* genes, the expression of mRNAs increased by 6.5-fold and 5.5-fold in S2 juveniles and by 7.2- and 6.3-fold in S3 juveniles compared to the level found in S1 juvenile. In contrast to the anterior part of juveniles, the expression of a*qp7* mRNA was found to be 51.0- and 54.7-fold higher in S2 and S3 juveniles, respectively, compared to the level found in S1 juveniles. In cases of *aqp8* and *aqp12* genes, again, not much of variations in the expression of mRNAs were seen among different juvenile stages. Whereas, in the case of *aqp11*, the mRNA level was found to be 13.4- and 19.2-fold higher in S2 and S3 juveniles, respectively, compared to the level found in S1 juveniles.

The pattern of relative expression of different AQP transporter proteins in three different juvenile stages (S1, S2, and S3) of stinging catfish showed almost a similar trend comparable to the relative expression of mRNAs of corresponding *aqp* genes (Fig. 3a & b). In general, the levels of expression of different AQP proteins were found to be higher in S2 and S2 juveniles compared to the levels found in S1 juveniles of stinging catfish. For AQP1, the level of expression was 4.5- and 3.0-fold higher in the anterior region of S2 and S3 juveniles, respectively, compared to the level found in the anterior region of S1 juvenile, and 3.1- and 3.5-fold higher in the posterior region of S2 and S3 juveniles, respectively, compared to the level found in the posterior region of S1 juvenile. For AQP3, the level of expression was 2.4- and 2.9-fold higher in S2 and S3 juveniles, respectively, compared to the level found in the anterior region of S1 juvenile, and 1.7- and 2.1-fold higher in the posterior region of S2 and S3 juveniles, respectively, compared to the level found in the posterior region of S1 juvenile. Similarly, for AQP4, the level of expression in the anterior region of S1 and S2 juveniles were found to be 2.8- and 3.1-fold higher, respectively, compared to the level found in the anterior region of S1 juveniles, and 2.2- and 2.8-fold higher in the posterior region of S2 and S3 juveniles, respectively, compared to the level found in the posterior region of S1 juvenile. Likewise, in the case of AQP7, the fold increase in its expression was found to be 2.9- and 3.3-fold higher in the anterior region and 3.2- and 3.5-fold higher in the posterior region of S2 and S3 juveniles, respectively, compared to S1 juvenile. Whereas in cases of AQP8 and AQP12 proteins, the significant fold increases were seen in S2 and S3 juveniles only in the anterior region compared to S1 juveniles, but without any significant difference in their expression patterns among three stages in the posterior region of juveniles. However, in the case of AQP11, the levels of expression were 3.1- and 3.6-fold higher in the anterior region and 2.4- and 3.4-fold higher in the posterior region of S2 and S3 juveniles, respectively, compared to the level in S1 juvenile.

Expression of aqp transcripts and related proteins in S2 juvenile of stinging catfish during exposure to hypertonicity

The changes in the expression of *aqp* transcripts were studied both in the anterior and posterior parts of 14-day old juvenile (S2) stinging catfish while exposed to a hypertonic environment (300 mOsmol/L NaCl) for a period of 48 h (Fig. 4). Wide variations in the expression of different *aqp* genes were noticed in the anterior part of the S2 juvenile of stinging catfish throughout the period of 48 h exposure to hypertonicity (Fig. 4a). The mRNAs for *aqp1*, *aqp4*, *aqp8, aqp11*, and *aqp12* genes were found to be significantly upregulated within 6 h of exposure to hypertonicity, followed by a further increase at later

stages of exposure. The mRNAs for *aqp1*, *aqp4*, *aqp8, aqp11* and *aqp12* increased maximally by 43.0-fold after 12 h, 48.2-fold after 24 h, 45.9-fold after 24 h, 11.3-fold after 24 h, 4.9-fold after 12 h of exposure, respectively. Whereas, in the cases of *aqp1b* and *aqp3*, the expression levels of mRNAs were found to be downregulated during hypertonic exposure. In the case of *aqp7*, there was an initial downregulation of mRNA expression at an early stage of treatment, followed by an upregulation by 2.5-fold after 12 h of hypertonic exposure.

In the posterior region of the S2 juvenile, the expression of mRNAs for *aqp1* and *aqp3* genes were seen to decrease significantly throughout the period of exposure to hypertonicity compared to 0 h control (Fig. 4b). Whereas, in the cases of *aqp1b*, *aqp4*, *aqp7*, *aqp8*, *aqp11*, and *aqp12*, the mRNA levels increased significantly during hypertonic exposure and remained increased throughout the period of exposure with maximum rises of 49.5-fold after 48 h, 36.7-fold after 24 h, 27.8-fold after 24 h, 7.3-fold after 48 h, 11.9-fold after 12 h, and 14.4-fold after 24 h, respectively.

Parallel to the changes in the expression of different mRNA transcripts for *aqp* genes, the different AQP proteins also showed differential expression patterns in the anterior region of S2 juveniles at different time intervals of hypertonic exposure, as revealed by Western Blot analysis (Figs. 5a & b). In the anterior region, the relative band intensities of AQP1, AQP4, AQP7, AQP8, AQP11 and AQP12 increased significantly with a maximum rise of 7.7-fold at 24 h, 8.1-fold at 24 h, 4.1-fold at 12 h, 7.9-fold at 24 h, 2.9-fold at 24 h and 2.1-fold at 12 h of treatment, respectively. On the other hand, the expression of AQP3 was seen to remain downregulated throughout the period of hypertonic exposure as compared to 0 h control.

Upon exposure to hypertonicity, the relative expression of different AQP proteins in the posterior part of S2 juvenile was seen to increase significantly except for AQP3, which showed a decreasing trend in its expression throughout the period of hypertonic exposure (Figs. 5a & 5b). The AQP1, AQP4, AQP7, AQP8, AQP11, and AQP12 proteins expression increased maximally by 7.9-fold at 48 h, 4.5-fold at 24 h, 4.7-fold at 12 h, 4.2-fold at 48 h, 2.5-fold at 12 h, 3.5-fold at 12 h, respectively.

Discussion

In this study, it was observed that the expression of different AQP proteins varies in different juvenile stages of stinging catfish (*H. fossilis*), thus establishing the fact that these proteins are important components in the regulation of osmolarity under anisotonicity at early stages of development. Starting from its semi-absorbed yolk sac larval stage (S1), the different isoforms of AQPs were found to express at varying degrees in each successive stages with differential patterns of expression in the anterior and posterior regions of stinging catfish juveniles. Since freshwater fish have hypertonic blood compared to their external environment, there will be a passive osmotic influx of water and diffusive loss of ions, like Na^+ and Cl^-, and they also have the capacity to adapt to having a low integument permeability, active uptake of ions in the branchial chambers (mainly gills), lowering of drinking rate and production of the high volume of hypotonic urine. In adult fish, the maintenance of the hydromineral balance of the body is

achieved by specialized tissues and organs, such as the gills, intestine, and urinary system, including the kidney and urinary bladder. The embryos and larvae of teleost fish also face ionic and osmotic gradients between their body fluids and the external environment. However, in the early developmental stages, the osmoregulatory organs are either under-developed or absent (Holliday 1969; Varsamos et al. 2005). Therefore, osmoregulation in early developmental stages would be associated with either to have a limitation in the passive flux of water and ions, which are mainly achieved by lowering the permeability of the embryonic membranes, or by active ion and water homeostasis mechanisms mainly by regulating the channel proteins until the development of adult osmoregulatory organs. In newly hatched larvae, osmoregulation is mainly achieved by the integument or skin since other osmoregulatory organs are not properly developed. While the larvae are slowly transforming from a yolk-sac and free feeding forms to metamorphosed juveniles, other organs like gills, kidney, and intestine take over the osmoregulatory functions (Varsamos et al. 2005). Therefore, during this study, the entire body of different stages of juveniles was divided into anterior and posterior regions with the intention to investigate about differential expression of different AQP genes in both the regions at different developmental stages. The anterior region, including the gills, anterior part of the gastrointestinal tract, brain and some parts of skin were different from the posterior region which contained the kidney, a major portion of the gastrointestinal tract, and most of the skin and muscle. The S1 stage larvae were still attached to their yolk sac indicating their semi-free-feeding state, and so most of their osmoregulatory functions were chiefly integumentary. However, as the larvae got metamorphosed and transitioned into adult-like juveniles, their preferred organs of osmoregulation changed, which probably resulted in more expression of different AQP proteins as a consequence of the upregulation of corresponding genes. In the anterior region of stinging catfish juveniles, the expression of *aqp 1, 1b, 3, 4, 7, 8, 11*and *12* genes were found to increase with the progress of developmental stages. In the posterior region, the expression of *aqp1, 1b, 3, 7, 11* genes were also shown to increase significantly in the S2 and S3 juveniles compared to the S1 juvenile, with the exception of *aqp12* genes, which got downregulated at later stages of development.

When teleost fish are placed in a hypertonic environment, they usually hypo-osmoregulate, leading to an ion invasion and dehydration. Adaptation to a saline environment is known to cause low permeability (in reverse directions) of the integument, activation of osmoregulatory mechanisms combined with a high drinking rate and an active ion uptake along with the digestive tract, followed by an osmotic intake of water, active ion excretion through the gills, and release of a limited amount of isotonic urine (Varsamos et al. 2005). Under different environmental stress-related threats, AQPs are known to play a key role in maintaining water homeostasis and balance (Luu et al. 2005; Wan et al. 2001; Galmes et al. 2007; Ranganathan et al. 2017).

Upon exposure to hypertonicity, most of the *aqp* genes in S2 juvenile of stinging catfish were found to get upregulated, except for a few, which got downregulated in both the anterior and posterior regions of the juvenile with certain gene-specific variations in their expression patterns during 48 h of hypertonic exposure. The isoforms, which were significantly upregulated in the anterior region of S2 juvenile, included *aqp1, 4, 8, 11*, and *12*, and in the posterior region, the *aqp 1b, 4, 7, 8, 11*, and *12* were seen to significantly upregulate during hypertonicity treatment. The *aqp3* gene was seen to downregulate in the

whole fish during hypertonic treatment. It was suggested that NaCl has the ability to regulate the expression of *aqp* genes by inducing the hypertonicity response element (HRE) at the promoter region of the *aqp* gene via the activation of MAPK signaling pathway (Umenishi and Schrier 2002, 2003). The same mechanisms might have also been followed in the activation of *aqp* genes that were observed in the early developmental stages of stinging catfish as a biochemical adaptive strategy to survive under hypertonic environment. Comparable to the expression of different *aqp* genes, an almost similar pattern of expressions of different AQP proteins was also seen in both the regions of S2 juvenile during hypertonic exposure. But the fold changes of expressions of different AQP proteins were much lower than the corresponding mRNA expressions in both the regions of S2 juvenile. Hence, it may be contemplated that some translational and posttranslational regulatory mechanisms exist in this stinging catfish for regulating the process of expression of different *aqp* genes and their translated products under osmotic stress also at different developmental stages. Additionally, degradation of some amount of mRNAs might have also occurred during the process of hypertonic treatment. Such differences in the abundance of *aqp* transcripts and the encoded proteins were also reported earlier in certain plant species (Aharon et al. 2003; Almeida-Rodriguez et al. 2010). In fact, due to protein turnover and posttranslational modifications, all of the *aqp* transcripts are not converted into functional proteins (Aroca et al. 2005; Yu et al. 2005). Moreover, salinity stress can also affect membrane trafficking of AQPs, showing internalization of AQPs in the membrane (Ueda et al. 2016; Kapilan et al. 2018).

Salinity-induced upregulation and downregulation of specific isoforms of AQPs have been reported in different marine and freshwater fish species such as Atlantic salmon (Tipsmark et al. 2010, Engelund et al. 2013), climbing perch (Ip et al. 2013), European eel (Cutler and Cramb 2002, Lignot et al. 2002), Japanese eel (Aoki et al. 2003), Japanese medaka (Madsen et al. 2014), marine medaka (Kim et al. 2014), rainbow wrasse (Brunelli et al. 2010), river pufferfish (Jeong et al. 2014), seabass (Giffard-Mena et al. 2007), silver seabream (Deane et al. 2011) roughskin sculpin (Ma et al. 2020), etc. establishing their osmoregulatory roles. Regulation of the expressions of *aqp* genes can occur through many signaling pathways and through complicated transcriptional, translational and posttranscriptional controls such as methylation, phosphorylation, heteromerization, and protonation etc., hence it is very difficult to distinguish a standard expression pattern for each AQP isoform (Maurel et al. 2001; Hachez et al. 2006). Salinity and other environmental factors are known to regulate *aqp* expression in a cell-specific manner via hormones. These regulatory mechanisms have been demonstrated to affect the AQP trafficking through the secretory pathway to reach the plasma membrane or opening and closing of the pores. However, the effectiveness of AQPs in osmoregulation can vary depending on the physiological condition of the organism, tissue type, stages of development as well as the duration and intensity of stress (Alexandersson et al. 2005; Galmes et al. 2007), and the type of AQP isoform (Jang et al. 2004).

In this study, it was observed that the entire aquaporin family found in this fish has revealed a complex pattern of transcriptional and translational responses to the salinity stress, with sometimes opposite patterns among different isoforms at different regions of the juveniles. During salinity stress, specific AQP isoforms may be expressed in certain tissues, while other isoforms are expressed all through the organism. Every aquaporin isoform discovered so far has been grouped depending upon its functions,

which include transporting gases and other solute molecules in addition to water. Considering the fact that aquaporins transport water and additional molecules, it is quite challenging to understand the nature of these complex changes in their expression pattern in the same organism during stress. However, this may be considered as the mechanism by which an overall ion and water homeostasis is maintained in their body. Here, the aquaglyceroporin AQP3, which transports urea and glycerol along with water, has been significantly reduced at both the transcriptional and translational levels under salinity stress in juveniles. These complex expression patterns of classical water channel AQPs (AQP1 and 4), and other non-exclusive or unorthodox AQPs (AQP3, 7, 8, 11, and 12) in stinging catfish juveniles at different body regions suggest that water homeostasis is maintained by increasing or by reducing the cell-to-cell water transport via AQPs under the hypersaline condition in a coordinated manner.

The results of the current study have the potential to provide a foundation for the in-depth study on the role of AQPs in the adaptive mechanisms in the stinging catfish in adverse environmental conditions. Since juvenile stages of this catfish are proved to be efficient osmoregulator and saline tolerant, their large-scale production at coastal aquaculture farms can be encouraged to boost the economy. Moreover, future prospective include gene manipulation of AQPs in freshwater catfishes to make the organism more fit to survive in adverse conditions, making it a largely beneficial tool for the freshwater fish culture industry.

Declarations

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Competing Interest

The authors have no relevant financial or non-financial interests to disclose.

Authors' contribution

Manas Das conceptualized and designed the study, analyzed the data, and acquired funding. Nirmalendu Saha conceptualized, designed, analyzed the data, and wrote and edited the manuscript. Priyambada Chutia contributed in the acquisition of data and wrote the manuscript. Lalit Mohan Goswami contributed in the acquisition of data. All authors read and approved the final manuscript.

Data availability

Datasets are available from the corresponding author on reasonable request.

Ethics approval

The study was approved by the Institutional Animal Ethical Committee (IAEC) of Gauhati University, Guwahati, India under Ref: IAEC/Per/2019/PP-IAEC/2019-036.

Consent for publication

Not applicable

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Figures

Figure 1

Post-hatched larval and juvenile stages of *H. fossilis*: **(a, b** & **c**) Stage-1 (S1), 7 days old larvae; (**d**) Stage-2 (S2), 14 days old juvenile (**e**) Stage-3 (S3), 21 days old juvenile

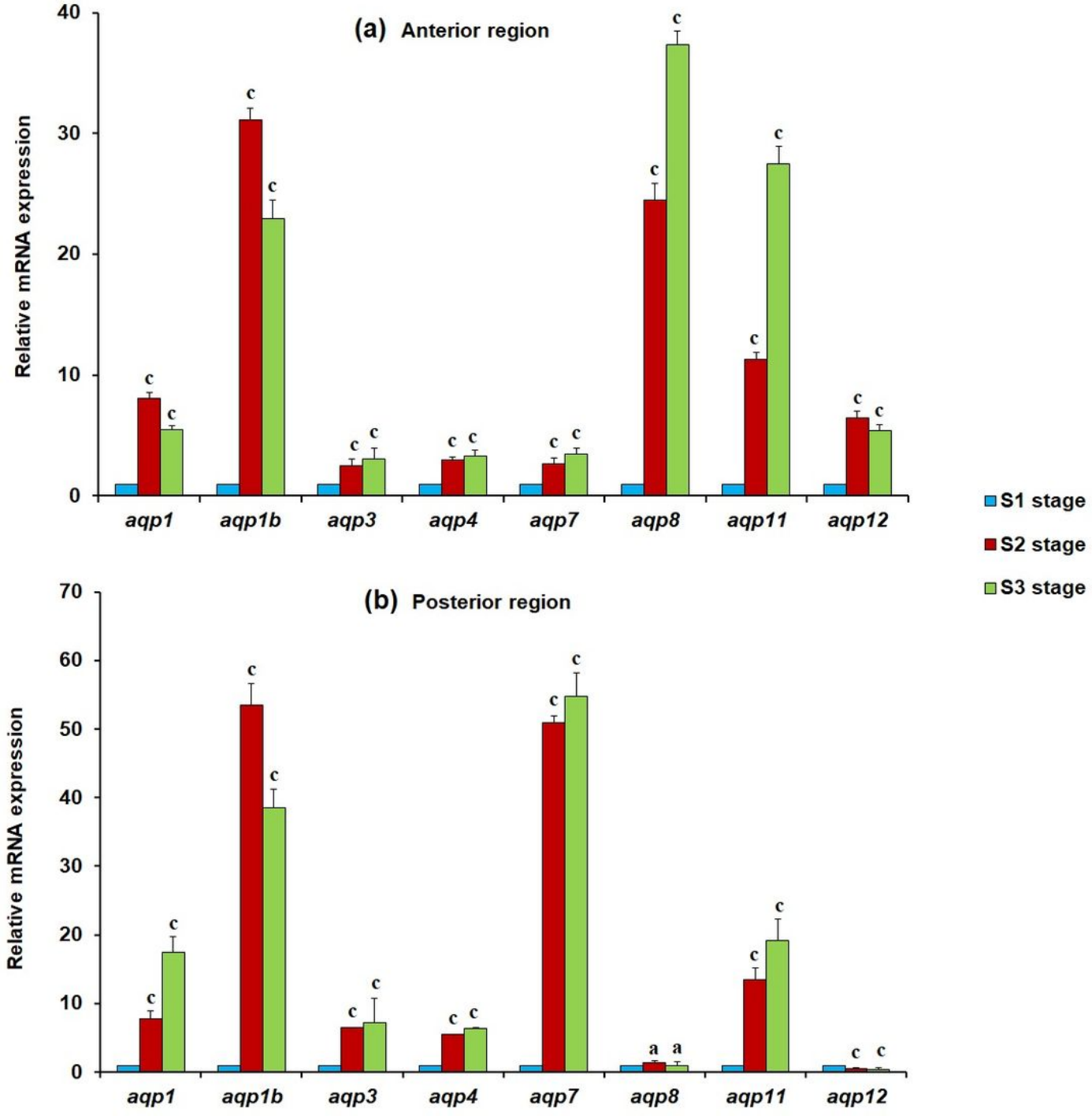

Figure 2

The patterns of mRNA expression of different *aqp* genes in the **(a)** anterior and **(b)** posterior regions of S1, S2 and S3 juveniles of *H. fossilis*. Fold changes in the levels of expression of different *aqp* genes in S2, and S3 juveniles were compared with the levels found in S1 juveniles. Values are plotted as mean ± S.E.M. (n = 5).

a, b, c: *P* values significant at <0.05, <0.01 and <0.001 levels, respectively (One-way ANOVA)

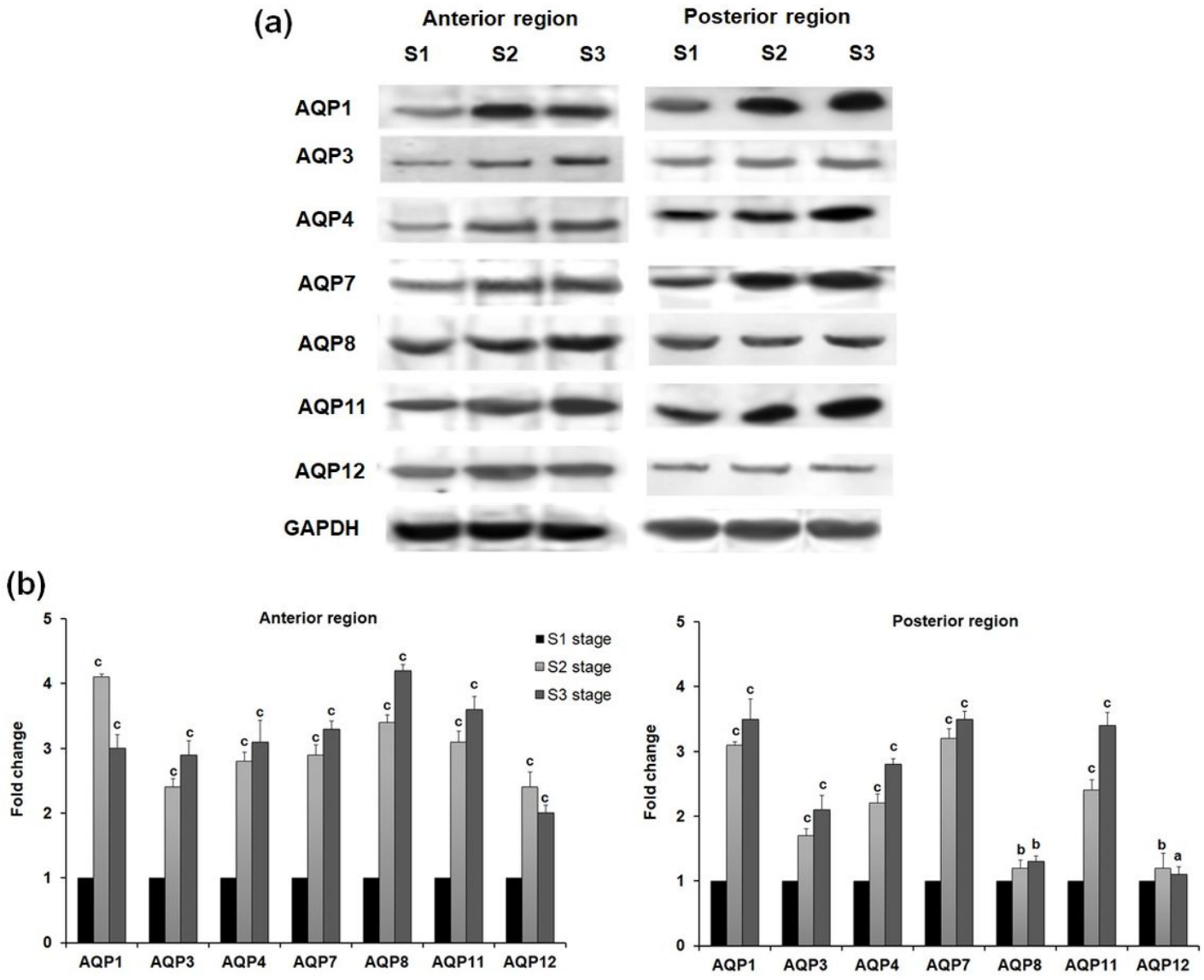

Figure 3

The patterns of expression of different AQP proteins in the anterior and posterior regions of S1, S2 and S3 juveniles of *H. fossilis*. **(a)** Representative plots of WB analysis of individual AQPs. **(b)** Densitometric analysis of AQP proteins expression in the S2 and S3 juveniles compared to S1 juveniles. Values are plotted as mean ± S.E.M. (n = 3).

a, b, c: *P* values significant at <0.05, <0.01 and <0.001 levels, respectively (One-way ANOVA)

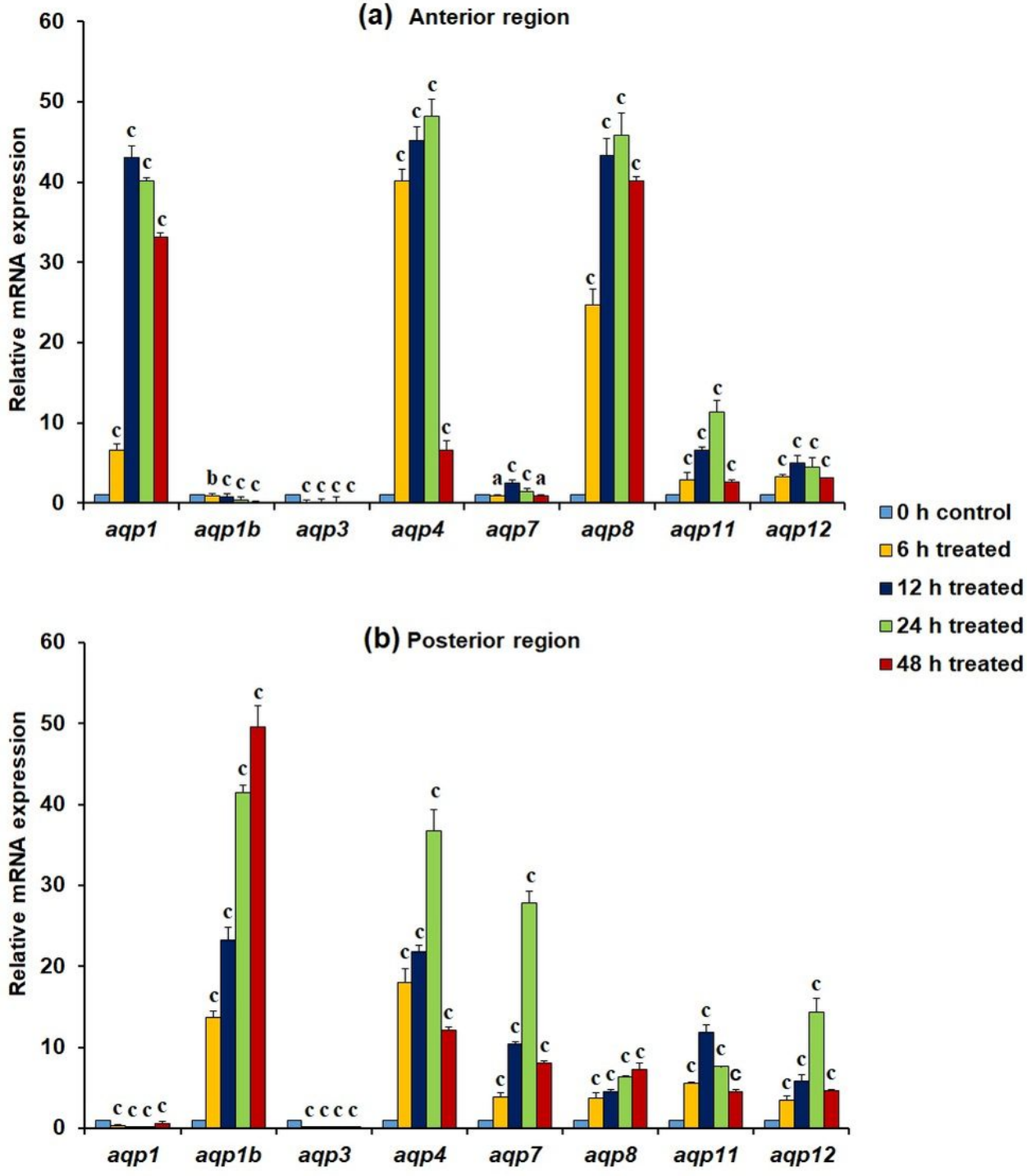

Figure 4

Changes in the levels of expression of mRNAs for different *aqp* genes in the **(a)** anterior and **(b)** posterior regions of S2 juvenile of *H. fossilis* while exposed to hypertonic environment (300 mOsmol/L NaCl) for 48 h. Values are plotted as mean ± S.E.M. (n = 5).

a, b, c: *P* values significant at <0.05, <0.01 and <0.001 levels, respectively (One-way ANOVA)

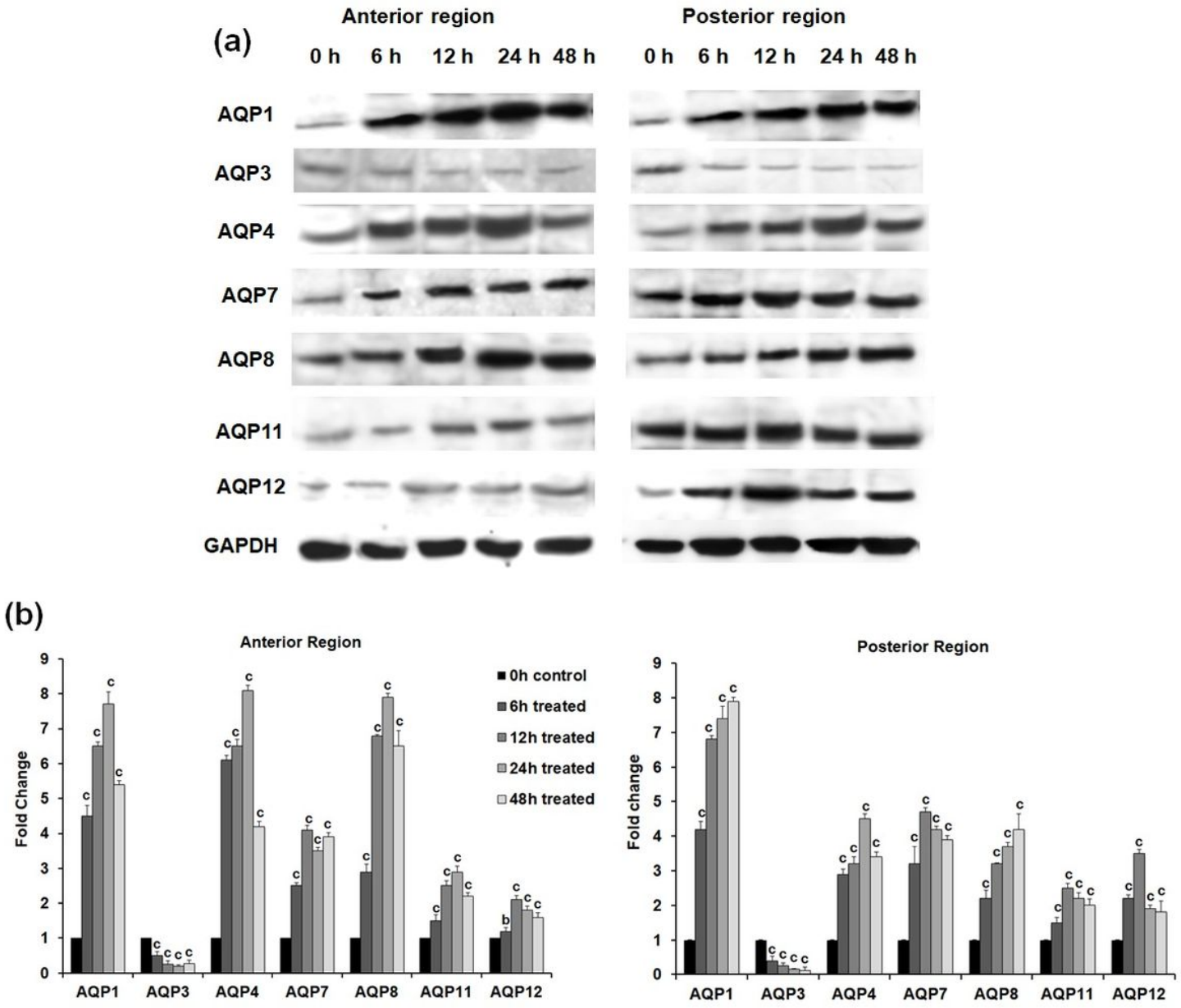

Figure 5

Changes in the levels of expression of different AQP proteins in the anterior and posterior regions of S2 juvenile of *H. fossilis* while exposed to hypertonic environment (300 mOsmol/L NaCl) for 48 h. **(a)** Representative plots of WB analysis of individual AQP proteins. (B) Densitometric analysis of AQP proteins expressions in the S2 juvenile at different time intervals of hypertonic exposure compared to 0 h controls. Values are plotted as mean ± S.E.M. (n = 3).

a, b, c: *P* values significant at <0.05, <0.01 and <0.001 levels, respectively (One-way ANOVA)