Journal of Bioresources 7(1): 28-36 (2020)
Print: ISSN 2394-4315
Online: ISSN 2582-2276

Original Research Article

Effect of Altered pH on the Transcription of Aquaporins in Stinging Catfish *Heteropneustes fossilis*

Priyambada Chutia, Manas Das* and Daisy Brahma

Department of Zoology, Animal Physiology and Biochemistry Laboratory, Gauhati University, Guwahati, Assam, India, 781014

***Corresponding author:** manasdasne@gmail.com

Received: October 27, 2019; revised: February 17, 2020; accepted: February 24, 2020

DOI: 10.17605/OSF.IO/B5XJ7

Abstract: Living in a world of water, aquatic animals face the problem of directly interacting with their aqueous environment that differs markedly in osmolality from their internal physiology. Aquaporins are a superfamily of transmembrane channel proteins that transport water and some other molecules into and out of the cell and are found throughout the living biota. These proteins are important for maintaining normal fluid composition, cell volume and osmoregulation and so they are abundantly found as different isoforms and paralogs expressed in different tissues of eukaryotic organisms. pH of a water body can be altered by various environmental factors including pollutants. Both cytosolic and environmental pH is found to regulate the functionality and expression of aquaporins. In this study, we investigated the effect of altered environmental pH on the transcription of certain isoforms of aquaporins in the catfish *Heteropneustes fossilis*, which is a hardy fish that can inhabit swamps, shallow ponds and polluted water. Adult catfishes were grouped into 3 groups of 6 individuals each and exposed to pH 6.4, 8.4 as well as control pH 7.4 with the addition of HCl and NaOH to induce altered pH into freshly collected water. *H. fossilis* gills, kidney and liver were dissected, total mRNA isolated and the template abundance was quantified by qPCR analysis. *H. fossilis aqp1, 3, 8, 11* and *12* showed sensitivity towards acidic and alkaline pH. Aquaporin isoforms are mostly upregulated at the altered pH, tends to increase with increasing pH except *aqp12* in the liver of *H. fossilis*. In comparison to the physiological pH of 7.4, at pH 8.4 most isoforms were upregulated two to eighty folds depending upon the organ in concern. It was also found that longer the exposure time, greater was the effect of altered pH on the transcription of aquaporins, radically altering the mRNA levels by the end of the experiment.

Key words: Aquaporins, catfish, *Heteropneustes fossilis*, osmoregulation, pH

Introduction

All life on earth sustains on water. Whether it is unicellular or multicellular organism, water forms the medium of life and is vital to the physiology of all living cells. Cells of all living organisms facilitate and control the flux of water into and out of the cell by means of a family of water-specific transmembrane protein channels called aquaporins. Members of the aquaporin family have been found in archaea, eubacteria and eukaryotes, including fungi, animals and plants. Apart from transporting water, some isoforms are also involved in the exchange of other molecules like ammonia, urea, glycerol, CO_2, arsenite etc. Aquaporins are basically classified according to their permeation preference for water, glycerol, or other small solutes and gases, molecular structure and phylogeny (Stahlberg *et al.,* 2001; King *et al.,* 2004; Takata *et al.,* 2004; Zardoya, 2005; Tingaud-Sequeira *et al.,* 2010). Therefore, aquaporins are grossly divided into: Classical water selective aquaporins (AQP 0,1,2,4,5,14, and 15); Aquaglyceroporins (AQP 3,7,9,10,13; transport glycerol and urea additionally) and

Unorthodox Aquaporins or Superaquaporins that include AQP 6,8,11,12,16 (Dong *et al.,* 2016) due to their different characteristics. Vertebrate superfamily contains 17 classes of aquaporins (AQP0 - AQP16; Finn *et al.,* 2014). Mammals are reported to have 13 aquaporin isoforms (AQPs 0-12). Zebrafish (*Danio rerio)* contains 18 AQP paralogs; Pufferfish (*Fugu rubripes)* contains 17 isoforms (Tingaud-Sequeira *et al.,* 2010), Atlantic salmon has 42 AQP paralogs distributed in 12 AQP classes (Finn *et al.,* 2014).

The aquaporin protein exists as a homotetramer in the cell membrane. Each monomer consists of six membrane spanning alpha-helices that have a central water-transporting pore. Therefore, unlike ion channels where four subunits surround a single pore, each monomer in an AQP tetramer has its own pore. Both C and N termini of the AQP protein face the cytosol. Most members of the aquaporin family consist of a conserved NPA (Asn-Pro-Ala) motif that is characteristic feature of this family (Sui *et al.,* 2001, Murata *et al.,* 2000, Krane *et al.,* 2003).

Aquaporins play a vital role in the survival of fishes as their natural environment is exclusively water. Both marine and fresh water fishes express aquaporins which are regulated in a way to allow them to survive in their respective environments with different levels of salinity. Aquaporin isoforms are either upregulated or downregulated in response to the changing environmental conditions in which the fish dwells on. Regulation of aquaporin expressions in animals and plants during environmental stresses provide much insight into the role of this membrane protein in maintaining the internal milieu of an organism even under physiological stress.

Rain water is naturally acidic due to dissolved atmospheric CO_2 and its acidity may rise even more due to reactions with SO_2 and NO_2 from burning fossil fuels. These acid rains can significantly bring down the pH of a water body. On the other hand, a lake or pond can become alkaline if it is fed with water that had passed over rocks and soil containing carbonate, bicarbonate and hydroxide compounds. Absence or reduction of buffering will eventually affect the aquatic life present in such water bodies. Changing pH has been shown to modulate the expression level and functionality of aquaporins in both plants and animals. Most eukaryotic aquaporins show upregulation with increasing pH as well as show differential permeability to water and other molecules. The aquaglyceroporin AQP3 shows reversible pH dependent conductivity of water and glycerol (Zeuthen and Klaerke, 1999). At a physiological pH, AQP3 acts as a glycerol and water channel, but predominantly as a glycerol channel at pH values around 6.1. AQP0 water permeability at neutral pH is approximately 40 times lower than that of AQP1 (Chandy *et al.,* 1997, Yang *et al.,* 1997), but AQP0 water conductance doubles under mildly acidic conditions while reducing at a higher pH (Németh-Cahalan and Hall, 2000). In the case of aquaporins in plant roots, a pH-dependent closure of the water pores has been reported (Tournaire-Roux *et al.,* 2003). AQP1 is upregulated at a higher pH with two-fold higher mRNA levels at pH 8 than those at pH 6 (Zhai *et al.,* 2018). Plant plasma membrane aquaporins are gated by pH (Tournaire-Roux *et al.,* 2003), divalent cations (Alleva *et al.,* 2006, Verdoucq *et al.,* 2008), and phosphorylation (Johansson *et al.,* 1996, 1998). Extracellular pH regulates the expression of aquaporin 1 genes via SPIB transcription factor that binds to an upstream enhancer region in the promoter of the *aqp 1* gene (Zhai *et al.,* 2018).

In the present study, mRNA transcription level of different aquaporin isoforms are investigated in the catfish *Heteropneustes fossilis* under acidic and alkaline pH revealing the patterns different isoforms display under altered environmental pH. At pH 8.4 aquaporins showed many fold upregulation especially in the gills and kidneys. At pH 6.4, aquaporins either didn't show much significant difference than physiological pH of 7.4 or were downregulated to a certain extent. The *H. fossilis* is a hardy air breathing fish species that shows excellent competency in adapting against environmental challenges such as altered pH, osmolality, hyperammonia and desiccation. Aquaporins may play a role in the success of this fish to survive under such adverse conditions. Nevertheless, altered environmental pH affects the transcript abundance of aquaporins in the catfish *H. fossilis*.

Materials and methods

Animal and maintenance

Air breathing stinging catfish (*Heteropneustes fossilis*) weighing 35±10 g were purchased from a local fish market of Guwahati, Assam. The fishes were acclimatized in the Aquaculture and Biodiversity Centre laboratory, Gauhati University for a month, maintained under natural photoperiod at 26±2^{0}C, and pH 7.4 ± 0.1. Fishes were fed ad libitum with fish feed containing ground up dried fish and flour. Experiment was conducted after there were zero mortality rates and normal food consumption, hence complete acclimatization. Food was withdrawn 24 hrs prior to experiments.

Experimental set up

Two groups of fish of similar sizes having six in each group were placed in two large containers having 5L water with a low pH of 6.4 and high pH of 8.4 respectively. Another group of fishes was kept in normal pH 7.4 which served as the control batch. The acidity and alkalinity of the water was induced by the addition of HCl and NaOH respectively. pH altered water was replaced every day at a fixed time. Fishes were treated for 96 hrs, 5 days and 10 days after which they were anaesthetized in neutralized 3-aminobenzoic acid ethyl ester (MS-222, 0.2 g.l^{-1}) followed by killing by decapitation. Gills, liver and kidney tissues were dissected out, plunged into liquid nitrogen and stored at - 80°C.

Total RNA isolation and cDNA synthesis:

Total RNA was extracted from liver, kidney and gills of *H. fossilis* using the RNeasy Mini Kit (Qiagen, Germany); or using RNAiso Plus (Takara, Japan) following the method of Rio *et al.,* (2010). Isolated total RNA was quantified in a Qubit® 3.0 Flurometer (ThermoFisher Scientific). First strand cDNA was synthesized from 500 ng of total RNA in a volume of 20 μL using High Capacity cDNA Reverse transcription kit (ThermoFisher Scientific) following the manufacturer's protocol. For quantitative real time PCR studies, the cDNA samples were diluted 50 times with sterile miliQ water.

Quantitative real time PCR

In order to investigate the transcript abundance of different *aqp* isoforms in liver, kidney and gills of *H. fossilis* exposed to low (6.4) and high (8.4) pH, quantitative real time PCR was performed. The relative quantities of *aqp1, aqp3, aqp8, aqp11* and *aqp12* mRNA transcripts, in each sample, were normalized to an endogenous gene *H. fossilis* β-actin (*hfact* β). All the *aqp* primers were designed using the Primer 3 software v 0.4.0 (National Human Genome Research Institute, USA) and their details are furnished in table1. Quantitative real time PCR analyses were performed with a Rotor Gene Q SYBR Green PCR (Qiagen). In a 25 μl reaction volume, 12.5 μl QuantiNova^{TM} 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 was used; and qPCR reactions were done at the following thermal cycling conditions: 95^0 C for 10 min, followed by 45 cycles of denaturation at 95^0 C for 15s, annealing at different temperatures for each primer set in the range of 51^{0}C- 57^0 C (Table 1) for 30 s and extension at 72^0 C for 45 s. Further, amplification specificity was assessed by melting curve analysis, determined post PCR with continuous fluorescence acquisition (72–95^{0}C) at a temperature transition rate of 1^0 / step. The relative expressions of the *aqp* transcripts in the treated groups were expressed as the fold change to non treated control by using the standard $2^{-\Delta\Delta Ct}$ method (Livak *et al.,* 2001, Kubista *et al.,* 2006; Schmittgen and Livak 2008). Each reaction was performed in triplicates.

Results

Transcription of *aqp1* gene in gills, kidney and liver at altered pH following 96 hour, 5 days and 10 days exposure were as follows: it showed upregulation in gills by 1.79, 1.8 and 1.15 folds respectively at pH 6.4; while upregulated at a much higher rate at pH 8.4 such as by 4.64 folds in 96 hr, 22.5 folds in 5 days and 33.9 folds in 10 days (Fig. 1a). In kidney (Fig. 1b), there is no significant difference in transcript abundance of *aqp1* till 5 days at a lower pH but at a high pH it increased by

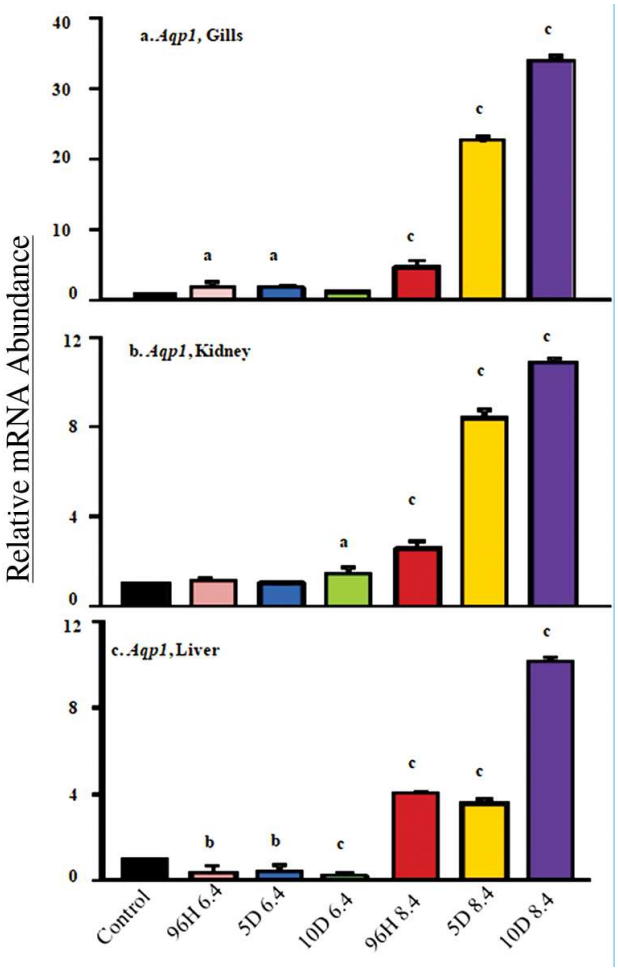

Fig. 1. Relative *Aqp1* mRNA abundance in gills (a), kidney (b) and liver (c) of *H. fossilis* under pH 7.4 (control), acidic pH 6.4 and alkaline pH 8.4 following 96 hour, 5 days and 10 days exposure as determined by quantitative real time PCR. Levels of *aqp1* transcripts in exposed groups are expressed as fold changes relative to control group after being normalized against *hfactβ* standard. Data shown as mean values ± S.E (n=3) of relative concentrations. Letters a, b and c represent statistically significant difference between respective control and treated groups where P <0.05, 0.01 and 0.001 respectively.

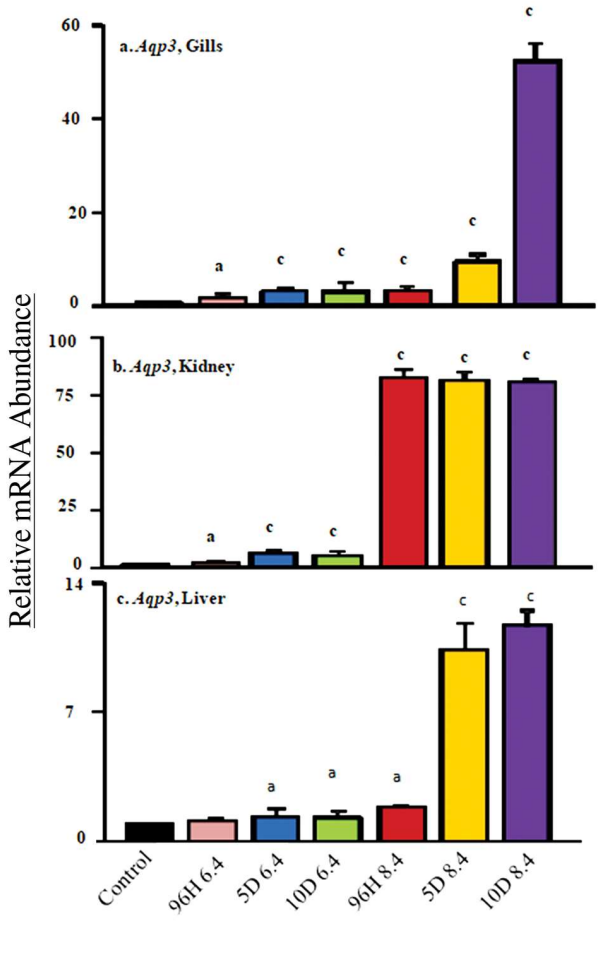

Fig. 2. Relative *Aqp3* mRNA abundance in gills (a), kidney (b) and liver (c) of *H. fossilis* under pH 7.4 (control), acidic pH 6.4 and alkaline pH 8.4 following 96 hour, 5 days and 10 days exposure as determined by quantitative real time PCR. Levels of *aqp3* transcripts in exposed groups are expressed as fold changes relative to control group after being normalized against *hfactβ* standard. Data shown as mean values ± S.E.M (n=3) of relative concentrations. Letters a, b and c represent statistically significant difference between respective control and treated groups where P <0.05, 0.01 and 0.001 respectively.

2.57 folds in 96 hrs, 8.4 folds in 5 days and 10.9 folds in 10 days. In liver, *aqp1* is downregulated by 0.381, 0.44 and 0.27 folds at 96 hrs, 5 days and 10 days at pH 6.4; but upregulated by 4.06, 3.59 and 10.16 folds at pH 8.4 (Fig. 1c). Transcription of *aqp3* in gills (Fig. 2a) is upregulated by 1.86, 3.31 and 3.21 folds at 96 hr, 5days and 10 days respectively at pH 6.4; by 3.34, 9.43 and 52.183 folds at pH 8.4. In kidney, *aqp3* transcript level is increased by 1.85, 6.3 and 4.92 folds at pH 6.4; and drastically increased by 82.85, 81.29 and 80.6 at pH 8.4 compared to the control (Fig. 2b). As shown in (Fig. 2c), in liver at pH 6.4, significant difference was not observed between control and treated groups initially at 96 hrs, but raised to 1.3 folds by 10th day; however *aqp3* was upregulated at once at pH 8.4 by 1.88, 10.37 and 11.7 folds in 96 hr, 5 days and 10 days respectively. As shown in (Fig. 3a), transcription of *aqp8* in *H. fossilis* at altered pH following 96 hour, 5 days and 10

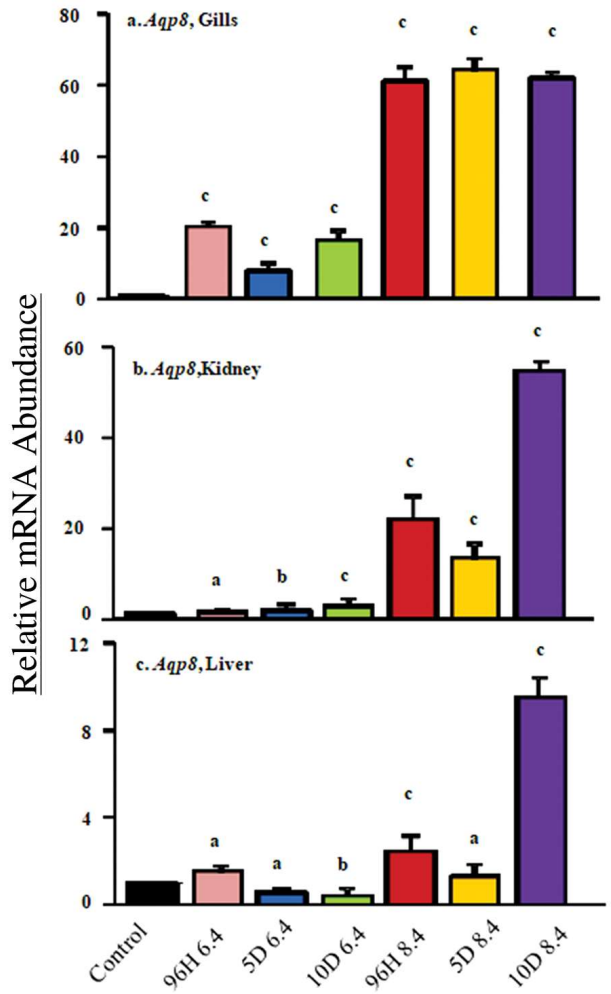

Fig. 3. Relative *Aqp8* mRNA abundance in gills (a), kidney (b) and liver (c) of *H. fossilis* under pH 7.4 (control), acidic pH 6.4 and alkaline pH 8.4 following 96 hour, 5 days and 10 days exposure as determined by quantitative real time PCR. Levels of *aqp8* transcripts in exposed groups are expressed as fold changes relative to control group after being normalized against *hfactβ* standard. Data shown as mean values ± S.E (n=3) of relative concentrations. Letters a, b and c represent statistically significant difference between respective control and treated groups where P <0.05, 0.01 and 0.001 respectively.

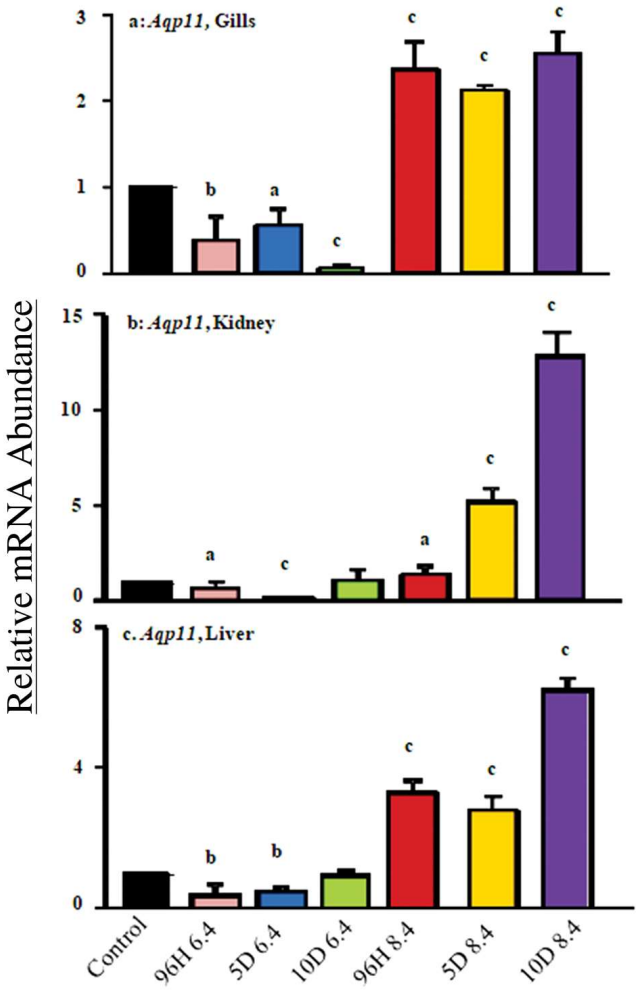

Fig. 4. Relative *Aqp11* mRNA abundance in gills (a), kidney (b) and liver (c) of *H. fossilis* under pH 7.4 (control), acidic pH 6.4 and alkaline pH 8.4 following 96 hour, 5 days and 10 days exposure as determined by quantitative real time PCR. Levels of *aqp11* transcripts in exposed groups are expressed as fold changes relative to control group after being normalized against *hfactβ* standard. Data shown as mean values ± S.E.(n=3) of relative concentrations. Letters a, b and c represent statistically significant difference between respective control and treated groups where P <0.05, 0.01 and 0.001 respectively.

days treatment shows increase in gills by 20.19, 7.72 and 16.6 folds (pH 6.4); whereas by 61.37, 64.21 and 61.9 folds at pH 8.4. In kidney, transcription raised by 1.44, 1.85 and 2.67 folds at pH 6.4; and by 22.08, 13.18, 54.78 folds at pH 8.4 (Fig. 3b). In liver, there was upregulation initially by 1.49 folds but it was decreased to 0.435 folds by the 10th day at pH 6.4. At pH 8.4, *aqp8* is upregulated by 2.44, 1.296 and 9.54 folds for 96 hrs, 5 days and 10 days respectively in liver (Fig. 3c). As shown in (Fig. 4), in Gills, kidney and liver *aqp11* showed downregulation at the acidic pH and showed increasing transcription as the pH rises. Levels of mRNA in comparison to the physiological pH of 7.4 were as follows: at pH 6.4, gills exhibited 0.39, 0.56, and 0.065 fold downregulation at 96 hrs, 5 days, and 10 days respectively. At pH 8.4 however, upregulation by 2.367, 2.12 and 2.557 folds were observed (Fig. 4a). In kidney, downregulation at pH 6.4 was observed

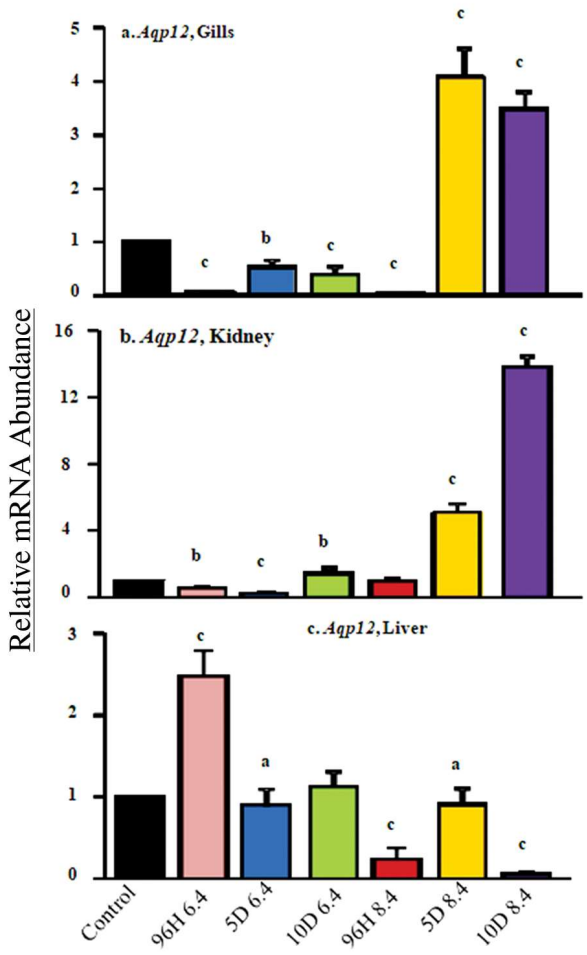

Fig. 5. Relative *Aqp12* mRNA abundance in gills (a), kidney (b) and liver (c) of *H. fossilis* under pH 7.4 (control), acidic pH 6.4 and alkaline pH 8.4 following 96 hour, 5 days and 10 days exposure as determined by quantitative real time PCR. Levels of *aqp12* transcripts in exposed groups are expressed as fold changes relative to control group after being normalized against *hfactβ* standard. Data shown as mean values ± S.E (n=3) of relative concentrations. Letters a, b and c represent statistically significant difference between respective control and treated groups where P <0.05, 0.01 and 0.001 respectively.

with 0.663, 0.129 folds at 96hr and 5 days but on 10th day returned close to control value with 1.1 fold relative abundance. Transcripts were gradually increasing by 1.4, 5.23 and 12.87 folds at 96 hrs, 5 days, and 10 days respectively at pH 8.4 (Fig. 4b). Similarly, liver also exhibited a downregulation with 0.371, 0.514 and 0.922 relative abundance at pH 6.4. *Aqp11* increased by 3.32, 2.78 and 6.224 folds at the higher pH of 8.4 (Fig. 4c). Compared to a control pH of 7.4, acidic pH 6.4 led to a

Table 1. Primer sequences of *H. fossilis* aquaporin isoforms

Primer	Sequence 5'→3'	Accession no	Annealing Temperature (°C)
Aqp1 F	TGTCAGGACCTCCGACTGAT	MF100768	53
Aqp 1R	TCTACTGCAAGTCCGAGGGA		
Aqp3 F	GCCACACTTGGAGTCTTGGT	MG545607	51
Aqp3 R	TGGACCTTCCGAGAATGCAG		
Aqp8 F	TCAGTAATGCGTCTGGAGTG	MG545608	53.8
Aqp8 R	CGCCGTGGTCAGAAACACAG		
Aqp11 F	CAACTGGTAGCGTGAACGTG	MK689683	52
Aqp11 R	CACCACTGCAGGATGTCTGT		
Aqp12 F	ACTCAGTGCAGTTCATCGCT	MG545609	56.2
Aqp12 R	GCTAAGGTCCGCAAGGTGAT		
hfactβ F	CAGCTGAGCGTGAAATCGTG	FJ409641	51-57
hfactβ R	TCCAGAGAGGATGAGGAGGC		

downregulation of *aqp12* transcription in gills and kidney but not in the liver (Fig. 5). It was observed that gills (Fig. 5a) showed decreasing mRNA levels of 0.08, 0.532 and 0.39 fold at 96 hrs, 5 days and 10 days of treatment. At pH 8.4 it was abruptly rising from 0.041 folds at 96hrs to 4.08 and 3.49 fold abundance. In the kidney also similar pattern was observed (Fig. 5b). At pH 6.4, at 96 hrs, 5 days and 10 days transcripts were 0.5, 0.227 and 1.39 compared to control. At pH 8.4, kidney exhibited upregulation by 1.01, 5.08 and 13.824 folds. However a low pH induced upregulation of *aqp12* in the liver and a subsequent downregulation at a high pH (Fig. 5c). At pH 6.4, transcript abundance was increasing by 2.5 to 1.13 folds and at pH 8.4 it was downregulated by 0.231, 0.91 and 0.0547 folds.

Discussion

Not much is known about the pH dependent transcription of aquaporin genes in fish. Nonetheless, a few studies addressed pH dependent functionality of these channel proteins in different fishes. Besides, there is no available study on the transcription rates of aquaporins under pH stress in *H. fossilis* and this is the first report on this fish. In the present study, different isoforms of *H. fossilis* aquaporins showed sensitivity towards both acidic and alkaline pH. Transcription of aquaporin isoforms changed at the altered pH, in a trend

that observed increase with increasing pH except in the liver of *H. fossilis*. In comparison to the physiological pH 7.4, at pH 8.4 most isoforms were upregulated two to tens of folds depending upon the organ in concern. Moreover, extensive treatment for 10 days induced maximum transcription rate which provides evidence that the longer the exposure time, the greater will be the effect of altered pH on the transcription of these genes. In the kidney and gills of *H. fossilis* uniformity was observed for the mRNA levels of *aqp1, 3, 8, 11* and *12* under varying pH. The classical *aqp1* was not drastically regulated at a lower pH and it remained somewhat stable in these organs, but in the liver acidic pH significantly downregulates *aqp1*. However, for the high pH of 8.4, *aqp1* was unanimously showing significant upregulation in all the tissues in concern. A rise in mRNA transcripts of *aqp1* upon increasing the pH was also observed by Zhai *et al.,* 2018 in HEK-293T cells with two-fold higher mRNA levels at pH 8 than those at pH 6. They also reported that the *AQP1* promoter contains a pH-sensitive region upstream of the *AQP1* gene ("2300 to 2200" bp) that upregulates *AQP1* expression in response to increasing pH via the action of the SPIB transcription factor.

Aqp3 was upregulated for the either range of pH in all the three tissues. Even though at pH 6.4 there was only slight upregulation, an abrupt rise in transcripts was observed (up to 82.85 folds in kidney) at the high pH 8.4. Similarly, a many fold rise in transcripts of *aqp8* was observed in the gills (64.216 folds) and kidney (54.78 folds) in alkaline pH 8.4. In the liver as well, after a decrease in transcript at lower pH, it was again upregulated to 9.54 folds by the 10^{th} day. *Aqp11* mRNA levels decreased in all the tissues at a low pH and then it began rising as the pH increased, as far as 12.87 folds in kidney on the 10^{th} day. Compared to a control pH of 7.4, acidic pH 6.4 led to a downregulation of *aqp12* in gills and kidney but not the liver where it was showing significant upregulation. Conversely, *aqp12* was downregulated at high pH in liver but in gills and kidney it was upregulated to 13.824 folds by the 10^{th} day (in kidney). This sporadic regulation of mRNA levels in different aquaporins upon pH changes indicates that aquaporins are of huge importance for the catfish to adapt in pH associated environmental stresses. The change in water pH might have caused the ion regulatory and acid-base disturbances originating at the gill leading to the altered internal pH, electrolyte and osmotic balances causing drastic changes in transcription rates of these aquaporins.

Although the regulation of aquaporin genes at transcript levels has not been addressed much, several studies do support regulation at protein function level by altering pH. Fish aquaporins are gated by pH and they show varying water conductivity at different pH. Paralog specific pH sensitivities are quite common. Teleost AQP0a, such as Atlantic salmon AQP0a2, zebrafish AQP0a, and killifish AQP0a, show maximum water channel activity at alkaline pH, whereas teleost AQP0 b-type, such as Atlantic salmon AQP0b1 and zebrafish AQP0b are more permeable at acidic pH (Virkki *et al.,* 2001). The other two paralogs of AQP0 in Atlantic salmon also shows specific pH sensitivity, such as AQP0a1 is more permeable at neutral pH, whereas AQP0b2 does not display pH sensitivity. Likewise, mammalian AQP0 also display an acidic pH permeation preference (Chauvigne *et al.,* 2015). In the lens or in oocytes, if the external pH decreases from 7.5 to 6.5 it causes a two to fourfold increase in water permeability of mammalian AQP0 (Varadaraj *et al,.* 2005). Even plant AQP activity is modulated by pH. pH dependent modification of the aquaporin structure leading to a change in water conductivity has been reported in plants by Fischer and Kaldenhoff (2008). AQP3 (Zeuthen & Klaerke, 1999), AQP6 (Yasui *et al.,* 1999), and bovine AQP0 (Ne′meth-Cahalan & Hall, 2000), were shown to be sensitive to pH. Water and glycerol permeability of human and eel AQP3 is inhibited at a low pH (Zeuthen & Klaerke, 1999). Rat AQP4 is not acidic pH sensitive but exhibits maximum water permeability at alkaline pH (Ne′meth-Cahalan and Hall, 2000).

In this study, different isoforms of *H. fossilis* aquaporin have been shown to exhibit pH sensitivity at transcriptional level. However, water permeability under altered pH for this fish was not performed. Due to pollution and other factors, pH of the water bodies undergoes drastic

changes affecting the aquatic animals and plants. Hardy fish like the *H. fossilis* can survive in these adverse conditions without much difficulty. Fine tuning of water homeostasis appears to be of crucial importance for survival in environments with altered pH, salinity, drought and other such challenges. It is expected that since the catfish is quite resilient to environmental stresses, it maintains homeostasis through the regulation of proteins required for sustaining normal body physiology, and aquaporins play a significant role amongst them.

Acknowledgement

The authors are thankful to the Head, Department of Zoology, Gauhati University; University Grants Commission Special Assistance Programme (UGC SAP), for providing the necessary facilities and equipments for conducting the purpose of the study. The authors acknowledge the Department of Science and Technology India, Science and Engineering Research Board, Early Career Research (DST SERB ECR) Grant with Sanction Number: ECR12016/000809 dated 7th March 2017 for provision of financial support. The funders had no role in the process of study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

Alleva K, Niemietz C M, Sutka M, Maurel C, Parisi M, Tyerman S D and Amodeo G. 2006. Plasma membrane of *Beta vulgaris* storage root shows high water channel activity regulated by cytoplasmic pH and a dual range of calcium concentrations. J. Exp. Bot. 57, 609-621.

Chandy G, Zampighi G A, Kreman M, and Hall J E. 1997. Comparison of the water transporting properties of MIP and AQP1.J. Membr. Biol. 159, 29-39.

Chauvigne′ F, Zapater C, Stavang J A, Taranger G L, Cerda‘ J, and Finn R N. 2015. The pH sensitivity of Aqp0 channels intetraploid and diploid teleosts. FASEB J. 29: 2172–2184.

Dong C, Chen L, FengJ,Xu J, Mahboob S, Al-Ghanim K *et al.*, 2016. Genome wide identification, phylogeny, and expression of Aquaporin genes in common carp (*Cyprinus Carpio*). PLoS ONE 11(12).

Finn R N, Chauvigné F, Hlidberg J B, Cutler C P, and Cerdá J. 2014. The lineage-specific evolution of aquaporin gene clusters facilitated tetrapod terrestrial adaptation. PLoS One 9(11).

Fischer M and Kaldenhoff R. 2008. On the pH regulation of plant aquaporins. The J. Biol. Chem. 283 (49) : 33889-33892.

Johansson I, Karlsson M, Shukla V K, Chrispeels M J, Larsson C and Kjellbom P. 1998. Water transport activity of the plasma membrane aquaporin PM28A is regulated by phosphorylation. Plant Cell. 10: 451-459.

Johansson I, Larsson C, Ek B and Kjellbom P. 1996. The major integral proteins of spinach leaf plasma membranes are putative aquaporins and are phosphorylated in response to Ca2+ and apoplastic water potential. Plant Cell. 8: 1181-1191.

King L S, Kozono D, and Agre P. 2004. From structure to disease: the evolving tale of aquaporin biology. Nat. Rev. Mol. Cell Biol. 5: 687-698.

Krane C M, and Kishore B K. 2003. Aquaporins: The membrane water channels of the Biological world. Biologist. 50(2): 81-86.

Kubista M, Andrade J, Bengtsson M, Forootan A, Jonák J, Lind K, Sindelka R, Sjöback R, Sjögreen B, Strömbom L, Ståhlberg A and Zoric N. 2006. The real-time polymerase chain reaction. Molecular aspects of medicine. 27. 95-125.

Livak K J and Schmittgen T D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta Ct}$ methods, Methods. 25 (4). 2001, Pages 402-408,

Murata K, Mitsuoka K, Hirai T, Walz T, Agre P, Heymann J B, Engel A &Fujiyoshi Y. 2000. Structural determinants of water permeation through aquaporin-1. Nature. 407: 599-605.

Ne′meth-Cahalan K L and Hall J E. 2000. pH and calcium regulate the water permeability of aquaporin 0. J. Biol. Chem. 275:6777-6782.

Ne′meth-Cahalan K L, Kalman K and Hall J H. 2004. Molecular basis of pH and Ca^{2+} regulation of aquaporin water permeability. The J. General Physiol. 123(5): 573-580.

Rio DC, Ares M Jr, Hannon GJ and Nilsen TW. 2010. Purification of RNA using TRIzol (TRI reagent). Cold Spring HarbProtoc. 2010(6):pdb.prot. 5439.

Schmittgen T D and Livak K J. 2008. Analyzing real-time PCR data by the comparative C(T) method. Nat Protocols 3(6): 1101-1108.

Stahlberg H, Heymann B, Mitsuoka K, Fuyijoshi Y and Engel A. 2001. "The aquaporin superfamily: structure and function,"in *Aquaporins*, eds S. Hohmann, S. Nielsen, and P. Agre (New York: Academic Press). Pp. 40-119.

Sui H, Han B G, Lee J K, Walian P. and Jap B K. 2001. Structural basis of water-specific transport through the AQP1 water channel. Nature. 414: 872-878.

Takata K., Matsuzaki T, and Tajika Y. 2004. Aquaporins: water channel proteins of the cell membrane. Prog.Histochem.Cytochem. 39: 1-83.

Tingaud-Sequeira A, Calusinska M, Finn R N, Chauvigné F, Lozano J and Cerda' J. 2010. The zebrafish genome encodes the largest vertebrate repertoire of functional aquaporins with dual paralogy and substrate specificities similar to mammals. BMC Evol. Biol. 10: 38.

Tournaire-Roux C, Sutka M, Javot H, Gout E, Gerbeau P, Luu D T, Bligny R and Maurel C. 2003. Cytosolic pH regulates root water transport during anoxic stress through gating of aquaporins. Nature. 425: 393-397.

Varadaraj K, Kumari S, Shiels A, Mathias R T. 2005. Regulation of aquaporin water permeability in the lens. Invest. Ophthalmol. Vis. Sci. 2005;46(4):1393-1402.

Verdoucq L, Grondin A and Maurel C. 2008. Structure-function analysis of plant aquaporin AtPIP2;1 gating by divalent cations and protons. Biochem. J. 415: 409-416.

Virkki L V, Cooper G J, and Boron W F. 2001. Cloning and functional expression of an MIP (AQP0) homolog from killifish (*Fundulus heteroclitus*) lens. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 281:6, R1994-R2003.

Yang B, and Verkman A S. 1997. Water and glycerol permeability of aquaporins 1-5 and MIP determined quantitatively by expression of epitope- tagged constructs in *Xenopus* oocytes. J. Biol. Chem. 272: 16140-16146.

Yasui M, Kwon T H, Knepper M A, Nielsen S and Agre P. 1999. Aquaporin-6: an intracellular vesicle water channel protein in renal epithelia. Proc. Natl Acad. Sci. USA. 96: 5808-5813.

Zardoya R. 2005. Phylogeny and evolution of the major intrinsic protein family.Biol. Cell. 97: 397-414.

Zeuthen T and Klaerke D A. 1999. Transport of water and glycerol in aquaporin 3 Is gated by H^+. J. Biol. Chem. 274:21631-21636.

Zhai Y, Xu H, Shen Q, Schaefer F, Schmitt C P, Chen J, Liu H, Liu J and Liu Jiaojiao. 2018. pH mediated upregulation of *AQP1* gene expression through the SpiB transcription factor. BMC Molecular Biol. 19:4.