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SPECIALTY SECTION

This article was submitted to
Aquatic Physiology,
a section of the journal
Frontiers in Marine Science

RECEIVED 02 August 2022

ACCEPTED 24 August 2022

PUBLISHED 12 September 2022

CITATION

Huang M, Zhang L-J, Wu M-X,
Peng G-F and Zhang Y-L (2022)
Aquaporins1 and 3 in the tissues of
Paramisgurnus dabryanus and their
expression profiles in response to
ammonia and drought.
Front. Mar. Sci. 9:1009679.
doi: 10.3389/fmars.2022.1009679

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Aquaporins1 and 3 in the tissues of *Paramisgurnus dabryanus* and their expression profiles in response to ammonia and drought

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Aquaporins (Aqp)1 and Aqp3 are the representatives of two types of aquaporins (classical aquaporin and aquaglyceroporin) and have been proven to potentially facilitate ammonia detoxification in air-breathing fishes. *Paramisgurnus dabryanus* is one of East Asia's most important commercially farmed species and can breathe air directly through its hindgut. Besides, excellent ammonia tolerance was reported in this species. However, the mechanisms of its tolerance of high ammonia are still unclear. To explore the physiological roles of Aqp1 and Aqp3 in *Paramisgurnus dabryanus*, we detected the expression pattern of these two Aqps in several tissues and their transcriptional changes in response to different levels (30, and 70 mmol L⁻¹ NH₄Cl) and periods (0, 12, 48, and 96 h) of ammonia exposure and different periods (0, 12, 48, and 96 h) of drought stress. The *aqp1* mRNA showed a high abundance in the brain and the osmoregulatory tissues, such as the intestine and kidney. While *aqp3* was mainly expressed in the epithelium of skin and gills that were directly exposed to the environment. The expression level of *aqp3* in gills was more responsive than *aqp1* to ammonia challenges, although both of them were up-regulated. Considering the wide difference between *aqp1* and *aqp3* in the skin, *aqp3* increased significantly after 12 h of air exposure. The changes of the two *aqps* in the intestine of *P. dabryanus* were relatively stable after ammonia stress. After drought stress, intestinal *aqp1* increased significantly, while *aqp3* decreased. Combined with the effects of tissue location and environmental stress on Aqps, it was suggested that Aqp1 was mainly involved in osmotic regulation, providing water as raw material for body metabolism. Accordingly, our results suggest that Aqp3 has the positive function of assisting ammonia transportation and epidermal moisturizing.

KEYWORDS

aquaporins, air-breathing fish, ammonia loading, drought stress, gene expression

Introduction

Aquaporins (Aqp) are a superfamily member of integral membrane proteins and mainly function as membranal transport channels for water and glycerol (Cerdà and Finn, 2010). At present, 17 Aqps (Aqp0-16) have been identified in animals (Finn and Cerdà, 2015), while the classifications and physiological functions of Aqp13-16 are still unclear (Finn and Cerdà, 2015; Madsen et al., 2015). The studies of the physiological functions of Aqps were mostly found in mammals, including the transport of water, glycerol, urea, and several gases (e.g., CO₂, NO, NH₃, and O₂). The previous results indicated that Aqp1, 3, 8, and 9 played an important role in regulating membranal NH₃ permeability and consequently affected the ammonia transport in mammals (Saparov et al., 2007; Musa-Aziz et al., 2009; Herrera and Garvin, 2011; Geyer et al., 2013). For aquatic organisms, the most relevant studies focused on the effects of Aqps on regulating osmotic pressure (Deane et al., 2011; Engelund and Madsen, 2011). Only a few studies reported that Aqp1 and 3 were very related to ammonia tolerance in fish species (Chen et al., 2010; Kolarevic et al., 2012; Ip et al., 2013; Talbot et al., 2015). However, the effects and regulation mechanisms of Aqp1 and 3 on teleosts adapted to high environmental and/or endogenous ammonia are still unclear. Therefore, there is a great need to reveal the effects of Aqp1 and 3 on regulating ammonia tolerance in fish species because the results will help to analyze the mechanisms of fish adapted to deteriorating or drought environments.

Compared with mammals, the information on the physiological function of Aqps in teleosts is very poor. Whole-genome duplication in zebrafish (*Danio rerio*) obtained 20 Aqps (including isoforms) and the function of these Aqps was shown to be similar to mammals (Tingaud-Sequeira et al., 2010). Subsequently, functional Aqps were identified in several teleost species, such as *Dicentrarchus labrax* (Giffard-Mena et al., 2011), *Sparus sarba* (Deane et al., 2011), salmon (Kolarevic et al., 2012; Macqueen and Johnston, 2014), *Oryzias dancena* (Kim et al., 2014), *Anabas testudineus* (Ip et al., 2013), *Protopterus annectens* (Chng et al., 2016), *Misgurnus mizolepis* (Lee et al., 2017), *Lateolabrax maculatus* (Shen et al., 2019), and *Trachidermus fasciatus* (Ma et al., 2020). It follows that Aqps are widely distributed in teleosts. However, research on the physiological function of Aqps in teleosts is still limited. Recent reports have shown that Aqp1aa and Aqp1al located in the gill of zebrafish played an important role in gas transport and significantly increased the membranal NH₃ permeability (Chen et al., 2010; Talbot et al., 2015). For *Salmo salar*, 22 days of ammonia exposure induced a reduction of *aqp3a* mRNA expression quantity, while up-regulation of *aqp3a* mRNA expression quantity was observed when the exposure time was prolonged to 105 days (Kolarevic et al., 2012), suggesting that Aqp3a could facilitate ammonia transport in fish species. Similarly, *aqp1aa*

mRNA quantity in the gill and skin of *A. testudineus* decreased remarkably during ammonia exposure. This obvious down-regulation could be used to inhibit external ammonia uptake (Ip et al., 2013). Although the reports about the effects of Aqps on ammonia transport in fish are very poor, all the results of previous studies reveal that several Aqps, especially Aqp1 and 3, are closely related to the adaptation to internal and/or external ammonia loading in teleosts.

P. dabryanus is an important economic aquaculture variety in East Asia (Liu et al., 2018) and has the characteristics of low oxygen tolerance, fast growth, high nutrition, and delicious taste (Dong et al., 2018). Compared with other fishes, *P. dabryanus* has great advantages in aquaculture management and long-distance transportation (Zaccone et al., 2018). As a typical air-breathing fish (Zhang et al., 2016), the gills, skin, and intestines of *P. dabryanus* are the main sites that contribute to the alleviation of osmotic stress and harbor mechanisms to respire. Moreover, *P. dabryanus* is an excellent model for the study of the mechanisms controlling the acquisition, retention, and metabolic waste excretion due to its characteristics of high tolerance to the harsh environment (Zhang et al., 2017; Zhang et al., 2019; Zhang et al., 2021; Huang et al., 2022). However, the regulation mechanism of air-breathing fish facing a harsh environment is not clear. Further study of the function and molecular regulation mechanisms of Aqps in the process of environmental stress is not only the theoretical basis of fish physiology and ecology but also an important issue to improve the utilization rate of water in the aquaculture industry. Therefore, the present study aims to determine the tissue distribution of Aqp1 and Aqp3 and their transcriptional changes in response to high levels of exogenous and endogenous ammonia loading in *P. dabryanus*. These results provide theoretical support for a comprehensive understanding of the potential function of Aqps in biological processes and the coordination role of Aqps in maintaining physiological homeostasis. An understanding of the involvement of Aqps in ammonia defense in teleosts, especially in air-breathing species, leads to improved information on the air-breathing fish adapted to high ammonia environments. The present paper will provide a broader perspective on ammonia defensive strategies in teleosts and is helpful for molecular-assisted breeding of ammonia-resistant varieties.

Materials and methods

Ethics statement

This study was carried out by the principles of the Basel Declaration and Recommendations of the Guide for the Care and Use of Laboratory Animals published by the Animal Care Committee of Anhui Agricultural University (Hefei, China).

The protocol was approved by the Animal Care Committee of Anhui Agricultural University.

Animals

Fish specimens of *P. dabryanus* (18–22 g) were obtained from a local Hefei aquatic market. Fish were kept in dechlorinated tap water at 22–24 °C in fiberglass tanks for at least 2 weeks regime before the experiments. *P. dabryanus* with similar specifications was selected from the tank (70 cm × 40 cm × 40 cm) and placed in a square plastic basin (30 cm × 20 cm × 20 cm).

Experimental design and sample collection

Seven somatic tissues (gill, intestine, skin, muscle, liver, kidney, and brain) were derived from healthy individuals for the tissue distribution assay. The ammonia loading group was configured with NH₄Cl and set into two concentration groups of 30 mmol L⁻¹ and 70 mmol L⁻¹, using NaOH (4 mol L⁻¹) to adjust the pH to 7.2–7.5. During the exposure test, the NH₄Cl solution was totally exchanged every 24 hours to maintain the ammonia concentration. In the drought stress group, the selected *P. dabryanus* were directly placed in the same temporary incubator and added 100 ml of distilled water to keep the body moist during exposure, and the temperature was kept at (25.0 ± 1.0) °C. The 100 ml of distilled water was totally exchanged every 8 hours to maintain the humidity. Fish reared in temporary tanks were used as a control group. There were 3 parallel groups at each exposure time (12, 48, and 96 h) for each group, and 4 fish in each parallel group. After the temporary cultivation, the experimental fish were anesthetized with MS-222 (200 mg L⁻¹). The fish were dissected and the tissues were taken, and the fish was frozen and stored at -80 °C for subsequent use.

RNA extraction and cDNA synthesis

The total RNA of the sample was extracted using the Ultrapure RNA Kit (DNase I), and the concentration and

purity of RNA were measured using a spectrophotometer. The extracted RNA was checked using 1% agarose electrophoretically and a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA) to verify integrity. RNA samples were kept at -20 °C. The HiScriptIII RT SuperMix for qPCR (+gDNA wiper) kit was used to reverse transcribe the RNA into cDNA. DNase treatment was performed using template RNA (2 μl), 4× gDNA Wiper Mix (4 μl), and RNase-free ddH₂O (10 μl) which were configured into a 16 μl mixture at 42 °C for 2 min. The reverse transcription reaction was carried out with 5 HiScriptII qRT SuperMix (4 μl) directly added to the reaction solution of 16 μl, which was kept at 37°C for 15 minutes and then at 85°C for 5 seconds.

Real-time quantitative PCR

The gene sequence was obtained using primers designed from the highly conserved regions of the transcriptome sequencing sequence in our laboratory. All primers in this experiment were synthesized by Sangon Biotech (Shanghai, China) (Table 1). The cDNA of each tissue was used as a template, and the mixed solution was configured as 2×AceQ qPCR SYBR Green Master Mix (10 μl), forward primers (0.4 μl), reverse primer (0.4 μl), template cDNA (2 μl), and ddH₂O (7.2 μl). The following qPCR conditions were used: 5 minutes of pre-denaturation at 95°C, 10 seconds of denaturation at 95°C, 30 seconds of annealing at 60°C, and 40 cycles. Relative mRNA levels were calculated using the 2^{-ΔΔCt} method as described by Livak and Schmittgen (2001), with the reference gene *β-actin* being used as an internal standard to normalize the results.

Data analysis

The variance homogeneity of the data was examined using Levene's test. One-way (exposure time, different groups) and two-way (exposure time × NH₄Cl concentration) analysis of variance (ANOVA) was used to compare mRNA expression levels. Tukey's (homogeneity of variance) and Games-Howell's (inhomogeneity of variance) multiple tests were performed

TABLE 1 List of primers used in this study.

Gene	Primer	Primer sequence	Tm (°C)	Product length (bp)
<i>aqp1</i>	F	GCTGGTTGGCATGACCTCTTC	61.5	121
	R	AGTGGCGATGGACAAACCGAAAG	60.4	
<i>aqp3</i>	F	TCCATCATTGGCGTGATTGTGTACC	59.6	134
	R	AGGCTAGATCCATCCTTACTGGTGAC	59.8	
<i>β-actin</i>	F	AGACCTTCAACACCCCTGCC	60.6	132
	R	AACCCTCGTAGATGGGCACG	60.2	

All the primers were designed based on transcriptome assemblies (Shang et al., 2021) and synthesized by Sangon Biotech (Shanghai, China).

when significant differences were found at the 0.05 level. The *t*-test was performed to compare the differences between the 30 mmol L⁻¹ and 70 mmol L⁻¹ NH₄Cl groups at the same exposure time. Statistical analyses were performed using SPSS software (SPSS Inc. Chicago, IL, USA).

Results

The distribution of *aqp1* and *aqp3* in the tissues of *P. dabryanus*

The expression levels of *aqp1* and *aqp3* mRNA of *P. dabryanus* are shown in Figure 1. The *aqp1* expression level in the brain was the richest, up to 1448-fold of the skin, whereas the expression levels in the intestine and kidney were 166 and 129-fold of the control, respectively ($P < 0.05$, Figure 1A). The *aqp1* expression was relatively low in the liver, muscle, gills, and skin ($P > 0.05$, Figure 1A). The transcript of *aqp3* in the skin was 7503-fold higher than that in the liver ($P < 0.05$, Figure 1B). Aside from the skin, *aqp3* mRNA was primarily expressed in the gills, which was 2027-fold higher than the control ($P < 0.05$, Figure 1B). *aqp3* was relatively lower in muscle and intestinal tissues, but still 279-fold and 178-fold higher than that in control tissue ($P < 0.05$, Figure 1B). The *aqp3* mRNA expression level was the lowest in the kidney, brain, and liver ($P > 0.05$, Figure 1B).

Changes in *aqp1* and *aqp3* expression after ammonia stress

Two-way ANOVA results showed there were significant interactions between different NH₄Cl concentrations and exposure times on *aqp1* and *aqp3* expression in the gills, skin, and intestine (except *aqp3* in gills) of *P. dabryanus* ($P < 0.05$,

Table 2). The expression of *aqp1* showed an opposite pattern in different groups in gill. It was significantly increased and reached 3.34-fold at 96 h in the 30 mmol L⁻¹ group, while it decreased in the 70 mmol L⁻¹ group ($P < 0.05$, Figure 2A). *aqp1* was significantly induced in the skin and increased to 3.78-fold in the 70 mmol L⁻¹ group at 12 h ($P < 0.05$, Figure 2A). Intestinal *aqp1* only changed significantly at 12 h ($P < 0.05$). It was increased in the low concentration group while decreased in the high concentration group ($P > 0.05$, Figure 2A). It is worth noting that the changes in the expression levels of AQP1 and 3 in the skin were only related to the concentration of NH₄Cl, and were not significantly affected by exposure time ($P > 0.05$, Table 2). *aqp3* was significantly influenced by NH₄Cl ($P < 0.05$, Table 2) in the gills, and significantly up-regulated to 4.74 and 5.22-fold at 96 h of 30 mmol L⁻¹ and 70 mmol L⁻¹ groups, respectively ($P < 0.05$, Figure 2B). The transcription of *aqp3* in skin reached a maximum value of 4.11-fold at 48 h in the 30 mmol L⁻¹ group ($P < 0.05$, Figure 2B). Intestinal *aqp3* increased significantly in the early period ($P < 0.05$, Figure 2B). However, in the high concentration group, it was down-regulated in the early period ($P < 0.05$, Figure 2B).

aqp1 and *aqp3* expression changes after air exposure

The expression levels of *aqp1* and *aqp3* in the gills of *P. dabryanus* showed a similar pattern under drought conditions, and gradually decreased with the exposure time ($P < 0.05$, Figure 3A). Transcripts of *aqp1* and *aqp3* showed opposite changes in the skin. During the early exposure time, *aqp1* had no significant change, but higher mRNA levels were observed as the time went by ($P < 0.05$, Figure 3A). Transcription of *aqp3* increased significantly to 1.82-fold in a short period and then began to decline significantly ($P < 0.05$, Figure 3B). The changes

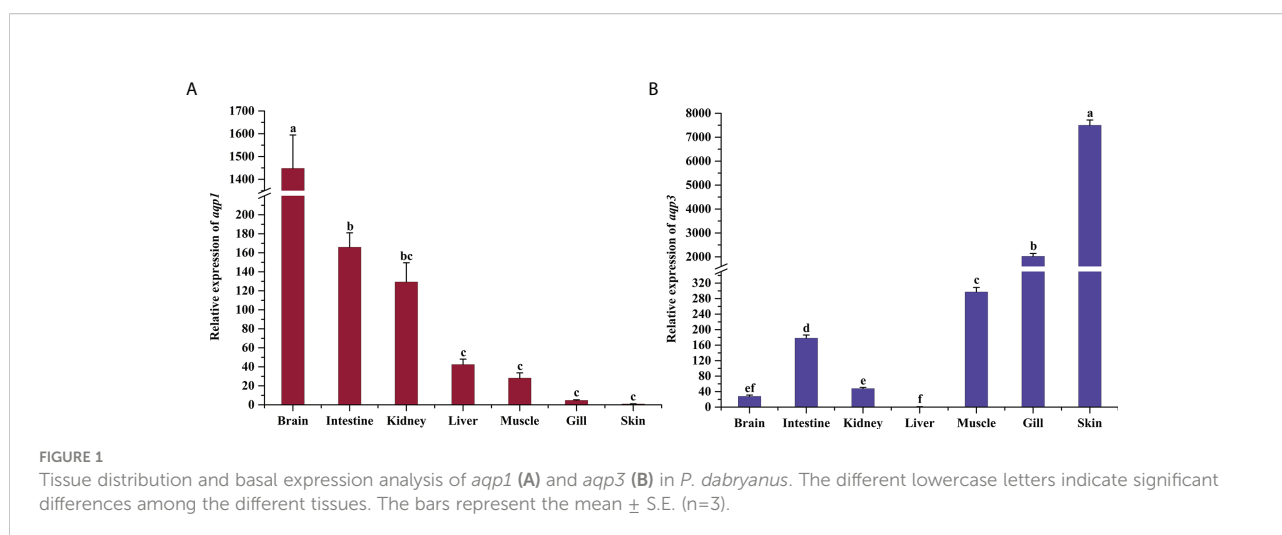


TABLE 2 Two-way ANOVA *P* value in an ammonia exposure trial.

Significance		<i>aqp1</i>	<i>aqp3</i>
Gill	N	0.002	<0.001
	ET	<0.001	0.733
	N × ET	0.001	0.575
Skin	N	0.025	<0.001
	ET	0.669	0.081
	N × ET	<0.001	<0.001
Intestine	N	0.023	0.036
	ET	0.004	0.023
	N × ET	0.001	<0.001

N, NH₄Cl concentrations (30 mmol L⁻¹, 70 mmol L⁻¹); ET, exposure time.

in intestinal *aqp1* were more rapid than those of *aqp3*. The mRNA level of *aqp1* increased significantly (4.8-fold) in the early time and remained significant at 96 h ($P < 0.05$, Figure 3A), whereas the mRNA level of *aqp3* decreased and was significantly lower than the control group ($P < 0.05$, Figure 3B).

Discussion

Tissue expression assay of *P. dabryanus aqp1* and *aqp3*

In the present study, transcripts encoding *aqp1* were detectable in the brain, intestine, gills, kidney, and other tissues of *P. dabryanus*, which play an important role in osmotic regulation, while *aqp3* was present in high levels in the skin, gills, and intestine that regulate respiration and osmotic pressure. The two distinct Aqps in the European eel (*Anguilla anguilla*) also have a similar distribution pattern. *aqp1* was highly expressed in the brain, eye, and heart, but much lower in the esophagus, stomach, intestine, skeletal muscles, gills, and kidney. Aquaglyceroporin was mainly expressed in the intestine and kidney, but rarely in the brain (Martinez et al., 2005a).

The expression level of *aqp1* in the brain of *P. dabryanus* was the highest, coinciding with the mammalian species, which plays an important role in cerebral edema, hydrocephalus, and the migration of brain tumors (Li et al., 2020). As the regulatory center, brain cells rich in Aqp1 can maintain cell matter exchange and energy production through rapid water transportation. *M. mizolepis* Aqp1a is also mainly distributed in heart and brain tissues, requiring high energy consumption (Lee et al., 2017). *P. dabryanus* brain *aqp3* mRNA abundance was low, which might be due to its substrate permeability to NH₃ and CO₂ (Geyer et al., 2013). NH₃ is neurotoxic to brain cells and can trigger severe blood-brain barrier breakdown, neuroinflammation, mitochondrial dysfunction, and affect the concentration of NO and Ca⁺ in cells, thereby leading to the excessive production of reactive oxygen species (ROS) (Jo et al.,

2021). Excessive CO₂ can cause increased cerebral blood volume, resulting in cerebral edema and increased intracranial pressure (Deng et al., 2020). A low level of Aqp3 in the brain can avoid the toxic damage of NH₃ and CO₂. The expression of *aqp3* in *P. dabryanus* skin was very surprising. Similarly, it also has obvious advantages in external exposed organs such as the fins, gills, and eyes of *M. mizolepis* (Lee et al., 2017). Different from the first two species, the content of Aqp3 in the spleen and gills of Mozambique tilapia (*Oreochromis mossambicus*) was higher than that in the skin (Watanabe et al., 2005). For air-breathing fish like *P. dabryanus*, the skin acts as a transporting epithelium for gas, ions, and nutrients. Thus, the abundance of Aqp3 in the skin enables the epidermis to play a more active physiological role (Glover et al., 2013).

Gills are the primary water transport pathway, accounting for approximately 90% of total body water influx (Foguesatto et al., 2017). The expression level of branchial *aqp1* in *P. dabryanus* was relatively low, which may be due to its role in concert with other homologs (e.g., Aqp3 in this study). Immunolocalization of the gill epithelium of *Coris julis* showed that Aqp1 was located in chloride cells, while Aqp3 was in chloride cells and accessory cells (Brunelli et al., 2010). Cutler and Cramb (2002) believed that Aqp3 may involve the discharge of ammonia and urea in eel gills, in addition to discharging excess water to prevent cell swelling and bursting.

P. dabryanus aqp3 mRNA exists sporadically in the kidney, as well as in spiny dogfish, *Squalus acanthias*, and these findings are consistent with our results (Cutler et al., 2022). After seawater domestication, *aqp3* expression levels in the kidneys of European eel, Atlantic salmon (*Salmo salar*), and European bass (*Dicentrarchus labrax*) were significantly increased (Engelund and Madsen, 2011). In contrast to the European yellow eel, European silver eel Aqp1 is mainly distributed in the apical brush border of renal epithelial cells, with a small amount in vascular endothelium (Martinez et al., 2005a). Taken together, Aqp3 has potential functional roles in water retention, glycerol supplementation, and urea transportation in the teleost kidney. The expression level of *aqp1* in the intestine of European eels in seawater was significantly increased by 10-25-fold compared to that in freshwater (Martinez et al., 2005b). European eel intestine Aqp3 was distributed in macrophage-like cells and goblet cells, and the number of goblet cells increased greatly and became wider and more round after seawater acclimatization (Lignot et al., 2002). The distribution characteristics of Aqp3 in the rectum indicated the potential role of Aqp3 in mucus secretion for keeping the surface of the intestine moist. The piscine liver performs basic metabolic functions, including bile formation and excretion, and is also known as the main detoxification organ, participating in the contribution of macrophages (Mokhtar, 2018). It has been reported that hepatic Aqp3 may be involved in both mammalian Kuffer cell migration and secretion of pro-inflammatory cytokines, reflecting its involvement in immune response (Rodríguez et al., 2011). The

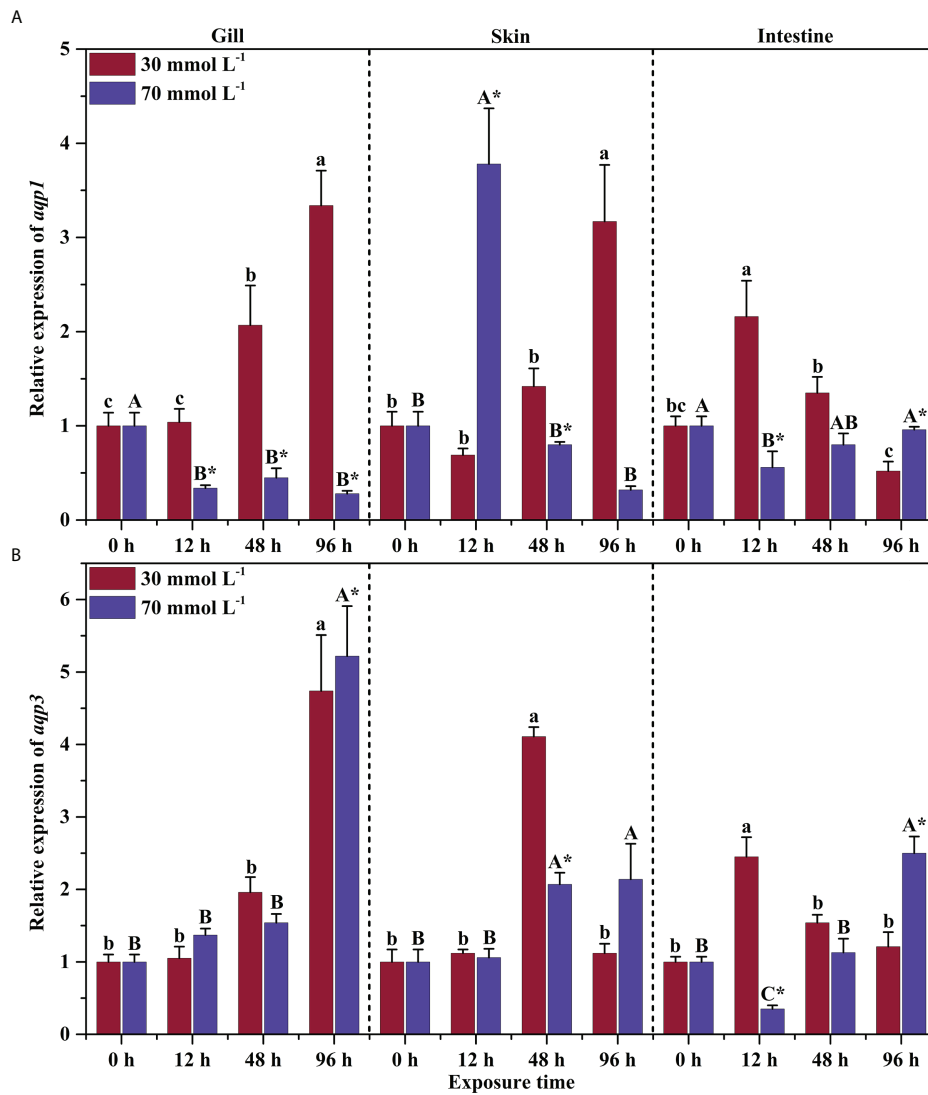


FIGURE 2 Effects of ammonia exposure on the relative expression of *aqp1* (A) and *aqp3* (B) in different tissues of *P. dabryanus*. The different lowercase letters in the same tissue are significant differences among the various periods of 30 mmol L⁻¹ NH₄Cl exposure. The different capital letters in the same tissue are significant differences among the various periods of 70 mmol L⁻¹ NH₄Cl exposure. *Significant difference between 30 mmol L⁻¹ and 70 mmol L⁻¹ NH₄Cl exposure groups. The bars represent the mean ± S.E. (n=3).

expression level of *aqp3* in the liver of *P. dabryanus* was relatively lower compared with *aqp1*. Injection of LPS or Poly (I:C) can significantly increase *aqp1* and *aqp3* transcript levels in mud loach liver (Lee et al., 2017). Immune challenge-induced changes indicate that the two types of aquaporins have an important role in responding to inflammatory diseases or conditions. Transcripts encoding Aqp1 and Aqp3 were low in the muscle of *P. dabryanus*, which might be due to the presence of other homologous. Aqp4 was found to be abundant in the skeletal muscle of mammals using double immunofluorescence (Vizzaccaro et al., 2018). Abundant Aqp1 exists in tissues with high energy consumption, such as the human heart and skeletal

muscle, but little Aqp3 was observed (Abdel-Sater, 2018). The co-expression of *aqp4* and *aqp3* in skeletal muscle may represent the need for water and non-ionic solute transportation.

Expression changes of *aqp1* and *aqp3* after ammonia and drought stress

The abundance of *aqp3* mRNA in the gills of yellow and silver eels decreased by 76% and 97%, respectively, after 3 weeks of domestication in seawater (Cutler and Cramb, 2002). The expression of *P. dabryanus* gill *aqp3* was always up-regulated

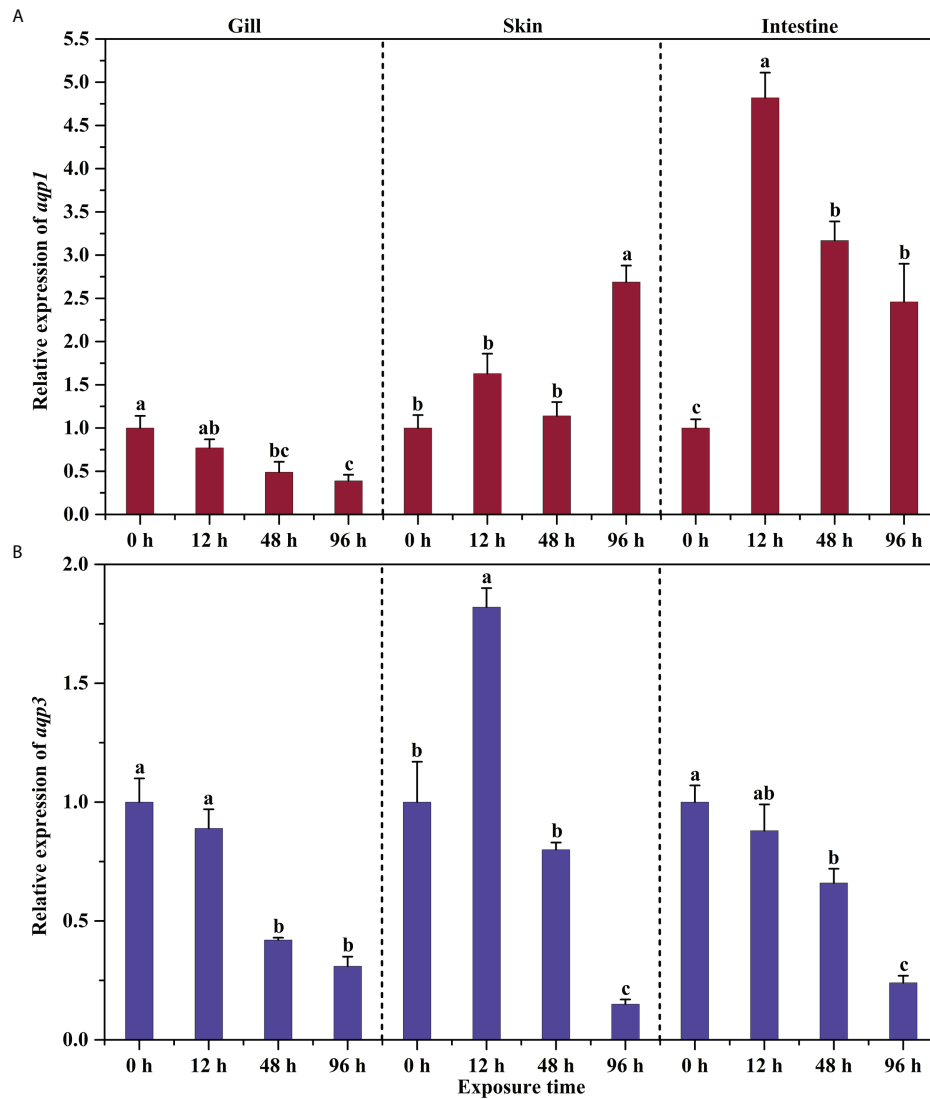


FIGURE 3
 Effects of aerial exposure on the relative expression of *aqp1* (A) and *aqp3* (B) in different tissues of *P. dabryanus*. The different lowercase letters in the same tissue are significant differences among the various periods of aerial exposure. The bars represent the mean \pm S.E. (n=3).

under ammonia stress even when *aqp1* was inhibited in high ammonia groups (70 mmol L⁻¹). These findings suggest that *aqp3* can not only regulate water flux but also facilitate ammonia excretion. The transcription levels and protein abundance of African lungfish (*Protopterus annectens*) gill *aqp1* and *aqp3* were both significantly down-regulated during the aestivation stage (Chng et al., 2016). This changing profile was also represented in *P. dabryanus* gill when exposed to air. This may be due to the closure of gill function during drought and aestivation, and the down-regulation of Aqp can reduce water loss of the gill epithelium.

Aqp3 and the homolog *Aqp3l* were detected and mainly expressed in the gills, skin, and eyes of zebrafish (Hamdi et al.,

2009). Our ammonia loading study of *P. dabryanus* showed that skin *aqp1* expression level was significantly up-regulated, whereas the *aqp1aa* mRNA of climbing perch (*Anabas testudineus*) under 100 mmol L⁻¹ NH₄Cl reached the opposite conclusion (Ip et al., 2013). The different results may be caused by the different concentrations of NH₄Cl. The excessive concentration of NH₄Cl has a toxic effect on climbing perch, leading to the direct down-regulation of the *aqp1* transcription level.

Air-breathing fish excrete nitrogen-containing waste through their skin when their gills are no longer effective under drought stress (Glover et al., 2013). Both the gills and skin *aqp1a* abundance of climbing perch were increased after

drought stress (Ip et al., 2013). The results of *P. dabryanus* in common with climbing perch, considering air exposure, could lead to water loss. Thus, Aqp1 might not be the main aquaporin for osmotic regulation. Hamdi et al. (2009) found that aquaglyceroporins (Aqp9a, Aqp9b, Aqp3, Aqp31, and Aqp10) have more effective trafficking of water or glycerin to the plasma membrane when heterologously expressed in *Xenopus* oocytes. The decrease of *aqp3* transcription in the skin may be a feedback regulation mechanism of *P. dabryanus* loach to alleviate water loss and skin dryness caused by drought exposure. In addition, the inhibition of *aqp1* and *aqp3* transcription can reduce metabolizable energy requirements to a certain extent.

The intestine acts as the organ for nutrient absorption and osmotic regulation in the teleost. The water absorption rate of the hindgut is usually higher than in the prosogaster. The *aqp1* gene expression in both freshwater and seawater eel intestines tends to be higher in the prosogaster than in the hindgut, and the *aqp1* in the intestines of sea eel is significantly higher than in freshwater eel (Aoki et al., 2003). At least 8 aquaporin isoforms have been identified in the small intestine of mammals (Aqp1, Aqp2, Aqp3, Aqp4, Aqp7, Aqp8, Aqp10, and Aqp11) (Zhu et al., 2016). Although the expression level of Sockeye salmon (*Oncorhynchus nerka*) *aqp8* and seawater eel *aqp1* in the intestine were significantly up-regulated after seawater domestication, there was no significant change in *aqp3* in the intestine and esophagus (Cutler et al., 2007; Choi et al., 2013). A concentration of 100 mmol L⁻¹ NH₄Cl had no significant effect on *aqp1aa* transcripts in the intestinal tract of climbing perch (Ip et al., 2013). However, in high ammonia conditions, *P. dabryanus* intestinal *aqp3* was significantly increased. Therefore, it can be inferred that *aqp3* is associated with mucus secretion and ammonia excretion of intestinal cells, while *aqp1* plays a greater role in water absorption of the intestinal surface.

After 1 day of exposure to air, *aqp1* mRNA expression in the prosogaster and hindgut of climbing perch increased sharply to 4.95-fold and 2.03-fold, respectively (Ip et al., 2013). This was consistent with our results, where *P. dabryanus* intestinal *aqp1* was up-regulated to 4.82-fold after 12 h of drought stress, while *aqp3* was relatively down-regulated. It is speculated that the significant up-regulation of *aqp1* in the intestine was due to the absorption of water from intestinal food to compensate for the dehydration caused by drought, while the down-regulation of *aqp3* may be due to the reduced secretion of intestinal mucus.

Conclusion

P. dabryanus aqp1 and *aqp3* mRNA showed broad distribution in multiple tissues, including breath-responsive tissues as well as osmoregulatory tissues. Hence, the diversity of Aqps distribution and expression possibly suggests potential functional roles in response to environmental stimuli. The transcription of *aqp1* and *aqp3* genes was differentially

modulated by high ammonia challenges. In particular, the *aqp3* expression level in the gills was more responsive to ammonia loading than that of *aqp1*. Taken together, environmental deterioration resulted in significant modulation of *P. dabryanus* Aqp genes, and the expression profiles during ammonia loading and drought challenge indicated that, in addition to their well-established osmoregulatory functions, *aqp1* and *aqp3* appear to play important roles in the ammonia defensive process.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was reviewed and approved by The Animal Care Committee of Anhui Agricultural University.

Author contributions

MH wrote the paper. MH, L-JZ, M-XW, and G-FP performed the experiments and analyzed the data. Y-LZ, reviewed and edited the manuscript. All authors have read and approved the final manuscript.

Funding

This work was funded by the National Natural Science Foundation of China (No. 32101248) and the Provincial Natural Science Research Project of Anhui Provincial Higher University Education (No. YJS20210227).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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