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Gill remodeling responses of the clown knifefish (*Chitala ornata*) to temperature and hypoxic stress

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ABSTRACT

Gill remodeling ability of clown knifefish exposed to different temperature levels (27°C and 33°C) and dissolved oxygen levels (hypoxia - 25% and 30% air saturation (at 27°C and 33°C), and normoxia - 95% air saturation) were performed applying vertical section to estimate lamellar surface area (SA), gill filament volume, lamellar volume, harmonic mean water blood thickness and calculate anatomic diffusion factor (ADF). The initial lamellar SA and harmonic mean water blood thickness of Chitala (33.12 ± 1.09 g) were 51.43 ± 3.10 mm^2g^{-1} and 3.59 ± 0.15 µm in normoxia, respectively. The lamellae SA increased strongly after one month of exposure to elevated temperature and hypoxia. A significant reduction in the harmonic mean water–blood barrier thickness was observed following one month of hypoxia, whereas temperature effects were observed significantly after two months. The value of lamellar SA in the hypoxic group at 33°C (47.02 ± 2.44 mm^2g^{-1}) was twice as high as that of the normoxic group at 27°C (22.38 ± 1.06 mm^2g^{-1}) while the ADF in the hypoxic group at 33°C was nearly 4-fold higher than the normoxic group at 27°C. Findings in Chitala suggest that gill remodeling represents an ancient adaptation that has existed for over 300 million years.

1. INTRODUCTION

The fish gills are known as a multipurpose organ that plays vital roles in physically responding to internal and external changes. Fish gills serve as the primary site for gas exchange, osmoregulation, acid–base balance, and nitrogenous waste excretion, functioning analogously to a kidney (Evans et al., 2005; Díaz et al., 2009). The capacity of fish gill epithelia to remodel under environmental stressors has been widely documented. For instance, Tuurala et al. (1998) and Ong et al. (2007) observed responses to cold water and terrestrial air exposure, whereas Sollid et al. (2003, 2005) and Sollid & Nilsson (2006) reported remodeling under temperature and oxygen fluctuations, with similar findings later confirmed by Matey et al. (2008) and Mitrovic et al. (2009). This gill transformation, termed the increase or decrease interlamellar cell mass (ILCM) (Sollid et al., 2003) was first reported in water-breathing fish crucian carp (*Carassius carassius*), showing that a completely lacking protruding lamelae fish in 8°C normoxia transformed the morphometry of the gills to normal

looking with bare lamellae when the fish was exposed to hypoxia (6-8% air saturation) (Sollid et al., 2003). It was later demonstrated by Sollid et al. (2005) that goldfish (*Carassius auratus*) are capable of altering gill morphology when exposed to warm water (>25 °C) and hypoxia. Recently, the exercise also induced gill remodeling in goldfish and crucian carp (Brauner et al., 2011; Fu et al., 2011). Moreover, it was reported that there was a similar morphology of gill remodeling occurring in salmonids induced by aluminum (Nilsson et al., 2012). A reduction in the interlamellar cell mass (ILCM) is accompanied by an increase in gill surface area, resulting in a lower critical oxygen tension (Pcrit) and thereby enhancing oxygen uptake efficiency (Sollid et al., 2003; Fu et al., 2011).

For air-breathing fish species, it has also been emphasised that the gills are able to transform their morphology occurring in anabantoid (dwarf gourami) (Huang and Lin, 2011) and killifish (Ong et al., 2007) when exposed to acid water and emerge from water to land, respectively. Brauner et al. (2004) have found that an obligated air-breathing fish (*Arapaima gigas*) possesses protruded lamellar gills at the early stage of its life as an exclusive water-breathing fish species and without protruded gills when the fish start to use the air-breathing organ after 100 g. This phenomenon cannot be reversed in this fish species. Recently, a tropical air-breathing fish species (*Pangasianodon hypopthalmus*) has been found to is able to increase or reduce the ILCM under the effect of high temperature (33°C), hypoxia (35% air saturation), and exercise (Phuong et al., 2017, 2018). In general, gill remodeling was recorded in many fish species; however, estimating surface area and volume to numerically present these changes is rare. The clown knifefish (*Chitala ornata*) is a facultative air-breathing fish whose air-breathing organ is a gas bladder (Smith, 1945; Dehadrai, 1962; Poulsen et al., 2004; Vidthayanon, 2012). This species belongs to Osteoglossomorpha, one of the oldest groups of Osteoglossocephala devision. According to Viet (2015), the production of *Chitala* has risen sharply, with an estimated 500 tons cultured annually in the Mekong Delta. According to So and Tuan (2022), by 2020, *Chitala* farming had expanded to 86 hectares of intensive culture, yielding about 6,880 tons of fish. The present paper applied the stereological method that revealed precise estimation of filament and lamellar surface area and volume (da Costa et al., 2007; Phuong et al., 2017, 2018) on *Chitala ornata*. The authors presented the hypothesis that *Chitala ornata* can transform gill morphology under the effect of temperature and hypoxia, supporting the hypothesis that gill remodeling is an evolutionarily conserved trait (Near et al., 2012). In this study, temperature range of 27°C (average present temperature) and 33°C (higher than the highest predicted future temperature) (Li et al., 2013; IPCC, 2014) and hypoxic levels of 27°C and 33°C (P_{crit} level of *Chitala*, Tuong et al., 2018) were chosen.

2. MATERIALS AND METHODS

2.1. Fish

Chitala ornata were sourced from a local fish farm in Hau Giang Province and maintained in a 4 m³ oxygenated tank at the Laboratory of the College of Aquaculture and Fisheries, Can Tho University, Viet Nam. *Chitala* were fed a floating commercial diet containing 43% protein (Stella S3, Nutreco Company).

2.2. Experimental protocol

After two weeks of recovery from transportation, 120 clown knifefish (33.12 ± 1.13 g) were randomly chosen and stocked into four tanks (2 m^3) of recirculating aquaculture system (RAS) at 27°C. Environmental parameters were held in ranges of dissolved oxygen >95%, pH ~ 7.6, NH_3^- <0.02 mg l^{-1}, NO_2^-<0.5 mg l^{-1}, NO_3^-<90 mg l^{-1}, and PCO_2<0.5 mgl^{-1} (Boyd, 2015). Fish were fed a commercial diet containing 43% protein (Stella S3, Nutreco Company) twice daily to apparent satiation. After 3 days in normoxia and at 27°C, the gills of six fish were sampled and stored in 4% formalin-phosphate buffer solution (4% formalin PBS) for the zero-day sample. Then the temperature and the oxygen level were adjusted to create four treatments including: 27°C under normoxic (95% O_2 saturation) and hypoxic (25% O_2 saturation) conditions), 33°C under normoxic (95% O_2 saturation) and hypoxic (30% air saturation) conditions (Tuong et al., 2018). Next, fish gills were sampled after one and two months (N = 6 for each treatment). Fish body mass and length were also recorded. Hypoxic conditions were established by bubbling nitrogen gas into the fish tanks, with oxygen levels monitored and controlled using an Oxyguard Pacific system (Oxyguard, Denmark). To prevent air oxygen diffusion, the water surface of experimental tanks were covered with a floated plastic film, which was designed with a hole at the middle (40-cm diameter) allowing fish to feed and to take air-breathings.

2.3. Processing of gill samples and stereological procedure

Gill samples of *Chitala* were processed, embedded, and sectioned using previously established methods (da Costa et al., 2007). Gill samples were sectioned using the Cavalieri estimation method and vertical uniform random (VUR) sections as described by Baddeley et al. (1986). Gill samples were taken from the left or right side of each fish at random. Each side of the fish possesses five gill arches, with the fifth arch being completely reduced. Therefore, two gill arches-either the first or second, and the third or fourth-were selected, and the cartilage and hard components were carefully removed. The remaining gill tissues were weighed and then dehydrated through a graded alcohol series. Subsequently, the tissues were embedded in methyl methacrylate (Technovit 7100; Heraeus Kulzer, Germany). Each embedding block contained four gill samples from two fish, oriented so that the horizontal plane was parallel to the lateral opercular surface of the gill tissues, while the vertical axis was perpendicular to this plane. Each gill tissue was subsequently rotated 40° from the previous one. Following the procedure described by da Costa et al. (2007), each block was vertically sectioned to obtain eight 3-µm sections, with equal spacing between consecutive sections. The sections were stained with hematoxylin and eosin (H&E) and then dried at 55°C for 24 hours. The samples were then analyzed using newCast stereological software VIS (Visiopharm Integrator System, Olympus, Denmark).

Reference volumes of the gill component, lamellae surface areas, and thickness of the water–blood barrier were first estimated. Gill filament and lamellar reference volumes were determined via stereological point counting at 20X magnification (Gundersen et al., 1988). A sine-weight test line was used to estimate the lamellae surface areas under the lens of 20X. Points were recorded when the test lines intersected the outer surface of the lamellae, following the stereological method described by Gundersen et al. (1988). Following the method described by Gundersen et al. (1988) and Fernandes et al. (2012), the thickness of the water–blood barrier was estimated by randomly drawing probing lines from the inner margin of the lamellar blood spaces to the outer surface of the lamellae under 60X oil-immersion magnification. The harmonic mean was used to represent barrier thickness.

2.4. Calculations

Equations used	where
Secondary lamellae surface area (mm²) $S = \frac{2\sum I}{\frac{l}{p}.\sum P}.V_{SL}$	S (sec lamella): Surface area of secondary lamella t: Thickness of sections a/p: Representative area per counting point l/p: Representative length per counting test $\sum$ I: Total intersection counted $\sum$ P: Total counting point hit secondary lamella
Secondary lamellae volume (mm³) $V = \frac{1}{SSF} \times \frac{1}{ASF} \times \sum_{i=1}^{n} Pi\ (sec\ lamella) \times (\frac{a}{p}) \times Ts$	$\frac{1}{SSF}$: Selection sampling fraction $\frac{1}{ASF}$: Area sampling fraction V (sec lamella)/V_{SL}: second lamella volume $\sum_{i=1}^{n} Pi\ (\text{sec}\ lamella)$: Total counting points hitting the secondary lamella tissue $(\frac{a}{p})$: Representative surface area per counting point Ts: Thickness of sections
Gill filament volume (mm³) $V(gill) = \frac{1}{SSF} \times \frac{1}{ASF} \times \sum_{i=1}^{n} Pi\ (gill) \times (\frac{a}{p}) \times Ts$	$\frac{1}{SSF}$: Selection sampling fraction $\frac{1}{ASF}$: Area sampling fraction V (gill): Reference gill volume $\sum_{i=1}^{n} Pi\ (gill)$: Total counting point counted in primary filaments and secondary lamella $(\frac{a}{p})$: Representative surface area per counting point Ts: Thickness of sections

Equations used	where
Harmonic mean water-blood barrier thickness (µm) $\tau_h = \frac{2}{3} l_h$	τ_h :The harmonic mean thickness of lamellae l_h : The harmonic mean intercept length
Anatomic diffusion factor ($cm^2 \mu m^{-1} kg^{-1}$) $ADF = SA_{L(Total)}/\tau_h$	SA_L(Total): Lamellar surface area τ_h :The harmonic mean thickness of lamellae
Coefficient of error (CE) was used to estimate the precision of the Cavalieri reference volume: $CE(\sum P) = \frac{\sqrt{\text{Total variance of } \sum P}}{\sum P}$, Total variance of $\sum P$ = Noise + Var_{SURS} ($\sum$area) Noise = $0.0724*(b/\sqrt{a}) \times \sqrt{n \times \sum P}$, Var_{SURS} ($\sum$area) = $\frac{3(A-Noise)-4B+C}{240}$	A=$P_i \times P_i$; B=$P_i \times P_{i+1}$; C=$P_i \times P_{i+2}$; mean CE=$((CE_1^2+CE_2^2+\ldots+Ce_n^2)/n)^{1/2}$ and CV=s.d/mean. Noise is the point counting error variance, $b/\sqrt{a}$ is the average profile shape of the observed tissues according to the nomogram of Gundersen and Jensen (1987), n is the number of examined sections, $\sum P$ is the total number of points hitting the observed tissue, Var_{SURS} ($\sum$area) is variance of the total SA and CV is the coefficient of variance.

2.5. Statistics

Data was analyzed using SPSS 18.0. The effects of temperature, oxygen level, and their interaction on lamellar surface area, gill filament volume, water–blood barrier thickness, and anatomical diffusion factor were analyzed using two-way ANOVA. A p-value of less than 5% ($p < 0.05$) was determined to be significant. All data were shown as mean ± SEM (standard error mean).

3. RESULTS AND DISCUSSION

3.1. Gill surface area

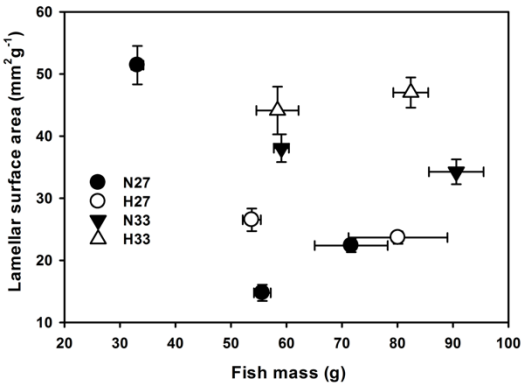

Figure 1. Surface area of respiratory lamellae in *Chitala* exposed to different temperatures and oxygen conditions

There was a significant effect of high temperature and oxygen levels on the lamellar surface area of *Chitala ornata* (Figure 1; Table 1, 2). Lamellar surface area at the initial was 51.43 ± 3.1 mm^2g^{-1} from fish of 33.13 ± 1.09 g. After one month, at 27°C lamellar surface area decreased to 14.77 ± 1.31 and 26.53 ± 1.81 mm^2g^{-1} for normoxia and hypoxia, respectively. At 33°C, the surface area was 38.07 ± 2.24 and 44.13 ± 3.83 mm^2g^{-1} for normoxia and hypoxia, respectively. After two months, lamellar surface area was significantly affected by temperature ($p = 0.000$), oxygen level ($p = 0.010$), and interaction of temperature and oxygen level ($p = 0.029$) (Figure 1, Table 2). During the two-month period, temperature showed a stronger effect on lamellar surface area (Tables 1 and 2).

3.2. Volume of gill parts

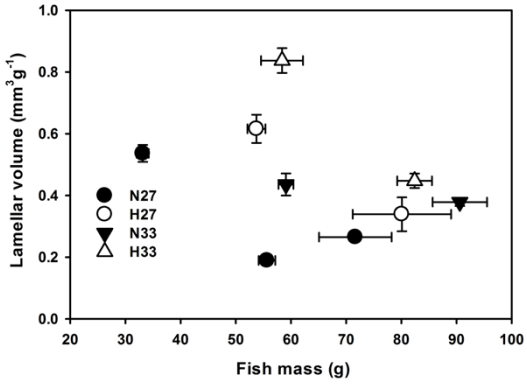

Figure 2. Respiratory lamellae volume in *Chitala* exposed to different temperatures and/or oxygen conditions

Gill filament volume was initially 3.40 ± 0.17 mm^3g^{-1}, and significantly affected by the oxygen levels after one month (Table 1, 2). However, after two months, neither temperature nor oxygen affected gill filament volume (Tables 1 and 2). Lamellar volume was strongly affected by temperature and oxygen level after one month. The initial lamellar volume was 0.54 ± 0.03mm^3g^{-1} decreasing to 0.019 ± 0.01mm^3g^{-1} at 27°C and

normoxia after one month and 0.26 ± 0.01 mm^3g^{-1} after two months. Lamellar volume in hypoxia at 27 and 33°C accounted for 0.62 and 0.84 mm^3g^{-1} after one month and took 9.5 and 13% of total gill volume. There was no temperature and oxygen level interaction effect nor gill volume, filament, nor lamellar volume after one and two months (Figure 2, Tables 1 and 2).

3.3. Water-blood barrier diffusion pathway

The harmonic mean thickness of the water–blood interface initially averaged 3.59 ± 0.15µm. The oxygen level affected the harmonic mean water-blood thickness significantly after one month (7.31, 7.09 µm for 27 and 33 °C in normoxia and 5.22, 5.17 µm for 27 and 33°C in hypoxia, respectively). This thickness was not significantly influenced by temperature or by the interaction between temperature and hypoxia after one month. However, temperature, oxygen level, and temperature and oxygen level interaction showed a significant effect on this thickness, while the hypoxia at 33 °C caused the most reduction of thickness (4.11 µm) after two months (Figure 3, Tables 1, 2).

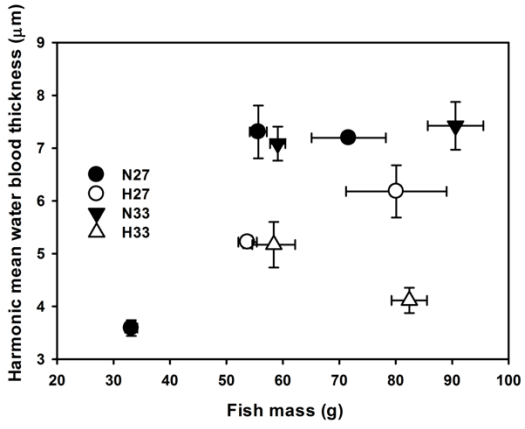

Figure 3. Harmonic mean water–blood barrier thickness in *Chitala* subjected to varying temperatures and/or oxygen conditions

3.4. Anatomic diffusion factor

Both temperature and oxygen level showed the effect on anatomic diffusion factor (ADF). Temperature affected on lamellar surface area more strongly than the oxygen level while the oxygen level showed a stronger effect on the harmomic mean water-blood thickness . The lowest ADF was found in normoxia 27 °C of 2.07 to 3.11 $mm^2g^{-1}µm^{-1}$, whereas the highest one was found in hypoxia 33 °C of 8.80 to 11.56 $mm^2g^{-1}µm^{-1}$ after one and two months, respectively (Figure 4).

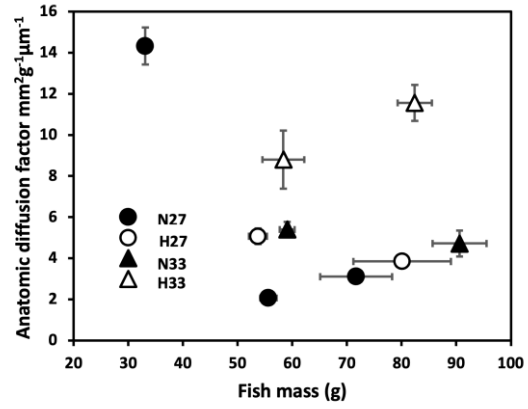

Figure 4. Anatomic diffusion factor in *Chitala* under varying temperatures and/or oxygen conditions

Figure 5. One side gill arches of *Chitala ornata*

Table 1. Lamellar surface area (mm^2g^{-1}), gill filament volume (mm^3g^{-1}), lamellar volume (mm^3g^{-1}), harmonic mean (HM) water blood thickness (µm), and anatomic diffusion factor ($mm^2g^{-1}µm^{-1}$) in *Chitala* subjected to varying temperatures and/or oxygen conditions. Data are expressed as mean ± standard error of the mean (S.E.M)

Time (month)	Treatment	Mass (g)	Lamellar surface area/mass mm^2g^{-1}	Gill filament volume/mass mm^3g^{-1}	Lamellar volume/mass mm^3g^{-1}	Harmonic mean water blood thickness µm	Anatomic diffusion factor mm^2g^{-1} $µm^{-1}$
0	N27(n=24)	33.12±1.09	51.43±3.10	3.40±0.17	0.54±0.03	3.59±0.15	14.32±0.90
1st	N27 (n=5)	55.64±1.50	14.77±1.31	3.95±0.47	0.19±0.01	7.31±0.50	2.07±0.31
	H27 (n=5)	53.74±1.63	26.53±1.81	6.57±0.55	0.62±0.05	5.22±0.12	5.07±0.38
	N33 (n=5)	59.08±1.37	38.07±2.24	4.51±0.19	0.44±0.04	7.09±0.32	5.40±0.37
	H33 (n=4)	58.38±3.81	44.13±3.83	6.46±0.12	0.84±0.04	5.17±0.43	8.80±1.41
2nd	N27 (n=4)	71.68±6.57	22.38±1.06	3.72±0.19	0.26±0.01	7.20±0.08	3.11±0.17
	H27 (n=4)	80.10±8.91	23.66±1.00	4.74±0.49	0.34±0.06	6.18±0.50	3.85±0.10
	N33 (n=4)	90.60±4.92	34.27±2.00	4.17±0.13	0.38±0.01	7.42±0.45	4.72±0.63
	H33 (n=5)	82.40±3.15	47.02±2.44	5.07±0.39	0.45±0.02	4.11±0.24	11.56±0.87

Table 2. Comparison of gill parameter responses of *Chitala ornata* under different temperature and oxygen conditions ($P < 0.05$ indicates significant effects and interactions)

	2-way ANOVA	Lamellar surface area/mass (mm^2g^{-1})	Gill filament volume/mass (mm^2g^{-1})	Lamellar volume/mass (mm^2g^{-1})	HM water blood thickness (µm)	Anatomic diffusion factor (mm^2g^{-1} $µm^{-1}$)
1st month	Temperature	0.000	0.656	0.000	0.718	0.000
	Oxygen	0.008	0.000	0.000	0.000	0.000
	Temp*O_2	0.342	0.515	0.778	0.817	0.765
2nd month	Temperature	0.000	0.392	0.014	0.020	0.000
	Oxygen	0.010	0.046	0.090	0.000	0.000
	Temp*O_2	0.029	0.900	0.954	0.006	0.000

Table 3. Comparative analysis of lamellar surface area, water–blood barrier thickness, and anatomical diffusion factor (ADF) across different fish species

Species	Lamellar surface area (mm^2g^{-1})	HM water blood thickness (µm)	ADF ($mm^2µm^{-1}g^{-1}$)	References
Water-breathing				
Oncorhynchus mykiss	240	6	40	Hughes *et al*., 1973
Hoplias malabaricus	240	3.16	75.9	Fernandes *et al*., 1994
Chaenocephalus aceratus	120	6	20	Hughes, 1972
Tinca tinca	250	3.0	83.33	Hughes, 1972
Obligate air-breathing				
Anabas testudineus	94	10	9.4	Hughes *et al*., 1973
Arapaima gigas	77	7.76	9.9	da Costa *et al*., 2007
Arapaima gigas				
Channa punctata	72	2.03	35	Hughes & Munshi, 1973
Facultative air-breathing				
Saccobronchus fossilis	58	3.58	16	Hughes & Munshi, 1973
Pangasianodon hypophthalmus	30.2*	1.97	36.13	Phuong *et al*., 2018 Body mass: 100 g; *: body mass 276 g
Chitala ornata	22.38	7.2	3.11	Present study (Normoxia - 27°C)
Chitala ornata	47.02	4.11	11.56	Present study (Hypoxia - 33°C)

4. DISCUSSION

Chitala ornata has quite small gills with five pairs of long gill arches, whereas the fifth arches are reduced without any filaments (Figures 5 and 6). Lamellar surface area of *Chitala* was 44.13 to 47.02 mm^2g^{-1} for high temperature (33°C) in hypoxia (30% air saturation) from one to two months, respectively. This SA was suitable for air-breathing species has been reported (da Costa et al., 2007) and is 4 times lower than SA in Pangasius in the same condition (Phuong et al., 2017). Clown knifefish (Tuong et al., 2018) have been shown they be inactive and calm, and their standard metabolic rate (SMR) was quite small; however, increasing oxygen uptake with high temperature apparently shows an increase in oxygen metabolic need. The results of the present experiment revealed that the gill organ of *Chitala ornata* is transformed and plastic influenced by temperature and oxygen levels, which caused gill lamellae filled with ILCM (interlamellar cell mass) (Figure 6) and significant decrease of harmonic diffusion thickness when fish were exposed to high temperature (33°C) and low oxygen level (hypoxia), respectively. Lamellar surface area showed significant differences between 27°C and 33°C, and normoxia and hypoxia (14.77 ± 1.31 and 44.13 ± 3.83 mm^2g^{-1} for normoxia 27°C and hypoxia 33°C, respectively) (Figure 6, Table 1). The value of hypoxia 33°C of *Chitala* was almost five times lower than that of *Pangasianodon hypopthalmus* (Phuong et al., 2017) (noting that Pangasius owns an unusual large gill SA) and quite low compared to other water-breathing fish species (Table 3). As the description of Fick's law of diffusion, there are two factors that influent the diffusion capacity, which are surface area and diffusion thickness. Additionally, Hughes (1972); Hughes and Morgan (1973) emphasized that the diffusion thickness was as important as surface area, whereas the diffusion capacity increases with the increase of the respiratory surface area and the decrease of the diffusion thickness. The ADF was directly proportional to respiratory gill surface area and inversely proportional to diffusion thickness, which represents the relationship of these two components contributing to the diffusion capacity of particularly respiratory media (Perry, 1978). The calculation of the respiratory gill ADF of *Chitala* increased with the increase of temperature and the decrease of dissolved oxygen level (3.11 and 11.56 $mm^2g^{-1}\mu m^{-1}$ for normoxia 27°C and hypoxia 33°C, respectively) (Fig. 4). Moreover, there was a stronger effect of the temperature on the respiratory gill surface area compared to the hypoxia whereas stronger effect of hypoxia on diffusion thickness (Table 1, 2). It has been reported that the increase of gill surface area was a functional support to oxygen demand rather than ambient water oxygen levels (Nillson et al., 2012; Fu et al., 2011; Perry et al., 2012; Phuong et al., 2017; Phuong et al., 2018). Tuong et al. (2018) recently showed that the oxygen uptake of *Chitala ornata* increased with the increase of temperature from 27 to 33°C, clearly supporting that *Chitala ornata* increases diffusion capacity as well as lamellar surface area to respond to the higher need of oxygen metabolic rate. The ADF of *Chitala* was in optimal ranges found in air-breathing fish species (Table 3) with an unusual exception of Tra catfish (*Pangasianodon hypopthalmus*) (Phuong et al., 2017; Phuong et al., 2018). The gill morphological response of *Chitala ornata* to the temperature was significant after one month in terms of increasing respiratory surface area, and the gill morphological response to hypoxia was almost two months in terms of decreasing diffusion distance (Table 1, 2). These results reveal that the increase in respiratory surface area was due to faster reduction of ILCM, while the thinner diffusion thickness mechanism took a longer time.

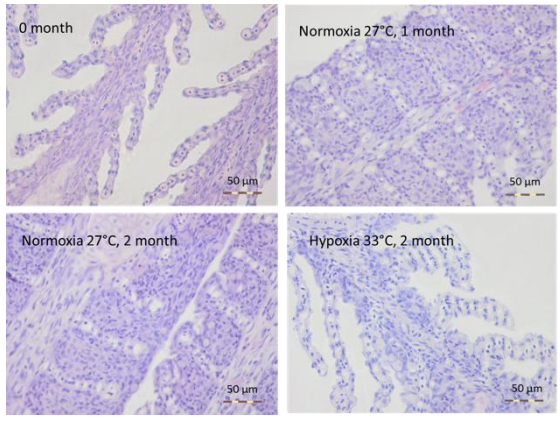

Figure 6. Gill filaments of *Chitala* observed under light microscopy at normoxia (27 °C) and hypoxia (33 °C) after 0, 1, and 2 months of exposure

A mode of gill remodeling, characterized by either a reduction or an increase in the interlamellar cell mass (ILCM), was also observed in *Chitala* (Figure 6). This provides further evidence that gill remodeling is likely an ancient and widespread trait among fish species, as reported by Nilsson, (2007) and Nilsson et al. (2012). There are similar mechanisms found in cyprinidae (crucian carp, goldfish, common carp and Qinghai carp) (Sollid et

al., 2003; Metz et al., 2003; Sollid and Nilsson, 2005, Matey et al., 2008), Cyprinodontidae (killfish) (Ong et al., 2007), Teleost (eel) (Tuurala et al., 1998) and recently Siluriformes (tropical Tra catfish) (Phuong et al., 2017; Phuong et al., 2018). The discussion of how widespread gill remodeling is and for how long it has existed have been argued intensively by Nilsson (2007) and Nilsson et al. (2012), showing that there was at least 150 million years ago that these mentioned fish species had a common ancestor. It has also been argued that there may be further when including European eel (*Anguilla anguilla*), which is one of the oldest groups of Teleost (Nilsson, 2007). However, the degree of gill remodeling (filled with ILCM) of European eel observed in micrographs was much less compared to crucian carp, Tra catfish, or *Chitala*. A tropical species of air-breathing catfish, *P. hypophthalmus,* has been documented to have gill lamellae filled with ILCM at cold temperature (27°C) and transformed to respond to higher temperature (33°C), combining with hypoxia rather than the low oxygen level at cold temperature (Phuong et al., 2017; Phuong et al., 2018). This fish species belongs to Siluriformes, having a common suborder Ostophysi with Cyprinidae, while *Chitala* and Araipaima belong to Osteoglossomorpha (Near et al., 2012). Then these four species had a common ancestor of Osteoglossocephala, a group of Teleostei (Near et al., 2012). Therefore, based on Actinterygian time-calibrated phylogeny, it can be more consistent to state that the gill remodeling mechanism is an ancient trait shared among widespread fish species existing at least 300 million years ago.

5. CONCLUSIONS

C. ornata is an ancient facultative air-breathing species possessing an air-bladder to sustain during hypoxia. Gills of *Chitala* primarily represent air-breathing fish species normally thought to be reduced through evolution. However, gill remodeling in *Chitala* in many aspects enables fish to be able to adapt to changes in the environment. The temperature increase (higher than the predicted level (Li et al., 2013)) induced ILCM decrease faster than hypoxia, whereas hypoxia induced an air-breathing response immediately (Tuong et al., 2018) and a diffusion thickness thinner. These responses of *Chitala* may indicate that this fish species tends to respond to oxygen metabolic demand rather than oxygen available in the environment. Moreover, the discovery of gill remodeling in *Chitala* was intriguing, supporting that gill remodeling is owned by broad fish species (including water-breathing and air-breathing species) and is inherited from ancestors hundreds of millions of years ago. However, studies on widespread and intensive fish gill remodeling, including the mechanism of ILCM increase or decrease and effects of other environmental factors, should be investigated further to draw any firm conclusion.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest regarding the publication of this article.

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