Journal of King Saud University – Science 31 (2019) 713–723

Contents lists available at ScienceDirect

Journal of King Saud University – Science

journal homepage: www.sciencedirect.com

Original article

Water quality tolerance and gill morphohistology of pure and reciprocal crosses of *Pangasianodon hypophthalmus* and *Clarias gariepinus*

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ARTICLE INFO

Article history:
Received 23 December 2017
Accepted 15 January 2018
Available online 7 February 2018

Keywords:
Gill lamellar
African catfish
Asian catfish
Morphotype
Anoxia

ABSTRACT

Tolerance to degrading water qualities in relation to the gill morphohistology was investigated in pure and reciprocal crosses of two important catfishes. Progenies of *Clarias gariepinus* (CG), *Pangasianodon hypophthalmus* (PH), Pangapinus (♀PH×♂CG) and the two observed morphotypes (Clarias-like and Panga-like) of Clariothalmus (♀CG×♂PH) were obtained from the same breeding history. These fishes were cultured in a static system without aeration and at high stocking density for a maximum period of two weeks. Mortality and water quality were monitored daily. Total mortality was observed 24 h post exposure (1DPE) in the Panga-like Clariothalmus, while the Pangapinus and pure *P. hypophthalmus* did not survive beyond the third day (3DPE). However, total mortality was not recorded in the Pure *C. gariepinus* and Clarias-like Clariothalmus even after 14DPE. Morphohistology of the gills of the latter fishes showed the presence of an accessory breeding organ in the form of branched bulbous dendritic-like structures and less occurrence/severity of many histopathological conditions observed during the challenge experiment. However, this was not the case with the other fish groups. It was concluded that the architecture and the level of susceptibility of the gill influenced the tolerance ability of the different fishes to poor water quality.

1. Introduction

Intergeneric crosses in fish have been attempted with the aim of increasing genetic and phenotypic variation for the purpose of research and commercial production. Progenies of many distance hybridization are often composed of different phenotypic characters (Zhao et al., 2015) which is suggestive of significant differences in genetic composition and inheritance pattern (Okomoda et al., 2017a). Hence, they not only differ in their external phenotypic character but also could have significant variation in their physiology, and performance characteristic. However, the intra-cross comparison between different morphotype of the same hybrid has been limited to only phenotypic, genetic cytogenetic (Zhong et al., 2012) and erythrocyte characterization (Okomoda et al., 2017b).

Intergeneric crosses between Asian catfish *Pangasianodon hypophthalmus* (Sauvage, 1878) and African catfish *Clarias gariepinus* (Burchell, 1822) was recently attempted to produced novel aquaculture candidate for culture and solve some breeding challenges associated with the production of the pure crosses (Okomoda et al., 2017a,c). The resultant progeny of the cross with higher growth rate (♀*C. gariepinus* × ♂*P. hypophthalmus* aka Clariothalmus) consisted of two distinct morphotypes (Clarias-like and Panga-like). While the reciprocal cross (♀*P. hypophthalmus* × ♂*C. gariepinus* aka Pangapinus) had only one morphotype (Panga-like). A twenty-four hours challenge test conducted was suggestive that some of the progenies of the Clariothalmus had low tolerance to dissolve oxygen when compared to the other crosses (Okomoda et al., 2017c). However, no strong scientific backing could be advanced for this observation aside the assumption of genetic incompatibility (Okomoda et al., 2017a). More so, in view of the aquaculture potential of the novel hybrid, there is need to study

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.jksus.2018.01.003
1018-3647/

in detail the tolerance of the fish to deteriorating water quality if commercial production is envisaged.

Characterization of the morphohistology of the internal organs of fish is pivotal to understanding the fundamental of its physiology. For instance, the nature and structure of the respiratory organs of fish have a significant consequence on its ability to tolerate poor water condition. The gill of *C. gariepinus* is equipped with an air-breathing organ known as suprabranchial organ (Vandewalle and Chardon, 1991, Ahmed et al., 2008) while those of *P. hypophthalmus* do not have this organ but possesses a vascularized swim bladder (Browman and Kramer, 1985; Okomoda et al., 2017c). The efficiency of the air-breathing organ of the latter makes it more tolerant to anoxic water than the former. Although there is an increasing interest in the anatomical and histological aspects of the gills of fish in the light of its application in the evaluation of fish's health (Strzyzewska et al., 2016), however, data on many ichthyofaunal species are still very scarce. More so, the Clariothalmus and Pangapinus been a novel progeny have no available or documented information on the nature and structure of its gills. Assessing the morphohistology of the gills of the different morphotypes of the hybrids progenies may provide important insight into their perceived responses to degrading water quality, hence, the aim of this study.

2. Materials and methods

Fingerlings of pure and reciprocal crosses of *C. gariepinus* and *P. hypophthalmus* (two months old) were obtained from four breeding trials following the method described by Okomoda et al. (2017c). Based on the different morphotype observed in the treatments, a novel water quality challenge evaluation procedure was designed. The experimental set designed was 5 × 4 × 2 i.e. five fish groups, four replicates and two experimental setups (treatment and control setup). For the treatment setup, one hundred litter of water was vigorously aerated overnight in a 5 × 1 × 1 m^3 fiberglass tank. From this, fifteen aquariums (23.0 × 12.5 × 12.5 cm^3) meant for the treatment setup were half filled with aerated water (2 L) to ensure uniformity in water quality at the start of the study. To ensure a high stocking density, ten fish (10 g ± 2.3) per morphotype of each cross were then distributed evenly into the aquarium (in triplicate). The treatment setup was maintained without aeration or water change for two weeks.

For the control setup, same aquarium size, number and size of fish as described in the treatment setup was used. However, this setup was continuously aerated and the water changed on daily bases. Fishes in both the treatment and control setup were starved throughout the experimental period. The water quality from all system was monitored using YSI professional plus multiparameter water quality meter (Model 13 M10065, Made in the USA). Water quality and mortality were initially recorded six hours into the challenge test and subsequently on daily bases (twice daily for treatment and control group) until total mortality was observed in the treatment group or the study was terminated at two weeks. Triplicate data of the mortality and water quality result were analyzed for descriptive statistics and presented in line graphs using the Microsoft Excel software. The odds ratio of mortality in this study was also determined according to Altman (1991) using the MedCalc (2017) online software bvba (Seoul, Republic of Korea).

The forth replicate of the treatment group were used for the gill histological examination. However, to ensure efficient observation, fishes selected for anatomic assessment were older (four months of age) and larger (between 30 and 100 g) than those used for histological assessment (2months old with a mean weight of 10 g ± 2.3). Ten samples were used for morphohistological examination. Samples for anatomic examination were tranquilized with 150 mg/1 solutions of tricaine methane sulphonate (MS222) (Wagner et al., 1997) and then sacrificed. The fishes were then incised by the opercular cavity to expose the gills and any observable attached accessory respiratory organ. Organs were examined in situ and washed under slowly running tap water to remove blood stain from the tissue. The ventral and dorsal view of the gills were then photographed using a Sony camera (Cyber-shot 16.2MP Model number: DSC-TX10 50i) fitted to a triple camera stand.

Samples for histological examination were fixed in 10% buffered formaldehyde for 48 h, after which gills samples were collected for analysis. The gills were subjected to routine histological techniques (dehydration, diaphanization, and paraffin embedding) and microsectioned at 5 μm following the method adopted by Solomon et al. (2017). Samples were stained with Harris's hematoxylin and eosin (H&E), following routine procedures. For the slide microphotography procedure, a Nikon Eclipse 80i compound microscope was used, and the images were processed using NIS element basic research software (at 40 × magnification). Histopathological condition observed in the gills were noted and scored according to

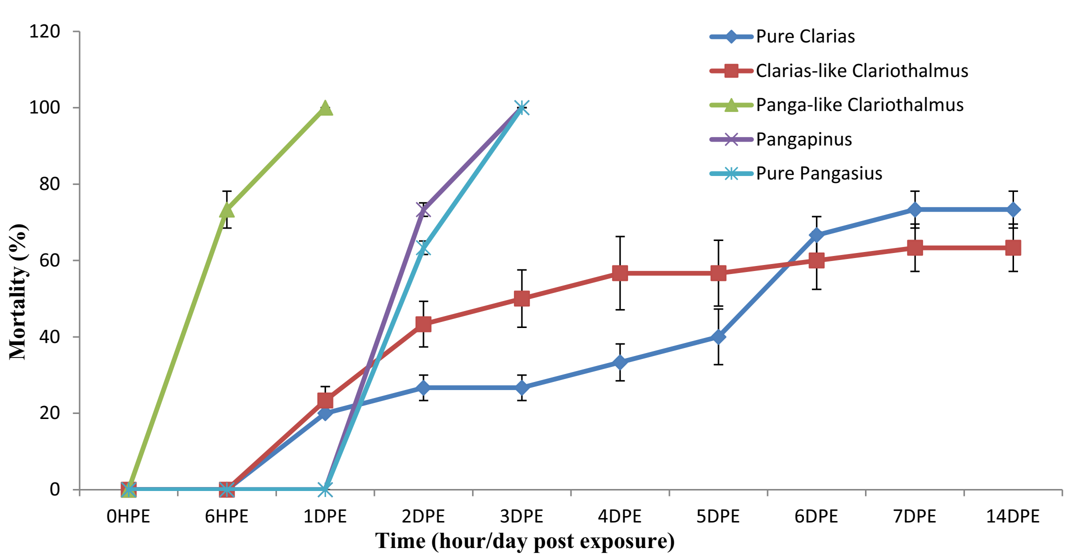


Fig. 1. Mortality in the culture system of the treated groups of fishes during the challenge test.

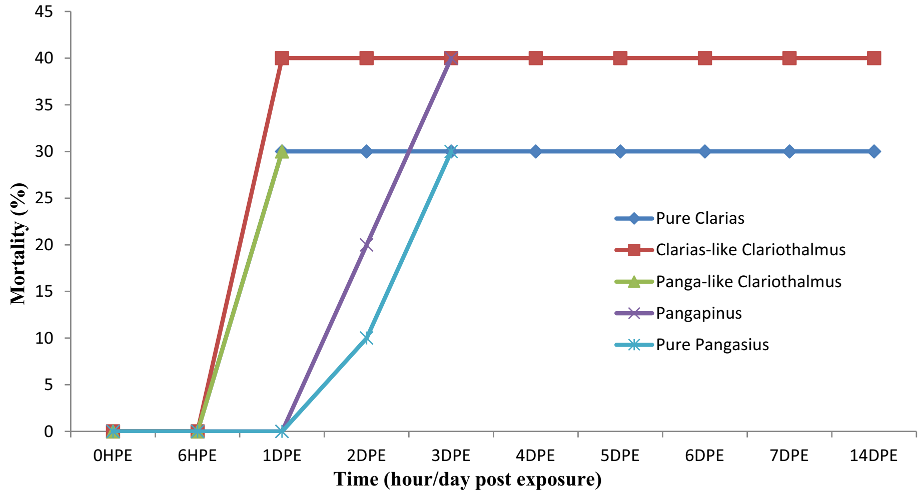
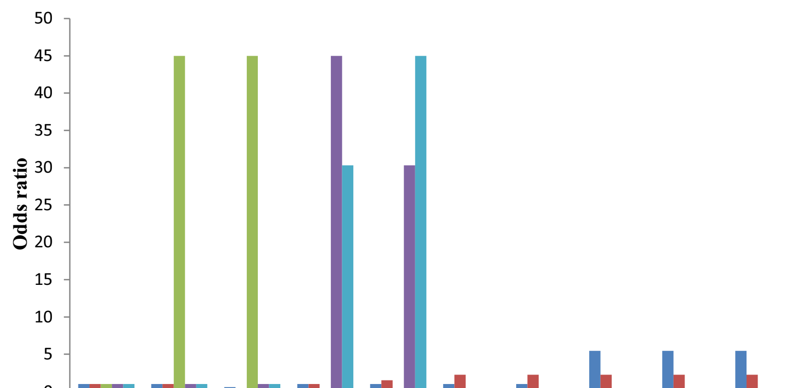


Fig. 2. Mortality of control fishes under continuous aeration.



	0HPE	6HPE	1DPE	2DPE	3DPE	4DPE	5DPE	6DPE	7DPE	14DPE
Pure Clarias	1	1	0.58	1	1	1	1	5.44	5.44	5.44
Clarias-like Clariothalmus	1	1	0.38	1	1.5	2.25	2.25	2.25	2.25	2.25
Panga-like Clariothalmus	1	45	45							
Pangapinus	1	1	1	45	30.33					
Pure Pangasius	1	1	1	30.33	45					

Time (hour/day post exposure)

Fig. 3. Odds ratio of mortality per time of exposure of the fishes to low dissolved oxygen.

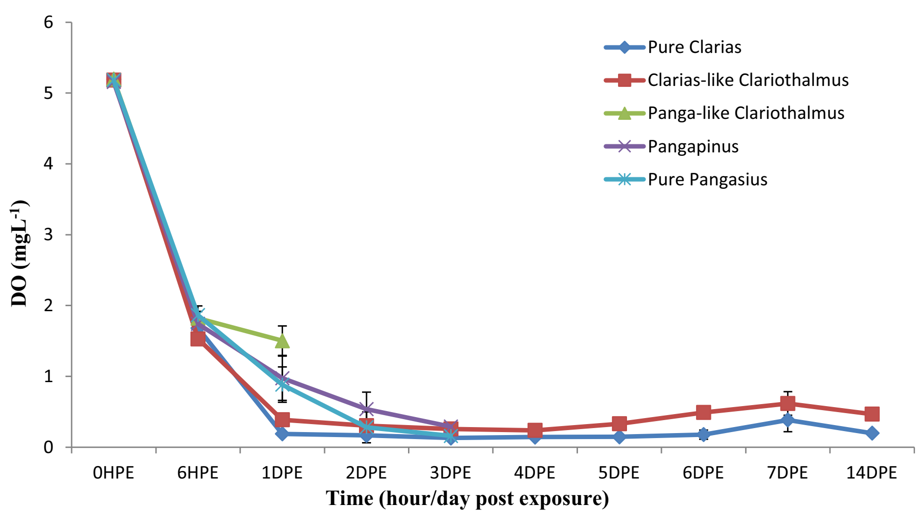


Fig. 4. Dissolved oxygen changes in the culture system of the treated groups of fishes during the challenge test.

the method described by Dutta et al. (1993) and Aghamirkarimi et al. (2017).

3. Result and discussion

Studies on fish tolerance to deterioration in water quality are scarce and seldom reported despite their importance. This is likely because of the difficulty in the experimental setup which may require subjecting fishes to a constant value of water quality over a period. Unlike experiment for temperature and salinity tolerance, it is practically difficult and many times impossible to keep water quality parameters such as DO, pH, and NH_3 constant for a prolonged time because of the fish activities. In this study, we designed a novel method in which high stocking density ensured depletion of DO over time. Also, increase in NH_3 and pH was

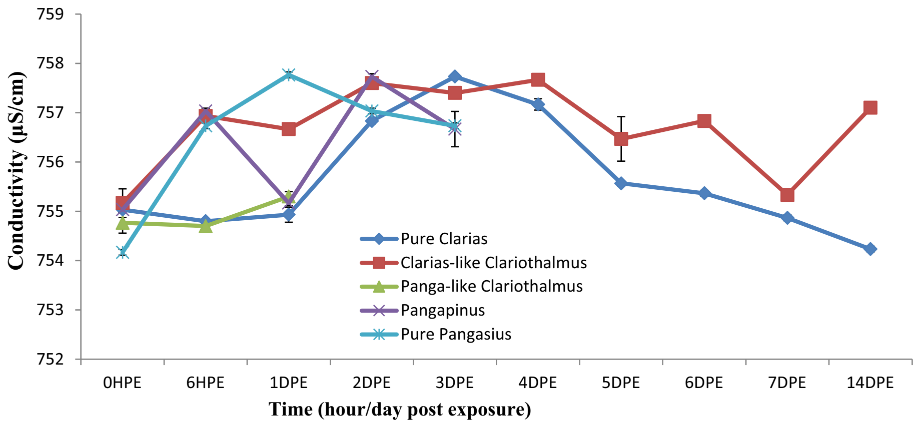


Fig. 5. Conductivity changes in the culture system of the treated groups of fishes during the challenge test.

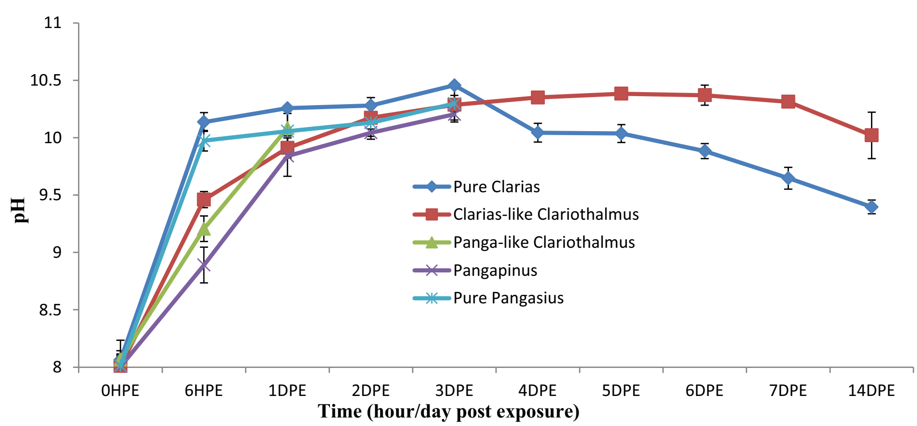


Fig. 6. pH changes in the culture system of the treated groups of fishes during the challenge test.

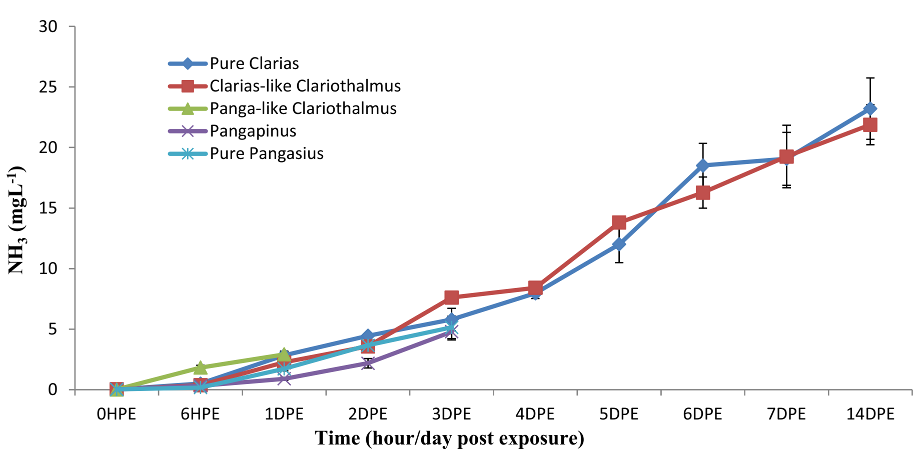


Fig. 7. NH_3 changes in the culture sytem of the treated groups of fishes during the challenge test.

hypothesized with prolonged excretion of the fish in the static nature of the treatment group (without a water change). The same stocking density was used in the control group; hence, mortality only could have resulted from the effect of high stocking density and starvation. Since these two factors are also interplaying in the treatment group in addition to poor water quality, the odd ratio calculated therefore isolates the effect of water quality on performance from those caused by these other factors. The result of this study (Figs. 1 and 2) suggests three categories of fish tolerance namely (i) the less tolerant group i.e. the Panga-like Clariothalmus progenies (ii) the fairly tolerant group i.e. the Pangapinus and *P. hypophthalmus* progenies and lastly (iii) the extremely tolerant group i.e. the Clarias-like Clariothalmus and *C. gariepinus*. This is justified by the odds ratio value recorded during the study period for the pure and reciprocal progenies (Fig. 3). Okomoda et al. (2017a) had earlier highlighted, that the progeny of the ♀*C. gariepinus* × ♂*P. hypophthalmus* (Clariothalmus) hybrids were more sensitive than other crosses to a degradation of water quality. This

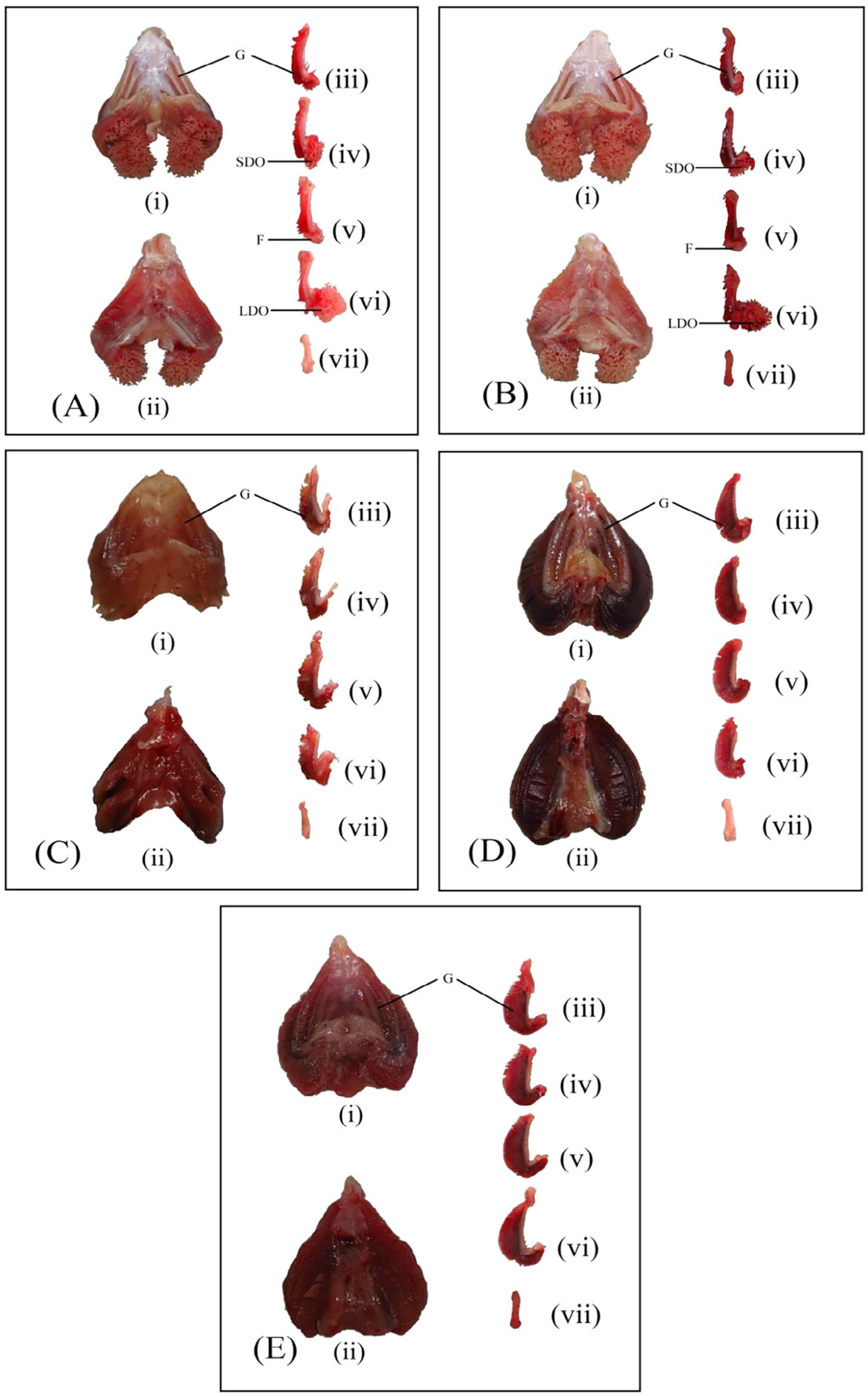


Fig. 8. Morphology of the gills of *Clarias gariepinus* (A), Clarias-like Clariothalmus (B), Panga-like Clariothalmus (C), Pangapinus (D), and *Pangasianodon hypophthalmus* (E). Keys: G = gills; SDO = small part of dendritic organ; F = gill fan; LDO = large part of dendritic organ, (i) = Dorsal view of gills; (ii) = ventral view of gills; (iii) = First gill arch; (iv) = second gill arch; (v) = third gill arch; (vi) = fourth gill arch and (vii) = fifth rudimental gill arch.

study, however, revealed that a certain portion of the same crosses (Clarias-like Clariothalmus) is resistant to deteriorating water quality just like the maternal parent. However, all the progenies of the Pangapinus were fairly tolerant like the maternal parent. The differences in tolerance of these fishes may be linked to the different pattern of inheritance which is evident in the appearance of different morphotypes of the same crosses.

Tucker and D'Abramo (2008) had earlier, shown that undesirable pH (very high or low) could be detrimental to fish survival reared in aquaculture ponds. Knepp and Arkin (1973) had also stated that ammonia level for favorable rearing condition of fish should be below 0.2 mg/l. Hence, the combined effect of increased pH and un-ionized ammonia in the absence of oxygen became lethal to the fish over time. However, *C. gariepinus* was able to survive prolonged exposure to this lethargic condition for two weeks. According to Toko et al. (2007), *C. gariepinus* could survive and even growth at a normal rate at oxygen concentration between 0.9 ppm to 1.2 ppm. After six hours of exposure in the treatment group, most water quality parameters recorded were already below the recommended ranges by Boyd (1982) to support the survival and development of aquatic life in any culture system (Figs. 4–7). However, since none of the deteriorating water qualities observed in this study could be isolated as the sole causative of the mortality in the treatment group, it is important to kept water quality optimum at all time if commercialization of the novel hybrid is to be achieved. Despite the high tolerance of the Clarias-like Clariothalmus and *C. gariepinus,* mortality was noticed earlier than the fairly tolerant Pangapinus/*P. hypophthalmus* progenies both in the control and treatment group (Figs. 1 and 2). This observation is strongly linked to the high rate of aggressive behaviour and cannibalism. Aside food availability and size differentiation, cannibalism and aggression are strongly influenced by stocking density (Hecht and Appelbaum, 1987; Al-Hafedh and Ali, 2003; Brummett et al., 2007; Solomon and Okomoda, 2012; Olufeagba and Okomoda, 2016). The finding of this study is suggestive that Pangapinus/*P. hypophthalmus* progenies could withstand higher stocking density for culture than the Clarias-like Clariothalmus/*C. gariepinus* progenies provided water quality is kept optimum. The number of death recorded in the control group of Panga-like Clariothalmus progenies culture under optimum water quality further justifies the assumption of reduced fitness in this fish. However, with a total mortality occurring within twenty-four hours in the Panga-like Clariothalmus progenies in the treat-

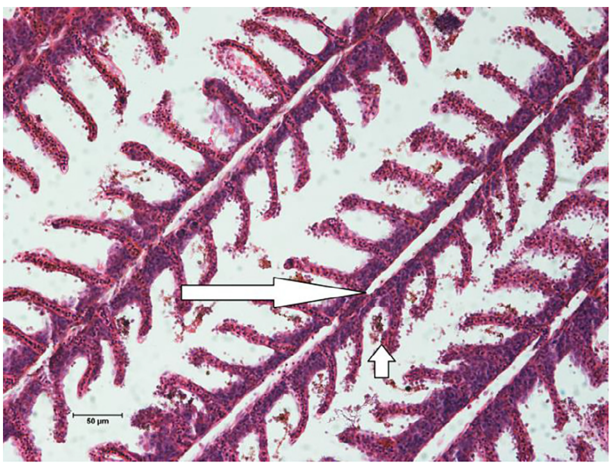


Fig. 9. Photomicrographs of a typical normal gill from the control groups of fishes. Primary lamellar (long arrow); secondary lamellar (short arrow).

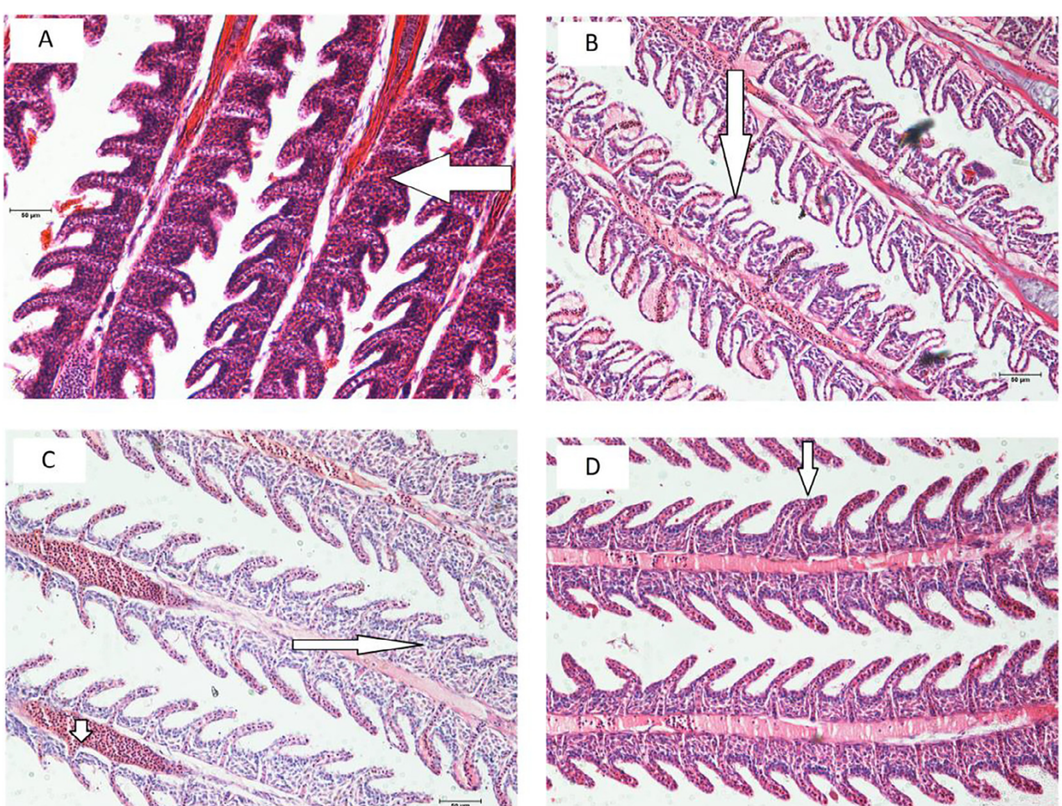


Fig. 10. Photomicrographs of the gill of *Clarias gariepinus*. (a) partial hyperplasia of the epithelial cells (arrow); (b) epithelia lifting (arrow); (c) partial hyperplasia of the epithelial cells (long arrow), blood congestion in primary lamellar (small arrow); (d) fusion of 3 lamellae (arrow). Scale bar 50 μm. Hematoxylin/Eosin stain.

ment group, the issue of survival might outweigh any other advantage this fish may have over the parent species whenever water quality is not optimal (Okomoda et al., 2017c).

Most bony fishes have been previously reported to possess four pairs of gills arches (Eiras-Stofella et al., 2001; Eiras-Stofella and Fank-de-Carvalho, 2002). In addition, a fifth rudimentary gill, without gill filaments was observed in the entire fish group in this study (Fig. 8). The Clarias-like Clariothalmus/*C. gariepinus* progenies also had accessory respiratory organ structurally similar to those previously reported in other catfishes (Maina and Maloiy, 1986; Vandewalle and Chardon, 1991; Ahmed et al., 2008). This is in the form of branched bulbous dendritic-like structures. In fishes such as Bowfin *Amia calva* (Gervais and Tufts, 1998), *Arapaima gigas* (Brauner et al., 2004) and *P. hypophthalmus* (Browman and Kramer, 1985; Okomoda et al., 2017c), a vascularized swim bladder help take in oxygen-rich water at the interface. However, the efficacy of the accessory respiratory organ of the Clarias-like Clariothalmus/*C. gariepinus* progenies explains why they could survive extremely low dissolve oxygen for prolonged period. The dendritic part of the air-breathing organ of the Clarias-like Clariothalmus/*C. gariepinus* progenies are derived from the 2nd and 4th gill arches (Fig. 8) which is accordance with the findings of other catfishes (Moussa, 1956; Munshi, 1961; Harder, 1975; Lewis, 1979; Johnston et al., 1983; Maina and Maloiy, 1986; Chandra and Banerjee, 2003; Zayed and Mohamed, 2004). However, the dendritic organ of *Anabantoidei, Perciformes, Channa punctata* and *Channa striatus* originated only from the 1st gill arch (Harder, 1975; Munshi, 1962). These variations may be attributed to the differences in the mechanisms of respiration of the different fishes. The gill filaments and lamellae of Panga-like fishes are longer and larger than those of the Clarias-like fishes, indicating a larger surface area. Similarly, the findings of Moron and Fernandes (1996), Fernandes and Mazon (2003) suggest that large gill respiratory surface is usual characteristics of fishes that are exclusive water-breather. More so, it is well established that teleosts which undergo aerial respiration have reduced gill surface area (Schottle, 1931; Dubale, 1951; Carter, 1957; Hughes and Morgan, 1973) as observed in the Clarias-like progenies.

The physiological response of fish to changes in water chemistry is an adaptation to maintaining a relatively stable internal system (Mancera et al., 1993; Carmona et al., 2004). The extent of changes in the gills can serve as a biomarker to evaluate whether the environmental imbalances experienced has an acute or chronic effect on the fish (Winkaler et al., 2001; Tkatcheva, et al., 2004). Based on the classification by Takashima and Hibiya (1995), Bernet et al. (1999). Myers and Fournie (2002), Bais and Lokhande (2012), the acute lesions observed in this study include epithelial lifting and swelling (Figs. 9–14, Table 1). The chronic lesions, however, include hyperplasia, aneurysm, and telangiectasia. Several authors have opined that the fusion of secondary lamellae in many fishes are physiological responses to increased levels of ammonia and nitrite concentration in the culture system (Svobodova et al., 1993; Cardoso et al., 1996; Frances et al., 1998; Svobodova et al., 2005; Das et al., 2004). Some other authors have linked low oxygen content in water (Scott and Rogers, 1980; Fernandes and Mazon, 2003), pH changes (Capkin et al., 2009), and irritation of the gills by parasites to hyperplasia as a result of increased mucus production in this organ (Speare et al., 1991; Thiyagarayah et al., 1996, Lease et al., 2003, Svobodova et al., 2005). In theory, it is believed that this physiological change increases the distance between the blood capillary and lamellae surface, hence, reducing the intake of the causative chemical. However, this cell proliferation leads to respiratory dysfunction, as it also affects gas exchange because of the decrease in the gill surface

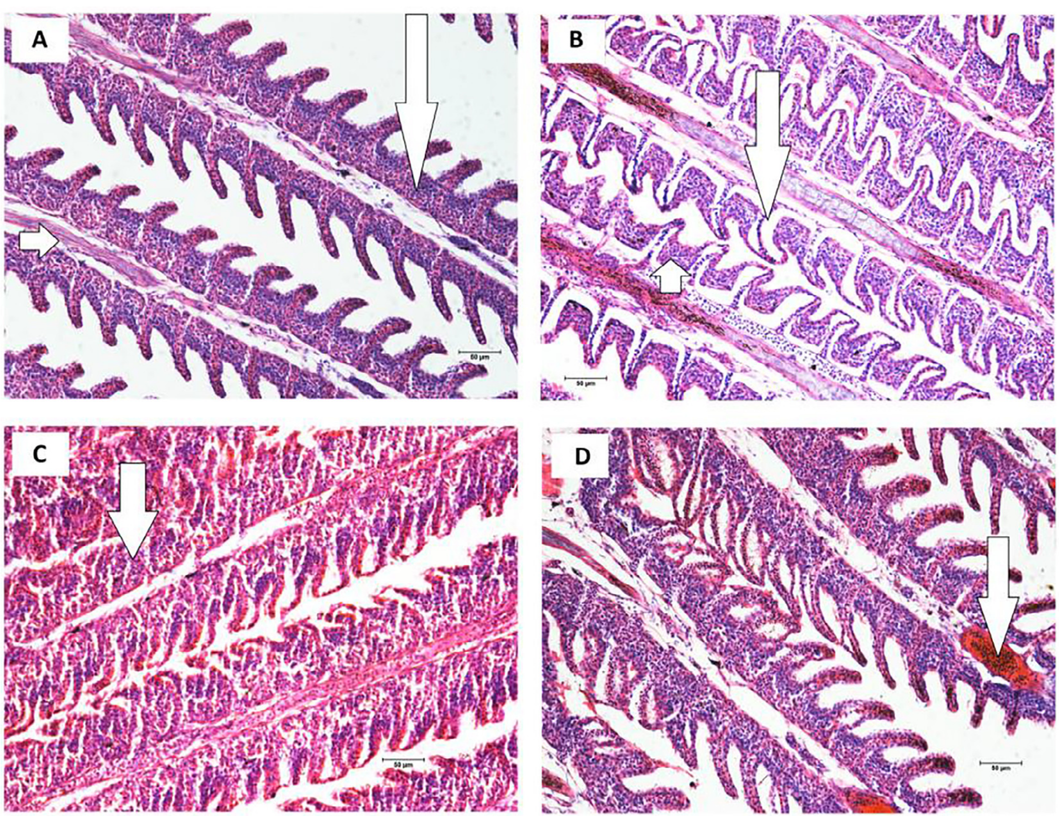


Fig. 11. Photomicrographs of the gill of Clarias-like Clariothalmus. (a) partial hyperplasia of the epithelial cells (long arrow); expanded primary lamellar (short arrow); (b) epithelial lifting (long arrow); partial hyperplasia of the epithelial cells (short arrow); (c) complete fusion of several lamellae (arrow); (d) blood congestion in primary lamellar arrow). Scale bar 50 μm. Hematoxylin/Eosin stain.

area and disrupting the fish osmoregulation process (Mallat, 1985; Poleksic and Mitrovic-Tutundzic, 1994; Takashima and Hibiya, 1995; Liebel, 2013). The presence of an accessory respiratory organ in the Clarias-like fishes may explain why these fishes are less vulnerable to this condition as compared to the Panga-like fishes.

Parasitic infections of gills can occur due to environmental factors such as in the pond such as stocking density and/or stress (Roberts, 1989; Nowak et al., 2004). More so, increased organic pollution and its decomposition increase the vulnerability of the fish gills to pathogenic and parasitic infections (Klontz, 1995; Poleksic et al., 1999; Nikolic et al., 2003; Cirkovic, 2003). It is hypothesized that these organic matters accumulate between the gill filaments and constitute an excellent medium for parasitic infections (Turnbull, 1993; Abbas, 2006; Roberts, 2001). However, the reaction of the gill tissue to mitigate parasitic infections are limited to focal hyperplasia and mucous cells proliferation (Liebel, 2013), hence, this would not affect the normal function of the gills and as such non-lethargic. Parasites were prevalent more in the Panga-like fishes as compared to the Clarias-like fishes in the present study. This may be explained by the presence of a longer and larger gill filaments and lamellae in the former compared to those found in the latter. Epithelia lifting and telangiectasia of the respiratory lamellae have been associated with the acidification of water (Roberts, 2001); however, these conditions were noticed even with increasing pH levels of the culture system in the present study. Some authors have also linked this phenomenon to increased ammonia level among other chemical causatives (Arellano et al., 1999; Pane et al., 2004; Schwaiger et al., 2004; Authman and Abbas, 2007; Monteiro et al., 2008). However, these conditions are symptoms of disorders of osmoregulation (Movahedinia et al., 2012) and were found commonly in all treated groups of fish in the present study.

4. Conclusion

In conclusion, the susceptibility to water quality and the severity of the histopathological conditions in the gills of Panga-like Clariothalmus within a short time of exposure as against the observation in other groups in this study is an indication of genetic incompatibility of this hybrid progeny as compared to the others. The findings of this study have also justified the hypothesis of differences in the inheritance pattern of the fishes (even within the

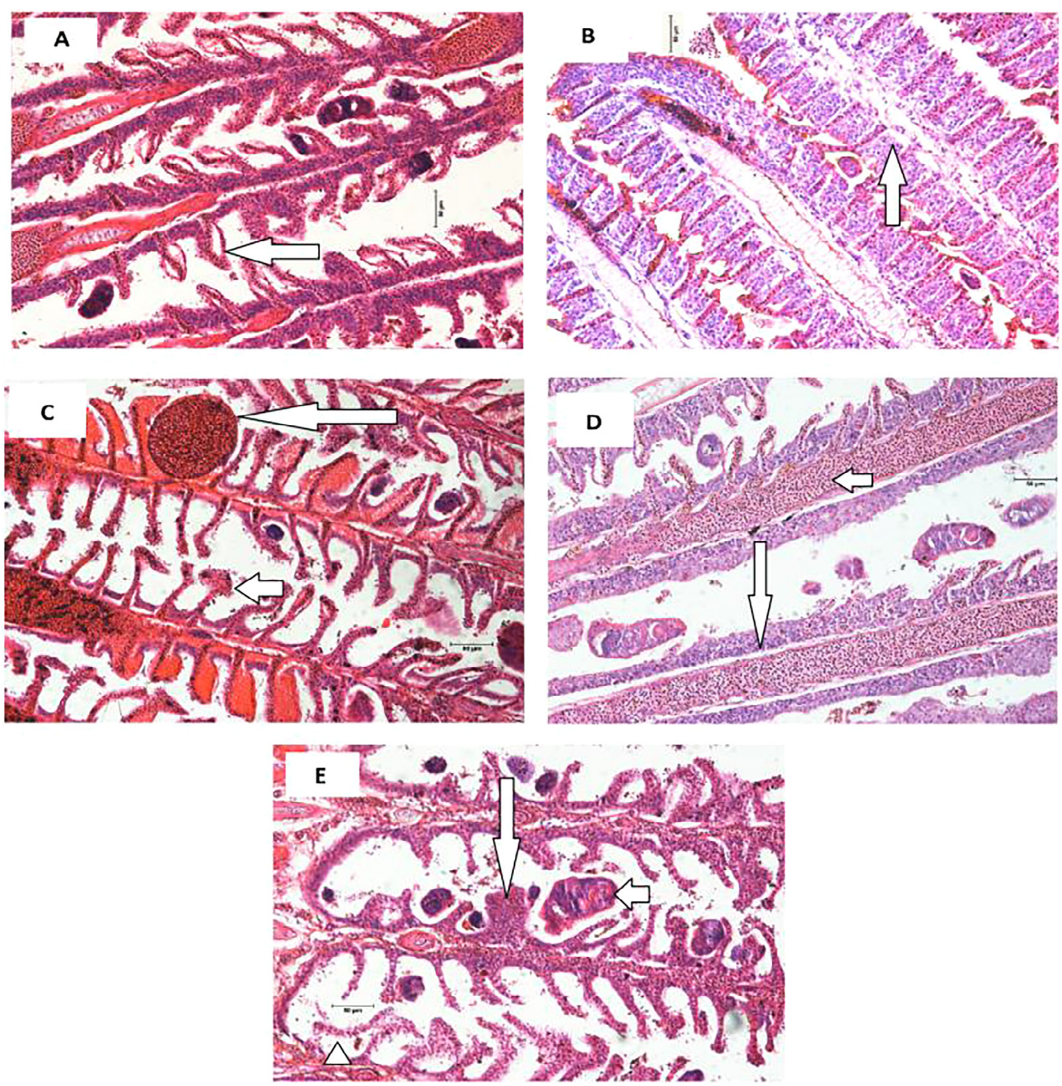


Fig. 12. Photomicrographs of the gill of Panga-like Clariothalmus. (a) epithelial lifting; (b) Complete hyperplasia of the secondary lamellae (arrow); (c) aneurisms in secondary lamellae (long arrow), Telangiectasis at the secondary lamellae (short arrow); (d) blood congestion in primary lamellae (short arrow), complete fusion of several lamellae. (e) fusion of 3 lamellae (long arrow), ecto-parasite in the gills (short arrow), secondary lamellae disorganization (arrow head). Scale bar 50 μm. Hematoxylin/Eosin stain.

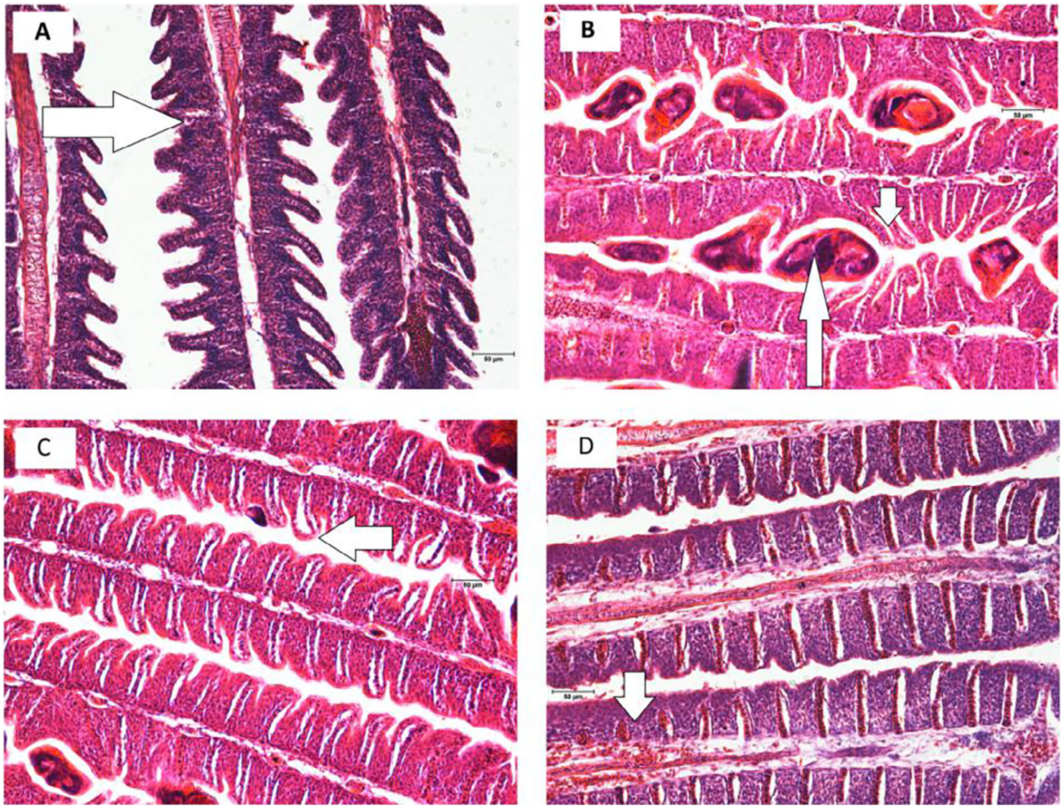


Fig. 13. Photomicrographs of the gill of Pangapinus. (a) Partial hyperplasia of the epithelial cells (arrow); (b) parasite in the gills (long arrow), fusion of few lamellae (short arrow); (c) epithelial lifting (arrow); (d) complete hyperplasia of the epithelial cells (arrow). Scale bar 50 μm. Hematoxylin/Eosin stain.

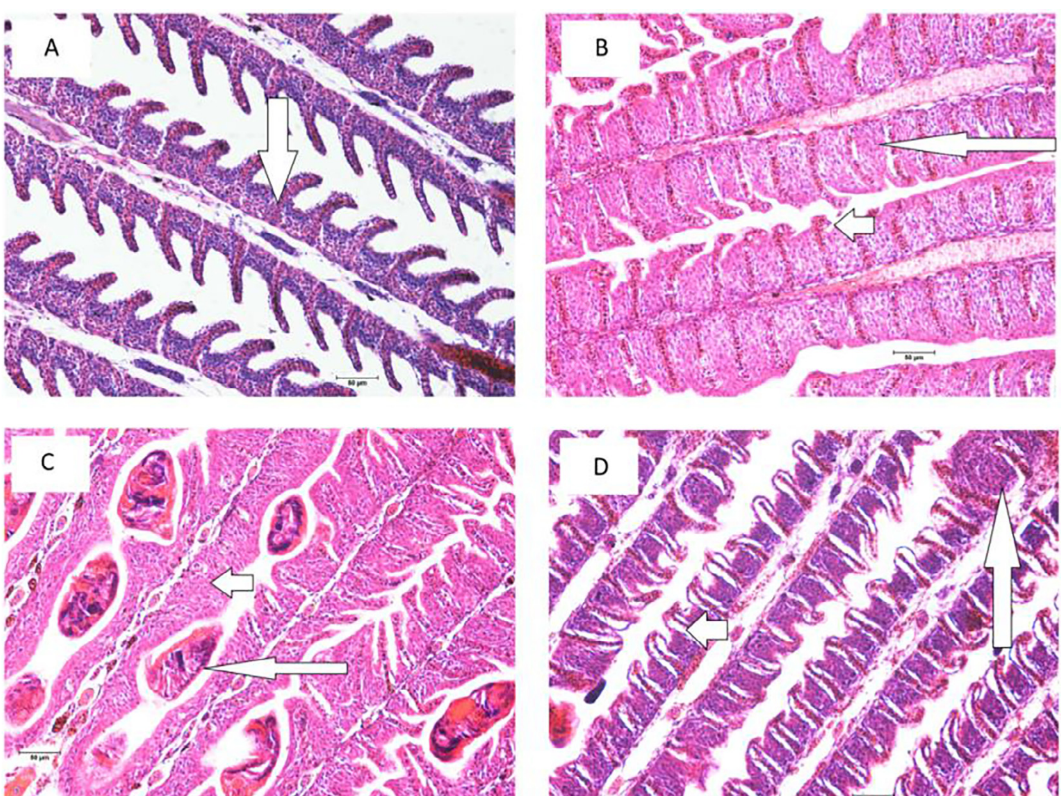


Fig. 14. Photomicrographs of the gill of *Pangasianodon hypophthalmus*. (a) partial hyperplasia of the epithelial cells (arrow); (b) complete fusion of the lamellae (long arrow), telangiectasis at the secondary lamellae (short arrow); (c) hyperplasia of the lamellae (short arrow), parasitic cyst (long arrow), (d) epithelial lifting (short arrow), parasitic cyst (long arrow). Scale bar 50 μm. Hematoxylin/Eosin stain.

Table 1
Histopathological scores of the gills of pure and reciprocal hybrids *Pangasianodon hypophthalmus* and *Clarias gariepinus* under normal rearing condition.

Parameter	♀CG × ♂CG	♀CG × ♂PH		♀PH × ♂CG	♀PH × ♂PH
		Clarias-like	Panga-like		
Hyperplasia	+	+	+++	+++	+++
SL Detachment	–	–	++	+	++
Aneurysm	–	–	+++	+++	++
Ectoparasite	–	–	+++	+++	+++
Blood congregation in PL	+	+	++	++	++
Epithelia lifting	++	++	++	++	++
Telangiectasia	–	–	++	+	+

Keys: –, No significant observed microscopic changes.
+, Mild changes (10 percent changes in 40 × objective microscope view).
++, Moderate changes (20 percent changes in 40 × objective microscope view).
+++, Severe changes (more than 20 percent changes in 40 × objective microscope view).
PL, Primary lamellae.
SL, Secondary lamellae

same cross). Hence, future studies can be done to characterise the nature of hybrids gotten in this study using cytogenetic and genetic approaches.

Conflict of interest

We declare that no fund was received for the conduct of this research; hence, we have no conflict of interest what so ever (financial or otherwise).

Acknowledgements

The authors are indebted to the School of Fisheries and Aquaculture Science, Universiti Malaysia Terengganu, Malaysia for providing *P. hypophthalmus* broodstock used in this study. We also acknowledge the assistance of all technical staffs of the PPSPA hatchery department during the breeding trial of this study. This study is part of the first author's Ph. D research.

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