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Identification of retinal degeneration of African Catfish *C.gariepinus* suffer from abnormal pectoral fins originating from cultivation in pond

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Objective of this study was to investigate the difference of retinal structure between *C.gariepinus* suffer from abnormal pectoral fin and the complete pectoral fins fish. Samples (w=±150g/individual; TL= ±27cm) took from the fish farmer from the Leran Village, Manyar Sub-district, District of Gresik, Province of East Java, Indonesia in harvested time (Periode of July-August 2018). HE slides made from left eye organ. There were two groups compared, measured by Dot Slide Scanning Microscope OlyVIA. The variables measured were T*otal Retinal Thickness*, *Retinal Pigment Epithelium (RPE), Outer Segment (OS), Middle Layer (ML),* and *Inner Layer (IL).* The results showed that *C.gariepinus* suffer from abnormal pectoral fin has degenerated *Retinal Pigment Epithelium* (RPE;72.94±18.88µm), which significantly different (*P<0.05*) from the normal fish (108.10±13.29µm). *Total Retinal Thickness*, *Outer Segment (OS), Middle Layer (ML*), and *Inner Layer (IL)* in fish suffer from abnormal pectoral fins were remains lower than normal fish but not significantly different. The role of *RPE* for the retinal function was crucial for homeostasis of the neural retina and visual system. Degenerated RPE will affect their normal function as photoreceptor sheath and reduce the versatility of *RPE.* From the results, it shows that pectoral fin loss has a relationship with abnormal eyes, and there is morphological integrity between pectoral fins and eyes organ.

Keywords: pectoral fin loss, photoreceptors, morphological integrity, degeneration, eye

INTRODUCTION

Skeletal abnormalities often found in fish farming activities (Gjerde et al., 2005; Bardon et al., 2009; Cobcroft and Battaglene, 2013) and incidence of the skeletal anomaly in farmed fish still become the most common problems in aquatic farming today (Boglione et al., 2013b; Estivals, 2015). This problem reduced economic value ((Boglione et al., 2013a; Cheng et al., 2018). But the proper solution to prevent their onset in farmed fish still become the big question. It is needed to improve our knowledge to reduce farmed fish skeletal anomaly rising in aquaculture systems. Commonly known that three factors influence the body architecture of the fish, *i.e*., genetics, environment, and interaction between genetics and environment. From three points mentioned, the genetics factor rarely to study (Boglione et al*.,* 2013).

African catfish *C.gariepinus*, a freshwater commodity which is widely cultured in Indonesia (Soedibyo et al., 2017). This commodity is now facing skeletal anomaly in various kinds that threatening their productivity decline. Based on

field observation, there was a unique and distinct abnormality in the form of paired pectoral fins lost. The pectoral fins did not grow as normal fish. It is commonly often found among normal fish at the age of maintenance for about three months when fish are ready to be harvested by the fish farmer. Individual observation in rearing business of the *C.gariepinus* Manyar District, Gresik Regency, ten years ago, the number of abnormal pectoral fins fishes was quite tiny, ranging from 2 individual to 3 individual in a harvested volume of 2-3 quintals. However, recently its frequency has increased dramatically, abnormal pectoral fins fish can be found about ten up to twenty individual in 1quintal of fish harvested. So far the character of pectoral fins abnormality, as happened in *C.gariepinus*, has not been studied so much that this research needs further investigation. The aim of this study is to find out more clearly about pectoral fins abnormalities in *C.gariepinus*.

The pectoral fins organ of the fish is formed during late gastrulation (Tickle, 2015; Moody, 2007). They came from mesodermal cells layer which differentiates into fin buds. Fin buds are a mass of mesenchymal cells that migrate to the location of the pectoral fin due to the action of the *T-box5* gene (Albalat et al., 2010; Ahn et al., 2002; Gurrieri et al., 2002; Parrie et al., 2013; Ruvinsky, 2000; Tamura et al., 1999). Beside pectoral fins, Tbx5 gen in *Danio rerio* also expressed in eye organ (Begemann and Ingham, 2000). It is necessary to conduct a further investigation about pectoral fins loss in farmed fish. Association between locomotor and oculomotor, indicates that the eye organ is associated with the pectoral fin when the fish moves (Mandecki & Domenici, 2015). Thus, it is interesting to know more about how the structure of the eye organ in *C.gariepinus* without pectoral fins. Eyes have various parts, and one of them called retina. Retina plays essential roles in various visual mechanisms and metabolisms. Changes in retinal structure reduce the visual ability of fish (Li & Dowling, 1997; Link et al., 2011). The change of retinal structure might reduce the strength of the fish' visual (Menke et al., 2011; Boulton et al., 2001).

MATERIALS AND METHODS

Collection of C.gariepinus suffer from abnormal pectoral fins

The *C.gariepinus* have reared in outdoor concrete ponds collected from the fish farmer in Leran Village, Manyar District, Gresik Regency, East Java Province, Indonesia, in the harvest period July-August 2018. Fish are checked manually for pectoral fins one by one in that time. Individual without pectoral fins separated from the harvested-fish population and used as samples.

Sample determination

Sorting process based on sex and fish weight was carried out in both groups, namely abnormal pectoral fins fish and normal fish as the control. Determined six individuals as samples in each group, taken randomly from the male fish containers. Each sample is weighted (g), measured by *Total Length (TL, cm)* and *Standard Length (SL, cm).* The meristic characteristics paired of fins both pectoral and ventral fins. The dimension of samples presented in Table 1 and the gross morphology of fish was shown (Fig.1)

Isolation and fixation of eye organs

The fish killed and as soon as possible the eye organ is isolated (left side) using sectio set, carefully avoiding the eyeball breaking. The organ was fixed with Bouin's solution (1:10) for 16 hours in a sample bottle that had been labeled according to the origin of the fish, is from abnormal or normal fish.

Hematoxylin-Eosin (HE) staining

The organs that have done fixed in Bouin's solution are selected the best and refer to the location of the part of the organ to be studied. Organs are cut with a thickness of 2-3mm and inserted in a cassette that has occurred given a gross code of the researcher. On a tape, the network is processed with an Automatic Tissue Tex Processor for 90 minutes until the device alarm sounds. Followed by the process of blocking and cutting the network. The tissue is removed from the *Tissue Tex* Processor machine and then blocked with liquid paraffin according to the network code. Then the tissue is cut with Microtome 3-5µm thickness. Tissue incisions are placed on the glass of sterile objects and arranged in the best possible position.

The deparaffinization process is carried out by placing the slide object that has contained incisions in the electric oven for 30 minutes (t = 70-80ºC). After that, the slides containing tissue incisions inserted in two xylol tubes of 20 duration each. Subsequently immersed in four tubes containing alcohol for 3 minutes, and finally put into running water for 15 minutes.

The coloring stage begins with painting with Harris Hematoxyline main paint for 10-15 minutes then washed with running water for 15 minutes. Slides dipped in 1% alcohol as much as five times. Slides stained with lithium carbonate Ammonium for 3-5 minutes. The last part is staining with Eosin for 10-15 minutes.

Immersion in multilevel alcohol starts from 70% alcohol for 3 minutes and then soaking in 80% alcohol for 3 minutes. After that, the slides soaked in 96% alcohol for 3 minutes and finally soaked in absolute alcohol for 3 minutes. The slides immersed in two xylol tubes with a duration of 15 minutes each to make slides more clear. The last stage is mounting, closing the tissue incision with glass cover and enthelan. Then the slide is dried, and if it has dried completely, the slide is ready to be observed.

There were five variables, i.e., *total retinal thickness* (µm), *Retinal Pigment Epithelial (RPE) thickness* (µm)*, Outer Segment* (µm)*, Middle layer* (µm), and *Inner Layer* (µm). *Total retinal thickness* measured from Choroid to Inner Limiting Membrane (ILM).*Retinal Pigment Epithelial (RPE)* is the brownish layer stay between Choroid and *Outer Segment (OS)*, It's thickness measured from the base of RPE to the tip of Apical Microvilli of RPE. *Measurement of the Outer Segment (OS)* by subtracting the thickness of the outer layer (the distance between the Outer Layer Membrane and the Choroid) and the thickness of the PE layer. *The middle layer* is the Inner Nuclear Layer (INL) layer, a layer that lies between OLM and the Inner Plexiform Layer (IPL). *The inner layer* is a layer consisting of IPL, Ganglion Cells, and Internal Limiting Membrane (ILM). These three layers are difficult to determine their limits explicitly so that they are measured in one unit as Inner Layer (IL). Measuring aids are used, namely Dot Slide Scanning Microscope and OlyVIA program For Windows 2010. Each variable was calculated on average and deviation per sample group (abnormal pectoral fins, normal pectoral fins) *(mean ± SD*).

Statistical Analysis

The two groups were analyzed by *Student t-test (α = 0.05) one-tail* if the two groups were significantly different. Determination of *C.gariepinus* suffer from abnormal pectoral fin experienced a deterioration of the retinal structure so that its structure decreased compared to the normal retinal fish. Photographs of *C.gariepinus*eye and retinal tissues were taken by scanning objects (M = 40x), using computer-assisted shooting (Windows 2010) and the Microsoft Paint program.

RESULTS

This study used homogenous fish in each group have been compared. Morphometric and meristics characters of two group were relatively the same in term of total body weight (g), Total Length (cm), Standard Length (cm), and the present of a paired ventral fins. The phenotype of paired pectoral fins were entirely different, where the one group is normal pectoral fins, and the other have no paired pectoral fins as shown in Table 1. The gross morphology of the two groups of research objects shown in Fig.1.In normal condition, pectoral fins presented in end base of left-right operculum each side of the body. Normal fish have two types of paired fins both pectoral fins and ventral fins. When pectoral fins lost, the morphological anatomy of fish become changed. Fish did not have any pectoral fins in its body so paired fins presented ventral fins only.

The normal fish' sagittal section of eyes (Fig. 2) possessed three major counterparts *i.e* tunica fibrous, tunica vasculosa, and tunica nervous (retina) which commonly similar to *C.gariepinus* suffer from abnormal pectoral fins.The tunica fibrous encompasses cornea (e) and sclera (f). The tunica fasculosa encompasses choroid (d), choroid rete, and iris (b). Retina (c) presents in the inner layer of eye tissues, curved along the inner eye cavity whichis composed of many distinct layers (Menke et al., 2011). The thickness of the retinal layer varies depending on its position in the eye cavity; there is a thin and thick one. The thin retina is located at the edge, at the end of the cavity close to the iris and eye lens. A thick retina is in several places called macula. Brownish curved layer on the retina is the *Retinal Pigment Epithelial (RPE).*

Partial of retinal enlargement (in rectangle) obtained by the structure as seen in Fig.3 and Fig.5. From anterior to posterior, retinal building consist of Internal Limiting Membrane (ILM), Ganglia Cell Layers (GCL), Internal Nuclear Layer (INL), Internal Plexiform Layer (IPL), External Plexiform Layer (EPL), Outer Nuclear Layer (ONL), Outer Limiting Membrane (OLM), *Outer Segment (OS),Retinal Pigment Epithelium (RPE),* and Choroid. Fig. 3 is the structural histology of normal African catfish *C.gariepinus* (control) while Fig. 5 is from abnormal pectoral fins individual.

Table1; Comparison of morphometric and meristic aspects between abnormal pectoral fins fish and normal pectoral fins

Aspects compared	Abnormal fish (n=6)	Normal fish (n=6)
Mean of body weight (g)	148.33±35.45	146.67±44.57
Mean of Total Length (cm)	27.27±1.32	27.80±3.12
Mean of Standard Length(cm)	24.20±1.48	24.25±2.68
A paired of Pectoral Fins	absent	complete
Apaired of Ventral fins	complete	complete

Figure 1; Normal African catfish *C.gariepinus* with a normal paired of pectoral fins (up) compared to fish suffer from abnormal pectoral fins (down)

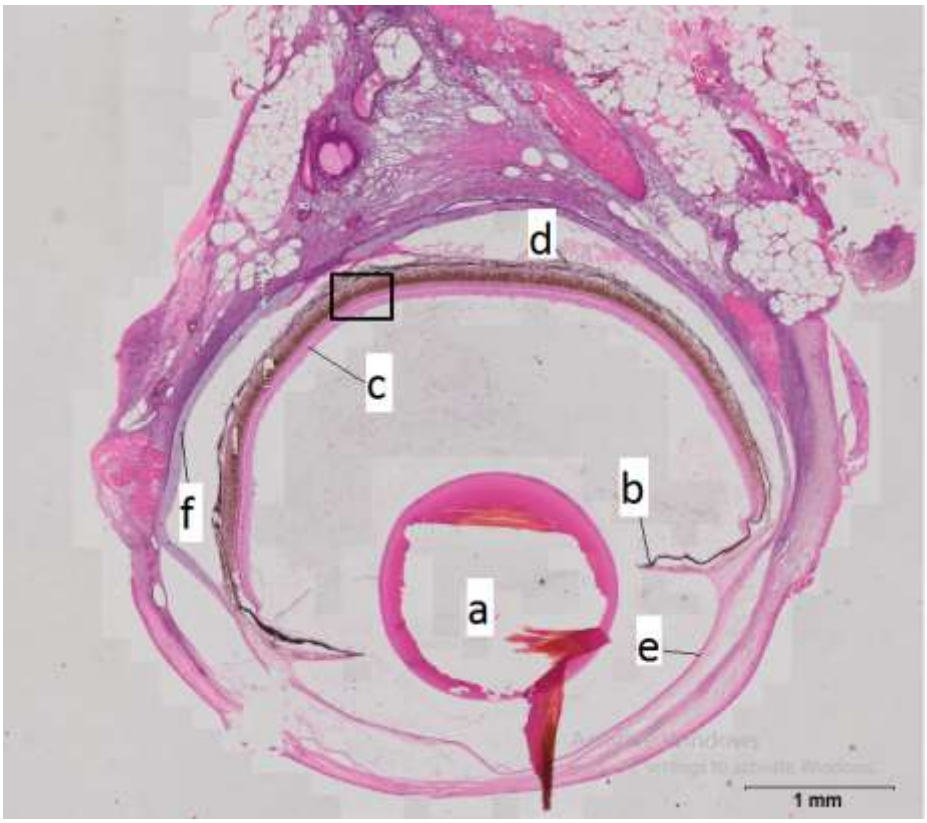

Figure 2;Histological structure of African catfish *C.gariepinus*with normal pectoral fin (a. lens b. iris c. retina d. choroid e. cornea f. sclera; black rectangle is an area of the retina which enlarged below)

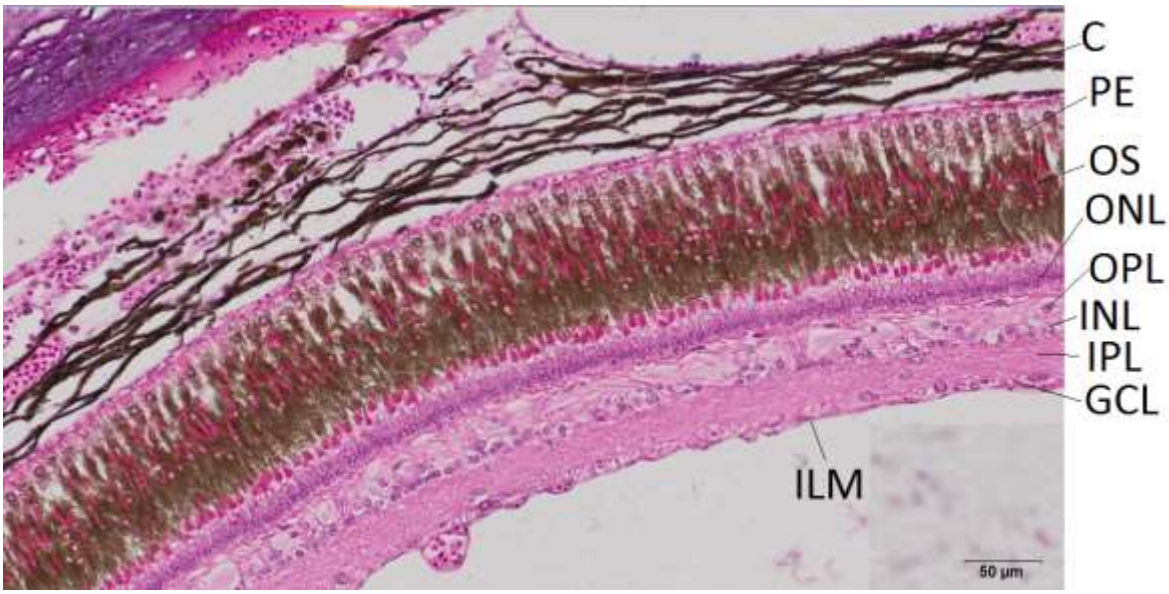

Figure 3;Histological structure of retina from rectangle area. This is the retinal African catfish *C.gariepinus*with normal pectoral fin (*PE: Pigment Epithelium OS: Outer Segment of Photoreceptors* ONL: Outer Nuclear Layer OPL: Outer Plexiform Layer INL: Inner Nuclear Layer IPL: Inner Plexiform Layer GCL: Ganglion Cell Layer ILM: Inner Limiting Layer

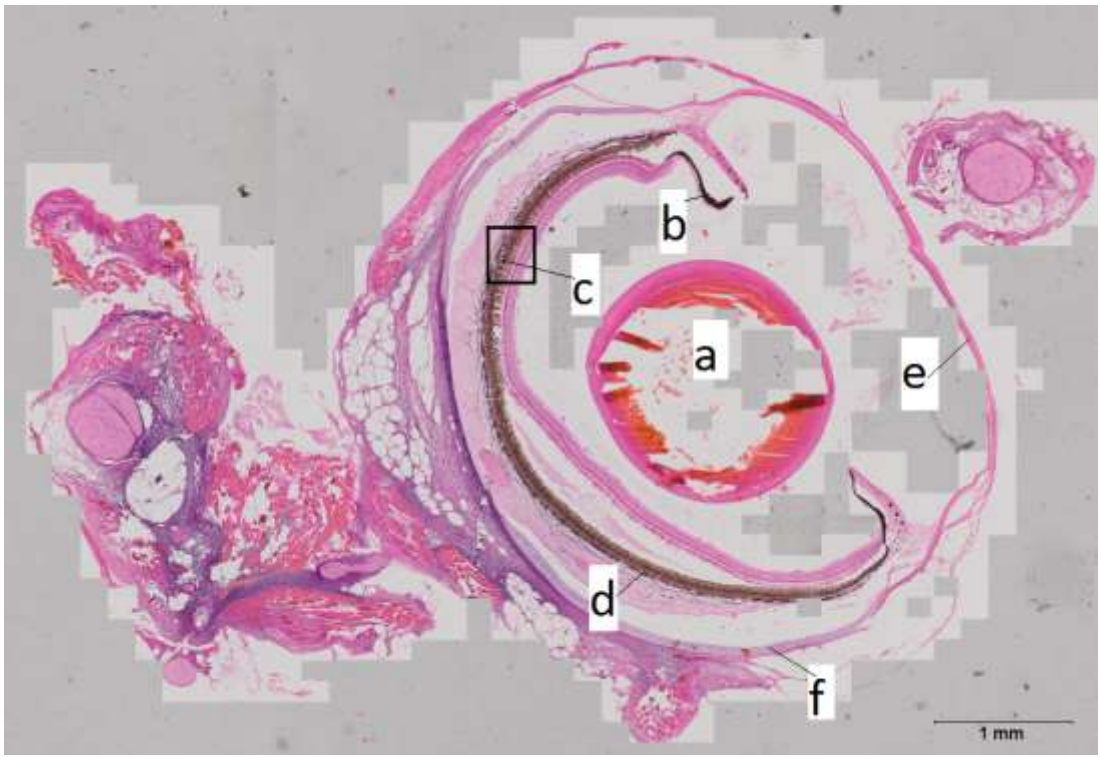

Figure 4; Histological structure of African catfish *C.gariepinus* suffer from abnormal pectoral fin (b. iris c. retina d. choroid e. cornea f. sclera; black rectangle is an area of the retina which enlarged below)

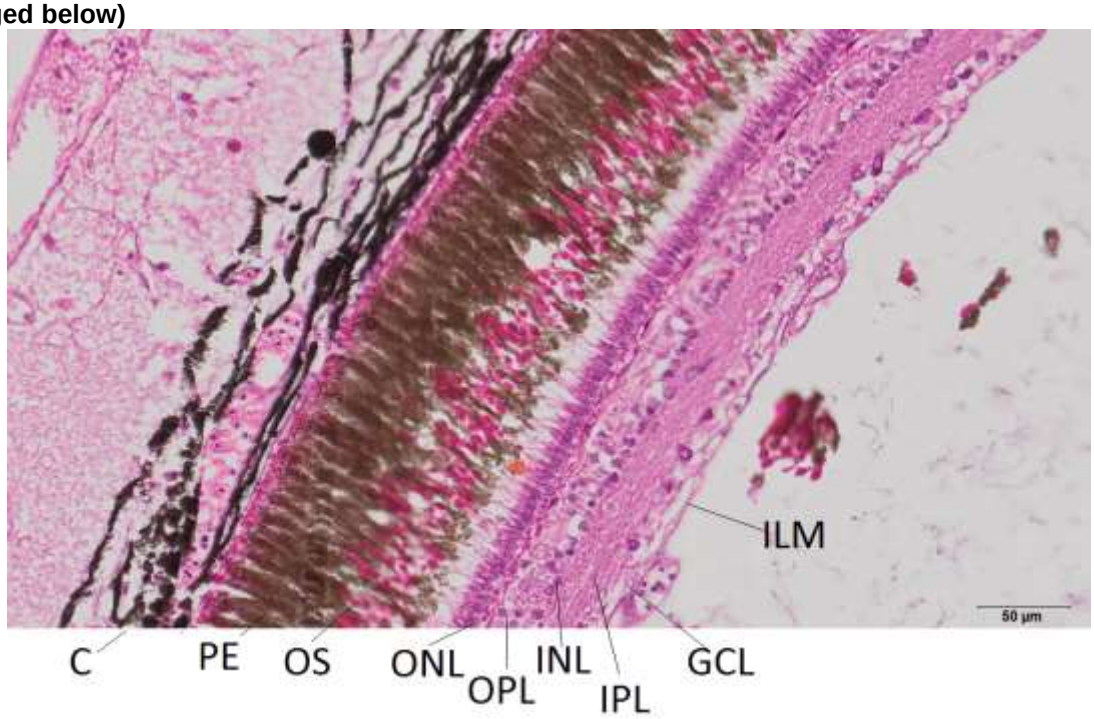

Figure 5.Histological structure of retina from rectangle area in Fig.2. This is the retinal African catfish *C.gariepinus* suffer from abnormal pectoral fin. *PE: Pigment Epithelium OS: Outer Segment of Photoreceptors* ONL: Outer Nuclear Layer OPL: Outer Plexiform Layer INL: Inner Nuclear Layer IPL: Inner Plexiform Layer GCL: Ganglion Cell Layer ILM: Inner Limiting Layer

Table 2; The retinal thickness of *C.gariepinus* suffer from abnormal pectoral fins compared to the Control (normal pectoral fins *C.gariepinus)*

The main part of retina's layer (µm)	Abnormal pectoral fins	Normal pectoral fins
Total retinal thickness	232.71±35.90	278.27±52.26
1st layer thickness	140.82± 22.98	168.31±29.39
2nd layer thickness	41.32±8.05	46.32±10.90
3rd layer thickness	48.82±13.30	55.90±16.42
Retinal Pigment Epithelial (RPE)	72.94±18.88*	108.10±13.29
Outer Segment Photoreceptor (OS)	67.88±4.10	60.21±16.10

Value *(mean±SD*). *significant with *Student t-Test (α=0,05) one-tail*

It can be seen from both that the obvious difference is the thickness or height of Pigment Epithelium (RPE) where the normal fish have dense and thick but the abnormal fish is the opposite. As a consequence, the *Outer Segment (OS)* or disc Photoreceptor, wide opened in African catfish *C.gariepinus* suffer from abnormal pectoral fins while OS in normal fish covered almost entirely with PE microvilli. These facts were supported by Table 2. From Table 2, it showed that African catfish *C.gariepinus* suffer from abnormal pectoral fins had Retinal Pigment Epithelial (RPE) *C.gariepinus* lower (72,94±18,88µm) and quite different *(P<0.05)* from the normal fish (108,10±13,29µm).

4. Discussion

The function of the eye as a visual organ is essential for fish life, including *C. gariepinus*. When the optical system works appropriately, various stimuli from their surrounding cultivation environment detect and respond correctly. So that the eye can function adequately, the constituent components of the eye must be healthy. One of the main elements of the eye is the retina which plays a vital role in the visual mechanism. The retina is the thickest part of the eyeball that curves along the globe. A healthy and good retina will guarantee normal visual function of the eye. The normal retina is the key to the success of fish in standard visual mechanisms and vice versa.

The retina in principle is composed of two main parts, namely the pigmented layer and the neural layer. *Retinal Pigment Epithelium (RPE)* is included in the pigmented layer, which is located posteriorly precisely between Choroid and Outer Segment Photoreceptors (Boulton & Dayhaw-Baker, 2001). Melanin pigments cause the RPE to show a brownish color that is distributed throughout the RPE range, but the highest distribution is in the periphery (Shmidt & Peiss, 1986). This pigment serves a great function in absorbing excess light which enters the eyeball, prevents internal reflection, and stores vitamin A. also *Retinal Pigment Epithelium (RPE)*makes a strong defense between cells laterally to protect the blood vessels below. Another function of the *RPE* is to perform daily phagocytosis of *Outer Segment (OS)* photoreceptor cells, absorption, processing, transportation, the release of Vitamin A (retinol) and several visual cycles (retinoid) (Bok, 1993), and play an essential role as immune modulation (Strauss, 2011).

Studies of retinal structure associated with loss of pectoral fins in aquaculture have not been studied before. The findings of this study reveal that abnormal pectoral fins are associated with retinal degeneration where each retinal structure decreases in thickness from normal. Shown in Fig. 5,*Retinal Pigment Epithelium (RPE)* from the pectoral fins lost to *C. gariepinus* has decreased and appears to shrink from normal conditions. As a result, the degenerate retina contains an outer segment layer of open photoreceptor cells without a sheath which appears in bright red above the *RPE* under Inner Segment (IS). In contrast to the normal retina, the retinal Outer Segment layer does not appear or appear slightly because most Discs are encased in *Retinal Pigment Epithelium (RPE*), even almost all covered (Fig. 3).

RPE is in the posterior part of the retina, which is in the photosensitive area, adheres to and attaches to both rod and cone photoreceptor cells. At that position, the *RPE* can carry out the main strategic function as a light absorbent and play a role in the viewing process. When photons enter the eye and reach the retina, the photons will pass through the anterior part which is not photosensitive and then propagate to the photosensitive part of the Outer Segment (OS). In the OS, Photon will be bound by rhodopsin in the OS, and photoreceptor cells occur and stereochemical changes occur 11-cis retinal

becomes all-trans-retinal. All-retinal trans is then metabolized into all-trans retinol then sent to RPE. In the RPE, retinol is re-isomerized to 11-cis retinal and then sent back to the OS. A retinal 11-cis deposit is available in the OS so that it can continue to be used at any time if needed.

Retinal Pigment Epithelium (RPE) consists of two main substructure namely the Basolateral surface attached to Bruch (BM) and Apical Microvilli Membrane (Finnemann & Chang, 2008). Decreasing Retina Pigment Epithelium (RPE) structure experienced by Catfish suffering from abnormal pectoral fins, the shortening part is Apical Microvilli, while Basal surface is still normal. The end discs of the light-sensitive Outer Segment of photoreceptor (OS) cells sink in the distal part of the basolateral surface while the OS stem is tightly wrapped by Apical Microvilli which hangs above the Basal surface. Apical Microvilli size in this species, *C.gariepinus* is long and can wrap almost all of the Outer Segment cells. When the apical microvilli is short, the retinal pigment epithelium (RPE) cannot completely close the outer segment (OS) of the photoreceptor cell and the implication is that the function of *retinal pigment epithelium (RPE)* as light absorption decreases. If the function of light absorption is not optimal, the light entering the photoreceptor cells becomes excessive, so that the temperature increases around it. Increasing the temperature of photoreceptor cells will be dangerous, especially for blood vessels in the choroid.

Previous studies have found that a decrease in the *Retinal Pigment Epithelium (RPE)* structure is caused by two main factors, age and genetic (the relationship between *Retinal Pigment Epithelium (RPE)* degeneration and loss of pectoral fin in *C.gariepinus* can be explained from insights into embryonic development. Development of the retina started from gastrulation and neural plate formation (Bharti et al., 2006). The pectoral fin begins to develop when mesenchymal cells migrate to the lateral mesoderm plate and form the base of the pectoral fins. *Retinal Pigment Epithelium (RPE)* originates from multipotent optics near the retina requires molecular signals namely Bone Morphogenetic Proteins (BMPs), Hedgehog proteins, Fibroblast Growth Factors (FGFs), and activin (Bharti et al., 2006). On the other hand, pectoral fins develop from mesenchymal cells migrate to the lateral mesoderm plate and up the pectoral fin with Transcription Factor T -Box5 and support from several signaling molecules such as retinoic acid, Bone Morphogenetic Protein (BMP), Hedgehog proteins, or Fibroblast Growth Factors (FGFs) (Tickle, 2015). Their actions are mainly in determining the three dimensions of spatiotemporal factors in dorso-ventral, proximo-distal, and anterior-posterior.

CONCLUSION

C. gariepinus fish with abnormal pectoral fins, that is, missing pectoral fins, have a histological structure of the eye that is indicated to increase degeneration when compared to normal or complete pectoral fins eye tissue. It also concludes that there is a morphological relationship between the pectoral fin organs and eye organs in fish.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

F designed and performed the experiments and also wrote the manuscript. DS analyzed the data mainly of histological data. S, UY, and FI reviewed the manuscript. All authors read and approved the final version.

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