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Research Article

Locomotion Index and Retinal Thickness of the Eye *Anguilla bicolor bicolor* in its Developmental Stage

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ABSTRACT
When migrating to the deep sea, the eye of the eel which functions for locomotion will adapt to the deep environment. This study is the first study to identify the locomotion index and the retinal structure at the stage glass eel, elver, yellow, and silver eel Anguilla bicolor bicolor. Character and habitat information for each stage of eel can be used as a conservation strategy. Seventeen eel fish samples were collected from the Pasir Puncu River in Purworejo. Observation of the Locomotion Index is done by comparing the Eye Index (EI), Pectoral Fin Index (PFI), Anal Fin Index (AFI), and Dorsal Fin Index (DFI) at several stages of development of eel, while histologically the observations were carried out with analyzing the retina structure and the thickness of Rod and Cone Layer (RCL), Outer Nuclear Layer (ONL), Inner Nuclear Layer (INL), Ganglion Layer (GL) and Nerve Fiber Layer (NFL). Based on these observations it can be seen that the retina (RC, ONL, INL, GL, NFL) will have increased from yellow to the silver stage, this is due to adaptation eels that adapt to new habitats. The locomotion index shows that the more developed the pectoral fins, the greater the thickness of the NFL, which indicates the greater the number of nerves used for adaptation in the ocean. In the early elver stage, eels tend to swim on the surface of the water in brackish waters. At the yellow eel stage, the eels have started swimming on the bottom of the water that is rich in the substrate with darker environmental characteristics, while at the silver stage, the eel will begin to adjust to the darker deep sea.

Key words: *Eye; Retina; Silver eel; Yellow eel.*

Introduction

When the eel reaches sexual maturity, there are physiological and morphological changes to support long migration journeys to the high seas. At this stage morphological changes usually occur both externally and internally including enlargement size of eye diameter (Pankhurst, 1982; Beullens et al., 1997; Septriani et al., 2019), changes in integumentary structure and color (Pankhurst and Lythgoe, 1983; Fontaine, 1995), differentiation in the lateral line (Zacchei and Tavolaro, 2009), swimbladder modification (Yamada et al., 2005), and degeneration digestive system and tract, changes in the sensitivity of the pigment in the retina, to adapt in the dark and increase cone cells (Pankhurst and Lythgoe, 1983; Bowmaker et al., 2008).

Changes in eye diameter and eye structure indicate adaptive behavior to changes in habitat from the riverbed to the sea deep release, it also allows the function of the eye that is used to avoid predators. Changes in the size of the eel's eye diameter from the yellow eel phase to silver eel fish eel *A. bicolor bicolor* from Segara Anakan, Indonesia have been studied by Septriani et al., (2019), but the development of locomotion and retinal thickness at several stages' development has not been studied in detail. Therefore, this study aims to examine the locomotion index and retinal thickness of the eye *A. bicolor bicolor* at the glass eel, elver, yellow eel, and silver eel stages.

Materials and Methods

1. Fish Collection

This study will use 17 samples of eel obtained from the Pasir Puncu River in Purworejo. Here are the details of the 17 eels to be caught; 3 from the glass eel, 6 from the elver stage, 5 from the yellow stage, and 3 from the silver stage. The captured eels were immediately put into a 10% formalin solution to bring to the laboratory Animal Structure and Development, Faculty of Biology, Universitas Gadjah Mada.

2. Morphometric Measurement

Measurements of the captured eels will be carried out, according to Arai et al., 2012. The data took about body mass (BW), total length (TL), eye diameter, pectoral fin, dorsal fin, and anal fin were measured and saved.

3. Histological Preparation

The eye organs of the eel were prepared by following the standard paraffin method. The eye was taken and fixed with Bouin's solution overnight, then performed decalcification, followed by washing with 70% alcohol fixative solution. The next step is tissue dehydration using gradient ethanol followed by immersion in toluene solution to purify the tissue. When the tissue is ready, it will be infiltrated with paraffin, embedding, trimming, and trimming to a thickness of 5 µm. After that, the network will be colored with Ehrlich Hematoxylin Eosin (HE) dye and MAF dye.

4. Data analysis

The data of locomotion indices that have been collected were analyzed by Kruskal Wallis and continued with Mann Whitney U-Test with confidence level P 0.05. Histology of the eye will be analyzed qualitative descriptive by comparing the eye index at several stages of development of the eel, then histological observations were carried out by analyzing the retina structure and the thickness of Rod and Cone Layer (RCL), Outer Nuclear Layer (ONL), Inner Nuclear Layer (INL), Ganglion Layer (GL) and Nerve Fiber Layer (NFL) using Image J Software.

Results and Discussion

Locomotion Index

The eyes, pectoral fins, anal fins, and dorsal fins are locomotion organs in fish. Which will coordinate with each other in their movements. The eye index, pectoral fin index, and dorsal fin index are used as a reference for the development of the movement of eels in the sea related to habitat change.

The movement and locomotion of fish, it is influenced by the eyes, pectoral fins, dorsal fins, and anal fins. The dorsal fin contributes to the stability of the vertical position, by increasing the lateral surface during swimming. During swimming their position is stabilized by motor neurons that control the trunk muscles (Grillner, 2011).

TL, BW, and locomotion index measurements in this study, including EI, PFI, AFI, and DFI are as follows (Table 1).

TL, BW, EI, PFI, AFI, and DFI based on the developmental stage of *A. bicolor bicolor* was shown in Figure 1.

Based on the Spearman correlation analysis, a high correlation value is obtained (> 0.500) between the developmental stages of eel and locomotion organs (eyes, pectoral, dorsal, and anal fins). Meanwhile, in Kruskals analysis Wallis showed the significance of total length, body weight, eye index, fins pectoral, and anal fins at each stage of elver, yellow, and silver. Mann Whitney follow-up test U-Test showed that the total length, body weight, EI, PFI, AFI, and DFI of the silver phase was significantly higher compared to the elver phase, but not significant with the yellow phase. Increased eye index on the development of eel according to research Pankhurst, 1982; Beullens et al., 1997; Durif et al., 2005; Okamura et al., 2007; Hagihara et al., 2012;

Septriani et al., 2019 on eel species *A. australis*, *A. dieffenbachii*, *A. anguilla*, *A. japonica*, *A. marmorata* and *A. celebesensis*, *A. bicolor bicolor*. Changes in eye diameter and eye structure indicate adaptive behavior to changes in habitat from changing riverbeds to the deep high seas it also allows it to be used to avoid predators.

Table 1. TL, BW, EI, PFI, DFI, and AFI of *A. bicolor bicolor* on glass eel, elver, yellow and silver stage

Stage	TL (mm)	BW (gr)	EI	PFI	DFI	AFI
Glass Eel	50	0.10	-	-	-	-
Glass Eel	55	0.13	-	-	-	-
Glass Eel	55	0.14	-	-	-	-
Elver	88	-	0.51	3.41		
Elver	64	0.28	-	-		
Elver	63	0.173	-	-		
Elver	185.3	9	1.08	2.97	1.13	0.81
Elver	210	10	1.65	3.62	1.09	1.00
Elver	200	6.1	2.45	2.69	1.09	1.10
Yellow	345	48	2.33	3.19	1.16	1.16
Yellow	403.7	65	2.66	2.75	1.39	1.39
Yellow	350	52	5.83	3.31	1.97	1.86
Yellow	320	49	2.48	2.72	1.42	1.30
Yellow	350	60	6.3	2.93	1.86	1.77
Silver	430	157	15.79	4.98	1.34	1.42
Silver	418	150	16.90	5.00	2.34	2.06
Silver	450	145	18.84	6.11	2.67	2.53

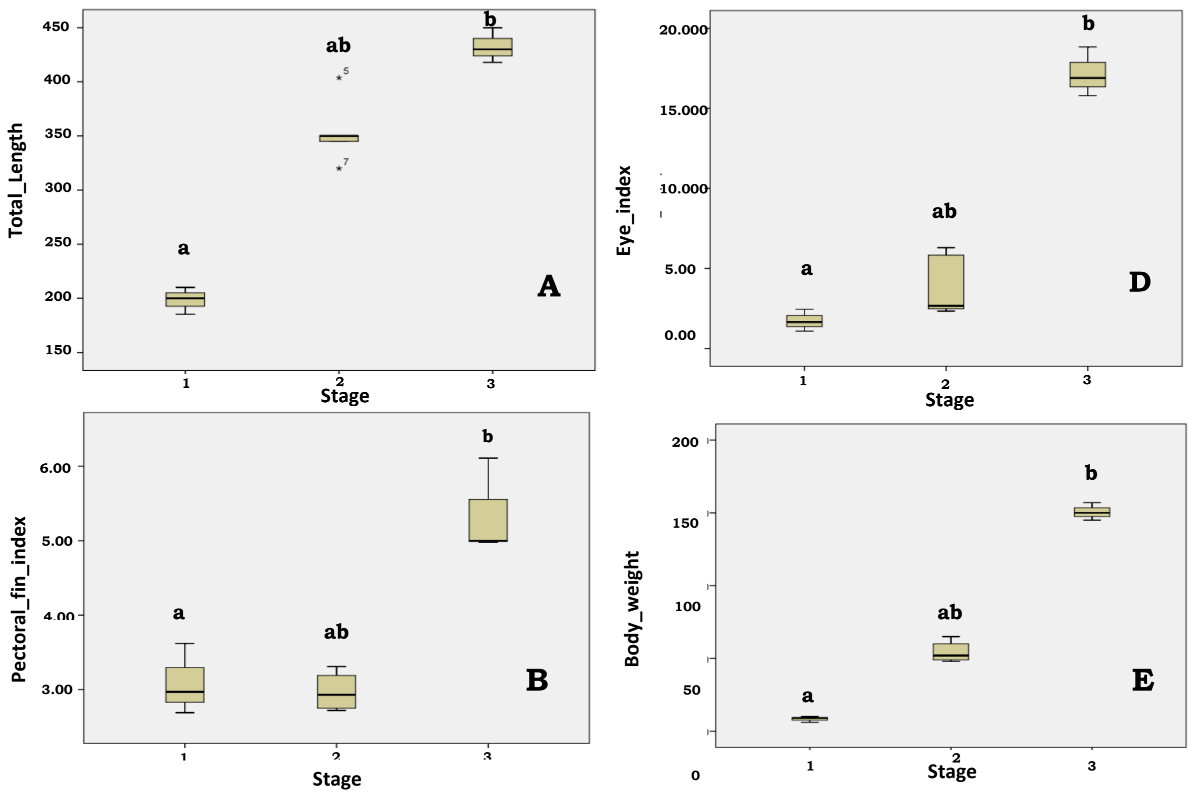

Figure 1. (A) Total length, (B) Pectoral Fin Index, (D) Eye Index, (E) Body Weight. Note step 1=elver, 2=yellow, 3=silver.

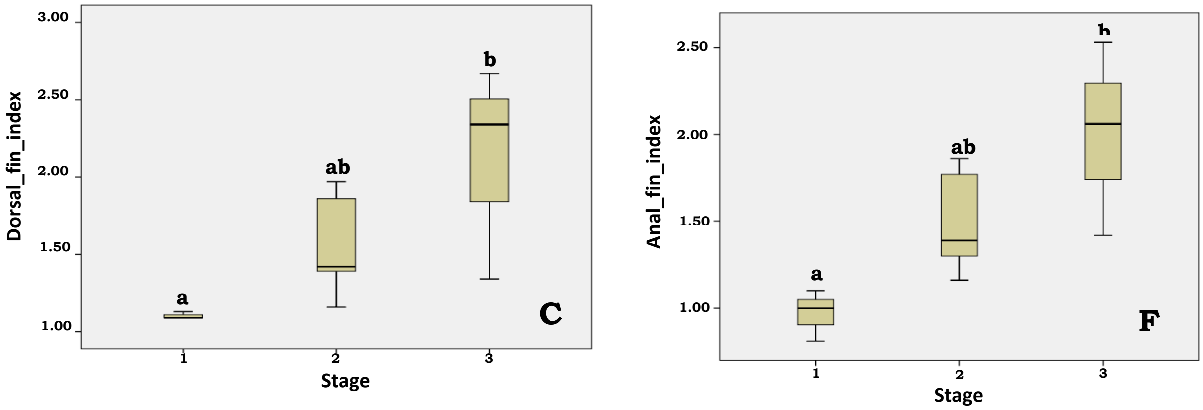

Figure 1. Continue (C) Dorsal Fin Index, and (F) Anal Fin Index on its developmental stage; (1) elver, (2) yellow, (3) silver stage of *A. bicolor bicolor.* (a), (ab), (b) same characters mean insignificantly different. Note step 1=elver, 2=yellow, 3=silver

In addition to the eye, fins also play a role in locomotion, especially the pectoral fins. The pectoral fins increase swimming speed and maneuvering, and are activated in short undulatory bursts. The angle of the surface of the pectoral fins changes when it moves vertically. These fins can generate lift and movement up and down by redirecting the head and body. The back of the pectoral fin is active as a cover to redirect the head and body to the start-up and down movement (Wilga and Lauder, 1999).

Structure of the Eye and Retinal Thickness

This study is the first to reveal the histology of fish eyes eels *A. bicolor bicolor* based on the stage of development. The histological structure of the eye *A. bicolor bicolor* was shown in (Figure 2, Figure 3, Figure 4, Figure 5, Figure 6).

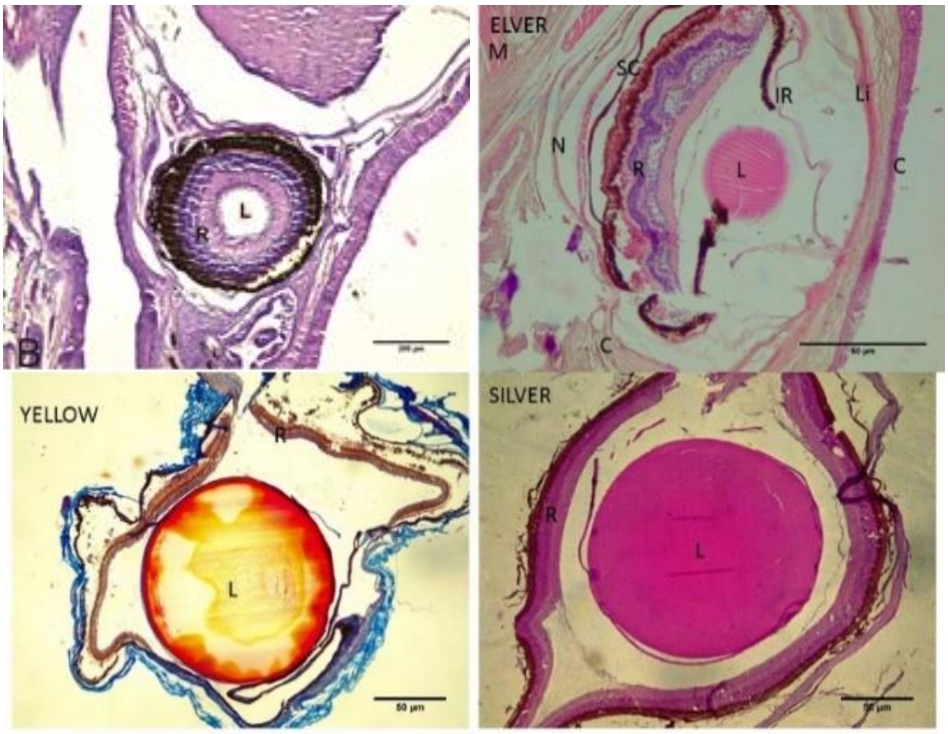

Figure 2. Histological structure of the eye *A. bicolor bicolor* at the glass eel stage, elver, yellow, and silver. Line scale: 200 µm. Abbreviations: L: lens, R: retina, IR: iris, Li: limbus, C: cornea, Sc: sclera, N: nerve.

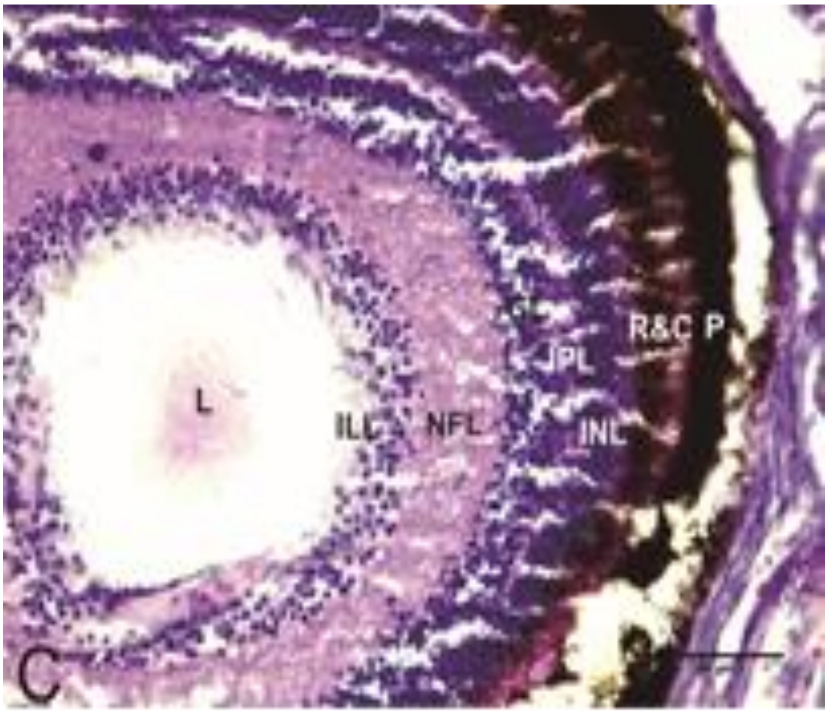

Figure 3. Histological structure of the eye of the eel (*A. bicolor bicolor*) at the glass eel stage. Line Scale: µ 50 m. Abbreviation: L: lens, R: retina, ILL: inner line layer, NFL: nerve fiber layer, GL: ganglionic layer, IPL: inner plexiform layer, INL: inner nuclear layer, R&C: rod and cone cells, P: pigment epithelium. Hematoxylin-eosin staining.

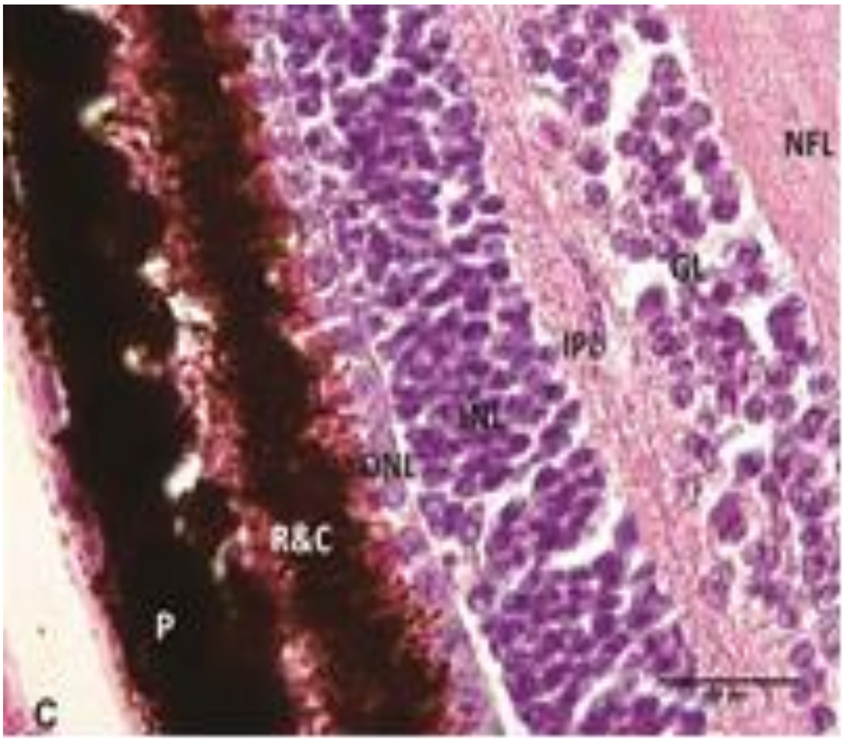

Figure 4. Histological structure of the eye *A. bicolor bicolor* in the early elver stage. Line Scale: 50 µm. Abbreviation ILL: inner line layer, NFL: nerve fiber layer, GL: ganglionic layer, IPL: inner plexiform layer, INL: inner nuclear layer, ONL: outer nuclear layer, R&C: rod and cone cells, P: pigment epithelium. Hematoxylin-eosin staining.

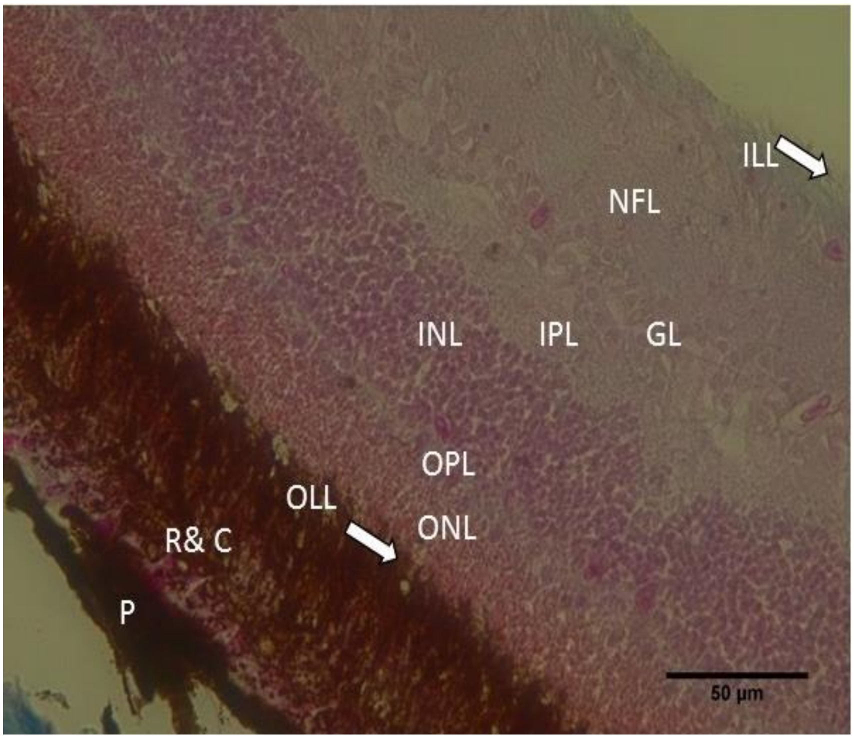

Figure 5. Histological structure of the eye of the eel (A. bicolor bicolor) at the yellow eel stage. Line scale: 50 µm. Abbreviation: ILL: inner line layer, NFL: nerve fiber layer, GL: ganglionic layer, IPL: inner plexiform layer, INL: inner nuclear layer, OPL: outer plexiform layer, ONL: outer nuclear layer, OLL: outer line layer R&C: rod and cone cells, P: pigment epithelium. Mallory acid fuchsin staining.

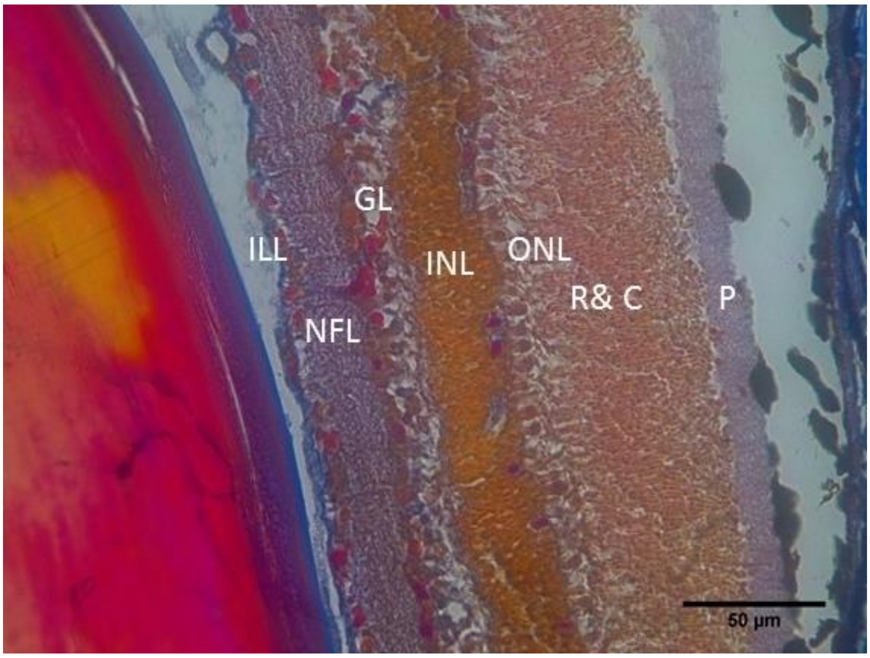

Figure 6. Histological structure of the eye of the eel (*A. bicolor bicolor*) at the silver eel stage. Line scale: 50µm. ILL: inner line layer, NFL: nerve fiber layer, GL: ganglionic layer, IPL: inner plexiform layer, INL: inner nuclear layer, OPL: outer plexiform layer, ONL: outer nuclear layer, OLL: outer line layer R&C: rod and cone cells, P: pigment epithelium. Hematoxylin-eosin staining.

A retinal layer of the eye *A. bicolor bicolor* consists of Pigmented Layer (PL) which has a function for supporting retina neural, Rod Containing Layer (RCL) which contains rods and cones as photoreceptors, Outer Line Layer (OLL) which holds cones and rods in between muller cell, Outer Nuclear Layer (ONL) which containing cell body and nuclear of the rods and cones, Outer Plexiform Layer (OPL) which containing fibers and synapses of the bipolar neurons and rod and cones, Inner Nuclear Layer (INL) which containing cell body from neuron bipolar which integrate signal from rod and cones, Inner Plexiform Layer (IPL) which containing fibers and synapse of the bipolar neurons of the next layers. Ganglionic layer (GL) which containing ganglionic cells. Nerve Fiber Layer (NFL) which containing axons of the ganglion cells which form nervous optics (Mescher, 2017). The thickness of NFL, GL, INL, ONL, and RCL was shown in figure 7.

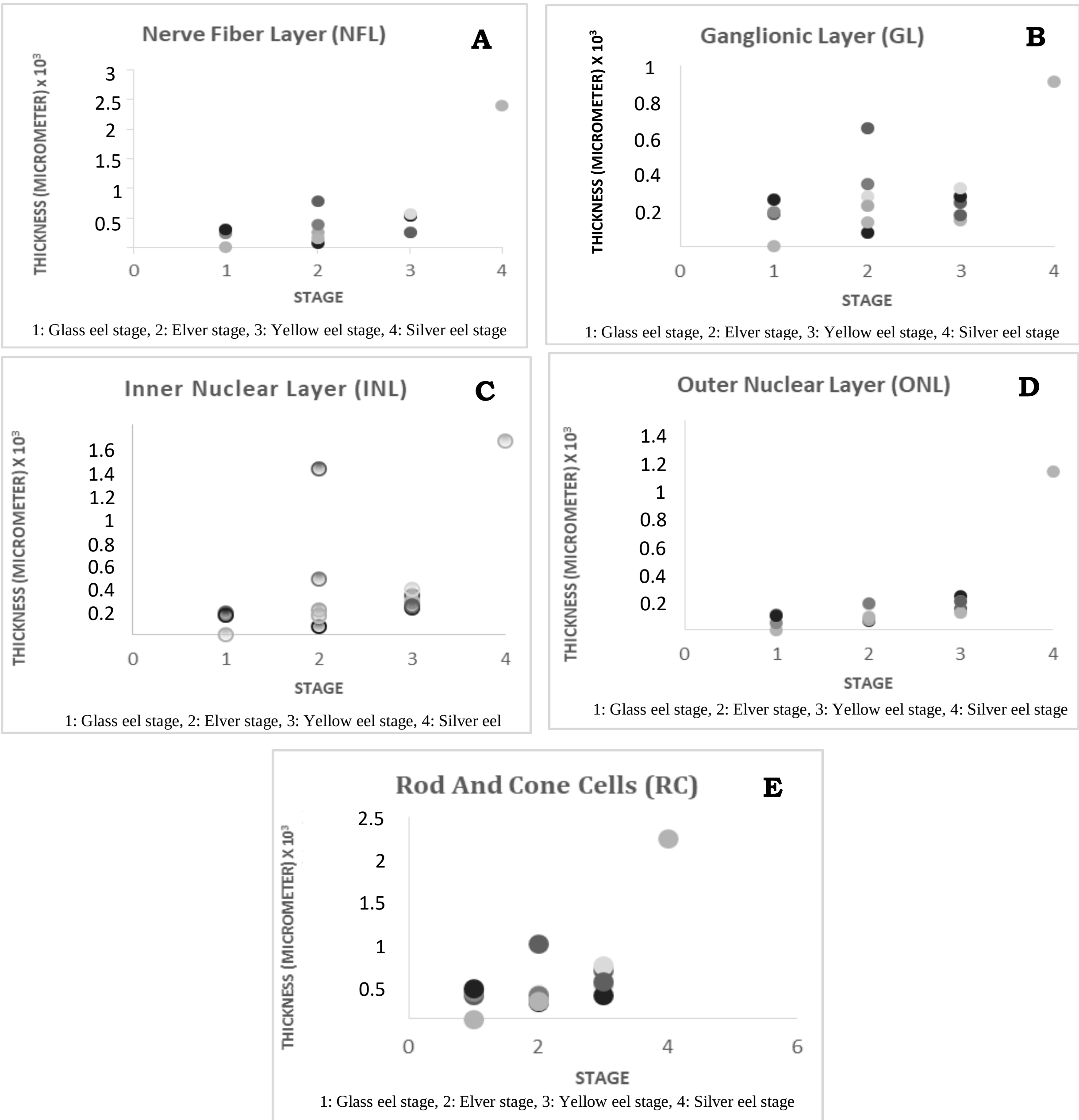

Figure 7. The thickness of (A) NFL, (B) GL, (C) INL, (D) ONL, and (E) RCL of *A. bicolor bicolor* on glass eel, elver, yellow, and silver stage development.

Based on these observations it can be seen that the retina layer from glass eel to elver stage has increased, while the elver stage to yellow stage is insignificantly different (based on Mann Whitney analysis, P> 0.05), the yellow to the silver stage will have increased significantly.

Vision helps organisms in searching for prey and detecting predators. The ability of this viewer is very determined by the structure and function of retinal nerves (Evans and Browman, 2004). The development of the retina in the visual system must be optimized for the species to survive successfully (Fritsches et al., 2003). As a form of adaptation to the environment, freshwater eels undergo changes in the retina of the eye. They could change sensitivity spectral during migration from freshwater to environment sea in good by replacing chromophore type, or by expressing different opsin genes for resolve change environment light (Wang et al., 2011).

The correlation between Locomotion and Retinal Thickness was calculated for determining the locomotion organ's contribution during retinal thickness development (Table 2).

Table 2. Correlation between INL, ONL, GL, RC, NFL with EI, PFI, DFI, and AFI of *A. bicolor bicolor*

	INL	ONL	GL	RC	NFL
EI	0.358	0.53	0.489	0.68	0.762
PFI	0.747	0.04	0.83	0.85	0.906
DFI	0.057	0.112	0.014	0.016	0.001
AFI	0.007	0.116	0.001	0.003	0.021

The highest correlation value from the calculation between locomotion and retinal index is PFI and NFL is 0.906. The lowest correlation occurs between DFI and NFL and between AFI and GL is 0.001. It might be suggested that pectoral fins contribute to the largest locomotion role and the nerve layer's development. Amount adaptation system vision and locomotion allow fish to resolve constraints happening in the environment.

RCL in this study showed that from yellow to silver there is an increase in rods and cones. Rods are very sensitive to a dark environment, so the number will experience an increase as it grows, while the cone cells begin to shrink even hamper is no longer visible (Pankhurst and Lythgoe, 1983; Bowmaker et al., 2008). However, in this study, we didn't differentiate the abundance of rods and cone cells.

NFL in this study showed that from yellow to silver there is an increase in nerve. Retinal nerve development in a species can become an indicator of pressures or threats that individuals face throughout their early life (Fritsches et al 2003).

GL in this study also showed a significant increase from yellow to silver. The number of ganglion cells per unit large in the retina provides the ' barrier ' for subtlety image that reaches the brain. Photons must pass the whole neural retina before being captured and transduced to become impulse nerves by photopigments contained in the segment outside rods and cones cell photoreceptors. Information light then walks return through the retina as impulse neurons and is processed by horizontal, bipolar, and amacrine cells before continuing to ganglion cells

Visual information of the walking retina to the brain along nerve optics as potential action walk and through axon ganglion cells. Details and discrimination visual image (predator or prey) that reaches brain through axon this will depend on ability processing signal from intensity light around, and the retina must have sufficient sharpness for a complete relevant object by ecological. Sensitivity is range intensity and long waves that can be transduced by the retina. Sharpness refers to the resolution spatial retina (Wang et al., 2011).

In conclusion, the beginning fish larval stage depends on transparency to avoid detection and the use of cue mechanoreceptors for reducing the possibility of capture by predators (Wang et al., 2011). While, in the early elver stage, eels tend to swim on the surface of the water in brackish waters. At the yellow eel stage, the eels have started swimming on the bottom of the water that is rich in the substrate with darker environmental characteristics, while at this stage the silver eel will begin to adjust to the darker deep sea.

Conclusion

Based on these observations it can be seen that the retina (RC, ONL, INL, GL, NFL) will have increased from yellow to the silver stage, this is due to adaptation eels that adapt to new habitats. The more developed stage the greater the thickness of the retina layers. Based on the correlation, the locomotion index shows that the more developed the pectoral fins, the greater the thickness of the NFL, which indicates the greater the number of nerves used for adaptation in the ocean.

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