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Morphological Variation of Blue Panchax (*Aplocheilus panchax*) Lives in Different Habitat Assessed Using Truss Morphometric

Diah Mustikasari, Suhestri Suryaningsih, Agus Nuryanto*

Faculty of Biology, Universitas Jenderal Soedirman, Indonesia
*Corresponding author: agus.nuryanto@unsoed.ac.id

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Abstract. Blue panchax (*Aplocheilus panchax* Hamilton, 1822) lives in broad ranges of habitat from open waters to closed waters, including at ex-tin mining pits in Bangka Island, Indonesia. Variable habitats might cause morphological variations due to different ecological factors. This study aimed to assess the morphological variation of blue panchax collected from different habitats using truss morphometric characters. Fish samples (70 individuals) were collected from abandoned ex-tin mining pits of different ages and a river in Bangka Island. Twenty-nine truss characters were analyzed using the Kruskal-Wallis test and post hoc with Dunn's test . The results showed that almost all of the body parts of blue panchax found in ex-tin mining pits and rivers were significantly different (p-value < 0.05), except some truss characters of caudal peduncle. The post hoc of Dunn's test showed a positive correlation between habitats and truss characters (adj. sig < 0.05). Some truss characters of killifish were different between ex-tin mining pits with different ages chronosequence, and some other characters were different between pits and river. This study provides the first data about the morphological variation of blue panchax in ex-tin mining pits of different ages. The data is valuable as a scientific basis of further utilization of ex-tin mining pits in the areas.

Key words: *Aplocheilus panchax*; morphometry; pits; river

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INTRODUCTION

Blue panchax (*Aplocheilus panchax*), locally known as ikan Kepala Timah, is one of the killifish species from the Order Cyprinodontiformes, Family Aplocheilidae, and Genus *Aplocheilus*. Member of Genus *Aplocheilus* is widely distributed across the Indo-Malayan Islands, including Indonesia, the Indo-China region, and India (Vasil'eva et al., 2013; Dekar et al., 2018). *Aplocheilus panchax* is a species of the genus *Aplocheilus*. It is an endemic species to the Oriental Region (Costa, 2013; Sedlacek et al., 2014; Furness, 2015; Costa, 2016; Beck et al., 2017).

Blue panchax can live in a broad range of habitats (Manna et al., 2011). It can survive in open and closed waters such as lakes or pits of ex-tin mining, including newly formed and old pits. According to Kurniawan et al. (2019) and Irawan et al. (2014), the newly formed ex-tin mining pits are extreme ecosystems with very low pH values and dissolved oxygen (DO), but with high heavy metal content. Conversely, the older abandoned tin mining pits have a better water quality. Nevertheless, a previous study by Kurniawan (2019) had proved that blue panchax was reported to live in newly abandoned tin mining pits in Bangka Island though have deplorable water quality conditions.

A study had shown that ecological characteristics have impacted fish genotype (Nguyen et al., 2017) and have a further effect on their morphology (Baillie et al., 2016; Endo & Watanabe, 2020). Other studies also proved that fish live in different habitats, showed variable morphologies, and, in extreme condition, might form different ecotypes (Rajeswari et al., 2017). Morphological variation among individual fish can be assessed using conventional and truss morphometric characters (Pazhayamadom et al., 2014; Mojekwu and Anumudu, 2015; Rawat et al., 2017). According to Ariyanto et al. (2011), truss morphometric provides a comprehensive, systematic, and fairly high-accuracy geometric picture of fish body shapes. So, this method can be used to distinguish between individual fish more precisely than standard morphometric. It has been proven that truss morphometric is an efficient technique to differentiate fish individuals than conventional morphometric (Ihya et al. 2020; Nabila et al. 2019; Pambudi et al. 2019)

It is assumed that different ecological factors among different ages of ex-tin mining pits and rivers in Bangka might cause morphological differences among blue panchax collected from such a diverse ecosystem. There is no study assessing the morphological variation of blue panchax inhabits different ages of abandoned tin mining pits and rivers in Bangka Province. The only research was about the existence and factors affecting blue panchax in the abandoned ex-tin mining pits (Mustikasari et al., 2020; Kurniawan et al., 2019). Therefore, this is the first research about the morphological variation of blue panchax live in different ages of ex-tin mining pits and river in Bangka Island. The data is vital for the management of the further utilization of ex-tin mining pits in the areas. This study aimed to assess the morphological variation of blue panchax collected from different habitats using truss morphometric characters.

METHODS

Study sites

The study was conducted in Pangkalpinang City and Bangka Regency of Bangka Belitung Archipelago Province, Indonesia (Figure 1). Fish samples were collected from ex-tin mining pits with chronosequences abandoned after mining activities. The pits were clustered into six different ages, i.e., Station A and Station B (< 5 years old), Station C and Station D (5-15 years), Station E and Station F (15 - 25 years), Station G (25 - 50 years), Station H (50 - 100 years), Station I and Station J (> 100 years), and Limbung River Stream of Bangka Regency as Station K. The sampling site condition is shown in Figure 2.

Sample collection and preservation

The 70 fish samples were collected at 09.00 am - 1.00 pm from ex-tin mining pits and a river using nets with mesh size of about 0.4 mm. Fresh individuals were placed in the labeled plastics bottle filled with 40% formalin. For permanent preservation, the samples were preserved with absolute ethanol. In the laboratory, the morphometric characters were measured by a ruler with an accuracy of 0.5 mm.

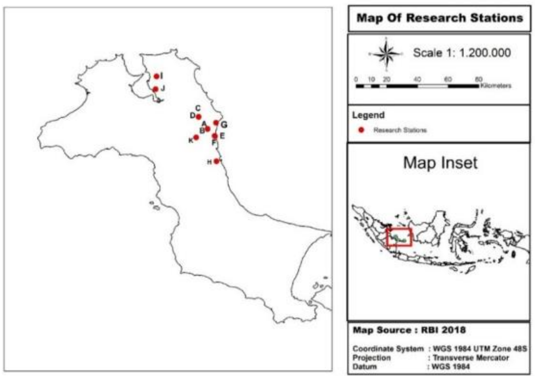

Figure 1. Map of research stations. Station A and Station B were pits with age < 5 years, Station C and Station D (5-15 years), Station E and Station F (15-25 years), Station G (25-50 years), Station H (50-100 years), Station I and Station J (> 100 years), while Station K was Limbung River Stream.

Figure 2. Waters condition of research stations, (a) Station A and (b) Station B were pits with age < 5 years; (c) Station C and (d) Station D (5-15 years); (e) Station E and (f) Station F (15-25 years); (g) Station G (25-50 years); (h) Station H (50-100 years); (i) Station I and (j) Station J (> 100 years); and (k) Station K was Limbung River Stream (private documentations).

Morphometric measurement

Fish morphology was measured using truss network measurement. Truss morphometric was used to measure 29 diagonal distances among truss points and the truss characteristics encoded from A1 to D5 (Figure 3), while the description of each truss characteristics is presented in Table 2. The truss morphometric characters were analyzed using the Kruskal-Wallis test in SPSS Program version 25 to know significant differences of truss characters among individuals collected at different habitats. Dunn's test was used for the post hoc of Kruskal-Wallis.

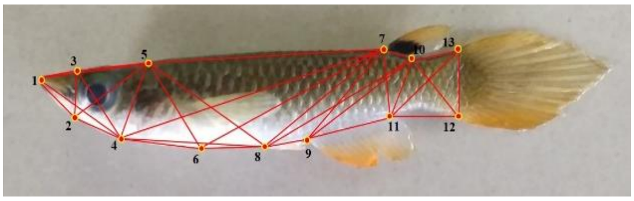

Figure 3. The truss network characteristics of Kepala Timah fish (*Apocheilus panchax*) (private documentations).

Table 1. Truss characteristics of Kepala Timah fish (*A. panchax*) and their descriptions

Part of Body	Code	Descriptions
Head	A1 (1 to 2)	distance between the snout or premaxilla and the pelvic maxilla (lower jaw)
	A2 (1 to 3)	distance between the snout and dorsal maxilla or anterior eye diameter (upper jaw)
	A3 (1 to 4)	distance between the snout and the pelvic operculum
	A4 (1 to 5)	distance between the snout and the dorsal operculum
	A5 (2 to 3)	distance between pelvic maxilla and dorsal maxilla or anterior eye diameter
	A6 (2 to 4)	distance between the pelvic maxilla and the pelvic operculum
	A7 (2 to 5)	distance between the pelvic maxilla and the dorsal operculum
	A8 (3 to 4)	distance between the dorsal maxilla or anterior eye diameter and the pelvic operculum
	A9 (3 to 5)	distance between the dorsal maxilla or anterior eye diameter and the dorsal operculum
	A10 (4 to 5)	distance between the pelvic operculum to the dorsal operculum
Anterior Body	B1 (4 to 6)	distance between the pelvic operculum and lower body-pectoral fin
	B2 (4 to 7)	distance between the pelvic operculum and the anterior dorsal fin
	B3 (5 to 6)	distance between the dorsal operculum and lower body-pectoral fin
	B4 (5 to 7)	distance between the dorsal operculum and the anterior dorsal fin
	B5 (5 to 8)	distance between the dorsal operculum and ventral or pelvic fin
	B6 (6 to 8)	distance between the lower body-pectoral fin and ventral or pelvic fin
	B7 (7 to 8)	distance between the anterior dorsal fin and ventral or pelvic fin
Posterior Body	C1 (7 to 9)	distance between the anterior dorsal fin and anterior anal fin
	C2 (7 to 10)	distance between the anterior and the posterior dorsal fin
	C3 (7 to 11)	distance between the anterior dorsal fin and posterior anal fin
	C4 (8 to 10)	distance between the ventral or pelvic fin and the posterior dorsal fin
	C5 (9 to 10)	distance between the anterior anal fin and the posterior dorsal fin
	C6 (9 to 11)	distance between anterior and posterior anal fin
	C7 (10 to 11)	distance between the posterior dorsal fin and rear anal fin
Caudal Peduncle	D1 (10 to 12)	distance between the posterior dorsal fin and pelvic-posterior caudal peduncle
	D2 (10 to 13)	distance between the posterior dorsal fin and dorsal-posterior caudal peduncle
	D3 (11 to 12)	distance between the posterior anal fin and pelvic-posterior caudal peduncle
	D4 (11 to 13)	distance between the posterior anal fin and dorsal-posterior caudal peduncle
	D5 (12 to 13)	the caudal peduncles' height

RESULTS AND DISCUSSION

The general morphology of killifish collected from different aged ex-tin mining pits and river is shown in Figure 4.. It can be realized from Figure 4 that killifish individuals collected at the different ecosystems showed different colorations. Killifish individuals live in abandoned tin mining pits (Figure 4a-d) have brighter colors than fish in the river (Figure 4e). It can also be seen in Figure 4 that among different pits ages, killifish individual shows different body color. Nevertheless, body-color brightness does not correlate with pits ages. It is shown in Figure 4b that killifish collected from older than 5 to15 years old pits has the brightest body color and more colorful than individuals collected in less than one to 5 years and older than 15 years pits (Figure 4a, 4c, and 4d).

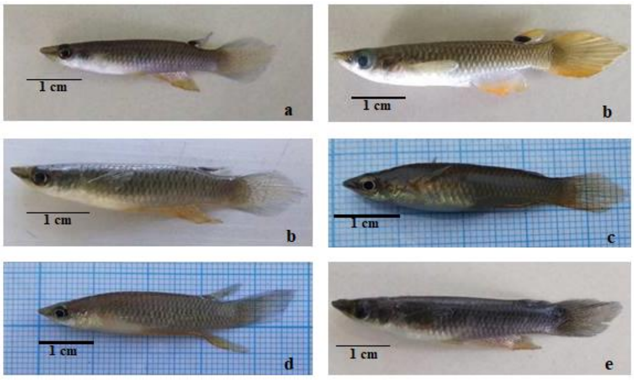

Figure 4. General morphology of killifish collected from different habitats. (a) pits with age of < 5 years, (b) pits with age of 5-15 years, (c) pits with age of 15-25 years, (d) pits with age of 25-50 years, (e) Limbung River.

It is suggested that morphological differences among individuals of killifish live in different habitats is because of differences in ecological factors among the habitats. That argument rose based on the fact that sampling locations have distinct environmental parameters (Table 3). According to Nguyen et al. (2017), ecological factors might affect fish genotype, and according to Baillie et al. (2016) and Endo and Watanabe (2020), ecological characteristics have a further effect on fish morphology. Other studies also proved that fish live in different habitats show variable morphologies, and in extreme condition might form different ecotypes (Rajeswari et al., 2017).

Truss analysis was a measurement based on the ratio of truss character with head length or standard length. According to Paknejad et al. (2014), the truss morphometric network study effectively provides information about an organism's shape. It can describe the body shape of the fish more properly. Truss morphometric is a reliable method for morphological differentiation among fish samples. Therefore, it is expected that truss morphometric analysis could differentiate killifish collected from different habitats, such as among different aged ex-tin mining pits and between ex-tin mining pit habitats as closed waters with the river as open waters. The results of Dunn's test among truss morphometric characters of killifish from different habitats are presented in Table 2.

As can be seen in Table 2, that almost all of the truss characters of killifish showed significant differences (p-value < 0.05) among habitats, except on four characters (D1, D2, D4, and D5) that are parts of the caudal peduncle. These four characters indicated that the caudal peduncle could not differentiate among individuals collected at different habitats. The other parts of the blue panchax fish's body showed significant differences based on the Kruskal-Wallis test. Dunn's test showed that some of these truss characters showed a positive correlation between different locations (adj. sig. < 0.05). The truss characters of A1, A5, B3, B7, C1, C2, C3, and C5 only showed the differences between ex-tin mining pits with different ages chronosequence. The truss characters of A2, A3, A4, A6, A7, A8, A9, A10, B1, B2, B4, B5, B6, C4, C6, C7, and D3 described the differences among pits with different ages and also between pits as closed water and the river as representative of open water.

The differences in morphometric among killifish can be caused by habitat characteristics where they live. They might differ in biotic and abiotic factors such as food availability, salinity, temperature, radiation, water depth, current flow, and other environmental factors that can affect the morphometric of the fish in each location (Sen et al., 2011; Kashefi et al., 2012; Muchlisin, 2013). Besides, the condition of ex-tin mining pits as closed water containing many elements such as metals and heavy metals (Kurniawan, 2017; Kurniawan et al., 2019) might contribute to the metabolism process impacted to morphometric characters of fish. It can happen in Bangka Island rivers too that the contamination of elements contributed to biological activity and morphometric characters of organisms in the waters. According to Lestari et al. (2018), human activities directly affecting rivers' water quality and freshwater fish diversity. According to Nuryanto et al. (2012) and Nuryanto et al. (2015), the existence of fish in rivers could be due to the different Physico-chemical characters of the rivers, especially its dissolved oxygen, carbon dioxide level, temperature, acidity (pH), substrates, organic and inorganic materials, water volume, the width of the river, and geology factor. These factors will naturally gradually change the microhabitat and impact of fish's diversity and characteristics in the river. According to Sari and Zakaria (2017), physical aspects (e.g., temperature), chemical factors (e.g., dissolved oxygen and acidity of the water), and biological factors (e.g., amount and type of food or food availability) can influence morphology and sexuality of fish.

Therefore, the differences in ecological characteristics can affect morphological characteristics (Baillie et al., 2016; Endo and Watanabe, 2020). Habitat variation affecting fish's community structures and previous studies had proven a positive correlation between fish morphology and their environment (Gebrekiros, 2016). The correlation between ecological parameters and morphological characters explains that the water quality, especially pH value in ex-tin mining pits or river, can influence the morphometric standard and truss characters where the low water quality can inhibit the fish growth. These conditions can be seen in ex-tin mining pits that pits with age ranged between 1 and 5 years old was acidic, while pits with age between 25 and 50 years old was neutral. The neutral pH can support biological life such as, plankton, and improves the physicochemical parameters, which in turn could promote fish's growth.

Table 2. The results of the Kruskal-Wallis test and Dunn's test of truss characteristics

Truss Parameters	Mean	Sig. of Kruskal-Wallis Test	Pairwise comparison from different research stations (adj. sig < 0.05 of Dunn's Test)***
A1*	0.3584	0.000	A-E (.009); A-F (.013); E-G (.016); F-G (.026)
A2*	0.3094	0.000	D-G (.038); E-F (.010); E-G (.038); E-K (.000); F-G (.005); G-K (.000)
A3*	0.6899	0.000	A-F (.001); A-K (.008); C-F (.002); C-K (.018); E-F (.000); E-K (.001); D-F (.025)
A4*	0.9393	0.000	A-D (.018); A-E (.005); A-G (.000); B-G (.010); E-K (.034); F-G (.002); G-K (.000)
A5*	0.3031	0.000	D-E (.001); D-F (.000)
A6*	0.3440	0.000	A-F (.036); C-F (.001); C-K (.006); E-F (.000); E-G (.035); E-K (.001); D-F (.040)
A7*	0.4199	0.000	A-F (.028); A-G (.000); B-G (.000); C-G (.012); D-G (.001); E-G (.001); G-K (.018)
A8*	0.5314	0.000	D-E (.024); D-F (.030); D-K (.003)
A9*	0.6687	0.000	A-F (.001); B-F (.025); D-F (.001); D-K (.020); E-F (.000); E-K (.008)
A10*	0.6296	0.000	D-G (.006); D-K (.001); E-F (.016); E-G (.000); E-K (.000)
B1*	0.1316	0.002	D-K (.042)
B2*	0.6257	0.000	A-E (.010); A-K (.000); B-E (.036); B-K (.000); C-K (.000); D-K (.046); G-K (.008)
B3*	0.1886	0.000	A-G (.024); D-G (.001); E-G (.008); F-G (.014)
B4*	0.5636	0.001	C-K (.021); F-K (.002)
B5*	0.2923	0.003	G-K (.044)
B6*	0.1791	0.000	A-D (.024); B-D (.027); C-G (.003); D-E (.002); D-F (.028); D-G (.000); G-K (.002)
B7*	0.4053	0.002	E-F (.000)
C1*	0.2776	0.000	A-C (.005); A-G (.002); C-D (.046); D-G (.014)
C2*	0.0701	0.000	A-G (.001); B-G (.001); D-G (.008)
C3*	0.2950	0.000	A-C (.021); A-G (.002); D-G (.009); E-G (.042)
C4*	0.4410	0.000	D-F (.042); E-F (.030); E-G (.036); F-K (.008); G-K (.010)
C5*	0.3120	0.000	A-G (.009); C-D (.043); C-E (.031); D-G (.001); E-G (.001)
C6*	0.2440	0.000	A-G (.001); A-K (.000); C-G (.037); C-K (.014); D-G (.013); D-K (.004); E-G (.049); E-K (.019)
C7*	0.1421	0.000	A-G (.022); B-G (.005); C-G (.009); D-G (.002); E-G (.016); G-K (.001)
D1**	0.1734	0.099	
D2**	0.1294	0.206	
D3*	0.1586	0.026	B-K (.004)
D4**	0.2111	0.557	
D5**	0.1264	0.160	

*= sig. < 0.05 means significant differences of the parameters by Kruskal-Wallis test, and then they were continued to post hoc analysis with Dunn's test

**=sig. > 0.05 means no significant differences of the parameters by Kruskal-Wallis test, and then they weren't continued to post hoc analysis with Dunn's test

***=pairwise comparisons of pits with adj. sig. < 0.05 of Dunn's test means the significant differences of two pits for each truss parameter. The Bonferroni correction for multiple tests has adjusted the significance value.

Killifish body length differences among pits with chronosequence ages are positively correlated with the pits' water quality. The first indicator of water quality in ex-tin mining pits is pH value, where pit with age of < 10 years has an acidic condition with a pH value of about 3 (Kurniawan et al., 2019). The acidification of habitat due to anthropogenic activities can impact the biological and ecological processes

(Kleinhappel et al., 2019). In acidic conditions, the biological activity such as plankton growth can be inhibited, causing plankton's appearance in the habitat to be minimum. The acidification in freshwaters reduces species' richness in general, including plankton, and it has negative consequences for aquatic organisms such as fish (Rychła et al., 2011; Hasler et al., 2018). The presence of plankton as nutrition for fish was significant for their growth, so directly and indirectly, the intensity of sunlight and pH value contributed to blue panchax fish's morphometric characters.

The low pH value directly can influence the metabolism of fish. Some study explained that the low pH value of acidic condition contribute to metabolism changes and inhibition of growth. According to Mota et al. (2018), fish exposure to acidic media experiencing various adverse effects, either on physiological and cytological conditions of fishes. Moreover, according to Srineetha et al. (2013) and Kwong et al. (2014), physiological impact of acidic environment includes ionocytes fluctuation, white blood cell production, and increases mucus production. Further impacts of low pH are increasing blood viscosity and affecting fish respiration through lowering total and rate of oxygen intake by fish.

The correlation between low pH and reducing growth and feed intake metabolism could impact growth, appetite, food conversion efficiency, a disruption to physiological homeostasis, blood acidosis, and blood plasma pH (Abbink et al., 2012; Kennedy and Picard, 2012). This condition impacted the morphometric characters of killifish in ex-tin mining pits, especially in pits with age < 5 years with low pH value. The acidic conditions of ex-tin mining pits, especially in pits with age < 5 years, caused this ecosystem not to have any biological life, organic substance, and nutrition to support the blue panchax fish maximum life and its growth. For a long time that more than 15 years, the chronosequence effect in ex-tin mining pits caused the pH value to change to normal conditions (pH 7). The change of pH of chronosequence impacted the other changes such as DO, BOD, C-organic, total nitrogen, total phosphate, and others (Kurniawan et al., 2019). The consequence of changes can cause an increase in biological life, activity, and nutrition. The existence of biological substances can be the fundamental factor in supporting the blue panchax fish's life besides the chemical and physical aspects of water quality. Therefore, it is reasonable if this study observed that killifish inhabit less than 5 years old ex-tin mining pits have the shortest body length and body length tend to increase related to pits ages with the longest fish body was reach at pits ages between 25 and 50 years old.

Killifish were not found in ex-tin mining pits with age > 50 years. That condition contradicted the report by Raja et al. (2015) and Karuppaiah dan Ramesh (2016) that the members of Aplocheilidae could live in a broad range of water quality (Table 3). Simultaneously, ex-tin mining pits older than 50 years have some water quality parameters within ranges values reported by both groups of researchers. It is suggested that the other ecological factors in ex-tin mining pits with age > 50 years have influenced the existence of killifish in these habitats. It has been reported by Mustikasari et al. (2020) that the presence of killifish in ex-tin mining pits was strongly related to water quality.

Metal and heavy metal content in water could accumulate on fish tissue and organs through serial food chains (Rajeshkumar and Li, 2018; Kurniawan and Mustikasari, 2019). Heavy metal accumulation might disturb fish metabolism and inhibits fish growth. Under extreme conditions the accumulation of heavy metals in fishs' organs can cause mortality (Afshan et al., 2014).

Table 3. Water quality of habitat for Family Aplocheilidae

Parameters	Value						
	(a)	(b)	(c)	(d)	(e)	(f)	(g)
Temperature (ºC)	21-25	25-28	26-30	28-30	25-32	30-32	19-32
pH	6.8-7.5	7.1-7.8	7.5-7.9	7.3-7.9	7.3-8.1	7.5-8.1	7.0-9.23
DO ($mg.l^{-1}$)	4.8-11.6	4.1-6.4	3.0-3.8	2.0-3.8	0.2-0.3	0.2-0.3	0.02-14.4
COD ($mg.l^{-1}$)	9-13	12-62	110-150	100-150	160-250	173-299	12.6-71.2
BOD ($mg.l^{-1}$)	3.5-12.2	9.5-16.8	96-338	110-338	53-300	45-300	0.01-10.16
Hardness ($mg.l^{-1}$)	33-98	-	-	-	-	-	34-356
Alkalinity ($mg.l^{-1}$)	18-77	4.1-6.4	2.0-3.8	3.0-3.9	1.1-2	1.1-2.9	120-360
Conductivity ($mhos.cm^{-1}$)	42-88	108-270	250-329	280-2000	850-2900	950-2900	240-1560
Turbidity (NTU)	-	14-22	16-25	21-30	26-32	29-32	-
TDS (ppm)	-	-	-	-	-	-	135-1451.6

Sources: (a) Reservoir Bhavanisagar, Tamil Nadu, India (Raja et al., 2015); (b) Dam Viagra, (c) Anaipatti River, (d) Solavandhan River, (e) Arapalaiyam River, (f) Anna Nagar River, India (Karuppaiah dan Ramesh, 2016); (g) Mysore Wetland, India (Prasad et al., 2009).

Previous studies have shown that fish morphology was affected by habitat characteristics (Baillie et al., 2016; Endo and Watanabe, 2020). Habitat characteristics might cause specific of body shape and coloration in fish (Rajeswari et al., 2017). However, there is no study assessing morphological variation in the abandoned ex-tin mining pits. Previous studies by Mustikasari et al. (2020) and Kurniawan et al. (2019) were only discussing blue panchax in the abandoned ex-tin mining pits and its ecological factors. Therefore, this study provides the first data about the morphological variation of blue panchax live in different aged ex-tin mining pits and river in Bangka Island. The data is vital for the management of the further utilization of ex-tin mining pits in the areas. In a broader sense, this study provides a scientific basis that variable sizes and colors of fish production can be obtained through ecological manipulations.

CONCLUSION

It can be concluded that blue panchax fish collected from different ex-tin mining pits and Limbung River showed morphological variation. The variations are positively related to the ecological characteristics of each habitat.

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