Morphological characteristics and length-weight relationship of endemic wild betta in Sumatra Island

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Abstract. *Betta raja* is an endemic fish found on the island of Sumatra. Its natural habitat is found in Jambi, Riau and North Sumatra provinces. The three locations are geographically separated and it is suspected that *B. raja* has developed physiological adaptations in each habitat, thus presenting differences in morphology. Morphological identification studies may prove that the speciation process has occurred. The purpose of the study was to analyze the morphometric and meristic characters of *B.* cf *raja* on the island of Sumatra. Determination of observation stations for fish sampling were based on information related to the distribution of *B.* cf. *raja* in Sumatra. Morphometric and meristic characters were determined for each fish caught (15 individuals per location). Data processing used SPSS software version 22 and MVSP version 3.22. Based on the results of the Kruskal-Wallis test, there were 2 characters that showed non-significant values (DFR and TS) and 23 characters that had significant differences. The results of PCA analysis showed that some morphological characters of fish clustered in Riau and North Sumatra populations. Furthermore, some morphological characters between the Jambi and North Sumatra populations are clustered. The length-weight relationship of *B. raja* is negative allometric. It is concluded that differences in habitat location also produce differences in morphological characters and length-weight relationships of *B. raja* due to the process of physiological adaptation. It is suspected that the Riau and North Sumatra populations are still one species, while the Jambi population is different. Molecular taxonomic studies are further needed.
Key Words: growth pattern, meristic, morphometric, native species.

Introduction. *Betta raja* is one of the wild fish endemic to Sumatra Island (Dahruddin et al 2021). Sumatran endemic fishes are reported to have declining populations and face the threat of extinction due to the impacts of habitat loss, pollution, overfishing (Prianto et al 2017), and development activities (Ohee et al 2021). Iskandar et al (2021) reported that 22 native and endemic fish species in Indonesia are threatened with extinction and 16 species are endangered. Based on IUCN Redlist data, *B. raja* has been categorized as Least Concern (Low 2019). The morphological characteristics of *B. raja* are as follows: 6.8 cm maximum standard length, dorsal fin 7-10 rays, anal fin 25-28 rays, literal scales (30-32), and head length 33.9-37.4% of standard length (Fishbase 2023).

There has not been much research conducted on *B. raja*. Manullang & Khairul (2020) are known to have studied the biological aspects of *B. raja* in Bandar Tinggi Village, Labuhanbatu Regency, North Sumatra. However, based on the identification results, there is still some doubt, given the similarity with *B. fusca*. Research on ecomorphology, length-weight relationships, and genetic variation within the scope of molecular ecology on *B. raja* in Sumatra Island has never been conducted.

B. raja habitat in Sumatra is found in Rokan Hulu Regency (Riau Province) and Muaro Jambi Regency (Jambi Province). *B. raja* populations live in different habitats and are geographically separated, so each population will develop physiological adaptations. According to Bhagawati et al (2013), morphological traits are physical traits of all species and serve to distinguish one from another. Furthermore, fish morphological characters are a form of adaptation to the environment, can be caused by separate geographical conditions, so they can be used as a basis for studying genetic variation (Medrado et al 2008; Panarari-Antunes et al 2011; Vidal & García-Marín 2011).

Martinez et al (2018) state that habitat plays a role in shaping genetic diversity in fish, and it can be used to determine its conservation status. Genetic diversity can be investigated through a genomic approach and also a phenomic approach. In general, studies analyzing phenotypic or morphological characters of fish use morphometric and meristic character measurements. Morphometric and meristic studies have been widely used for taxonomic and conservation purposes. According to Muchlisin et al (2020), endemic fish need to be preserved and conservation efforts must be made immediately. Endemic fish have an important ecological role in the ecosystem in which they live (Wargasasmita 2017).

Material and Method

Description of the study sites. The research locations were selected based on the purposive sampling method, namely based on public information about where *B. raja* were found on the island of Sumatra. Thus, 3 observation stations were determined: Station 1 (Jambi Province), Station 2 (Riau Province), and Station 3 (North Sumatra Province) (Figure 1).

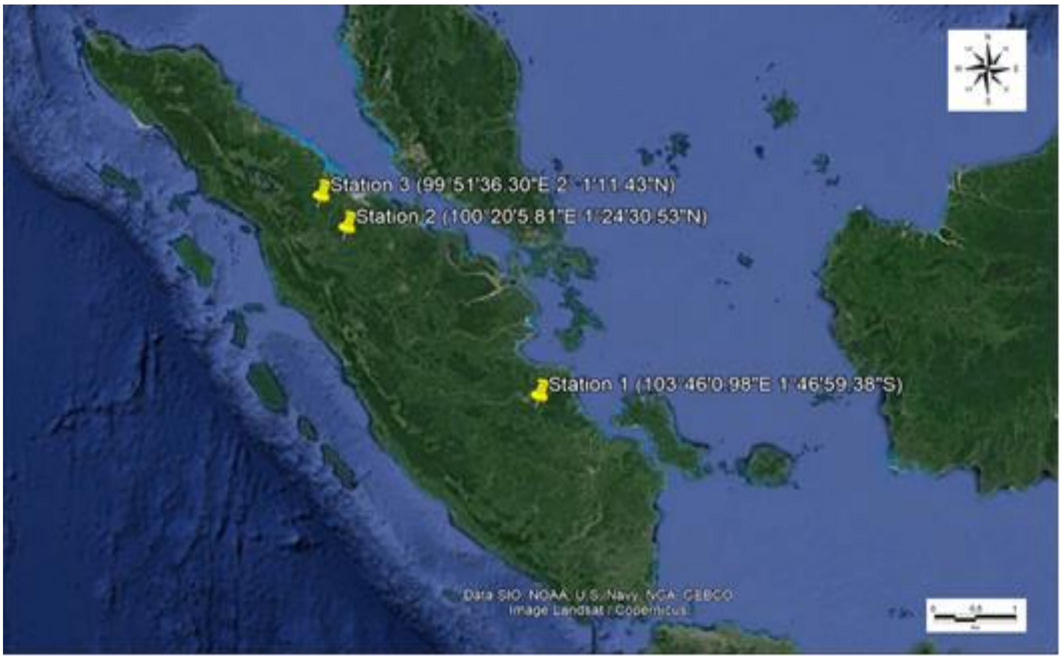

Figure 1. Map of research locations.

Research procedures. B. raja sampling was conducted in July-August 2023. Fish were caught using a fishing rod with earthworm (*Lumbricus terrestris*) as hook bait. The number of fish retained from each study site was 15 individuals. The morphological measurement method (morphometric and meristic characters) on B. raja refers to the research of Sholihati et al (2014) (Tables 1 and 2).

Table 1

Morphometric character measurements

No	Morphometric characters	Description	Code
1	Standard length	The distance from the front of the head to the base of the caudal fin	SL
2	Predorsal length	The distance between the leading edge of the head and the first rays of the dorsal fin	PreDL
3	Postdorsal length	The distance between the back of the last rays of the dorsal fin to the base of the tail fin	PDL
4	Preanal length	The distance from the tip of the head to the base of the first anal fin	PreAL
5	Body depth at dorsal fin origin	The distance from the base of the first dorsal fin to the ventral side	BDD
6	Body depth at pectoral fin origin	The distance between the dorsal part which is parallel to the base of the pectoral fin to the ventral part	BDP
7	Caudal peduncle depth	The lowest or minimum distance at the tail bar	CPD
8	Head length	The distance from the leading edge of the head to the tip of the operculum	HL
9	Orbit diameter	Maximum distance from the edge of the eye to the edge of the other edge	OD
10	Snout length	The distance between the front of the head to the front of the eyes	SNL
11	Postorbital length	The distance between the back of the edge of the eye to the tip of the operculum	PL
12	Interorbital width	The distance on the dorsal side from the edge of one eye to the edge of the other eye	IW
13	Dorsal fin base length	The distance from the base of the dorsal fin from the base of the dorsal fin rays to the last rays of the dorsal fin	DFBL
14	Anal fin base length	The distance from the base of the anal fin from the base of the anal fin rays to the last radius of the anal fin	AFBL
15	Pelvic fin length	The distance from the base of the ventral fin to the longest tip of the ventral fin	PVFL
16	Pectoral fin length	The distance between the base of the pectoral fin to the longest tip of the pectoral fin	PCFL

Table 2

Morphometric character measurements

No	Meristic characters	Description	Code
1	Transversal scales	Number of scales from beginning of the dorsal fin slanted down anterior to lateral scales, then from lateral scales slanted downward rear to the anal fin base	TS
2	Lateral scales	Number of scales behind the operculum to the beginning of the base of the caudal fin	LS
3	Predorsal scales	Number of scales from the base of the dorsal fin to the head	PreDS
4	Postdorsal scales	Number of scales from the back of the dorsal fin to the surface of the base of the caudal fin	PDS
5	Subdorsal scales	The number of scales from the bottom of the dorsal fin from the tip of the fin to the end of the dorsal fin	SDS
6	Dorsal fin rays	Number of dorsal fin rays	DFR
7	Anal fin rays	Number of anal fin rays	AFR
8	Pectoral fin rays	Number of pectoral fin rays	PCFR
9	Pelvic fin rays	Number of ventral fin rays	PVFR

Morphological data analysis. Morphometric and meristic characters were analyzed using the Kruskal-Wallis test to identify characters that differed significantly from the overall population being compared (Nofrita 2017). The formula used was as follows:

$$H = \frac{12}{n\,(n+1)} = \sum_{n=1}^{1} \frac{Ri^2}{ni} - 3(n+1)$$

Where: H - Kruskal-Wallis value; n - number of samples; R - number of ranges for all groups.

Principal component analysis (PCA). The PCA analysis was in the form of morphological data that had been matched with standard lengths and transformed with log10. Furthermore, the data were processed using the Multi-Variate Statistical Package (MVSP) version 3.1 program to obtain PCA ordination plots. The equation used in PCA analysis is as follows:

$$\begin{bmatrix} a1.1 & a1.2 \\ a2.1 & a2.2 \end{bmatrix} \begin{bmatrix} x \\ y \end{bmatrix} = \lambda \begin{bmatrix} x \\ y \end{bmatrix}$$

Where: a1.1 to a2.2 - data matrix.

Length-weight relationship. Fish length-weight relationship data were analyzed using the following formula (Le Cren 1951):

$W=aL^b$

Where: W - total weight (g); L - total length (mm); a and b - constant values.

Data were analyzed using Microsoft Excel 2013 to obtain a graph of the relationship between the length and weight of fish.

Statistical analysis. To determine if the value of b shows isometry based on the category for the length-weight relationship, a t test was conducted with the SPSS software.

Results and Discussion

Morphological characteristics. The measurement results of the population from Jambi Province were higher than those for the population of North Sumatra and Riau provinces. The results of morphometric character measurements of *B. raja* were transformed (log10) first for data uniformity, while the meristic character data were not. Based on the results of the Kruskal-Wallis analysis, 2 characters showed non-significant results (TS and DFR), while 23 characters showed significant results (SL, PreDL, PDL, BDD, BDP, CPD, HL, OD, SNL, PL, IW, DFBL, AFBL, PVFL, PCFL, LS, PreDS, PDS, SDS, DFR, AFD, PCFR, and PVFR). The complete Kruskal-Wallis test results can be seen in Table 3.

The differences in morphological characters of *B. raja* in the three locations are thought to be due to environmental factors and geographically separated habitats. According to Poulet et al (2004) and Nofrita (2017), one of the factors affecting morphological variation in fish is differences in environmental conditions. The same species occupying different habitats can undergo morphological changes as an adaptation to their respective environments. Based on the results of Keeley et al (2007), there are morphological differences in the form of fin, head length and mouth size in *Oncorhynchus mykiss* caused by differences in environmental factors. According to Rawat et al (2017), the number of dorsal fin rays and lateral line scales are key to distinguishing species in the genus *Betta*. Morphological variation in wild betta can occur due to geographic isolation (Syarif et al 2020). Syarif et al (2021) found several measurements such as total length, caudal fin length, body length, and head height as distinguishing morphological characteristics for wild betta species.

Table 3

Kruskal-Wallis test results of three populations of *Betta* cf. *raja* based on Log 10 base data

Code	*N*	*Mean*	*Standard deviation*	*Minimum*	*Maximum*	*Test result*
			Morphometric			
SL	45	1.717	0.081	1.583	1.861	0.000*
PreDL	45	1.531	0.087	1.35	1.68	0.000*
PDL	45	1.115	0.107	0.92	1.29	0.000*
PreAL	45	1.415	0.072	1.29	1.54	0.000*
BDD	45	1.297	0.064	1.18	1.44	0.000*
BDP	45	1.237	0.136	1.07	1.51	0.000*
CPD	45	0.995	0.087	0.83	1.17	0.000*
HL	45	1.237	0.083	1.09	1.41	0.000*
OD	45	0.725	0.075	0.62	0.87	0.000*
SNL	45	0.703	0.109	0.55	0.90	0.000*
PL	45	0.891	0.121	0.70	1.08	0.000*
IW	45	0.802	0.110	0.62	1.01	0.000*
DFBL	45	1.250	0.174	1.06	1.48	0.002*
AFBL	45	1.582	0.087	1.38	1.81	0.001*
PVFL	45	1.296	0.117	1.11	1.58	0.000*
PCFL	45	1.025	0.074	0.87	1.15	0.000*
			Meristic			
TS	45	21.088	2.284	13	26	0.350^{ns}
LS	45	29	1.965	25	33	0.000*
PreDS	45	25.2	2.006	21	30	0.009*
PDS	45	10.444	1.271	8	13	0.008*
SDS	45	12.044	2.610	8	18	0.000*
DFR	45	6.689	0.9	4	8	0.070^{ns}
AFR	45	23.889	1.465	20	27	0.001*
PCFR	45	8.08	3.188	4	18	0.000*
PVFR	45	6.089	1.505	3	9	0.000*

Note: N - population; ns - non-significant; * - significant; SL - standard length; PreDL - predorsal length; PDL - postdorsal length; PreAL - preanal length; BDD - body depth at dorsal fin origin; BDP - body depth at pectoral fin origin; CPD - caudal peduncle depth; HL - head length; OD - orbit diameter; SNL - snout length; PL - postorbital length; IW - interorbital width; DFBL - dorsal fin base length; AFBL - anal fin base length; PVFL - pelvic fin length; PCFL - pectoral fin length; TS - transversal scales; LS - lateral scales; PreDS - predorsal scales; PDS - postdorsal scales; SDS - subdorsal scales; DFR - dorsal fin rays; AFR - anal fin rays; PCFR - pectoral fin rays; PVFR - pelvic fin rays.

Furthermore, PCA analysis results are presented in Figure 2. Some of the morphological characters of *B. raja* are clustered in the Riau population with North Sumatra. Furthermore, the Jambi population also partially appears to cluster with the North Sumatra population. It is concluded based on the results of the PCA analysis that differences in location affect the differences in morphological characters of *B. raja* populations in each habitat. It is suspected that fish develop physiological adaptations that form morphological characters with different appearances.

Differences in habitat affect morphological characteristics between fish species due to the adaptation process and can cause differences in genetic variation (Turan et al 2004; Panarari-Antunes et al 2011). There are many differences in morphological characters both morphometric and meristic in the three *B. raja* populations found. This can be caused by physiological adaptation in each natural habitat. The almost uniformly significant differences in morphological characteristics for each population could indicate different species. Differences can be compared with results from previous research. Hui & Ng (2005) determined meristics and morphometric characteristics of wild *Betta* in Sumatra. They obtained the following results. The SL for *B. cracens* is between 38.2-57.4 mm, for *B. fusca* between 53.1-59.7 mm, for *B. raja* between 45.9-63.9 mm and for *B. rubra* between 33.1-35 mm. The number of pectoral fin rays for each of the species, respectively, is 13, 12, 12-13 and 13. The number of anal fin rays for each species, respectively, is 26-27, 23-25, 23-26, and 24-26. The number of dorsal fin rays for each species, respectively, is 8-9, 7-8, 7-10, 7-8. The predorsal length for each species is, respectively, 65-67.1, 68.5-70.2, 63.2-69.9 and 62.9-67.6 mm. The caudal peduncle depth for each species is, respectively, 15.1-16.7, 19.2-20.4, 18.3-23.2, 13.1-15.3 mm. The body depth at dorsal-fin origin for each species is, respectively, 21.2-24.2, 30.9-31.7, 28.1-33.5, 23.2-25.8 mm. The pelvic fin length for each species, respectively, is 36.1-43.4, 38.6-39.2, 37.4-69.8, and 28.1-38.4 mm. the anal-fin base length for each species is, respectively, 53.4-55.7, 47.8-51.4, 48.9-54.8 and 49.2-52.8 mm. According to Hebert et al (2003), it is necessary to identify the species with molecular biology techniques.

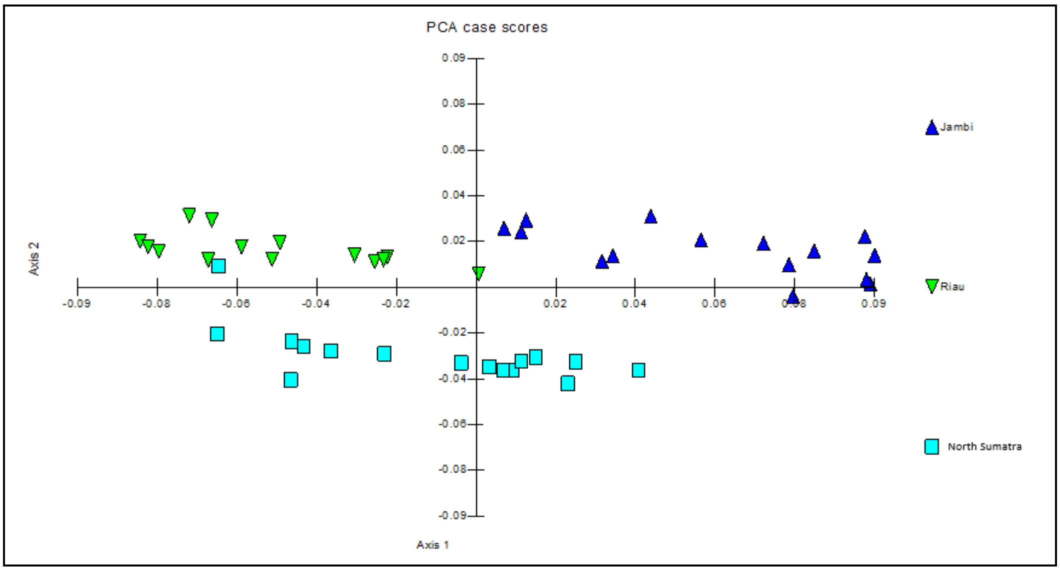

Figure 2. Component plot of morphological characters of *Betta* cf. *raja* based on Principal Component Analysis.

Length-weight relationship. The length-weight relationship in the three populations of *B. raja* was negative allometric and positive allometric. The results of the fish length-weight relationship analysis can be seen in Figures 3, 4, and 5.

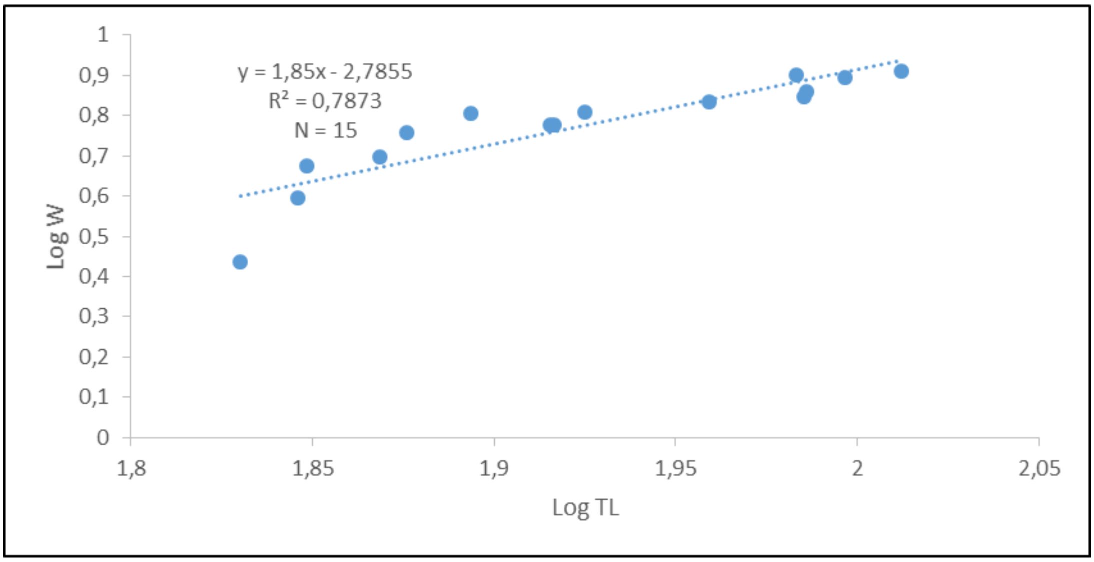

Figure 3. Length-weight relationship of *Betta* cf. *raja* Jambi population.

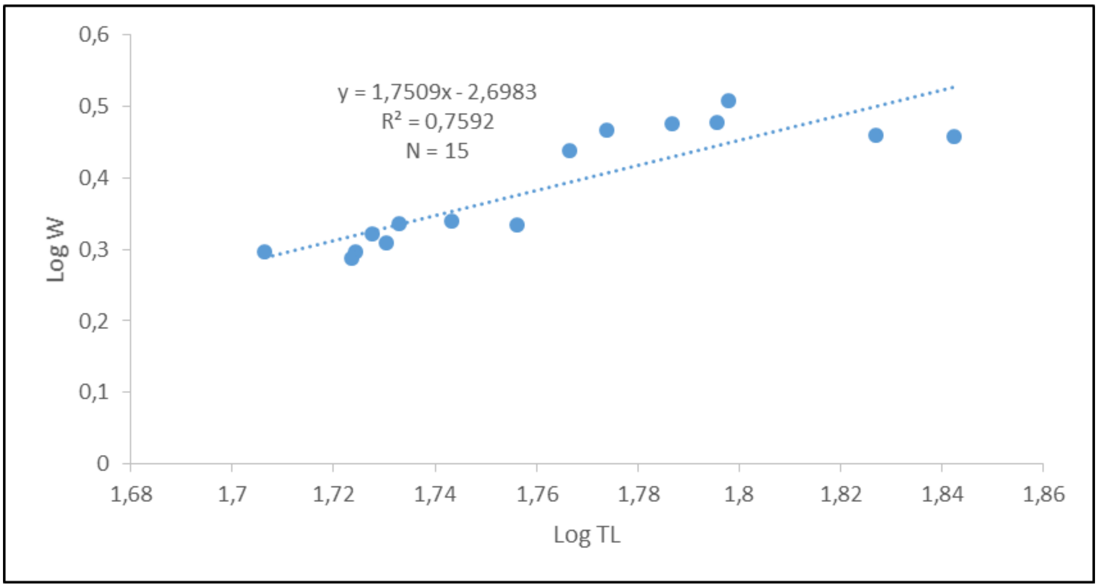

Figure 4. Length-weight relationship of *Betta* cf. *raja* Riau population.

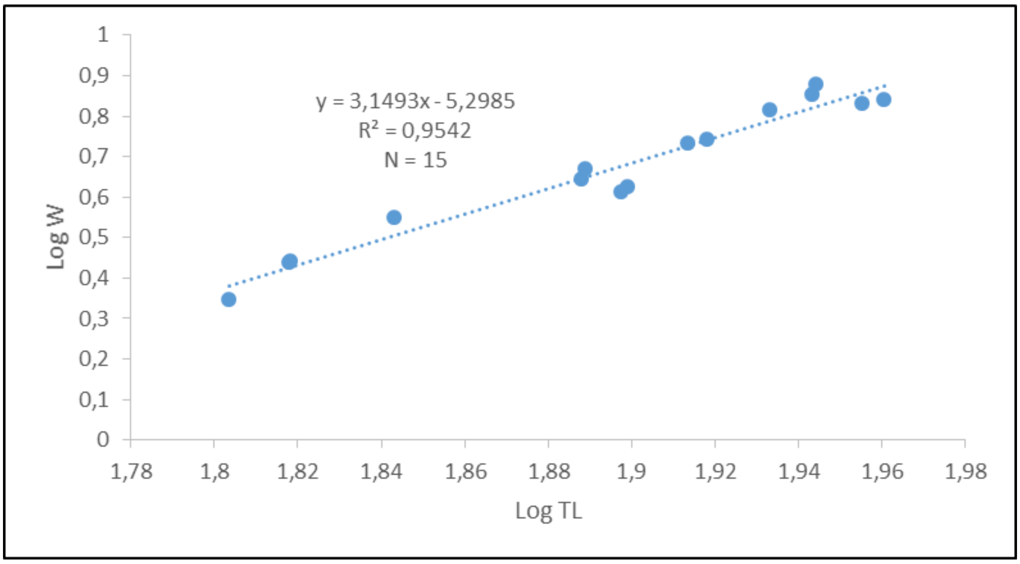

Figure 5. Length-weight relationship of *Betta* cf. *raja* North Sumatra population.

The length-weight relationship of *B.* cf. *raja* shows a value of b>3 (negative allometric) for stations 1 and 2 and a value of b>3 (positive allometric) for Station 3. According to Mulfizar et al (2012), the value of b depends on physiological conditions, environmental factors such as temperature, pH, and geographical location. According to Isa et al (2010), several factors that affect the growth of fish are habitat, fish activity, eating habits, and season. Related to the environmental conditions of *B. raja*, the Jambi area has peat swamps, Riau has tributaries and North Sumatra has clearer and rocky areas where the fish live. Based on observations in the Jambi region, *B. raja* likes slightly darker waters, with a reddish brown color. In these conditions, the fish found were larger than those from clearer water.

The growth rate of wild betta is influenced by water color, being faster in dark water (Rahmawati et al 2016). *B. raja* prefers habitats with dark water color presumably to make it easier to forage, avoiding predators at the same time.

T test results. Further tests were carried out to determine the level of confidence in the data of the results of the length-weight relationship for the value of b (Table 4).

Table 4

The results of the t test analysis

Station	*Population*	*Value of a*	*Value of b*	*R^2*	*t test Sig.*	*Category*
1	15	1.85	2.78	0.79	0.56	NA
2	15	1.75	2.70	0.76	0.56	NA
3	15	3.15	5.30	0.95	0.56	PA

Note: R^2 - coefficient of determination; NA - negative allometry; PA - positive allometry.

The t test results show a value of 0.56. If the significance value of t>0.01, then H_0 is accepted (there is no significant influence from one independent variable on the dependent variable). The environment and morphological characteristics of different fish species may affect the results of length-weight relationship analysis. According to Ndiaye et al (2015), when the length-weight relationship of fish has a high value, the increase in length is followed by weight gain. Mehanna & Farouk (2021) state that some aspects that affect the length-weight relationship of fish are habitat, physiological species, growth stage, sex, gonad maturity level, food, and health.

Conclusions. Based on the results of data analysis, there are many differences in morphological characters, both morphometric and meristic, between *Betta* cf. *raja* populations found. This is due to physiological adaptations in each natural habitat. Differences in morphological characteristics indicate different species. However, to determine this taxonomic difference, molecular research must be carried out, namely analysis of genetic variation and DNA barcoding to validate morphological observations that are doubtful.

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Conflict of Interest. The authors declare that there is no conflict of interest.

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