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Research article

Sex differentiation via the relationship between some morphological measurements of *Mystus albolineatus* caught from Cai Rang - Can Tho and Long Phu - Soc Trang

Phi Ngoc Nguyen, Thu Thi Anh Phan, Thoai Kim Nguyen, Vuong Van Ly, Thao Thi Thu Thai, Quang Minh Dinh*

Department of Biology, School of Education, Can Tho University, Xuan Khanh ward, Ninh Kieu district, Can Tho 900000, Vietnam

Abstract

This study supplements the gender determination methodology based on the relationship of some morphological indicators in *Mystus albolineatus*. A total of 229 specimens were caught by trawl nets at Cai Rang – Can Tho and Long Phu – Soc Trang in four months from April to July 2023. After collection, the samples were transferred to the laboratory to determine the total length (TL), standard length (SL), eye diameter (ED), eye distance (DE), body height (BH), head length (HL), and mouth diameter (MD) before surgery to determine the sex based on the gonad. The results showed that the TL-SL regression relationship could be used to determine the sex of this fish. Besides, TL-BH and TL-MD growth relations were relatively balanced in females versus males. In addition, based on SL, DE, and MD, it was also possible to determine whether the sampling time was the wet or dry seasons. These findings form the basis for the initial study of the biological characteristics of this fish in the Mekong Delta.

Keywords: *Gender differentiation, Mekong Delta, Morphology, Mystus albolineatus.*

***Corresponding author:** Quang Minh Dinh, Department of Biology, School of Education, Can Tho University, Xuan Khanh ward, Ninh Kieu district, Can Tho 900000, Vietnam. Email: dmquang@ctu.edu.vn

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INTRODUCTION

Mystus albolineatus is one species of the Bagridae family with commercial importance in the Mekong Delta. Long, fatty fins and a silvery gray body with a thin white stripe running down the middle are characteristics of this fish (Tran et al., 2013). This fish's maximum body length was about 35 cm (Baird et al., 1999). *Mystus albolineatus* typically inhabits regions with both flowing water (rivers) and standing water (lakes). The mating season for this species lasts from July to September in the United States (Breder and Rosen, 1966). The biology of this fish is currently little understood in both Vietnam and the rest of the world. Numerous authors are interested in and can identify morphological traits to approach fish biology (Nguyen and Dinh, 2021; Phan et al., 2021; Nguyen and Dinh, 2023). They are providing a foundation for more research on the biological traits of this fish from there. On the other hand, the Mekong Delta is currently seeing heavy levels of aquaculture, and several species face the threat of overexploitation (Trinh and Tran, 2012).

The fish sex ratio is affected by several variables, including salinity and habitat temperature, according to Vu (2000). This ratio also varies by species, according to Tran et al. (2013), depending on their developmental stage and reproductive habits. Fish sex discrimination is a significant topic in the biology and ecology of fish. In the *Periophthalmodon septemradiatus* species, males have more colorful and longer dorsal fins than females, making it simple to discern between the sexes based on exterior morphological color (Dinh et al., 2018). However, the secondary genital spine characteristics of several species of the Bagridae family, including the striped pincer *Mystus mysticetus* (Nguyen and Duong, 2020), are used to determine the sex of the animal. Females have pink, oval-shaped missing genitals, while the male has long genitalia and a pointed genital spine that is darker red than the female. However, the genital spines of this fish are tiny, so it is difficult to see with the naked eye. Therefore, this study aimed to provide a new way of sex determination in this fish without killing fish by measuring and determining the relationship of morphological features between them. In the world and Vietnam, there have been several studies to implement this method in many different fish species, such as *Zacco koreanus* (Kim et al., 2008), *Heterotis niloticus* (Obi, 2010), *Glossogobius sparsipapillus* (Dinh et al., 2021a), *Glossogobius giuris* (Dinh et al., 2021b), *Ellochelon vaigiensis* (Nguyen et al., 2022), *Mystus mysticetus* (Phan et al., 2022), *Caragobius urolepis* (Nguyen et al., 2023). These studies show that this method can be applied to determine sex in *Mystus albolineatus*. Limiting the killing of fish when conducting research contributes to better protection of this fish species. Besides, this study supports the validity of this method for determining sex and lays the groundwork for future investigations into the biology of this fish.

MATERIALS AND METHODS

Sampling and analysis

During a period of 4 months from April to July 2023, 229 (121 females and 108 males) samples of *M. albolineatus* fish were collected periodically once a month at two sites, including Cai Rang - Can Tho (CRCT) and Long Phu - Soc Trang (LPST) (Figure 1). Fish samples were collected continuously

for 48 hours using fishermen's 1.5 cm bottom net. Individuals were randomly collected with various sizes. Before being transported to the Animal Laboratory, Department of Biology Teacher Education, Can Tho University, the samples were stored in a 4% formalin solution. Then, fish samples were identified based on the description of Tran et al. (2013). The morphometric features include total length (TL), standard length (SL), eye diameter (ED), eye distance (DE), body height (BH), head length (HL), and mouth diameter (MD) (Figure 2).

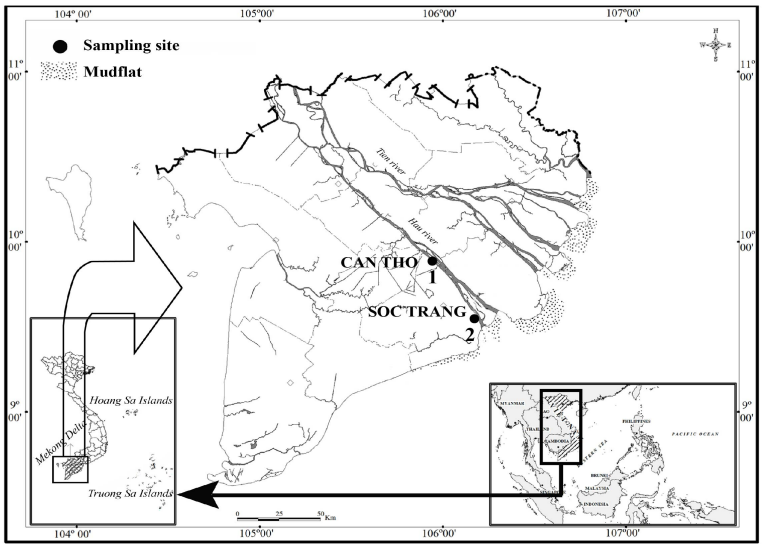

Figure 1 Map of sampling locations (●: Sampling location; 1: Cai Rang - Can Tho; 2: Long Phu - Soc Trang; this figure was modified from Dinh (2018))

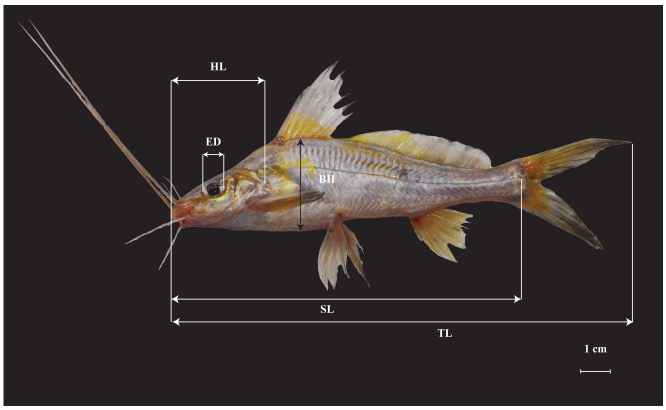

Figure 2 Measuring parameters of *M. albolineatus* (TL: total length; SL: standard length; ED: eye diameter; DE: eye distance; BH: body height; HL: head length; Source: Nguyen and Dinh (2023)).

Statistical Analysis

According to Minos et al. (2008), the relationship between total length (TL) and other measurement parameters of fish was determined based on the formula Y=a x TLb. Y was the morphological size (SL, ED, DE, BH, HL, and MD), and a and b were the regression coefficients (a was the intercept, and b was the slope). Morphological variables were classified into positive growth (A+) if b >1, negative growth (A-) if <1, and isometry (I) if b ≈1. This test also confirmed if b varied by sex, season, and sampling location. The data in this study were analyzed using SPSS v21 with a significance level of 5%.

RESULTS

The data analyzed of *Mystus albolineatus* over four months in LPST and CRCT showed different morphological characteristics depending on gender, season, and specimen location. In addition, the determination value of these regressions was high ($r^2 > 0.666$ for all cases in Table 1 - 3), showing a positive relationship between TL and SL, TL, DE, ED, BH, HL, and MD. In female fish, the regression ratio between TL and SL was placed in group A+ (Table 1). This shows that in females, the development of normal length was faster than the total length of the fish. Conversely, males had a relatively slow growth of normal length compared to females due to regressive relationships in group A- (Table 1). There were similar growth patterns for the rest of the relationship, including TL-ED, TL-DE, TL-BH, TL-HL, and TL-MD in both males and females. Specifically, TL-ED, DE, and HL in both sexes tended to develop more slowly than the total length (A-); only TL-BH and MD in males and females developed relatively fast (A+). From these results, different growth patterns of males and females could be seen based on the evolution of SL.

Table 1 Regression relationship of the total length (TL) to standard lengths (SL), eye diameter (ED), eye distance (DE), body height (BH), head length (HL), and mouth diameter (MD) of *Mystus albolineatus* regarding sex

Index	*B*	SE	a	SE	n	r^2	Growth pattern	t_s	P
Female									
SL	1.03	0.04	0.82	0.07	121	0.88	A+	29.37	0.00
ED	0.87	0.07	0.06	0.01	121	0.61	A-	13.45	0.00
DE	0.94	0.06	0.10	0.02	121	0.65	A-	14.73	0.00
BH	1.22	0.07	0.12	0.02	121	0.74	A+	18.21	0.00
HL	0.92	0.04	0.29	0.03	121	0.80	A-	22.00	0.00
MD	1.06	0.07	0.08	0.01	121	0.67	A+	15.65	0.00
Male									
SL	1.00	0.03	0.88	0.07	108	0.89	A-	29.26	0.00
ED	0.82	0.09	0.07	0.01	108	0.47	A-	9.67	0.00
DE	0.97	0.06	0.10	0.01	108	0.73	A-	16.71	0.00
BH	1.19	0.08	0.12	0.02	108	0.67	A+	14.65	0.00
HL	0.96	0.04	0.27	0.03	108	0.84	A-	23.98	0.00
MD	1.01	0.06	0.09	0.01	108	0.76	A+	18.31	0.00

Furthermore, the analysis results of the relationship between seasonal morphology indicators (dry and wet) showed that *Mystus albolineatus* has significant differences, as shown in Table 2. The regression of TL-SL exhibited a difference between the two seasons. Specifically, this relationship was classified as A- in the dry season and A+ in the wet season. This suggested that the standard length in the wet season had grown more substantial than in the dry season. On the other hand, the results of the TL-DE relationship in the dry season showed that the eye gap grew faster than the total length (A+, Table 2) and, in the wet season, tended to grow more slowly than the overall length(A-, Table 2). In addition, the regression ratio of TL-MD was classified as A+ in the dry season and A- in the wet season, which suggests that the growth of the mouth diameter was relatively slow in the wet season (Table 2).

Furthermore, the analysis results of the relationship between seasonal morphology indicators (dry and wet) showed that *Mystus albolineatus* has significant differences, as shown in Table 2. The regression of TL-SL exhibited a difference between the two seasons. Specifically, this relationship was classified as A- in the dry season and A+ in the wet season. This suggested that the standard length in the wet season had grown more substantial than in the dry season. On the other hand, the results of the TL-DE relationship in the dry season showed that the eye gap grew faster than the total length (A+, Table 2) and, in the wet season, tended to grow more slowly than the overall length(A-, Table 2). In addition, the regression ratio of TL-MD was classified as A+ in the dry season and A- in the wet season, which suggests that the growth of the mouth diameter was relatively slow in the wet season (Table 2).

Table 2 Regression relationship of the total length (TL) to standard lengths (SL), eye diameter (ED), eye distance (DE), body height (BH), head length (HL), and mouth diameter (MD) of *Mystus albolineatus* regarding season

Index	b	SE	a	SE	n	r^2	Growth pattern	t_s	P
Dry season									
SL	1.00	0.03	0.88	0.07	118	0.88	A-	29.35	0.00
ED	0.97	0.08	0.05	0.01	118	0.57	A-	12.46	0.00
DE	1.07	0.07	0.08	0.01	118	0.70	A+	16.15	0.00
BH	1.01	0.08	0.18	0.03	118	0.59	A+	12.82	0.00
HL	0.93	0.04	0.28	0.03	118	0.81	A-	22.24	0.00
MD	1.14	0.06	0.07	0.01	118	0.75	A+	18.66	0.00
Wet season									
SL	1.02	0.04	0.84	0.08	111	0.86	A+	26.08	0.00
ED	0.83	0.07	0.06	0.01	111	0.57	A-	12.01	0.00
DE	0.84	0.06	0.13	0.02	111	0.65	A-	14.02	0.00
BH	1.34	0.07	0.09	0.01	111	0.77	A+	19.14	0.00
HL	0.96	0.04	0.27	0.03	111	0.82	A-	22.26	0.00
MD	0.93	0.07	0.11	0.02	111	0.63	A-	13.68	0.00

The regression relationship of morphological values varied not only by sex and season and sampling location. The analysis of morphological parameters in the CRCT area showed that the regression relationship between TL-DE and TL-MD was classified into group A+, indicating that the dominant DE and MD developed faster than TL (Table 3). In contrast, these relationships represented a regression outcome of A- at LPST. The results showed that the ratio of DE and MD in CRCT was higher than in LPST. In CRCT, the area had rivers, and the current was tight, so the eye's distance and mouth width were larger to observe and get food, while in LPST, the flow was more static.

Table 3 Regression relationship of total length (TL) with standard length (SL), eyes diameter (ED), eyes distance (DE), body height (BH), head length (HL), and mouth width (MD) of *Mystus albolineatus* regarding sampling site

Index	b	SE	a	SE	n	r^2	Growth pattern	t_s	P
Cai Rang, Can Tho									
SL	1.03	0.04	0.82	0.07	110	0.88	A+	27.86	0.00
ED	0.95	0.09	0.05	0.01	110	0.51	A-	10.65	0.00
DE	1.15	0.07	0.06	0.01	110	0.70	A+	15.75	0.00
BH	1.06	0.09	0.16	0.03	110	0.59	A+	12.47	0.00
HL	0.96	0.05	0.27	0.03	110	0.80	A-	20.40	0.00
MD	1.15	0.07	0.06	0.01	110	0.70	A+	15.77	0.00
Long Phu, Soc Trang									
SL	1.00	0.03	0.87	0.07	119	0.89	A+	30.36	0.00
ED	0.77	0.06	0.07	0.01	119	0.57	A-	12.42	0.00
DE	0.80	0.05	0.14	0.02	119	0.71	A-	16.75	0.00
BH	1.32	0.07	0.09	0.01	119	0.77	A+	19.98	0.00
HL	0.92	0.04	0.29	0.03	119	0.84	A-	24.95	0.00
MD	0.95	0.05	0.10	0.01	119	0.74	A-	18.17	0.00

DISCUSSION

Using the TL-SL relationship in the study could determine the sex of fish by simply determining the length of TL and SL without killing the fish. This was important in the study of the biology of species. The TL-SL rates, helping to determine a size for catching full-grown fishes, had high productivity for the next generation of fish since the considered norms did not affect fish during collecting and analyzing samples. Due to the behavior and reproductive process, during development, there could be differences in growth rate among morphological characteristics of fish (Kim et al., 2008). In some species, this variation depended on the sex of the fish; for example, in *Glossogobius sparsipapillus* (Dinh et al., 2021a), males were more elongated than females, indicating that body length tends to grow stronger than the body height of the fish. In addition, in some other species of fish that have been studied, TL-SL relationships are also used to determine the sex of fish, such as *Mystus mysticetus* (Phan et al., 2022); *Ellochelon vaigiensis* (Nguyen et al., 2022). Besides, some other fish species can determine sex based on morphological relationships, such as *Zacco koreanus* (Kim et al., 2008), *Heterotis niloticus* (Obi, 2010), *Glossogobius giuris* (Dinh et al., 2021b), and *Caragobius urolepis* (Nguyen et al., 2023). In a previous study of *Mystus mystycetus*, Nguyen and Duong (2020) determined the sex of the fish at the right time of the spawning season, so sampling would be straightforward to decide on due to the differences in the appearance of the males and the females for the high body (BH) and head depth (HD),... but for those periods of reproductive sampling, it is difficult to distinguish, so using the regression relationship between TL-SL to determine the sex of the fish is one of the methods that should be applied.

The change of TL-SL morphology of the species was also influenced by seasons, typically the dry and wet seasons. In previous studies, not only the BH morphology index was affected by season, but also other indices, typically DE and MD. This difference might be due to the significant difference in rainfall and river water volume in the Mekong Delta region. During the rainy season, considerable rainfall could change the system of poorly adapted organisms, thereby changing the composition of fish food. On the other hand, in the wet

season, the amount of water in the Mekong River was quite large, and the flow became stronger. That also affects the morphological characteristics of the fish. However, this change was not so evident in some species. In the same distribution area as *Mystus albolineatus* in this study, *Mystus mysticetus* (Phan et al., 2022) also showed a significant difference in the TL-SL morphological relationship. This difference was not observed for *Glossogobius sparsipapillus* (Dinh et al., 2021a).

In addition, different populations could also partly affect the morphological development of fish. At each site, typical environmental factors such as water volume, flow rate, salinity, etc. could affect fish. The two research sites in this study were relatively far apart and had different environmental characteristics. In CRCT, there was fresh water all year round and stable flow, whereas, in LPST, there was saltwater intrusion and tidal influence. For example, the analysis of the relationship between the morphological parameters of *M. mysticetus* showed a change in morphology between the two sites, Cai Rang - Can Tho and Long Phu - Soc Trang (Phan et al., 2022). A study of *Glossogobius giuris* (Dinh et al., 2021b) also displayed similar variation between these sites

CONCLUSIONS

The results of the analysis of morphological parameters of *Mystus albolineatus* played a role in sex determination. Based on the relationship between the total length and the standard length, it is possible to determine the sex of this fish. This has important implications for the area's research and management of this fish. Moreover, the regression relationships of morphological parameters also help to determine the fishing time in the dry or wet seasons (TL-SL, TL-DE, and TL-MD) and the fishing area (TL-DE and TL-MD). This study provides a new way of sex determination for this fish and the basis for further research.

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AUTHOR CONTRIBUTIONS

Nguyen Ngoc Phi; Investigation, methodology, formal analysis, manuscript preparation
Phan Thi Anh Thu; Investigation, methodology, formal analysis, manuscript preparation
Nguyen Kim Thoai; Investigation, methodology, formal analysis, manuscript preparation
Ly Van Vuong; Investigation, methodology, formal analysis, manuscript preparation
Thai Thi Thu Thao; Investigation, methodology, formal analysis, manuscript preparation
Quang Minh Dinh; Investigation, methodology, formal analysis, manuscript preparation

CONFLICT OF INTEREST

We have no conflict of interest.

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