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**Research article**

Sex discrimination based on morphological traits in *Mystus mysticetus* Roberts, 1992

Thu Quynh Phan, Anh Ngoc Tran, Lam Thi Thao Vo, Quang Minh Dinh*

Department of Biology, School of Education, Can Tho University, Can Tho 900000, Vietnam

Abstract

This study supplemented the way of sex determination of fish based on the regression relationship of some morphological indicators of *Mystus mysticetus*. A total of 425 fish specimens were collected by trawl nets at Cai Rang - Can Tho and Long Phu - Soc Trang from January to July 2022. After collection, the fish were transferred to the laboratory to determine their total length (TL), standard length (SL), eye diameter (ED), eye distance (DE), body height (BD), head length (HL), and mouth width (MD) before anatomy for sex determination based on gonads. The regression analysis results of TL-SL, TL-ED, TL-DE, TL-BD, TL-HL, and TL-MD showed that SL, DE, and MD could be used to determine the sex of this fish species because SL and DE were dominant in growth in males, and MD was prevalent in females. In addition, SL could be used to determine the sampling time and HL to determine the distribution environment.

Keywords: Fish body height, Fish head length, Mekong Delta, *Mystus mysticetus*

Corresponding author: Quang Minh Dinh, Department of Biology, School of Education, Can Tho University, Can Tho 900000, Vietnam. Email: dmquang@ctu.edu.vn

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INTRODUCTION

Mystus mysticetus Roberts, 1992 is one of the common species of the Bagridae family. They are commonly distributed along the Chao Phraya and Mekong river basins (Kottelat et al., 1993; Vidthayanon et al., 1997; Dinh et al., 2011; Le et al., 2018). The fish has an elongated body, the front part is round, and the back part is flattened. When viewed horizontally, the fish's snout is pointed, and when viewed from above, the fish's snout is oval. The mouth adjacent to the bottom has a width equal to the distance between the eyes. The eyes are large, and the distance between the eyes is almost flat. They have four pairs of long antennae. The gill membrane is developed but not attached to the gill waist (Tran et al., 2013). The characteristic morphology of *M. mysticetus* is the appearance of three dark longitudinal stripes running vertically below the lateral line, the back of the body has a dark black spot on the operculum (Kottelat, 2001; Tran et al., 2013). This fish has a close distribution environment with other pelagic species, such as *M. multiradiatus*. They move into wetlands during the wet season and return to the river in November and December in the lower Mekong (Breder and Rosen, 1966). This fish has morphological diversity according to habitat and sex between regions in the Mekong Delta (Tran and Yen, 2020). According to Vo et al. (2022), *M. mysticetus* spawns mainly from January to July in the Hau River of the Mekong Delta.

Sex discrimination plays a vital role in the ecology and biology of fish species, especially in the Mekong Delta, a large delta in Vietnam and Southeast Asia with an ichthyofauna of ~200 different fish species, ranging from brackish to freshwater (Tran et al., 2013). Many of them must be killed and their gonads removed to identify their sex, which is a time-consuming activity (Dinh and Le, 2017; Dinh et al., 2021a; Ho et al., 2021; Dinh et al., 2022a; Dinh et al., 2022b). Therefore, it is necessary to find an alternative way of doing it without killing fish and in a shorter time. Some authors in recent times have used the relationship between total length (*TL*) and standard length (*SL*), head length (*HL*), and body depth (*BD*) to identify the sex of *Ellochelon vaigiensis* fish (Nguyen et al., 2022). Furthermore, the *TL-SL*, *TL-HL*, and *TL-BD* regressions help estimate fish sampling time (Dinh et al., 2021b; Nguyen et al., 2022). No studies have used the relationship of the above morphological indicators in *Mystus mysticetus* to distinguish sex. Therefore, we conducted the study at two locations in Cai Rang - Can Tho and Long Phu - Soc Trang to contribute to the creation of an alternative way of identifying the sex of this fish specimens and some other with similar characteristics.

MATERIALS AND METHODS

Study sites and fish collection

This study was conducted from January 2022 to July 2022 in Cai Rang - Can Tho (the freshwater region) and Long Phu - Soc Trang (the brackish water region in the dry season because of salinization) (Figure 1). Samples of *M. mysticetus* of different sizes were collected monthly from these sites using trawl nets by local fishermen. As we used dead fish, no animal ethics approval was required. The fish samples obtained were identified based on morphological characteristics from the description of Tran et al. (2013). After that, they were

transported to Can Tho University for research in the laboratory. Here, the sex of the fish samples was determined based on the characteristics of the gonads as described by Vo et al. (2022), and fish morphometrics such as total length (TL), standard length (SL), eye diameter (ED), eye distance (DE), body depth (BD), head length (HL) and mouth width (MD). The experiment was conducted on a commercial pig farm in the.

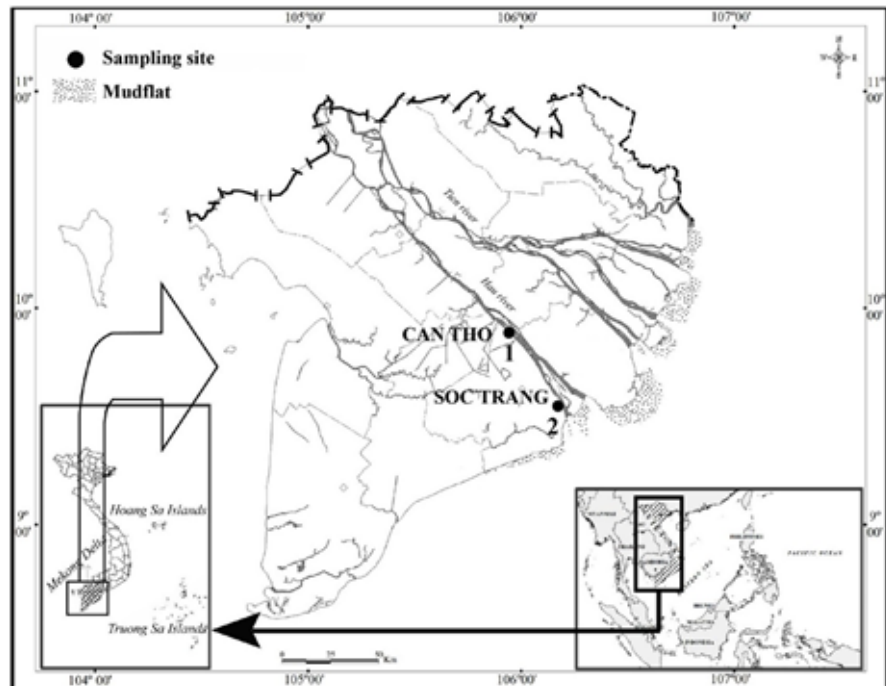


Figure 1 Map of sampling locations (●: Sampling location; 1: Cai Rang - Can Tho; 2: Long Phu - Soc Trang; this figure was modified from Dinh (2018)).

Data analysis

The relationship between fish total length (TL) and some morphometrics (Y) was determined based on the formula $\text{Log}_{10}Y = a \times \log_{10}(TL) + b$; where Y was one of this morphometrics, e.g., the standard length (SL), eye diameter (ED), eye distance (DE), body depth (BD), head length (HL), and mouth width (MD) (Minos et al., 2008). The letters a and b are the regression coefficients (a is the intercept, and b is the SL lope). The t-test was used to compare b with the value one as the basis for determining the growth pattern of each variable Y ($A-$, I , and $A+$). If $b > 1$, then each variable Y respectively belongs to the group of positive unequal growth ($A+$) and vice versa, the group of negative unequal growth ($A-$). The corresponding variable Y belongs to the growth group with the same sign (I) when $b \approx 1$. Besides, the t-test was also used to test the change of coefficient b by gender and season. The data in this study were processed and analyzed using SPSS v21 software with a significance level of 5%.

RESULTS

After seven months of sampling, a total of 425 (158 females and 267 males) *M. mysticetus* were collected to determine morphological changes. The regression analysis results of morphological values (*TL-SL*, *TL-ED*, *TL-DE*, *TL-BD*, *TL-HL*, and *TL-MD*) showed a shift by sex, season, and location. Differences were found in the regression of *TL-SL* between females and males because this relationship was classified into two groups. In females, *TL-SL* regression was classified into group A-, showing that the increase of *SL* in females was relatively slower than *TL*. In contrast, in males, *TL-SL* was classified into group A+ (Table 1). This result showed that the development process of *SL* in males was relatively faster than *TL*, indicating that the development of *SL* was different between males and females in this fish species (Table 1). The *TL-DE* regression of males and females was classified into two groups. Specifically, this relationship was classified as A- in females and I in males (Table 1). This analysis showed that, in females, the development of *DE* was relatively slower than the *TL*. Meanwhile, the development of *DE* and *TL* of the males were similar. Thereby, it was seen that the increase in *DE* between males and females was not equivalent (Table 1). Considering the regression relationship between *TL* and *MD*, this fish was classified into two groups. Specifically, female analysis results were classified into group A+ (Table 1). This result showed that the development of *MD* in females was relatively faster than *TL*. For this regression relationship, males were arranged into group A- (Table 1). The increase of *MD* was relatively slower than the *TL* in males. It has been shown that there is a difference in the development of *MD* between males and females in this species (Table 1). The *TL-ED* regression of females and males was classified into group A- (Table 1), suggesting that the increase of *ED* was relatively slower than the *TL* in this fish. Similarly, *TL-BD* regression of females and males was classified into group A-, showing that the stem height developed relatively slowly compared to the *TL* (Table 1). Thereby, it was revealed that the growth of *BD* between males and females in this species was relatively uniform (Table 1). The regression relationship of *TL* and *HL* in females and males was classified into group A+. As a result of this analysis, it was found that the growth of *HL* was relatively faster than the *TL* of fish. Thereby, the development of *HL* in this fish was similar between females and males (Table 1). The *TL-SL*, *TL-DE*, and *TL-MD* relationships have shown that sex determination in this fish can be based on the development process of total length, standard length, eye distance, and mouth width.

Table 1 Regression relationship of total length (*TL*) with standard length (*SL*), eye diameter (*ED*), eye distance (*DE*), body height (*BD*), head length (*HL*) and mouth width (*MD*) of *Mystus mysticetus* by sex.

Index	Growth pattern	<i>b</i>	<i>SEb</i>	<i>a</i>	<i>SEa</i>	<i>n</i>	<i>F</i>	<i>p</i>	<i>ts</i>	<i>p</i>
Female										
<i>SL</i>	A-	9.920	0.206	-15.281	0.517	158	2314.710	0.00	43.30	0.00
<i>ED</i>	A-	0.411	0.029	-0.489	0.073	158	198.431	0.00	-20.3	0.00
<i>DE</i>	A-	0.818	0.088	-0.945	0.220	158	86.844	0.00	-2.07	0.04
<i>BD</i>	A-	0.255	0.177	1.521	0.442	158	2.090	0.00	-4.21	0.00
<i>HL</i>	A+	1.989	0.135	-2.152	0.339	158	216.637	0.00	7.33	0.00
<i>MD</i>	A+	1.783	0.248	-2.640	0.622	158	51.642	0.00	3.16	0.00
Male										
<i>SL</i>	A+	9.330	0.225	-13.634	0.544	267	1723.882	0.00	37.02	0.00
<i>ED</i>	A-	0.373	0.043	-0.370	0.103	267	76.013	0.00	-14.58	0.00
<i>DE</i>	I	0.918	0.090	-1.281	0.219	267	103.519	0.00	-0.91	0.36
<i>BD</i>	A-	0.712	0.109	0.120	0.264	267	42.750	0.00	-2.64	0.01
<i>HL</i>	A+	2.108	0.142	-2.609	0.343	267	220.708	0.00	7.80	0.00
<i>MD</i>	A-	0.469	0.216	0.791	0.523	267	4.706	0.00	-2.46	0.01

The regression relationship of morphological values varied not only by sex but also by season. The relationship between the morphological indices of *TL* and *SL* in fish in the dry and wet seasons was classified into two groups. Specifically, the analysis results in the dry season showed that the *SL* of the *M. mysticetus* grew relatively slower than the *TL* (A-, Table 2). While in the wet season, *SL* growth and *TL* of fish were similar in the relationship of indices between *TL* and *SL* (I, Table 2). Thereby, it was found that the *SL* in the wet season grew faster than in the dry season in this fish (Table 2). The regression relationship of *TL-BD* was included in group A- in both seasons (Table 2). This result showed that stem height growth was relatively *SLow* compared to the *TL* and did not change with the seasons. Similarly, considering the correlation of *TL* and *HL* in the dry season, it has been shown that the *TL-HL* correlation results in striped carp in the dry season and the wet season have been classified into group A- (Table 2). Thereby, it was shown that the *TL* and *SL* in the dry and wet seasons had similar growth (Table 2).

Table 2 Regression relationship of total length (*TL*) with standard length (*SL*), body height (*BD*), and head length (*HL*) of *Mystus mysticetus* by season.

Index	Growth pattern	<i>b</i>	<i>SEb</i>	<i>a</i>	<i>SEa</i>	<i>n</i>	<i>p</i>	<i>ts</i>	<i>p</i>	
Dry season										
<i>SL</i>	A-	0.965	0.013	0.846	0.028	303	0.00	-2.69	0.01	
<i>BD</i>	A-	0.380	0.072	0.782	0.139	303	0.00	-8.61	0.00	
<i>HL</i>	A-	0.882	0.048	0.301	0.036	303	0.00	-2.46	0.02	
Wet season										
<i>SL</i>	I	1.017	0.020	0.746	0.037	122	0.00	0.85	0.40	
<i>BD</i>	A-	0.377	0.086	0.797	0.173	122	0.00	-7.24	0.00	
<i>HL</i>	A-	0.797	0.062	0.372	0.058	122	0.00	-3.27	0.01	

In addition, the regression relationship of *TL-SL* was different between the two sampling sites. Specifically, the relationship between the total length and standard length of fish in Cai Rang - Can Tho has been classified into group A- (Table 3), showing that in Cai Rang - Can Tho, the growth of the *SL* was relatively slower than the *TL*. Meanwhile, in Long Phu - Soc Trang, *TL-SL* regression of fish was included in group I (Table 3), suggesting that the development of *SL* and *TL* of fish in Long Phu - Soc Trang were similar. For the regression relationship *TL-BD* of *M. mysticetus* in Cai Rang - Can Tho and Long Phu - Soc Trang were included in group A- (Table 3). The analysis results showed that the growth of the *BD* of the fish at the two sampling sites was relatively slower compared to the *TL*. The regression relationship between *TL* and *HL* was classified into two different groups. Specifically, this relationship fell into I type at Cai Rang - Can Tho and A- one at Long Phu - Soc Trang (Table 3), showing that the *HL* at Cai Rang - Can Tho was longer than at Long Phu - Soc Trang.

Table 3 Regression relationship of total length (*TL*) with standard length (*SL*), body height (*BD*), and head length (*HL*) of *M. mysticetus* by sampling sites.

Index	Growth pattern	<i>b</i>	<i>SEb</i>	<i>A</i>	<i>SEa</i>	<i>n</i>	<i>p</i>	<i>ts</i>	<i>P</i>
Cai Rang									
<i>SL</i>	A-	0.956	0.013	0.868	0.029	215	0.00	-3.38	0.01
<i>BD</i>	A-	0.491	0.063	0.594	0.094	215	0.00	-8.08	0.00
<i>HL</i>	I	0.912	0.052	0.276	0.036	215	0.00	-1.69	0.09
Long Phu									
<i>SL</i>	I	1.021	0.018	0.738	0.033	210	0.00	1.17	0.25
<i>BD</i>	A-	0.252	0.096	1.075	0.253	210	0.00	-7.79	0.00
<i>HL</i>	A-	0.797	0.057	0.374	0.052	210	0.00	-3.56	0.00

DISCUSSION

The results of this study showed that morphological relationships could be used to determine the sex of fish. This is because males and females displayed different life activities, e.g., the female was responsible for laying and protecting the eggs, while the male was responsible for building the nest, which caused some differences in some morphological indicators of the fish. However, the relationships between morphological indicators based on which sex can be determined are different depending on the fish species. Thus, our study found that the *TL-SL* regression relationship is relevant for sex determination in *Glossogobius sparsipapillus* in the Mekong Delta (Dinh et al., 2021b), like in *Pagrus pagrus* in Greece (Minos et al., 2008). Meanwhile, in some other fish species, sex determination was still can be based on other relationships such as *TL-HL* in *Glossogobius giuris* (Dinh et al., 2021c), or *TL-BD* in *Ellochelon vaigiensis* (Nguyen et al., 2022). The *TL-SL* is also used in distinguishing fish sex was also found in *Heterotis niloticus* (Obi, 2010), *Zacco koreanus* (Kim et al., 2008), suggesting that the regression relationships of *TL* and *SL/HL/BD* could be played as an alternative method in fish sex determining.

When environmental conditions change, the fish values also change in response to that change. The change in the TL - SL regression relationship in this fish suggested that environmental conditions influenced the *M. mysticetus*. This result shows that the ratio of SL to TL in the wet season was larger than in the dry season, so the tail length of fish in the wet season was smaller. It is possible that during the wet season, the water in the river flows strongly, so the fish reduce the size of their tail to limit water pressure. The change in the regression relationship of seasonal morphological parameters has been studied in many other fish species such as *Glossogobius sparsipapillus* (Dinh et al., 2021b), or *Ellochelon vaigiensis* (Nguyen et al., 2022).

From the analysis of the relationship between the morphological indicators of *M. mysticetus*, it has been shown that there are morphological changes between the two locations Cai Rang - Can Tho and Long Phu - Soc Trang. The two regression relationships in this affected fish species were TL - SL and TL - HL . Meanwhile, there was no difference between the two study sites in TL - BD . This change between the two study sites may be due to the change in salinity of the Long Phu - Soc Trang area, while Cai Rang - Can Tho had stable fresh water all year round. A study on the *Glossogobius giuris* (Dinh et al., 2021c) showed similar variability between these sites. These results indicated that when studying the changes in fish between different ecological regions in terms of salinity, TL , SL , and HL were essential indicators that needed to be monitored.

CONCLUSIONS

The analysis results showed that TL , SL , DE , and MD could be used to identify the sex of *M. mysticetus* fish. It was also possible to use SL to determine the best timing for fishing this species because SL is the largest during the wet season. In addition, HL could be used to determine the distribution environment of this fish because it is longer in freshwater specimens, like in those from Cai Rang - Can Tho.

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AUTHOR CONTRIBUTIONS

Thu Huynh Phan; Investigation, methodology, formal analysis, manuscript preparation

Anh Ngoc Tran; Investigation, methodology, formal analysis, manuscript preparation

Lam Thi Thao Vo; Investigation, methodology, formal analysis, manuscript preparation

Quang Minh Dinh; Conceptualization and design the experiment, investigation, supervision, editing and finalization

CONFLICT OF INTEREST

We have no conflict of interest.

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