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RESEARCH ARTICLE
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Morphoanatomic Characteristics of Two-Spot Catfish (*Mystus nigriceps*) Caught in the Klawing River, Indonesia

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ABSTRACT

The population of Two-spot Catfish *Mystus nigriceps* had been detected decreased as a result of overfishing. This highlighted the necessity of finding ways protecting the fish in order to prevent its extinction. This study set out to ascertain the morphoanatomical characteristics of the fish's reproductive process. Random sampling method was employed in this study, and samples of fish were obtained from three different stations namely Jetis, Bokol, and Kedungbenda. The samples were then subjected to morphoanatomical indices, such as the gonado-somatic GSI, hepato-somatic HSI, and viscero-somatic VSI indexes. In addition, sex ratio measurements were also measured in this study. The results indicated that GSI of the male and female fish at each station ranged from 22.66-29.09% and from 26.03-36.78%, according to the data. The VSI of fish varied from 13.83-17.76%; 15.36-17.17%, and the HSI of male and female fish ranged from 11.81-15.18%; 12.69-16.56%. The results also show that the sex ratio of two-spots catfish found in the Klawing River shows a significant balance at each station, namely 1:1, where male two-spots catfish are 50% and females are 50%. This result indicates that the reproductive process of the two-spots catfish is in good condition. Water quality parameters in accordance with standards. It has been found in this study that two-point catfish meet the conditions for reproduction since they have reached the gonadal maturity phase. One of the factors that significantly affects the maturity of the ovaries and testes is the living habitat of the fish. Based on the results of morphoanatomical observations, it can be concluded that the condition of Two-Spot Catfish (*Mystus nigriceps*) obtained from the capture in the Klawing River is in a mature gonadal condition and ready for spawning.

Keywords: Morphoanatomical Index, Klawing Rivers, Gonadal Maturity, Mystus nigriceps.

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INTRODUCTION

Rivers is a source of water for the community, which is used for various daily needs and activities, such as households, agriculture, industry, mineral mining and tourism (Saprudin et al., 2022; Rahayu and Sari, 2023). One of the open river waters broadly utilized by the community is the Klawing River. As one of the tributaries of the Serayu River, the Klawing River is laid in Purbalingga District, Central Java Province of Indonesia. The usage of the Klawing River by the surrounding community is generally for fisheries, sand and stone mining, ranch woodlands and intensive farming (Pramono et al., 2019). The Klawing River is also one of the important waters on Javaisland, since 213 species of freshwater fish on the island, some of them are found in the Klawing River and are endemic species (Suryaningsih et al., 2018). Some families of freshwater fishes, regularly found in the Klawing River, include

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Cyprinids, Bagrids, Mastacembelids, Anabantids, Cichlids, Channids, Eleotridids, Beleontinids, Osphronemids, and Poecilids. (Pramono et al., 2018; Suryaningsih et al., 2018). Bagrid fish also present as one of the economic values in the river.

Two-spots catfish, Mystus nigriceps belonging to Bagrids family is a popular fish in the community. This fish has a high economic value so it was caught by the community both for consumption and ornamental fish (Pramono et al., 2019; Anggitasari et al., 2019). The increasing demand for two-spot catfish has encouraged overexploitation. This is due to the supply of two-spots catfish for consumption, until now, it still relies on catching in the wild (Syafrialdi et al., 2020). This would certainly affect the sustainability of the population and increase the risk of extinction of the fish (Pramono et al., 2019). According to the Directorate General of Capture Fisheries (2010), of the Ministry of Marine and Fisheries Affairs, Republic of Indonesia, there has been a decline in the population and production of Bagrids, including the two-spot catfish in the wild, caused by overfishing. Excessive fishing of two-spots catfish will damage the community structure and fish size (Sumaila and Tai, 2020). This can result in resource depletion, reduced biological growth rates, and low biomass levels (Vollrath et al., 2024). The impact of continuous overfishing can cause a critical dispensation, where the fish population is no longer able to maintain its survival. So that fish will experience extinction (Wittayakorn-puripunpinyoo, 2017; Pham et al., 2023).

Conservation of the two-spot catfish resources, which is compatible also for other fishes species, needs to be improved to avoid its extinction. Pramono et al. (2018) explained that in the last decade, the Klawing River basin has now developed various industries and the intensity of sand and stone mining has increased. Human activities around the Klawing River could destroy the natural habitat and even result in the extinction of fish that live in it. Habitat degradation that continues without serious protection efforts will very likely lead to extinction. The domestication of fish will be successful by applying scientific knowledge of well-known aspects of reproduction (Kurniawan et al., 2019; Priyadi et al., 2024). Sulistyo et al. (2023) suggest that some efforts to protect a species from extinction might be pursued through domestication, and afterward aquaculture. Domestication of this fish will be successful if the reproductive process is studied well. Reproduction is an important physiological system in the life cycle of organisms including fish (Pasquet 2018; Zohar 2021). The reproductive ability of fish varies according to environmental conditions.

Fish reproduction is indirectly explained by several aspects of the reproductive process. Aspects of fish reproduction can be seen from observations of morphology, morphoanatomy and histology (Mujtahidah et al., 2019; Parawangsa et al., 2022). The morphoanatomical index describes the relationship between the internal performance of reproductive organs and their supporters during gametogenesis (Ali et al., 2023). This morphoanatomical index is used as a method for predicting fish reproductive performance. The morphoanatomical indices in question include the gonadosomatic index (IGS), hepatosomatic index (IHS), visceasomatic index (IVS) (Gurkan et al., 2021; Wang et al., 2022). Observation of morphoanatomical indices involves observing the reproductive organs, namely the gonads, liver and visceral (Windarti et al., 2021). This research was conducted to evaluate the reproductive performance of two-spots catfish through sex ratio and morphoanatomical indices such as the gonadosomatic index (IGS), hepatosomatic index (IHS), and visceasomatic index (IVS).

MATERIALS & METHODS

Ethical Approval

The test animal in this study, namely Catfish (*Mystus nigriceps*), was given good and consistent treatment. This research procedure was approved by the Ethical Clearance that applies at the Faculty of Fisheries and Marine Sciences, Jenderal Soedirman University.

Fish Sampling

A random sampling was carried out, to obtain the fish, in Klawing River, divided into 3 different sampling stations, namely Station 1 (Jetis village), Station 2 (Bokol village), and Station 3 (Kedungbenda village). A fisherman was hired to catch fish with a cast net, randomly thrown to the middle or the edge of the river in each station. The operation of cast net was triplicated in each station during May and June 2023. Samples of 40 individuals were transported to the laboratory, for further analysis, in ice-filled boxes. Two-spots catfish sampling station can be seen in Fig. 1.

Sex Ratio

Determining the sex of fish is based on primary sexual characteristics based on the shape of the gonads. The sex ratio can be calculated based on the ratio of the number of male and female two-spots catfish caught during sampling. The sex ratio is calculated using the Effendie (1979) formula as follows:

$$\text{Sex Ratio (\%)} = \frac{Number\ of\ male\ fish}{Number\ of\ male\ fish\ +\ Number\ of\ female\ fish} x\ 100$$

Data Collection

Two-spots catfish samples were separated one by one into males and females. Two-spots catfish samples were then measured for total length and standard length measured using mm blocks and weight weighed using digital scales. The total length of the two-spots catfish is measured using a ruler from the tip of the snout to the tip of the tail fin and the standard length is measured from the tip of the snout to the base of the tail fin. Two-spots catfish were dissected using surgical tools, then the gonads, liver and visceral organs were taken and then weighed using analytical scales to get accurate results. The data on these organs is the target for calculating morphoanatomical indices. Morphology of Two-spot catfish can be seen in Fig. 2.

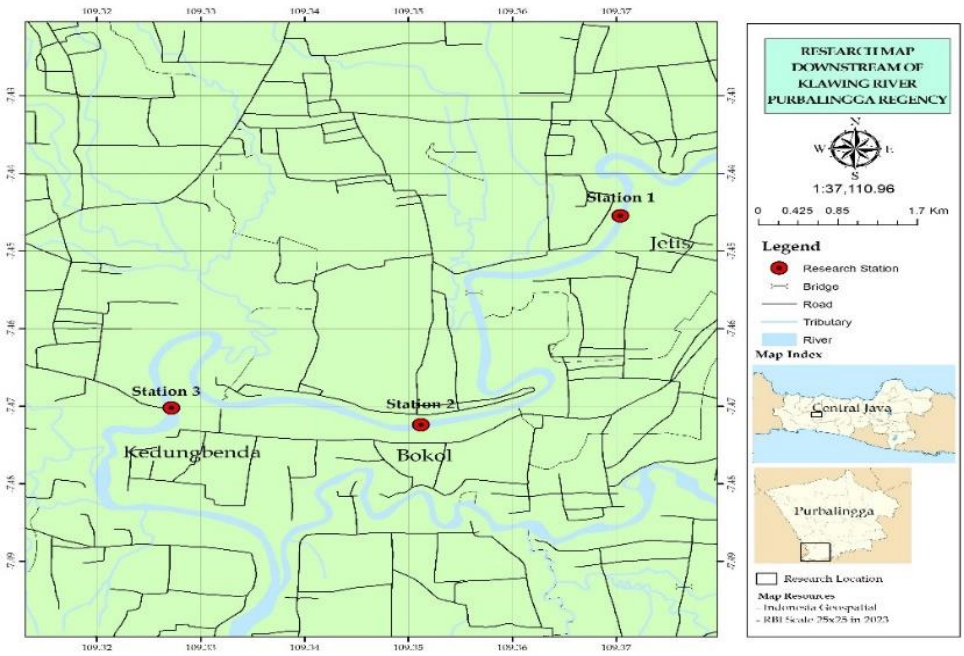

Fig. 1: Two-spots catfish sampling station.

Fig. 2: Morphology of Two-spot catfish caught from Klawing River, Central Java, Indonesia.

Morphoanatomical Indexes

Morphoanatomical Indexes were expressed as Gonado-somatic (GSI), Hepato-somatic (HSI), and Visceral-somatic (VSI) Indexes. They were calculated according to the following formulas (Sulistyo et al., 2000):

Gonado-somatic Index GSI (%) = (Effendie 1979)

$$\frac{gonad\ weight\ (g)}{body\ weight\ (g)}\ x\ 100$$

Hepato-somatic Index HSI (%) = (Brusle and Anadon, 1996)

$$\frac{liver\ weight\ (g)}{body\ weight\ (g)}\ x\ 100$$

Visceral-somatic Index VSI (%) = (Sulistyo, 1998)

$$\frac{visceral\ weight\ (g)}{body\ weight\ (g)}\ x\ 100$$

Water Quality Parameters

Measurement of air quality parameters is carried out in situ including physical and chemical parameters. Measurements were carried out once at each station upstream and downstream of the Klawing river. Physical parameters include temperature, brightness, depth and current speed and chemical parameters measured include pH and Dissolved oxygen (DO). The tools used in water quality parameters are shown in Table 1.

Table 1: Water quality measurement tools

Parameter	Unit	Methods/Tools	Source
Physics			
Temperature	˚C	Digital (Water Quality Control)	PP No. 82 Tahun 2001
Brightness	Cm	*Secchi disc*	PP No. 82 Tahun 2001
Depth	M	Scale piles	PP No. 82 Tahun 2001
Current speed	m/s	Digital (*Current meter*)	PP No. 82 Tahun 2001
Chemistry			
pH	-	Digital (Water Quality Control)	PP No. 82 Tahun 2001
Dissolved oxygen	mg/L	Digital (Water Quality Control)	PP No. 82 Tahun 2001

Data Analysis

Quantitative data of the Gonado-somatic Index (GSI), Viscero-somatic Index (VSI), Hepato-somatic Index (HSI), and regression correlation between those data were tabulated and analyzed with ANOVA at 0.05 level of significance. Significant analysis results were followed by the LSD test. Qualitative data were analyzed descriptively.

RESULTS

Sex Ratio

Based on the catches of two-spots catfish at 3 stations on the Klawing River, a total of 30 two-spots catfish were obtained, consisting of 15 male two-spots catfish and 15 female two-spots catfish. The results of the sex ratio show a balanced value between males and females with a ratio of 1:1, with a percentage of males of 50% and females of 50% at each station. The results of observations of the sex ratio of two-spots catfish in the Klawing River can be seen from Table 2.

Table 2: Sex ratio of two-spots catfish in the Klaing River

Station	Frequency		Percentage		Total
	Male	Female	Male	Female	
Jetis village	5	5	50	50	10
Bokol village	5	5	50	50	10
Kedungbenda village	5	5	50	50	10
Total	15	15	50	50	30

Gonado-somatic Index (GSI)

The gonadal-somatic indices of fish collected at all sampling stations showed statistically significant

differences (P<0.05). The highest value of female GSI was found from Bokol station being 36.78±6.00%, followed by Kedungbenda and Jetis stations presenting 28.13±4.98% and 26.03±6.13%, respectively. The male fish with the highest GSI, 29.09±29.09%, was found in the Jetis station. Bokol and Kedungbenda stations follow, with 27.71±2,69% and 22.66±5.44%, respectively. Fig. 3 shows the variation in GSI between male and female fish.

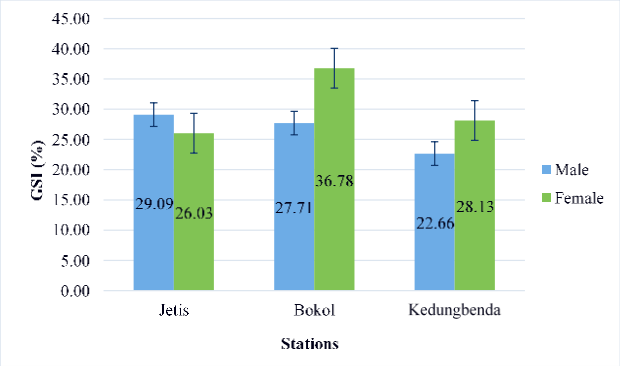

Fig. 3: Gonado-somatic Index of Two-spot Catfish from Klawing River.

Hepato-somatic Index (HSI)

All stations exhibited statistically significant differences (P<0.05) in HSI readings. Bokol station presented the highest HSI of female fish at 16.56±1.10%, Kedungbenda and Jetis stations followed with 16.02±2.18% and 12.69±1,83%, respectively. Bokol station made the highest HSI of male fish at 15.18±1.82%, followed by the Jetis and Kedungbenda stations at 12.21±1.96% and 11.81% ± 0.98, respectively. Fig. 4 shows the variation in HSI between the male and female fish.

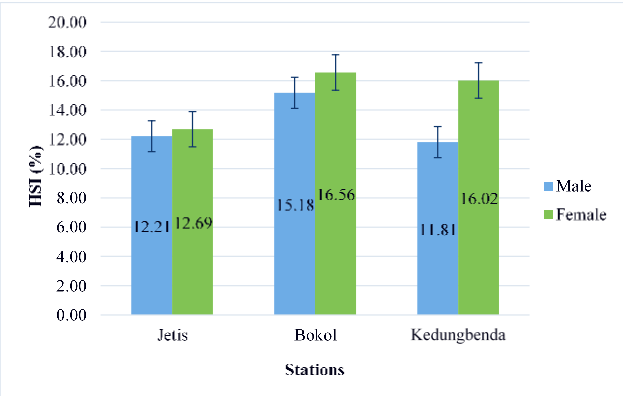

Fig. 4: Hepato-somatic Index of Two-spot Catfish from Klawing River.

Viscero-somatic (VSI)

All stations exposed statistically significant differences (P<0.05) in the VSI assessments. The female fish's VSI exhibited the greatest percentages in the Bokol station (17.17±1.61%), followed by the Jetis and Kedungbenda stations (17.14±1.51% and 15.36±2.30%, respectively). Jetis station displayed the greatest VSI (17.76±4.06%) for male fish, followed by Bokol and Kedungbenda stations (17.33±2.48% and 13.83±2.26%, respectively). Fig. 5 shows the variation in the VSI of the male and female fish.

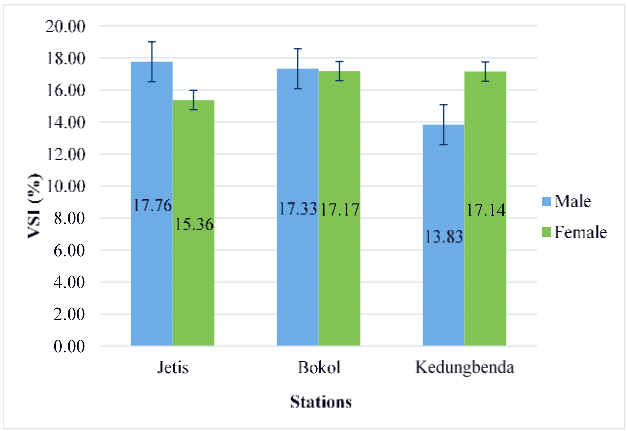

Fig. 5: Viscro-somatic Index of Two-spot Catfish from Klawing River.

Correlation between GSI and HSI

With R^2 = 0.388, there was a positive correlation between the GSI and the HSI (y = 0.229x + 7.5384). HSI increased in response to a rise in GSI of 38.8%, and vice-versa. Numerous additional factors also impacted the remaining 61.2%. Fig. 6 illustrates the relationship between GSI and HSI.

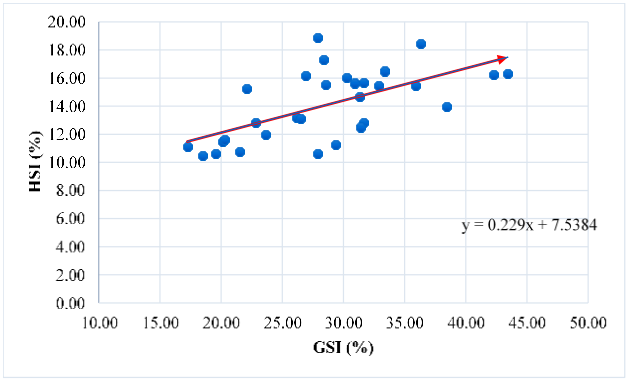

Fig. 6: Correlation between GSI and HSI of Two-spot Catfish from Klawing River.

Correlation between GSI and VSI

VSI and GSI showed a positive correlation (y = 0.2801x + 8.4318; R^2 = 0.463). The rise in the VSI was impacted by a 46.3% increase in GSI, and vice-versa. Numerous additional factors would affect the remaining 63.7%. Fig. 7 shows the relationship between the VSI and the GSI.

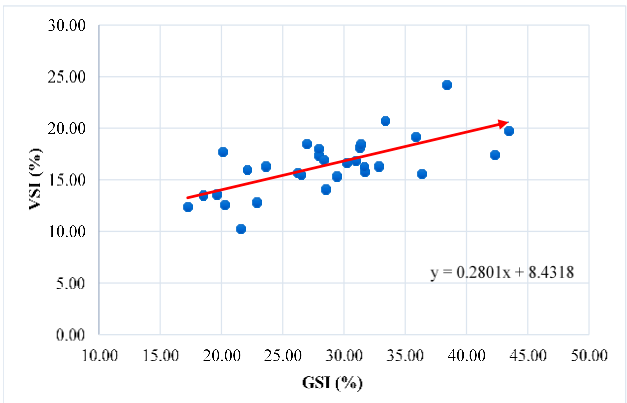

Fig. 7: Correlation between GSI and VSI of Two-spot Catfish from Klawing River.

Correlation between HSI and VSI

A favorable correlation between VSI and HIS (y = 0.52x + 9.1104; R^2 = 0.2155) was revealed. VSI would increase by a 21.5% increase in HSI, and vice-versa. Several more factors affected the remaining 78.5%. Fig. 8 illustrates the relationship between HSI and VSI.

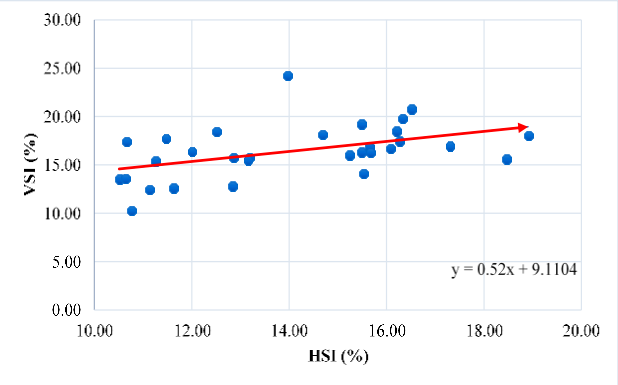

Fig. 8: Correlation between HSI and VSI of Two-spot Catfish from Klawing River.

Water Quality Parameters

The Klawing River's water quality parameters were still within an acceptable range for fish life, according to the measurement results. The parameters observed include the water's temperature, varying between 29 and 29.4°C; pH, which fluctuated between 6 and 9, and dissolved oxygen, which diverged between 3.8 and 5.3mg/L. These parameters showed that the water quality was within the river water quality criteria. Table 3 displays the water quality parameters of Klawing River.

DISCUSSION

The sex ratio is basic information in assessing the reproductive potential of fish populations (Jega et al., 2017; Rohmayani et al., 2018). Our research found that the sex ratio of two-spots catfish found in the Klawing River shows a significant balance at each station, namely 1:1, where male two-spots catfish are 50% and females are 50%. The balanced sex ratio of two-spots catfish illustrates the ideality of a population in maintaining its sustainability (Elvyra et al., 2023). According to Rinandha et al. (2020), a balanced sex ratio indicates that one male fish will fertilize one female fish. This balance causes spawning to take place well (Elvyra et al., 2023). Our research indicates that the sex ratio of male to female two-spots catfish may be balanced due to migration patterns of spawning fish. This statement is supported by Rinandha et al. (2020) that the difference in the number of male and female fish caught is closely related to fish migration behavior patterns, both for spawning and foraging. The sex ratio comparison is not absolute. This is influenced by distribution patterns caused by food availability, population density, and the balance of the food chain. Deviations from ideal conditions are caused by behavioral factors of the fish themselves, differences in mortality and growth rates (Fitrafiani, 2019).

The results of this study revealed that the two-spot catfish had spent through the gonadal maturity phase signified by the GSI of both male and female collected from each station. The present study demonstrated the GSI of two spots catfish ranging from 22.66 to 29.09% and 26.03 to 36.78% in male and female, respectively. This finding confirms previous study by Sulistyo (2008) which described that fish with a GSI of at least 19% predictably has mature gonads and is ready for spawning. In addition, Affandi et al. (2011) found that the higher GSI the more mature *Mystus nigriceps* from Klawing River Purbalingga, Jawa Tengah. Rizzo and Bazzoli (2020) further claimed that a high GSI, correlates with fish gonad maturity, at the mature stage and thereafter declining following spawning. The present findings indicated that female fish developed a higher GSI than males. The difference in GSI of males and females was in line with differences in gonadal structure and growth, where the growth and development of the ovaries tends to always be faster than the testes (Agustin et al., 2020; Mukti et al., 2020). Aja et al. (2023) claimed that female's ovary development is faster than male testicular development. In addition, because ovaria developed into mature reproductive organs that eventually fill the entire abdominal cavity, the morphological distinctions between ovaria and testes were reasonably intricate as observed in barred loach, *Nemacheilus fasciatus* (Nurhidayat et al., 2017). The profusion of food sources in the aquatic environment provided energy for the gonad's development and reproduction (Kantun et al., 2018), as evidenced by the high GSI at each station under observation in this study.

The HSI of two-spots catfish reflects the amount of energy stored during the maturation phase of the gonads. In this study it was found that HSI varied between 11.81-15.18%, and 12.69-16.56% in males and females respectively. This value, however, correlated positively with 38.8% with the GSI, meaning that a rise in the GSI would also increase the HSI. Mojumder et al. (2020), additionally Saleh and Ali (2017), declared that high GSI with lower HSI coupled with mobilization of liver reserves for gonadal maturation. This was by the study by Jan and Jan (2017) which stated that the HSI gradually decreased with ovarian maturation. Nonetheless, other facts revealed that HSI was positively correlated with GSI, meaning that

Table 3: Water Quality Parameters of Klawing River during the study

Parameters	Stations			Quality Standard
	Bokol	Jetis	Kedungbenda	
Temperature (°C)	29.1	29.4	29	28-32 (PP No. 82, 2001)*
pH	7.47	7.98	7.98	6-9 (PP No. 82, 2001)*
Current speed (m/s)	0.4	0.4	0.3	0.4 (Permen KP RI No.75, 2016)**
Brightness (cm)	52.5	40	45	200 (PP No. 82, 2001)*
Depth (m)	4.7	6.2	4.6	10 (Permen KP RI No.75, 2016)**
Dissolved Oxygen (mg/L)	5.3	3.8	4.2	3-6 (PP No. 82, 2001)*

*Pemerintah Republik Indonesia (2001); ** Pemerintah Republik Indonesia (2016).

the HSI continued to increase along with GSI (Hismayasari et al., 2015). The current data also showed that the HSI of female fish was superior to that of males. This occurred as a result of the liver organ's significant function in the reproductive process of female fish, which is directly related to the development of eggs, as also stated by Sulistyo et al. (2000). Besides being closely related to the reproductive phase, the increase in HSI related to the abundance of food in the environment. Where the abundance of food sources in the environment triggered an increase in appetite and fat reserves in the liver. The increase in fat reserves in the liver went then to reproduce, and the fat supplied the energy to carry out spawning (Tresnati et al., 2019).

The study's VSI for current fish correlated with the energy availability required for the gonad development and spawning processes. The findings indicated that the VSI female ranged from 15.36 to 17.17%, while the male ranged from 13.83 to 17.76%. This number had a 46.3% positive correlation with GSI, meaning that an increase in VSI impacted GSI. Windarti et al. (2015), discussed that the decrease in VSI would follow by an increase in GSI. This was because during gonadal maturation there tended to be a transfer of visceral organ mass, especially fat, into fat deposits in the gonads. Fat accumulation in the ovaries remained important as an energy source during embryogenesis and larval development.

Fish stored energized material after spawning in visceral tissue as a reserve for the next reproductive process (Kpelly et al., 2022). VSI, in the current study, correlated positively with HSI (21.5%). As described by Tessaro et al. (2014), meaning that the increase in HSI affected VSI, and vice-versa. Manor et al. (2015) detailed that gonadal development was supported by energy stored in the liver, muscle, and visceral tissues. Aragón-Flores et al. (2017) pronounced that protein, lipids, and carbohydrates constituted enormous energy-sustaining biological activities. Torsabo et al. (2022), compared with non-reproductive fish, noted that fish in the process of reproduction had a higher fat content.

Differences in environmental conditions, especially the availability of nutrients and water quality detected at each sampling station of two-spot catfish, also affected the morphoanatomical index of fish. According to Abdollahpour et al. (2022), hitherto by Tresnati et al. (2019), that water quality and nutrient availability would be important parameters in supporting fish growth and reproduction. This was following Delomas and Dabrowski (2018) that water quality parameters, specifically water temperature, would essentially affect egg development and fish reproduction. Mazzeo et al. (2014) highlighted that inappropriate water quality would hinder gonad development, hinder growth, and prevent successful spawning.

Conclusion

Two-spot catfish at each study station had reached the mature gonad phase, according to morphoanatomical observations. The GSI, HSI, and VSI values (male; female) varied from 22.66±5.44% - 29.09±7.16%; 26.03±6.13% - 36.78±6.00% (GSI), 11.81±0.98% - 15.18±1.82%; 12.69±1,83% - 16.56±1.10% (HSI), and 13.83±2.26% - 17.76±4.06%; 15.36±2.30% - 17.17±1.61% (VSI), which served as indicators of mature gonad Two-spot catfish. The fish is about to spawn since there was a positive relationship between morphoanatomical index values.

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Author's Contribution: RPCP: Writing - Original Draft, Conceptualization, data analyze. IS: principal investigator. NVH: - Review & Editing, H: Writing - Original Draft. M and RF: Writing - Review. All authors critically revised the manuscript and approved the final version.

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