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Study of Hepatosomatic Index, Gonadosomatic Index and Fecundity of Tengra Fish (*Mystus gulio*)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The tengra fish (*Mystus gulio*) is an important commercial species found in the coastal regions of West Bengal, India and Bangladesh. *M. gulio* is an important species within its ecosystem and has significance in local fisheries. Its adaptability to both freshwater and brackish environments makes it a resilient species. This study investigated the hepatosomatic index (HSI), gonadosomatic index (GSI), and fecundity of *M. gulio* to better understand its reproductive biology. Specimens were collected monthly from the Meghna River estuary between February and June 2023. The GSI was

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found to be highest in August (9.95±0.15%), indicating the peak spawning season. The HSI showed an inverse relationship with GSI, being lowest in March. Fecundity ranged from 88,495 to 264,104 eggs, with a mean of 171,581±17,855. Fecundity was positively correlated with total length, body weight, and gonad weight. Statistical analysis revealed significant differences in GSI and HSI between sexes and age groups (virgin vs 1+ year). Regression models showed that fecundity was highly dependent on gonad weight (r2=0.809) compared to length (r2=0.047) and weight (r2=0.216). *M. gulio* is an important brackish water fish with good taste and nutritional value. While it is optimally exploited from the East Coast of India, its culture potential is being explored through polyculture experiments and research on seed production and farming techniques. These findings provide valuable insights into the reproductive dynamics of *M. gulio* and can inform management strategies for sustainable fisheries. However, further research is needed to fully understand the reproductive biology of this catfish across its range to inform sustainable management strategies.

Keywords: Fecundity; Gonadosomatic Index (GSI); Hepatosomatic Index (HIS); Mystus gulio; reproductive biology.

1. INTRODUCTION

The Tengra fish, *M. gulio*, often known as "nona tengra" in the local region, is a small euryhaline catfish belonging to the Bagridae family. It is generally referred to as the long-whiskered catfish and is commonly found in the coastal waters of Bangladesh and India. *M. gulio*, can reach a maximum length of approximately 30 cm and a weight of up to 160 gm. Its body is elongated and compressed, typically exhibiting a greyish or brownish color with lighter underbellies. This species is carnivorous, primarily feeding on a protein-rich diet consisting of smaller fish, crustaceans, and other aquatic organisms, which supports its growth and health in various freshwater and brackish environments [1] Small indigenous fish species (SIS) are highly sought after and command premium pricing in the market due to their rich protein content, abundance of micronutrients, vitamins, and minerals (Ross *et al*., 2003), and low-fat composition. Therefore, they are a perfect selection for aquaculture in Southeast Asia. Nevertheless, the limited production of seeds in hatcheries is hindering the expansion of the culture [2]. Catfish account for approximately 1.46% of the total fish production in Bangladesh [3]. This species significantly enhances our coastal fishing industry. There is an increasing demand for it in both the domestic and foreign markets. The presence of this particular fish species in our country is solely reliant on its natural capture. The phenomenon is consistently present, with an increase in activity from June to August, coinciding with the rainy season [4].

The majority of this fish's habitat is brackish water, although Talwar and Jhingran [5] noted that it does occasionally swim into freshwater. It has been documented in marine and estuarine environments by Mukherjee et al. (2002). Its presence has been confirmed in many water bodies in Bangladesh, including canals, beels, haors, oxbow lakes, rivers, and estuaries [6,7]. Sundarban delta, Ganges-Brahmaputra estuary, India and Bangladesh). While Hamilton (1822) first recorded *M. gulio* in the upper Gangetic estuary, the species is now known to inhabit a broad variety of marine environments, including the oceans, estuaries, and tidal waters as far afield as the Malay Archipelago, Sri Lanka, Sumatra, and Siam (Day, 1878). There have been reports of its distribution along the coastlines of estuaries and tidal waters in several countries, including Bangladesh, India, Sri Lanka, Vietnam, Pakistan, Java, Thailand, Malaysia, and Myanmar [8].

M. gulio is a fish species that is not picky in its food choices, has a wide-ranging diet, and is very hungry. The organism consumes a diverse range of food sources, such as zooplankton, aquatic insects, crustaceans, annelids, and small forage fish. Nevertheless, the research discovered that *M. gulio* also ingested non-food objects of human origin, including coconut husk fibers, eggshells, chicken feathers, brick fragments, sand particles, and plastic fibers. The existence of these non-edible objects signifies the highly deteriorated environmental circumstances in the Ulhas River estuary and Thane Creek, where the fish were collected for examination. The high levels of pollution and contamination in Thane Creek pose a threat to the existence of *M. gulio*, notwithstanding its ability to tolerate pollution [9]. *M. gulio* primarily inhabits brackish water systems like estuaries, lagoons, and mangrove swamps with a mud or clay substratum. It forms schools of 10 to 25

individuals and feeds on small fishes, crustaceans, and aquatic plants. The aim of this study was to investigate the hepatosomatic index (HSI), gonadosomatic index (GSI), and fecundity of the Tengra fish (*M. gulio*) and analyze the statistical correlations between these parameters. The study sought to provide insights into the reproductive biology and maturity stages of the Tengra fish, which is an important commercial species in the region.

2. MATERIALS AND METHODS

2.1 Sampling

A total of 73 species were obtained from several local marketplaces in Uluberia, Howrah, and Belgharia, Kolkata. The fish samples were collected in collaboration with local fishermen using traditional fishing gear like nets, bubu, pengilar, and waring. Sampling was conducted during the rainy season. This suggests that the appropriate time and methods for sampling tengra fish should be determined in consultation with local fishermen and based on the species' natural habitat and behavior. A deliberate choice was made between male and female animals. The investigation utilized female specimens.

2.2 Fecundity

The term "fecundity" has been employed to describe the fish's reproductive strategy and the recruitment or stages of oocytes. Fecundity refers to the number of eggs produced by a female fish. The fisheries literature has utilized different definitions to quantify the quantity of eggs produced per female. Historically, potential fecundity has been estimated using either gravimetric or volumetric approaches [10,11]. Although the methods are basic, inexpensive, and yield solid results, the job is nevertheless demanding and requires a lot of physical effort [12]. The fecundity was calculated using the gravimetric technique. The ovaries of the female subjects were dissected and measured in terms of weight (W). Subsequently, three samples were extracted from distinct regions of the ovary: the upper section (w1), the middle area (w2), and the lower section (w3). Each piece was weighed and subsequently immersed in a 4% formalin solution for a duration sufficient to facilitate the separation of the eggs. Subsequently, the eggs were manually enumerated and designated as n1, n2, and n3 for the aforementioned areas, respectively. The following formula was used to calculate the fecundity:

$$\textbf{Fecundity} = \frac{W(g)\,X\,(n1 + n2 + n3)}{(w1(g) + w2(g) + w3\,(g)}$$

Where,

W(g)= Total weight of fish
w1(g)= Weight of top section of ovary
w2(g)= Weight of middle section of ovary
w3(g)= Weight of bottom section of ovary

n1= Number of eggs counted in the top section

n2= Number of eggs counted in the middle section

n3= Number of eggs counted in the bottom section

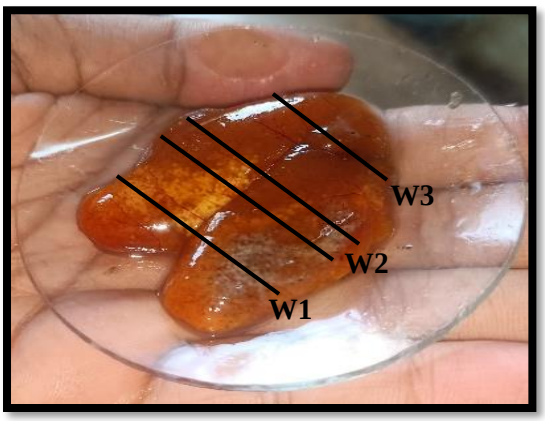

Fig. 1a. Ovary of *M. gulio* (w1, w2 & w3 marked

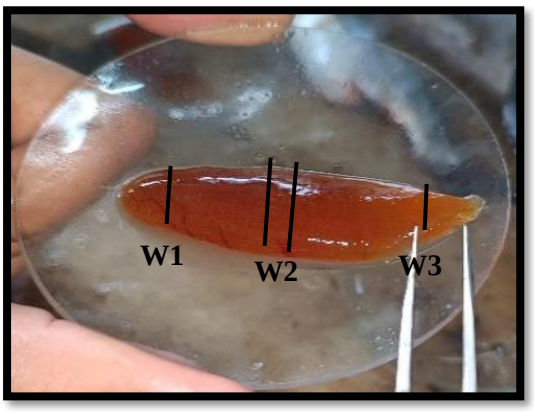

Fig. 1b. Ovary of *M. gulio* (w1, w2 & w3 marked

2.3 Gonadosomatic Index (GSI)

The Gonadosomatic index (GSI) is the ratio of gonad weight to total body weight, expressed as a percentage. It is used to assess the reproductive status of fish. The GSI is a useful tool for estimating the reproductive cycle of a species on a yearly, biweekly, or monthly basis. Identifying the species' spawning season at the field level is an extremely useful strategy. GSI (Gonadosomatic Index) indicates that the size of a gonad increases in proportion to the ratio of the gonad's mass (GW) to the animal's total mass (BW) during development. The gonadosomatic index of each fish was determined. The selected specimen was a live, mature female. The measurements of the body's overall length and weight were recorded. The mature ovaries were ultimately revealed during the dissection and meticulously removed in an undamaged state. Once the weight of the gonad was established, the GSI value for the female specimen was calculated. The gonadosomatic index was determined using the formula:

$$\mathbf{GSI}\ (\%) = \frac{(\text{Gonad weight (g) X 100})}{\text{Body weight (g)}}$$

2.4 Hepatosomatic Index (HSI)

The HSI is the ratio of liver weight to total body weight, expressed as a percentage. It is an indicator of the energy reserves in the fish body during gonadal development. The hepatosomatic index (HSI) was computed to investigate the relationship between its fluctuations and the gonadosomatic index (GSI). The HSI was computed using the Rajaguru technique (1992) by determining the hepatopancreas weight as a proportion of the fish's total live weight. Where LW represents the weight of the liver and TW represents the overall weight of the fish. The following formula was used:

$$\mathbf{HSI}\ (\%) = \frac{(\text{Liver weight (g) X 100})}{\text{Body weight (g)}}$$

Fig. 2. In-situ liver dissection

Fig. 3. In-situ gonad dissection

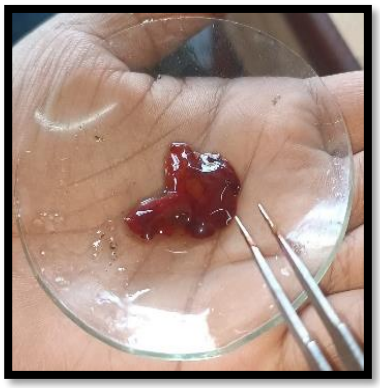

Fig. 4. Liver

Fig. 5. Liver being weighed

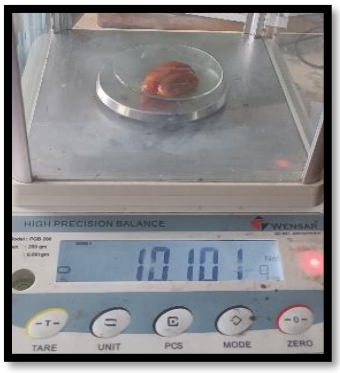

Fig. 6. Gonad being weighed

3. RESULTS

GSI is a measure of the gonadal mass as a proportion of the total body mass, and is used as an indicator of fish spawning periodicity and maturity. Studies have found that GSI values are typically highest during the peak spawning season for various fish species. The peak spawning season for this species occurs in August. There is a strong positive correlation between fecundity (number of eggs produced) and gonad weight. The female fish typically have a higher GSI (10-25% of body weight) compared to males (5-10% of body weight). The HSI tends to be inversely related to the GSI, as the liver stores energy that is later used for gonad development. Fecundity, or the number of eggs produced by a female fish, has been studied for several fish species. Fecundity is often positively correlated with the fish's total length, body weight, and gonad weight.

Table 1. Total weight, gonad weight and liver weight of *M. gulio*

Samples	Total Weight (gm)	Gonad Weight(gm)	Liver Weight(gm)
1	30	1.117	0.396
2	45	2.94	0.672
3	52	3.74	0.918
4	37	5.97	0.37
5	39	9.202	0.708
6	25	5.041	0.375
7	50	10.421	0.987
8	58	17.137	0.859
9	35	10.1	0.685
10	30	6.215	0.478
11	31	6.278	0.599
12	25	6.294	0.494
13	26	4.84	0.374
14	25	4.402	0.542
15	27	5.16	0.386
16	41	8.774	0.857
17	45	9.265	0.825

Table 2. GSI, HSI and fecundity of *M. gulio*

G.S.I.	H.S.I.	Fecundity
3.723333333	1.32	1864.434944
6.533333333	1.493333333	4812.238806
7.192307692	1.765384615	5907.819549
16.13513514	1	9915.010989
23.59487179	1.815384615	14613.50728
20.164	1.5	7907.66309
20.842	1.974	15965.09909
29.54655172	1.481034483	26710.80832
28.85714286	1.957142857	15828.96711
20.71666667	1.593333333	9499.015852
20.2516129	1.932258065	9853.781076
25.176	1.976	9866.025862
18.61538462	1.438461538	7603.340961
17.608	2.168	7029.625551
19.11111111	1.42962963	8083.467492
21.4	2.090243902	13681.08342
20.58888889	1.833333333	14508.33013

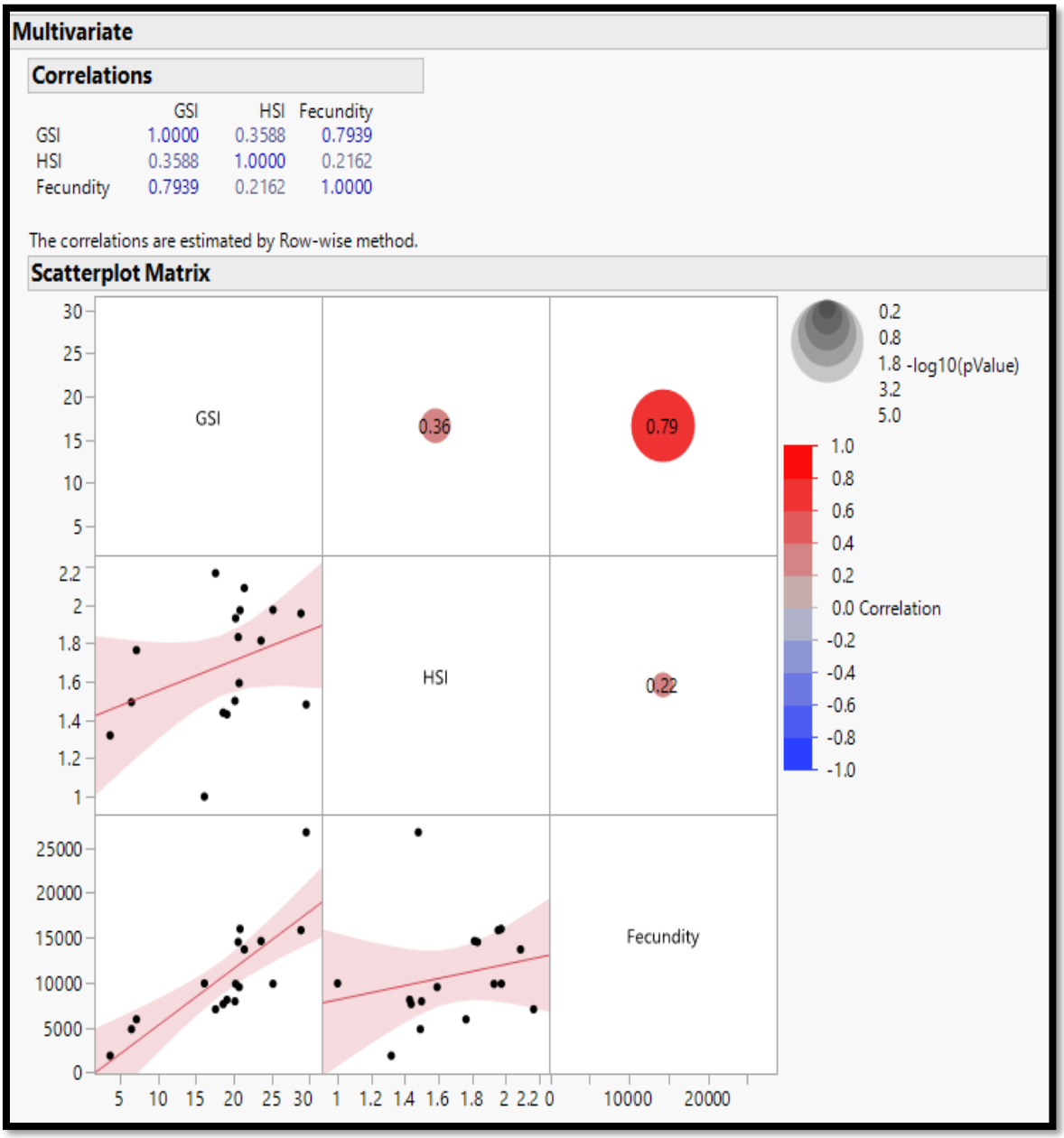

Fig. 7. Correlation and scatterplot matrix

The ovary was a pair of elongated sacs fused at the posterior end. The fused ovary often resembles a 'Y' shape. The immature ovary was a small, translucent, elongated, and tube-like structure. Oocytes were invisible to the naked eye. When they reached a mature stage and filled three-fourths of the body cavity, ovaries started to get bigger. Oocytes in the ovary were clearly visible and had clear blood vessels. Ova were yellowish and transparent. The whole length of the body cavity was filled with ripe ovaries, which were yellow. Ova were fairly distinct and round, and the ovarian wall was thin.

From the samples collected, we measured the Total Weight, Gonad Weight and the Liver Weight of the fish species and calculated the GSI, HSI and Fecundity represented in Table 1. and Table 2.

Following the calculation of the GSI, HSI, and Fecundity, a statistical software called JMP was utilised to determine any positive or negative correlation among these three measures. The software's results are displayed in Fig. 7, which clearly depicts the multivariate correlation, scatter plot, regression value, and significance.

4. DISCUSSION

The words reproductive strategies, spawning pattern, ovarian organization, and fecundity are defined in [13] Murua and Saborido-Rey, 2003). Fecundity refers to the possible yearly production of advanced yolked oocytes per female, without considering any losses due to atretic (degenerative) processes [13]. Fecundity, defined as the aggregate quantity of eggs generated by a female fish, is a crucial factor in comprehending the reproductive capacity of a species. The estimation of fecundity in M. gulio involved the enumeration of mature eggs present in both ovaries. In order to gain a deeper understanding of the reproductive strategy of this species, Lal et al. [14] investigated the correlation between the number of oocytes, fork length (FL), and body weight. Studies on *Mystus gulio* have documented a wide range of fecundity, with estimates varying from approximately 5,950 to over 141,210 eggs, depending on the size and health of the fish [15]. The relationship between fecundity and various factors such as body weight, gonad weight, and environmental conditions is crucial for effective fishery management and aquaculture practices. Understanding these relationships helps in predicting reproductive success and managing breeding programs effectively [16]. The fecundity, which refers to the number of eggs produced by a female fish, of *M. gulio* varied between 3,891 and 168,358, with an average of 32,909. The study revealed a positive correlation between fecundity and the fish's overall length, body weight, gonad length, and gonad weight. The statistical analysis revealed that these associations were very significant at a level of $p<0.05$ [16]. Estimating fecundity often involves determining the number of vitellogenic oocytes present, which represents the potential fecundity. Vitellogenesis is the process by which mature oocytes in the ovary accumulate yolk. The maturation phase in most fish species is seasonal, and spawning occurs only once throughout each yearly reproductive cycle. Hence, any variable that influences the vitellogenic cycle can have a substantial impact on an individual's reproductive success, including the quantity of eggs produced, the pace of hatching, and the viability of the embryos. Consequently, this has the capacity to thrive for the entire population of the species. Oogenesis primarily involves the liver producing yolk droplets and the oocytes absorbing the yolk precursor protein vitellogenin [17]. The oogenesis is affected by environmental cues and controlled by many hormones (Billard *et al*., 1978). In their study, Sarker *et al*. [4] observed that the weight range of 90-110g in fishes had the highest average fecundity, with a mean value of 22546 (ranging from 19741 to 23009). The lowest average fecundity was 10982 (10831-12481) among fish weighing between 15-20 grams. The highest mean fecundity of 21378 (19611-22825) was observed in ovaries weighing between 16-18g. This study found that the reproductive capacity of fish was positively correlated with their size, weight, and gonad weight.

The Gonadosomatic Index (GSI) is a critical measure used to assess the reproductive status of fish. It is calculated as the ratio of the weight of the gonads to the total body weight of the fish, expressed as a percentage. In *M. gulio*, studies indicate that the GSI varies significantly throughout the year, reflecting the seasonal reproductive cycle typical of many teleost fish. Research has shown that the GSI is particularly high during the breeding season, which is essential for understanding the timing of spawning and reproductive health. Research has demonstrated that there might be substantial variations in GSI (gonadosomatic index) between males and females, suggesting disparities in their reproductive tactics. The GSI (Gonadosomatic Index) was employed to determine the maturity phases and estimate the fertility of *M. gulio*, as stated by Lal et al. [14]. The research conducted on *M. gulio* determined that it exhibits a broad spawning season spanning from March to November. This species experiences a single peak in spawning activity during the month of July, as evidenced by the gonadosomatic index (GSI) and the width of its eggs. The gonadosomatic index (GSI), defined as the ratio of gonad weight to body weight, reached its highest value during the peak spawning period in July, as reported by Islam et al. [16]. In their study, Sarker *et al*. (2002) observed that the GSI values exhibited an upward trend starting from March and reaching their highest point in July. Subsequently, there was a progressive decline in GSI values until November. The rise in GSI (Gonadosomatic Index) values in females over the period from March to July signifies the maturation of the gonads, while a sharp decline in December shows the spent stage of the fish. The monthly fluctuations in the GSI provide more evidence for the spawning season and also indicate the main phases of reproductive cycles. DeVlaming [18] states that the brain stimulates the pituitary gland to secrete gonadotropins,

which are peptide hormones, in response to conditions such as temperature and/or photoperiod. These hormones regulate the reproductive function in vertebrates [19]. Furthermore, as stated by Harmin and Crim [20], they also promote the process of meiotic maturation and ovulation. The hypothalamus produces brain eurohormones such as gonadotropin-releasing hormone (GnRH) to regulate the secretory activities of the pituitary gland. Once oogenesis is initiated, the gonadotropins are released into the bloodstream and go to the ovaries to promote the development of oocytes and ultimately trigger ovulation. Furthermore, they stimulate the synthesis of oestrogens, specifically estradiol, by the follicle cells [21]. After being released into the bloodstream, estradiol binds with steroid-binding proteins or albumins to prevent it from causing harm [22]. Estradiol has been found to stimulate the liver to produce vitellogenin, as indicated by various investigations (MacKay and Lazier, 1993). Vitellogenin, a serum protein found only in females, has been discovered as the primary precursor of egg yolk in most oviparous vertebrates. It contains phosphate, lipids, carbohydrates, calcium, and iron [23]. Estradiol permeates the liver cells and forms a strong connection with the oestrogen receptor (ER) protein, effectively retaining it within the target cells [24]. The transcription of the vitellogenin locus is activated afterwards as a consequence [25]. Increasing the dosage of estradiol leads to a higher binding affinity of the hormone to the ER [26]. Estradiol stimulation of hepatic cells induces the growth of rough endoplasmic reticulum and Golgi apparatus. These structures are responsible for modifying the vitellogenin precursor [27]. After being released into the bloodstream, vitellogenin migrates towards the ovaries. It enters the follicle by passing through a network of tiny blood vessels in the thecal layer. Vitellogenin is released from the capillaries and moves to the surface of the oocyte through channels between the follicle cells. With the help of gonadotropin, vitellogenin is then taken up by receptor-mediated endocytosis into yolk platelets, where it is quickly broken down into the yolk proteins lipovitellin and phosvitin. Multiple investigations [28] have discovered the presence of yolk precursor in the plasma of vitellogenic females of various teleosts. According to various studies using radiolabeled [32P]- and [3H] leucine, vitellogenin is the sole protein containing phosphorus that is present in the blood of oviparous mammals [29]. The yolk can be synthesized by the oocyte itself through autosynthesis, or it can be generated using material from external sources through heterosynthesis. In vertebrates, the production of eggs through heterosynthetic generation is more common.

The HSI is another important index that provides insights into the overall health and metabolic condition of fish. It is calculated similarly to GSI, using the liver weight relative to total body weight. A higher HSI often indicates better nutritional status and energy reserves, which are crucial for reproduction. In *M. gulio*, the average HSI has been reported to be around 1.5, with variations observed based on environmental conditions and reproductive phases [30]. The relationship between HSI and GSI is often inverse, suggesting that energy allocation shifts towards reproductive efforts during the breeding season [31-33]. The Scatterplot matrix reveals a clear and statistically significant positive association between the GSI and Fecundity variables. Studies have found that GSI values are typically highest during the peak spawning season for various fish species. Fecundity is often positively correlated with the fish's total length, body weight, and gonad weight. Additionally, it indicates a little degree of positive correlation between GSI, HSI, and HSI, Fecundity. The primary objective of this study was to determine whether there is a positive link between HSI and fecundity, indicating the significant function of the liver in the development of gonads and vitellogenesis in *M. gulio*. Prior studies have demonstrated a positive association between GSI and Fecundity, but no research has been conducted to establish any correlation between HSI and Fecundity. This is significant since a healthy liver in the species undoubtedly contributes to its gonad growth.

5. CONCLUSION

In conclusion, this study focuses on the statistical association and analysis of the hepatosomatic index (HSI), gonadosomatic index (GSI), and fecundity of tengra fish. The GSI and HSI readings are crucial for comprehending the reproductive and nutritional condition of Tengra fish. During the peak season, a high GSI indicates ideal conditions for reproduction, whereas the negative correlation with HSI reveals that liver health is affected by reproductive activity. HSI, which is a measure of liver size, is known to play a crucial role in the growth of gonads and maturation of eggs. The data clearly indicate that the liver plays a crucial

part in the process of vitellogenesis, and maintaining its health significantly enhances fecundity. The fecundity of tengra fish is significantly influenced by body size and weight, highlighting the importance of these factors in reproductive biology. We achieved our objective and established a positive association between HSI and Fecundity, thereby confirming our hypothesis.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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