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Physiological biomarkers reveal environmental stress and reproductive impairment in lempuk fish (*Gobiopterus* sp.) from Lake Ranu Grati, Indonesia

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ABSTRACT

The endemic lempuk fish (*Gobiopterus* sp.), inhabiting Lake Ranu Grati, East Java, Indonesia, is increasingly threatened by environmental degradation and anthropogenic disturbances, highlighting the need for reliable biological indicators to assess ecosystem health and support conservation strategies. This study evaluated physiological stress biomarkers as indicators of environmental stress and investigated their relationship with fish health and reproductive performance to support ecological monitoring, domestication, and F1 seed production of *Gobiopterus* sp. Fish were collected from two sampling sites representing contrasting environmental conditions in Lake Ranu Grati, where water quality and physiological responses were simultaneously assessed. Physiological stress biomarkers were assessed using reactive oxygen species (ROS), superoxide dismutase (SOD), catalase (CAT) and Cortisol. Reproductive performance was evaluated based on fertilization rate (FR), hatching rate (HR), and survival rate (SR). Relationships among variables were analyzed using Spearman correlation and principal component analysis (PCA). The results demonstrated that fish collected from the environmentally less favorable site exhibited higher ROS, SOD, CAT, and Cortisol levels accompanied by lower fertilization, hatching, and survival rates. Correlation analysis showed that reproductive performance declined with increasing physiological stress, whereas broodstock health parameters remained within physiological tolerance limits. Further PCA analysis differentiated physiological responses across sampling sites, with ROS and cortisol making the largest contributions to the observed variation. These findings demonstrate the potential of physiological biomarkers as early indicators of environmental quality decline and provide scientific support for the ecological monitoring, domestication, and conservation of the endemic *Gobiopterus* sp.

Keywords: physiological stres, environmental, reproductive performance, lempuk fish, domestication.

INTRODUCTION

Freshwater ecosystems provide vital ecosystem services and harbor exceptionally high levels of global biodiversity, despite covering less than 1% of Earth's surface (Dudgeon et al., 2006). These ecosystems supply water resources, regulate biogeochemical cycles, support fisheries, and provide critical habitats for various endemic species, thereby playing a fundamental role in maintaining ecological integrity and human well-being (Tickner et al., 2020). However, increasing anthropogenic activities including habitat degradation, eutrophication, pollution, and climate-driven environmental changes have accelerated the degradation of freshwater ecosystems and contributed to the global decline of freshwater biodiversity (Reid et al., 2019). Consequently, there is a growing need for reliable biological indicators to detect ecological disturbances early and to support effective environmental monitoring and conservation planning. Endemic fish species are particularly vulnerable to these disturbances due to their limited geographic ranges, narrow ecological niches, and limited capacity to adapt to rapidly changing environmental conditions (Carosi et al., 2019).

Lake Ranu Grati is a volcanic freshwater lake located in East Java, Indonesia, serving various ecological and socio-economic functions, including fisheries, irrigation, tourism, and the conservation of endemic aquatic biodiversity (Alfarisi et al., 2025). As one of the few natural habitats supporting the endemic lempuk fish, the lake's ecological condition is closely linked to the survival of this endemic fish population and the ecosystem services it provides. Therefore, maintaining water quality and ecosystem integrity is crucial not only for biodiversity conservation but also for supporting sustainable fisheries management and domestication programs. The lempuk fish is a species endemic to Lake Ranu Grati in East Java, Indonesia. It plays a vital role in maintaining the balance of the aquatic ecosystem while serving as an economically valuable fishery commodity for local communities (Anitasari et al., 2024). However, in recent years, the lempuk fish population has declined due to increasing anthropogenic pressure and environmental degradation (Prakash and Verma, 2022).

Various studies have demonstrated that changes in environmental quality particularly temperature, potential of hydrogen (pH), and dissolved oxygen (DO) levels directly influence the physiological and reproductive status of fish (Mariu et al., 2023). These parameters are among the most sensitive indicators of the decline in freshwater ecosystem quality. Elevated temperatures and reduced DO levels can trigger the excessive formation of reactive oxygen species (ROS), leading to oxidative stress (Sachdev et al., 2021). As a defense mechanism, fish increase the activity of antioxidant systems involving enzymes such as superoxide dismutase (SOD) and cortisol, alongside elevated cortisol levels as a physiological adaptive response to environmental stress (Aliko et al., 2018). These physiological responses have recently gained increasing attention as sensitive biological indicators because they provide early-warning signals of environmental deterioration before visible ecological damage or population decline becomes evident (Steckl and Ray, 2018). Unlike conventional physicochemical measurements, physiological biomarkers integrate the cumulative effects of multiple environmental stressors, making them valuable tools for environmental monitoring and ecological assessment or freshwater ecosystems(Abdallah et al., 2024). In various fish species, increases in these stress biomarkers have been linked to metabolic disturbances, cellular damage, reduced gonad quality, and diminished reproductive performance (Chowdhury and Saikia, 2020). Furthermore, the physiological condition of broodstock plays a crucial role in determining fertilization rates (FR), hatching rates (HR), and survival rates (SR), all of which are influenced by gamete quality, the stage of gonadal maturity, and environmental stability (Engdaw and Geremew, 2024).

Ecological pressures resulting from eutrophication and habitat degradation are known to inhibit the natural reproduction of endemic freshwater fish and accelerate their population decline (Huang & Han, 2025). Although the relationships among environmental quality and physiological stress have been extensively studied in various fish species, most studies have focused on commercially cultured fish or have examined physiological stress biomarkers independently (Alavi et al., 2021). Previous studies on lempuk fish have primarily focused on morphology, feeding ecology, gonadal development, and domestication (Hertika et al., 2024), whereas the integration of physiological stress biomarkers with fish health and reproductive performance as indicators of environmental quality remains unexplored. Therefore, the mechanisms linking environmental stress, physiological responses, and reproductive success in endemic lempuk fish have not been comprehensively understood.

To address this knowledge gap, the present study employed an integrative approach by correlating physiological stress biomarkers (ROS, SOD, CAT, cortisol) with fish health and reproductive performance. Rather than evaluating physiological responses solely from an aquaculture perspective, this study positions these biomarkers as biological indicators of environmental quality, thereby linking ecosystem condition with fish health and reproductive success. Recent studies have emphasize that comprehensive assessment of aquatic ecosystem health must integrate conventional water quality measurements with physiological and biochemical biomarkers, as these complementary indicators provide a more holistic understanding of environmental conditions. Water quality directly influences fish physiological performance, reproductive processes, and the long-term sustainability of aquatic ecosystems (Nyamweha et al., 2026), while advancements in environmental monitoring have enhanced the ability to detect ecological disturbances at an early stage (Oladosu et al., 2026). Furthermore, increasing attention has been focused on oxidative stress biomarkers and antioxidant defense systems as sensitive biological indicators for evaluating environmental stress and ecological health in aquatic organisms, thereby supporting their application in environmental monitoring and conservation research(Cokrowati et al., 2025). Therefore, the study aims to evaluate the relationships among physiological stress biomarkers and reproductive performance in lempuk fish (*Gobiopterus* sp.) under environmental conditions associated with domestication.

MATERIALS AND METHODS

Study area

The research was conducted for 4 months from December 2025 to March 2026 at Ranu Grati Lake, Indonesia. Field sampling was conducted over two sampling periods, with the first sampling conducted in December 2025 and the second sampling conducted in February 2026. The location of the research carried out was divided into 2 different sampling point located on each side of Ranu Grati, representing different environmental characteristics. Sampling point 1 is located in the northern part of the lake. Sampling point 2 is situated in the southeastern part of the lake. The geographical coordinates for the sampling station in Ranu Grati are presented in Figure 1.

Samples collection

This research process was carried out in situ and ex situ. A total of 40 endemic lempuk fish (*Gobiopterus* sp.) were collected from Lake Ranu Grati, East Java, Indonesia, Twenty fish (n = 20) were collected during each sampling period with (n = 10 fish per station), with five females and five males collected at each station, using seine nets and dip nets (0.5 cm mesh size). Subsequently, evaluations were conducted regarding water quality parameters, physiological stress biomarkers, fish health, and reproductive performance. All sampling activities were carried out with the assistance of local fishermen. Laboratory analyses were performed at the Fish Culture Laboratory, Fish Reproduction Division, Faculty of Fisheries and Marine Sciences, Universitas Brawijaya. Reproductive performance was assessed using FR, HR, and SR. Broodstock health was evaluated based on body length, body weight, and gonad maturity index. Water quality parameters, including temperature, pH, and DO, using a water quality checker, pH meter, and DO meter. Table 1 shows the methods and instruments used for the measurement.

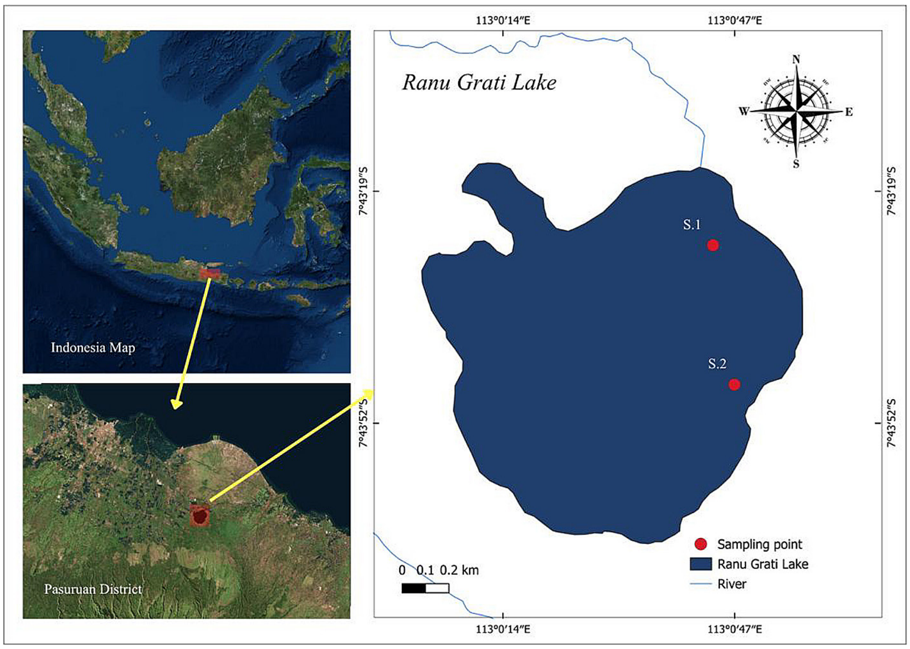

Figure 1. Location of sampling point in Ranu Grati Lake

Physiological stress biomarker analysis

Physiological stress biomarkers, including reactive oxygen species (ROS), superoxide dismutase (SOD), and cortisol, were analyzed using commercial ELISA kits. Blood plasma and tissue homogenates were prepared from 40 fish (20 males and 20 females), and biomarker concentrations were quantified following the manufacturers' protocols with minor modifications (Moniruzzaman et al., 2022). Absorbance was measured at a wavelength of 450 nm using a microplate reader (Bio-Rad, USA).

Reproductive performance analysis

Reproductive performance was determined through *fertilization rate* (FR), *hatching rate* (HR), and *survival rate* (SR), calculated using the following equations (Haque, 2021).

$$FR\ (\%) = \frac{Number\ of\ fertilized\ eggs}{Total\ number\ of\ eggs\ used\ in\ fertilization} \times 100 \quad (1)$$

$$HR(\%) = \frac{Number\ of\ Hatched\ eggs}{Total\ number\ of\ fertilized\ eggs} \times 100 \quad (2)$$

$$SR(\%) = \frac{Number\ of\ final\ larvae}{Total\ number\ of\ hatched\ larvae} \times 100 \quad (3)$$

where: FR – fertilization rate (%), HR – hatching rate (%), SR – survival rate (%).

Fish health analysis

Fish health was evaluated using gonad maturity index, body length, and body weight measurements. The formula for the gonad maturity index is as follows (Nikolsky, 1963):

$$GMI = \left(\frac{Wg}{Wt}\right) \times 100 \quad (4)$$

where: GMI – gonad maturity index (%), Wg – gonad weight (g), Wt – total body weight (g).

Data analysis

The difference between the December 2025 and February 2026 sampling periods was evaluated using the Mann–Whitney U test. Since the objective of this analysis was to assess the effect of sampling time, observations from both sampling periods were pooled because no significant temporal differences were detected (p>0.05). The pooled dataset consisted of 20 pooled observations representing pooled observations from female and male fish collected at Sampling Point I and II was subsequently used for Spearman rank correlation and principal component analysis (PCA). PCA was performed using PAST version 4.03 to explore multivariate relationships among oxidative stress biomarkers to variations in reproductive performance and fish health parameters. Spearman correlation was used to evaluate the monotonic relationship between biomarkers and biological parameters, as the data did not meet the assumption of normality. The strength of the correlation was interpreted based on (Schober and Schwarte, 2018), and statistical significance was set at $p < 0.05$.

RESULT AND DISCUSSION

Identification of water quality in Ranu Grati Lake

Ranu Grati Lake supports multiple ecological and socio-economic activities, including fisheries, tourism, and conservation, contributing to spatial variations in environmental conditions (Hertika et al., 2025). Water quality measurements revealed significant differences between the two sampling sites (Table 2). Sampling site 2 exhibited a higher average water temperature (31.1 °C), lower dissolved oxygen (DO) concentration (5.4 mg/L), and lower pH (7.1) compared to sampling site 1 (30.6 °C, 6.0 mg/L, and pH 7.5, respectively), indicating less favorable environmental conditions. The rise in water temperature observed at sampling site 2 is

Table 1. Methods and instruments for assessing water quality

Parameter	Unit	Method/Instrument
Temperature	°C	Dissolved oxygen analyzer type DO9100
Potential of hydrogen (pH)	-	Wter quality tester tipe EZ-9901
Dissolved oxygen (DO)	mg/L	Dissolved oxygen analyzer type DO9100

ecologically significant because temperature directly influences the physicochemical characteristics of freshwater ecosystems. Higher temperatures reduce oxygen solubility in water while simultaneously increasing the metabolic activity of aquatic organisms and microorganisms. Consequently, oxygen consumption rises while oxygen availability declines, resulting in lower dissolved oxygen concentrations (Robinson, 2019). Furthermore, increased microbial decomposition of organic matter produces carbon dioxide (CO_2), contributing to a decrease in water pH (Khatoon et al., 2017). These combined environmental changes create suboptimal conditions that can disrupt physiological homeostasis in aquatic organisms and trigger an oxidative stress response (Astria et al., 2025).

The decline in environmental quality at sampling site 2 may also have been exacerbated by the seasonal sapon phenomenon reported by local fishermen prior to the second sampling period. This phenomenon is characterized by the vertical mixing of the water column, which brings oxygen-poor bottom water and accumulated organic matter to the surface. Such mixing can cause rapid fluctuations in water temperature, dissolved oxygen, and pH, while simultaneously increasing biological oxygen demand due to accelerated microbial decomposition (Difinubun et al., 2023). Consequently, these unstable environmental conditions likely impose additional physiological stress on the endemic lempuk fish by increasing the production of reactive oxygen species (ROS) and triggering antioxidant and endocrine stress responses (Prates, 2025).

Overall, the consistently higher temperatures and lower DO and pH levels at sampling site 2 indicate that this location experiences greater environmental stress compared to sampling site 1. These differences provide an ecological basis for interpreting the elevated physiological stress biomarkers and reduced reproductive performance observed in fish collected from the site with higher environmental disturbance. The result of the water quality analysis study in Ranu Grati Lake are presented in Table 2.

Oxidative stress biomarkers

Physiological stress biomarkers including ROS, SOD, CAT, and cortisol were consistently higher in fish collected from sampling site 2 compared to those from sampling site 1, in both males and females (Table 3). Elevated levels of these biomarkers indicate that fish inhabiting sampling site 2 experienced greater physiological stress; this aligns with poorer environmental conditions, characterized by higher water temperatures, lower dissolved oxygen levels, and lower pH. Such environmental conditions increase metabolic oxygen demand while simultaneously reducing oxygen availability, thereby disrupting cellular homeostasis and triggering excessive mitochondrial electron leakage, which leads to the overproduction of reactive oxygen species (ROS) (Menon et al., 2023).

Reactive oxygen species are continuously produced during normal aerobic metabolism. However, under unfavorable environmental conditions their production may exceed the antioxidant capacity of the organism, leading to oxidative stress. The higher ROS concentrations observed at sampling site 2 were accompanied by increased SOD and CAT activities, indicating activation of the antioxidant defense system in response to excessive free radical formation. Superoxide dismutase functions as the first enzymatic defense by converting superoxide radicals into hydrogen peroxide, whereas catalase subsequently decomposes hydrogen peroxide into water and (Tsalafouta, 2014), thereby preventing the formation of highly reactive hydroxyl radicals that can damage cellular components (Balasch and Tort, 2019). The simultaneous increase in ROS, SOD, and CAT therefore reflects an adaptive physiological response aimed at maintaining cellular homeostasis under environmental stress. In addition to antioxidant activation, fish collected from sampling site 2 exhibited higher cortisol concentrations, suggesting stimulation of the hypothalamic–pituitary–interrenal (HPI) axis as part of the endocrine stress response. Cortisol plays a central role in maintaining energy homeostasis by mobilizing metabolic reserves required for survival

Table 2. Water quality parameters in Ranu Grati Lake

Parameter	Sampling Point 1	Sampling Point 2	Satuan
Temperature	30.6	31.1	°C
pH	7.5	7.1	-
DO	6	5.4	mg/L

under stressful conditions(Zabixullaevich et al., 2025). However, prolonged elevation of cortisol may divert energy allocation from growth and reproduction toward physiological maintenance, consequently reducing reproductive capacity and gamete quality (Aliko et al., 2018).

Persistent oxidative stress may also induce lipid peroxidation, protein oxidation, DNA damage, and apoptosis of reproductive cells, ultimately impairing sperm motility, oocyte quality, fertilization success, and embryonic development (Khalil et al., 2019; Ma et al., 2025). Similar relationships between elevated oxidative stress biomarkers and reduced reproductive success have been reported in several freshwater fish species exposed to thermal stress and hypoxic conditions (Rumondang et al., 2024; Pintus and Ros-Santaella, 2021). Under these environmental conditions, oxidative stress impairs mitochondrial function and ATP production, thereby reducing the energy available for gamete maturation, fertilization, and embryonic development (May-Panloup et al., 2021). Consequently, fertilized eggs become more susceptible to developmental abnormalities, reduced hatching rates, and low larval viability. Therefore, the low FR, HR, and SR values observed at sampling site 2 are likely linked to the accumulation of oxidative damage caused by unfavorable environmental conditions, rather than differences in gonad maturity or broodstock size.

Interestingly, although physiological stress biomarkers differed markedly between sampling sites, broodstock morphometric characteristics remained relatively similar, suggesting that physiological responses occurred earlier than measurable changes in body condition. This finding highlights the high sensitivity of oxidative stress biomarkers as early-warning indicators of environmental deterioration and demonstrates their potential application for ecological monitoring and conservation of the endemic lempuk fish.

Reproductive performance

Reproductive performance declined at sampling point 2, indicated by lower fertilization rates (FR), hatching rates (HR), and survival rates (SR) at Table 4. The decline in reproductive performance is likely linked to oxidative damage triggered by excessive ROS production. Under stressful environmental conditions, ROS accumulation can overwhelm the antioxidant capacity provided by SOD and CAT, leading to lipid peroxidation, protein oxidation, DNA damage, and apoptosis in reproductive cells, which ultimately reduces gamete quality and fertilization success (Dcunha et al., 2022; Afzal et al., 2023). Similar findings were reported by Pintus and Ros-Santaella, (2021), who demonstrated that oxidative stress reduces sperm motility, membrane integrity, and fertilization success in several teleost fish species. Likewise, (Rumondang et al., 2024) observed that elevated ROS levels resulting from thermal stress significantly reduced egg quality and hatching success by impairing embryonic development. The consistency of these findings indicates that oxidative stress is a key physiological mechanism linking environmental degradation to reproductive impairment in freshwater fish.

Table 3. Value of oxidative stress in sampling point 1 and sampling point 2

	Sampling Point 1				Sampling Point 2			
	ROS	SOD	CORTISOL	CAT	ROS	SOD	CORTISOL	CAT
B1	10.35	5.75	2.15	32.4	11.25	5.9	2.4	32.4
B2	6.35	6.45	11.45	42.65	6.6	6.55	11.7	42.65
B3	12.55	5.05	17.6	40.8	12.95	5.25	18	40.8
B4	13.6	7.65	19.5	40.3	14	7.85	19.85	40.3
B5	6.1	7.15	26.3	34.5	6.45	7.35	26.7	34.5
J1	4.5	11.4	24.6	42.55	4.85	11.7	24.95	42.55
J2	13.25	8.45	20.9	42	13.55	8.65	21.3	42
J3	12.45	7.7	25.2	37.9	12.75	7.9	25.55	37.9
J4	4.45	7.55	26.7	42.45	4.75	7.55	27.1	42.45
J5	8.65	7.15	21.7	38.7	8.95	7.35	22.05	38.7

Note: These values represent pooled observations from the December 2025 and February 2026 sampling periods. B1–B5: female fish, and J1–J5: male fish.

Following fertilization, oxidative stress can continue to affect embryonic development by disrupting mitochondrial respiration and reducing ATP production; both processes are crucial for rapid cell division and tissue differentiation (Almansa et al., 2020). Excessive ROS levels also increase oxidative damage to embryonic DNA and cell membranes, resulting in developmental abnormalities, delayed hatching, and reduced hatchability (Marsico et al., 2023). The proposed mechanism is consistent with experimental studies using zebrafish (*Danio rerio*) which demonstrated that excessive ROS disrupts mitochondrial function, induces DNA damages, and impairs embryonic development, ultimately reducing hatching success (Zhao et al., 2016)

The larval stage appears highly vulnerable to environmental stress, as evidenced by the lower survival rates observed at sampling site 2. Newly hatched larvae possess relatively immature antioxidant defense systems, limiting their capacity to neutralize excess ROS. Consequently, oxidative stress disrupts mitochondrial energy metabolism, inhibits growth, impairs swimming ability, and reduces resilience to environmental stressors, ultimately leading to increased larval mortality (Gusnirwan et al., 2024). Similar responses have been reported in Nile tilapia (*Oreochromis niloticus*), where chronic oxidative stress significantly reduced larval survival and growth performance under hypoxic conditions (Odetayo et al., 2023). These parallels suggest that oxidative stress exerts a consistent impact on the early life stages of various freshwater fish species.

Overall, these findings indicate that reproductive performance is more sensitive to declining environmental quality than the morphometric characteristics of broodstock. The strong association between oxidative stress biomarkers and reproductive parameters suggests that FR, HR, and SR alongside ROS, SOD, CAT, and cortisol can serve as complementary biological indicators for assessing environmental quality and supporting conservation and domestication programs for the endemic lempuk fish.

Table 4. Value of egg performance (fertilization, hatching rate, and survival rate) in sampling point 1 and sampling point 2

Parameter	Sampling Point I	Sampling Point II
FR (%)	81	75.70
HR (%)	77.65	75.25
SR (%)	59.65	57.85

Note: These values represent pooled observations from the December 2025 and February 2026 sampling periods. FR: fertilization, HR: hatching rate, SR: survival rate.

Gonad maturity index

Female lempuk fish observed during both sampling periods were predominantly at gonad maturity stages III–IV and IV; this indicates that most of the broodstock had reached reproductive maturity and were ready to spawn a condition characterized by vitellogenesis, oocyte enlargement, and increased gonadal metabolic activity (Fekri et al., 2019). Histologically, these stages correspond to the development of yolk-rich, mature oocytes prior to spawning (Latifa et al., 2024). In contrast, males were primarily classified at stages I–II and II–III, reflecting early-to-intermediate stages of spermatogenesis, during which spermatogonia gradually develop into mature spermatozoa (Anitasari et al., 2024). Although gonadal conditions were generally mature, fish collected from sampling site 2 exhibited higher levels of physiological stress biomarkers (ROS, SOD, CAT, and cortisol); this suggests that environmental stress can affect gamete quality without significantly impacting gonadal maturity. Consequently, the lower fertilization rate (FR), hatching rate (HR), and survival rate (SR) observed at sampling site 2 were likely attributable to physiological stress rather than differences in gonadal maturity (Marsela et al., 2025). These findings demonstrate that gonadal maturity alone is insufficient to predict reproductive success under stressful environmental conditions. Elevated oxidative stress and cortisol levels can compromise oocyte and sperm quality despite morphological gonadal maturity, ultimately reducing reproductive performance. Therefore, integrating physiological stress biomarkers with assessments of gonadal maturity provides a more comprehensive evaluation of reproductive potential and environmental quality for the endemic lempuk fish. The level of gonad maturity index are presented in Table 5.

Fish health

Body length and body weight of male and female lempuk fish were relatively similar between sampling point in Table 6. Male fish measured 3.1–3.4 cm in length and weighed 0.19–0.24 g,

Table 5. The level of gonad maturity

	Sampling I		Sampling II	
	Sampling Point I	Sampling Point II	Sampling Point I	Sampling Point Ii
B1	III	III-IV	III	IV
B2	III-IV	IV	IV	IV
B3	III-IV	III	IV	III
B4	IV	III-IV	IV	III
B5	III-IV	IV	III	III-IV
J1	I-II	I-II	I-II	I-II
J2	II	I-II	III	II
J3	II	I-II	III	I-II
J4	I-II	I-II	I-II	II
J5	II	I-II	III	II

Note: B1–B5: female fish, and J1–J5: male fish.

whereas females measured 2.55–3.05 cm and weighed 0.17–0.22 g. Limited variation in morphometric characteristics indicates that environmental differences between sampling sites were not significant enough to cause measurable changes in somatic growth. Despite having relatively similar body sizes, fish collected from sampling site 2 exhibited significantly higher levels of ROS, SOD, CAT, and cortisol, as well as poorer reproductive performance, compared to those from sampling site 1. These findings indicate that physiological stress responses manifest earlier than detectable changes in morphometric characteristics. Environmental stress initially alters biochemical and endocrine processes to maintain cellular homeostasis, whereas changes in body length and weight generally require prolonged exposure to become apparent (Ulusu, 2026). Consequently, oxidative stress biomarkers serve as more sensitive indicators of declining environmental quality than conventional morphometric measurements (Valavanidis et al., 2006). Similar observations have been reported in other teleost fish, where oxidative stress increased significantly due to adverse environmental conditions without causing an immediate decline in body growth. For instance, Mehanna and Farouk, (2021) reported that fish exposed to environmental stress showed elevated oxidative stress biomarkers while maintaining relatively stable body length and weight; this indicates that physiological disturbances precede measurable changes in somatic growth. A similar pattern has been observed in gobies (family Gobiidae), including (*Glossogobius giuris*), where environmental degradation primarily affects physiological condition and reproductive capacity before impacting morphometric

Table 6. Body length and weight values of female and male fish

	Length		Weight	
	Sampling Point I	Sampling Point II	Sampling Point I	Sampling Point II
B1	2.55	2.7	0.19	0.195
B2	2.7	2.55	0.2	0.175
B3	2.75	3.05	0.195	0.22
B4	2.6	2.85	0.19	0.18
B5	2.7	2.5	0.195	0.17
J1	3.3	3.3	0.23	0.225
J2	3.15	3.35	0.195	0.235
J3	3.15	3.1	0.19	0.215
J4	3.4	3.2	0.24	0.21
J5	3.3	3.3	0.215	0.215

Note: B1–B5: female fish, and J1–J5: male fish.

characteristics (Ghosh et al., 2021). Thus, these findings demonstrate that body length and weight are less sensitive indicators of short-term environmental stress, whereas physiological biomarkers respond rapidly to changes in environmental quality. This supports the use of ROS, SOD, CAT, and cortisol as early-warning biomarkers for assessing the health status of the endemic lempuk fish and provides a stronger scientific basis for environmental monitoring and conservation programs at Ranu Grati Lake.

Premilinary analysis of sampling period

Prior to correlation and multivariate analyses, differences between the December and February sampling periods were evaluated using the Mann–Whitney U test. No significant differences were detected for the measured variables (all $p > 0.05$) (Table 7). Therefore, pooling of observations was considered appropriate, and data from both sampling periods were combined into a pooled dataset. The pooled dataset consisted of 20 pooled observations obtained by averaging measurements from the December 2025 and February 2026 sampling periods for each corresponding female and male fish at Sampling Point I and II. This pooled dataset was subsequently used for Spearman correlation and PCA.

Correlation analysis

Spearman correlation analysis revealed relationships ranging from very weak to strong (Table 8 and Table 9). Most correlations between physiological stress biomarkers and fish health parameters were weak and statistically non-significant ($p > 0.05$). However, SOD activity was positively correlated with body length (rs = 0.542, $p = 0.013$), whereas body length was negatively correlated with GSI (rs= −0.617, $p = 0.004$), indicating a difference in energy allocation between somatic growth and reproduction (Difinubun et al., 2023). Body length and body weight showed the strongest positive correlation (rs = 0.851, $p < 0.001$), consistent with general fish growth patterns (Froese, 2006). Overall, these results indicate that physiological stress biomarkers respond more sensitively to environmental variations than morphometric characteristics do. Overall, these findings indicate that physiological stress biomarkers respond earlier than morphometric characteristics and serve as sensitive indicators of environmental quality degradation in the endemic lempuk fish (Sigacheva and Skuratovskaya, 2022).

Relation of physiological biomarker with fish health

The PCA was performed as an exploratory multivariate approach to examine relationships among physiological stress biomarkers (ROS, SOD, CAT, and Cortisol), fish health and reproductive performance (Figure 2). PCA analysis revealed an ordination pattern in which cortisol and ROS were more closely associated with the distribution of observations than other physiological variables. Previous studies have reported that environmental

Table 7. Mann–Whitney U test

UJI two test						
ROS	SOD	CAT	Cortisol	Length	Weight	GMI
0.578	0.644	0.403	0.699	0.986	0.644	1.000

Table 8. Spearman's rank correlation coefficients (rs)

Parmeter	ROS	CAT	SOD	Cortisol	Length	Weight	IGS
ROS	-						
CAT	-0.234	-					
SOD	0.232	0.244	-				
Cortisol	-0.449	0.054	0.49285	-			
Length	-0.193	0.392	0.54182	0.452	-		
Weight	-0.290	0.356	0.22733	0.310	0.851	-	
GMI	0.193	-0.064	-0.304	-0.341	-0.617	-0.394	-

Table 9. Corresponding *p*-values

Parmeter	ROS	CAT	SOD	Cortisol	Length	Weight	IGS
ROS	-						
CAT	0.320	-					
SOD	0.3241	0.29857	-				
Cortisol	0.046	0.818	0.027	-			
Length	0.413	0.086	0.013	0.045	-		
Weight	0.213	0.122	0.335	0.183	0.0000019	-	
GMI	0.414	0.786	-0.304	0.14084	0.0037	0.084	-

stress is associated with an endocrine response involving cortisol secretion and oxidative stress characterized by increased ROS production (Rankin and Jensen, 1993). The patterns observed in this PCA are consistent with those reports; however, since PCA is an exploratory statistical method, it does not establish a cause-and-effect relationship or confirm that the biomarkers serve as universal indicators of environmental stress.

The association of cortisol with PC1 is biologically plausible because previous studies have show that activation of the hypothalamus-pituitary-interrenal (HPI) axis under environmental stress is commonly accompanied by elevated cortisol concentrations (Nesan and Vijayan, 2016). Elevated cortisol concentrations facilitate metabolic adjustments by diverting energy away from growth and reproduction to maintain homeostasis under unfavorable environmental conditions (Formicki et al., 2025). Similarly, the association of ROS with PC2 may reflect differences in environmental conditions between the sampling sites, including the relatively higher water temperature and lower dissolved oxygen concentration and lower pH recorded at sampling site 2. However, PCA alone cannot determine whether these environmental variables directly caused the observed physiological responses (Umeno et al., 2017).

The relatively low contribution of body length, body weight, and GSI indicates that these variables explained less of the multivariate variation observed in tte present dataset. Previous studies have similarly reported that physiological responses to environmental stress may become evident before detectable changes occur in morphometric or reproductive characteristics (Sánchez-Vázquez et al., 2019). Overall, PCA provided an exploratory overview of the multivariate relationships among physiological

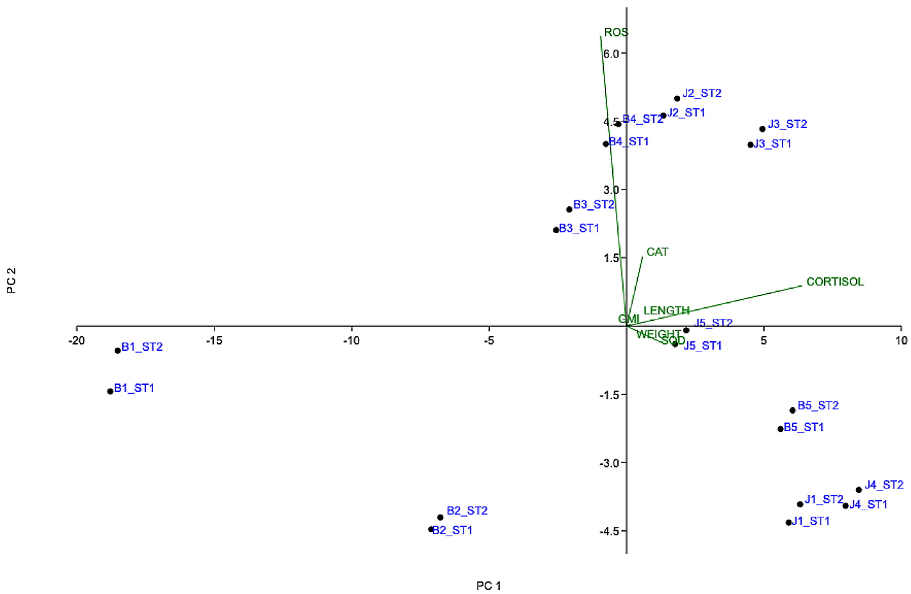

Figure 2. PCA biplot of physiological stress biomarkers, fish health, and GMI; B – female; J – male; ST1 – Sampling Station I; ST2 – Sampling Station II. Each point represents one pooled observation

biomarkers, fish health, and reproductive performance within the present dataset. Because the analysis was conducted using pooled observations and a relatively limited sample size, the resulting ordination patterns should be interpreted cautiously and should not be considered definitive evidence of biological mechanisms or universally applicable indicators of environmental stress. Further validation using larger datasets with individual-level observations is required to confirm the robustness and broader applicability of these findings.

CONCLUSIONS

Physiological stress in lempuk fish is characterized by elevated levels of ROS, SOD, CAT, and cortisol in both sexes. These physiological stress biomarkers correlate with variations in fish health indicators but do not indicate a significant decline in broodstock health. However, the increase in physiological stress coincides with low fertilization, hatching, and survival rates, suggesting that reproductive performance is more responsive to physiological stress than broodstock health.

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