

Dimorphism in oxidative stress markers in brain and testis of catfish broodstocks of *Clarias gariepinus* and *Heterobranchus longifilis*

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ABSTRACT

This study investigated species- and sex-related variations in oxidative status in brain and testis of *Heterobranchus longifilis* and *Clarias gariepinus*. Forty (40) catfish broodstocks each of *Clarias gariepinus* and *Heterobranchus longifilis* (20 males and 20 females per species) weighing 2.54 ± 0.12 and 2.11 ± 0.15 kg, respectively, were procured from reputable breeder farm in Ado Ekiti environs. Testicular and brain homogenates were obtained and assay for Antioxidant indices including catalase, superoxide dismutase, glutathione peroxidase, total antioxidant activity and lipid peroxidation. The results showed that superoxide dismutase and lipid peroxidation ($p < 0.05$) were higher in the left testis, while glutathione peroxidase and total antioxidant activity ($p < 0.05$) were higher in right testis of *Heterobranchus longifilis* catfish broodstocks. Glutathione peroxidase and superoxide dismutase ($p < 0.05$) were higher in right testis and lipid peroxidation ($p < 0.05$) was higher in left testis of *Clarias gariepinus* catfish broodstocks. Lipid peroxidation in female brain was significantly ($p < 0.05$) higher compare to males in *Clarias gariepinus* catfish broodstocks. Superoxide dismutase, glutathione peroxidase, lipid peroxidation and total antioxidants activity of *Heterobranchus longifilis* catfish broodstocks were significantly ($p < 0.05$) higher in the brain of males compared to females. In conclusion, variations in antioxidant enzyme activity and lipid peroxidation

observed have potential implications for reproductive health, fertility, and overall broodstock management.

Keywords: catfish, breeding, brain, oxidative stress markers, species- and sex-related variations.

INTRODUCTION

The fastest growing animal production sector in the whole world is Aquaculture due to heightened interest in fish as an animal source of protein (Iheanacho et al., 2018). *Clarias gariepinus* and *Heterobranchus longifilis* are two commercially important catfish species widely distributed in Africa especially in Nigeria. They exhibit differences in their ecological niches, reproductive strategies, and physiological responses. *Clarias gariepinus* and *Heterobranchus longifilis* are highly priced and widely accepted fish cultured species due to their taste, fast growth rate and ability to adapt to environmental conditions in small ponds. In the tropics, particularly Nigeria, where it is widely cultivated, *Heterobranchus longifilis* is a commercial fish meal with high levels of protein (Eriegha and Ekokotu, 2017). *Heterobranchus longifilis* has some benefits over *Clarias gariepinus* such as high growth rates, feed conversion, high yield but *Clarias gariepinus* mature early and had high fecundity (Quyến et al., 2022).

Oxidative stress is defined as the imbalance between the generation of reactive oxygen species (ROS) and the antioxidant defense systems (Chowdhury and Saikia, 2020). Oxidative stress is a major determinate in a wide range physiological and pathological processes, notably influencing reproduction and brain function. Genetic variability refers to the variation in DNA sequences among individuals of the same species (Suleiman et al., 2023) and can have a major impact on how individuals in the same species react to oxidative stress. Genetic diversity significantly influences an organism's reaction to external challenges, such as oxidative stress (Zhou et al., 2023). Oxidative damage can negatively impact reproductive functions by affecting sperm quality, oocyte development, and hormone regulation (Jenila et al., 2023).

Exploring genetic variations in oxidative stress markers in *Clarias gariepinus* and *Heterobranchus longifilis* might shed light on their adaptability and reproductive ability (Haymer and Khedkar, 2022). The brain's high metabolic rate and quantity of polyunsaturated fatty acids make it vulnerable to ROS damage. However, the testis, which generates sperm, is very susceptible to oxidative stress, which can affect the fertility and quality of sperm. Reproductive performance in catfish broodstocks may be impacted by genetic variations in oxidative stress indicators in the brain and testis.

Male infertility has been linked to oxidative stress (Aitken, 2018), which also includes reproductive toxicity particularly from exposure to environmental

pollutants and testicular dysfunction (Vaos and Zavras, 2017). It is believed that oxidative stress during the spermatid stage is the cause of functional problems in the spermatozoa later on, especially their ability to recognize sperm eggs (Bromfield et al., 2017). Differences in antioxidant enzyme activities and lipid peroxidation levels can influence sperm quality, oocyte development, hormone regulation, and overall reproductive performance (Mehrim et al., 2022, Tao et al., 2023). The brain and testis were chosen as the target tissues for this study because they play important roles in reproduction and are susceptible to oxidative stress (Mansour et al., 2023). Studying these species- and sex-related variations, we can gain insights into their adaptability, reproductive potential, and develop strategies to improve their productivity.

Understanding these species- and sex-related variations can aid in the development of breeding programs for improved reproductive success and productivity. This study aims to investigate the species- and sex-related variations in oxidative stress markers in the brain and testis of catfish broodstocks of *Clarias gariepinus* and *Heterobranchus longifilis*.

MATERIALS AND METHODS

Ethics approval and consent to participate

All experimental procedures involving animals were conducted in accordance with the established conditions and guidelines for the use of animals in research. Ethical approval for this study was granted by the Research and Ethics Committee of The Federal Polytechnic, Ado-Ekiti, Ekiti State, Nigeria (Approval No: AP/REC/2022/0435).

Experimental animals and management

Forty (40) catfish broodstocks of *Heterobranchus longifilis* and *Clarias gariepinus* (20 males and 20 females each species) were purchased from respectable breeder farms in the Nigerian states of Ondo, Ekiti, and Ogun Osun. The weights of the broodstocks for *Heterobranchus longifilis* and *Clarias gariepinus* were 2.11 ± 0.15 kg and 2.54 ± 0.12 kg, respectively. Selection of broodstocks was based on genital readiness; males were identified by the presence of reddish and pointed genital papillae, while gravid females were selected based on abdominal distension and the presence of well-developed oocytes. From the farms to the Fisheries laboratory, brood stocks were moved in plastic troughs. After a week of acclimation and conditioning in different tanks, the brood stocks were fed commercial pelleted feed containing 40% crude protein at a weight of 3% twice a day at 9:00 and 18:00. Water quality parameters are as indicated; dissolved oxygen (4.16 mg/l), total dissolved

solids (77.46 mg/l), total suspended solids (0.67 mg/l), biochemical oxygen demand (10.24 mg/l), chemical oxygen demand (21.76 mg/l).

Testicular tissue preparation and oxidative stress assay

The male catfish were killed by cervical dislocation, which, resulted in rapid unconsciousness, and death was verified by the stoppage of circulation. The testes were then carefully separated and freed of any connective tissues. They were sorted into right and left testes and then homogenized in phosphate buffer for biochemical analysis. Testicular homogenates were centrifuged at 20,000RPM for 30 minutes to test for antioxidant enzymes, lactate dehydrogenase, total protein, total antioxidant activity, and lipid peroxidation.

Brain tissues preparation and oxidative stress assay

Each fish's skull was carefully dissected and its brain removed. The whole brain was taken within one minute and homogenized in 10% w/v cold potassium phosphate buffer (50 mM, pH 7.4). The homogenate was centrifuged at 20,000 RPM for 30 minutes to measure antioxidant enzymes, total protein, antioxidant activity, and lipid peroxidation. The protein and lactate dehydrogenase content of the samples were determined using commercial test kits from Fortress Diagnostics Limited UK with product codes BXC0242 and BXC0173, respectively. The activities of antioxidant enzymes catalase, superoxide dismutase, and glutathione peroxidase, as well as total antioxidant activity and lipid peroxidation, were measured in accordance with Jimoh (2019). Each fish represented an independent biological replicate. All biochemical assays were performed in duplicate to ensure analytical reliability, and mean values were used for statistical analysis.

Total Antioxidant activity

A reactive mixture comprising 0.5 mL of Na-Benzoate (10 mmol/L), 0.2 mL of H₂O₂ (10 mmol/L), 0.49 mL of phosphate buffer (100 mmol/L, pH = 7.4) (made by combining 19.5 mL of KH₂PO₄ (100 mmol/L) with 80.5 mL of Na₂HPO₄ (100 mmol/L), and 0.2 mL of Fe-EDTA complex (2 mmol/L) (freshly made by combining equal volumes of EDTA (2 mmol/L) and ammonium ferrous sulfate (2 mmol/L) and letting it stand at 25 °C for 60 minutes). Ten microliters of the serum were added to the latter reactive mixture, which was then incubated for sixty minutes at 37 °C. Following a 10-minute incubation period at 100 °C, 1 mL of glacial acetic acid (20 mmol/L) and 1 mL of thiobarbituric acid (0.8% w/v in 100 mL of 50 mmol/L NaOH) were added, and the absorbance at 532 nm was determined spectrophotometrically. The following formula was used to determine total antioxidant activity:

$$\text{Total antioxidant activity (mmol/L)} = (\text{CUA}) (K - A) / (K - \text{UA})$$

where CUA is the concentration of uric acid (mmol/L); K is the absorbance of the control ($K1 - K0$); A is the absorbance of the sample ($A1 - A0$); UA is the absorbance of uric acid solution ($UA1 - UA0$).

Lipid Peroxidation

There were 1.0 mL of TCA (0.67%) and 1.0 mL of serum in the reaction mixture, which had a total volume of 3.0 mL. Every test tube was immersed in a kettle of boiling water for forty-five minutes. After being transferred to the cold bath, the tubes were centrifuged for ten minutes at 2500 rpm. The quantity of malondialdehyde (MDA) generated in each sample was ascertained by measuring the optical density of the supernatant at 532 nm.

Glutathione Peroxidase (GPx) Assay

The 0.5 ml 0.4 M buffer (pH 7.0), 0.2 ml enzyme source, 0.2 ml 2 mM GSH, and 0.1 ml 0.2 mM H₂O₂ were added, and the tube was incubated at room temperature for 10 minutes. A control tube was also included, which included all the reagents except the enzyme source. After adding 0.5 ml of 10% TCA to stop the reaction, the mixture was centrifuged for five minutes at 4000 rpm, and the amount of GSH in the 0.5 ml of supernatant was calculated. The activity was expressed as g/min/mg protein using GSH consumption.

Catalase (CAT) Assay

The test equipment contained 1.0 ml of 0.059 M H₂O₂ and 1.9 ml of 0.05 M buffer (pH 7.0). The reaction began with the addition of 0.1 mL of enzyme source (homogenate). The reduction in absorbance at 240 nm was monitored every 1 minute for 5 minutes, and activity was calculated as nmoles of H₂O₂ decomposed per minute per mg of protein.

Superoxide Dismutase (SOD) Assay

0.02 ml of the enzyme source, 0.86 ml of pure water, and 2.1 ml of 50 mM buffer. 0.02 ml of 10 mM pyrogallol was used to start the reaction, and the absorbance change was measured at 420 nm. In a standard test method of 3 ml, one unit of SOD was defined as the quantity of enzyme needed to 50% block the auto-oxidation of pyrogallol. Units/min/mg protein were used to express the particular activity.

Statistical analysis

Data obtained were analyzed using a T-test at a significance level of $p=0.05$, employing the General Linear Model (GLM) procedure of IBM SPSS Version 25 after multivariate analysis showed no interaction between species and sex.

RESULTS

Oxidative stress markers in testis of *Clarias gariepinus* broodstocks is presented in Table 1. The superoxide dismutase (SOD), glutathione peroxidase (GPx) and lipid peroxidation showed significant difference ($p < 0.05$) between the left and right testis of *Clarias gariepinus*. The SOD and GPx were significantly ($p < 0.05$) higher in right testis compared to the left testis while lipid peroxidation is higher in left testis than the right testis of the fish. There was no significant ($p > 0.05$) difference observed in catalase and total antioxidant activity of the fish.

Table 1. Oxidative stress markers in testis of *Clarias gariepinus* broodstocks

<i>Clarias gariepinus</i>	Left testis	Right testis
Catalase (nm H ₂ O ₂ /min/mg protein)	10.30±1.20	9.77±0.33
Superoxide dismutase (U/min/mg protein)	2.44±0.97 ^b	4.00±1.39 ^a
Glutathione peroxidase (mg GSH/min/mg protein)	9.04±1.65 ^b	19.67±8.43 ^a
Lipid peroxidation (x10 ⁻⁵ MDA/mg protein)	3.68±2.49 ^a	1.43±0.05 ^b
Total antioxidant activity (mmol/L)	4.67±1.53	2.39±0.55

The oxidative stress markers in testis of *Heterobranchus longifilis* broodstocks is presented in Table 2. The SOD and lipid peroxidation were significantly ($p < 0.05$) higher in the left testis of *Heterobranchus longifilis* broodstocks, while GPx and total antioxidant activity were higher in right testis of the fish. There was no significant ($p > 0.05$) difference in the catalase in testis of the *Heterobranchus longifilis* broodstocks fish.

Table 2. Oxidative stress markers in testis of *Heterobranchus longifilis* broodstocks

<i>Heterobranchus longifilis</i>	Left testis	Right testis
Catalase (nm H ₂ O ₂ /min/mg protein)	8.22±0.65	9.80±1.06
Superoxide dismutase (U/min/mg protein)	4.50±1.99 ^a	2.00±1.05 ^b
Glutathione peroxidase (mg GSH/min/mg protein)	7.46±1.86 ^b	12.38±5.65 ^a
Lipid peroxidation (x10 ⁻⁵ MDA/mg protein)	1.15±0.43 ^a	0.83±0.26 ^b
Total antioxidant activity (mmol/L)	3.10±2.05 ^b	4.67±1.17 ^a

The oxidative stress markers in brain of *Clarias gariepinus* broodstocks is shown in Table 3.

Table 3. Oxidative stress markers in brain of *Clarias gariepinus* broodstocks

<i>Clarias gariepinus</i>	Female	Male
Catalase (nm H ₂ O ₂ /min/mg protein)	9.94±1.79	11.22±1.90
Superoxide dismutase (U/min/mg protein)	3.70±1.11	2.76±0.53
Glutathione peroxidase (mg GSH/min/mg protein)	9.94±2.09	14.18±4.07
Lipid peroxidation (x10 ⁻⁵ MDA/mg protein)	6.54±4.97 ^a	1.64±0.33 ^b
Total antioxidant activity (mmol/L)	9.22±6.36	6.06±2.45

It was observed that the lipid peroxidation in the brain of female was significantly ($p < 0.05$) higher than the male counterparts. Moreover, there was no significant ($p > 0.05$) difference in catalase, SOD, GPx and total antioxidants activity of both genders of *Clarias gariepinus*.

The oxidative stress markers in brain of *Heterobranchus longifilis* is shown in Table 4. There was significant difference ($p < 0.05$) in SOD, GPx, lipid peroxidation and total antioxidant activity of both genders in *Heterobranchus longifilis*.

Table 4. Oxidative stress markers in brain of *Heterobranchus longifilis* broodstocks

<i>Heterobranchus longifilis</i>	Female	Male
Catalase (nm H ₂ O ₂ /min/mg protein)	10.41±1.32	12.07±1.29
Superoxide dismutase (U/min/mg protein)	4.09±0.26 ^b	7.51±2.40 ^a
Glutathione peroxidase (mg GSH/min/mg protein)	8.29±1.27 ^b	14.62±3.08 ^a
Lipid peroxidation (x10 ⁻⁵ MDA/mg protein)	2.17±0.50 ^b	3.39±1.19 ^a
Total antioxidant activity (mmol/L)	2.90±2.33 ^b	7.45±3.01 ^a

The SOD, GPx, lipid peroxidation and total antioxidants activity were significant ($p < 0.05$) higher in the brain of the male compared to females. There was no significant difference in catalase of both genders.

DISCUSSION

Aquaculture hybrid production had allowed improved culture parameters such as high growth rates, fillet yield and low mortality which had influenced economic conditions majorly in the tropics. It was opined that progeny from the same broodstocks source could contain high species- and sex-related variations or similarities as it was reported by Zhang et al. (2012). In the present study, the genetic variations were investigated in oxidative stress markers in testis of *Clarias gariepinus* and *Heterobranchus longifilis*. SOD was higher in right testis of *Clarias gariepinus*, while, SOD was higher in left testis of *Heterobranchus longifilis*. GPx is higher in the right testis of both fish species, but lipid peroxidation is higher in left testis of both fish species. The work emphasizes the significance of examining the effects of oxidative stress on various fish organs and contributes to the expanding body of research on oxidative stress in fish. The study discovered that the left and right testis of *Clarias gariepinus* had significantly different SOD, GPx, and lipid peroxidation activities. Specifically, SOD and GPx activities were higher in the right testis than the left testis. However, there was no significant differences observed in catalase and total antioxidant activity of the fish. The results indicates that oxidative stress may be affecting the testes of the fish, with higher levels of SOD and GPx indicating a higher defense to oxidative stress. Tyor and Pahwa (2018) reported that *Clarias gariepinus* from the River Yamuna in the Delhi

region showed cellular abnormalities and testicular oxidative stress, could explain differences in the expression of the enzymes obtained in this study. While exposure to mercury and lambda-cyhalothrin has been associated with the induction of oxidative stress and decrease SOD and GPx activity in the testicles of *Clarias gariepinus* (Ibrahim et al., 2019, Ezenwosu et al., 2021). The findings of this study suggest that higher SOD and GPx activity in right testis in *Clarias gariepinus* confer greater protection against oxidative assault, and accounts lower lipid peroxidation in the right testis in comparison to left testis. SOD and GPx are two important antioxidant enzymes that play a role in regulating testicular and epididymal redox balance in fish (Khaled et al., 2023). SOD and GPx are key enzymes involved in regulating redox balance, protecting lipids, and supporting spermatogenesis in the testis of fish. SOD and GPx work together to minimize oxidative stress in the testis by neutralizing ROS and preventing damage to cells and tissues. They are also involved in responding to stressors and lowering oxidative stress, which can have detrimental effects on fish health (Afzal et al., 2023). SOD and GPx help to regulate the redox balance in the testis by converting superoxide anions and hydrogen peroxide into less harmful molecules (Albogami, 2023). And could explain the higher lipid peroxidation in left testis *Heterobranchus longifilis* despite its higher SOD, while higher GPx in right testis led to lower lipid peroxidation.

Lipid peroxidation is a process that occurs when free radicals attack the polyunsaturated fatty acids in cell membranes, leading to the formation of lipid peroxides. Lipid peroxidation can reduce the antioxidant capacity of the testis by depleting antioxidants (Slaninova et al., 2009), and disrupt the redox balance in the testis by generating ROS and lowering the activity of antioxidant enzymes such as SOD and GPx (Hoseinifar et al., 2020).

The study found that there were notable differences in SOD and lipid peroxidation between the left and right testis of *Heterobranchus longifilis* broodstocks. SOD and lipid peroxidation levels were higher in the left testis than in the right testis. In contrast, the fish's right testis had higher levels of GPx and total antioxidant activity. There was no significant change in catalase levels between the fish's left and right testis. The findings imply that oxidative stress is harming the fish's testes, with greater levels of SOD and lipid peroxidation indicating an oxidative stress response (Jimoh et al., 2021).

The differences in SOD and lipid peroxidation between the left and right testis of *Heterobranchus longifilis* broodstocks in this study may have several implications for testicular function and fertility, the response to stressors and antioxidant capacity of the testes. The higher lipid peroxidation in the left testis may indicate the presence of testicular oxidative stress in that organ (Tyor and Pahwa, 2018, Jimoh et al., 2021), and could have negative effects on testicular function and fertility. The observed differences in SOD and lipid peroxidation between the left and right testis may indicate a redox imbalance

in the testes of the fish (Tyor and Pahwa, 2018, Owolabi and Abdulkareem, 2021). This could lead to oxidative damage and cellular deformities in the testis, which could affect testicular function and fertility. The differences in SOD and lipid peroxidation between the left and right testis may be due to a differential response to stressors such as pollutants and other oxidative stressors (Guerriero et al., 2014). This could imply differential susceptibility of the testes to oxidative stress. The higher levels of GPx and total antioxidant activity in the right testis may indicate a higher antioxidant capacity in that organ (Meng et al., 2021). This may contribute to protecting the right testis from oxidative damage and maintain its function. The study found significant differences in the activity levels of SOD, GPx, and lipid peroxidation between the left and right testes of *Clarias gariepinus* and *Heterobranchus longifilis*. This suggests that there may be asymmetrical oxidative stress responses within the testes of these catfish species. The findings highlight the importance of understanding oxidative stress mechanisms in reproductive tissues, as they may influence the reproductive health and management of these catfish species. Additional research is needed to clarify the mechanisms and their potential implications for breeding programs and aquaculture management.

The brain regulates important systemic operations through the activity of neurons located throughout the body (Schwartz, 2016). Exposure to toxic components can have a significant impact on brain homeostasis and, as a result, the entire system (Jaishankar et al., 2014). The finding showed that there was no significant difference in catalase, SOD, GPx, and total antioxidant activity between male and female of *Clarias gariepinus* broodstock. However, it was observed that lipid peroxidation in the brain of female fish was significantly higher compared to male fish. of *Clarias gariepinus* broodstock. This suggests that female fish might be more exposed to oxidative damage in the brain compared to male fish. There are several other factors that can cause differences in antioxidant activity between the male and female of *Clarias gariepinus* broodstock. A similar study found that there were significant differences in metabolic and antioxidant status between male and female of *Clarias gariepinus* broodstock (Jimoh et al., 2021). Catalase, SOD, and GPx are major antioxidant enzymes that defend against oxidative stressed induced cellular and molecular damage (Demirci-Cekic et al., 2022), by neutralizing ROS and decrease oxidative damage to cells and tissues and helps to maintain the proper balance of oxidants and antioxidants. The study found that there was no significant difference in catalase activity between male and female fish brain of *Heterobranchus longifilis* broodstock. However, SOD, GPx, lipid peroxidation, and total antioxidant activity were observed to be significantly elevated in the brain among males compared to females' brain of *Heterobranchus longifilis* broodstock. These results suggest that there may be notably differences in oxidative stress and antioxidant defense between male

and female fish brains of *Heterobranchus longifilis* broodstock. This suggests that the oxidative stress response may vary between male and female individuals of this species. Exposure to stressors such as pollutants and other oxidative stressors can affect antioxidant activity in fish (Vranković et al., 2021). Changes in catalase, SOD, GPx, and total antioxidant activity can indicate a response to stressors and provide insight into the health status of the fish. Monitoring these markers can provide valuable information about the health status of fish and help to identify potential health problems. The observed differences in SOD, GPx, lipid peroxidation, and total antioxidant activity between male and female fish brains of *Heterobranchus longifilis* broodstock may have implications for brain function and health (Sobočanec et al., 2003, Shim and Kim, 2013, Lee et al., 2020). Oxidative stress and lipid peroxidation can cause cellular deformities and reduced function in the brain, while key antioxidant enzymes like the SOD and GPx can protect against oxidative damage and maintain brain health. The observed differences in SOD, GPx, lipid peroxidation, and total antioxidant activity may be due to sexual dimorphism, which is the physical and physiological differences among males and females within the same species (Mruk et al., 2002, Shim and Kim, 2013). Differences in hormonal profiles, metabolism, and other factors may contribute to the observed differences. The observed differences in SOD, GPx, lipid peroxidation, and total antioxidant activity may indicate a differential response to stressors such as pollutants and other oxidative stressors (Owolabi and Abdulkareem, 2021). Elevated level of lipid peroxidation were observed in the brain of female catfish compared to males in this study, suggesting potential sex-specific susceptibility to oxidative stress. The higher lipid peroxidation observed in the brain of female fish suggests increased susceptibility to oxidative stress. This may have implications for physiological and reproductive functions; however, this relationship remains speculative and requires further investigation through dedicated reproductive studies. Male and female fish may have different antioxidant defense systems and responses to stressors, which could affect their susceptibility to oxidative stress and other health problems. The result of this study contradict that of Sobocanec et al. (2003) that found no significant sex or age differences in brain SOD levels between male and female mice while Strehlow et al. (2003) observed decreased superoxide production accompanied by increased SOD activity observed in the brain of female rats treated with estrogen (Wusu et al., 2021). Previous studies suggest that hormonal differences influence antioxidant activity in the brain of mice, rats, and fish. However, further research is required to understand the underlying mechanisms and their implications for fish health.

CONCLUSION

Findings from the study emphasize the influence of oxidative stress markers on reproductive physiology in *Clarias gariepinus* and *Heterobranchus longifilis*. The study confirms that testicular location and gender influence oxidative stress responses. Right testes generally exhibit higher antioxidant activity, suggesting that selecting broodstocks with strong antioxidant defense mechanisms could enhance reproductive success. Breeding programs should prioritize fish with higher superoxide dismutase and glutathione peroxidase activity in their reproductive organs for better fertility and sperm quality. Higher lipid peroxidation in female brains and left testes suggests a vulnerability to oxidative stress, which could have potential implications for reproductive physiology. Environmental stressors (e.g., poor water quality, pollutants) could exacerbate oxidative damage. Maintaining optimal water quality (dissolved oxygen, pH balance, temperature control) and reducing exposure to pollutants can minimize oxidative stress and improve overall reproductive health. Higher antioxidant enzyme activity correlates with lower lipid peroxidation, implying that dietary antioxidants can play a protective role against oxidative damage. Formulating fish feed with antioxidant-rich supplements (such as vitamins C and E, selenium) can enhance reproductive efficiency, particularly in broodstocks with higher lipid peroxidation levels. Routine monitoring of oxidative stress markers in hatchery operations can serve as an early detection system for reproductive issues, allowing for timely interventions such as dietary adjustments, improved water management, or selective breeding measures. Future research should focus on further optimizing antioxidant strategies and understanding genetic factors influencing oxidative stress resilience.

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