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A Review of Copper Sulphate-Induced Hormonal Dysregulation in the Reproductive Physiology of Freshwater Teleosts

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Abstract: Copper sulphate is highly valued for its fungicidal and algaecidal qualities, making it one of the most commonly used agrochemicals and aquaculture treatments in the world. In spite of the fact that it has a number of applications, copper sulphate has become a substantial contaminant in freshwater systems. This is mostly the result of runoff from agricultural land, effluent discharge, and excessive usage in aquaculture. The emphasis of this study is on the intricate ways in which copper sulphate influences the reproductive physiology of freshwater teleost fishes. This group of fishes accounts for a significant amount of the biological variety found in freshwater environments and is essential for maintaining ecological equilibrium and economic subsistence. The processes of hormonal dysregulation, which disrupt the endocrine system that controls gonadal development, gametogenesis, and reproductive behaviours, have given a lot of attention. These mechanisms are responsible for the failure of reproductive success. Copper's interaction with the hypothalamic-pituitary-gonadal (HPG) axis, its generation of oxidative stress, its interference with steroidogenesis, and its disruption of hormone signalling cascades are all investigated in great detail. This review further explores the ramifications of these hormonal imbalances on the dynamics of fish populations and aquatic ecosystems. It also highlights upcoming research paths and analyses the obstacles that are now being faced in identifying and mitigating the effects of copper toxicity for fish populations.

Keywords: Copper sulphate, freshwater teleosts, hormonal dysregulation, reproductive physiology, endocrine disruption, hypothalamic-pituitary-gonadal axis, sex steroids, oxidative stress

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INTRODUCTION

Copper is a micronutrient that is essential for a wide variety of biological processes that are carried out by a wide variety of taxonomic groups, including freshwater teleost fishes. It plays an essential part in enzymatic activities such as the action of cytochrome c oxidase in the electron transport chain, the antioxidant defence that is mediated by superoxide dismutase, and other metalloprotein functions that are essential to the maintenance of cellular homeostasis and metabolism [1]. The potential toxicity of copper when quantities surpass physiological limits is a counterweight to the requirement of copper at trace levels. This equilibrium is being progressively upset by human impacts, which are causing the balance to be broken. Copper concentrations in freshwater ecosystems across the globe have dramatically increased as a result of a number of factors, including industrial effluents; agricultural runoff, mining operations, and the extensive use of copper-based compounds for example copper sulphate [2]. The increased concentrations provide significant difficulties for aquatic life, in particular for fish species that are very sensitive to changes in the chemical composition of the water sources [3].

Due to the fact that it has antibacterial qualities, copper sulphate (CuSO4) is widely used in aquaculture as an efficient fungicide, algaecide, and parasite control agent. Within the realm of agriculture, it functions as a fungicide and bactericide, therefore safeguarding crops against a wide range of dangerous organisms [4]. However, the continual use of copper sulphate and its discharge without discrimination have led to its buildup in aquatic habitats, which often results in situations in which resident biota are exposed to copper sulphate for an extended period of time [5]. When it comes to freshwater systems, the bioavailability of copper ions is affected by a number of physicochemical parameters [6]. These factors include pH, hardness, dissolved organic carbon, and competing ions, all of which have the power to change the toxicity profile of copper ions. Copper continues to be a powerful stressor, capable of producing oxidative damage, altering ionoregulation, and compromising essential physiological systems in fish. This is despite the fact that copper is a complex substance [7].

Copper poisoning may have a major impact on the reproductive system, which is one of the most susceptible and ecologically relevant targets available. The reproductive health of freshwater teleost fishes is orchestrated by the hypothalamic-pituitary-gonadal (HPG) axis, a highly integrated neuroendocrine network that governs reproductive timing, gametogenesis, steroidogenesis, and reproductive behaviours through the secretion and regulation of gonadotropin-releasing hormones, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and sex steroids such as oestradiol and testosterone [8] [9]. Copper is one of the environmental toxicants that may cause this axis to be disrupted, which can result in a cascade of negative consequences such as altered hormone levels, poor gamete development, decreased spawning success, and limited offspring survivability. These physiological deficits may translate into effects at the population level, which in turn threatens biodiversity, ecological stability, and the productivity of fisheries [10].

In order to provide a comprehensive summary of the existing information about the effects of copper sulphate exposure on the reproductive physiology of freshwater teleost fishes, this review will be comprehensive [11]. It will investigate the biochemical and molecular processes that are responsible for endocrine disruption. These mechanisms include oxidative stress induced damage to reproductive tissues, interference with steroidogenic enzymes, and altered gene expression within the HPG axis [14]. On the other hand, behavioural investigations that highlight alterations in mating rituals and spawning behaviours will be studied with histopathological data that record gonadal degeneration, follicular atresia, and testicular injury. Within the perspective of wider environmental and conservation problems, the ecological repercussions of these reproductive deficiencies will be contextualized [12].

Through the incorporation of data from laboratory tests, field research, and molecular investigations, the purpose of this thorough study is to shed light on the complex nature of the effects that copper sulphate has on fish reproductive [15]. A discussion of the present gaps in knowledge, the proposal of methodological considerations for future study, and the suggestion of practical ways for minimising copper contamination in freshwater ecosystems will also be included. In the end, the purpose of this synthesis is to provide information that can be used to guide environmental policies and aquaculture practices on the subject of protecting aquatic biodiversity and assuring sustainable fisheries resources in the face of continued challenges caused by human activity [16].

COPPER SULPHATE CONTAMINATION IN FRESHWATER SYSTEMS

Copper sulphate contamination in freshwater ecosystems has become a critical environmental concern due to its extensive use and persistence [17]. This section explores the sources of copper sulphate contamination, its chemical behavior in aquatic environments, factors affecting its bioavailability and toxicity, and the implications for freshwater teleost fishes [18].

Sources of Copper Sulphate in Freshwater Environments

Copper sulphate ($CuSO_4$) enters freshwater systems through multiple anthropogenic pathways:

- **Agricultural Runoff:** The widespread application of copper sulphate as a fungicide and bactericide in crop protection leads to its runoff into nearby water bodies, particularly during rain events. Irrigation return flows also contribute to copper inputs.
- **Aquaculture Practices:** Copper sulphate is commonly used in aquaculture ponds and tanks to control fungal infections and parasitic infestations. Repeated treatments result in accumulation within the water and sediment, creating chronic exposure scenarios [20].
- **Industrial and Municipal Wastewater:** Effluents from industries such as mining, electroplating, and chemical manufacturing, as well as sewage treatment plants, often contain elevated levels of copper, including copper sulphate, which enter freshwater bodies.
- **Atmospheric Deposition:** Although less significant than direct discharge, atmospheric deposition from industrial emissions can also contribute trace amounts of copper to surface waters [19].

Chemical Speciation and Forms of Copper in Water

Upon entering aquatic environments, copper exists in various chemical forms, which dictate its mobility, bioavailability, and toxicity:

- **Free Ionic Copper (Cu^{2+}):** The most bioavailable and toxic form, free Cu^{2+} readily interacts with biological membranes, enzymes, and DNA, causing oxidative damage and disrupting cellular function.
- **Complexed Copper:** Copper ions often bind with organic ligands (such as humic and fulvic acids) or inorganic ligands (carbonates, hydroxides), reducing the concentration of free ions. These complexes can vary in stability and bioavailability.
- **Particulate and Sediment-Bound Copper:** Copper adsorbed onto suspended particulates or sediments is less bioavailable but can act as a reservoir, releasing copper ions under changing environmental conditions such as pH shifts or redox reactions [21] [22].

Environmental Factors Influencing Copper Bioavailability and Toxicity

Copper's toxicity in freshwater systems is not solely dependent on its concentration but is strongly influenced by physicochemical water parameters:

- **Water pH:** Acidic waters (low pH) increase the solubility of copper, leading to higher concentrations of free Cu^{2+} ions and enhanced toxicity. Conversely, alkaline conditions reduce copper availability.
- **Water Hardness:** Higher hardness, characterized by elevated concentrations of calcium and magnesium ions, mitigates copper toxicity by competing for binding sites on biological membranes and forming less

toxic copper complexes.

- **Dissolved Organic Carbon (DOC):** Organic molecules in water can bind copper ions, forming complexes that reduce free copper concentrations. The quality and quantity of DOC influence the extent of copper complexation.
- **Temperature and Salinity:** Elevated temperatures can increase copper toxicity by enhancing metabolic rates, while salinity affects copper speciation, particularly in estuarine environments [23] [25].

Bioavailability and Uptake of Copper in Freshwater Teleost Fishes

Freshwater teleosts are particularly susceptible to copper contamination due to their physiology:

- **Gill Permeability:** Gills are highly vascularized and have large surface areas facilitating gas exchange but also providing a direct pathway for copper ions to enter the bloodstream. Copper exposure can damage gill epithelia, impairing respiratory and osmoregulatory functions.
- **Accumulation and Tissue Distribution:** Copper accumulates in vital organs such as the liver, kidney, and gonads. The liver acts as a detoxification center, but chronic accumulation can cause hepatotoxicity and systemic physiological disturbances.
- **Sublethal Effects:** Even at concentrations below acute toxicity thresholds, copper can induce oxidative stress, disrupt ion regulation, and impair endocrine function, especially affecting reproduction [26] [28].

Global Trends and Ecological Implications

Copper sulphate contamination and its effects have been documented worldwide:

- **Chronic Exposure Incidences:** Long-term exposure to copper at environmentally relevant concentrations has been reported in rivers, lakes, and aquaculture systems across Asia, Europe, North America, and Africa.
- **Impact on Fish Populations:** Sublethal copper toxicity impairs critical physiological functions, leading to reduced growth, compromised immune responses, altered reproductive success, and increased mortality in sensitive species [29].
- **Bioindicator Role of Fish:** Due to their sensitivity to metal pollution and their position in aquatic food webs, freshwater fish serve as effective bioindicators, reflecting the health and contamination status of their habitats.

Understanding copper speciation and bioavailability in freshwater environments is fundamental to assessing the ecological risks posed by copper sulphate contamination. Environmental variables modulate the extent of copper toxicity, while the vulnerability of teleost fishes highlights the importance of monitoring and managing copper inputs to preserve aquatic biodiversity and sustain fisheries [27].

OVERVIEW OF REPRODUCTIVE PHYSIOLOGY IN FRESHWATER TELEOSTS

The reproductive physiology of freshwater teleost fishes is a highly coordinated and complex process primarily regulated by the hypothalamic-pituitary-gonadal (HPG) axis. This neuroendocrine system integrates internal physiological states with external environmental signals to control the timing,

progression, and success of reproduction [8]. Understanding the functioning of the HPG axis is crucial for comprehending how environmental stressors, such as copper sulphate contamination, interfere with reproductive health and fitness in these species [30].

The Hypothalamus: Initiator of the Reproductive Cascade

At the apex of the HPG axis lies the hypothalamus, a specialized brain region that serves as the principal regulatory center for reproductive function. The hypothalamus synthesizes and secretes gonadotropin-releasing hormones (GnRHs), neuropeptides that are released in a pulsatile manner into the hypophyseal portal circulation [32]. These GnRH molecules act as the key signaling messengers that stimulate the anterior pituitary gland to release gonadotropins. In teleosts, multiple forms of GnRH may exist, each with distinct roles in reproductive regulation, reflecting evolutionary adaptations to diverse ecological niches [31].

The Pituitary Gland: The Hormonal Relay Station

Upon receiving stimulation from GnRH, the anterior pituitary gland secretes two principal gonadotropins: luteinizing hormone (LH) and follicle-stimulating hormone (FSH) [34]. These glycoprotein hormones serve as critical regulators of gonadal activity:

- **Follicle-Stimulating Hormone (FSH):** Primarily involved in early gametogenesis, FSH promotes the proliferation and maturation of germ cells. In females, it stimulates ovarian follicle development and vitellogenesis (yolk deposition), while in males, it supports spermatogonial proliferation.
- **Luteinizing Hormone (LH):** LH plays a vital role in final gamete maturation, ovulation in females, and steroidogenesis. It triggers the production of sex steroids and is often involved in regulating spawning behaviors and timing.

The release of LH and FSH is finely tuned by feedback from gonadal steroids and neuropeptides, ensuring a tightly regulated reproductive cycle [35].

The Gonads: The Effector Organs of Reproduction

The gonads ovaries in females and testes in males are the end effectors of the HPG axis. They respond to gonadotropin stimulation by producing sex steroid hormones and generating mature gametes:

- **Sex Steroids:** Key steroids include testosterone, estradiol, and 11-ketotestosterone. Testosterone is a precursor to estradiol and also directly influences male secondary sexual characteristics and reproductive behaviors. Estradiol, a primary estrogen, regulates vitellogenesis and female reproductive tissue development. 11-ketotestosterone is a potent androgen predominant in many teleosts, critical for male reproductive functions.
- **Gamete Development:** Spermatogenesis in testes and oogenesis in ovaries proceed through multiple stages controlled by hormonal cues. These processes are energy-intensive and require precise hormonal orchestration.
- **Feedback Regulation:** Sex steroids exert negative or positive feedback on the hypothalamus and pituitary to modulate GnRH and gonadotropin secretion, maintaining hormonal homeostasis and

reproductive readiness [36].

Integration of Environmental and Physiological Signals

Successful reproduction depends not only on endogenous hormonal control but also on the integration of environmental signals such as photoperiod, temperature, water chemistry, and social cues [37] [38]. The hypothalamus serves as the sensory interface, detecting changes in these external factors and adjusting GnRH secretion accordingly to synchronize reproduction with favorable conditions.

Vulnerability of the HPG Axis to Environmental Toxicants

The sophisticated hormonal interplay within the HPG axis renders it susceptible to disruption by environmental contaminants, including copper sulphate. Copper ions can interfere at multiple levels:

- **Hypothalamic Effects:** Altered GnRH synthesis or release, possibly through neurotoxicity or oxidative stress.
- **Pituitary Dysfunction:** Inhibited synthesis or secretion of LH and FSH, affecting downstream gonadal responses.
- **Gonadal Toxicity:** Direct damage to germ cells and steroidogenic tissues, impaired steroid hormone biosynthesis, and altered expression of key enzymes and receptors.

These disruptions lead to impaired gametogenesis, hormonal imbalances, behavioral changes, and reduced fecundity, with potential consequences for fish populations and aquatic ecosystem health [39].

MECHANISMS OF COPPER SULPHATE-INDUCED HORMONAL DYSREGULATION

Disruption of the Hypothalamic-Pituitary-Gonadal Axis

Copper sulphate exposure interferes with the HPG axis at the hypothalamic, pituitary, and gonadal levels. At the hypothalamus, copper may impair the synthesis or release of GnRH through neurotoxic effects or oxidative damage to neurons responsible for hormone production. This disruption translates into altered stimulation of the pituitary gland, leading to reduced secretion of LH and FSH [40].

The pituitary's capacity to secrete gonadotropins can be directly inhibited by copper, which may bind to receptors or interfere with intracellular signaling pathways. Furthermore, copper-induced oxidative stress can damage pituitary cells, diminishing hormone output.

At the gonadal level, copper sulphate disrupts steroidogenic pathways, reducing the enzymatic activities required for the biosynthesis of sex steroids. This includes inhibition of key enzymes such as aromatase, 17β-hydroxysteroid dehydrogenase, and cholesterol side-chain cleavage enzymes. The resultant decline in testosterone and estradiol disrupts gamete maturation and feedback regulation of the HPG axis, creating a vicious cycle of endocrine dysfunction.

Oxidative Stress and Endocrine Dysfunction

Copper's redox-active nature catalyzes the generation of reactive oxygen species (ROS), overwhelming

antioxidant defenses and causing oxidative stress. Elevated ROS levels lead to lipid peroxidation, protein oxidation, and DNA damage within endocrine tissues, impairing their function.

Oxidative damage to Leydig and Sertoli cells in testes or follicular cells in ovaries compromises hormone synthesis and secretion. Antioxidant enzyme systems such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) are often downregulated in copper-exposed fish, exacerbating oxidative injury [41].

Oxidative stress also modulates signal transduction pathways, altering transcription factors and hormone receptor expression critical for normal endocrine function. This contributes to reduced hormone receptor sensitivity and diminished cellular responsiveness to circulating hormones.

Interference with Steroidogenesis and Hormone Signaling

Copper exposure alters the expression of genes encoding steroidogenic enzymes, steroid hormone receptors, and hormone transport proteins. Downregulation of steroidogenesis-related genes leads to decreased production of sex steroids.

Copper may also disrupt membrane receptor function, impairing hormone binding and signal transduction. For example, alterations in androgen and estrogen receptor expression reduce tissue sensitivity to circulating hormones, impacting gamete development and secondary sexual characteristics.

In addition, copper can influence non-genomic pathways by affecting membrane ion channels, calcium signaling, and kinase activity, which further modulate hormone action [38].

CONSEQUENCES OF HORMONAL DYSREGULATION ON REPRODUCTIVE PHYSIOLOGY

Gonadal Development and Gametogenesis

Disrupted hormone levels impede the proper development of gonadal tissues. In males, decreased testosterone and LH levels lead to reduced spermatogenesis, testicular atrophy, and impaired sperm maturation. In females, reduced estradiol and FSH impair oocyte development, follicular growth, and vitellogenesis.

Histological studies reveal increased oocyte atresia, degeneration of seminiferous tubules, vacuolization, necrosis, and fibrosis. Such structural damage compromises gamete quality and quantity, directly reducing reproductive capacity [35] [38].

Reproductive Behaviors and Spawning

Reproductive hormones regulate complex behaviors such as courtship, mating rituals, nest building, and parental care. Copper sulphate-induced hormonal imbalances reduce the expression of these behaviors. Affected fishes exhibit decreased mating displays, altered swimming patterns, delayed spawning, and reduced parental investment, all of which undermine reproductive success [39].

Fertility and Fecundity

Cumulative hormonal and gonadal impairments culminate in reduced fertility and fecundity. This includes lowered egg production, poor sperm quality, increased embryo mortality, and decreased hatchability. These effects threaten population sustainability, especially in species with narrow reproductive windows or seasonal breeding cycles.

ECOLOGICAL AND AQUACULTURAL IMPLICATIONS

Hormonal dysregulation induced by copper sulphate contamination presents profound ecological and economic challenges that extend beyond individual fish health. In natural freshwater ecosystems, impaired reproductive function leads to diminished fish populations, which in turn destabilizes intricate food webs and alters community dynamics. Predatory prey relationships, nutrient cycling, and overall ecosystem productivity can be severely compromised when key fish species experience reproductive failure. Such disruptions can cascade through trophic levels, affecting biodiversity and the resilience of aquatic habitats. In the realm of aquaculture, the consequences are equally significant; reduced fertility and spawning success diminish stock replenishment rates, leading to economic losses for farmers and hatcheries. This not only impacts the supply chain but also degrades the genetic quality of breeding populations, undermining selective breeding and conservation efforts. Moreover, copper contamination rarely acts in isolation [40]. It often coincides with additional environmental stressors such as fluctuating water temperatures, hypoxic conditions, and exposure to other chemical pollutants, which can synergistically exacerbate endocrine system disruptions. These combined stressors may amplify hormonal imbalances, increasing the vulnerability of fish to reproductive impairment and mortality. Therefore, a comprehensive understanding of the multifactorial interactions between copper toxicity and co-occurring environmental challenges is essential. Such knowledge is critical to inform effective ecological risk assessments and to design integrated management strategies that safeguard aquatic biodiversity, enhance aquaculture sustainability, and ensure the health of freshwater ecosystems in the face of mounting anthropogenic pressures [41].

DETECTION AND MONITORING OF HORMONAL DISRUPTION

Recent advances in biochemical and molecular technologies have revolutionized the detection and understanding of copper-induced endocrine disruption in freshwater teleost fishes, allowing for earlier and more precise identification of reproductive toxicity. Sensitive hormone assays enable quantification of key reproductive hormones such as estradiol, testosterone, and gonadotropins, revealing subtle imbalances caused by copper exposure before overt physiological damage manifests. Complementing hormonal measurements, gene expression profiling through techniques like quantitative PCR and RNA sequencing provides insights into the molecular mechanisms underlying endocrine disruption by monitoring changes in the transcription of genes involved in steroidogenesis, hormone receptors, and the hypothalamic-pituitary-gonadal (HPG) axis regulatory pathways [32]. Additionally, the use of specific biomarkers such as vitellogenin induction in males or antioxidant enzyme activity offers robust indicators of early toxicological stress. When combined with traditional histopathological analyses of gonadal tissues, these endocrine and molecular assessments significantly enhance diagnostic accuracy, enabling researchers to correlate structural damage with functional hormonal disturbances. Beyond laboratory settings, non-invasive behavioral monitoring serves as a practical and cost-effective approach to detect sublethal effects of copper toxicity in natural and aquaculture environments. Behavioral endpoints, including altered spawning activity,

courtship behaviors, and swimming patterns, provide real-time indicators of reproductive impairment that complement biochemical data. The integration of these multidisciplinary methods forms a comprehensive framework for environmental monitoring programs, facilitating early warning systems and improving the assessment of ecological risks posed by copper pollution. Moreover, these approaches underpin the development of science-based regulatory policies aimed at establishing safe copper exposure thresholds, thereby contributing to the protection of aquatic biodiversity and sustainable fisheries management [34].

MITIGATION STRATEGIES AND FUTURE RESEARCH DIRECTIONS

Mitigating copper sulphate's adverse effects requires multifaceted approaches:

- Regulatory control of copper usage and effluent discharge.
- Water treatment technologies such as phytoremediation, chemical precipitation, and biofilters to reduce copper bioavailability.
- Use of antioxidants and protective agents in aquaculture to ameliorate oxidative damage.
- Research into genetic and epigenetic mechanisms underlying copper-induced endocrine disruption.
- Long-term ecological studies assessing population-level impacts.
- Interaction studies with other environmental contaminants and stressors.

Future research should prioritize molecular mechanisms, cross-generational effects, and development of environmentally safe alternatives to copper sulphate [35] [38].

CONCLUSION

Copper sulphate, while indispensable as an essential trace element, transforms into a formidable endocrine disruptor when present at elevated concentrations in freshwater environments. Its toxicological impact on freshwater teleost fishes is multifaceted, primarily mediated through disruption of the hypothalamic-pituitary-gonadal (HPG) axis, which governs reproductive endocrine function. By impairing steroidogenesis the biosynthesis of critical sex hormones and generating oxidative stress within reproductive tissues, copper sulphate precipitates widespread hormonal imbalances. This biochemical perturbations manifest as altered gametogenesis, diminished reproductive behaviors, and ultimately compromised fecundity. The resulting reproductive failure poses significant threats not only to individual fish but also to population sustainability and ecosystem stability. Recognizing the severity of these effects underscores the urgent need for comprehensive research that elucidates the mechanistic pathways of copper-induced endocrine disruption. Such understanding is foundational to developing effective environmental conservation strategies and advancing sustainable aquaculture practices that minimize copper exposure. Furthermore, integrated approaches encompassing rigorous environmental monitoring, stringent regulatory frameworks, and innovative remediation technologies are essential to curtail the ecological footprint of copper pollution. Through these concerted efforts, it is possible to safeguard freshwater biodiversity, preserve the reproductive integrity of aquatic fauna, and maintain the resilience and productivity of freshwater ecosystems in the face of escalating anthropogenic pressures.

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