



Discussion

Engineered nanoparticles and fish reproduction: Mechanistic pathways and environmental implications

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ABSTRACT

The increasing production and environmental release of engineered nanoparticles (ENPs) have raised concerns regarding their impacts on aquatic organisms and ecosystem sustainability. We examine the environmental fate, transformation, bioavailability, and reproductive toxicity of ENPs in fish, focusing on silver, zinc oxide, titanium dioxide, copper oxide, and carbon-based nanomaterials. Current evidence indicates that ENPs impair reproduction through interconnected mechanisms involving oxidative stress, hypothalamic–pituitary–gonadal (HPG) axis disruption, apoptosis, autophagy, gonadal histopathology, and neuroendocrine dysfunction. Environmental processes such as aggregation, dissolution, and surface modification further influence nanoparticle bioavailability and toxicity, complicating ecological risk prediction. We propose an integrated mechanistic framework to explain how molecular and cellular disturbances translate into altered hormone regulation, impaired gametogenesis, reduced fertility, compromised offspring viability, and broader ecological consequences. Critical knowledge gaps include the mismatch between environmentally reported and experimentally applied concentrations, the scarcity of long-term and multigenerational studies, and the limited integration of omics-based findings with reproductive endpoints. Emerging evidence on life-stage sensitivity and mixture toxicity is also discussed. Finally, we outline future research and translational perspectives that emphasize environmentally realistic exposure scenarios, standardized testing strategies, adverse outcome pathway development, omics-driven investigations, and safer-by-design nanomaterials. This framework supports improved environmental risk assessment and the sustainable application of nanotechnology in aquatic ecosystems.

1. Introduction

Engineered nanoparticles (ENPs) are increasingly used in industrial, agricultural, biomedical, and aquaculture applications owing to their unique physicochemical properties, including high surface area, enhanced reactivity, and tunable functionality. These characteristics have facilitated their use in water treatment, disease control, drug delivery, and antimicrobial applications. Nanotechnology has also contributed to sustainable environmental solutions, particularly in wastewater remediation, pollutant removal, and water quality improvement, highlighting its potential role in environmental management [1]. However, the rapid expansion of nanotechnology has also increased the release of ENPs into aquatic environments through industrial effluents, agricultural runoff, wastewater discharge, and consumer products, raising concerns regarding their environmental fate and

ecological impacts [2,3]. While nanotechnology offers several sustainable solutions, unintended exposure of aquatic organisms to ENPs has emerged as a vital environmental challenge requiring careful evaluation.

Fish are widely recognized as valuable bioindicators of aquatic pollution because they occupy different trophic levels, readily accumulate contaminants, and exhibit measurable physiological, behavioural, and reproductive responses to environmental stressors [4,5]. Among the various toxicological endpoints, reproduction is particularly significant because impairments in reproductive performance can directly affect population sustainability and ecosystem stability. Current studies suggest that ENP exposure can adversely influence reproductive processes in fish, leading to altered fertility, reduced reproductive success, developmental abnormalities, and potential long-term ecological consequences [6,7].

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Although numerous studies have examined the reproductive effects of ENPs, the available literature remains fragmented. Most investigations focus on individual toxicological endpoints or specific nanoparticle types, while limited attention has been given to integrating molecular, physiological, and ecological responses within a unified framework. Furthermore, many studies employ laboratory exposure conditions that may not accurately reflect environmentally realistic scenarios, making ecological risk prediction challenging. Important questions also remain regarding chronic exposure, multigenerational effects, species-specific sensitivity, and the influence of environmental factors on nanoparticle behavior and toxicity [8,9]. This discussion article critically examines current knowledge on ENP-induced reproductive toxicity in fish by integrating evidence across molecular, physiological, and ecological levels. It evaluates the environmental relevance of existing findings, identifies key knowledge gaps, and proposes future research priorities to improve ecological risk assessment and support the development of safer and more sustainable nanomaterials.

2. Sources, environmental fate, and bioavailability of ENPs

ENPs enter aquatic environments from a wide range of anthropogenic sources, including industrial discharges, agricultural runoff, wastewater effluents, and biomedical applications. Their increasing production and usage enhance the likelihood of environmental release, making aquatic systems major sinks for these materials [10,11]. Once introduced, ENPs can reach water bodies through direct discharge or indirectly via soil leaching and atmospheric deposition, where they interact with diverse physicochemical components of the aquatic milieu. In these environments, ENPs rarely remain in their original pristine form; instead, they undergo dynamic transformations that significantly influence their behaviour and toxicity. Processes such as aggregation, dissolution into ionic forms, and the formation of surface coatings through interaction with organic and inorganic molecules, often referred to as corona formation, alter their stability, mobility, and reactivity [10,12]. Physicochemical characteristics such as particle size, surface charge, coating composition, and dissolution behaviour can strongly influence nanoparticle transport, environmental persistence, cellular uptake, and tissue distribution. Smaller particles generally exhibit greater surface reactivity and biological accessibility, whereas aggregation and surface modifications may alter their bioavailability and toxic potential [13,14]. These transformations can either enhance or reduce their persistence and biological interactions, thereby complicating predictions of their ecological impact. The bioavailability of ENPs in fish is governed by these transformations as well as exposure routes, which primarily include uptake from water through gills, ingestion via contaminated diet, and interaction with sediment-bound nanoparticles. Once internalized, ENPs can translocate to various tissues, including reproductive organs, where they may exert toxic effects. Thus, understanding the sources, environmental fate, and bioavailability of ENPs is essential for accurately assessing their risks to piscine health and reproduction.

A significant challenge in assessing the reproductive toxicity of ENPs lies in the disparity between concentrations encountered in natural aquatic systems and those commonly used in laboratory experiments. Available monitoring data and predictive models indicate that ENPs generally occur in surface waters at concentrations ranging from nanograms per litre to low micrograms per litre. For example, silver (Ag), zinc oxide (ZnO), and carbon-based nanoparticles are typically detected at low nanogram-per-litre to several hundred nanogram-per-litre levels, whereas titanium dioxide (TiO₂) nanoparticles may reach low microgram-per-litre concentrations under certain environmental conditions. Higher concentrations may occur in localized contamination hotspots associated with wastewater treatment plant discharges, urban runoff, and industrial effluents [3,10,11,15,16].

In contrast, experimental studies investigating fish reproductive toxicity often employ ENP concentrations ranging from 0.1 to

100 mg L⁻¹, several orders of magnitude higher than those reported in aquatic environments [7,17]. Although such concentrations are valuable for elucidating toxicological mechanisms, they may not fully reflect chronic environmentally relevant exposure scenarios, highlighting a notable limitation in extrapolating laboratory findings to ecological risk assessment. Environmental variables such as pH, salinity, dissolved organic matter, and sediment interactions can modify nanoparticle aggregation, dissolution, bioavailability, and toxicity, thereby influencing biological responses. Consequently, future research should emphasize long-term and multigenerational studies conducted at environmentally realistic concentrations and under ecologically relevant conditions. Such approaches will improve ecological risk assessments and provide a more reliable understanding of the potential impacts of ENPs on fish reproduction and population sustainability.

2.1. Major ENP classes associated with reproductive toxicity

ENPs comprise a diverse group of nanomaterials with distinct physicochemical properties, environmental behaviours, and toxicological profiles. Although AgNPs and ZnONPs have been the primary focus of reproductive toxicity studies, recent investigations suggest that other ENPs, including TiO₂ NPs, CuO NPs, and carbon-based nanomaterials, can also adversely affect fish reproduction. TiO₂ NPs induce oxidative stress, hematological alterations, and biochemical disturbances in fish, while in vitro studies demonstrate impaired sperm motility and reduced sperm quality, suggesting adverse effects on fertilization and reproductive performance [18–20]. Similarly, CuO nanoparticles disrupt steroidogenesis, alter hypothalamic–pituitary–gonadal (HPG) axis regulation, modify reproductive hormone levels, and induce gonadal histopathological changes [21,22]. Carbon-based nanomaterials, including graphene derivatives and carbon nanotubes, can promote oxidative stress and inflammatory responses through interactions with biological membranes and cellular macromolecules, thereby contributing to reproductive toxicity [23]. Despite differences in composition and environmental behaviour, these nanomaterials share common mechanisms of toxicity involving oxidative stress, endocrine disruption, cellular damage, and impaired reproductive function. Expanding research beyond AgNPs and ZnO NPs is therefore essential for a comprehensive understanding of nanomaterial-associated reproductive risks in aquatic organisms. The major engineered nanoparticles associated with reproductive toxicity, their exposure routes, mechanisms of action, and reported reproductive effects of ENPs in fish are summarized in Table 1.

3. Mechanistic insights into ENP-induced reproductive toxicity

ENPs induce reproductive toxicity in fish through multiple interconnected mechanisms operating at cellular, molecular, and endocrine levels. Central pathways include oxidative stress-mediated damage, disruption of the HPG axis, and activation of apoptosis and autophagy, thereby leading to gonadal dysfunction. The integrated pathways and outcomes of engineered nanoparticle exposure in aquatic systems are summarized in Fig. 1. The following sub-sections outline these key mechanisms and their implications for reproductive health.

3.1. Oxidative stress and cellular damage

A central mechanism underlying ENP-induced reproductive toxicity in fish is the generation of oxidative stress. Exposure to ENPs has been consistently associated with elevated production of ROS, which can overwhelm endogenous antioxidant defence systems and disrupt cellular redox balance. This oxidative imbalance leads to lipid peroxidation, compromising the integrity and functionality of cellular and organelle membranes within gonadal tissues [7,17]. In addition to membrane damage, excessive ROS can induce significant genotoxic effects, including DNA strand breaks and oxidative base modifications,

Table 1

Major engineered nanoparticles, exposure routes, mechanisms of toxicity, and reported reproductive effects in fish.

Nanoparticle type	Major exposure routes	Principal mechanisms of toxicity	Major reproductive effects
AgNPs	Waterborne uptake through gills, dietary ingestion	Oxidative stress, endocrine disruption, apoptosis, steroidogenic impairment	Impaired gametogenesis, altered steroidogenesis, gonadal damage, reduced fertility [7, 17,24]
ZnO NPs	Waterborne uptake, gills, gastrointestinal tract	Oxidative stress, autophagy, apoptosis	Oocyte degeneration, impaired spermatogenesis, reduced reproductive performance [7,25, 26]
TiO ₂ NPs	Waterborne exposure, dietary uptake	Oxidative stress, hematological and biochemical disturbances	Reduced sperm motility, altered sperm quality, potential impairment of fertilization success [18–20]
CuO NPs	Waterborne uptake through gills and ingestion	HPG axis disruption, steroidogenic impairment, oxidative stress	Altered reproductive hormone levels, gonadal histopathological changes, reduced reproductive capacity [21,22]
Carbon-based nanomaterials (graphene derivatives, carbon nanotubes)	Waterborne exposure, dietary ingestion	Oxidative stress, inflammation, cellular damage	Potential reproductive toxicity through endocrine disruption and gonadal cellular injury [23]

which interfere with normal gene expression and cell cycle progression in germ cells. Such damage is particularly critical in spermatogenic and oogenic cells, where genomic integrity is essential for successful reproduction. Moreover, oxidative stress can impair mitochondrial function, reducing energy production and activating downstream pathways such as apoptosis and autophagy, eventually leading to cell death [26].

Beyond direct cellular damage, ROS function as vital signaling molecules that regulate several redox-sensitive pathways, including Nrf2/Keap1, mitogen-activated protein kinase (MAPK), and nuclear factor-kappa B (NF- κ B). Excessive ROS production induced by ENPs can dysregulate these pathways, resulting in altered expression of genes involved in antioxidant defense, inflammation, apoptosis, and steroidogenesis [17,27]. Persistent activation of oxidative stress signaling also contributes to mitochondrial dysfunction, cytochrome-c release, and caspase activation, thereby promoting apoptosis in reproductive cells. These findings suggest that oxidative stress acts not only as a source of cellular injury but also as a key upstream regulator of endocrine and reproductive dysfunction in fish. Consequently, these processes result in structural and functional deterioration of reproductive tissues, thereby diminishing fertility and reproductive performance in fish exposed to ENPs.

3.2. Endocrine disruption (HPG axis)

ENPs can interfere with the endocrine regulation of reproduction in fish by disrupting the HPG axis, the central endocrine pathway governing gametogenesis, gonadal maturation, and steroidogenesis. In

teleosts, GnRH stimulates the secretion of two functionally distinct pituitary gonadotropins, FSH and LH, which regulate reproductive processes through the production of sex steroids. In males, 11-ketotestosterone (11-KT) is the principal androgen controlling spermatogenesis and secondary sexual characteristics, with FSH promoting spermatogenesis through androgen production [28]. In females, 17 β -estradiol (E2) regulates vitellogenesis and oocyte growth, whereas 17 α ,20 β -dihydroxy-4-pregnen-3-one (17 α ,20 β -DHP) functions as the maturation-inducing hormone responsible for final oocyte maturation and ovulation [29].

Exposure to ENPs can disrupt this finely regulated endocrine network by altering GnRH signaling, gonadotropin secretion, and gonadal steroidogenesis, eventually leading to impaired reproductive capacity. For example, copper-based nanoparticles can disrupt the HPG axis, alter reproductive hormone levels, and induce gonadal histopathological changes. In yellow catfish, *Pelteobagrus fulvidraco*, exposure to copper nanoparticles dysregulated the HPG axis and altered gonadal histology [22], whereas CuO nanoparticles induced reproductive toxicity and gonadal damage in *D. rerio* [21]. Similarly, exposure to chitosan-conjugated eurycomanone nanoparticles altered ovarian development and reproductive responses in a female fish model, demonstrating the susceptibility of endocrine-regulated reproductive processes to nanoparticle-induced perturbations [30]. HPG-axis dysfunction is closely linked to the cellular disturbances described in Section 3.1. ENP-induced alterations in redox homeostasis can impair hypothalamic GnRH release, disrupt pituitary gonadotropin secretion, and suppress the activity of steroidogenic enzymes, including aromatase (CYP19A1), 3 β -hydroxysteroid dehydrogenase, and 17 β -hydroxysteroid dehydrogenase, thereby reducing the synthesis of 11-ketotestosterone, estradiol, and other reproductive steroids [22,24]. Such endocrine disturbances compromise spermatogenesis, vitellogenesis, gamete maturation, and spawning success, thereby diminishing reproductive performance. In addition to direct effects on the HPG axis, ENPs may influence the hypothalamic–pituitary–interrenal (HPI) axis, where altered cortisol homeostasis can further disrupt reproductive endocrine signaling [31]. The interplay between oxidative stress, endocrine disruption, and steroidogenic impairment suggests that ENPs affect fish reproduction through multiple interconnected pathways rather than isolated mechanisms. This integrated perspective provides a more comprehensive understanding of ENP-mediated reproductive toxicity and its potential consequences for fish populations and aquatic ecosystem sustainability.

3.3. Apoptosis, autophagy, and cell signaling

ENPs can disrupt reproductive function in fish by activating programmed cell death pathways and altering intracellular signaling networks in germ cells. Current findings indicate that ENP exposure triggers apoptosis through mitochondrial dysfunction, activation of caspases, and upregulation of pro-apoptotic genes, leading to the loss of spermatogenic and oogenic cells. In parallel, ENPs can induce autophagy as a stress response; however, excessive or dysregulated autophagy may further contribute to cellular degeneration rather than protection. For example, ZnO NPs simultaneously activate apoptosis and autophagy pathways in developing oocytes, ultimately impairing their maturation and viability [26].

At the molecular level, these effects are mediated through the disruption of key signaling pathways involved in cell survival and proliferation, including oxidative stress-sensitive pathways and metabolic regulatory networks. Advanced studies integrating transcriptomic and metabolomic analyses have demonstrated that exposure to AgNPs alters the expression of genes associated with cell cycle regulation, energy metabolism, and stress responses in testicular cells, thereby compromising spermatogenesis [32]. Such interference with finely regulated signaling cascades can lead to impaired germ cell development and reduced reproductive output. These molecular alterations are further

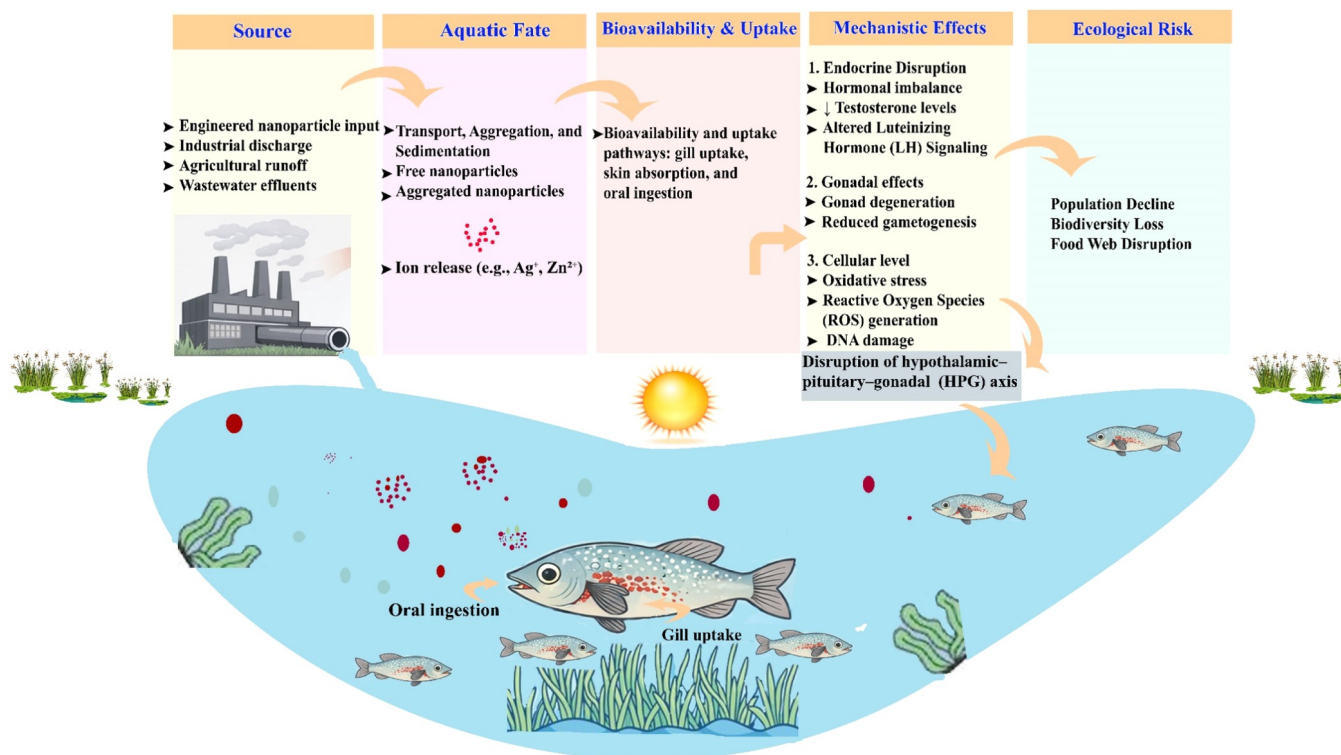


Fig. 1. Schematic overview of ENP pathways in aquatic systems, illustrating environmental sources, transport and transformation processes (aggregation, sedimentation, and ion release), bioavailability and uptake through gill, skin, and oral routes, and subsequent biological effects in fish. ENP exposure can induce oxidative stress, disruption of the HPG axis, gonadal damage, and impaired gametogenesis, resulting in reproductive dysfunction and ecological consequences, including reduced population fitness, biodiversity loss, and food-web disruption.

reflected in physiological and reproductive endpoints, including disrupted steroid hormone synthesis, reduced gonadosomatic index, impaired gametogenesis, decreased fecundity and fertilization success, and compromised offspring viability, thereby providing a mechanistic link between omics-based changes and reproductive dysfunction. These findings highlight the importance of apoptosis, autophagy, and cell-signaling dysregulation in mediating ENP-induced reproductive impairment. Emerging evidence also suggests that epigenetic regulation may influence reproductive function through mechanisms such as DNA methylation, histone modifications, and non-coding RNAs, which modulate the expression of genes involved in gametogenesis and endocrine signaling. Although evidence for ENP-induced epigenetic alterations in fish reproduction remains limited, these mechanisms may contribute to long-term and transgenerational reproductive effects and warrant further investigation [33].

3.4. Gonadal histopathology

Histopathological alterations in gonadal tissues provide clear evidence of ENP-induced reproductive toxicity in fish. In the testis, exposure to ENPs has been associated with structural disorganization of seminiferous tubules, degeneration of Sertoli and Leydig cells, and impaired progression of spermatogenesis. Such changes can reduce sperm quality and quantity by disrupting the supportive and endocrine functions essential for germ cell development [25,34]. In particular, damage to Sertoli cells affects the microenvironment required for germ cell maturation, while Leydig cell dysfunction can alter testosterone synthesis, further compromising reproductive capacity.

In female fish, ENP exposure can adversely affect ovarian structure and function, leading to impaired oocyte maturation and increased incidence of follicular atresia. Nanoparticle-induced oxidative stress and cellular damage can disrupt normal folliculogenesis, resulting in degeneration of developing oocytes and reduced fecundity [7,26]. These

histopathological changes reflect the cumulative impact of cellular and molecular disruptions caused by ENPs, ultimately leading to diminished reproductive performance. Gonadal histopathological alterations provide a direct link between molecular toxicity mechanisms and reproductive dysfunction.

3.5. Neuroendocrine and behavioural effects

Beyond direct gonadal toxicity, ENPs can disrupt the neuroendocrine regulation of reproduction by interfering with the brain–gonad axis in fish. This axis integrates neural and hormonal signals, where the hypothalamus regulates GnRH secretion, ultimately controlling pituitary and gonadal functions. ENP-induced neurotoxicity may also affect dopaminergic and serotonergic pathways that regulate hypothalamic GnRH release, thereby contributing to HPG axis dysfunction and impaired reproductive function. However, direct evidence linking neurotransmitter alterations to reproductive toxicity in fish remains limited and warrants further investigation. For instance, AgNPs disrupt neuroendocrine pathways linked to stress regulation, modifying cortisol responses through disruption of the HPI axis, which can indirectly influence reproductive processes [31].

Additionally, ENP exposure alters behavioural patterns such as reduced locomotion, impaired mating behaviour, and changes in social interactions, which are critical for successful reproduction. Studies in zebrafish indicate that ZnONPs can affect brain function and glial activity, suggesting a link between neurotoxicity and reproductive dysfunction [35]. Neuroendocrine disruption may be further amplified under combined exposure to ENPs and co-occurring environmental contaminants, where interactions between the HPG and HPI axes can enhance cortisol-mediated endocrine imbalance and behavioural alterations, potentially exacerbating reproductive impairment. These neurobehavioral alterations may impair reproductive success not only through hormonal dysregulation but also by affecting courtship and

spawning behaviours. Neuroendocrine disturbances represent an important yet underexplored component of ENP-induced reproductive toxicity that warrants further investigation.

3.6. Integrated mechanistic framework linking oxidative stress, endocrine disruption, apoptosis, and histopathology

ENPs initiate a cascade of interconnected toxicological events rather than isolated biological responses. Following uptake through the gills, gastrointestinal tract, or skin, ENPs can enter cells through various endocytic pathways and interact with intracellular organelles, including mitochondria, lysosomes, and the endoplasmic reticulum, thereby influencing cellular homeostasis and stress responses [36]. After cellular internalization, ENPs accumulate in metabolically active tissues, including the brain, liver, and gonads, where they trigger cellular stress responses and disrupt redox regulation [3,7]. The resulting molecular alterations affect signaling pathways such as Nrf2/Keap1, MAPK, and NF- κ B, leading to changes in the expression of genes associated with antioxidant defense, inflammation, steroidogenesis, and cell survival [17,27]. These disturbances interfere with HPG-axis signaling and impair steroidogenic enzymes, resulting in altered secretion of gonadotropins and sex steroids that are essential for normal reproductive function [22,24,37].

Concurrently, mitochondrial dysfunction promotes activation of intrinsic apoptotic pathways through cytochrome-c release, caspase activation, and dysregulated autophagy, leading to degeneration of spermatogenic and oogenic cells [7,26]. At the tissue level, the cumulative effects of endocrine imbalance and germ-cell loss manifest as degeneration of seminiferous tubules, follicular atresia, disruption of gonadal architecture, impaired steroid-producing cell function, and reduced gonadosomatic indices [25,34]. These pathological alterations translate into organism-level outcomes, including reduced sperm quality, impaired oocyte maturation, decreased fertility, altered reproductive behaviour, reduced offspring viability, and impaired reproductive success [6,7]. As a result, chronic reproductive impairment may contribute to reduced population recruitment and broader ecological consequences in aquatic ecosystems. Collectively, these interconnected mechanisms demonstrate that ENP-induced reproductive toxicity

cannot be attributed to a single pathway. However, the magnitude and biological significance of these effects vary across developmental stages, exposure scenarios, and nanoparticle mixtures, which are discussed in the following section. An integrative conceptual framework linking ENP uptake, oxidative stress, endocrine disruption, apoptosis, gonadal pathology, and ecological consequences is presented in Fig. 2

While the mechanistic pathways described above are supported by growing experimental evidence, their relative contribution to reproductive impairment is likely to vary among nanoparticle types, species, developmental stages, and exposure scenarios. We suggest that future research should move beyond identifying individual toxicity pathways and focus on understanding how these mechanisms interact to influence reproductive outcomes. Such an approach may provide a more realistic representation of ENP-induced reproductive toxicity and improve the interpretation of mechanistic biomarkers in ecological risk assessment.

4. Emerging evidence from experimental studies

While mechanistic evidence provides important insights into the pathways underlying reproductive toxicity, emerging studies indicate that life stage, exposure duration, and interactions among contaminants can substantially influence toxicological outcomes. Early developmental stages, particularly embryos and larvae, are especially vulnerable due to their underdeveloped defence systems and rapid cellular differentiation. Exposure during embryogenesis impairs normal development, induce oxidative stress, and disrupt organ formation, ultimately affecting survival and future reproductive capacity [7]. In contrast, adult fish exhibit pronounced gonadal and endocrine disturbances upon ENP exposure, including impaired spermatogenesis, altered steroidogenesis, and reduced fertility [25,34]. Representative experimental studies demonstrating the reproductive effects of different ENPs in fish are summarized in Table 2.

A significant advancement in recent research is the recognition of mixture toxicity, which more closely reflects real-world environmental conditions. Combined exposure to AgNPs and ZnONPs produces additive or synergistic effects, intensifying oxidative stress, cellular damage, and reproductive dysfunction compared to single nanoparticle exposure [17,42]. These interactions challenge toxicity predictions and highlight

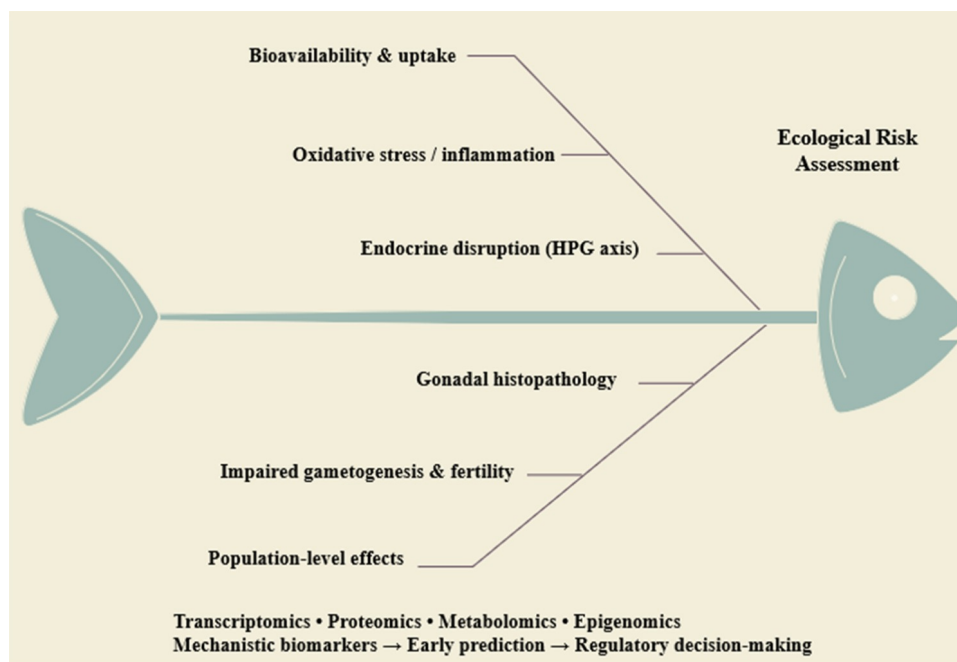


Fig. 2. Integrative conceptual framework illustrating the mechanisms of ENP-induced reproductive toxicity in fish and their implications for ecological risk assessment.

Table 2

Representative experimental studies reporting reproductive effects of ENPs in fish.

ENP Type	Fish Species	Exposure Concentration	Major Reproductive Effects	Reference
ZnO NPs	Zebrafish	0.69 and 3.45 mg L ⁻¹	Reduced fertility, impaired oocyte development, activation of apoptosis and autophagy	[26]
ZnO NPs	Common carp	10–40 mg L ⁻¹	Testicular degeneration, impaired spermatogenesis, altered steroidogenesis	[25]
ZnO NPs	Japanese medaka	Experimental exposure	Sex-dependent bioaccumulation, altered sexual behavior, reduced reproductive performance	[38]
CuO NPs	Guppy	10–100 µg L ⁻¹	Delayed parturition, reduced reproductive success, decreased offspring survival	[39]
Copper nanoparticles (Cu NPs)	Catfish	Experimental exposure	Testicular damage and impaired spermatogenic activity	[40]
CuO NPs	Zebrafish	Experimental exposure	Gonadal alterations and reduced reproductive performance	[21]
TiO ₂ NPs	Zebrafish	0.1–1.0 mg L ⁻¹	Reduced egg production and altered reproductive performance	[41]

the need for more ecologically relevant experimental designs. Emerging findings using advanced analytical tools have revealed that ENPs can disrupt multiple biological pathways simultaneously, including metabolic regulation, gene expression, and cellular homeostasis. Integrative approaches, such as transcriptomics and metabolomics, have provided deeper insights into how nanoparticles alter reproductive physiology at the molecular level, particularly in testicular tissues [32]. Taken together, these findings underscore the importance of considering life-stage sensitivity, combined exposures, and mechanistic interactions when evaluating the reproductive effects of ENPs in aquatic organisms. However, direct comparisons among studies remain challenging because of differences in nanoparticle properties, exposure conditions, species selection, and reproductive endpoints. We propose that greater standardization of experimental designs and reproductive biomarkers would improve cross-study comparability, strengthen the interpretation of toxicological responses, and enhance the reliability of ecological risk assessment.

5. Ecological implications of ENP-induced reproductive toxicity

The reproductive toxicity of ENPs extends beyond individual fish and may have significant consequences for population dynamics and aquatic ecosystem stability. Reduced gamete quality, impaired fertilization, altered reproductive behaviour, and decreased offspring viability may lower recruitment success and contribute to population declines under chronic exposure conditions, thereby reducing population resilience [6–8]. At the ecosystem level, species-specific sensitivity to ENPs may alter community composition, reduce biodiversity, and disrupt trophic interactions and ecosystem functioning. Comparative studies have demonstrated substantial interspecies variation in nanoparticle toxicity, indicating that ecological risk may differ among fish taxa and developmental stages [9]. Furthermore, bioaccumulation and trophic transfer of ENPs, together with their interactions with co-occurring contaminants, may amplify ecological risks beyond those predicted from single-particle exposures [2,3,10].

The increasing use of ENPs in aquaculture for disease control, water treatment, and feed supplementation also warrants careful evaluation. Although these applications offer considerable benefits, excessive exposure may impair broodstock fertility, hatchability, and larval survival, potentially compromising production sustainability. Environmentally realistic, long-term, and population-level studies are essential to improve ecological risk assessment and support the safe and sustainable application of nanotechnology in aquatic ecosystems [8,43].

6. Critical experimental gaps

Despite growing mechanistic understanding, several critical uncertainties continue to limit the accurate assessment of ENP-induced reproductive toxicity and its ecological consequences. One of the major gaps lies in the scarcity of long-term and multigenerational studies. Most existing investigations are short-term and focus on acute or

subacute exposures, which may not adequately capture the cumulative and heritable effects of ENPs on reproductive fitness across successive generations. As reproduction is inherently a long-term biological process, the absence of such studies limits the ability to predict population-level consequences. In addition, species-specific differences in sensitivity, life-stage-dependent responses, and the effects of nanoparticle mixtures remain insufficiently characterized, creating uncertainty in the extrapolation of laboratory findings across taxa and environmental scenarios. Another vital limitation is the insufficient integration of molecular, physiological, and ecological endpoints. Although transcriptomic, proteomic, and metabolomic approaches have improved understanding of the cellular mechanisms underlying ENP toxicity, these data are often not linked to reproductive performance, offspring fitness, or population-level outcomes. As a result, the ecological significance of many mechanistic responses remains unclear. Establishing stronger connections between molecular biomarkers and higher levels of biological organization would improve the predictive capacity of reproductive toxicity assessments.

Furthermore, the incorporation of mechanistic evidence into ecological risk assessment frameworks remains limited. Integrating molecular responses with adverse outcome pathways (AOPs), reproductive endpoints, and population-level models would facilitate a more comprehensive understanding of how ENP-induced effects propagate across biological scales. Greater integration of mechanistic, physiological, and ecological datasets is therefore needed to strengthen predictive frameworks and improve the interpretation of reproductive toxicity across different levels of biological organization. Addressing these knowledge gaps will be essential for advancing future research and supporting more robust environmental risk assessment of engineered nanoparticles in aquatic ecosystems. An important question that remains unresolved is whether commonly reported mechanistic responses, such as oxidative stress and endocrine disruption, consistently translate into long-term reproductive impairment under environmentally relevant exposure scenarios. Clarifying this relationship will be essential for distinguishing transient physiological responses from adverse effects with ecological significance.

7. Future research and translational perspectives

Future research should prioritize the integration of mechanistic, physiological, and ecological evidence to improve the prediction of ENP-induced reproductive effects across different levels of biological organization. Particular emphasis should be placed on linking molecular biomarkers with reproductive performance, offspring fitness, and population-level responses. Such an approach would strengthen the ecological relevance of mechanistic findings and facilitate their incorporation into environmental risk assessment frameworks.

Strengthening regulatory frameworks is equally imperative for the effective management of ENP-associated risks. Although protocols for assessing nanoparticle toxicity in aquatic organisms are advancing, standardized approaches for reproductive endpoints remain limited.

Establishing harmonized safety thresholds, monitoring strategies, and testing guidelines will facilitate evidence-based regulatory decision-making. Standardized characterization and reporting of nanoparticle properties, including hydrodynamic size, zeta potential, dissolution rate, and dispersion stability in exposure media, are essential for improving reproducibility, enabling cross-study comparisons, and enhancing ecological risk assessment [44,45]. Incorporating mechanistic evidence into ecological risk models, particularly through the application of Adverse Outcome Pathways (AOPs), can strengthen the linkage between molecular initiating events and adverse reproductive effects at individual and population levels, thereby improving the predictive value of environmental risk assessment and management strategies [46,47].

The development of safer and more sustainable nanomaterials also represents a key future direction. Approaches such as green synthesis, safer-by-design engineering, surface functionalization, biocompatible coatings, and controlled-release technologies offer opportunities to reduce nanoparticle toxicity while preserving their desired functional properties [48,49]. Nevertheless, safety evaluations should extend beyond physicochemical characterization and incorporate reproductive and ecological endpoints throughout the material development process. Such integration would facilitate the identification of potential adverse effects at an early stage and support the design of environmentally responsible nanomaterials.

Emerging studies indicate that nanotechnology may also support reproductive health through targeted delivery systems; for example, nanoparticle-assisted delivery of bioactive compounds has shown potential for enhancing male reproductive function, highlighting the need to balance therapeutic applications with environmental safety considerations [42]. For aquaculture applications, these innovations could improve disease management and productivity without adversely affecting fish health or reproductive performance. Nanotechnology offers opportunities for sustainable environmental management through applications in wastewater treatment, pollutant removal, and water quality improvement [50]. Recent advances in predictive toxicology and AI-based models can improve nanoparticle risk assessment by predicting toxicity from physicochemical and biological data. Integrating these approaches with AOP frameworks may facilitate early hazard identification and support the development of safer-by-design nanomaterials, thereby promoting the sustainable application of nanotechnology in aquatic ecosystems.

8. Conclusion

ENPs pose an emerging challenge to fish reproductive health through multiple interconnected mechanisms involving endocrine disruption, apoptosis, autophagy, neuroendocrine dysfunction, and gonadal pathology. These responses originate from early cellular disturbances and propagate across molecular, physiological, and reproductive levels, thereby impairing reproductive performance and potentially threatening population sustainability in aquatic ecosystems. The principal contribution of this discussion article lies in presenting an integrated conceptual framework that connects mechanistic evidence with ecological implications across multiple classes of ENPs. By bringing together findings from molecular, physiological, and ecological perspectives, we provide a broader context for understanding reproductive toxicity and identify key gaps that currently limit ecological risk prediction and regulatory assessment.

Based on the available evidence, we propose that ENP-induced reproductive toxicity should be viewed as the outcome of interacting molecular, endocrine, and cellular pathways rather than the consequence of a single dominant mechanism. We further suggest that understanding these mechanistic interactions is essential for improving the interpretation of toxicological responses and strengthening ecological risk assessment. In our view, greater integration of mechanistic evidence with reproductive and ecological endpoints will enhance the ability to predict the long-term consequences of nanoparticle exposure for aquatic

organisms and populations. Overall, we envision that closer integration of mechanistic toxicology, ecological risk assessment, and regulatory science will facilitate the development of safer and more sustainable nanotechnologies. Such an interdisciplinary framework can support evidence-based environmental management while helping to safeguard the reproductive integrity and biodiversity of aquatic ecosystems.

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CRedit authorship contribution statement

Prashanth Konkal: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Formal analysis, Data curation, Conceptualization. **Taranath T. C. :** Validation, Data curation. **Bheemanagouda N. Patil:** Validation, Data curation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data Availability

No datasets were generated or analysed during the current study.

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