Chapter 4

The Effects of Micro & Nano Pollution on Fish Reproduction 8

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Abstract

The changing world and the increasing use of nanomaterials in industry have caused industrial nano wastes to enter the water and oceans in recent years. By a similar mechanism, plastic wastes that enter the waters erode over time and turn into microplastics. Fish, which encounter micro & nano pollutants (MNP) in their habitats, ingest these materials through the food chain, respiration and direct contact. Although water pollution attracts attention in the media and society as a result of mass fish deaths, disruptions in the reproductive cycles of fish exposed to MNPs and anomalies in juvenile development stages threaten the future of fish population in aquatic ecosystems. Fish that experience limitations in their ability to reproduce or produce offspring with anomalies are at risk of facing extinction within a short timeframe. In this chapter, the effects of various industrial nanomaterials and microplastics on fish reproductive systems are discussed and an awareness is tried to be created.

1. Sources of Microplastics and Nanoparticles in Aquatic Ecosystems

Nanomaterials are one of a kind structural properties due to their dimensions, which typically range from 1 to 100 nanometers (Biswas and Wu 2005). By reducing materials to nanoscale dimensions compared to their normal size, they exhibit distinct properties. Nanomaterials possess a significantly higher surface area due to their size, enhancing their effectiveness compared to their bulk counterparts. Incorporating structures like nanotubes or nanosheets can impart desirable properties such as enhanced strength or flexibility to the final product. Furthermore, nanomaterials display

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varying electrical and thermal conductivity properties based on their size and structure, making them suitable for applications in electronic devices and energy storage systems. With their expansive surface areas and reactive groups, nanomaterials find utility in diverse fields such as catalysts for chemical reactions, sensors, and medical applications (Neuberger, Schöpf et al. 2005, Mathew and Juang 2007, Ghazanfari, Kashefi et al. 2016, Koo, Ismail et al. 2019).

Nanomaterials find applications in a wide range of industries, including electronics, drug development, materials science, and energy storage. As research in nanotechnology and nanoscience continues to advance, new nanomaterials will be developed and utilized. Industrial nanomaterials are specifically designed using nanotechnology for use in industrial applications, and they exhibit diverse properties. Carbon nanotubes, for instance, have numerous uses in areas such as electronics, energy storage, and material reinforcement. On the other hand, nanocomposites enhance the mechanical, thermal, or electrical properties of polymers, resulting in more durable and functional materials. The continued development and utilization of nanomaterials contribute to advancements and innovations in various industries. (Darwish, Mostafa et al. 2022, Khan, Mubarak et al. 2022, Mishra, Devi et al. 2022, Öztürk and Ömür 2022). Nanoparticles like gold, silver, and platinum possess valuable catalytic properties and a large surface area, enabling their application in a wide range of fields. These tiny particles find utility in sensors, electronic and optical devices, as well as health and agricultural applications (Rastogi, Kumari et al. 2022, Husain, Nandi et al. 2023). TiO2 nanoparticles are semiconducting materials known for their remarkable photocatalytic activity. As a result, they are extensively employed in a variety of applications such as solar energy units, sunscreens, coatings, and air and water pollution control (Jiang, Zhou et al. 2022, Nandiyanto, Salsabila et al. 2023). Quantum dots, a type of semiconductor nanomaterial, possess the unique ability to absorb and emit light at precise wavelengths. This distinctive property makes them valuable for a range of applications including LED displays, solar panels, and biomedical imaging (Abdellatif, Younis et al. 2022, Xu, Niu et al. 2022, Jang and Jang 2023). Microplastics refer to tiny particles composed of different types of plastic materials, typically measuring less than 5 mm in size. These particles may originate from the degradation of plastic waste or be generated during the production, usage, or processing of specific products (Zhang, Xu et al. 2022). The degradation of large plastic waste in marine and natural environments can occur over time through various factors such as exposure to sunlight, wave movements, and mechanical abrasion. As a result of this degradation process, microplastics are

formed and released into the environment (Fan, Zou et al. 2022). Garments made from synthetic fibers, such as polyester or acrylic, are widely used and are known for their plastic-based composition. When these garments are washed, microplastic particles can be released from the fiber materials and enter the water system. Eventually, these microplastics may find their way into natural environments through the sewage system (Chen, Chen et al. 2022). Over time, plastic-based packaging products like plastic bottles, bags, and packaging materials can undergo degradation and transform into microplastics. These microplastics can originate from various sources, including landfills, leaks from garbage, or plastics that have been accidentally discarded in natural environments (Chen, Xu et al. 2023). Certain cosmetic products and cleaning agents, such as exfoliating peels and toothpastes, may include particles that contain microplastics. When these products are used, the microplastics can enter the water system and subsequently disperse into the environment (Zhou, Ashokkumar et al. 2023) (Fig 1).



Figure 1. The sources of MNPs in water and oceans.

The presence and buildup of microplastics in natural environments and water resources can pose a potential threat to ecosystems and living organisms. Hence, it is crucial to prioritize initiatives that aim to raise awareness about microplastic pollution and promote the reduction of plastic consumption. It's worth noting that microplastics and nanoparticles have the potential to contaminate various consumable items, including food and beverages, underlining the significance of addressing this issue for the well-being of both humans and the environment (Vitali, Peters et al. 2023). The escalating issue of nanoparticle and microplastic pollution in water, as discussed earlier, raises concerns about the potential impact on aquatic organisms. Research indicates that nanomaterials and microplastics primarily pose risks to the intestinal system and gills of these organisms (Alak, Uçar et al. 2022, Nabi, Ahmad et al. 2022). While studies on the effects of micronano pollutants (MNPs) on fish reproductive systems are still ongoing, there is growing evidence indicating potential impacts. Recent research compiled in this section aims to shed light on this issue, highlighting the alarming problem that these pollutants pose to the survival and potential extinction of various living organisms.

2. Effects of Nano Pollutants on Fish Reproductive System

For a considerable period, water pollution resulting from industrial growth and inadequate disposal of industrial waste into rivers and oceans was chiefly associated with visible waste materials. Nonetheless, recent studies has shed light on the presence of MNPs in water pollution, prompting scientists to investigate the potential ramifications of these pollutants. Remarkable findings have emerged from the diligent efforts of scientists studying fish reproduction and its relation to MNPs in aquatic environments.

Deepa, Mamta et al. (2022) conducted a study highlighting the detrimental effects of carbon nanotubes on testicular tissue in *Common carp*. Their findings revealed an increase in reactive oxygen species levels within the testicular cells, leading to cellular apoptotic changes. Another study by Deepa, Murugananthkumar et al. (2019) reported that ZnO nanoparticles resulted in the downregulation of steroid enzymes in testicular tissue. Additionally, they observed that increasing doses of ZnO nanoparticles led to oxidative stress and damage in the testicular tissue of *Cyprinus carpio* fish.

When Asian striped catfish (*Mystus vittatus*), which is known for their resilient structure, were exposed to ZnS nanomaterials, abnormalities were observed in testicular cell development. Additionally, damage to the spermatozoa membrane was detected. (Bhattacharjee and Chatterjee 2016). Cu nanoparticles were introduced to *Clarias bathrachus*, a catfish species, resulting in damage to the basal lamina of the testicular tissue. This damage led to the presence of different types of spermatogonia and enlargement of spermatocytes (Murugananthkumar, Rajesh et al. 2016).

Zebrafish (*Danio rerio*) is widely recognized as the primary experimental fish in aquatic studies. This versatile fish species is commonly employed for modeling human conditions and is also extensively utilized in studies examining the potential toxicity of nanoparticles. In zebrafish studies conducted by (Kotil, Akbulut et al. 2017), it was observed that TiO2 nanoparticles led to mitochondrial degeneration and necrosis specifically in Sertoli cells. Similarly, Ma, Lu et al. (2018) discovered that silver nanoparticles induced the formation of reactive oxygen species (ROS) in the testes and ovaries after 5 weeks or longer exposure, subsequently triggering mitochondria-dependent apoptosis in the cells. In a study conducted by Sumi and Chitra (2020), it was found that exposure to C60 fullerene nanoparticles resulted in decreased testosterone levels in male fish and estrogen levels in female fish, specifically in their research with *Anabas testudineus*. They concluded that such exposure to C60 fullerene nanoparticles caused reproductive toxicity in *Anabas testu-dineus* fish.

Nanotoxicity studies focusing on females are equally crucial in understanding the effects on reproduction. While males primarily experience damage to testicular tissue and spermatozoa, females face additional risks, including damage to the ovary, oocyte, and even the potential harm to larvae in live-bearing species. Notably, since a male can fertilize multiple females, a decrease in the male population may be "partially" tolerable. However, a decline in the number of females can lead to irreversible damage, significantly endangering the species' continuity. This highlights the significance of considering the broader impact on females in nanotoxicity studies for the preservation of species.

Studies investigating nano-toxicity in various species of female fish have indicated that silver (Ag) nanoparticles can inhibit the release of steroid hormones from ovarian cells of *Oryzias melastigma* (Degger, Anna et al. 2015). Ag nanoparticles were also found to affect meiosis and cause oxidative stress in Zebrafish gonads (Szudrowicz, Kamaszewski et al. 2022). In a study conducted on zebrafish, Dayal, Thakur et al. (2016) stated that gold nanoparticles cause damage to the ovarian structure. Similarly, TiO2 nanoparticles cause 29.5% egg loss in females at 13 weeks of exposure (Wang, Zhu et al. 2011).

Nanomaterials can be produced in two forms: chemical and biological (green synthesis). The general opinion is that green synthesis nanoparticles are less toxic to biological systems(Nadaroglu, Güngör et al. 2017). In a study proving this information, Sarkar, Netam et al. (2014)stated that plant derived silver nanoparticles have no toxic effect on Zebrafish. Therefore, it is necessary

to leave an open door for the toxicity of these industrial nanomaterials to those of biological origin. Numerous studies have demonstrated the toxicity of silver nanoparticles, revealing that they not only accumulate in the mother fish but can also be transferred to the fry during larval development, as highlighted in the study by Yan and Wang (2022). This maternal transfer of nanoparticles poses a significant risk, impacting not only the current generation but also potentially impacting future generations. Similarly, in a related study conducted by Shi, Zhang et al. (2018), it was observed that selenium nanoparticles induced malformations in the offspring.

3. Effects of Microplastics on Fish Reproductive System

Fish can come into contact with microplastics through various routes, including inhalation, ingestion through water, or direct consumption as food. The potential impacts of microplastics on fish can be categorized as; i. digestive problems by the accumulation in the intestinal tract, ii. toxic effects iii. food chain bioaccumulation iv. behavioral changes such as feeding, swimming behavior, and reproduction. (Andrady 2011, Wang, Tan et al. 2016, Pinheiro, Oliveira et al. 2017, Bessa, Barría et al. 2018).

When investigating the reproductive system in fish, one frequently mentioned type of microplastic is polystyrene microplastics. These microplastics consist of small particles made from a polymer known as polystyrene and are utilized in various industries. One prominent use of polystyrene foam is found in packaging materials due to its lightweight and insulating properties (Abidin, Sanny et al. 2022). Additionally, polystyrene-based materials are commonly employed for thermal and sound insulation in buildings, including walls, ceilings, and roofs (Ramli Sulong, Mustapa et al. 2019). They have also been utilized in medical devices and laboratory equipment (Loos, Syrovets et al. 2014), as well as electronic devices, computer cases, and cell phone casings (Sen, Zhao et al. 2004).

The utilization of polystyrene microplastics in various applications has faced criticism due to their detrimental environmental impacts. These tiny particles can persist in the environment for thousands of years and are prone to ingestion by marine organisms in aquatic ecosystems. This ingestion poses a significant threat to marine organisms, as the effects of polystyrene microplastics can propagate through the food chain. The environmental impact of microplastics, including their potential to exacerbate pollution in marine and aquatic ecosystems, has raised significant concerns. Consequently, numerous countries and companies have taken measures to reduce or completely eliminate the use of polystyrene microplastics in response to these environmental concerns (Wagner 2020).

Microplastics remain in aquatic ecosystems for a long time. Therefore, studies have shown that long-term exposure to polystyrene microplastics introduced into aquatic ecosystems causes ROS formation in both the ovaries and testes of zebrafish. In addition, they emphasized that increasing doses of polystyrene microplastics activate the p53 mediated apoptotic pathway in gonads. (Qiang and Cheng 2021). Another study conducted on zebrafish highlighted the impact of polystyrene microplastics, emphasizing their role in increasing the bioaccumulation of microcystin-LR and causing endocrinological disorders through gonadal damage. (Lin, Luo et al. 2023). In a separate endocrinological study involving male Oryzias melastigma fish, the presence of polystyrene microplastics resulted in a significant estrogenic effect (Wang, Li et al. 2022). Although chronic studies have revealed that microplastics cause endocrinological disorders and gonadal damage, the low transgenerational effects are promising for now (Qiang, Lo et al. 2020). Despite the transgenerational effect being generally limited, the reproductive development of growing Oryzias latipes juveniles demonstrates a different outcome. The exposure of larvae to polyethylene microplastics resulted in a noteworthy decrease in the gene expression of 11-beta-dehydrogenase isozyme 2 (HSO11 β 2), which is directly linked to sperm quality during the developmental stage. This decrease led to poor sperm quality in the affected individuals (DiBona, Haley et al. 2022). Although it is encouraging that microplastics are not transmitted to the next generation, the increasing concentration of microplastics in freshwater and oceans is posing limitations on fish reproduction.

4. Conclusion

The emergence of various industrial products has been driven by increasing population and consumption needs. This industrial development has also led to the rise of new technologies, including nanotechnology, which has found applications in numerous sectors such as healthcare, transportation, technology, construction, food, and agriculture. Nanotechnology has played a transformative role, providing easy and cost-effective solutions in technological products, acting as a preservative or enhancer in agriculture, enhancing strength in mechanical and mechatronic fields, serving as colorants or coatings in textiles, and acting as targeted carriers in the field of healthcare. However, these widespread applications have also resulted in a significant amount of nano waste being generated in developed and developing countries. Sadly, the ultimate destination for much of this nano waste, which combines with soil, air, and water, has been the seas and oceans. On the other hand, the emergence of microplastics presents a slightly different challenge. MNPs can have a direct effect on the reproductive systems of fish. The physical

degeneration of the gonads causes a decrease in the quality of sperm and ovum and a decrease in the number of offspring. On the other hand, MNPs, which have also been found to cause estrogenic effects in male fish, also bring about changes in gender characteristics. Although not in microplastics, nano-pollutants also affect the next generation, causing offspring born with anomalies or developmental problems. Exposure to MNPs (magnetic nanoparticles) during the developmental stages of naturally-born offspring has been found to have detrimental effects on their development. Studies in this field indicate that the extent of damage is dependent on the dosage of MNPs. Fish, being among the last organisms affected by nano-pollution in the oceans, will likely experience harm only when the concentration of MNPs required to cause damage is already present. By that point, algae, plankton, and other marine creatures may have already perished. Even if MNPs do not directly impact fish, they can still disrupt aquatic ecosystems, leading to disturbances in the marine ecosystem and ultimately resulting in secondary factors contributing to fish mortality. In his review, (Dedman 2022) showed a few of the studies examining the effects of nano-pollution on marine ecosystems. The results from this section are simply shown in Figure 2.

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Figure 2. Effect of MNPs on males, females and larvae

In conclusion, while MNPs may not currently pose as significant of a threat as other factors such as global warming or chemical wastes, the ongoing industrial development raises concerns about their potential to become a major source of pollution in the coming decades. Without proper measures and precautions, MNPs could emerge as a significant contributor to environmental pollution. This, in turn, could lead to detrimental consequences such as fish mortality, reproductive impairments, and even species extinctions. It is crucial to address and mitigate the potential risks associated with MNPs to prevent such outcomes from occurring in the future.

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