

## Chapter

# Mind the Gut: Overview on the Microbiota-Gut-Brain Axis Intricacies and Insights from the Zebrafish Model

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## Abstract

The gut-brain axis represents a complex bidirectional communication network between the central nervous system and the gastrointestinal tract, notably through its microbiome. This axis involves multiple signaling pathways, including neural, hormonal, immunological, and metabolic processes, mediated by the intestinal microbiome that are highly conserved among vertebrates. These microorganisms play fundamental roles in modulating the immune system, digestion, and brain function through their interaction with the enteric nervous system, often referred to as the “second brain.” Changes in the microbial community – known as dysbiosis – due to inflammatory diseases or extrinsic factors, such as diet and pollutants, can result in impairments in the development, physiology, behavior, and overall health of organisms, in addition to being identified as a contributing factor to the emergence of neurological and neurodegenerative diseases. Through studies using animal models such as zebrafish, new scientific advances can be achieved, driving the development of innovative strategies to prevent and treat human diseases, as well as a better understanding of the link between dysbiosis and neurological disorders. This approach addresses central issues of the United Nations Sustainable Development Goals, specifically those aimed at promoting health and well-being. Thus, this chapter aims to provide a comprehensive overview of the functions and implications of the microbiome-gut-brain axis in maintaining homeostasis, with a particular focus on the potential deleterious effects of dysbiosis and the insights gained in the last decades from using zebrafish as an experimental model.

**Keywords:** dysbiosis, microbiota-gut-brain Axis (MGBA), microbiome, neurobehavior, zebrafish

## 1. Introduction

Humans and other animals are remarkably complex systems whose functioning, well-being, and homeostasis are shaped by a delicate interplay of intrinsic and extrinsic factors. Among the intrinsic factors, genetics, metabolism, and the immune

system play critical roles, while extrinsic influences, such as environment, diet, and lifestyle, further modulate most biological processes. Collectively, these elements converge to shape an individual's health and overall well-being.

In recent years, microbiome research has unveiled how microbial communities (including bacteria, fungi, and viruses residing in and on the human body) serve as key mediators between built-in and outside components, influencing everything from immunity to metabolism. Among these microbial ecosystems, the gut microbiota (i.e., the bacteria inhabiting the digestive system) has emerged as a mighty player, exerting profound effects on human health. These microorganisms play essential roles in immune modulation, digestion, and even brain function, underscoring their far-reaching impact [1]. This rapidly evolving field highlights the profound interconnectedness between human biology and the microbial world, offering transformative insights for health, disease, and personalized medicine.

Contemporary research evidence suggests a strong biological connection between the mind and the digestive system [2, 3]. Studies at the forefront of knowledge have demonstrated an intricate connection between dysbiosis – an imbalance in the composition of the microbiome – and the emergence of psycho-neurological diseases, such as depression, autism spectrum disorder (ASD), and neurodegenerative diseases [4, 5]. Thus, this connection between the microbiome and the nervous system, mediated by the microbiome-gut-brain (MGB) axis, represents a promising area that has been increasingly explored. In addition, it enables a greater understanding of how exposure to chemical compounds or habits, such as a healthy diet and proper hydration, can influence physical health and psychological well-being.

Through studies using animal models such as zebrafish (*Danio rerio*), which allow the recapitulation of changes in the microbiome-gut-brain axis, new scientific advances can be achieved. Such findings include the development of treatments based on the modulation of the microbiome or screening for compounds that have an adverse effect on the intestinal microbial community, which can induce neurological and inflammatory diseases, paving the way for innovative strategies for the prevention and treatment of human diseases [4, 6, 7].

Thus, research that seeks to elucidate the relationship between disruption of the microbiome-gut-brain axis and the appearance of atypical physiological and behavioral manifestations has the potential to revolutionize areas such as neuroscience, immunology, psychiatry, and even personalized medicine, contributing to a more integrative understanding of human health, as well as potentially teaching us that, with minor changes in our diet or lifestyle, we can enjoy a calmer mental state, improve immunity, and reduce the risk of developing neurological diseases such as Parkinson's and Alzheimer's. Ultimately, the balance between the mind and gut may be the key to achieving more vigorous health.

Therefore, this chapter aims to provide a comprehensive overview of the functions and implications of the microbiome-gut-brain axis in maintaining homeostasis, with a particular focus on the systems-level relationships, potential deleterious effects of dysbiosis, and the insights gained from using zebrafish as an experimental model in this field of research.

## **2. The microbiota-gut-brain (MGB) axis**

The gut-brain axis has long been known as essential for maintaining homeostasis. The intricate and complex interaction between the gut and the brain has become

an increasingly important topic in contemporary science due to its profound health implications [8]. Nonetheless, the gut microbiota, a vast community of trillions of microorganisms, has been recognized over the past two decades as an essential component in the regulation of the gut-brain functions. This progress has underscored the importance of a distinct MGB axis, a framework now widely acknowledged in the scientific literature. Mounting evidence has unveiled the bidirectional communication between the gut microbiome and the central nervous system (CNS), further solidifying the critical influence of this axis on physiology and behavior [2, 3, 8].

The MGB axis operates through bidirectional communication. This crosstalk occurs via multiple pathways, including immune signaling, vagus nerve transmission, and the enteric nervous system, as well as through microbial metabolic activity, such as the production of short-chain fatty acids, branched-chain amino acids, and peptidoglycans. Together, these mechanisms integrate gut microbial signals with CNS processes, underscoring the pivotal role of this axis in host physiology [9].

As reviewed by Cryan *et al.* [9], multiple lines of evidence support the idea that the microbiota plays a significant role in modulating the gut-brain axis. First, studies using germ-free animals have demonstrated that the absence of gut microbes results in noticeable alterations in brain development and function. Second, behavioral changes observed in animals administered specific bacterial strains, and the confirmation of similar effects in human studies, highlight the potential for cross-species translatability. Third, population-based studies, such as those following the Walkerton outbreak in Canada (*Escherichia coli* contamination), have demonstrated that exposure to infections can lead to lasting gut-brain symptoms. These findings are supported by animal models, where even low-level infections can influence behavior without triggering an immune response. Fourth, preclinical research has demonstrated that administering antibiotics, whether early in life or during adulthood, can lead to long-term alterations in the brain, spinal cord, and enteric nervous system. Finally, clinical observations of hepatic encephalopathy reveal that targeting the gut microbiota with antibiotics can significantly improve neurological symptoms. Collectively, this understanding shaped and strengthened the concept of the MGB axis, which is now a central focus in neuroscience and microbiome research.

Due to its critical role, the microbiome-gut-brain axis emerges as a promising platform for developing novel therapeutics interventions. Such treatments could address a wide spectrum of disorders, from conditions affecting mental health and cognitive function to obesity and various gastrointestinal (GI) illnesses, notably inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS).

Zebrafish possess a functionally conserved MGB axis that shares fundamental mechanisms with humans, while offering unique experimental advantages [10]. As in humans, the zebrafish MGB axis operates through bidirectional communication pathways involving microbial metabolites, immune signaling, and neural connections. These shared pathways similarly influence neurodevelopment, stress responses, and behavior in both species. The zebrafish gut microbiota, like its human counterpart, produces neuroactive compounds that can modulate CNS function, demonstrating remarkable evolutionary conservation of these interactions. Importantly, key microbial metabolites, such as butyrate and serotonin precursors, exhibit comparable neuroactive effects across both species. However, zebrafish exhibit faster microbiota colonization and simpler neural circuitry, allowing more precise observation of direct microbiota-brain effects without the complexity of mammalian cortical modulation [10, 11]. Despite these differences, the high degree of functional conservation makes the zebrafish a powerful model for studying fundamental mechanisms of the MGB

axis, which remain relevant to human biology, particularly in early neurodevelopment and stress-related disorders. The species' genetic tractability and transparency further enhance its utility for dissecting causal relationships in gut-brain communication that are difficult to study in humans.

Overall, the zebrafish is a highly valuable model for translational research. Both larval and adult zebrafish offer unique insights into disease mechanisms and drug responses, while their sensitivity to neuroactive compounds mirrors human pharmacodynamics in key aspects. The expanding molecular toolkit available for zebrafish manipulation continues to refine its applicability in modeling pathologies with increasing precision. As the use of the zebrafish model continues to expand, its contribution to identifying therapeutic targets, validating biomarkers, and guiding the development of safe, high-efficacy drugs will become increasingly indispensable, securing its role as a cornerstone in translational science [12–14].

### **3. Microbiome and the enteric nervous system**

Our planet is mainly a microbial world. The number of microbial species on the Earth vastly exceeds that of all other life forms combined. Thus, our existence is profoundly shaped by microbes, which are ancient, invisible life forms that have thrived for hundreds of millions of years, long before humans emerged. Therefore, there has not been a moment in our history when our bodies have not been in constant dialog with these microscopic partners, receiving their signals and responding in ways that continue to define our biology, health, and even our minds.

The microbiota is defined as the set of microorganisms that inhabit the body. In the context of human health, the microbiota is often associated with the colon microbiota, which harbors the majority (~95%) of the body's bacteria. In contrast, the microbiome encompasses both microorganisms and their genes, as well as the biological interactions they facilitate. Collectively referred to as the “second human genome,” the intestinal microbiome is considered by some experts to be a separate “organ” with distinct metabolic and immunological activity [15].

Interestingly, there are about 10 times more microbial cells in the gut than in the entire human body, comprising approximately 100 trillion microscopic organisms, within up to 5000 different species, with an approximate mass of about 1 kilogram [8, 15, 16]. The estimates, while potentially overestimated, are impressive. Some divergences have emerged in recent years, such as the work of Sender *et al.* [17], which revisited the question of the ratio of bacterial to host cells in humans, finding—based on more accurate and updated quantitative criteria—that it is much closer to 1:1, despite often presented as common knowledge that bacteria outnumber human cells by a ratio of at least 10:1.

Most of them are harmless or beneficial to the host, acting as protection against enteropathogens, extracting nutrients and energy from the diet, and contributing to immune functions. Therefore, understanding how the gut microbiota affects health and disease requires a shift in focus from individual pathogens to an ecological approach that considers the community as a whole [8].

Viewed through this lens, it has become clear that the microbiota plays a central role in shaping human health and disease, serving as a central regulator of host physiology. To integrate this idea, the concept of the holobiont has been developed to describe the ecological unit comprising both the host species and its symbiotic microbiota [18].

Although each person's microbiota profile is distinct, studies suggest that all healthy adults share a group of intestinal bacterial species, which would constitute a "core microbiota." It is estimated that each person harbors thousands of phylotypes at the species level, with the two most prominent phyla being Firmicutes and Bacteroides, representing at least  $\frac{3}{4}$  of the microbiome, while the most prevalent species are *Faecalibacterium prausnitzii*, *Roseburia intestinalis*, and *Bacteroides uniformis* [8, 19]. According to combined data from the Human Microbiome Project and MetaHIT, at least 2776 prokaryotic species have been identified in human fecal samples. These species span 11 distinct phyla, with *Proteobacteria*, *Firmicutes*, *Actinobacteria*, and *Bacteroidetes* collectively representing over 90% of the gut microbiome [20, 21]. In contrast, *Fusobacteria* and *Verrucomicrobia* are present in much lower abundances [22].

A vast and dynamic microbial diversity has been observed, varying significantly over time and across different human populations [8]. Several factors can influence the microbiota composition during an individual's life, including infections, mode of birth delivery, use of antibiotics, nutritional provision, environmental stressors, and host genetics. Additionally, microbial diversity tends to diminish with aging [9].

In zebrafish, on the other hand, studies have shown that, under controlled laboratory conditions, the diversity and abundance of the most common microbial phyla tend to be relatively uniform, reflecting a stable core microbiota. However, this apparent uniformity can be significantly influenced by a range of variables, including the origin of the animals, their genetic background or lineage, dietary composition, and various environmental factors such as housing systems, water sources, and microbial exposure. According to Borrelli *et al.* [23], an analysis of gut bacterial communities reveals that they are dominated by *Fusobacteria*, followed by *Proteobacteria*, *Firmicutes*, *Actinobacteria*, and *Cyanobacteria*. This diversity is also evident in the literature, as illustrated by Davis *et al.* [11], who found that, at 6 days post-fertilization (dpf) in zebrafish larvae, the phyla *Proteobacteria* and *Cyanobacteria* were the most prevalent. Teng *et al.* [24] found *Proteobacteria*, *Bacteroidetes*, *Firmicutes*, and *Fusobacteria* as the most abundant in the juvenile zebrafish intestine, while Shi *et al.* [25] reported the *Firmicutes*, *Proteobacteria*, *Fusobacteriota*, and *Bacteroidota* as most abundant phyla in zebrafish guts under non-treated conditions.

Since the gut is the microenvironment where most of the interactions between the microbiota, their metabolites, and the host happen, it soon got clear to the scientists that such interaction prevalently happens through a network of neurons known as the enteric nervous system (ENS), positioned to respond directly or indirectly to the microbiota and its metabolites [9]. This interconnection, known as the MGB axis, represents a tangled physiological network of bidirectional communication involving neuroendocrine, immunological, and neural pathways. Notably, the gut serves as a crucial interface where microorganisms, their metabolites, and the immune and nervous systems interact [26].

This "small brain," specifically, is a complex network of more than 100 million nerve cells that extends through the gastrointestinal tract, popularly known as the "brain in the gut" or "second brain" due to its autonomy and vast communication with the CNS [27]. The ENS is structured into two ganglionated plexi, the submucosal and myenteric plexus, containing neurons and enteric glial cells, connected by a dense network of nerve fibers and glial processes [28]. It is primarily responsible for regulating intestinal motility, the secretion of digestive enzymes, immune function, the growth and integrity of the mucosa, intestinal permeability, blood flow in the intestine, and interacting directly with the microbiome [28].

ENS is currently recognized as playing a crucial role in general health and gastrointestinal physiology, affecting inflammatory and behavioral processes, as well as being related to diseases such as irritable bowel syndrome and neuropsychiatric disorders, through the microbiome-gut-brain axis [1, 19, 27, 29–31]. Several recent studies have demonstrated that the microbiota also plays an important role in the development of brain function, pathology of neurobehavioral, psychiatric, and neurodevelopmental disorders, some of which depend on activation of the vagus nerve [16, 32–36].

Cryan *et al.* [9], Hyland and Cryan [37], and other groups have reviewed the mechanisms by which the microbiota influences ENS development and function, which includes activation of pattern recognition receptors (PRRs), including toll-like receptors (TLRs), in particular, TLR2 and TLR4, which are involved in the recognition of microbial molecules. While much attention has been given to how the microbiota influences ENS development and function, it is now becoming clear that the ENS can, in turn, regulate the composition and activity of the gut microbiota [38]. Additionally, studies in germ-free animals have shown that bacterial colonization of the gut is critical for the development and maturation of the enteric and central nervous systems [30, 39].

The brain and gastrointestinal tract are both essential sensory systems that identify, relay, process, and react to cues from the body's internal and external surroundings. At the interface of this sensory function, immune cells in the gut and brain consistently track environmental factors, eliciting responses that inform the body's physiological state [40]. Thus, understanding aspects related to the microbiome-gut-brain axis provides valuable insights into its roles in maintaining homeostasis, overall health, mental well-being, and the sustainable balance of the intestinal microbiota.

#### **4. Dysbiosis and its downstream effects**

Dysbiosis is broadly understood as the disruption of the normal balance between the microbiota and the host, marked by the change in the community diversity—usually characterized by the loss of beneficial microorganisms and expansion of potentially harmful ones—or abundance shift of specific microbial species [32, 35, 41]. Parallel and clinically oriented definitions can also be represented by compositional and functional alterations in the microbiota in individuals with disease compared to healthy subjects [42]. However, it should be noted that, more often than not, unhealthy outcomes evolve as a consequence of dysbiosis rather than being directly caused by it.

One of the most valuable benefits conferred by a balanced microbiota is the ability to repulse pathogens, which is known as colonization resistance [43]. This phenomenon is part of the intrinsic roles the microbiota play in supporting the balance and well-functioning of the whole system. While facing minor microbiota changes, individuals may begin to exhibit early symptoms of related diseases, such as immunosuppression, an inflammatory response, or even changes in behavior and well-being.

During inflammation triggered by or resulting from dysbiosis, immune and non-immune cells form complex networks of interaction to regulate such responses in the central nervous system, enteric system, and gastrointestinal tract [40]. Many neurological disorders are associated with gut inflammation and inflammatory markers in the blood, which are linked to deficits in brain function and behavior. Specifically,

inflammatory bowel diseases are a group of inflammatory conditions unrelated to infection, and their prevalence is increasing significantly in industrialized countries. Advances in the understanding of these comorbidities and their relationship with dysbiosis have been made through chemical induction models of the disease. Some of the compounds most frequently used to induce dysbiosis in animals are sodium dextran sulfate (DSS) and 2,4,6-trinitrobenzene sulfonic acid (TNBS) [44]. Although the exact mechanism of DSS-induced colitis remains unclear, it is thought to have a deleterious effect on intestinal epithelial cells, leading to the disruption of barrier integrity and the translocation of commensal bacteria, which then activate innate immune responses [7, 44].

In addition to chemicals, other environmental factors can also exert a negative effect on the intestinal microbial profile. In this sense, diet appears to have one of the most potent influences on the intestinal microbiome. In modulating dietary dysbiosis, prolonged exposure to a high-fat diet induces a significant increase in the expression of markers of pro-inflammatory, apoptotic, and proliferative processes in the tissues of the intestine, liver, and brain [45]. Long-term consumption of a high-fat diet can even cause activation of the Wnt/ $\beta$ -catenin signaling pathway in brain tissue. Fish fed with such a diet were shown to present anxiety, aggression, and hyperactivity [46], which confirms that an imbalance in intestinal homeostasis and the microbiome can lead to non-traumatic brain injury and stimulate a regenerative response.

In the literature, considerable attention is devoted to investigations focused on understanding the association between the microbiota and a wide range of human pathologies, including metabolic, inflammatory, neurological diseases, and even cancer [41]. Despite this growing attention, further studies are needed to deepen our understanding of the specific mechanisms linking the microbiota to various pathologies and to develop effective therapeutic solutions. These investigations should focus on how the microbiota directly contributes to the development or exacerbation of these conditions.

Lastly, the assessment of how dysbiosis can be correlated to health impairments was driven by advancements in sequencing techniques. Usually, the dysbiosis is evaluated by sequencing the V3 and V4 regions of the 16S rRNA gene. This is the most commonly used target, as it serves as a universal marker for bacterial identification and classification. The gene contains highly conserved regions and hypervariable regions, providing quantitative information to classify microbial communities.

## **5. Zebrafish as a model for microbiome-gut-brain axis research**

Animal models have been fundamental in the study of the cellular and molecular mechanisms underlying human diseases, providing the basis for understanding animal physiology and developing new therapeutic strategies [44]. Specifically, regarding the MGB axis, the use of relevant laboratory animals with varying levels of microbial complexity presents a promising opportunity to better understand the effects of host-microbiota interactions in regulating health and disease states. Furthermore, manipulation of the composition of the gut microbiota, the use of probiotic treatments, and even the creation of germ-free strains in vertebrates and invertebrates allow for functional and mechanistic studies aimed at determining causality in disease-associated biological changes [41].

In this context, zebrafish have emerged in recent decades as a valuable laboratory animal for studying the MGB axis, with significant implications in the areas

of translational medicine, toxicology, disease modeling, and behavioral studies. This fish, which serves as an alternative model, offers numerous advantages that make it an ideal candidate for unraveling the complex interactions between the gut microbiome, the nervous, and immune systems [47–50]. Furthermore, since the first applications by George Streisinger in the early 1980s, zebrafish have become a prominent organism for understanding the role of leukocytes in controlling sterile or microbial-induced inflammation, for modeling various human diseases, as well as in pharmacology through studies that contribute to toxicological assessment and preclinical drug discovery [51–53].

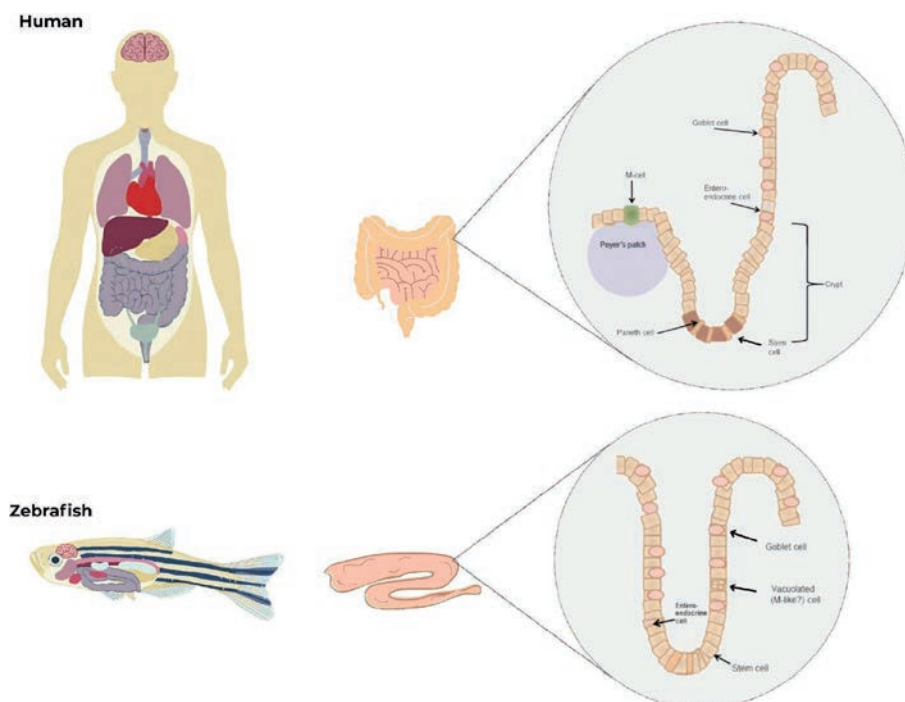
The zebrafish is a small freshwater fish native to South Asia (3–4 cm in length), which is generally considered a juvenile at 45 dpf and an adult at 90 dpf. Females spawn every 5–7 days, laying an average of 300 eggs per week, and reproduce year-round. The eggs are fertilized *ex vivo*, and the transparent embryos and larvae develop ideally at an average temperature of 28°C [54].

Zebrafish embryogenesis is rapid with a distinct head-tail axis evident by 10 hours post-fertilization (hpf), and the process is characterized by the period of cleavage (45–90 min), blastula (4 h), gastrulation (10 h), segmentation (10.5–20 h), pharyngeal period (24–36 h), and hatching (48–96 h) [55]. Most major organs, including blood vessels, brain, heart, liver, intestine, and eyes, are formed by two dpf, becoming fully functional within a week [56]. The process of organ formation, such as the kidneys, heart, blood-brain barriers, and gastrointestinal system, also occurs similarly in mammals [57].

Specifically regarding the zebrafish gastrointestinal tract, its intestine forms a folded tube that fills most of the abdominal cavity. While it possesses only three intestinal segments, unlike the five found in mammals, there is significant functional similarity [58, 59]. Instead of a stomach, teleost fish have an anterior segment, called the intestinal bulb, characterized by an expanded lumen and strong expression of digestive enzymes [58]. The intestinal bulb is followed by the midgut, which contains goblet and enteroendocrine cells, and then a short posterior section, which is devoid of absorptive enterocytes, analogous to the colon in mammals [59].

The intestinal epithelium of zebrafish exhibits similarities to that of mammals (**Figure 1**). Intestinal epithelial cells travel from the base of the folds up to the tip, culminating in their elimination through apoptosis. As in mammals, the renewal of these epithelial cells depends on the microbiota [61]. There are also similarities in the mechanisms that control differentiation toward the secretory lineage, such as the conservation of Delta-Notch signaling to induce goblet cell differentiation and the conserved inhibition of Kruppel-like factor 4 (KLF4), a master regulator of goblet cell differentiation, by retinoic acid [62, 63]. Immune cells can also be detected within the intestine, where T cells are present from 3 weeks post-fertilization [64]. Zebrafish also exhibit several differences in their intestinal epithelial architecture compared to mammals. For example, the epithelium lacks Lieberkühn crypts, and only three types of differentiated epithelial cells have been identified in zebrafish: enterocytes, goblet cells, and enteroendocrine cells [65].

Microbial colonization of the zebrafish begins around three dpf, when larvae hatch from the axenic environment within the chorion [59]. By 2 weeks of age, the zebrafish microbiome is relatively well established, with a community that resembles that found in adults. The core microbiota undergoes specific changes over time, being dominated by *Proteobacteria* (76–82%), a notable difference from mammals, where *Firmicutes* and *Bacteroidetes* are the dominant phyla. Qualitatively, six of the eleven bacterial phyla found in zebrafish are detected in the mouse gut microbiota, and five



**Figure 1.**  
 Compared anatomy of the human and zebrafish intestinal tracts and its epithelial structure. Zebrafish do not have Paneth cells, crypts, and organized lymphoid structures such as mesenteric lymph nodes, isolated lymphoid follicles or Peyer's patches. Comparison of cell types and intestinal structures (detail) was adapted from Brugman [60].

of these are also shared by the adult human microbiota [66]. On the other hand, the microbiota of zebrafish and mammals respond similarly to various stimuli, including toxins or high dietary levels of fat, which allows for a significant understanding of the subject from studies using the zebrafish model [41].

Regarding general aspects of the immune response, zebrafish exhibit broad conservation in the response of cytokines, receptors, and components of several signaling pathways, primarily those known for recruiting cells of the innate immune system, such as neutrophils and macrophages. A phylogenetic study of cytosolic NOD-like receptors (NLRs) in zebrafish revealed three distinct families: NLR-A, orthologs of mammalian NOD-like receptors; NLR-B, orthologs of mammalian NLRP receptors; and NLR-C, a group present exclusively in teleost fish [67, 68].

Given the in-depth knowledge of zebrafish biology and physiology, as well as their high homology with humans, it is possible to establish models of both inflammatory bowel and neurological diseases. In the context of inflammatory bowel diseases, zebrafish have emerged as a functional model to study the inflammatory response through disruption of the MGB axis, and also allow us to explore how this altered signaling network contributes to metabolic and neurological disorders. In the latter case, research with zebrafish allows researchers to study neurodegenerative diseases – such as Parkinson's and Alzheimer's – as well as biological responses, including anxiety, stress, or hyperactivity, as they exhibit behavior comparable to that of mammals, but are easier to handle and reproduce through behavioral tests, such as the exploration test [23, 69].

The research focused on the zebrafish MGB axis, evaluation of dysbiosis, and its consequences have been extensively studied using zebrafish in recent years. An overall summary of contemporary research in the main areas is presented below.

### **5.1 Experimentally induced shifts in the microbiota**

The study of experimentally induced dysbiosis has been useful to elucidate the effects and mechanisms related to the alteration in microbial diversity associated with chemical exposure or rearing settings. In that regard, dysbiosis can be tested primarily by drug administration, different diet regimes, or exposure to environmental contaminants.

It has been well established in the literature that overnutrition and calorie-dense foods have the potential to alter lipid metabolism, induce inflammation, and alter gut microbiota. The approach, consisting of providing high-fat diets to zebrafish and, after a medium to long period, assessing the effects on dysbiosis and inflammatory markers, is now often applied in experimental research. Some examples to illustrate this methodology are presented here. For example, Arias-Jayo *et al.* [45] established a high-fat diet in a zebrafish colony over a period of 25 days. The exposure led to microbial dysbiosis, marked by an increased relative abundance of the phylum *Bacteroidetes*, with additional activation of NF- $\kappa$ B signaling, which confirmed the onset of intestinal inflammation, also evidenced by the upregulation of genes involved in receptor signaling, antimicrobial responses, and the inflammatory cascade. The authors find that the intestinal barrier was compromised, accompanied by elevated mucin production from goblet cells.

In addition, Qiao *et al.* [70] also reported the decreased abundance of *Proteobacteria* in the zebrafish fed with a high-fat diet (15% fat compared to 7% fat in the control group). Interestingly, when authors further investigate the causal relationship between the microbiota and lipid accumulation in colonized three dpf germ-free zebrafish and the intestinal microbiota of adult zebrafish from the high-fat diet experiment, they identify that such microbiota reshaping leads to detrimental lipid accumulation.

Recently, zebrafish have been widely used to investigate potential agents of dysbiosis, particularly in the form of pollutants, besides drugs and diets. This area connecting environmental toxicology with dysbiosis and physiological impairments in zebrafish has been very fruitful. Examples of studies that illustrate such line of research are the investigation of pollutants such as (nano)microplastics, where significant alterations in the gut microbiome and tissue metabolic profiles were observed, with most of these alterations linked to inflammation, lipid metabolism, and oxidative stress [31, 71]. Also, nanomaterials, like quantum dots, induced behavioral abnormalities in zebrafish, associated with inhibited acetylcholinesterase activity, increased dopaminase activity, and neuronal apoptosis. In the intestine, the exposure resulted in disrupted mucosal barriers, increased permeability, and triggered oxidative stress and apoptosis, as well as a reduction in gut microbial diversity [72]. Finally, literature findings indicate that exposure to per- and polyfluoroalkyl substances (PFAS) can induce hepatic immunotoxicity, associated with intestinal microbiota dysbiosis, in adult zebrafish [73].

Due to the elevated use of psychoactive therapeutic drugs, their release into the environment has also been of crucial concern. Flunitrazepam, for example, is a medication that causes severe depression of the CNS and is used sporadically to reduce anxiety attacks and sleep disorders. In that regard, Lin *et al.* [48] investigated

how a 30-day exposure to the benzodiazepine drug flunitrazepam (FLZ, 0.2 and 5 µg/L) induces neurotoxicity. The authors found that FLZ disrupted the balance of gut microbiota and caused an increase in pathogenic bacteria, such as *Paracoccus* and *Aeromonas*, leading to pathological damage to the intestine. Moreover, intestinal pro-inflammatory factors (i.e., IL-1 $\beta$  and TNF- $\alpha$ ) were upregulated, while tight junction proteins, Occludin and Zonula Occludens 1, were found to be downregulated.

Wang *et al.* [49] also studied the mechanisms of toxicity of triclosan, a broad-spectrum bactericide highly used in the industry, on the gut-brain axis. For that purpose, zebrafish were exposed to 250 µg/L of triclosan from 30 to 90 dpf. The chronic exposure to triclosan affected the zebrafish development and caused neurobehavioral abnormalities by inhibiting acetylcholinesterase and dopamine activity, promoting brain neuron apoptosis, and accelerating diencephalic lesions. Moreover, the exposure led to gut microbiota dysbiosis and decreased diversity, characterized by an increase in pathogenic bacteria and a decrease in probiotics in the adult zebrafish gut, resulting in numerous pathological damages, including inflammatory infiltration, thinning of the intestinal wall, and an increase in goblet cells within the villus.

Novel classes of environmental pollutants, such as the (nano)microplastics, have been implicated in zebrafish dysbiosis. To investigate the adverse effects of 50 µm and 100 nm (nano)microplastics on the zebrafish gut microbiota, antioxidant activity, and innate immune response, Pei *et al.* [71] exposed adult zebrafish to 100 and 1000 µg/mL of the xenobiotic. The exposure led to significant changes in the richness and diversity of gut microbiota, inducing oxidative damage through the increased activity biomarkers such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px). It altered the level of most pro- and anti-inflammatory factors, including IL-8, NF- $\kappa$ B, IL-10 (increased), and TNF- $\alpha$  (decreased). Still, in that regard (i.e., nanotoxicology), Cao *et al.* [72] tested if and how quantum dots (Black Phosphorus Quantum Dots - BPQDs, 5, 50, and 500 µg/mL) could induce toxicity and dysbiosis in zebrafish. The authors observed that, following 35 days of exposure, the substance caused locomotor behavior abnormalities, inhibited acetylcholinesterase activity, induced dopaminase activity, and promoted apoptosis in the brain tissue. Meanwhile, BPQDs caused damage to the physical and chemical barriers in zebrafish intestinal tissue, which increased the permeability of the intestinal mucosa, and induced oxidative stress and apoptosis. Additionally, dysbiosis was observed as a result of the decreased diversity. Specifically, the relative abundance of *Firmicutes*, *Bacteroidetes*, and *Actinobacteria* decreased, while the relative abundance of *Proteobacteria* and *Clostridiobacteria* increased.

Another example of how contaminants of emerging concern (CEC) could impact the MGB axis is illustrated by the study of Huang *et al.* [73] when assessing the perfluorooctane sulfonate (PFOS) toxicity for 21 days. In summary, the results support the role of PFOS in changing the relative abundance of *Plesiomonas*, *Aeromonas*, *Cetobacterium*, *Shewanella*, and *Vibrio*. Furthermore, PFAS exposure upregulated the expression of *hepcidin*, *muc*, and the immune-related genes *mpo* and *saa*, while downregulating the tight junction-associated gene *occ* in the intestine.

## 5.2 Use of probiotic treatments

Some bacteria are termed psychobiotics because of their ability to alleviate stress and anxiety-related behaviors, thereby enhancing mood and cognition [4]. This approach, which involves introducing specific strains to promote well-being and restore homeostasis, falls within the broader field of probiotics.

To test the effects of probiotic treatments, Olorocisimo *et al.* [4] tested three Lactobacillaceae isolates, namely, *Lactobacillus paracasei*, *Lactobacillus delbrueckii*, and *Lactobacillus casei*, which were inoculated twice a day into fish tanks containing 10 6–7 month-old female fish each, at a final concentration of  $1.6 \times 10^6$  CFU/mL per aquarium for 2 weeks. Authors reported a considerable decrease in anxiety-like behavior in *L. delbrueckii*-exposed fish, as assessed by the novel object exploration and novel tank diving tests. This observation resulted from the fact that the *L. delbrueckii* group spent most of the time in the upper half of the novel tank and had the shortest latency time. Besides the behavioral tests, the expression patterns of genes commonly associated with anxiety reveal that glutamic acid decarboxylase (*gad1*) was upregulated in the gut of *L. delbrueckii* group, as well as in the brain of *L. casei*-exposed group. To confirm that such an effect is due to a change in bacterial taxa abundance, microbial sequencing revealed that the *L. delbrueckii*-treated group showed a lower percentage of *Firmicutes* and a higher percentage of *Actinobacteria* than controls. Genus-level analysis revealed that zebrafish exposed to *L. delbrueckii* exhibited a marked increase in the abundance of *Lactobacillus* sp., *Verrucomicrobium* sp., *Kaistia* sp., *Methylobacterium* sp., *Singulispira* sp., *Gordonia* sp., and *Mycobacterium* sp. Conversely, the genera *Legionella*, *Planctomyces*, *Flavobacterium*, and *Prevotella* showed a reduction in abundance compared to controls.

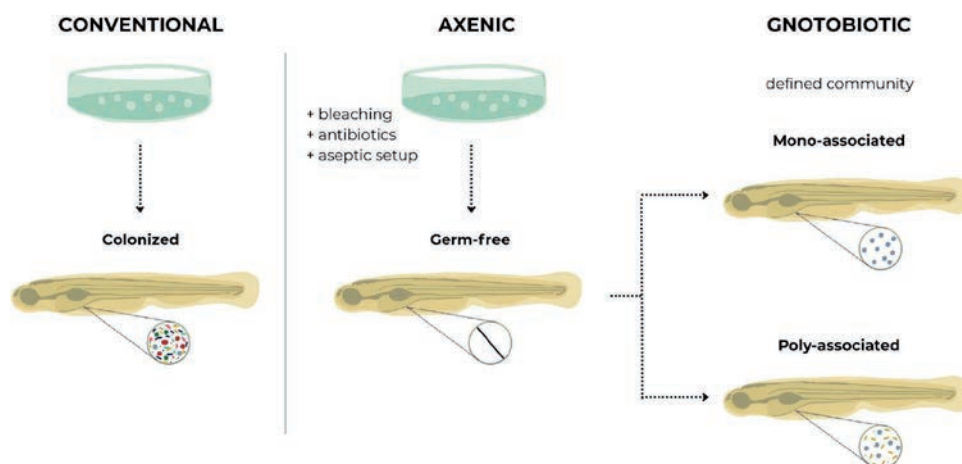
Another study focused on investigating the probiotic modulation outcomes in zebrafish behavior was the Borrelli's *et al.* [23] study. After 28 days of dietary administration with the probiotic *Lactobacillus rhamnosus* IMC 501 it was found differences in shoaling behavior, brain expression levels of *bdnf* and of genes involved in serotonin signaling and metabolism (i.e., *tph1a*, *tph1b*, and *htr1aa*) between control and treated zebrafish group. In addition, there was a reported modulation in the microbial communities with a significant increase of *Firmicutes* and a trending reduction of *Proteobacteria*.

### 5.3 Axenic (germ-free) and gnotobiotic strains

Zebrafish reproduction is well known, and embryo development occurs outside the progenitor body, which provides crucial advantages to early life studies. More than that, the interior of the chorion is free of microorganisms, providing an excellent opportunity to use this species to answer scientific questions where a germ-free status may be required. Given the comparative simplicity of producing germ-free zebrafish, this species represents a compelling model for studies focused on gnotobiotics.

In the scientific context, axenic status refers to a condition in which an organism or culture is established and sustained in the strict absence of any other contaminating organisms, that is, it refers to a state of being free from any association or contamination with other organisms. In comparison to the axenic strain, there are the gnotobiotic colonies (from Greek *gnōtos* 'known' + biotic). This term describes animals in which the microbial status is fully known. Within this broad definition, one can consider two main subclasses: (I) mono-associated, that is, animals colonized with only one defined microbial species; and (II) poly-associated, that is, colonized with a defined (but reduced) community of microbes. It is important to note that these two approaches are correlated, as the starting point for all gnotobiotic zebrafish research is the derivation of germ-free embryos (**Figure 2**) [74, 75].

Since zebrafish behavior can be easily tracked in the laboratory, scientists can now associate alterations in these patterns with microbial changes, linking these effects to neurodegenerative disorders' phenotypes. One study led by Weitekamp and



**Figure 2.**  
 Colonization status of zebrafish. The chorion acts as a protective barrier that is largely impermeable to microbes in the surrounding water. This characteristic makes zebrafish embryos an excellent model for studying host-microbiome interactions, as researchers can easily generate and maintain germ-free (axenic) fish and colonies with defined communities of microorganisms (gnotobiotic).

Kvasnicka [76] demonstrates a clear relationship between zebrafish dysbiosis and behavioral alterations, specifically hyperactivity. Axenic zebrafish larvae exhibited increased locomotor activity. This phenotype was reversed when associated with particular host-derived bacterial species or their mixtures, suggesting that specific microbes are essential for normal neurobehavioral development. Moreover, the findings highlight that the composition and complexity of the intestinal microbiota play a critical role in modulating zebrafish behavior, with implications for experimental reproducibility in behavioral studies involving pharmacology and toxicology.

In another study, scientists tested whether the microbial composition could influence the zebrafish's developmental neurobehavioral toxicity due to exposure to the hazardous chemical benzo[a]pyrene (B[a]P). Stagaman *et al.* [6] applied a gnotobiotic zebrafish experiment, up to 9 dpf, and found that gut microbiome state altered the relationship between B[a]P exposure concentration and behavioral response.

Additionally, indirect effects resulting from the disruption of beneficial host-microbiota interactions may also contribute to the toxicity of nanoparticles, notably silver nanoparticles, which have antimicrobial capacity. In that context, Brinkmann *et al.* [77] compared the acute toxicity of silver and zinc oxide nanoparticles (nAg and nZnO) to zebrafish larvae that were either germ-free or colonized by microbiota. Over 2 days of exposure, germ-free zebrafish larvae were more sensitive to nAg than microbially colonized larvae, whereas silver ion toxicity did not differ between germ-free and colonized larvae.

## 6. Final considerations and perspectives

In a world where novel chemicals are continuously being produced and the exposome (i.e., the totality of environmental exposures over a lifetime) is continually expanding, microbiome research and its effects on the gut-brain axis highlight the urgent need to understand how emerging contaminants and lifestyle habits impact host physiology through microbiome disruption.

Furthermore, although significant progress has been made in terms of scientific knowledge, we still have a long journey to go to understand the full, body-wide impact of a shifting microbiome. Because we have co-evolved with our microbiota, they play a crucial part in setting up and running all our other bodily systems, meaning that microbial communities are not passive guests but active regulators of host physiology, particularly influencing immune maturation, neurodevelopment via the gut-brain axis, and metabolic processes. Disruption of this microbial ecosystem, through factors such as antibiotics, poor diet, or environmental contaminants, can lead to systemic dysregulation and contribute to a range of disorders. This understanding positions the microbiota as a system-wide modulator of health and disease, emphasizing its relevance in avant-garde scientific research seeking to find a deeper understanding of disease progression and homeostasis.

Building on this perspective, it is important to recognize that while there is growing evidence linking microbiota alterations to psychiatric, neurological, and neurodegenerative diseases, definitive causal relationships remain unproven and require cautious interpretation. As toxicology shifts toward a systems-level, mechanism-driven approach, integrating microbiome research in biomedical research will be essential to unravel how exposures shape host health through microbial pathways. Continued multidisciplinary efforts are crucial for moving beyond speculation and translating microbiome insights into practical strategies for disease prevention and health promotion.

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## **Authors contribution**

All authors have made substantial intellectual contributions and approved it for publication.

## **Conflict of interest**

The authors declare no conflicts of interest.

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