Critical Review

The Gut Microbiome and Aquatic Toxicology: An Emerging Concept for Environmental Health

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Abstract: The microbiome plays an essential role in the health and onset of diseases in all animals, including humans. The microbiome has emerged as a central theme in environmental toxicology because microbes interact with the host immune system in addition to its role in chemical detoxification. Pathophysiological changes in the gastrointestinal tissue caused by ingested chemicals and metabolites generated from microbial biodegradation can lead to systemic adverse effects. The present critical review dissects what we know about the impacts of environmental contaminants on the microbiome of aquatic species, with special emphasis on the gut microbiome. We highlight some of the known major gut epithelium proteins in vertebrate hosts that are targets for chemical perturbation, proteins that also directly cross-talk with the microbiome. These proteins may act as molecular initiators for altered gut function, and we propose a general framework for an adverse outcome pathway that considers gut dysbiosis as a major contributing factor to adverse apical endpoints. We present 2 case studies, nanomaterials and hydrocarbons, with special emphasis on the *Deepwater Horizon* oil spill, to illustrate how investigations into the microbiome can improve understanding of adverse outcomes. Lastly, we present strategies to functionally relate chemical-induced gut dysbiosis with adverse outcomes because this is required to demonstrate cause–effect relationships. Further investigations into the toxicant–microbiome relationship may prove to be a major breakthrough for improving animal and human health. *Environ Toxicol Chem* 2018;37:2758–2775. © 2018 SETAC

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THE IMPORTANCE OF THE MICROBIOME IN HEALTH AND DISEASE

A microbiome is defined as any collection of microbiota (bacteria, archaea, viruses, and eukaryotes). The immediate environment of these microorganisms is also typically included in the definition of the microbiome because biotic and abiotic characteristics of the surrounding environment can influence the composition of the microbiome (Marchesi and Ravel 2015). Microbiomes are ubiquitous, occurring in our environment (e.g., soil, water, air microbiomes) as well as in association with organisms (e.g., gastrointestinal, lung, skin microbiomes).

Microbiomes that establish symbiotic relationships with organisms often offer important biological services to the host. These symbiotic microbiomes are often referred to as functional microbiomes because they perform important biological functions for the host. Although the majority of the microbiome research has focused on the gastrointestinal microbiomes (esophagus, stomach, gut), there are numerous other tissues that contain a functional microbiome including the skin, respiratory (mouth, lungs, gills), and reproductive tissues (Cho and Blaser 2012). Thus, these assemblages show tissue-specific diversity and function and are susceptible to modulation from the outside environment.

Microbial communities present in the tissues of humans, animals, and plants play an essential role in physiological homeostasis. These tissue-associated microbiomes are important for nutrient processing and uptake, providing immune defenses from pathogenic microbes, and for the

biotransformation of toxicants (Hollister et al. 2014; Claus et al. 2016). Disruption of the microbiome has been associated with a number of diseases including inflammatory bowel disease, diabetes, obesity, chronic obstructive pulmonary disease, cystic fibrosis, asthma, and vaginal pathological conditions (Fettweis et al. 2011; Huang et al. 2013; Kostic et al. 2014; Surette 2014; Hartstra et al. 2015; Huang and Boushey 2015). However, it is not always clear whether microbiome dysbiosis is the root cause, a contributor, or a response to the environmental conditions associated with these diseases.

ASSESSING STRUCTURE AND FUNCTION OF THE MICROBIOME: NEW TOOLS OF THE TRADE

In the past, exploration of the microbiome was limited to selective culturing of pathogenic bacteria because the density and diversity of most microbiomes precluded general culture and identification. There was initially little interest in nonpathogenic bacteria until recently, when it became apparent that microbiomes play an essential role in the physiology of humans and animals (Hiergeist et al. 2015). As a result, emerging technologies have been optimized to determine the composition and function of the microbiome. For example, the microbiota can play a functional role in the metabolism of carbohydrates, amino acids, and lipids, as well as sulfur and nitrogen metabolism and alkane degradation. Currently, next-generation sequencing platforms are the technology of choice for the majority of microbiome studies. For strictly compositional analysis, investigators typically construct libraries targeting the hypervariable regions of the phylogenetically conserved 16S ribosomal RNA (rRNA). Universal primers are used in conserved regions to amplify these hypervariable regions, followed by sequencing and assignment of taxonomy as an operational taxonomic unit because sequencing resolution to the genus or species level is not always possible using this approach. This approach is more cost-effective than whole-genome or transcriptome-based approaches because the targeted amplicons allow for focus on a single short-length gene for each bacterial species. As a result, total reads required for a representative sampling of the microbiome are comparatively low, facilitating the use of more cost-effective platforms. Sequence results from 16S rRNA sequencing typically go through quality control procedures followed by assignment of operational taxonomic units, which can be used to determine the composition of microbiome samples. Numerous pipelines have been developed to help with this process, including Quantitative Insights into Microbial Ecology and Mothur (Schloss et al. 2009; Caporaso et al. 2010). Although many studies vary in their specific approach to sequencing and analyzing 16S-based microbiome data sets, Benjamino et al. (2018) provide a general protocol for this analysis within a toxicological context.

A limitation of the 16S rRNA–based approach is that only a very small part of the bacterial genome is used to identify the species, which only allows for determination of relative species abundance and provides little information about the functions of the species that are present. This approach also misses bacterial

plasmids, which may also present an interesting mechanism for toxicant resistance. To bridge this gap, investigators have devised methods for linking 16S rRNA composition data with what is known about the essential functions of specific bacterial operational taxonomic units, using tools such as Phylogenetic Investigation of Communities by Reconstruction of Observed States (Langille et al. 2013). This computational program uses knowledge of bacterial evolution and function to estimate the contributions of gene families to a metagenome using 16S rRNA sequencing data. In doing so, biological insight can be achieved on the enrichment of processes that involve the microbiome.

An increasing number of studies have moved to shotgun-based genomic and transcriptomic approaches that combine both bacterial community compositional analysis and gene-level information regarding essential functions performed by bacterial communities. These approaches are more expensive; however, they provide valuable information about which genes are present within a community (metagenomics) or which genes are being modulated within a specific experimental design or scenario (metatranscriptomics). Although analysis of these data is more complicated and requires specially designed pipelines like MEGAN, SAMSA, or MetaTrans, these types of approaches are necessary to better characterize the functionality of a specific microbial community (Huson et al. 2007; Martinez et al. 2016; Westreich et al. 2016). Further, recently developed tools such as PALADIN (Westbrook et al. 2017) can be used to predict functional protein products from the metagenomics data, and computational software continues to improve at a rapid rate, overcoming challenges accompanying these complex data sets to better address the functional aspects of the microbiome. Figure 1 outlines the role of each sequencing strategy in addressing questions about the microbiome. We point out that the proteome and the metabolome are also integral to this flow of information and that microbial composition and abundance are directly related to the type and the concentration of metabolites that are produced in the gut. As such, although assessment through metagenomics and metatranscriptomics can be used to predict the impacts of environmental stressors on microbiome function, investigators have also turned to metabolomics to determine if changes in composition or function at the gene level translate to alterations in levels of metabolites that are produced and/or metabolized by these microbiota and known to be associated with disease. Mass spectrometry– and nuclear magnetic resonance–based approaches have emerged as the go-to technologies for both targeted and nontargeted assessment of the metabolome in the gastrointestinal lumen (Saric et al. 2007; Theriot et al. 2014; Sinha et al. 2016).

DIVERSITY OF MICROBIAL COMMUNITIES AMONG HOST SPECIES

Data supporting or refuting the presence of core phyla for each host species have been presented in the literature, but there continues to be some skepticism regarding the existence of these core microbial phyla. Much of this notion stems from the idea that hosts have coevolved with microbes, such that a core set of microbes may be expected in all healthy individuals in
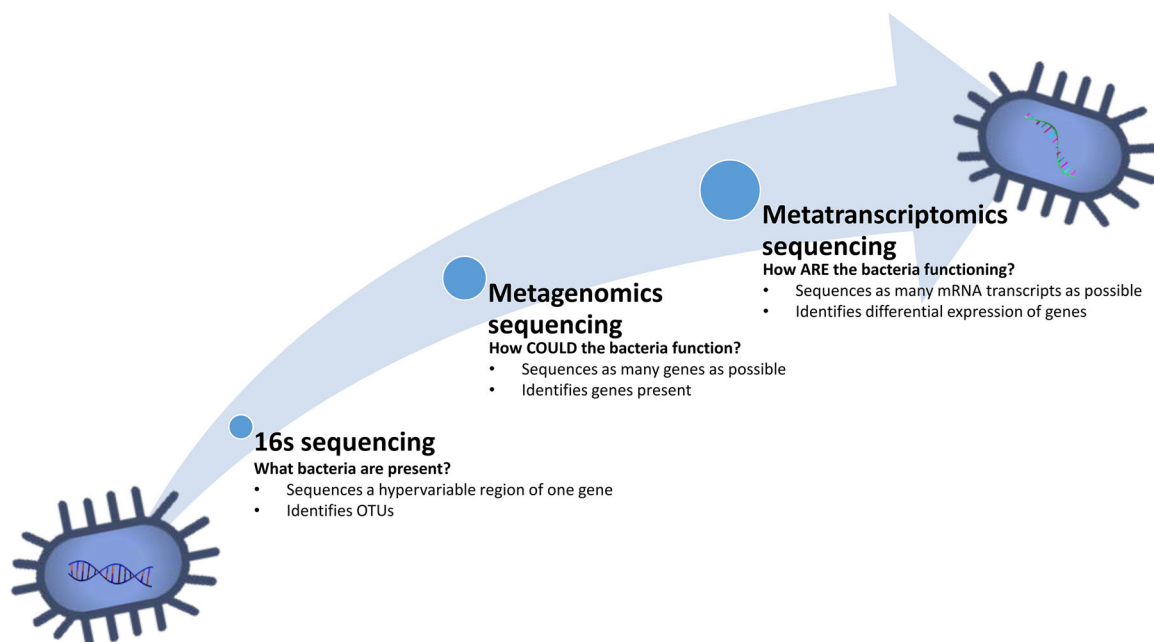
Metatranscriptomics sequencing
How ARE the bacteria functioning?
- Sequences as many mRNA transcripts as possible
- Identifies differential expression of genes

Metagenomics sequencing
How COULD the bacteria function?
- Sequences as many genes as possible
- Identifies genes present

16s sequencing
What bacteria are present?
- Sequences a hypervariable region of one gene
- Identifies OTUs

FIGURE 1: DNA sequencing can address many questions related to the microbiome. OTU = operational taxonomic unit.

a population or species (Lloyd-Price et al. 2016). Studies have therefore attempted to identify a core microbiome across various species, including humans, rodents, and fish (Ley et al. 2008; Patterson and Turnbaugh 2014). These "core micro-biomes" (Arumugam et al. 2011) vary based on the species and geographical location, among other factors, and in many cases the variation between organisms of the same species is so great that it may match the variability in microbial composition between colocalized species (Burke et al. 2011; Lozupone et al. 2012; Ottman et al. 2012). Thus, it is becoming clear that microbiomes can show unique individual characteristics that have been shaped over development, life history, and their immediate environment (i.e., exposome). In addition, recent studies have indicated that though the composition of an individual microbiome can vary greatly, multiple bacterial species can occupy the same functional niche (i.e., functional redundancy) in the gastrointestinal ecosystem, which further highlights the importance of studying microbial function over composition (Burke et al. 2011).

Interspecies variation

A strong consensus for a core phyla assemblage in the mammalian gut has not been reached, but in general, Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, Ver-rucomicrobia, and Fusobacteria are phyla described to be predominant in the class Mammalia (Ley et al. 2008; D'Argenio et al. 2014; Patterson and Turnbaugh 2014; Bashiardes et al. 2016; Hugon et al. 2017). For example, laboratory mice gut microbiota are reportedly dominated by Bacteroidetes, Firmi-cutes, Actinobacteria, and Proteobacteria (Wu et al. 2016). Differences in species composition in mammalian microbiomes are expected, based on metagenomics studies that show that approximately one-third of gut microbial genes in humans are

found in all healthy people, leaving approximately two-thirds to vary between individuals.

The characterization of microbiomes of aquatic organisms such as teleost fishes has been less of a focus compared to that of mammals. In *Danio rerio* (zebrafish), the gut microbiome is dominated by 2 phyla, Proteobacteria and Fusobacteria; and in *Ictalurus punctatus* (channel catfish), *Micropterus salmoides* (largemouth bass) and *Lepomis macrochirus* (bluegill), the gut microbiomes are characterized by the dominance of those 2 phyla as well, with Bacteroidetes, Cyanobacteria, Firmicutes, and Tenericutes also playing important roles (Roeselers et al. 2011; Larsen et al. 2014; Gaulke et al. 2016). Moreover, although studies of juvenile *Sander lucioperca* (pike perch) and *Lates calcarifer* (Asian seabass) report that the gut microbiota is dominated by both Proteobacteria and Firmicutes, there also appear to be noteworthy species-specific characteristics, such as high prevalence of Actinobacteria (pike perch only) and Bacteroidetes (Asian seabass only; Xia et al. 2014; Dulski et al. 2018). In a study of both wild and cultured species, the gut microbiome of 12 bony fishes and 3 shark species was analyzed, and the 2 most abundant phyla in most samples were Proteobacteria and Firmicutes, with all samples contain-ing 3 to 98% Proteobacteria and 1.3 to 45% Firmicutes (Givens et al. 2015). In addition, Actinobacteria, Bacteroidetes, and Fusobacteria were present but lower in abundance in all 15 species; and 13 of 15 species had Spirochaetes and Tenericutes phyla present in their gut microbiome (Givens et al. 2015). By surveying a wide variety of cartilaginous and bony fishes, both wild and cultured, that study demonstrated a conservation of several main phyla in the fish gut microbiome, while also demonstrating the immense variation in the presence of phyla abundance within species (Givens et al. 2015). Although studies using mammalian models have moved toward functional studies of the microbiome, many studies

using fish remain in the descriptive and characterization phases of the research.

Intraspecies variation

Individual and species variation in the microbiome poses another challenge for microbiome research. Variation between individuals of a population can be so great that it may not be possible to define the phylogenetic composition of a common or "normal" microbiome in a host species, which can hamper the ability to identify deviations from this composition or "abnormal" microbiomes (Patterson and Turnbaugh 2014; Silbergeld 2017). Some have pointed out that an individual's microbiome is so unique that it is a fingerprint of individuality (Cryan and O'Mahony 2011). For example, in a study with pike perch gut microbiomes, it was determined that the core microbiome of one fish was drastically different from that of another fish, yet all animals appeared healthy (Dulski et al. 2018). In a survey of 15 different fish species, several species, including *Sphyraena barracuda* (barracuda), were widely varied in their microbial structure across individuals, but all presented as healthy (Givens et al. 2015). In addition, there may be many "healthy" community structures of a gut microbiome that produce similar or equally beneficial effects on the host by production of the same enzymes and nutrients even if the operational taxonomic units are not the same (Patterson and Turnbaugh 2014). Furthermore, differences in sex, age, disease status, and geography may affect microbiome health and bias results (Patterson and Turnbaugh 2014; Chi et al. 2016; Silbergeld 2017). In addition, the region of the gut from which samples are collected is influential for the operational taxonomic units detected (Kovatcheva-Datchary et al. 2013). Importantly for fish species, there is variability in gut microbiomes between farmed and wild species (Givens et al. 2015). However, as a fish develops, it has been reported that the differences between the environmental microbiome and the fish gut microbiome diverge, suggesting that location may not play as significant a role as previously suggested (Stephens et al. 2016). In a national survey of zebrafish from different laboratories in the United States, location did not appear to be the most significant predictor of microbial community structure, suggesting that selective factors within the host for a microbiome may play a larger role than the environment (Roeselers et al. 2011).

Functional assessment of the microbiome

Community abundance in the gut microbiome varies across species and within individuals of the same species, and therefore, the field is moving toward examination of how the function of the gut microbiome varies between and within species. Often, studies report major phyla that dominate the microbiome, but the functionality of species within one phyla can be drastically varied; therefore, these reports of "core microbiota" are not necessarily indicative of differences in function between individuals of the same species and between species. Through a metagenomics study of algae, Burke et al. (2011) reported that although 16S sequences only revealed a 15%

similarity between samples, the functional profiles of individuals were 70% similar, drawing skepticism on the importance of species diversity metrics alone. Instead of the core microbiome of phyla traditionally discussed, the authors framed the "core functional microbiome" as the most important factor for host function (Burke et al. 2011). This supports the theory that there are multiple "healthy" microbial profiles for any one individual that may interact with the host in a similar way and that although the diversity of the microbes may not converge into one core profile, the functional profile becomes similar over the life span (Lloyd-Price et al. 2016; Flemer et al. 2017). Studies using metagenomics and metatranscriptomics sequencing illustrate the movement away from operational taxonomic units to focus on functional aspects of the microbiota, and studies on the microbiome are expected to shift to a functional, rather than a compositional, nature (Mai et al. 2016). It is also important to note that microbes interact with both the host and each other, which is often overlooked in studies (Mai et al. 2016); and this will also change functional aspects of the microbiome–host interaction. Select studies have attempted to bridge this gap by developing networks of interactions between bacteria or by determining how the addition of a bacterium through a probiotic leads to alterations in the abundance of other phyla (Gaulke et al. 2016).

THE IMPORTANCE OF THE MICROBIOME IN ENVIRONMENTAL TOXICOLOGY

Given the important role that the microbiome has in wildlife and human health (Cho and Blaser 2012), it is important to understand how chemicals perturb the microbiome–host relationship because the microbiome is expected to act as the conduit between chemical exposures and adverse effects. Studies now indicate that microbiome–host relationships can be modulated by chemical exposures (Jin Y et al. 2017). Thus, because of the ability of the microbiota to mediate the biotransformation for a wide variety of chemicals, there is now the recognition that microbial communities can influence fundamental properties of toxicants in situ that include individual dose and availability. This can have long-term implications for adaptation of organisms in highly contaminated environments. As the field advances, the role of microbial communities in diverse aquatic organisms will become better defined in light of the evolutionary process.

Similar to transcriptomics approaches that have been proposed in environmental biomonitoring scenarios (Feswick et al. 2017), microbial community composition can serve as an important bioindicator of exposures in animals. Indeed, earlier studies have proposed that gut bacterial structure can provide useful information on community-level responses to short- and long-term metal pollution in terrestrial isopods (Lapanje et al. 2007). Given that there is a close association between microbiota and disease, changes in microbial community composition and function may serve to indicate exposure source and chemical type (i.e., microbiome fingerprint) and to predict adverse effects on wildlife and human health. If such functional relationships can be established, microbial biomarkers can then

be developed and sampled routinely in individuals collected from polluted environments.

In the following sections, we describe targets of chemicals that, when perturbed, may disrupt microbiome–host interactions. These impacted relationships between a host's physiology and a microbiome may explain in part adverse effects observed later in life; to illustrate this point, we present a generic adverse outcome pathway (AOP) that incorporates the microbiome with these specific targets in mind. We also present 2 case studies in aquatic organisms (nanomaterials and hydrocarbons) that demonstrate how different types of environmental pollutants of concern may induce microbial community shifts associated with adverse health outcomes. Lastly, we suggest experiments moving forward that can strengthen the links between chemicals and specific disease-causing bacteria.

HOST–MICROBIOME INTERACTIONS: IMPLICATIONS FOR ENVIRONMENTAL TOXICOLOGY

The gut is colonized by trillions of microbes that aid in digestion, modulate immune responses, and generate a variety of beneficial biological products through metabolic activities. Microbial metabolites are sensed by the host and can thus play a key role in microbiome–host interactions (Holmes et al. 2011). However, the repertoire of diet-derived, microbially produced bioactive metabolites in the gut is not completely documented. Most studied microbial metabolites include microbial

fermentation of dietary carbohydrates to generate short-chain fatty acids and tryptophan metabolites, microbial conversion of primary bile acids to secondary bile acids, and microbial conversion of choline and L-carnitine to trimethylamine (Figure 2).

The microbiota is a source of nutritional signals, many of which have pleiotropic effects on the host and are energy substrates for gut epithelium. The short-chain fatty acids are the C1 to C6 organic fatty acids that are formed in the gut of mammals by microbial fermentation of carbohydrates. Acetate (C2), propionate (C3), and butyrate (C4) account for 83% of short-chain fatty acids and are produced in an approximate ratio of 3:1:1 (total concentration 50–150 mM; Rivière et al. 2016; Rooks and Garrett 2016). Metabolically, they are the most important microbial end products of the human colon fermentation process because they display several physiological effects. Generally, short-chain fatty acids are epigenetic regulators of host physiology and have profound effects on the health of the host, promoting anti-inflammatory effects, improving colonic blood flow and oxygen uptake, providing energy sources for various organs (e.g., muscle, brain, and intestinal cells), decreasing the pH of the colon (by increasing mineral absorption and decreasing ammonia absorption), lowering blood cholesterol, improving insulin sensitivity, and promoting satiety (Rivière et al. 2016). Although the exact underlying mechanisms of action of short-chain fatty acids have not been fully elucidated, there are at least 2 potential systems for molecular signaling by short-chain fatty acids: 1) inhibition of
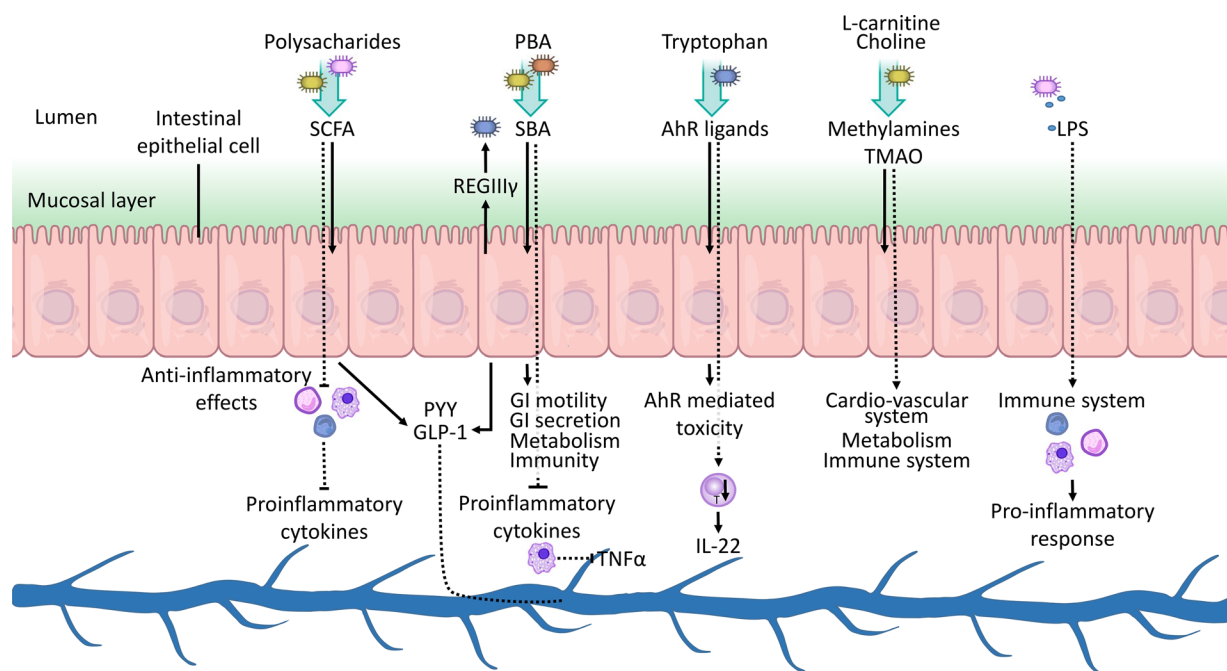

FIGURE 2: Diagram of the different interactions the gut microbiome can have with the host gastrointestinal system. The gut is colonized by trillions of microbes that aid in digestion, modulate immune responses, and generate a variety of beneficial biological products through metabolic activities. Microbial metabolites are sensed by the host and can thus play a key role in microbiome–host interactions. Most studied microbial metabolites include microbial conversion of choline and L-carnitine to trimethylamine, microbial fermentation of dietary carbohydrates to generate short-chain fatty acids, and microbial conversion of primary bile acid to secondary bile acids. AhR = aryl hydrocarbon receptor; GI = gastrointestinal; GLP-1 = glucagon-like peptide 1; IL-22 = interleukin-22; LPS = lipopolysaccharide; PBA = primary bile acid; PYY = peptide YY; REGIIIγ = regenerating islet-derived protein 3 gamma; SBA = secondary bile acid; SCFA = short-chain fatty acid; TMAO = trimethylamine-*N*-oxide; TNFα = tumor necrosis factor alpha.

histone deacetylases, and 2) activation of specific G protein–coupled receptors. Histone deacetylases are enzymes that remove the acetyl group from lysine located on histones, which regulate gene expression. In addition, studies with macrophages indicate that short-chain fatty acid-induced inhibition of histone deacetylase is a crucial regulator of nuclear factor κB (NF-κB) activity and proinflammatory innate immune responses (Tremaroli and Bäckhed 2012).

Most importantly, studies show that there can be anti-inflammatory effects of histone deacetylase inhibition by short-chain fatty acids to macrophages (Kendrick et al. 2010; Tolhurst et al. 2012; Tremaroli and Bäckhed 2012; Chang et al. 2014; Rooks and Garrett 2016). The microbial short-chain fatty acids are thus involved in mediating the microbiota–gut–brain axis during appetite regulation. Also, short-chain fatty acid-dependent G protein–coupled receptor activation regulates immune function and promotes anti-inflammatory cell phenotype, via inhibiting NF-κB, a molecule that is an important transcription factor in gut and immune homeostasis (Usami et al. 2008). Specific activation of G protein–coupled receptors also has significant effects in the gastrointestinal system including the following: 1) maintenance of mucosal immunity (increased transcription of mucin genes; Willemsen et al. 2003; Gaudier et al. 2004); 2) prevention of colitis and colon carcinogenesis through increased expression of anti-inflammatory molecules by monocytes (Singh et al. 2014), and interleukin-10, (IL-10), interleukin-18 (IL-18) producing cells (Macia et al. 2015); 3) downregulation of the production of pro-inflammatory cytokines (e.g., tumor necrotic factor alpha [TNFα]) (Vinolo et al. 2011); 4) regulating peripheral regulatory T cells (Treg) (Furusawa et al. 2013); and 5) suppressing chemotaxis and the expression of inflammatory genes in neutrophils (Vinolo et al. 2011). In terms of relevance to toxicology, there are multiple examples of how these critical microbiome–host interactions and anti-inflammatory actions of short-chain fatty acids can be perturbed by xenobiotics (e.g., metals, air pollutants). For example, in vivo exposure of mice to cadmium or environmental particulate matter was reported to significantly change the microbial profile (e.g., reduction of Bacteroidetes growth), which resulted in a decrease of the levels of short-chain fatty acids such as the anti-inflammatory butyrate, which signifies that exposure to xenobiotics could perturb the gut microbiome and promote gut inflammatory diseases (Kish et al. 2013; Liu et al. 2014; Lu et al. 2015). In general, the decrease of short-chain fatty acids by xenobiotics can be caused by the interaction with microbial metabolism or simply by changing the Firmicutes to Bacteroidetes ratio (Yang et al. 2015).

Another group of bioactive compounds produced by the microbiome is composed of tryptophan metabolites (e.g., indole, indole-3-acetate, and tryptamine; Jin et al. 2014). These compounds are converted from the dietary amino acid tryptophan in the lumen of the gut primarily by bacteria within the genus *Lactobacillus* (Relman 2017). Tryptamine and indole-3-acetate are aryl-hydrocarbon receptor (AhR) agonists, whereas indole is an AhR antagonist (Jin et al. 2014; Hubbard et al. 2015; Noakes 2015). The AhR is a ligand-inducible transcription factor/receptor that is highly expressed by epithelial cells, tumors,

immune cells, and both the interleukin-17 (IL-17)/IL-22–producing and the IL-17/IL-22-nonproducing subsets of peripheral γδ T cells (Esser and Rannug 2015). It also strongly interacts with anthropogenic xenobiotics (e.g., benzo[*a*]pyrene, polyaromatic hydrocarbons [PAHs]), many of which are frequently found in municipal areas or in surface waters (e.g., oil spills, urban runoffs). Because of the presence of AhR in immune cells, indoles (e.g., indole-3-acetic acid) can affect adaptive immunity of the host, down-regulating the differentiation of T lymphocytes into proinflammatory T-helper 17 cells (Wilck et al. 2017) and promoting the AhR-dependent production of IL-22 in innate lymphoid cells (Qiu et al. 2012), the cytokine responsible for protecting against intestinal inflammation (Jin et al. 2014; Shanahan et al. 2017). These tryptophan metabolites are crucial for appropriate AhR signaling, host–microbial mutualism, resistance to colonization, and protection from mucosal inflammation-mediated toxicity (Lee et al. 2012). For example, it was reported in investigations using an intestinal cell model that indole inhibits 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)–induced CYP1A1 expression, decreasing the toxic effects of TCDD AhR-dependent effects of xenobiotics (Jin et al. 2014).

In addition to dietary bioactive microbial metabolites, intestinal bacteria can transform the host-secreted bile acids to secondary bile acids through the enzymatic activity of 7α-dehydroxylate (Cyp7a1), which is a highly active enzyme in several species of *Clostridium* (Holmes et al. 2011). Gut microbiota can also modify the profile of bioactive molecules through production of secondary bile acids (e.g., deoxycholic acid, lithocholic acid; Sears and Garrett 2014). Secondary bile acids can activate surface receptors (TGR5) and the farnesoid nuclear receptor (FXR), which has several downstream effects on gastrointestinal motility and secretion, central signaling (satiety), metabolism, and immunity (Shanahan et al. 2017). The receptor TGR5 can reduce inflammation by antagonizing TNFα and NF-κB-dependent induction of proinflammatory cytokines in macrophages and the intestine; this protects against gut dysbiosis and diseases such as colitis, inflammatory bowel disease, Crohn's disease, and atherosclerosis (Chiang 2013; Yoneno et al. 2013). Dysregulation of the production of secondary bile acids by xenobiotics might have significant effects on tissues expressing TGR5 (e.g., brown adipocytes, macrophages/monocytes, hepatic Kupffer cells, gallbladder epithelium, and intestinal cells). Further, nonphysiologically up-regulated levels of secondary bile acids can increase insulin sensitivity by stimulating mitochondrial energy metabolism (Watanabe et al. 2011) and by the production of glucagon-like peptide 1 (GLP-1) in L-cells, which causes the secretion of insulin and regulates glucose homeostasis (Thomas et al. 2009). The gut microbiome may therefore contribute to the level of obesity and type 2 diabetes by influencing lipid and glucose metabolism though the composition of bile acid pools and the modulation of FXR and TGR5 signaling. Further, unbalanced bile acid levels have an indirect conditioning influence on the composition of the microbiota by regulating the expression of host-derived antimicrobial factors, such as regenerating islet-derived protein 3 gamma and influencing barrier function and inflammasome

activity (Shanahan et al. 2017). Altered bile acid profiles have been observed in patients with diabetes or obesity, further highlighting a possible involvement of bile acid metabolism in the pathogenesis of metabolic diseases (Gu et al. 2017). The levels of primary and secondary bile acids can also be reduced in response to chemical exposure, as was shown in a study with rats exposed to antibiotics, where a decrease in bile acids was related to a population shift in the gut microbiome and reduction in liver bile acid production and/or transport (Sun et al. 2013). Importantly, in the study, it was determined that deceases in secondary bile acids and subsequent effects on the host attributable to a tested xenobiotic were consistent with gut microbiota suppression, demonstrating the toxicological importance of secondary bile acids.

Another group of biologically active microbial metabolites are methylamines (e.g., methylamine, dimethylamine, trimethylamine, trimethylamine-*N*-oxide [TMAO]). Methylamines can be metabolized from choline and L-carnitine by gut microbiota and have been shown to be involved in many diseases such as obesity, diabetes, cardiovascular diseases, colorectal cancer, and atherosclerotic processes (Holmes et al. 2011; Wang et al. 2011; Xu et al. 2015). Methylamine, specifically TMAO, is the main metabolite of interest in this group and represents another microbial metabolite linking the microbiome to the innate immunity of the host. This methylamine can regulate the surface expression of macrophage scavenger receptors known to participate in the development of atherosclerosis (CD36, SR-A1) and enhance the level of cholesterol in macrophages, an early cellular hallmark in the atherosclerotic process (Wang et al. 2011). A high-fat diet can lead to the formation of intestinal microbiota which convert dietary choline into methylamines, reducing circulating plasma levels of phosphatidylcholine, producing similar effects as a choline-deficient diet and causing nonalcoholic steatohepatitis (Dumas et al. 2006). Microbiota-induced choline deficiency therefore results in triglyceride accumulation in hepatocytes and hepatic secretion of very-low-density lipoprotein, and the increase in the plasma levels of trimethylamine and its hepatic metabolite TMAO have been linked to atherosclerosis and cardiovascular disease (Schnabl and Brenner 2014). Although there is a lack of studies that would determine the effects of toxicants on methylamine production and their subsequent role in host health, it is known that their production is influenced by pharmaceutically (e.g., antibiotics, resveratrol, meldonium) targeting bacteria that utilize or produce TMAO (Velasquez et al. 2016).

One important point to make is that the aforementioned studies are in mammals, and this raises questions as to whether the mode of action of microbial signaling molecules can be translated to the majority of aquatic species. Many of these targets are evolutionarily conserved and have corresponding orthologs in fish. For example, AhRs are present across a large spectrum of species including mammals, birds, amphibians, fish, cartilaginous fishes, and invertebrates (Hahn 2002). Similarly, other targets (G protein–coupled receptors, TGR5, FXR, NF-κB) and responsive gut peptides (peptide YY, GLP-1) have orthologs in a wide breadth of aquatic organisms (Plisetskaya and Mommsen 1996; Conlon 2002; Fredriksson and Schioth 2005;

Savan and Sakai 2006; Reschly et al. 2008; Hov et al. 2010; Fink et al. 2015; Hodgkinson et al. 2015). Thus, studies are needed that determine whether or not these mechanisms are conserved in aquatic organisms or whether microorganisms in the gut act through different pathways to modulate immune signaling.

A final point is that, because of recent advances in metabolomics, new bioactive microbial metabolites are being discovered at a rapid pace; however, data on their potential systemic effects and mode of action are lacking. Microbial metabolites associated with diseases are reviewed by Holmes et al. (2011), but in many cases the functions of these metabolites are unknown. Microbial metabolites can affect broad sets of intestinal genes, as documented in a genome-wide study of intestinal tissue or isolated intestinal cell transcripts from mice reared in either the absence or the presence of microbiota (Camp et al. 2014). That study showed that intestinal cells alter their transcriptional response by modulating hundreds of genes following microbial colonization. It is clear that there are multiple targets for chemicals to mediate effects within the host–microbiome cascade, with many new targets and metabolic pathways yet to be discovered.

CASE STUDIES INVESTIGATING THE MICROBIOME IN AQUATIC TOXICOLOGY

The effects of some environmental chemical contaminants on the microbiome of aquatic organisms have been investigated on a limited basis (Table 1). Examples include triclosan, the heavy metal cadmium, PAHs, nanomaterials, and the fungicide imazalil (Gaulke et al. 2016; Brown-Peterson et al. 2017; Jin C et al. 2017; Zhai et al. 2017), to name a few. Studies have identified a number of operational taxonomic units from phyla to genera that can change in abundance in the gut following contaminant exposures (Table 1). Because the majority of aquatic animal studies have focused on microbial community structure, with very few exploring functional significance, we include what is known about the role of some genera/species in Table 1. As mentioned (see *Assessing Structure and Function of the Microbiome: New Tools of the Trade*), the functional aspects of the microbiome for teleost fishes, as well as invertebrates, remain an exciting avenue of research to come.

In the present section, we present 2 case studies that include 1) nanoparticles (NPs), and 2) hydrocarbons with reference to the *Deepwater Horizon* oil spill to illustrate how investigations into the microbiome offer insight into adverse outcomes. Because of our experience with fish as a group, we focus on this taxon but recognize that there are significant efforts under way to characterize microbiota in invertebrate marine organisms (Hentschel et al. 2012; Kelly et al. 2014). Nanoparticles are a unique contaminant in terms of the microbiome because these particles of concern can modulate the microbiota as a result of their small size and emergent properties compared to chemical contaminants. In addition, although the focus of the present review is placed on the toxicity of environmental chemicals to gut microbiome, data pertaining to other tissue microbiomes or in vitro microbial

TABLE 1: Examples of some of the most abundant genera in the fish gut that are affected by chemicals and environmental contaminants[a]

Fish species	Chemical: description	Groups discussed	Effect of chemical on group	Functional significance (group)	Phyla	Functional significance (phyla)	Reference
Southern flounder (Paralichthys lethostigma)	PAHs: environmental pollutants produced from partial combustion of organic material	Clostridia	−	Often pathogenic	Firmicutes	Carbohydrate metabolism	Brown-Peterson et al. (2017)
		Owenweeksia hongkongensis (species)	−	Metabolizes carbohydrates, amino acids, and lipids; sulfur and nitrogen metabolism	Bacteroidetes	Degrade high–molecular weight organic matter like protein, polysaccharides, and carbohydrates	
		Sphingobacteria (genus)	+	Pathogenic on rare occasions			
		Alphaproteobacteria (class)	−	Pathogenic, fix nitrogen	Proteobacteria	Pathogenic, nitrogen fixation	
		Gammaproteobacteria (class)	+	Pathogenic, methane oxidation, CO_2 fixation, trough photosynthesis			
		Deltaproteobacteria (class)	+	Sulfur-reducing			
		Epsilonproteobacteria	+	Possible pathogen			
		Oceanospirillales (order)	+	n-Alkane and cycloalkane degradation			
		Alcanivorax (genus)	+	Alkane degradation			
		Arcobacter (genus)	+	Rarely pathogenic, sulfur oxidation			
		Donghicola (genus)	+	Present in seawater			
		Rhodovacteraceae (family)	+	Sulfur and carbon biogeochemical cycling			
Nile tilapia (Oreochromis niloticus)	Cadmium: a metal that is a silver-white color in its elemental state, carcinogenic, and mostly a by-product of zinc mining and smelting	Pseudoalteromonas	+	—	Bacteroidetes	Degrade high–molecular weight organic matter like protein, polysaccharides, and carbohydrates	Zhai et al. (2017)
		Bacteroidetes (phylum)	+	See functional significance of phyla			
		Flavobacterium (genus)	+	Pathogenic, degrades macromolecules	Fusobacteria	Pathogenic	
		Fusobacteria (phylum)	−	See functional significance of phyla			
		Cetobacterium (genus)	−	Produce vitamin B_{12}	Proteobacteria	Pathogenic, nitrogen fixation	
		Plesiomonas (genus)	−	Fermentation of lactose			
		Deefgea (genus)	+	Facultative anaerobe			
		Pseudomonas (genus)	+	Diverse, often pathogenic			
		Cellvibrio (genus)	+	Plant polysaccharide degradation			
		Acinetobacter (genus)	+	Pathogenic, bioremediation			
Zebrafish (Danio rerio)	Oxytetracycline and sulfamethoxazole: both compounds used as antibiotics	Proteobacteria (phylum)	−	See functional significance of phyla	Proteobacteria	Pathogenic, some groups fix nitrogen	Zhou et al. (2018)
		Planctomycetes (phylum)	−	See functional significance of phyla	Planctomycetes	Oxidize ammonia to dinitrogen without oxygen	
		Fusobacteria (phylum)	+	See functional significance of phyla	Fusobacteria	Pathogenic	
		Bacteroidetes (phylum)	−	See functional significance of phyla	Bacteroidetes	Degrade high–molecular weight organic matter like protein, polysaccharides, and carbohydrates	
		CKC4 (phylum)	+	—	CKC4	—	

(Continued)

TABLE 1: (Continued)

Fish species	Chemical: description	Groups discussed	Effect of chemical on group	Functional significance (group)	Phyla	Functional significance (phyla)	Reference
Fathead minnows (Pimephales promelas)	Triclosan: chemical often used as an antibiotic/antimicrobial	CK-1C4-19	+	—	Proteobacteria	Pathogenic, some groups fix nitrogen	Narrowe et al. (2015)
		Hydrogenophaga (genus)	+	Hydrogen oxidation			
		Thauera (genus)	+	Degradation of aromatic compounds			
		Methylobacterium (genus)	+	Pathogenic, synthesize carotenoids			
Zebrafish (Danio rerio)	Silver nanoparticles: small particles of silver possessing antimicrobial properties	Acidovorax (genus)	+	Pathogenic	Fusobacteria	Can be pathogenic, some species produce vitamin B_{12}	Merrifield et al. (2013)
		Cetobacterium somerae (species)	−	Produces vitamin B_{12}			
Zebrafish (Danio rerio)	Imazalil: fungicide used to keep plants/crops fungus-free	Bacteroides (genus)	−	Pathogenic	Bacteroidetes	Degrade high-molecular weight organic matter like protein, polysaccharides, and carbohydrates	Jin C et al. (2017)
		Alistipes (genus)	−	—			
		Rhodobacter (genus)	−	Anoxygenic photosynthesis and carbon/nitrogen fixation	Proteobacteria	Pathogenic, some groups fix nitrogen	
		Akkermansia (genus)	−	Degrades mucus	Verrucomicrobia	Some species oxidize methane	

[a]The effect of chemical on the microbiota is provided (+ = increased presence in the gut; − = decreased presence of the organism in the gut following chemical treatment). The functional significance of the group is also indicated based on manual compilation of information from the literature. The phylum and its functional significance for CK-1C4-19 and the functional significance of CkC4 are unknown because they have not been extensively studied. To the best of our knowledge, functional data are also lacking for Pseudoaltermonas and Alistipes.
PAH = polycyclic aromatic hydrocarbon.

communities are also included in these case studies because they improve our understanding of the potential toxic effects on the gut microbiome following exposure to these emerging contaminants of concern.

Nanomaterials

Nanomaterials are classified as compounds with at least one dimension between 1 and 100 nm and continue to be an emerging contaminant of concern in lieu of a booming nanotechnology industry. These particles are used in a significant number of consumer and personal care products, including sunscreens, toothpaste, and food items such as chewing gum and Kool-Aid (Weir et al. 2012). Nanomaterials are also used for a variety of industrial purposes and are present in coatings, electronics, textiles, and filters (Piccinno et al. 2012). The widespread application of nanomaterials presents several routes for environmental release and contamination (Keller and Lazareva 2013), ensuring that these chemicals require continued attention in toxicological studies. Nanomaterials have unique properties, such as nanoscale dimensions and high surface area-to-volume ratios that may confer mechanisms of dysbiosis in host microbiomes. In addition, several types of nanomaterials have antimicrobial properties, including nano-titanium dioxide (nano-TiO_2), nano-zinc oxide (nano-ZnO), carbon nanomaterials, and nano-silver (nano-Ag; Brunet et al. 2009; Rai et al. 2009; Marambio-Jones and Hoek 2010; Musee et al. 2011; Sirelkhatim et al. 2015). The antimicrobial behavior of these nanomaterials is the primary reason they are added to products (e.g., clothing, sterile surfaces, water filters; Vance et al. 2015); however, this spurs new questions regarding their effects on important microbial communities. This is an emerging and relatively underexplored area of research because few studies quantify the effects of nanomaterials on the gut microbiome.

The addition of nanomaterials to food, food packaging, and other domestic products presents a potential for environmental exposure; and methods for safety assessments of these chemicals are still in development (Bouwmeester et al. 2014). Organic matter, metal, and metal oxides comprise the majority of domestically related nanomaterials (Bouwmeester et al. 2014) and thus are more likely to be environmentally released (Keller and Lazareva 2013) and will be the focus of this part of the review.

Metal oxide nanomaterials and the gut microbiome

Metal oxide nanomaterials such as nano-TiO_2, nano-silicon dioxide (nano-SiO_2), and nano-ZnO are produced at the highest levels globally (Vance et al. 2015). Commonly TiO_2 and ZnO are used as a pigment in foods, cosmetics, and coatings (Weir et al. 2012; Peters et al. 2014) and as a bactericide in food packaging (Chawengkijwanich and Hayata 2008; Espitia et al. 2012). Nano-SiO_2 is used primarily in protective coatings and environmental treatment but is also present in dietary supplements (Vance et al. 2015). Although humans are more likely to be exposed to metal oxide nanomaterials because of their presence in processed food items, some studies have suggested that they are also

bioavailable to aquatic organisms, with the oral route as the most likely route of exposure (Johnston et al. 2010).

Despite the widespread presence of metal oxide nanomaterials in food items and the high likelihood of exposure through gastrointestinal association with these compounds, there are few studies reporting on their effects in the gut microbiome. Taylor et al. (2015) found significant phenotypic changes in the microbial community of a model colon after exposure to environmentally relevant concentrations of 3 metal oxide nanomaterials (nano-TiO_2, nano-ZnO, and nano-cerium dioxide), including changes in cellular hydrophobicity, cell size, surface charge, and metabolism of the exposed microbiome communities. A similar study conducted by Waller et al. (2017) using food-grade TiO_2 (a mixture of nano-sized and bulk particles) observed phenotypic changes in the exposed microbial community comparable to those seen by Taylor et al. (2015) but also reported a significant decrease in microbial cell concentration (58.6%) and a slight difference in protein content of the extracellular polymeric substance, a matrix of high–molecular weight polymers essential for biofilm formation.

In addition to in vitro effects, metal oxide nanomaterials can impact the gut microbiome in vivo. In an in vivo study with zebrafish (Chen et al. 2018), coexposure to nano-TiO_2 and bisphenol A induced dysbiosis in the gut microbiome, and the nano-TiO_2 exposure was associated with a significant increase in the relative abundance of Firmicutes and Bacteroidetes compared to controls. Feng et al. (2017) observed changes in gut microbiome structure and metabolic profiles in hens exposed to high concentrations of nano-ZnO (>25 mg/kg), with notable impact on microbiome diversity at the highest treatment concentration, the relative abundance of several bacterial groups (class Bacilli and phyla Fusobacteria, Proteobacteria, and Firmicutes), and metabolite levels (most notably glucose, lactate, choline, and methionine) in treated hens compared to controls. An in vivo study conducted in piglets found that low levels of dietary nano-Zn impacted the diversity and richness of the gut microbiome, with location-specific alterations in the relative abundance of intestinal Firmicutes and Bacteroidetes (Xia et al. 2017). Overall, it seems that metal oxide nanomaterials have the potential to disrupt the host gut microbiome both in vitro and in vivo, but it remains unclear as to whether environmentally relevant amounts of these compounds may elicit microbiome-level effects in aquatic systems.

Other metal nanomaterials and the gut microbiome

Like metal oxide nanomaterials, metal nanomaterials are present in many commercially available products and are likely to be released into the environment. Nano-Ag is the most abundant metal-based nanomaterial in commercial products (Vance et al. 2015) and is utilized primarily for its antimicrobial properties (Rai et al. 2009). According to the 2018 Consumer Products Inventory, these types of NPs are present in textiles, water filters, food containers, and even certain domestic products such as dietary supplements and toothpaste (Project on Emerging Technologies 2018). Although little is known about

the environmental transport and fate of nano-Ag, research indicates that nano-Ag can leach from products and enter into aquatic environments (Benn and Westerhoff 2008), where silver ions and conjugates are formed rapidly. Recent models predict its presence in wastewater effluent in the low parts per billion range (Keller and Lazareva 2013). In addition to nano-Ag, copper nanomaterials (nano-Cu) present another potentially toxic metal-based nanomaterial group. Although not as widely used as nano-Ag, nano-Cu also displays antimicrobial properties and is found in low concentrations in the environment (Keller and Lazareva 2013). Mammalian studies investigating the potential impact of nano-Ag exposure on the gut microbiome report conflicting results. Some studies using rodents have found that oral exposure to nano-Ag was associated with an altered ratio between Firmicutes and Bacteroidetes phyla (Van Den Brûle et al. 2015; Williams et al. 2015) and increased prevalence of bacteria in the family Enterobacteriaceae and the genus *Lactobacillus* (Williams et al. 2015). Other mammalian studies have not seen the same results following nano-Ag exposure; for example, Wilding et al. (2016) reported that nano-Ag exposure did not induce any changes in the gut microbiome of mice, and Hadrup et al. (2012) reported no significant changes in the ratio between Firmicutes and Bacteroidetes in Wistar rats exposed to nano-Ag. As stated by Wilding et al. (2016), the differences in observations reported by these in vivo studies may be attributable to differences in exposure duration, experimental design, and dosing. In any case, future work is required to answer the questions presented by these conflicting studies. Although in vitro data are lacking, one in vitro study conducted by Das et al. (2014) with a cultured human fecal microbial community found that nano-Ag exposure caused changes in microbial respiration, fatty acid profiles, and phylogenetic composition.

Toxicity of nano-Ag to the gut microbiome has also been assessed in nonmammalian models. A study with Japanese quail (Sawosz et al. 2007) found that waterborne exposure to nano-Ag increased lactic acid–producing bacteria in the gut microbiome. In addition, a study with zebrafish found that dietary exposure to both nano-Cu and nano-Ag impacted the diversity of the gut microbiome. Nano-Cu exposure induced the most significant changes, causing complete suppression of common gut bacterial species (namely *Cetobacterium somerae*), whereas nano-Ag exposure induced only minor changes in bacterial diversity. A study with *Drosophila melanogaster* reported a significant reduction in the diversity of the gut microbiota of larvae exposed to nano-Ag, specifically an increase in *Lactobacillus brevis* and *Acetobacter* compared to control groups (Han et al. 2014). Surprisingly, nano-Cu-treated experimental groups did not show the same changes in bacterial diversity as seen in the nano-Ag treatment groups, which indicates that the sensitivity to the nanomaterials may be host species-specific.

Carbon nanomaterials and the gut microbiome

Carbon nanomaterials are an emerging class of nanomaterials consisting primarily of cylindrical single-walled and multiwalled nanotubes and spherical fullerenes. Although

currently not as widely produced as metal and metal oxide nanomaterials, their unique properties, coupled with their overtly low toxicity, has made them a major player in the nanomaterial industry (De Volder et al. 2013). Although detection and quantification of these materials are difficult, recent models predict their environmental release and partitioning into surficial sediments (Schierz et al. 2014), where they may be potentially bioavailable to aquatic organisms.

To date, there are few studies investigating the relationship between dietary exposure to carbon nanomaterials and dysbiosis of the gut microbiome. An in vitro study conducted with microbes common to the human gut microbiome (*Lactobacillus acidophilus*, *Escherichia coli*, *Staphylococcus aureus*, and *Enterococcus fecalis*) found that single-walled and multi-walled carbon nanotubes have broad-spectrum antibacterial effects through the lysis of bacterial cell walls and membranes (Chen et al. 2013). Another in vitro study conducted by Zhu et al. (2014) found decreased viability in *E. coli*, *S. aureus*, *Bacillus subtilis*, and *Ochobactrum* species after exposure to single-walled carbon nanotubes of varying lengths, along with changes in membrane fatty acid composition of *S. aureus* and *B. subtilis* (Zhu et al. 2014). Li et al. (2018) found that orally administered fullerenol NPs caused marked changes in the structure and composition of the gut microbiota, with significant enrichment in bacterial groups involved in the production of short-chain fatty acids, such as *Lactobacillus*.

Although this is a relatively new area of research, there is some evidence indicating that dietary exposure to carbon nanomaterials may induce changes in microbial groups involved in lipid synthesis and metabolism, and additional research is necessary to explore this possibility.

Deepwater Horizon *effects on fish microbiomes*

In the aftermath of the 2010 *Deepwater Horizon* oil spill, there were many reports demonstrating that the incursion of oil altered the microbial population spectrum of water and sediment significantly as a function of both time and distance from the oil release (Hazen et al. 2010; Kostka et al. 2011; Dubinsky et al. 2013; Gutierrez et al. 2013; Looper et al. 2013; Mason et al. 2014). However, there was relatively little research aimed at understanding the effects of oil contamination on the microbiomes of fish species in the affected area (Barron 2012; Whitehead et al. 2012; Barron et al. 2013; Brewton et al. 2013; Brown-Peterson et al. 2017). This was somewhat surprising, given that there is increasing evidence that exogenous factors can have significant effects on the microbiome of organisms (Carlson et al. 2015; Gaulke et al. 2016). Microbiota shifts attributable to contaminants can have detrimental impacts on the health status of the host (Lefever et al. 2016; Jin Y et al. 2017) and immune function (Kelly and Salinas 2017); thus, it is important to characterize more completely the long-term health consequences that are related to changes in the microbiome.

Following published reports of bacteria-induced lesions in red snapper (*Lutjanus campechanus*) following the *Deepwater Horizon* oil spill (Murawski et al. 2014), researchers examined the microbiome of wild-caught snapper from opportunist cruises off

the Louisiana coast (Arias et al. 2013). Using culture-based techniques, the researchers identified 179 isolates from skin and 43 species isolated from mucus from 60 individual fish. The researchers examined the prevalence of 2 fish pathogens, *Vibrio vulnificus* and *Photobacterium damselae* in red snapper populations. The genera *Vibrio* and *Photobacterium* were both highly represented in the samples, contributing 32 and 23%, respectively, of the total number of isolates. The authors interpreted these results to indicate that these taxa are normally present in red snapper and, because none of the caught fish exhibited signs of poor health, were unlikely to be directly responsible for any observed lesions in other individuals. However, it is important to note that no independent markers of health were reported, and the fish were caught in an area that was also potentially affected by oil from the *Deepwater Horizon*, meaning that linkages between skin microbiomes, health status, and oil exposure were difficult to draw with any firm conclusions.

A second study by the same group later examined the effects of oil and season on the skin microbiome of *Fundulus grandis* collected from oiled and nonoiled marsh sites in Barataria Bay, Louisiana, USA, in 2011. Here, using ribosomal intergenetic spacer analysis, Larsen et al. (2015) showed no evidence of difference in skin microbial populations among fish collected in oiled and nonoiled sites. The skin microbiome was different from the water microbiome, providing evidence that skin microbial populations are not simply reflections of bacteria in the water column. However, shifts in microbial composition were observed across seasons, indicating that there are external stimuli that can affect the skin microbiome of *F. grandis* that are not chemical-specific. The lack of evidence of shifts associated with oiled versus nonoiled sites was surprising, although it should be noted that no independent assessment of PAH contamination in the selected sites was presented. However, it is possible that either historical or transient oil exposure was affecting the results.

More recently, 2 controlled laboratory experiments have examined the effect on oil-contaminated sediment exposure on the gill and intestinal microbiomes of juvenile southern flounder (*Paralichthys lethostigma*). Initially, juvenile southern flounder were exposed to oil-contaminated sediment for 30 d under flow-through conditions, and microbiomes of gill and intestine were analyzed by 16S sequencing (Brown-Peterson et al. 2015). The researchers observed significant shifts in population structures for lower gill, upper gill, and intestine. In general, the lower gill was most strongly affected among tissues, whereas the top gill and intestine were less impacted by oil exposure. Of particular interest was that there was a strong increase in the prevalence of the hydrocarbon degrading bacteria genus *Alcanivorax* in flounder exposed to oil-contaminated sediment, demonstrating that the microbiome–host interaction "responds" in some way to chemical stressors. It is unclear if this is an adaptive response of the host to the oil or whether the bacterial communities are outcompeting other residents in an oil-rich environment.

In a follow-up study, Bayha et al. (2017) extended this to examine the effect of oil-induced microbiome shifts on disease resistance in southern flounder. Flounder were exposed to control or contaminated sediments for 4 d and then challenged

with a known fish pathogenic bacterium, *Vibrio anguillarum*, and followed for several days. At 24 h after the bacterial challenge, there was again a significant difference in the microbiome of the different organs. Most noticeably, the flounder that were exposed to oil had a significant increase in the prevalence of the hydrocarbon-degrading bacteria *Alcanivorax*, and there was a significant difference in the ability of the fish pathogen *V. anguillarum* to colonize the gills of challenged fish. In fish that were exposed to oil-contaminated sediments prior to the bacterial challenge *V. anguillarum* was able to colonize the gills, whereas fish that were placed on uncontaminated sediment prior to the challenge were able to defend against the pathogen. This effect was linked to an oil-induced down-regulation in the expression of the immune gene immunoglobulin M, implying that there is a strong linkage between oil exposure, organ-specific microbiomes, and health outcomes.

This conclusion is particularly interesting in light of what is known from the biomedical research community about interactions between the AhR and the intestinal microbiome. There are intriguing data that there is a functional linkage between intestinal microbiota, the AhR, and host health (Zhang et al. 2017). For example, several AhR ligands or agonists, including tryptophan metabolites, are produced by intestinal microbiota, which have been shown to affect the AhR–Il22 axis (Zelante et al. 2013). This raises the interesting possibility that environmental exposure to hydrocarbons, such as oil from the *Deepwater Horizon* incident, may be affecting exposed organisms via specific mechanisms that are mediated by specific signaling mechanisms. In addition to the direct exposure effects, which are becoming more clear and well characterized, the contaminants may be causing indirect effects, through altering the activity of the AhR pathway.

The hypothesis that some chemicals can exert effects on the microbiome via AhR signaling is supported by studies in rodent models. Murray et al. (2016) showed that AhR$^{-/-}$ mice have different microbiomes from AhR heterozygotes. In this experiment, mixed-genotype littermates were cohoused for 6 mo, then separated by genotype and maintained under identical conditions for 18 d. Following this segregation, 16S sequence data indicated a modest but significant shift in the bacterial diversity in the cecum of the different genotypes. Most noticeably, AhR$^{-/-}$ mice had an increase in the prevalence of segmented filamentous bacteria in the cecum. Inferred metabolic pathway analysis also indicated that different microbial populations were present in the 2 genotypes, as did the different metabolic profiles produced. In another study, AhR$^{-/-}$ and AhR heterozygote mice were exposed to 2,3,7,8-tetrachlorodibenzofuran (TCDF; 24 μg/kg dietary exposure for 5 d), and the effects on the intestinal microbiome and metabolism were investigated (Zhang et al. 2015). The study showed that dietary TCDF shifted the ratio of Firmicutes to Bacteroidetes and triggered gut inflammation, presumably attributable to the activation of bacterial fermentation, suggesting that these events are AhR-mediated. In addition, principal component analysis showed that in AhR heterozygotes exposure to TCDF produces a dramatic and significant shift in total microbiome population, while no such difference

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was apparent in AhR$^{-/-}$ mice. Exposure to TCDF also induced a significant decrease in the presence of segmented filamentous bacteria and a significant increase in expression of IL-1b, TNF, and Lcn-2 in the ileum, while in the AhR$^{-/-}$ mice this effect was abolished. Similarly, TCDF-driven reductions in certain bile salts (fibroblast growth factor-15, Fxr, and small heterodimer partner) that were present in AhR heterozygotes were nonsignificant in AhR$^{-/-}$ mice. Taken together, these papers provide strong indications that the AhR ligand pathway is closely linked with intestinal microbiomes and should be further examined. Although this linkage has only so far been demonstrated in mice, the fact that other researchers have shown that oil can cause severe effects on the microbiome of exposed fish implies that the interaction of oil exposure, microbiome shifts, and AhR-linked pathways is likely to be a fruitful future avenue of research.

ADVERSE OUTCOME PATHWAYS AND THE MICROBIOME

In Figure 3, we present a framework for incorporating the gut microbiome into an AOP. An oral route of exposure is perhaps the most relevant when linking gut dysbiosis and chemicals because aquatic organisms are exposed to environmental chemicals through the water and food. Water-soluble chemicals or those adhered to food particles can be ingested into the gut, where they can interact with gut epithelial receptors before or after microbial transformation. For example, there are a number of pesticides that act on estrogen receptors to elicit estrogenic responses in tissues (Seeger et al. 2016), including in the gut. Indeed, the mammalian gastrointestinal system expresses a vast repertoire of receptors for environmental chemicals and endocrine disruptors. A specific example includes the ingestion of PAHs bound to food, which activates AhR in the gastrointestinal tract. There can also be active uptake of the chemical via endocytosis-mediated events or passive transport of the chemical through the gut epithelium (not depicted in the figure but one process that can also act as a molecular initiating event

[MIE]). These events can occur with the parent compound, or they can occur following bioactivation or biotransformation by the gut microbiome; this process can be a significant mechanism prior to an MIE at the host–chemical interface (Lapanje et al. 2007). Lastly, there is the possibility that the chemical also binds microbial enzymes directly, leading to secondary changes in their metabolic outputs.

Following the MIE, the host epithelium is expected to respond on a cellular level in a unique way to each specific chemical, which may include the activation of immune responses attributable to localized chemical-induced cell damage. Activated inflammatory response can include stimulation of cytokines, interleukins, and other inflammatory pathways as immune cells infiltrate the gut epithelium to mitigate the damage. It is important to recognize that the responses between microbiome and host are dynamic, complex, and reciprocal. Activation of cellular responses (e.g., immune or stress response) in gut epithelial cells can have profound effects on the microbiome; microbial diversity and species richness are also expected to be modulated by postinflammatory and protective mechanisms in the gut epithelium. Altered microbial diversity and richness can lead to changes in the microbial metabolites produced within the gastrointestinal tract, and this in turn can have direct consequences for the host, causing exacerbated inflammation, impaired nutrient uptake, gut leakiness, and eventually programmed cell death and necrosis. Mechanisms underlying these events can include transcriptional and protein regulation of molecules needed for epithelial protection, the cell cycle, and DNA repair or specific xenobiotic pathways for the chemical.

Because these events coalesce, gut dysbiosis is exacerbated and can induce systemic effects within the organism. Poor nutrition and impaired metabolism can ensue as inflammation in the gut impairs transporter-mediated uptake of nutrients and vitamins. Microbial metabolites considered to be damaging to the organism may enter into the circulatory system of the host, affecting multiple organs within the organism (Blacher et al. 2017). Poor overall health of the organism can lead to
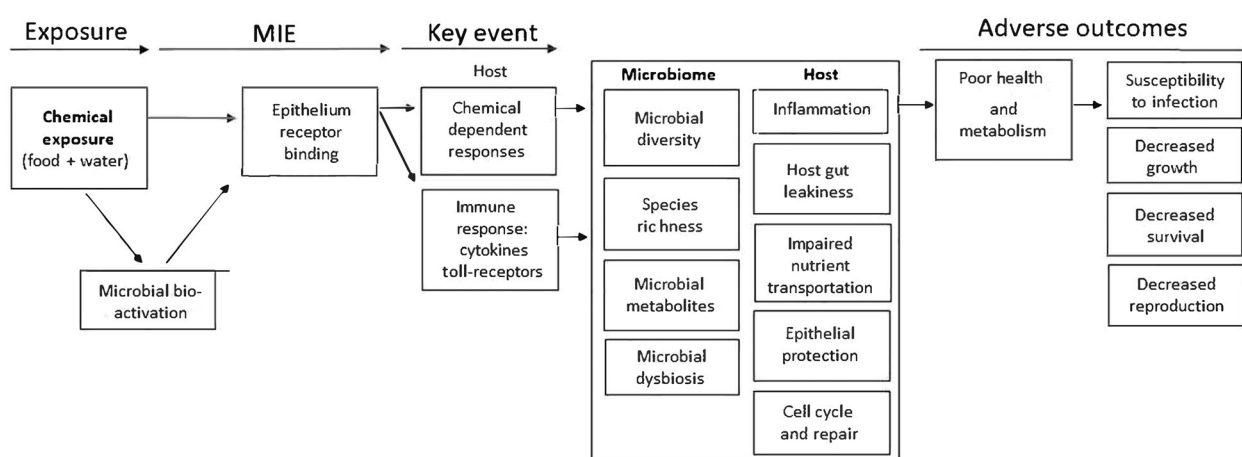

FIGURE 3: Proposed outcome framework for chemicals that affect the microbiome. Ingested chemicals can be biotransformed by the microbiome or they can act directly on the host epithelium to exert adverse effects in the host. MIE = molecular initiating event.

population-level effects that may include increased susceptibility to infection, decreased growth, and decreased survival. This general framework for AOPs related to chemical-induced gut dysbiosis can be included into larger frameworks that integrate quantitative AOPs. We also point out that this framework is not comprehensive because there are likely a number of MIEs and key events that remain undefined; these MIEs will be dependent on the chemical ingested.

WHAT IS NEXT? DEMONSTRATING THE LINK BETWEEN MICROBIAL SHIFTS AND TOXICANTS

Research continues to address questions about how exogenous contaminants affect the microbiome of organisms and whether the altered microbiome affects the health status of the fish. However, it becomes increasingly important to discern which microbial species are contributing directly to the gut dysbiosis and any health-related issues. Strategies have been developed to determine the cause-and-effect relationship between specific microbiome changes and gut inflammation. Culturomics has been proposed as a high-throughput method to isolate and identify specific microbial communities, allowing for further in vitro investigation into effects or interactions with the host immune system (Tidjani Alou et al. 2017). The idea is to leverage different culture media and conditions (i.e., temperature, nutrients, oxygen) to isolate a wide variety of microbial species from fecal matter to perform functional assays (e.g., activation assays with Toll-like receptors). This approach, of course, is only possible with those bacteria that can be cultured successfully outside of the gut. A second strategy is to use functional genomics, leveraging expression quantitative trait loci and data on single-nucleotide polymorphisms to define microbe–host interactions (Luca et al. 2017). In this approach, genome-wide association studies have correlated microbial variability to human disease phenotypes. Moreover, efforts move toward "a gut on a chip" in humans, which can be potentially developed for aquatic organisms and used to examine microbe–host interactions. A third strategy includes probiotic manipulation or fecal transplant experiments, resulting in reduction or overexpression of sensitive microbial communities associated with an adverse outcome. Lastly, developing a diversity of gnotobiotic animals to understand the role of their microbial communities in health will also be an exciting step forward.

These experimental strategies can be employed to test hypotheses that specific microbial species are associated with an adverse outcome. Within the context of toxicology, the final step would be to demonstrate an association between the chemical exposure and the proliferation, survivability, or functional output (metabolites) of a targeted microbial species. One of the key challenges is that, when manipulating the microbiome, it is expected that one will also alter the physiology of the host organism. Thus, determining the contribution of the altered microbiome versus host from the effects of the "agent that altered the microbiome" is a nontrivial challenge that requires innovative ways to differentiate.

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

Research has now established that the microbiome is an integrated component of wildlife and human health. Studies that examine the microbiome in the context of aquatic toxicology are increasing at a rapid rate, and there are unique challenges for toxicology when it comes to understanding the role of the microbiome in environmental and animal health. Major questions to be addressed moving forward include the following: 1) What microbiome communities exist in aquatic organisms—do species in the same geographical region have more similar microbiomes compared to close evolutionary relatives living in different habitats? 2) What are the molecular mechanisms by which host genetic variation affects microbiome composition? 3) What is the capacity of the microbiome to transform environmental pollutants? Can aquatic species use their microbiome to adapt to contaminated environments? 4) How do environmental factors that include climate change and acidification affect microbiomes and the balance between host and microbe? 5) How are microbial communities shaped in long migrant species, for example, those species that seek specialized habitats for reproduction? How do microbiomes drive development? 6) How do dose, diet, and individual genetic variability influence the microbiota?

Addressing these questions is expected to spur exciting research in the future. We have learned that aquatic organisms have diverse and complex microbiomes that can often differ from species to species. Elucidating the role of the microbial phenotype in adaption to polluted habitats will be a significant advance for understanding how aquatic organisms interact with their environment.

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