11-1-2018

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

Ondrej Adamovsky
*University of Florida*

Amanda Buerger
*University of Florida*

Alexis M. Wormington
*University of Florida*

Naomi Ector
*University of Florida*

Robert J. Griffitt
*University of Southern Mississippi*, Joe.Griffitt@usm.edu

***See next page for additional authors***

Environmental Toxicology and Chemistry

Invited Critical Review

# The gut microbiome and aquatic toxicology: An emerging concept for environmental health

**Ondrej Adamovsky, Amanda Buerger, Alexis M. Wormington, Naomi Ector, Robert J. Griffitt, Joseph H. Bisesi Jr, Christopher J. Martyniuk**

*Environ Toxicol Chem.*, **Accepted Article** • DOI: 10.1002/etc.4249

**Accepted Article**

**Invited Critical Review** **Environmental Toxicology and Chemistry**
**DOI 10.1002/etc.4249**

# The gut microbiome and aquatic toxicology: An emerging concept for environmental health

Ondrej Adamovsky[1,2,3*], Amanda Buerger[2,4*], Alexis M. Wormington[2,4], Naomi Ector[1], Robert J. Griffitt[5], Joseph H. Bisesi Jr[2,4,†], Christopher J. Martyniuk[1,2,6†]

[1]Department of Physiological Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL, 32611, USA

[2]Center for Environmental and Human Toxicology, University of Florida, Gainesville, FL, 32611, USA

[3]Research Centre for Toxic Compounds in the Environment (RECETOX), Faculty of Science, Masaryk University, Brno, Czech Republic

[4]Department of Environmental and Global Health, University of Florida, Gainesville, FL, 32610, USA

[5]Division of Coastal Sciences, School of Ocean Science and Engineering, University of Southern Mississippi, Gulfport, MS, 39564, USA

[6]Genetics Institute, University of Florida, Gainesville, FL, 32611 USA

*These authors contributed equally

†Correspondence: Joseph Bisesi and Christopher J. Martyniuk

email: jbisesi@phhp.ufl.edu, cmartyn@ufl.edu

Tel.: +1 352 294 4642; fax: +1 352 392 4707



**Submitted 10 May 2018; Returned for Revisions 8 August 2018; Accepted 8 August 2018**

**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, as 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. This 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 two 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, as 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. 

**Key words:** gut dysbiosis, short chain fatty acids, inflammation, adverse outcome pathway, polycyclic aromatic hydrocarbons, nanomaterials

This article is protected by copyright. All rights reserved

## 1. 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, as 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. While 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, Gao et al. 2014, Claus, Guillou 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, Alves et al. 2011, Huang,

Charlson et al. 2013, Kostic, Xavier et al. 2014, Surette 2014, Hartstra, Bouter 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.

**2. 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, as the density and diversity of most microbiomes precluded general culture and identification. There was initially little interest in non-pathogenic bacteria until recently, when it became apparent that microbiomes play an essential role in the physiology of humans and animals (Hiergeist, Gläsner 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 sulphur 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 (OTU), due to the fact that 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 OTUs, 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 (QIIME) and Mothur (Schloss, Westcott et al. 2009, Caporaso, Kuczynski et al. 2010). While many studies vary in their specific approach to sequencing and analyzing 16S based microbiome datasets, Benjamino et al. 2018 provides a general protocol for this analysis within a toxicological context (Benjamino, Beka et al. 2018).

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 mechanisms 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 OTUs, using tools such as Phylogenetic Investigation of Communities by Reconstruction of Observed States (PICRUSt)(Langille, Zaneveld 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 (meta-transcriptomics). While 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, Auch et al. 2007, Martinez, Pozuelo et al. 2016, Westreich, Korf et al. 2016). Further, recently developed tools such as PALADIN (Westbrook, Ramsdell 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 datasets 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 microbial composition and abundance is directly related to type and the concentration of metabolites that are produced in the gut. As such, while assessment of 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 a gene level translates 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 (NMR) based approaches have emerged as the go-to technology for both targeted and non-targeted assessment of metabolome in the gastrointestinal lumen (Saric, Wang et al. 2007, Theriot, Koenigsknecht et al. 2014, Sinha, Ahn et al. 2016).

## 3. Diversity of microbial communities among host species

Data supporting/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 co-evolved with microbes, such that a core set of microbes may be expected in all healthy individuals in a population or species (Lloyd-Price, Abu-Ali et al. 2016). Studies have therefore attempted to identify a core microbiome across various species, including humans, rodents and fish (Ley, Hamady et al. 2008, Patterson and Turnbaugh 2014). These "core microbiomes" (Arumugam, Raes 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 co-localized species [15, 26, 27]. 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). Additionally, 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, Steinberg et al. 2011).

### *3.1. Inter-species Variation*

A strong consensus for a core phyla assemblage in the mammalian gut has not been reached, but in general, Firmicutes, Bacteriodetes, Proteobacteria, Actinobacteria, Verrucomicrobia and Fusobacteria are phyla described to be predominant in the class Mammalia (Ley, Hamady et al. 2008, D'Argenio, Casaburi et al. 2014, Patterson and Turnbaugh 2014,

Bashiardes, Zilberman-Schapira et al. 2016, Hugon, Lagier et al. 2017). For example, laboratory mice gut microbiota are reportedly dominated by Bacteroidetes, Firmicutes, Actinobacteria, and Proteobacteria (Wu, Wen 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 two 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 two phyla as well, with Bacteriodetes, Cyanobacteria, Firmicutes and Tenericutes also playing important roles (Roeselers, Mittge et al. 2011, Larsen, Mohammed et al. 2014, Gaulke, Barton et al. 2016). Moreover, while studies of juvenile *Sander lucioperca* (pikeperch) and *Lates calcarifer* (Asian seabass) report that the gut microbiota is dominated by both Proteobacteria and Firmicutes, there also appears to be noteworthy species-specific characteristics, such as high prevalence of Actinobacteria (pikeperch only) and Bacteriodetes (Asian seabass only) (Xia, Lin et al. 2014, Dulski, Zakęś et al. 2018). In a study of both wild and cultured species, the gut microbiome of twelve bony fishes and three shark species was analyzed, and the two most abundant phyla in most samples were Proteobacteria and Firmicutes, with all samples containing 3-98% Proteobacteria and 1.3-45% Firmicutes (Givens, Ransom et al. 2015). Additionally, Actinobacteria, Bacteriodetes, and Fusobacteria were present, but lower in abundance in all fifteen species, and 13/15 species had

Spirochaetes and Tenericutes phyla present in their gut microbiome (Givens, Ransom et al. 2015). By surveying a wide variety of cartilaginous and bony fishes, both wild and cultured, this 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 [25]. While studies using mammalian models have moved towards functional studies of the microbiome, many studies using fish remain in the descriptive and characterization phases of the research.

*3.2. Intra-species 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 pikeperch gut microbiomes, it was determined that the core microbiome of one fish was drastically different from the other fish in the study, yet all animals appeared healthy (Dulski, Zakęś et al. 2018). In a survey of fifteen different fish species, several species, including the *Sphyraena barracuda* (barracuda), were widely varied in their microbial structure across individuals, but all presented as healthy (Givens, Ransom et al. 2015). Additionally, 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 OTUs are not the same [13] (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, Bian et al. 2016, Silbergeld 2017). Additionally, the region of the gut from which samples are collected is influential in the OTUs detected (Kovatcheva-Datchary, Tremaroli et al. 2013). Importantly for fish species, there is variability in gut microbiomes between farmed and wild species (Givens, Ransom 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 of a role as previously suggested (Stephens, Burns et al. 2016). In a national survey of zebrafish from different labs 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, Mittge et al. 2011).

*3.3. 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 towards 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, and therefore these reports of "core microbiota" are not necessary 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

(Burke, Steinberg et al. 2011). Instead of the core microbiome of phyla traditionally discussed, the authors frame the "core functional microbiome" as the most important factor for host function (Burke, Steinberg 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 while the diversity of the microbes may not converge into one core profile, the functional profile becomes similar over the life-span (Lloyd-Price, Abu-Ali et al. 2016, Flemer, Gaci et al. 2017). Studies using metagenomics and metatranscriptomics sequencing illustrate the movement away from OTUs to focus on functional aspects of the microbiota, and studies on the microbiome are expected to shift to a functional rather than compositional nature (Mai, Prosperi 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, Prosperi 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, Barton et al. 2016).

**4. 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, as 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, Wu et al. 2017). Thus, due to 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, Munkittrick et al. 2017), microbial community composition can serve as an important bio-indicator 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, Rupnik 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, chemical type (i.e. microbiome fingerprint) and may be used to predict adverse effects on wildlife/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 two 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.

## 5. 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, Li 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 (SCFAs), tryptophan metabolites, microbial conversion of primary bile acid 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 SCFAs are the C1–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 SCFAs and are produced in an approximate ratio 3:1:1 (total concentration of 50–150 mM) (Rivière, Selak et al. 2016, Rooks and Garrett 2016). Metabolically, they are the most important microbial end products of the human colon fermentation process, as they display several physiological effects. SCFAs are generally 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, Selak et al. 2016). Although the exact underlying mechanisms of action of SCFA have not been fully elucidated, there are at least two potential systems for molecular signaling by SCFAs: i) inhibition of histone deacetylases and ii) activations of a specific G-protein coupled receptors (GPCRs). Histone deacetylases (HDAC) are enzymes that remove the acetyl group from lysine located on histones which regulate gene expression. In addition, studies with macrophages indicate that SCFAs-induced inhibition of HDAC is a crucial regulator of nuclear factor κB (NF-κB) activity and pro-inflammatory innate immune responses (Tremaroli and Bäckhed 2012).

Most importantly, studies show that there can be anti-inflammatory effects of HDAC inhibition by SCFAs to macrophages (Kendrick, O'boyle et al. 2010, Chang, Hao et al. 2014) (Rooks and Garrett 2016) (Tolhurst, Heffron et al. 2012, Tremaroli and Bäckhed 2012). The microbial SCFAs are thus involved in mediating the microbiota–gut–brain axis during appetite regulation. SCFA dependent GPRs activation also regulate immune function and promote anti-inflammatory cell phenotype, via inhibiting NF-κB, a molecule that is an important transcription factor in gut and immune homeostasis (Usami, Kishimoto et al. 2008). GPCRs specific activation also has significant effects in the GI system including the following: (i) maintenance of mucosal immunity (increased transcription of mucin genes) (Willemsen, Koetsier et al. 2003, Gaudier, Jarry et al. 2004) (Singh, Gurav et al. 2014) (Macia, Tan et al. 2015), inflammatory cytokines (e.g. tumor necrotic factor alfa - TNFα) (Vinolo, Rodrigues et al. 2011)$T_{reg}$) (Furusawa, Obata et al. 2013) v) suppressing chemotaxis and the expression of inflammatory genes in neutrophils (Vinolo, Rodrigues 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 SCFAs 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 the decrease of the levels of SCFA such as the anti-inflammatory butyrate, which signifies that exposure to xenobiotics could perturb the gut microbiome and promote gut inflammatory diseases (Kish, Hotte et al. 2013, Liu, Li et al. 2014, Lu, Mahbub et al. 2015) . In general, the decrease of the SCFA by xenobiotic can be caused by the interaction with microbial metabolism or simply by changing *Firmicutes*/*Bacteroidetes* ratio) (Yang, Santisteban et al. 2015).

Another group of bioactive compounds produced by the microbiome are tryptophan metabolites (e.g. indole, indole-3-acetate, and tryptamine) (Jin, Lee 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, Lee et al. 2014, Hubbard, Murray et al. 2015, Noakes 2015). AhR is a ligand-inducible transcription factor/receptor that is highly expressed by epithelial cells, tumors, immune cells and both the IL-17/IL-22–producing and IL-17/IL-22– nonproducing subsets of peripheral γδ T cells (Esser and Rannug 2015). AhR also strongly interacts with anthropogenic xenobiotics (e.g. benzo[a]pyrene, polyaromatic hydrocarbons), many of which are frequently found in municipal areas or in surface waters (e.g. oil spills, urban runoffs). Due to a presence of AhR in immune cells, indoles (e.g. indole-3-acetic acid can affect adaptive immunity of the host, downregulating the differentiation of T-lymphocytes into proinflammatory T-helper ($T_H$) 17 cells (Wilck, Matus et al. 2017) and promoting the AhR-dependent production of interleukin-22 in innate lymphoid cells (Qiu, Heller

et al. 2012), the cytokine responsible for protecting against intestinal inflammation (Jin, Lee et al. 2014, Shanahan, van Sinderen et al. 2017). These tryptophan metabolites are crucial for appropriate AhR signaling, host-microbial mutualism, resistance to colonization and for protection from mucosal inflammation mediated toxicity (Lee, Cella et al. 2012). For example, it was reported in investigations using an intestinal cell model that indole inhibits TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin)–induced CYP1A1 expression, decreasing the toxic effects of TCDD AhR-dependent effects of xenobiotics (Jin, Lee et al. 2014).

In addition to dietary bioactive microbial metabolites, intestinal bacteria can transform the host-secreted bile acids to secondary bile acids (SBAs) through the enzymatic activity of 7α-dehydroxylate (Cyp7a1), which is a highly active enzyme in several species of *Clostridium* (Holmes, Li et al. 2011). Gut microbiota can also modify the profile of bioactive molecules through production of SBA (e.g. deoxycholic acid, lithocolic 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 GI motility and secretion, central signaling (satiety), metabolism, and immunity (Shanahan, van Sinderen et al. 2017). 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, Hisamatsu et al. 2013). Dysregulation of the production of SBAs by xenobiotics might have significant effects on tissues expressing TGR5 (e.g brown adipocytes, macrophages/monocytes and hepatic Kupffer cells, gallbladder epithelium, and intestinal cells). Further, non-physiologically upregulated levels of SBAs can increase insulin sensitivity by stimulating

mitochondrial energy metabolism (Watanabe, Horai 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, Gioiello 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 acids 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 (REGIIIγ), and influencing barrier function and inflammasome activity (Shanahan, van Sinderen 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, Wang 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, Schnackenberg et al. 2013). Importantly, in the study, it was determined that deceases in SBAs and subsequent effects on host due to a tested xenobiotic were consistent with gut microbiota suppression, demonstrating the toxicological importance of SBAs.

Another group of biologically active microbial metabolites are methylamines (e.g. methylamine, dimethylamine, trimethylamine, trimethylamine-N-oxide). 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, Li et al. 2011, Wang, Klipfell et al. 2011, Xu, Wang et al.

2015). Methylamine, specifically trimethylamine-N-oxide (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. TMAO 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, Klipfell 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 of a choline-deficient diet and causing nonalcoholic steatohepatitis (NASH) (Dumas, Barton 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 trimethylamine-N-oxide have been linked to atherosclerosis and cardiovascular disease (Schnabl and Brenner 2014). Although there are a lack of studies that would determine the effects of toxicants on the methylamine production and their subsequent role on 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, Ramezani 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 the targets outlined above are evolutionary conserved, and have corresponding orthologs in fish. For example, AhR are present across a large spectrum of species including mammals, birds, amphibians, fish, cartilaginous fishes and invertebrates (Hahn 2002). Similarly, other targets (GPRCs, TGR5, FXR, NfkB) and responsive

gut peptides (PYY, GLP1) have orthologs in a wide breadth of aquatic organisms (Plisetskaya and Mommsen 1996, Conlon 2002, Fredriksson and Schioth 2005, Savan and Sakai 2006, Reschly, Ai et al. 2008, Hov, Keitel et al. 2010, Fink, Benard et al. 2015, Hodgkinson, Grayfer et al. 2015). Thus, studies are needed that determine whether or not these mechanisms are conserved in aquatic organisms, or whether micro-organisms in the gut act through different pathways to modulate immune signaling.

A final point is that, due to 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 in a study by Holmes at al. (2011), but in many cases, the functions of these metabolites are unknown (Holmes, Li et al. 2011). 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 either in the absence or presence of microbiota (Camp, Frank et al. 2014). This 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 can mediate effects within the host-microbiome cascade, with many new targets and metabolic pathways have yet to be discovered.

**6. Case studies investigating the microbiome in aquatic toxicology**

The effects of some environmental chemical contaminants on the microbiome of aquatic organisms has been investigated on a limited basis (Table 1). Examples include triclosan, the heavy metal cadmium, polycyclic aromatic hydrocarbons, nanomaterials and the fungicide imazalil (Gaulke, Barton et al. 2016, Brown-Peterson, Krasnec et al. 2017, Jin, Luo et al. 2017,

Zhai, Yu et al. 2017) to name a few. Studies have identified a number of OTUs from phyla to genera that can change in abundance in the gut, following contaminant exposures (Table1). As 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 in Section 2, the functional aspects of the microbiome for teleost fishes, as well as invertebrates, remains an exciting avenue of research to come.

Below, we present two case studies that include (1) nanoparticles and (2) hydrocarbons with reference to the Deepwater Horizon oil spill to illustrate how investigations into the microbiome offer insight into adverse outcomes. Due to our experience with fish as a group, we focus on this taxa, but recognize that there are significant efforts underway to characterize microbiota in invertebrate marine organisms (Hentschel, Piel et al. 2012, Kelly, Williams et al. 2014). Nanoparticles are a unique contaminant in terms of the microbiome, as these particles of concern can modulate the microbiota due to their small size and emergent properties compared to chemical contaminants. Additionally, although the focus of this review is placed on the toxicity of environmental chemicals to gut microbiome, data pertaining to other tissue microbiomes or *in vitro* microbial communities are also included in these case studies, as they improve our understanding as to the potential toxic effects on the gut microbiome following exposure to these emerging contaminants of concern.

*6.1.1 Nanomaterials*

Nanomaterials are classified as compounds with at least one dimension between 1 and 100 nanometers 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, Westerhoff et al. 2012). Nanomaterials are also used for a variety of industrial purposes, and are present in coatings, electronics, textiles, and filters (Piccinno, Gottschalk 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. Additionally, several types of nanomaterials have antimicrobial properties, including nano-titanium dioxide (nano-$TiO_2$), nano-zinc oxide (nano-ZnO), carbon nanomaterials, nano-silver (nano-Ag) (Brunet, Lyon et al. 2009, Rai, Yadav et al. 2009, Marambio-Jones and Hoek 2010, Musee, Thwala et al. 2011, Sirelkhatim, Mahmud 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, Kuiken 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 as 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 for these chemicals are still in development (Bouwmeester, Brandhoff et al. 2014). Organic, metal, and metal oxides comprise the majority of domestic-related nanomaterials (Bouwmeester, Brandhoff et al. 2014); thus are more likely to be environmentally released (Keller and Lazareva 2013), and will therefore be the focus of this part of the review.

*6.1.2 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, Kuiken et al. 2015). $TiO_2$ and ZnO are used commonly as a pigment in foods, cosmetics, and coatings (Weir, Westerhoff et al. 2012, Peters, van Bemmel et al. 2014) and as a bactericide in food packaging (Chawengkijwanich and Hayata 2008, Espitia, Soares et al. 2012). Nano-$SiO_2$ is used primarily in protective coatings and environmental treatment, but is also present in dietary supplements (Vance, Kuiken et al. 2015). Although humans are more likely to be exposed to metal oxide nanomaterials due to 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, Scown 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. (Taylor, Marcus et al. 2015) found significant phenotypic changes in the microbial community of a model colon after an exposure to environmentally relevant concentrations of three 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. (Waller, Chen 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 in Taylor et al. (Taylor, Marcus 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 also impact the gut microbiome *in vivo*. In an *in vivo* study with zebrafish (Chen, Guo et al. 2018), co-exposure 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 were glucose, lactate, choline, and methionine) in treated hens compared to controls (Feng, Min et al. 2017). 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, Lai 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.

*6.1.3. 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. Silver nanoparticles (nano-Ag) are the most abundant metal-based nanomaterial in commercial products (Vance, Kuiken et

al. 2015) and are utilized primarily for their antimicrobial properties (Rai, Yadav et al. 2009). According to the 2018 Consumer Products Inventory, these types of nanoparticles are present in textiles, water filters, food containers, and even certain domestic products such as dietary supplements and toothpaste (http://www.nanotechproject.org/cpi/). 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 ppb 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 Bacteroides phyla (Van Den Brûle, Ambroise et al. 2015, Williams, Milner et al. 2015) and increased prevalence of bacteria in the family Enterobacteriaceae and genus *Lactobacillus* (Williams, Milner et al. 2015). Other mammalian studies have not seen the same results following nano-Ag exposure – for example, Wilding et al. (Wilding, Bassis et al. 2016) reported that nano-Ag exposure did not induce any changes in the gut microbiome of mice, and Hadrup et al. (Hadrup, Loeschner et al. 2012) reported no significant changes in the ratio between Firmicutes and Bacteroides in Wistar rats exposed to nano-Ag. As stated by Wilding et al. (Wilding, Bassis et al. 2016), the differences in observations reported by these *in vivo* studies may be due 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 is lacking, one *in vitro* study conducted by Das et al. (Das, McDonald 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 non-mammalian models. A study with Japanese quail (Sawosz, Binek et al. 2007) found that waterborne exposure to nano-Ag increased lactic acid-producing bacteria in the gut microbiome. Additionally, a study with zebrafish found that dietary exposure to nano-Cu and nano-Ag both impacted the diversity of the gut microbiome. Nano-Cu exposure induced the most significant changes, causing complete suppression of common gut bacterial species (namely *C. somerae*), while 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, Geller 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.

*6.1.4. Carbon Nanomaterials and the Gut Microbiome*

Carbon nanomaterials are an emerging class of nanomaterials consisting primarily of cylindrical single and multi-walled 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, Tawfick et al. 2013). Although detection and quantification of these materials are

difficult, recent models predict their environmental release and partitioning into surficial sediments (Schierz, Espinasse 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 and multi-walled carbon nanotubes have broad spectrum antibacterial effects through the lysis of bacterial cell walls and membranes (Chen, Wang 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, Xia et al. 2014). Li et al. (2018) (Li, Lei et al. 2018) found that orally administered fullerenol nanoparticles 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.

*6.2. 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, Dubinsky et al. 2010, Kostka, Prakash et al. 2011, Dubinsky, Conrad et al. 2013, Gutierrez, Singleton et al. 2013, Looper, Cotto et al. 2013, Mason, Scott 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, Dubansky et al. 2012, Barron, Hemmer et al. 2013, Brewton, Fulford et al. 2013, Brown-Peterson, Krasnec 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, Hyde et al. 2015, Gaulke, Barton et al. 2016). Microbiota shifts due to contaminants can have detrimental impacts on the health status of the host (Lefever, Xu et al. 2016, Jin, Wu 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, Hogarth et al. 2014), researchers examined the microbiome of wild-caught snapper from opportunist cruises off the Louisiana coast (Arias, Koenders 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 two fish pathogens, *Vibrio vulnificus* and *Photobacteria 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 in this study, no independent markers of health were reported, and the fish were caught in an area that was also potentially affected by oil from *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 non-oiled marsh sites in Barataria Bay, LA in 2011. Here, using Ribosomal Intergenetic Spacer Analysis (RISA), Larsen et al., (2015) (Larsen, Bullard et al. 2015) showed no evidence of difference in skin microbial populations on fish collected in oiled and non-oiled 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 is not chemical-specific. The lack of evidence of shifts associated with oiled vs. non-oiled 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 were affecting the results.

More recently, two controlled laboratory experiments have attempted to examine 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 days under flow-through conditions, and microbiomes of gill and intestine analyzed by 16S sequencing (Brown-Peterson, Krasnec et al. 2015). Here, 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, while the top gill and intestine were less impacted by oil exposure. Of particular interest here 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 response is an adaptive response of the host to the oil or whether the bacterial communities are outcompeting other residence in an oil-rich environment.

In a follow up study Bayha et al. (2017) (Bayha, Ortell 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 days, and then were challenged with a known fish pathogenic bacterium, *Vibrio anguillarum*, and followed for several days. At 24 hours 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, while in the fish that were placed on uncontaminated sediment prior to the challenge the fish 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 receptor and intestinal microbiome. There is intriguing data that there is a functional linkage between intestinal microbiota, the AhR, and host health (Zhang, Nichols 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, Iannitti 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) (Murray, Nichols et al. 2016) showed that AhR-/- mice have different microbiomes than those that are AhR heterozygotes. In this experiment, mixed genotype littermates were co-housed for 6 months, then separated by genotype and maintained under identical conditions for 18 days. Following this segregation, 16S sequence data indicated a modest but significant shift in the bacterial diversity in the caecum of the different genotypes. Most noticeably, AhR -/- mice had an increase in the prevalence of segmented filamentous bacteria in the caecum. Inferred metabolic pathway analysis also indicated different microbial populations were present in the two 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 ug/kg dietary exposure for 5 days)

and the effect on the intestinal microbiome and metabolism were investigated (Zhang, Nichols et al. 2015). The study showed that dietary TCDF shifted the ratio of Firmicutes to Bacteroidetes and triggered gut inflammation, presumably due to the activation of bacterial fermentation, suggesting that these events are AhR-mediated. In addition, PCA analysis showed that in AhR heterozygotes exposure to TCDF produces a dramatic and significant shift in total microbiome population, while no such difference 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 (Fgf15, Fxr, and Shp) that were present in AhR heterozygotes were non-significant in AhR -/- mice. Taken together, these papers provide strong indicates that the AhR ligand pathway is closely linked with intestinal microbiomes and should be further examined. While 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.

## 7. Adverse outcome pathways and the microbiome

In Figure 3, we present a framework for incorporating the gut microbiome into an adverse outcome pathway (AOP). An oral route of exposure is perhaps the most relevant when linking gut dysbiosis and chemicals, as 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 these chemicals 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, Klawonn 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 polycyclic aromatic hydrocarbons bound to food which activates AhR in the GI 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 molecular initiating event, or MIEs). These events can occur with the parent compound, or they can occur following bio-activation or biotransformation by the gut microbiome; this process can be a significant mechanism prior to a MIE at the host-chemical interface [78]. 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 due 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 is also expected to be modulated by post-inflammatory and protective mechanisms in the gut epithelium. Altered microbial diversity and richness can lead to changes in the microbial metabolites produced within the GI 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, cell cycle, and DNA repair or specific xenobiotic pathways for the chemical.

As 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 in the circulatory system of the host, affecting multiple organs within the organism (Blacher, Levy et al. 2017). Poor overall health of the organism can lead to population-level effects that may include increased susceptibility to infection, decreased growth, and decreased survival. This general framework for Adverse Outcome Pathways 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, as there are likely a number of MIEs and key events that remain undefined; these MIEs will be dependent upon the chemical ingested.

**8. What's 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, Million 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 in order 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 QTL and data on single nucleotide polymorphisms to define microbial-host interactions (Luca, Kupfer et al. 2017). In this approach, genome-wide association studies have correlated microbial variability to human disease phenotypes. Moreover, efforts move towards "a gut on a chip" in humans, which can be potentially developed for aquatic organisms and used to examine microbial-host interactions. A third strategy includes probiotic manipulation or fecal transplant experiments, resulting in reduction/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 to 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 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 non-trivial challenge that requires innovative ways to differentiate.

## 9. 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-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 does dose, diet, and individual genetic variability influence the microbiota?

Addressing these questions are 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.

**Data Accessibility**

Not applicable.

**Acknowledgement**

The authors have no conflict of interest to disclose. We thank funding sources that include research infrastructure projects from Czech Ministry of Education (LM2015051 and CZ.02.1.01/0.0/0.0/16_013/0001761) and the European Union program Skłodowska-Curie actions - Global Fellowship Programme (no.707241) to Adamovsky, The National Science Foundation: CBET- to Bisesi, University of Florida College of Public Health and Health Professions Fellowship Deans Scholarship to Buerger.

References

Arias, C. R., K. Koenders and A. M. Larsen (2013). "Predominant bacteria associated with red snapper from the northern Gulf of Mexico." Journal of aquatic animal health 25(4): 281-289.

Arumugam, M., J. Raes, E. Pelletier, D. Le Paslier, T. Yamada, D. R. Mende, G. R. Fernandes, J. Tap, T. Bruls and J.-M. Batto (2011). "Enterotypes of the human gut microbiome." nature 473(7346): 174.

Barron, M. G. (2012). "Ecological impacts of the deepwater horizon oil spill: implications for immunotoxicity." Toxicol Pathol 40(2): 315-320.

Barron, M. G., M. J. Hemmer and C. R. Jackson (2013). "Development of aquatic toxicity benchmarks for oil products using species sensitivity distributions." Integrated environmental assessment and management 9(4): 610-615.

Bashiardes, S., G. Zilberman-Schapira and E. Elinav (2016). "Use of metatranscriptomics in microbiome research." Bioinformatics and biology insights 10: BBI. S34610.

Bayha, K. M., N. Ortell, C. N. Ryan, K. J. Griffitt, M. Krasnec, J. Sena, T. Ramaraj, R. Takeshita, G. D. Mayer and F. Schilkey (2017). "Crude oil impairs immune function and increases susceptibility to pathogenic bacteria in southern flounder." PloS one 12(5): e0176559.

Benjamino, J., L. Beka and J. Graf (2018). "Microbiome analyses for toxicological studies." Current Protocols in Toxicology: e53.

Benn, T. M. and P. Westerhoff (2008). "Nanoparticle silver released into water from commercially available sock fabrics." Environmental science & technology 42(11): 4133-4139.

Blacher, E., M. Levy, E. Tatirovsky and E. Elinav (2017). "Microbiome-Modulated Metabolites at the Interface of Host Immunity." J Immunol 198(2): 572-580.

Bouwmeester, H., P. Brandhoff, H. J. Marvin, S. Weigel and R. J. Peters (2014). "State of the safety assessment and current use of nanomaterials in food and food production." Trends in food science & technology 40(2): 200-210.

Brewton, R. A., R. Fulford and R. J. Griffitt (2013). "Gene expression and growth as indicators of effects of the BP Deepwater Horizon oil spill on spotted seatrout (Cynoscion nebulosus)." J Toxicol Environ Health A 76(21): 1198-1209.

Brown-Peterson, N. J., M. Krasnec, R. Takeshita, C. N. Ryan, K. J. Griffitt, C. Lay, G. D. Mayer, K. M. Bayha, W. E. Hawkins and I. Lipton (2015). "A multiple endpoint analysis of the effects of chronic exposure to sediment contaminated with Deepwater Horizon oil on juvenile Southern flounder and their associated microbiomes." Aquatic Toxicology 165: 197-209.

Brown-Peterson, N. J., M. O. Krasnec, C. R. Lay, J. M. Morris and R. J. Griffitt (2017). "Responses of juvenile southern flounder exposed to Deepwater Horizon oil-contaminated sediments." Environ Toxicol Chem 36(4): 1067-1076.

Brunet, L. n., D. Y. Lyon, E. M. Hotze, P. J. Alvarez and M. R. Wiesner (2009). "Comparative photoactivity and antibacterial properties of C60 fullerenes and titanium dioxide nanoparticles." Environmental science & technology 43(12): 4355-4360.

Burke, C., P. Steinberg, D. Rusch, S. Kjelleberg and T. Thomas (2011). "Bacterial community assembly based on functional genes rather than species." Proceedings of the National Academy of Sciences 108(34): 14288-14293.

Camp, J. G., C. L. Frank, C. R. Lickwar, H. Guturu, T. Rube, A. M. Wenger, J. Chen, G. Bejerano, G. E. Crawford and J. F. Rawls (2014). "Microbiota modulate transcription in the intestinal epithelium without remodeling the accessible chromatin landscape." Genome research 24(9): 1504-1516.

Caporaso, J. G., J. Kuczynski, J. Stombaugh, K. Bittinger, F. D. Bushman, E. K. Costello, N. Fierer, A. G. Pena, J. K. Goodrich and J. I. Gordon (2010). "QIIME allows analysis of high-throughput community sequencing data." Nature methods 7(5): 335.

Carlson, J. M., E. R. Hyde, J. F. Petrosino, A. B. Manage and T. P. Primm (2015). "The host effects of Gambusia affinis with an antibiotic-disrupted microbiome." Comp Biochem Physiol C Toxicol Pharmacol 178: 163-168.
Chang, P. V., L. Hao, S. Offermanns and R. Medzhitov (2014). "The microbial metabolite butyrate regulates intestinal macrophage function via histone deacetylase inhibition." Proceedings of the National Academy of Sciences 111(6): 2247-2252.
Chawengkijwanich, C. and Y. Hayata (2008). "Development of TiO2 powder-coated food packaging film and its ability to inactivate Escherichia coli in vitro and in actual tests." International journal of food microbiology 123(3): 288-292.
Chen, H., B. Wang, D. Gao, M. Guan, L. Zheng, H. Ouyang, Z. Chai, Y. Zhao and W. Feng (2013). "Broad-Spectrum Antibacterial Activity of Carbon Nanotubes to Human Gut Bacteria." Small 9(16): 2735-2746.
Chen, L., Y. Guo, C. Hu, P. K. Lam, J. C. Lam and B. Zhou (2018). "Dysbiosis of gut microbiota by chronic coexposure to titanium dioxide nanoparticles and bisphenol A: Implications for host health in zebrafish." Environmental Pollution 234: 307-317.
Chi, L., X. Bian, B. Gao, H. Ru, P. Tu and K. Lu (2016). "Sex-specific effects of arsenic exposure on the trajectory and function of the gut microbiome." Chemical research in toxicology 29(6): 949-951.
Chiang, J. Y. (2013). "Bile acid metabolism and signaling." Comprehensive Physiology.
Cho, I. and M. J. Blaser (2012). "The human microbiome: at the interface of health and disease." Nature Reviews Genetics 13(4): 260.
Claus, S. P., H. Guillou and S. Ellero-Simatos (2016). "The gut microbiota: a major player in the toxicity of environmental pollutants?" Npj biofilms and microbiomes 2: 16003.
Conlon, J. M. (2002). "The origin and evolution of peptide YY (PYY) and pancreatic polypeptide (PP)." Peptides 23(2): 269-278.
Cryan, J. F. and S. O'mahony (2011). "The microbiome-gut-brain axis: from bowel to behavior." Neurogastroenterology & Motility 23(3): 187-192.
D'Argenio, V., G. Casaburi, V. Precone and F. Salvatore (2014). "Comparative metagenomic analysis of human gut microbiome composition using two different bioinformatic pipelines." BioMed research international 2014.
Das, P., J. A. McDonald, E. O. Petrof, E. Allen-Vercoe and V. K. Walker (2014). "Nanosilver-mediated change in human intestinal microbiota." Journal of Nanomedicine & Nanotechnology 5(5): 1.
De Volder, M. F., S. H. Tawfick, R. H. Baughman and A. J. Hart (2013). "Carbon nanotubes: present and future commercial applications." science 339(6119): 535-539.
Dubinsky, E. A., M. E. Conrad, R. Chakraborty, M. Bill, S. E. Borglin, J. T. Hollibaugh, O. U. Mason, Y. M. Piceno, F. C. Reid and W. T. Stringfellow (2013). "Succession of hydrocarbon-degrading bacteria in the aftermath of the Deepwater Horizon oil spill in the Gulf of Mexico." Environmental science & technology 47(19): 10860-10867.
Dulski, T., Z. Zakęś and S. Ciesielski (2018). "Characterization of the gut microbiota in early life stages of pikeperch Sander lucioperca." Journal of fish biology 92(1): 94-104.
Dumas, M.-E., R. H. Barton, A. Toye, O. Cloarec, C. Blancher, A. Rothwell, J. Fearnside, R. Tatoud, V. Blanc and J. C. Lindon (2006). "Metabolic profiling reveals a contribution of gut microbiota to fatty liver phenotype in insulin-resistant mice." Proceedings of the national academy of sciences 103(33): 12511-12516.
Espitia, P. J. P., N. d. F. F. Soares, J. S. dos Reis Coimbra, N. J. de Andrade, R. S. Cruz and E. A. A. Medeiros (2012). "Zinc oxide nanoparticles: synthesis, antimicrobial activity and food packaging applications." Food and Bioprocess Technology 5(5): 1447-1464.
Esser, C. and A. Rannug (2015). "The aryl hydrocarbon receptor in barrier organ physiology, immunology, and toxicology." Pharmacological reviews 67(2): 259-279.

Feng, Y., L. Min, W. Zhang, J. Liu, Z. Hou, M. Chu, L. Li, W. Shen, Y. Zhao and H. Zhang (2017). "Zinc oxide nanoparticles influence microflora in ileal digesta and correlate well with blood metabolites." Frontiers in microbiology 8: 992.

Feswick, A., K. R. Munkittrick and C. J. Martyniuk (2017). "Estrogen-responsive gene networks in the teleost liver: What are the key molecular indicators?" Environmental toxicology and pharmacology 56: 366-374.

Fettweis, J. M., J. P. Alves, J. F. Borzelleca, J. P. Brooks, C. J. Friedline, Y. Gao, X. Gao, P. Girerd, M. D. Harwich and S. L. Hendricks (2011). "The vaginal microbiome: disease, genetics and the environment."

Fink, I. R., E. L. Benard, T. Hermsen, A. H. Meijer, M. Forlenza and G. F. Wiegertjes (2015). "Molecular and functional characterization of the scavenger receptor CD36 in zebrafish and common carp." Molecular immunology 63(2): 381-393.

Flemer, B., N. Gaci, G. Borrel, I. R. Sanderson, P. P. Chaudhary, W. Tottey, P. W. O'Toole and J.-F. Brugere (2017). "Fecal microbiota variation across the lifespan of the healthy laboratory rat." Gut microbes 8(5): 428-439.

Fredriksson, R. and H. B. Schioth (2005). "The repertoire of G-protein coupled receptors in fully sequenced genomes." Molecular pharmacology.

Furusawa, Y., Y. Obata, S. Fukuda, T. A. Endo, G. Nakato, D. Takahashi, Y. Nakanishi, C. Uetake, K. Kato and T. Kato (2013). "Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells." Nature 504(7480): 446.

Gaudier, E., A. Jarry, H. Blottiere, P. De Coppet, M. Buisine, J. Aubert, C. Laboisse, C. Cherbut and C. Hoebler (2004). "Butyrate specifically modulates MUC gene expression in intestinal epithelial goblet cells deprived of glucose." American Journal of Physiology-Gastrointestinal and Liver Physiology 287(6): G1168-G1174.

Gaulke, C. A., C. L. Barton, S. Proffitt, R. L. Tanguay and T. J. Sharpton (2016). "Triclosan exposure is associated with rapid restructuring of the microbiome in adult zebrafish." PLoS One 11(5): e0154632.

Givens, C. E., B. Ransom, N. Bano and J. T. Hollibaugh (2015). "Comparison of the gut microbiomes of 12 bony fish and 3 shark species." Marine Ecology Progress Series 518: 209-223.

Gu, Y., X. Wang, J. Li, Y. Zhang, H. Zhong, R. Liu, D. Zhang, Q. Feng, X. Xie and J. Hong (2017). "Analyses of gut microbiota and plasma bile acids enable stratification of patients for antidiabetic treatment." Nature communications 8(1): 1785.

Gutierrez, T., D. R. Singleton, D. Berry, T. Yang, M. D. Aitken and A. Teske (2013). "Hydrocarbon-degrading bacteria enriched by the Deepwater Horizon oil spill identified by cultivation and DNA-SIP." The ISME journal 7(11): 2091.

Hadrup, N., K. Loeschner, A. Bergström, A. Wilcks, X. Gao, U. Vogel, H. L. Frandsen, E. H. Larsen, H. R. Lam and A. Mortensen (2012). "Subacute oral toxicity investigation of nanoparticulate and ionic silver in rats." Archives of toxicology 86(4): 543-551.

Hahn, M. E. (2002). "Aryl hydrocarbon receptors: diversity and evolution1." Chemico-biological interactions 141(1-2): 131-160.

Han, X., B. Geller, K. Moniz, P. Das, A. K. Chippindale and V. K. Walker (2014). "Monitoring the developmental impact of copper and silver nanoparticle exposure in Drosophila and their microbiomes." Science of the Total Environment 487: 822-829.

Hartstra, A. V., K. E. Bouter, F. Bäckhed and M. Nieuwdorp (2015). "Insights into the role of the microbiome in obesity and type 2 diabetes." Diabetes care 38(1): 159-165.

Hazen, T. C., E. A. Dubinsky, T. Z. DeSantis, G. L. Andersen, Y. M. Piceno, N. Singh, J. K. Jansson, A. Probst, S. E. Borglin and J. L. Fortney (2010). "Deep-sea oil plume enriches indigenous oil-degrading bacteria." Science 330(6001): 204-208.

Hentschel, U., J. Piel, S. M. Degnan and M. W. Taylor (2012). "Genomic insights into the marine sponge microbiome." Nature Reviews Microbiology 10(9): 641.
Hiergeist, A., J. Gläsner, U. Reischl and A. Gessner (2015). "Analyses of intestinal microbiota: culture versus sequencing." ILAR journal 56(2): 228-240.
Hodgkinson, J. W., L. Grayfer and M. Belosevic (2015). "Biology of bony fish macrophages." Biology 4(4): 881-906.
Hollister, E. B., C. Gao and J. Versalovic (2014). "Compositional and functional features of the gastrointestinal microbiome and their effects on human health." Gastroenterology 146(6): 1449-1458.
Holmes, E., J. V. Li, T. Athanasiou, H. Ashrafian and J. K. Nicholson (2011). "Understanding the role of gut microbiome–host metabolic signal disruption in health and disease." Trends in microbiology 19(7): 349-359.
Hov, J. R., V. Keitel, J. K. Laerdahl, L. Spomer, E. Ellinghaus, A. ElSharawy, E. Melum, K. M. Boberg, T. Manke and T. Balschun (2010). "Mutational characterization of the bile acid receptor TGR5 in primary sclerosing cholangitis." PloS one 5(8): e12403.
Huang, Y. J. and H. A. Boushey (2015). "The microbiome in asthma." Journal of Allergy and Clinical Immunology 135(1): 25-30.
Huang, Y. J., E. S. Charlson, R. G. Collman, S. Colombini-Hatch, F. D. Martinez and R. M. Senior (2013). "The role of the lung microbiome in health and disease. A National Heart, Lung, and Blood Institute workshop report." American journal of respiratory and critical care medicine 187(12): 1382-1387.
Hubbard, T. D., I. A. Murray and G. H. Perdew (2015). "Indole and tryptophan metabolism: endogenous and dietary routes to Ah receptor activation." Drug Metabolism and Disposition 43(10): 1522-1535.
Hugon, P., J.-C. Lagier, P. Colson, F. Bittar and D. Raoult (2017). "Repertoire of human gut microbes." Microbial pathogenesis 106: 103-112.
Huson, D. H., A. F. Auch, J. Qi and S. C. Schuster (2007). "MEGAN analysis of metagenomic data." Genome research 17(3): 377-386.
Jin, C., T. Luo, Z. Zhu, Z. Pan, J. Yang, W. Wang, Z. Fu and Y. Jin (2017). "Imazalil exposure induces gut microbiota dysbiosis and hepatic metabolism disorder in zebrafish." Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 202: 85-93.
Jin, U.-H., S.-O. Lee, G. Sridharan, K. Lee, L. A. Davidson, A. Jayaraman, R. S. Chapkin, R. Alaniz and S. Safe (2014). "Microbiome-derived tryptophan metabolites and their aryl hydrocarbon receptor-dependent agonist and antagonist activities." Molecular pharmacology 85(5): 777-788.
Jin, Y., S. Wu, Z. Zeng and Z. Fu (2017). "Effects of environmental pollutants on gut microbiota." Environ Pollut 222: 1-9.
Johnston, B. D., T. M. Scown, J. Moger, S. A. Cumberland, M. Baalousha, K. Linge, R. van Aerle, K. Jarvis, J. R. Lead and C. R. Tyler (2010). "Bioavailability of nanoscale metal oxides TiO2, CeO2, and ZnO to fish." Environmental science & technology 44(3): 1144-1151.
Keller, A. A. and A. Lazareva (2013). "Predicted releases of engineered nanomaterials: from global to regional to local." Environmental Science & Technology Letters 1(1): 65-70.
Kelly, C. and I. Salinas (2017). "Under Pressure: Interactions between Commensal Microbiota and the Teleost Immune System." Front Immunol 8: 559.
Kelly, L. W., G. J. Williams, K. L. Barott, C. A. Carlson, E. A. Dinsdale, R. A. Edwards, A. F. Haas, M. Haynes, Y. W. Lim and T. McDole (2014). "Local genomic adaptation of coral reef-associated microbiomes to gradients of natural variability and anthropogenic stressors." Proceedings of the National Academy of Sciences 111(28): 10227-10232.

Kendrick, S. F., G. O'boyle, J. Mann, M. Zeybel, J. Palmer, D. E. Jones and C. P. Day (2010). "Acetate, the key modulator of inflammatory responses in acute alcoholic hepatitis." Hepatology 51(6): 1988-1997.
Kish, L., N. Hotte, G. G. Kaplan, R. Vincent, R. Tso, M. Gänzle, K. P. Rioux, A. Thiesen, H. W. Barkema and E. Wine (2013). "Environmental particulate matter induces murine intestinal inflammatory responses and alters the gut microbiome." PloS one 8(4): e62220.
Kostic, A. D., R. J. Xavier and D. Gevers (2014). "The microbiome in inflammatory bowel disease: current status and the future ahead." Gastroenterology 146(6): 1489-1499.
Kostka, J. E., O. Prakash, W. A. Overholt, S. J. Green, G. Freyer, A. Canion, J. Delgardio, N. Norton, T. C. Hazen and M. Huettel (2011). "Hydrocarbon-degrading bacteria and the bacterial community response in Gulf of Mexico beach sands impacted by the Deepwater Horizon oil spill." Applied and environmental microbiology 77(22): 7962-7974.
Kovatcheva-Datchary, P., V. Tremaroli and F. Bäckhed (2013). The gut microbiota. The prokaryotes, Springer: 3-24.
Langille, M. G., J. Zaneveld, J. G. Caporaso, D. McDonald, D. Knights, J. A. Reyes, J. C. Clemente, D. E. Burkepile, R. L. V. Thurber and R. Knight (2013). "Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences." Nature biotechnology 31(9): 814.
Lapanje, A., M. Rupnik and D. Drobne (2007). "Gut bacterial community structure (Porcellio Scaber, Isopoda, Crustacea) as a measure of community level response to long-term and short-term metal pollution." Environmental toxicology and chemistry 26(4): 755-763.
Larsen, A., H. Mohammed and C. Arias (2014). "Characterization of the gut microbiota of three commercially valuable warmwater fish species." Journal of applied microbiology 116(6): 1396-1404.
Larsen, A. M., S. A. Bullard, M. Womble and C. R. Arias (2015). "Community structure of skin microbiome of gulf killifish, Fundulus grandis, is driven by seasonality and not exposure to oiled sediments in a Louisiana salt marsh." Microbial ecology 70(2): 534-544.
Lee, J. S., M. Cella, K. G. McDonald, C. Garlanda, G. D. Kennedy, M. Nukaya, A. Mantovani, R. Kopan, C. A. Bradfield and R. D. Newberry (2012). "AHR drives the development of gut ILC22 cells and postnatal lymphoid tissues via pathways dependent on and independent of Notch." Nature immunology 13(2): 144.
Lefever, D. E., J. Xu, Y. Chen, G. Huang, N. Tamas and T. L. Guo (2016). "TCDD modulation of gut microbiome correlated with liver and immune toxicity in streptozotocin (STZ)-induced hyperglycemic mice." Toxicol Appl Pharmacol 304: 48-58.
Ley, R. E., M. Hamady, C. Lozupone, P. J. Turnbaugh, R. R. Ramey, J. S. Bircher, M. L. Schlegel, T. A. Tucker, M. D. Schrenzel and R. Knight (2008). "Evolution of mammals and their gut microbes." Science 320(5883): 1647-1651.
Li, J., R. Lei, X. Li, F. Xiong, Q. Zhang, Y. Zhou, S. Yang, Y. Chang, K. Chen and W. Gu (2018). "The antihyperlipidemic effects of fullerenol nanoparticles via adjusting the gut microbiota in vivo." Particle and fibre toxicology 15(1): 5.
Liu, Y., Y. Li, K. Liu and J. Shen (2014). "Exposing to cadmium stress cause profound toxic effect on microbiota of the mice intestinal tract." PloS one 9(2): e85323.
Lloyd-Price, J., G. Abu-Ali and C. Huttenhower (2016). "The healthy human microbiome." Genome medicine 8(1): 51.
Looper, J. K., A. Cotto, B.-Y. Kim, M.-K. Lee, M. R. Liles, S. M. N. Chadhain and A. Son (2013). "Microbial community analysis of Deepwater Horizon oil-spill impacted sites along the Gulf coast using functional and phylogenetic markers." Environmental Science: Processes & Impacts 15(11): 2068-2079.
Lu, K., R. Mahbub and J. G. Fox (2015). "Xenobiotics: interaction with the intestinal microflora." ILAR journal 56(2): 218-227.

Luca, F., S. S. Kupfer, D. Knights, A. Khoruts and R. Blekhman (2017). "Functional Genomics of Host–Microbiome Interactions in Humans." Trends in Genetics.
Macia, L., J. Tan, A. T. Vieira, K. Leach, D. Stanley, S. Luong, M. Maruya, C. I. McKenzie, A. Hijikata and C. Wong (2015). "Metabolite-sensing receptors GPR43 and GPR109A facilitate dietary fibre-induced gut homeostasis through regulation of the inflammasome." Nature communications 6: ncomms7734.
Mai, V., M. Prosperi and L. Yaghjyan (2016). "Moving microbiota research toward establishing causal associations that represent viable targets for effective public health interventions." Annals of epidemiology 26(5): 306-310.
Marambio-Jones, C. and E. M. Hoek (2010). "A review of the antibacterial effects of silver nanomaterials and potential implications for human health and the environment." Journal of Nanoparticle Research 12(5): 1531-1551.
Marchesi, J. R. and J. Ravel (2015). The vocabulary of microbiome research: a proposal, BioMed Central.
Martinez, X., M. Pozuelo, V. Pascal, D. Campos, I. Gut, M. Gut, F. Azpiroz, F. Guarner and C. Manichanh (2016). "MetaTrans: an open-source pipeline for metatranscriptomics." Scientific reports 6: 26447.
Mason, O. U., N. M. Scott, A. Gonzalez, A. Robbins-Pianka, J. Bælum, J. Kimbrel, N. J. Bouskill, E. Prestat, S. Borglin and D. C. Joyner (2014). "Metagenomics reveals sediment microbial community response to Deepwater Horizon oil spill." The ISME journal 8(7): 1464.
Murawski, S. A., W. T. Hogarth, E. B. Peebles and L. Barbeiri (2014). "Prevalence of External Skin Lesions and Polycyclic Aromatic Hydrocarbon Concentrations in Gulf of Mexico Fishes, Post-Deepwater Horizon." Transactions of the American Fisheries Society 143(4): 1084-1097.
Murray, I. A., R. G. Nichols, L. Zhang, A. D. Patterson and G. H. Perdew (2016). "Expression of the aryl hydrocarbon receptor contributes to the establishment of intestinal microbial community structure in mice." Scientific reports 6: 33969.
Musee, N., M. Thwala and N. Nota (2011). "The antibacterial effects of engineered nanomaterials: implications for wastewater treatment plants." Journal of Environmental Monitoring 13(5): 1164-1183.
Noakes, R. (2015). "The aryl hydrocarbon receptor: a review of its role in the physiology and pathology of the integument and its relationship to the tryptophan metabolism." International Journal of Tryptophan Research 8: IJTR. S19985.
Patterson, A. D. and P. J. Turnbaugh (2014). "Microbial determinants of biochemical individuality and their impact on toxicology and pharmacology." Cell metabolism 20(5): 761-768.
Peters, R. J., G. van Bemmel, Z. Herrera-Rivera, H. P. Helsper, H. J. Marvin, S. Weigel, P. C. Tromp, A. G. Oomen, A. G. Rietveld and H. Bouwmeester (2014). "Characterization of titanium dioxide nanoparticles in food products: analytical methods to define nanoparticles." Journal of agricultural and food chemistry 62(27): 6285-6293.
Piccinno, F., F. Gottschalk, S. Seeger and B. Nowack (2012). "Industrial production quantities and uses of ten engineered nanomaterials in Europe and the world." Journal of Nanoparticle Research 14(9): 1109.
Plisetskaya, E. M. and T. P. Mommsen (1996). Glucagon and glucagon-like peptides in fishes. International review of cytology, Elsevier. 168: 187-257.
Qiu, J., J. J. Heller, X. Guo, E. C. Zong-ming, K. Fish, Y.-X. Fu and L. Zhou (2012). "The aryl hydrocarbon receptor regulates gut immunity through modulation of innate lymphoid cells." Immunity 36(1): 92-104.
Rai, M., A. Yadav and A. Gade (2009). "Silver nanoparticles as a new generation of antimicrobials." Biotechnology advances 27(1): 76-83.
Relman, D. A. (2017). "Microbiota: A high-pressure situation for bacteria." Nature 551(7682): 571.

Reschly, E. J., N. Ai, S. Ekins, W. J. Welsh, L. R. Hagey, A. F. Hofmann and M. D. Krasowski (2008). "Evolution of the bile salt nuclear receptor FXR in vertebrates." Journal of lipid research 49(7): 1577-1587.

Rivière, A., M. Selak, D. Lantin, F. Leroy and L. De Vuyst (2016). "Bifidobacteria and butyrate-producing colon bacteria: importance and strategies for their stimulation in the human gut." Frontiers in microbiology 7: 979.

Roeselers, G., E. K. Mittge, W. Z. Stephens, D. M. Parichy, C. M. Cavanaugh, K. Guillemin and J. F. Rawls (2011). "Evidence for a core gut microbiota in the zebrafish." The ISME journal 5(10): 1595.

Rooks, M. G. and W. S. Garrett (2016). "Gut microbiota, metabolites and host immunity." Nature Reviews Immunology 16(6): 341.

Saric, J., Y. Wang, J. Li, M. Coen, J. Utzinger, J. R. Marchesi, J. Keiser, K. Veselkov, J. C. Lindon and J. K. Nicholson (2007). "Species variation in the fecal metabolome gives insight into differential gastrointestinal function." Journal of proteome research 7(01): 352-360.

Savan, R. and M. Sakai (2006). "Genomics of fish cytokines." Comparative Biochemistry and Physiology Part D: Genomics and Proteomics 1(1): 89-101.

Sawosz, E., M. Binek, M. Grodzik, M. Zielińska, P. Sysa, M. Szmidt, T. Niemiec and A. Chwalibog (2007). "Influence of hydrocolloidal silver nanoparticles on gastrointestinal microflora and morphology of enterocytes of quails." Archives of Animal Nutrition 61(6): 444-451.

Schierz, A., B. Espinasse, M. R. Wiesner, J. H. Bisesi, T. Sabo-Attwood and P. L. Ferguson (2014). "Fate of single walled carbon nanotubes in wetland ecosystems." Environmental Science: Nano 1(6): 574-583.

Schloss, P. D., S. L. Westcott, T. Ryabin, J. R. Hall, M. Hartmann, E. B. Hollister, R. A. Lesniewski, B. B. Oakley, D. H. Parks and C. J. Robinson (2009). "Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities." Applied and environmental microbiology 75(23): 7537-7541.

Schnabl, B. and D. A. Brenner (2014). "Interactions between the intestinal microbiome and liver diseases." Gastroenterology 146(6): 1513-1524.

Sears, C. L. and W. S. Garrett (2014). "Microbes, microbiota, and colon cancer." Cell host & microbe 15(3): 317-328.

Seeger, B., F. Klawonn, B. N. Bekale and P. Steinberg (2016). "Mixture Effects of Estrogenic Pesticides at the Human Estrogen Receptor α and β." PloS one 11(1): e0147490.

Shanahan, F., D. van Sinderen, P. W. O'toole and C. Stanton (2017). "Feeding the microbiota: transducer of nutrient signals for the host." Gut: gutjnl-2017-313872.

Silbergeld, E. K. (2017). "The Microbiome: Modulator of Pharmacological and Toxicological Exposures and Responses." Toxicologic pathology 45(1): 190-194.

Singh, N., A. Gurav, S. Sivaprakasam, E. Brady, R. Padia, H. Shi, M. Thangaraju, P. D. Prasad, S. Manicassamy and D. H. Munn (2014). "Activation of Gpr109a, receptor for niacin and the commensal metabolite butyrate, suppresses colonic inflammation and carcinogenesis." Immunity 40(1): 128-139.

Sinha, R., J. Ahn, J. N. Sampson, J. Shi, G. Yu, X. Xiong, R. B. Hayes and J. J. Goedert (2016). "Fecal microbiota, fecal metabolome, and colorectal cancer interrelations." PloS one 11(3): e0152126.

Sirelkhatim, A., S. Mahmud, A. Seeni, N. H. M. Kaus, L. C. Ann, S. K. M. Bakhori, H. Hasan and D. Mohamad (2015). "Review on zinc oxide nanoparticles: antibacterial activity and toxicity mechanism." Nano-Micro Letters 7(3): 219-242.

Stephens, W. Z., A. R. Burns, K. Stagaman, S. Wong, J. F. Rawls, K. Guillemin and B. J. Bohannan (2016). "The composition of the zebrafish intestinal microbial community varies across development." The ISME journal 10(3): 644.

Sun, J., L. K. Schnackenberg, S. Khare, X. Yang, J. Greenhaw, W. Salminen, D. L. Mendrick and R. D. Beger (2013). "Evaluating effects of penicillin treatment on the metabolome of rats." Journal of Chromatography B 932: 134-143.

Surette, M. G. (2014). "The cystic fibrosis lung microbiome." Annals of the American Thoracic Society 11(Supplement 1): S61-S65.

Taylor, A. A., I. M. Marcus, R. L. Guysi and S. L. Walker (2015). "Metal oxide nanoparticles induce minimal phenotypic changes in a model colon gut microbiota." Environmental Engineering Science 32(7): 602-612.

Theriot, C. M., M. J. Koenigsknecht, P. E. Carlson Jr, G. E. Hatton, A. M. Nelson, B. Li, G. B. Huffnagle, J. Z. Li and V. B. Young (2014). "Antibiotic-induced shifts in the mouse gut microbiome and metabolome increase susceptibility to Clostridium difficile infection." Nature communications 5: 3114.

Thomas, C., A. Gioiello, L. Noriega, A. Strehle, J. Oury, G. Rizzo, A. Macchiarulo, H. Yamamoto, C. Mataki and M. Pruzanski (2009). "TGR5-mediated bile acid sensing controls glucose homeostasis." Cell metabolism 10(3): 167-177.

Tidjani Alou, M., M. Million, S. I. Traore, D. Mouelhi, S. Khelaifia, D. Bachar, A. Caputo, J. Delerce, S. Brah and D. Alhousseini (2017). "Gut Bacteria Missing in Severe Acute Malnutrition, Can We Identify Potential Probiotics by Culturomics?" Frontiers in microbiology 8: 899.

Tolhurst, G., H. Heffron, Y. S. Lam, H. E. Parker, A. M. Habib, E. Diakogiannaki, J. Cameron, J. Grosse, F. Reimann and F. M. Gribble (2012). "Short-chain fatty acids stimulate glucagon-like peptide-1 secretion via the G-protein–coupled receptor FFAR2." Diabetes 61(2): 364-371.

Tremaroli, V. and F. Bäckhed (2012). "Functional interactions between the gut microbiota and host metabolism." Nature 489(7415): 242.

Usami, M., K. Kishimoto, A. Ohata, M. Miyoshi, M. Aoyama, Y. Fueda and J. Kotani (2008). "Butyrate and trichostatin A attenuate nuclear factor κB activation and tumor necrosis factor α secretion and increase prostaglandin E2 secretion in human peripheral blood mononuclear cells." Nutrition research 28(5): 321-328.

Van Den Brûle, S., J. Ambroise, H. Lecloux, C. Levard, R. Soulas, P.-J. De Temmerman, M. Palmai-Pallag, E. Marbaix and D. Lison (2015). "Dietary silver nanoparticles can disturb the gut microbiota in mice." Particle and fibre toxicology 13(1): 38.

Vance, M. E., T. Kuiken, E. P. Vejerano, S. P. McGinnis, M. F. Hochella Jr, D. Rejeski and M. S. Hull (2015). "Nanotechnology in the real world: Redeveloping the nanomaterial consumer products inventory." Beilstein journal of nanotechnology 6: 1769.

Velasquez, M. T., A. Ramezani, A. Manal and D. S. Raj (2016). "Trimethylamine N-oxide: the good, the bad and the unknown." Toxins 8(11): 326.

Vinolo, M. A., H. G. Rodrigues, E. Hatanaka, F. T. Sato, S. C. Sampaio and R. Curi (2011). "Suppressive effect of short-chain fatty acids on production of proinflammatory mediators by neutrophils." The Journal of nutritional biochemistry 22(9): 849-855.

Waller, T., C. Chen and S. L. Walker (2017). "Food and industrial grade titanium dioxide impacts gut microbiota." Environmental Engineering Science 34(8): 537-550.

Wang, Z., E. Klipfell, B. J. Bennett, R. Koeth, B. S. Levison, B. DuGar, A. E. Feldstein, E. B. Britt, X. Fu and Y.-M. Chung (2011). "Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease." Nature 472(7341): 57.

Watanabe, M., Y. Horai, S. M. Houten, K. Morimoto, T. Sugizaki, E. Arita, C. Mataki, H. Sato, Y. Tanigawara and K. Schoonjans (2011). "Lowering bile acid pool size with a synthetic farnesoid X receptor (FXR) agonist induces obesity and diabetes through reduced energy expenditure." Journal of Biological Chemistry 286(30): 26913-26920.

Weir, A., P. Westerhoff, L. Fabricius, K. Hristovski and N. Von Goetz (2012). "Titanium dioxide nanoparticles in food and personal care products." Environmental science & technology 46(4): 2242-2250.
Westbrook, A., J. Ramsdell, T. Schuelke, L. Normington, R. D. Bergeron, W. K. Thomas and M. D. MacManes (2017). "PALADIN: protein alignment for functional profiling whole metagenome shotgun data." Bioinformatics 33(10): 1473-1478.
Westreich, S. T., I. Korf, D. A. Mills and D. G. Lemay (2016). "SAMSA: a comprehensive metatranscriptome analysis pipeline." BMC bioinformatics 17(1): 399.
Whitehead, A., B. Dubansky, C. Bodinier, T. I. Garcia, S. Miles, C. Pilley, V. Raghunathan, J. L. Roach, N. Walker, R. B. Walter, C. D. Rice and F. Galvez (2012). "Genomic and physiological footprint of the Deepwater Horizon oil spill on resident marsh fishes." Proc Natl Acad Sci U S A 109(50): 20298-20302.
Wilck, N., M. G. Matus, S. M. Kearney, S. W. Olesen, K. Forslund, H. Bartolomaeus, S. Haase, A. Mähler, A. Balogh and L. Markó (2017). "Salt-responsive gut commensal modulates T H 17 axis and disease." Nature 551(7682): 585.
Wilding, L. A., C. M. Bassis, K. Walacavage, S. Hashway, P. R. Leroueil, M. Morishita, A. D. Maynard, M. A. Philbert and I. L. Bergin (2016). "Repeated dose (28-day) administration of silver nanoparticles of varied size and coating does not significantly alter the indigenous murine gut microbiome." Nanotoxicology 10(5): 513-520.
Willemsen, L., M. Koetsier, S. Van Deventer and E. Van Tol (2003). "Short chain fatty acids stimulate epithelial mucin 2 expression through differential effects on prostaglandin E1 and E2 production by intestinal myofibroblasts." Gut 52(10): 1442-1447.
Williams, K., J. Milner, M. D. Boudreau, K. Gokulan, C. E. Cerniglia and S. Khare (2015). "Effects of subchronic exposure of silver nanoparticles on intestinal microbiota and gut-associated immune responses in the ileum of Sprague-Dawley rats." Nanotoxicology 9(3): 279-289.
Wu, J., X. W. Wen, C. Faulk, K. Boehnke, H. Zhang, D. C. Dolinoy and C. Xi (2016). "Perinatal lead exposure alters gut microbiota composition and results in sex-specific bodyweight increases in adult mice." Toxicological Sciences 151(2): 324-333.
Xia, J. H., G. Lin, G. H. Fu, Z. Y. Wan, M. Lee, L. Wang, X. J. Liu and G. H. Yue (2014). "The intestinal microbiome of fish under starvation." BMC genomics 15(1): 266.
Xia, T., W. Lai, M. Han, M. Han, X. Ma and L. Zhang (2017). "Dietary ZnO nanoparticles alters intestinal microbiota and inflammation response in weaned piglets." Oncotarget 8(39): 64878.
Xu, R., Q. Wang and L. Li (2015). "A genome-wide systems analysis reveals strong link between colorectal cancer and trimethylamine N-oxide (TMAO), a gut microbial metabolite of dietary meat and fat." BMC genomics 16(7): S4.
Yang, T., M. M. Santisteban, V. Rodriguez, E. Li, N. Ahmari, J. M. Carvajal, M. Zadeh, M. Gong, Y. Qi and J. Zubcevic (2015). "Gut dysbiosis is linked to hypertension." Hypertension 65(6): 1331-1340.
Yoneno, K., T. Hisamatsu, K. Shimamura, N. Kamada, R. Ichikawa, M. T. Kitazume, M. Mori, M. Uo, Y. Namikawa and K. Matsuoka (2013). "TGR5 signalling inhibits the production of pro-inflammatory cytokines by in vitro differentiated inflammatory and intestinal macrophages in Crohn's disease." Immunology 139(1): 19-29.
Zelante, T., R. G. Iannitti, C. Cunha, A. De Luca, G. Giovannini, G. Pieraccini, R. Zecchi, C. D'angelo, C. Massi-Benedetti and F. Fallarino (2013). "Tryptophan catabolites from microbiota engage aryl hydrocarbon receptor and balance mucosal reactivity via interleukin-22." Immunity 39(2): 372-385.
Zhai, Q., L. Yu, T. Li, J. Zhu, C. Zhang, J. Zhao, H. Zhang and W. Chen (2017). "Effect of dietary probiotic supplementation on intestinal microbiota and physiological conditions of Nile tilapia

(Oreochromis niloticus) under waterborne cadmium exposure." Antonie van Leeuwenhoek 110(4): 501-513.
Zhang, L., R. G. Nichols, J. Correll, I. A. Murray, N. Tanaka, P. B. Smith, T. D. Hubbard, A. Sebastian, I. Albert and E. Hatzakis (2015). "Persistent organic pollutants modify gut microbiota–host metabolic homeostasis in mice through aryl hydrocarbon receptor activation." Environmental health perspectives 123(7): 679.
Zhang, L., R. G. Nichols and A. D. Patterson (2017). "The aryl hydrocarbon receptor as a moderator of host-microbiota communication." Current Opinion in Toxicology 2: 30-35.
Zhu, B., X. Xia, N. Xia, S. Zhang and X. Guo (2014). "Modification of fatty acids in membranes of bacteria: implication for an adaptive mechanism to the toxicity of carbon nanotubes." Environmental science & technology 48(7): 4086-4095.

**Figure Captions**

**Figure 1. Addressing questions related to the microbiome.**

**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 (SCFAs), and microbial conversion of primary bile acid to secondary bile acids.

**Figure 3. Proposed outcome framework for chemicals that affect the microbiome.** Ingested chemicals can be bio-transformed by the microbiome or act directly on the host epithelium to exert adverse effects in the host.

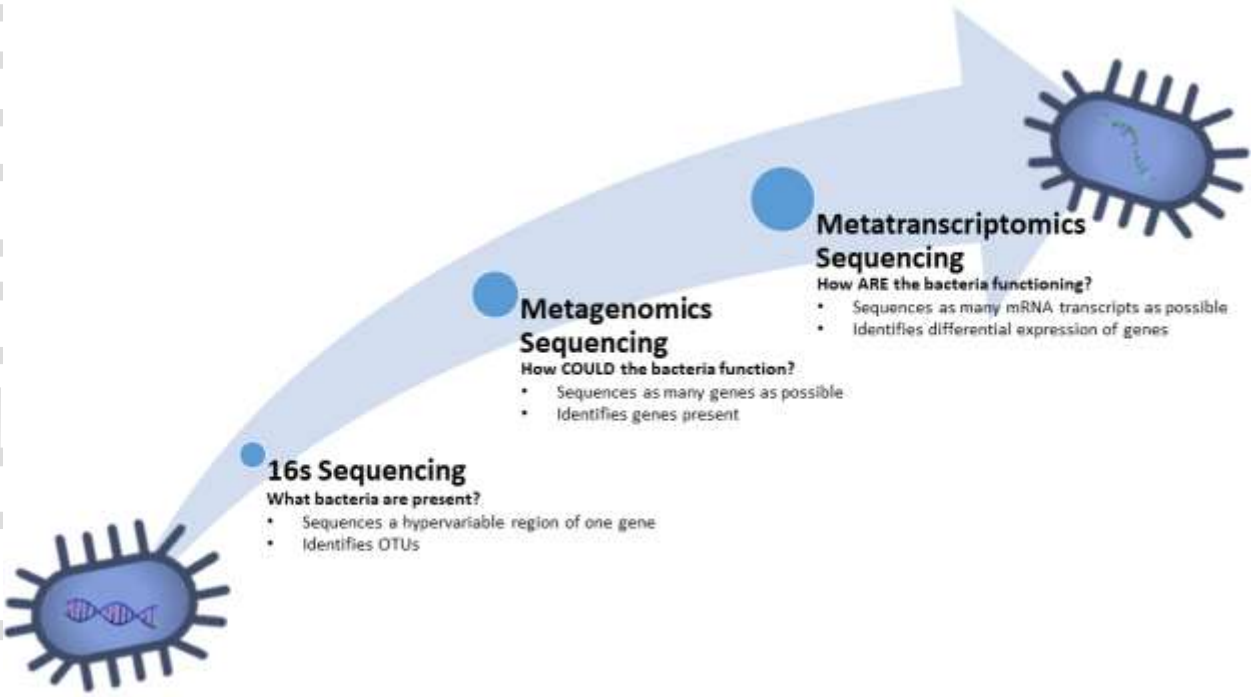


**Figure 1**

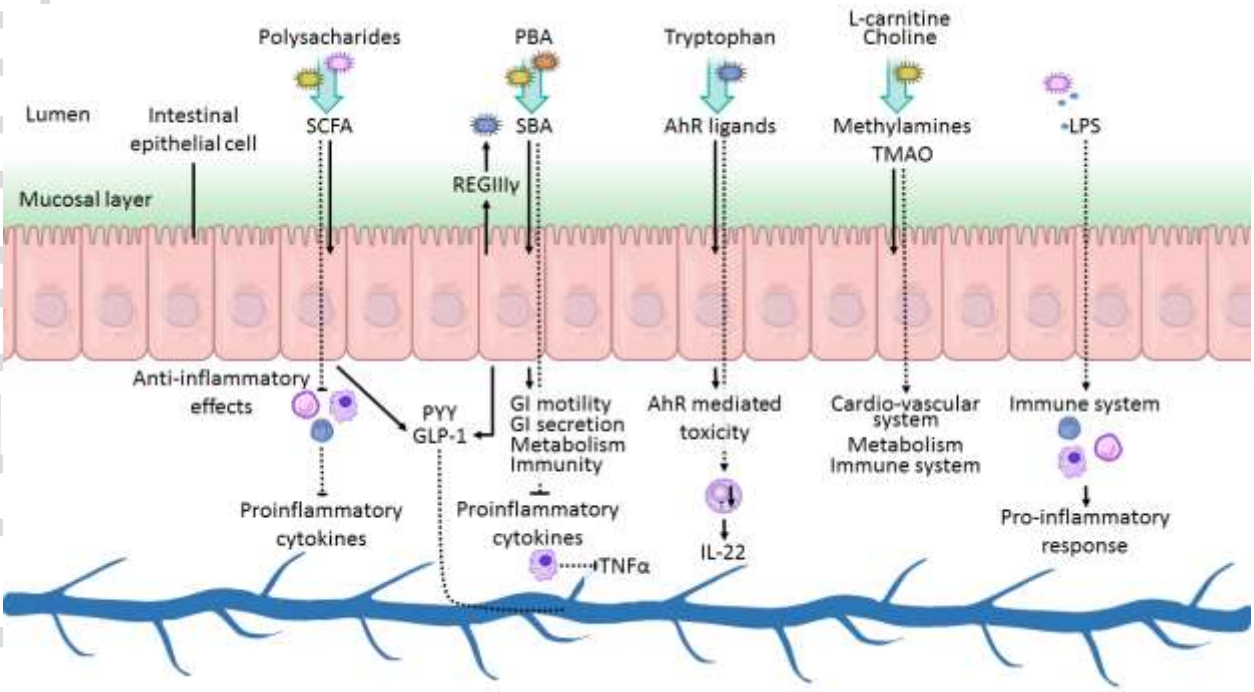


**Figure 2**

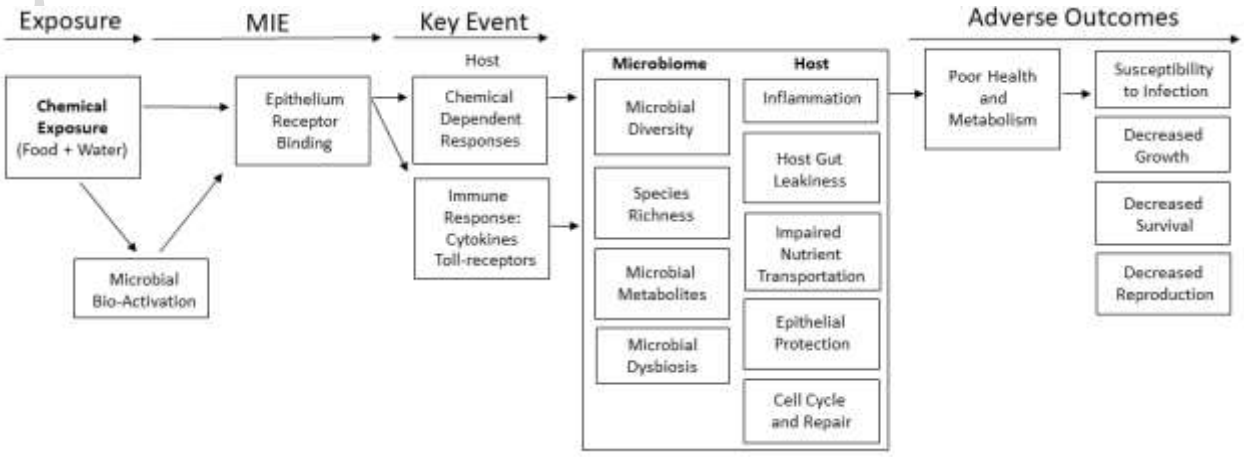


**Figure 3**

**Table 1:** Examples of some of the most abundant genera in the fish gut that are affected by chemicals and environmental contaminants. The effect of chemical on the microbiota is provided (plus sign indicates increased presence in the gut while negative sign indicates 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 literature. PAH = polycyclic aromatic hydrocarbon. The phylum and its functional significance for CK-1C4- 19 and the functional significance of *CKC4* is unknown as it has not been extensively studied. To the best of our knowledge, functional data are also lacking for *Pseudoaltermonas* and *Alistipes.*

| Fish Species | Chemical: description | Groups discussed in paper | Effect of Chemical on Group | Functional Significance (Group) | Phyla | Functional significance (Phyla) | Citation |
|---|---|---|---|---|---|---|---|
| Southern Flounder *(Paralichthys lethostigma)* | PAHs:<br><br>Environmental pollutants produced from partial combustion of organic material. | *Clostridia* | - | Often pathogenic | *Firmicutes* | Carbohydrate metabolism | [1] |
| | | *Owenweeksia Hongkongensis (species)* | - | Metabolizes carbohydrates, amino acids, and lipids. Sulphur 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, sulphur oxidation | | | |
| | | *Donghicola (Genus)* | + | Present in seawater | | | |
| | | *Rhodovacteraceae (Family)* | + | Sulfur and carbon biogeochemical cycling | | | |
| | | *Pseudoaltermonas* | + | --------- | | | |
| Nile tilapia (*Oreochromis niloticus*) | Cadmium:<br><br>A metal that is a silver-white color in its' elemental state. Cadmium is | *Bacteroidetes* (phylum) | + | See functional significance of phyla. | *Bacteroidetes* | Degrade high molecular weight organic matter like protein, polysaccharides, and carbohydrates. | [2] |
| | | *Flavobacterium (genus)* | + | Pathogenic, degrades macromolecules | | | |
| | | *Fusobacteria* (phylum) | - | See functional significance of phyla. | *Fusobacteria* | Pathogenic | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | carcinogenic and mostly a by-product of zinc mining and smelting. | *Cetobacterium (genus)* | - | Produce vitamin B12 | | | |
| | | *Plesiomonas (genus)* | - | Fermentation of lactose | *Proteobacteria* | Pathogenic, nitrogen fixation | |
| | | *Deefgea (genus)* | - | Facultative anaerobe | | | |
| | | *Pseudomonas (genus)* | + | Diverse, often pathogenic | | | |
| | | *Cellvibrio (genus)* | + | plant polysaccharide degradation | | | |
| | | *Acinetobacter (genus)* | + | Pathogenic, bioremediation | | | |
| Zebrafish (*Danio rerio*) | Oxytetracycline And Sulfamethoxazole:<br><br>-both compounds used as antibiotics | *Proteobacteria (phylum)* | - | See functional significance of phyla. | *Proteobacteria* | Pathogenic, some groups fix nitrogen | [3] |
| | | *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* | -- | |
| Fathead minnows (*Pimephales promelas)* | Triclosan:<br><br>Chemical often used as an antibiotic/ antimicrobial | *CK-1C4- 19* | + | -- | -- | -- | [4] |
| | | *Hydrogenophaga (genus)* | + | Hydrogen oxidation | *Proteobacteria* | Pathogenic, some groups fix nitrogen | |
| | | *Thauera (genus)* | + | Degradation of aromatic compounds | | | |
| | | *Methylobacterium (genus)* | + | Pathogenic, synthesize carotenoids | | | |
| | | *Acidovorax (genus)* | + | Pathogenic | | | |
| Zebrafish (*Danio rerio*) | Silver nano particles:<br><br>Small particles of silver possessing antimicrobial properties. | Cetobacterium somerae (species) | - | Produces vitamin B12 | *Fusobacteria* | Can be pathogenic, some species produces vitamin B12 | [5] |
| Zebrafish (*Danio rerio*) | Imazalil:<br><br>Fungicide used to keep plants/ crops fungus free. | *Bacteroides (genus)* | - | Pathogenic | Bacteroidetes | Degrade high molecular weight organic matter like protein, polysaccharides, and carbohydrates. | [6] |
| | | *Alistipes (genus)* | - | --------- | | | |
| | | *Rhodobacter (genus)* | - | anoxygenic photosynthesis and carbon/ nitrogen fixation | *Proteobacteria* | Pathogenic, some groups fix nitrogen | |
| | | *Akkermansia (genus)* | - | -degrades mucous | *Verrucomicrobia* | Some species oxidize methane | |

[1] Brown-Peterson NJ, Krasnec MO, Lay CR, Morris JM, Griffitt RJ. 2017. Responses of juvenile southern flounder exposed to Deepwater Horizon oil-contaminated sediments. *Environmental toxicology and chemistry* 36:1067-1076.
[2] Zhai Q, Yu L, Li T, Zhu J, Zhang C, Zhao J, Zhang H, Chen W. 2017. Effect of dietary probiotic supplementation on intestinal microbiota and physiological conditions of Nile tilapia (Oreochromis niloticus) under waterborne cadmium exposure. *Antonie van Leeuwenhoek* 110:501-513.
[3] Zhou L, Limbu SM, Shen M, Zhai W, Qiao F, He A, Du Z-Y, Zhang M. 2018. Environmental concentrations of antibiotics impair zebrafish gut health. *Environmental Pollution* 235:245-254.
[4] Narrowe AB, Albuthi-Lantz M, Smith EP, Bower KJ, Roane TM, Vajda AM, Miller CS. 2015. Perturbation and restoration of the fathead minnow gut microbiome after low-level triclosan exposure. *Microbiome* 3:6.
[5] Merrifield DL, Shaw BJ, Harper GM, Saoud IP, Davies SJ, Handy RD, Henry TB. 2013. Ingestion of metal-nanoparticle contaminated food disrupts endogenous microbiota in zebrafish (Danio rerio). *Environmental pollution* 174:157-163.
[6] Jin C, Luo T, Zhu Z, Pan Z, Yang J, Wang W, Fu Z, Jin Y. 2017. Imazalil exposure induces gut microbiota dysbiosis and hepatic metabolism disorder in zebrafish. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* 202:85-93.