Published in final edited form as:

Curr Opin Microbiol. 2017 August ; 38: 137–141. doi:10.1016/j.mib.2017.05.011.

The scales of the zebrafish: host-microbiota interactions from proteins to populations

Adam R. Burns¹ and Karen Guillemin^{2,3,*}

¹Institute of Ecology and Evolution, University of Oregon, Eugene, Oregon 97403, United States

²Institute of Molecular Biology, University of Oregon, Eugene, Oregon 97403, United States

³Humans and the Microbiome Program, Canadian Institute for Advanced Research, Toronto, Ontario M5G 1Z8, Canada

Abstract

The interactions between animal hosts and their associated microbiota can be studied at multiple spatial and conceptual scales, with each providing unique perspectives on the processes structuring host-microbe systems. Recently, zebrafish, *Danio rerio*, has emerged as a powerful model in which to study these interactions at many different scales. Controlled but simplified gnotobiotic experiments enable discovery of the molecules and cellular dynamics that shape host-microbe system development, whereas population level investigations of bacterial dispersal and transmission are beginning to reveal the processes shaping microbiota assembly across hosts. Here we review recent examples of these studies and discuss how the results can be integrated to better understand host-microbiota systems.

Abstract

Graphical abstract

* To whom correspondence should be addressed: kguillem@uoregon.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Conflict of Interest Statement

None of the authors have any financial or non-financial competing interests to declare.

Introduction

Animals and their resident microorganisms form complex biological systems of multiple interacting elements. The potential importance of these interactions in determining the health of humans and other animals has motivated efforts to better manipulate and predict their behavior. Understanding these interactions is challenging, because they operate at multiple scales, from the level of molecular interactions between microbial and host cells to the transmission of microorganisms across both populations and generations of hosts. Studies of other complex biological systems and naturally occurring ecological communities have concluded that the scale at which they are observed can fundamentally alter interpretations about the nature and importance of various mechanisms and processes driving the system [1,2]. Thus, any approach that focuses solely on interactions occurring at a single scale, such as either direct cell to cell interactions within a single host or changes in microbiota composition across populations of hosts, will likely lead to an incomplete or even misleading understanding of these systems as a whole.

In recent years, the zebrafish, *Danio rerio*, has emerged as an excellent model system in which multiple levels of interactions can be analyzed together to build a comprehensive understanding of how different mechanisms act across scales. For cellular level studies of host-microbe interactions, the zebrafish model affords sophisticated genetic tools and resources for marking and manipulating different cell types. Equally important is the ease with which hundreds of individual embryos can be derived germ-free and reared gnotobiotically with a defined or absent microbiota [3]. The optical transparency of zebrafish larvae provide the unique opportunity to observe the spatial and temporal dynamics of microbial populations alongside the host's response [4]. At the population level of host-microbe studies, the characteristic variability of animal microbiomes can be overcome through the large degree of biological replication afforded by zebrafish's high fecundity, allowing for robust analysis of the effects of host factors on the composition of the microbiota. Finally, combining these features with the control over the hosts' physical environment, zebrafish husbandry opens up opportunities to study broad scale interactions occurring across multiple individual hosts. Like any model system, the zebrafish has its limitations when it comes to modeling human biology. Zebrafish are aquatic animals that interact with their microbial world through the medium of water rather than air and terrestrial surfaces. They lack lungs and mammary glands, limiting their utility for modeling host-microbe interactions that are shaped by these organs, and they only develop their adaptive immune systems during juvenile stages. They are cold blooded and typically reared at 28 degrees Celsius, thus restricting their capacity to be colonized by microbes specialized to warm blooded animal temperatures. Despite these limitations, they have proven to be a powerful model to explore the interactions between animal hosts and their microbial partners at multiple scales. In this review, we discuss how using the zebrafish to study host-microbe interactions across multiple scales has led to novel findings and promising avenues of future research.

Molecular interactions between microbial and host cells

The zebrafish is a powerful model for studying host-microbe interactions at the tissue, cell, and molecular level to identify mechanisms that maintain homeostasis between hosts and their resident microorganisms and the consequences of this interplay on host organ development and differentiation. Comparisons of germ-free larval zebrafish with those colonized with a complex microbiota have repeatedly demonstrated effects of resident microorganisms on host intestinal cell differentiation [5], epithelial cell proliferation [6,7], intestinal absorption of fatty acids [8], and immune responses [6,9,10]. In addition to inducing changes in the intestinal track and the immune system, both of which come into frequent contact with microorganisms and microbial products, there is mounting evidence of microorganism-dependent impacts on the development of other organs. For example, our group found that microbiota are required for normal expansion of pancreatic β -cells in larval zebrafish and we discovered a single protein produced by specific zebrafish gut bacteria that is sufficient for this expansion in germ-free larvae [11]. Furthermore, homologs of this protein, which we named Beta cell expansion factor A or BefA, are found in microorganisms isolated from human microbiota and show a similar ability to induce β -cell expansion in zebrafish, suggesting this function has a conserved origin. The discovery of BefA provides a clear example of how the gnotobiotic zebrafish system can be used to simultaneously uncover the consequences of host-microbe interactions on animal physiology as well as identify the specific molecular mechanisms underlying those interactions.

Interactions between defined microbiota and their hosts

Zebrafish have also proven useful in studying the colonization and dynamics of populations of microorganisms in hosts. By combining gnotobiotic husbandry with a high-throughput transposon mutagenesis screen of zebrafish-associated bacterial strains, Stephens et al. [12] characterized the bacterial gene functions predictive of colonization and population sizes *in vivo*. Doing so highlighted the importance of bacterial motility in particular, both in the form of chemotaxis and flagellar-mediated motility and adhesion, to intestinal colonization and invasion of already established populations. An additional study utilizing gnotobiotic zebrafish further supported the importance of bacterial adhesion in the establishment of specific bacterial strains, particularly those with potential probiotic properties [13].

The optical transparency of larval zebrafish provides unique opportunities to further study the motility and spatial distributions of bacterial populations *in vivo*. Early work imaging *Mycobacteria marinum* infection of macrophages fundamentally changed the idea of tuberculous granulomas as static structures of host immune containment and demonstrated that they play a role in pathogen dissemination [14,15]. The adoption of light sheet microscopy for live imaging of bacterial dynamics in zebrafish has allowed for measurements of bacterial populations along the entire intestine with high temporal and spatial resolution [4]. Using these techniques to characterize colonization dynamics of fluorescently labeled strains has highlighted a diversity of bacterial colonization strategies. For example, *Aeromonas veronii*, a common resident of the zebrafish intestine, was found to form clustered aggregates of cells that grew at faster rates than individual planktonic cells, suggesting a strategic advantage to growth in aggregates [16]. Interestingly, a second *Vibrio* species that exhibited a highly planktonic growth, was found to competitively exclude

Aeromonas when the two were co-colonized [17]. This exclusion was largely mediated by the different responses of the species to host intestinal motility: the *Aeromonas* aggregates were regularly expelled from the intestine by peristalsis whereas the more diffuse populations of *Vibrio* were able to resist these perturbations and prevent the recovery of *Aeromonas*. The important role of the host in this competitive exclusion was demonstrated by the finding that both species were able to persist together in *ret* mutant zebrafish lacking normal intestinal motility. These studies reveal potential trade-offs between planktonic versus aggregated bacterial cell strategies for growth and persistence in the intestine and possibly inter-host transmission.

Interactions among taxa of bacteria not only alter their independent growth dynamics, but also their potential effects on their hosts. A screen of multiple species of zebrafish-derived bacteria in monoassociation with larval zebrafish revealed variation in the overall neutrophil response [18]. This relationship between neutrophil response and bacterial abundance also differed among species, with some, such as *Vibrio* sp., exhibiting a positive monotonic relationship, others, such as *Shewanella* sp., exhibiting a negative relationship, and finally some, such as *Aeromonas* sp., showing no clear relationship. Interestingly, each species had disproportional effects on neutrophil response, such that predicting the host response to a complex microbiota requires knowing both the abundance and per capita effect of each species. To a first approximation, these same rules were found to apply to much more complex microbiota that assembled in a mutant zebrafish line, *sox10*, that has defective peristalsis and develops spontaneous intestinal inflammation [19]. The extent of inflammation varied across mutant siblings reared in a shared aquatic environment and could be explained by the relative abundances of pro-inflammatory *Vibrio* sp. and anti-inflammatory *Escherichia* sp. in individual intestines. These studies suggest that inferences from simple, defined communities can be scaled up to the complex ones that naturally inhabit vertebrates.

Interactions between complex microbiota and their hosts

The resident microbiota of zebrafish consist of hundreds of microbial taxa and is typically dominated by members of the Proteobacteria phylum, followed by Fusobacteria and Actinobacteria [20–22]. In addition, zebrafish can also be colonized by a wider range of microorganisms than those normally found associated with them, including taxa isolated from mammalian and human hosts [23]. This property has facilitated analysis of potential human probiotics, including several strains of *Lactobacillus*, in larval gnotobiotic zebrafish [24]. Foundational work utilizing zebrafish as a model to study host-microbe interactions has shown that the composition and distribution of microbial lineages in the zebrafish microbiota is in large part selected by the zebrafish host. Following the reciprocal transplant of mouse microbiota into germ-free zebrafish recipients, bacterial taxa from the donor mouse microbiota were able to successfully colonize larval zebrafish, but the relative abundances of these strains was altered to match the relative abundance of shared lineages in conventional zebrafish microbiota [20]. Thus, despite differences in the microbial inoculum, the zebrafish gut environment selects for a specific distribution of bacterial lineages. The importance of host habitat selection is further supported by the observation that zebrafish raised in laboratory facilities still retain strong, broad level similarities in microbiota

composition to wild caught zebrafish, and distinct from other closely related fishes, despite large differences in geography and environmental exposure [21].

Some of the most dramatic changes that happen in animals occur during their development from embryos to fully mature adults. Initial small scale studies found that zebrafish development was a strong predictor of bacterial diversity [25]. A larger, more densely sampled study found similar changes in bacterial diversity and an increase in inter-individual variation from larval to adult stages, suggesting developmental changes in the processes by which hosts select their microbiota [22]. In addition, the zebrafish microbiota became less similar to the microbial communities in the surrounding environment and food over time. To further investigate this relationship between host development, microbiota, and diet, Wong et al. analyzed the intestinal microbiota of zebrafish maintained on one of three constant diets that differed in their fat content [26]. Dietary fat levels had distinct effects on the intestinal microbiota that changed throughout host development and manifested through changes in the assemblages of multiple bacterial species. Furthermore, dietary fat levels also had a substantial effect on environmental communities of bacteria, suggesting that diet may also change hosts' environmental exposures.

Zebrafish have provided a powerful high-throughput system in which to screen and study the effects of environmental exposures on vertebrates for many of the same reasons that they make attractive models to study host-microbe interactions [27], and this can further be extended to study the additional effects on the microbiota. For example, Gaulke *et al.* characterized the microbiota of zebrafish exposed to the antibacterial agent triclosan, and detected significant changes in the composition and diversity of the intestinal microbiota [28]. In addition, triclosan exposure altered the correlation networks of microbial taxa, increasing their connectedness. This suggests that alterations to a host and its environment can not only alter the composition of an individual's microbiota, but also the nature of the interactions among its resident microbial taxa.

Interactions among microbiota and populations of hosts

Animal microbiota also have the potential to be influenced by processes occurring beyond an individual host, through the migration of microorganisms from biotic and abiotic sources. For example, transmission among hosts is already known to be a key process determining the distributions and spread of pathogens in animals, and zebrafish have increasingly been used as a model to study the colonization and transmission of *Vibrio cholera* [29], *Salmonella* [30], and *Mycobacterium* [31]. Key to the use of zebrafish as a model for pathogen transmission is the ability to raise and house large populations under controlled environmental conditions and the ability to manipulate their exposure to microorganisms in their environment and to other individuals.

Recently, research utilizing zebrafish has attempted to expand the study of microbiota assembly from an individual host-centric to a population-based investigation. Toward this goal we employed a neutral modeling framework to test the null hypothesis that differences among bacterial species and hosts were unimportant to microbiota assembly [32]. Our analysis demonstrated that passive migration and stochastic demographic processes were sufficient to explain a large amount of variation in the microbiota of a large population of

zebrafish, however the relative importance of these processes decreased with host development. A major component of this development is the maturation of the adaptive immune system, which does not become fully active in zebrafish until they are juveniles [33]. Due to its potential ability to selectively identify and target specific microorganisms, we hypothesized that the adaptive immune system would limit the transmission of microbes from other hosts. Indeed, we found that *ragF* mutant zebrafish lacking a functioning adaptive immune system were associated with more neutrally assembled microbiota [34]. However, differences in the composition of the microbiota between wild type and mutant *ragF* zebrafish were subtle and weaker overall than the effect of cohousing, a phenomena that has been observed in other model systems. This suggests that although adaptive immunity may constrain colonization of zebrafish, the effects of transmission overall may be great enough to overwhelm individual host factors.

Conclusion

Increasingly expanding the scale at which host-microbiota systems are observed has revealed what appears to be the inherently noisy and variable nature of these systems. At very fine scales, studying molecular and cell-cell interactions, the impacts of an individual bacterial taxa or molecule on the host can be measured as a binary response. In these situations, the variable nature of the microbiota is merely manifested as statistical “noise” that is more an obstacle than a feature of the system. However, beyond these relatively reductionist experimental approaches, we have seen an increased role of stochastic effects, from the potentially random influence of population bottlenecks during the initial colonization of the intestine [12], to the importance of passive bacterial transmission in determining the overall composition of intestinal microbiota [32,34]. Furthermore, where fine scale gnotobiotic studies have identified specific bacterial factors inducing changes in zebrafish hosts [5,7–9,11], broader scale studies can inform the circumstances under which those interactions even have the potential to occur (for example, by determining whether or not the bacterium is even able to colonize a zebrafish [17]), and to what degree the overall effect on the host can be predicted by the population size of individual bacterial species [18,19].

A major challenge for the microbiome field today is translating broad patterns gleaned from studies of highly variable human populations into therapeutic actions for individuals. By serving as a model to study host-microbe interactions from the molecular to the population scales, zebrafish provide a potential path towards tackling this challenge. Live imaging techniques, like those described above, enable studies of temporal and spatial dynamics within an individual zebrafish, and the flexible and controllable husbandry of zebrafish allows for the design of experiments to study fine scale interactions occurring among populations of zebrafish and between zebrafish and their environment. By exploiting the zebrafish model in this way, future research can connect insights on multiple scales at the same time, thereby providing a more comprehensive understanding of host-microbe systems as a whole.

Acknowledgments

This work was supported by the National Institutes of Health under award number P50GM098911. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

References

1. Chase JM, Myers JA. Disentangling the importance of ecological niches from stochastic processes across scales. *Philos Trans R Soc London B Biol Sci*. 2011; 366
2. Münkemüller T, Gallien L, Lavergne S, Renaud J, Roquet C, Abdulkhak S, Dullinger S, Garraud L, Guisan A, Lenoir J, et al. Scale decisions can reverse conclusions on community assembly processes. *Glob Ecol Biogeogr*. 2014; 23:620–632. [PubMed: 24791149]
3. Melancon E, Gomez De La Torre Canny S, Sichel S, Kelly M, Wiles TJ, Rawls JF, Eisen JS, Guillemin K. Chapter 3 — Best practices for germ-free derivation and gnotobiotic zebrafish husbandry. *Methods Cell Biol*. 2017; 138:61–100. [PubMed: 28129860]
4. Taormina MJ, Jemielita M, Zac Stephens W, Burns AR, Troll JV, Parthasarathy R, Guillemin K. Investigating bacterial-animal symbioses with light sheet microscopy. *Biol Bull*. 2012; 223:7–20. [PubMed: 22983029]
5. Bates JM, Mittge E, Kuhlman J, Baden KN, Cheesman SE, Guillemin K. Distinct signals from the microbiota promote different aspects of zebrafish gut differentiation. *Dev Biol*. 2006; 297:374–386. [PubMed: 16781702]
6. Rawls JF, Samuel BS, Gordon JI. Gnotobiotic zebrafish reveal evolutionarily conserved responses to the gut microbiota. *Proc Natl Acad Sci U S A*. 2004; 101:4596–601. [PubMed: 15070763]
7. Cheesman SE, Neal JT, Mittge E, Seredick BM, Guillemin K. Epithelial cell proliferation in the developing zebrafish intestine is regulated by the Wnt pathway and microbial signaling via Myd88. *Proc Natl Acad Sci U S A*. 2011; 108(Suppl):4570–7. [PubMed: 20921418]
8. Semova I, Carten JD, Stombaugh J, Mackey LC, Knight R, Farber SA, Rawls JF. Microbiota Regulate Intestinal Absorption and Metabolism of Fatty Acids in the Zebrafish. *Cell Host Microbe*. 2012; 12:277–288. [PubMed: 22980325]
9. Bates JM, Akerlund J, Mittge E, Guillemin K. Intestinal Alkaline Phosphatase Detoxifies Lipopolysaccharide and Prevents Inflammation in Zebrafish in Response to the Gut Microbiota. *Cell Host Microbe*. 2007; 2:371–382. [PubMed: 18078689]
10. Kanther M, Tomkovich S, Xiaolun S, Grosser MR, Koo J, Flynn EJ, Jobin C, Rawls JF. Commensal microbiota stimulate systemic neutrophil migration through induction of Serum amyloid A. *Cell Microbiol*. 2014; 16:1053–1067. [PubMed: 24373309]
- 11*. Hill JH, Franzosa EA, Huttenhower C, Guillemin K. A conserved bacterial protein induces pancreatic beta cell expansion during zebrafish development. *Elife*. 2016; 5:18–29. By combining gnotobiotic zebrafish experiments with comparative bacterial genomics, the authors show that early developmental expansion of pancreatic β cells is microbiota dependent and they identify a conserved bacterial protein that is sufficient to promote β cell proliferation.
12. Stephens WZ, Wiles TJ, Martinez ES, Jemielita M, Burns AR, Parthasarathy R, Bohannon BJM, Guillemin K. Identification of Population Bottlenecks and Colonization Factors during Assembly of Bacterial Communities within the Zebrafish Intestine. *MBio*. 2015; 6:e01163–15. [PubMed: 26507229]
13. Rendueles O, Ferrières L, Frétau M, Bégaud E, Herbomel P, Levraud J-P, Ghigo J-M, Backhed F, Ley R, Sonnenburg J, et al. A New Zebrafish Model of Oro-Intestinal Pathogen Colonization Reveals a Key Role for Adhesion in Protection by Probiotic Bacteria. *PLoS Pathog*. 2012; 8:e1002815. [PubMed: 22911651]
14. Davis JM, Clay H, Lewis JL, Ghori N, Herbomel P, Ramakrishnan L. Real-Time Visualization of Mycobacterium-Macrophage Interactions Leading to Initiation of Granuloma Formation in Zebrafish Embryos. *Immunity*. 2002; 17:693–702. [PubMed: 12479816]
15. Ramakrishnan L. The Zebrafish Guide to Tuberculosis Immunity and Treatment. *Cold Spring Harb Symp Quant Biol*. 2013; 78:179–192. [PubMed: 24643219]

16. Jemielita M, Taormina MJ, Burns AR, Hampton JS, Rolig AS, Guillemin K, Parthasarathy R. Spatial and Temporal Features of the Growth of a Bacterial Species Colonizing the Zebrafish Gut. *MBio*. 2014; 5:e01751–14. [PubMed: 25516613]
- 17*. Wiles TJ, Jemielita M, Baker RP, Schlomann BH, Logan SL, Ganz J, Melancon E, Eisen JS, Guillemin K, Parthasarathy R, et al. Host Gut Motility Promotes Competitive Exclusion within a Model Intestinal Microbiota. *PLOS Biol*. 2016; 14:e1002517. This study uses live imaging of two competing bacterial populations in the zebrafish intestine to reveal how bacterial interactions are modulated by the host environment. [PubMed: 27458727]
- 18*. Rolig AS, Parthasarathy R, Burns AR, Bohannon BJM, Guillemin K. Individual Members of the Microbiota Disproportionately Modulate Host Innate Immune Responses. *Cell Host Microbe*. 2015; 18:613–620. This study measure the relationship between host neutrophil and colonizing bacterial populations in gnotobiotic larval zebrafish and show that the overall host response can be predicted using a quantitative model that incorporates the different *per capita* effects of each bacterial species individually. [PubMed: 26567512]
- 19*. Rolig AS, Mittge EK, Ganz J, Troll JV, Melancon E, Wiles TJ, Alligood K, Stephens WZ, Eisen JS, Guillemin K. The enteric nervous system promotes intestinal health by constraining microbiota composition. *PLOS Biol*. 2017; 15:e2000689. This study characterizes dysbiosis in a zebrafish model of Hirschsprungs disease that develops spontaneous intestinal inflammation. The authors associate neutrophil populations and abundance of bacterial taxa to identify specific bacterial genera that drive or protect against inflammatory disease. [PubMed: 28207737]
- 20*. Rawls JF, Mahowald MA, Ley RE, Gordon JI. Reciprocal Gut Microbiota Transplants from Zebrafish and Mice to Germ-free Recipients Reveal Host Habitat Selection. *Cell*. 2006; 127:423–433. In this foundational work, the authors performed a reciprocal transplant of intestinal microbiota between germ-free mice and zebrafish. The resulting microbiota were comprised of microbial lineages originating from donor hosts but restructured to match the distributions found in the conventional microbiota of the recipient, demonstrating the overall importance of host habitat selection in explaining differences between host species. [PubMed: 17055441]
21. Roeselers G, Mittge EK, Stephens WZ, Parichy DM, Cavanaugh CM, Guillemin K, Rawls JF. Evidence for a core gut microbiota in the zebrafish. *ISME J*. 2011; 5:1595–1608. [PubMed: 21472014]
- 22*. Stephens WZ, Burns AR, Stagaman K, Wong S, Rawls JF, Guillemin K, Bohannon BJM. The composition of the zebrafish intestinal microbial community varies across development. *ISME J*. 2016; 10:644–54. This study provides a large and comprehensive survey of the zebrafish intestinal microbiota at various important stages of zebrafish development, and in so doing documents characteristic changes in microbial composition, diversity, and inter-individual variation. [PubMed: 26339860]
- 23*. Toh MC, Goodyear M, Daigneault M, Allen-Vercos E, Van Raay TJ. Colonizing the Embryonic Zebrafish Gut with Anaerobic Bacteria Derived from the Human Gastrointestinal Tract. *Zebrafish*. 2013; 10:194–198. Despite differences in the intestinal environment of zebrafish and human hosts, the authors of this study were able to colonize germ-free zebrafish with several human derived isolates, demonstrating the feasibility of studying members of human microbiota in the controlled environment of the zebrafish model system. [PubMed: 23530761]
24. Russo P, Iturria I, Mohedano ML, Caggianiello G, Rainieri S, Fiocco D, Angel Pardo M, López P, Spano G. Zebrafish gut colonization by mCherry-labelled lactic acid bacteria. *Appl Microbiol Biotechnol*. 2015; 99:3479–3490. [PubMed: 25586576]
25. Yan Q, van der Gast CJ, Yu Y. Bacterial community assembly and turnover within the intestines of developing zebrafish. *PLoS One*. 2012; 7:e30603. [PubMed: 22276219]
26. Wong S, Zac Stephens W, Burns AR, Stagaman K, David LA, Bohannon BJM, Guillemin K, Rawls JF. Ontogenetic differences in dietary fat influence microbiota assembly in the zebrafish gut. *MBio*. 2015; 6:e00687–15. [PubMed: 26419876]
27. Garcia GR, Noyes PD, Tanguay RL. Advancements in zebrafish applications for 21st century toxicology. *Pharmacol Ther*. 2016; 161:11–21. [PubMed: 27016469]
- 28*. Gaulke CA, Barton CL, Proffitt S, Tanguay RL, Sharpton TJ. Triclosan exposure is associated with rapid restructuring of the microbiome in adult zebrafish. *PLoS One*. 2016; 11:e0154632. This study uses zebrafish to characterize how a host's chemical environment impacts its

microbiome. The authors demonstrate that exposure to the antibiotic agent triclosan alters the diversity and composition of the microbiota and increases the connectedness of microbial correlation networks. [PubMed: 27191725]

29. Runft DL, Mitchell KC, Abuaita BH, Allen JP, Bajer S, Ginsburg K, Neely MN, Withey JH. Zebrafish as a natural host model for *Vibrio cholerae* colonization and transmission. *Appl Environ Microbiol.* 2014; 80:1710–7. [PubMed: 24375135]
30. Howlader DR, Sinha R, Nag D, Majumder N, Mukherjee P, Bhaumik U, Maiti S, Withey JH, Koley H. Zebrafish as a novel model for non-typhoidal *Salmonella* pathogenesis, transmission and vaccine efficacy. *Vaccine.* 2016; 34:5099–5106. [PubMed: 27614779]
31. Peterson TS, Ferguson JA, Watral VG, Mutoji KN, Ennis DG, Kent ML. *Paramecium caudatum* enhances transmission and infectivity of *Mycobacterium marinum* and *M. chelonae* in zebrafish *Danio rerio*. *Dis Aquat Organ.* 2013; 106:229–39. [PubMed: 24192000]
- 32*. Burns AR, Stephens WZ, Stagaman K, Wong S, Rawls JF, Guillemin K, Bohannan BJ. Contribution of neutral processes to the assembly of gut microbial communities in the zebrafish over host development. *ISME J.* 2016; 10:655–664. Using a neutral model of community assembly, the authors are able to infer the contribution of passive migration and stochastic demographic processes to shaping the zebrafish intestinal microbiota. While these processes are important early in development, their relative contribution decreases over time, suggesting the importance of host habitat selection increases as the host develops into adulthood. [PubMed: 26296066]
33. Lam S, Chua H, Gong Z, Lam T, Sin Y. Development and maturation of the immune system in zebrafish, *Danio rerio*: a gene expression profiling, in situ hybridization and immunological study. *Dev Comp Immunol.* 2004; 28:9–28. [PubMed: 12962979]
34. Stagaman K, Burns AR, Guillemin K, Bohannan BJ. The role of adaptive immunity as an ecological filter on the gut microbiota in zebrafish. *ISME J.* 2017; doi: 10.1038/ismej.2017.28

Highlights

- Interactions between animal hosts and their microbiota occur at multiple scales
- The zebrafish is a powerful model to study many scales of host-microbe interactions
- Gnotobiotic experiments provide fine scale resolution to identify molecular interactions
- Live imaging reveals dynamics of host-microbe interactions with cellular resolution
- Population level studies explore the host and microbe traits that shape microbiota