UNIVERSITY OF CALIFORNIA, SAN DIEGO

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Piggyback-the-Winner: lytic to temperate switching of viral communities

A dissertation submitted in partial satisfaction of the requirements for the degree

Doctor of Philosophy

in

Biology

by

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2016

The Dissertation of Benjamin William Knowles is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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2016

DEDICATION

I would like to dedicate this thesis to my Lady, Pearl Quijada. I'd dedicate it to the Cat too, but am loath to endorse her badness. I couldn't have done it without you.

EPIGRAPH

“Is this a hickey or a bruise?”

- Katy Perry

“They don’t think it be like it do, but it does.”

- Oscar Gamble

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

PtW	Piggyback-the-Winner
KtW	Kill-the-Winner
PtL	Piggyback-the-Loser
VMR	Virus to Microbe Ratio
VLP	Virus-like Particle
FLC	Fraction of Lysogenic Cells
FCIC	Fraction of Chemically Inducible Cells
DOC	Dissolved Organic Carbon
H’	Shannon Diversity
RR-B	Robust Regression with Bootstrapped Confidence Interval
SE	Standard Error of the Mean
PI	Prediction Interval
CI	Confidence Interval
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
N	Number of Bacteria (math model)
V	Number of Viruses (math model)
K	Carrying Capacity (math model)
Φ	Viral Adsorption Coefficient (math model)
β	Burst Size (math model)
r	Host Intrinsic Growth Rate (math model)
m	Viral Density-Independent Decay Rate (math model)

d	Host Density-Independent Death Rate (math model)
DNA	Deoxyribonucleic Acid
SARS	Severe Acute Respiratory Syndrome
FDR	False Discovery Rate
GAAS	Genome relative Abundance and Average Size tool
OTU	Operational Taxonomic Unit
ORF	Open Reading Frame
BLAST	Basic Local Alignment Search Tool (nucleotide x nucleotide)
BLASTx	Basic Local Alignment Search Tool (nucleotide x protein)
NCBI	National Center for Biotechnology Information
Ig-like	Immunoglobulin-like

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGMENTS

The major work in this thesis has 43 authors. The second work has a lot too, and counting. Obviously, my transformation from field ecologist to scientist through the Cell and Molecular Biology program, and through the Rohwer Lab in particular, took many hands. I am grateful for the guidance of my committee (Forest Rohwer, Rob Edwards, Stuart Sandin, Anca Segall, and Eric Allen) over the course of my training.

I would like to thank the San Diego State BioMath Group for their endless patience and sincere generosity. I have never met such a brilliant and engaged group of people. Math on a Friday afternoon was the most fun part of my week for the last three years: enough said. In particular, Jim Nulton has been amazing. He made me a minor deity of my own Lotka-Volterra world by teaching me how to use math to make explicit statements about how I think the world functions. He also wisely taught me how to avoid the narcissistic trap of becoming fascinated by my own logic by teaching me at the same time that every law in that world is a conceit that must be approached with care, if not fear. Barb Bailey has been a force of bubbly, happy nature in my life. She has always been there to dig me out of my own statistical catastrophes. Barb has never given up trying to teach me statistics, even when all the signs show its futility.

I have been fortunate enough to be forged in the fires of the Rohwer Lab. I can imagine no more stimulating environment, especially in the first years of my PhD under the mentorship of the maverick Texan Mike Furlan. The air is charged with politics, personalities, and science. I didn't know what growth was before I joined that milieu. I have learned from everyone, and appreciate every lesson. Thank you all for your part in my growth. I feel prepared to swim in any waters because of you.

The best for last. Forest Rohwer is the best scientist, the best mentor, and the best leader I have ever met. I had no idea when I joined his lab how masterfully he would induct me into the mysteries of the world, into the craft of science, over the length of my PhD. I don't know why Forest took me in, but I am profoundly grateful that he did. He taught me the value of trying harder, the joy of daring to fail, the importance of digging and digging and digging. I've learnt that science is a magic alloy of wild creativity and dogged perseverance. I'm hooked. Forest is a kid down at the tide-pool staring into the wonder of nature. I hope to spend a long time down there with him.

Chapter 1 has been published in the book "Life in Our Phage World" as a perspective chapter Knowles B (2014) 'March from the sea: a brief history of environmental phage ecology from marine to human ecosystems.' It provides a lively discussion of the historical arc and biases in the field of viral ecology. It was written with profound input from Merry Youle, and the dissertation author is the author of this work.

Chapter 2 is an early draft of a manuscript that has been published in the journal *Nature*: Knowles B, Silveira CB, Bailey BA, Barott K, Cantu VA, Cobián-Güemes AG, Coutinho FH, Dinsdale EA, Felts B, Furby KA, George EE, Green

KT, Gregoracci GB, Haas AF, Haggerty JM, Hester ER, Hisakawa N, Kelly LW, Lim YW, Little M, Luque A, McDole-Somera T, McNair K, de Oliveira LS, Quistad SD, Robinett NL, Sala E, Salamon P, Sanchez SE, Sandin S, Silva GGZ, Smith J, Sullivan C, Thompson C, Vermeij MJA, Youle M, Young C, Zgliczynski B, Brainard R, Edwards RA, Nulton J, Thompson F, and F Rohwer (2016) 'Lytic to temperate switching of viral communities.' New evidence is presented that challenges established concepts of viral lytic and lysogenic dynamics and establishes a novel model of viral activity. The dissertation author is a primary author and investigator of this manuscript.

Chapter 3 has been prepared for submission. Chapter 3, in full, is prepared for submission. Knowles B, Bailey B, Edwards R, Felts B, Grasis J, Haas A, Kelly LW, Luque A, Nulton J, Paul L, Robinett N, Segall A, and F Rohwer. Examination of induction-based evidence for host density-dependence of lysogeny suggests potentially novel drivers of natural viral communities. The dissertation author was the primary author and investigator on this manuscript.

Chapter 4 may one day be a Perspective paper on the implications of the work in the preceding chapters. The dissertation author is the primary author and investigator of this manuscript.

VITA

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Viral ecology; predator-prey dynamics; food webs; density-dependent dynamics; bloom formation and decline; lysogenic vs lytic switches; viral biogeochemical impacts

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2016	Haas AF, Fairoz MFM, Kelly LW, Nelson CE, Dinsdale EA, Edwards RA, Giles S, Hatay M, Hisakawa N, **Knowles B**, Lim YW, Maughan H, Pantos O, Roach T, Sanchez SE, Silveira CB, Sandin S, Smith JE, and F Rohwer (accepted; May publication) Global microbialization of coral reefs. *Nature Microbiology.*
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2014	**Knowles B** (2014) March from the sea: a brief history of environmental phage ecology from marine to human ecosystems. In F Rohwer, M Youle, Maughan H, Hisakawa N (eds.) *Life in Our Phage World*, Wholon Publishing, San Diego.
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2015	Year of the Phage Conference, San Diego USA. Knowles et al. Piggyback-the-Winner: Viral communities transition towards lysogeny at high host availability (poster presentation).
2013	Environmental Virology Workshop, Tucson USA.
2012	International Coral Reef Symposium, Cairns Australia. Knowles et al. The global distribution of relative nutrient scarcity (poster presentation).
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2015	Microbial virus- and protist-predation dynamics along a disturbance gradient in the near-shore waters of Curaçao, South Caribbean Sea (shore-based, April 2015).

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MEDALS AND AWARDS

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ABSTRACT OF THE DISSERTATION

Piggyback-the-Winner: lytic to temperate switching of viral communities

by

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Doctor of Philosophy in Biology

University of California, San Diego, 2016

San Diego State University, 2016

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There is a commonly accepted paradigm in viral ecology that lytic dynamics are the default pathway for viruses and that lysogeny is a refuge for viruses when lytic dynamics are unlikely to succeed (e.g., when host densities are low). This was established early in the development of the field of viral ecology (Chapter 1). Here, an analysis of viral and microbial counts and viral metagenomes collected from across the Pacific and Atlantic Oceans (Chapter 2), as well as an exhaustive literature analysis of published frequencies of mitomycin C inducible cells (a proxy for lysogeny) addition (Chapter 3) shows a lack of support for the established paradigm. Rather, the increased success of lysogenic viruses at high host density is supported, and no significant trends in mitomycin C inducible cells were observed. From the latter study, a hitherto uncharacterized driver of viral dynamics is laid out to guide future studies. Taken together, these analyses suggest that lysogeny becomes increasingly prevalent at high host densities, and that there may be novel drivers of inducibility that can allow viral ecology to transcend traditional drivers like host density and growth rate.

INTRODUCTION OF THE DISSERTATION

Viral ecology is a young field. It only came into being in the late 1980s and 1990s with a flurry of papers showing that viruses are abundant in the environment (Bergh et al. 1989), and that their widespread predation of host microbes had a profound influence on global biogeochemical cycles (Thingstad and Lignell 1997; Wilhelm and Suttle 1999; Proctor and Fuhrman 1990). Concurrent work on the prevalence and impact of lysogeny showed it to be insignificant in comparison to lysis and was especially low in high host density and growth rate systems (Jiang and Paul 1998; Jiang and Paul 1996). These findings led to the establishment, early in the growth of viral ecology, of a paradigm that suggested that lytic activity is the default pathway for viruses, especially promoted under high host densities and growth rates, and that lysogeny represents a refuge for viruses when lytic dynamics are not favored such as when hosts are rare or starved. This view of lytic/lysogenic switches suggests that viral predators exert control of host diversity and densities through mass-action driven Kill-the-Winner lytic dynamics during blooms (Bratbak et al. 1990; Thingstad 2000), overlooking the ecological potential for lysogeny to support and maintain host diversity and densities through horizontal gene transfer and development of immunity (Thingstad et al. 2014; Knowles et al. 2016). The work presented here challenges the established paradigm and suggests that viral dynamics in the environment are likely more complex than it implies.

Chapter 1 of this dissertation is a brief history of viral ecology that discusses the fundamental discoveries in the field over time. In addition to providing an appreciation for the research and researchers that laid the foundation for the field, the development of the established lytic/lysogenic switch can be traced through this chapter. Within historical context, the established paradigm makes sense. Lotka-Volterra models, central to ecological predator-prey dynamics since the early 1900s suggest that elevated densities of predators and/or prey should lead to increased predation rates (Lotka 1925), consistent with the observation of lytic collapse of host blooms and viral-mediated host recycling through the viral shunt (Bratbak et al. 1990; Bratbak, Egge, and Heldal 1993; Wilhelm and Suttle 1999; Thingstad and Lignell 1997). In terms of environmental lysogeny, early mitomycin C induction studies showed highly variable rates of lysogeny that appeared to be higher in coastal waters than in the open ocean (Jiang and Paul 1996), suggesting a host density- or growth rate-dependence to this viral lifestyle. These findings were subsequently amalgamated into the established lytic/lysogenic switch.

In Chapter 2, a global-scale survey of microbial and viral abundances showed a sub-linear relationship between microbial and viral densities (B Knowles et al. 2016). This was inconsistent with canonical lytic models that predict a negative relationship between viruses and hosts based on model organisms (Weitz and Dushoff 2008). It was, however, consistent with models showing suppressed lytic activity at high host densities because of the generation of host immunity to infection (Thingstad et al. 2014). When probed for mechanisms that could explain this seeming suppression of lytic activity at high density in the environment, no evidence for immunity was found. Rather evidence for enhanced temperate viral success at high densities was observed, contrary to the established switch. A survey of published viral and microbial densities suggested that this was a widespread dynamic. Experimental manipulation of host growth rates showed a inverse relationship between host density and virus: microbe ratio, indicating that rather than killing numerous or rapidly growing ‘winning’ hosts (‘Kill-the-Winner’; Thingstad 2000), the viruses integrate with them. We named this new dynamic, where viruses lysogenize their hosts precisely when they would be expected to kill them ‘Piggyback-the-Winner’. This new dynamic has implications for the formation and maintenance of microbial blooms and associated ecosystem decline as discussed in Chapters 2 and 4.

Given that discovery of a novel lytic/lysogenic switch in Chapter 2, in Chapter 3 the evidence is examined for density-dependence of lysogeny as measured by mitomycin C induction (yielding the Frequency of Chemically Inducible Cells as a proxy for lysogeny; FCIC) by a survey of published datasets (Jiang and Paul 1994; Jiang and Paul 1996). Rather than showing a negative relationship between FCIC and host density, as suggested by the literature (e.g., Payet and Suttle 2013; Brum et al. 2015; Maurice et al. 2013), highly variable and insignificant relationships were observed at the across-study and within-study levels. Further, significant artifacts of excluding FCIC values ≤ 0 were found.

While issues were found in the estimation of environmental lysogeny through induction, these issues allowed the profiling of a driver that potentially drives FCIC distribution, allowing the possibility to investigate hitherto unknown factors that could drive viral dynamics.

The findings of the preceding chapters are further discussed in a more accessible manner in Chapter 4. This perspective chapter lays out in an accessible manner what the Piggyback-the-Winner world is like, how it works, how it diverges from the canonical Kill-the-Winner world, and how it changes how we conceive of ecological processes.

Taken together, the research herein will generate a renewed interest in the drivers of the lytic/temperate switch in the environment, with potential to deepen our understanding of the complexity and diversity of viral dynamics.

REFERENCES

Bergh, O, K Y Borsheim, G Bratbak, and M Heldal. 1989. “High Abundance of Viruses Found in Aquatic Environments.” *Nature* 340 (6233): 467–68. doi:10.1038/340467a0.

Bratbak, G, J K Egge, and M Heldal. 1993. “Viral Mortality of the Marine Alga Emiliania Huxleyi (Haptophyceae) and Termination of Algal Blooms.” *Marine Ecology Progress Series* 93: 39–48.

Bratbak, G, M Heldal, S Norland, and T F Thingstad. 1990. “Viruses as Partners in Spring Bloom Microbial Trophodynamics.” *Applied and Environmental Microbiology* 56 (5). United States: 1400–1405.

Brum, Jennifer R, Bonnie L Hurwitz, Oscar Schofield, Hugh W Ducklow, and Matthew B Sullivan. 2015. “Seasonal Time Bombs: Dominant Temperate Viruses Affect Southern Ocean Microbial Dynamics.” *ISME J*, August. International Society for Microbial Ecology, 1–13. http://dx.doi.org/10.1038/ismej.2015.125.

Jiang, S C, and J H Paul. 1998. “Significance of Lysogeny in the Marine Environment: Studies with Isolates and a Model of Lysogenic Phage Production.” *Microbial Ecology* 35 (3-4). Springer: 235–43.

Jiang, Sunny C, and John H Paul. 1994. “Seasonal and Diel Abundance of Viruses and Occurrence of Lysogeny/bacteriocinogeny in the Marine Environment.” *Marine Ecology-Progress Series* 104: 163.

———. 1996. “Occurrence of Lysogenic Bacteria in Marine Microbial Communities as Determined by Prophage Induction.” *Marine Ecology-Progress Series* 142: 27.

Knowles, B, C B Silveira, B A Bailey, K Barott, V A Cantu, A G Cobián-Güemes, F H Coutinho, et al. 2016. “Lytic to Temperate Switching of Viral Communities.” *Nature* advance online publication (March). Nature Publishing Group, a division of Macmillan Publishers Limited. All Rights Reserved. http://dx.doi.org/10.1038/nature17193.

Lotka, Alfred J. 1925. “Elements of Physical Biology.”

Maurice, C. F., C. Bouvier, R. Wit, and T. Bouvier. 2013. “Linking the Lytic and Lysogenic Bacteriophage Cycles to Environmental Conditions, Host

Physiology and Their Variability in Coastal Lagoons.” *Environmental Microbiology* 15 (9). Wiley Online Library: 2463–75.

Payet, Jérôme, and Curtis A Suttle. 2013. “To Kill or Not to Kill: The Balance between Lytic and Lysogenic Viral Infection Is Driven by Trophic Status.” *Limnology and Oceanography* 58 (2): 465–74. doi:10.4319/lo.2013.58.2.0465.

Proctor, L M, and J A Fuhrman. 1990. “Viral Mortality of Marine Bacteria and Cyanobacteria.” *Nature* 343: 60–62.

Thingstad, T F, and R Lignell. 1997. “Theoretical Models for the Control of Bacterial Growth Rate, Abundance, Diversity and Carbon Demand.” *Aquatic Microbial Ecology* 13 (1). Inter-Research: 19–27.

Thingstad, T Frede. 2000. “Elements of a Theory for the Mechanisms Controlling Abundance, Diversity, and Biogeochemical Role of Lytic Bacterial Viruses in Aquatic Systems.” *Limnology and Oceanography* 45 (6). Wiley Online Library: 1320–28.

Thingstad, T Frede, Selina Våge, Julia E Storesund, Ruth-Anne Sandaa, and Jarl Giske. 2014. “A Theoretical Analysis of How Strain-Specific Viruses Can Control Microbial Species Diversity.” *Proceedings of the National Academy of Sciences* 111 (21). National Acad Sciences: 7813–18.

Weitz, Joshua S, and Jonathan Dushoff. 2008. “Alternative Stable States in Host--Phage Dynamics.” *Theoretical Ecology* 1 (1). Springer: 13–19.

Wilhelm, Steven W, and Curtis A Suttle. 1999. “Viruses and Nutrient Cycles in the Sea Viruses Play Critical Roles in the Structure and Function of Aquatic Food Webs.” *Bioscience* 49 (10). Oxford University Press: 781–88.

CHAPTER 1

March from the sea: a brief history of environmental phage ecology from marine to human system.

ABSTRACT

Environmental phage ecology has gone from obscure neglect to mainstream science over the past twenty-five years. This trajectory has been heavily influenced by methodological innovation, as researchers invent new means to probe phage communities through visualization or genomic and proteomic decoding. The advance of phage ecology has mainly been played out in marine systems where the major current tenets of the field have been threshed out: abundances in the 1980s and 1990s, lytic and lysogenic dynamics in the 1990s, connectivity and metabolic functionality in the 2000s. As such, there is a heavy marine bias to our knowledge. However, the methods and vision are in place to leverage our knowledge of how phage are involved in aspects of life ranging from biogeochemistry and evolution to host manipulation to positive effect in non-marine, unconventional environments. One such environment is the human body, and phage ecology is now poised to enter the human environment with profound potential impacts on our understanding of disease and treatment. Ultimately, the history of phage ecology shows that invisibility does not mean insignificance, and that our understanding of the world is still wonderfully incomplete.

You won't find what you've never seen

It seems strange to us now, with our current conception of the ocean as a bustling metropolis swarming with microbes and viruses and protists (and the occasional whale), but until recently the ocean was viewed as a sparsely populated desert (Figure 1.1). This perception came from the work of pioneering environmental microbiologists who concluded there were very low numbers of microbes in the sea based on what they could grow in the lab. That they saw anything at all is amazing. From its foundation, environmental microbiology was hindered by the necessity for researchers to invent basic techniques from scratch. For example, in the 1940s researchers had to resort to developing their own culture media in order to study or count microbes. However, while culture-based approaches have allowed researchers to domesticate and study environmental microbes in the lab for almost a century, using counts of cultured microbes to assess microbial abundance in the ocean suffers profoundly from the fact that only a tiny fraction (approximately 1%) of microbes can be cultured using current methods. As a result of this unrecognized bias, now known as the Great Plate Count Anomaly, researchers radically underestimated marine microbial abundance for most of the last century.

In science, direct measurements should trump indirect estimates. However the perception of vacant oceans was so thoroughly entrenched between the 1940s and 1970s that direct microscopy evidence contradicting indirect culture-based enumerations was discounted (Jannasch, Jones 1959). Tragically for phage biology, this seeming dearth of microbes in the sea led researchers to conclude that marine phage could not possibly be ecologically significant as there were not enough prey to support them—a view formally codified by the father of marine microbiology, Claude Zobell, in his defining 1946 textbook (ZoBell 1946). Within this paradigm, for almost the entire twentieth century environmental phage ecology was relegated to lab-based studies of only some phages that could be cultured.

Advances with isolates

Although today we would not call it *environmental* phage biology due to its dependence on lab-based microbial and phage domestication, important work was conducted between the 1940s and 1980s on phage-host pairs from diverse environments using culture-based approaches. For example, the *Pseudoalteromonas* phage PM2 was isolated and cultured from seawater off the Chilean coast in the late 1960s and was the subject of hundreds of publications (Mannisto et al. 1999). Studies using phage and bacterial isolates from aquatic systems provided insights into ecological questions such as the effects of phage infection on host virulence (Barksdale, Pappenheimer 1954), impact of temperature on phage adsorption (Seeley, Primrose 1980), specificity of phage host selection (Markel, Fowler, Eklund 1975), and community composition of polluted waters (Tartera, Jofre 1987). Similar questions were investigated in soil (Crosse, Hingorani 1958; Kowalski et al. 1974) and even in the leaves of apple trees (Ritchie, Klos 1977). Answers gained during this period would allow researchers to better understand what they were observing when phage ecology became truly environmental in the 1980s.

Discoveries by epifluorescence

Despite these culture-based advances, the field yearned for a means to scrutinize phage and microbes in their natural environment. That revolutionary advance arrived in the late 1970s with the advent of an epifluorescence microscopy technique that allowed researchers to directly count microbes in seawater (Hobbie, Daley, Jasper 1977). Everything changed: there were microbes everywhere and in massive abundances, approximately 10^6 microbes per ml throughout the oceans (reviewed in Wommack, Colwell 2000; Suttle 2007). The prevailing worldview was proven to be radically inaccurate.

Although this brought marine microbes to the attention of the scientific community, phage were unable to claim their place in the emerging microbial paradigm in the 1980s because phage virions and phage genomes were too small to be detected using contemporary visualization techniques. However, given the immense numbers of microbes just discovered, the indications were strong that there must also be abundant phage preying on them. Given this reasonable expectation, the hunt was on over the next decade for a method to accurately quantify phage in natural waters.

At first things did not seem promising when initial direct counts yielded only 10^4 viruses per ml in the ocean (Torrella, Morita 1979), 100 times less than the abundance of their hosts. Perhaps Zobell was right after all. Environmental phage ecology limped out of the 1970s in obscurity. It took another decade before reliable counts by Øivind Bergh and colleagues, published in a 1989 paper with the understated title of *High Abundance of Viruses found in Aquatic Environments*, reported 10^8 viruses per ml of seawater (Bergh et al. 1989). Using epifluorescence microscopy techniques refined by Jed Fuhrman and Rachel Noble in 1998, it is now possible to rapidly count the viruses accurately and robustly in environmental samples without the need for laborious electron microscopy work (Figure 1.1, Noble, Fuhrman 1998). Counts conducted with this approach, used in myriad studies all over the world, have revealed that phages are the most abundant organisms on the planet identified to date. Ocean surveys have shown average viral abundances of approximately 10^7 per ml in the ocean; thus marine phages outnumber their hosts by a factor of ten. Although this is fairly well accepted, there is no consensus as to why this ratio occurs, or why microbial and phage abundances in the ocean tend to be magically consistent, varying by only an order of magnitude or so across very different conditions.

By the end of the 1980s it was clear that the ocean is nothing like a desert.[1] An average ml of seawater contains the population of urban Rio de Janeiro in phage alone, not to mention thousands of protists and millions of bacteria and other microbes. And in that average ml of seawater these organisms are moving around, scavenging particles, photosynthesizing, reproducing, eating each other while trying to avoid being eaten, with many dying every day. Every

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[1] This is a metaphorical desert. Even real deserts are not deserted, and bear little actual resemblance to metaphorical deserts. This reflects the main point of this section: life is more pervasive than we imagine it to be, figuratively or scientifically.

ml of seawater is a riotous Carnival (Figure 1.1). No one would consider Rio de Janeiro a desert, and yet the perception of the open ocean as such has been difficult to dispel from popular and scientific mindsets and is still taught in some universities today, perhaps due to the difficulty of comprehending such riotous activity in a minuscule world we cannot directly perceive.

Massive bacteriocide by rapacious phages

Oceanographic work throughout the early and mid-1980s showed that while protists kill a large proportion of bacteria on a daily basis, a comparable amount of bacterial mortality remained unaccounted for. This was one of the deeper mysteries in marine science for over a decade. Phage were not considered credible suspects in this killing until it was shown how supremely abundant they are in the sea. At that point, researchers turned to oceanographic radiolabeled uptake techniques developed in microbial ecology to ask whether phage could be the unknown agents in this massive daily bacteriocide. They then rapidly showed that not only do phage kill as many Bacteria as do grazing protists in some locations (Fuhrman, Noble 1995), thereby causing a decline in bacterial productivity (Suttle, Chan, Cottrell 1990), but they can also infect up to 70% of the bacterial community (Proctor, Fuhrman 1990), and kill up to ~50% of Bacteria daily at some sites (Jiang, Paul 1994). That amounts to over 500,000 phage-mediated bacteriocidal events per ml per day and equates to approximately 10^{23} viral infection events per second in the global ocean, lytic and lysogenic infections combined (Suttle 2007). Viral infection is surely the most frequently occurring interaction between organisms on the planet. Given an estimated 10^{30} phage in the ocean, one phage in every hundred thousand is actively infecting a host every minute. Busy.

Further, radiotracer research in the early 1990s turned up something totally unexpected: the absence of phages was sometimes associated with large reductions in microbial productivity (reviewed in (Wilhelm, Suttle 1999)). It makes intuitive sense that phage-induced *reductions* of bacterial productivity arise via lysis of significant portions of the host community, but phage bacteriocide leading to *increased* bacterial production did not. And yet the observation was widespread in empirical and modeled data from a number of research groups around the world. It was quickly suggested by researchers such as Frede Thingstad and Farooq Azam (who had described the microbial loop a decade earlier (Azam et al. 1983)), that this enhanced productivity was fueled by the cellular debris and metabolites liberated by host lysis—a process labeled the viral shunt by Steven Wilhelm and Curtis Suttle in 1999 after almost a decade of conjecture and the gathering of evidence.

The viral shunt model has far ranging implications for ecosystem function (reviewed in (Suttle 2007; Weitz, Wilhelm 2012)). According to this model, bacterial lysis liberates organic carbon and other nutrients (e.g., nucleotides, amino acids) that are immediately recycled through the microbial community instead of being devoured as intact cells by protists that are, in turn, eaten by larger organisms. Released nutrients that are not readily assimilated into microbial metabolism such as refractory dissolved organic carbon are lost from the productive surface waters, falling as marine snow to deep ocean communities.

The tragic price of success

In 2000, Frede Thingstad published the “Kill-the-Winner” hypothesis that modeled the effects of specialist phage predation and generalist protistan bactivory on bacterial communities (Thingstad 2000). He described predation by phages as scaling with the abundance of their specific prey according to an idealized Lotka-Volterra model. In contrast, generalist protistan predators were considered to consume all prey as they encounter them. In the model, microbial species that become common (the ‘winners’) face ever-increasing phage predation pressure as they become more numerous. Likewise protistan predation also grows as the increasing abundance of a prey species brings higher encounter rates. Thus, abundant species face compounded protistan and phage predation, while rare species are in low-density numerical refugia. However, the specificity of phage predation combined with the rapid increase of the phage population on the heels of the increase in their specific prey leads ultimately to a collapse of the prey population, quickly followed by the marked decline in the phage predators dependent on them. This rise and fall does not perturb the protist population that simply transitions to consuming the new dominant microbe.

This constant turnover of dominant species implied in the kill-the-winner model leads to increased diversity of microbial communities by promoting increased evenness among prey populations and by intermittent periodic predatory pressure on individual species. The ability of the kill-the-winner hypothesis to elegantly explain phenomena such as the commonly observed 10:1 virus:microbe ratio and bacteria-phage coevolutionary diversification led to its adoption by phage ecologists. However, confirmation of this hypothesis awaited the advent of metagenomics. It is now known that while the overarching oscillations in dominant and rare microbial organisms suggested by kill-the-winner occur, this cycling occurs at the strain level, rather than at the level of species as originally envisioned in the model (Rodriguez-Brito et al. 2010). Thus the species composition of an environment can appear stable while the strain composition of those species cycles as evolving phage predation tactics are countered by ongoing innovations in host resistance mechanisms. The kill-the-winner hypothesis ultimately helps clarify the key tradeoff of microbial life: how to maximize reproduction by balancing competitive acquisition of resources and defense against infection (discussed further in Thingstad et al. 2014). Being a successful bacterium is not a secure occupation.

Phage lysogeny and transduction

When the massive magnitude and impact of phage predation were established at the birth of phage ecology in the early 1990s, predation became fixed—probably erroneously—in our minds as the dominant role of phage. Phage became the killers of the sea akin to their pathogenic viral cousins in the hospitals. However, research on the less overt effects of marine phage lysogeny, almost solely by John Paul and colleagues, formed a counter plot to that lytic-centric commentary. In a series of papers throughout the early and mid-1990s, they reported the results of adding the induction agent mitomycin C to seawater sampled from a variety of regions ranging from coral reefs to the open ocean (reviewed in (Paul et al. 2002)). They found that ~30% to 50% of the microbes in the ocean are lysogens (as defined by carrying a prophage that responds to mitomycin C induction). In some samples, it looked like all the microbes harbored at least one inducible prophage. Lysogeny was particularly high in stable and oligotrophic areas such as the open ocean and lower in disturbed, seasonal, and eutrophic areas. This has been borne out by studies in temperate (Maurice et al. 2010), but not Antarctic, lakes (Laybourn-Parry, Marshall, Madan 2007). However, high rates of lysogeny observed in soil show that eutrophic conditions and lysogeny are not mutually exclusive (Ghosh et al. 2008), and in fact lysogeny may be favored in soil compared to aquatic environments (Marsh, Wellington 1994). What determines the preponderance of lytic versus lysogenic activity in an environmental community? What drives the relative abundances of lytic versus temperate phages and what determines the lysogenic/lytic decisions made by the latter are still very controversial, with varied results from studies in different systems, possibly due to the widespread reliance on artifact-prone DNA-damaging induction. Also of import, when Paul and colleagues investigated rates of transduction (virus-mediated gene exchange) in marine environments, they estimated that 10^{14} transduction events occur every year in Tampa Bay alone (Jiang, Paul 1998a). That extrapolates to 10^{28} base pairs of DNA being transferred every year in the global ocean (Paul et al. 2002).

While the prevalence of lysogeny and transduction are both high in the marine environment, demonstrating their effect is very challenging and lends itself to underestimation. For example, when researchers study lytic dynamics, there are abundant phage to count as an outcome to successful infection. Lysogenic infection may have no similarly overt signature, appearing at one level of observation as if there was no infection at all. This makes phage success hard to assess and define. This decoupling between infection and observable effect (i.e., host lysis) is apparent in that although lysogeny is rampant in the ocean, spontaneous lysogen induction accounts for only 0.02% of detectible free phage (Jiang, Paul 1998b). Although the work of Paul and colleagues allowed us a glimpse into the world of phage mediated gene flow and lysogeny, it would take the advent of metagenomic sequencing a decade later to really see these processes directly.

Enter the sequencer

In 1999, the Pittsburg Phage Group led by Roger Hendrix and Graham Hatfull published a paper with the oft-quoted subtitle “all the world’s a phage” (Hendrix et al. 1999). Sequence homologies observed across a large number of phage genomes, both lysogenic and lytic, indicated a high degree of relatedness between disparate phage groups. Further, they suggested not only that the tailed phages comprise one lineage, but also that phage genomes may be mosaic constructs that over evolutionary time recruit elements from shared gene pools resulting from host overlap. Genes were on the move between phages that shared hosts. Phage genomes, transmutable through mosaicism and recombination, reflected a more complex shared evolutionary past. The power of comparative genomics has made the field of phage biology more exciting and, from the perspective of a Darwinian vertical heritability, stranger than ever.

Inspired to extend the work of the Pittsburg Phage Group to environmental phages, the San Diego Phage Group sequenced the alleged ‘first’ marine phage genome in 2000, that of Roseophage SIO1, a predator of *Roseobacter* (Rohwer et al. 2000). This work complemented the publication of the *actual* first marine phage genome and proteome the prior year by Dennis Bamford and colleagues, that of *Pseudoalteromonas* phage PM2 (Kivela et al. 1999; Mannisto et al. 1999), and identified evolutionary linkages between seemingly unrelated phages from different environments. It is clear, environmental boundaries clear to us do not constrain phage. Now that the ability to sequence environmental phage was established, the push was on to expand sequencing approaches from cultured strains like SIO1 and PM2 to environmental communities. This stimulated the development of one of the most important tools in phage ecology today: shotgun metagenomics.

Sequencers with shotguns

Research done in the 1990s that amplified and sequenced conserved phage genes demonstrated that environmental phage were diverse, but such studies were limited to groups of closely related phages (reviewed in (Short, Suttle 1999)). Intriguing as these findings were, this approach could not be extended to include less related phages as phage are of radically disparate lineages. They have no universal gene in common, nothing comparable to the handy ribosomal DNA with which the three domains of cellular life were resolved (Woese, Kandler, Wheelis 1990). The very first shotgun metagenome ever, a virome published in 2002 (years ahead of the first microbial metagenome) by the San Diego Phage Group, provided our first direct look at the diversity of viruses in any environment (Breitbart et al. 2002). The diversity observed was incredible: up to 7,000 phage types in 200 L of surface seawater. For comparison, there are only 5,000 to 6,000 species of mammals on the entire planet. Phage diversity was also radically patchy, varying from ~300 to 7,000 viral types species per 200 L in different samples from nearby locations.

While the phages were locally diverse, some—or at least some phage genes—are widely dispersed (Angly et al. 2006), found all over the world in almost every biome imaginable, as demonstrated for two highly conserved DNA polymerases named Hector and Paris (Breitbart, Miyake, Rohwer 2004). This echoed earlier reports of related algal virus polymerases in antipodal samples from Antarctica and British Columbia (Short, Suttle 2002). Some phage are so cosmopolitan that members of the phage communities in lakes, sediment, and soil can also infect marine microbes (Sano et al. 2004). However, there is also evidence of localized adaptation that precludes phage from infecting potential hosts from neighboring trees while allowing infection of hosts from within the same tree (Koskella et al. 2011). Tree to tree infective exclusion in a world that allows marine-soil permissiveness of phage infection: boundaries to phage infection is not intuitive. This patchiness makes assessing the size of the global virome non-trivial and suggests that we cannot estimate the total phage diversity on Earth by assessing the diversity in various biomes and then calculating their sum. Ultimately, it appears that global phage diversity is not as large as the findings of Breitbart et al (Breitbart 2002) would suggest (Ignacio-Espinoza, Solonenko, Sullivan 2013). Armed with shotgun metagenomics as we are now, we can look back on sixty years of being shackled to culture-based observations and appreciate the formidable challenges faced by culture-bound researchers like Moebus. Working within those constraints, he found that phage could not infect microbes isolated from even less than 200 miles away, whereas now, examining viral communities directly, we know that phage are infective across biomes and across the globe.

Archaeal viruses in the shadows

Science has acknowledged the high abundance of bacteriophage hosts in the environment since the 1970s, but archaeal abundances remain underestimated. Even though we now realize that Archaea are not constrained to 'extreme' environments such acid hot springs, salty solar salterns, and hydrothermal vents in the perennially dark marine depths, their roles in environmental processes (Chaban, Ng, Jarrell 2006; Lipp et al. 2008), including the human environment (Probst, Auerbach, Moissl-Eichinger 2013), is generally disregarded. As a result, while our appreciation for phage and their ecological significance has bloomed, archaeal viruses remain a mostly unexplored realm promising surprising rewards for those who dare to enter. For example, the most extreme instance of genomic mosaicism known to date is from an archaeal virus found almost by chance in a hot, acidic lake by a research team led by Ken Steadman (Diemer, Stedman 2012). This virus, a chimera of sequences from DNA and RNA viruses, has radically broadened the known scope of mosaicism in viral genomes. Further analysis suggested that this phenomenon may be widespread, but overlooked, in other environments.

The Dark Matter

Phage metagenomics also disclosed the genetic composition of phage communities, and things got even more interesting. The vast majority of the sequences in the first viral metagenome were unknown, i.e., were reads that do not have recognizable similarity to any genes in the predominantly microbial genome databases microbiologists had laboriously created (Breitbart et al. 2002). Despite the dramatic increase in the number of sequenced genomes now included in the databases, the percentage of phage unknowns has not decreased significantly. Phage genomes definitely remain the deepest reservoir of unknown functional potential on earth—the beckoning genetic Dark Matter.

Phage metabolism

The first viral metagenome also showed that phage genomes do not encode only ‘phage’ genes required for genome replication and virion assembly. Phages also carry some unexpected, but clearly recognizable, genes of known metabolic function. First discovered were the photosynthesis genes (*psbA* and *psbD*) found in a cyanophage that encode the key photosystem II reaction center proteins D1 and D2, respectively (Mann et al. 2003). Soon thereafter, others confirmed that not only were these genes present in many lytic cyanophages (Sullivan et al. 2006), in at least some cases their expression during infection helped to maintain photosynthesis for the duration and thus enhanced phage fecundity (Lindell et al. 2005; Clokie et al. 2006). Similarly, phage genomes also contain photosystem I genes functionally organized in cassettes that, when combined, probably encode a whole photosystem I reaction complex (Sharon et al. 2009). These complexes may be mosaic constructs from different hosts (Mazor et al. 2012). Not only are these phage genes encoding metabolic proteins, but some at least travel back-and-forth between phage and host over evolutionary time. While sojourning within a phage, they evolve more rapidly and under different selection pressures, sometimes yielding improved functionality (Lindell et al. 2004; Frank et al. 2013). Subsequent metagenomic work by Sullivan et al. found an almost ubiquitous prevalence of *psbA* in cyanophage isolates, with half of the strains investigated having both *psbA* and *psbD* (Sullivan et al. 2006). Moreover, their data provided evidence of repeated host-phage and phage-phage exchange of these genes, further demonstrating the genetic connections between phages with overlapping host ranges.

At the time of their publication, many metagenomes are made publically available and archived in standard formats. As a result, there now exists an abundance of metagenomic data accessible to any ecologist, data that readily lends itself to meta-analysis and synthesis. Although still hampered by the large proportion of phage genes without functional annotations in reference databases, these datasets nevertheless allow us to probe molecular facets of phage ecology and behavior in previously unimaginable depth. Since the mid-2000s, for example, there has been a string of papers characterizing the diversity and function of some of the metabolic genes that phage carry around in their genomes, largely based on publically available sequences (reviewed in (Breitbart et al. 2007; Breitbart 2012). Phage manipulate not only photosynthesis but numerous other aspects of host metabolism to their own benefit by encoding functional auxiliary metabolic genes (Breitbart 2012), e.g., genes involved in energetic and nucleotide metabolism through the pentose phosphate pathway (Thompson et al. 2011), post-translational protein modification by peptide deformylase (Sharon et al. 2011; Frank et al. 2013), and in the deep sea, where sulfur metabolism is of crucial importance, elemental sulfur oxidation (Anantharaman et al. 2014). The conclusion is clear: phage play a significant and varied role (Dinsdale et al. 2008b) in microbial metabolic processes, likely complementing and extending microbial function in every environment examined, and we've only just begun digging.

Connecting the ecological dots

While environmental phage ecology has made great strides since its nascence in the late 1980s, exciting challenges remain. As a science of connections between unseen organisms, progress in this field is clearly tied closely to methodological innovation. There are currently a slew of new methods available that will enable us to address interesting but previously unanswerable questions in the near future.

Metagenomic characterization of environmental phage communities is still hampered by the high percentage of unknown reads. This must be remedied in some manner before we can see the full scope of phage genetic potential. Given the inherent challenges here, there has been a strong incentive to develop alternative approaches to assign putative functions to some of these unknowns. Methods used so far include protein clustering (Hurwitz, Sullivan 2013) and artificial neural networks trained on known datasets (Seguritan et al. 2012). Researchers have also refined high throughput means such as viral tagging to identify host-phage pairs (Deng et al. 2012), helping us understand host specificity and overlap, and thus map the common genetic pools connecting phages. Determination of phage host ranges is essential for charting routes for gene flow via host overlap in the environment as well as determining the degree of their host specificity in natural environments. Much of this work uses innovative tagging techniques coupled to flow cytometry to sort infected cell-phage pairs that can then be sequenced using new DNA amplification techniques (Duhaime et al. 2012).

Metagenomics goes rogue

After publishing the first virome using marine samples, the San Diego Phage Group went on an interdisciplinary, cross-country environmental phage metagenome excursion. They sequenced viromes from a wild diversity of environments (Table 1). By the conclusion of this scientific March from the Sea, the value of culture-independent metagenomics for phage exploration in all conceivable environments was established.

The human body is also an environment, or rather multiple environments, all of which are amenable to investigation by ecological metagenomic methods. For example, Yijun Ruan, one of the primary biomedical researchers who identified and characterized SARS in Singapore in 2003, has used metagenomics techniques to explore the composition, ecology, and clinical impacts of microbes and viruses in air and wastewater (Tringe et al. 2008; Rosario et al. 2009). Metagenomics characterized the time course for acquisition and development of our gut virome, with the diversity of the phage community being low in newborns and increasing with time (Breitbart et al. 2008). Further, it has been shown that while our gastrointestinal microbiota are remarkably functionally conserved between individuals, the resident phage communities we each house are functionally far more unique (Reyes et al. 2010), and appear to respond to diet (Minot et al. 2011).

Metagenomics also revealed that phages exist in human environments that researchers and clinicians had thought to be sterile, environments such as the healthy human lung. The lung seemed to be sterile because no bacterial isolates from lungs were culturable. Culture-independent methods such as metagenomics have overturned this view and demonstrated that lungs, too, are ecological landscapes with niches, succession, and other ecological properties (Willner et al. 2009). The parallels between the ‘sterile’ lung and the ‘desert’ ocean of Claude Zobell are striking and, to a potential patient, alarming. The appreciation of the human body as an ecosystem housing microbial and viral communities creates a new paradigm for understanding disease and also new possibilities for disease treatment. Viewing polymicrobial diseases such as cystic fibrosis in an ecological context leads directly to innovative clinical protocols (Conrad et al. 2013) with exciting potential to improve human health.

In our quest for health, phage may already be our partners in diverse ways. For example, some phages use Ig-like domains of capsid decoration proteins to adhere to the mucus membranes that protect our tissues from the environment (Barr et al. 2013). There they take advantage of the greater abundance of Bacteria that makes for higher rates of host encounter and lysis—thus reproductive success for the phage and potentially reduced pathogen invasion for us. Even though there is no more ‘self’ a system than the immune system, part of our immune system that guards mucosal surfaces such as those found in the gut and lungs may be harnessing a non-human-derived immunity through phage. More overtly, in this era of antibiotic-resistant bacterial infections, we will almost certainly witness a renewed commitment to the development of phage therapies, now guided by the knowledge and methodologies resulting from decades of marine phage research. From the ocean of marine phage dynamics to phage-host interactions within the human ecosystem, the march goes on.

The future

Environmental phage ecology has made enormous strides in the past 25 years and is positioned now to tackle many remaining exciting challenges. While earlier observational studies brought awareness of the vast scope of phage activity in nature, we currently lack a mechanistic understanding of many important processes. For example, while observations suggest that factors such as microbial abundance, perturbation, season, and biogeography are determinants of phage abundance, rigorous experimental evidence detailing their interacting roles is scarce. While marine virus: microbe ratios appear to follow depth and latitudinal trends, the ultimate drivers of these, too, remain unknown. Similarly, despite research confirming abundant lysogeny in the marine environment, the ecological factors that select for temperate phage, that determine their choice of lysis or lysogeny during each infection, and that cause their ultimate induction are all unknown. Experimentally sorting out this, and other aspects of phage ecology, is especially challenging as phage affects host that then affects phage in an endless rotation of complex circular interactions.

Phage ecology has had a strong marine bias with but lesser excursions into environments such as sediments, soils, lakes, hot springs, animals, and plants. Facilitated by the rapid decline of sequencing costs, deeper probing of more biomes lies ahead. The results obtained will complement, expand, and rewrite much of what we have learned from the marine environment. Shifting the focus from individual biomes to the global scale we see yet more unexplored territory. That some phages (or phage genes) are already known to be present in what we perceive as different environments raises numerous questions about phage mobility both globally and between biomes, as well as about phage gene mobility between different hosts. These investigations have the power to reconfigure our understanding of the ecological divisions and connections in nature. Boundaries we perceive or intellectually construct are not always real.

Even within the marine environment there is a major pro-DNA bias to our understanding of phage. Double-stranded DNA phages, especially the tailed phages (order *Caudovirales*) dominate the sequence space we have investigated so far. The RNA viruses that comprise a large proportion—perhaps half, perhaps even more—of marine viruses remain often overlooked (Steward et al. 2013). We cannot say our vision of marine phage ecology is complete until all phage genotypes are incorporated into our science. This raises methodological challenges as RNA viruses are much more difficult to study and both RNA and single-stranded DNA genomes are not compatible or optimal with many of our most common methods.

Although they are arguably outside the scope of *bacteriophage* ecology, archaeal viruses currently represent a large gap in our understanding of environmental viral dynamics. Archaeal viruses are some of the most genomically diverse viruses on the planet, their virion architectures are unimaginably creative, but their ecological impacts remain almost completely unknown. Deeper probing of archaeal viruses could eliminate a large hole in our knowledge of viral ecology and increase our appreciation of the impact and diverse forms of viral genetic exchange in the environment.

Finally, research on the human holobiont as an ecosystem that has only just begun has exciting potential. Work to date has altered our understanding of human immune function and suggested other potential roles of phage in human development and health. The abundance and diversity of human-associated phages is radically altering our perception of humanness and individuality. This is sure to be a controversial and insightful field going forward, yet one with promise to improve human life by harnessing ecological knowledge gained in environmental systems (Reyes et al. 2012).

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REFERENCES

Anantharaman, K, MB Duhaime, JA Breier, KA Wendt, BM Toner, GJ Dick. 2014. Sulfur oxidation genes in diverse deep-sea viruses. Science 344:757-760.

Anderson, NG, GB Cline, WW Harris, JG Green. 1967. Isolation of viral particles from large fluid volumes. In: G Berg, editor. Transmission of Viruses by the Water Route: Interscience Publishers. p. 75-88.

Angly, FE, B Felts, M Breitbart, et al. 2006. The marine viromes of four oceanic regions. PLoS Biol 4:e368.

Azam, F, T Fenchel, JG Field, JS Gray, LA Meyerreil, F Thingstad. 1983. The ecological role of water-column microbes in the sea. Mar Ecol Prog Ser 10:257-263.

Barksdale, WL, AM Pappenheimer. 1954. Phage host relationships in nontoxigenic and toxigenic diphtheria-bacilli. J Bacteriol 67:220- 232.

Barr, JJ, R Auro, M Furlan, et al. 2013. Bacteriophage adhering to mucus provide a non-host-derived immunity. Proc Natl Acad Sci USA 110:10771-10776.

Bergh, O, KY Borsheim, G Bratbak, M Heldal. 1989. High abundance of viruses found in aquatic environments. Nature 340:467-468. 2-52.

Breitbart, M. 2012. Marine viruses: Truth or dare. Ann Rev Mar Sci 4:425-448.

Breitbart, M, B Felts, S Kelley, JM Mahaffy, J Nulton, P Salamon, F Rohwer. 2004. Diversity and population structure of a near-shore marine-sediment viral community. Proc Biol Sci 271:565-574.

Breitbart, M, M Haynes, S Kelley, et al. 2008. Viral diversity and dynamics in an infant gut. Res Microbiol 159:367-373.

Breitbart, M, JH Miyake, F Rohwer. 2004. Global distribution of nearly identical phage-encoded DNA sequences. FEMS Microbiol Lett 236:249-256.

Breitbart, M, F Rohwer. 2005. Method for discovering novel DNA viruses in blood using viral particle selection and shotgun sequencing. Biotechniques 39:729-736.

Breitbart, M, P Salamon, B Andresen, JM Mahaffy, AM Segall, D Mead, F Azam, F Rohwer. 2002. Genomic analysis of uncultured marine viral communities. Proc Natl Acad Sci USA 99:14250-14255.

Breitbart, M, LR Thompson, CA Suttle, MB Sullivan. 2007. Exploring the vast diversity of marine viruses. Oceanography (Wash D C) 20:135-139.

Chaban, B, SY Ng, KF Jarrell. 2006. Archaeal habitats--from the extreme to the ordinary. Can J Microbiol 52:73-116.

Clokie, MR, J Shan, S Bailey, Y Jia, HM Krisch, S West, NH Mann. 2006. Transcription of a ‘photosynthetic’ T4-type phage during infection of a marine cyanobacterium. Environ Microbiol 8:827-835.

Conrad, D, M Haynes, P Salamon, PB Rainey, M Youle, F Rohwer. 2013. Cystic fibrosis therapy: a community ecology perspective. Am J Respir Cell Mol Biol 48:150-156.

Crosse, JE, MK Hingorani. 1958. Method for isolating Pseudomonas morsprunorun phages from the soil. Nature 181:60-61.

Deng, L, A Gregory, S Yilmaz, BT Poulos, P Hugenholtz, MB Sullivan. 2012. Contrasting life strategies of viruses that infect photo- and heterotrophic Bacteria, as Revealed by viral tagging. Mbio 3:e00373-00312.

Deng, L, JC Ignacio-Espinoza, AC Gregory, BT Poulos, JS Weitz, P Hugenholtz, MB Sullivan. 2014. Viral tagging reveals discrete populations in Synechococcus viral genome sequence space. Nature advance online publication.

Desnues, C, B Rodriguez-Brito, S Rayhawk, et al. 2008. Biodiversity and biogeography of phages in modern stromatolites and thrombolites. Nature 452:340-343.

Diemer, GS, KM Stedman. 2012. A novel virus genome discovered in an extreme environment suggests recombination between unrelated groups of RNA and DNA viruses. Biol Direct 7:13.

Dinsdale, EA, RA Edwards, D Hall, F Angly, M Breitbart, JM Brulc, M Furlan, C Desnues, M Haynes, L Li. 2008a. Functional metagenomic profiling of nine biomes. Nature 452:629-632.

Dinsdale, EA, O Pantos, S Smriga, et al. 2008b. Microbial ecology of four coral atolls in the Northern Line Islands. Plos One 3:1-17.

Duhaime, MB, L Deng, BT Poulos, MB Sullivan. 2012. Towards quantitative metagenomics of wild viruses and other ultra-low concentration DNA samples: A rigorous assessment and optimization of the linker amplification method. Environ Microbiol 14:2526-2537.

Frank, JA, D Lorimer, M Youle, P Witte, T Craig, J Abendroth, F Rohwer, RA Edwards, AM Segall, AB Burgin. 2013. Structure and function of a cyanophage-encoded peptide deformylase. Isme Journal 7:1150-1160.

Fierer, N, M Breitbart, J Nulton, et al. 2007. Metagenomic and small-subunit rRNA analyses reveal the genetic diversity of bacteria, archaea, fungi, and viruses in soil. Appl Environ Microbiol 73:7059-7066.

Fuhrman, JA, RT Noble. 1995. Viruses and protists cause similar bacterial mortality in coastal seawater. Limnol Oceanogr 40:1236-1242.

Ghosh, D, K Roy, KE Williamson, DC White, KE Wommack, KL Sublette, M Radosevich. 2008. Prevalence of lysogeny among soil bacteria and presence of 16S rRNA and trzN genes in viral-community DNA. Appl Environ Microbiol 74:495-502.

Hendrix, RW, MCM Smith, RN Burns, ME Ford, GF Hatfull. 1999. Evolutionary relationships among diverse bacteriophages and prophages: All the world's a phage. Proc Natl Acad Sci USA 96:2192-2197.

Hobbie, JE, RJ Daley, S Jasper. 1977. Use of nuclepore filters for counting bacteria by fluorescence microscopy. Appl Environ Microbiol 33:1225-1228.

Hurwitz, BL, MB Sullivan. 2013. The Pacific Ocean Virome (POV): A marine viral metagenomic dataset and associated protein clusters for quantitative viral ecology. Plos One 8:12.

Ignacio-Espinoza, J, SA Solonenko, MB Sullivan. 2013. The global virome: Not as big as we thought? Curr Opin Virol 3:566-571.

Jannasch, HW, GE Jones. 1959. Bacterial populations in sea water as determined by different methods of enumeration. Limnol Oceanogr 4:128-139.

Jiang, SC, JH Paul. 1994. Seasonal and diel abundance of viruses and occurrence of lysogeny/bacteriocinogeny in the marine environment. Mar Ecol Prog Ser 104:163-172. Jiang, SC, JH Paul. 1998a. Gene transfer by transduction in the marine environment. Appl Environ Microbiol 64:2780-2787.

Jiang, SC, JH Paul. 1998b. Significance of lysogeny in the marine environment: Studies with isolates and a model of lysogenic phage production. Microb Ecol 35:235-243.

Keen, EC. 2014. Tradeoffs in bacteriophage life histories. Bacteriophage 4:e28365.

Kivela, HM, RH Mannisto, N Kalkkinen, DH Bamford. 1999. Purification and protein composition of PM2, the first lipid-containing bacterial virus to be isolated. Virology 262:364-374.

Koskella, B, JN Thompson, GM Preston, A Buckling. 2011. Local biotic environment shapes the spatial scale of bacteriophage adaptation to bacteria. Am Nat 177:440-451.

Kowalski, M, GE Ham, Frederic.Lr, IC Anderson. 1974. Relationship between strains of Rhizobium japonicum and their bacteriophages from soil and nodules of field-grown soybeans. Soil Sci 118:221-228.

Laybourn-Parry, J, WA Marshall, NJ Madan. 2007. Viral dynamics and patterns of lysogeny in saline Antarctic lakes. Polar Biol 30:351-358.

Lindell, D, JD Jaffe, ZI Johnson, GM Church, SW Chisholm. 2005. Photosynthesis genes in marine viruses yield proteins during host infection. Nature 438:86-89.

Lindell, D, MB Sullivan, ZI Johnson, AC Tolonen, F Rohwer, SW Chisholm. 2004. Transfer of photosynthesis genes to and from Prochlorococcus viruses. Proc Natl Acad Sci USA 101:11013-11018.

Lipp, JS, Y Morono, F Inagaki, K-U Hinrichs. 2008. Significant contribution of Archaea to extant biomass in marine subsurface sediments. Nature 454:991-994.

Mann, NH, A Cook, A Millard, S Bailey, M Clokie. 2003. Marine ecosystems: Bacterial photosynthesis genes in a virus. Nature 424:741-741.

Mannisto, RH, HM Kivela, L Paulin, DH Bamford, JKH Bamford. 1999. The complete genome sequence of PM2, the first lipid-containing bacterial virus to be isolated. Virology 262:355-363.

Marhaver, KL, RA Edwards, F Rohwer. 2008. Viral communities associated with healthy and bleaching corals. Environ Microbiol 10:2277- 2286.

Markel, DE, MJ Fowler, C Eklund. 1975. Phage-host specificity texts using Levinea phages and isolates of Levinea spp and Citrobacer Freundii. Int J Syst Bacteriol 25:215-218.

Marsh, P, EMH Wellington. 1994. Phage-host interactions in soil. FEMS Microbiol Ecol 15:99-107.

Maurice, CF, T Bouvier, J Comte, F Guillemette, PA del Giorgio. 2010. Seasonal variations of phage life strategies and bacterial physiological states in three northern temperate lakes. Environ Microbiol 12:628-641.

Mazor, Y, I Greenberg, H Toporik, O Beja, N Nelson. 2012. The evolution of photosystem I in light of phage-encoded reaction centres. Philos Trans R Soc Lond B Biol Sci 367:3400-3405.

Minot, S, R Sinha, J Chen, H Li, SA Keilbaugh, GD Wu, JD Lewis, FD Bushman. 2011. The human gut virome: Inter-individual variation and dynamic response to diet. Genome Res 21:1616-1625.

Moebus, K. 1980. A method for the detection of bacteriophages from ocean water. Helgol Mar Res 34:1-14.

Moebus, K, H Nattkemper. 1981. Bacteriophage sensitivity patterns among bacteria isolated from marine waters. Helgol Mar Res 34:375- 385.

Noble, RT, JA Fuhrman. 1998. Use of SYBR Green I for rapid epifluorescence counts of marine viruses and bacteria. Aquat Microb Ecol 14:113-118.

Paul, JH, MB Sullivan, AM Segall, F Rohwer. 2002. Marine phage genomics. Comp Biochem Physiol B Biochem Mol Biol 133:463-476.

Probst, AJ, AK Auerbach, C Moissl-Eichinger. 2013. Archaea on human skin. Plos One 8:e65388.

Proctor, LM, JA Fuhrman. 1990. Viral mortality of marine bacteria and cyanobacteria. Nature 343:60-62.

Reyes, A, M Haynes, N Hanson, FE Angly, AC Heath, F Rohwer, JI Gordon. 2010. Viruses in the faecal microbiota of monozygotic twins and their mothers. Nature 466:334-338.

Reyes, A, NP Semenkovich, K Whiteson, F Rohwer, JI Gordon. 2012. Going viral: Next-generation sequencing applied to phage populations in the human gut. Nat Rev Microbiol 10:607-617.

Ritchie, DF, EJ Klos. 1977. Isolation of Erwinia amylovora bacteriophage from aerial parts of apple trees. Phytopathology 67:101-104.

Rodriguez-Brito, B, LL Li, L Wegley, et al. 2010. Viral and microbial community dynamics in four aquatic environments. ISME J 4:739-751.

Rohwer, F, A Segall, G Steward, V Seguritan, M Breitbart, F Wolven, F Azam. 2000. The complete genomic sequence of the marine phage Roseophage SIO1 shares homology with nonmarine phages. Limnol Oceanogr 45:408-418. Rosario, K, C Nilsson, YW Lim, YJ Ruan, M Breitbart. 2009. Metagenomic analysis of viruses in reclaimed water. Environ Microbiol 11:2806- 2820.

Sano, E, S Carlson, L Wegley, F Rohwer. 2004. Movement of viruses between biomes. Appl Environ Microbiol 70:5842-5846.

Seeley, ND, SB Primrose. 1980. Effect of temperature on the ecology of aquatic bacteriophages. J Gen Virol 46:87-95.

Seguritan, V, N Alves, M Arnoult, A Raymond, D Lorimer, AB Burgin, P Salamon, AM Segall. 2012. Artificial neural networks trained to detect viral and phage structural proteins. Plos Comput Biol 8:22.

Sharon, I, A Alperovitch, F Rohwer, M Haynes, F Glaser, N Atamna-Ismaeel, RY Pinter, F Partensky, EV Koonin, YI Wolf. 2009. Photosystem I gene cassettes are present in marine virus genomes. Nature 461:258-262.

Sharon, I, N Battchikova, EM Aro, C Giglione, T Meinnel, F Glaser, RY Pinter, M Breitbart, F Rohwer, O Beja. 2011. Comparative metagenomics of microbial traits within oceanic viral communities. ISME J 5:1178-1190.

Short, SM, CA Suttle. 1999. Use of the polymerase chain reaction and denaturing gradient gel electrophoresis to study diversity in natural virus communities. Hydrobiologia 401:19-32.

Short, SM, CA Suttle. 2002. Sequence analysis of marine virus communities reveals that groups of related algal viruses are widely distributed in nature. Appl Environ Microbiol 68:1290-1296.

Sullivan, MB, D Lindell, JA Lee, LR Thompson, JP Bielawski, SW Chisholm. 2006. Prevalence and evolution of core photosystem II genes in marine cyanobacterial viruses and their hosts. Plos Biology 4:1344-1357.

Suttle, CA. 2007. Marine viruses - major players in the global ecosystem. Nat Rev Microbiol 5:801-812.

Suttle, CA, AM Chan, MT Cottrell. 1990. Infection of phytoplankton by viruses and reduction of primary productivity. Nature 347:467-469. 2-54

Tartera, C, J Jofre. 1987. Bacteriophages active against Bacteroides fragilis in sewage polluted waters. Appl Environ Microbiol 53:1632- 1637.

Thingstad, TF. 2000. Elements of a theory for the mechanisms controlling abundance, diversity, and biogeochemical role of lytic bacterial viruses in aquatic systems. Limnol Oceanogr 45:1320-1328.

Thingstad, TF, S Vage, JE Storesund, RA Sandaa, J Giske. 2014. A theoretical analysis of how strain-specific viruses can control microbial species diversity. Proc Natl Acad Sci USA 111:7813-7818.

Thompson, LR, Q Zeng, L Kelly, KH Huang, AU Singer, J Stubbe, SW Chisholm. 2011. Phage auxiliary metabolic genes and the redirection of cyanobacterial host carbon metabolism. Proc Natl Acad Sci USA 108:E757-E764.

Thurber, RLV, KL Barott, D Hall, et al. 2008. Metagenomic analysis indicates that stressors induce production of herpes-like viruses in the coral Porites compressa. Proc Natl Acad Sci USA 105:18413-18418.

Torrella, F, RY Morita. 1979. Evidence by electron-micrographs for a high incidence of bacteriophage particles in the waters of Yaquina Bay, Oregon - Ecological and taxonomical implications. Appl Environ Microbiol 37:774-778. Tringe, SG, T Zhang, XG Liu, et al. 2008. The airborne metagenome in an indoor urban environment. Plos One 3:10.

Waldor, MK, JJ Mekalanos. 1996. Lysogenic conversion by a filamentous phage encoding cholera toxin. Science 272:1910-1914.

Weitz, JS, SW Wilhelm. 2012. Ocean viruses and their dynamical effects on microbial communities and biogeochemical cycles. F1000 Biol Rep 4:17.

Wilcox, RM, JA Fuhrman. 1994. Bacterial viruses in coastal seawater: lytic rather than lysogenic production. Mar Ecol Prog Ser 114:35-35.

Wilhelm, SW, CA Suttle. 1999. Viruses and Nutrient Cycles in the Sea - Viruses play critical roles in the structure and function of aquatic food webs. Bioscience 49:781-788.

Willner, D, M Furlan, M Haynes, R Schmieder, FE Angly, J Silva, S Tammadoni, B Nosrat, D Conrad, F Rohwer. 2009. Metagenomic analysis of respiratory tract DNA viral communities in cystic fibrosis and non-cystic fibrosis individuals. Plos One 4:e7370.

Willner, D, RV Thurber, F Rohwer. 2009. Metagenomic signatures of 86 microbial and viral metagenomes. Environ Microbiol 11:1752- 1766.

Woese, CR, O Kandler, ML Wheelis. 1990. Towards a natural system of organisms - Proposal for the domains Archaea, Bacteria, and Eucarya. Proc Natl Acad Sci USA 87:4576-4579.

Wommack, KE, RR Colwell. 2000. Virioplankton: Viruses in aquatic ecosystems. Microbiol Mol Biol Rev 64:69-114.

Zhang, Y, C Huang, J Yang, N Jiao. 2011. Interactions between marine microorganisms and their phages. Chin Sci Bull 56:1770-1777.

ZoBell, CE. 1946. Marine microbiology, a monograph on hydrobacteriology. Waltham, Mass.: Chronica Botanica Company.

FIGURES AND TABLES

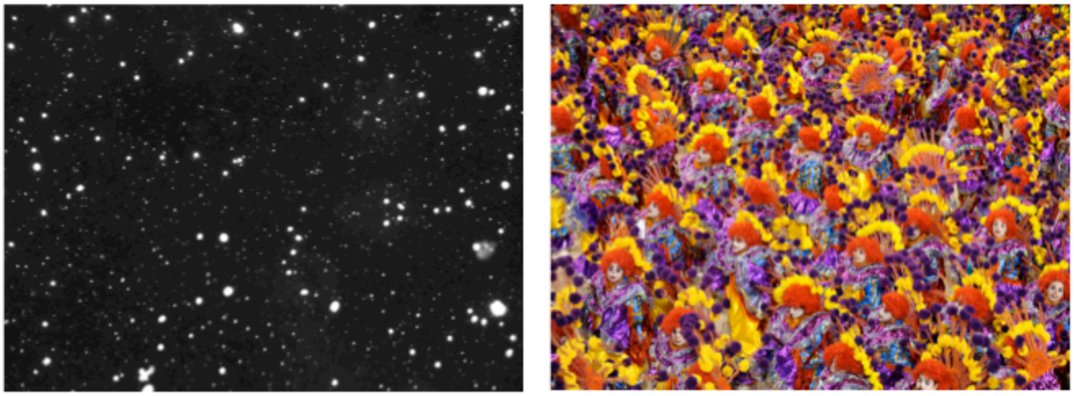


Figure 1.1: Every milliliter of seawater contains the urban population of Rio de Janeiro in viruses. **a**) The microbes (large objects) and virus-like particles (dots) found in 1/8th of a microliter of seawater from the coral reef at Guam with genomic content stained using Sybr Gold (Noble, Fuhrman 1998) and imaged at ~800X magnification (Credit: Ben Knowles), compared to **b**), the density of clowns in Rio de Janeiro during Carnivale.

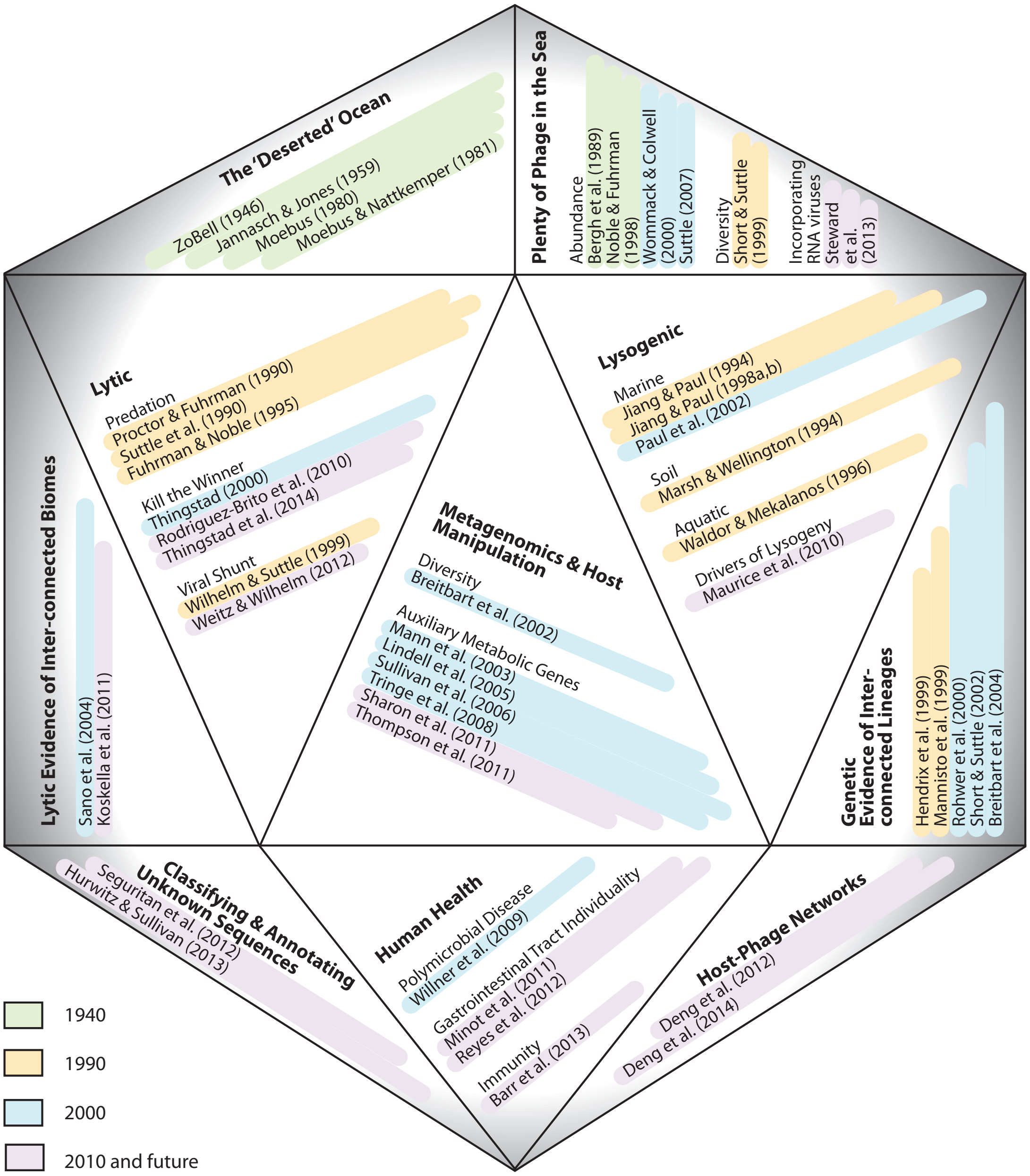
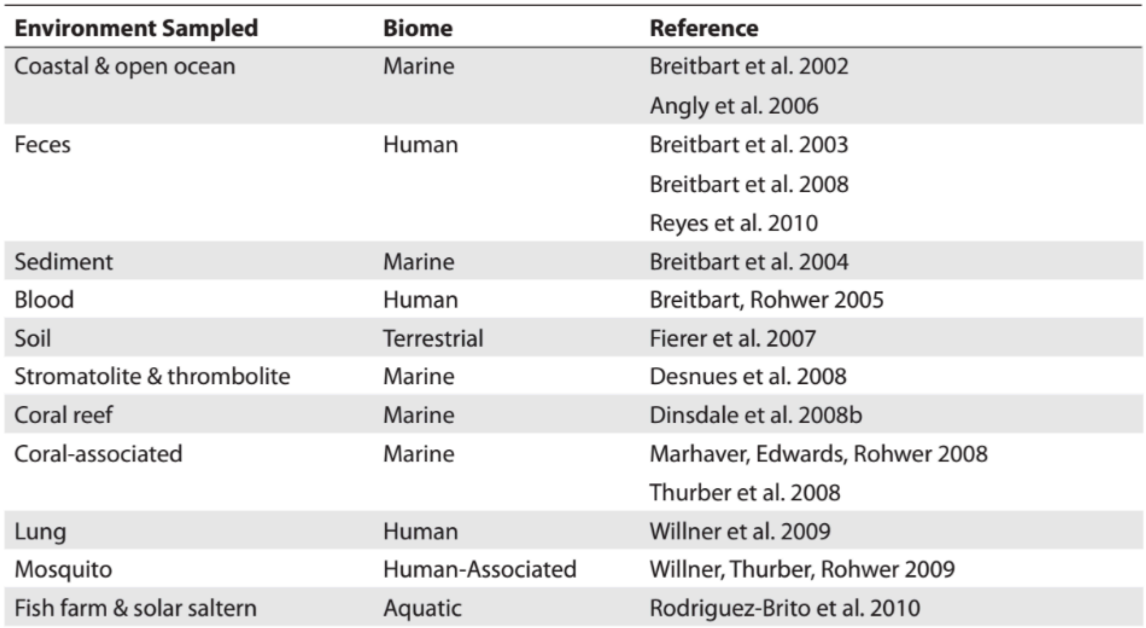


Figure 1.2: Viral ecology from the early 1900s to present day.
A conceptualized timeline of phage ecology from the desert ocean of Zobell to the environmental and metagenomic analyses of today. Adjacent triangles are conceptually related.

Table 1.1: Initial viral ecology studies using shotgun metagenomics.
Environments probed using metagenomics by members of the San Diego Phage Group throughout the 2000s, ranked by publication date of the first paper for that environment.



Environment Sampled	Biome	Reference
Coastal & open ocean	Marine	Breitbart et al. 2002 Angly et al. 2006
Feces	Human	Breitbart et al. 2003 Breitbart et al. 2008 Reyes et al. 2010
Sediment	Marine	Breitbart et al. 2004
Blood	Human	Breitbart, Rohwer 2005
Soil	Terrestrial	Fierer et al. 2007
Stromatolite & thrombolite	Marine	Desnues et al. 2008
Coral reef	Marine	Dinsdale et al. 2008b
Coral-associated	Marine	Marhaver, Edwards, Rohwer 2008 Thurber et al. 2008
Lung	Human	Willner et al. 2009
Mosquito	Human-Associated	Willner, Thurber, Rohwer 2009
Fish farm & solar saltern	Aquatic	Rodriguez-Brito et al. 2010

Chapter 1 has been published in the book “Life in Our Phage World” (Wholon Publishing, San Diego) as a perspective chapter Knowles B (2014) ‘March from the sea: a brief history of environmental phage ecology from marine to human ecosystems.’ It provides a lively discussion of the historical arc and biases in the field of viral ecology. It was written with profound input from Merry Youle., The dissertation author is the author of this work.

CHAPTER 2

Piggyback-the-Winner: lytic to lysogenic switches of viral communities

INTRODUCTION

Microbial viruses infect about 10^{23} cells per second in the world's oceans and the majority of microbial cells are infected at any given time (Suttle 2007, Proctor and Fuhrman 1990). What determines the proportion of lytic versus lysogenic infections is not well understood, despite the known importance of lytic/lysogenic fate in driving ecological and biogeochemical outcomes (Suttle 2007, Wilcox and Fuhrman 1994, Payet and Suttle 2013, Wommack and Colwell 2000, Thingstad 2000). Kill-the-Winner (KtW) models of lytic infection predict that density- and frequency-dependent viral predation suppresses blooms of rapidly growing hosts, increasing host diversity (Thingstad 2000, Rodriguez-Valera et al. 2009, Rodriguez-Brito et al. 2010, Weitz and Dushoff 2008, Thingstad et al. 2014). A limited number of studies provide empirical support for these predictions (Rodriguez-Brito et al. 2010, Weinbauer et al. 1998, Bratbak et al. 1993, Evans and Brussaard 2012, Needham et al. 2013). In contrast, temperate phage dynamics in the environment are much less studied and the relationship between lysogeny and host density is unclear. However, provirus induction studies indicate that lysogeny is more frequent with low host density (Jiang and Paul 1998, Paul 2008, Paul and Weinbauer 2010). As such, it is established that viral communities transition from lysogeny to lytic dominance as host densities rise (Payet and Suttle 2013, Thingstad et al. 2014, Bratbak et al 1993, Needham et al. 2013, Jing and Paul 1998, Maurice et al 2010, Maurice et al 2013).

Coral reefs offer a unique opportunity to probe the relationship between microbial host densities and the relative frequency of lytic versus temperate viral life cycles. Anthropogenic stressors can shunt these ecosystems into degradative regimes that result in changes in microbial and microbial community composition, especially with rising microbial energy demand and densities, a state described as Microbialized (McDole et al. 2012, Dinsdale et al. 2008, Smith et al. 2006, Thurber et al. 2009, Kelly et al. 2012). On heavily microbialized reefs, microbial abundances increase 5- to 10-fold, which increases predicted virus-host encounter rates (Dinsdale et al 2008, Alongi et al. 2015). Density-dependent lytic KtW models predict that reef microbialization correlates with increased lytic viral predation, demonstrated through an increased virus to microbe ratio (Weitz and Dushoff 2008, Thingstad 2000). In contrast, here we use four independent analyses - direct counts, literature meta-analyses, experiments, and viral community metagenomics - to show that increased host density is instead accompanied by a transition from lytic to temperate dynamics. On this basis we propose an extension of the KtW models, the Piggyback-the-Winner (PtW) model, which reflects the increased contribution of temperate viruses in ecosystems with high host abundance, yielding more microbes, fewer viruses.

METHODS

Viral and microbial counts

Seawater was collected in 2 L diver-deployed niskin bottles at approximately 10 m depth within 30 cm of the benthos on coral reefs across the Pacific and Atlantic Oceans (Haas et al. 2014). Samples were fixed with 2% paraformaldehyde within four hours of collection. Pacific Ocean samples were filtered and stained with SYBR Gold (Life Technologies, USA), mounted on slides and analyzed by epifluorescence microscopy (Haas et al. 2014). Atlantic Ocean samples were flash frozen and stored in liquid nitrogen until analysis on a BD FACSCalibur flow cytometer (Brussaard et al. 2010). Investigators were blinded when conducting all counts in this study (environmental or experimental), with sites or incubation samples imaged and analysed in a random order and identified only after analysis.

Predator-prey modeling

Steady state solutions to the dynamic model of Weitz and Dushoff (2008) were calculated under varying carrying capacities (K). The chemostat model of Thingstad et al. (2014) was run for varying K, and the final point in the evolution of the system plotted.

A standard lytic model (modified in Weitz and Dushoff 2008) that incorporates a logistic or trophic-state dependence for the microbe growth rate r is given by the equations:

$$\delta N/\delta t = r \bullet N \bullet (1 - N / K) - (d \bullet N) - (\phi \bullet N \bullet V)$$

$$\delta V/\delta t = (\beta \bullet \phi \bullet N \bullet V) - (m \bullet V)$$

where d and m are, respectively, the trophic-independent death rates for microbes and phage, N and V are, respectively, microbial host and viral abundances, β is the burst size, and ϕ is the adsorption coefficient. This corresponds to the Weitz-Dushoff model with their parameter *a* set equal to 0.

In this case the specific viral production rate per microbe is given by the product $\beta \square \phi$. In the new PtW model of viral-host interactions proposed here we replace this product with the quantity $\beta \square \phi \square N / K$, suppressing viral production as the system moves away from K (i.e. N/K becomes smaller) to simulate augmentation of lysogeny in eutrophic conditions. In this case $\beta \square \phi$ has the interpretation as the maximum value for the specific viral production rate per microbe. Steady state solutions of host and viral densities in the PtW model generated herein were calculated across a range of K (Figure 1b). All models are available as Matlab scripts from: https://github.com/benjaminwilliamknowles/Piggyback-the-Winner.

Meta-analysis of cell and viral abundances

The relationships between published VLP and cell abundances from disparate environments were probed from 20 studies (Payet and Suttle 2013, Evans and Brussaard 2012, Amossé et al. 2013, Barr et al. 2013, Bettarel et al. 2006, Bouvier et al. 2011, Glud and Middelboe 2004, Furlan 2009, Hewson et al. 2001, Kim et al. 2011, Lisle and Priscu 2004, Laybourn-Parry et al. 2006, Madan et al.2005, Maurice et al. 2011, Mei and Danovaro 2004, Parsons et al. 2012, Parsons et al. 2014, Rinta-Kanto et al. 2004, Schapira et al. 2009, Payet et al. 2014). When abundances were not available, we used the WebPlotDigitizer tool to recover data from graphs (http://arohatgi.info/WebPlotDigitizer/app/). Samples were grouped by habitat: animal-associated, polar lakes, coastal/estuarine, coral reefs, deep ocean, drinking water, open ocean, sediment, soil, soil water and temperate lake/river. We similarly extracted data from published studies and tested the relationship between cell abundance and the frequency of lysogenic cells as studied by mitomycin C induction in previous studies from the Adriatic Basin, Arctic Shelf, Mid Atlantic Ridge and Tampa Bay (Payet and Suttle 2013, Muck et al. 2014, Bongiorni et al. 2005, Williamson et al. 2002).

Metaviromic sampling and processing

Viral metagenomic samples were collected at 24 reefs, a subset of sites sampled for counts as previously described (Haas et al. 2014). Pacific viral concentrates were treated with 250 µl of chloroform per 50 ml of concentrate to destroy microbes and purified using CsCl step gradient ultracentrifugation (Haas et al. 2014). Viral DNA was extracted using the formamide/phenol/chloroform isoamyl alcohol technique (Haas et al. 2014) and amplified using the Linker Amplified Sequencing Library approach (Duhaime et al. 2012) and sequenced on an Illumina MySeq platform (Illumina, USA). Atlantic viral concentrates were passed through a 0.22 *µ*m filter and 250 µl of chloroform per 50 ml of concentrate was added to remove microbes, followed by ultra centrifugation for further concentration. DNA from Atlantic sites was extracted by phenol/chloroform/isoamyl alcohol technique, amplified using multiple displacement amplification (Thurber et al. 2009) and sequenced on an Ion Torrent sequencer (Life Sciences, USA). Microbial metagenomes were prepared by DNA extraction from the >0.22 μm fraction of the microbial community using Nucleospin Tissue Extraction kits (Macherey Nagel, Germany; Haas et al. 2014) and sequencing on an Illumina MySeq platform (Illumina, USA).

Bioinformatics

Sequences less than 100 bp and with mean quality scores less than 25 were removed using PrinSeq (Schmieder and Edwards 2011a). Acceptable sequences were then dereplicated with TagCleaner (Schmieder et al. 2010) and potential contaminants matching lambda or human DNA sequences removed with DeconSeq (Schmieder and Edwards 2011b). Focusing on prokaryotic reads, microbial metagenomes were taxonomically annotated based on k-mer similarity using FOCUS (Silva et al. 2014). Rank-abundance tables were then used to calculate species-level Shannon (base *e*) taxonomic diversity. For the virome analysis, protein sequences of all integrase, excisionase, and competence gene sequences on the NCBI RefSeq database (http://www.ncbi.nlm.nih.gov/refseq/) were downloaded and made into BLAST databases (makeblastdb command; BLAST version 2.2.29+, ncbi.nlm.nih.gov/blast/executables/ blast+). The Virulence Factors of Pathogenic Bacteria Database (http://www.mgc.ac.cn/VFs/ main.htm) was used as a protein database for pathogenicity genes. The percentage of each sequence library composed of integrase, excisionase, competence, or pathogenicity genes was computed as the number of sequences with > 60 bp match at a 40% identity to database sequences identified using BLASTx, normalized by the total number of sequences in the virome. CRISPRs were identified in microbiomes using the CRISPR Recognition Tool (https://github.com/ajmazurie/CRT) and hits normalized to parts per million against total reads. The fraction of known prophage-like reads in the viromes, normalized by total sequences, was assessed by a stringent (e-value 10^{-10}) BLAST against known prophages in cultured bacteria downloaded from NCBI (Hosts (number of prophage): *Escherichia coli* (36), *Shigella flexneri* (31), *Salmonella enterica* (16), *Staphylococcus aureus* (14), *Xylella fastidiosa* (12), *Yersinia pseudotuberculosis* (11), *Yersinia pestis* (9), *Shewanella baltica* (8), *Streptococcus pyogenes* (7), *Pseudomonas syringae* (7), *Salmonella typhimurium* (6), *Xanthomonas campestris* (5), *Mycobacterium tuberculosis* (4), *Yersinia enterocolitica* (3), *Streptococcus agalactiae* (3), *Stenotrophomonas maltophilia* (3), *Pseudomonas putida* (3), *Staphylococcus haemolyticus* (2), *Streptomyces avermitilis* (1), *Streptococcus uberis* (1), *Listeria monocytogenes* (1), *Caulobacter sp.* (1). For functional diversity analysis, reads of each virome were assembled using MIRA (Chevreux et al. 1999) followed by ORF calling using FragGenScan (Rho et al. 2010) and ORF clusterization at 85% identity using CD-HIT (Fu et al. 2012) to build protein cluster databases. We then performed BLASTx of reads against clusters databases to access the number of reads assigned to each protein cluster. An OTU-like table was built using each cluster as a rank unit and read counts as abundance. Shannon (base *e*) indexes were calculated using the VEGAN package in R (http://cran.r-project.org/web/packages/vegan/index.html). Average viral genome size estimates were performed using GAAS (Angly et al. 2009) and virome clustering was performed using crAss (Dutilh et al. 2012).

Bioinformatic code availability

The following codes and parameters were used for each step of the viral functional diversity analysis:

Assembly parameters used in Mira: minimum overlap = 30 and minimum relative score = 90.

FragGeneScan code: ./run_FragGeneScan.pl -genome=[seq_file_name] -out=[output_file_name] -complete=0 -train=illumina_10.

CD-HIT code: cd-hit-est –i [input fastafilename.faa] -o [outputfilename]_95 -c 0.95 -n 8.

CD-HIT output was used as database for BLASTx with virome reads, and output format 6 was parsed with the following python script to create rank-abundance tables:

```
f="BlastOutput.txt"
myfile=open(f)
h={};temp=""
for line in myfile:
```

```
    line=line.split()
    if temp!=line[0]:
        if line[1] not in h:
            h[line[1]]=0
        h[line[1]]+=1
    temp=line[0]
myfile.close()
```

Incubation experiments

Water samples were collected at Palmyra Atoll, a pristine coral reef in the central Pacific, and Mission Bay, a degraded embayment in San Diego, CA. Samples were twice filtered through 0.8 *μ*m pre-combusted GF/F filters to remove protists. Palmyra water was subsampled in 100 ml aliquots and distributed in 12 Whirl-Pak bags (Cole-Parmer, USA), divided into two experimental groups and one control, each one containing four randomly chosen replicate bags. For the two experimental groups we added a DOC cocktail containing 48 different labile carbon sources (Sanchez et al. 2015) at the final concentration of 500 *μ*M or 60 *μ*M (+ DOC treatment; Extended Data Figure 3a), while no DOC was added to the control group (- DOC treatment; Extended Data Figure 3a). Viral decay in microbe-free incubation bags was monitored as an additional control with 0.22 *μ*m double-filtered water samples (Extended Data Figure 3b). 1 ml samples were taken at times 0 h, 24 h, 48 h, 72 h, and 120 h from each bag for cell and viral counts. Mission Bay water was filtered and separated in three groups as above. 250 ml aliquots were distributed in each bag and incubated with 0 μM, 1 μM or 100 μM final DOC concentrations. Samples were taken at times 12 h, 24 h, 48 h, and 72 h for counts. All incubations were performed in the dark at 25 °C. Samples were fixed and analyzed by epifluorescence microscopy as described above.

Statistical analysis

Significance was determined using an alpha of 0.05 when direct counts data were compared, and using an alpha of 0.1 when analyzing counts versus bioinformatic analyses to account for the disparate nature of these datasets (although 95% prediction intervals are also shown). The relationship between microbial density and microbial diversity, CRISPR sequences, and competence genes were tested for significant deviation from a slope of 0 by linear regression. The relationship between VLP and microbial densities in Figures 1a, 2 (all except the final panel showing VMR), 3a, and 3c were tested for slopes significantly different to 1 by t-tests that tested the null hypothesis that the slope is not equal to 1 against the two-sided alternative; the corresponding p-value is given for this test. The fdrtool package in R was used to provide False Discovery Rate-corrected (FDR) p-values for the multiple comparisons conducted in Figure 2 (Extended Data Table 1). Conclusions were similar between FDR and uncorrected analyses. Experimental data in Figure 3 was complemented by average counts taken from previous studies (Wilcox and Fuhrman 1994, Hennes et al. 1995) using the WebPlotDigitizer tool. Data was taken from the nutrient added treatment of Hennes et al. (1995) as it was described as showing ‘lytic’ dynamics (Figures 3c and 3d). Data from the 30%, 20%, 10%, and 3% dilutions by Wilcox and Fuhrman (1994) were used as they had encounter rates (the product of viral and host densities) ~10^{12} or less at the beginning of the incubations, described as the cutoff below which lytic dynamics were not sustained. A thin plate spline was applied to experimental and literature values for visualization and interpretation (Figures 3b and 3d). While some published datasets used in Figures 2 and 3 violated the assumptions of linear regression, this analysis was used for comparability. Robust regressions were used in Figure 4 (Figures 4a, 4b, 4d, and Extended Data Figure 4a) analyses in order to accommodate high-leverage samples on parametric statistical models, allowing all samples to be retained in the analysis. Results are presented for robust regression estimation using Tukey’s biweight and corresponding bootstrapped 90th percentile and 95th percentile confidence intervals (CIs) for the slope using 1000 bootstrap replications. It should be noted for Figure 4a that even though 95th CI covers 0 (percentile intervals: -0.114, 2.848), the 90th CI does not cover 0 indicating that there is evidence at the 0.1 confidence level that the slope is positive. For subsequent analyses in Figure 4, 95th CIs do not straddle 0, showing that there is evidence at the 0.05 confidence level that the slope is negative (Figure 4c) or positive (Figures 4b and 4d). To account for error in the y-axis we also performed Model II Regression analyses with data shown in figures 1, 2 and 4 using the package lmodel2 in R (Extended Data Table 3). It should be noted, however, that these results should be treated with caution, as error variance and goodness of fit metrics are not obtainable for this analysis.

RESULTS

Viral and microbial abundance

Microbial and viral abundances were measured in 223 Pacific and Atlantic coral reef samples (Figure 1a). The density of Virus-Like Particles (VLPs) was significantly higher than that of the microbes ($t = -19.61$, d.f. = 236.96, $p < 2.20 \times 10^{-16}$; Welch two sample t-test) and ranged from 9.03×10^5 to 3.86×10^7 ($7.08 \times 10^6 \pm 3.01 \times 10^5$, mean ± SE) VLPs ml^{-1} versus 8.08×10^4 to 6.75×10^6 ($1.09 \times 10^6 \pm 5.53 \times 10^4$, mean ± SE) microbes ml^{-1}. The log-log plot of these VLP and microbe abundances had a slope < 1 ($m = 0.59$, $R^2 = 0.50$, d.f. = 221, $F_{(1, 221)} = 219.50$, $t = 14.82$, p [t-test; $m \neq 1$] = 4.08×10^{-21}; slope significantly different from $m = 1$ by linear regression with t-test; Figure 1a), indicating a downward concave relationship between these variables. As a result, the virus to microbe ratio (VMR) decreased significantly (analyzed against host density, both log-transformed; $m = -0.37$; $R^2 = 0.29$, d.f. = 221, $F_{(1, 221)} = 90.63$; $t = -9.52$, $p < 2.00 \times 10^{-16}$; linear regression) from a ratio of 25 to 2 VLPs per microbe (7.44 ± 0.24, mean ± SE) as microbial abundance increased from $\sim 1 \times 10^5$ to greater than 6×10^6.

Recent models were used to contrast our counts with predicted viral-host relationships (Weitz and Dushoff 2008, Thingstad 2000). Weitz and Dushoff (2008), in which burst size is proportional to density-dependent microbial growth rate, predicts a negative correlation between viral and host density as viral predation causes declining host density with rising density-dependent host growth rate (Figure 1b; details of steady state solution in Materials and Methods). The KtW-like model by Thingstad et al. (2014), that incorporates terms for nested resistance to viral infection amongst multiple host strains (Thingstad et al. 2014, Needham et al. 2013, Flores et al. 2013), predicts an approximately downward concave relationship between viral and host abundances with the increasing dominance of slow growing, resistant hosts suppressing lytic dynamics as host density rises (Figure 1b). The *Piggyback-the-Winner* (PtW) model introduced here predicts a relationship between VLP and host densities similar to Thingstad et al. (2014), but lytic dynamics are suppressed at high host density and density-dependent growth rate due to the increased prevalence of lysogeny (modeled as lower specific viral production rates per infection) and super-infection exclusion rather than resistance.

Diversity and functional composition of microbial communities

Viral predation is thought to stimulate species-level host diversity through lineage-specific predation targeting dominant lineages, promoting community evenness (Rodriguez-Valera et al. 2009, Weinbauer et al. 2007, Weinbauer and Rassoulzadegan 2004, Thingstad and Lignell 1997). However, a weak and significantly negative relationship between host density and taxonomic diversity was observed in Pacific microbiomes (Figure 1c; microbial abundance log-transformed; $m = -0.29$, $R^2 = 0.09$, d.f. = 64, $F_{(1,64)} = 6.55$, $t = -2.60$, $p = 0.01$; linear regression). This also indicates that lytic dynamics are suppressed when both density-dependent (i.e., total encounter rates) and frequency-dependent (i.e., the relative density of a given host) both favor lytic activity.

The recent Thingstad et al. (2014) model suggests that elevated host densities lead to an increase in host resistance to viral infection. However, we found weak relationships and no support for increased host resistance via CRISPRs or potential horizontal transfer of resistance (% competence genes) in the mixed microbial community metagenomes (CRISPRs: Figure 1d; $m = -26.17$, $R^2 = 0.03$, d.f. = 64, $F_{(1,64)} = 2.08$, $t = -1.44$, $p = 0.15$; % competence genes: Extended Data Figure 1a; $m = -0.25$, $R^2 = 0.08$, d.f. = 64, $F_{(1,64)} = 5.75$, $t = -2.40$, $p = 0.02$; microbial abundance log-transformed and linear regressions in both analysis). These data indicate that immunity to viral infection does not change with host density as predicted by Thingstad et al. (2014), and is not promoted by horizontal transfer of resistance genes as predicted in King-of-the-Mountain dynamics (Thingstad et al. 2014, Giovannoni et al. 2013). Thus, an alternative mechanism, such as increasing resistance via strain-level diversification through lysogeny, likely drives the observed decrease in VMR with increasing microbial abundance. This is in accordance with changes in strain diversity predicted by Thingstad et al. (2014; Extended Data Figure 1b). The convergence of these predictions suggests that PtW lysogeny, with its implicit super-infection immunity dynamic, could complement the nested infection design of the Thingstad et al. (2014) model in future studies of resistance/growth tradeoffs.

Viral and host abundances in other ecosystems

Data from 20 independent studies were compiled for a meta-analysis to determine the generality of the *more microbes, fewer viruses* observation. These studies spanned five orders of magnitude of microbial and VLP density (Figure 2; summary statistics in Extended Data Table 1). Analysis of log-transformed microbial and VLP abundances yielded slopes of significantly less than 1 in eight of the eleven environments. VMR therefore declined with increasing microbial density in disparate coastal and estuarine, coral reef, deep ocean, open ocean, temperate lake, animal-associated, sediment, and soil systems, consistent with our coral reef observations. This trend was also observed in the cystic fibrosis lung (James et al. 2014). Together, these results show that *more microbes, fewer viruses* is a common phenomenon. When viewed across the full range of host densities, peak VMR values were observed at ~10^6 microbes ml^{-1} or g^{-1} of sample. VMR declines as host density decreases or increases from this value (Figure 2, final panel).

The relationship between microbial and viral densities was further examined through an analysis of published values of the fraction of lysogenic cells determined by mitomycin C induction (Payet and Suttle 2013, Muck et al. 2014, Bongiorni et al. 2005, Williamson et al. 2002). Although a sometimes-significant negative relationship exists at a within-study level, examination across the full range of host abundances studied revealed no significant slope (Extended Data Figure 2). The paradigm that low host density favours lysogeny is not well supported by induction data when viewed globally; there is reason to reexamine the drivers of lysogeny with lines of evidence independent of established methods.

Experimental manipulation of host growth rate

Our counts data contrast with predicted density-dependent lytic predation. Further, the models examined in Figure 1b predict different relationships between microbial density, density-dependent host growth rate and viral lytic activity (measured as VMR). The actual relationship between these variables was probed with incubation experiments using seawater sampled from a pristine coral reef (Palmyra; 120 hour time series) and a degraded embayment (Mission Bay; 72 hour time series). Data were pooled within sites as high variability led to a lack of significant impact of dissolved organic carbon addition on host density ($t = 0.82$, d.f. = 32.18, $p = 0.42$; Welch two sample t-test with microbial abundance log-transformed; Extended Data 3a) or VMR ($t = 0.17$, d.f. = 27.70, $p = 0.87$; Welch two sample t-test).

The experimental data matched our field observations: slopes significantly < 1 were observed between log-transformed VLP and microbial densities in both incubations (Palmyra: $m = 0.63$, $R^2 = 0.43$, d.f. = 23, $F_{(1,23)} = 17.63$, $t = 4.20$, p [t-test; $m \neq 1$] = 2.25×10^{-2}; Mission Bay: $m = 0.56$, $R^2 = 0.82$, d.f. = 10, $F_{(1,10)} = 44.18$, $t = 6.65$, p [t-test; $m \neq 1$] = 3.59×10^{-4}; linear regression; Figure 3a). These incubations are therefore more similar to the data set characterized by Wilcox and Fuhrman (1994) as non-lytic ($m = 0.13$, $R^2 = 0.02$, d.f. = 26, $F_{(1,26)} = 0.41$, t =

0.64, p [t-test; m ≠ 1] = 2.65×10^{-4}; Figure 3c) than the putatively lytic data set of Hennes et al. (1995; m = 1.19, R^2 = 0.39, d.f. = 4, $F_{(1,4)}$ = 2.52, t = 1.59, p [t-test; m ≠ 1] = 0.81; Figure 3c). Hennes et al. (1995) and Wilcox and Fuhrman (1994) attribute their lytic (where VMR rose ~40) and non-lytic dynamics to elevated and lowered microbial densities, respectively. In contrast, we did not observe a similar rise in VMR despite exceeding an order of magnitude higher host densities (Figure 3b and 3d) and five times faster growth rates than Hennes et al. (1995; 9.71×10^6 and 1.77×10^6 cells h^{-1} in Palmyra and Mission Bay incubations, respectively; Hennes et al. 1995: 1.74×10^6 cells h^{-1}). These protist predator-free incubations (i.e., 0.8 µm filtered) showed no significant increase in VMR with increasing host density, indicating that viral-host interactions alone are sufficient to drive the approximately downward concave relationship between VLP and host densities (Figure 3a and 3b).

Temperate genes, diversity, and virulence

Metagenomes of viral communities (viromes) from 24 Pacific and Atlantic coral reefs were sequenced (Extended Data Table 2). High variability and high leverage points were observed in the relationship of all viral bioinformatic metrics and host density, requiring the use of robust regression followed by bootstrap confidence interval estimation (RR-B) due to its insensitivity to high leverage, peripheral values. The percent abundance of viral integrase, excisionase, and prophage reads increased significantly with microbial density (Figures 4a, 4b, and Extended Data Figure 4a) at the ≥ 90% confidence level (% integrase, m =

1.23, 90th percentile Confidence Interval (CI; 0.01, 2.69), Figure 4a; % excisionase, m = 0.04, 90th CI (0.01, 0.11), Figure 4b; % prophage, m = 0.13, 90th CI (0.03, 0.44), Extended Data Figure 4a; RR-B; R^2 are not appropriate for robust regressions and are omitted).

Increased cell density was associated with a significant decline in functional diversity of the viral communities, an indicator of temperate viral communities (McDaniel et al. 2014), as measured by the Shannon (H') index of putative coding genes in the viromes (m = -3.54, 90th CI (-6.14, -1.73); RR-B against log-transformed host density; Figure 4c). Furthermore, the lower diversity, more temperate viral communities carry more pathogenicity genes than the more diverse and lytic viral communities found at lower host densities (m = 1.09, 90th CI (0.46, 3.01); RR-B against log-transformed host density; Figure 4d). Further, while we have conservatively used linear regression to analyze these relationships, the data strongly suggest an exponential relationship between host density and % integrase, % excisionase, and % pathogenicity genes, and a decay function between host density and viral functional diversity. These observed trends were not a result of overall viral community genome size reduction, as the average viral genome size determined by GAAS was unaffected by microbial abundance (m = -9190, $R^2 = 0.04$, d.f. = 22, $F_{(1,22)} = 1.04$, t = -1.02, p = 0.32; linear regression of genome length (bp) against log-transformed host density; mean estimated viral genome length = 42.07 ± 2.45 kb, mean ± SE). Rather, viral communities changed with increasing temperateness; viral communities clustered geographically as low-cell-density Atlantic viromes grouped away from Pacific viromes (Extended Data Figure 4b).

DISCUSSION

The observed decline in the virus to microbe ratio (VMR) with elevated host abundances on microbialized coral reefs (Figure 1) is consistent with lowered lytic activity at high host density (Figure 1b). This trend common, being found in eight of eleven (> 70 %) other disparate environments studied to date (Figure 2). No support was found for possible drivers of this suppressed lytic activity such as competitive exclusion of viral predators by heterotrophic protists (Thingstad 2000, Fuhrman 2009, Weitz et al. 2015; Figure 3a), the rise or transfer of resistance to viral infection (Thingstad et al. 2014, Giovannoni et al. 2013; Figure 1d and Extended Data Figure 1b), greater species-level host diversity (Wommack and Colwell 2000; Figure 1c), or increasing viral decay (Wommack and Colwell 2000; Extended Data Figure 3b). Rather, multiple independent bioinformatic analyses of our viromes from this study, reinforced by viromes from other ecosystems (Reyes et al. 2010, Breitbart et al. 2003, Angly et al. 2006, Breitbart et al. 2004, Brum et al. 2015), indicated an increased relative abundance of temperate viruses in communities with high microbial densities (Figure 4). Tests of alternatives to PtW showed weak or ambiguous relationships while correlations supporting PtW were remarkably strong (e.g., R^2 of < 0.07 in Figures 1c, 1d, and Extended Data Figure 2, compared with R^2 > 0.56 in Figures 1a and 3a), despite data being gathered from locations across the globe. Further, all four independent lines of evidence examined here - direct counts, literature meta-analyses, experiments, and viral community metagenomics - provide significant support for PtW.

In contrast to our findings, the established paradigm in viral ecology predicts that lytic dynamics dominate at high host density, lysogeny at low densities (Payet and Suttle 2013, Jiang and Paul 1998, Maurice et al. 2013). We are therefore proposing an extension of these Kill-the-Winner (KtW) models, *Piggyback-the-Winner* (PtW), wherein temperateness is favored at high host densities as viruses exploit their hosts through lysogeny rather than killing them.

The characteristic narwhal-shaped distribution that results when VMR is plotted against microbe density in all environments investigated to date (Figure 2, final panel) suggests that host densities observed in the ocean (~5×10^5 to 1×10^6 cells ml^{-1}) favor lytic KtW dynamics. Lower and higher host densities may drive a lysogenic dynamic. Thus we predict that a Piggyback-the-Losers (PtL) dynamic extends the lytic-to-temperate shift to communities with low host densities communities, complementing high density PtW dynamics. Implications of this shift for all ecosystems include loss of lytic top-down control of microbial populations, acquisition by lysogens of phage-encoded functions including pathogenicity genes, and the decoupling of microbial taxa and function (Kelly et al. 2012, Kelly et al. 2014). Which viral-host dynamic prevails within a system, PtL, KtW, or PtW, has major ecological effects on trophic webs as the effects of viral lysis of microbial hosts cascades through multiple ecosystem levels (Weitz et al. 2015, Silveira et al. 2015).

SUMMARY POINTS

- Piggyback-the-Winner (PtW) extends lytic Kill-the-Winner dynamics by incorporating the global-scale switch from lytic to temperate behavior in viral communities as microbial host density increases.
- This model has important implications for how we think of viral-host interactions across ecosystems from coral reefs to the human body, soil, lakes, the deep ocean, and sediment.
- If an ecosystem has the potential for numerous viruses and hosts, then we predict that there will be more lysogeny and commensal relationships between the two groups.
- Lysogens are expected to be protected from other viral infections and their proviruses will provide additional metabolic function.
- PtW dynamics have the capacity to shunt systems into autocatalytic regimes of sustained microbialization and lessened lytic viral predation.
- This appears to be a ubiquitous phenomena underpinning processes from ecosystem degradation to dysbiotic disease progression.

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REFERENCES

Alongi, D. M. *et al.* Phytoplankton, bacterioplankton and virioplankton structure and function across the southern Great Barrier Reef shelf. *J. Mar. Syst.* **142,** 25–39 (2015).

Amossé, J. *et al.* The flows of nitrogen, bacteria and viruses from the soil to water compartments are influenced by earthworm activity and organic fertilization (compost vs. vermicompost). *Soil Biol. Biochem.* **66,** 197–203 (2013).

Angly, F. E. *et al.* The marine viromes of four oceanic regions. *PLoS Biol.* **4,** 2121–2131 (2006).

Angly, F. E. *et al.* The GAAS Metagenomic Tool and Its Estimations of Viral and Microbial Average Genome Size in Four Major Biomes. *PLoS Comput. Biol.* **5,** e1000593 (2009).

Barr, J. J. *et al.* Bacteriophage adhering to mucus provide a non-host-derived immunity. *Proc. Natl. Acad. Sci.* **110,** 10771–10776 (2013).

Bettarel, Y., Bouvy, M., Dumont, C. & Sime-Ngando, T. Virus-bacterium interactions in water and sediment of West African inland aquatic systems. *Appl. Environ. Microbiol.* **72,** 5274–5282 (2006).

Bongiorni, L., Magagnini, M., Armeni, M., Noble, R. & Danovaro, R. Viral production, decay rates, and life strategies along a trophic gradient in the North Adriatic Sea. *Appl. Environ. Microbiol.* **71,** 6644–6650 (2005).

Bouvier, T. & Maurice, C. F. A single-cell analysis of virioplankton adsorption, infection, and intracellular abundance in different bacterioplankton physiologic categories. *Microb. Ecol.* **62,** 669–78 (2011).

Bratbak, G., Egge, J. K. & Heldal, M. Viral mortality of the marine alga Emiliania huxleyi (Haptophyceae) and termination of algal blooms. *Mar. Ecol. Prog. Ser.* **93,** 39–48 (1993).

Breitbart, M. *et al.* Metagenomic analyses of an uncultured viral community from human feces. *J. Bacteriol.* **185,** 6220–6223 (2003).

Breitbart, M. *et al.* Diversity and population structure of a near--shore marine--sediment viral community. *Proc. R. Soc. London B Biol. Sci.* **271,** 565–574 (2004).

Brum, J. R., Hurwitz, B. L., Schofield, O., Ducklow, H. W. & Sullivan, M. B. Seasonal time bombs: dominant temperate viruses affect Southern Ocean microbial dynamics. *ISME J* 1–13 (2015). at <http://dx.doi.org/10.1038/ismej.2015.125>

Brussaard, C. P. D., Payet, J. P., Winter, C. & Weinbauer, M. G. Quantification of aquatic viruses by flow cytometry. *Man. Aquat. Viral Ecol.* **11,** 102–109 (2010).

Chevreux, B., Wetter, T. & Suhai, S. Genome sequence assembly using trace signals and additional sequence information. in *German conference on bioinformatics* 45–56 (1999).

Dinsdale, E. A. *et al.* Microbial ecology of four coral atolls in the Northern Line Islands. *PLoS One* **3,** e15841–17 (2008).

Duhaime, M. B., Deng, L., Poulos, B. T. & Sullivan, M. B. Towards quantitative metagenomics of wild viruses and other ultra-low concentration DNA samples: a rigorous assessment and optimization of the linker amplification method. *Environ. Microbiol.* **14,** 2526–37 (2012).

Dutilh, B. E. *et al.* Reference-independent comparative metagenomics using cross-assembly: CrAss. *Bioinformatics* **28,** 3225–3231 (2012).

Evans, C. & Brussaard, C. P. D. Viral lysis and microzooplankton grazing of phytoplankton throughout the Southern Ocean. *Limnol. Oceanogr.* **57,** 1826–1837 (2012).

Flores, C. O., Valverde, S. & Weitz, J. S. Multi-scale structure and geographic drivers of cross-infection within marine bacteria and phages. *ISME J.* **7,** 520–532 (2013).

Fu, L., Niu, B., Zhu, Z., Wu, S. & Li, W. CD-HIT: accelerated for clustering the next-generation sequencing data. *Bioinformatics* **28,** 3150–2 (2012).

Fuhrman, J. A. Microbial community structure and its functional implications. *Nature* **459,** 193–199 (2009).

Furlan, M. Viral and microbial dynamics in the human respiratory tract. (San Diego State University, 2009).

Giovannoni, S., Temperton, B. & Zhao, Y. Giovannoni et al. reply. *Nature* **499,** E4–E5 (2013).

Glud, R. N. & Middelboe, M. Virus and bacteria dynamics of a coastal sediment: Implication for benthic carbon cycling. *Limnol. Oceanogr.* **49,** 2073–2081 (2004).

Haas, A. F. *et al.* Unraveling the unseen players in the ocean - a field guide to water chemistry and marine microbiology. *J. Vis. Exp.* **21,** 1–16 (2014).

Hennes, K., Suttle, C. A. & Chan, A. M. Fluorescently Labeled Virus Probes Show that Natural Virus Populations Can Control the Structure of Marine Microbial Communities. *Appl. Environ. Microbiol.* **61,** 3623–3627 (1995).

Hewson, I., O'neil, J. M., Fuhrman, J. A. & Dennison, W. C. Virus-like particle distribution and abundance in sediments and overlying waters along eutrophication gradients in two subtropical estuaries. *Limnol. Oceanogr.* **46,** 1734–46 (2001).

James, C. E. *et al.* Lytic activity by temperate phages of *Pseudomonas aeruginosa* in long-term cystic fibrosis chronic lung infections. *ISME J.* 1–8 (2014). doi:10.1038/ismej.2014.223

Jiang, S. C. & Paul, J. H. Significance of lysogeny in the marine environment: studies with isolates and a model of lysogenic phage production. *Microb. Ecol.* **35,** 235–243 (1998).

Kelly, L. W. *et al.* Black reefs: iron-induced phase shifts on coral reefs. *ISME J.* **6,** 638–649 (2012).

Kelly, L. W. *et al.* Local genomic adaptation of coral reef-associated microbiomes to gradients of natural variability and anthropogenic stressors. *Proc. Natl. Acad. Sci. U. S. A.* **111,** 10227–10232 (2014).

Kim, M.-S., Park, E.-J., Roh, S. W. & Bae, J.-W. Diversity and abundance of single-stranded DNA viruses in human feces. *Appl. Environ. Microbiol.* **77,** 8062–70 (2011).

Laybourn-Parry, J., Marshall, W. A. & Madan, N. J. Viral dynamics and patterns of lysogeny in saline Antarctic lakes. *Polar Biol.* **30,** 351–358 (2006).

Lisle, J. T. & Priscu, J. C. The occurrence of lysogenic bacteria and microbial aggregates in the lakes of the McMurdo Dry Valleys, Antarctica. *Microb. Ecol.* **47,** 427–39 (2004).

Madan, N. J., Marshall, W. A. & Laybourn-Parry, J. Virus and microbial loop dynamics over an annual cycle in three contrasting Antarctic lakes. *Freshw. Biol.* **50,** 1291–1300 (2005).

Maurice, C. F., Bouvier, T., Comte, J., Guillemette, F. & Del Giorgio, P. A. Seasonal variations of phage life strategies and bacterial physiological states in three northern temperate lakes. *Environ. Microbiol.* **12,** 628–641 (2010).

Maurice, C. F., Bouvier, C., Wit, R. & Bouvier, T. Linking the lytic and lysogenic bacteriophage cycles to environmental conditions, host physiology and their variability in coastal lagoons. *Environ. Microbiol.* **15,** 2463–2475 (2013).

Maurice, C. F. *et al.* Disentangling the relative influence of bacterioplankton phylogeny and metabolism on lysogeny in reservoirs and lagoons. *ISME J.* **5,** 831–842 (2011).

McDaniel, L. D., Rosario, K., Breitbart, M. & Paul, J. H. Comparative metagenomics: natural populations of induced prophages demonstrate highly unique, lower diversity viral sequences. *Environ. Microbiol.* **16,** 570–585 (2014).

McDole, T. *et al.* Assessing coral reefs on a Pacific-wide scale using the microbialization score. *PLoS One* **7,** e432331–10 (2012).

Mei, M. L. & Danovaro, R. Virus production and life strategies in aquatic sediments. *Limnol. Oceanogr.* **49,** 459–470 (2004).

Muck, S. *et al.* Fracture zones in the Mid Atlantic Ridge lead to alterations in prokaryotic and viral parameters in deep-water masses. *Front. Microbiol.* **5,** 264 (2014).

Needham, D. M. *et al.* Short-term observations of marine bacterial and viral communities: patterns, connections and resilience. *ISME J.* **7,** 1274–1285 (2013).

Parsons, R. J., Breitbart, M., Lomas, M. W. & Carlson, C. A. Ocean time-series reveals recurring seasonal patterns of virioplankton dynamics in the northwestern Sargasso Sea. *ISME J.* **6,** 273–284 (2012).

Parsons, R. J. *et al.* Marine bacterioplankton community turnover within seasonally hypoxic waters of a subtropical sound: Devil's Hole, Bermuda.

Environ. Microbiol. 3481–3499 (2014). doi:10.1111/1462-2920.12445

Paul, J. H. Prophages in marine bacteria: dangerous molecular time bombs or the key to survival in the seas? *ISME J.* **2,** 579–589 (2008).

Paul, J. H. & Weinbauer, M. Detection of lysogeny in marine environments. *Man. Aquat. Viral Ecol.* **4,** 30–33 (2010).

Payet, J. P., McMinds, R., Burkepile, D. E. & Vega Thurber, R. L. Unprecedented evidence for high viral abundance and lytic activity in coral reef waters of the South Pacific Ocean. *Front. Microbiol.* **5,** 1–16 (2014).

Payet, J. & Suttle, C. A. To kill or not to kill: The balance between lytic and lysogenic viral infection is driven by trophic status. *Limnol. Oceanogr.* **58,** 465–474 (2013).

Proctor, L. M. & Fuhrman, J. A. Viral mortality of marine bacteria and cyanobacteria. *Nature* **343,** 60–62 (1990).

Reyes, A. *et al.* Viruses in the faecal microbiota of monozygotic twins and their mothers. *Nature* **466,** 334–338 (2010).

Rho, M., Tang, H. & Ye, Y. FragGeneScan: predicting genes in short and error-prone reads. *Nucleic Acids Res.* **38,** e191–12 (2010).

Rinta-Kanto, J. M., Lehtola, M. J., Vartiainen, T. & Martikainen, P. J. Rapid enumeration of virus-like particles in drinking water samples using SYBR green I-staining. *Water Res.* **38,** 2614–8 (2004).

Rodriguez-Brito, B. *et al.* Viral and microbial community dynamics in four aquatic environments. *ISME J.* **4,** 739–751 (2010).

Rodriguez-Valera, F. *et al.* Explaining microbial population genomics through phage predation. *Nat Rev Micro* **7,** 828–836 (2009).

Sanchez, S. E. *et al.* Phage Phenomics: Physiological approaches to characterize novel viral proteins. *J. Vis. Exp. JoVE* e52854, doi:10.3791/52854 (2015).

Schapira, M. *et al.* Distribution of heterotrophic bacteria and virus-like particles along a salinity gradient in a hypersaline coastal lagoon. *Aquat. Microb. Ecol.* **54,** 171–183 (2009).

Schmieder, R., Lim, Y. W., Rohwer, F. & Edwards, R. TagCleaner: Identification and removal of tag sequences from genomic and metagenomic datasets. *BMC Bioinformatics* **11,** 341 (2010).

Schmieder, R. & Edwards, R. Quality control and preprocessing of metagenomic datasets. *Bioinformatics* **27,** 863–4 (2011a).

Schmieder, R. & Edwards, R. Fast identification and removal of sequence contamination from genomic and metagenomic datasets. *PLoS One* **6,** e17288 (2011b).

Silva, G. G. Z., Cuevas, D. A., Dutilh, B. E. & Edwards, R. a. FOCUS: an alignment-free model to identify organisms in metagenomes using non-negative least squares. *PeerJ* **2,** e425 (2014).

Silveira, C. B. *et al.* Microbial and sponge loops modify fish production in phase-shifting coral reefs. *Environ. Microbiol.* **17,** 3832–3846 (2015).

Smith, J. E. *et al.* Indirect effects of algae on coral: algae-mediated, microbe-induced coral mortality. *Ecol. Lett.* **9,** 835–845 (2006).

Suttle, C. A. Marine viruses — major players in the global ecosystem. *Nat. Rev. Microbiol.* **5,** 801–812 (2007).

Thingstad, T. F. Elements of a theory for the mechanisms controlling abundance, diversity, and biogeochemical role of lytic bacterial viruses in aquatic systems. *Limnol. Oceanogr.* **45,** 1320–1328 (2000).

Thingstad, T. F. & Lignell, R. Theoretical models for the control of bacterial growth rate, abundance, diversity and carbon demand. *Aquat. Microb. Ecol.* **13,** 19–27 (1997).

Thingstad, T. F., Våge, S., Storesund, J. E., Sandaa, R.-A. & Giske, J. A theoretical analysis of how strain-specific viruses can control microbial species diversity. *Proc. Natl. Acad. Sci.* **111,** 7813–7818 (2014).

Thurber, R. *et al.* Metagenomic analysis of stressed coral holobionts. *Environ. Microbiol.* **11,** 2148–2163 (2009).

Wilcox, R. M. & Fuhrman, J. A. Bacterial viruses in coastal seawater: lytic rather than lysogenic production. *Mar. Ecol. Ser.* **114,** 35–45 (1994).

Wommack, K. E. & Colwell, R. R. Virioplankton: viruses in aquatic ecosystems.

Microbiol. Mol. Biol. Rev. **64,** 69–114 (2000).

Weitz, J. S. & Dushoff, J. Alternative stable states in host--phage dynamics. *Theor. Ecol.* **1,** 13–19 (2008).

Weinbauer, M. G. & Hofle, M. G. Significance of viral lysis and flagellate grazing as factors controlling bacterioplankton production in a eutrophic lake. *Appl. Environ. Microbiol.* **64,** 431–438 (1998).

Weinbauer, M. G., Rassoulzadegan, F. Are viruses driving microbial diversification and diversity? *Environ. Microbiol.* **6,** 1–11 (2004).

Weinbauer, M. G. *et al.* Synergistic and antagonistic effects of viral lysis and protistan grazing on bacterial biomass, production and diversity. *Environ. Microbiol.* **9,** 777–788 (2007).

Weitz, J. S. *et al.* A multitrophic model to quantify the effects of marine viruses on microbial food webs and ecosystem processes. *ISME J.* **9,** 1352–1364 (2015).

Williamson, S. J., Houchin, L. A., McDaniel, L. & Paul, J. H. Seasonal variation in lysogeny as depicted by prophage induction in Tampa Bay, Florida. *Appl. Environ. Microbiol.* **68,** 4307–4314 (2002).

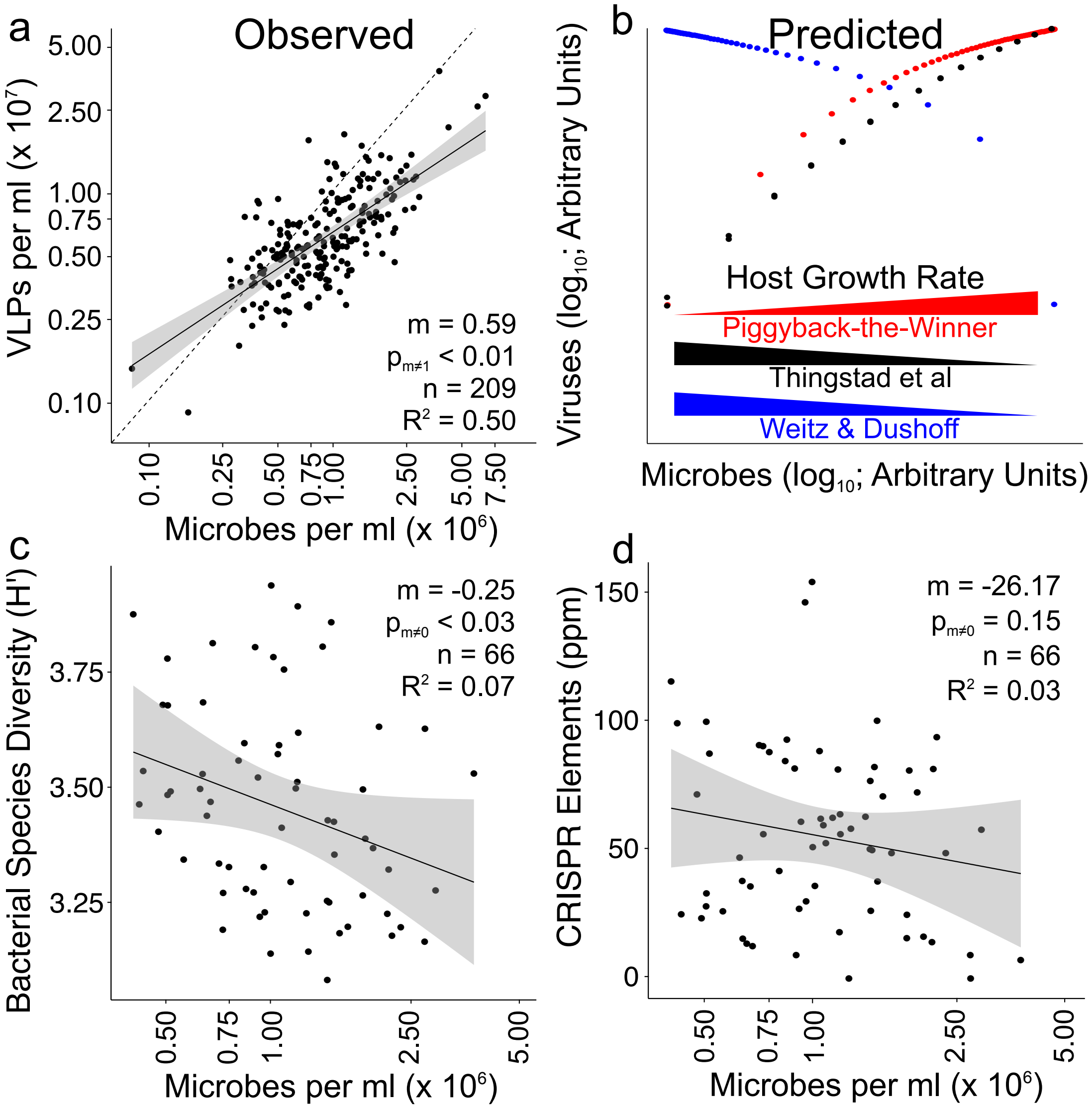


Figure 2.1: Virus-like particle (VLP) relative abundance declines with increasing host density despite lower microbial diversity and similar host sensitivity to infection, contrary to predictions of lytic models.

a, Log-transformed VLP versus microbial densities have an $m < 1$ relationship ($n = 223$ independent measures); the dashed reference line depicts a 10:1 relationship. **b**, Steady-state microbial and viral abundances and schematic microbial growth rate predicted by three modified Lotka–Volterra models: Piggyback-the-Winner (red), Thingstad *et al.* (2014; black), and Weitz and Dushoff (2008; blue). **c**, Shannon microbial species diversity versus host density (H′; $n = 66$ independent measures). **d**, Abundance of CRISPR elements in the microbial metagenomes ($n = 66$ independent measures). All slopes (m), R^2, and P values describe linear regressions testing against a slope of 0, except **a** which shows the P value from a two-sided t-test against a slope $\neq 1$. Black best-fit lines with grey 99% prediction intervals from linear regressions are shown (**a**, **c**, and **d**).

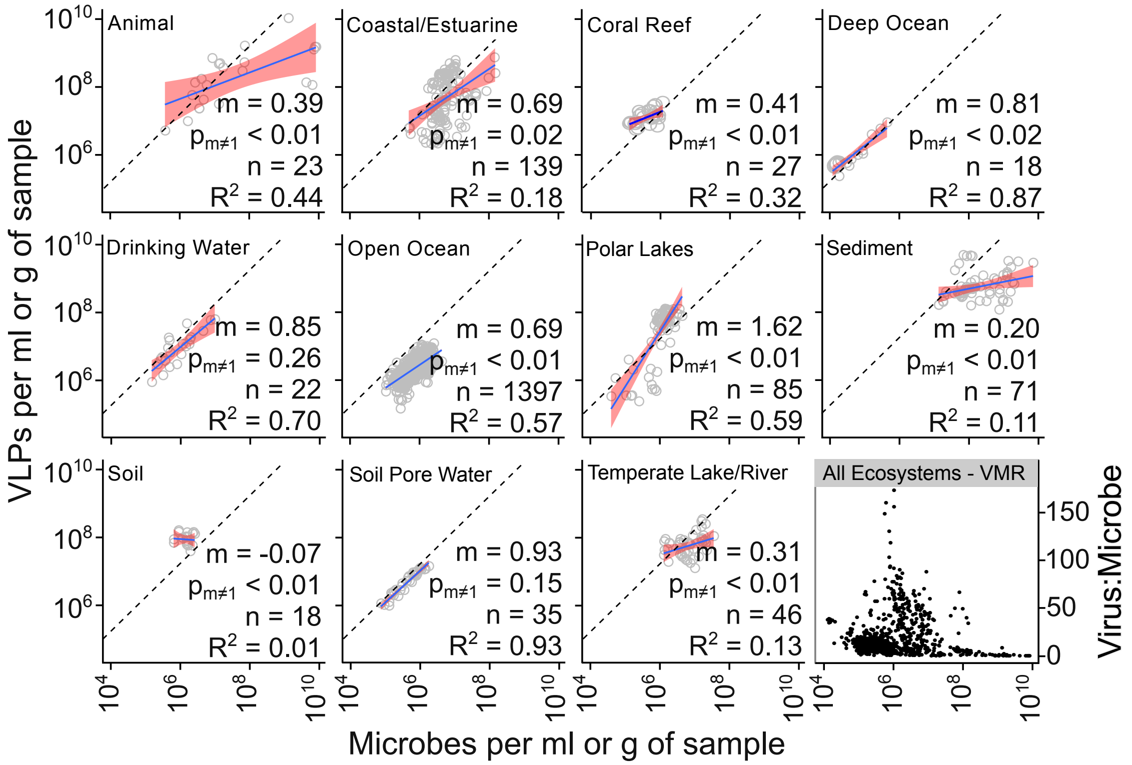


Figure 2.2: The relative decline in virus-like particles (VLPs) with increasing host density is common in disparate environmental systems.

Published microbial and VLP densities, and calculated virus to microbe ratio (with all environments pooled; final panel) are plotted by ecosystem. *n* = 23, 139, 27, 18, 22, 1397, 85, 71, 18, 35, and 46 independent measures for Animal-associated, Coastal/estuarine, Coral reef, Deep ocean, Drinking water, Open ocean, Polar lakes, Sediment, Soil, Soil pore water, and Temperate lake/river environments, respectively; pooled *n*=1,881. Dashed lines depict 10:1 linear relationships; blue lines of best fit and pink 99% prediction intervals from linear regression are shown. All slopes (*m*) and R^2 values describe linear regressions, and *P* values are derived from a two-sided *t*-test against a slope ≠ 1; details, including false-detection rate corrected values in Extended Data Table 1.

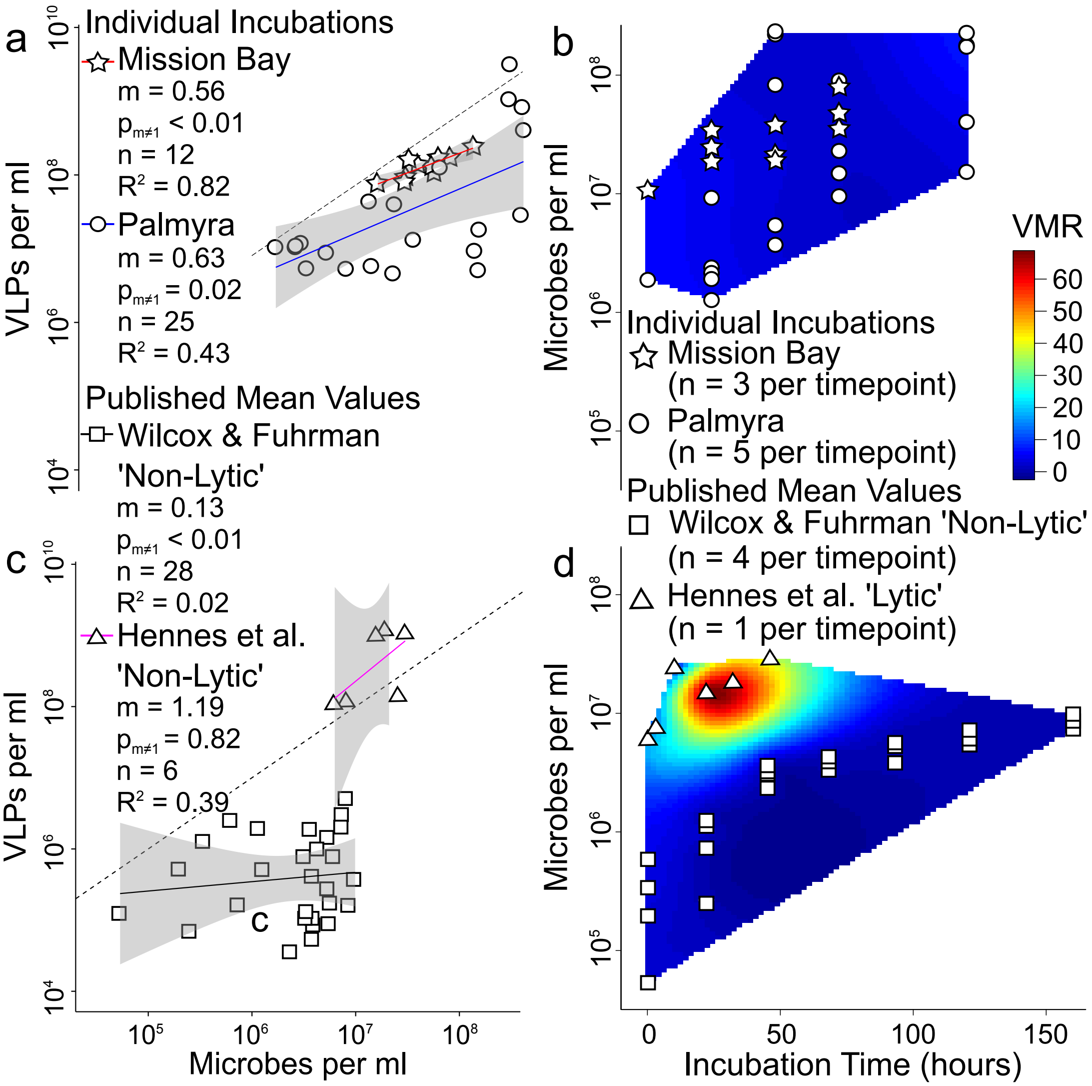


Figure 2.3: Density dependence does not drive viral predation.

a, **c**, Viral and host densities (individual counts shown) follow an $m < 1$ distribution despite high host densities (**a**; Mission Bay (stars) and Palmyra (circles) incubations; n = 12 and 25, respectively, from repeated measures), compared to putatively ‘lytic’ (slope = 1) and ‘non-lytic’ (m < 1) published data (**c**; Hennes *et al.* (1995) (triangles) and Wilcox and Fuhrman (1994) (squares), respectively; mean values shown; n = 6 and 28, respectively, from repeated measures). **b**, **d**, Microbe density and VMR over time in Mission Bay and Palmyra (**b**; individual values; n = 3 and 5 per time-point, respectively, except for time zero, when n = 1), and published putative lytic and non-lytic incubations (**d**; mean values; n = 1 and 4 per time-point, respectively) plotted over a thin plate spline. **a**, **c**, Dashed 10:1 lines, solid lines of best fit, with 99% prediction intervals in grey; all slopes (m) and R^2 values describe linear regressions, and P values are derived from a two-sided t-test against a slope $\neq 1$. Individual incubation data are shown in Extended Data Figure 2.3. Mission Bay and Palmyra incubation experiments were each conducted once.

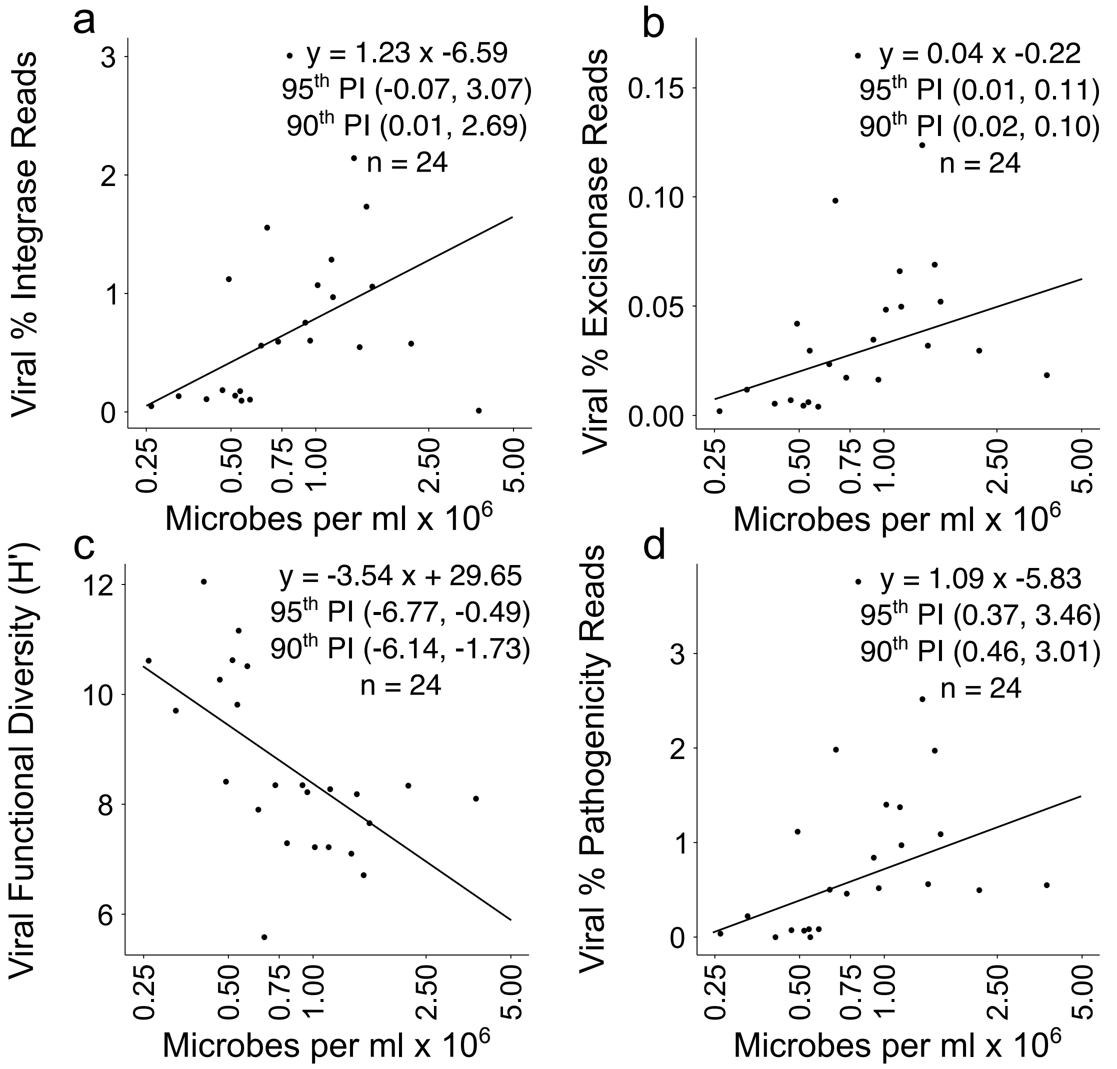
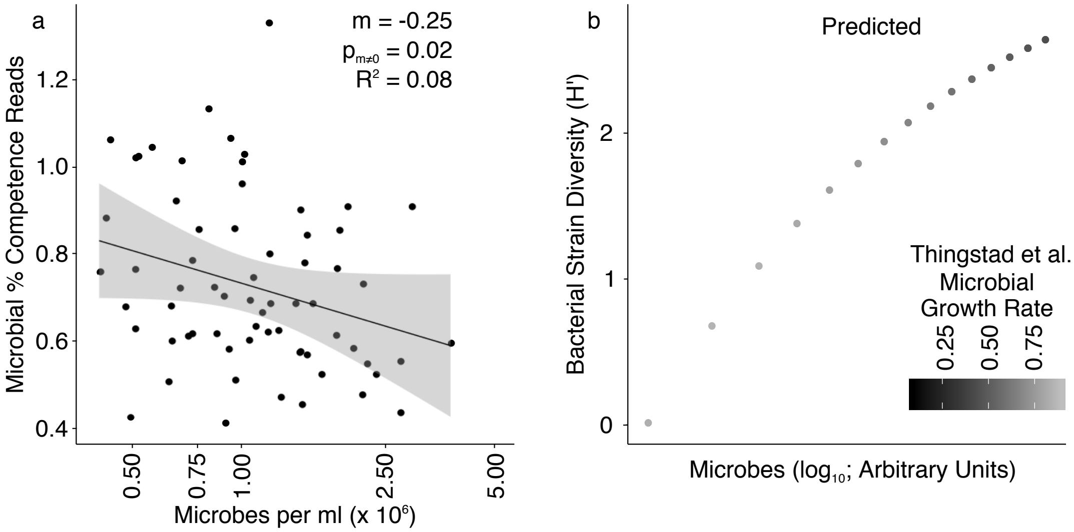
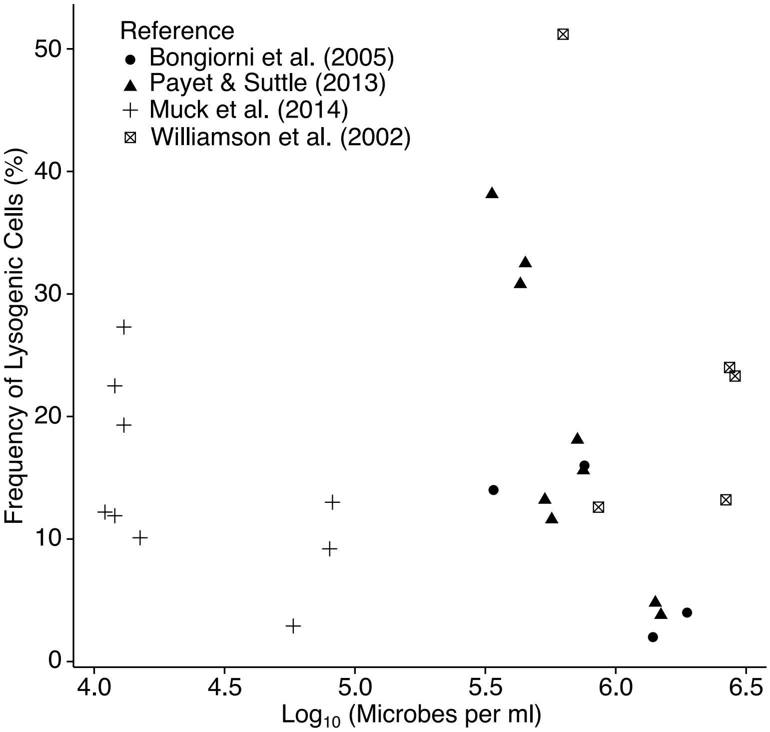


Figure 2.4: Temperate features in viromes increase with host density.
a–d, The relationship between log-transformed microbial density and the percent abundance of integrase (a), excisionase (b), and virulence reads in viromes (d), normalized by total sequences in each sample, and Shannon (H′) viral functional diversity (c) (*n* = 24 independent measures for all analyses). The linear equations and lines of best fit from robust regression and bootstrapped 95% and 90% confidence intervals (CIs) for the slopes are shown. Goodness of fit metrics are inappropriate for robust regression and are omitted.



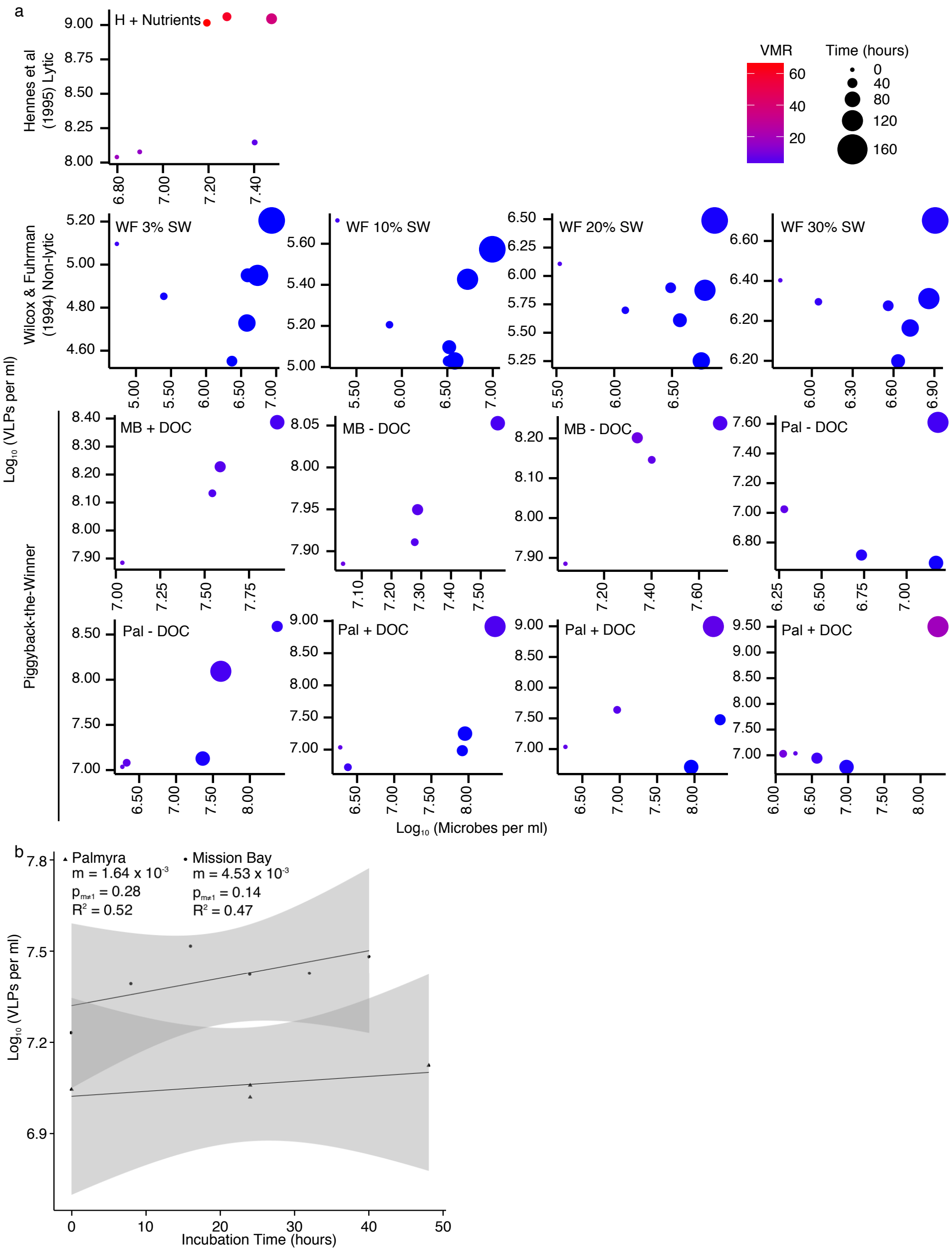
Extended Data Figure 2.1: The observed decline in virus to microberatio with increasing host density is not supported by horizontal transfer (for example, of resistance genes) under conditions where strain diversity is predicted to rise.

a, Host competence gene composition likely does not facilitate the expected rise in resistance to viral infection (*n* = 66; *m* = –0.25, *t* = –2.40, d.f. = 64, *P* = 0.02; R^2 = 0.08; microbial abundance log-transformed; linear regression). b, Lysogeny may provide strain diversification similar to the co-evolutionary diversification predicted by Thingstad *et al.* (2014) nested-infection chemostat model.



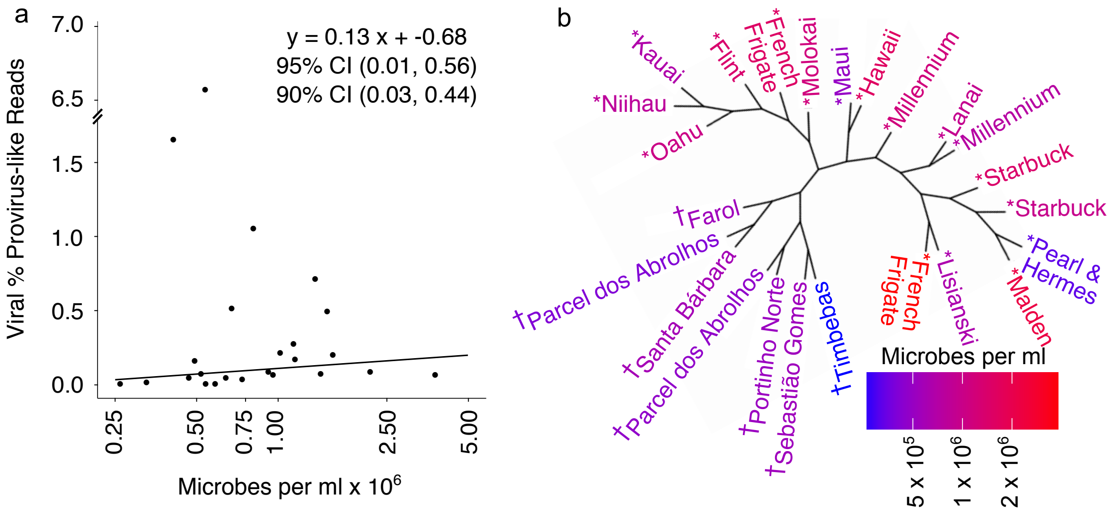
Extended Data Figure 2.2: Meta-analysis of the frequency of lysogenic cells (FLC) from mitomycin C induction experiments yields ambiguous results.

FLC from four published studies is plotted against total cell abundance. Although a sometimes-significant negative relationship exists at a within-study level (microbial abundance log-transformed; Muck*et al.* (2014), n=9, m=−10.79, t=−1.76, d.f.=7, P=0.12; R^2=0.31; Bongiorni *et al.* (2005), n = 4, m = −17.23, t = −1.91, d.f. = 2, P = 0.20; R^2= 0.65; Payet and Suttle (2013), n = 9, m = −48.31, t = −4.80, d.f. = 7, $P = 1.96 \times 10^{-3}$; R^2= 0.77; Williamson *et al.* (2002), n = 5, m = −26.08,t = −1.08, d.f. = 3, P = 0.36; R^2= 0.28; linear regression of each data set examined independently), when examined altogether across the full range of host abundances studied, no significant slope was observed (microbial abundance log-transformed; n = 27, m = −0.11, t = −0.04, d.f. = 25,P = 0.97; $R^2 = 5.94 \times 10^{-5}$; linear regression of pooled data).



Extended Data Figure 2.3: Decline in virus to microbe ratio (VMR) observed in incubations with elevated host density over time, contrasted with published values and viral decay.

a, Log-transformed VLP densityin experimental incubations is plotted against microbial host density over time (dot size) with VMR indicated by dot colour. Data from Mission Bay (MB) and Palmyra (Pal) water with DOC added (+ DOC) or not (– DOC) is complemented by the nutrient-added 'lytic' system of Hennes *et al.* (1995; H + Nutrients) as well as the 'non-lytic' dilutions (3%, 10%, 20%, and 30% final concentration seawater diluted by 0.02 µm filtered seawater) of Wilcox and Fuhrman (1994); WF 3% SW, WF 10% SW, WF 20% SW, WF 30% SW). n = 1 all incubations and published mean values.b, Significant viral decay was not observed in cell-free viral decay controls in incubation experiments (Palmyra: $n = 4$, $m = 1.64 \times 10^{-3}$ $t = 1.48$, d.f. = 2, $P = 0.28$; $R^2 = 0.52$; Mission Bay: $n = 6$, $m = 4.53 \times 10^{-3}$, $t = 1.87$, d.f. = 4, $P = 0.14$; $R^2 = 0.47$; linear regression with log-transformed viral density).



Extended Data Figure 2.4: Temperateness of viral communities increases with host density and viral functional composition change.

a, The relative composition of provirus-like reads, normalized by total sequences in each sample, increases with host density in viral metagenomes (host density log-transformed; *n* = 24 independent measures). The linear equations and line of best fit from robust regression and bootstrapped 95% and 90% confidence intervals (CIs) for the slope are shown. Goodnessof fit metrics are inappropriate for robust regression and are omitted. b, Viromes clustered by functional similarity (crAss cross-assembly), showing higher host density Pacific viromes (*) grouped away from lower host density Atlantic viromes (†); site names coloured by host density.

Extended Data Table 2.1: Summary of linear regression analyses of published microbial and viral counts.

Ecosystem	**Intercept**	**Slope**	**R^2**	**p-value ($m \neq 1$)**	**FDR**
Animal	5.34366	0.38784	0.4374	**0.00000**	**0.00000**
Coastal/Estuarine	3.03460	0.68960	0.1780	**0.01548**	**0.02123**
Coral Reef	4.84280	0.40500	0.3197	**0.00003**	**0.00007**
Deep Ocean	2.25335	0.80947	0.8713	**0.02618**	**0.03200**
Drinking Water	1.84750	0.8549	0.6967	0.26342	0.26342
Open Ocean	2.63034	0.68802	0.5708	**0.00000**	**0.00000**
Polar Lakes	-2.2782	1.6215	0.5933	**0.00006**	**0.00011**
Sediment	7.07985	0.20243	0.1062	**0.00000**	**0.00000**
Soil	8.40482	-0.07364	0.0059	**0.00037**	**0.00059**
Soil Pore Water	1.42619	0.93485	0.9328	0.14518	0.15970
Temperate Lake and River	5.75600	0.3124	0.1341	**0.00000**	**0.00000**

Extended Data Table 2.2: Summary information on the post-quality control viromes analysed.

Site	Reef area	Year	Sequencing Technology	Size (reads)	Size (bp)
Millenium	Southern Line Islands	2013	Illumina	396,009	108,504,986
French Frigate	Northwestern Hawaiian Islands	2013	Illumina	27,811	7,620,127
French Frigate	Northwestern Hawaiian Islands	2013	Illumina	341,762	93,642,528
Flint	Southern Line Islands	2013	Illumina	26,649	7,288,052
Hawaii	Main Hawaiian Islands	2013	Illumina	169,145	46,345,548
Kauai	Main Hawaiian Islands	2013	Illumina	53,601	14,659,196
Lanai	Main Hawaiian Islands	2013	Illumina	216,678	59,369,605
Lilianski	Northwestern Hawaiian Islands	2013	Illumina	197,754	54,184,414
Maui	Main Hawaiian Islands	2013	Illumina	210,548	57,689,978
Malden	Southern Line Islands	2013	Illumina	273,248	74,869,874
Millennium	Southern Line Islands	2013	Illumina	205,734	56,370,772
Molokai	Main Hawaiian Islands	2013	Illumina	131,338	35,986,536
Niihau	Main Hawaiian Islands	2013	Illumina	62,462	17,086,857
Oahu	Main Hawaiian Islands	2013	Illumina	104,948	28,702,185
Pearl & Hermes	Northwestern Hawaiian Islands	2013	Illumina	896,672	245,686,926
Starbuck	Southern Line Islands	2013	Illumina	209,210	57,322,864
Starbuck	Southern Line Islands	2013	Illumina	158,006	43,293,430
Farol	Abrolhos	2011	Ion Torrent	5,309,048	1,111,783,405
Parcel dos Abrolhos	Abrolhos	2011	Ion Torrent	3,345,804	656,547,520
Parcel dos Abrolhos	Abrolhos	2012	Ion Torrent	870,631	182,798,753
Portinho Norte	Abrolhos	2012	Ion Torrent	718,794	143,135,114
Sebastião Gomes	Abrolhos	2012	Ion Torrent	317,401	67,968,285
Santa Barbara	Abrolhos	2011	Ion Torrent	323,883	68,006,354
Timbebas	Abrolhos	2012	Ion Torrent	933,142	149,255,589

Extended Data Table 2.3: Summary of model II OLS, MA, and SMA regression analyses.

Variables tested	Method	Intercept	Slope	Slope Confidence Intervals (2.5 % / 97.5 %)	P-perm
Log10(VLP abundance) ~	OLS	3.270594	0.5909753	0.5123641/0.6695865	0.01
Log10(Microbial abundance)	MA	2.154908	0.7784587	0.6796619/0.8877203	0.01
Figure 1a	SMA	1.805399	0.8371912	0.7622627/0.9194850	NA
Microbial Species Diversity (H') ~	OLS	5.201590	-0.2899249	-0.5162249/-0.06362489	0.01
Log10(Microbial abundance)	MA	8.577445	-0.8499407	-1.9640454/-0.31929823	0.01
Figure 1c	SMA	9.189515	-0.9514762	-1.2043178/-0.75171774	NA
CRISPR elements (ppm) ~	OLS	212.9163	-26.1718	-62.41016/10.06656	0.07
Log10(Microbial abundance)	MA	5063.2712	-830.7903	2159.95654/-348.39257	0.07
Figure 1d	SMA	944.0547	-147.4593	-188.08521/-115.60849	NA
Competence gene abundance	OLS	2.224755	-0.2484529	-0.455530/-0.04137585	0.01
~ Log10(Microbial abundance)	MA	4.438175	-0.6156341	-1.349562/-0.17225329	0.01
Extended Data Figure 1a	SMA	5.945424	-0.8656695	-1.097170/-0.68301541	NA
Log10(VLP abundance) ~	OLS	5.343661	0.3878362	0.1882105/0.5874618	0.01
Log10(Microbial abundance)	MA	4.767191	0.4639165	0.2410987/0.7324118	0.01
Figure 2h - Animal	SMA	3.838768	0.5864464	0.4198659/0.8191172	NA
Log10(VLP abundance) ~	OLS	3.034594	0.6896393	0.4393031/0.9399756	0.01
Log10(Microbial abundance)	MA	-11.009437	2.7827455	2.0144638/4.2863025	0.01
Figure 2a – Coastal/estuarine	SMA	-3.304430	1.6344001	1.4031244/1.9037969	NA
Log10(VLP abundance) ~	OLS	4.842831	0.4049663	0.1616585/0.6482740	0.02
Log10(Microbial abundance)	MA	3.943664	0.5655113	0.2599340/0.9794991	0.02
Figure 2b – Coral reefs	SMA	3.099839	0.7161750	0.5130687/0.9996842	NA
Log10(VLP abundance) ~	OLS	2.253353	0.8094707	0.6445947/0.9743466	0.01
Log10(Microbial abundance)	MA	2.031353	0.8584923	0.6967851/1.0509648	0.01
Figure 2c- Deep ocean	SMA	1.991944	0.8671945	0.7178530/1.0476048	NA
Log10(VLP abundance) ~	OLS	1.8475372	0.8548761	0.5918003/1.117952	0.01
Log10(Microbial abundance)	MA	0.8154567	1.0290240	0.7527892/1.410628	0.01
Figure 2f – Drinking water	SMA	0.8442242	1.0241699	0.7943422/1.320494	NA
Log10(VLP abundance) ~	OLS	2.630340	0.6880182	0.6566834/0.7193530	0.01
Log10(Microbial abundance)	MA	1.560510	0.8836194	0.8441542/0.9246933	0.01
Figure 2d – Open ocean	SMA	1.412589	0.9106645	0.8798687/0.9425383	NA
Log10(VLP abundance) ~	OLS	-2.278235	1.621498	1.328398/1.914598	0.01
Log10(Microbial abundance)	MA	-7.623837	2.514119	2.120363/3.053842	0.01
Figure 2e – Antarctic lakes	SMA	-5.174698	2.105156	1.832362/2.418562	NA
Log10(VLP abundance) ~	OLS	7.079846	0.2024254	0.06135500/0.3434959	0.01
Log10(Microbial abundance)	MA	6.275650	0.3000129	0.09877745/0.5263175	0.01
Figure 2i - Sediment	SMA	3.628034	0.6212953	0.49603921/0.7781801	NA
Log10(VLP abundance) ~	OLS	8.404815	-0.07364098	-0.581317 0.4340350	0.45
Log10(Microbial abundance)	MA	11.689048	-0.60574792	NA/NA	0.45
Figure 2i - Soil	SMA	13.880157	-0.96074842	-1.594310/-0.5789576	NA
Log10(VLP abundance) ~	OLS	1.426192	0.9348495	0.8460122/1.023687	0.01
Log10(Microbial abundance)	MA	1.246901	0.9668013	0.8788657/1.063210	0.01
Figure 2g – Soil pore water	SMA	1.240642	0.9679167	0.8831477/1.060822	NA
Log10(VLP abundance) ~	OLS	5.756037	0.3124330	0.07122673/0.5536392	0.02
Log10(Microbial abundance)	MA	3.565635	0.6552208	0.22182386/1.3743755	0.02
Figure 2h – Temperate lakes	SMA	2.300832	0.8531567	0.64539226/1.1278047	NA
Integrase abundance ~	OLS	-4.124376	0.8290249	-0.3506466/2.008696	0.07
Log10(Microbial abundance)	MA	-48.478845	8.3292824	3.3669820/-20.091584	0.07
Figure 4a	SMA	-15.743870	2.7938596	1.8530297/4.212373	NA
Excisionase abundance ~	OLS	-0.2354934	0.04659967	-0.01650558/0.1097049	0.07
Log10(Microbial abundance)	MA	-0.2412107	0.04756645	-0.01678433/0.1123127	0.07
Figure 4b	SMA	-0.8477914	0.15013811	0.09975581/0.2259663	NA
Provirus-like sequences ~	OLS	5.291734	-0.8009354	-3.159957/1.558086	0.17
Log10(Microbial abundance)	MA	208.253969	-35.1214691	18.023316/-8.862742	0.17
Figure 4c	SMA	32.460430	-5.3951110	-8.247333/-3.529289	NA
Genetic diversity (H') ~	OLS	29.31875	-3.492783	-5.583645/-1.401920	0.01
Log10(Microbial abundance)	MA	66.09613	-9.711770	-24.038458/-6.056465	0.01
Figure 4d	SMA	43.42959	-5.878899	-8.330507/-4.148782	NA
Pathogenicity gene abundance ~	OLS	-6.065022	1.172082	-0.2624953/2.60666	0.06
Log10(Microbial abundance)	MA	-54.762269	9.406695	4.1780142/-43.26440	0.06
Figure 5e	SMA	-19.534564	3.449756	2.3015745/5.17073	NA

Chapter 2 is a prior version of a manuscript that has been published in the journal *Nature*: Knowles B, Silveira CB, Bailey BA, Barott K, Cantu VA, Cobián-Güemes AG, Coutinho FH, Dinsdale EA, Felts B, Furby KA, George EE, Green KT, Gregoracci GB, Haas AF, Haggerty JM, Hester ER, Hisakawa N, Kelly LW, Lim YW, Little M, Luque A, McDole-Somera T, McNair K, de Oliveira LS, Quistad SD, Robinett NL, Sala E, Salamon P, Sanchez SE, Sandin S, Silva GGZ, Smith J, Sullivan C, Thompson C, Vermeij MJA, Youle M, Young C, Zgliczynski B, Brainard R, Edwards RA, Nulton J, Thompson F, and F Rohwer (2016) ‘Lytic to temperate switching of viral communities.’ The dissertation author is a primary author and investigator of this manuscript.

CHAPTER 3

Examination of induction-based evidence for host density-dependence of lysogeny suggests potentially novel drivers of natural viral communities

INTRODUCTION

Conditions favoring lysogeny in the environment are controversial. Recent work using metagenomics and microbial and viral counts suggests that the lysogeny is promoted when host density and growth rate are high (B Knowles et al. 2016). In contrast, studies estimating lysogeny via induction of integrated viruses by addition of the antibiotic compound mitomycin C have shown the fraction of chemically-inducible cells (FCIC, also known as the frequency of lysogenic cells; FLC) to be inversely proportional to host density and growth rate (Jiang and Paul 1998; Maurice et al. 2013; Payet and Suttle 2013; Paul and Weinbauer 2010; Paul 2008). As a result, the established paradigm in viral ecology is that lysogeny functions mainly as a low-density refuge for temperate viruses during periods of retarded host growth that complements lytic activity when conditions and host densities recover (Payet and Suttle 2013; Weinbauer and Hofle 1998; Evans and Brussaard 2012; Jiang and Paul 1998; Paul 2008; Maurice et al. 2013).

This view of lytic/lysogenic switches describes how viral predators exert control of host diversity and densities through Kill-the-Winner lytic dynamics during blooms (Thingstad 2000) and drive biogeochemical recycling in ecosystems (Wilhelm and Suttle 1999; Suttle 2007; Fuhrman 1999; Weitz et al. 2015; Weitz and Wilhelm 2012). However, it overlooks the ecological potential for lysogeny to support host diversity and densities through development of immunity (T Frede Thingstad et al. 2014; Våge, Storesund, and Thingstad 2013) and horizontal gene transfer (B Knowles et al. 2016; Linda W Kelly et al. 2014; Hurwitz and U'Ren 2016; Roitman et al. 2015; Hevroni et al. 2015) that could allow sustained elevated microbial densities in degraded ecosystems through Piggyback-the-Winner dynamics (Haas et al. 2016; McDole et al. 2012; Linda W Kelly et al. 2014; Dinsdale et al. 2008; B Knowles et al. 2016).

Although there are now two decades of observational studies on host density-dependency of FCIC in the environment (since the publication of Jiang and Paul (1994); discussed in Paul (2008) and Paul and Weinbauer (2010)), there exists no quantitative assessment of the breadth of support for the established paradigm based on this work. A meta-analysis of published studies showed high variability and a lack of support for host density-dependence in FCIC at both global across-study and within-study levels. Although it is reasonable to exclude lysogeny estimates ≤ 0 because negative lysogeny cannot exist, the FCIC proxy for lysogeny commonly shows values that fall in this range, comprising a third of all FCIC values sampled herein. Even when this negative 'unheard third' is excluded from analysis, significantly altering conclusions drawn from inductions, FCIC values from natural communities are remarkably low compared to environmental isolates. Variability between within-site replicates was comparable to that observed across sites in published datasets, possibly confounding technical and biological variability in those studies. Dilution experiments probing the effects of host density and growth rate on FCIC estimates showed elevated variability in FCIC. Taken together these results suggest that lysogeny and its proxy, FCIC, are not globally equitable. These issues provide tantalizing clues as to what drives FCIC, coupled to lysogeny or not that may ultimately facilitate the identification of new viral dynamics. Potential new avenues of inquiry are outlined here to help future studies capitalize on this opportunity.

METHODS

Published values

Published values of the frequency of chemically-inducible cells (FCIC; %) were taken from 38 studies mined from Google Scholar that reported host density and corresponding FCIC estimated using induction via the addition of mitomycin C (Table 1) (Bettarel et al. 2006; Breitbart et al. 2004; Colombet et al. 2006; Laybourn-Parry et al. 2013; Lisle and Priscu 2004; Lymer and Lindström 2010; Maurice et al. 2010; Maurice et al. 2011; Palesse et al. 2014; Ram and Sime-Ngando 2010; Ram et al. 2013; Ram et al. 2014; Tapper and Hicks 1998; Thomas et al. 2011; Bettarel et al. 2008; Bettarel et al. 2011; Bongiorni et al. 2005; Bouvy et al. 2012; Brum et al. 2015; Cochran and Paul 1998; Cochran, Kellogg, and Paul 1998; Evans and Brussaard 2012; Jiang and Paul 1996; Laybourn-Parry, Marshall, and Madan 2007; Long et al. 2008; Maurice et al. 2013; Muck et al. 2014; Nguyen-Kim et al. 2015; Ortmann, Lawrence, and Suttle 2002; Payet and Suttle 2013; Weinbauer and Suttle 1999; Weinbauer, Brettar, and Höfle 2003; Williamson et al. 2002; Mei and Danovaro 2004; Montanié et al. 2014; Ghosh et al. 2008; Williamson et al. 2007; Weinbauer and Suttle 1996). Where necessary, values were extracted from figures using the webplotdigitizer Chrome extension tool (http://arohatgi.info/WebPlotDigitizer/app/). Most papers do not report FCIC if induced viral densities did not exceed untreated viral densities (Paul and Weinbauer 2010). Published FCIC data sets were therefore analyzed with values ≤ 0 excluded in order to ensure a consistent analysis. All within-study and global across-study linear regressions were conducted using data at the greatest resolution possible; individual measures were used whenever accessible and mean values used otherwise (Table 1).

FCIC estimation

Mitomycin C from a stock less than 1 month old was added to environmental samples to a final concentration of 1 μg ml^{-1}, consistent with the most environmental induction studies and as prescribed for high density nearshore samples (Paul and Weinbauer 2010). Samples, with corresponding mitomycin C-minus controls, were then incubated for 18 - 24 hours at room temperature in the dark, and viral densities then compared between mitomycin C-positive treatments and -negative controls. The FCIC was then calculated after Paul and Weinbauer (2010) using a burst size of 25 in these calculations.

In situ studies

Freshwater and saltwater sites around San Diego, California, USA were sampled with sterile 50 ml polypropylene tubes (Famosa Slough 32.751882°N 117.229039°W, Spanish Landing 32.728260°N 117.209929°W, Vacation Road 32.773537°N 117.232739°W, Chollas Reservoir 32.737363°N 117.062441°W, Old Mission Dam 32.839547°N 117.043067°W, and Lake Murray 32.784481°N 117.042490°W). Subsamples were aliquoted into 2 ml technical replicates in 24-well plates (technical replicates are ecological pseudoreplicates as they are intentionally not independent samples; Corning, USA). The three technical replicate fractions required to estimate FCIC (initial cell counts, final mitomycin C-added viral counts, and final mitomycin C-minus control viral counts) for each technical replicate were transferred within one syringe-draw in order to keep technical replicate fractions as coupled as possible. Mitomycin C-added and -minus samples were segregated in different plates to preclude airborne antibiotic impacts on controls. After incubation, 1 ml samples were drawn from each 2 ml well, fixed with 2 % final concentration paraformaldehyde for 30 minutes and then flash frozen in liquid nitrogen (Haas et al. 2014; B Knowles et al. 2016). Samples were thawed at room temperature immediately prior to staining with 2X Sybr Gold nucleic acid stain (Life Technologies, USA) for 30 minutes and filtered onto 0.02 um Anodisc filters (Whatman, USA; Haas et al. 2014; Knowles et al. 2016). Filters were mounted on slides and imaged on an Olympus 100X oil microscope and counts conducted using Image Pro software (Media Cybernetics, USA).

Dilution experiments

Sites sampled in the observational study above were resampled for manipulative dilution experiments. All sampling and aliquoting procedures were the same as above. However, rather than aliquot 2 ml of subsampled site water into each well of the 24-well plates, technical replicates were mixed with either 0.02 μm-filtered site water or artificial seawater buffer (Tropic Marin, Germany) at saltwater sites (Spanish Landing, Vacation Road, Famosa Slough) or Hydra Media buffer (Grasis et al. 2014) at freshwater sites (Old Mission Dam, Chollas Reservoir, Lake Murray) to produce undiluted, 25 %, 50 %, and 75 % dilutions of unfiltered water samples (undiluted, 75 %, 50 %, and 25 % unfiltered site water, respectively). Host densities in each dilution when mitomycin C incubations began were estimated as host density in undiluted samples multiplied by the dilution factor. Both artificial seawater and freshwater diluents maintain chemical buffering of site water but do not contain nutrients to sustain or enhance host growth. As a result of this, host density was diluted, with likely concomitant simplification of community composition and elevation of the dose of inducing agent per cell present, while the frequency of lysogeny and host growth rate were presumably unchanged. Dilution with site water rehabilitates carrying capacity with dilution, allowing host growth to increase proportionate to dilution in addition to the changes induced by buffer dilution. Undiluted samples (0 % dilutions) were considered ‘correct’ estimates of FCIC for comparison because they are equivalent to samples used for publication in FCIC studies. Mitomycin C addition, incubation, sample fixation, storage, and processing were the same as above, but slides were imaged using an Olympus 60X oil microscope.

Statistical analysis

All statistical analyses were conducted with an *a priori* permissive alpha of 0.1 (90 % confidence) due to prior knowledge of high variability in FCIC. Linear regressions were conducted using the lm() function in R (Figures 2 and 3). Published data sets showed a negative relationship between n and R^2, suggesting that the analysis of means, as the majority of data available for this study, facilitated finding relationships between FCIC and host density (Table 1).

As FCIC values generated here were intentionally not independent (i.e., were technical, not biological, replicates) a conservative bootstrapping, rather than parametric, approach was used to generate means and 90 % confidence intervals (Figure 4). These parameters were estimated using the boot_out() function in R with 10,000 iterations on data with all FCIC values included as well as with FCIC values ≤ 0 excluded consistent with the literature. Similarly, non-independent technical replicate dilution datasets (Figure 5) were analysed using a mixed model approach with within-site repeated measures.

RESULTS

The global distribution of FCIC studies

Two main clusters of FCIC studies exist: saltwater studies in the Gulf of Mexico and freshwater studies in France (Figure 1). Thus, while a large number of studies have delved into the prevalence of lysogeny through chemical induction, these studies have focused on concentrated areas with approximately 20 % of all FCIC studies stemming from each of these clusters (21.05 % from saltwater systems centered on the Gulf of Mexico, 18.42 % from freshwater in France). Of the 38 studies surveyed here, 35 were in aquatic environments (21 saltwater and 14 freshwater studies; 92.11 % of all studies combined), while three were focused on sediments and two on soils. This lead to 91.06 % of published data points coming from aquatic rather than particulate environments (326 points from fresh and salt water combined compared to 32 from pooled sediment and soil).

Host density-dependence of FCIC across studies in published datasets

A survey of published FCIC values, segregated into freshwater, saltwater, sediment, and soil environments, revealed a high degree of variability when FCIC was plotted against host density (Figure 2; Extended Data Figure 1). Global regressions in freshwater, saltwater, and sediment environments showed a lack of significant effect of host density on FCIC (Figure 2; freshwater: $p = 0.13$, n = 147, m, = -0.17, $R^2 = 0.02$; saltwater: $p = 1.00$, n = 179, m, = 5.13×10^{-5}, $R^2 =$

3.93×10^{-9}; sediment: $p = 0.32$, n = 19, m, = -0.10, $R^2 = 0.06$; linear regressions with FCIC and host density log-transformed). The only environment with a slope significantly different to zero was soil, where a positive relationship was observed ($p < 0.01$, n = 13, m, = 0.20, $R^2 = 0.56$; linear regressions with FCIC and host density log-transformed). Global regression lines explained 56 %, 6 %, 2 %, and < 1 % of the variability between FCIC and host density in soil (2 studies), sediment (3 studies), freshwater (14 studies), and saltwater (21 studies) systems, respectively (Figure 2). Similar to the within-study analysis, the more studied the system, the higher the variability unexplained by host density as a predictor.

Host density-dependence of FCIC within studies in published datasets

The median range in FCIC values in individual studies was 14.55, 25.63, 3.90, and 36.55 % respectively in freshwater, saltwater, sediment, and soil environments (freshwater: 1.10, 8.33, 14.55, 60.77, 90.71; saltwater: 2.90, 13.80, 25.63, 35.20, 90.90; sediment: 3.28, 3.59, 3.90, 5.17, 6.43; soil: 11.00, 23.78, 36.55, 49.33, 62.10; minimum, 25th, 50th (median), 75th percentiles, and maximum ranges in each environment; Figure 2). While the most common trends linking FCIC and host density were negative in freshwater, saltwater, and sediment environments, 35.71 %, 28.57 %, and 33.33 % of studies in these environments, respectively, showed positive trends (slopes of linear regressions with FCIC and host density log-transformed; Figure 3a, Extended Data Figure 1) and both studies in soil had positive slopes. This ambiguity is also reflected in the prevalence of insignificant relationships between FCIC and host density in individual studies (Figure 3a, Table 1, Extended Data Figure 1; *p*-values of linear regressions with FCIC and host density log-transformed), where only nine significant relationships were observed at the permissive 90% confidence level in 38 analyses (76.32 % of analyses showed insignificant slopes; Table 1). Finally, environments with higher sampling effort to date, either as individual data points or as number of studies, such as fresh and salt water, have lower R^2 values than lesser-sampled environments like sediment and soil (Table 1, Figure 2).

The distribution of published FCIC values

FCIC data from all studies shows a truncated normal distribution centered approximately around zero % FCIC when pooled together, where excluded values would be expected to fill out the lower range of the distribution (Figure 3b). The most common FCIC value across all environments is 0 - 5 % FCIC in these skewn datasets. Freshwater, saltwater, and sediment environments had 0 - 5 % FCIC as the most common observation, while soil was once again dissimilar to those environments and had a 20 - 25 % FCIC peak.

The frequency of FCIC values ≤ 0

In order to assess the frequency and distribution of FCIC values ≤ 0, technical replicates were collected and subjected to chemical induction from three saltwater and three freshwater sites. A high degree of variation in FCIC was observed within and between sites (Figure 4a), with within-site average FCIC values from -2.17 % to 9.61 % observed (Chollas Reservoir: 1.34 (0.03, 2.66);

Famosa Slough: 4.62 (-1.21, 8.15); Lake Murray: -2.17 (-6.88, 1.20); Old Mission Dam: 9.61 (5.68, 13.19); Spanish Landing: 4.38 (-0.54, 8.69); Vacation Road: 3.34 (2.03, 4.77); all sites pooled: 3.52 (1.67, 5.35); all measures bootstrapped mean % FCIC (90 % CI); Figures 4a and 4b). Four of these sites showed higher variability between technical replicates than was observed in a quarter of published freshwater and saltwater environmental studies (1.10, 10.20, 24.55, 52.50, 90.90 % FCIC; minimum, 25th, 50th (median), 75th quartiles, and maximum ranges; shown in Figure 5c) with ranges up to 14.51 % observed (Chollas Reservoir: 3.54; Famosa Slough: 13.31; Lake Murray: 11.14; Old Mission Dam: 11.59; Spanish Landing: 14.51; Vacation Road: 4.35; ranges % FCIC; Figure 4a). Spanish Landing, the site with the highest variability in FCIC, showed both negative and positive values (FCIC ranged from -4.17 to 10.34 %; coefficient of variation: 1.44), with a remarkably consistent host density across replicates (ranging from 5.09×10^6 to 6.55×10^6 cells per ml; coefficient of variation: 0.11; Figure 4a). FCIC values ≤ 0 were observed in four of six sites and spanned the full range of host densities, indicating that 'unsuccessful' induction events or inhibition are not host density- or site-dependent (Figure 4a).

When all data points were considered, half of all sites showed no significant evidence of lysogeny (i.e., FCIC values with bootstrapped 90 % confidence intervals that included zero; Famosa Slough, Lake Murray, and Spanish Landing; Figure 4b). However, when values ≤ 0 were excluded, this conclusion was reversed; it appeared that significant levels of lysogeny were detected at all sites (Figure 4b) as means increased and confidence intervals constricted in those sites, an effect observed without significant effect in Chollas Reservoir samples also (Chollas Reservoir: 1.79 (0.62, 2.95); Famosa Slough: 7.67 (6.99, 8.35); Lake Murray: 1.29 (1.11, 1.47); Old Mission Dam: 9.60 (5.45, 13.19); Spanish Landing: 7.22 (5.01, 9.43); Vacation Road: 3.34 (2.03, 4.77); all sites pooled: 5.49 (4.02, 7.05); all measures bootstrapped mean % FCIC (90 % CI) with FCIC values ≤ 0 excluded; Figures 4a and 4b). Every site with values ≤ 0 were therefore biased by exclusion of these values. Similar to the published FCIC datasets, most FCIC values from our survey fell between 0 and 5 % (Figure 4c). When all data points are considered (Figure 4c), a normal distribution centered around zero is observed, indicating the true distribution and extent of the excluded data in Figure 2c.

Experimental manipulation and FCIC

Host density is not a significant driver of FCIC or of the generation of FCIC values ≤ 0. However, previous work had shown FCIC to vary with host growth rate (Payet and Suttle 2013), and potentially increased sensitivity to mitomycin C treatment in rapidly growing hosts (Stopar et al. 2004). To probe the potential interactions between inducibility, host density, and host growth rate we estimated FCIC in communities in technical replicates diluted either with nutrient free buffers (low growth) or filtered site water (high growth). It was expected that higher dilutions would lead to increased FCIC estimates due to the higher mitomycin C does per microbe, compounded by increased sensitivity to mitomycin C, especially in the higher nutrient site water dilutions.

However, rather than showing monotonic changes in FCIC with dilution in either dilution experiments, dilution actually led to an inconsistent and dramatic increase in FCIC variability (Figures 5a and 5b) compared to the undiluted technical replicates (Figure 5c). Sites showed ranges of 39.19, 16.17, 22.70, 19.58, 38.39, and 15.39 % FCIC in buffer dilutions at Chollas Reservoir, Famosa Slough, Lake Murray, Old Mission Dam, Spanish Landing, and Vacation Road, respectively. Similarly ranges of 32.73, 24.03, 13.19, 16.88, 17.86, and 56.36 % FCIC were observed in sitewater dilutions from the same sites (Figure 5c). Variability was inconsistent with dilution or diluent in most sites (Figure 5a, 5b). Further, variation within the diluted technical replicates from four of six sites (Chollas Reservoir, Spanish Landing, and Vacation Road) exceeded that observed across sites in half of the published freshwater and saltwater studies (Figure 5c); variability in local dynamics appears to equal the impact of broader ecological drivers on FCIC. This large variability, coupled with low sample size precluded finding significant changes in FCIC across dilutions ($p > 0.1$ for all dilutions; linear mixed effects model with FCIC and estimated cells per ml log-transformed). Despite this lack of statistical support, dilutions showed biologically meaningful departures from the ‘correct’ undiluted sample; dilution lead to an up to 39.19 % (mean 15.88 % FCIC) overestimate of lysogeny, measured as deviation from the undiluted samples (Figures 5a and 5b). As above, FCIC values showed a normal distribution during dilutions (Figure 5d). A third of the FCIC values measured under natural (Figure 4a) experimental manipulation (Figure 5a, 5b) conditions were ≤ 0 (25 out of 74 values; 33.78 %) and would normally have been excluded (see Figure 3b).

DISCUSSION

Host density as a driver of FCIC in published datasets

A literature survey of 38 published mitomycin C lysogen induction studies showed high variability and a global lack of host density dependence in lysogeny (FCIC) in freshwater, saltwater, and sediment environments and a significant increase in FCIC with host density in soils (Figure 2). Further, there was no consensus in the relationships identified in different studies between host density and FCIC (Figure 3a; Table 1; Extended Data Figure 1). Rather than relationships between host density and FCIC becoming more constrained with increased sampling, variability rose with higher sampling effort both at the within-study and across-study levels, even though the vast majority of studies focused on two geographic areas (Table 1; Extended Data Figure 2; Figure 1). This shows a comprehensive, multi-level lack of support for the current paradigm in environmental virology that suggests lysogeny is inversely proportional to host density (Payet and Suttle 2013; Weinbauer and Hofle 1998; Evans and Brussaard 2012; Jiang and Paul 1998; Paul 2008; Maurice et al. 2013). There are more important drivers of environmental lysogeny left to explore.

Removing the 'Unheard Third' of FCIC values ≤ 0

FCIC Values ≤ 0 were observed across the range host densities and sites here (Figures 4a and 5a), and made up a third of the values herein (25 of 74 values in Figures 4 and 5), suggesting that this 'unheard third' likely permeates the literature. This has great potential to bias interpretation of FCIC values: when the ‘unheard third’ was included in the analysis presented in Figure 4b; inclusion of these values showed there was no evidence of lysogeny in the majority of sites when exclusion suggested the opposite. The incorporation of this unheard third has the potential to alter our understanding of environmental lysogeny as measured through induction. Further, the exclusion of FCIC values ≤ 0 in the literature suggests that the accuracy of induction as a measure of lysogeny (which cannot be < 0) is not trusted outside of the range of positive values. However, retaining the unheard third in future studies will allow the opportunity to calibrate FCIC as a measure of lysogeny, and allow the accurate back-calculation of more robust estimates from already-published work.

Induction in mixed communities vs. isolates

Previous work with bacterial isolates has shown that 43 % (Jiang and Paul 1994), 25 - 62.5 % (Jiang and Paul 1998), and 71 % (Stopar et al. 2004) of bacterial isolates are inducible with mitomycin C. Two thirds of viruses with known lifestyles and sequenced genomes are temperate (McNair, Bailey, and Edwards 2012). The lack of significant presence of lysogeny found at the majority of sites in this study (Figures 4 and 5), and the observation that the most common published FCIC values fall between 0 % and 5 % (Figure 3b), suggests that mitomycin C induction provides an underestimate of lysogeny in natural communities. Unfortunately, this is likely obscured in the literature by exclusion of the unheard third (Figure 4b. This decoupling of isolated and mixed-community bacterial induction rates may be explained as an artifact induced by increased sensitivity of rapidly growing isolates to mitomycin C induction (Stopar et al. 2004), suggesting that decoupling between lysogeny and its FCIC proxy is possible. Experimental investigation of the linkages between actual lysogeny and the interactions between mitomycin C induction and host density (Paul and Weinbauer 2010), identity (Maurice et al. 2011), and growth rate (Stopar et al. 2004), will facilitate calibration of FCIC as a proxy for lysogeny in mixed communities as may use of more diverse inducers (Jiang and Paul 1994; Cochran, Kellogg, and Paul 1998; Wommack and Colwell 2000).

Stochastic effects of dilution on FCIC

We induced lysogens in diluted samples in order to determine if there was any systematic impact of host density on FCIC estimation in slow (buffer diluent; nutrients not restored with dilution) and fast (site water diluent; nutrients restored with dilution) growing communities, assuming that actual lysogeny did not change across dilutions as inductions were immediately commenced. The most marked effect of dilution, with either diluent, was a large increase in variability in FCIC between replicates compared to between undiluted replicates (Figure 5a, 5b vs Figure 4a). Variability in both diluents was higher between technical replicates across dilutions from within sites (up to 56.36 % range in FCIC) than was observed in the majority of studies using independent samples across sites (~ 20 % median range of FCIC observed within studies), possibly due to a decoupling between lysogeny and FCIC. The variability was non-uniform in response to mitomycin C dose per cell (dilution led to a linear increase in dose of mitomycin C per cell; Figure 5a, 5b). Dilution has consistently been shown to result in lowered taxonomic diversity, but variable changes in functional capability (Franklin et al. 2001; Philippot et al. 2013; Peter et al. 2011; Roger et al. 2016). As such, stochastic functional changes in the microbial communities with dilution are consistent with the patterns observed in FCIC (Figure 5), and warrant further investigation.

Dilution stochasticity and viral production assays:

Dilution is a common technique in the estimation of lytic and lysogenic viral production (e.g., Wilhelm, Brigden, and Suttle 2002; Weinbauer and Suttle 1996; Thomas et al. 2011; Maurice et al. 2010). Similar to the dilution experiments conducted here, this approach assumes that lysogeny does not change with dilution. However, here we show that FCIC actually varies considerably with dilution, especially in a manner inconsistent with dilution factor. If this variability is real, and rates of lysogeny change during dilution, then the estimates of viral production derived from dilution may contain methodological artifacts. In contrast, if rates of lysogeny remain constant during the estimation of viral production, then dilution reveals a decoupling of lysogeny and the FCIC proxy, subverting lysogeny studies by confounding technical and biological variability. Pairing FCIC and viral production estimates using the dilution approach in future studies would allow the de-convolution of these potential artifacts as well as provide complementary information on the conditions that drive decoupling of FCIC from lysogeny and the impacts of altered viral lifecycles on viral production.

The Lurking Variable that drives of FCIC

While host density does not drive the distribution of the FCIC proxy, it shows a remarkably constrained and consistent distribution in published studies (Figure 3b), undiluted samples (Figure 4c), and diluted samples (Figure 5c). As a non-random parameter, FCIC appears to be an informative metric that may be driven by environmental condition(s); this study provides some clues to the nature of those conditions. This 'lurking variable' can be characterized as: (i) something that varies significantly between sites, even those with similar abiotic conditions (e.g., temperature, salinity); (ii) shows a gradation between the particulate environments of soil and sediment, is stable in each of these systems, and is variable in the non-particulate aquatic environments between; (iii) varies stochastically with dilution (i.e., with taxonomic diversity); (iv) is conserved across microbial densities, and (v) is not likely host density or growth rate. A number of variables could fit this description, with taxonomic and functional composition chief among them. However, while techniques such as metagenomics can allow functional profiling and identification of prophage elements (Roux et al. 2015; Arndt et al. 2016), casual assignment of which functions are important, or determination of the prevalence of lysogeny is challenging. However, a growing array of complementary traditional (e.g., counts, induction, radiotracers) and cutting edge (e.g., metagenomics, bioinformatics) techniques are available in the search for this lurking variable now that its profile has been defined.

SUMMARY POINTS

- The Fraction of Chemically Inducible Cells (FCIC) is not host density dependent.
- Analysis of FCIC studies does not ultimately provide support for that paradigm that lysogeny is a low host density refuge for viruses.
- Up to one third of FCIC values may be excluded from published studies as they are ≤ 0, but the distribution of these values is unknown.
- Exclusion of this ‘unheard third’ is demonstrated to provided artificial evidence of lysogeny where none is actually found when unabridged datasets are analyzed.
- Even with this bias, evidence of lysogeny in mixed communities is markedly lower than expected from studies of environmental isolates, indicating that mitomycin C induction is not equivalent in mixed communities and individual strains.
- Dilution led to increased variability in FCIC with implications for viral production assessment using dilution techniques.
- However, the issues identified in this paper actually present opportunities to better understand viral dynamics: a new driver has appeared.
- By facilitating the profiling of this hitherto unknown driver of FCIC (coupled to lysogeny or not, FCIC likely remains an informative metric), the observed inconsistencies in FCIC provide tantalizing evidence of novel

dynamics that may allow viral ecology to transcend established drivers such as host density and growth rate in future research.

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REFERENCES

Arndt, David, Jason R Grant, Ana Marcu, Tanvir Sajed, Allison Pon, Yongjie Liang, and David S Wishart. 2016. "PHASTER: A Better, Faster Version of the PHAST Phage Search Tool." *Nucleic Acids Research*. Oxford Univ Press, gkw387.

Bettarel, Yvan, Robert Arfi, Thierry Bouvier, Marc Bouvy, Enora Briand, Jonathan Colombet, Daniel Corbin, and Télesphore Sime-Ngando. 2008. "Virioplankton Distribution and Activity in a Tropical Eutrophicated Bay." *Estuarine, Coastal and Shelf Science* 80 (3). Elsevier: 425–29.

Bettarel, Yvan, Thierry Bouvier, Corinne Bouvier, Claire Carré, Anne Desnues, Isabelle Domaizon, Stéphan Jacquet, Agnès Robin, and Télesphore Sime-Ngando. 2011. "Ecological Traits of Planktonic Viruses and Prokaryotes along a Full-Salinity Gradient." *FEMS Microbiology Ecology* 76 (2). The Oxford University Press: 360–72.

Bettarel, Yvan, Marc Bouvy, Claire Dumont, and Telesphore Sime-Ngando. 2006. "Virus-Bacterium Interactions in Water and Sediment of West African Inland Aquatic Systems." *Applied and Environmental Microbiology* 72 (8). United States: 5274–82. doi:10.1128/AEM.00863-06.

Bongiorni, Lucia, Mirko Magagnini, Monica Armeni, Roberto Danovaro, and Rachel Noble. 2005. "Viral Production , Decay Rates , and Life Strategies along a Trophic Gradient in the North Adriatic Sea Viral Production , Decay Rates , and Life Strategies along a Trophic Gradient in the North Adriatic Sea." doi:10.1128/AEM.71.11.6644.

Bouvy, Marc, Marine Combe, Yvan Bettarel, Christine Dupuy, Emma Rochelle-Newall, and Loic Charpy. 2012. "Uncoupled Viral and Bacterial Distributions in Coral Reef Waters of Tuamotu Archipelago (French Polynesia)." *Marine Pollution Bulletin* 65 (10). Elsevier: 506–15.

Breitbart, Mya, Linda Wegley, Steven Leeds, Tom Schoenfeld, and Forest Rohwer. 2004. "Phage Community Dynamics in Hot Springs." *Applied and Environmental Microbiology* 70 (3). American Society for Microbiology: 1633–40. doi:10.1128/AEM.70.3.1633-1640.2004.

Brum, Jennifer R, Bonnie L Hurwitz, Oscar Schofield, Hugh W Ducklow, and Matthew B Sullivan. 2015. "Seasonal Time Bombs: Dominant Temperate Viruses Affect Southern Ocean Microbial Dynamics." *ISME J*, August. International Society for Microbial Ecology, 1–13.

http://dx.doi.org/10.1038/ismej.2015.125.

Cochran, Pamela K, Christina A Kellogg, and John H Paul. 1998. “Prophage Induction of Indigenous Marine Lysogenic Bacteria by Environmental Pollutants.” *Marine Ecology-Progress Series* 164: 125.

Cochran, Pamela K, and John H Paul. 1998. “Seasonal Abundance of Lysogenic Bacteria in a Subtropical Estuary.” *Applied and Environmental Microbiology* 64 (6). Am Soc Microbiol: 2308–12.

Colombet, J, Télesphore Sime-Ngando, H M Cauchie, G Fonty, L Hoffmann, and G Demeure. 2006. “Depth-Related Gradients of Viral Activity in Lake Pavin.” *Applied and Environmental Microbiology* 72 (6). Am Soc Microbiol: 4440–45.

Dinsdale, Elizabeth A, Olga Pantos, Steven Smriga, Robert A Edwards, Florent Angly, Linda Wegley, Mark Hatay, et al. 2008. “Microbial Ecology of Four Coral Atolls in the Northern Line Islands.” *PLoS ONE* 3 (2): e15841–17. doi:10.1371/journal.pone.0001584.

Evans, Claire, and Corina P D Brussaard. 2012. “Viral Lysis and Microzooplankton Grazing of Phytoplankton throughout the Southern Ocean.” *Limnology and Oceanography* 57 (6). Wiley Online Library: 1826–37.

Franklin, Rima B, Jay L Garland, Carl H Bolster, and Aaron L Mills. 2001. “Impact of Dilution on Microbial Community Structure and Functional Potential: Comparison of Numerical Simulations and Batch Culture Experiments.” *Applied and Environmental Microbiology* 67 (2). Am Soc Microbiol: 702–12.

Fuhrman, Jed A. 1999. “Marine Viruses and Their Biogeochemical and Ecological Effects.” *Nature* 399 (6736). Nature Publishing Group: 541–48.

Ghosh, Dhritiman, Krishnakali Roy, Kurt E Williamson, David C White, K Eric Wommack, Kerry L Sublette, and Mark Radosevich. 2008. “Prevalence of Lysogeny among Soil Bacteria and Presence of 16S rRNA and trzN Genes in Viral-Community DNA.” *Applied and Environmental Microbiology* 74 (2). Am Soc Microbiol: 495–502.

Grasis, Juris A, Tim Lachnit, Friederike Anton-Erxleben, Yan Wei Lim, Robert Schmieder, Sebastian Fraune, S�ren Franzenburg, et al. 2014. “Species-Specific Viromes in the Ancestral Holobiont <italic>Hydra</italic>.” *PLoS ONE* 9 (10). Public Library of Science: e109952. http://dx.doi.org/10.1371%2Fjournal.pone.0109952.

Haas, Andreas F, Mohamed F M Fairoz, Linda W Kelly, Craig E Nelson, Elizabeth A Dinsdale, Robert A Edwards, Steve Giles, et al. 2016. “Global Microbialization of Coral Reefs.” *Nature Microbiology* 1. Nature Publishing Group: 16042.

Haas, Andreas F, Ben Knowles, Yan Wei Lim, Tracey McDole Somera, Linda Wegley Kelly, Mark Hatay, and Forest Rohwer. 2014. “Unraveling the Unseen Players in the Ocean - a Field Guide to Water Chemistry and Marine Microbiology.” *Journal of Visualized Experiments : JoVE* 21 (93): 1–16. doi:10.3791/52131.

Hevroni, Gur, Hagay Enav, Forest Rohwer, and Oded Béjà. 2015. “Diversity of Viral Photosystem-I psaA Genes.” *The ISME Journal* 9 (8). Nature Publishing Group: 1892–98.

Hurwitz, Bonnie L, and Jana M U’Ren. 2016. “Viral Metabolic Reprogramming in Marine Ecosystems.” *Current Opinion in Microbiology* 31. Elsevier: 161–68.

Jiang, S C, and J H Paul. 1998. “Significance of Lysogeny in the Marine Environment: Studies with Isolates and a Model of Lysogenic Phage Production.” *Microbial Ecology* 35 (3-4). Springer: 235–43.

Jiang, Sunny C, and John H Paul. 1994. “Seasonal and Diel Abundance of Viruses and Occurrence of Lysogeny/bacteriocinogeny in the Marine Environment.” *Marine Ecology-Progress Series* 104: 163.

———. 1996. “Occurrence of Lysogenic Bacteria in Marine Microbial Communities as Determined by Prophage Induction.” *Marine Ecology-Progress Series* 142: 27.

Kelly, Linda W, Gareth J Williams, Katie L Barott, Craig A Carlson, Elizabeth A Dinsdale, Robert A Edwards, Andreas F Haas, et al. 2014. “Local Genomic Adaptation of Coral Reef-Associated Microbiomes to Gradients of Natural Variability and Anthropogenic Stressors.” *Proceedings of the National Academy of Sciences of the United States of America* 111 (28). United States: 10227–32. doi:10.1073/pnas.1403319111.

Knowles, B, C B Silveira, B A Bailey, K Barott, V A Cantu, A G Cobián-Güemes, F H Coutinho, et al. 2016. “Lytic to Temperate Switching of Viral Communities.” *Nature* advance online publication (March). Nature Publishing Group, a division of Macmillan Publishers Limited. All Rights Reserved. http://dx.doi.org/10.1038/nature17193.

Laybourn-Parry, Johanna, Alexandre M Anesio, Nanette Madan, and Christin Säwström. 2013. “Virus Dynamics in a Large Epishelf Lake (Beaver Lake, Antarctica).” *Freshwater Biology* 58 (7). Wiley Online Library: 1484–93.

Laybourn-Parry, Johanna, William A Marshall, and Nanette J Madan. 2007. “Viral Dynamics and Patterns of Lysogeny in Saline Antarctic Lakes.” *Polar Biology* 30 (3). Springer: 351–58.

Lisle, J T, and J C Priscu. 2004. “The Occurrence of Lysogenic Bacteria and Microbial Aggregates in the Lakes of the McMurdo Dry Valleys, Antarctica.” *Microbial Ecology* 47 (4): 427–39. doi:10.1007/s00248-003-1007-x.

Long, Amy, Lauren D McDaniel, Jennifer Mobberley, and John H Paul. 2008. “Comparison of Lysogeny (Prophage Induction) in Heterotrophic Bacterial and Synechococcus Populations in the Gulf of Mexico and Mississippi River Plume.” *The ISME Journal* 2 (2). Nature Publishing Group: 132–44.

Lymer, David, and Eva S Lindström. 2010. “Changing Phosphorus Concentration and Subsequent Prophage Induction Alter Composition of a Freshwater Viral Assemblage.” *Freshwater Biology* 55 (9). Wiley Online Library: 1984–96.

Maurice, C F, Thierry Bouvier, J Comte, F Guillemette, and P A Del Giorgio. 2010. “Seasonal Variations of Phage Life Strategies and Bacterial Physiological States in Three Northern Temperate Lakes.” *Environmental Microbiology* 12 (3). Wiley Online Library: 628–41.

Maurice, C. F., C. Bouvier, R. Wit, and T. Bouvier. 2013. “Linking the Lytic and Lysogenic Bacteriophage Cycles to Environmental Conditions, Host Physiology and Their Variability in Coastal Lagoons.” *Environmental Microbiology* 15 (9). Wiley Online Library: 2463–75.

Maurice, Corinne F, David Mouillot, Yvan Bettarel, Rutger De Wit, Hugo Sarmento, and Thierry Bouvier. 2011. “Disentangling the Relative Influence of Bacterioplankton Phylogeny and Metabolism on Lysogeny in Reservoirs and Lagoons.” *The ISME Journal* 5 (5). Nature Publishing Group: 831–42.

McDole, Tracey, James Nulton, Katie Barott, Ben Felts, Carol Hand, Mark Hatay, Hochul Lee, et al. 2012. “Assessing Coral Reefs on a Pacific-Wide Scale Using the Microbialization Score.” *PLoS One* 7 (9): e432331–10. doi:10.1371/journal.pone.0043233.

McNair, Katelyn, Barbara A Bailey, and Robert A Edwards. 2012. “PHACTS, a

Computational Approach to Classifying the Lifestyle of Phages." *Bioinformatics* 28 (5). Oxford Univ Press: 614–18.

Mei, M. L., and R. Danovaro. 2004. "Virus Production and Life Strategies in Aquatic Sediments." *Limnology and Oceanography* 49 (2): 459–70. doi:10.4319/lo.2004.49.2.0459.

Montanié, Hélene, Pascaline Ory, Francis Orvain, Daniel Delmas, Christine Dupuy, and Hans J Hartmann. 2014. "Microbial Interactions in Marine Water Amended by Eroded Benthic Biofilm: A Case Study from an Intertidal Mudflat." *Journal of Sea Research* 92. Elsevier: 74–85.

Muck, Simone, Thomas Griessler, Nicole Köstner, Adam Klimiuk, Christian Winter, and Gerhard J Herndl. 2014. "Fracture Zones in the Mid Atlantic Ridge Lead to Alterations in Prokaryotic and Viral Parameters in Deep-Water Masses." *Frontiers in Microbiology* 5. Frontiers Media SA: 264. doi:10.3389/fmicb.2014.00264.

Nguyen-Kim, Hanh, Yvan Bettarel, Thierry Bouvier, Corinne Bouvier, Hai Doan-Nhu, Lam Nguyen-Ngoc, Thuy Nguyen-Thanh, Huy Tran-Quang, and Justine Brune. 2015. "Coral Mucus Is a Hot Spot for Viral Infections." *Applied and Environmental Microbiology* 81 (17). Am Soc Microbiol: 5773–83.

Ortmann, A C, J E Lawrence, and C A Suttle. 2002. "Lysogeny and Lytic Viral Production during a Bloom of the Cyanobacterium Synechococcus Spp." *Microbial Ecology* 43 (2). Springer: 225–31.

Palesse, S, Jonathan Colombet, A S Pradeep Ram, and Télesphore Sime-Ngando. 2014. "Linking Host Prokaryotic Physiology to Viral Lifestyle Dynamics in a Temperate Freshwater Lake (Lake Pavin, France)." *Microbial Ecology* 68 (4). Springer: 740–50.

Paul, John H. 2008. "Prophages in Marine Bacteria: Dangerous Molecular Time Bombs or the Key to Survival in the Seas?" *The ISME Journal* 2 (6). Nature Publishing Group: 579–89.

Paul, John H, and Markus Weinbauer. 2010. "Detection of Lysogeny in Marine Environments." *Manual of Aquatic Viral Ecology* 4: 30–33.

Payet, Jérôme, and Curtis A Suttle. 2013. "To Kill or Not to Kill: The Balance between Lytic and Lysogenic Viral Infection Is Driven by Trophic Status." *Limnology and Oceanography* 58 (2): 465–74. doi:10.4319/lo.2013.58.2.0465.

Peter, Hannes, Sara Beier, Stefan Bertilsson, Eva S Lindström, Silke Langenheder, and Lars J Tranvik. 2011. "Function-Specific Response to Depletion of Microbial Diversity." *The ISME Journal* 5 (2). Nature Publishing Group: 351–61.

Philippot, Laurent, Aymé Spor, Catherine Hénault, David Bru, Florian Bizouard, Christopher M Jones, Amadou Sarr, and Pierre-Alain Maron. 2013. "Loss in Microbial Diversity Affects Nitrogen Cycling in Soil." *The ISME Journal* 7 (8). Nature Publishing Group: 1609–19.

Pradeep Ram, A S, and Télesphore Sime-Ngando. 2010. "Resources Drive Trade-off between Viral Lifestyles in the Plankton: Evidence from Freshwater Microbial Microcosms." *Environmental Microbiology* 12 (2). Wiley Online Library: 467–79.

Ram, A S Pradeep, S Palesse, Jonathan Colombet, M Sabart, F Perriere, and T Sime-Ngando. 2013. "Variable Viral and Grazer Control of Prokaryotic Growth Efficiency in Temperate Freshwater Lakes (French Massif Central)." *Microbial Ecology* 66 (4). Springer: 906–16.

Ram, Angia Sriram Pradeep, Stephanie Palesse, Jonathan Colombet, Antoine Thouvenot, and Telesphore Sime-Ngando. 2014. "The Relative Importance of Viral Lysis and Nanoflagellate Grazing for Prokaryote Mortality in Temperate Lakes." *Freshwater Biology* 59 (2). Wiley Online Library: 300–311.

Roger, Fabian, Stefan Bertilsson, Silke Langenheder, Omneya Ahmed, and Lars Gamfeldt. 2016. "Multiple Dimensions of Bacterial Diversity Unrelated to Functioning, Stability and Multifunctionality." *PeerJ PrePrints* 4. PeerJ Inc. San Francisco, USA: e1688v1.

Roitman, Sheila, José Flores-Uribe, Alon Philosof, Ben Knowles, Forest Rohwer, J Cesar Ignacio-Espinoza, Matthew B Sullivan, et al. 2015. "Closing the Gaps on the Viral Photosystem-I psaDCAB Gene Organization." *Environmental Microbiology* 17 (12). Wiley Online Library: 5100–5108.

Roux, Simon, Francois Enault, Bonnie L Hurwitz, and Matthew B Sullivan. 2015. "VirSorter: Mining Viral Signal from Microbial Genomic Data." *PeerJ* 3. PeerJ Inc.: e985.

Stopar, D, A Černe, M Žigman, M Poljšak-Prijatelj, and V Turk. 2004. "Viral Abundance and a High Proportion of Lysogens Suggest That Viruses Are

Important Members of the Microbial Community in the Gulf of Trieste." *Microbial Ecology* 47 (1). Springer: 1–8.

Suttle, Curtis A. 2007. "Marine Viruses — Major Players in the Global Ecosystem." *Nature Reviews Microbiology* 5 (10): 801–12. doi:10.1038/nrmicro1750.

Tapper, Mark A, and Randall E Hicks. 1998. "Temperate Viruses and Lysogeny in Lake Superior Bacterioplankton." *Limnology and Oceanography* 43 (1). Wiley Online Library: 95–103.

Thingstad, T Frede. 2000. "Elements of a Theory for the Mechanisms Controlling Abundance, Diversity, and Biogeochemical Role of Lytic Bacterial Viruses in Aquatic Systems." *Limnology and Oceanography* 45 (6). Wiley Online Library: 1320–28.

Thingstad, T Frede, Selina Våge, Julia E Storesund, Ruth-Anne Sandaa, and Jarl Giske. 2014. "A Theoretical Analysis of How Strain-Specific Viruses Can Control Microbial Species Diversity." *Proceedings of the National Academy of Sciences* 111 (21). National Acad Sciences: 7813–18.

Thomas, Rozenn, Lyria Berdjeb, Télesphore Sime-Ngando, and Stéphan Jacquet. 2011. "Viral Abundance, Production, Decay Rates and Life Strategies (Lysogeny versus Lysis) in Lake Bourget (France)." *Environmental Microbiology* 13 (3). Wiley Online Library: 616–30.

Våge, Selina, Julia E. Storesund, and T. Frede Thingstad. 2013. "Adding a Cost of Resistance Description Extends the Ability of Virus-Host Model to Explain Observed Patterns in Structure and Function of Pelagic Microbial Communities." *Environmental Microbiology* 15 (6): 1842–52. doi:10.1111/1462-2920.12077.

Weinbauer, M G, and M G Hofle. 1998. "Significance of Viral Lysis and Flagellate Grazing as Factors Controlling Bacterioplankton Production in a Eutrophic Lake." *Applied and Environmental Microbiology* 64 (2). United States: 431–38.

Weinbauer, Markus G, Ingrid Brettar, and Manfred G Höfle. 2003. "Lysogeny and Virus-Induced Mortality of Bacterioplankton in Surface, Deep, and Anoxic Marine Waters." *Limnology and Oceanography* 48 (4). Wiley Online Library: 1457–65.

Weinbauer, Markus G, and C A Suttle. 1999. "Lysogeny and Prophage Induction in Coastal and Offshore Bacterial Communities." *Aquatic Microbial Ecology* 18 (3): 217–25.

Weinbauer, Markus G, and Curtis A Suttle. 1996. "Potential Significance of Lysogeny to Bacteriophage Production and Bacterial Mortality in Coastal Waters of the Gulf of Mexico." *Applied and Environmental Microbiology* 62 (12). Am Soc Microbiol: 4374–80.

Weitz, Joshua S, and Jonathan Dushoff. 2008. "Alternative Stable States in Host--Phage Dynamics." *Theoretical Ecology* 1 (1). Springer: 13–19.

Weitz, Joshua S, Charles A Stock, Steven W Wilhelm, Lydia Bourouiba, Maureen L Coleman, Alison Buchan, Michael J Follows, et al. 2015. "A Multitrophic Model to Quantify the Effects of Marine Viruses on Microbial Food Webs and Ecosystem Processes." *The ISME Journal* 9 (6). Nature Publishing Group: 1352–64.

Weitz, Joshua S, and Steven W Wilhelm. 2012. "Ocean Viruses and Their Effects on Microbial Communities and Biogeochemical Cycles." *F1000 Biology Reports* 4 (17): 17. doi:10.3410/B4-17.

Wilhelm, S W, S M Brigden, and C A Suttle. 2002. "A Dilution Technique for the Direct Measurement of Viral Production: A Comparison in Stratified and Tidally Mixed Coastal Waters." *Microbial Ecology* 43 (1). Springer: 168–73.

Wilhelm, Steven W, and Curtis A Suttle. 1999. "Viruses and Nutrient Cycles in the Sea Viruses Play Critical Roles in the Structure and Function of Aquatic Food Webs." *Bioscience* 49 (10). Oxford University Press: 781–88.

Williamson, Kurt E, Mark Radosevich, David W Smith, and K Eric Wommack. 2007. "Incidence of Lysogeny within Temperate and Extreme Soil Environments." *Environmental Microbiology* 9 (10). Wiley Online Library: 2563–74.

Williamson, S J, L A Houchin, L McDaniel, and J H Paul. 2002. "Seasonal Variation in Lysogeny as Depicted by Prophage Induction in Tampa Bay, Florida." *Applied and Environmental Microbiology* 68 (9). Am Soc Microbiol: 4307–14.

Wommack, K Eric, and Rita R Colwell. 2000. "Virioplankton: Viruses in Aquatic Ecosystems." *Microbiology and Molecular Biology Reviews* 64 (1). Am Soc Microbiol: 69–114.

FIGURES AND TABLES

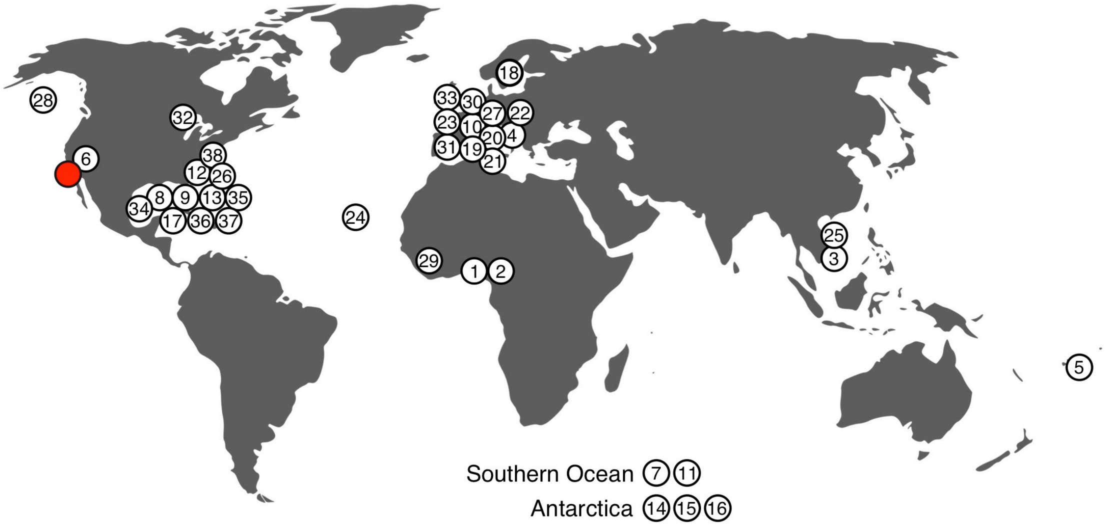


Figure 3.1: Schematic map of locations of the thirty-eight published chemically induced lysogen studies subjected to meta-analysis.

Studies were numbered by reference, showing clustering of saltwater studies in the Caribbean and freshwater studies in Western Europe. The red circle indicates the current study, with samples taken from freshwater and saltwater sites around San Diego, USA. Study numbers correspond to the superscripts shown in Table 3.1. Map downloaded from http://www.deviantart.com/art/Worldmap-113203288.

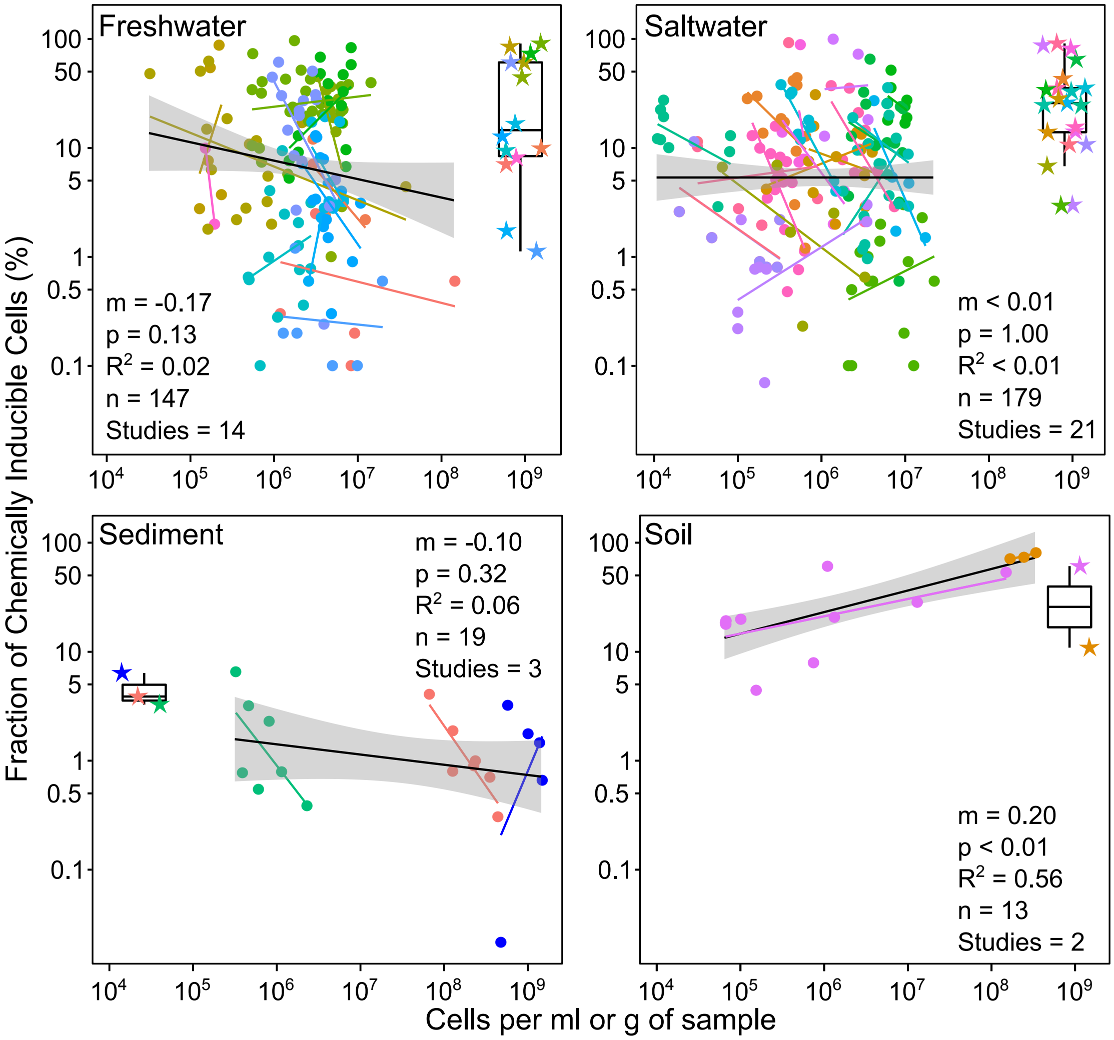


Figure 3.2**:** Reported fractions of chemically inducible cells (%; FCIC) in freshwater, saltwater, and sediment environments.

FCIC in freshwater, saltwater, and sediment environments show a lack of negative host density-dependence (cells per ml or g of sample; FCIC and host density log transformed). A lack of significant relationships between FCIC and host density were observed in freshwater, saltwater, and sediment environments, and a significant rise in FCIC with host density occurs in soils. Global lines of best fit (black) and 90% prediction intervals (grey) are overlain in each environment and summary statistics of global linear regressions shown on plots (*m*, *p*, R^2, *n*, and number of studies). Data points and associated linear regression lines of best fit from individual studies are color-coded (see Table 1 for details of each study). Prediction intervals are not shown for individual studies. Box plots show the distribution of ranges (stars) from individual studies within each environment (stars color-coded by study). Boxes show the 25th, 50th (median), and 75th percentiles while whiskers range from minimum to maximum values. Outliers are present in the marine plot; the lower whisker extends to 1.5 × the interquartile range below the 25th percentile. Boxplots are shown in sediment and soil environments for consistency despite low sample numbers. Data points from each reference are plotted individually in Extended Data Figure 1.

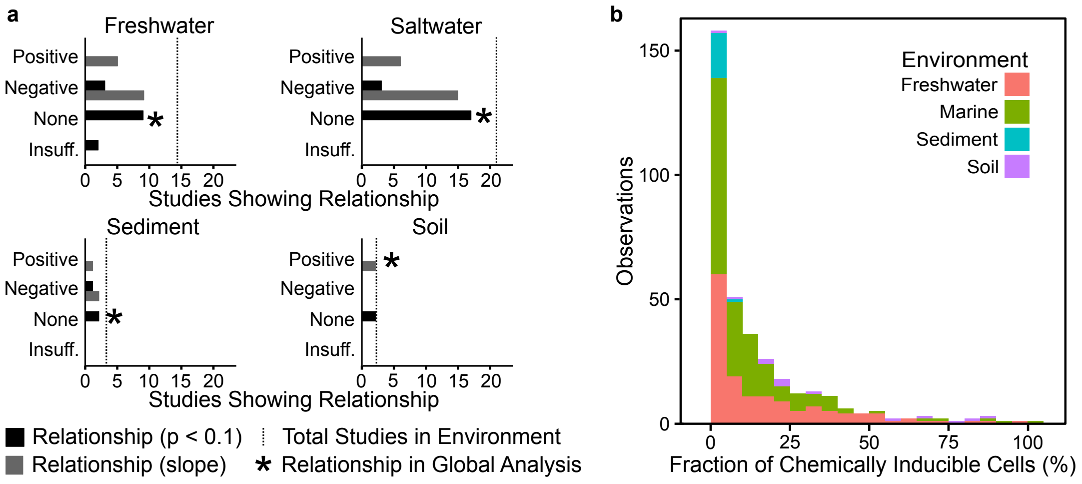


Figure 3.3: Ambiguous relationships between FCIC and host density at the within-study level and the truncated distribution of published FCIC values.

(a) Summary of the number of positive, negative, and flat relationships observed in individual studies within these environments. Significant relationships are shown as black bars (studies with $n < 3$ are shown as insufficient data ‘Insuff.’), slopes are shown as grey bars, and stars reflect the significant conclusions drawn from the global analysis in Figure 2. Dashed vertical lines show the total number of studies in a given environment, showing freshwater and saltwater environments to be more extensively studied than sediments or soils. (b) Stacked histogram of FCIC values (in 5 % bins) with the number of values in each bin, colour coded by environment, shows that the most common published FCIC values are between 0 % and 5 %, with evidence of a large number of FCIC values truncated ≤ 0. Data points from each reference are plotted individually in Extended Data Figure 3.1.

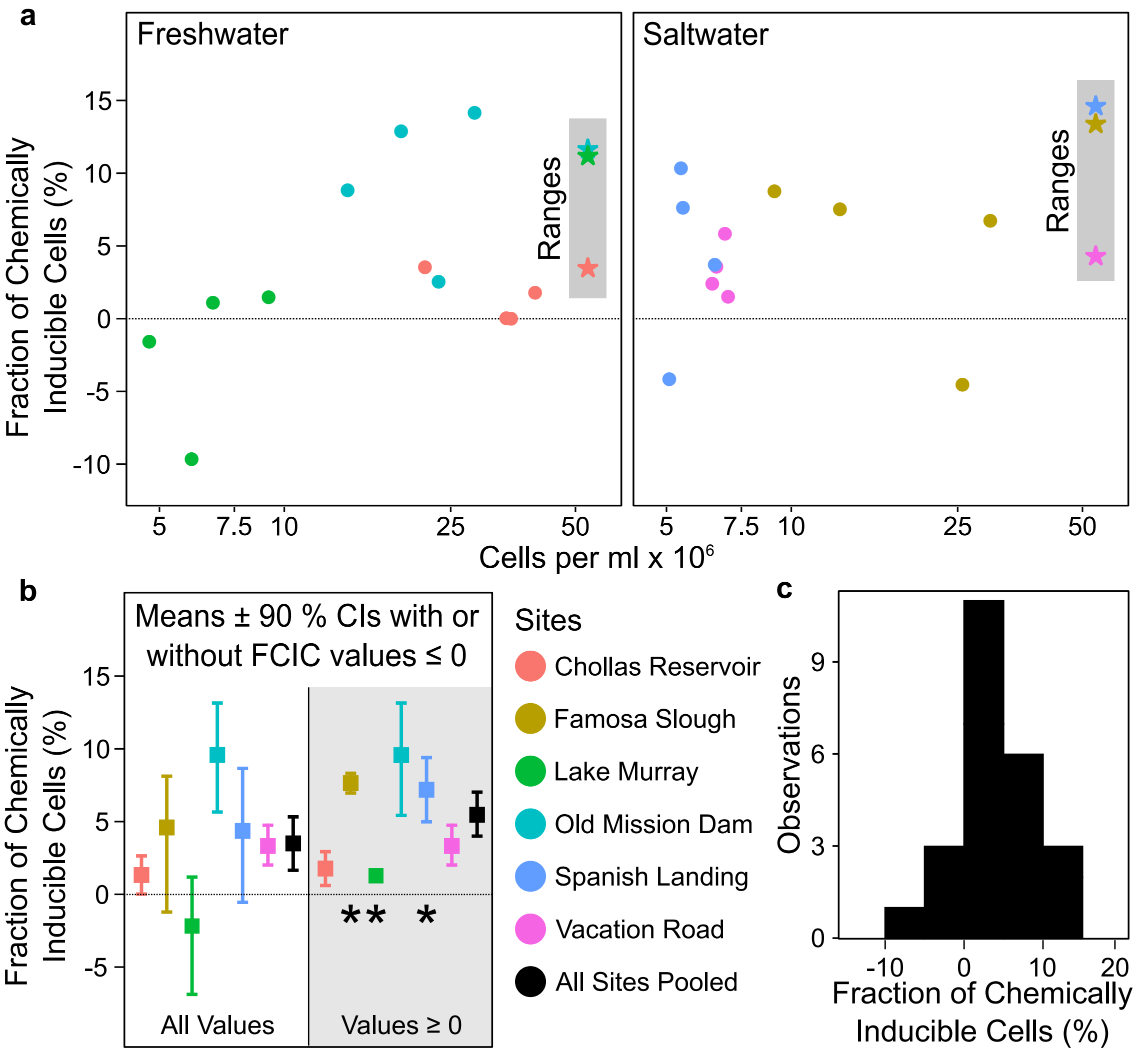


Figure 3.4: FCIC shows high variability between technical replicates and within and between sites, with significant impacts of excluding values ≤ 0 in most sites.

FCIC from technical replicates (n = 4) from three freshwater and three saltwater sites (color-coded) show high variability when plotted against log-transformed host density. Ranges of FCIC values from within technical replicates are shown in each environment (grey shaded area with stars color-coded by site). Inhibition (i.e., FCIC values < 0) occurred across the full range of host densities examined. (b) No significant evidence of lysogeny (i.e., FCIC was not significantly different to 0; 90 % confidence interval crosses the dashed 0 line) was observed in four of six sites, but this conclusion was reversed (stars; 90 % CI crosses the dashed 0 line) when FCIC values ≤ 0 were excluded. The maximum 90 % confidence interval was ± 10.38 % FCIC at Spanish Landing. Means (squares) and 90 % confidence intervals (whiskers) are shown, color-coded by site. Means rose and CIs constricted in all sites where values were excluded (Chollas Reservoir, Famosa Slough, Lake Murray, and Spanish Landing; two freshwater and two saltwater sites), as did global means and CIs (black square and whiskers). (c) The distribution of replicate FCIC values from the environment shows an approximately normal distribution centred around zero.

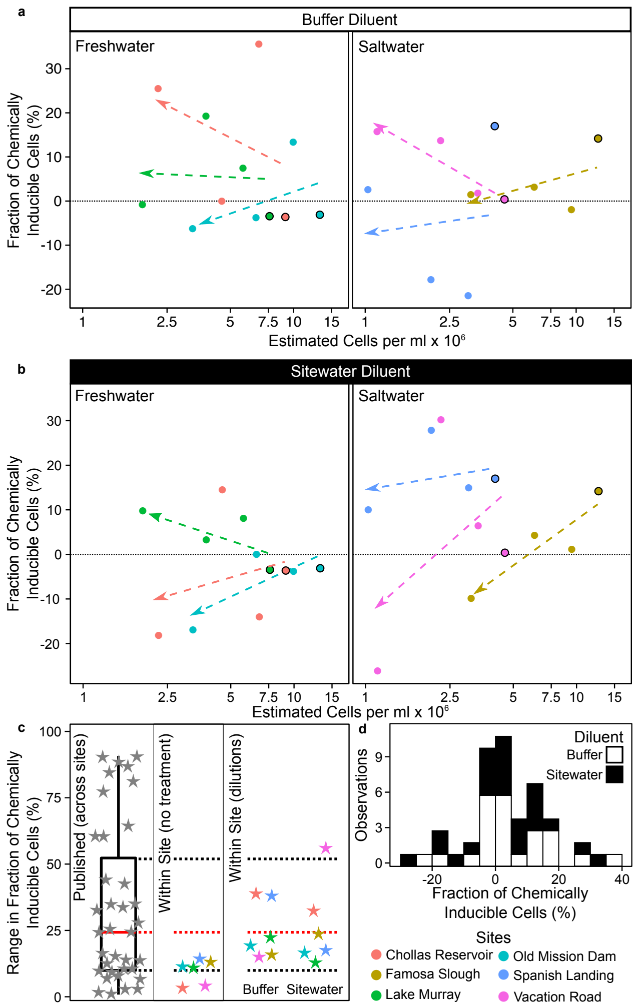
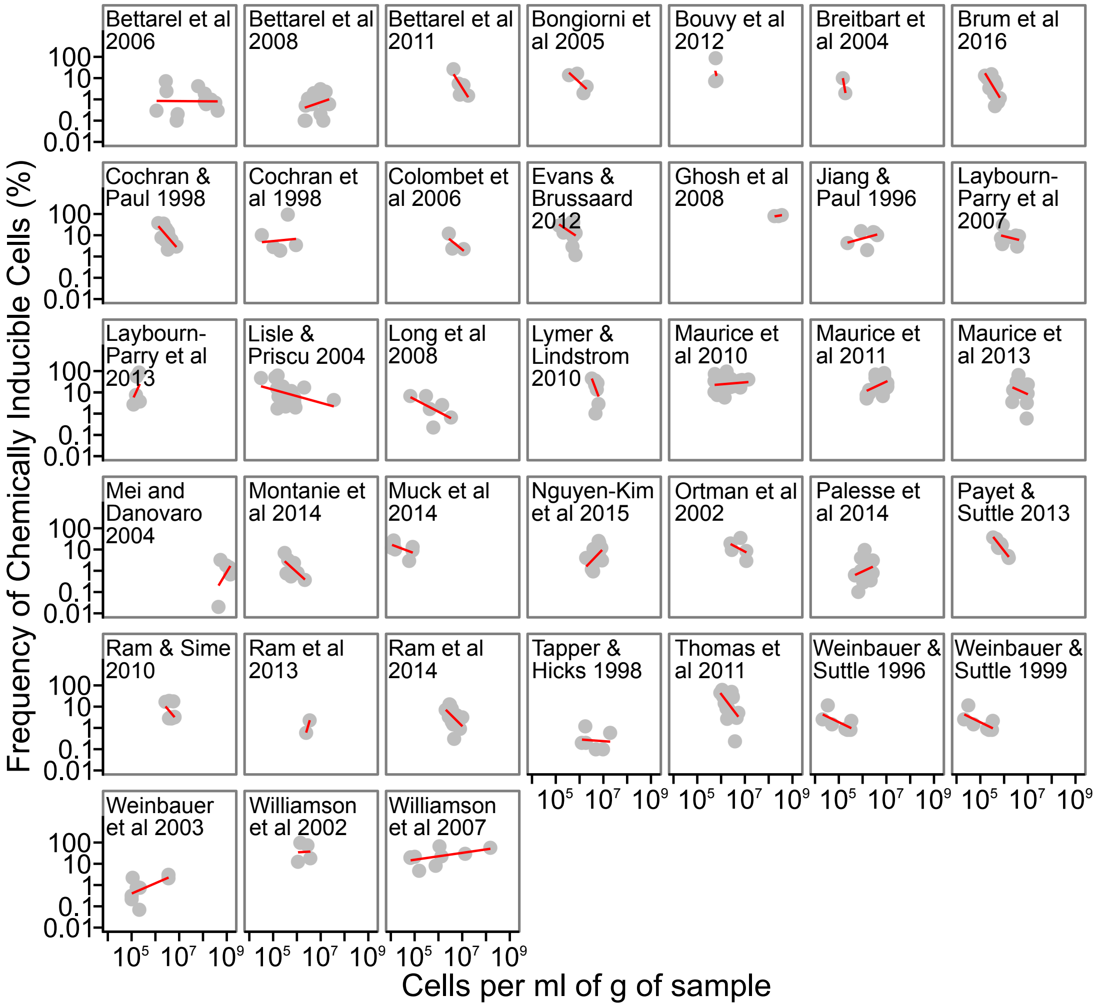


Figure 3.5: Variability in FCIC rises with dilution.
Dilution with either 0.02 µm-filtered buffers (**a**) or site water (**b**) generates variable FCIC values (sites split by environment for display). Dashed heuristic arrows of best fit across technical replicates are shown, color-coded by site, with arrow heads pointing towards more diluted samples. The 'correct' undiluted samples are indicated with black rings. Inhibition (i.e., FCIC values < 0) occurred across the full range of host densities examined. (**c**) Ranges from published studies, and undiluted and diluted samples processed here, show the high variability of diluted technical replicates. A boxplot of FCIC ranges from individual studies pooled from investigations of FCIC in freshwater and saltwater samples (grey stars) is shown, with the published median value (red line) and 25th and 75th percentiles (box hinges) extended across the undiluted and diluted samples analyzed here (dashed lines). The majority (66 %) of the within-site undiluted technical replicates show ranges in the 25th - 50th percentiles of published studies using biological replicates and addressing FCIC across sites. Higher variability is observed at the within-site level when technical replicates are diluted; 33 % of sites show a FCIC range greater than observed in the majority of published FCIC studies (i.e., their ranges are greater than the median published range) and the range in FCIC observed with dilution in one site is greater than in 75 % of published studies conducted with biological replication across sites. (**d**) A histogram showing the distribution of replicate FCIC values from the dilution experiments shows an approximately normal distribution centered around zero, either as an aggregate or in each diluent treatment (buffers: white; site water: black).

Table 3.1: Summary statistics of published studies.

Studies showing number of measures (*n*; mean or individual values), goodness of fit (R^2), slope (*m*) and significance (*p*) for least squares regression line testing for difference of slope from zero between FCIC and host densities (both log-transformed). Bold p-values indicate slopes significantly different from zero at the 90% confidence level. R^2 and p-value statistics were not computed for studies with n < 3. Asterisks mark studies conducted in more than one environment with FCIC values split for analysis. Superscripts indicate the study number used in Figure 3.1.

Environment	Reference	Mean or individual	n	R^2	m	p
Freshwater	Bettarel et al. (2006)[1,*]	mean	6	0.04	-0.19	0.70
Freshwater	Breitbart et al. (2004)[6]	individual	2	-	-6.40	-
Freshwater	Colombet et al. (2006)[10]	mean	3	0.51	-0.91	0.49
Freshwater	Laybourn-Parry et al. (2013)[15]	mean	5	0.14	2.43	0.54
Freshwater	Lisle and Priscu (2004)[16]	individual	16	0.17	-0.31	0.12
Freshwater	Lymer and Lindstrom (2010)[18]	mean	9	0.20	-2.97	0.23
Freshwater	Maurice et al. (2010)[19]	mean	26	0.02	0.09	0.52
Freshwater	Maurice et al. (2011)[20,*]	mean	16	0.27	0.76	**0.04**
Freshwater	Palesse et al. (2014)[27]	mean	14	0.07	0.52	0.37
Freshwater	Ram and Sime-Ngando (2010)[29]	mean	11	0.17	-1.33	0.22
Freshwater	Ram et al. (2013)[30]	mean	2	-	3.86	-
Freshwater	Ram et al. (2014)[31]	mean	15	0.21	-1.11	**0.09**
Freshwater	Tapper and Hicks (1998)[32]	mean	6	0.01	-0.08	0.87
Freshwater	Thomas et al. (2011)[33]	mean	16	0.27	-1.48	**0.04**
Saltwater	Bettarel et al. (2008)[2]	mean	15	0.07	0.39	0.35
Saltwater	Bettarel et al. (2011)[3]	mean	5	0.64	-1.77	0.10
Saltwater	Bongiorni et al. (2005)[4]	mean	4	0.61	-1.04	0.22
Saltwater	Bouvy et al. (2012)[5]	mean	3	0.04	-4.62	0.87
Saltwater	Brum et al. (2016)[7]	mean	17	0.45	-1.84	**0.00**
Saltwater	Cochran and Paul (1998)[8]	mean	12	0.42	-1.30	**0.02**
Saltwater	Cochran et al. (1998)[9]	mean	6	0.01	0.11	0.88
Saltwater	Evans and Brussaard (2012)[11]	mean	12	0.13	-0.75	0.24
Saltwater	Jiang and Paul (1996)[13]	mean	6	0.16	0.31	0.44
Saltwater	Laybourn-Parry et al. (2007)[14]	mean	8	0.08	-0.28	0.49
Saltwater	Long et al. (2008)[17]	mean	6	0.35	-0.58	0.22
Saltwater	Maurice et al. (2011)[20,*]	mean	8	0.14	-0.58	0.36
Saltwater	Maurice et al. (2013)[21]	mean	13	0.05	-0.50	0.45
Saltwater	Muck et al. (2014)[24]	mean	9	0.32	-0.42	0.11
Saltwater	Nguyen-Kim et al. (2015)[25]	mean	14	0.24	1.17	**0.08**
Saltwater	Ortmann et al. (2002)[26]	mean	5	0.22	-0.59	0.43
Saltwater	Payet and Suttle (2013)[28]	individual	9	0.88	-1.43	**0.00**
Saltwater	Weinbauer and Suttle (1996)[34]	mean	7	0.01	-0.08	**0.07**
Saltwater	Weinbauer and Suttle (1999)[35]	mean	7	0.44	-0.54	0.10
Saltwater	Weinbauer et al. (2003)[36]	mean	9	0.32	0.49	0.11
Saltwater	Williamson et al. (2002)[37]	mean	4	0.00	0.07	0.96
Sediment	Bettarel et al. (2006)[1,*]	mean	7	0.80	-1.13	**0.01**
Sediment	Mei and Danovaro (2004)[22]	mean	5	0.22	1.87	0.42
Sediment	Montanie et al. (2014)[23]	mean	7	0.43	-1.01	0.11
Soil	Ghosh et al. (2008)[12]	mean	3	0.92	0.18	0.18
Soil	Williamson et al. (2007)[38]	mean	10	0.28	0.16	0.12



Extended Data Figure 3.1: Fraction of chemically inducible cells across host densities by study. Lines of best fit for each study are shown (red; FCIC and host density log-transformed).

Chapter 3, in full, is prepared for submission. Knowles B, Bailey B, Edwards R, Felts B, Grasis J, Haas A, Kelly LW, Luque A, Nulton J, Paul L, Robinett N, Segall A, and F Rohwer. Examination of induction-based evidence for host density-dependence of lysogeny suggests potentially novel drivers of natural viral communities. The dissertation author was the primary author and investigator on this manuscript.

CHAPTER 4

Life in the Piggyback-the-Winner World

Overview

Viral infection of microbial hosts is one of the most common biological interactions on Earth (Suttle 2007). Liberation of cellular metabolites during lysis of infected cells is a major driver of global biogeochemical cycling (Fuhrman 1999; Wilhelm and Suttle 1999; Suttle 2007). As such, processes affecting the rates of lysis that occur in a given system are ecologically important. As lytic and lysogenic viral life cycles result in different lysis patterns, the balance of lytic to lysogenic infection outcomes has profound impacts on ecosystem function. The viral lytic lifestyle is thought to be favored under conditions where hosts are abundant and rapidly growing, while the exclusively viral lifecycle of lysogeny is seen as a refuge for temperate viruses that have rare or ailing hosts. However, recent work suggests that high density systems may, in fact, favor temperate viruses capable of lysogenizing their numerous and proliferating hosts.

In addition to changing fates of energy and matter within ecosystem circuits, this challenges how we conceive of ecosystem function. The viral ability to confer immunity to lytic infection on its host when forming a lysogen provides a novel dynamic in ecology wherein prey may exist in high-density numerical refuges and exploit anthropogenic disturbance regimes that alleviate bottom-up limitation and promote microbial overgrowth. Such Microbialized systems experience a slew of impacts from pathogenicity to energetic overloading that less degraded, Viralized, systems are sheltered from due to higher viral lytic surveillance of microbial densities.

Viralized and Microbialized systems allowed the elucidation of two complementary but distinct virus-host predator-prey dynamics; Kill-the-Winner and Piggyback-the-Winner. While viruses kill hosts on a host density- and growth-dependent basis under Kill-the-Winner, they parasitize, promote and exploit their hosts in high growth and densities under Piggyback-the-Winner dynamics. When viewed across the full range of host densities studied to date, a spectrum of lytic to lysogenic switching is observed, with low density Piggyback-the-Loser through Kill-the-Winner to high density Piggyback-the-Winner dynamics. These switches show tightly constrained and rapid transitions into the lytic Kill-the-Winner densities, a distribution that resembles a narwhal with its prominently horned profile. Narwhal Switches of the dominant viral lifestyle across the full range of host densities shows that viral-host predator-prey dynamics are more complex than previously thought. Here we examine two viral Worlds that result from these Narwhal Switches, the Kill-the-Winner and Piggyback-the-Winner worlds.

The Kill-the-Winner World

Ecological models of how viruses impact bacterial hosts in the environment in terms of predation pressure, generation of diversity, and regulation of community composition were developed in the late 1990s and early 2000s (Thingstad and Lignell 1997; Thingstad 2000). These Lotka-Volterra-like models suggested that rapidly growing prey (‘Winners’) are subject to a combination of viral (specialist) and protistan (generalist) predation commensurate with their growth rate once their densities rise enough to facilitate chance predator-prey encounters. This mechanism should result in the rapid top-down suppression of any blooming prey lineages (thus ‘Kill-the-Winner’) until their densities could no longer support lytic dynamics allowing prey to exist in a low-density numerical refuge. A new Winner may then arise. As such, Kill-the-Winner (KtW) dynamics provide a resilience mechanism predicted to maintain relatively low densities of prey lineages and prevent sustained dominance of community composition by an oligarchy of lineages, promoting evenness of prey communities. Further, prey may be able to escape viral surveillance by mutation of characteristics required for viral infection (e.g., the cell surface receptors as modeled by Thingstad et al. (2014)). Sustained KtW viral surveillance likely drives prey diversification through these two mechanisms.

Kill-the-Winner makes intuitive sense: simplified, rapidly growing host populations can support high levels of viral reproduction as well as rapid transmission of viruses throughout sensitive host populations. It has been shown that viral burst sizes (the number of viral progeny released per successful infection) are a positive function of host growth rate (Wommack and Colwell 2000). In addition to being host density-dependent (i.e., driven by how often prey are encountered based on the density of predators and prey in a system), specialist predation in mixed communities can also be host-diversity frequency-dependent (i.e., driven by how often *appropriate* prey are encountered based on prey community composition). Communities simplified by the rapid growth of a small number of lineages, as occurs during blooms, should create a density- and frequency-dependent ‘lytic wonderland’ for viral top-down control and KtW suppression of these lineages.

Indeed, Kill-the-Winner is supported by the observation of collapse of seasonal blooms by lytic viruses (Bratbak et al. 1990; Bratbak, Egge, and Heldal 1993) that show Lotka-Volttera-like oscillations of predator and prey densities. Further, Kill-the-Winner explains why blooms are ephemeral phenomena and why there are markedly stable and seemingly homeostatic bacterial densities in the environment (Azam et al. 1983). However, and despite its wide acceptance, relatively few studies have demonstrated KtW dynamics in more complex communities outside of blooms. This is largely because of methodological constraints on testing the model: it only recently that the required resolution of microbial and viral density and community composition data have been available, especially as it appears that KtW dynamics may apply most closely to strain-level dynamics rather than to the original species level (Needham et al. 2013; Rodriguez-Brito et al. 2010).

The post-collapse Kill-the-Winner World

In communities where host densities are unstable, lytic dynamics appear to optimize the viral productivity of any episodic increase in host density or growth rate. However, this is a short-term plan. Collapsing the prey population leads to a period of Malthusian famine for the viruses after their hosts are numerically exhausted and exist in low-density numerical refuges (Malthus 1878).

After bloom suppression, lytic viruses are likely confronted with high competition from their copious residual siblings (i.e., there is a high virus:microbe ratio) as well as low encounter rates with appropriate hosts - worse, hosts that are no longer winners - while exposure in the extracellular milieu saps their infectivity in a matter of days (Wommack and Colwell 2000). Rather than being viewed as a Resilience Police Force enforcing the law of density-dependence on their prey, lytic viruses are perhaps best seen as Pollyanna-ish paupers getting by on predominantly rare, ephemerally abundant, hosts while attrition winnows their population.

However, Malthusian catastrophes are avoidable through innovation. Indeed, one of the major points of the Kill-the-Winner model is not about the killing, it is that prey must escape predation. Kill-the-Winner has morphed into a mechanistic model on how a Winner is formed or reformed. Rather than focusing on Kill-the-Winner scenarios, therefore, modeling the means through which viruses evolve to avoid these periods of famine, such as host range diversification or the generation of immunity to infection (Flores et al. 2011; Thingstad et al. 2014) provide promising avenues for future research. These modeling efforts could be challenged with data from viral-tagging that can yield real-world virus-host networks and probe altered infection profiles over time (Deng et al. 2012; Deng et al. 2014; Weitz et al. 2013) or from time-series correlations between predators and prey (Needham et al. 2013; Steele et al. 2011).

The Piggyback-the-Winner World

Piggyback-the-Winner is an empirically derived model of virus-host predator-prey dynamics in high host density systems. It posits that at high host growth rate and density, temperate viruses are favored over lytic viruses; exactly when it viruses would be expected to kill their hosts, they instead parasitize them. As explained above, lytic models suggest that high microbial prey densities should be ephemeral phenomena due to top-down control. However, in ecosystems from degraded coral reefs to exacerbated cystic fibrosis lungs and the gastro-intestinal tract, sustained high microbial densities are the norm (Knowles et al. 2016; Willner et al. 2009; Mills et al. 2013). While examination of purely lytic models (either mathematical; Weitz and Dushoff 2008; or microbiological models such as T4 and *Escherichia coli*) almost begs the question how any host strain that has encountered its viral predators can exist at all, Piggyback-the-Winner provides a mechanism by which these observed densities are sustained.

In the Piggyback-the-Winner World, like the Kill-the-Winner World, elevated host growth leads to higher host densities and thus a probability of infection events due to random density-dependent encounters between predators and prey according to the logic of density-dependent models (e.g., Lotka 1925; Volterra 1926). This creates a pressing selective barrier for the hosts: if they are to survive (i.e., escape a Kill-the-Winner fate) they must become immune to lytic infection, and quickly so. While there are likely myriad ways to navigate this bottleneck, evasion of lytic viruses is thought to take three common forms in natural communities: (i) hiding from the viruses in a diverse host community, (ii) host generated immunity, or (ii) using viral competitors against each other.

A lineage really becomes a Winner when frequency-dependent processes stop working for it, and start to work against it. If a population within a mixed community is in a low-density numerical refuge, it can benefit from its low density first by being rare and second by having a low frequency compared to the total number of microbes in the community. This second effect occurs because of the probability of inappropriate adsorption occurring between the predators of that population and a microbe of another lineage unable to support viable infections of that virus. In this way, rare populations can ‘hide in the woods’ of complex communities from their killers. This advantage is discarded upon becoming a Winner; the associated growth and increase in densities of the winning lineage change the frequency-dependence of appropriate encounter against the nascent Winners and they are more likely to be infected. Bloom ‘lytic wonderlands’ are extreme examples of this.

Another means to escape the lytic fate is for hosts to develop their own immunity through mechanisms such as mutation of cell surface receptors or CRISPRs (Barrangou et al. 2007; Bondy-Denomy and Davidson 2014; Labrie, Samson, and Moineau 2010; Våge, Storesund, and Thingstad 2013; Thingstad et al. 2014). This must be accomplished rapidly to prevent the onset of the lytic default pathway.

However, CRISPRs are not universally distributed throughout host lineages, and do not show the vast diversity expected of an agile immune function, suggesting that they may not be able to confer immunity across a broad spectrum of hosts and that they may not operate on immediate timescales (Touchon et al. 2011; Touchon and Rocha 2010; Takeuchi et al. 2012). No evidence of elevated CRISPR content was observed in high density hosts, suggesting that this means of immunity was not involved in the inferred suppression of lytic dynamics in those systems (Knowles et al. 2016).

Cell surface receptor mutations engineered by hosts before, or by viruses after, infection are diverse in terms of the proteins targeted as well as the possible changes in these proteins (Abedon 2012; Bondy-Denomy et al. 2016; Labrie, Samson, and Moineau 2010). As a result, they are difficult to measure in mixed communities. While this mechanism may provide resistance to viral infection, it likely provides a fitness cost to the host (as modeled by Våge, Storesund, and Thingstad (2013) and Thingstad et al. (2014)) and may take more than one generation to implement.

In contrast, viral mediated immunity conferred by integrated temperate viruses may be more rapidly instituted than mutated cell surface proteins and offer a broader spectrum immunity than the highly specific CRISPRs, and may come with gain of function instead of fitness costs (e.g., virulence factors). Evidence of more temperateness of viral communities in high host density systems suggests that this is the means by which microbes escape, or at least delay, the lytic fate in these systems and find refuge in the Piggyback-the-Winner World (Knowles et al. 2016).

Viruses are molecular competitors. They compete with each other in both the extra-cellular milieu to find hosts and they compete within cells to dominate the replication machinery co-opted from the host at the expense of each other and often the host (Six and Klug 1973; La Scola et al. 2008). These competitive forces can structure ecosystems (Yau et al. 2011). Piggyback-the-Winner dynamics suggest that hosts may take advantage of virus-virus competition and inhibition, recruiting temperate viruses to integrate with them and protect them as a lysogen. Indeed, the rampant infections observed after spiking host growth rate in the Piggyback-the-Winner experiments followed by a lack of lytic activity are consistent with this provirus recruitment hypothesis (Knowles et al. 2016). This would work for both viral and cellular parties, with both contributing to the success of the lysogen. The live-in ‘guard-virus’ could enjoy the security of its tenured status as it patrols the cell for foreign DNA without the need to engage in the uncertain process of reinfection while replicating safely within its host. The host could enjoy a newfound impregnability to lytic insult as well as likely benefitting from viral-encoded factors such as virulence genes. This shared fate strategy has the potential to transform high host growth and density systems from ‘suicide by virus’ to a high-density numerical refuge from viral, and possibly protist, predation.

However, if the lysogenic collaboration ends, it ends acrimoniously and probably not well for either party. Dynamical experiments suggest that if the host is to retain its resident virus, growth must be sustained, as a slump in growth appears to result in viral induction. The termination of the piggybacking phase would result in host death and the virus facing a Malthusian catastrophe similar to that of its lytic counterparts after bloom collapse. Extending the collaborative phase of Piggyback-the-Winner is likely of tantamount importance to both parties.

Piggyback-the-Winner and resilience

Kill-the-Winner provides a rapid and autocatalytic resilience mechanism; the faster the bloom, the sooner the crash. In contrast, Piggyback-the-Winner appears to stabilize blooms - as long as hosts maintain rapid growth and can therefore hide in their high-density refuge. By precluding suppression via lytic infection and protist predation, through super-infection immunity and virulence factors, respectively, PtW may exacerbate ecosystem perturbations. However, viral induction likely occurs if the growth rate of the lysogen declines. It is therefore imperative to Winners under PtW dynamics to maintain growth. If growth can be sustained, Winners may win for extended periods of time, leading to dominance of ecosystems by an oligarchy of Winners in the absence of Kill-the-Winner system turnover. These winners are known to have enlarged genome sizes and capacity for horizontal gene transfer as observed on coral reefs (Kelly et al. 2012; Kelly et al. 2014), suggesting that winners may be able to sustain growth and thus numerical dominance by gaining required metabolic functions to optimize fitness as conditions vary.

As a result, Piggyback-the-Winner dynamics are expected to provide minimal initial resilience compared to Kill-the-Winner dynamics. However, once PtW has allowed microbial overgrowth to occur, it appears as if PtW then strongly stabilizes ecosystems in a Microbialized state. Further, as viruses decay in the order of days (Wommack and Colwell 2000), lytic viruses disadvantaged by PtW dynamics may rapidly extirpated from the system. This would then lead to a free-viral pool relatively enriched in temperate viruses, as observed by Knowles et al. (2016), and depleted in the lytic viruses that could alleviate the Microbialized phenotype. It is therefore expected that a strong hysteresis would rapidly be established against returning ecosystems to their pre-Microbialized state, especially in systems experiencing sustained human-associated eutrophication that can maintain the growth required for PtW lysogens.

Piggyback-the-Winner food webs

Current conceptions of food webs suggest that viral and protist predators compete for prey, mainly bacteria. These predation dynamics lead to a bifurcated fate for infected prey who either lysed and enter the Viral Shunt (Wilhelm and Suttle 1999) or are grazed and enter the Microbial Loop (Azam et al. 1983). While viral and protistan predators have been studied extensively, their combined effects have rarely been considered, as researchers tend to focus on the impacts of one or the other. The few papers that have considered both protist and bacteriophage predation rates are aimed at contrasting the relative importance of the guilds under different conditions (Fuhrman and Noble 1995). There are myriad possible interactions between bacterial viruses and protists. However, few of these interactions have been studied, and in particular, how these interactions affect their trophic ecology remains unresolved (although see Örmälä-Odegrip et al. 2015).

Temperate Piggyback-the-Winner viral communities associated with high host densities show elevated fractions of virulence factors (Knowles et al. 2016). This presents the strong possibility that lysogenic conversion of bacterial strains through stimulation of PtW dynamics can allow lysogens to evade predation by protists through horizontal gene transfer of viral-encoded virulence factors (Escoll et al. 2013; Waldor and Mekalanos 1996). This has the potential to short circuit protist grazing-based trophic dynamics with cascading impacts on food webs by inhibiting the Microbial Loop, possibly contributing to the global trophic downgrading associated with human environmental impacts (Estes et al. 2011) and producing a Microbialized phenotype (Haas et al. 2016; McDole et al. 2012). If this is true, then PtW dynamics may rewire the trophic structures of degraded systems. Further, while a Viralized to Microbialized spectrum has been identified with respect to viruses and their prey across host densities (Knowles et al. 2016), a similar Eukaryotized phenotype may be observed in healthy sites where heterotrophic nano-flagellates suppress bacterial densities. Thus a prey density-dependent Eukaryotized to Microbialized spectrum may exist from healthy to degraded ecosystems.

Viral life history theory and Piggyback-the-Winner

Ecological tradeoffs have been neglected in viral ecology due to the difficulty of capturing these traits. In canonical macrobial ecological *r*- to K-selection space (MacArthur and Wilson 2015), there exists a conceptual continuum between organisms are able to exploit uncrowded niches through the production of high quantities of offspring (*r*-selected) and those inhabiting more crowded niches where the production of high offspring quality is favored (K-selected). Ecosystem impacts and degradation generally moves community profiles ‘backward’ in succession from K-selected climax composition towards the dominance of more weedy *r*-selected lineages (Sandin and Sala 2012).

Viruses may be tentatively binned into lytic *r*-selected and lysogenic K-selected categories (Bordería and Elena 2002) based on the rapid proliferation of lytic lineages and the longer-term strategy of lysogens. Piggyback-the-Winner predicts that degraded ecosystems that are dominated by early-successional *r*-like microbial communities (Dinsdale et al. 2008; McDole et al. 2012; Somera et al. 2016) are associated with more K-like viral dynamics. In the within-cell host landscape it appears that high resource availability (similar to the log-phase of the Verhulst logistic curve that *r*-strategists exploit) may stimulate K-like integration. However, at the between-host-cell level, conditions are physically crowded which is more similar to a K-selecting landscape and is consistent with increased prevalence of lysogeny. This suggests that viruses can act on a more ecosystem level than merely at a within-cell level, and that lysogens and lysogenization may be more attuned to the landscape experienced by the lysogen rather than just that of the virus. The mechanisms by which this could occur are unknown, as viruses have not been found to be able to sense the extracellular environment.

There are likely feedbacks within these systems. Lysogeny can favor pathogenic lineages like the early successional microbes observed on degraded reefs (Dinsdale et al. 2008), facilitating the microbial overgrowth of these systems. As a result, there is likely a bi-directional coupling between the observed degraded phenotype and lysogeny.

Why has Piggyback-the-Winner not been seen before?

Piggyback-the-Winner applies to high host density systems. To date the open ocean is the most extensively studied environment in viral ecology. This system appears to have the relatively low host densities particularly suitable for Kill-the-Winner dynamics (Knowles et al. 2016). In contrast, high-density systems such as soil, lungs, and the gastrointestinal tract have been relatively understudied, precluding deep analysis. PtW was observed only with the in-depth study of coral reef-associated viral and microbial dynamics, where host densities in excess of open ocean samples, coupled with a global distribution of counts that are prohibitively challenging to obtain from human samples like the lung and gastrointestinal tract. Subsequent analysis of published values from more isolated datasets in other environments was then able to reinforce the universality of PtW dynamics using this scaffold.

In addition to *where* viral ecology was studied, *how* it is studied also precluded identifying PtW dynamics. In particular, two preexisting datasets suggest lytic to lysogenic switches contrary to PtW. These are estimates of lysogeny and of viral production generated by chemical induction of lysogens and by dilutions, respectively.

Chemical induction has been applied to environmental samples in order to assess what fraction of the mixed communities present are lysogens since the mid-1990s (Jiang and Paul 1994; Jiang and Paul 1996; Jiang and Paul 1998). These studies noted that the fraction of lysogenic strains was higher in oligotrophic conditions than in eutrophic nearshore conditions, although estaurine samples also showed high fractions of lysogens at times (Jiang and Paul 1998; Jiang and Paul 1996). This observation has since framed the dialogue around what conditions favor lysogeny (e.g., Maurice et al. (2013); Brum et al. (2015)).

Regardless of known issues with the use of chemical induction to estimate rates of lysogeny in mixed samples as discussed by environmental induction pioneers Paul and Weinbauer (2010), there appears to be minimal support for the idea of trophic dependency of lysogeny as measured by induction. A preliminary (Knowles et al. 2016) and subsequent exhaustive analysis of thirty-eight (Knowles et al., in prep.) published chemical induction datasets found no significant relationship between the induced fraction and host density when examined at the within environment (freshwater, saltwater, sediment, soil) or within-study level. While this presents the opportunity to identify drivers of lysogeny beyond canonical host density and growth rate factors, the established perception that lysogeny is more frequent at low host densities derived from a handful of induction studies provides a major barrier to identifying new dynamics like PtW.

Contemporary with the induction studies of Jiang and Paul, Wilcox and Fuhrman (1994) conducted a series of dilution studies to tease apart the relative impacts of lytic and lysogenic viral activity. This crucial experiment rested on two premises: (i) that dilution eliminated lytic activity by lowering encounter rates below the level required to support infection, and (ii) that dilution does not lead to changes in viral activity - that the lytic/lysogenic profile of the viral community is maintained across dilutions. This study found that when lytic dynamics are shut down, viral communities do not recover consistent with the dominance of lytic dynamics (Wilcox and Fuhrman 1994). This was a fundamental finding during the formation of viral ecology. However, Piggyback-the-Winner suggests that dilution leads to an increase in lysogeny, violating the second premise. In this light, rather than the lack of viral recovery demonstrating a scarcity of lysogenic activity, it may actually be an artifact of its increased importance. The challenges in measuring lysogeny, and the corresponding ease of measuring lytic dynamics, may have altered our perception of how prevalent lysogeny is in the environment.

Viral production is usually estimated using a dilution technique similar to Wilcox and Fuhrman (1994) and with same premises as those stated above (Wilhelm, Brigden, and Suttle 2002). Data from this assay show higher viral production per volume of sample under high host density conditions, suggesting that there is greater lytic activity in higher density samples (De Corte et al. 2012; Payet and Suttle 2013). However, as this metric is not measuring the number of viruses produced per host (S W Wilhelm, Brigden, and Suttle 2002), it is difficult to interpret higher production as being indicative of more or less lysogeny. Further, dilution experiments conducted as part of an examination of the drivers of the inducible fraction of cells (Knowles et al., in prep.) showed that either dilution alters lysogenic profiles of communities or that the induced fraction decouples from lysogeny in dilutions. If the former is correct, then viral production estimated by the dilution technique may be confounded by altered viral infection dynamics, consistent with PtW dynamics (Knowles et al. 2016). If the latter is true, then FCIC is a poor proxy for lysogeny. In either case, there is only questionable evidence against PtW dynamics provided by these datasets. As with the induction literature above, the strength of available evidence supporting the paradigm that lytic dynamics increase with host density is inconsistent with how strongly the paradigm has been accepted, stifling the identification of new dynamics.

Conclusion

The Piggyback-the-Winner model presents a novel view of virus-host predator-prey dynamics as well as new hypotheses to be challenged. Rather than high host densities being a transient state, Piggyback-the-Winner explains how Microbialized systems can exist as well as suggesting that rather than lytic viruses crashing blooms, temperate viruses may actually do this once the growth of their hosts slows and they exit their Piggybacking phase. This emphasizes a unique aspect of the Piggyback-the-Winner world: bottom-up control must be suspended in order for viral top-down control to be delayed, either in terms of nutrients delivered or nutrients assimilated using potential viral-encoded functions through horizontal gene transfer. Such a situation is occurring in degraded and animal-associated systems. These systems have progressed to a Microbialized state that may be exceedingly stable and preclude movement back to the Viralized state observed in more oligotrophic systems. The continuum between the Kill-the-Winner and Piggyback-the-Winner Worlds, appears to be driven by a change in viral life cycle selection captured by Narwhal Switches. As viruses are the one of the major predatory guilds on the planet, and as it seems that lysogenic cycles may have been mistaken for lytic dynamics, it appears that parasitism may be an almost ubiquitous phenomenon. Parasitism, the new normal of the Piggyback-the-Winner World, presents opportunities and challenges across ecology from coral reefs to the human lung in terms of how to model and measure the Piggyback-the-Winner world now that it has been glimpsed. Nature is not red in tooth and claw; it is wily and parasitic.

REFERENCES

Abedon, Stephen T. 2012. “Bacterial ‘immunity’against Bacteriophages.” *Bacteriophage* 2 (1). Taylor & Francis: 50–54.

Azam, F, T Fenchel, J G Field, J S Gray, L Meyer-Reil, and F Thingstad. 1983. “The Ecological Role of Water-Column Microbes in the Sea.” *Mar. Ecol. Prog. Ser.* 10: 257–63.

Barrangou, Rodolphe, Christophe Fremaux, Hélène Deveau, Melissa Richards, Patrick Boyaval, Sylvain Moineau, Dennis A Romero, and Philippe Horvath. 2007. “CRISPR Provides Acquired Resistance against Viruses in Prokaryotes.” *Science* 315 (5819). American Association for the Advancement of Science: 1709–12.

Bondy-Denomy, Joseph, and Alan R Davidson. 2014. “To Acquire or Resist: The Complex Biological Effects of CRISPR--Cas Systems.” *Trends in Microbiology* 22 (4). Elsevier: 218–25.

Bondy-Denomy, Joseph, Jason Qian, Edze R Westra, Angus Buckling, David S Guttman, Alan R Davidson, and Karen L Maxwell. 2016. “Prophages Mediate Defense against Phage Infection through Diverse Mechanisms.”

Bordería, Antonio V, and Santiago F Elena. 2002. “R- and K-Selection in Experimental Populations of Vesicular Stomatitis Virus.” *Infection, Genetics and Evolution* 2 (2): 137–43. doi:http://dx.doi.org/10.1016/S1567-1348(02)00094-1.

Bratbak, G, J K Egge, and M Heldal. 1993. “Viral Mortality of the Marine Alga Emiliania Huxleyi (Haptophyceae) and Termination of Algal Blooms.” *Marine Ecology Progress Series* 93: 39–48.

Bratbak, G, M Heldal, S Norland, and T F Thingstad. 1990. “Viruses as Partners in Spring Bloom Microbial Trophodynamics.” *Applied and Environmental Microbiology* 56 (5). United States: 1400–1405.

Brum, Jennifer R, Bonnie L Hurwitz, Oscar Schofield, Hugh W Ducklow, and Matthew B Sullivan. 2015. “Seasonal Time Bombs: Dominant Temperate Viruses Affect Southern Ocean Microbial Dynamics.” *ISME J*, August. International Society for Microbial Ecology, 1–13. http://dx.doi.org/10.1038/ismej.2015.125.

De Corte, Daniele, Eva Sintes, Taichi Yokokawa, Thomas Reinthaler, and

Gerhard J Herndl. 2012. “Links between Viruses and Prokaryotes throughout the Water Column along a North Atlantic Latitudinal Transect.” *ISME J* 6 (8). International Society for Microbial Ecology: 1566–77. http://dx.doi.org/10.1038/ismej.2011.214.

Deng, Li, Ann Gregory, Suzan Yilmaz, Bonnie T Poulos, Philip Hugenholtz, and Matthew B Sullivan. 2012. “Contrasting Life Strategies of Viruses That Infect Photo-and Heterotrophic Bacteria, as Revealed by Viral Tagging.” *MBio* 3 (6). Am Soc Microbiol: e00373–12.

Deng, Li, J Cesar Ignacio-Espinoza, Ann C Gregory, Bonnie T Poulos, Joshua S Weitz, Philip Hugenholtz, and Matthew B Sullivan. 2014. “Viral Tagging Reveals Discrete Populations in Synechococcus Viral Genome Sequence Space.” *Nature* 513 (7517). Nature Publishing Group: 242–45.

Dinsdale, Elizabeth A, Olga Pantos, Steven Smriga, Robert A Edwards, Florent Angly, Linda Wegley, Mark Hatay, et al. 2008. “Microbial Ecology of Four Coral Atolls in the Northern Line Islands.” *PLoS ONE* 3 (2): e15841–17. doi:10.1371/journal.pone.0001584.

Escoll, Pedro, Monica Rolando, Laura Gomez-Valero, and Carmen Buchrieser. 2013. “From Amoeba to Macrophages: Exploring the Molecular Mechanisms of Legionella Pneumophila Infection in Both Hosts.” In *Molecular Mechanisms in Legionella Pathogenesis*, 1–34. Springer.

Estes, James A, John Terborgh, Justin S Brashares, Mary E Power, Joel Berger, William J Bond, Stephen R Carpenter, et al. 2011. “Trophic Downgrading of Planet Earth.” *Science* 333 (6040). American Association for the Advancement of Science: 301–6.

Flores, Cesar O, Justin R Meyer, Sergi Valverde, Lauren Farr, and Joshua S Weitz. 2011. “Statistical Structure of Host--Phage Interactions.” *Proceedings of the National Academy of Sciences* 108 (28). National Acad Sciences: E288–97.

Fuhrman, Jed A. 1999. “Marine Viruses and Their Biogeochemical and Ecological Effects.” *Nature* 399 (6736). Nature Publishing Group: 541–48.

Fuhrman, Jed A, and Rachel T Noble. 1995. “Viruses and Protists Cause Similar Bacterial Mortality in Coastal Seawater.” *Limnology and Oceanography* 40 (7): 1236–42. doi:10.4319/lo.1995.40.7.1236.

Haas, Andreas F, Mohamed F M Fairoz, Linda W Kelly, Craig E Nelson, Elizabeth A Dinsdale, Robert A Edwards, Steve Giles, et al. 2016. “Global Microbialization of Coral Reefs.” *Nature Microbiology* 1. Nature Publishing Group: 16042.

Jiang, S C, and J H Paul. 1998. “Significance of Lysogeny in the Marine Environment: Studies with Isolates and a Model of Lysogenic Phage Production.” *Microbial Ecology* 35 (3-4). Springer: 235–43.

Jiang, Sunny C, and John H Paul. 1994. “Seasonal and Diel Abundance of Viruses and Occurrence of Lysogeny/bacteriocinogeny in the Marine Environment.” *Marine Ecology-Progress Series* 104: 163.

———. 1996. “Occurrence of Lysogenic Bacteria in Marine Microbial Communities as Determined by Prophage Induction.” *Marine Ecology-Progress Series* 142: 27.

Kelly, Linda W, Gareth J Williams, Katie L Barott, Craig A Carlson, Elizabeth A Dinsdale, Robert A Edwards, Andreas F Haas, et al. 2014. “Local Genomic Adaptation of Coral Reef-Associated Microbiomes to Gradients of Natural Variability and Anthropogenic Stressors.” *Proceedings of the National Academy of Sciences of the United States of America* 111 (28). United States: 10227–32. doi:10.1073/pnas.1403319111.

Kelly, Linda Wegley, Katie L Barott, Elizabeth Dinsdale, Alan M Friedlander, Bahador Nosrat, David Obura, Enric Sala, et al. 2012. “Black Reefs: Iron-Induced Phase Shifts on Coral Reefs.” *The ISME Journal* 6 (3). England: 638–49. doi:10.1038/ismej.2011.114.

Knowles, B, C B Silveira, B A Bailey, K Barott, V A Cantu, A G Cobián-Güemes, F H Coutinho, et al. 2016. “Lytic to Temperate Switching of Viral Communities.” *Nature* advance online publication (March). Nature Publishing Group, a division of Macmillan Publishers Limited. All Rights Reserved. http://dx.doi.org/10.1038/nature17193.

Knowles B, Bailey B, Edwards R, Felts B, Grasis J, Haas A, Wegley Kelly L, Luque A, Nulton J, Paul L, Robinett N, Segall A, and Forest Rohwer. In Preparation. “Examination of induction-based evidence for host density-dependence of lysogeny suggests potentially novel drivers of natural viral communities”.

La Scola, Bernard, Christelle Desnues, Isabelle Pagnier, Catherine Robert, Lina Barrassi, Ghislain Fournous, Michele Merchat, et al. 2008. “The Virophage as a Unique Parasite of the Giant Mimivirus.” *Nature* 455 (7209). Nature

Publishing Group: 100–104.

Labrie, Simon J, Julie E Samson, and Sylvain Moineau. 2010. “Bacteriophage Resistance Mechanisms.” *Nature Reviews Microbiology* 8 (5). Nature Publishing Group: 317–27.

Lotka, Alfred J. 1925. “Elements of Physical Biology.”

MacArthur, Robert H, and Edward O Wilson. 2015. *Theory of Island Biogeography.(MPB-1)*. Vol. 1. Princeton University Press.

Malthus, Thomas Robert. 1878. *An Essay on the Principle of Population*. London, Reeves and Turner.

Maurice, C. F., C. Bouvier, R. Wit, and T. Bouvier. 2013. “Linking the Lytic and Lysogenic Bacteriophage Cycles to Environmental Conditions, Host Physiology and Their Variability in Coastal Lagoons.” *Environmental Microbiology* 15 (9). Wiley Online Library: 2463–75.

McDole, Tracey, James Nulton, Katie Barott, Ben Felts, Carol Hand, Mark Hatay, Hochul Lee, et al. 2012. “Assessing Coral Reefs on a Pacific-Wide Scale Using the Microbialization Score.” *PLoS One* 7 (9): e432331–10. doi:10.1371/journal.pone.0043233.

Mills, Susan, Fergus Shanahan, Catherine Stanton, Colin Hill, Aidan Coffey, and R Paul Ross. 2013. “Movers and Shakers: Influence of Bacteriophages in Shaping the Mammalian Gut Microbiota.” *Gut Microbes* 4 (1). Landes Bioscience: 4–16. doi:10.4161/gmic.22371.

Needham, David M, Cheryl-Emiliane T Chow, Jacob A Cram, Rohan Sachdeva, Alma Parada, and Jed A Fuhrman. 2013. “Short-Term Observations of Marine Bacterial and Viral Communities: Patterns, Connections and Resilience.” *The ISME Journal* 7 (7). Nature Publishing Group: 1274–85. doi:10.1038/ismej.2013.19.

Örmälä-Odegrip, Anni-Maria, Ville Ojala, Teppo Hiltunen, Ji Zhang, Jaana K H Bamford, and Jouni Laakso. 2015. “Protist Predation Can Select for Bacteria with Lowered Susceptibility to Infection by Lytic Phages.” *BMC Evolutionary Biology* 15 (1). BioMed Central: 1.

Paul, John H, and Markus Weinbauer. 2010. “Detection of Lysogeny in Marine Environments.” *Manual of Aquatic Viral Ecology* 4: 30–33.

Payet, Jérôme, and Curtis A Suttle. 2013. “To Kill or Not to Kill: The Balance between Lytic and Lysogenic Viral Infection Is Driven by Trophic Status.” *Limnology and Oceanography* 58 (2): 465–74. doi:10.4319/lo.2013.58.2.0465.

Rodriguez-Brito, Beltran, LinLin Li, Linda Wegley, Mike Furlan, Florent Angly, Mya Breitbart, John Buchanan, et al. 2010. “Viral and Microbial Community Dynamics in Four Aquatic Environments.” *The ISME Journal* 4 (6). Nature Publishing Group: 739–51.

Sandin, Stuart A, and Enric Sala. 2012. “Using Successional Theory to Measure Marine Ecosystem Health.” *Evolutionary Ecology* 26 (2). Springer: 435–48.

Six, Erich W, and Carol A.Connelly Klug. 1973. “Bacteriophage P4: A Satellite Virus Depending on a Helper such as Prophage P2.” *Virology* 51 (2): 327–44. doi:http://dx.doi.org/10.1016/0042-6822(73)90432-7.

Somera, Tracey McDole, Barbara Bailey, Katie Barott, Juris Grasis, Mark Hatay, Brett J Hilton, Nao Hisakawa, et al. 2016. “Energetic Differences between Bacterioplankton Trophic Groups and Coral Reef Resistance.” In *Proc. R. Soc. B*, 283:20160467.

Steele, Joshua A, Peter D Countway, Li Xia, Patrick D Vigil, J Michael Beman, Diane Y Kim, Cheryl-Emiliane T Chow, et al. 2011. “Marine Bacterial, Archaeal and Protistan Association Networks Reveal Ecological Linkages.” *The ISME Journal* 5 (9). Nature Publishing Group: 1414–25.

Suttle, Curtis A. 2007. “Marine Viruses — Major Players in the Global Ecosystem.” *Nature Reviews Microbiology* 5 (10): 801–12. doi:10.1038/nrmicro1750.

Takeuchi, Nobuto, Yuri I Wolf, Kira S Makarova, and Eugene V Koonin. 2012. “Nature and Intensity of Selection Pressure on CRISPR-Associated Genes.” *Journal of Bacteriology* 194 (5). Am Soc Microbiol: 1216–25.

Thingstad, T F, and R Lignell. 1997. “Theoretical Models for the Control of Bacterial Growth Rate, Abundance, Diversity and Carbon Demand.” *Aquatic Microbial Ecology* 13 (1). Inter-Research: 19–27.

Thingstad, T Frede. 2000. “Elements of a Theory for the Mechanisms Controlling Abundance, Diversity, and Biogeochemical Role of Lytic Bacterial Viruses in Aquatic Systems.” *Limnology and Oceanography* 45 (6). Wiley Online Library: 1320–28.

Thingstad, T Frede, Selina Våge, Julia E Storesund, Ruth-Anne Sandaa, and Jarl Giske. 2014. “A Theoretical Analysis of How Strain-Specific Viruses Can Control Microbial Species Diversity.” *Proceedings of the National Academy of Sciences* 111 (21). National Acad Sciences: 7813–18.

Touchon, Marie, Sophie Charpentier, Olivier Clermont, Eduardo P C Rocha, Erick Denamur, and Catherine Branger. 2011. “CRISPR Distribution within the Escherichia Coli Species Is Not Suggestive of Immunity-Associated Diversifying Selection.” *Journal of Bacteriology* 193 (10). Am Soc Microbiol: 2460–67.

Touchon, Marie, and Eduardo P C Rocha. 2010. “The Small, Slow and Specialized CRISPR and Anti-CRISPR of Escherichia and Salmonella.” *PloS One* 5 (6). United States: e11126. doi:10.1371/journal.pone.0011126.

Våge, Selina, Julia E. Storesund, and T. Frede Thingstad. 2013. “Adding a Cost of Resistance Description Extends the Ability of Virus-Host Model to Explain Observed Patterns in Structure and Function of Pelagic Microbial Communities.” *Environmental Microbiology* 15 (6): 1842–52. doi:10.1111/1462-2920.12077.

Volterra, Vito. 1926. “Fluctuations in the Abundance of a Species Considered Mathematically.” *Nature* 118: 558–60.

Waldor, Matthew K, and John J Mekalanos. 1996. “Lysogenic Conversion by a Filamentous Phage Encoding Cholera Toxin.” *Science* 272 (5270). The American Association for the Advancement of Science: 1910.

Weitz, Joshua S, Timothée Poisot, Justin R Meyer, Cesar O Flores, Sergi Valverde, Matthew B Sullivan, and Michael E Hochberg. 2013. “Phage--Bacteria Infection Networks.” *Trends in Microbiology* 21 (2). Elsevier: 82–91.

Wilcox, Robin M, and Jed A Fuhrman. 1994. “Bacterial Viruses in Coastal Seawater: Lytic rather than Lysogenic Production.” *Marine Ecology-Progress Series* 114. INTER RESEARCH: 35–45.

Wilhelm, S W, S M Brigden, and C A Suttle. 2002. “A Dilution Technique for the Direct Measurement of Viral Production: A Comparison in Stratified and Tidally Mixed Coastal Waters.” *Microbial Ecology* 43 (1). Springer: 168–73.

Wilhelm, Steven W, and Curtis A Suttle. 1999. “Viruses and Nutrient Cycles in the Sea Viruses Play Critical Roles in the Structure and Function of Aquatic

Food Webs.” *Bioscience* 49 (10). Oxford University Press: 781–88.

Willner, Dana, Mike Furlan, Matthew Haynes, Robert Schmieder, Florent E Angly, Joas Silva, Sassan Tammadoni, Bahador Nosrat, Douglas Conrad, and Forest Rohwer. 2009. “Metagenomic Analysis of Respiratory Tract DNA Viral Communities in Cystic Fibrosis and Non-Cystic Fibrosis Individuals.” *PLoS ONE* 4 (10). Public Library of Science: e7370. doi:10.1371/journal.pone.0007370.

Wommack, K Eric, and Rita R Colwell. 2000. “Virioplankton: Viruses in Aquatic Ecosystems.” *Microbiology and Molecular Biology Reviews* 64 (1). Am Soc Microbiol: 69–114.

Yau, Sheree, Federico M Lauro, Matthew Z DeMaere, Mark V Brown, Torsten Thomas, Mark J Raftery, Cynthia Andrews-Pfannkoch, et al. 2011. “Virophage Control of Antarctic Algal Host--Virus Dynamics.” *Proceedings of the National Academy of Sciences* 108 (15). National Acad Sciences: 6163–68.

CONCLUSION OF THE DISSERTATION

Thresholds in viral dynamics

The work presented in this thesis challenges the accepted paradigm of viral ecology that lytic predation is the default viral pathway, promoted when hosts are abundant or fast growing (i.e., ‘winning’) and that lysogeny is a fall-back viruses exploit only when host densities or growth are not favorable to lysis. The evidence of alternative dynamics assembled through the research here represents an extension of these dynamics. The most profound insight here is not that the dynamics we describe disagree with the established paradigm. Rather it is that transitions exist between established Kill-the-Winner and alternative dynamics, characterized in Chapter 2 as ‘Piggyback-the-Winner’ and ‘Piggyback-the-Loser’ and that unexpected and novel drivers of viral dynamics exist, characterized as the ‘Lurking Variable’ driving the distribution of Fraction of Lysogenic Cells (FCIC) characterized in Chapter 3. From first assuming the established paradigm to be correct, the research herein has illustrated that viral dynamics are more complex than hitherto known.

Lytic to lysogenic switches and viral predation

Increased microbial densities, whether sustained or ephemeral, are a hallmark of degraded ecosystems (Stauffer et al. 2012; McDole et al. 2012; Barott and Rohwer 2012). This is incongruous with ecological models of predator-prey dynamics suggesting that predation should rapidly suppress these blooms (Weitz et al. 2015; Thingstad 2000). While in macro-ecological systems prey population explosions may be explained by the decoupling of prey and predator population cycles (i.e., predators require longer generation times to catch up with prey with shorter generation times; May 2007), this is not appropriate for microbial and viral systems. This is evidenced by the ability to the model virus T4 to clear cultures of log-growth *Escherichia coli*. Recent attempts to incorporate viral- and microbial-specific phenomena like the host-mediated generation of immunity to infection into predator-prey models have shown an attenuation of viral predation at high host densities that can occur within microbial lifespans and allow bloom formation and maintenance (Thingstad et al. 2014; Våge, Storesund, and Thingstad 2013). However, no evidence of increased resistance to infection (measured as CRISPR content) was observed in microbial metagenomes from high host density systems. Rather, evidence of elevated prevalence of lysogeny, which can also confer a viral-mediated immunity to infection, was observed (B Knowles et al. 2016). As a result of this switch towards lysogeny, viral activity may sustain, rather than suppress, microbial blooms.

Lytic to lysogenic switches and ecosystem processes

Viral predatory dynamics promote ecosystem productivity by liberating cellular metabolites and debris during host lysis, forming the Viral Shunt (Thingstad and Lignell 1997; Wilhelm and Suttle 1999; Suttle 2007). Suppression of the Viral Shunt should result in increased export of microbial energy and matter up through the food web as protists prey upon microbial prey, forming the basis of the Microbial Loop (Azam et al. 1983). In addition to the viral predation discussed above, this should also suppress microbial densities (Fuhrman and Noble 1995; Thingstad 2000). However, the analysis in Chapter 2 suggests that lysogenic viruses can confer on their hosts the ability to evade this predation by horizontal transfer of virulence factors similar to Cholera toxin (Waldor and Mekalanos 1996). It therefore appears that lysogenization of hosts my have the benefit of halting both viral and protist predation, sustaining blooms (Knowles et al. 2016). This dynamic has the potential to substantially alter the flow of energy and matter in the environment with energy and matter becoming localized in the microbial, and potentially pathogenic, fraction (Dinsdale et al. 2008; Barott and Rohwer 2012).

Evidence for lysogeny and mitomycin C induction

As discussed in the Introduction and in Chapters 1 and 2, the established lytic/lysogenic switch rests on two lines of evidence: (i) mathematical models of predator-prey dynamics (Weitz et al. 2015; Weitz and Dushoff 2008; Thingstad 2000), and (ii) estimations of the lysogeny through mitomycin C-mediated prophage induction (termed Fraction of Chemically Inducible Cells here; FCIC; Jiang and Paul 1996; Jiang and Paul 1994; Maurice et al. 2013; Maurice et al. 2011; Payet and Suttle 2013). Mitomycin C-based studies are characterized as showing a negative relationship between host density and lysogeny (Maurice et al. 2010; Payet and Suttle 2013; Brum et al. 2015), seemingly at odds with the Piggyback-the-Winner model developed in Chapter 2. In order to reconcile this discordance, a literature survey of published mitomycin C induction datasets was conducted (Chapter 3). These analyses indicated that the evidence against the Piggyback-the-Winner model afforded by FCIC datasets was not strong. However, FCIC offers potential insight into viral dynamics once the lack of dependence of FCIC on the canonical drivers host density or growth is discarded, and it is examined in isolation. From the inconsistencies observed in the FCIC datasets, published and derived here, it can be seen that FCIC varies stochastically with dilution, shows a gradation from soil to sediment and is variable in aquatic ecosystems in between, is inconsistent with abiotic drivers such as temperature or salinity, and can be conserved across host densities and growth rates. These characteristics may allow the characterization of a hitherto unconsidered, and potentially novel, driver of viral dynamics. This is likely to be a rewarding direction for future studies.

Future directions

The ecosystem impacts implied by the lytic to lysogenic switching of viral communities outlined in Chapter 2 require further investigation. While it appears that heterotrophic protists and lytic viral communities may be disadvantaged by Piggyback-the-Winner dynamics, this is yet to be experimentally confirmed. There is likely an emergent hysteresis of such a switch in determining ecosystem fate: once the vital rates of lytic viruses and grazing protists have been suppressed and lysogens selected for, even if host growth or density fall, it is unknown whether these disadvantaged, and perhaps rare, communities can then help ecosystems return to their pre-Piggyback-the-Winner transition state. However, transitions across the Piggyback-the-Loser Kill-the-Winner Piggyback-the-Winner spectrum are yet to be probed experimentally. It is possible that microbialized steady states in which the viral shunt and microbial loop have been suppressed are exceedingly stable, but highly unproductive. Developing a deep understanding of the full food web and ecosystem-wide impacts of Piggyback-the-Winner dynamics may provide insight into the future functioning of ecosystems. Finally, the determining what drives FCIC promises to be a fascinating avenue of future study, with the potential to allow viral ecology to transcend canonical drivers of viral dynamics like host density and growth rate as outlined in Chapter 3. This lurking variable may stimulate a reimagining of the drivers of viral dynamics in unforeseeable ways through future work.

REFERENCES

Azam, F, T Fenchel, J G Field, J S Gray, L Meyer-Reil, and F Thingstad. 1983. “The Ecological Role of Water-Column Microbes in the Sea.” *Mar. Ecol. Prog. Ser.* 10: 257–63.

Barott, Katie L, and Forest L Rohwer. 2012. “Unseen Players Shape Benthic Competition on Coral Reefs.” *Trends in Microbiology* 20 (12). Elsevier Current Trends: 621–28.

Brum, Jennifer R, Bonnie L Hurwitz, Oscar Schofield, Hugh W Ducklow, and Matthew B Sullivan. 2015. “Seasonal Time Bombs: Dominant Temperate Viruses Affect Southern Ocean Microbial Dynamics.” *ISME J*, August. International Society for Microbial Ecology, 1–13. http://dx.doi.org/10.1038/ismej.2015.125.

Dinsdale, Elizabeth A, Olga Pantos, Steven Smriga, Robert A Edwards, Florent Angly, Linda Wegley, Mark Hatay, et al. 2008. “Microbial Ecology of Four Coral Atolls in the Northern Line Islands.” *PLoS ONE* 3 (2): e15841–17. doi:10.1371/journal.pone.0001584.

Fuhrman, Jed A, and Rachel T Noble. 1995. “Viruses and Protists Cause Similar Bacterial Mortality in Coastal Seawater.” *Limnology and Oceanography* 40 (7): 1236–42. doi:10.4319/lo.1995.40.7.1236.

Jiang, Sunny C, and John H Paul. 1994. “Seasonal and Diel Abundance of Viruses and Occurrence of Lysogeny/bacteriocinogeny in the Marine Environment.” *Marine Ecology-Progress Series* 104: 163.

———. 1996. “Occurrence of Lysogenic Bacteria in Marine Microbial Communities as Determined by Prophage Induction.” *Marine Ecology-Progress Series* 142: 27.

Knowles, B, C B Silveira, B A Bailey, K Barott, V A Cantu, A G Cobián-Güemes, F H Coutinho, et al. 2016. “Lytic to Temperate Switching of Viral Communities.” *Nature* advance online publication (March). Nature Publishing Group, a division of Macmillan Publishers Limited. All Rights Reserved. http://dx.doi.org/10.1038/nature17193.

Maurice, C F, Thierry Bouvier, J Comte, F Guillemette, and P A Del Giorgio. 2010. “Seasonal Variations of Phage Life Strategies and Bacterial Physiological States in Three Northern Temperate Lakes.” *Environmental*

Microbiology 12 (3). Wiley Online Library: 628–41.

Maurice, C. F., C. Bouvier, R. Wit, and T. Bouvier. 2013. “Linking the Lytic and Lysogenic Bacteriophage Cycles to Environmental Conditions, Host Physiology and Their Variability in Coastal Lagoons.” *Environmental Microbiology* 15 (9). Wiley Online Library: 2463–75.

Maurice, Corinne F, David Mouillot, Yvan Bettarel, Rutger De Wit, Hugo Sarmento, and Thierry Bouvier. 2011. “Disentangling the Relative Influence of Bacterioplankton Phylogeny and Metabolism on Lysogeny in Reservoirs and Lagoons.” *The ISME Journal* 5 (5). Nature Publishing Group: 831–42.

May, Robert. 2007. *Theoretical Ecology: Principles and Applications*. Oxford University Press on Demand.

McDole, Tracey, James Nulton, Katie Barott, Ben Felts, Carol Hand, Mark Hatay, Hochul Lee, et al. 2012. “Assessing Coral Reefs on a Pacific-Wide Scale Using the Microbialization Score.” *PLoS One* 7 (9): e432331–10. doi:10.1371/journal.pone.0043233.

Payet, Jérôme, and Curtis A Suttle. 2013. “To Kill or Not to Kill: The Balance between Lytic and Lysogenic Viral Infection Is Driven by Trophic Status.” *Limnology and Oceanography* 58 (2): 465–74. doi:10.4319/lo.2013.58.2.0465.

Stauffer, Beth A, Alyssa G Gellene, Astrid Schnetzer, Erica L Seubert, Carl Oberg, Gaurav S Sukhatme, and David A Caron. 2012. “An Oceanographic, Meteorological, and Biological ‘perfect Storm’yields a Massive Fish Kill.” *Marine Ecology Progress Series* 468: 231–43.

Suttle, Curtis A. 2007. “Marine Viruses — Major Players in the Global Ecosystem.” *Nature Reviews Microbiology* 5 (10): 801–12. doi:10.1038/nrmicro1750.

Thingstad, T F, and R Lignell. 1997. “Theoretical Models for the Control of Bacterial Growth Rate, Abundance, Diversity and Carbon Demand.” *Aquatic Microbial Ecology* 13 (1). Inter-Research: 19–27.

Thingstad, T Frede. 2000. “Elements of a Theory for the Mechanisms Controlling Abundance, Diversity, and Biogeochemical Role of Lytic Bacterial Viruses in Aquatic Systems.” *Limnology and Oceanography* 45 (6). Wiley Online Library: 1320–28.

Thingstad, T Frede, Selina Våge, Julia E Storesund, Ruth-Anne Sandaa, and Jarl Giske. 2014. “A Theoretical Analysis of How Strain-Specific Viruses Can Control Microbial Species Diversity.” *Proceedings of the National Academy of Sciences* 111 (21). National Acad Sciences: 7813–18.

Våge, Selina, Julia E. Storesund, and T. Frede Thingstad. 2013. “Adding a Cost of Resistance Description Extends the Ability of Virus-Host Model to Explain Observed Patterns in Structure and Function of Pelagic Microbial Communities.” *Environmental Microbiology* 15 (6): 1842–52. doi:10.1111/1462-2920.12077.

Waldor, Matthew K, and John J Mekalanos. 1996. “Lysogenic Conversion by a Filamentous Phage Encoding Cholera Toxin.” *Science* 272 (5270). The American Association for the Advancement of Science: 1910.

Weitz, Joshua S, and Jonathan Dushoff. 2008. “Alternative Stable States in Host--Phage Dynamics.” *Theoretical Ecology* 1 (1). Springer: 13–19.

Weitz, Joshua S, Charles A Stock, Steven W Wilhelm, Lydia Bourouiba, Maureen L Coleman, Alison Buchan, Michael J Follows, et al. 2015. “A Multitrophic Model to Quantify the Effects of Marine Viruses on Microbial Food Webs and Ecosystem Processes.” *The ISME Journal* 9 (6). Nature Publishing Group: 1352–64.

Wilhelm, Steven W, and Curtis A Suttle. 1999. “Viruses and Nutrient Cycles in the Sea Viruses Play Critical Roles in the Structure and Function of Aquatic Food Webs.” *Bioscience* 49 (10). Oxford University Press: 781–88.