

Re-examination of the relationship between marine virus and microbial cell abundances

Charles H. Wigington,¹ Derek Sonderegger,² Corina P.D. Brussaard,^{3,4} Alison Buchan,⁵ Jan F. Finke,⁶ Jed A. Fuhrman,⁷ Jay T. Lennon,⁸ Mathias Middelboe,⁹ Curtis A. Suttle,^{6,10,11} Charles Stock,¹² William H. Wilson,¹³ K. Eric Wommack,¹⁴ Steven W. Wilhelm,^{5,*} and Joshua S. Weitz^{1,15,†}

¹ School of Biology, Georgia Institute of Technology, Atlanta, GA 30332

² Department of Mathematics and Statistics, Northern Arizona University, Flagstaff, AZ

³ Department of Biological Oceanography, Royal Netherlands Institute for Sea Research (NIOZ), Texel, The Netherlands.

⁴ Department of Aquatic Microbiology, Institute for Biodiversity and Ecosystem Dynamics (IBED), University of Amsterdam, The Netherlands.

⁵ Department of Microbiology, The University of Tennessee, Knoxville, TN

⁶ Department of Earth, Ocean and Atmospheric Sciences, University of British Columbia, Vancouver BC V6T 1Z4

⁷ Dept of Biological Sciences, University of Southern California, Los Angeles 90089

⁸ Department of Biology, Indiana University, Bloomington, IN 47405

⁹ Marine Biological Section, Department of Biology, University of Copenhagen, Helsingør, Denmark

¹⁰ Departments of Microbiology and Immunology, and Botany, University of British Columbia, Vancouver BC V6T 1Z4

¹¹ Program in Integrated Microbial Diversity, Canadian Institute for Advanced Research, Toronto ON M5G 1Z8

¹² Geophysical Fluid Dynamics Laboratory Princeton, NJ 08540-6649

¹³ Sir Alister Hardy Foundation for Ocean Science, The Laboratory, Citadel Hill, Plymouth, UK

¹⁴ Plant and Soil Sciences Delaware Biotechnology Institute Newark, DE

¹⁵ School of Physics, Georgia Institute of Technology, Atlanta, GA 30332

(Dated: December 21, 2015)

Supplementary Text 1 – Operational definitions of viral and microbial abundances

The operational definitions “near-surface” and “sub-surface” are used to indicate predominantly euphotic and aphotic ocean depths [1]. We use the term virus abundance throughout this manuscript to denote estimates derived from culture-independent methods, including epifluorescence microscopy [2] or flow cytometry [3]. Viruses measured in these methods are generally thought to represent bacteriophage, consistent with the numerical dominance of bacteria in seawater [4]. Yet, currently available methods have potential limitations. For example, ssDNA viruses [5, 6], RNA viruses [7, 8], and giant viruses [9] are under-counted when estimates are made via epifluorescence microscopy with standard DNA based stains.

*Electronic address: wilhelm@utk.edu

†Electronic address: jsweitz@gatech.edu

Year	Observation	Reference
1894	Marine bacteria are first discussed by Certes, Fischer and Russell	Certes [10], Fischer [11], Russell [12], Fischer [13]
1915,1917	Bacteriophage are discovered	Twort [14], d'Hérelle [15]
1925	The presence of bacteriophage in seawater is noted	Arloing and Chavanne [16]
1946	ZoBell reports that bacteriophage occur only sporadically and in the littoral zone and concludes there is insufficient evidence for viruses to be considered as key to limiting open ocean bacteria	ZoBell [17], Carlucci and Pramer [18]
1947	The presence of bacteriophage described in the oceans	Kriss [19]
1979	Using transmission electronic microscopy, up to 10^4 ml ⁻¹ bacteriophage particles are observed in coastal water, an observations that sparked the rebirth of virus ecology a decade later.	Torrella and Morita [20]
1989	"Rebirth" of virus ecology across a series off papers begins with a report of virus and bacteria abundances for which VMRs range from 0.2 (Raunefjorden) to 50 (North Atlantic)	Bergh et al. [21]
1990	Report of virus particles ranging from 10^6 - 10^{11} per liter, infecting up to 7% of heterotrophic bacteria and each infected cell containing 10-100 mature virions	Proctor and Fuhrman [22]
1991-1993	Estimates of virus abundance exceeding bacteria abundance by 5-10 fold from a series of papers (this observation noted in Fuhrman and Suttle [23])	Hara et al. [24], Paul et al. [25], Wommack et al. [26], Cochlan et al. [27], Paul et al. [28]
1995	Maranger and Bird [29] survey 22 Quebec lakes and collect literature from 14 studies [21, 24, 26-28, 30-35] and report VMR higher in freshwater (20-25) than marine systems (1-5).	Maranger and Bird [29]
2000	Wommack and Colwell suggest that VMR typically ranges between 3 and 10, and note that VMR decreases as microbial abundance increases.	Wommack and Colwell [36]
2000	A VMR "roughly equal to 10" (attributed to Maranger and Bird [29] is designated as a target for parameterizing the Kill-the-Winner theory of virus-microbe interactions.	Thingstad [37]
2004	Consistency in VMR is attributed to the idea that most viruses are phage that infect bacteria. Notes a VMR of 10 in marine systems and attributes to Maranger and Bird [29].	Weinbauer [4]
2004	Chibani-Chennoufi and colleagues advance the notion that VMR is 10:1 in the ocean and that this is justified by the claim that each bacterial species can be infected by 10 different phage.	Chibani-Chennoufi et al. [38]
2008	VMR ratios reviewed in several publications that collate information from multiple studies, with a 10:1 consensus despite noted variation.	Clasen et al. [39], Wilhelm and Matteson [40]
2011	VMR reviewed across several regimes, with evidence for a linear relationship between viruses and microbes in the water column and a nonlinear relationship in sediment.	Danovaro et al. [41]
2014	The BioNumbers database, intended to facilitate quantitative analysis in the biosciences, lists VMR as 10.	Milo et al. [42]

TABLE S1: Origins and emerging consensus of the 10:1 ratio of virus abundance to microbial cell abundance in aquatic systems - from freshwater lakes to the global oceans.

Study	$\leq 100\text{m}$	$> 100\text{m}$	Total
ARCTICSBI	292	0	292
BATS	626	756	1382
BEDFORDBASIN	188	0	188
CASES03-04	199	46	245
ELA	85	0	85
FECYCLE1	31	0	31
FECYCLE2	15	0	15
GEOTRACES	141	631	772
GEOTRACES_LEG3	78	351	429
GREENLAND2012	78	46	124
INDIANOCEAN2006	42	10	52
KH04_5	159	383	542
KH05_2	117	238	355
MOVE	84	0	84
NASB2005	31	0	31
NORTHSEA2001	164	27	191
POWOW	9	0	9
RAUNEFJORD2000	95	0	95
SOG	67	0	67
STRATIPHYT1	89	24	113
STRATIPHYT2	59	34	93
SWAT	31	0	31
TABASCO	12	0	12
TROUT	47	0	47
USC MO	182	204	386
Total	2921	2750	5671

TABLE S2: Number of data points per study.

Model	$\leq 100\text{m}$		$>100\text{m}$	
	R^2	AIC	R^2	AIC
10:1	-0.16	-15305.83	-0.25	-14492.09
Power Law	0.15	-16301.81	0.64	-18313.82
Constrained Power Law	0.39	-17292.11	0.66	-18513.48
Power Law by Study	0.79	-20293.10	0.72	-18972.81

TABLE S3: Information theoretic comparison of alternative models of the relationship between virus and microbial cell abundances. The values of the Aikake Information Criteria (AIC) are defined in the Materials and Materials and Methods. The value of R^2 for each model denotes the relative amount of variance explained. Negative values of R^2 mean that a model explains less variance than does the overall mean.

Study	Intercept	Std. Error	Group
ARCTICSBI	4.552594	0.1580157	A
FECYCLE1	4.552594	0.1837236	A
FECYCLE2	4.552594	0.1961546	A
MOVE	4.552594	0.1982541	A
Raunefjord	4.552594	0.1716625	A
StratiphytI	4.552594	0.1543207	A
USCMO	4.552594	0.1806831	A
KH05.2	4.513041	0.1683358	A
SOG	4.480697	0.1869220	A
POWOW	4.408948	0.2060002	B
StratiphytII	4.389658	0.1765001	B
KHO4	4.339907	0.1688285	B
CASES0304	4.339902	0.1669256	B
BEDFORDBASIN	4.336256	0.1825271	B
BATS	4.332784	0.1647363	B
ELA	4.332784	0.1885707	B
GEOTRACES	4.332784	0.1580225	B
GEOTRACES_LEG3	4.332784	0.1679548	B
GREENLAND2012	4.332784	0.1759093	B
INDIANOCEAN2006	4.332784	0.1820002	B
NASB2005	4.332784	0.1876671	B
NORTHSEA2001	4.332784	0.1770218	B
SWAT	4.332784	0.1945882	B
TABASCO	4.332784	0.2047635	B
TROUT	4.332784	0.2019569	B

TABLE S4: Variation in the estimate of the intercept, $\alpha_0^{(i)}$, for each study and associated standard error for the constrained power-law model as applied to surface ocean data. The common intercept in this model is $\alpha_0 = 4.44$ and the common slope is 0.42. The group column denotes whether the study-specific intercept exceeds that of the common intercept (denoted as group A) or is below that of the common intercept (denoted as group B). The table is sorted according to the lab-specific intercept estimates.

Study	≤ 100 m		> 100 m	
	R^2	p -value	R^2	p -value
ARCTICSBI	0.441	<1e-05		
BATS	0.045	<1e-05	0.504	<1e-05
BEDFORDBASIN	0.537	<1e-05		
CASES03-04	0.541	<1e-055	0.072	0.0718
ELA	0.343	<1e-05		
FECYCLE	0.146	0.0341		
FECYCLE2	0.004	0.813		
GEOTRACES	0.163	<1e-05	0.706	<1e-05
GEOTRACES_LEG3	0.043	0.0695	0.396	<1e-05
GREENLAND2012	0.868	<1e-05	0.333	2.7e-05
INDIANOCEAN2006	0.068	0.0955	0.288	0.11
KH04.5	0.325	<1e-05	0.703	<1e-05
KH05.2	0.122	0.000112	0.836	<1e-05
MOVE	0.24	<1e-05		
NASB2005	0.382	0.00021		
NORTHSEA2001	0.542	<1e-05	0.51	2.85e-05
POWOW	0.136	0.329		
RAUNEFJORD2000	0.349	<1e-05		
SOG	0.788	<1e-05		
STRATIPHYT1	0.448	<1e-05	0.471	0.000214
STRATIPHYT2	0.768	<1e-05	0.731	<1e-05
SWAT	0.026	0.389		
TABASCO	0.371	0.0354		
TROUT	0.687	<1e-05		
USC MO	0.229	<1e-05	0.462	<1e-05

TABLE S5: Explanatory power and significance of power-law fits for the model in which the power-law exponent is allowed to vary between studies. Empty cells in a row denote the absence of samples collected at depths > 100 m for the study denoted in the left-most column.

Study	≤ 100 m		> 100 m	
	α_0	α_1	α_0	α_1
ARCTICSBI	2.13	0.97		
BATS	4.81	0.31	2.49	0.72
BEDFORDBASIN	1.32	0.91		
CASES03-04	2.40	0.77	2.80	0.65
ELA	2.38	0.66		
FECYCLE	1.29	1.05		
FECYCLE2	5.36	0.38		
GEOTRACES	4.40	0.41	3.63	0.52
GEOTRACES_LEG3	4.09	0.45	3.43	0.53
GREENLAND2012	0.98	0.97	2.05	0.76
INDIANOCEAN2006	4.97	0.28	2.75	0.66
KH04_5	4.04	0.48	3.00	0.64
KH05_2	4.66	0.40	2.48	0.76
MOVE	5.06	0.45		
NASB2005	1.80	0.69		
NORTHSEA2001	0.74	1.00	1.59	0.84
POWOW	5.14	0.30		
RAUNEFJORD2000	4.30	0.48		
SOG	-0.68	1.25		
STRATIPHYT1	3.42	0.71	4.53	0.45
STRATIPHYT2	2.93	0.68	2.96	0.67
SWAT	6.36	0.11		
TABASCO	3.73	0.49		
TROUT	1.83	0.78		
USC MO	4.37	0.49	2.18	0.79

TABLE S6: Power-law exponents, α_1 , and intercepts, α_0 , for each study from the mixed model allowing study-specific slopes and intercepts. Empty cells in a row denote the absence of samples collected at depths > 100 m for the study denoted in the left-most column.

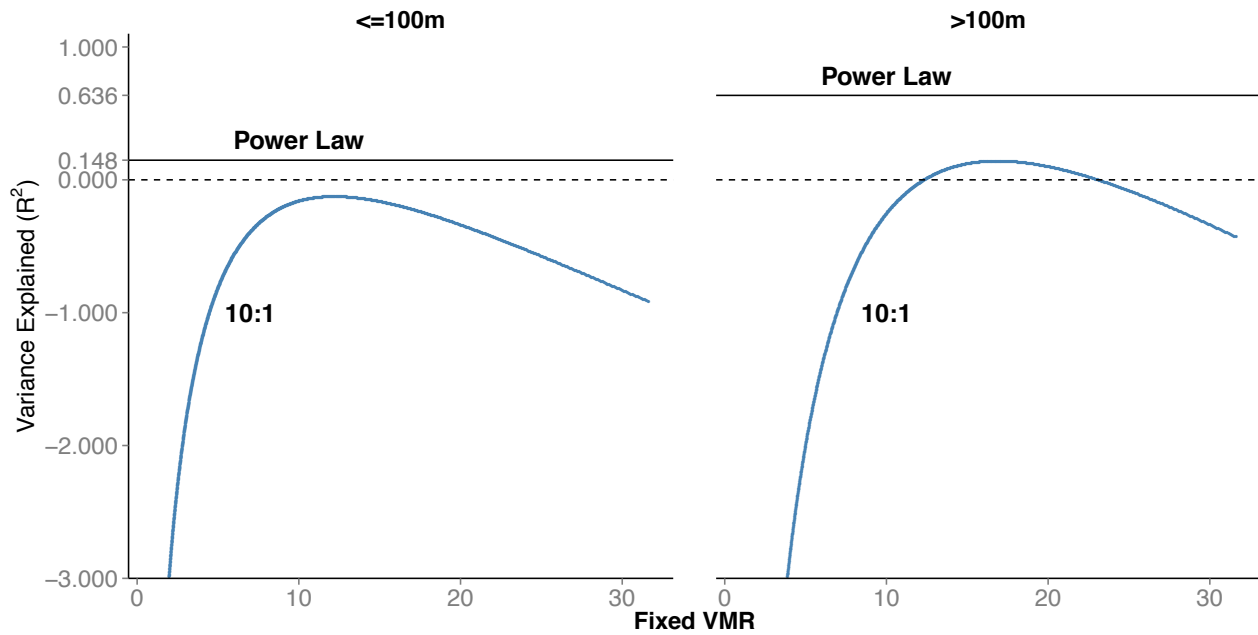


FIG. S1: Explanatory power of fixed VPR models in the surface ocean (left) and deeper water column (right). The x-axis denotes the value r in the model $V = rM$ where V denotes virus abundance and M denotes microbial abundance. The y-axis denotes the fraction of variance explained, R^2 . Here, $R^2 = 1 - \text{SSE}_{\text{model}}/\text{SSE}_{\text{total}}$ where $\text{SSE}_{\text{model}}$ is the sum of squared errors for the model and $\text{SSE}_{\text{total}}$ is the sum of total squared errors.

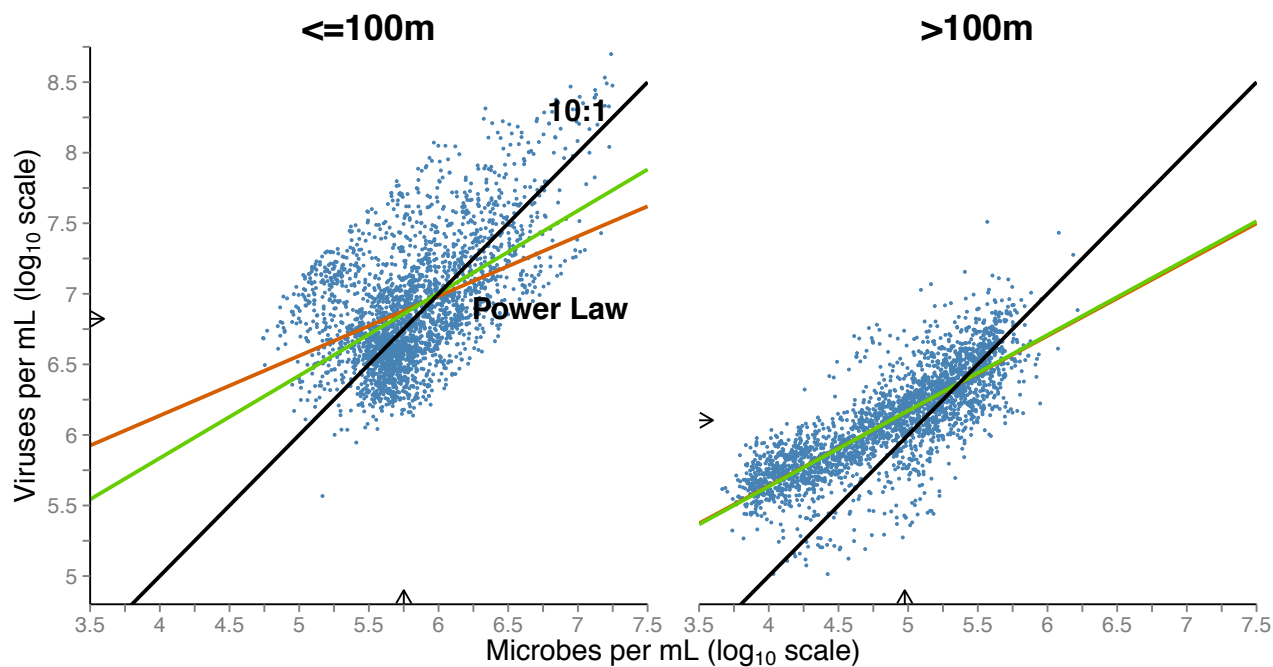


FIG. S2: Explanatory power of fixed VPR models in the near-surface and sub-surface with and without outliers. The three lines in each panel denote the 10:1 line (black), power-law fit (red) and power-law fit when removing outliers (green). The R^2 value for the power law fit for surface data excluding outliers is 0.30, has a slope of 0.58 and an intercept of 3.50. The R^2 value for the power law fit for sub-surface data excluding outliers is 0.65, has a slope of 0.54 and an intercept of 3.49.

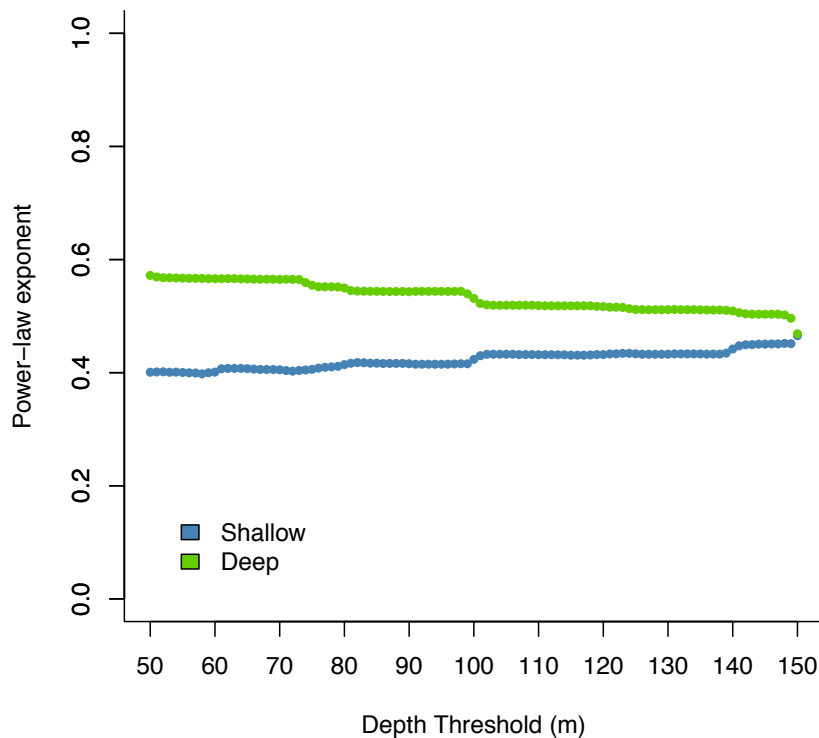


FIG. S3: Variation in estimated power-law exponent as a function of sampling depth cutoff, over the range 50m to 150m. In all cases power-law exponents were measured on log transformed data (see Materials and Methods). The slope varies from 0.40 – 0.47 for near-surface samples, as compared to the CI of 0.39 – 0.46 when using 100m cutoffs, i.e., nearly coinciding with the original uncertainty in the estimated slope. The slope varies from 0.47 – 0.57 for sub-surface samples, as compared to the CI of 0.52 – 0.55 when using 100m cutoffs. This represents an approximately 10% change in slope estimate. The trend in slope with changes in cutoff depth reflects the difference between near- and sub-surface scaling relationships which are shallower and steeper, respectively. Irrespective of cutoff, we conclude that power-law exponents are sublinear, close to that when estimated using a 100m cutoff.

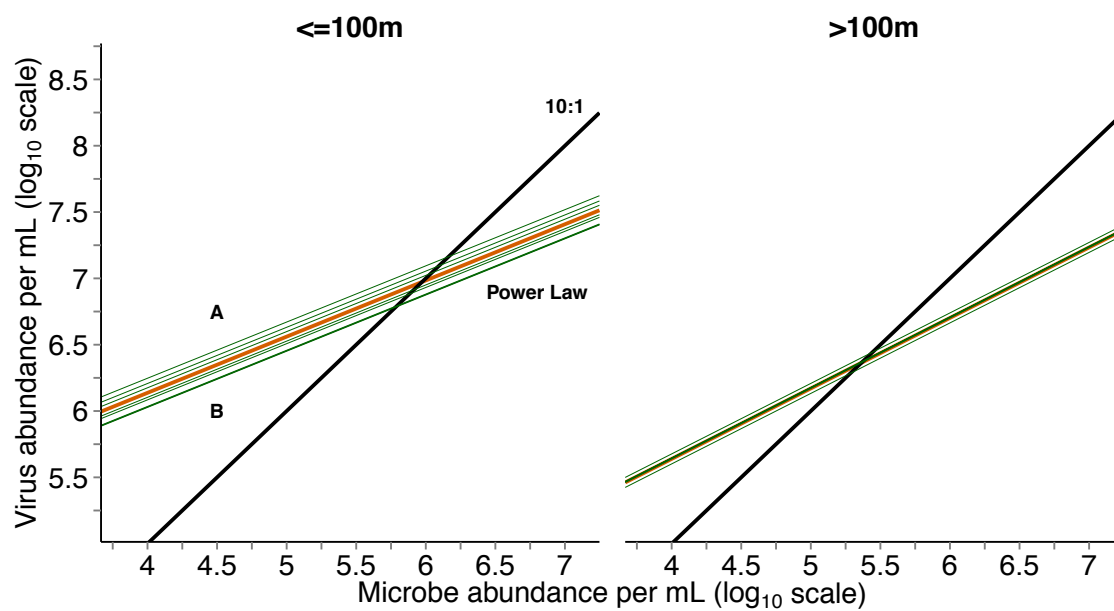


FIG. S4: Constrained regression model for samples taken at depths $\leq 100\text{m}$ (left) and $> 100\text{m}$ (right) where the intercept for each study was permitted to vary (see Materials and Methods). Blue line denotes the 10:1 relationships, the red line denotes the best-fitting power-law model, and the remainder of lines denote the variable intercept model with intercept values reported in Table S4.

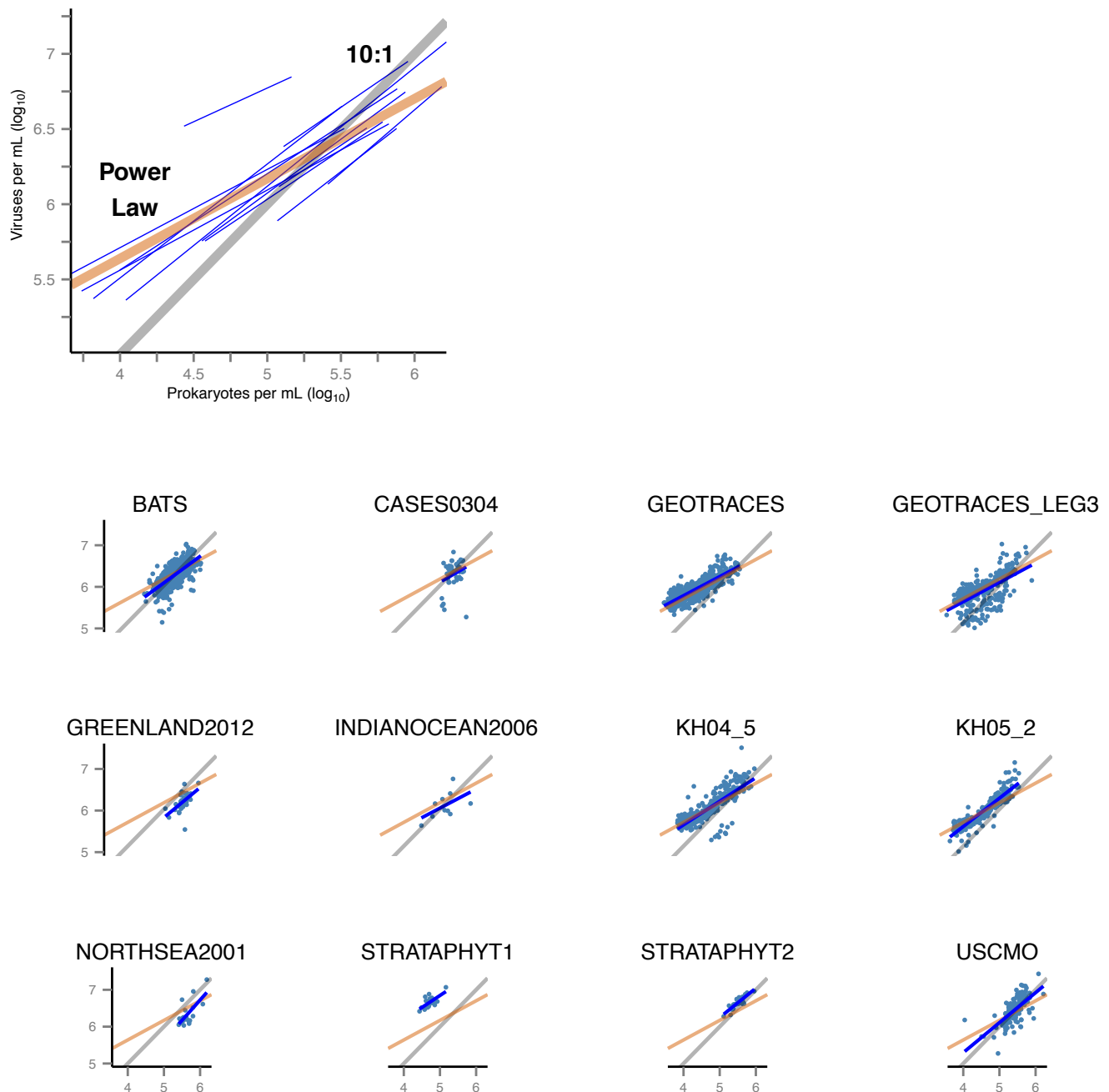


FIG. S5: Virus-microbe relationships given the variable slope and intercept mixed-effects model for samples taken at depths greater than 100m. (Upper-left) Best-fit power-law for each study (blue lines) plotted along with the best-fit power-law of the entire dataset (red line) and the 10:1 line (grey line). (Individual panels) Best-fit power-law model (blue line) on log-transformed data (blue points) for each study, with the power-law model regression (red) and 10:1 line (black) as reference. The power-law exponents and associated confidence intervals are shown in Figure S6,

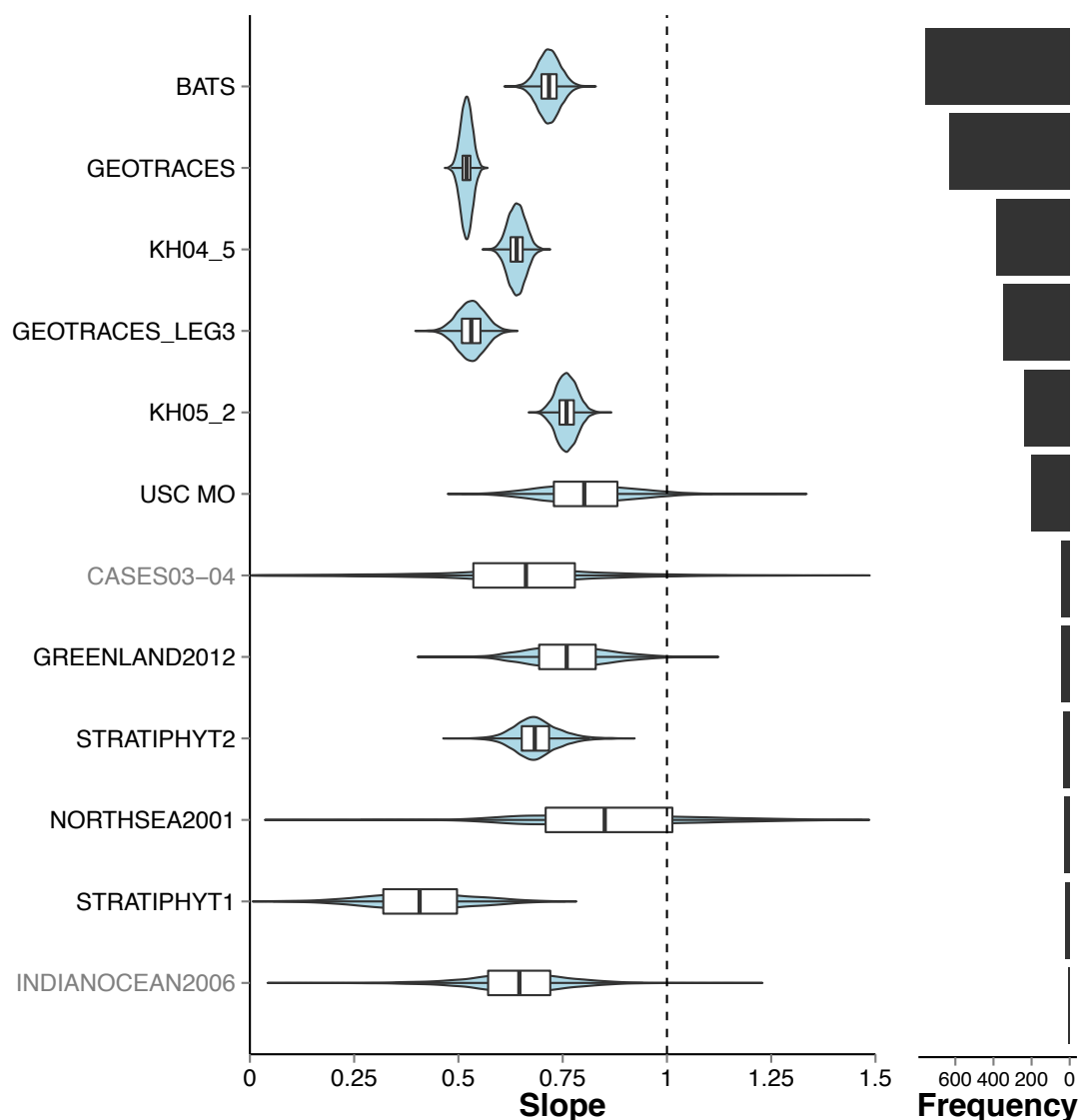


FIG. S6: Study-specific 95% confidence intervals of power-law exponents for relationships between virus and microbial cell abundance from samples taken at depths greater than 100m. The confidence intervals are plotting using “violin” plots including the median (center black line), 75% distribution (white bars) and 95% distribution (black line), with the distribution overlaid (blue shaded area). The number of points included as part of each study is displayed on the right-most bar plots. Study labels in black indicate those studies whose linear regression had a p-value less than .05/12 while labels in gray indicate a p-value above this threshold.

- 1 A. Morel and J. Berthon. Surface pigments, algal biomass profiles, and potential production of the euphotic layer: Relationships reinvestigated in view of remote-sensing applications. *Limnology and Oceanography*, 34(8):1545–1562, 1989.
- 2 C.A. Suttle and J.A. Fuhrman. Enumeration of virus particles in aquatic or sediment samples by epifluorescence microscopy. In S. W. Wilhelm, M. G. Weinbauer, and C.A. Suttle, editors, *Manual of Aquatic Viral Ecology*, pages 145–153, Waco, TX, 2010. American Society of Limnology and Oceanography.
- 3 C.P.D. Brussaard, J.P. Payet, C. Winter, and M.G. Weinbauer. Quantification of aquatic viruses by flow cytometry. In S.W. Wilhelm and M.G. Weinbauer, editors, *Manual of Aquatic Viral Ecology*, pages 102–109, 2010.
- 4 M.G. Weinbauer. Ecology of prokaryotic viruses. *FEMS Microbiology Reviews*, 28(2):127–181, 2004.
- 5 Y. Tomaru and K. Nagasaki. Flow cytometric detection and enumeration of DNA and RNA viruses infecting marine eukaryotic microalgae. *Journal of Oceanography*, 63(2):215–221, 2007.
- 6 J.M. Labonté and C.A. Suttle. Previously unknown and highly divergent ssDNA viruses populate the oceans. *The ISME Journal*, 7(11):2169–2177, 2013.
- 7 C.P.D. Brussaard, D. Marie, and G. Bratbak. Flow cytometric detection of viruses. *Journal of Virological Methods*, 85:175–182, 2000.
- 8 G.F. Steward, A.I. Culley, J.A. Mueller, E.M. Wood-charlson, M. Belcaid, and G. Poisson. Are we missing half of the viruses in the ocean? *ISME J.*, 7:672–679, 2012.
- 9 D. Raoult, S. Audic, C. Robert, C. Abergel, P. Renesto, H. Ogata, B. La Scola, M. Suzan, and J. Claverie. The 1.2-megabase genome sequence of mimivirus. *Science*, 306:1344–1350, 2004.
- 10 A. Certes. Sur la culture, à labri des germes atmosphériques, des eaux et des sédiments rapportés par les expéditions du travailleur et du talisman 1882 - 1883. *Comptes Rendus Acad Sci*, (98):4, 1884.
- 11 B. Fischer. Bakteriologische untersuchungen auf einer reise nach west- indien. *Zeitschrift für Hygiene und Infektionskrankheiten*, (1):44, 1886.
- 12 H.L. Russell. Untersuchungen ber im golf von neapel lebende bakterien. *Zeitschrift für Hygiene und Infektionskrankheiten*, 11:42, 1891.
- 13 B. Fischer. *Die Bakterien des Meeres nach den Untersuchungen der Plankton-Expedition unter gleichzeitiger Berücksichtigung einiger älterer und neuerer Untersuchungen*. Lipsius & Tischer, Keil, 1894.
- 14 T.W. Twort. An investigation on the nature of ultra-microscopic viruses. *Lancet*, 2:1241–1243, 1915.
- 15 F. d'Hérelle. Sur un microbe invisible antagoniste des bacilles dysentériques. *Cr. R. Acad. Sci. Paris*, 165, 1917.
- 16 F. Arloing and Chavanne. On the influence of the environment on the bacteriophage, electrolytes and the concentration of H ions. *Comptes Rendus Des Séances De La Société De Biologie Et De Ses Filiales*, 93:531–532, 1925.
- 17 C.E. ZoBell. *Marine microbiology, a monograph on hydrobacteriology*. Marine microbiology. A monograph on hydrobacteriology. 1946.
- 18 A.F. Carlucci and D. Pramer. An evaluation of factors affecting the survival of Escherichia-coli in sea water. *Applied Microbiology*, 8(4):251–254, 1960.
- 19 Rukina E.A. Kriss, A.E. *Bacteriophage in the sea. (In Russian)*, volume 57. Dok Akad Nauk SSSR, 1947.
- 20 F. Torrella and R.Y. Morita. Evidence by electron-micrographs for a high-incidence of bacteriophage particles in the waters of Yaquina Bay, Oregon - ecological and taxonomical implications. *Applied and Environmental Microbiology*, 37(4):774–778, 1979.
- 21 O. Bergh, K. Y. Borsheim, G. Bratbak, and M. Haldal. High abundance of viruses found in aquatic environments. *Nature*, 340(6233):467–468, 1989.
- 22 L.M. Proctor and J. A. Fuhrman. Viral mortality of marine-bacteria and cyanobacteria. *Nature*, 343(6253):60–62, 1990.
- 23 J.A. Fuhrman and C.A. Suttle. Viruses in marine planktonic systems. *Oceanography*, 6(2):51–63, 1993.
- 24 S. Hara, K. Terauchi, and I. Koike. Abundance of viruses in marine waters - assessment by epifluorescence and transmission electron-microscopy. *Applied and Environmental Microbiology*, 57(9):2731–2734, 1991.
- 25 J.H. Paul, S. C. Jiang, and J. B. Rose. Concentration of viruses and dissolved DNA from aquatic environments by vortex flow filtration. *Applied and Environmental Microbiology*, 57(8):2197–2204, 1991.
- 26 K.E. Wommack, R.T. Hill, M. Kessel, E. Russekcohen, and R.R. Colwell. Distribution of viruses in the Chesapeake Bay. *Applied and Environmental Microbiology*, 58(9):2965–2970, 1992.
- 27 W.P. Cochlan, J. Wikner, G.F. Steward, D.C. Smith, and F. Azam. Spatial-distribution of viruses, bacteria and chlorophyll-a in neritic, oceanic and estuarine environments. *Marine Ecology Progress Series*, 92(1-2):77–87, 1993.
- 28 J.H. Paul, J. B. Rose, S. C. Jiang, C. A. Kellogg, and L. Dickson. Distribution of viral abundance in the reef environment of Key Largo, Florida. *Applied and Environmental Microbiology*, 59(3):718–724, 1993.
- 29 R. Maranger and D.F. Bird. Viral abundance in aquatic systems - a comparison between marine and fresh-waters. *Marine Ecology Progress Series*, 121(1-3):217–226, 1995.
- 30 G. Bratbak, M. Haldal, S. Norland, and T.F. Thingstad. Viruses as partners in spring bloom microbial trophodynamics. *Applied and Environmental Microbiology*, 56(5):1400–1405, 1990.
- 31 M. Haldal and G. Bratbak. Production and decay of viruses in aquatic environments. *Marine Ecology Progress Series*, 72: 205–212, 1991.
- 32 D.C. Smith, G.F. Steward, F. Azam, and J.T. Hollibaugh. Virus and bacteria abundance in the Drake Passage during January and August 1991. *Antarctic Journal of the United States*, 27:125–127., 1992.
- 33 J. Boehme, M.E. Frischer, S.C. Jiang, C.A. Kellogg, S. Pichard, J.B. Rose, C. Steinway, and J.H. Paul. Viruses, bacteri-

- oplankton, and phytoplankton in the southeastern Gulf-of-Mexico - distribution and contribution to oceanic DNA pools. *Marine Ecology Progress Series*, 97(1):1–10, 1993.
- 34** M.G. Weinbauer, D. Fuks, and P. Peduzzi. Distribution of viruses and dissolved DNA along a coastal trophic gradient in the Northern Adriatic Sea. *Applied and Environmental Microbiology*, 59(12):4074–4082, 1993.
- 35** S.C. Jiang and J.H. Paul. Seasonal and diel abundance of viruses and occurrence of lysogeny/bacteriocinogeny in the marine-environment. *Marine Ecology Progress Series*, 104(1-2):163–172, 1994.
- 36** K. E. Wommack and R. R. Colwell. Virioplankton: Viruses in aquatic ecosystems. *Microbiology and Molecular Biology Reviews*, 64(1):69–114, 2000.
- 37** T.F. Thingstad. Elements of a theory for the mechanisms controlling abundance, diversity, and biogeochemical role of lytic bacterial viruses in aquatic systems. *Limnology and Oceanography*, 45:1320–1328, 2000.
- 38** S. Chibani-Chennoufi, A. Bruttin, M.L. Dillmann, and H. Brussow. Phage-host interaction: an ecological perspective. *Journal of Bacteriology*, 186(12):3677–3686, 2004.
- 39** J.L. Clasen, S.M. Brigden, J.P. Payet, and C.A. Suttle. Evidence that viral abundance across oceans and lakes is driven by different biological factors. *Freshwater Biology*, 53(6):1090–1100, 2008.
- 40** S. W. Wilhelm and A. R. Matteson. Freshwater and marine viroplankton: a brief overview of commonalities and differences. *Freshwater Biology*, 53(6):1076–1089, 2008.
- 41** R. Danovaro, C. Corinaldesi, A. Dell’Anno, J.A. Fuhrman, J.J. Middelburg, R.T. Noble, and C.A. Suttle. Marine viruses and global climate change. *FEMS Microbiology Reviews*, 35(6):993–1034, 2011.
- 42** R. Milo, P. Jorgensen, U. Moran, G. Weber, and M. Springer. BioNumbers-the database of key numbers in molecular and cell biology. *Nucleic Acids Research*, 38:D750–D753, 2010.