Supplemental Appendices: Mojica KDA, Brussaard CPD. 2026. Marine viruses and their role in marine ecosystems and carbon cycling. *Annu. Rev. Mar. Sci.* 18. https://doi.org/10.1146/annurev-marine-040324-020244

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Supplemental Appendices

Supplemental Appendix A: MDA

Most of the published viral mediated mortality rates for natural marine phytoplankton are obtained using the modified dilution assay (MDA) [1-3]. This approach has been pivotal in showcasing the importance of viral-mediated mortality across various phytoplankton groups and highlighting the comparative impact of viruses versus grazing [1, 4, 5]. Additionally, it provides simultaneous estimates of phytoplankton viral lysis, microzooplankton grazing, and gross growth rates (division rates). MDA experiments are typically initiated in the morning to ensure that the incubation period encompasses most of the light cycle, facilitating optimal photosynthesis, supporting any light-dependent adsorption kinetics, as well as providing the energy and organic carbon necessary for viral proliferation.

Like any method, the MDA has some limitations, however, it remains the only assay that provides direct rates of virus-mediated mortality for phytoplankton without the need for conversion factors [6-9]. The MDA is limited to phytoplankton populations with sufficiently high cell abundances to permit effective dilution—typically those in the <20 µm size classes—and to phytoplankton exhibiting measurable viral lysis within the 24-hour incubation period. This size fraction represents an abundant and stable component of phytoplankton assemblages, accounting for 50–90% of the total phytoplankton biomass and contributing substantially to global primary production [10-15]. Similarly, microzooplankton, rather than mesozooplankton, account for most of the global grazing pressure – particularly for this size fraction [16-19]. Thus, provides essential insights into the significant role viruses play in shaping marine phytoplankton communities, while simultaneously offering a valuable comparative perspective on the relative impacts of viral lysis and microzooplankton grazing.

We conducted a meta-analysis of the MDA experiments conducted over the last 22 years, examining trends in viral lysis rates across different phytoplankton classes (*Prochlorococcus, Synechococcus,* picoeukaryotes <3 µm and nanoeukaryotes 3-20 µm) season, and oceanic regions (tropical/subtropical and temperate/polar) (Figure 2*a*, Supplemental Table 1).

1.1 Data compilation

The web of science and google scholar were used to search for peer-reviewed articles that employed the modified dilution assay to measure phytoplankton mortality rates in marine ecosystems. Results were further screened for studies that focused on population-based estimations of mortality rather than bulk community rates (*i.e.*, Chl *a*) using the 4-point dilution series. Data were extracted directly from each article or from accompanying plots using the software plot digitalizer (https://plotdigitizer.com/). On occasion, data was provided by the author upon request. Due to the limited number of studies time series data and mesocosm studies were included. For mesocosm experiments only control mesocosm data was included. Data was initially binned into 10 different phytoplankton classes based on size and fluorescence properties (Supplemental Table 1).

Supplemental Table 1. Median cell diameter and sample size for the 10 different phytoplankton classes distinguished from FCM measurements.

Phytoplankton class	Median diameter (µm)	Sample Size (N)
Prochlorococcus	0.75	26
Synechococcus	0.95	75
Pico IA	0.90	43
Pico IB	1.25	38
Pico II	2.00	86

Pico III	2.50	27
Nano I	3.50	63
Cryptophytes	6.00	19
Nano II	6.50	32
Nano III	9.00	22
Nano IV	15.00	19

1.2 Data

MA18_Brussaard_MDA_data.xlsx

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1.3 Calculations

1.2.2 Total standing stock lysed

The total loss of cells (L_T) was calculated for each phytoplankton size class (Supplemental Table 1) according to [4]. Specifically,

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$$L_T(d^{-1}) = \frac{Total\ Morality}{(Gross\ Growth - Total\ Mortality)} \times \left[X_0 \times e^{(Gross\ growth - Total\ mortality) \times T} - X_0\right]$$

Wherein:

Total mortality is the sum of grazing and viral lysis rates obtained from MDA
Gross growth rate obtained from the MDA
X_o is the phytoplankton abundance (mL^{-1})
T is the time (days) over which the MDA experiment was conducted (*i.e.*, 1 for 24 h incubations).

The total loss of cells for each mortality process was then calculated as the relative proportion of total mortality.

Total loss due to viral lysis (L_v) and grazing (L_g) was calculated as,

$$L_v = \left(\frac{Virus}{Total\ Mortality}\right) \times L_T$$

$$L_g = \left(\frac{Grazing}{Total\ Mortality}\right) \times L_T$$

Standing stock lysed was then calculated as:

$$SS_{lysed} = \frac{Lv}{X_0}$$

1.2.3 Phytoplankton biomass and carbon

Cell volumes were calculated using the median cell diameter for each phytoplankton size class (Supplemental Table 1), assuming spherical cell shapes. Standing stock carbon, carbon lysed, and carbon grazed were subsequently estimated using size-specific carbon-to-volume conversion factors: 237 fgC μm^{-3} for pico-sized plankton [20], and 196.5 fgC μm^{-3} for nano-sized plankton [21].

1.3.3 Global carbon lysed

Global average carbon concentrations ($\mu gC\ L^{-1}$) for pico- and nanoplankton were estimated by dividing total biomass (converted from PgC to µgC) by total ocean volume (L). The regression equation derived from measured C_{lysed} as a function of standing stock carbon (SSC) (Figure 6*b*) was applied to estimate carbon lysed concentrations from these biomass concentration as follows:

$$C_{lysed} = a \times Biomass\ concentration^b$$

where $a = 0.0434$ and $b = 1.0186$. Carbon lysed concentrations were then converted back to global carbon totals (PgC) by multiplying by the ocean volume. Global carbon lysed was calculated for the upper 200 m ocean layer using global biomass estimates of 2.2 PgC for picoplankton and 1.8 PgC for nanoplankton [22], assuming a global ocean surface area of $3.619 \times 10^{14}\ m^2$ [23].

1.3 Analysis of mortality rates obtained from MDA experiments

1.3.1 Methods

A generalized linear model (GLM) was used to assess the effects of season, phytoplankton class, and depth layer (*i.e.*, ML or DCM) on the rate of viral lysis in phytoplankton populations. Statistical analysis was

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performed using R [24] statistical software (v4.3.1) within the RStudio (v2023.09.1) interface supplemented with vegan [25] and emmeans [26] packages. The GLM model was initially structured using a gamma distribution to include main effects and interaction terms. To determine the most appropriate transformation, different link functions (identity, log, and inverse) were tested. The log link function was selected based on Akaike Information Criterion (AIC), residual diagnostics, and overall model performance.

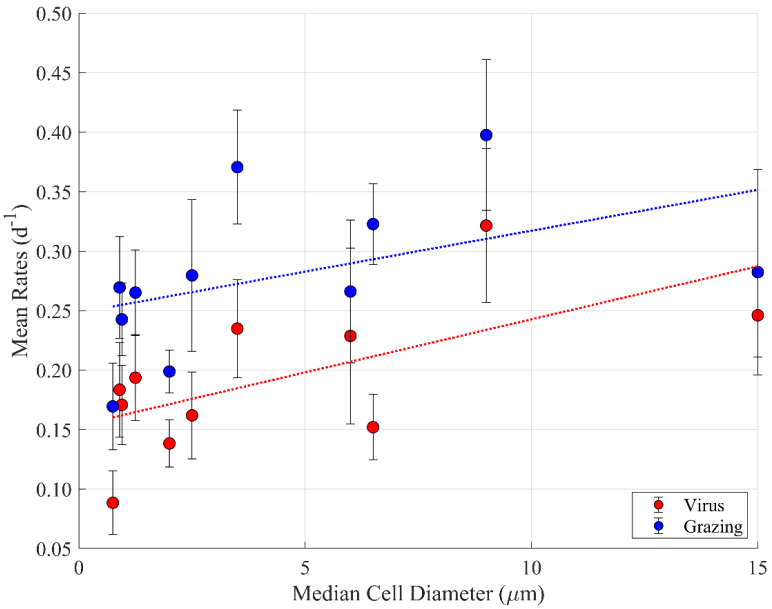
The initial GLM model revealed that the inclusion of depth layer did not significantly contribute to explaining variation in viral lysis rates and therefore to retain the most parsimonious model, this variable was removed. A second model was then run to assess the effect of region, which was categorized into two levels based on absolute latitude: Tropical/Subtropical (absolute latitude <40°) and Temperate/Polar (absolute latitude ≥40°). Models with and without region were then evaluated using ANOVA and AIC.

Region was included in the final model, as it significantly contributed to explaining variability, as evaluated using ANOVA and AIC comparisons between models with and without region. Interaction terms were also removed, as their inclusion did not improve model performance based on AIC evaluation.

Model residuals were assessed for dispersion, and no evidence of overdispersion was found, indicating an adequate fit of the model to viral lysis data. Post-hoc pairwise comparisons were then conducted using estimated marginal means (emmeans), with contrasts performed to identify significant differences between seasons and phytoplankton classes. To control for Type I error, multiple comparisons were adjusted using Tukey's Honest Significant Difference (HSD) correction.

1.3.2 Results

1.3.2.1 Grazing and viral lysis rates



Supplemental Figure 1. Mean loss rates generally scaled with phytoplankton cell diameter. Correlation coefficients were 0.6 for viruses and 0.5 for grazing.

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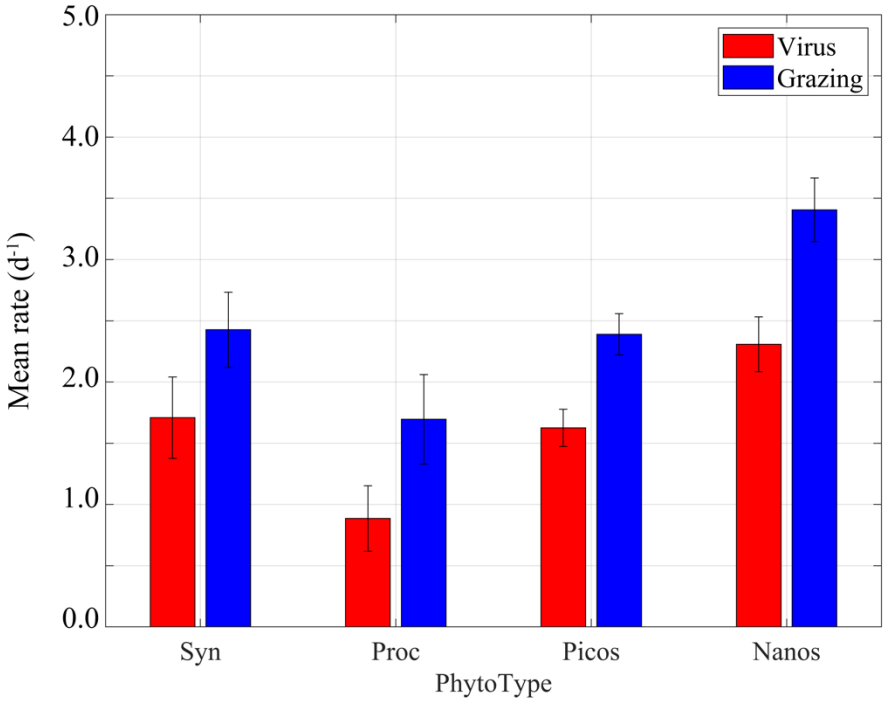
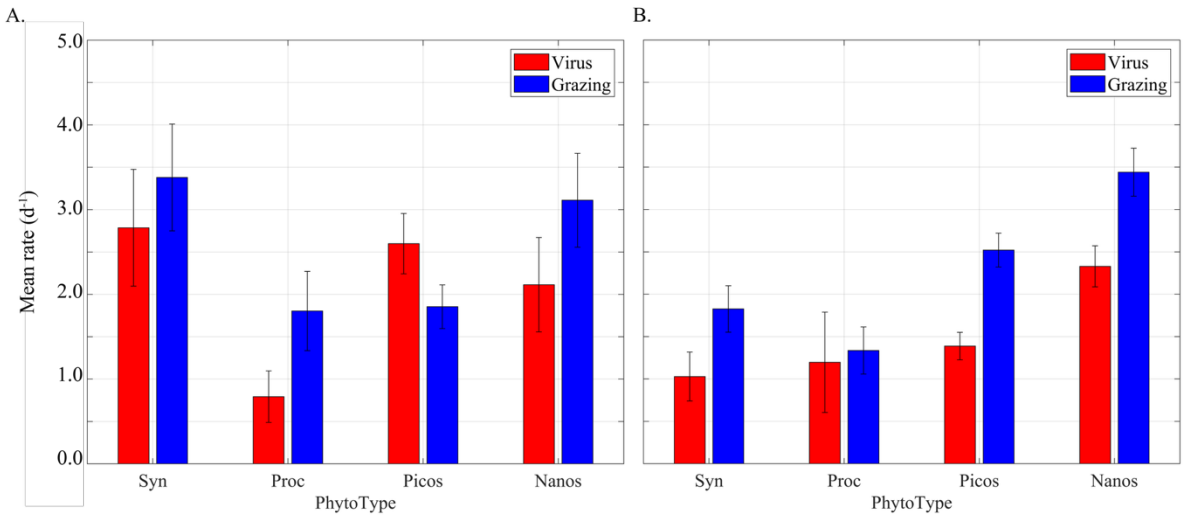


Supplemental Figure 2. Mean viral lysis and grazing rates for each phytoplankton class averaged across the entire dataset. Error bars indicate standard error.

In general, mortality rates scale with cell size (Supplemental Figure 1). Averaged across the entire dataset, mean viral lysis rates were 0.09 ± 0.03 d^{-1} for *Prochlorococcus*, 0.17 ± 0.03 d^{-1} for *Synechococcus*, 0.16 ± 0.02 d^{-1} for Picos, and 0.23 ± 0.02 d^{-1} for Nanos (Supplemental Figure 2). A Kruskal-Wallis non-parametric analysis revealed that grazing rates were significantly higher than viral lysis rates for all groups: 0.17 ± 0.04 d^{-1} for *Prochlorococcus* ($p = 0.006$), 0.24 ± 0.03 d^{-1} for *Synechococcus* ($p = 0.002$), 0.24 ± 0.02 d^{-1} for Picos ($p < 0.001$), and 0.34 ± 0.03 d^{-1} for Nanos ($p = 0.002$). Further analysis, however, demonstrated that the relative contribution of these mortality processes could vary significantly across regions (Supplemental Figure 3).



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Supplemental Figure 3. Mean viral lysis and grazing rates for each phytoplankton class averaged across the (A) tropical/subtropical (absolute latitude <40) and (B) temperate/polar (absolute latitude >40) regions. Error bars indicate standard error.

1.4.2.2 General linear regression analysis of viral lysis rates

GLR analysis revealed that season (*i.e.*, spring, summer, autumn), phytoplankton class (*Prochlorococcus*, *Synechococcus*, Picoeukaryotes (0.8–3 µm) and Climate Region (Tropical/subtropical and Temperate/Polar) significantly affect virus mortality rates measured by the modified dilution approach (Figure 3, Supplemental Table 2). Pairwise comparisons showed that viral lysis rates were significantly higher in the summer compared to the spring, as well as in the tropical/subtropical region compared to the temperate/polar regions (Supplemental Table 3). Among phytoplankton classes, Nanoeukaryotes exhibited the highest mortality rates followed by picoeukaryotes and *Synechococcus*. There is also some evidence suggesting that picoeukaryotes and *Synechococcus* had higher viral lysis rates than *Prochlorococcus*, although this difference was not statistically significant (p-value 0.08 and 0.09, respectively). No significant interaction was found between season and phytoplankton class and viral lysis rates were not significantly different between those obtained from the deep chlorophyll maximum (DCM) and the mixed layer (ML) populations. Moreover, despite observed latitude and seasonal trends, no significant relationship was between viral lysis rates and environmental conditions (*e.g.*, depth, nutrient concentrations, temperature, oxygen, Chl *a*), suggesting that rates are primarily modulated by host dynamics.

Supplemental Table 2. Results of the GLM for the effect of season, phytoplankton class, and region on viral lysis rates obtained from the modified dilution assay. The intercept represents the estimate of viral lysis rates when all categorical predictors are at the reference levels (*i.e.*, Season:spring, Region: temperate/polar, and Phytoplankton class:*Prochlorococcus)*.

Predictor	**Coefficient (β)**	**Standard Error**	**t-value**	**P-value**	**Significance Symbol**
Intercept	-0.041	0.048	-0.849	0.397	
Season (Autumn)	0.027	0.038	0.704	0.482	
Season (Summer)	0.088	0.024	3.609	0.000	**
Region (trop/subt)	0.097	0.026	3.791	0.000	**
Phyto (Nano)	0.178	0.046	3.894	0.000	**
Phyto (Picos)	0.105	0.044	2.386	0.017	*
Phyto (Syn)	0.109	0.046	2.339	0.020	*

Signif. codes: '**' 0.001 '*' 0.05 '·' 0.1

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Supplemental Table 3. Results from post-hoc pairwise comparisons of intra-group differences in viral lysis rates. For each comparison, results were averaged over the remaining two categorical variables. Estimates are provided on the log scale (not the response scale). P-values were adjusted for multiple comparisons using Tukey's Honest Significant Difference (HSD) method to control for Type I error.

Comparison	Mean Difference	Standard Error	df	t-ratio	p-value
Season					
Spring - Autumn	-0.027	0.038	432	-0.704	0.761
Spring - Summer	-0.088	0.024	432	-3.609	0.001
Autumn - Summer	-0.061	0.035	432	-1.713	0.202
Pytoplankton class					
Proc - Nanos	-0.178	0.046	432	-3.894	0.001
Proc - Picos	-0.105	0.044	432	-2.386	0.081
Proc - Syn	-0.109	0.047	432	-2.339	0.091
Nanos - Picos	0.072	0.022	432	3.247	0.007
Nanos - Syn	0.069	0.029	432	2.350	0.089
Picos - Syn	-0.004	0.028	432	-0.126	0.999
Region					
Temp - Trop	-0.097	0.026	432	-3.791	0.000

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Supplemental Appendix B: VP

The viral reduction, or viral production, assay [1-3] is the most common method used to quantitatively assess lytic viral production and lysogeny within heterotrophic prokaryotic (HP) populations in various marine environments.

2.1 Data compilation

The web of science and google scholar were used to search for peer-reviewed articles that employed the virus reduction approach to measure viral production in heterotrophic prokaryote communities in marine ecosystems. Results were screened to focus on studies that focused on field-based measurements using one of the three following virus reduction approaches – Dilution with virus-free water, viral reduction over 0.22 µm membrane filter/vacuum pump setup, and tangential flow filtration. Only induction studies utilizing mitomycin C as an inducing agent were included. Data were extracted directly from each article or from accompanying plots using the software plot digitalizer (https://plotdigitizer.com/). On occasion, data was provided by the author upon request. Data were characterized according to climate region, depth zone, environment and season. Climate region was based on latitudinal range: tropical (0 - 23.5°), subtropics (23.5 – 40°), temperate (40 – 60°), and polar (60-90°). Depth zones were defined as epipelagic (<200 m), mesopelagic (200 – 1000 m), bathypelagic (1000 – 4000 m), abyssopelagic (4000 -6000 m) and hadalpelagic (>6000 m). The Gulf of Mexico (GoM) was classified as a sea based on its characterization as a marginal or coastal sea. Viral production data (N = 592) was compiled from 50 studies which provided a dataset that spans across climate zones (tropical, subtropical, temperate, and polar), marine habitats (ocean, coastal, sea, estuarine, and bays), seasons (spring, summer, autumn, winter), and depth zones (epipelagic, mesopelagic, bathypelagic, hadalpelagic, and abyssalpelagic). Moreover, these studies also included information on BS (N = 137) and lysogenic infection (N = 236).

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MA18_Brussaard_VP_data.xlsx

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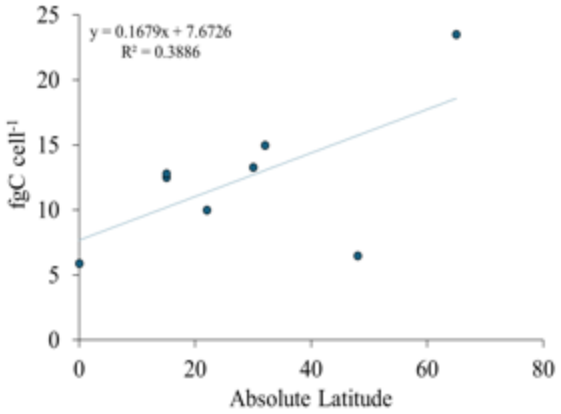
2.3 Data handling and preliminary analysis

2.3.1 Heterotrophic Prokaryotic Production

To maximize data coverage for statistical analysis, several key variables were derived from values reported in the literature. For example, heterotrophic prokaryote production (HPP) was reported as leucine incorporation rates (pmol leucine L^{-1} d^{-1}), carbon uptake (µg C L^{-1} d^{-1}), cell production (cells mL^{-1} d^{-1}), or growth rate (d^{-1}). Since the focus of this review is on carbon-based rates, all reported values were converted to carbon uptake. This required the application of appropriate carbon conversion factors. Although direct measurements of the cellular carbon content of heterotrophic prokaryotes are limited [4-6], available data for ocean communities were analyzed and found to generally increase with latitude (Supplemental Figure 4). The final carbon conversion factors used are listed in Supplemental Table 4; due to the scarcity of data for coastal regions, average values derived primarily from sea environments were applied across all nearshore sites. Leucine incorporation rates were converted to carbon using the empirical conversion factor of 1.5 kg C mol Leu^{-1} [7].



Supplemental Figure 4. Heterotrophic prokaryote cellular content in ocean samples generally varied linearly with absolute latitude. Data compiled from [4-6].

Supplemental Table 4. Heterotrophic prokaryote carbon content used in calculations of Mojica & Brussaard 2025. Ocean estimates were derived from Supplemental Figure 4.

Ocean	fgC $cell^{-1}$
tropics (0-23.5°)	9.73
subtropics (23.5-40°)	12.64
temperate (40-60°)	16.07
polar (>60°)	20.27
Nearshore environments*	30.16

* Average value from Fukuda *[4]* for coastal environments was used for all seas, coastal, bays and estuary environments of current study.

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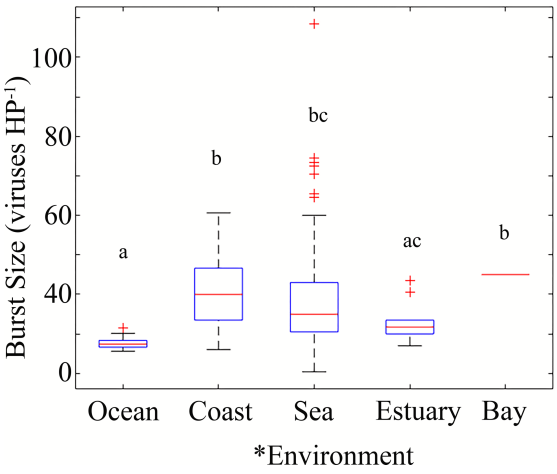
2.3.2 Burst Size

Burst size estimates are essential to convert viral production and prophage induction rates (viruses produced per unit time) into viral mediated mortality (bacterial lysed per unit time). Burst sizes vary across various marine environments and trophic status of ecosystems [8]. Here we further evaluate the potential for burst size estimates from the compiled data set (N=137) to vary across different types of marine environments. A non-parametric Kruskal-Wallis test was used to evaluate overall differences, followed by post-hoc pairwise comparisons using the Wilcoxon rank-sum test with continuity correction. The Holm method was applied to adjust for multiple comparisons. The alpha level for all significance tests was set at 0.05.



Supplemental Figure 5. Boxplot of burst size estimates across different types of marine environments. Bars with different letters are significantly different (P<0.05), as tested by Kruskal-wallis analysis of means followed by post-hoc comparison of Wilcoxon rank sum test.

The type of marine environment had a significant effect on BS estimates (Supplemental Figure 5). Specifically, oceans (N = 13) had the lowest mean BS (Avg±Stdev; 15.4 ± 3.3), which was comparable only to estuary environments (N = 20; 25.9 ± 10.2) (Supplemental Table 5). Bay environments showed the highest mean BS (50 viruses per heterotrophic prokaryote; N = 5), which was similar to coast and sea environments. Coast environments (N = 29) had a mean BS of 40.9 ± 16.1, while sea environments (N = 70) exhibited high variability (39 ± 29.8). Notably, the Adriatic Sea (N = 13) and the South China Sea (N = 13) had the highest BS values (63.7 ± 33.4 and 63.8 ± 40.4, respectively), whereas the Red Sea (N = 5) had the lowest (14 ± 15.3). Rounded average BS values for each environment (Supplemental Table 5) were then used to calculate viral-mediated mortality (VMM) rates and %lysogens. No single environmental driver could be linked to the observed variability in BS across the dataset (*i.e.*, no general trends were found between BS and salinity, temperature, P, HPA, HPP). However, Parada et al. (2006) suggest that these relationships can be masked at broad spatial scales and only emerge when examined at finer spatiotemporal resolutions [8].

Supplemental Table 5. Viral burst size estimates for heterotrophic prokaryote host used in calculations of Mojica & Brussaard 2025.

Environment Type	N	Burst Size (BS)
Ocean	13	15
Coastal	29	41
Sea	70	39
Estuary	20	26
Bay	5	50

2.4 Calculations

2.4.1 Percentage lysogens

Data for lysogenic infection was recorded in the literature either as prophage induction rates (VPC: viruses mL^{-1} d^{-1}) from experiments using mitomycin C, or as the percentage of lysogens within a heterotrophic community. To maximize data coverage, all prophage induction rates were converted to % lysogens according to:

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$$\%Lysogens = \frac{VPC}{BS \times HPA} \times 100$$

where BS is the burst size, estimated based on the type of marine environment (Supplemental Table 5) from which the sample was collected, and HPA was the *in-situ* abundance of heterotrophic prokaryotes (mL^{-1}).

2.4.2 Standing stock lysed (SS_{lysed})

SS_{lysed} was calculated as the percentage of heterotrophic prokaryotes cells lysed by lytic viral infection, using the following equation:

$$SS_{lysed} = \frac{VP}{BS \times SSC} \times 100$$

where VP is the viral production, BS is the burst size (environment-specific; see Supplemental Table 5), and HPA is the *in-situ* abundance of heterotrophic prokaryotes (mL^{-1}).

2.4.3 Global carbon lysed

Global average carbon concentrations (µgC L^{-1}) for heterotrophic prokaryotes were estimated by dividing total biomass (converted from PgC to µgC) by total ocean volume (L). The regression equation derived from measured C_{lysed} as a function of standing stock carbon (SSC) (Figure 6*c*) was applied to estimate carbon lysed concentrations from these biomass concentration as follows:

$$C_{lysed} = a \times Biomass\ concentration^{b}$$

where $a = 0.5489$ and $b = 0.7156$. Carbon lysed concentrations were then converted back to global carbon totals (PgC) by multiplying by the ocean volume. Global carbon lysed was calculated for the upper 200 m ocean layer using global biomass estimates for heterotrophic bacteria of 3.7 PgC [9], assuming a global ocean surface area of 3.619×10^{14} m^2 [10].

2.5 Statistical analysis of viral lysis rates obtained from virus reduction experiments

2.5.1 Methods

All statistical analysis was conducted using the statistical software R [11]. In order to test for significant variance in mean viral production rates obtained from the virus reduction approach (*e.g.,* [12]) across different seasons, marine environments, and climate regions a Kruskal-Wallis test was applied to log-transformed viral production rates. Post-hoc analysis of pair-wise comparisons using Wilcoxon rank sum test with continuity correction was conducted, using the holm correction method for multiple comparisons. Alpha for all significance test was set at 0.05.

Ordinary least squares (OLS) regression analysis was used to identify key predictors of viral production rates and the percentage of lysogens in marine environments using a global dataset of rates obtained from the virus reduction approach. Data exploration and modeling were performed using usdm package [13], mgcv [14], and ggeffects [15], among others, for visualizing data. Data exploration followed of Zuur et al. [16]. Log transformations were applied where necessary to improve homogeneity of variance and reduce the effect of outliers. To address collinearity, correlation biplots were used to identify highly correlated variables ($r > 0.7$), leading to the removal of total available carbon and growth rate due to redundancy with HPA and HPP, respectively. Residual collinearity was further evaluated using Variance Inflation Factors

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(VIF), applying an upper threshold of 10 (actual values ≤ 4). Covariates were also examined for systematic variation across categorical factors (Season, Climate, and Environment) using boxplots, which revealed strong collinearity between Environment (*i.e.,* Ocean, Coastal, Estuary, Bay, Sea) and multiple continuous variables (HPA, HPP and PO_4).

To investigate the factors influencing viral production rates and the percentage of lysogens within heterotrophic prokaryote communities, a series of ordinary least squares (OLS) regression models were fitted, testing different combinations of log-transformed continuous predictors (host prokaryote abundance [HPA], host prokaryote production [HPP], virus-host contact rate [VPR], depth, phosphate [PO_4], temperature, and salinity) and categorical factors (Climate Region: 4 levels; Season: 4 levels; Marine Environment Type: 5 levels).

Model selection was guided by the Akaike Information Criterion (AIC) and ANOVA, comparing simpler models with interaction-inclusive models to balance complexity and explanatory power. Standard diagnostic checks were performed to validate OLS assumptions, including residual versus fitted value plots (to assess homoscedasticity), quantile-quantile (Q-Q) plots (to evaluate normality of residuals), and autocorrelation function (ACF) plots (to test for residual independence).

For the viral production, model predictions were further examined for systematic overprediction or underprediction across categorical levels. This additional evaluation aimed to identify potential extrapolation errors or instability in underrepresented climate regions, assessing robustness in ecological forecasting applications. Additionally, to improve numerical stability, ensure comparability of effect sizes, and reduce collinearity, all continuous predictors in the viral production model were standardized using z-score transformation, *i.e.*, $((x-\mu)/\sigma)$. This transformation mitigates issues related to differences in scale between variables and enhanced interpretability in models that included multiple interacting terms.

For the lysogen model, post-hoc analysis was conducted for categorical variables using Tukey's Honest Significant Difference (HSD) test, adjusting for multiple comparisons to between groups.

2.5.2 Results

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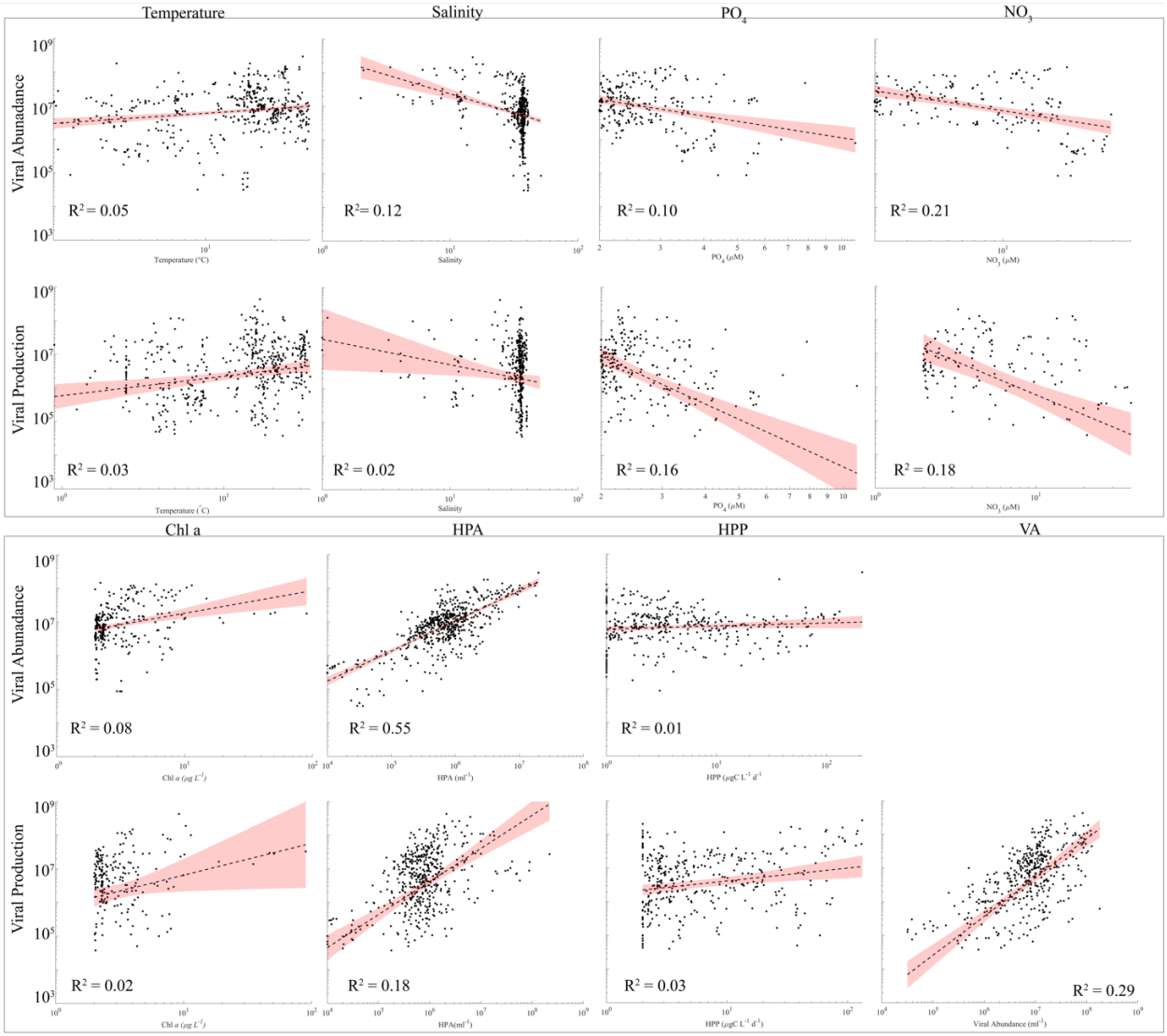

Supplemental Figure 6. Linear regressions showed that no individual physicochemical variable strongly predicted viral abundance (VA) or viral production (VP). To avoid implying causality or directionality, we therefore characterized the associations among viral abundance, host abundance, and viral production using correlation coefficients rather than regression coefficients.

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Analysis of means revealed two distinct clusters of marine environments. The first cluster, comprising Oceans and Seas, exhibited the lowest mean viral production rates of $1.54 \pm 0.25 \times 10^7$ mL^{-1} d^{-1} and $0.97 \pm 0.13 \times 10^7$ mL^{-1} d^{-1}, respectively. In contrast, Coasts, Estuaries, and Bays had mean rates at least two-fold higher, at $2.9 \pm 0.83 \times 10^7$ mL^{-1} d^{-1}, $3.04 \pm 0.47 \times 10^7$ mL^{-1} d^{-1}, and $3.7 \pm 0.94 \times 10^7$ mL^{-1} d^{-1}, respectively. These results suggest that terrestrial influence positively affects the rate of virus-mediated mortality within heterotrophic prokaryote communities.

Season also influenced viral production (VP) rates, with the highest rates observed in the spring and summer ($2.61 \pm 0.38 \times 10^7$ mL^{-1} d^{-1} and $2.22 \pm 0.30 \times 10^7$ mL^{-1} d^{-1}, respectively). Mean rates were significantly lower in the autumn at $0.59 \pm 0.12 \times 10^7$ mL^{-1} d^{-1}, with a further slight decrease during winter to $0.57 \pm 0.07 \times 10^7$ mL^{-1} d^{-1}. Despite these seasonal differences, only minor variations were observed among the different climate regions. The most significant difference was between the polar region, with the lowest mean rate of $0.64 \pm 0.10 \times 10^7$ mL^{-1} d^{-1}, and the temperate region, with $2.49 \pm 0.32 \times 10^7$ mL^{-1} d^{-1}. Tropical and subtropical regions exhibited intermediate rates of $2.06 \pm 0.32 \times 10^7$ mL^{-1} d^{-1} and $1.78 \pm 0.32 \times 10^7$ mL^{-1} d^{-1}, respectively. Despite compiling data from 50 separate studies, the spatiotemporal resolution remained insufficient to separately assess the effect of season within specific environments (*e.g.*, bays) or to isolate the influence of climate region independent of seasonal and environmental variation - limiting our ability to resolve finer-scale effects.

Ordinary least squares regression analysis revealed

$$VP = f(HPA, HPP, PO4, Climate\ Region) + \varepsilon$$

that rate of viral production (VP) is a function of host abundance, host production, nutrient concentrations (PO_4 used here as a proxy due to the highest number of measured values compared to other nutrients), and climate region – along with their interactions. This model explained the highest proportion of variance (adjusted R^2 = 76%) while using a relatively small number of predictors. Although HPP was initially excluded via stepwise regression based on the Akaike Information Criterion (AIC), its reintegration significantly improved the explanatory power of the model and compliance with OLS assumptions. Despite the overall significance of the model, individual t-tests for main effects and interactions were not statistically significant, even though the main effects-only model (Supplemental Table 6) showed that PO_4 and HPA were individually significant in explaining VP. The substantial improvement in R^2 when interactions were included suggests that viral production is influenced by multiple interacting factors, rather than being driven by a single dominant predictor (Supplemental Figure 6). Since collinearity was assessed and removed, the lack of significance in individual coefficients further suggests that the variance in VP is explained by interactions among variables rather than by any single predictor alone. The higher R^2 in the interaction model compared to the main-effects model indicates that these interactions capture additional variance that would otherwise remain unexplained. This implies a context-dependent relationship, where the effect of one predictor (*e.g.,* HPA, HPP, or PO_4) depends on environmental conditions within a given climate region. The individual linear regressions shown in Supplemental Figure 7 provide further evidence to support the presence of complex interactions. Due to model complexity and data limitations in certain climate regions, the model accurately predicted viral production within observed tropical conditions but exhibited unrealistic predictions when applied to unobserved predictor combinations. This suggests that while the model performs well within the measured data range, its extrapolation ability—particularly in tropical regions—is constrained by limited quantitative observations. Although individual coefficients were not statistically significant, the model suggests that interactions among predictors meaningfully contribute to explaining VP variability. These findings highlight the importance of studying viral production within an integrated ecological framework rather than through isolated factors. Future research should focus on expanding quantitative observations of viral production, particularly with coupled physicochemical datasets, to improve statistical power, enhance model robustness, and refine predictive capacity.

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Supplement Table 6. Results of the OLS regression for the main effects (no interactions model) of heterotrophic prokaryote abundance (HPA), heterotrophic prokaryote production (HPP), PO_4 and Climate Region on viral production rates obtained from the viral reduction approach. The intercept represents the estimate of viral production when climate is at the reference level, *i.e.*, Tropical. Adjusted R^2 for this model was 35.9% with a p-value of $2.1x10^{-8}$. All regression estimates are presented on the log-log scale (not the response scale), meaning that coefficients describe relative (percentage) changes rather than absolute changes.

Predictor	**Coefficient (β)**	**Standard Error**	**t-value**	**P-value**	**Significance Symbol**
(Intercept)	1.647	1.556	1.058	0.293	
HPA	0.705	0.331	2.129	0.036	*
HPP	-0.124	0.180	-0.693	0.490	
PO_4	2.117	0.647	3.275	0.002	*
Climate (Subtropical)	1.136	0.577	1.968	0.052	.
Climate (Temperate)	0.775	0.577	1.345	0.182	
Climate (Polar)	0.102	0.600	0.170	0.865	

Signif. codes: '**' 0.001 '*' 0.05 '' 0.1

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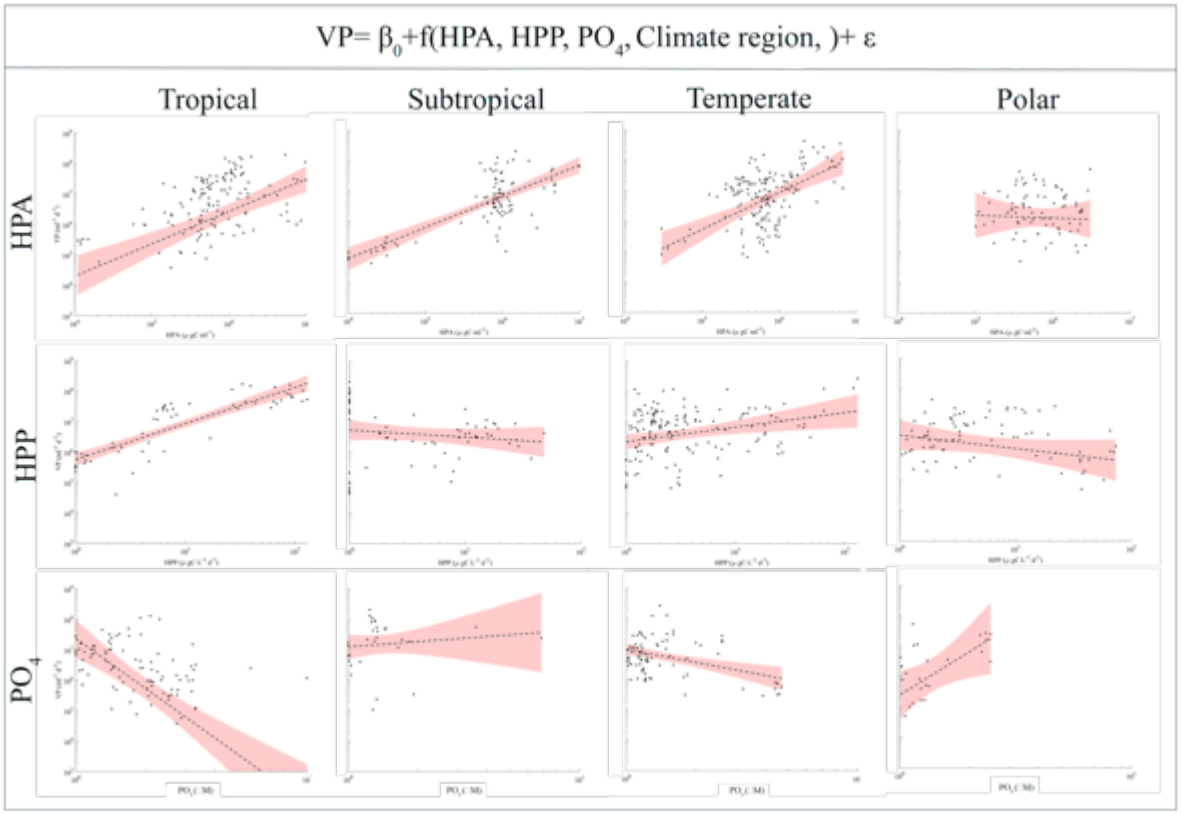

Supplemental Figure 7. Ordinary least squares regression analysis revealed that viral production (VP) is a function of heterotrophic prokaryote abundance (HPA), heterotrophic prokaryote production (HPP), phosphate concentrations (PO_4), and climate region, along with their interactions. Individual linear regressions illustrate complex interactive effects of individual predictors on VP, which vary across climate regions.

To investigate the factors influencing the percentage of lysogens within heterotrophic prokaryote communities, a series of ordinary least squares (OLS) regression models were fitted to assess variability explained by host dynamics (HPA, HPP and virus-to-host contact rate, VPR), environmental variables (salinity, temperature, phosphate, and depth), and categorical factors (season, climate, and environment type). The initial model, focused solely on host-related factors, identified HPA as the only significant predictor. However, it explained little variability (adjusted $R^2 = 0.04$), suggesting host dynamics alone are not the primary regulators of lysogeny. Expanding the model to include environmental variables revealed depth as the strongest predictor, yet in a reduced model with HPA, Depth, and their interactions, neither variable contributed significantly. Recognizing the potential influence of ecosystem-scale differences, categorical factors for Climate Region, Season, and Marine Environment Type were introduced. The final model, which included only Depth and Environment Type (Supplemental Figure 8, Supplemental Table 7), was identified as the best fit. ANOVA confirmed that it performed as well as the full interaction model, reinforcing the dominant role of depth and habitat type in shaping lysogeny patterns. However, despite its significance, the model explained only a modest proportion of variability (adjusted $R^2 = 19.2\%$), indicating that additional key factors remain unidentified.

Post-hoc analysis revealed that lysogeny was significantly higher in seas compared to oceanic and coastal environments (Supplemental Table 8). There was also some evidence of higher lysogeny rates in bays

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compared to oceans, though this difference was only marginally significant (p = 0.0791) in Tukey's test, suggesting a potential effect that warrants further investigation.

Lysogeny is thought to be common among HP hosts, *i.e.*, between 18-40% of HPA contain at least one prophage [17, 18]. Our meta-analysis revealed depth to be an important factor regulating lysogeny – together with type of marine environment - supporting the general consensus that lysogeny is considered as a survival strategy for viruses to survive periods of low host abundance and productivity [19-30]. However, the absence of a strong biological or physicochemical predictor (outside of depth) and the relatively low explained variance underscores a current gap in our understanding of lysogenic viral infection in marine environments. Future studies should explore additional ecological and molecular drivers, including microbial community composition, host metabolic states, and viral genetic mechanisms.

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Supplemental Table 7. Results of the OLS regression for the effects of depth and Environment on the % lysogens within a heterotrophic prokaryote community. The intercept represents the estimate of %lysogens when Environment is at the reference level, *i.e.*, Ocean. Adjusted R^2 for this model was 20.8% with a p-value of $1.5x10^{-10}$. All regression estimates are presented on the log-log scale (not the response scale).

Predictor	**Coefficient (β)**	**Standard Error**	**t-value**	**P-value**	**Significance Symbol**
(Intercept)	0.145	0.090	1.618	0.107	
Depth	0.191	0.047	4.088	0.000	**
Environment (Bay)	0.565	0.198	2.861	0.005	*
Environment (Coastal)	0.146	0.122	1.196	0.233	
Environment (Estuary)	0.291	0.189	1.544	0.124	
Environment (Sea)	0.568	0.085	6.673	0.000	**

Signif. codes: '**' 0.001 '*' 0.05 '·' 0.1

Supplemental Table 8. Results from post-hoc pairwise comparisons of intra-group differences in the percentage of lysogens within heterotrophic prokaryote communities. For each comparison, results were averaged over the remaining two categorical variables. All regression estimates are presented on the log-log scale (not the response scale). P-values were adjusted for multiple comparisons using Tukey's Honest Significant Difference (HSD) method to control for Type I error.

Comparison	Mean Difference	Standard Error	df	t-ratio	p-value
Environment					
Ocean - Bay	-0.565	0.198	212	-2.861	0.037
Ocean - Coastal	-0.146	0.122	212	-1.196	0.754
Ocean - Estuary	-0.291	0.189	212	-1.544	0.535
Ocean - Sea	-0.568	0.085	212	-6.673	0.000
Bay - Coastal	0.419	0.211	212	1.991	0.274
Bay - Estuary	0.274	0.250	212	1.098	0.808
Bay - Sea	-0.003	0.200	212	-0.016	1.000
Coastal - Estuary	-0.145	0.203	212	-0.718	0.952
Coastal - Sea	-0.423	0.127	212	-3.334	0.009
Estuary - Sea	-0.277	0.191	212	-1.451	0.596

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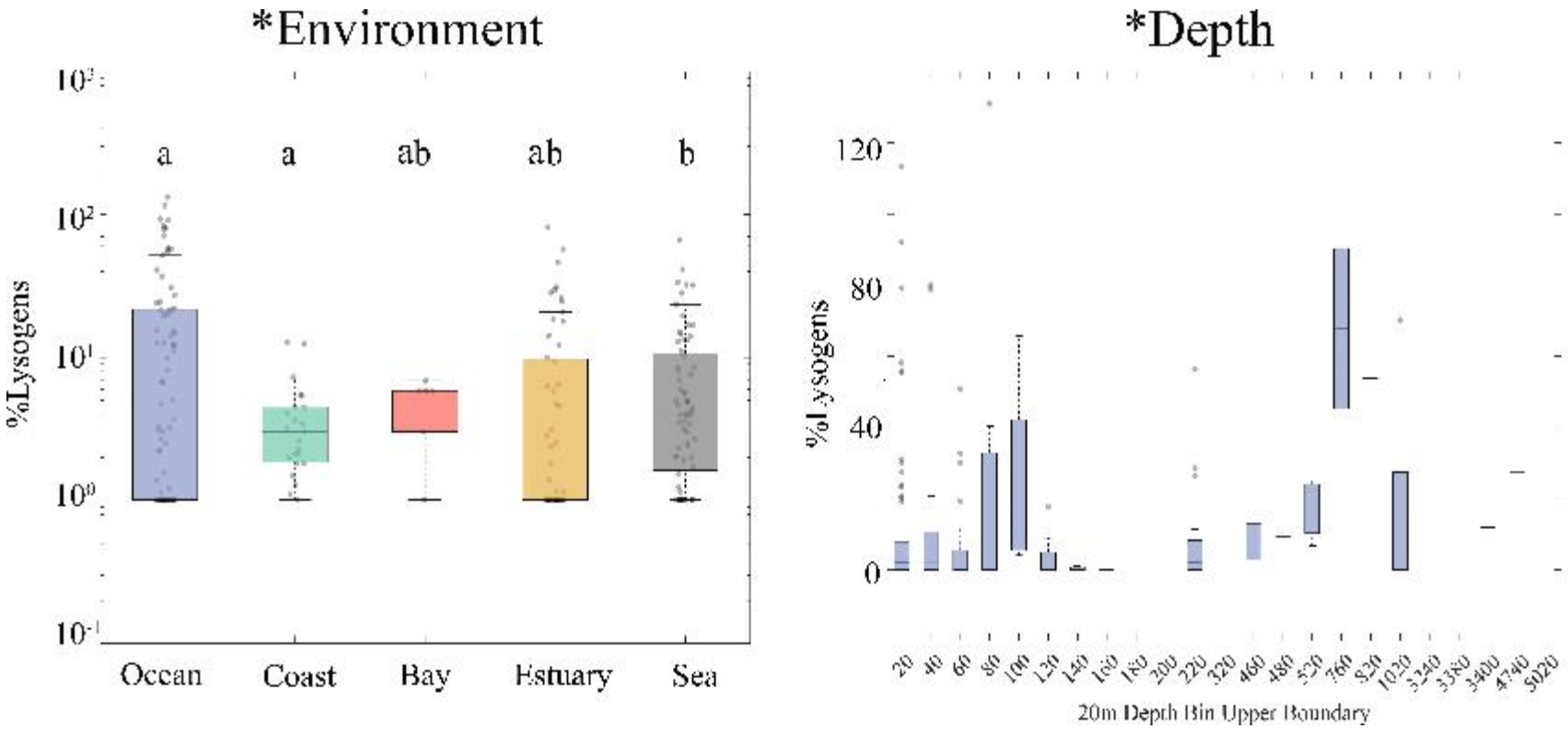
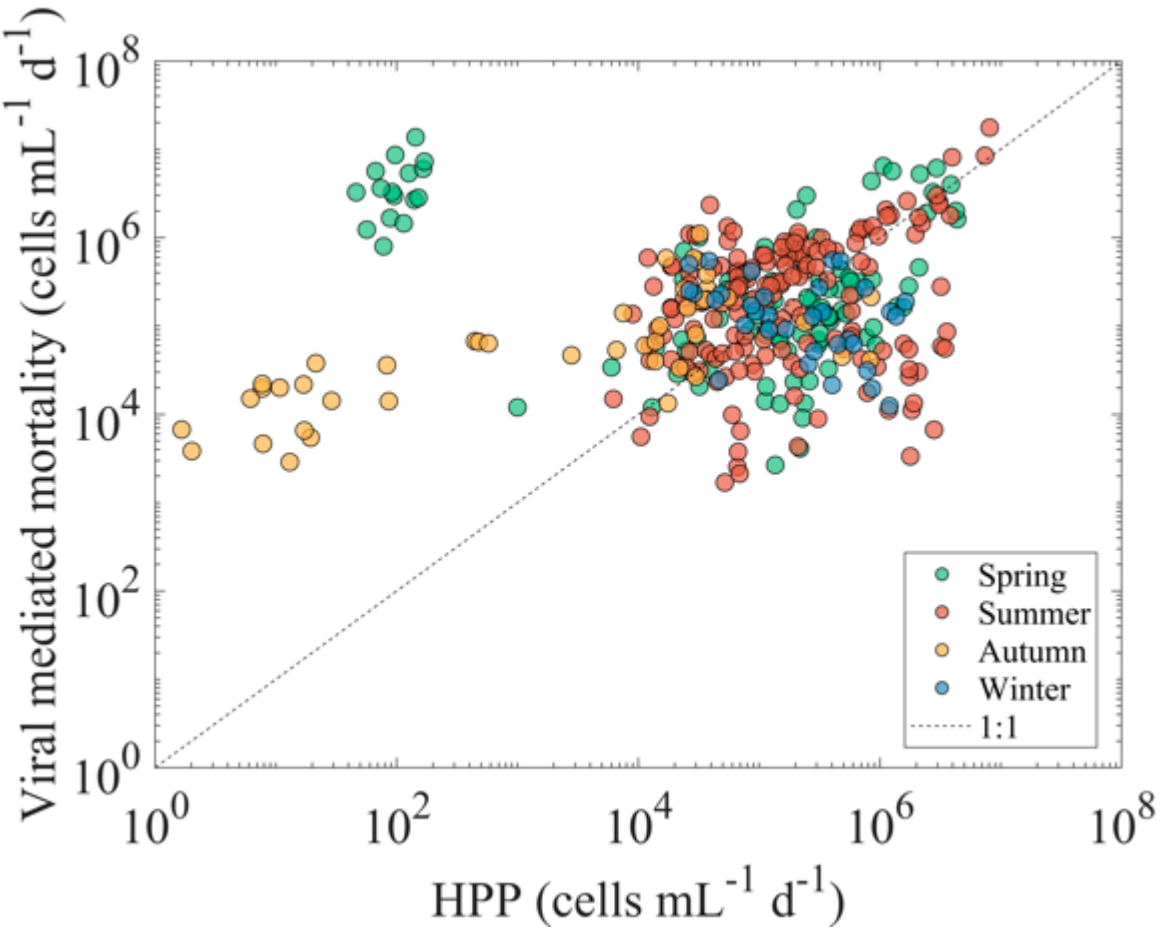


Supplemental Figure 8. Lysogeny was best described as a function of marine environment and depth.



Supplemental Figure 9. Viral mediated mortality (VMM) is tightly coupled with heterotrophic prokaryote production (HPP) except in the spring and autumn when at times VMM exceeded HPP.

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