Cyanophage infecting the oceanic cyanobacterium, *Prochlorococcus*

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Prochlorococcus **is the numerically dominant phototroph in the tropical and sub-tropical oceans, accounting for half of the photosynthetic biomass in some areas[1,2]. Here we report the first isolation of cyanophage that infect *Prochlorococcus*, and show that while some are host-strain specific, others cross-infect with closely related marine *Synechococcus* as well as between high-light (HL) and low-light (LL) adapted *Prochlorococcus* isolates, suggesting a mechanism for horizontal gene transfer (HGT). HL *Prochlorococcus* hosts yielded *Podoviridae* exclusively, which were extremely host-specific, whereas LL *Prochlorococcus* and all strains of *Synechococcus* yielded primarily broad host range *Myoviridae.* Finally, both *Prochlorococcus* and *Synechococcus* strain-specific cyanophage titers were low ($<10^3$ ml^{-1}) in stratified oligotrophic waters even where total cyanobacterial abundances are high ($>10^5$ cells ml^{-1}). These low titers in areas of high total host cell abundance appear to be a feature of open ocean ecosystems. We hypothesize that gradients in cyanobacterial population diversity, growth rates, and/or the incidence of lysogeny underlie these trends.**

Phage are thought to evolve by the exchange of genes drawn from a common gene pool through differential access imposed by host range limitations[3]. Similarly, HGT, important in microbial evolution[4,5], can be mediated by phage[6] and is likely responsible for many of the differences in the genomes of closely related microbes[5]. Recent detailed analyses of molecular phylogenies constructed for marine *Prochlorococcus* and *Synechococcus*[7,8] (Fig. 1) show that these genera form a single group within the marine picophytoplankton clade[9] (>96% identity in 16S rDNA sequences), yet display microdiversity in the form of 10 well defined sub-groups[8]. We have used members of these two groups to study whether phage isolated on a particular host strain cross-infect other hosts, and if so, whether the probability of cross-infection is related to rDNA-based evolutionary distance between the hosts.

Analyses of host range were conducted (Fig. 1) with 44 cyanophage, isolated as previously described[10] from a variety of water depths and locations (see Supplementary Information) using 20 different host strains chosen to represent the genetic diversity of *Prochlorococcus* and *Synechococcus*[8]. While we did not examine how these patterns would change if phage were propagated on different hosts, this would undoubtedly add another layer of complexity due to host range modifications as a result of methylation of phage DNA[6]. Like those that infect other marine bacteria[11] and *Synechococcus*[10-14], our *Prochlorococcus* cyanophage isolates fell into three morphological families: *Myoviridae*, *Siphoviridae*, and *Podoviridae*[15].

As would be predicted[10-14], *Podoviridae* were extremely host specific with only 2 cross-infections out of a possible 300 (Fig. 1). Similarly, the two *Siphoviridae* isolated were specific to their hosts. In instances of extreme host specificity, *in situ* host abundance would need to be high enough to facilitate phage-host contact. It is noteworthy in this regard that members of the HL *Prochlorococcus* cluster, which yielded the most host-specific cyanophage, have high relative abundances *in situ*[16]. The *Myoviridae* exhibited much broader host ranges, with 102 cross-infections out of a possible 539. They not only cross-infected among and between *Prochlorococcus* ecotypes but also between *Prochlorococcus* and *Synechococcus*. Those isolated with *Synechococcus* host strains have broader host ranges and are more likely to cross-infect LL than HL *Prochlorococcus* strains. The LL *Prochlorococcus* are less diverged from *Synechococcus* than HL *Prochlorococcus*[7,8], suggesting a relationship, in this instance, between the probability of cross-infection and rDNA relatedness of hosts. Finally, we tested the *Myoviridae* for cross-infection against marine bacterial isolates closely related to *Pseudoalteromonas* which are known to be broadly susceptible to diverse bacteriophage (bacterial strains HER1320, HER1321, HER1327, HER1328)[11]. None of the *Myoviridae* cyanophage infected these bacteria.

Phage morphotypes isolated were determined, to some degree, by the host used for isolation (Fig. 1). For example, 10 of 10 cyanophage isolated using HL *Prochlorococcus* strains were *Podoviridae.* In contrast, all but two cyanophage isolated on *Synechococcus* were *Myoviridae*, a bias that has been reported by others[14], and over half of those isolated on LL *Prochlorococcus* belonged to this morphotype. We further substantiated these trends by examining lysates (as opposed to plaque purified isolates) from a range of host strains, geographic locations, and depths: of 58 *Synechococcus* lysates 93% contained *Myoviridae*, of 43 LL *Prochlorococcus* lysates 65% contained *Myoviridae* and of 107 HL *Prochlorococcus* lysates 98% contained *Podoviridae* (see Supplementary Information).

Maximum cyanophage titers, using a variety of *Synechococcus* hosts, are usually found to be within an order of magnitude of the total *Synechococcus* abundance[10,14,17,18], and can be as high as 10^6 phage ml^{-1}. Suttle and Chan[17] have shown, for example, that along a transect in which total *Synechococcus* abundance decreased from 10^5 cells ml^{-1} to 250 cells ml^{-1}, maximum cyanophage titers remained at least as high as the total number of *Synechococcus*. We wondered whether titers of *Prochlorococcus* cyanophage in the Sargasso Sea, where *Prochlorococcus* cells are abundant (10^5 cells ml^{-1}) would be comparable to those measured in coastal oceans for *Synechococcus* where total *Synechococcus* host abundances are of similar magnitude. We assayed cyanophage titers in a depth profile in the Sargasso Sea at the end of seasonal stratification using 11 strains of *Prochlorococcus* (Fig. 2), choosing at least one host strain from each of the six phylogenetic clusters that span the rDNA-based genetic diversity of our culture collection[8].

Three *Prochlorococcus* host strains (MIT 9303, MIT 9313, SS120) yielded low or no cyanophage. Other hosts yielded titers which reached a maximum at 70 m (NATL2A-phage) or 100 m (MIT 9302-, MIT 9515-, MED4-, MIT 9211-, NATL1A-phage) near the depth of maximum *Prochlorococcus* abundance (Fig. 2). All *Prochlorococcus* cyanophage titers were low (<350 cyanophage ml^{-1}) compared to those reported for *Synechococcus* in coastal regions (~10^4-10^6 cyanophage ml^{-1}) even though total host abundances were similar between these regions (~10^5 cells ml^{-1})[10,14,17,18]. *Prochlorococcus* cyanophage titers are comparable to those of *Synechococcus* from oligotrophic waters in the Gulf of Mexico — but in that instance the total *Synechococcus* abundance was also low (<250 cells ml^{-1})[17].

Cyanophage titers were also examined along a surface water transect from coastal (mesotrophic) to open ocean (oligotrophic) in the Atlantic Ocean to better understand the relationship between maximum phage titer and total host abundance along a trophic gradient. Titers were assayed with twelve strains of *Synechococcus* and *Prochlorococcus* that represented the known rDNA-based genetic diversity at the time we began the study[8] (but see ref. 19). *Synechococcus* cyanophage titers decreased by an order of magnitude or greater in surface waters between the coastal and open ocean (Sargasso) sites, while total *Synechococcus* abundance decreased from 3x10^4 to 7x10^3 cells ml^{-1} (Fig. 3). *Prochlorococcus* hosts did not yield cyanophage in coastal samples where there are no *Prochlorococcus* cells, and yielded relatively low titers (0 to 1.5x10^3 phage ml^{-1}) at the Shelf, Slope, and Sargasso stations where total *Prochlorococcus* abundance was between 4.5x10^4 and 1.4x10^5 cells ml $^{-1}$. Even though total *Prochlorococcus* abundance at the Sargasso site was similar to that of *Synechococcus* at the coastal site (Fig. 3i, j), *Prochlorococcus* and *Synechococcus* cyanophage titers were significantly lower at the open ocean site (Fig. 3a-h). Moreover, regardless of the host used, titers never exceeded 3x10^3 cyanophage ml^{-1} at any depth throughout the photic zone even though total *Prochlorococcus* abundances exceeded 10^5 cells ml^{-1} (see Supplementary Information). Thus it appears that cyanophage titers at the end of summer stratification are relatively low in open ocean ecosystems, where the total possible host cell abundances are relatively high. Low titers lead to reduced contact rates and lowered mortality rates[6].

Although it is difficult to draw definitive conclusions about causality from such trends because of the complexity of the phage-host interaction, there are some factors that might be implicated. If, for example, host strain microdiversity increased along the transect, and cross-infection ability did not increase concurrently, this would lead to lower phage titers yielded by a suite of host strains[20]. Indeed, we know the relative abundance of *Synechococcus* ecotypes changes from coastal to oligotrophic waters [16]. However, we observed a systematic decrease in cyanophage titers for all 5 *Synechococcus* hosts (note WH 8020 yielded no plaques) at the extremes of this transect (the coastal and Sargasso sites; Fig. 3a-e). If changing host abundance alone explained the change in titers, and if our suite of host strains is representative of natural diversity, then one might expect at least one host-strain to yield increasing titers along the gradient (eg. for the "open ocean strain" WH 8102). This was not observed.

Another possible explanation for decreasing phage titers as one goes from coastal to open ocean ecosystems is decreased nutrient availability along the transect[21] resulting in sub-optimal growth of host cells in the Sargasso Sea[22] relative to *Synechococcus* at the coastal site[23]. Viral production is correlated with host growth rates in chemostats[24] and in the field[25], which could result from nutrient limitation causing physiological changes in the host that stall the lytic process of obligately lytic phage[6], or favour lysogeny in temperate phage[18,26,27]. Although temperate phage have not been identified for marine *Prochlorococcus* or *Synechococcus*, INT family site-specific recombinases exist in the genomes of *Prochlorococcus* MED4 and MIT 9313 and *Synechococcus* WH 8102 (http://www.jgi.doe.gov/JGI_microbial/html/index.html) suggesting that prophage were once integrated into these host genomes[28,29].

The phage-host system described here should continue to be a useful framework for advancing our understanding of the ecology and evolution of phage-host interactions in marine ecosystems. We have known for some time that cyanophage must play a role in maintaining genetic diversity among hosts[10,17]. The broad host ranges reported here indicate further their potential for mediating horizontal gene transfer, which may help explain the extensive microdiversity[8,19,28,29] seen in these two groups of marine cyanobacteria. The extent to which this potential is realized should become clear as more and more host and phage genomes are sequenced. Of significance also is the coupling between phage morphology and host type. Experiments designed to characterize phage resistance across variable hosts and phage (eg. identification of receptors and restriction and modification systems) should elucidate the underlying mechanisms responsible for these patterns. Finally our analyses of cyanophage titers along a coastal-open ocean transect suggest that the underlying processes responsible for the production of free cyanophage differ along trophic gradients in the oceans. To fully explain these observations will require the development of approaches that allow one to determine which phage can infect which host(s) in a given community, and an understanding of the relative roles of lytic and lysogenic phases of the viral life cycle in aquatic systems.

Methods

Sample collection: Water samples for cyanophage titers, cyanophage isolations and cyanobacterial abundances were collected at the Bermuda Atlantic Time Series Station on 26 Sept 1999 and four sites along a transect from Woods Hole to the Western Sargasso Sea on 5, 16, 17 and 22 Sept 2001 (see Supplementary Information). Water for cyanophage isolations was filtered (0.4 ? m, Poretics cat.#13028 in 1999 or 0.2 ? m, Osmonics cat#K02CP04700 in 2001) and stored at 4?C in the dark in acid-washed polycarbonate (1999) or glass (2001) bottles until analysis (up to 15 months later). Cyanophage titers remain stable for at least one year[27]. Control experiments showed that titers were stable over a 15 month period (see Supplementary Information).

Culturing conditions: *Prochlorococcus* and *Synechococcus* strains were maintained in "75% Pro99" medium, a modification of the "Pro2" medium[30] with a 75% seawater base and the following final concentrations of N and P: 800 ? M NH_4Cl, 50 ? M NaH_2PO_4. Cultures were grown at 19-21?C under constant light 8-12 ? E m^{-2} s^{-1} for LL *Prochlorococcus* and *Synechococcus*; 35-45 ? E m^{-2} s^{-1} for HL *Prochlorococcus*.

Cyanophage isolations: *Prochlorococcus* cyanophage isolations were done initially using an axenic strain of *Prochlorococcus* (MED4ax; M. Saito & JBW, unpub.). Exponentially growing cells were transferred to fresh medium (1 ml:20 ml) and inoculated with 1 ml of 0.4 ? m filtered seawater. The time course of auto-fluorescence (chlorophyll biomass) of these cultures was then followed with a Turner Designs 10-AU fluorometer. Cultures showing reduced fluorescence relative to controls were filtered and examined for phage particles as previously described[10,12]. Lysates were stored at 4?C in the dark. Subsequent isolations using 19 additional host strains were done using the same procedures scaled down to small volumes. Cyanophage isolates used in this study were plaque purified twice before use, classified using morphology described by the ICTV[15], and named according to suggestions made for cyanophage[18].

Cyanophage host-range: Host range analyses were conducted over a period of ~2 years. Each interaction between a cyanophage and its potential host cell was performed with exponentially growing cells in triplicate on at least two different occasions. Marine bacterial strains were purchased from the Felix d'Herelle Reference Center for Bacterial Viruses (contact Dr. Hans Ackermann: ackermann@mcb.ulaval.ca). Several of the *Synechococcus* cyanophage used in this study ("Syn" phages; S-PM2 and S-WHM1) had been previously examined for host range cross-infectivity[10,13] and were maintained as lysates at 4?C in the dark while host cyanobacterial cultures were serially transferred in late exponential and early stationary phase. 103 of 108 cross-infections using these stored cyanophage yielded similar host-range results in this study (Fig. 1). Of the differences observed, 4 of 5 were for one cyanophage isolate (Syn10) suggesting it might have evolved an extended host-range mutation. Host-range can be altered through DNA modifications that can occur during propagation of a phage on an alternate host. Overall, these results suggest that cyanophage susceptibility of these host strains and the cross-infectivity of the cyanophage remained relatively stable throughout the 10+ years of storage and culture maintenance.

Host cell and cyanophage quantification: Host cell abundance was measured using a modified Becton-Dickinson FACScan flow cytometer[7]. Cyanophage titers were quantified using most probable number (MPN) assays (1999) or plaque assay (2001). MPN assays were monitored for lysis relative to controls for 2-3 weeks depending upon the host strain used. For the plaque assays, we plated the host strain in soft agarose (0.4% final concentration; GIBCO BRL, Life Technologies Cat. # 5517-014) along with the phage being titered; lawns of cells appeared 8-28 days after inoculation, depending upon the strain (M. Saito, MBS, JBW, unpubl.). Plaques were counted daily until they no longer appeared (3-14 days after the first plaques). Titers measured using both assays were not significantly different (t-test assuming equal variances, a= 0.10).

Host dependency of measured titers: To see if our standard suite of host cells used in our assays was giving us a representative picture of the maximum phage titer obtainable in a given sample, we used every cultured isolate of *Prochlorococcus* in our collection with a unique ITS rDNA sequence (23 isolates) to assay the cyanophage titer of a 50m water sample from the Red Sea. The range of titers yielded was representative of the range we measured using our subset of *Prochlorococcus* isolates (see Supplementary Information).

1. Liu, H., Nolla, H. A. & Campbell, L. *Prochlorococcus* growth rate and contribution to primary production in the equatorial and subtropical North Pacific Ocean. *Aquatic Microbial Ecology* **12**, 39-47 (1997).

2. Partensky, F., Hess, W. R. & Vaulot, D. *Prochlorococcus*, a marine photosynthetic prokaryote of global significance. *Microbiol. Molc. Biol. Rev.* **63**, 106-127 (1999).

3. Hendrix, R. W., Smith, M. C., Burns, R. N., Ford, M. E. & Hatfull, G. F. Evolutionary relationships among diverse bacteriophages and prophages: All the world's a phage. *Proc. Natl. Acad. Sci. USA* **96**, 2192-7. (1999).

4. Woese, C. R. Interpreting the universal phylogenetic tree. *Proc. Natl. Acad. Sci. USA* **97**, 8392-6 (2000).

5. Ochman, H., Lawrence, J. G. & Groisman, E. A. Lateral gene transfer and the nature of bacterial innovation. *Nature* **405**, 299-304 (2000).

6. Stent, G. S. *Molecular biology of bacterial viruses* (W.H. Freeman and Company, San Francisco, 1963).

7. Moore, L. R., Rocap, G. & Chisholm, S. W. Physiology and molecular phylogeny of coexisting *Prochlorococcus* ecotypes. *Nature* **393**, 464 (1998).

8. Rocap, G., Distel, D., Waterbury, J. B. & Chisholm, S. W. Resolution of *Prochlorococcus* and *Synechococcus* ecotypes by using 16S-23S ribosomal DNA internal transcribed spacer sequences. *Applied Environmental Microbiol.* **68** (2002).

9. Urbach, E. & Chisholm, S. W. Genetic diversity in *Prochlorococcus* populations flow cytometrically sorted from the Sargasso Sea and Gulf Stream. *Limnology and Oceanography* **43**, 1615-1630 (1998).

10. Waterbury, J. B. & Valois, F. W. Resistance to co-occurring phages enables marine *Synechococcus* communities to coexist with cyanophage abundant in seawater. *Applied Environmental Microbiol.* **59**, 3393-3399 (1993).

11. Wichels, A. et al. Bacteriophage diversity in the North Sea. *Applied Environmental Microbiol.* **64**, 4128-33 (1998).

12. Suttle, C. A. & Chan, A. M. Marine cyanophages infecting oceanic and coastal strains of *Synechococcus*: abundance, morphology, cross-infectivity and growth characteristics. *Mar. Ecol. Prog. Ser.* **92**, 99-109 (1993).

13. Wilson, W. H., Joint, I. R., Carr, N. G. & Mann, N. H. Isolation and molecular characterization of five marine cyanophages propogated on *Synechococcus* sp. strain WH 7803. *Applied Environmental Microbiol.* **59**, 3736-3743 (1993).

14. Lu, J., Chen, F. & Hodson, R. E. Distribution, isolation, host specificity, and diversity of cyanophages infecting marine *Synechococcus* spp. in river estuaries. *Applied Environmental Microbiol.* **67**, 3285-90. (2001).

15. Murphy, F. A. et al. *Virus Taxonomy: Classification and Nomenclature of Viruses* (Springer, Vienna, Austria, 1995).

16. Scanlan, D. J. & West, N. J. Molecular ecology of the marine cyanobacterial genera *Prochlorococcus* and *Synechococcus*. *FEMS Microb. Ecol.* **40**, 1-12 (2002).

17. Suttle, C. A. & Chan, A. M. Dynamics and distribution of cyanophages and their effects on marine *Synechococcus* spp. *Applied Environmental Microbiol.* **60**, 3167-3174 (1994).

18. Suttle, C. A. in *The Ecology of Cyanobacteria* (eds. Whitton, B. A. & Potts, M.) 563-589 (Kluwer Academic Publishers, Netherlands, 2000).

19. Fuller, N. J. et al. Clade-specific 16S ribosomal DNA oligonucleotides reveal the predominance of a single marine *Synechococcus* clade throughout a stratified water column in the Red Sea. *Applied Environmental Microbiol.* **69**, 2430-43 (2003).

20. Thingstad, T. F. Elements of a theory for the mechanisms controlling abundance, diverstiy, and biogeochemical role of lytic bacterial viruses in aquatic ecosystems. *Limnology and Oceanography* **45**, 1320-1328 (2000).

21. Cavender-Bares, K. K., Karl, D. M. & Chisholm, S. W. Nutrient gradients in the western North Atlantic Ocean: Relationship to microbial community structure, and comparison to patterns in the Pacific Ocean. *Deep-Sea Research, Part I* **48**, 2373-2395 (2001).

22. Mann, E. L., Ahlgren, N., Moffett, J. W. & Chishol, S. W. Copper toxicity and cyanobacteria ecology in the Sargasso Sea. *Limnology and Oceanography* **47**, 976-988 (2002).

23. Waterbury, J. B., Watson, S. W., Valois, F. W. & Franks, D. G. Biological and ecological characterization of the marine unicellular cyanobacterium *Synechococcus*. *Can. Bull. Fish. Aquat. Sci.* **214**, 71-120 (1986).

24. Bohannan, B. J. M. & Lenski, R. E. Linking genetic change to community evolution: insights from studies of bacteria and bacteriophage. *Ecol. Letters* **3**, 362-377 (2000).

25. Steward, G., Smith, D. C. & Azam, F. Abundance and production of bacteria and viruses in the Bering and Chukchi Seas. *Mar. Ecol. Prog. Ser.* **131**, 287-300 (1996).

26. Rohwer, F. et al. The complete genomic sequence of the marine phage Roseophage SIO1 shares homology with nonmarine phages. *Limnology and Oceanography* **45**, 408-418 (2000).

27. Wilson, W. H., Carr, N. G. & Mann, N. H. The effect of phosphate status on the kinetics of cyanophage infection in the oceanic cyanobacterium *Synechococcus* sp. WH7803. *J. Phycology* **32**, 506-516 (1996).

28. Rocap, G. et al. Microbial niche differentiation viewed from whole genome comparison of two *Prochlorococcus* ecotypes. *Nature* **in press** (2003).

29. Palenik, B. et al. The genome of a motile marine *Synechococcus*. *Nature* **in press** (2003).

30. Moore, L. R. & Chisholm, S. W. Photophysiology of the marine cyanobacterium *Prochlorococcus*: Ecotypic differences among cultured isolates. *Limnology and Oceanography* **44**, 628-638 (1999).

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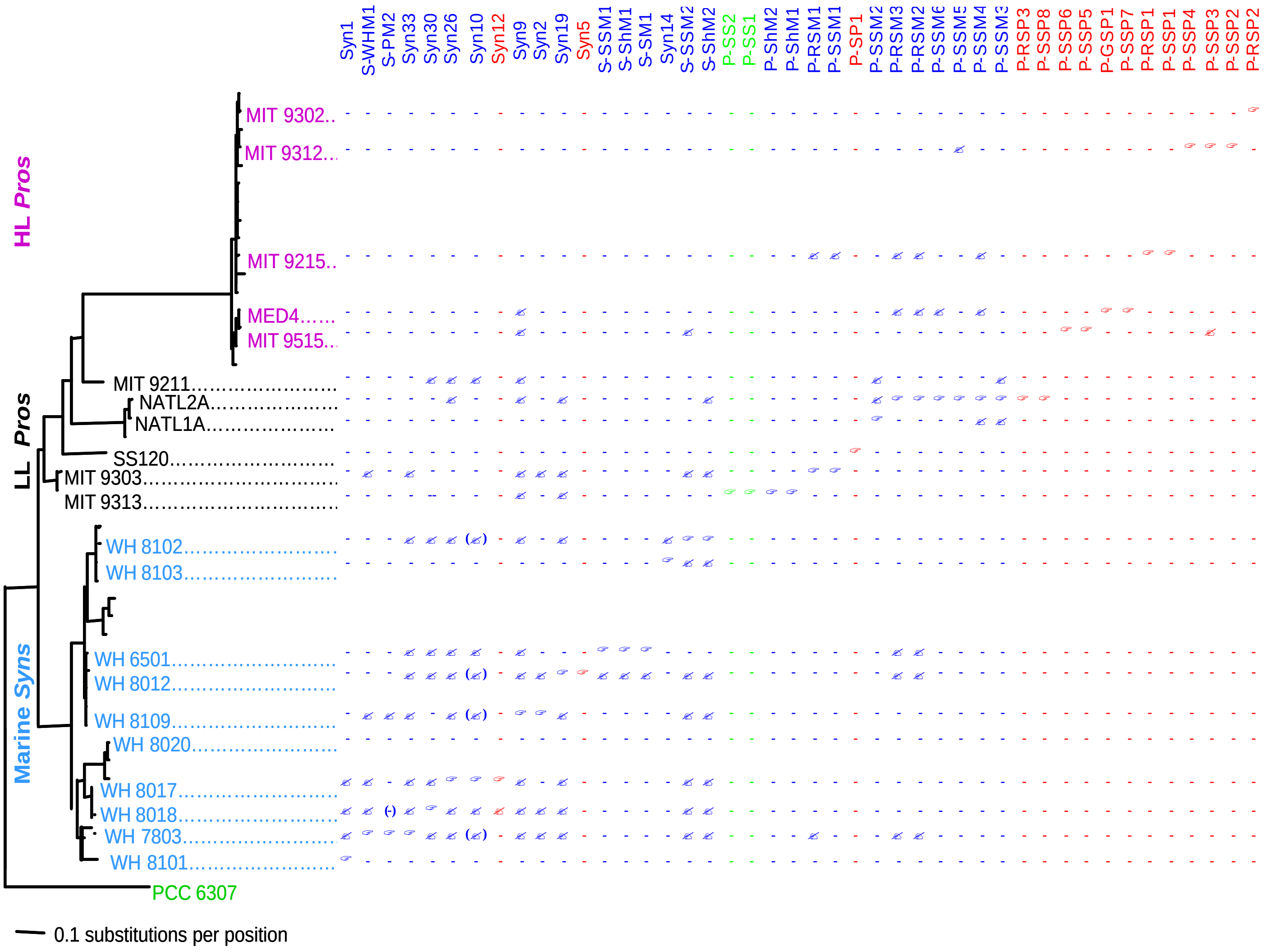
Figure 1: Host-ranges of 44 clonal cyanophage exposed to marine *Prochlorococcus* and *Synechococcus* cultured isolates. The evolutionary relationships between the 21 host strains are shown in the phylogenetic tree inferred using 16S-23S rDNA spacer regions[8]. For the cyanophage, Red = *Podoviridae*, blue = *Myoviridae*, green = *Siphoviridae*; (solid circles “?”) = host strain used to isolate a particular cyanophage; (open circle “o”) = cross-infection of cyanophage with another host; (dash “?”) no infection (i.e. lysis); those symbols in parentheses “()” denote the results that did not match earlier studies[10,13] with these phage and hosts (see methods section for details).

Figure 2: Cyanophage titers, measured using *Prochlorococcus* hosts strains, as a function of depth at the Bermuda Atlantic Time Series Station in the Sargasso Sea on 26 Sept. 1999. Nine of the cyanophage used in host range analyses were isolated from this depth profile (see Supplementary Information). (a) Titers measured using HL-adapted *Prochlorococcus* hosts, (b) Titers measured using LL-adapted *Prochlorococcus* hosts, (c) total *Prochlorococcus* and *Synechococcus* cell abundances (*Prochlorococcus* cells x 10^4 ml^{-1} and *Synechococcus* cells x 10^3 ml^{-1}) and Sigma-T (s_t— a proxy for water density used to measure the depth of the mixed layer). Titers were undetectable for LL-adapted *Prochlorococcus* strains SS120, MIT9303, and MIT9313. Error bars represent the standard deviation of assays.

Figure 3: Cyanophage titers measured on *Synechococcus* and *Prochlorococcus* host cells along a surface water transect from coastal (Coast – Woods Hole, MA) to open ocean (Sargasso) conducted in September 2001. Note that the magnitudes of the y-axes are different for (a-e) and (f-j). Ten of the cyanophage used in host range analyses were isolated from along this transect (see Supplementary Information). Cyanophage titers (a-h) represent the averages and standard deviation of triplicate plaque assays, and cell concentrations (i-j) represent average and standard deviation of duplicate flow cytometry assays. Where no bar is shown, there were no plaques (a,c,e-h), or no cells (i). No plaques were observed at any of the surface samples along the transect for *Synechococcus* strain WH 8020 and *Prochlorococcus* strains MIT 9313, SS120, MIT 9211 (data not shown).

Fig. 1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P-SSM4
P-SSM3
P-RSP3
P-SSP8
P-SSP6
P-SSP5
P-GSP1
P-SSP7
P-RSP1
P-SSP1
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HL *Pros*
MIT 9302
MIT 9312
MIT 9215
MED4
MIT 9515
LL *Pros*
MIT 9211
NATL2A
NATL1A
SS120
MIT 9303
MIT 9313
Marine *Syns*
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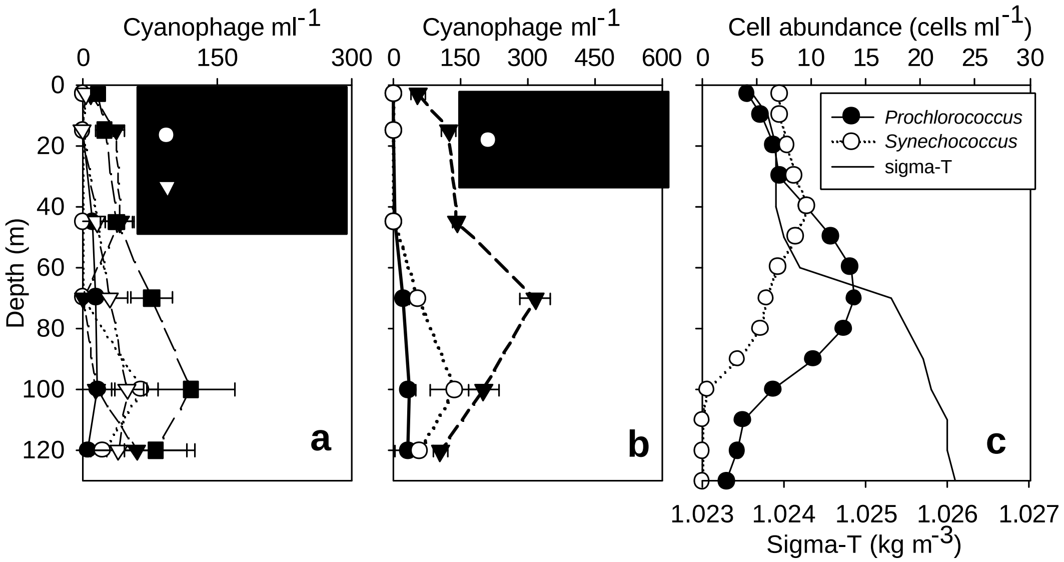
Cyanophage ml^{-1}
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150
300
Cyanophage ml^{-1}
0
150
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Cell abundance (cells ml^{-1})
0
5
10
15
20
25
30
Depth (m)
0
20
40
60
80
100
120
a
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Prochlorococcus
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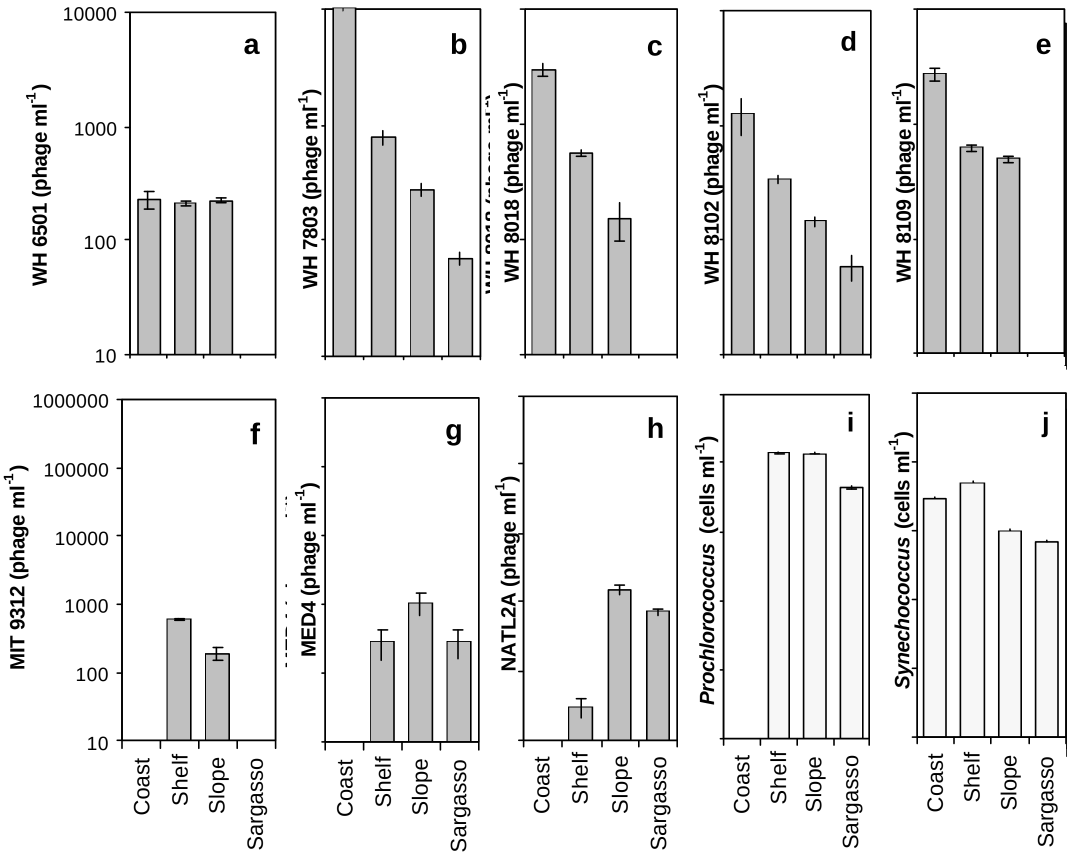
Sigma-T (kg m^{-3})

Figure 2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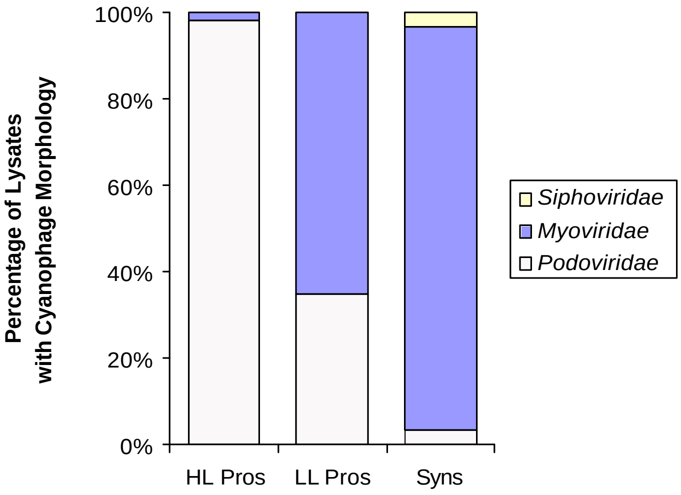
WH 6501 (phage ml^{-1})
WH 7803 (phage ml^{-1})
WH 8018 (phage ml^{-1})
WH 8102 (phage ml^{-1})
WH 8109 (phage ml^{-1})
MIT 9312 (phage ml^{-1})
MED4 (phage ml^{-1})
NATL2A (phage ml^{-1})
Prochlorococcus (cells ml^{-1})
Synechococcus (cells ml^{-1})
10000
1000
100
10
1000000
100000
10000
1000
100
10
Coast
Shelf
Slope
Sargasso

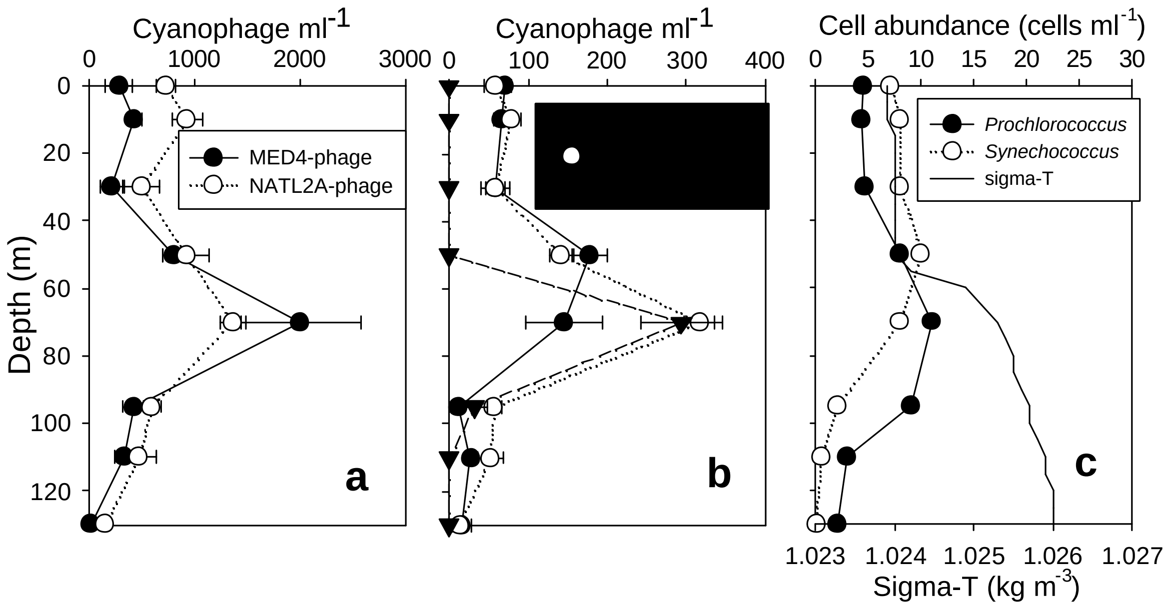
Figure 3

Supplementary Table 1: Detailed information about the *Prochlorococcus* and *Synechococcus* cyanophage isolates used in the host-range analyses in this study. TEM Morphology designations as follows: “P” = *Podoviridae*, “M” = *Myoviridae*, “S” = *Siphoviridae*, “+” indicates positive stained particles. Morphometric data for cyanophage are approximated where possible from negatively stained (except where indicated by ‘+’) images without internal standards, “n.a.” suggests no tail was observed.

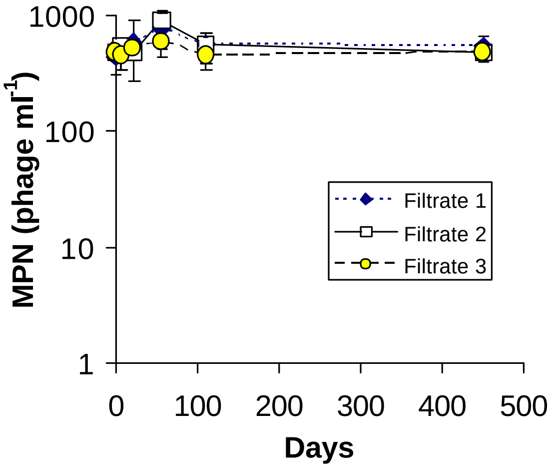
Phage Name	*Locale*	*Latitude and Longitude*	*Depth (m)*	*Date Collected*	*TEM Morphology*	*Head Diameter (nm)*	*Tail Length x Width (nm)*	*Reference*
P-SSP1	BATS	31?48’N, 64?16’W	100	6 June 2000	P+	45+	n.a.	This study
P-RSP1	Red Sea	29?28’N, 34?53’E	0	15 July 2000	P	55	6 x 10	This study
P-RSP2	Red Sea	29?28’N, 34?53’E	0	15 July 2000	P+	40+	n.a.	This study
P-SSP2	BATS	31?48’N, 64?16’W	120	29 Sep 1999	P+	40+	n.a.	This study
P-SSP3	BATS	31?48’N, 64?16’W	100	29 Sep 1999	P	50	n.a.	This study
P-SSP4	BATS	31?48’N, 64?16’W	70	26 Sep 1999	P	50	n.a.	This study
P-SSP5	BATS	31?48’N, 64?16’W	120	29 Sep 1999	P	55	6 x 10	This study
P-SSP6	BATS	31?48’N, 64?16’W	100	26 Sep 1999	P	55	n.a.	This study
P-SSP7	BATS	31?48’N, 64?16’W	100	26 Sep 1999	P	50	n.a.	This study
P-GSP1	Gulf Stream	38?21’N, 66?49’W	40	6 Oct 1999	P	45	n.a.	This study
P-SSP8	BATS	31?48’N, 64?16’W	100	26 Sep 1999	P+	50	n.a.	This study
P-RSP3	Red Sea	29?28’N, 34?55’E	50	13 Sep 2000	P+	50	n.a.	This study
P-SP1	Slope	37?40’N, 73?30’W	83	17 Sep 2001	P	60	8 x 12	This study
Syn-12	Gulf Stream	36?58’N, 73?42’W	0	Dec 1990	P	45	8 x 10	Waterbury & Valois 1993
Syn-5	Sargasso Sea	34?06’N, 61?01’W	0	July 1990	P			Waterbury & Valois 1993
P-SSM1	BATS	31?48’N, 64?16’W	100	6 June 2000	M+	60+	160 x 20	This study
P-RSM1	Red Sea	29?28’N, 34?53’E	0	15 July 2000	M	80	110 x 27	This study
P-ShM1	Shelf	39?60’N, 71?48’W	40	16 Sep 2001	M	75	120 x 18	This study
P-ShM2	Shelf	39?60’N, 71?48’W	0	16 Sep 2001	M+			This study
P-SSM2	BATS	31?48’N, 64?16’W	100	6 June 2000	M	85	110 x 25	This study
P-SSM3	BATS	31?48’N, 64?16’W	100	6 June 2000	M	95	105 x 25	This study
P-SSM4	BATS	31?48’N, 64?16’W	10	6 June 2000	M	80	160 x 20	This study
P-SSM5	BATS	31?48’N, 64?16’W	15	26 Sep 1999	M+	60+	20 x 80+	This study
P-SSM6	BATS	31?48’N, 64?16’W	40	29 Sep 1999	M	90	150 x 28	This study
P-RSM2	Red Sea	29?28’N, 34?55’E	50	13 Sep 2000	M	75	170 x 23	This study
P-RSM3	Red Sea	29?28’N, 34?55’E	50	13 Sep 2000	M	80	175 x 23	This study
S-SM1	Slope	37?40’N, 73?30’W	0	17 Sep 2001	M	90	80+ x 25	This study
S-ShM1	Shelf	39?60’N, 71?48’W	0	16 Sep 2001	M	80	120 x 25	This study
S-SSM1	Sargasso Sea	34?24’N, 72?03’W	70	22 Sep 2001	M	95	150 x 25	This study
Syn-2	Sargasso Sea	34?06’N, 61?01’W	0	July 1990	M	66	149 x 17	Waterbury & Valois 1993
Syn-9	Woods Hole	41?31'N, 71?40'W	0	Oct 1990	M	87	153 x 19	Waterbury & Valois 1993
Syn-19	Sargasso Sea	34?06’N, 61?01’W	0	July 1990	M			Waterbury & Valois 1993
Syn-10	Gulf Stream	36?58’N, 73?42’W	0	Dec 1990	M	100	145 x 19	Waterbury & Valois 1993
Syn-26	NE Providence Channel	25?53’N, 77?34’W	0	Jan 1992	M			Waterbury & Valois 1993
Syn-30	NE Providence Channel	25?53’N, 77?34’W	0	Jan 1992	M			Waterbury & Valois 1993
Syn-33	Gulf Stream	25?51’N, 79?26’W	0	Jan 1995	M			Waterbury & Valois 1993
S-PM2	English Channel	50?18’N, 4?12’W	0	23 Sep 1992	M	90	165 x 20	Wilson et al. 1993
S-WHM1	Woods Hole	41?31'N, 71?40'W	0	11 Aug 1992	M	88	108 x 23	Wilson et al. 1993
Syn-1	Woods Hole	41?31'N, 71?40'W	0	August 1990	M			Waterbury & Valois 1993
S-ShM2	Shelf	39?60’N, 71?48’W	0	16 Sep 2001	M+	70+	100 x 30	This study
S-SSM2	Sargasso	34?24’N, 72?03’W	0	22 Sep 2001	M	80	225 x 22	This study
Syn-14	Gulf Stream	36?58’N, 73?42’W	0	Dec 1990	M	93	136 x 21	Waterbury & Valois 1993
P-SS1	Slope	37?40’N, 73?30’W	60	17 Sep 2001	S+	45+ x 90	280 x 15	This study
P-SS2	Slope	37?40’N, 73?30’W	83	17 Sep 2001	S	50 x 100	260 x 12	This study



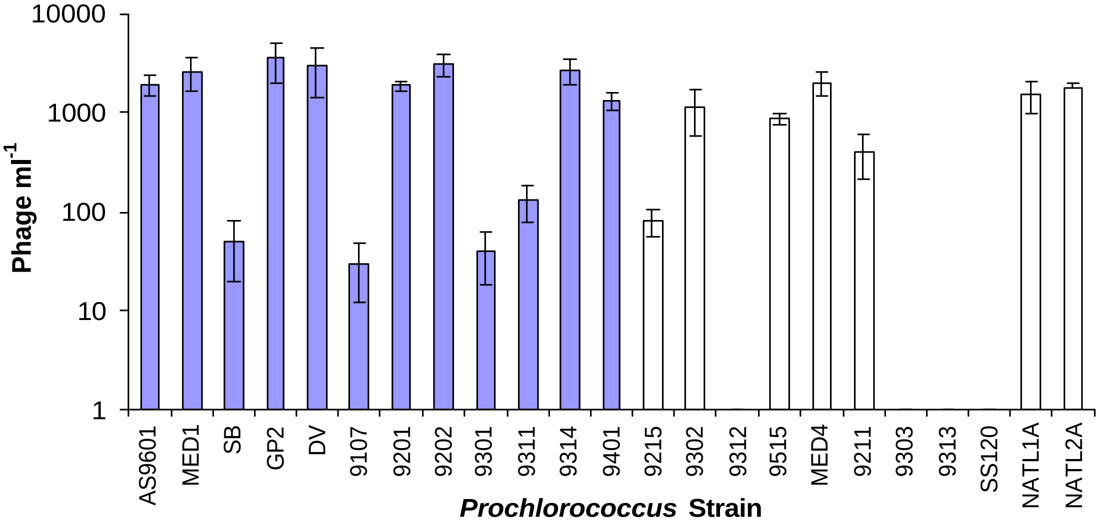
Supplementary Figure 1: Proportion of *Prochlorococcus* and *Synechococcus* cyanobacterial lysates with a given cyanophage morphology. Data represent presence or absence of a given morphology in each lysate and do not include observations of doubly plaque purified cyanophage isolates used in the host range analyses. The following number of observations were made for each category from the lysates: HL *Prochlorococcus* = 107, LL *Prochlorococcus* = 43, *Synechococcus* = 58.



Supplementary Figure 2: Cyanophage titers, measured using *Prochlorococcus* and *Synechococcus* hosts strains, as a function of depth in the oligotrophic western Sargasso Sea (34?24’N, 72?03’W) on 22 Sept. 2001. (a) titers measured using *Prochlorococcus* hosts, (b) titers measured using *Synechococcus* hosts, (c) total *Prochlorococcus* and *Synechococcus* cell abundances (*Prochlorococcus* cells x 10^4 ml^{-1} and *Synechococcus* cells x 10^3 ml^{-1}) and Sigma-T (s_t— a proxy for water density used to measure the depth of the mixed layer). Titers were insignificant for host strains *Prochlorococcus* MIT9312, MIT9211, MIT9313, SS120 and *Synechococcus* WH6501, WH8018, WH8101. Error bars represent the standard deviation of assays.



Supplementary Figure 3: Water collected from Dyer's Dock, Woods Hole, MA on 3 Oct 2000 was immediately sub-sampled to prepare three separate filtrates to test the effects of long term storage on cyanophage titers using our storage methods. Cyanophage titers were measured using *Synechococcus* WH8012 in a MPN assay periodically (3 Oct 2000, 10 Oct 2000, 24 Oct 2000, 28 Nov 2000, 22 Jan 2001, 28 Dec 2001) over the course of 15 months. Data presented are the average and standard deviation of triplicate MPN assays from each subsample.



Supplementary Figure 4: Every isolate in the MIT *Prochlorococcus* culture collection with a unique ITS rDNA sequence was used in plaque assays to determine the strain-specific cyanophage titer from a water sample taken from 50 m depth in the Red Sea on 13 Sep 2000. These data were used to explore, for this one sample only, whether or not the strains we were using to assay phage titers throughout our studies (white bars) were yielding results that might be deemed "typical" with respect to the rest of the host cells in our collection (dark bars). The results show that none of the other host strains yield significantly higher titers than those used in this study. Data shown are average and standard deviation of triplicate plaque assays.