

Supporting Information

Vardi et al. 10.1073/pnas.1208895109

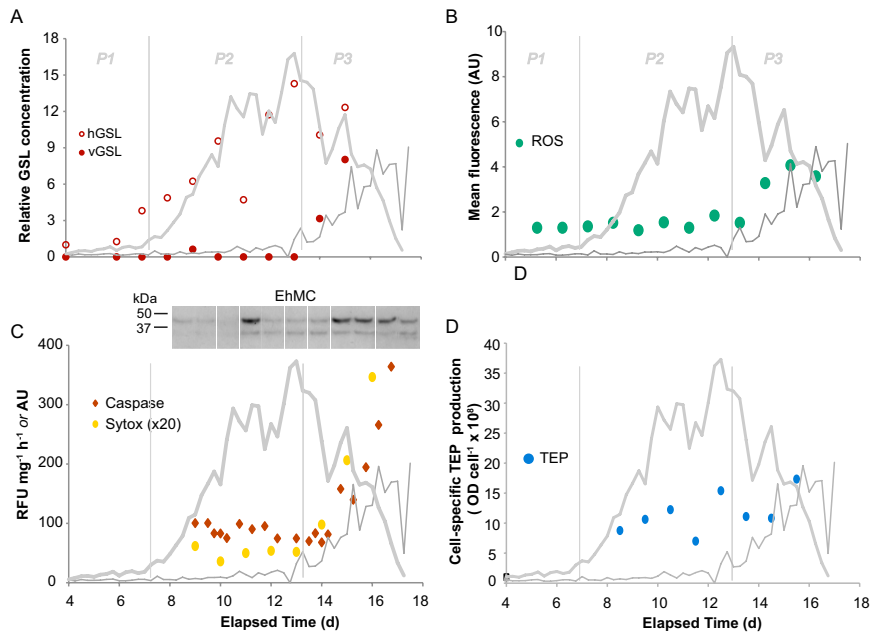


Fig. S1. Cellular response of natural *Emiliana huxleyi* populations to double-stranded, DNA-containing coccolithovirus (EhV) infection of replicate mesocosm bag 1. The traces of host and viral abundances are provided as thick gray and thin gray lines, respectively. Time courses of the following are given: (A) Host-specific glycosphingolipids (hGSLs) and viral glycosphingolipids (vGSLs), provided as the concentration relative to hGSL at 4 d; analytical error of vGSL measurements is $\sim 10\%$ (1), and thus the observed increases in glycosphingolipids during infection are distinct from background levels. (B) Levels of cellular reactive oxygen species (ROS) detected by in vivo fluorescent staining with 5-(and-6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate and flow cytometry. (C) Caspase-specific activity ($\text{RFU} \cdot \text{mg}^{-1} \cdot \text{h}^{-1}$) and cell death (SYTOX staining; arbitrary units). (Inset) Metacaspase protein expression via immunoreactivity to polyclonal *E. huxleyi* metacaspase (EhMC) antisera; all lanes were run on the same gel, had identical exposure times, and are positioned to correspond with elapsed time on the x-axis. (D) Cell-specific transparent exopolymer particle (TEP) production, as measured by alcian blue staining and optical density. Bloom phases are indicated by vertical gray lines (P1, prebloom; P2, bloom; P3, crash/demise). RFU, relative fluorescence unit.

1. Vardi A, et al. (2009) Viral glycosphingolipids induce lytic infection and cell death in marine phytoplankton. *Science* 326(5954):861–865.

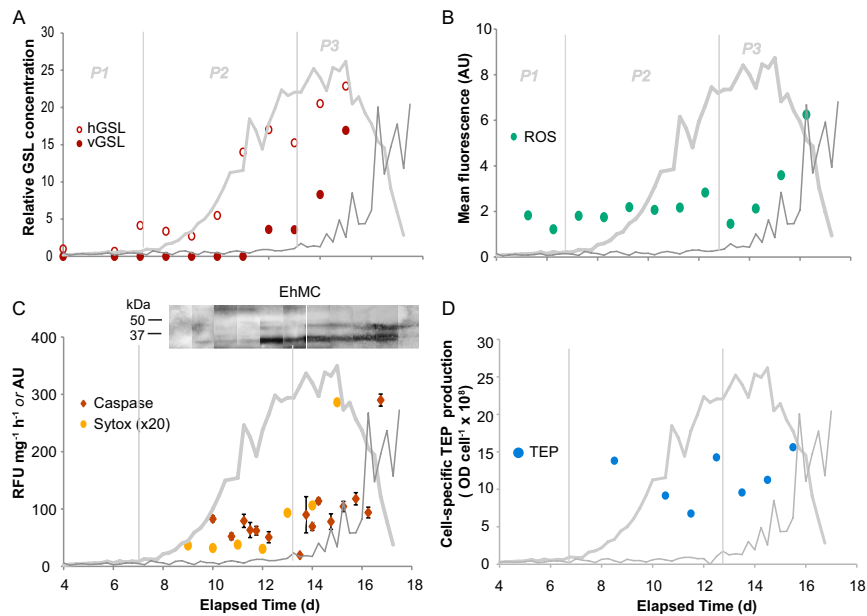


Fig. 52. Cellular response of natural *Emiliana huxleyi* populations to double-stranded, DNA-containing coccolithovirus (EhV) infection for replicate mesocosm bag 5. The traces of host and viral abundances are provided as thick gray and thin gray lines, respectively. Time courses of the following are given: (A) Host-specific glycosphingolipids (hGSLs) and viral glycosphingolipids (vGSLs), provided as the concentration relative to hGSL at 4 d; analytical error of vGSL measurements are approximately 10% (1), and thus the observed increases in glycosphingolipids during infection are distinct from background levels. (B) Levels of cellular reactive oxygen species (ROS), detected by *in vivo* fluorescent staining with 5-(and-6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate and flow cytometry. (C) Caspase-specific activity ($\text{RFU}\cdot\text{mg}^{-1}\cdot\text{h}^{-1}$) and cell death (SYTOX staining; arbitrary units). (Inset) Metacaspase protein expression via immunoreactivity to polyclonal *E. huxleyi* metacaspase (EhMC) antisera; all lanes were run on the same gel, had identical exposure times, and are positioned to correspond with elapsed time on the x-axis. (D) Cell-specific transparent exopolymer particle (TEP) production, as measured by alcian blue staining and optical density. Bloom phases are indicated by vertical gray lines (P1, prebloom; P2, bloom; P3, crash/demise). RFU, relative fluorescence unit.

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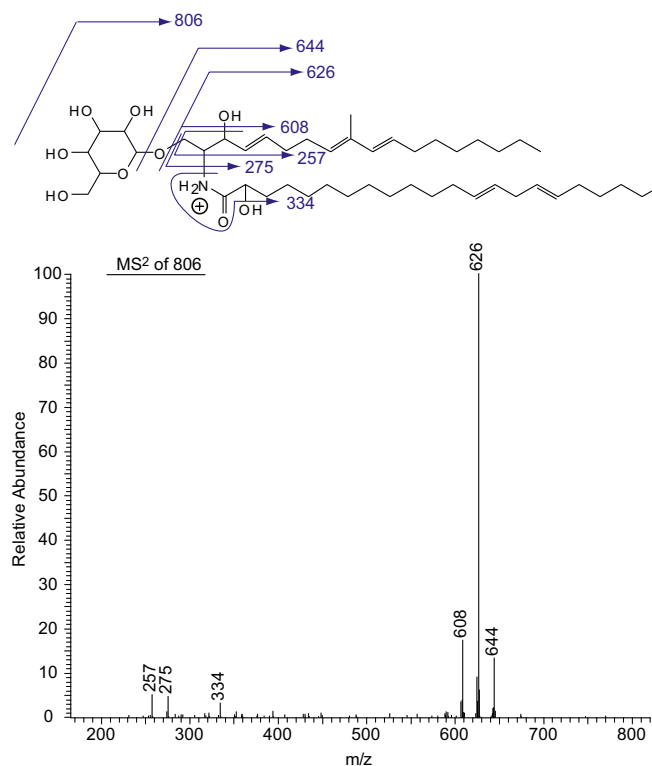


Fig. S3. Tentative structure and ion-trap MS^2 for the host-specific glycosphingolipid (hGSL) molecule. The mass spectrum is that of the 806- m/z molecular ion and shows characteristic glycosyl loss ions (m/z 644, 626, and 608), an amino fatty acid ion (m/z 334), and long-chain base (LCB) ions (m/z 275 and 257). The LCB ions are distinct from those reported by Vardi et al. (1) for the monounsaturated viral glycosphingolipids in that they are $-NH_3^+$. As explained by Ohashi et al. (2), polyunsaturated LCB ions can form conjugated dienes, which retain a positive charge center upon elimination of ammonia. Positions of double bonds on both the fatty acid and LCB moieties are tentative, as is the methyl branch on the LCB. The LCB ions bear very strong resemblance to those reported by Ohashi et al. (2), and thus we have adapted their LCB structure in the figure. Basic elements of the hGSL structure were also supported by ultra-high-resolution Fourier transform ion cyclotron resonance MS. Observed m/z of the molecular ion was 806.61337, which supports a molecular formula of $C_{47}H_{84}O_9N^+$ ($\delta = -0.855$ ppm). Similarly, the minus sugar ions were observed as m/z 644.56024, 626.55122, and 608.54035, which supports formulas of $C_{41}H_{74}O_4N^+$ ($\delta = -1.546$ ppm), $C_{41}H_{72}O_3N^+$ ($\delta = 0.875$ ppm), and $C_{41}H_{70}O_2N^+$ ($\delta = 0.399$ ppm). The amino fatty acid ion was observed to be m/z 334.31066, yielding $C_{22}H_{40}ON^+$ ($\delta = 0.714$ ppm). Finally, m/z 's for the LCB ions were 275.23703 ($\delta = 0.318$ ppm) and 257.22646 ($\delta = 0.332$ ppm), which implicates the $C_{19}H_{31}O^+$ and $C_{19}H_{29}$ LCB structures above.

1. Vardi A, et al. (2009) Viral glycosphingolipids induce lytic infection and cell death in marine phytoplankton. *Science* 326(5954):861–865.
2. Ohashi Y, et al. (2000) Squid nerve sphingomyelin containing an unusual sphingoid base. *J Lipid Res* 41(7):1118–1124.