Supporting Information

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SI Methods

Virome Generation. Metagenomic viral DNA extractions: Viral particles were isolated using cesium chloride (CsCl) gradient centrifugation. Coral slurries were centrifuged at 3,000 rpm for 15 min to remove coral debris, and the supernatant was placed in a new 50 mL conical tube. CsCl gradients were made with FASW, and loaded in the following densities: 1.7, 1.5, and 1.35 mg·mL^{-1}, respectively. To each gradient, ≈9 mL of sample supernatant was added. Gradients were then centrifuged at 82,000 × *g* for 2 hours in a Beckman Ultracentrifuge at 4°C. The 1.5–1.35 mg·mL^{-1} fraction was removed with an 18-gauge needle on a sterile syringe and run through an additional gradient to ensure that all bacteria and debris were removed. A subsample of the resulting fraction was analyzed for the presence of contaminating eukaryotic and microbial cells using Sybr Gold (Invitrogen) staining and epifluorescence microscopy as described in ref. 1. The viral fraction was then DNase I treated to remove any residual free DNA. Viral DNA was extracted using formamide and CTAB, as described in ref. 1. To verify the absence of contaminating eukaryotic and microbial DNA 16S and 18S PCR was conducted on all samples before sequencing as described in ref. 2. No bands were detected.

Virome DNA from each time point and treatment was isolated and extracted to generate twenty-five temporal samples, which were subsampled, amplified, and then pooled by treatment for metagenomic library construction (Fig. S1). Once purified, 1 ng of viral genomic DNA from all samples underwent amplification, using GenomiPhi from GE LifeSciences (Quebec, Canada) and repurification with a DNeasy Blood and Tissue Kit from QIAGEN (Valencia, CA). Each of the metagenomic libraries included equal amounts of the amplified DNA from the 1, 4, 16, and 64 h isolates of each corresponding treatment, except the reference sample time zero, which was a pool of all of the time 0 temporal samples (Fig. S1). Approximately 5 μg of total genomic DNA for each library was sent to 454 Life Sciences (Branford, CT) for pyrosequencing using GS20 technology (Fig. S1).

Each of the reads was parsed, stored, assigned a number, and archived at the San Diego State Center for Universal Microbial Sequencing (http://scums.sdsu.edu). Each viral metagenome can be accessed through this website under the accession nos. 4440374.3, 4440375.3, 4440370.3, 4440371.3, 4440377.3, 4440376.3, but are also found at National Center for Biotechnology Information as genome projects IDs: 28427, 28429, 28431, 28433, 28435, and 28437.

Repeat stressor experiment: All parameters were identical to the initial experiment except that in the Temperature treatment, water was increased to 3°C above ambient instead of 5° C. Also, in the ocean acidity stressor, seawater pH was reduced to 7.8 to reflect a more likely environmental change. Salinity, temperature and pH were measured at every sampling.

Herpes-like thymidylate synthase contig assembly. Sequences from each metagenome that were annotated as herpes-like were assembled using SeqMan from DNASTAR Inc. (Madison, WI) using 99% similarity, 35 base pair overlaps, and a minimum sequence read length of 80 base pairs. Contigs were generated and consensus viral sequences were identified using PSI-BLAST to the nonredundant database at National Center for Biotechnology Information. Primer sets were generated and used first for conventional PCR and then real-time PCR, cloning, and Sanger sequencing.

PCR and putative herpes-like thymidylate synthase gene sequence cloning. For the putative herpes-like virus thymidylate synthase gene, PCR was carried out in 50 μL of standard reactions containing 200 nM primers and 25 ng of total viral DNA. Touch down thermocycling was conducted using a 3 min 95°C hot start and 30 cycles of the following: 95°C for 1 min, 60°C (−0.5°C) for 30 seconds, and 72°C for 1 min. A 10-min extension at 72°C completed the PCR. Samples were run on a 1 or 1.5% agarose gel. PCR reactions were purified using a PCR AccuPrep Kit from BioNeer and cloned using a TopoTA Kit from Invitrogen. Sequencing was conducted at the CSUPERB Microchemical Core Facility. Sequences were trimmed by hand and homology identified using PSI-BLAST to the nonredundant database at National Center for Biotechnology Information.

Fig. S1. Coral collections: *Porites compressa* specimens were collected on February 22, 2006 (metagenomic experiment) and February 27, 2007 (repeated stressor experiment) directly off Reef Point (≈21°.25′ N, 157°47 W) on Coconut Island, Kane'ohe Bay, O'ahu, Hawai'i. Samples were collected 25 m off shore, at 1-meter depth and 5 meters apart. Coral heads of ≈20 cm in diameter were removed with hammer and chisel at the base of the colony to avoid polyp damage. Specimens were placed in a 3 L seawater cured bucket, carried to shore, and immediately transferred to large open outdoor aquaria that contained continuous and rapidly flowing seawater.

Environmental stressor experiments for viromes: The 3 coral heads (*A–C*) were each broken into 5 ''fingers'' of approximately equal size (≈30–40 grams), and 3 parts or ''nubbins'' along each finger were sampled before placement in tanks creating the Time Zero reference samples (Fig. S1). One coral finger from each head was then placed in a glass aquarium (12.5 × 5 × 8), containing treated sand filtered seawater. The Aquarium Control sample received no treatment but similar manipulations (placement in tank, removal from tank etc…). Water for the controls was 5 L of sand-filtered seawater from the HIMB aquaria facility. Temperature treatment was a 1-time increase (not ramped) of the tank seawater temperature from local ambient, 24°C, to a maximum of 30°C. Nutrient treatment consisted of a 10 μM excess of: nitrate ($Ca(NO_3)_2$), nitrite ($NaNO_2$), ammonium (NH_4Cl), and phosphate (KH_2PO_4). Dissolved organic carbon loading was addition of 25 $mg \cdot L^{-1}$ glucose, and pH treatment was a reduction of ambient pH from 8.0 to 6.8 using hydrochloric acid and bicarbonate. Ambient conditions included lower than average air temperatures and high precipitation rates. Aquarium treatment water/stressor was replenished every 24 h.

At each time point (1, 4, 16, and 64 h) all 3 fingers were sequentially removed from each tank and placed on sterile aluminum foil. Then 3 distinct, 1–2 gram, ''nubbins'' (tissue and skeleton) were cut away with a sterile razor blade, and the finger was immediately returned to the tank. This resulted in 9 different tissue samples for each treatment at each time point. Each nubbin was rinsed with filtered (0.02 μm) autoclaved sea water (FASW), placed in a precleaned mortar and pestle in the presence of ≈10 mL FASW, crushed into a slurry, and rinsed into a 50 mL of BD Falcon tube with an additional 10 mL of FASW. Approximately 0.2 volumes of molecular biology grade chloroform was added to the slurry to kill any microbes or eukaryotes present, mixed vigorously for several minutes and placed at 4°C until shipping. This was repeated for every coral (*A–C*) to generate a total pool of 9 nubbins at each time point (1, 4, 16, 64 h) for the aquarium control, temperature, DOC, pH, and nutrient treatments. Samples were stored at 4°C and shipped on ice to San Diego State University for processing and pooling before pyrosequencing. Metagenomic libraries were generated by pooling DNA in equal amounts from each of the viromes (each of the different 9 nubbin pools). Real-time PCR was conducted on each of the 3 coral sets (*A–C*) at each time point for every treatment (5 treatments, 5 time points) for a total of 25 samples in triplicate. All samples were then shipped on ice to San Diego State University for processing.

Saimirine herpesvirus 1
Saimirine herpesvirus 2
Ateline herpesvirus 2
P. Compressa HLV
Cercopithecine herpesvirus 1
Human herpesvirus 3
Equid herpesvirus 2
Cercopithecine herpesvirus 2
Human herpesvirus 8
Heliothis zea virus 1
Macropodid herpesvirus 1
Kordia aligicida OT-1 virus
Nematostella
Vibrio sp. Med 222 phage
Vibrio shilonii Ak1 phage
Vibrio harveyi phage
100.0 85.8 33.3 11.6 29.8 11.2 14.6 68.6 NA 19.4 58.7 75.3 86.6 100.0
167.1
160 140 120 100 80 60 40 20 0
Amino Acid Substitutions (x100)
Bootstrap Trials = 10,000, seed = 111

Fig. S2. Coral associated herpes-like viral sequence used for real-time PCR. Sequences from several viral, bacterial, and anemone thymidylate synthase proteins were aligned to the putative Coral HLV sequence using CLUSTAL W and MegAlign from DNASTAR. Blosum 30, PAM 350, and Gonnot 250 matrixes all produced the same tree. Bootstrap analysis was performed using 10,000 iterations.

Table S1. Metagenome characteristics and similarity statistics when compared against the non-redundant ($e < 10^{-4}$) database at NCBI using BLASTn. Each sequence with a similarity in the NR was given a taxonomic assignment (viral, bacterial, eukaryotic) based on its best (smallest e value) similarity

Sample	Reads	Approx. read length	Known, %	GC content, %	Viral, %	Bacterial, %	Eukaryotic, %
Time zero	39,270	101.32	2.12	42.25	1.87	64.14	33.14
Control	39,340	103.7	5.21	46.48	2.89	90.18	6.05
Temperature	39,036	113.38	2.01	48.27	0.98	58.09	40.05
DOC	35,680	102.18	1.88	42.49	8.71	71.24	19.55
pH	50,368	104.73	1.57	43	3.37	68.67	27.44
Nutrient	34,433	107.18	1.83	45.1	6.92	76.82	15.32

Table S2. Coral-associated viral metagenomes were compared for similarity to four previously isolated marine water viromes

Sample	Kingman Reef	Christmas Reef	Palmyra Reef	Tabuaren Reef
Time zero	10.53	10.88	11.10	6.52
Control	2.49	3.65	5.57	1.13
Temperature	29.07	20.12	38.14	25.44
DOC	1.58	2.46	1.74	0.77
pH	11.91	9.63	16.29	9.59
Nutrient	17.41	11.81	23.04	14.27

Percentage similarities between each coral library and reef water viral library were calculated using BLASTn ($e < 10^{-4}$).

Table S3. Examples of combined viral metagenome coverage to 2,020 fully sequenced viral genomes from NCBI

Virus	Accession no.	Genome size, kb	Family	Similarities	Coverage
A. polyphaga mimivirus	NC_006450	1181.4	Mimivirus	952	0.09
A. tigrinum virus	NC_005832	106.33	Iridoviridae	64	0.07
A. moorei entomopoxvirus	NC_002520	232.4	Poxviridae	333	0.16
Bovine herpesvirus 1	NC_001847	135.3	Herpesviridae	1071	**0.87**
Bovine herpesvirus 5	NC_005261	137.82	Herpesviridae	360	0.29
Canarypox virus	NC_005309	359.85	Poxviridae	168	0.05
Cercopithecine herpesvirus 1	NC_004812	156.8	Herpesviridae	666	**0.47**
Cercopithecine herpesvirus 16	NC_007653	156.5	Herpesviridae	976	**0.69**
Cercopithecine herpesvirus 2	NC_006560	150.72	Herpesviridae	1282	**0.94**
E. siliculosus virus	NC_002687	335.59	Phycodnaviridae	174	0.06
E. huxleyi virus 86	NC_007346	407.34	Phycodnaviridae	725	0.20
Equid herpesvirus 2	NC_001650	184.43	Herpesviridae	176	0.10
Human herpesvirus 1	NC_001806	152.26	Herpesviridae	216	0.16
Human herpesvirus 2	NC_001798	154.746	Herpesviridae	283	0.20
M. sanguinipes entomopoxvirus	NC_001993	236.12	Poxviridae	169	0.08
P. bursaria Chlorella virus 1	NC_000852	330.74	Phycodnaviridae	414	0.14
Shrimp spot syndrome virus	NC_003225	305.11	Nimaviridae	125	0.05
Singapore grouper iridovirus	NC_006549	140.13	Iridoviridae	169	0.13
Suid herpesvirus 1	NC_006151	143.46	Herpesviridae	862	**0.66**
Tupaia herpesvirus	NC_002794	195.86	Herpesviridae	238	0.13

All coral-associated viral metegenomes were combined and the number of sequence similarities ($e < 10^{-6}$) to each genome was calculated. Frequency tables were generated for each 2,500-bp bin across each reference genome. First, the number of synonymous nucleotides between the metagenome and the reference genome was counted. Coverage was then calculated by dividing this number by the total length of reference the genome. Any genome with coverage equal to or greater than 0.05 is listed. Coverage values in bold denotes the 5 highest values.

Table S4. Herpes-like viral sequences in the reference genomes of two Cnidarians

Nematostella Draft	Hydra ESTs
5507928	74131562
5500715	74132680
5503168	68410998
5522046	
5497885	
5517497	
5512689	
5521542	
5498193	
5515317	
5515317	
XP_001626845	
EDO34745	
XP_001641920	
EDO49857	
XP_001636448	
EDO44385	
XP_001629245	
XP_001627817	
XP_001626452	
XP_001624299	
XP_001621368	
XP_001617849	
EDO25749	
EDO29268	
EDO32199	
EDO34352	
EDO35717	
EDO37182	