Microb Ecol (2011) 62:48–57
DOI 10.1007/s00248-011-9856-1

ENVIRONMENTAL MICROBIOLOGY

Nucleopolyhedrovirus Detection and Distribution in Terrestrial, Freshwater, and Marine Habitats of Appledore Island, Gulf of Maine

Ian Hewson · Julia M. Brown · Shari A. Gitlin · Devin F. Doud

Received: 11 December 2010 / Accepted: 5 April 2011 / Published online: 21 April 2011

Abstract Viruses in aquatic ecosystems comprise those produced by both autochthonous and allochthonous host taxa. However, there is little information on the diversity and abundance of viruses of allochthonous origin, particularly from non-anthropogenic sources, in freshwater and marine ecosystems. We investigated the presence of nucleopolyhedroviruses (NPV) (*Baculovirus*), which commonly infect terrestrial lepidopteran taxa, across the landscape of Appledore Island, Gulf of Maine. PCR and qPCR primers were developed around a 294-bp fragment of the polyhedrin (*polH*) gene, which is the major constituent protein of NPV multivirion polyhedral occlusion bodies. *polH* was successfully amplified from several aquatic habitats, and recovered *polH* sequences were most similar to known lepidopteran NPV. Using quantitative PCR designed around a cluster of detected sequences, we detected *polH* in Appledore Island soils, supratidal freshwater ponds, nearshore sediments, near- and offshore plankton, and in floatsam. This diverse set of locations suggests that NPVs are widely dispersed along the terrestrial—marine continuum and that free polyhedra may be washed into ponds and eventually to sea. The putative hosts of detected NPVs were webworms (*Hyphantria* sp.) which form dense nests in late summer on the dominant Appledore Island vegetation (*Prunus virginiana*). Our data indicate that viruses of terrestrial origin (i.e., allochthonous viruses) may be dispersed widely in coastal marine habitats. The dispersal of NPV *polH* and detection within offshore net plankton (>64 μm) demonstrates that terrestrial viruses may interact with larger particles and plankton of coastal marine ecosystem, which further suggests that viral genomic information may be transported between biomes.

Introduction

Viruses of terrestrial arthopods have been observed for over a century [1, 2]. They affect a wide suite of *Hexactapoda* where in some cases they cause substantial mortality (e.g., colony collapse disorder in bees [3, 4]). One of the most extensively studied groups of arthropod viruses in terrestrial ecosystems are *Baculovirus*. Nucleopolyhedroviruses (NPVs), which along with granuloviruses comprise a suborder of baculoviruses, are amongst the most widely studied viruses. They are dsDNA viruses with genomes of 120–140 kbp that infect well over 600 species of terrestrial insects, notably lepidopterans [5]. The death of an NPV-infected host results in the release of the occlusion bodies [6]. Horizontal transfer of the virus occurs upon ingestion of the occlusion bodies on leaf surfaces or leaf litter by another host [7]. NPVs can also be transmitted vertically from parents to larvae [8]. The formation of degradation-resistant occlusion bodies therefore circumvents the high decay of viruses when free of tissues and provides a vector when host densities are rare. NPVs are generally host specific, and consequently have been investigated for use in agriculture for pest control.

The occlusion bodies that are characteristic of NPVs are comprised primarily of polyhedrin, encoded for by the typically highly conserved gene *polH* [9]. The highly

---

Electronic supplementary material The online version of this article (doi:10.1007/s00248-011-9856-1) contains supplementary material, which is available to authorized users.

I. Hewson (✉) · J. M. Brown · S. A. Gitlin · D. F. Doud
Department of Microbiology, Cornell University,
Wing Hall 403,
Ithaca, NY 14853, USA
e-mail: hewson@cornell.edu
URL: http://microbe.calsnet.cornell.edu/~hewsonlab/

conserved nature of the *polH* gene has led to numerous studies examining specific groups of virus across terrestrial and aquatic habitats. Nucleopolyhedrovirus infection of *Spodoptera littoralis* was examined when primers were designed around conserved regions of the polyhedrin genes of *Autographa californica* nucleopolyhedrovirus and *Lymantria dispar* nucleopolyhedrovirus [10]. The tight conservation of the *polH* gene suggests that it may be an appropriate target for study of NPV infection in other taxa, or even surveying wild viral populations for the presence of NPVs. The exception to the tight conservation of *polH* amongst nucleopolyhedroviruses is in *Penaeus monodon* nucleopolyhedrovirus, which bears little nucleotide resemblance to *polH* from other NPVs [11]. Its distinctive nucleotide sequence permits its detection by PCR in the environment and amongst alternate hosts against a backdrop of mixed viral communities [8].

Viruses are believed to decay rapidly in seawater, where approximately 2–4% h^{-1} of viruses disappear in incubations treated with KCN [12]. The cause of viral decay is believed to be largely heat-labile organic matter [13], including proteases and nucleases. Sediments on the other hand may provide a more preservative environment than overlying waters. While viruses are rapidly produced in sediment incubations where the balance between production and decay are shifted [14], cyanophage have been detected deep within the sediment column in areas with high sedimentation rates [15]. The behavior of allochthonous terrestrial viruses in marine habitats is well understood for pathogenic human viruses like poliovirus and hepatitis [16–18], and their dynamics in sediments and resuspension has been recently investigated in coastal waters [19]. A recent metagenomic survey of RNA viruses within Lake Neewood reported two previously undescribed viruses: one related to the human disease-causing Banna virus, and another related to the Israeli acute paralysis virus which has been implicated in colony collapse disorder in bees [20]. The authors hypothesized that freshwater habitats may represent repositories of viruses shed from allochthonous sources including humans, domestic, and wild animals.

The behavior of nucleopolyhedroviruses of terrestrial origin in marine habitats has not been previously investigated. The causes of NPV decay in terrestrial systems may include inactivation by plant exudates [21] and other charged substances free in the environment [22], ultraviolet light [23], and heat-sensitive materials in moisture [24]. In freshwater ecosystems, England et al. [25, 26] used PCR primers designed around the polyhedrin promotor-P10 promotor-*lacZ* region of *A. californica* to detect extracted lepidopteran NPV DNA in the water column and sediments of freshwater microcosms, which persisted up to 24 h. Further investigation [27] used the same assay to examine persistence of intact virions within aquatic microcosms and found that they could be detected up to 1 year after inoculation. These very long survival times of intact viruses has been suggested as a potential dispersal mechanism for flying insects, including the mosquito *Culex nigripalpus* which is infected by *C. Nigripalpus* nucleopolyhedrovirus [28]. The short survival of free viral DNA yet very long survival of polyhedra in pond water suggests that NPVs may persist for long periods in seawater.

The aim of the present study was to examine the dispersal of NPVs that originate in terrestrial ecosystems into aquatic and marine habitats of the Gulf of Maine. First, we examined the diversity of *polH* genes amplified from plankton samples using primers designed around insect NPVs, then used quantitative PCR using primers designed around detected sequences as a tool to examine potential origin of these NPVs amongst island insects. We then surveyed a broader range of habitats to determine the extent to which terrestrial NPVs may disperse into aquatic habitats. We found NPVs that likely originate from the webworm (*Hyphantria* sp.) exist in soils, freshwater habitats, exopolymeric substance and macroalgal floatsam (wrack), and open-ocean plankton suggesting wide dispersal of NPVs across habitats.

Methods

Design of Nucleopolyhedrovirus *polH* PCR Primers

We designed nested PCR primers around a 294-bp fragment of the polyhedrin (*polH*) gene sequences of several lepidopteran NPVs (Supplemental Table 1). We chose to use only lepidopteran NPV *polH* since we sought viruses of terrestrial origin, and the only NPV *polH* sequence of a crustacean shares no homology with *polH* from other NPVs [11]. Initial amplification with primers polH_1F and polH_2R (Table 1) of environmental DNA yielded multiple

Table 1 Primers and probe sequences used for detection of *polH* across samples

Primer Name	Sequence 5′-3′
polH_1F	CAG(A/G)GAAACTTGGACCCG
polH_2R	GAAGTT(C/T)TCCCA(A/G/T)ATGAC(A/G)CG
polH_3R	T(C/G)TTC(A/G)AA(A/C/G)GAGTTGGTGTA
polH_5F	GGACAGCCTCCCCATTGTTA
polH_6R	GTTGTTGCAACCGACGTACA
polH_7Pr	[FAM]AGATTCCTAGCCCAACACGCTCTGC [TAMRA]

Primers polH_1F, polH_2R, and polH_3R were used in nested, degenerate PCR, while primers polH_5F, polH6R, and probe polH_7Pr were in quantitative PCR

FAM 6-carboxyfluorescein, *TAMRA* carboxytetramethylrhodamine sequences that most closely matched bacterial gene fragments, hence polH_3R was designed to increase specificity of the primer set and permit amplification of rare targets within mixed assemblage template DNA. Primers were designed using Primer3 [29], and were compared to the non-redundant database at National Center for Biotechnology Information (NCBI) by BLASTn [30]. In order to permit detection of *polH* in natural samples, a TaqMan© primer and probe set (polH_5F, polH_6R, and polH_7Pr) was designed around a 96-bp fragment within the region of *polH* amplified with polH_1F and polH_3R. The dual TAMRA- and FAM-labeled probe was designed based on sequences derived from the survey of environmental DNA.

PCR Amplification of *polH* from Aquatic Samples

The presence of nucleopolyhedroviruses was examined by screening samples of environmental DNA collected from the Gulf of Maine (Fig. 1). Plankton samples were collected using a 64-µm mesh size net that was towed in surface waters for ~5 min, and then transferred into Whirlpak bags. Seston from ponds was collected by transferring 25 ml of material directly into a Whirlpak. Exopolymeric substance floatsam (“EPS foam”) was collected after a heavy weather event from rocks on the windward side of Appledore Island using a sterile transfer pipette, and stored in a whirlpak. All samples were kept frozen prior to processing in the lab at Cornell.

DNA was extracted from samples using the ZR Tissue & Insect DNA Miniprep kit (Zymo Research) following manufacturer's protocols. In each case ~100 mg of wet zooplankton, seston, wrack, or EPS foam was transferred into bead-beating tubes using sterile transfer pipettes. Extracted DNA (50 µl) was stored at −20°C prior to PCR. Nested degenerate PCR was conducted on 2-µl extracted DNA in 50-µl reactions containing 1× PCR Buffer (Invitrogen), 2.5 mM MgCl2, 0.2 mM dNTPs (Promega PCR Nucleotide Mix), 2 µM each of primers polH_1F and polH_2R, 2 ng μl^{-1} BSA (Sigma 7030), and 5U Taq Polymerase (Invitrogen). Thermal cycling comprised of an initial heating step at 95°C for 3 min, followed by 30 cycles of 95°C denature for 30 s, 55°C anneal for 30 s, 71°C extension for 45 s, and followed by a 71°C final extension for 7 min. The second round of PCR consisted of the same reagents as the first round, except polH_2R was replaced by polH_3R, and 2 µl of first-round PCR product served as template in the second round.

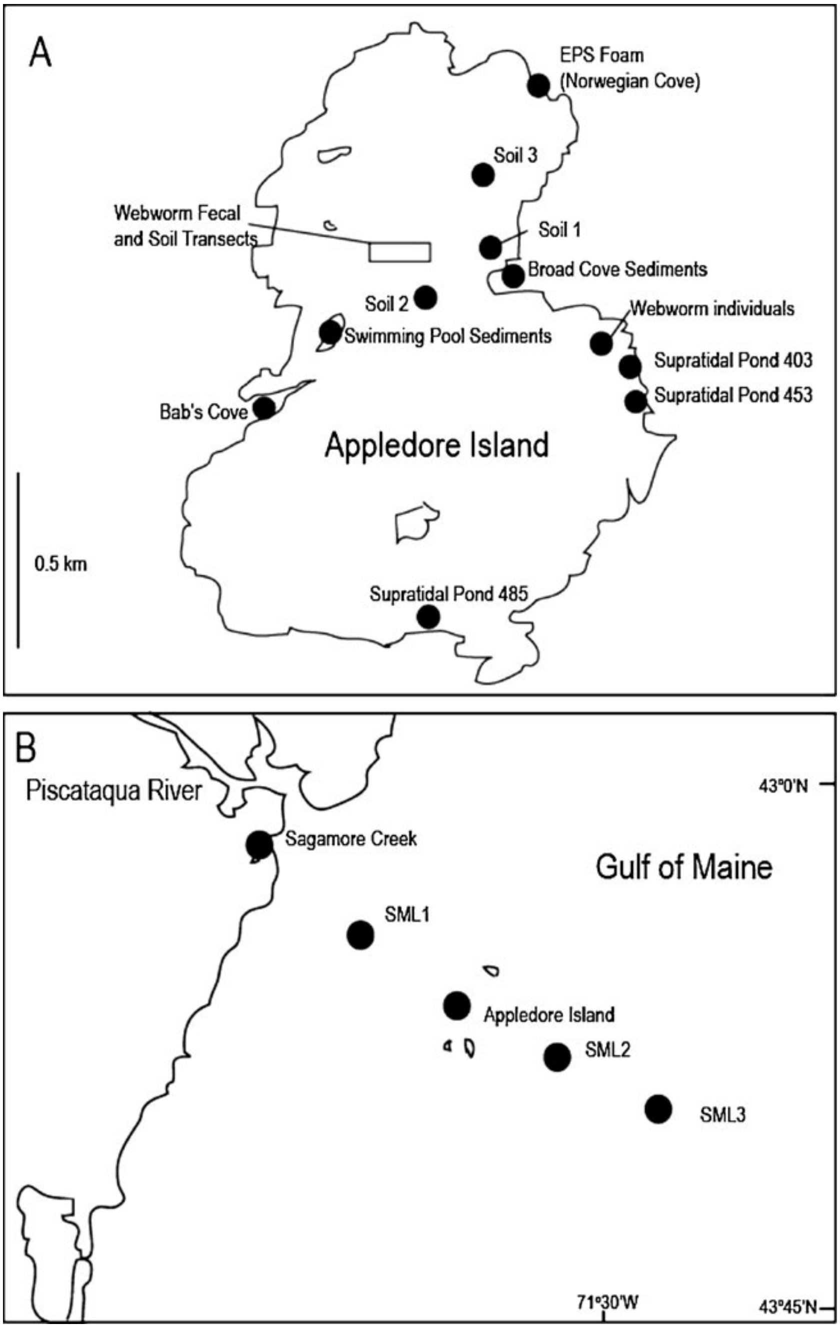
PCR products were electrophoresed on a 1% agarose gel in 1X TBE at 6 Vcm^{-1} for 1 h, which was subsequently stained with SYBR Gold (0.1 µl ml^{-1}) and visualized on a UV light transilluminator. The presence or absence of PCR products at ~300 bp on the gel was scored. Products at 300 bp were excised with a clean razor blade and gel slices were placed into 2-ml microcentrifuge tubes. DNA in the slices were extracted using the ZR Gel Recovery Kit (Zymo Research), igated into pGem-T Easy II Vectors (Promega) following the manufacturer's protocols and transformed into Z-competent cells (Zymo Research). Plasmids were prepared using the Zyppy Plasmid Prep Kit (Zymo Research), and sequenced at the Core Laboratories Center at Cornell University. Sequences matching *polH* by BLASTx analysis [30] against the non-redundant database at NCBI were aligned and bootstrapped against other NPV *polH* genes using ClustalX [31]. Sequences derived in this study have been deposited at GenBank under accession nos JF756079-JF756085.

Investigation into Fall Webworms (*Hyphantria* sp.) as a Source of NPVs

Two webworm nests were sampled to determine the possible origin and distribution of NPVs. Fall webworms (*Hyphantria* sp.) were collected from nests using forceps and placed into 0.5-ml microcentrifuge tubes, which were frozen on liquid N_2. Fecal matter from two nests was collected by placing a 10-cm diameter funnel below tents and shaking the tent gently to dislodge fecal pellets. The material in the funnel was collected into 15-ml centrifuge tubes. Soil cores (1 cm^3) were collected along a transect starting beneath each tent. Samples were collected at 0 (immediately beneath the nest), 1, 2, and 5 m from the tent. Soil cores were extruded and placed into sterile 15-ml centrifuge tubes prior to freezing in liquid nitrogen. Insect DNA was extracted using the ZR Tissue & Insect DNA Miniprep kit. Soil and fecal samples were extracted using the ZR Soil DNA Miniprep kit (Zymo Research).

Quantitative PCR (qPCR) was used as a sensitive method to detect *polH* in environmental samples. Extracted DNA was subject to qPCR of *polH* using primers polH_5F, polH_6R, and probe polH_7Pr (Table 1). qPCR reactions (25 µl) comprised 1× TaqMan Master Mix with no UNG and ROX passive dye (Invitrogen), 80 nM of each primer and probe, and 2-µl template DNA. Thermal cycling conditions comprised of an initial step at 50°C for 10 min, followed by a hot start at 95°C for 5 min. Data was collected at the end of each of 60 cycles of 95°C denature for 15 s followed by 55°C anneal and extension for 60 s. Each reaction was conducted in duplicate, using an oligonucleotide standard over eight orders of magnitude. Because of uncertainty of extraction efficiency and lack of standardization between disparate template types, qPCR was only used as a detection method and results are not presented as quantities. Samples for which one of two technical replicate qPCR reactions were positive but the second was not positive were scored as “detected (DET)” per previous studies [32].

Figure 1 **A** Map of Appledore Island showing locations sampled for detection and quantification of nucleopolyhedrovirus *polH* genes; **B** map of location of offshore stations and Sagamore Creek



Survey of *polH* in Habitats of the Isles of Shoals by Quantitative PCR

To examine the wider distribution of detected *polH* sequences around the Isles of Shoals, samples of soils, sediments, and plankton were collected at several locations around the island (Table 3). Firstly, soil cores were collected from three locations on the island, and subtidal sediment collected from three habitats using cut-off syringes, which were then extruded into sterile 15-ml centrifuge tubes and frozen. Seston was collected from three supratidal ponds around the island's periphery, and plankton in the 1.0–0.2-μm size fraction was collected from a further ten ponds by filtration of 20–30 ml water in each pond. Plankton from Bab's cove was collected using a 64-μm plankton net towed in surface waters. Bacterioplankton from Bab's cove and a further four locations in the vicinity of the Shoals archipelago (stations SML1, SML2, and SML3) were collected by filtering 20 L water through 10 μm polycarbonate (Isopore) filters onto 0.22-μm Durapore filters (Millipore). All samples were flash-frozen after collection prior to transport to the laboratory at Cornell. Samples were extracted using the ZR Tissue & Insect Kit following the manufacturer's instructions. The extracted nucleic acids were then subjected to qPCR detection of NPV *polH* genes using primers polH_5F, polH_6R, and probe polH_7Pr (Table 1) per the webworm investigation.

Results and Discussion

PCR Amplification of *polH* Across Habitats

We amplified products in the predicted size range from 4 of 10 environmental samples by conventional PCR (Tables 2 and 3). Quantitative PCR was used to confirm the presence of *polH* within amplicons prepared from several samples, which also had products at 300 bp examined by gel electrophoresis. All samples tested which had PCR products of 300 bp demonstrated amplification with our qPCR primer and probe sets (Table 3). To further confirm that amplicons contained *polH* genes, we pooled several amplicons (from Appledore Island Supratidal Ponds 485 and 403, and several bulk tow samples from the Shoals Marine lab) and randomly sequenced clones from combined samples. While we obtained several sequences that did not match *polH* within amplicon pools, we retrieved a total of five *polH*-like sequences that were most similar to *Agrotis ipsilon* NPV (99% nucleotide identity), and two sequences most similar to *Plusia orichalcea* NPV (83% nucleotide identity; Fig. 2).

The observation of *polH* sequences with similarity to lepidopteran NPVs in several diverse locations suggests that these are distributed widely in aquatic habitats. Because we pooled our samples for sequencing the exact origin of the sequences are not resolved. However, qPCR of the *polH* gene applied to PCR amplicons of these samples demonstrated the presence of NPV *polH* within the EPS foam, and applied to extracted DNA detected the presence of NPV *polH* in the other habitats. EPS foam occurs above the intertidal line on the windward side of Appledore Island after heavy weather events. It presumably comprises exopolymeric substances produced by autotrophs and other proteinaceous material that is agitated by weather events and blown onshore [33]. Hence, NPV *polH* in these samples may originate from terrestrial insects that are advected offshore during rainfall events, or from insects inhabiting beach wrack material. It is also not surprising that sequences retrieved were closest to known lepidopteran NPVs, since primer sequences were designed based on an alignment of primarily moth NPVs. Moreover, these observations indicate that sequences retrieved from aquatic environments represent allochthonous viruses, since the typical hosts of NPVs (caterpillar larvae of lepidopterans) are not present in water.

Investigation into *Hyphantria* sp. as A Potential NPV Host

Quantitative *PCR* detection of *polH* in *Hyphantria* sp. was successful, with all but one individual having detectable *polH*. Fecal matter falling from *Hyphantria* sp. nests, and the distribution of *polH* in soils underneath *Prunus virginiana* trees were investigated at two locations to further resolve *Hyphantria* as a potential host of detected NPV *polH* (Table 3). Near-detection threshold (i.e., detected in two of three analytical replicate reactions) *polH* was detected in the fecal matter of only one tree. Only soils

Table 2 Detection of nucleopolyhedrovirus *polH* by conventional PCR and quantitative PCR in seawater samples

Sampling location	Sample type	Sampling date	PCR detection	qPCR detection
Appledore Island, Gulf of Maine	Plankton, >64 μm	6-Jun-09	Na	DET
		7-Jun-09	−	DET
		24-Jun-09	Na	DET
		4-Jul-09	−	DET
		6-Jul-09	−	DET
		7-Jul-09	+	DET
Sagamore Creek, Gulf of Maine	Plankton, 10–0.2 μm	27-Jun-10	Na	+
Station SML1, Gulf of Maine	Plankton, >64 μm	26-Jun-09	−	−
	Plankton, 10–0.2 μm	26-Jun-09	Na	−
Station SML2, Gulf of Maine	Plankton, >64 μm	25-Jun-09	Na	DET
	Plankton, 10–0.2 μm	25-Jun-09	Na	+
Station SML3, Gulf of Maine	Plankton, >64 μm	25-Jun-09	−	DET
	Plankton, 10–0.2 μm	25-Jun-09	Na	+

Detection by PCR is indicated when a product of ~300 bp was detected and confirmed by application of qPCR to band-isolated amplicon

Na indicate that amplification was not attempted, *DET* detected amplicon in one of two analytical replicates

Table 3 Detection of *polH* from samples across the landscape of Appledore Island

Sample type	Sampling date	PCR detection	qPCR detection
EPS foam	22-Jun-09	+	+
Supratidal pond 403 seston	19-May-09	+	DET
Supratidal pond 453 seston	19-May-09	−	−
Supratidal pond 585 seston	19-May-09	+	−
Soil sample 1	2-Jul-09	Na	+
Soil sample 2	2-Jul-09	Na	−
Soil sample 3	2-Jul-09	Na	+
Sediment, Broad Cove	2-Jul-09	Na	+
Sediment, swimming pool	2-Jul-09	Na	+
Sediment, Babs Cove	2-Jul-09	Na	+
Hyphantria sp. fecal matter, tree 1	20-Jul-10	Na	−
Hyphantria sp. fecal matter, tree 2	20-Jul-10	Na	DET
Soil, 0 m from tree 1	20-Jul-10	Na	−
Soil, 1 m from tree 1	20-Jul-10	Na	−
Soil, 2 m from tree 1	20-Jul-10	Na	−
Soil, 5 m from tree 1	20-Jul-10	Na	−
Soil, 0 m from tree 2	20-Jul-10	Na	+
Soil, 1 m from tree 2	20-Jul-10	Na	+
Soil, 2 m from tree 2	20-Jul-10	Na	+
Soil, 5 m from tree 2	20-Jul-10	Na	−

Locations are also indicated on Fig. 1. Detection by PCR is indicated when a product of ~300 bp was detected and confirmed by application of qPCR to band-isolated amplicon

Na indicate that amplification was not attempted, *DET* detected amplicon in one of two analytical replicates

beneath the tree where fecal matter was *polH*-positive contained *polH*. Interestingly, *polH* was detected only within the first 2 m underneath the *polH*-positive tent, but not at 5 m.

Our data indicate that *Hyphantria* sp. (webworm) are the likely host of NPV *polH* sequences recovered on Appledore Island. Although moths which resemble *Agrotis* sp. were observed in net-collected flying insects (data not shown), their larvae were anecdotally not abundant on the island (the *A. ipsilon* NPV affects only the caterpillar stage of the host [34]). *Hyphantria* sp. instars, on the other hand, formed dense colonies in web tents. The dominant vegetation cover on Appledore Island is *P. virginiana* (Chokecherry). A limited visual survey in August 2010 indicated that at least two tent colonies were on each chokecherry tree, where tents appeared in mid July and persisted until late August. Lepidopteran NPVs are transported by several means, but primarily by instar consumption of materials that bear remains of infected individuals [6, 35]. The lack of large signals of *polH* NPV in material falling from nests, and detection only in one of two tents surveyed, suggests that fecal matter is not a significant route of transfer to soils. Our collection does not discriminate between fecal matter and broken-down remains of adults; hence, the presence of *polH* NPV in only one tent and in soils underneath that tent suggests that NPVs may fall to soils by either mechanism. Not all caterpillars assayed were NPV *polH*-positive, which suggests that within a nest there may be differences in the proportion of infected to uninfected caterpillars.

Soil and Sediment Distribution of NPV *polH* on Appledore Island

Amongst the terrestrial and freshwater samples that were *polH* positive by conventional PCR, only the sample of exopolymeric substance (EPS) foam was positive for *polH* by qPCR, and only one of the three supratidal ponds sampled was positive (Table 3). We investigated soils at three locations on the island that were away from *Hyphantria* nests in July 2009 (Fig. 1). Of these, we detected *polH* in two by qPCR. In addition to soils, we investigated three nearshore sediment locations around the island. *polH* was detected by qPCR in all three sediment samples.

The wide distribution of NPV *polH* around Appledore Island suggests that the dispersal of lepidopteran viruses is not limited to habitats adjacent to their putative hosts. The three soil samples analyzed from 2009 were not immediately below chokecherry trees, which suggest that if the detected sequences are indeed *Hyphantria* sp. viruses, they may be moved around in soils by biological or physical means. During the sampling period from mid May through early July, Appledore Island experienced significant rainfall events (mean rainfall was 0.46 cm day^{-1} for the 56 days from May 15 through July 9, where rain occurred on

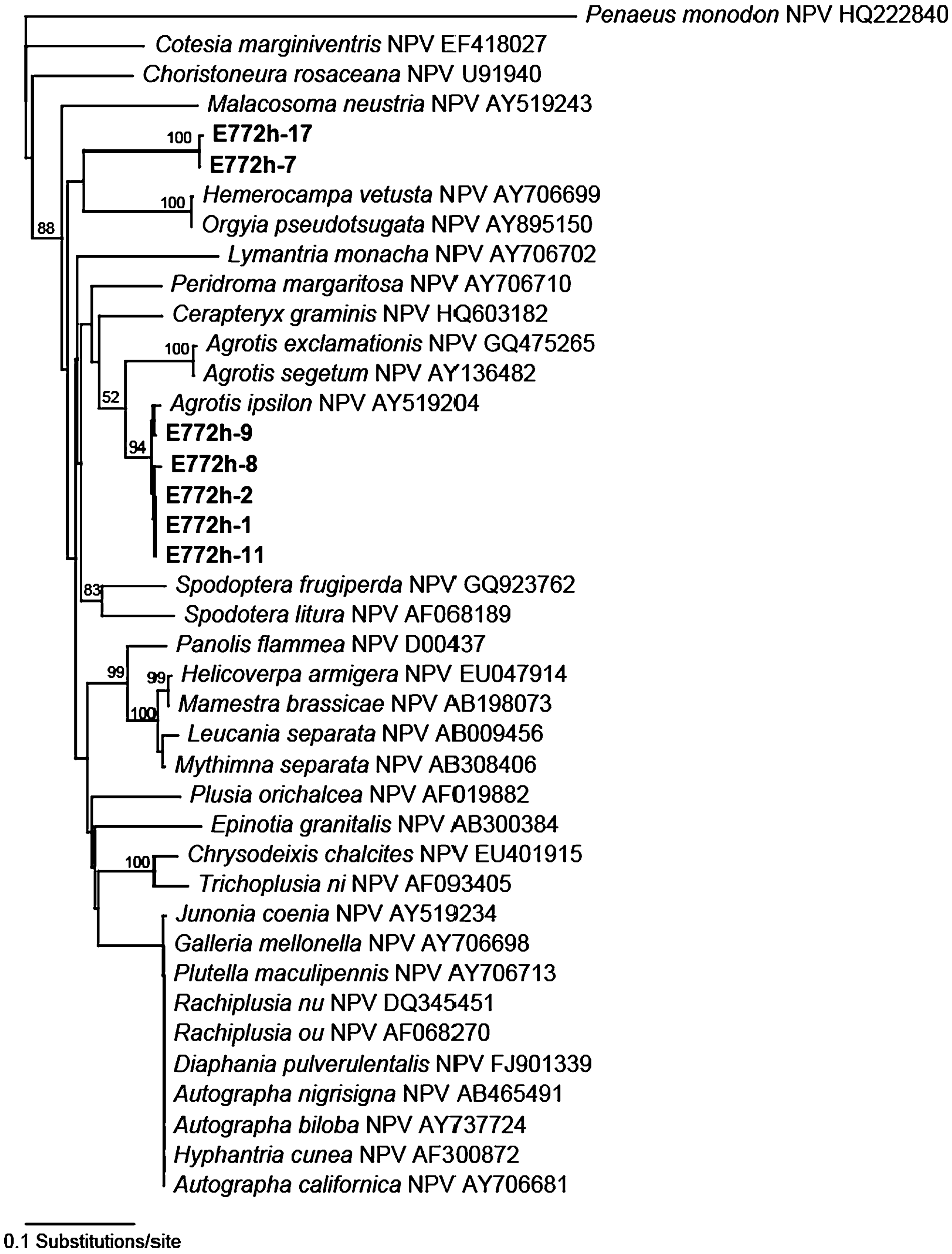


Figure 2 Phylogenetic representation of nucleopolyhedrovirus polyhedrin (*polH*) sequences derived from aquatic samples (*bold*) and representative *polH* in GenBank. The tree was generated upon the 294-bp nucleotide sequence alignment (ClustalX) and using the neighbor joining method. The tree is rooted to *Penaeus monodon* baculovirus *polH* which is highly divergent from other NPV *polH*. Bootstrap values based on 1,000 replicates are given at each node

29 days), which may have caused viruses to be washed from soil locations. The presence of NPV *polH* in sediments adjacent to Appledore Island further suggests that NPVs may be washed into the ocean and sink to sediments. Because NPV *polH* was detected in the EPS foam sample from adjacent Norwegian Cove by qPCR, these data may demonstrate that viruses may be in greater occurrence on the windward side of Appledore Island. Furthermore, the detection by both PCR and qPCR of NPV *polH* in seston of Pond 403, which is on a rock ledge adjacent to where *Hyphantria* sp. individuals were collected, and which receives freshwater inputs from that catchment, demonstrates that NPV may be transported from soils seaward through rainfall events. However, we cannot discount active transport of NPVs to the supratidal ponds, since Pond 403 is also visited by seagulls (*Larus* spp.) and by flying insects (*Trichocorixa* sp.) who may also transport viruses from terrestrial habitats. Similar transport by waterbirds of invertebrate taxa between habitats has been noted elsewhere [36].

Distribution of NPV *polH* in Marine Plankton

We detected the presence of *polH* in only one sample of net plankton (>64 µm) by conventional PCR. *polH* was detected by qPCR only in a small number of samples, notably within the 10–0.2-μm size fraction of plankton at two stations seaward of Appledore Island, and in Sagamore Creek. Other samples had limit-of-detection (i.e., DET) signals of *polH* in net plankton collected in Bab's Cove, Appledore Island, and at the two offshore stations in the Gulf of Maine.

We investigated the presence of NPV *polH* in plankton samples adjacent to Appledore Island initially to determine if NPVs similar to lepidopteran viruses were present in zooplankton populations, which could be due to either infection or consumption of infected particles. We speculated that NPVs may be consumed by zooplankton since they resemble larger bacteria (1–5 μm) [37]. However, our data suggest that NPVs in waters adjacent to Appledore Island are likely washed to sea rather than within zooplankton, since the highest abundance was measured in the 10–0.2-μm size fraction, and were less abundant in the >64-μm size fraction. The wide dispersal of NPVs to sea from Appledore suggests that they may be a suitable tracer of runoff into the coastal zone in lepidopteran-rich regions. Polyhedra are resistant to environmental degradation [25, 38], hence they may persist for longer periods of time than other commonly used tracers of allochthonous inputs.

Our data demonstrate that allochthonous viruses from non-anthropogenic sources may be transported to coastal marine ecosystems putatively by runoff. Previous studies have focused on primarily pathogenic taxa and their fate within seawater [16, 18, 39]. To our knowledge, this is the first report focusing on non-human or pathogenic allochthonous viruses in marine ecosystems. Several coastal metaviriomic sequencing efforts have indicated the presence of viruses matching most closely terrestrial insect viruses [40–42], however these observations may be the consequence of poor coverage of eukaryotic marine viral genomes. We compared the genomes of NPVs used in design of *polH* primers currently sequenced marine metaviriomes [42–47] at the Community Cyberinfrastructure for Advanced Microbial Ecology Research and Analysis (CAMERA) server (http://camera.calit2.net/) by BLASTn and tBLASTx algorithms. We found no matches at e-values <1 between these NPV and environmentally derived viral shotgun sequences from any environment. While we detected NPV *polH* in coastal habitats, these viruses do not comprise a large proportion of virioplankton communities, and are unlikely to be included in metaviriomic sequencing efforts.

The detection of allochthonous viruses of terrestrial, non-anthropogenic origin in coastal marine waters suggests that viral nucleotide sequences are transported across biome boundaries. Because viruses are mostly believed to be host-species specific [48], it is unlikely that allochthonous viruses would infect distantly related taxa in seawater, like marine arthropods. However, potential consumption of allochthonous NPVs by native marine zooplankton and/or transduction of the genes they bear into native microorganisms [49, 50] may permit NPV-borne genes to enter the marine hosts. While this may not be a significant source of genetic diversity in the short term, over evolutionary time scales this mechanism may permit genetic information transfer across habitat boundaries. We cannot discount the possibility that our qPCR primer/probe set matches a *polH* gene fragment that is not present in NPVs amongst uncultivated microorganisms. However, *polH* shares no similarity with genes recovered from bacterioplankton in metagenomic surveys, nor genomes of cultivated organisms when compared using CAMERA.

Conclusions

Our data demonstrate that NPV *polH* genes of putatively terrestrial origin are distributed widely across the Appledore Island landscape, from terrestrial soils to the surrounding marine environment. Because *polH* sequences recovered from aquatic habitats match closely *polH* from lepidopteran NPVs, and because *polH* genes were detected within *Hyphantria* sp. caterpillars which form dense tents on the dominant vegetation on Appledore Island, we posit that this organism is the source of detected NPV *polH*. NPV *polH* was also detected in terrestrial-affected coastal and offshore waters of the Gulf of Maine, suggesting that the degradation-resistant occlusion bodies of NPV may be markers for terrestrial runoff into coastal marine habitats, even if they comprise a very small proportion of total aquatic viruses. Our study raises interesting questions about the persistence and decay of terrestrial viruses in aquatic ecosystems, and the transfer of genomic information between habitats with barriers to dispersal.

Acknowledgments The authors thank W. Bemis and the staff of the Shoals Marine Lab, Appledore Island A. Hajek, N. Felice, N. Scalfone, J. Means, E. Madsen, and J. Casey for field assistance and helpful conversations on the topic. This work was supported by NSF Grants DEB-1028898 and OCE-1049670, New York State Grant NYS-189489, and the Cornell Biogeochemistry and Biocomplexity Initiative.

References

1. Smith KM, Rivers CF (1956) Some viruses affecting insects of economic importance. Parasitology 46:235
2. Hukuhara T, Akutsu K, Watanabe H (1866) Nuclear polyhedroses of several *Insecta*. Jpn J Appl Entomol Zool 10:181–184
3. Cox-Foster DL, Conlan S, Holmes EC, Palacios G, Evans JD, Moran NA, Quan PL, Briese T, Hornig M, Geiser DM, Martinson V, vanEngelsdorp D, Kalkstein AL, Drysdale A, Hui J, Zhai JH, Cui LW, Hutchison SK, Simons JF, Egholm M, Pettis JS, Lipkin WI (2007) A metagenomic survey of microbes in honey bee colony collapse disorder. Science 318:283–287

4. Palacios G, Hui J, Quan PL, Kalkstein A, Honkavuori KS, Bussetti AV, Conlan S, Evans J, Chen YP, vanEngelsdorp D, Efrat H, Pettis J, Cox-Foster D, Holmes EC, Briese T, Lipkin WI (2008) Genetic analysis of Israel acute paralysis virus: distinct clusters are circulating in the United States. J Virol 82:6209–6217
5. Blissard GW, Rohrmann GF (1990) Baculovirus diversity and molecular biology. In: Mittler TE (ed) Annual review of entomology, vol 35 X+641p. Annual Reviews Inc, Palo Alto, California, USA, Illus pp. 127–156
6. Schmidt L (1960) Investigations in 1959 on microorganisms pathogenic to the gipsy moth. I. The causes for the larval mortality of the moth in its different instars. Plant Prot 1959:45–51
7. Georgievska L, De Vries RSM, Gao PJ, Sun XL, Cory JS, Vlak JM, Van Der Werf W (2009) Transmission of wild-type and recombinant HaSNPV among larvae of *Helicoverpa armigera* (Lepidoptera: Noctuidae) on cotton. Environ Entomol 39:459–467
8. Gangnonngiw W, Laisutisan K, Sriurairatana S, Senapin S, Chuchird N, Limsuwan C, Chaivisuthangkura P, Flegel TW (2009) Monodon baculovirus (MBV) infects the freshwater prawn *Macrobrachium rosenbergii* cultivated in Thailand. Virus Res 148:24–30
9. Rohrmann GF (1986) Polyhedrin structure. J Gen Virol 67:1499–1513
10. Seufi AM (2008) Characterization of an Egyptian *Spodoptera littoralis* nucleopolyhedrovirus and a possible use of a highly conserved region from polyhedrin gene for nucleopolyhedrovirus detection. Virol J 5:13
11. Chaivisuthangkura P, Tawilert C, Tejangkura T, Rukpratanporn S, Longyant S, Sithigorngul W, Sithigorngul P (2008) Molecular isolation and characterization of a novel occlusion body protein gene from *Penaeus monodon* nucleopolyhedrovirus. Virology 381:261–267
12. Heldal M, Bratbak G (1991) Production and decay of viruses in aquatic environments. Mar Ecol Prog Ser 72:205–212
13. Noble RT, Fuhrman JA (1997) Virus decay and its causes in coastal waters. Appl Environ Microbiol 63:77–83
14. Hewson I, Fuhrman JA (2003) Viriobenthos production and virioplankton sorptive scavenging by suspended sediment particles in coastal and pelagic waters. Microb Ecol 46:337–347
15. Suttle CA (2000) Cyanophages and their role in the ecology of cyanobacteria. In: Whitton A, Potts M (eds) the ecology of cyanobacteria, vol 1. Kluwer, Amsterdam, pp 563–589
16. Abdelzaher AM, Wright ME, Ortega C, Solo-Gabriele HM, Miller G, Elmir S, Newman X, Shih P, Bonilla JA, Bonilla TD, Palmer CJ, Scott T, Lukasik J, Harwood VJ, McQuaig S, Sinigalliano C, Gidley M, Plano LRW, Zhu XF, Wang JD, Fleming LE (2010) Presence of pathogens and indicator microbes at a non-point source subtropical recreational marine beach. Appl Environ Microbiol 76:724–732
17. Griffith JF, Schiff KC, Lyon GS, Fuhrman JA (2010) Microbiological water quality at non-human influenced reference beaches in southern California during wet weather. Mar Poll Bull 60:500–508
18. Fuhrman JA, Liang XL, Noble RT (2005) Rapid detection of enteroviruses in small volumes of natural waters by real-time quantitative reverse transcriptase PCR. Appl Environ Microbiol 71:4523–4530
19. Skraber S, Schijven J, Italiaander R, Husman AMD (2009) Accumulation of enteric bacteriophage in fresh water sediments. J Water Health 7:372–379
20. Djikeng A, Kuzmickas R, Anderson NG, Spiro DJ (2009) Metagenomic analysis of RNA viruses in a fresh water lake. PLoS ONE 4:e7264
21. Stevenson PC, D'Cunha RF, Grzywacz D (2010) Inactivation of baculovirus by isoflavonoids on chickpea (*Cicer arietinum*) leaf surfaces reduces the efficacy of nucleopolyhedrovirus against *Helicoverpa armigera*. J Chem Ecol 36:227–235
22. Shapiro M (2002) Effects of anion and cation inhibitors and carbonic anhydrase inhibitors upon the activity of the gypsy moth (*Lepidoptera*: *Lymantriidae*) nucleo-polyhedrovirus. J Econ Entomol 95:237–242
23. Shapiro M, Farrar RR, Domek J, Javaid I (2002) Effects of virus concentration and ultraviolet irradiation on the activity of corn earworm and beet armyworm (*Lepidoptera*: *Noctuidae*) nucleopolyhedroviruses. J Econ Entomol 95:243–249
24. Peng F, Fuxa JR, Richter AR, Johnson SJ (1999) Effects of heat-sensitive agents, soil type, moisture, and leaf surface on persistence of *Anticarsia gemmatalis* (*Lepidoptera*: *Noctuidae*) nucleopolyhedrovirus. Environ Entomol 28:330–338
25. England LS, Pollok J, Vincent M, Kreutzweiser D, Fick W, Trevors JT, Holmes SB (2005) Persistence of extracellular baculoviral DNA in aquatic microcosms: extraction, purification, and amplification by the polymerase chain reaction (PCR). Mol Cell Probes 19:75–80
26. England LS, Trevors JT, Holmes SB (2001) Extraction and detection of baculoviral DNA from lake water, detritus and forest litter. J Appl Microbiol 90:630–636
27. Holmes SB, Fick WE, Kreutzweiser DP, Ebling PM, England LS, Trevors JT (2008) Persistence of naturally occurring and genetically modified *Choristoneura fumiferana* nucleopolyhedroviruses in outdoor aquatic microcosms. Pest Manag Sci 64:1015–1023
28. Becnel JJ, White SE, Shapiro AM (2003) *Culex nigripalpus* nucleopolyhedrovirus (CuniNPV) infections in adult mosquitoes and possible mechanisms for dispersal. J Invertebr Pathol 83:181–183
29. Rozen S, Skaletsky H (2000) Primer3 on the WWW for general users and for biologist programmers. In: Krawertz S, Misener S (eds) Bioinformatics methods and protocols. Humana Press, Totowa, pp 365–386
30. Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acid Res 25:3389–3402
31. Thompson J, Higgins D, Gibson T (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acid Res 22:4673–4680
32. Foster RA, Subramaniam A, Mahaffey C, Carpenter EJ, Capone DG, Zehr JP (2007) Influence of the Amazon River plume on distributions of free-living and symbiotic cyanobacteria in the western tropical north Atlantic Ocean. Limnol Oceanogr 52:517–532
33. Passow U (2002) Production of transparent exopolymer particles (TEP) by phyto- and bacterioplankton. Mar Ecol Prog Ser 236:1–12
34. Boughton AJ, Harrison RL, Lewis LC, Bonning BC (1999) Characterization of a nucleopolyhedrovirus from the black cutworm, *Agrotis ipsilon* (*Lepidoptera*: *Noctuidae*). J Invertebr Pathol 74:289–294
35. Webb RE, White GB, Thorpe KW (2003) Augmenting nucleopolyhedrovirus load in gypsy moth (*Lepidoptera*: *Lymantriidae*) populations with egg mass treatments. J Entomol Sci 38:300–313
36. Green AJ, Jenkins KM, Bell D, Morris PJ, Kingsford RT (2008) The potential role of waterbirds in dispersing invertebrates and plants in arid Australia. Freshw Biol 53:380–392
37. Bonami JR, Aubert H, Mari J, Poulos BT, Lightner DV (1997) The polyhedra of the occluded baculoviruses of marine decapod crustacea: a unique structure, crystal organization, and proposed model. J Struct Biol 120:134–145
38. Mehrvar A (2009) Persistence of different geographical isolates of *Helicoverpa armigera* nucleopolyhedrovirus in two types of soils under different conditions. J Biol Sci 9:264–267

39. Noble RT, Griffith JF, Blackwood AD, Fuhrman JA, Gregory JB, Hernandez X, Liang XL, Bera AA, Schiff K (2006) Multitiered approach using quantitative PCR to track sources of fecal pollution affecting Santa Monica Bay, California. Appl Environ Microbiol 72:1604–1612
40. Culley AI, Lang AS, Suttle CA (2006) Metagenomic analysis of coastal RNA virus communities. Science 312:1795–1798
41. Culley A, Lang AS, Suttle CA (2003) High diversity of unknown picorna-like viruses in the sea. Nature 424:1054–1057
42. Bench SR, Hanson TE, Williamson KE, Ghosh D, Radosovich M, Wang K, Wommack KE (2007) Metagenomic characterization of Chesapeake bay virioplankton. Appl Environ Microbiol 73:7629–7641
43. Angly F, Felts B, Breitbart M, Salamon P, Edwards R, Carlson C, Chan A, Haynes M, Kelley S, Liu H, Mahaffy J, Mueller J, Nulton J, Olson R, Parsons R, Rayhawk S, Suttle C, Rohwer F (2006) The marine viromes of four oceanic regions. PLoS Biol 4:2121–2131
44. Breitbart M, Felts B, Kelley S, Mahaffy JM, Nulton J, Salamon P, Rohwer F (2004) Diversity and population structure of a near-shore marine-sediment viral community. Proc Royal Soc London B Biol Sci 271:565–574
45. Breitbart M, Salamon P, Andresen B, Mahaffy J, Segall A, Mead D, Azam F, Rohwer F (2002) Genomic analysis of uncultured marine viral communities. Proc Nat Acad USA 99:14250–14255
46. Breitbart M, Wegley L, Leeds S, Schoenfeld T, Rohwer F (2004) Phage community dynamics in hot springs. Appl Environ Microbiol 70:1633–1640
47. Desnues C, Rodriguez-Brito B, Rayhawk S, Kelley S, Tran T, Haynes M, Liu H, Furlan M, Wegley L, Chau B, Ruan YJ, Hall D, Angly FE, Edwards RA, Li LL, Thurber RV, Reid RP, Siefert J, Souza V, Valentine DL, Swan BK, Breitbart M, Rohwer F (2008) Biodiversity and biogeography of phages in modern stromatolites and thrombolites. Nature 452:340–U345
48. Martin E, Benson R (1988) Phages of Cyanobacteria. In: Calendar R (ed) The bacteriophages, vol 2. Plenum, New York, pp 607–645
49. Chiura HX (1997) Generalized gene transfer by virus-like particles from marine bacteria. Aquat Microb Ecol 13:75–83
50. Jiang SC, Paul JH (1998) Gene transfer by transduction in the marine environment. Appl Environ Microbiol 64:2780–2787