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Do viruses affect fecundity and survival of the copepod *Acartia tonsa* Dana?

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Naturally occurring viruses are extremely abundant in aquatic systems, and they infect bacteria, cyanobacteria, prokaryotic and eukaryotic phytoplankton, heterotrophic nanoflagellates, fish and mammals. Viral infections of single-celled organisms have been studied intensively in the past decade, but little is known about the effects of viruses on aquatic metazoans, other than for some economically important species. Because zooplankton assemblages are often dominated in number and biomass by copepods, we used them as model organisms to study the effects of naturally occurring viruses on higher trophic levels. We attempted to induce viral infection in laboratory-reared cultures of the estuarine copepod Acartia tonsa Dana by exposing them to elevated concentrations of natural viruses in seawater. We found no negative effects of such exposure on copepod fecundity, larval survival or adult survival.

INTRODUCTION

It has been nearly 50 years since the first marine bacteriophage was discovered (Spencer, 1955), but marine viruses were little studied until the mid-1980s. Since then, there has been a proliferation of research on viruses, demonstrating that they are extremely abundant in water (e.g. Bergh *et al.*, 1989; Suttle *et al.*, 1990; Hara *et al.*, 1991) and that they infect bacteria (e.g. Proctor and Fuhrman, 1992; Weinbauer, 2004) as well as a wide variety of prokaryotic and eukaryotic marine phytoplankters (e.g. Mayer and Taylor, 1979; Suttle *et al.*, 1990; Brussaard *et al.*, 1996; Short and Suttle, 2002). Viruses significantly contribute to biogeochemical cycling (Fuhrman, 1999; Wilhelm and Suttle, 1999) and can partly regulate the species composition of bacterioplankton (Fuhrman and Schwalbach, 2003) and phytoplankton (Bratbak *et al.*, 1990; Nagasaki *et al.*, 1994; Short and Suttle, 2003).

Little is known about marine viruses and their effects on population dynamics of higher trophic levels; however, some host–virus interactions are well studied. Since the first discovery of a crustacean-infecting virus (Vago, 1966), research has focused mainly on economically important species, chiefly shrimps and crabs. Virus infection has been documented in crustacean fishery species (Couch, 1978; Johnson, 1983; Overstreet *et al.*, 1997), as well as in aquaculture organisms (Lightner *et al.*, 1983; Edgerton and Owens, 1997).

Viruses have been transmitted to uninfected crustaceans in the laboratory and in aquaculture facilities using a variety of methods, including immersing larvae in water containing viruses (Johnson, 1983), inoculating uninfected animals with purified virus (Overstreet *et al.*, 1997), inoculating uninfected animals with homogenized virus-infected tissue (Mari and Bonami, 1988), inoculating uninfected animals with virus-infected hemolymph (Johnson, 1983), feeding virus-containing material to uninfected animals (Couch, 1978) and feeding virus-infected tissue to brine shrimp and in turn feeding the brine shrimp to uninfected animals (Overstreet *et al.*, 1988).

Viruses have been identified in field-collected crabs (Pappalardo *et al.*, 1986) and shrimp (Couch, 1978), and the prevalence of virus infection in natural populations can be as high as 50–80% (Johnson, 1983). The mechanisms of virus transmission among natural populations of marine crustaceans are unknown, although Couch (Couch, 1978) suggested that transmission of *Baculovirus penaei* occurs by cannibalism of infected shrimp by healthy shrimp.

Viral lesions have been demonstrated in cultured rotifers (Comps *et al.*, 1991), barnacles (Leibovitz and Koulish, 1989) and daphniids (Federici and Hazard, 1975; Bergoin *et al.*, 1984) suggesting marine zooplankton may also be vulnerable to infection by viruses. Copepods dominate the marine meso-zooplankton in terms of biomass and number of organisms (Conover, 1956; Baird and Ulanowicz,

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1989), and can occupy different trophic levels in marine food webs in that they may feed on primary producers (White and Roman, 1992) as well as on protozooplankton (Kleppel, 1993). In turn, copepod eggs, nauplii, and adults are a major food source for carnivores such as larval fish and invertebrates, including predatory copepods. If virus-zooplankton relationships are of interest, then it seems well considered to study copepods as host organisms, given their prominent and multi-tiered position in marine food webs.

There is no reason to presume copepods are immune to viral infection, given that viruses infect other crustaceans. Furthermore, copepods are susceptible to infection by fungi (Redfield and Vincent, 1979), ciliates (Turner *et al.*, 1979; Weissman *et al.*, 1993), bacteria (Carman and Dobbs, 1997) and diatoms (Ho and Perkins, 1985; Carman and Dobbs, 1997). The dearth of information on copepod–virus interactions is arguably the result of a lack of investigation, rather than a lack of infection of copepods by viruses. In the first experiments performed in this context, therefore, we sought to test the hypothesis that marine copepods are vulnerable to infection by viruses. We attempted to determine effects of viruses by measuring two life-history parameters—mortality and egg production—of copepods exposed in the laboratory to elevated concentrations of naturally occurring viruses.

METHOD

Laboratory cultures of copepods and phytoplankton

Acartia tonsa Dana were collected from the mouth of Chesapeake Bay in July 1995 and kept continuously in culture. Copepods were reared in gently aerated 1- and 2-L polymethylpentene beakers filled with autoclaved seawater. Beakers were kept in an incubator set at 20 ± 0.5°C and illuminated on a 12:12 Light:dark cycle by cool white fluorescent light bulbs. Copepods were fed the phytoplankton *Isochrysis galbana* and *Rhodomonas salina* (clones ISO and 1319, respectively, from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton, West Boothbay Harbor, Maine). Phytoplankton concentrations in the cultures were not strictly maintained, but 2–3 times weekly, copepods were fed $\sim 4 \times 10^7$ cells L^{-1} and 2×10^7 cells L^{-1} of *R. salina* and *I. galbana*, respectively. All experiment-related feedings (see below) were administered at approximately these concentrations (Drake, 1997). Cells were counted with an Improved Neubauer Hemacytometer. Phytoplankton were grown in F/2 media (minus silica) (Guillard and Ryther, 1962) in seawater (salinity 20–25) and maintained in the incubator.

Concentration of natural virus assemblage

Seawater was prefiltered (vacuum 60 mmHg) serially through (i) a glass fiber filter (Whatman GF/F or Gelman A/E) and (ii) a cellulose acetate or polyvinylidene difluoride filter into an acid-washed carboy on ice. For egg-production, egg-hatching and nauplii-survival experiments, as well as for preparing virus-like particle (VLP) concentrate for examination by electron microscopy, cellulose acetate filters with a pore size of 0.22 μm were used (Micron Separations, Inc.); for the serial-inoculation experiment, polyvinylidene difluoride filters with a pore size of 0.45 μm were used (Millipore Corporation). Twenty liters of prefiltered seawater was reduced to a volume of 100-200 mL using a MEMBREX Benchmark7 Gx Vortex Flow Filtration System with a 100 kDa filter. A 30-kDa filter was used for the first iteration of the serial-inoculation experiment. The fraction of seawater that passed through the 0.22-μm or 0.45-μm–pore size filter and was retained by the 100- or 30-kDa filter was considered the VLP concentrate. Filters with cut-offs of 100 kDa and 30 kDa retain particles with spherical diameters of ~0.01 μm and 0.003 μm, respectively (B. Herman, MEMBREX, Inc., personal communication). VLP concentrate was stored in the dark at room temperature until it was added to experimental beakers or microfuge tubes, which was within one hour of concentration.

Virus enumeration and confirmation

Virus-like particles (VLPs) were counted using the nucleic-acid stain YO-PRO (Hennes and Suttle, 1995) with an Olympus BX50 System Microscope with a BX-FLA epifluorescence attachment. To verify that the VLP concentrate contained viruses, concentrate was examined using a JEOL 100CX II transmission electron microscope. Each of three formvar-coated copper mesh grids was floated, formvar side down, on a drop of glutaraldehyde-fixed viral concentrate for 60 min, then rinsed 3 times with deionized water. Grids were stained for 10 s with uranyl acetate, then examined at an accelerating voltage of 80 000 kV and a magnification of 10 000–140 000.

Egg-production experiments

Three experiments were performed to determine the effects, if any, of virus concentrate on egg production (experiments 1, 2 and 5 in Table I; parameters measured in experiment 6 are discussed below under 'Serial-inoculation experiment'). Seawater used in the experiments was collected during the spring *A. tonsa* bloom, presumably when viruses infecting *A. tonsa* would be most abundant. In each experiment, 20 copepod eggs or first-stage nauplii were removed from cultures and placed in 1-L beakers filled with sterile seawater (control treatments; $n = 2$ or 3) or with sterile

Table I: Details of virus experiments with Acartia tonsa eggs, nauplii, copepodites and adults

Experiment number	Parameters measured	Location of water collection (date)	Water temperature (°C) (salinity)	VLP density in water before VFF (x 10^{10} L^{-1})	VLP density in experimental treatment (×10^{10} L^{-1})
1	Egg production	Lafayette River (25 April 1996)	18.0 (16.0)	Not determined	64.1
2	Egg production	Lafayette River (26 April 1996)	18.0 (14.0)	Not determined	69.5
3	Egg hatching	Chesapeake Bay mouth (7 May 1996)	15.0 (22.0)	10.2	657
4	Egg hatching	Chesapeake Bay mouth (8 May 1996)	15.0 (19.0)	14.2	712
	Nauplii survival				
5	Egg production	Chesapeake Bay mouth (31 May 1996)	17.8 (17.3)	5.0	12.9
	Egg hatching				359
	Nauplii survival				359
6	Egg production	Ocean View Beach, Chesapeake Bay (27 August 1997)	22.0 (25.0)	19.1	25.4
	Egg hatching				25.4
	Adult and copepodite survival				autoclaved sea water
7	Adult and copepodite survival	Chesapeake Bay mouth[a] (22 October 1995)	19.6 (25.0)	Not determined	0.07
8	Adult and copepodite survival	Chesapeake Bay mouth[a] (26 October 1995)	20.0 (28.0)	Not determined	0.03

VFF, vortex flow filtration, used to concentrate the virus-size fraction of seawater; VLP, virus-like particle.
[a]Location of copepod collection.

seawater plus VLP concentrate (virus treatments; $n = 2$ or 3; volume of concentrate per beaker = 36–57 mL to reach a final VLP concentration of 1.3–7.0 × 10^{11} L^{-1}). Sterile seawater was prepared by either autoclaving or microwaving (Sanborn *et al.*, 1982; Keller *et al.*, 1988). Salinity in the beakers was adjusted to 20 with autoclaved, deionized, distilled water and phytoplankton cells were added. In this and all other experiments, beakers were arranged randomly on a shelf in the incubator. Water was changed twice before copepods reached adulthood, each time replacing the water in all beakers with autoclaved seawater and new algae. Copepods in experimental beakers were exposed to virus concentrate from the initiation of the experiment to the time of the first water change, which was 4–7 days.

To measure egg production, one to three adult females were removed from each beaker and placed in an acrylic sleeve having a 202 μm-mesh bottom. The sleeve was immersed in a 250 mL polymethylpentene beaker filled with autoclaved seawater and *R. salina* (2 or 3 beakers per control and virus treatments). Egg production was monitored over 12-h periods (additional details in Drake, 1997).

Egg-hatching and nauplii-survival experiments

Experiments were performed to quantify the hatching and survival of eggs (experiments 3, 4 and 5) and survival of nauplii (experiments 4 and 5) in the presence of VLP concentrate (Table I). In each experiment, 10 copepod eggs or 5 first-stage nauplii were put in a sterile microcentrifuge tube with 50 μL of autoclaved seawater. To the control tubes (n = 3 or 4), 950 μL of autoclaved seawater or 950 μL of microwaved seawater or microwaved VLP concentrate was added. To experimental tubes (n = 3 or 4), 950 μL of VLP concentrate was added to reach a VLP concentration of 3.6–7.1 × 10^{12} L^{-1}. The salinity in the experimental tubes was not adjusted. Tubes were incubated for 36 h in the dark at 20°C, and then eggs or nauplii were checked for hatching or survival. Eggs that did not hatch during that time were monitored for an additional 48 h; eggs that did not hatch after a total of 84 h were assumed to be nonviable. Unaccounted for eggs and nauplii were excluded from calculations of hatching success and nauplii survival; 6 eggs and 3 nauplii were

unaccounted for out of totals of 280 and 100, respectively, used in all hatching and survival experiments.

Serial-inoculation experiment

In an attempt to build the titer of one or more viruses infecting *A. tonsa,* the virus-size fraction of seawater from laboratory copepod cultures was repeatedly concentrated and added to 400–600 copepod eggs, which ranged in age from 1 to 26.5 h old (experiment 6 in Table I). Initially, 20 L of seawater was collected from the Chesapeake Bay, and the virus-size fraction was concentrated (as described above). To each of three 1-L beakers, the following were added: 953 mL of autoclaved seawater, copepod eggs, phytoplankton and 47 mL of VLP concentrate to achieve a VLP concentration of 2.5×10^{11} L^{-1}. After seven days, adult copepods and copepodites were removed, stained with Rose Bengal (1:160 040 dilution w/v) and counted to determine their percent survival. The virus-size fraction of the three beakers' contents was then concentrated and 54 mL of VLP concentrate was added to each of three 1-L beakers containing fresh copepod eggs, autoclaved seawater and phytoplankton. This cycle of concentrating copepod-culture water and subsequently adding VLP concentrate (48–55 mL) to fresh copepod eggs from laboratory cultures was repeated five times, for a total of six iterations, with six or seven days between iterations. Initial VLP concentrations in the experimental beakers ranged from 4.6×10^{10} L^{-1} to 1.4×10^{11} L^{-1}. For the sixth iteration, a control was added. To each of three beakers in the control, chilled, autoclaved seawater (salinity = 20) was added *in lieu* of VLP concentrate. Water was changed in all beakers on day 7 and phytoplankton was added on days 7 and 11. On day 14, 10 adult female copepods were removed from each beaker and egg production was monitored over 12 h (see above). Remaining adult copepods and copepodites were stained with Rose Bengal and counted.

Other attempts to concentrate viruses that infect A. tonsa: pulverized copepods and copepod exudates

Approximately 1000 field-collected *A. tonsa*, primarily adults, were rinsed in autoclaved seawater, pulverized with a mortar and pestle and mixed with 500 mL of autoclaved seawater (experiment 7 in Table I). The mixture was kept in the dark at 20°C for 4 h, and then centrifuged for 16 min at ~745 × *g*. The supernatant, which had a VLP concentration of 7.0×10^{8} L^{-1}, was used immediately as the virus inoculum. Three hundred nauplii (0–33.3 h old) were removed from laboratory cultures and evenly divided among six 250-mL beakers containing either 250 mL of autoclaved seawater (control treatments; $n = 3$; 50 nauplii per beaker) or 210 mL of autoclaved seawater plus 40 mL of the virus inoculum (virus treatments; $n = 3$; 50 nauplii per beaker). Phytoplankton cells were added to each beaker. Water was changed once; water in all beakers was replaced with autoclaved seawater and fresh algae. After 15 days, the number of live adults and copepodites in each beaker was counted.

To determine whether copepod exudates include viruses that negatively affect copepod survival, field-collected *A. tonsa* were rinsed with autoclaved seawater and placed in a beaker with *R. salina* and autoclaved seawater (experiment 8 in Table I). The density of copepods was ~400 copepods L^{-1}. The beaker was kept on a laboratory bench. Twenty-four hours later, material in the beaker that passed through a 53 μm-mesh screen was used as the virus inoculum, which had a VLP concentration of 3.0×10^{8} L^{-1}; this mesh size excluded copepods but included fecal pellets. A total of 20 male and female adults and copepodites was removed from laboratory cultures and placed in each of 6 1-L beakers filled with either autoclaved seawater (control treatments; $n = 3$) or autoclaved seawater plus 250 mL of the virus inoculum (virus treatments; $n = 3$). Phytoplankton cells were added to each beaker. On day 4 of the experiment, *R. salina* was added to each beaker. On day 8 of the experiment, the number of live adults and copepodites in each beaker was counted.

RESULTS

Confirmation of viral concentration procedure

Examination of the VLP concentrate using transmission electron microscopy confirmed that it contained viruses. Many had angular heads with attached tails (data not shown). Head diameters ranged from 43 to 64 nm (data not shown).

Egg-production experiments

There was no significant difference in egg production between control and virus treatments in any of the three experiments (Fig. 1) ($P = 0.956$, *t*-test, SPSS version 10.1.0, experiment 1; $P = 0.917$, *t*-test, experiment 2; $P = 0.563$, one-way ANOVA, experiment 5).

Egg-hatching and nauplii-survival experiments

In two of the egg-hatching experiments (experiments 3 and 4), 100% of the eggs in both control and virus treatments hatched (Table II); given these results, no statistical analyses were performed. In the other trial (experiment 5), 93% of the eggs in the control treatment with autoclaved seawater hatched, and 100% in all other treatments hatched (Table II), results indicating no significant difference among treatment groups ($P = 0.053$, one-way ANOVA).

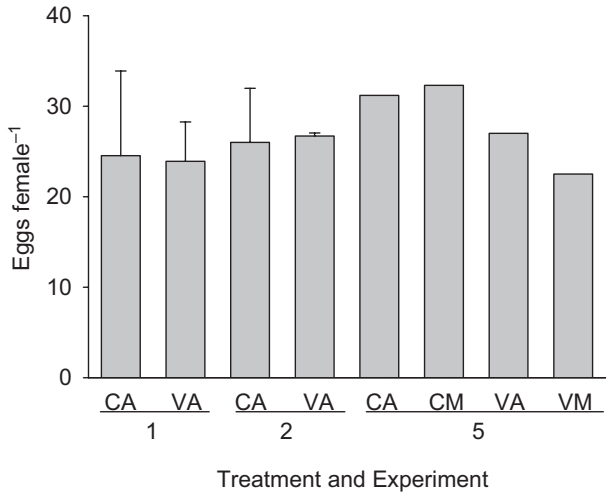

Fig. 1. Measurements of egg production in experiments 1, 2 and 5. Egg production was monitored over one nighttime 12-h period with one to three females in each replicate. Data are mean values ($n = 3$ replicates) ± 1 SE for experiments 1 and 2; $n = 2$ replicates for experiment 5. CA, control treatment of autoclaved seawater; CM, control treatment of microwaved seawater; VA, virus concentrate added to autoclaved seawater; VM, virus concentrate added to microwaved seawater.

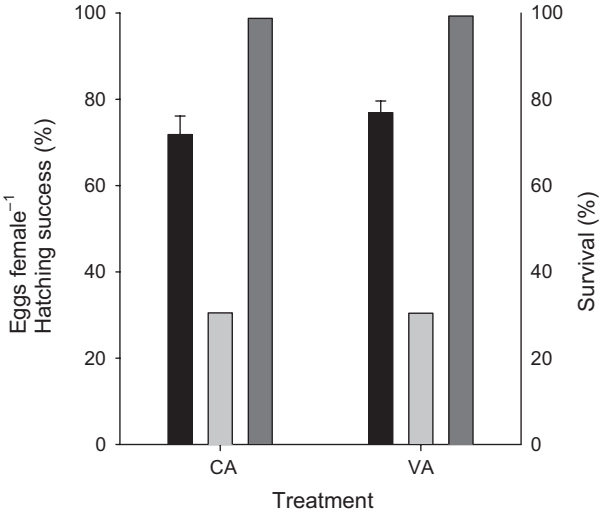

Fig. 2. Percent survival, egg production and egg hatching success in iteration six of the serial inoculation (experiment 6). Black bars represent percent survival of adults and copepodites; light-gray bars represent egg production; dark-gray bars represent hatching success. Percent survival of all copepods was measured 14 days after the beginning of the iteration. Data are mean values ($n = 3$ replicates) ± 1 SE. Egg production per female (10 females per replicate) was measured over one nighttime 12-h period. Twenty-five hours later, hatching success of the eggs was measured. Data are mean values ($n = 2$ replicates for control treatment; $n = 3$ replicates for virus treatment). CA, control treatment of autoclaved seawater; VA, virus concentrate added to autoclaved seawater.

In the first nauplii-survival experiment (experiment 4), survival was 100% in both the control and virus treatments (Table II). In the second run (experiment 5), survival was lower in the virus treatment with autoclaved seawater (93%) than all other treatments (100%; Table II). Again, there was no statistically significant difference among treatments in either experiment ($P = 0.441$, one-way ANOVA).

Serial-inoculation experiment

There was no significant inter-cycle difference in copepod survival during the first five iterations of experiment 6 (mean survival of adults and copepodites = 84–91%; coefficient of variation (CV) = 2–9%; $n = 3$; $P = 0.495$; one-way ANOVA, data not shown). Over time, VLP and bacteria abundances in the copepod water varied inversely with one another such that VLP concentrations decreased from 25 to 6×10^7 L^{-1} and bacteria concentrations increased from 3 to 18×10^6 L^{-1} (data not shown). Lastly, there was no difference between the control and virus treatment groups of the experiment's sixth iteration with respect to adult and copepodite survival ($P = 0.365$, *t*-test), egg production ($P = 0.994$, *t*-test) or egg hatching ($P = 0.441$, *t*-test) (Fig. 2).

Other attempts to concentrate viruses that infect A. tonsa

There was no difference in mean survival between control and virus treatments when pulverized copepods were used as virus inoculum (experiment 7) (control mean = 54%

Table II: Egg-hatching and nauplii-survival experiments

Experiment number	Parameters measured	Mean percentage of CA (*n*)	Mean percentage of CM (*n*)	Mean percentage of VA (*n*)	Mean percentage of VM (*n*)
3	Egg hatching	100 (4)	Treatment not performed	100 (4)	Treatment not performed
4	Egg hatching	100 (4)	Treatment not performed	100 (4)	Treatment not performed
	Nauplii survival	100 (4)	Treatment not performed	100 (4)	Treatment not performed
5	Egg hatching	93 (3)	100 (3)	100 (3)	100 (3)
	Nauplii survival	100 (3)	100 (3)	93 (3)	100 (3)

CA, control treatment of autoclaved seawater; CM, control treatment of microwaved seawater; n, number of replicates; VA, virus concentrate added to autoclaved seawater; VM, virus concentrate added to microwaved seawater. For egg-hatching experiments, 10 eggs were monitored in each replicate; for nauplii-survival experiments, 5 nauplii were monitored in each replicate.

survival; virus mean = 43% survival; $P = 0.341$, *t*-test, data not shown). Similarly, there was no difference in survival between control and virus treatments when exudates of copepods were used as virus inoculum (experiment 8). There were means of 176 (control) and 183 (virus treatment) copepodites and adults present at the end of the experiment ($P = 0.924$, *t*-test, data not shown).

DISCUSSION

These experiments demonstrated no support for the hypothesis that viruses infect and detrimentally affect the fecundity or larval longevity of *A. tonsa*. There are at least three ways to interpret these results. First, viruses specific for *A. tonsa* were not present in the concentrated seawater and infection was therefore not possible. While VLPs were present in high numbers in the viral concentrate, and transmission-electron microscopy confirmed at least some of these VLPs exhibited morphologies of viruses known to infect other organisms, they did not infect this species of copepod.

Second, viruses pathogenic to *A. tonsa* were present in the seawater concentrate but did not infect the copepods, their eggs or their larvae for one or more reasons. For example, other investigators have noted decreased infectivity of bacteria or phytoplankton following concentration of viruses from seawater (L. Proctor, personal communication, C. Brussaard, personal communication). Another reason viruses may have failed to infect is related to their low rates of host encounter. Wommack *et al.* (Wommack *et al.*, 1992) hypothesized that most viruses in seawater infect the most numerable organisms, the bacterioplankton. This hypothesis is supported by the size distribution and morphology of free viruses in seawater (Bergh *et al.*, 1989; Proctor and Fuhrman, 1990; Wommack *et al.*, 1992; Cochlan *et al.*, 1993). If the viruses concentrated from seawater contained mostly bacteriophage and few viruses infecting *A. tonsa*, then encounter rates of the latter with copepod eggs and larvae may have been further decreased through inactivation by adhering to particles such as plankton, bacteria or microaggregates (Mitchell and Jannasch, 1969; Bitton and Mitchell, 1974; Noble and Fuhrman, 1997). Note, however, that viruses can be transmitted via water under the artificial conditions of aquaculture facilities (Johnson, 1983; Overstreet *et al.*, 1997). Furthermore, despite virus-host encounter arguments against infection of large organisms, viruses infect animals 10–1000 times larger than copepods, including fish (Fryer and Bartholomew, 1996) and dolphins (Van Bressem *et al.*, 1994).

The third interpretation considers that viruses pathogenic to *A. tonsa* were present and indeed did infect the copepods, but the infection was not evident. Animal viruses can affect host cells in four ways—via latent, lytic or persistent infection and transformation of cells to tumor cells (Madigan *et al.*, 2003). In this case, the simplest explanation is viral infection was latent, i.e. viruses were present in host cells but did not negatively affect host metabolism. A lytic virus is adsorbed by the host, initiates host cell production of virus copies and then lyses the host cell, thereby releasing virus progeny. Lytic infection sometimes can proceed from latent infection. Persistent infections result in a continual release of viruses without killing the host cell. Lastly, tumor-inducing viruses cause uncontrolled cell growth. In a more complicated explanation, copepod eggs and larvae in these experiments may have been infected with a lytic, persistent or tumorous virus—or even all three—but infection did not manifest in mortality or diminished fecundity evident on the time scales measured. In light of these experiments' so-called 'negative results,' direct tests of this and the other scenarios above will require genetic probes specific for viruses infecting *A. tonsa*, molecular tools not yet able to be designed, as no such viruses have been isolated and sequenced.

We chose *A. tonsa* as a model metazoan because it is a prominent member of the zooplankton in Chesapeake Bay, and, by its numerical dominance, the copepod most likely to interact with viruses. By conducting experiments with replicate treatments, by repeating experiments, and by using a multiplicity of approaches to induce viral infection, we rigorously challenged *A. tonsa* with virus-laden water. We chose to run the experiments in warm seasons, rather than throughout the year, because increased summer temperatures yield higher rates of copepod reproduction (hence potential hosts). We stress these experiments represent a first approach to determine the effects of viruses on zooplankton; future research efforts should consider other species, including those not cultured, and different geographic regions.

We acknowledge these negative results are not as exciting as evidence of direct infection would be. Nonetheless, we contend they are useful—negative evidence that runs counter to an original hypothesis usually lends support to an alternative hypothesis (Hull, 1999). In this instance, an alternative hypothesis is that marine viruses infect small hosts (e.g. bacteria) in greater frequency than they infect larger hosts. These results are consistent with the paradigm that effects of viruses on marine organisms decrease as trophic level increases. That is, an estimated 10–50% of marine heterotrophic bacteria are lysed daily by viruses, while at the next trophic level, it is likely that 2–15% of primary production under nonbloom conditions is removed by viral lysis (Fuhrman, 1999). The decrease between trophic levels is roughly 4-fold. An extrapolation of that decrease to the next trophic level would yield

0.5–3.8% of copepod production lost to viral lysis. Perhaps virus effects on secondary producers are nonexistent, episodic or below the level of detection.

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