

Virus predation by sponges is a new nutrient-flow pathway in coral reef food webs

Abstract—The removal efficiency of viral particles by the coral reef sponge *Negombata magnifica* was measured. Virus particles were removed by the sponge at an average efficiency of $23.3\% \pm 2.9\%$. Significant amounts of nutrients are transported from virus particles to higher trophic levels via sponges.

Productivity in coral reefs is higher than would be expected from the limited nutrients available in its ambient oligotrophic environment. Basic to the understanding of this exceptional productivity is an analysis of the coral reef food web. One of the mechanisms proposed to explain this greater productivity is the high efficiency of nutrient recycling (Erez 1990).

One well-known nutrient recycling process in the sea is that of the microbial loop (Azam et al. 1983). Marine viruses were ignored in regard to having any significant role in the microbial food web until two decades ago, when their remarkable abundance in marine ecosystems was revealed (Bergh et al. 1989).

The discovery of these large virus communities instantly affected the accepted convention of the microbial loop (Fuhrman 1999; Wommack and Colwell 2000) because most free virus particles are pathogens of bacteria and small unicellular eukaryotes (Brussaard 2004). By infecting the latter organisms, viruses cause their lysis, and thus affect plankton community size and diversity. As a consequence of their activity, viruses enhance the transfer of nutrients from the particulate organic matter (POM) pool to the dissolved organic matter (DOM) pool (Wilhelm and Suttle 1999).

In addition to influencing marine food webs by causing lysis of cells, the possibility of direct energy and nutrients transfer from virus particles to higher trophic levels, by predation, has been overlooked. Although it was suggested by Gonzalez and Suttle (1993) that low-rate grazing activity of heterotrophic nanoflagellates (Protozoa) might exist, there has been no direct evidence for substantial viral removal by any organism.

Marine sponges constitute potentially good candidates for viral removal, as they are the only known filter feeders able to capture particles as small as bacteria at high efficiencies, as well as some DOM (Yahel et al. 2003). The aim of the present research was to test the hypothesis that sponges capture virus particles from the water as part of their diet. This was carried out by measuring the virus-removal efficiency by the sponge and estimating the potential amounts of carbon and nitrogen that are transferred to the sponge from this food source.

The Red Sea sponge *Negombata magnifica* was used as a model organism to measure the rate of viroplankton removal. All experiments were conducted in the laboratory at the Inter University Institute (IUI) in Elat, Israel. Fresh

seawater was pumped directly from the coral reef off the IUI to the laboratory to supply the sponges with a continuous water exchange.

To evaluate the removal efficiency (RE) of virus particles by sponges, we simultaneously sampled the water flowing into the sponge and flowing out of the sponge oscule (exhalant opening), based on previously described in situ methods (Reiswig 1971; Yahel et al. 2003). Seven sponges were mounted individually, each in a separate 4-liter glass aquarium. Each aquarium was supplied with fresh seawater at a rate of 1.5 L min^{-1} . Two capillary tubes (1 mm \varnothing) were directed toward the sponge using a micromanipulator. To sample outflow water, the end of the first tube was inserted a few millimeters into an osculum, with no physical contact with the sponge mass. To sample inflow water, the end of the second tube was placed about 1 cm away from the sampled osculum at a distance of about 5 mm above the sponge surface. The distal end of each tube was inserted into a separate collection vessel. From each vessel, we collected an aliquot of 1.5 mL seawater that was then mixed with glutaraldehyde (G-5882; Sigma) to a final concentration of 0.1% (v/v), frozen in liquid nitrogen, and kept at -80°C until further analysis. For each sponge, we calculated the RE by using its inflow/outflow pair in the following equation:

$$\text{RE} = 100(1 - C_2 \times C_1^{-1}) \quad (1)$$

where C_1 and C_2 are the virus particles concentration (mL^{-1}) in inflow and outflow water, respectively.

Viral concentrations were determined by flow cytometry following the protocol described in Marie et al. (1999). For each sample, a fivefold dilution was performed into TE buffer (Tris 10 mmol L^{-1} , pH 8.0, EDTA 5 mmol L^{-1}) and stained with 1/20,000 of SYBR Green-I (Molecular Probes). Samples were incubated for 15 min in the dark and analyzed using a FACSort flow cytometer (Becton Dickinson) equipped with a 488 nm-wavelength excitation and the standard filters setup. Data were recorded as listmode files and processed using CytoWin (http://www.sb-roscoff.fr/Phyto/index.php?option=com_content&task=view&id=72&Itemid=123, D. Vaulot).

Flow cytometry is a useful tool for the identification and counting of the smallest living particles in the sea that are part of the natural diet of sponges (Pile et al. 1996). One population of virus particles could be observed by flow cytometry after staining by SYBR Green-I (Fig. 1). All tested sponges removed virus particles from the water at an average efficiency of $23.3\% \pm 2.9\%$ ($n = 8$). Ambient virus abundance in the study site was found to be $870,000 \pm 60,000$ virus particles mL^{-1} .

The oxygen consumption and the filtration rate of 1 g wet weight (WW) *N. magnifica* were estimated as 37.3 ± 4.6 (standard error) $\text{nmol O}_2 \text{ min}^{-1}$ and $10.8 \pm 1 \text{ mL min}^{-1}$

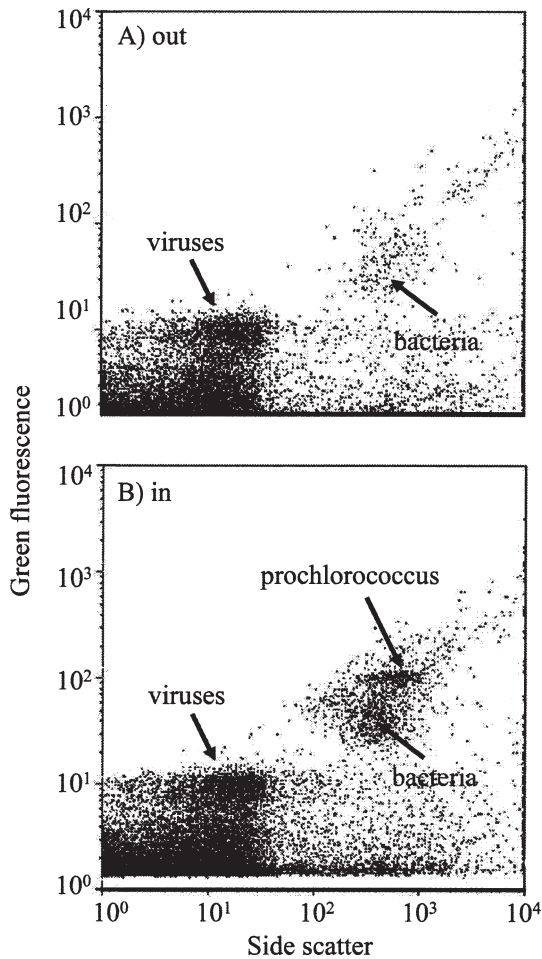


Fig. 1. Flow cytometric dot plots of side scatter versus green fluorescence for water samples collected for the sponge *N. magnifica* from the Red Sea, obtained after staining using SYBR Green-I. (A) The water flowing out from the sponge and (B) the water flowing in to it.

(Hadas et al., unpubl. data), both in the range found for other tropical marine sponges (Reiswig 1974; Yahel et al. 2003). Accordingly, the virus-removal capacity of a 1 g (WW) sponge is about 3.1×10^9 virus particles day^{-1} . Assuming an amount of 0.2 fg organic carbon per virus particle (Wilhelm and Suttle 1999) and a C : N ratio of not more than three (half of the virus biomass is the encapsulating protein), the amount of organic carbon and nitrogen transferred from the virus particles to the *N. magnifica* would be about $0.63 \mu\text{g C day}^{-1} \text{g (WW) sponge}^{-1}$ and $0.21 \mu\text{g N day}^{-1} \text{g (WW) sponge}^{-1}$.

These calculations show that viruses can potentially cover only a small fraction of the carbon required for a sponge's respiration ($550 \mu\text{g C day}^{-1} \text{g [WW] sponge}^{-1}$, using a conversion factor of 0.46 mg C per 1 mL O_2 respired [Thomassen and Riisgard 1995]). Although the viral contribution to a single sponge diet is probably low, the ubiquity of these organisms and their significant contribution to the benthic biomass in some tropical areas (Reiswig 1974; Barnes and Bell 2002) might result in a substantial total flow of nutrients from viroplankton to

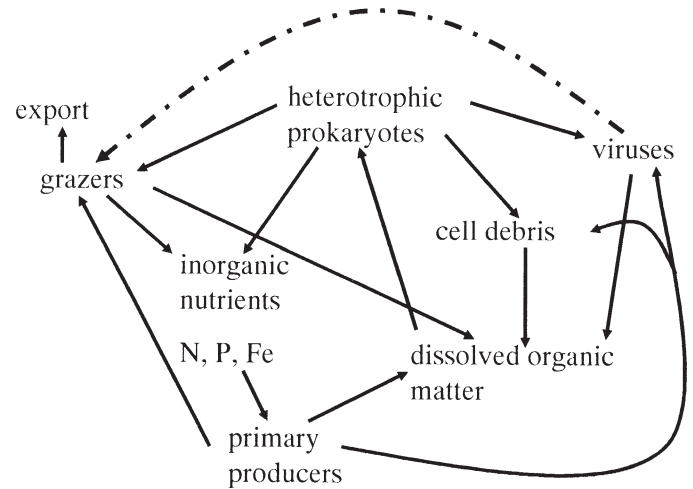


Fig. 2. Schematic diagram of a marine food web, adapted with permission from Fuhrman (1999). Solid arrows show the known nutrient flow in the marine food web; the dashed arrow is the new path, hypothesized from the results of the present research.

sponges, thus constituting a new energy flow pathway in the oligotrophic environment of the coral reefs (Fig. 2).

We hypothesize that this newly discovered nutrient pathway is not restricted only to sponges and that the full extent of the pathway is yet to be revealed. The ability to capture and ingest virus particles probably also exists in other marine organisms, especially in homologous taxa, such as choanoflagellates. These Protists are abundant in many marine environments (Fenchel 1982; Vors et al. 1995) and, because of the similarities between their filtration systems and that of the sponge (Maldonado 2004), they too might capture virus particles, thus greatly extending the significance of nutrient flow from viruses to higher trophic levels in the marine environment.

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Acknowledgments

This research was supported in part by the Rieger Foundation, the Israel Ministry of Energy and Infrastructure, and a research grant from the US-Israel BARD Foundation (8708-04). We thank the director and staff of the Inter University Institute in Elat, who enabled the performance of this research, and two anonymous reviewers for their valuable comments.

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Received: 12 April 2005

Accepted: 26 September 2005

Amended: 2 December 2005