SHORT COMMUNICATION

Vertical distribution of virus-like particles (VLP) and viruses infecting *Micromonas pusilla* during late summer in the southeastern Skagerrak, North Atlantic

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Abstract. Vertical profiles were made at one offshore station and one coastal station, on 4-5 September 1996, in the south-eastern Skagerrak. The surface water of the two stations differed significantly with respect to both temperature and salinity, as the outer station (A) was situated in high-saline water originating from the North Sea, while the low-saline surface water at the inner station (B) was influenced by the Baltic current. Virus-like particle (VLP) abundance was $5 \times 10^9 - 25 \times 10^9$ in the 0-50 m water column. Maximal VLP values were found in the surface water, although a lower number was detected in the low-saline surface water (0 m depth) at station B. Viruses infective to *Micromonas pusilla* were estimated to ~0.01% of the VLP number. The ambient concentrations of dissolved inorganic nutrients were typical for a stratified summer situation, i.e. generally low in the surface waters, although a raised ammonium concentration was associated with the sharp halocline at 5 m depth at station B, and all nutrient levels were increasing below 30 m depth.

Several investigations in recent years have found the abundance of marine viruses in the sea to be as high or higher than that of marine bacteria (e.g. Noble and Fuhrman, 1997). Experimental studies have shown that marine viruses may be an important factor regulating the bacterial and primary production rate in the sea (Suttle *et al.*, 1990; Suttle, 1992). Viruses lytic to microalgae may also be important factors in influencing the plankton successions and development of algal blooms (Peduzzi and Weinbauer, 1993; Bratbak *et al.*, 1995).

The prasinophyte *Micromonas pusilla* is one of a few microalgae against which lytic viruses have been observed and isolated (Mayer and Taylor, 1979; Cottrell and Suttle, 1991, 1995; Sahlsten, 1998). There is not much known about the vertical distribution of algal virus particles, or the infectivity of the viruses throughout the water column in the sea, and nothing has been known until now from Swedish coastal waters. Experimental studies of virus decay rates have shown, however, that the infectivity of viruses is destroyed faster in sunlight, particularly UV radiation, than in the dark, so one would expect most viruses in surface waters to be non-infective (Suttle and Chen, 1992).

The aim of the present investigation was to estimate the vertical distribution of marine viruses, i.e. virus-like particles (VLP), and to relate this to hydrographical data and composition and biomass distribution of the phytoplankton community during late summer in the southern Skagerrak. The work was specifically focused on the distribution of lytic microalgal viruses, which were infectious to the phytoflagellate *M. pusilla*.
Vertical profiles were made at two stations: one offshore station, A (N58°15'00", E10°50'00"), on 4 September, and one coastal station, B (N58°15'00", E11°13'00"), on 5 September 1996, in the south-eastern Skagerrak (Figure 1) using the R/V 'Arne Tiselius'. To monitor the change in the horizontal hydrography between the two stations, a horizontal CTD profile was continuously run in the cooling water of the ship, when moving in a transect from station A, westward to station B. The CTD was placed in a bucket with flowing sea water pumped from ~3 m depth, on 4 September. The surface water of the two stations differed significantly with respect to both temperature and salinity (Figure 2), as the outer station (A) was situated in high-saline water originating from the North Sea, while the low-saline surface water at the inner station (B) was influenced by the Baltic current. Below the surface, the profiles of temperature, salinity and in vivo chlorophyll a fluorescence (Figure 3A) were relatively similar between the stations, and changed very little in the time between the two casts, a few hours apart. The ambient concentrations of dissolved inorganic nutrients were typical for a stratified summer situation, i.e. generally low in the surface waters, although a raised ammonium concentration was associated with the sharp halocline at 5 m depth at station B, and all nutrient levels were increasing below 30 m depth (data not shown).

Concentrations of VLP, infective units and phytoplankton cell counts were estimated directly from the water samples collected at every 5 m depth, from 0 to 50 m depth, in Niskin bottles (1.8 l) mounted on a rosette equipped with CTD and an in situ fluorometer. At each station, two replicate CTD casts were run. Casts 1 and 2 at station A on 4 September were started at 14:30 and 17:00 h, respectively, while at station B on 5 September the two casts were started at 10:30 and 12:00 h, respectively.
Vertical distribution of VLP and viruses infecting *M*. *pusilla*

![Graph](https://academic.oup.com/plankt/article-abstract/20/11/2207/1546611)

**Fig. 2.** Horizontal profile (-3 m depth) of temperature (solid line) and salinity (dashed line), at a transect through Stations A and B on 4 September 1996.

Virus particles were collected on 0.02 μm pore size filters (Anodisc), stained with the cyanine-based dye Yo-Pro-1 according to Hennes and Suttle (1995), and quantified by epifluorescence microscopy. In all samples, the spherical 'virus-like' bright green fluorescing dots were counted and these are referred to as VLP. These particles looked similar to the homogeneous particles obtained from a lysed culture of *M*. *pusilla* infected by isolated viruses specific to this alga (Sahlsten, 1998). Two vertical profiles of VLP were counted from two separate casts, at each station. The abundance of VLP ranged between $5 \times 10^9$ and $25 \times 10^9$ VLP l$^{-1}$ in the 0- to 50-m-depth water column, with maximal values found in the surface water (Figure 3B), although a lower number was detected in the low-saline surface water (0 m depth) at station B. The epifluorescence counts of viruses found in the present study (VLP ranging from $4.9 \times 10^9$ to $25.0 \times 10^9$ l$^{-1}$) are in the same range as other virus estimates, made both by epifluorescence microscopy and by transmission electron microscopy from, for example, Japanese coastal and offshore waters (Hara *et al.*, 1991) as well as the Southern California Bight (Cochlan *et al.*, 1993). The variation in VLP counts between the casts, 1.5-2.5 h apart, is a factor of 2-3, which is a temporal fluctuation well within the range found by, for example, Bratbak *et al.* (1996) in Osterfjorden, Norway, in September 1993.

Estimates of the number of units infective to the prasinophyte *M*. *pusilla* (strain CCMP 490) were performed by inoculating samples in a dilution series of 10 parallels for each series of 10-fold dilutions up to $10^4$-fold dilution to algal cultures in microtitre plates (Suttle, 1993). The samples were incubated at 20°C in fluorescent light ($-5 \mu E \text{s}^{-1} \text{m}^{-2}$) at the laboratory, and lysing of the cultures was monitored by *in vivo* chlorophyll *a* fluorescence readings (Cytofluor™ 2300 Fluorescent Measurement System, Millipore). Numbers of infective units, i.e. supposed infective viral particles, per millilitre, were estimated by the MPN method using a BASIC program (Hurley and Roscoe, 1983). In all of the samples tested by inoculating to cultures of *M*. *pusilla*, lysis occurred in some of the
Fig. 3. Vertical distribution of (A) temperature (solid line), salinity (dashed line) and *in vivo* chlorophyll a fluorescence (dotted line, arbitrary units) (Cast 1 = thin lines, Cast 2 = bold lines), (B) virus abundance, VLP l⁻¹ (O = Cast 1; ● = Cast 2; error bars indicate SD, n = 5) and (C) infective units l⁻¹ (O = Cast 1; ● = Cast 2; error bars indicate 95% CI).
Vertical distribution of VLP and viruses infecting *M. pusilla*
dilutions within a few weeks. This means that in all water samples there existed
some infective units, supposed to be viruses, which were lytic to *M. pusilla*. The
abundance ranged from $2.5 \times 10^4$ to $2.0 \times 10^6$ l$^{-1}$, with the highest value found in
the surface water at station A (Figure 3C). Lysis occurred in all the dilution
experiments performed with algal samples inoculated with water, although the abundance of *M. pusilla* was low at both stations (see below). The observation
that all samples were found to contain infective units which were capable of lysing
*M. pusilla* confirms earlier data from the Kattegat and Skagerrak on ubiquitous
viruses lytic to *M. pusilla* (Sahlsten, 1998). Our maximal number of $2 \times 10^6$ infective units l$^{-1}$ found in the surface at station A is comparable to the lower end of
the range reported by Cottrell and Suttle (1991) from Texas coastal waters in
April 1993. Our number of infective units, calculated with the MPN technique,
was a very small fraction, ~0.01%, of the total number of VLP counted in epi-
fluorescence microscopy.

Picoplankton cells were quantified by epifluorescence microscopy (Kuylen-
stierna and Karlson, 1994). To obtain a reasonable statistical estimate, a lower
limit of the number of quantifiable cells was set at $4 \times 10^4$ cells l$^{-1}$ with the $\times100$
objective. The prasinophyte *M. pusilla*, which is the alga used for the infectivity
test, was detected at both stations, but the abundance was $<4 \times 10^4$ cells l$^{-1}$, which
was the statistical limit used.

This study demonstrates that the total number of VLP in the southern Skager-
rak in late summer varied with time and space, and most of the viruses seemed
to be concentrated in the upper productive layer of the water mass. All of the
virus particles, which are quantified by microscopy, are probably not infective,
due to adsorption to particulate matter or due to a defect in the synthesis of the
virus. As algal viruses may be both species and strain specific, as found for
*M. pusilla* viruses (Sahlsten, 1998), the estimate of 0.01% of the total viruses being
lytic to the species *M. pusilla* may be an underestimate, as it was estimated for
only one *M. pusilla* strain.

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