Chapter 6

Influence of irradiance on virus-algal host interactions

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The effect of different irradiance levels on the interactions between the algal host and its virus were investigated for two marine phytoplankton, Phaeocystis globosa Langerheim and Micromonas pusilla Manton and Parke. The algal cultures were acclimated at 25, 100, and 250 µmol photons m⁻² s⁻¹ (LL, ML, and HL, respectively), after which they were infected with a lytic virus (PgV and MpV) and monitored under the appropriate irradiance and in darkness. The effect of irradiance levels on the host-virus interactions was species-specific. For P. globosa, the LL-adapted cultures showed a 4 h prolonged latent period (11-16 h), which may be related to the subsaturated growth observed at this irradiance. The burst size was 50% reduced at LL and HL as compared to ML (525 PgV cell⁻¹). The fraction of infectious viruses was, however, unchanged. Viral replication was prevented when the LL P. globosa cultures were kept in darkness (up to 48h), but recovered when placed back into the light. PgV could still replicate in the dark for the ML- and HL-adapted cultures, but viral yield was reduced by 50 to 85%. For M. pusilla, the burst size (285 to 360 MpV cell⁻¹), the infectivity, and the latent period of MpV (7-11 h) were unaffected by the host’s photoacclimation. Conversely, darkness not only inhibited MpV replication but also resulted in substantial cell lysis of the noninfected cultures. Our study implies that irradiance level is an important factor controlling algal host-virus interactions, hence the phytoplankton population dynamics in the field.

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1. Introduction

Marine algal viruses have been shown to influence phytoplankton dynamics, the functioning of pelagic food webs and biogeochemical cycling (for review Wilhelm & Suttle 1999, Brussaard 2004a). There is, however, still little documentation on environmentally relevant factors that influence the interactions between the virus and its host. Viral replication is governed by the host cellular machinery; therefore the factors that affect the algal host’s physiology (e.g., nutrients, light) may influence the interactions between algal hosts and viruses.

In marine environments phytoplankton are constantly subjected to fluctuations in light intensities. These changes in irradiance can be generated, for instance, by cloud coverage or water mixing on a short time scale, but they can also be associated with stratification or seasonal variation in solar radiation on a longer time scale. In response to these light fluctuations, phytoplankton have evolved a large variety of physiological responses (photoacclimation) to optimize their growth (Falkowski & La Roche 1991). To date, the few existing studies investigating the effect of light on virus-host interactions were restricted to darkness and showed variable outcomes (Waters & Chan 1982, Van Etten et al. 1983, Bratbak et al. 1998, Suttle 2000). During the present study, the effect of different irradiance levels, including darkness, on the interactions between algae and their specific viruses were investigated for two ecologically important marine phytoplankton, *Phaeocystis globosa* Largerheim and *Micromonas pusilla* Manton and Parke.

Both *P. globosa* and *M. pusilla* are important for the structure of phytoplankton community and the functioning of the system where they occur (Brussaard et al. 1995, Not et al. 2004). These species can be subjected to virally induced mortality rates comparable to grazing losses (Evans et al. 2003, Baudoux et al. 2006). *Phaeocystis globosa* is known to generate dense and nearly monospecific spring blooms in temperate coastal waters when sufficient light and nutrients are available (Cadée & Hegeman 2002). *Phaeocystis globosa* is characterized by a polymorphic life cycle composed of unicellular flagellated cells (5 to 7 µm in diameter) and non-motile cells embedded in colonies (up to 1 cm). In contrast, *M. pusilla* does not form high-biomass blooms and only exists as small flagellated single cells (1 to 3 µm in diameter). *Micromonas pusilla* has a worldwide distribution and is described as a major component of the picophytoplankton community in many different coastal as well as oceanic waters (Kuyleniestierna & Karlson 1994, Thronsden & Zingone 1994, Not et al. 2004).

The different irradiance levels tested (0, 25, 100, and 250 µmol photons·m⁻²·s⁻¹) showed that light can strongly impact the interactions between the algal hosts and their specific virus. The effects of light intensity on viral infection were, however, species-specific. The viral growth cycle of the virus infecting *P. globosa* (PgV) revealed that both low and high irradiance reduced the burst size (number of produced viruses per host cell). The latent period (time until virus progeny is released from the host cell) was, furthermore, prolonged at low light. Darkness resulted in reduced burst sizes of PgV,
Partially restored to the original level upon return to the light. The lytic growth cycle of MpV was not affected by irradiance, but darkness stopped the viral production. These results imply that light can affect the ecological role of (algal) viruses very differently.

2. Material and methods

Phytoplankton and virus cultures. Unialgal cultures of the prymnesiophyte *Phaeocystis globosa* (Hariot) Largerheim strain G (Groningen University culture collection, The Netherlands) and the prasinophyte *Micromonas pusilla* (Butscher) Manton and Parke strain 1545 (Bigelow culture collection of marine phytoplankton, USA) were used during this study. Both algal cultures were grown in a 1:1 mixture of f/2 medium (Guillard 1975) and ESAW (Cottrell & Suttle 1991). The algae were cultured at 15°C under a light:dark cycle of 16:8 h at light intensities of 25, 100, and, 250 µmol photons m\(^{-2}\) s\(^{-1}\) (hereafter abbreviated LL, ML and HL for low, medium and high light intensities respectively). The algal cultures were adapted at the appropriate irradiance by repeated dilution (at least 5 volume changes) in fresh medium. Dilutions were performed to keep cultures in early exponential phase at cell abundance between 1 \(\times\) 10\(^4\) and 1 \(\times\) 10\(^6\) cells mL\(^{-1}\). The cultures' adaptation lasted until stabilization of their maximal growth rate (\(\mu_{\text{max}}\), Table 1).

The lytic viruses infecting *P. globosa* (PgV-07T, Baudoux & Brussaard 2005) and *M. pusilla* (MpV-02T) were both isolated from surface water of the North Sea according to Brussaard et al. (2004). In short, filtered (Whatman GF/F) natural seawater was added to respective algal host culture (10 – 20 % v/v) and incubated for 10 days at 15°C under a light dark cycle 16:8 h receiving 100 µmol photons m\(^{-2}\) s\(^{-1}\) (standard culture conditions of the hosts). Algal growth was monitored via in vivo chlorophyll fluorescence (Fo) using a Turner Designs fluorometer (model 10-AU). The cultures that showed signs of lysis as compared to noninfected controls were filtered through 0.2 µm pore-size cellulose acetate filters (Schleicher and Schuell GmbH, Dassel, Germany), after which the lysate was used to reinfect an exponentially growing algal host culture. Cultures were checked for the presence of algal viruses using FCM and transmission electron microscopy (TEM). After recurrent lysis and reinfection, viral isolates were made clonal by repeated end-point dilution.

Experimental set up. The three different light-adapted *P. globosa* and *M. pusilla* host cultures at a cell abundance of 1\(\times\)10\(^5\) cells mL\(^{-1}\) were split into four equal subcultures of 220 mL. Two of these subcultures were inoculated with the corresponding viral lysate (PgV-07T or MpV-02T) at an initial virus to host ratio of around 20. Most probable number (MPN) examination (see below) of the viral lysate showed that the multiplicity of infection (MOI) ranged between 10 and 20, which is sufficiently high to allow a one step infection cycle. The other subculture was a noninfected control and received an equal volume of medium. Each subculture was incubated at the respective light intensities for a period of 7 days. The cell abundance, chlorophyll a fluorescence (Fo) and the multiplication of the virus in each subculture were measured every day.
light regime (LL, ML, or HL) and sampled for algal and viral abundance, and photochemical efficiency (Fv/Fm) every 4 h for a total period of 70 h.

A parallel experiment was simultaneously conducted in the dark using 50 mL subcultures in triplicate. Earlier test showed that the use of a reduced volume (50 mL) as compared to the experiment presented in the previous paragraph (220 mL) did not affect the results. Darkness was achieved by wrapping the incubation flask completely into three layers of aluminium foil. All samples were incubated at the standard culture conditions. At T=0, 1, and 2 days, one of the dark triplicates was sampled for algal and viral counts and photochemical efficiency (Fv/Fm). A 5 mL aliquot of this sample was transferred into a borosilicate glass tube and exposed to the appropriate irradiance for 2 days (mixed by hand each day). After 2 days, subsamples of these tubes were taken for viral abundance and the natural chlorophyll autofluorescence Fo, as a measure of algal biomass.

**Analyses.** Algal abundance was monitored by flow cytometry using a Beckman Coulter EPICS XL-MCL flow cytometer equipped with a laser with an excitation wavelength of 488 nm (15mW) and emission bands for the chlorophyll autofluorescence (> 630 nm), phycoerythrin fluorescence (575 ± 20 nm), and green fluorescence (515 ± 20 nm). For the algal abundance, fresh samples were diluted up to 10-fold in 0.2 µm filtered sterile culture medium. Fluorescent microspheres of 0.95 µm (Invitrogen-Molecular Probes, Eugene, OR, USA) were added as an internal standard. The trigger was set on the red chlorophyll autofluorescence (RFL) and samples were analyzed for 1 min at a flow rate of 72 µL min⁻¹. Scatter and fluorescent signals were normalized to the signal of the internal standard beads.

For virus enumeration using flow cytometry (Brussaard 2004b), 1 mL samples were fixed with 25 % glutaraldehyde (0.5 % final concentration, EM grade, Sigma-Aldrich, St Louis, MO, USA) during 30 minutes at 4°C, flash frozen in liquid nitrogen and stored at -80°C until analysis. The thawed samples were diluted 100 to 1,000-fold in autoclaved 0.2 µm filtered TE-buffer (pH 8.0) and stained with the nucleic acid-specific dye SYBR Green I (Invitrogen-Molecular Probes, Eugene, OR, USA) for 10 min at 80°C. The trigger was set on the green fluorescence and the sample was delivered at a rate of 20 µL min⁻¹ and analyzed for 1 min. Virus counts were corrected for the blank consisting of TE-buffer with autoclaved 0.2 µm filtered seawater in the correct dilution.

The abundance of infectious PgV and MpV was estimated using the end-point dilution approach (Most Probable Number, MPN, Suttle 1993). Briefly, the freshly produced lysate was 10-fold serial diluted (5 replicates, 12 dilution levels) with an exponentially growing algal culture at the appropriate irradiance (LL, ML, or HL). The dilution series were incubated for 10 days at 15°C under the respective culture regime. Algal growth was monitored fluorometrically (Fo). Sample that underwent lysis were scored positive and the resulting MPN of infectious viruses was calculated using a MPN assay computer program (Hurley & Roscoe 1983).

The photochemical efficiency (Fv/Fm) of a 5 mL algal sample was measured using a Turner Designs model 10-AU fluorometer. After dark-acclimation of the algal cells for 5 min (Geider et al. 1993), the natural fluorescence (Fo) of the sample was
determined, after which the maximal fluorescence (Fm) was induced by adding DCMU (3',4'-dichlorophenyl-1,1-dimethylurea, Sigma-Aldrich, St Louis, MO, USA) to a final concentration of 10 µM. The variable fluorescence (Fv) was calculated as Fm minus Fo. Maximal photochemical efficiency of the PSII reaction center of the algal host was derived from the ratio of Fv over Fm. We occasionally (3 times) omitted unrealistically high Fv/Fm values originating from the ratio of very low Fo values as found for the cultures that underwent lysis at the end of the lytic cycle.

3. Results

3.1. Acclimation to different irradiance levels

The light acclimation of noninfected *P. globosa* resulted in a 2-fold reduced growth rate at LL (0.6 d⁻¹, Table 1) as compared to HL and ML (1.2 and 1.1 d⁻¹, respectively). The maximal growth rate of *M. pusilla* was also subsaturated at LL (0.5 d⁻¹, Table 1) whereas no difference could be detected between the ML and HL cultures (0.7 d⁻¹).

Besides differential maximal growth rates, the light scattering properties of the *P. globosa* cells increased in response to increasing irradiance (1.5 and 2-fold higher at HL than at LL for the SSC and FSC signals, respectively). In contrast, the cellular RFL and the culture’s photochemical efficiency (Fv/Fm) was comparable at all irradiance levels (Table 1). For *M. pusilla*, the cellular intrinsic characteristics also differed between the cultures, but in different way than for *P. globosa*. Increasing irradiance resulted in enhanced FSC signals (1.5-fold higher at HL than at LL) whereas the RFL decreased substantially (2.5-fold lower HL than LL). In contrast, the cellular SSC signals and the culture’s Fv/Fm signals were unaffected by the different irradiance regimes (Table 1).

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<th><em>P. globosa</em></th>
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<td>HL</td>
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<td>µmax (d⁻¹)</td>
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<td>Fv/Fm</td>
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*Table 1. Maximal growth rates (µmax, d⁻¹), photochemical efficiency (Fv/Fm) and flow cytometric characteristics (FSC, SSC, RFL) for *P. globosa* and *M. pusilla* cultures incubated at HL, ML and LL (being 250, 100, and 25 µmol photons m⁻² s⁻¹ respectively). All flow cytometric signals are standardized to an internal standard (0.95 µm beads). Values are means of duplicate samples. The coefficient of variation was at max. 6 % of the mean.*
3.2. Effect of irradiances on algal host - virus interactions

Viral infection of *P. globosa* resulted in a gradual loss in Fv/Fm signal and subsequent cell lysis (Fig. 1). Both processes were, however, dependent on the irradiance level the algal host was acclimated to. Cell lysis started 4 to 7 h post infection for the HL cultures, 11 to 15 h for the ML and 15 to 20 h for the LL. Full lysis of *P. globosa* cultures showed a similar pattern and was achieved after 30 h for the HL treatment, 45 h for the ML, and >70 h for the LL culture.

The latent period of PgV was 7 to 11 h at HL and ML, but it was prolonged at LL (11 to 16 h, Fig. 1E). The burst size of the HL and LL cultures, calculated as the ratio of the maximum net virus produced over net maximum decline of algal host, was half (265 and 260 PgV·cell⁻¹) that of the ML-adapted culture (525 PgV cell⁻¹). The fraction of infectious PgV produced was, however, 100% regardless of the light treatment.

*Figure 1.* Viral infection of *Phaeocystis globosa* grown at 250, 100, and 25 µmol photons m⁻² s⁻¹ (HL, ML, and LL respectively). Abundance and photochemical efficiency (Fv/Fm) of *P. globosa* in the (a, b) noninfected and (c, d) infected cultures, and abundance of *P. globosa* viruses PgV in (e) the infected cultures. Values are means of duplicate series, normalized to To.
As for *P. globosa*, the viral infection of the *M. pusilla* cultures induced a progressive decline in Fv/Fm and host cell abundance as compared to the noninfected algal hosts (Fig. 2). The time until full lysis was somewhat delayed for the LL treatment (>60 h) as compared to the 45 h for the HL and ML regimes. In contrast to PgV, the MpV growth cycle was not affected by the different irradiance levels; the latent period was 7 to 11 h and the burst size was 285 to 360 MpV cell\(^{-1}\). Like the PgV, the infectivity of the MpVs produced was maximal (100%) and unaffected by the different light regimes.

![Figure 2](image)

**Figure 2.** Viral infection of *Micromonas pusilla* grown at 250, 100, and 25 µmol photons m\(^{-2}\) s\(^{-1}\) (HL, ML, and LL respectively). Abundance and photochemical efficiency (Fv/Fm) of *M. pusilla* in the (a, b) noninfected and (c, d) the infected cultures, and abundance of *M. pusilla* viruses MpV in (e) the infected cultures. Values are means of duplicate series, normalized to To.
3.3. Darkness-induced effects on algal host-virus interactions

The growth of the noninfected *P. globosa* cultures halted upon transfer in complete darkness (Fig. 3A) but their Fv/Fm signal remained high (> 0.6, Fig. 3B). As observed for the virally infected cultures incubated in the light, the infected cultures, when placed in darkness, showed a faster decline in Fv/Fm signal and cell abundance in the culture adapted at increasing irradiance (Fig. 3D). Cell lysis was, however, only partial in all dark treatments. The PgV production in the ML and the LL was strongly reduced as compared to the HL-adapted culture (Fig. 3E). The LL culture did not show any PgV production when placed in the dark. The PgV yields in the HL and ML cultures transferred in the dark were both lower than in the respective light-incubated cultures (Fig. 1E). When the dark-incubated cultures were placed back to the appropriate irradiance after 2 days of darkness, additional algal cell lysis and PgV production was recorded in the ML and LL cultures. The final viral yield in the ML infected culture remained, however, 6-fold lower than in the light-incubated culture. In contrast, the final viral yield in the LL culture was comparable to that in the LL culture incubated in the light.

![Graphs showing effects of darkness on viral infection of *Phaeocystis globosa* acclimated at 250, 100, and 25 µmol photons m⁻² s⁻¹ (HL, ML, and LL respectively). Abundance and photochemical efficiency (Fv/Fm) of *P. globosa* in the (a, b) noninfected and (c, d) infected cultures and abundance of *P. globosa* viruses PgV in (e) the dark-infected cultures. Values are normalized to To.](image-url)
The growth of the noninfected *M. pusilla* in the dark was not only halted, as observed for *P. globosa*, but a large proportion (40%) of the cells died within 24 h (Fig. 4A). An independent repetition of this experiment gave similar results (data not shown). The remaining cells showed a drop of 20% in the Fv/Fm signal (Fig. 4B). Viral infection in the dark resulted in a stronger decline in Fv/Fm and in cell abundance than in the noninfected cultures (Figs. 4C, D). This additional decline, however, did not result in the production of MpV (Fig. 4E). When the dark-incubated cultures were placed back to the light, the Fo signal of noninfected *M. pusilla* increased again. In contrast, the Fo signal of the infected algal culture did not increase and still no MpV production was observed in any of the infected cultures.

**Figure 4.** Effect of darkness on viral infection of *Micromonas pusilla* acclimated at 250, 100, and 25 μmol photons m\(^{-2}\) s\(^{-1}\) (HL, ML, and LL respectively). Abundance and photochemical efficiency (Fv/Fm) of *M. pusilla* in the (a, b) noninfected and (c, d) infected cultures; and abundance of *M. pusilla* viruses (MpV) in (e) the dark-infected cultures. Values are means of duplicate series, normalized to To.
4. DISCUSSION

This study constitutes the first detailed report on how different irradiance levels influence the interactions between host and virus for two marine phytoplankton of ecological relevance, *P. globosa* and *M. pusilla*. Change in irradiance influenced species-specifically the studied algal hosts and convincingly influenced viral infection of *P. globosa*. Indeed, irradiance strongly affected the viral growth, latent period and burst size of PgV, whereas the viral infection of *M. pusilla* was most affected by darkness.

For *P. globosa*, the production of PgV was optimal at ML (100 µmol photons m$^{-2}$ s$^{-1}$) with a 7-11 h latent period and a burst size of 525 viruses cell$^{-1}$. This irradiance level corresponds to the so called “host standard culture conditions” at which a previous characterization of PgV-07T was executed (Baudoux & Brussaard 2005). The here reported latent period matches nicely that of the PgV group I to which PgV-07T belongs. The observed burst size was, however, higher than that reported earlier (approx. 300 PgVs cell$^{-1}$), which may originate from the use of a different algal strain in the present study. The acclimation of *P. globosa* at LL and HL (25 and 250 µmol photons m$^{-2}$ s$^{-1}$) negatively affected the PgV burst size (half the burst size at ML). In the LL-adapted cultures, the latent period was furthermore prolonged by 4 hours. These results suggest that viral replication was photolimited and photoinhibited in the LL and HL cultures, respectively.

The physiological adaptations of *P. globosa* host that influenced viral infection are unclear. The reduced burst size was associated with a subsaturated *P. globosa* growth rate in the LL-adapted culture whereas growth rate remained saturated in HL-adapted culture. This indicates that growth rate per se was not responsible for the reduced viral yield, contrarily as what has been suggested for *Paramecium Chlorella* host-virus systems (Van Etten et al. 1991). Nonetheless, the reduced host growth rate could still explain the prolonged latent period of PgV observed for the LL regime. Some of phenotypic adjustments of *P. globosa* at HL and LL, such as the changes in cellular light scattering signals FSC and SSC, may partially explain some of the observed difference in viral infection. The light scattering signals provide information on the structure, the internal granulometry and the biovolume of the cells. The considerable reduction of these parameters in the LL-adapted culture may be related to a decrease in cell volume as observed earlier (Buma et al. 1993, Moisan & Mitchell 1999). Such effect on cell volume may, in certain case, reduce the viral yield per cell due to packaging constraint (Brown et al. 2006). The elucidation of physiological reasons underlying such changes would require further investigations. Nevertheless, the here reported results have interesting ecological implications. Indeed, *P. globosa* found in shallow, turbulent and turbid coastal marine environments may experience a large range of light intensities. The lower PgV production obtained under high and low light intensity conditions can lead to a reduced encounter rate between virus and host which, in turn, can give the opportunity for *P. globosa* to thrive.
Even in complete darkness, we found that PgV could still be propagated when the host was acclimated at ML and HL, although the viral yield was reduced by 50 to 85%. On a physiological aspect, these observations imply that PgV production occurs only partially in the absence of photosynthesis as also reported for Chlorella sp. and Phaeocystis pouchetii (Van Etten et al. 1983, Bratbak et al. 1998). P. globosa acclimated at ML and HL should, thus, have sufficient energy stores or produce enough energy in the dark to support viral replication. Studies conducted on the viral infection of the raphidophyte Heterosigma akashiwo suggested that, in darkness, virus replication could use the ATP reserves or production via respiration or the energy generated by the cyclic photophosphorylation (Juneau et al. 2003, Lawrence & Suttle 2004). In contrast, the total inhibition of PgV replication in the LL cultures when placed in the dark suggests that the energy level of the LL-adapted host was too low for the viruses to replicate. The host’s ability to propagate viruses was, however, fully restored upon return to the light while photosynthesis proceeded de novo. Sedimentation and resuspension of P. globosa colonies as observed in the field (Cadée 1996; L. Peperzak pers. com.) may affect the viral infection processes. Under conditions of light depletion, settled P. globosa colonies are likely to shed their single cells (Peperzak et al. 2000), which can, in turn, be infected by viruses (Brussaard et al. 2005). Complete viral replication will, then again, be possible upon resuspension of the single cells into shallower waters with enhanced light intensities.

The light regimes here tested impacted viral infection of M. pusilla in a different way than P. globosa. The interactions between M. pusilla and MpV were unaffected by the different irradiance levels. The 7 to 11 h latent period observed during this study was comparable to those reported earlier (Waters & Chan 1982, Brussaard et al. 1999). The MpV burst size of 285 to 360 viruses cell\(^{-1}\) was, however, high as compared to the literature (49 to 230 MpV cell\(^{-1}\); Waters & Chan 1982, Brussaard et al. 1999, CB unpubl. data). The observed differential effect of light intensity on viral infection of the two host-virus model systems may be caused by the species-specific photoacclimation of M. pusilla and P. globosa. We indeed observed differential adjustment of their intrinsic cellular characteristics (FSC, SSC and RFL) and their growth. It is noteworthy that the effect of light limitation on growth rate was less severe for M. pusilla than P. globosa (only reduced by 25 % compared to 50% for P. globosa). Based on this parameter, M. pusilla seems to better accommodate the low irradiance than P. globosa, and interestingly, the characteristics of the MpV growth cycle are unaffected by LL. These observations strongly suggest that M. pusilla can experience a constant viral control over a large range of irradiance, which may prevent a sudden proliferation of this phytoplankter.

In spite of this tolerance to low irradiance, M. pusilla could not support viral replication in darkness. The photosynthetic processes did not take place in the dark; thereby it would be tempting to suggest that MpV replication is a photosynthesis-dependent process. An earlier study also suggested that viral infection of M. pusilla requires light to proceed (Waters & Chan 1982, Suttle 2000). Our study showed that, in
contrast to \textit{P. globosa}, darkness not only inhibited MpV replication, but resulted also in a substantial impairment of the host’s physiology. Already after 1 day of darkness, the noninfected culture showed significant cells lysis (40\% loss). We have as yet no explanation for this abrupt collapse of \textit{M. pusilla} culture, although this raises the option of apoptosis. Apoptotic features have been reported for other marine phytoplankton species but only upon long-term light deprivation (6 days, Berges \& Falkowski 1998). The impairment of \textit{M. pusilla} physiology makes it difficult to conclude whether MpV requires host photosynthesis to replicate. Upon return into light, the growth of the noninfected cells was restored but, in contrast to \textit{P. globosa}, the capacity of \textit{M. pusilla} to propagate MpV production was not recovered. Darkness irreversibly prevented the production of MpV. In nature, it is unlikely that \textit{M. pusilla} cells sink out of the euphotic zone considering their micrometer size range (Raven 1998). However, \textit{M. pusilla} distributes in polar and boreal latitudes (Throndsen 1970, Not et al. 2005) where cells may experience prolonged darkness due to quite frequent deep-mixing, particularly at spring and winter time. Based on our results we may expect a reduction or even a prevention of viral infection of \textit{M. pusilla} during prolonged dark conditions in natural environments due to a poor physiological condition of the algal host.

In summary, this study revealed that both \textit{P. globosa} and \textit{M. pusilla} are characterized by distinct strategies to accommodate different light intensities. The growth rate of \textit{P. globosa} was more affected by changing light intensities than that of \textit{M. pusilla}. \textit{Phaeocystis globosa}, however, survived darkness (up to 2 days) whereas \textit{M. pusilla} was severely impaired by dark incubations. This species-specific photoacclimation likely determined the differential effect of irradiance on viral infection. The changes in light intensities that phytoplankton naturally experience can thus be a significant process regulating viral lysis processes in the ocean. Further investigations on different algal host-virus model systems would be required to know whether the differential light effect can be related to the ecological strategies of the algal hosts.

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\textbf{Literature cited}


Geider RJ, La Roche J, Greene RM, Olaizola M (1993) Response of the photosynthetic apparatus of Phaeodactylum tricornutum (Bacillariophyceae) to nitrate,
phosphate, or iron starvation. J. Phycol. 29:755-766
Lawrence JE, Chan AM, Suttle CA (2002) Viruses causing lysis of the toxic bloom-forming alga Heterosigma akashiwo (Raphidophiceae) are widespread in coastal sediments of British Columbia, Canada. Limnol Oceanogr 47:545-550
Waters RE, Chan AT (1982) Micromonas pusilla virus: The virus growth cycle and
associated physiological events within the host cells; host range mutation. J Gen Virol 63:199-206