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# Effects of ultraviolet radiation on marine virus–phytoplankton interactions

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## Abstract

Ambient ultraviolet radiation (UVR) is harmful to many biological systems and increased UVR, due to a reduced ozone layer, may have many unforeseen consequences. Viruses are the most abundant biological particles in the sea and are thought to play an important role in the structure and functioning of aquatic ecosystems. Although an increasing number of studies have been published during the last 15 years, aquatic viral ecology is still in its infancy and little is known about the effect of environmental factors on virus life cycle and host–virus interactions. Using flow cytometry, we have investigated the effect of UVR (UVB intensity:  $0.22 \text{ W m}^{-2}$  and UVA/UVB ratio  $\sim 30$ ) on five different cultured marine phytoplankton host–virus systems (*CeV-Chrysochromulina ericina*, *EhV-Emiliania huxleyi*, *MpV-Micromonas pusilla*, *PpV-Phaeocystis pouchetti* and *PoV-Pyramimonas orientalis*). Viruses appear to be susceptible to UV, but also they might provide some protection to their hosts. It is shown that (i) some of the investigated microalgae that have been co-cultured with viruses are less sensitive (e.g. *P. pouchetii*, *M. pusilla*) to UVB stress compared to susceptible microalgae (i.e. virus-free cultures), (ii) different viruses have different sensitivities to UVB in terms of both their abundance patterns (no effect for most of them except *EhV*) and infectivity (from no effect for *PoV*, to complete inactivation for *PpV*), (iii) UVA has no effect on host–virus interactions. Our results show UVB to be a potentially important factor in the regulation of virus–host interactions in surface waters.

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**Keywords:** Virus; Phytoplankton; Ultraviolet B; Sensitivity

## 1. Introduction

With the recent evidence of significantly decreasing concentrations of ozone in the stratosphere and the concomitant increase in ultraviolet B radiation (UVB) for north and south temperate latitudes [1,2], many studies have stressed the effects of UV on marine phytoplankton and primary production [3–6]. Briefly, UV has been shown to have deleterious effects on photo-autotrophs including inhibition of photosynthesis and growth [3], decrease of primary production rates [7], inhibition of nutrient uptake [3], loss of pigmentation [8], inhibition of amino acid synthesis [9], mutagenesis and acute physiological stress that may ultimately lead to cell death [10]. These effects occur in spite of efficient means of algal defense such as avoid-

ance, screening, quenching and repair [11]. The role of UV on marine bacteria has been investigated in recent years providing evidence that UVB may be more damaging to bacterial DNA compared to that of eukaryotic plankton [12]. UVB may also inhibit bacterioplankton production [13–15], and affect bacterial community structure in near surface waters [16–18]. Impacts of UV on other microorganisms of significant importance in the ecology and biochemistry of the world's oceans, such as viruses and zooplankton, have received less attention than phyto- and bacterioplankton [12,19].

Marine viruses are now recognized to be the most abundant biological particles in the sea [20,21]. They can significantly affect primary production [22], playing a key role in population mortality [23], nutrient cycling [24], bacterial and algal biodiversity and distribution [25], algal blooms [26], dimethylsulfide release [27] and transfer of genetic material [28]. Environmental factors are likely to play critical roles in their biological impacts, life cycles and diversity in marine ecosystems. However, the influence of these environmental factors (temperature, light, UV, nu-

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Table 1

Characteristics of the different marine species of phytoplankton used in this study (taxonomy, cell size, and motility, sampling origin, general distribution)

Class	Genus and species	Size ( $\mu\text{m}$ )	Characteristics	Origin	Distribution
Prymnesiophyceae	<i>Chrysochromulina ericina</i>	4–8	motile, 2 flagella	Norwegian coastal waters	coastal, Europe
Prasinophyceae	<i>Micromonas pusilla</i>	1–3	motile, 1 flagellum	Eastern Pacific Ocean	ubiquitous
Prymnesiophyceae	<i>Emiliana huxleyi</i>	5–10	non-motile	Norwegian coastal waters	ubiquitous
Prymnesiophyceae	<i>Phaeocystis pouchetii</i>	4.5–8	motile, 2 flagella	Norwegian coastal waters	cold waters worldwide
Prasinophyceae	<i>Pyramimonas orientalis</i>	4–6	motile, 4 flagella	Norwegian coastal waters	coastal, ubiquitous

All strains were isolated in Norwegian coastal waters (University of Bergen) with the exception of *M. pusilla*, isolated in eastern Pacific waters (Curtis Suttle, University of British Columbia).

trients) on marine viruses and on virus–host interactions is still poorly understood (e.g. [29,30]).

It is now well established that UV constitutes a main cause of both the destruction and the loss of infectivity of marine bacteriophages and cyanophages in surface waters [31–34]. Infectivity of these phages has been shown to be extremely sensitive to solar radiation and damage to viral infectivity is proportional to the radiation received [35]. Conversely, photo-reactivation is likely to restore infectivity to a significant proportion of the damaged viruses [36–38]. It has been shown that some viruses, including the virus of the freshwater phytoplankton, *Chlorella* sp., also encode a DNA repair gene that permits host-independent DNA UV repair function [39,40]. We are not aware of any published studies on the potential effects of UV radiation (UVR) on the abundance and infectivity of a range of cultured viruses of marine phytoplankton species. The reason for this is that only a few laboratories around the world possess a variety of phytoplankton–virus systems in culture.

Using flow cytometry (FCM), we examined the effect of a 4-h daily moderate intensity of UVB provided alone ( $0.22 \pm 0.04 \text{ W m}^{-2}$ ) or with UVA (such as UVA/UVB  $\sim 30$ ) on a variety of different marine phytoplankton–virus systems available in culture. We found that there was a considerable interspecific variability in the sensitivity to UVB for both viruses and virus–host interactions, especially with regard to hosts co-cultured with viruses vs. susceptible hosts (i.e. virus-free cultures). The results provide new insights into the relationships between marine viruses and their marine phytoplankton hosts in response to a critical environmental factor.

## 2. Materials and methods

### 2.1. Cultures

Five marine phytoplankton–virus systems were studied: *Chrysochromulina ericina*-CeV, *Emiliana huxleyi*-EhV, *Micromonas pusilla*-MpV, *Phaeocystis pouchetii*-PpV and *Pyramimonas orientalis*-PoV. The marine microalgae employed in this study are important members of the pico- or nanoplanktonic community in many habitats around the

world and some of them are known as bloom-forming species. Most of the viruses of these different phytoplankters have been isolated recently and maintained in culture. The principal characteristics of both the microalgae and the viruses are provided in Tables 1 and 2.

### 2.2. Growth conditions

Six 350-ml cultures were grown on F/2 medium [45] in sterile and spherical 500-ml quartz flasks (Tamro MedLab AS, Oslo, Norway) placed in a tank designed especially for the experiment that was filled with cooled water. An external cooling device kept the temperature of the circulating water at 15°C for all the algal species except for *P. pouchetii* (10°C). Cultures were mixed carefully by hand two or three times a day. The system was designed to hold up to eight quartz flasks. Photosynthetic active radiation (PAR) was measured using a LI-COR light meter (Biosciences, Skytta, Norway). We used a PD105B-cos device (Delta-T Devices, Cambridge, UK) associated with a Fluke 8840A multimeter (Fluke, Washington, USA) for UV measurements. Both PAR and UVR were measured inside the flasks.

### 2.3. PAR and UV light conditions

UVR bulbs were placed at one side of the tank, made of Plexiglas XT which is transparent to UVR (Röhmi Chemische Fabrik, Kirchenallee, Germany) and PAR lamps at the other side, made of Plexiglas impervious to UV. PAR was provided by a set of Philips tubes (TLM series) for which light intensity could be manually controlled and it was set at about  $150 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$  throughout each experiment. Cultures were acclimated for 2–3 weeks to this light irradiance before UV light exposure. UV lamps were switched on for 4 h in the middle of the light period of a 14:10-h light:dark cycle to mimic the dose received in the near surface layer. UVB was provided by a Philips TL 20W/12 tube (280–320 nm) and UVA by a TL 20W/09N lamp (320–400 nm). These tubes were wrapped with wire netting to obtain desired intensities, i.e.  $0.22 \pm 0.04 \text{ W m}^{-2}$  for UVB and UVA/UVB  $\sim 30$  throughout each experiment, corresponding to a moderate intensity or a level equivalent to cloudy days in agreement

with what is typically recorded in the field, just above the water surface (e.g. [46,47]). Both PAR and UV intensities were controlled and maintained between the beginning and the end of each experiment for all treatments. Cellulose acetate was used to absorb UVC wavelengths and MYLAR-D to eliminate UVB. Total UVR was eliminated using a combination of Lee filter sheets 100 spring yellow and 130 clear (Lee Filters, Andover, Hants, UK).

#### 2.4. Type of experiments

The first experiment (Exp1 = virus-free) involved testing the effect of PAR, PAR+UVA and PAR+UVA+UVB on virus-free cultures. We verified that these cultures were virus-free using short-term exposures (< 1 min) of UVC radiation (Philips fluorescent tube type 57425 P/40 A6 T UV 15 W) applied to each separate replicate of the cultures. This manipulation was intended to cause induction of virus production in algae carrying proviruses, although the mechanisms of induction are unclear [41,44]. The presence of viruses in these cultures was never detected by FCM from a few hours to weeks (data not shown). The second experiment (Exp2 = combined) was performed under the same conditions as Exp1, with algae that were co-cultured with viruses (i.e. algal cultures that have recovered after viral infection and lysis and where the algae grow in balance with its virus [48]). Note that the same cultures as those in Exp1 were used for the infection experiment. Therefore, we were always working with the same strain for each algal species. The third experiment (Exp3 = infectivity) involved isolating the viruses studied in Exp2, which were then used to infect new susceptible host cultures. For Exp3, 30 ml of the culture was sampled and filtered through a GF/F (Whatman) filter to eliminate microalgae. The filtrate was centrifuged at 7500 rpm for 10 min with a Beckman J2-HS centrifuge. The supernatant, which was free of heterotrophic bacteria and of microalgal rests, but full of viruses (data not shown), was used to infect new virus-free cultures to investigate the degree of infectivity (i.e. the fraction of adsorbed virus particles that leads to infection and lysis) of the added viruses once they have been exposed to PAR or PAR+UVA or PAR+UVA+UVB. The concentration of viruses added in each of the experiments varied between  $1.7 \times 10^5$  and  $1.0 \times 10^6$  particles  $\text{ml}^{-1}$  and corresponded to a virus:host ratio of 1 to 10. The multiplicity of infection (MOI) was not mea-

sured but it was previously reported that the infectivity of viruses employed in this study is typically 1–10%, at least for PpV ([49] and other unpublished data). Thus, when the initial virus:host ratio (as based on FCM counts) is 10 the MOI can be expected to be up to 1. This infection potential also agrees with relatively low decay rates observed for lysates of MpV, PoV, PpV, EhV and CeV, which can be stored in the dark at 4°C for several months without losing the ability to lyse host cultures within a few days (data not shown). All experiments were conducted in duplicate.

#### 2.5. FCM analysis

Samples were obtained one or two times a day at 8–10-h intervals during daylight. Analyses were performed with a FACSCalibur flow cytometer (Becton Dickinson) equipped with an air-cooled laser providing 15 mW at 488 nm and with standard filter set-up. We used the method of analysis as described by Marie et al. [50] and as briefly outlined below. Analyses were performed at medium or high rate ( $\sim 30$  and  $70 \mu\text{l min}^{-1}$ , respectively) with the addition of 1- $\mu\text{m}$  fluorescent beads (Molecular Probes) in all samples. The enumeration of viruses was obtained from diluted samples in 0.02- $\mu\text{m}$  filtered TE (Tris-EDTA, pH 8) buffer 50–1000 times and heated for 10 min at 80°C (with the exception of PoV and MpV) after staining with the DNA dye SYBR<sup>®</sup>Green I (1/20 000 final concentration, Molecular Probes) in subdued light conditions. FCM listmode files were analyzed using CYTOWIN ([51], available at <http://www.sb-roscoff.fr/Phyto/cyto.html#cytwin>).

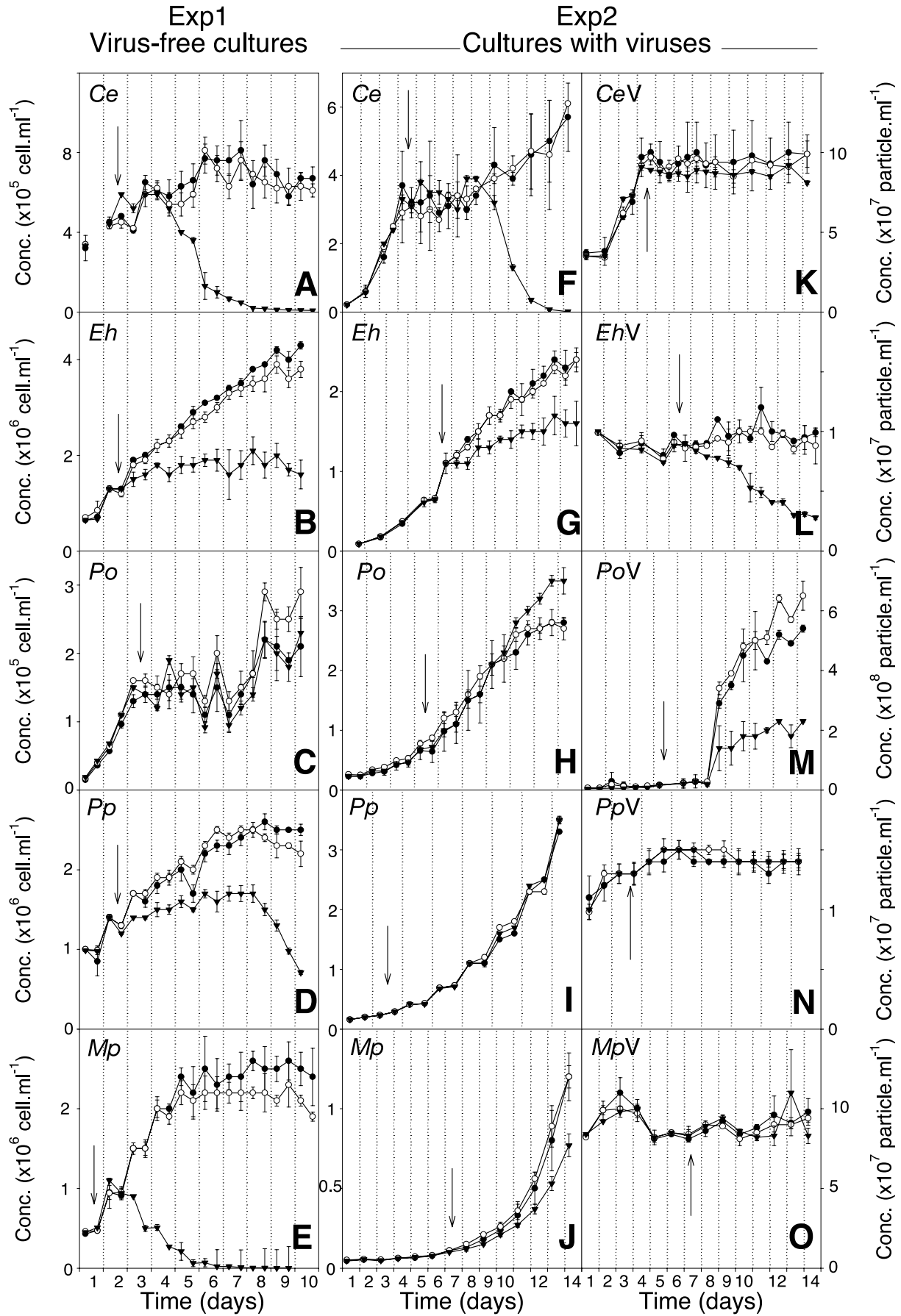
#### 2.6. Whole culture burst size estimation

Burst size, i.e. the number of viruses produced per lysed cell (or viruses released per lytic cycle), was estimated from FCM counts as the ratio of the maximum number of viruses produced to the maximum cell concentration reached by the specific host before cell decrease. This is an appropriate approach for estimating an average burst size when massive lysis occurs but cannot be used when the host growth is balanced by viral lysis and there is steady accumulation of virus particles with no corresponding decrease in host abundance. The estimate will not be affected by host growth between infection and lysis that may occur if the culture is infected with a low number of

Table 2

Characteristics of the eukaryotic marine phytoplankton viruses (virus identification and size, specific host, lytic parameters)

Virus name	Clone ID	Algal host (genus and species)	Virus size (nm)	Latent period (h)	Burst size (viruses/cell)	dsDNA genome (kb)	Reference
CeV	CeV-01B	<i>Chrysochromulina ericina</i>	160	14–19	1800–4100	510	[41]
EhV	EhV-99B1	<i>Emiliania huxleyi</i>	160–180	12–14	400–1000	415	[42]
MpV	MpV-SP1	<i>Micromonas pusilla</i>	130	7–14	70	200	[43]
PoV	PoV-01B	<i>Pyramimonas orientalis</i>	180–220	14–19	800–1000	560	[41]
PpV	PpV-01	<i>Phaeocystis pouchetii</i>	120	12–18	350–600	485	[44]



viruses and require two or three lytic cycles to obtain complete lysis, or delayed disintegration of lysed cells. Re-absorption of viruses or viral decay plays in fine a quantitatively minor role for an average estimation of the burst size.

### 3. Results

#### 3.1. Effects of UVR on virus-free algal cultures vs. algae co-cultured with viruses

Fig. 1 shows the variations over time of the concentration of phytoplankton cells and viral particles in response to the different treatments, in Exp1 (i.e. 'virus-free') and Exp2 (i.e. 'combined'). Both the algae and the viruses demonstrated different sensitivities to UVB, whereas no clear differences were recorded in population growth patterns in the PAR and the PAR+UVA treatments. From panel A to E of Fig. 1, corresponding to microalgae grown in the absence of viruses, we could observe a gradient from no effect of UVB on population growth (*P. orientalis*, C) to death (*C. ericina* and *M. pusilla*, A, E) with an intermediate effect for both *E. huxleyi* (B) and *P. pouchetii* (D). More interesting was the effect of UVB on the cultures previously infected by viruses, which subsequently recovered and were able to grow in the presence of viruses. Some of these cultures seemed to be less sensitive to the UVB treatment compared to the virus-free cultures. *C. ericina* began to decrease 3 days after the beginning of exposure to UVB (Fig. 1F). This response was 1–2 days later than in the experiment with the susceptible culture. Under UVB, *E. huxleyi* cell numbers still increased. However, this increase was at a lower rate than in the control (PAR treatment) and the UVA-treated culture (Fig. 1G). The pattern of response to UVB for both *P. pouchetii* and *M. pusilla* co-cultured with viruses (Fig. 1I,J) was different from the response for the sensitive cultures. UVB had no effect on the cell population in Exp2, while this treatment induced a rapid decrease of cell numbers in the virus-free culture (Fig. 1E). Finally, we saw no differences in growth patterns of *P. orientalis* (Fig. 1H) between the different treatments as observed for susceptible cultures.

As for microalgae, only the addition of UVB was associated with a change in the viral abundance (Exp2). Concentrations of *CeV* were very similar in all treatments (Fig. 1K) with an increase of particle numbers during the first 3 days of the experiment paralleling that of the host, *C. ericina*. After this period, the concentration of these

particles remained relatively constant with no significant differences between treatments. Similar patterns were recorded both for *PpV* and *MpV*, with no net increase in viruses (Fig. 1N,O). Concentrations of *EhV* were very similar in all treatments before UV exposure (Fig. 1L). However, after exposure there was no significant difference recorded in the concentration of the free viruses between the control and the UVA treatment. There was a clear and regular decrease of these particles in the UVB treatment, after only 24 h. *PoV* was also sensitive to UVB. However, no decrease was recorded in particle concentration (Fig. 1M). Instead, there was a clear increase in the concentration of *PoV* particles 2 days after the start of UV exposure while the cells were still growing. This increase was more marked in the PAR and PAR+UVA treatments with viruses reaching concentrations three to four times higher than in the UVB treatment. The reason for this sudden virus production is unclear, as it was not associated with cell lysis. However, it is clear that *P. orientalis* seemed to produce fewer viruses under UVB or there was a higher viral decay rate under UVB.

#### 3.2. Effects of UVR on the infectivity of viruses of marine phytoplankton

After 6 days of UV exposure (Exp2 = combined), viruses were isolated from each flask for infection of new susceptible (virus-free) cultures to investigate whether these viruses were (still) infective (Exp3 = infectivity). Fig. 2 shows the variation in the number of phytoplankton cells and viral particles during such infection over a few days. Cultures were infected with viruses in order to obtain a virus:host ratio between 1 and 10. All the cultures responded similarly during the first 2–3 days after infection with a clear increase in cell concentration except *P. orientalis* (Fig. 2A–E). Cultures infected with viruses that were pre-treated either with PAR or with PAR+UVA were characterized by a rapid decrease in cell numbers with concomitant virus production (occurring less than 24 h after infection as evidenced by zoom panels F'–J'). The fact that inoculation of cultures with the viruses did not result in rapid lysis with concomitant virus increase but rather in slow viral accumulation simultaneously with algal growth indicated that the infection rate and the resulting lysis rate was lower than the growth rate. This does not mean that no viral production occurred (there was viral production!) but rather that the decay rate was higher than the production rate and/or that some hosts could grow before complete lysis. Burst sizes were almost similar between PAR or PAR+UVA treatments for each system (Table 3). It is

←  
Fig. 1. A–E (Exp1): Time series obtained for virus-free (susceptible) cultures of the different marine phytoplankters. F–O (Exp2): Time series obtained for virus-resistant cultures (algae co-cultured with viruses) of the different marine phytoplankters (F–J) and the free viruses (K–O), grown as in Exp1. Arrows indicate the starting day of UV exposure. ●, ○ and ▼ represent PAR, PAR+UVA and PAR+UVA+UVB treatment, respectively. Values reported are means and the error bars represent the range of duplicate experiments. 'Conc.' means concentration.

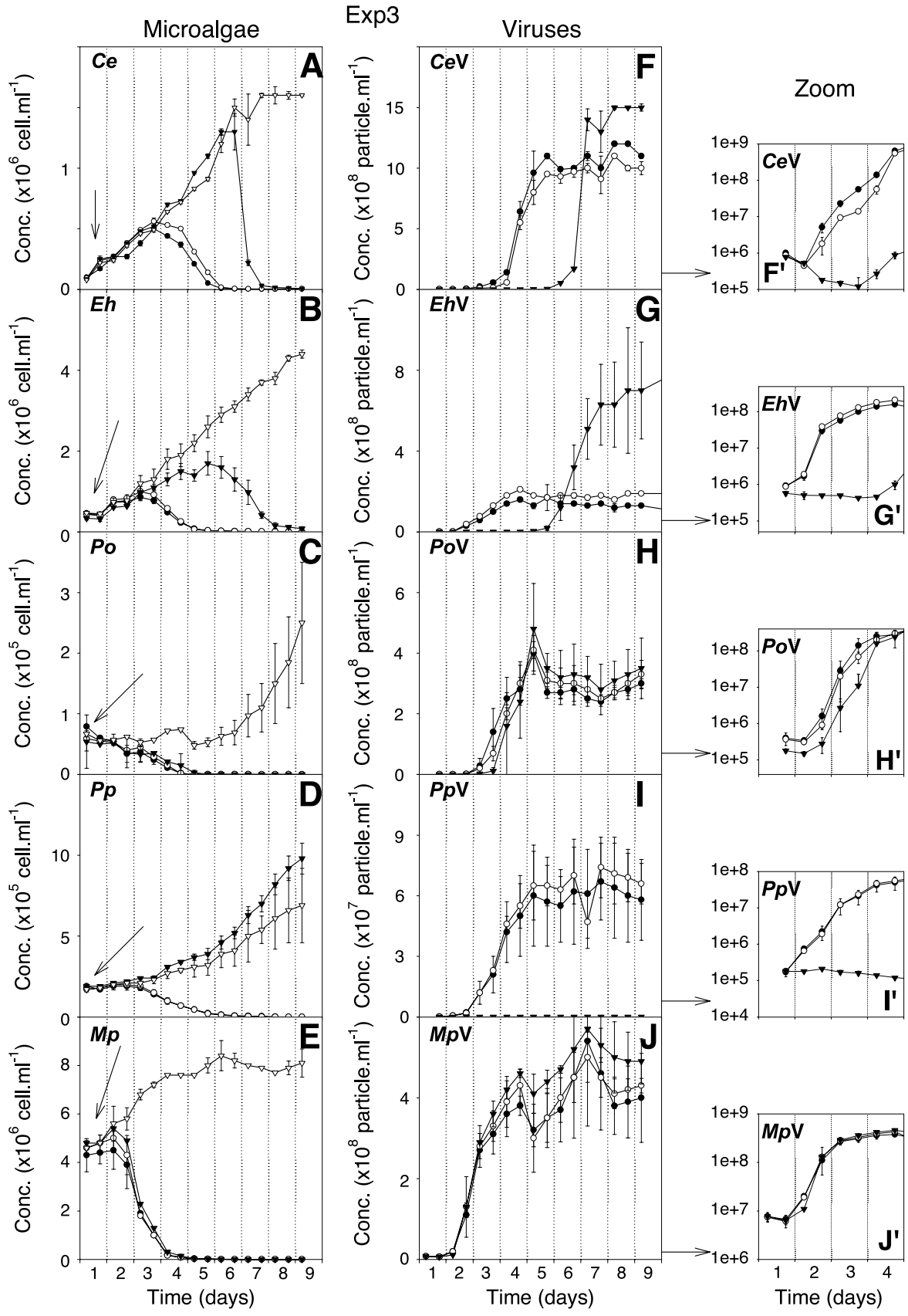


Table 3

Burst size (refer to Section 2.6) recorded during the infection experiments using PAR-, PAR+UVA-, and PAR+UVA+UVB-treated virus

	PAR	UVA	UVB
<i>Ce-CeV</i>	2400	1960	1150
<i>Eh-EhV</i>	200	210	450
<i>Mp-MpV</i>	85	85	85
<i>Po-PoV</i>	3540	5250	6480
<i>Pp-PpV</i>	350	370	–

noteworthy, however, that burst sizes calculated for *Ce-CeV* and *Po-PoV* differed according to the pretreatment. The ratio was significantly lower under PAR+UVA than PAR for *Ce-CeV*. It was exactly the reverse for *Po-PoV*.

By comparison to the apparent absence of viral inactivation by PAR or PAR+UVA, some important differences were recorded between cultures infected by UVB-treated viruses. A clear delay (i.e. a few days) in cell infection, cell lysis and virus production was observed for both *C. ericina* and *E. huxleyi* (Fig. 2A,B) suggesting that very few *CeV* and *EhV* were infective after the UVB treatment. This delay was more marked for *C. ericina* since cultures infected with UVB-treated viruses behaved like the control for almost 6–7 days before host cell numbers decreased. For *E. huxleyi*, cell lysis occurred only a few days after the addition of the virus lysate but at a lower rate compared to cultures infected with PAR- and UVA-treated viruses. Burst size was reduced by a factor of 2 for *Ce-CeV* after UVB exposure compared to the PAR or PAR+UVA treatments. In contrast, it was twofold greater in the case of *Eh-EhV* (Table 3). Note that the kinetics of *CeV* and *EhV* adsorption to their specific host were also very different compared to each other (see panels F' and G'). It is likely that when cultures were infected with these viruses, several rounds of replication were required in order to produce enough infective viruses to infect all cells and decimate the host population. Thus, the main differences between the cultures could have been the initial number of infective viruses, and the host cell abundance when lysis occurred. Whatever the pretreatment of *MpV* or *PoV*, there was a rapid lysis of *M. pusilla* and *P. orientalis* in each treatment with a concomitant production of viruses (Fig. 2C,H,E,J). This suggested that UVB exposure, at this intensity, did not affect the infectivity of these viruses. The burst size calculation revealed significant differences between the UVB-treated *PoV* viruses and the UVA and the PAR treatments (Table 3), with the same trend as that recorded for *Eh-EhV*. In contrast, the burst size was

exactly the same in all treatments for *Mp-MpV* (Table 3). The last observation was that *PpV* appears non-infective after a few days of UVB exposure. This apparent 100% loss of infectivity could be inferred from the absence of cell lysis for *P. pouchetii* and the lack of virus production (Fig. 2D,I,I'). Clearly, the effects of UVB on the burst size and viral production dynamics were different for each different algal host. Our results also suggested that there was no obvious connection between the loss of viral infectivity (inactivation) and the destruction of viral particles.

## 4. Discussion

### 4.1. General effects of UVR

During the last decade, an elegant body of work has revealed that DNA-containing viruses, which are common in marine environments, are damaged by solar UV radiation [12]. The persistence and infectivity of these particles in surface waters have been shown to be dependent on their capacity to restore UV-induced DNA damage by host cell reactivation mechanisms or photoreactivation [36] and possibly from the capacity of the viruses to encode gene(s) implicated in repair mechanisms to eliminate UV-induced DNA photoproducts [39,40]. As a result, and in contradiction to previous assumptions [35], it has been suggested that most of the pelagic viruses might be infective in surface waters because of efficient repair and mixing processes that reduce DNA damage accumulation [34,37,38]. To date, studies on the effects of UV radiation have been conducted with bacteriophages and cyanophages, with the exception of a few papers dealing with *Micromonas* sp. [31,52]. Hence, this is the first report of the effect of UV on a variety of viruses of ecologically significant marine phytoplankton. Three major results emerge from our experimental study, keeping in mind that we used a single intensity for PAR and UVR that corresponded, however, to that which would be found naturally in near surface waters. First, some algae co-living with viruses may be less sensitive to UVB stress compared to the same susceptible hosts. Second, viruses of marine phytoplankton have varying sensitivities to UVB. Third, our results indicate that UVA does not contribute to inactivation of marine viruses.

Our results show that some algal hosts that have been previously infected with viruses, and that have recovered from infection, are less stressed than sensitive cells by UVB. Although all experiments deserve to be repeated

←  
Fig. 2. Time series obtained for susceptible cultures (A–E) and the viruses (F–J) isolated from Exp2 under the different light and UV conditions and used to infect the susceptible cultures (Exp3). For virus time series, a zoom over the first 4 days of infection is provided (F'–J'). The control corresponds to cultures in which a 0.02- $\mu$ m pre-filtered virus lysate was added. Arrows indicate the time of virus introduction into the culture. For panels A–E, ●, ○, ▼ and ▽ represent the cultures infected with viruses pretreated with PAR, PAR+UVA and PAR+UVA+UVB and the control, respectively. For panels F–J, ●, ○ and ▼ represent viruses pretreated with PAR, PAR+UVA and PAR+UVA+UVB, respectively.

to provide more conclusive evidence, we think that algal viruses present in the cultures of Exp2 (by comparison with Exp1) were not likely responsible for absorption of a significant part of the damaging UV radiation that could have led to a significant attenuated level of UV reaching the algae (thereby indirectly protecting the algal hosts from UV damage). This assumption can be inferred from PAR and UV light measurements made throughout the study that revealed no significant differences between similar treatments in Exp1 and Exp2 (data not shown). In addition and following the same idea, we found no significant differences in FCM counts for both heterotrophic bacteria and bacteriophages between Exp1 and Exp2 for each treatment (data not shown). In the case of the relatively short delay recorded for *C. ericina* co-cultured with viruses before population decrease as well as the maintenance of growth for *E. huxleyi* co-cultured with viruses under UVB, all together compared to their respective virus-free cultures, there was a weak variation in the initial density of these different populations at the beginning of the experiments. Thus, it is not impossible that the difference growth patterns of the sensitive and the 'resistant' algae to infection may be due to differences in the stages of the population growth.

The difference of sensitivity we found suggests that strains that are resistant to viral infection may have ecological advantages compared to susceptible strains. Not only are they resistant to infection, but they are also less sensitive to UVB. How can we explain such a finding? Increased UV resistance of virus-infected cells might indicate that the viruses ferry some resistance gene(s) to the host. If so, we speculate that the virus may provide the host with gene(s) or induce cellular processes that increase their ability to survive stress (e.g. improved DNA repair mechanisms, increased cellular photoprotection, synthesis of enzymes removing free radicals, etc.). This remains to be demonstrated. We were able to stimulate virus production in resistant strains using short-term exposure of the resistant cultures to UVC (data not shown), suggesting that these cells may carry lysogenic viruses (or at least weaken the cells by UV and thus make them susceptible). Another possibility is that resistant algae may have evolved some mechanisms for protecting themselves from a new viral infection, for example, by altering the composition of the cell wall. Thirty years ago, Padan et al. [53] reported a change in the algal cell envelope that prevents cyanophage adsorption. At the same time, this may result in lower susceptibility to UVB irradiation. Recently, Middleboe et al. [54] showed that resistance of marine heterotrophic bacteria to viral infection may be associated with changes in receptor regions of the host's cell membrane and that such changes are likely to influence the ability of resistant cells to respond to their environment. These authors showed in particular that resistant bacteria had a competitive disadvantage relative to sensitive populations for assimilating nutrients. In our study, algae co-cultured

with viruses displayed lower growth rates (Fig. 1). This might be one possible cost of being resistant (e.g. [30]). More investigations are required to demonstrate the mechanisms of resistance.

#### 4.2. Inter-specific and virus variability

As recently reported for bacterioplankton [16,17], there was variability in the sensitivity of viruses to UVB, and in the recovery of viral infectivity from UVB stress. In addition, our results clearly revealed that there was a distinction between viral destruction and viral inactivation. Only *EhV* decreased in response to UVB, while other viral particles maintained their concentration levels. This may be explained as an increased decay rate, or as a decreased production rate caused by lower cell counts and the growth rate of *E. huxleyi* in this culture. The latter explanation assumes that the constant virus abundance in the PAR and the UVA cultures indicates that production equaled decay, and when the virus production drops under UVB stress, a net decrease in virus abundance will be observed. We found that UVB destroyed infectivity more quickly than virus particles. This disjunction between the loss of viral infectivity (inactivation) and the destruction of viral particles has already been previously reported [31] and may indicate the existence of two independent processes [55]. The inability of a virus to inject its genome into the host cell, mutations that make it unable to replicate, and the activity of host restriction enzymes are possible modes of viral inactivation [30].

The difference in UV sensitivity among viruses is intriguing. The fact that all viruses employed in this study were double-strand DNA viruses, with the same range of size and morphology (Phycodnaviridae) makes it difficult to explain the range in UVB sensitivity. Some possible explanations might be differences in the capsid structure, or specific genome properties of the viruses. Although there are clear differences in the genome sizes of the viruses studied here (Table 2), there was no correlation between the degree of sensitivity to UVB and viral genome size. The efficiency of gene-induced repair mechanisms is likely to explain the difference. Saanda and colleagues [41] have recently shown that viruses of marine phytoplankton like *C. ericina* or *P. orientalis* possess a larger genome size (> 500 kb) compared to other known phytoplankton viruses (typically *Chlorella* sp., < 400 kb [56]). This raises the question of whether this difference can be related to the presence of additional genes in viral genomes, especially given that increases in genome size are unlikely to be due to the presence of non-coding regions [41]. To date, only the virus of *Chlorella* has been shown to encode a DNA repair gene (i.e. a host-independent DNA UV repair function). Furuta et al. [39] showed that this virus possesses two separate DNA repair mechanisms: one that functions in the dark (virus-encoded enzyme) and one in the light (photoreactivation using host-encoded gene prod-

ucts). It is likely that such a combination significantly enhances survival and degree of infectivity of these viruses in aquatic surface waters. The presence of UV damage repair genes in *Chlorella* virus PBCV-1 (the phycodnaviridae type strain) suggests that other phytoplankton viruses may also encode UV damage repair gene(s). Thus, the explanation for the difference in sensitivity to UVB among virus types may be due to physiology, or to the difference in the host's capacity for DNA repair. The latter explanation is not supported by the results in Exp1 (*P. orientalis* is insensitive to UVB, while *M. pusilla* is sensitive). This remains to be demonstrated for the viruses employed in this study.

In contrast to the UVB effect, we did not find any effect of UVA compared to what was reported for some cyanophages [34]. Noble and Fuhrman [32] also observed that low levels of UVA contributed very little to the loss of infectivity of bacteriophages in contrast to UVB. This may be due to the relatively low UVA intensity applied in these experiments since it is known that UVA is involved in the recovery processes of some viruses [36,40]. A possible effect may also have escaped our attention as we did not measure viral infectivity directly. It is likely that the distinction between negative and positive effects of virus infectivity of marine phytoplankton may be very subtle. Also the comparison with results such as those of Garza and Suttle [34] is difficult since these authors reported that changes in the relative sensitivity to damaging radiation between cyanophage isolates and natural communities was the result of changes in the natural viral community. They hypothesized that increased resistance of cyanophages to solar irradiation could involve modifications leading to increased stability of the viral DNA (selection resulted in cyanophage communities that encode additional host-mediated repair mechanisms).

#### 4.3. Ecological considerations

The sensitivity of viruses to UVB has major implications on viral infectivity in seawater since it is well recognized that these wavelengths can penetrate to considerable depths, especially in oligotrophic waters [57]. On the one hand, a significant proportion of viruses may not be infective in surface waters [31,35,52]. However, on the other hand, photoreactivation [36] and cell-mediated reactivation can repair radiation-damaged virus DNA, and restore virus infectivity. Our data are consistent with reactivation, although they do not allow us to distinguish between host-mediated repair mechanisms or something more virus-specific. This also does not exclude other explanations. The simple observation that viruses demonstrate different sensitivities to UV radiation suggests indirect effects on phytoplankton community composition by lowering viral infectivity (leading to more frequent algal proliferation or blooms that last longer), or by reducing the ability of infectious viruses to contact host cells causing the host

cells to be less UV-resistant and subsequently more susceptible to dying. Viruses interfere with both blooming and non-blooming algae. The difference in viral impact on these two types may be attributed to the mechanisms that regulate the dynamics of viral infection [48,58] and the factors that determine the steady-state abundances of virus and host. In fact, the potential effect of UVB on marine algal viruses, and the differences demonstrated among viruses, may be one of the reasons why some phytoplankton escape viral control and form blooms. Indeed, how is it possible that phytoplankton populations are able to form blooms given the diversity of viral pathogens that seem to be present in the sea? For example, *E. huxleyi* and *Phaeocystis* sp. are both potentially important bloom-forming species [59,60], with blooms of *Phaeocystis* sp. typically occurring after those of *E. huxleyi* during spring and early summer. These blooms are controlled by viral activity [26,44]. In Exp1, we demonstrated that *E. huxleyi* was more sensitive to UVB than *P. pouchetii*. In turn, the viruses of *E. huxleyi* and *P. pouchetti* were strongly affected by UVB (Exp1 and 2). Our results suggest that these viruses may be potentially inactive because of the UVB stress in near surface waters, permitting the formation of blooms. This does not exclude other explanations such as the host density dependence for viral attack, protection of cells from viral adsorption by formation of mucus, cell cycle-dependent virus production [60], diel variation in viral decay [61,62] and facilitation of host survival via nutrient recycling [48]. By comparison, *C. ericina* and *P. orientalis* appear in low numbers in seawater [63]. The presence of *CeV* and *PoV* might have a regulatory effect on the two algal populations, preventing bloom formation. One reason for exhibiting this regulatory effect is that these viruses appear to be less sensitive to environmental stress, like UVB. Our experiments suggest that this explanation is plausible. Burst sizes that were consistent with previous studies (see Table 2) also revealed higher viral production for *C. ericina* and *P. orientalis* compared to *E. huxleyi* and *P. pouchetii*. This may be one mechanism that controls bloom formation that deserves further attention.

#### 5. Conclusion

We are well aware that a main drawback of this study was the use of intensity of irradiance, both for PAR and for UV. However, an effort was made to obtain intensities that are naturally found in the field. We did not use a saturating energy that would have led to complete algal growth inhibition, viral inactivation and/or destruction. The next step is to test different intensities of UV (both UVA and UVB) to measure the degree of sensitivity of the viruses of marine phytoplankton and the relationship between viruses and their hosts. The question of the role of UVA is also particularly intriguing (i.e. inactivation vs.

reactivation processes). The question of resistance should be investigated further to determine if cell resistance is due to morphological changes of the host, or if it is due to better UV repair mechanisms. The investigation of viral genomes for the purpose of finding specific genes implicated in UV-induced DNA damage repair is a priority. To date, only one freshwater virus infecting the eukaryotic alga *Chlorella* has been found to encode its own repair enzyme for excision of pyrimidine dimers [40]. There is no argument against similar repair systems existing in viruses of marine phytoplankton.

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