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1 **Interplay between the genetic clades of *Micromonas* and their viruses in the Western**
2 **English Channel**

3

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21

22 **Abstract**

23 The genus *Micromonas* comprises distinct genetic clades that commonly dominate eukaryotic
24 phytoplankton community from polar to tropical waters. This phytoplankter is also recurrently
25 infected by abundant and genetically diverse prasinoviruses. Here we report on the interplay
26 between prasinoviruses and *Micromonas* with regards to the genetic diversity of this host.
27 During one year, we monitored the abundance of 3 clades of *Micromonas* and their viruses in
28 the Western English Channel both in the environment, using clade-specific probes and flow
29 cytometry, and in the laboratory, using clonal isolates of *Micromonas* clades to assay for their
30 viruses by plaque-forming units. We showed that the seasonal fluctuations of *Micromonas*
31 clades were closely mirrored by the abundances of their corresponding viruses, indicating that
32 the members of *Micromonas* genus are susceptible to viral infection, regardless of their
33 genetic affiliation. The characterization of 45 viral strains revealed that *Micromonas* clades
34 are attacked by specific virus populations , which exhibit distinctive host clade specificity,
35 life history traits, and genetic diversity. However, some viruses can also cross-infect different
36 host clades suggesting a mechanism of horizontal gene transfer within the genus *Micromonas*
37 . This study provides novel insights into the impact of viral infection for the ecology and
38 evolution of the prominent phytoplankter *Micromonas*.

39

40 Key words: Mamiellophyceae, Phycodnaviridae, Prasinovirus, host specificities, life history
41 traits, DNA polymerase, English Channel

42

43 **Introduction**

44 Viruses are undoubtedly the most abundant biological entities in the ocean and they
45 intimately interact with every facet of the marine biosphere. Through these interactions,
46 viruses profoundly influence the global biogeochemical cycles by altering the structure and
47 the function of marine communities and by contributing to the cycling of major elements (for
48 review see Suttle, 2005, 2007; Sime-Ngando, 2014). In spite of their global-scale
49 implications, the nature and the dynamics of virus-host interactions in marine environments
50 are far from understood and many basic, yet fundamental, questions remain unsolved: Who
51 are the hosts infected by marine viruses? What are the infection strategies evolved by viruses?
52 How do virus-host interactions vary in time and space? In this respect, characterizing the
53 interplay of ecologically relevant virus-host model systems is a prerequisite for advancing our
54 understanding of virus impacts in nature.

55 Viruses that infect the cosmopolitan green picoalga *Micromonas* were first reported by
56 (Pienaar, 1976) and they were found to co-occur with their hosts in a wide range of marine
57 environments, from temperate to cold waters (e.g., Cottrell and Suttle, 1991, 1995a; Sahlsten,
58 1998; Zingone *et al.*, 1999). The majority of known *Micromonas* viruses belong to the genus
59 *Prasinovirus* in the Phycodnaviridae family. Virions are tailless, they exhibit icosahedral
60 capsids of 100 -125 nm in diameter and dsDNA genome of approx. 200 kb (Mayer and Taylor
61 1979; Cottrell and Suttle 1991; Zingone *et al.*, 1999). Yet, these prasinoviruses have variable
62 tolerance to chloroform (Martinez *et al.* 2015) suggesting ultrastructural divergence and they
63 exhibit significant level of genetic variation (Cottrell and Suttle, 1995b). These viruses
64 display a marked seasonal dynamics (Sahlsten, 1998; Zingone *et al.*, 1999) and they were
65 shown to cause considerable amount of mortality in their host population (Cottrell and Suttle,
66 1995a; Evans *et al.*, 2003). *Micromonas* strains show a great variability with respect to their
67 susceptibility to infection, indicating that viruses do not only quantitatively but also

68 qualitatively regulate their host populations (Sahlsten, 1998; Zingone *et al.*, 1999, 2006). So
69 far, the infection patterns and processes underlying these complex interactions are poorly
70 understood. Of particular interest, the relation between the susceptibility to infection and the
71 genetic diversity of *Micromonas* has seldom been investigated (Zingone *et al.*, 2006).

72 The ubiquitous genus *Micromonas* is genetically diverse and comprises an assemblage
73 of 3 (Guillou *et al.*, 2004) to 5 (Šlapeta *et al.*, 2006; Worden, 2006) discrete phylogenetic
74 clades (or lineages) of flagellated cells that correspond, at least, to 3 distinct species (Simon,
75 unpublished). These clades are often sympatric in marine ecosystems (Foulon *et al.*, 2008;
76 Šlapeta *et al.*, 2006). Yet, their relative contribution to total *Micromonas* abundance varies in
77 time and space, suggesting that they occupy specific niches (Foulon *et al.*, 2008). The factors
78 that regulate this clade dynamics are not clearly understood. In all likelihood, *Micromonas*
79 clades exhibit differential responses to abiotic factors but they might also respond differently
80 to predation risks, including those imposed by viruses.

81 To test whether *Micromonas* clades display differential susceptibility to virus
82 infection, we combined field and laboratory experiments on 45 novel viral isolates in order to
83 characterize the interactions between the genetic clades of *Micromonas* and their viruses in
84 the Western English Channel (WEC) throughout the year 2009. The WEC constitutes an ideal
85 study site where *Micromonas* is known to dominate the picophytoplankton community (Not *et*
86 *al.*, 2004). The three main *Micromonas* genetic types, designated as clades [A.ABC.12],
87 [B.E.3], and [C.D.5] (Worden, 2006) and here referred to as clades A, B, and C, respectively,
88 are recorded year round in this ecosystem (Foulon *et al.*, 2008). Our study revealed that
89 *Micromonas* clades interact with specific viral populations that display distinctive dynamics
90 and life history traits.

91

92 **Results and Discussion**

93

94 **Physico-chemistry at the sampling station**

95 Strong tidal mixing produces a permanently mixed water column at the long term
96 monitoring coastal station SOMLIT-Astan (48°46'N, 3°58'W, Marrec *et al.*, 2013). During the
97 sampling period (February to December 2009), the water temperature progressively increased
98 from 8.8°C in February to a maximum of 16.9°C in September while salinity varied between
99 34.8 (March) and 35.3 (September). Nutrient dynamics varied according to the classical
100 pattern observed in this area (Not *et al.*, 2004). Phosphate and nitrate concentrations showed
101 comparable dynamics with minima recorded in summer (0.16 and 0.7 µM, respectively) and
102 maxima during the winter period (0.53 and 11 µM, respectively). Detailed dynamics of
103 biogeochemical variables at SOMLIT-Astan are publicly available on the observatory website
104 (<http://somlit-db.epoc.u-bordeaux1.fr/bdd.php>).

105

106 **Dynamics of *Micromonas* clades in the Western English Channel**

107 The phytoplankton community at SOMLIT-Astan during this period was numerically
108 dominated by picophytoplanktonic cells, which comprised, on average, 90% of the total
109 community in 2009 (data not shown). Among these, the abundance of the picoeukaryotes
110 varied between 2.1 and 16.5×10^3 cells mL⁻¹ with *Micromonas* spp. accounting for 20% to
111 80% of the total counts as shown by a combination of flow cytometry (FCM) and TSA-FISH
112 analyses (Fig. 1A). As reported previously, this prominent genus displayed marked seasonal
113 dynamics with major peaks of abundance recorded in late spring (6.6×10^3 cells mL⁻¹), early
114 summer (6.3×10^3 cells mL⁻¹), and mid-autumn (4.4×10^3 cells mL⁻¹) (Foulon *et al.*, 2008;
115 Not *et al.*, 2004).

116 The 3 main *Micromonas* genetic clades (hereafter referred to as clades A, B, and C)
117 exhibit recurring seasonal dynamics in the WEC according to previous time series (Foulon *et*

118 *al.*, 2008). We confirmed this pattern of succession in 2009 (Fig. 1B). *Micromonas* clade A
119 numerically dominated the bloom from February to mid-May comprising 63 to 77% of the
120 total counts. From June to December, both genetic types A and B equally contributed to
121 *Micromonas* counts, although clade B cells showed a sudden, yet unexplained, drop in
122 abundance on June 16. Cells that belonged to clade C were the least abundant accounting, on
123 average, for $7 \pm 6\%$ of the total counts with somewhat higher contribution during the winter
124 period (14%) than the remainder of the year (5%).

125 The ecological processes that influence the shifts in the relative abundance of
126 *Micromonas* clades are unclear. It is traditionally believed that global-scale distribution and
127 diversity of species are mostly driven by ability to withstand abiotic controls (Wiens et al.
128 2011). Previous study on the global distribution of *Micromonas* clades suggested that clade B
129 tend to thrive in warmer coastal waters whereas clade C mostly occur during low-light
130 conditions (Foulon *et al.*, 2008). At local spatial extents, it is however well accepted that
131 species distribution is also influenced by biotic controls (Wiens et al. 2011). It is thus likely
132 that besides eco-physiological adaptations, biotic interactions also regulate the observed
133 *Micromonas* clade dynamics in the Western English Channel.

134 **Dynamics of *Micromonas* viruses in the Western English Channel**

135 Viruses that infect *Micromonas* (hereafter referred to as MicV), mainly prasinoviruses,
136 have been observed and isolated from different oceanic regions (Cottrell and Suttle, 1991,
137 1995a; Sahlsten, 1998; Zingone *et al.*, 1999; Short and Short, 2008). FCM analyses of
138 cultured isolates showed that these prasinoviruses consistently cluster within a well-defined
139 population based on their nucleic acid fluorescence (upon Sybr green I staining) and side
140 scatter properties (Fig. S1). In the WEC, this specific population accounted for a small but
141 significant proportion (on average 10%) of the total virus counts and displayed a marked
142 seasonal dynamics with concentration ranging from $3.4 \times 10^5 \text{ mL}^{-1}$ in February to 8.8×10^5

143 mL⁻¹ in July 2009 (Fig. 2A). This FCM population probably does not exclusively comprise
144 *Micromonas* viruses, but counts of this viral population covary strikingly well with
145 *Micromonas* abundances ($r=0.675$, $n=20$, $p<0.005$), suggesting that viruses might perhaps
146 control abundance of this genus during the sampling period.

147 To further investigate this hypothesis, we quantified infectious MicV in the WEC by
148 plaque assay using cultures of each 3 *Micromonas* clades. Significant differences in the
149 number of PFU were detected depending on the phylotype of the host culture (Fig. 2B). As
150 also recorded for their hosts, PFU obtained on *Micromonas* clade A and B (hereafter, PFU-A
151 and PFU-B, respectively) were substantially more abundant than PFU formed on clade C-
152 hosts (hereafter PFU-C). During the sampling period, PFU-A abundance increased
153 concomitantly with the development of *Micromonas* clade A. Their maximum abundance (8
154 $\times 10^2$ virus mL⁻¹) was observed 2 weeks after the peak of host abundance (Figs. 1B and 2B).
155 The monitoring of PFU-B showed low concentration during the winter-spring period but their
156 dynamics was tightly coupled to their host abundance from the summer period until the end of
157 the year, reaching a peak of 11×10^2 virus mL⁻¹ in autumn. Regarding PFU-C, infectious
158 particles were detected year round but their abundance was 10 to 100-fold lower than PFU-A
159 and -B with no clear relation to their specific host dynamics. Because of variable strain
160 susceptibilities to viral infection, it is difficult to compare our results to former PFU
161 monitoring on *Micromonas* host cell clades although maximal abundance were within the
162 same range ($1.0 - 4.6 \times 10^3$ PFU mL⁻¹, Cottrell and Suttle, 1991; Sahlsten, 1998; Zingone *et*
163 *al.*, 1999). For the same reason, this assay most likely underestimates the global abundance of
164 viruses that infect each *Micromonas* phylotype. Nonetheless, it provides unequivocal
165 evidence that *Micromonas*, regardless of their genetic clade, were subject to viral attack year
166 round in the WEC.

167

168 ***Micromonas* virus - host interactions**

169 The above results led us to question whether specific virus populations are associated to
170 *Micromonas* clades and, if so, how these virus populations interact with their hosts. To
171 tentatively address this question, 45 viruses (14-16 per host clade) were isolated throughout
172 the sampling period and they were characterized. These viruses were selected randomly from
173 a collection of 176 viral isolates obtained from plaques on *Micromonas* clade A, B, and C
174 along the year 2009. Regardless of the host on which they were isolated, MicVs exhibited
175 sizeable icosahedral capsids (110 to 130 nm in diameter) as determined by negative staining
176 using transmission electron microscopy, and large dsDNA genome, as suggested by their flow
177 cytometry signature upon Sybr green I staining (Fig. S1). We therefore assigned our isolates
178 to the virus family Phycodnaviridae and the genus *Prasinovirus*.

179

180 *Host specificities* - The host range of these 45 prasinoviruses was then determined by pair-
181 wise infection using 14 strains belonging to the 3 *Micromonas* clades (Table 1, Table S1). The
182 large majority of our isolates (35 MicVs) were strictly clade-specific. They infected host
183 strains that belonged only to the clade of their initial host. The remaining 10 viruses could
184 propagate at comparable yield on hosts belonging to clades A and B. Six viruses (out of 15)
185 isolated on a clade A strain could also infect clade B strains, and 4 viruses (out of 14) isolated
186 on a clade B strain could also infect clade A strains. Within each main pattern of specificity
187 (A, B, AB, and C), a remarkable clonal diversity was observed with 26 unique specificity
188 profiles that included variable virus infection types ranging from generalist to specialist.

189 In order to test the structure of this infection network, we applied a network-based
190 analysis according to Flores *et al.* (2011) and Weitz *et al.* (2013). As observed previously for
191 phage-bacteria infection networks (Flores *et al.*, 2011), the structure of *Micromonas* - virus
192 interactions was statistically modular. This statistical analysis applied at the whole matrix

193 scale and intra-module scale discriminated 3 modules, which comprised the viruses isolated
194 on hosts from clade A, clade B, and clade C, respectively (Fig. S2). Altogether, these results
195 suggest that *Micromonas* clade A, B, and C interact with distinct, yet diversified, viral
196 populations. The observed variability in strain-specificity indicates that viruses influence the
197 intraspecific diversity within each *Micromonas* clades. We also showed that host switches can
198 occur between the more closely related host clades (those that belong to clades A and B),
199 suggesting that viruses could also promote *Micromonas* diversity through horizontal gene
200 transfers within this genus.

201

202 *Virus infection strategies* - One-step growth experiments conducted on 4 to 5 representative
203 viruses per host clade indicated a similar clade-specific grouping of our isolates. This assay
204 provided estimates of the virus burst size and latent period, which can be related to the virus
205 life strategy. By analogy to the theory of the r- and K-selection (MacArthur, 1967),
206 opportunistic (the most r-selected) viruses are those that exhibit short generation times and
207 high burst sizes whereas less virulent (the most K-selected) viruses are those that induce low
208 mortality in their host population (Suttle, 2007) and they propagate through latent or chronic
209 infection as observed for specific *Ostreococcus* viruses (Thomas *et al.*, 2011). Based on these
210 parameters, we detected divergent life strategies in *Micromonas* viruses depending on the host
211 clades that they attack (Fig. 3). The viruses isolated on clade B hosts tended to be the most
212 virulent with short latent period and relatively high progeny production, regardless of their
213 infection range types (i.e. specialist or generalist) whereas the viruses isolated on clade C
214 strains tended to be the least virulent with generally extended latent period (up to 30 h) and
215 moderate to high burst size (Fig. 3). Viruses isolated on clade A hosts exhibited an
216 intermediate phenotype with moderate latent period and low burst sizes.

217 In contrast to past theories (trade-offs hypothesis, (Poulin, 1998)), generalism (i.e.,
218 viruses that propagate on several host clades) was not associated with any compensatory
219 effect on the burst size or on the latent period. The infection kinetics of the selected viral
220 isolates does not correlate to the growth rates of their host (Fig. S3). , The observed
221 differences in infection strategies could be explained by the dynamics of their respective host
222 clade in the studied area. For example, *Micromonas* clade C is present year round at low
223 abundance. It is thus tempting to speculate that viruses specific to this host clade had to
224 evolve long latent periods to be maintained within the host population and fairly high progeny
225 production to increase the probability of encounter with their host (Abedon *et al.*, 2001). By
226 contrast, *Micromonas* clade B displays a marked seasonal dynamics in the WEC with high
227 abundance recorded between mid-June and end-September. We could thus expect that
228 corresponding viruses had to evolve an opportunist lifestyle to efficiently exploit their
229 transient resources.

230

231 **Phylogenetic relationships between *Micromonas* viruses**

232 To investigate the phylogenetic relationships between these viral isolates, we
233 amplified a fragment of the gene encoding their DNA polymerase (*polB*, Chen and Suttle,
234 1996, Table S2). This sequence is well conserved among Phycodnaviridae, yet there is
235 sufficient sequence variability to build phylogenetic relationships between viruses from the
236 same genus (Bellec *et al.*, 2009). In spite of their affiliation to the Phycodnaviridae family, the
237 amplification of the *polB* gene using the AVS1/AVS2 primers failed for 8 of the 45 selected
238 isolates including B-, C- and AB-specific viruses. The reason for this failure is unclear. We
239 cannot exclude that the sequence targeted by the degenerated primers might be too divergent.
240 It is also possible that these polymerases carry an intein that makes the PCR product too long
241 to amplify easily under the tested conditions (Clerissi *et al.*, 2013).

242 The partial *polB* sequences of the remaining 37 isolates fell into 29 haplotypes (Fig.
243 4). The large majority of these haplotypes (24 out of 29) were new, i.e. different from *polB*
244 sequences in public databases (BLAST analyses, <http://www.ncbi.nlm.nih.gov/>). In most
245 cases, viral isolates that belonged to the same haplotype exhibited different specificity
246 patterns, suggesting that *polB* sequencing underestimates the functional diversity of
247 phycodnaviruses as reported previously (Baudoux and Brussaard, 2005; Bellec *et al.*, 2009;
248 Clerissi *et al.*, 2012). In the phylogenetic trees, *polB* sequences of viruses infecting clade C
249 hosts formed a well-supported cluster while viruses that infect clade A and clade B hosts
250 distributed into multiple branches suggesting a higher level of genetic diversity. Interestingly,
251 regardless of the host strain they infect, viruses that were highly specific (that infect one
252 single strain among all strains tested) fell apart from other viruses (i.e. RCC4232 and
253 RCC4240, 4242, 4243, Fig. 4 and Table 1). The prasinoviruses that infect *Ostreococcus tauri*
254 showed a similar phylogenetic pattern and tended to cluster with counterparts that exhibit
255 similar infection range types (Clerissi *et al.*, 2012).

256 This recurrent correlation between *polB* phylogeny and the number of hosts infected
257 by a given prasinovirus is somewhat puzzling. The *polB* gene is indeed used as a neutral
258 marker of evolution in Phycodnaviridae (Chen and Suttle, 1996; Dunigan *et al.*, 2006), that is,
259 this gene is well-conserved among this virus family whereas specificity markers are expected
260 to be more variable genes (Clerissi *et al.*, 2012). Furthermore, virus-host specificity is
261 typically determined during events upstream the virus DNA replication. Several studies
262 reported that the virus attachment on host cell surface determines the susceptibility of marine
263 plankton to infection (e.g., Tarutani *et al.*, 2006; Mizumoto *et al.*, 2007; Stoddard *et al.*,
264 2007). In other words, the evolution of resistance to viral infection is mostly due to changes in
265 host surface properties that limit viral attachment. Recently, Thomas *et al.* (2011) evidenced
266 an intriguing mode of resistance in the prasinophyte *Ostreococcus tauri*. This study

267 demonstrated that prasinoviruses could still attach on resistant strains but they did not produce
268 viral progeny. It is thus likely that the resistance mechanism was not due to a change in the
269 host receptor but rather occurred at the DNA entry or DNA replication stage, in which the
270 *polB* gene product would be involved. Alternatively, viral attachment and internalisation
271 might be a two-stage process as in reoviruses (Reiter *et al.*, 2011). The notion that DNA
272 replication machinery might be involved in host specificity is of course highly speculative but
273 the repeatedly observed link between *polB* clustering and prasinovirus infectivity range
274 certainly deserves appropriate investigation.

275

276 **Concluding remarks**

277 In the Western English Channel, a complex assemblage of viruses is associated to the
278 dominating picophytoplankton species *Micromonas spp.* Our study revealed, for the first time,
279 that the main *Micromonas* genetic clades (clade A, clade B, and clade C) are attacked by
280 specific virus populations which displayed distinctive dynamics and life history traits. The
281 apparently high variability in *Micromonas* virus specificities suggests that viruses maintain a
282 high genetic diversity within each of these clades. However, host switching can occur
283 (particularly for hosts that belong to the clade A and B) suggesting that virus could also
284 influence *Micromonas* diversity through horizontal gene transfers. The complete genome
285 sequencing of representative viruses and hosts should provide insightful information on the
286 extent of these putative genetic exchanges. It is of course critical to keep in mind that our
287 model-based approach only reflects a partial picture of the actual diversity of virus-host
288 interactions. Still, these approaches provide invaluable information to improve our
289 understanding of the mechanisms of virus-host interactions as well as the evolution and
290 outcomes of these interactions in natural environments.

291

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302

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