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A marine virus controls the green algae *Ulva lactuca* proliferation in the bay of Marseille

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Abstract

Background. *Ulva lactuca*, a green macro algae, is a worldwide problem due to green tides observed in Western Europe (particularly in Brittany, west of France) and in Eastern China. It is a nitrophilic algae and surprisingly *Ulva* proliferation is not observed in the bay of Marseille (Provence, south of France) despite abundant sources of nitrate due to the Rhône River.

Methods and Results. In this study we analyzed possible reasons why *Ulva* proliferation stops rapidly at the end of winter in the bay of Marseille. We show that nitrate concentration at Marseille is an average of $6 \pm 2 \mu\text{M}$ and this concentration is similar to what is observed in Brittany. *Ulva* collected in Brittany can grow and change its phenotype with sea water samples collected in Provence in winter but Breton *Ulva* become white (bleaching) with sea water samples collected in Provence in June 2018, 2019 and 2020. Breton *Ulva* bleaching is still observed after $0.2 \mu\text{m}$ sea water filtration and DAD HPLC shows a peak with a viral particle UV spectrum increasing with *Ulva* bleaching. Furthermore, epifluorescence microscopy analysis of SYBR-Gold pigment stain with $0.2 \mu\text{m}$ filtered sea water shows that virus-like particles increase to 6.5 million per ml with *Ulva* bleaching.

Conclusion. This study suggests that a virus in bay of Marseille sea water induces *Ulva* bleaching and controls its proliferation. Sea water containing this virus, which is not harmful for mammals or other species including algae that are not *Ulva*, may be used to control *Ulva* proliferation.

Keywords: *Ulva lactuca*; green tides; nitrate, sea water, virus.

41 Introduction

42 *Ulva lactuca* is a green macro alga that belongs to the phylum *Chlorophyta* [1]. *U. lactuca* called
43 “sea lettuce” was first described by Linnaeus in the Baltic Sea in the eighteen century and has a
44 belayed cell structure with a talus having a flat bladeliike appearance [2]. This alga is able to
45 grow both with a holdfast on rocks or free floating. *U. lactuca* has also the capacity to reproduce
46 with two methods, one being sexual and the other being from fragmentation of the talus [2].
47 These two methods provide a capacity to rapidly proliferate and to cover the water surface
48 decreasing the biodiversity even for other algae species [2]. *U. lactuca* is a polymorphic alga
49 regarding the degree of water salinity [3] or symbiosis with bacteria [1]. This polymorphism led
50 some authors to propose that different species may exist such as *U. armoricana*, *U. fasciata*, *U.*
51 *pertusa*, *U. prolifera*, *U. rigida*, *U. rotundata*, etc... but a genetic analysis revealed that these
52 different phenotypes were not due to genetic variations that would justify that different species
53 other than *U. lactuca* may exist [4]. These different phenotypes should be described as variants
54 or clades of *U. lactuca* [2]. We made the choice to use *Ulva* for the different *U. lactuca* clades
55 used in this study.

56
57 *Ulva* blooms are a great source of trouble in Brittany (west of France) because they damage
58 marine ecosystems, impair local tourism and *Ulva* are difficult to valorize when they are
59 collected on beaches [2]. *Ulva* accumulate on beaches and its biodegradation produce acidic
60 vapors (mainly H₂S) that are deadly for animals and potentially for human beings [2]. Another
61 human health problem is due to amoeba proliferation on beaches. Amoeba is the natural
62 scavenger for *Ulva* biodegradation and Amoebiasis is the second leading cause of death from
63 parasitic disease worldwide [5]. *Ulva* is a nitrophilic algae and the reason of its proliferation was
64 proposed to be due to intensive agriculture and fertilizers as a source of nitrate. However, the
65 first *Ulva* bloom to be described was at Belfast (North of Ireland) at the end of the nineteen
66 century and was due essentially to an increase of human wastes that are also rich in nitrates [6].
67 *Ulva* blooms were studied in the Laguna of Venice from 1930 and a correlation was established
68 with the increase of tourism and human wastes [7-8]. An isotope study following a cyanobacteria
69 proliferation in Florida revealed that the main source of nitrates was from human wastes instead
70 of fertilizers [9].

71
72 Although green tides due to *Ulva* proliferation are observed worldwide, the Mediterranean seas
73 appear to be protected from green tides due to *Ulva* proliferation or blooms. Even in the Laguna
74 of Venice, *Ulva* blooms occur only during short periods for springtime since 1990 despite
75 tourism and fertilizers carried through the Pô River [8]. *Ulva* blooms were never observed in the
76 bay of Marseille. Marseille is located nearby the mouth of the Rhône River, which is a big river
77 carrying large amounts of nitrate with a concentration up to 100 µM and an average flow rate of
78 9m³/sec [10]. This study selected three main hypotheses to explain the control of *Ulva*
79 proliferation. The first one is that the nitrate concentration in the bay of Marseille is much lower
80 compare to Brittany coastal water. The second one is that *Ulva* in Brittany could have

81 specificities to proliferate in Brittany and could not survive in Mediterranean seas. The third one
82 is that a pollutant or a living organism could limit *Ulva* proliferation in Provence.

83

84 **Materials & Methods**

85

86 *Sea water analyses and Ulva bleaching:* Sea water samples were collected in surface in June 15th
87 and June 18th 2018, April 15th, June 4th and June 10th 2019 and June 8th and June 12th 2020 (n=7)
88 in eight different spots in Brittany and Provence (Table 1). In Brittany (BR and PO) and in Provence
89 (RN, WF, RS) were open coastal sea water but TR in Brittany is a fjord, PR in Provence is a marina
90 and MU in Provence is a fjord (Calanque). Conductimetry was carried out with the INOLAB
91 device (Weither, Germany). Acidity was tested with a CRISON pH Meter (Barcelona, Catalonia,
92 Spain). The pH meter was calibrated before any measurement. Nitrate were measured with a
93 METRHOM chromatographic device (Berne, Switzerland) with a Metrosep column A supp
94 5 150/4 mm with 3.2 mM Na₂CO₃/1 mM NaHCO₃ as solvent. Sea Water was diluted 1/8 and a
95 standard was used to calibrate nitrates. *Ulva* were collected mainly for springtime in the Trieux
96 fjord (TR) in the north coast of Brittany (48°46' N, 3°6'O). The green tubular algae (figure 1)
97 formerly called *Enteromorpha* was collected in the same TR spot on November 2018. The
98 incubation was carried out for one month at 20°C and day light exposure with sea water collected
99 in June 2018 in the north bay (RN) of Marseille (43°18' N 5°16'E). For *Ulva* bleaching
100 experiment, *Ulva* were collected at TR in June 15th 2018, June 4th 2019 and June 8th 2020, then
101 immediately sent in Provence. When *Ulva* were shipped at Marseille, they were green and
102 healthy. They were cut in pieces of 1 cm² and put in sea water (40 ml) in tubes (n=25 for each
103 spot) closed with a tape and let at day light (12 hours) with an average temperature of 25°C for
104 one week. Controls were made with seawater collected in Brittany the very same day when *Ulva*
105 were collected. Other experiments were carried out with *Ulva* collected in Provence at MU on
106 April 15th 2019 and *Ulva* collected in South Brittany (PO) on June 8th 2020 (data not shown)

107

108 *Optical microscopy:* Optical microscopy (10X) was performed on healthy *Ulva lactuca* before
109 confluence, after acidic biodegradation and on white *Ulva* after five days with water sample
110 collected in the PR spot in the bay of Marseille (see Table 1). Pictures were carried out with a
111 camera Nikon D3100 coupled to a Nikon Eclipse Ti L100 microscope (Nikon, Tokyo, Japan).

112

113 *Diode Array Detection High Performance Liquid Chromatography (DAD HPLC):* Sea water
114 samples were filtered at 0.2 µm and were analyzed on a Beckman HPLC system gold device
115 with a reverse phase (C8) column using H₂O 0.1 % TFA (A) and CH₃CN 0.1%TFA (B). The
116 gradient was from 10 to 50 % B in 40 min, and then 10 min at 90 % B and 10 min at 10 % B.
117 Diode Array Detector Beckman device was coupled after the injector. Flow rate was 0.8 ml/min.

118

119 *Fluorescence microscopy after SYBR staining.* Mediterranean seas water without and with *Ulva*
120 sample was filtrated through 0.22 µm membrane filter (Millex cat n° SLGP033RS) to remove

121 cells and then through 0.02 μm Anodisc filters (Whitman cat n° WHA68096002) using a vacuum
 122 filtration system to collect viral particles. Then filters were stained with SYBR Gold dye (*N,N'*-
 123 dimethyl-*N*-[4-[(*E*)-(3-methyl-1,3-benzothiazol-2-ylidene)methyl]-1-phenylquinolin-1-ium-2-yl]-
 124 *N*-propylpropane-1,3-diamine) that binds reverently to DNA (Invitrogen cat n° S11494) at room
 125 temperature for 15 min in the dark, and washed three times with 500 μL of sterile 0.02 μm -
 126 filtered mQ water. Stained virus-like particles were observed with an epifluorescence
 127 Microscope Leica SP2.

128

129 Results

130

131 **Nitrate concentration is similar in Brittany and in Provence coastal water nearby the**
 132 **Rhône River:** *Ulva* is naturally present in the bay of Marseille and appears each year in winter.
 133 *Ulva* grows rapidly from February to March and disappears for springtime. *Ulva* proliferation
 134 was never reported in the bay of Marseille and a first hypothesis could be that the nitrate
 135 concentration is lower compared to sea water in Brittany. Five spots called RN, WF, RS, PR and
 136 Mu nearby Marseille were selected and sea water samples were collected in June 2018, 2019 and
 137 2020 and compared to three spots in Brittany (Table 1). Longitude and Latitude for the 8 spots
 138 are indicated in Table 1. TR and BR are in Northern Brittany coast near Paimpol while PO is in
 139 Southern Brittany coast near Nantes. From 2018, 2019 to 2020, *Ulva* collected in North Brittany
 140 (TR) was incubated with sea water from the five spots in Provence collected during springtime.
 141 The bay of Marseille is from 20 km of the mouth of Rhône River and North West winds (Mistral
 142 and Tramontane) blow regularly from Rhône River to Marseille. Table 1 shows that pH and
 143 conductimetry (related mainly to salinity) are lower in RN and WF regarding RS due probably to
 144 the influence of Rhône river. Table 1 shows that the concentration in nitrates in open coastal sea
 145 water is equivalent *in Brittany (BR and PO) and in Provence (RN, WF, RS)*. However, nitrate
 146 concentration can be much higher in Brittany fjord (TR) or in marina (PR) in Provence.

147

148

Table 1

Springtime Sea Waters from Brittany (TR, BR & PO) and Provence (RN, WF, RS, PR & MU).

(In Brittany BR and PO and in Provence RN, WF and RS were open coastal sea water but TR in Brittany is a fjord,
 PR in Provence is a marina and MU in Provence is a calanque)

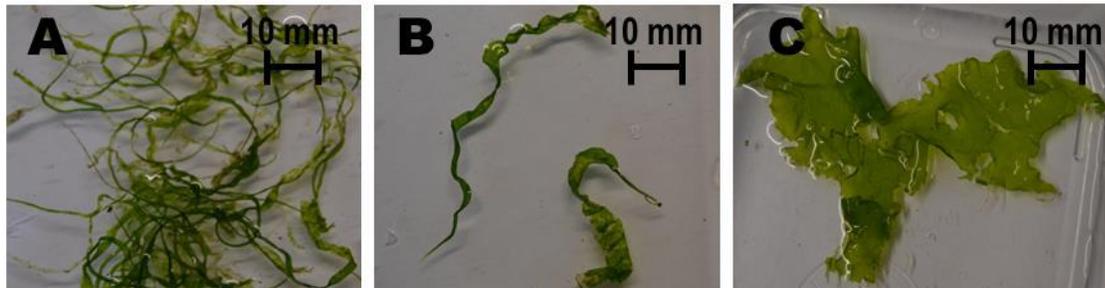
Location	TR	BR	PO	RN	WF	RS	PR	MU
Latitude (N)	48°46'	48°43'	47°06'	43°18'	43°14'	43°15'	43°14'	43°09'
Longitude	3°06'W	2°56'W	2°07'W	5°17'E	5°17'E	5°19'E	5°21'E	5°36'E
Nitrate (μM)	24 \pm 583	7 \pm 3	11 \pm 2	5 \pm 2	6 \pm 1	5 \pm 2	24 \pm 5	9 \pm 3
pH	7.9	8.0	8.1	7.9	8.0	8.0	8.0	8.0
Salinity (mS)	30.9	45.6	48.1	57.4	56.1	57.9	57.8	58.1
Bleaching* (%)	0	0	0	0	0	4 \pm 4	96 \pm 4	88 \pm 8

159 *Percentage of *in vitro* Breton *Ulva* bleaching with sea water samples collected for springtime in 2018, 2019 and
 160 2020 (n=7). All experiments were carried out with Breton *Ulva* collected in Trieuc fjord (TR) in northern Brittany in
 161 2018, 2019 and 2020 and 25 tubes for each sea water sample.

162

163

164 **Breton Ulva can grow in Mediterranean seas:** Breton *Ulva* can grow rapidly with sea waters
165 from Marseille (Fig 1). *Ulva* was collected in the Trieux fjord (North Brittany) with a green
166 tubular phenotype (Fig 1A) and changed its morphology after one month at $20 \pm 10^\circ\text{C}$ and day
167 light exposure (Fig 1C). This experiment illustrates the *Ulva* polymorphism that might be due
168 either to a difference in salinity as previously described [3] or other parameters such as day light.
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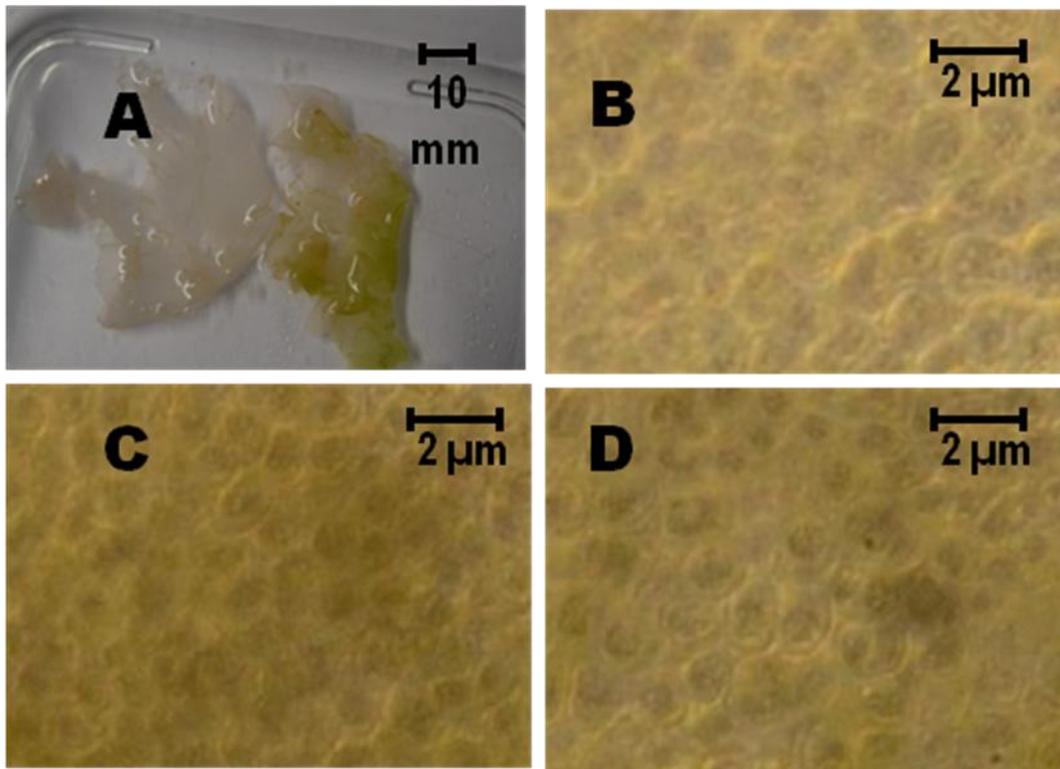


170
171
172 Figure 1: **Breton Ulva can grow in Mediterranean seas** A, *Ulva* with a green tubular phenotype was collected in
173 November 2018 in the Trieux fjord in the north coast of Brittany (TR see Table 1). B, the tubular morphology began
174 to change after incubation at $20 +10^\circ\text{C}$ and day light exposure with sea water collected in June 2018 in the RN spot
175 in bay of Marseille (see Table 1). C, after one month the transformation was completed. This experiment shows the
176 polymorphism of *Ulva* and the possibility for Breton *Ulva* to grow in Mediterranean seas water.
177

178 **Breton Ulva bleaching in sea water samples collected in Provence.** Natural biodegradation on
179 beaches occurs when *Ulva* reach confluence inducing anoxia characterized by production of H_2S
180 [2]. For this biodegradation, *Ulva* can become white due to dehydration. *Ulva* are intolerant to
181 severe desiccation and the induced sub lethal stress due to reduced photosynthetic performance
182 involves a loss of pigmentation [11]. However, this is a different phenomenon that we observed
183 with Breton *Ulva* in sea water collected in the PR spot in bay of Marseille. Breton *Ulva* were
184 turning white rapidly (bleaching) sometime in one day without dehydration. These experiments
185 were carried out in 2018, 2019 and 2020 with sea water samples from the five spots in Provence
186 and was reproducible each year (Table 1). No bleaching was observed with sea water collected in
187 Brittany for springtime when *Ulva* proliferation occurs at its highest level. Regarding the five
188 different spots in Provence, the number of tubes where bleaching was observed was not the
189 same. Bleaching was observed mainly at PR and MU spots (Table 1). At PR, *Ulva* proliferation
190 was observed only in winter while at MU *Ulva* was still observed at the beginning of springtime.
191 In tubes where *Ulva* could proliferate, confluence could be reached from one week to three
192 weeks and acidity was detected. As observed in natural conditions *Ulva* remained green under
193 biodegradation. No acidity was observed with *Ulva* bleaching and *Ulva* became white generally
194 after 5 days. In tubes where *Ulva* could not grow, *Ulva* were becoming white under day light at
195 20°C in five days with no acidity detected, as shown in Figure 2A. This bleaching was not
196 similar to white dehydrated *Ulva* observed in Brittany when *Ulva* was drying on the shore. Sea
197 water from PR collected in June 2018 was kept from one month and six months before to be
198 incubated again with Breton *Ulva* and bleaching was no longer observable after 6 months (data

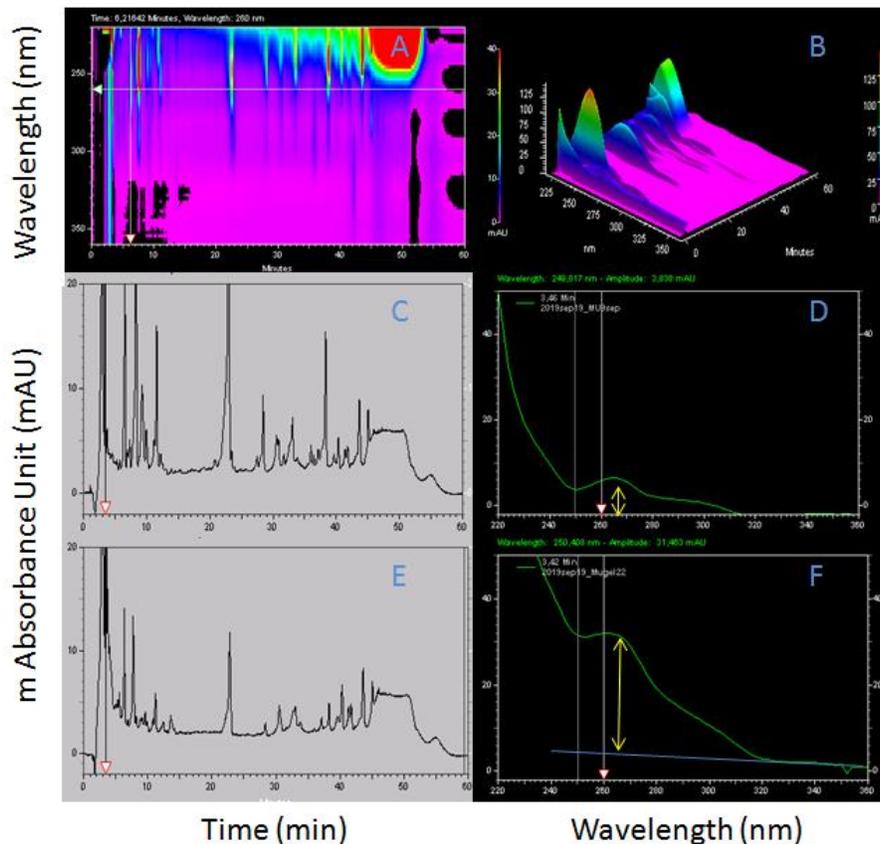
199 not shown). Chemical pollutants would have induced *Ulva* bleaching from D1 to D180. Other
200 Breton algae (mainly brown such as Wrack) were tested and no bleaching was observed with sea
201 waters from PR (data not shown). A pollutant would induce changes to these other algae and
202 bleaching appears to be specific to *Ulva*.

203
204 **Optical microscopy showed that bleaching was not due to *Ulva* sporulation.** *Ulva* bleaching
205 was characterized with *Ulva* tissue becoming white in solution (Figure 2A). Optical microscopy
206 showed that the tissue remained unaffected upon bleaching (Figure 2B) with a regular cell
207 appearance similar to healthy *Ulva* that have a tall made of tight cells with chloroplasts in
208 cytoplasm giving a green color to cells (Figure 2C). The white color in Figure 2B showed that
209 cells were dead but this death was not due to a macro predator or stress due to environmental
210 conditions as in dehydration that disrupted the tissue organization with liberation of chloroplasts
211 in the interstitial middle as shown in Figure 2D. This was not a sporulation that could give a
212 white color to cells. In that case, cells should become spherical with a large interstitial middle.
213 Finally, when sea water was filtrated at 0.2 μm , *Ulva* bleaching could still occur showing that
214 bleaching was not due to planktons, amoeba or bacteria having a size $> 0.2 \mu\text{m}$. Only a virus
215 having a size $< 0.2 \mu\text{m}$ could explain this rapid death of *Ulva* cells without tissue damage.



216
217 Figure 2: **Comparison of bleached *Ulva*, regular *Ulva* and anoxia *Ulva* with optical microscopy.** A *Ulva* became
218 white in five days following incubation at 20°C and day light exposure with sea water from PR spot (Table 1). B.
219 Optical microscopy (10X) of white *Ulva*. The white color is due to cellular death but *Ulva* tissue remains unaffected
220 with a regular cell wall organization (fibers). C. Optical microscopy (10X) of healthy *Ulva*. D. Optical microscopy
221 (10X) of *Ulva* after anoxia and acidic biodegradation. *Ulva* tissue is disrupted with release of chloroplasts in the
222 interstitial middle that remain green in spite of anoxia.

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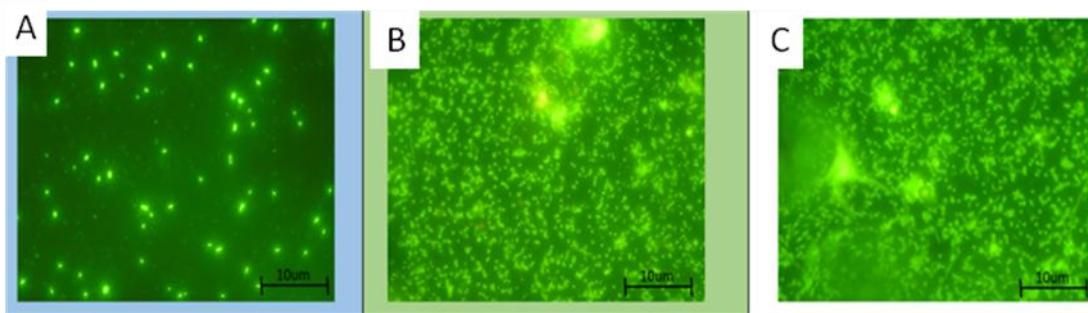


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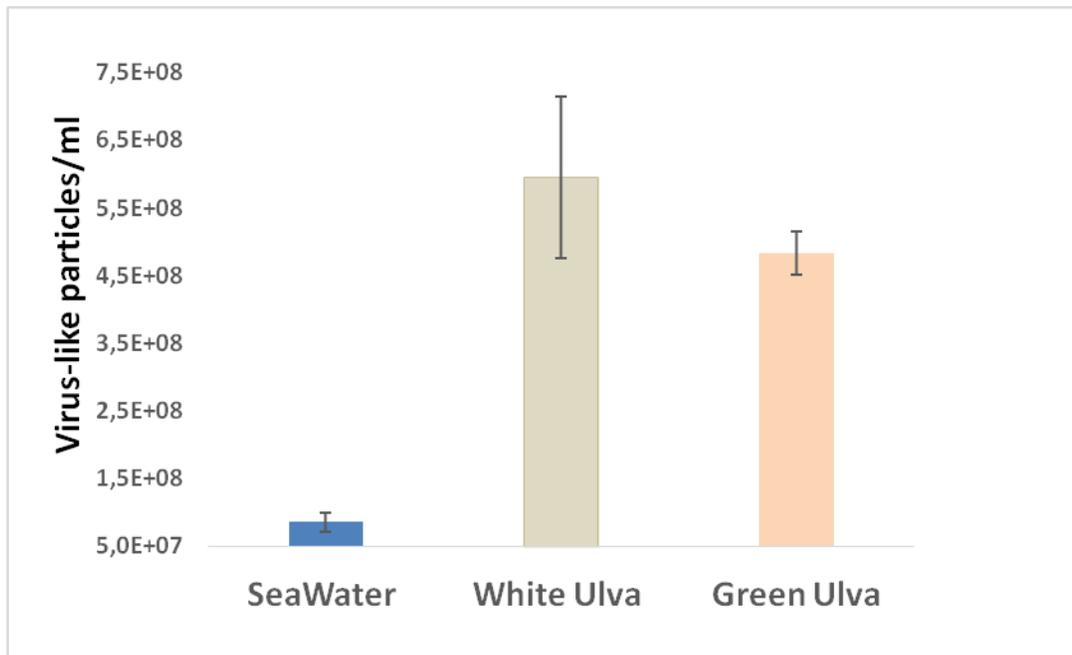
Figure 3: **Virus particles increase with bleached *Ulva***. Mediterranean sea water (500 μ l) filtrated at 0.2 μ m and analyzed with DAD HPLC. Panel A shows peak elution times regarding wavelengths from 220 to 360 nm, the vertical red arrow shows the peak at 6 min that correspond to free nucleic acids while the horizontal green arrows outline the 260 nm wavelength. Panel B shows a 3D view of peak elution times regarding both wavelengths and milli Absorption Units (mAU). Panel C shows peak elution times at 260 nm without *Ulva* and the vertical red arrow indicates the peak at 3.5 min due to virus. Panel D shows the UV absorption spectrum from 220 to 360 nm of the peak at 3.5 min due to virus without *Ulva* with a max. abs of 3.538 mAU at 366 nm. Panel E shows peak elution times at 260 nm with Breton *Ulva* for five days and the vertical red arrow indicates the peak at 3.5 min that might be due to viral-particles. Panel F shows the UV absorption spectrum from 220 to 360 nm of the peak at 3.5 min that could be due to viral particles with Breton *Ulva* for five days with a max. abs of 32 mAU at 366 nm. The blue line indicates the increase of background absorptions.

239 **Viral concentration increases in sea water with bleached *Ulva*** Mediterranean sea water
240 inducing bleaching was filtrated at 0.2 μ m and then analyzed with Diode Array Detection
241 coupled to High Performance Liquid Chromatography (DAD HPLC) providing a UV spectrum
242 of each peak eluting at different times (Figure 3). A majority of peaks eluting between 5 to 45
243 min were characterized by UV spectra having a maximum absorption at 243 nm (Figure 3A) and
244 were due to organic macromolecules called colloids [12]. A 3D view of the DAD HPLC run
245 showed that colloids were the major components of sea water filtrated at 0.2 μ m (Figure 3B).
246 Three peaks had different UV spectra. The peak indicated with a red arrow at 3.5 min (Figure
247 3C) was due to viral particles and was characterized by a first max. abs. at 266 nm (Figure 3D)

248 due to nucleic acids and aromatic amino acids [13-14]. The two other peaks were free nucleic
249 acids at 6 min (red arrow in Figure 3A) and free proteins at 45 min characterized respectively by
250 a max. abs. at 260 and 280 nm (Fig 3A&B). When Breton *Ulva* were added to Mediterranean
251 seas water for five days and after bleaching, the peak corresponding to virus increased
252 significantly (Figure 3E). UV spectra (Figure 3D and 3F) showed that the max. abs. at 266 nm
253 increased from 7 to 32 mAU. Interestingly, this peak increased while the colloid peaks decreased
254 (due probably because *Ulva* was using colloids as nutriments). We observed a natural decay of
255 30% in one month of the peak corresponding to virus with DAD HPLC (data not shown). We
256 observed also a good correlation with an increase of this virus peak in DAD HPLC and
257 apparition of viral lyses in September 2019 both at RN and WF (data not shown).
258



259
260 D



261
262 Figure 4: **Fluorescence microscopy after SYBR staining.** Sea water from Mediterranean seas inducing bleaching
263 was incubated without *Ulva* (panel A), with white *Ulva* (panel B) and green *Ulva* (panel C). Panel D shows the
264 Virus like particles amount. Sea water was filtrated at 0.2 µm.
265
266

267 **Bleached *Ulva* release Virus-like particle:** Provence water (MU) without and with *Ulva* was
268 filtrated at 0.2 μm and then stained with aromatic compounds called SYBR Gold pigment
269 binding on DNA and RNA. This pigment was currently used in virology to stain and visualize
270 virus like particles (VLPs) observed in seawater and other aquatic samples [15]. Fluorescence
271 microscopy after SYBR staining revealed a high viral production when *Ulva* was added to sea
272 water (Figure 4). This high viral production was already significant when *Ulva* was still green.
273 However, when *Ulva* became white the viral abundance reaches 6.5×10^8 viruses/ml, which was
274 an atypical viral high concentration (Figure 4D). This experiment showed that viruses were
275 actively produced and released with higher rates with *Ulva* bleaching.

276

277 **Discussion**

278

279 The average nitrate concentration in sea water worldwide and in Mediterranean seas is 1 μM . If
280 nitrate concentration was the reason for the absence of *Ulva* proliferation at Marseille, we should
281 have nitrate concentration up to 100 μM on Breton north coast where green tides are the most
282 important in Western Europe particularly for springtime but such is not the case excepted in river
283 or fjord (Table 1). Nitrate concentrations are variable regarding seasons. In Breton north coast
284 there is an average of 5 μM at the marine station of Roscoff that went to almost 10 μM in winter
285 and to 1 μM in summer in 2018 and 2019 [16]. Other parameters such as pH and conductimetry
286 are also variable regarding season at Rockoff [16].

287

288 Our data at BR in Brittany North coast (Table 1) are in the range of the nitrate concentration
289 observed at Roscoff and a same variability regarding season is observed at Marseille [16]. This
290 variability regarding seasons was also observed in Galicia at the west of Spain [17]. It is also
291 important to point out that the origin of *Ulva* green tides does not necessarily come from Brittany
292 coasts. *Ulva* proliferations are observed in the middle of North Atlantic and *Ulva* drift to Brittany
293 due to dominant western winds in the North Atlantic Ocean [18]. Chlorophyll anomalies appear
294 to be more and more frequent and the main cause of green tides could be due mainly to the
295 global warming [18]. A very interesting survey carried out in bay of Marseille in 2007 and 2008
296 by IFREMER confirms our results showing that nitrate concentration could be similar in coastal
297 seawater nearby Marseille and in North Brittany coast with nitrate concentration $> 8 \mu\text{M}$ in June
298 2008 at Marseille [19]. Although the nitrate concentration can be much higher in little rivers in
299 the Brittany, our study shows that the huge flow rate of the Rhône River induces also high
300 amounts of nitrates in the bay of Marseille.

301

302 Viruses are the most abundant biological entities in sea waters and can be found even in the
303 bathypelagic (1000 to 2000 m) zone. The Mediterranean seas appear to have the highest
304 concentration mainly in the epipelagic (5m) zone [20]. If prokaryotes and unicellular algae are
305 the main viral hosts, only 9% of these viruses are sequenced [13]. Viruses are well known to
306 control micro algae proliferation and begin to be discovered to control also macro algae

307 proliferation such as Kelp [21, 22]. In Mediterranean seas, the proliferation of the green micro
308 algae *Ostreococcus tauri* is controlled by a virus called OtV5 belonging to the *Prasinovirus*
309 family [23]. The sequence of our new virus does not fit with the *Prasinovirus* family (Lluesma et
310 al., manuscript in preparation).

311
312 Bleaching experiments in Table 1 were carried out only with *Ulva* collected in north Brittany
313 (TR). However, bleaching was also observed with *Ulva* collected in south Brittany (PO) and
314 *Ulva* collected at MU in Provence for springtime 2020 (data not shown). Since 2019 we are
315 doing seawater sampling in Provence every month and *Ulva* viral lyses can be different
316 regarding season in the bay of Marseille. Viral lyses that are very important in PR for springtime
317 become less important from September while viral lyses begin to be observed with water sample
318 from RN, WF and RS (data not shown). It might be due to the absence of *Ulva* in PR in summer
319 while *Ulva* proliferate in Rhône River and reach bay of Marseille. Detection of viral lyses
320 activities against *O. tauri* was observed only with seawater close to the area where the host strain
321 was isolated [24].

322
323 It is assumed that most viruses decay, or are destroyed rapidly in the environment. Algae viruses
324 may persist in the water through the establishment of latent infections where virus genomes
325 remain dormant and archived within host cells. From December no viral lyses can be detected in
326 the five spots that we selected in Provence (Table1). The decay of residual *Ulva* colonies both on
327 the Provençal coasts and in the Rhône River due to lower day lights is probably the reason of the
328 viral lyses decay. When sea waters reach 15°C at the end of winter at Marseille, *Ulva* is
329 reappearing in PR due to its high nitrate concentration (Table 1) and therefore this *Ulva* specific
330 virus. Winter remains both for Breton north coast and for Marseille the season where nitrate
331 concentrations are the highest [16, 19]. However, middle temperatures and sunny winter days at
332 Marseille makes possible for *Ulva* to shortly proliferate before apparition of viral lyses.

333
334

335 **Conclusions**

336
337 This study shows for the first time that a virus might control *Ulva* proliferation in the bay of
338 Marseille and describes the experiments that made possible to reveal the origin of the *Ulva*
339 bleaching with sea waters collected in Provence. The sequence and the characterization of this
340 new virus will be soon published (Lluesma et al, manuscript in preparation). Metagenomic
341 approaches that are independent of culturing and maintaining *Ulva* in the laboratory was the
342 methodology that helped to identify and recover the genetic information of this new virus [24].
343 The virus sequence will make possible to develop new tools such as droplet digital PCR to
344 estimating the viral absolute abundance [25]. This virus could be used for an innovative
345 ecological approach that might help to control *Ulva* proliferation and restore ecosystems in
346 Western Europe and Eastern China.

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