

1 **Dinophyceae use exudates as weapons against the parasite *Amoebophrya* sp.**
2 **(Syndiniales)**

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14 **Summary**

15 Parasites of the genus *Amoebophrya* sp. are important contributors to marine ecosystems and can be
16 determining factors in the demise of blooms of Dinophyceae, including microalgae commonly
17 responsible for toxic red tides. Yet they rarely lead to the total collapse of Dinophyceae blooms. The
18 addition of resistant Dinophyceae (*Alexandrium minutum* or *Scrippsiella donghaiensis*) or their exudate
19 into a well-established host-parasite culture (*Scrippsiella acuminata*-*Amoebophrya* sp.) mitigated the
20 success of the parasite and increased the survival of the sensitive host. Effect were mediated via water-
21 borne molecules without the need of a physical contact. Severity of the anti-parasitic defenses
22 fluctuated depending on the species, the strain and its concentration, but never totally prevented the
23 parasite transmission. The survival time of *Amoebophrya* sp. free-living stages (dinospores) decreased
24 in presence of *A. minutum* but not of *S. donghaiensis*. The progeny drastically decreased with both
25 species. Integrity of the membrane of dinospores was altered by *A. minutum* which provided a first
26 indication on the mode of action of these anti-parasitic molecules. These results demonstrate that
27 extracellular defenses are an effective strategy against parasites that does not only protect the
28 resistant cells but also have the potential to affect the whole surrounding community.

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31 Introduction

32 Parasites, which are supposed to account for half of the species richness, could make up the
33 unseen majority of species extinctions (Carlson *et al.*, 2017). Majority of parasites have essential
34 ecological roles by contributing to the resilience of ecosystems, limiting the invasion and emerging of
35 infectious diseases, and being critical to the biomass transfer between trophic levels (Johnson *et al.*,
36 2013; Dougherty *et al.*, 2016; Paseka *et al.*, 2020). In marine ecosystems, parasites have a predominant
37 role in the planktonic protists interactome inferred by sequence-based correlation networks (Lima-
38 Mendez *et al.*, 2015) and can represent up to 18% of interactions (Bjorbækmo *et al.*, 2020). Parasites
39 are important contributors to phytoplankton mortality and can even sometimes induce the demise of
40 microalgal blooms (Brussaard, 2004; Chambouvet *et al.*, 2008; Vardi *et al.*, 2009). Amongst marine
41 parasites, the Syndiniales *Amoebophryidae* (also called marine Alveolate group II, or MALVII) is a widely
42 distributed family (Guillou *et al.*, 2008; de Vargas *et al.*, 2015), ubiquitous in marine waters, including
43 ultra-oligotrophic ones (Siano *et al.*, 2011), and has been associated with the demise of toxic species
44 (Park *et al.*, 2002; Chambouvet *et al.*, 2008; Velo-Suárez *et al.*, 2013; Li *et al.*, 2014; Choi *et al.*, 2017)
45 in enriched coastal environments. Its life cycle is characterized by a free-swimming stage (zoospores,
46 also named dinospores) followed by two successive intracellular stages (trophont then sporont) that
47 eventually kills the host and release hundreds of dinospores. Dinospores are highly specialized and
48 short-lived flagellated unicellular forms that only survive in culture a few hours to few a few days (Park
49 *et al.*, 2002).

50 *Amoebophrya* spp. are specialist parasites that require a compatible host to complete their life
51 cycle. The overall consistency in the host spectrum observed within different strains of the same
52 species suggest a genetic determinism underlying their host specialization (Cai *et al.*, 2020). Many
53 factors can influence the parasitic population dynamic such as physical (e.g. temperature, water
54 column depth, physical mixing) and chemical (e.g. nutrients) parameters (Anderson & Harvey, 2020).
55 Optimal abiotic conditions for parasitic infection do not always induce the collapse of targeted
56 dinoflagellate blooms, implicating complex biotic interactions as fundamental player in the success of
57 the parasite (Anderson & Harvey, 2020). Modelling approaches also indicate that the parasitic control
58 of dinoflagellate blooms strongly depends on the structure (e.g. cell densities, grazing of free-living
59 stages of parasite stages, competition between cells) of the plankton community (Alves-de-Souza *et al.*
60 *et al.*, 2015). The co-existence between resistant and sensitive hosts could affect the parasitic
61 propagation through different mechanisms, including a dilution effect (Alves-de-Souza *et al.*, 2015;
62 Alacid *et al.*, 2016) or cell signaling between species.

63 Mechanisms of the host resistance against parasites are poorly known. Different strategies
64 have been described so far, including the production of resting stages (Chambouvet *et al.*, 2011a;
65 Pelusi *et al.*, 2020), the production of anti-parasitic metabolites produced internally (Pouneva, 2006;
66 Bai *et al.*, 2007; Place *et al.*, 2009; Rohrlack *et al.*, 2013; Scholz *et al.*, 2017), and sometimes released
67 into exudates (Scholz *et al.*, 2017). The release of anti-parasitic compounds (APC) is a strategy that can
68 be classified within the more general term of allelopathy. Allelochemicals refers to any secondary
69 metabolite exuded by a microalga that affect the growth of another co-occurring protist (Granéli &
70 Hansen, 2006). Whether and how the release of APC can influence the dynamic of parasite is a still an
71 opened question.

72 This study investigated whether co-occurring Dinophyceae, resistant to *Amoebophrya* sp., can
73 affect the dynamics of a parasite infecting a sensitive Dinophyceae host. For that, the infection
74 dynamics and the dinospore survival was monitored in the well-established parasitic couple *S.*
75 *acuminata* (ST147) infected by *Amoebophrya* sp. (A25) (Farhat *et al.*, 2020) in presence and absence

76 of two resistant dinoflagellate hosts: *Scrippsiella donghaiensis* and *Alexandrium minutum*. These
77 dinoflagellate species were selected for several reasons: (1) they can form recurrent dense blooms
78 (Chapelle *et al.*, 2014, 2015; Klouch *et al.*, 2016) and are potential competitors of *S. acuminata*, (2)
79 they co-occur with *S. acuminata* and its parasites *Amoebophrya* sp. in the same estuaries (Guillou *et*
80 *al.*, 2008; Cai *et al.*, 2020), (3) they are resistant to *Amoebophrya* sp. A25 (Cai *et al.*, 2020) and (4) the
81 species *A. minutum* is a producer of allelochemicals that can negatively affect competing protists (Long
82 *et al.*, 2018). The production of such compounds by the species *S. donghaiensis* has not been reported
83 so far.
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87 **Materials and methods**

88 **Biological material**

89 **Origin of strains and culture conditions**

90 All hosts and parasitic strains originated from coastal marine waters of the NE Atlantic Ocean
91 (Supporting Information Table S1). The parasite *Amoebophrya* sp. strain A25 was routinely maintained
92 using the sensitive *S. acuminata* clade STR1 (ST147) as host. Resistant dinoflagellates used in this study
93 were *A. minutum* (strains CCM11002, Am176, Da1257) and *S. donghaiensis* (strain Sc39 sampled during
94 an *A. minutum* bloom). Infected and uninfected host cultures were maintained in a medium prepared
95 with seawater from the Penzé estuary (27 PSU of salinity), stored in the dark for several months before
96 used, filtered on 0.22 µm, autoclaved and enriched by a modified F/2 medium (Guillard's Marine Water
97 Enrichment Solution, Sigma) and 5% (v/v) soil extract (Starr & Zeikus, 1993). Cultures used for the
98 Experiment 3 were prepared using a different medium (K, Keller et al. 1987, seawater from Argenton
99 at 35 of salinity) after acclimation of strains. In both cases, a final filtration using a 0.22 µm pore size
100 filter was processed after addition of nutritive solutions, under sterile conditions. Stock cultures and
101 experiments were performed under continuous light (90-140 µEinstein m⁻² s⁻¹, light bulb Sylvania
102 Aquastar F18W/174 or EASY LED universal light 438 mm) at 21 ± 1-2°C. All experiments were
103 performed with plastic flasks (CytoOne vented flasks in polystyrene).

104 Uninfected hosts were kept in exponential growth phase by diluting 5 volumes of stock culture into 8
105 volumes of fresh medium every 3-4 days. Infections were propagated by diluting 1:5 (vol:vol) of the
106 infected culture into healthy hosts *S. acuminata* (ST147) every 3-4 days. Physiological state of
107 uninfected microalgal cultures was routinely screened using a handheld Pulse Amplitude Modulation
108 (PAM) fluorometer (Aquapen-C AP-C 100, Photon Systems Instruments, Drassov, Czech Republic). As
109 a prerequisite, only healthy cultures with a maximum (dark-adapted) photosystem II quantum yield
110 (F_V/F_M) above 0.6 were used in experiments.

111 **Synchronization and collect of *Amoebophrya* dinospores**

112 Density and infectivity of dinospores decrease rapidly (days) after their release (Supporting
113 Information Table S2), therefore the use of freshly released dinospores helps to maximize infections
114 in the flask. To produce freshly released dinospores, cultures of parasites were synchronized (unless
115 specified) following the protocol detailed in [protocols.io dx.doi.org/10.17504/protocols.io.vrye57w](https://doi.org/10.17504/protocols.io.vrye57w).
116 During synchronization, infections were initiated with 3-days-old cultures of *Amoebophrya* from which
117 dinospores were collected after a gentle separation from the remaining host cells (*S. acuminata* ST147)
118 using gravity filtration through nylon filter (5 µm, Whatman). These dinospores were incubated with
119 the exponentially growing host *S. acuminata* (strain ST147) using a 1:2 parasite:host (vol:vol) ratio.
120 After 24 hours of incubation, infected hosts were collected by filtration through a 5-µm-nylon-filter,
121 then resuspended in an equal volume of fresh media, in order to remove remaining free-living
122 dinospores. Three days later, freshly liberated dinospores of the same age (i.e. synchronized) were
123 separated from remaining hosts by filtration as described before. In prior experiments, no effect of
124 dilutions on the dinospore survival over 24 hours was observed whether using fresh culture medium,
125 exudates from the healthy host ST147, or exudates from ST147xA25 infected culture (Supporting
126 Information Table S2). Hereafter, ST147 filtrate was used to adjust densities by dilution.

127 **Preparation of microalgal filtrates**

128 Exudates from exponentially growing microalgal strains were collected by filtration (0.2 µm, acetate
129 cellulose membrane, Minisart) using a gentle pressure process using a syringe. Exudates were diluted
130 in filtrate obtained from a growing culture of ST147 in order to adjust concentrations. In the present

131 study, dilution of exudates is expressed as equivalent to the microalgal density (corresponding to the
132 theoretical concentration of cells that would have been reached by the initial culture after a similar
133 dilution). Diluted exudates were then immediately used for experiments.

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135 **Cell counting methods**

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136 **Flow-cytometry (FCM): cell count and membrane permeability**

137 Cell densities and parameters (e.g. forward scatter, size scatter, fluorescence signals) were estimated
138 using a flow cytometer equipped with 488 nm and 405 nm lasers. A FACSAria flow cytometer (Becton
139 Dickinson) was used in experiments 1 and 2; a Novocyte Advanteon (ACEA Biosciences) was used in
140 Experiment 3. Dinophyceae were detected according to their red chlorophyll autofluorescence using
141 the 488 nm laser. Free-living (dinospores) and late stages of infection of *Amoebophrya* spp. emit a
142 bright green autofluorescence when excited under blue-violet light (Kim *et al.*, 2004; Kim, 2006;
143 Chambouvet *et al.*, 2011a), a proxy of the parasite survival (Coats & Park, 2002). This natural
144 autofluorescence was used to estimate the density of viable dinospores by FCM using the 405 nm laser.
145 Intact cell membranes are impermeable to the SytoxGreen (SYTOX Green nucleic acid stain,
146 Invitrogen), which only penetrates in cells having altered (i.e. permeable) membranes (DNA is then
147 stained, and this autofluorescence is detected by FCM using the 488 nm laser). The SytoxGreen (final
148 concentration of 0.05 μM) was incubated during 20 min in the dark before processing the samples.

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149 **Prevalence of infections (CARD-FISH)**

150 Samples for CARD-FISH were fixed with paraformaldehyde (1% final concentration) for 1 hour at 4°C
151 in the dark before filtration on a 0.8 μm , polycarbonate filter with a vacuum pump (< 200 mm Hg).
152 Filters were then dehydrated using successive 50%, 80% and 100% ethanol solutions, dried and stored
153 in the dark at -20°C. FISH-staining was then performed according to (Chambouvet *et al.*, 2008). The
154 prevalence was estimated under microscopy with an Olympus BX-51 epifluorescence microscope
155 (Olympus Optical) equipped with a mercury light source, a 11012v2-Wide Blue filters set (Chroma
156 Technology, VT, USA) and, with fluorescence filter sets for PI (excitation: 536 nm; emission: 617 nm)
157 and FITC (excitation: 495 nm; emission: 520 nm).

158 Prevalence was determined by averaging infection counts on a minimum of 80 cells per replicate.
159 Prevalence was divided in 3 categories: non-infected host cells, early stage (one or more dinospores of
160 *Amoebophrya* sp. are found in the cytoplasm) and advanced stages (intermediate and beehive stages)
161 as described in (Kayal *et al.*, 2020). The progeny (i.e., the number of dinospores per infected host) was
162 estimated by dividing the maximum concentration of dinospores by the concentration of infected host
163 at advanced stages.

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165 **Experimental set-ups**

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166 **Experiment 1: Co-cultures**

167 The dynamic of infection in co-cultures was compared when mixing the sensitive host (*S. acuminata*
168 ST147) with the parasite (*Amoebophrya* sp. A25) and one resistant host (*A. minutum* CCM11002 or *S.*
169 *donghaiensis* Sc39). Mixtures were prepared in triplicates, using a ratio of 1:1:1, with initial
170 concentrations of 4000 cells mL^{-1} for each strain. Controls consisted of flasks containing (i) only the
171 compatible host ST147 at 4000 cells mL^{-1} or (ii) the host (ST147) at 4000 cells mL^{-1} and its parasite A25
172 at a ratio of 1:1. An additional control consisted of mixing the host ST147 and one of the resistant host
173 (CCM11002 or Sc39) in parallel, replacing the parasite with 0.2 μm filtrate of the host culture. All
174 cultures and controls were started simultaneously, using the same mother cultures. Cell densities were
175 quantified once to twice per day by FCM. At the end of the experiment, samples were fixed with non-

176 acidic Lugol's solution (1% final concentration) for microscopic counts and differentiation between *S.*
177 *acuminata* and *A. minutum* cells.

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179 **Experiments 2 and 3: Evaluation of the effects of Dinophyceae filtrates on** 180 ***Amoebophrya***

181 Filtrates of microalgal cultures were used to analyze the effects of Dinophyceae exudates (from either
182 *A. minutum* or *S. donghaiensis*) on *Amoebophrya*. The Experiment 2 was organized into two parts, the
183 first one to estimate the effect of Dinophyceae exudates on the abundance of fluorescent dinospores
184 over time, then the second to analyze their potential to successfully infect and produce a second
185 generation of dinospores after 6 hours of contact with the filtrates.

186 First, dinospores from *Amoebophrya* sp. (A25) were exposed to serial dilutions of dinoflagellate
187 filtrates (equivalent to 1000 and 5000 and 10000 cells mL⁻¹) collected from three strains of *A. minutum*
188 (Da1257, Am176, CCM11002) and for one strain of *S. donghaiensis* (SC39). Densities of fluorescent
189 dinospores were monitored by FCM. The mortality rate (h⁻¹) of autofluorescent dinospores was
190 calculated over the first 3 hours according to equation 1, where N₁ and N₂ are the respective densities
191 of autofluorescent dinospores before and after 3 hours of exposure to the filtrates. Controls consisted
192 in the incubation of dinospores with exudates from the host ST147. Incubations for controls and using
193 the highest filtrate concentrations (10000 cells mL⁻¹) were performed in triplicates, while only one
194 replicate was performed for intermediate exudate concentrations.

195 **Equation 1: Mortality rate** $= \frac{\ln(N_1/N_2)}{3}$

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197 Then, dinospores previously exposed to the maximal concentration of exudates and in the control
198 conditions after 6 hours of incubation were used for the second part of the experiment. Exposed-
199 dinospores were mixed to the host strain ST147 at a dinospore:host ratio of 5:1 for dinospores exposed
200 to *A. minutum* filtrates, and at three different ratios (0.5:1, 1:1, and 5:1) for dinospores exposed to *S.*
201 *donghaiensis* filtrate. The production of dinospores was monitored twice per day during 5 days by FCM,
202 and the prevalence was analyzed after 47 hours of incubation by CARD-FISH in the controls and with
203 the CMM11002 and Sc39 filtrate treatments.

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205 Experiment 3 was performed to monitor the concentrations of fluorescent dinospores and their
206 membrane integrity over time when mixed with *A. minutum* exudates compared to the control.
207 Dinospores from three days-old parasite cultures (non-synchronized) of *Amoebophrya* sp. A25 were
208 harvested by filtration (5µm, cellulose acetate, Minisart). Dinospores were exposed in triplicate to *A.*
209 *minutum* CCM11002 filtrate at a final concentration of 5000 theoretical cells ml⁻¹ in six well plates
210 (CytoOne, made of polystyrene). In the control, dinospores were diluted in triplicate with *S. acuminata*
211 (ST147) filtrate. The dinospore concentrations and the permeability of their membranes was estimated
212 after 20, 40, 60 and 120 min of incubation with the filtrate.

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214 **Statistics**

215 All statistical analyses were performed using R software (R Foundation for Statistical Computing,
216 Vienna, 2011). Significant differences in the different endpoints (e.g. concentrations of microalgae,
217 concentrations of dinospores, prevalence) were assessed with a test of student or one-way ANOVA
218 followed by a post-hoc Tukey HSD (ANOVA-HSD) when meeting the homoscedasticity with a Bartlett
219 test and normality with a Shapiro-Wilk test. When homoscedasticity or normality could not be met, a

220 non-parametric Kruskal-Wallis test followed by a post-hoc Conover with a bonferroni adjustment was
221 applied (KWbf). All tests were performed with a significance level of p-value = 0.05.

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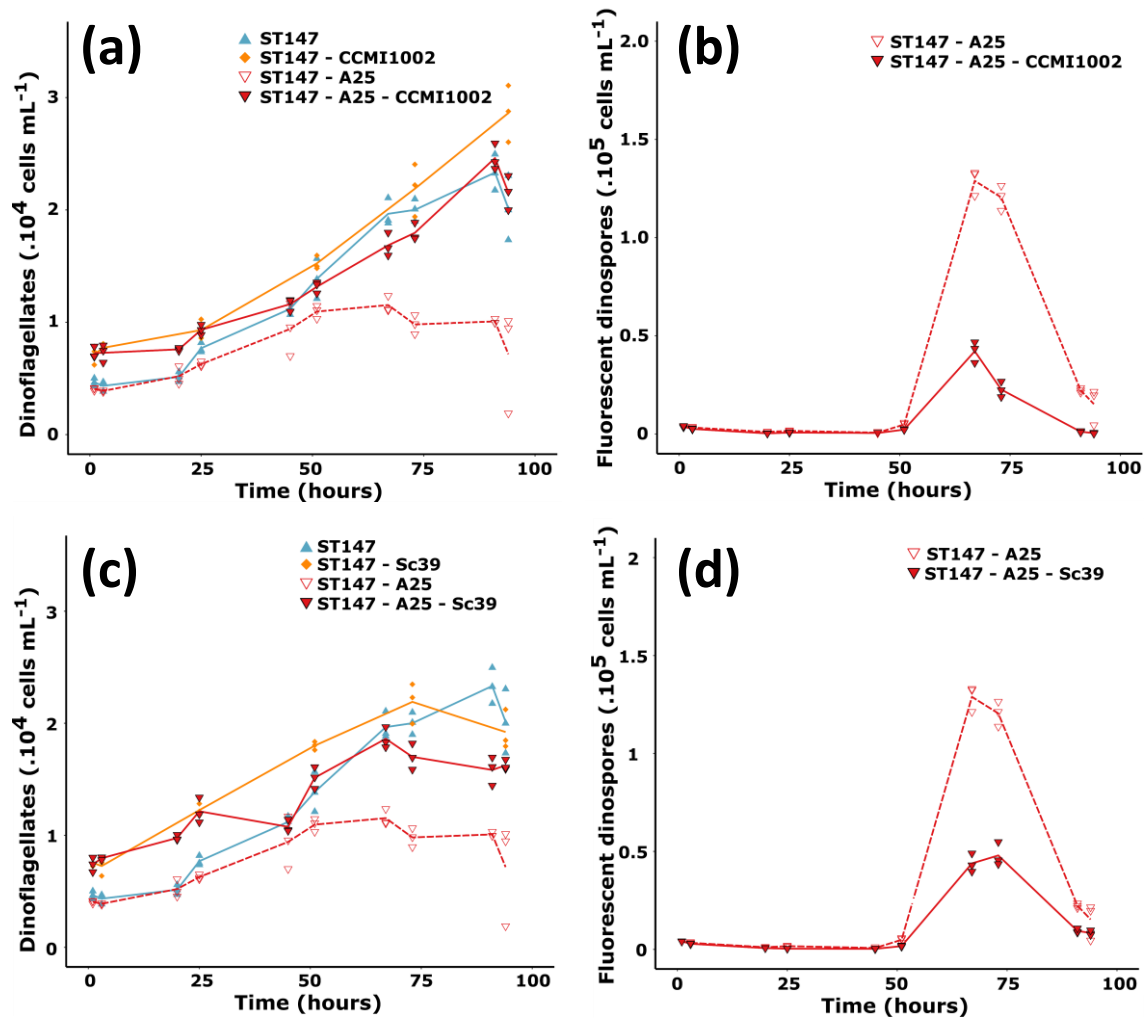
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224 **Results**

225 **Infections were mitigated by the presence of a resistant host**

226 Experiment 1 tested whether the co-presence of a resistant host (*A. minutum* or *S. donghaiensis*) could
227 modify the *Amoebophrya* infection dynamics on a sensitive host (*S. acuminata*). In controls and when
228 using fixed experimental culture conditions, a complete infection cycle lasted at least 51 hours, and
229 ended with the sudden released of freshly produced dinospores (Fig. 1). During that period, infected
230 host cells do not divide (Park *et al.*, 2002), which explain the lower net growth rates recorded 25 hours
231 after the parasite inoculation compared to the controls. Addition of a resistant host (CCMI1002 or
232 Sc39) did not modify the duration of the parasite development, but always resulted in a significant
233 decrease (> 60 %) of the dinospore production (Fig. 1). This observation could result from a deleterious
234 effect on the sensitive host, a direct effect on the dinospore survival/infectivity, or both. Co-cultivation
235 with *A. minutum* has a cost for *S. acuminata*. At the end of the experiment, densities of *S. acuminata*
236 in the co-culture without parasite was of 6900 ± 1400 cells mL⁻¹ while it reached 20000 ± 3000 cells
237 mL⁻¹ in the control.

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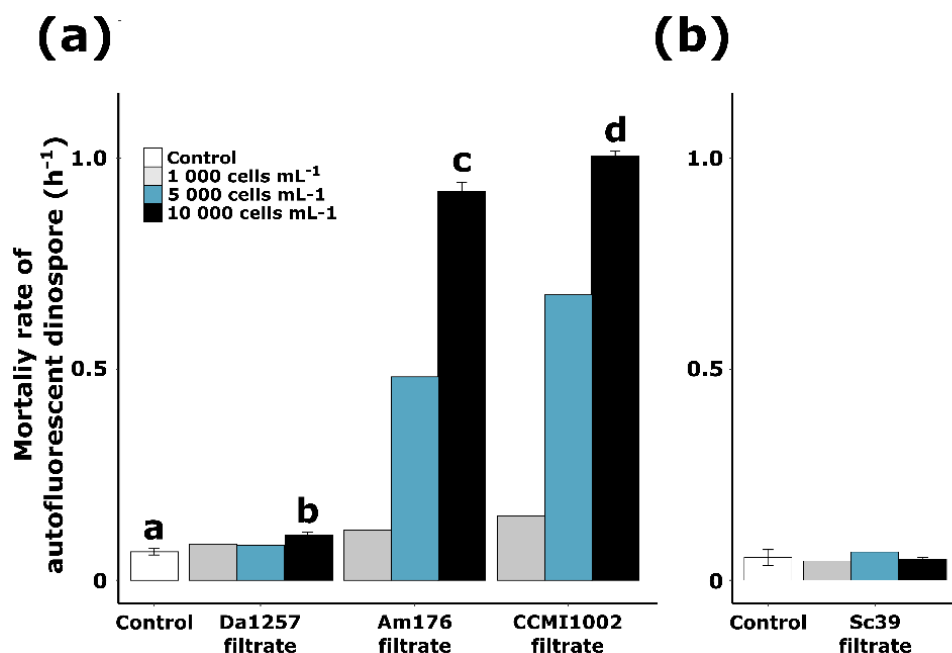
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241 Figure 1: Co-cultures of *Amoebophrya* sp. (A25) with its compatible host *S. acuminata* (ST147) and a secondary resistant host,
242 either *A. minutum* CCM1002 (a, b) or *S. donghaiensis* Sc39 (d, d). Densities of dinoflagellates (*S. acuminata* ST147 with *S.*
243 *donghaiensis* Sc39 or *A. minutum* CCM1002) are shown in (a) and (c). Densities of fluorescent dinospores are shown in (b) and
244 (d). Mean values are represented by lines, while replicate values are shown by the symbols. The same controls (ST147 and
245 ST147-A25) are shown for both species as experiments were performed at the meantime.

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Exudates from *A. minutum* decreased the density of autofluorescent dinospores

Natural autofluorescence of dinospores can be used as a proxy for their viability (Coats & Park, 2002). In controls, 25% of autofluorescent dinospores were lost after 6 hours, leading to a natural mortality rate of $0.07 \pm 0.01 \text{ h}^{-1}$ in tested cultures conditions (Fig. 2). Experiment 2 tested whether the resistant dinoflagellate exudates affected this mortality rate. If no significant effect using *S. donghaiensis* (Sc39) filtrate was observed, exposure to *A. minutum* filtrates resulted in a significant over-mortality (p-values < 0.02) compared to the control. This effect was however strain dependent, as illustrated by differing mortality rates between *A. minutum* strains (i.e. mortality of 0.11 ± 0.01 , 0.92 ± 0.02 and $1.00 \pm 0.01 \text{ h}^{-1}$ of fluorescent dinospores after 6h of exposure using DA1257, AM176, and CCM1002, respectively). After six hours, this exposure resulted in losses of 32 ± 1 , 96.1 ± 0.2 and $97.2 \pm 0.4 \%$ of the initial density of fluorescent dinospores after 6h of exposure.



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Figure 2: Maximal mortality rate of autofluorescent A25 dinospores in the different conditions. Dinospores were exposed to (a) *A. minutum* and (b) *S. donghaiensis* filtrates during two separate sets of experiment. Results are expressed as the value or the mean \pm standard deviation when triplicates were performed. Significant differences in the mortality rates are indicated by different letters. The complete dataset, with all sampling points (after 1, 3 and 6 hours) is provided in Supporting Information Fig. S1.

Exudates from *A. minutum* decreased *Amoebophrya* sp. infectivity

To test whether the loss of fluorescence (Experiment 2) was linked to a loss of infectivity, dinospores were challenged for six hours with exposure to exudates coming from three strains of *A. minutum* to fresh healthy host cultures. Densities were fixed for all treatments before the addition of exudates. However, because of the difference in mortality rates, the starting concentration of fluorescent dinospores differed over treatments (41000 ± 1400 dinospores mL⁻¹ in the control, and 36000 ± 800 , 2100 ± 100 , and 1500 ± 200 dinospores mL⁻¹ with exudates of Da1257, Am176, and CCM1002, respectively). The ability of the remaining autofluorescent dinospores to infect their host even at low and unfavorable ratios was then explored. Novel infections were still observed in all treatments (Fig. 3). Filtrates of *A. minutum* did not seem to affect the intracellular stage as new progeny was released

279 after 48 hours and the duration of infection was similar over treatments. Progeny (dinospore
280 production per infected host) was 100 times lower with CCMI1002 than in the control (Table 1). As a
281 result of lower prevalence and lower progeny, the maximal dinospore concentration were drastically
282 decreased in the CCMI1002 and AM176 treatment as compared to the control or DA1257 filtrate (p -
283 values $< 10^{-7}$).

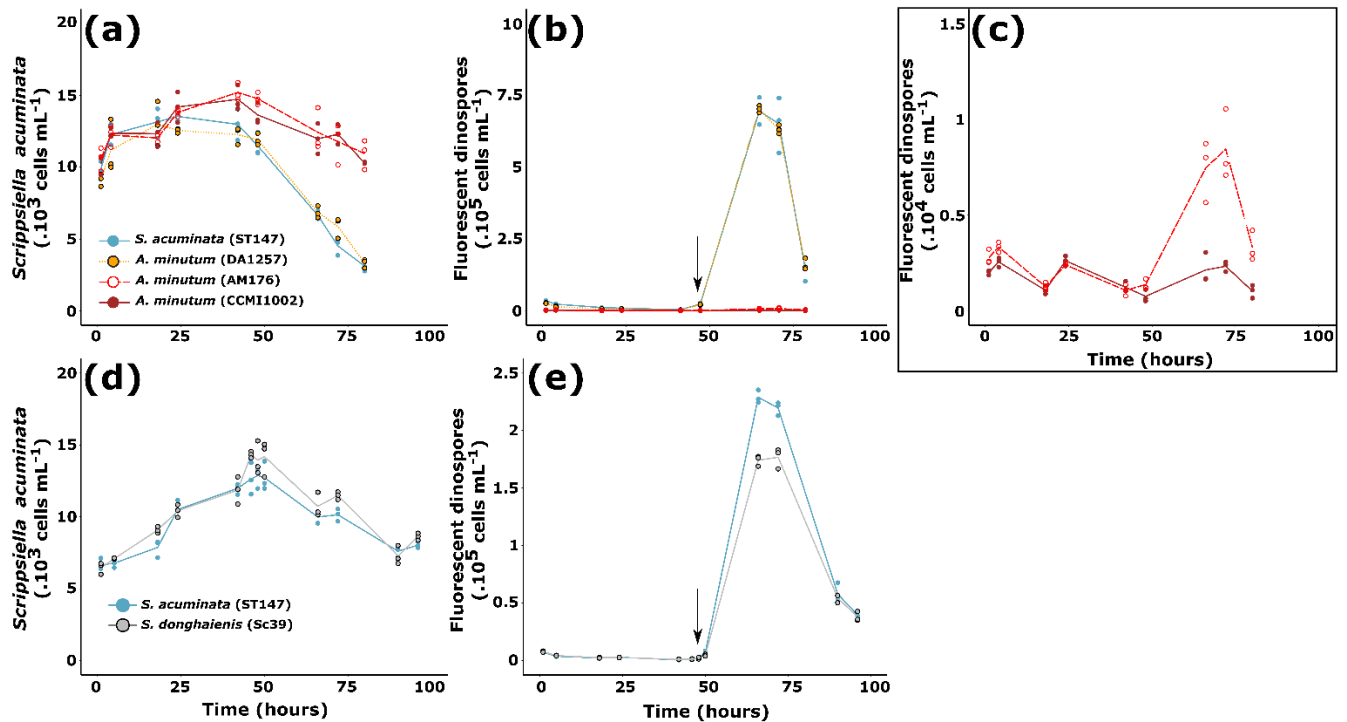
284 The growth of the compatible host (*S. acuminata* ST147) was suppressed by the dinospores from the
285 control or previously exposed to DA1257 filtrate. This suppression of the host growth in the control
286 was linked to the high prevalence ($61 \pm 6\%$ in the control; Table 1) of *Amoebophrya* sp. in host cells. In
287 comparison, the compatible host in contact with the dinospores previously exposed to AM176 or
288 CCMI1002 filtrates were still able to grow during the first 42 hours of incubation as the prevalence was
289 lower (approximately a 35 % in the CCMI1002 treatment; Table 1). Between 42 and 80 hours, a collapse
290 of the host population was observed in all conditions. The degree of the decline in host population was
291 likely correlated to the prevalence of cells at advanced stages of infection (Table 1). With the CCMI1002
292 treatment, $30 \pm 4\%$ of host cell losses were estimated (Fig. 3) against $75 \pm 2\%$ of host cell losses in the
293 control.

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295 The same experiment was conducted with Sc39, results from ratio 1:1 is shown (Fig 3d,e), results from
296 ratios 0.5:1 and 5:1 are available in Supporting Information Fig. S2. On contrary to *A. minutum* filtrates,
297 infections started with the same density of fluorescent dinospores in the controls and in Sc39
298 treatments, as no effect was observed on the autofluorescence of dinospores. Filtrates of *S.*
299 *donghaiensis* did not seem to affect the intracellular stage as novel infections were observed and the
300 duration of infection was similar to control conditions: the release of new progeny started between 48
301 and 50 hours (Fig. 3e). The previous treatment of dinospores with Sc39 filtrate did not significantly
302 affected the prevalence of *Amoebophrya* sp. in the host population nor affected the growth rate of the
303 host during the first 48 hours (Fig. 3d). With or without the previous treatment with *S. donghaiensis*
304 filtrate, a sharp decline of the host population, concomitant with release of new progeny, was
305 observed after 48 hours. Overall there were no statistical differences in the percentage of lysed host
306 cells between the treatments ST147 ($37 \pm 3\%$), and Sc39 ($38 \pm 4\%$). The main effect of pre-exposure of
307 dinospores to Sc39 filtrate was observed on the new generation of dinospores: the treatment induced
308 a significant decrease of 22% of the maximum concentration of the second generation of dinospores
309 (Fig. 3e and Supporting Information Fig. S2). This decrease did not seem to be linked to a lower
310 prevalence (Table 1) but was more likely related to a lower number of progeny per infected host, even
311 though the 3 fold decrease was not statistically significant when compared to the control (Table 1).

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316 Figure 3: Effect of *A. minutum* (a-c) and *S. donghaiensis* (d-e) filtrates (Theoretical cell concentration = 10^4 cells mL^{-1}) on
 317 infectivity of *Amoebophrya* sp. dinospores on its sensitive host *S. acuminata* (ST147). Cell densities of *S. acuminata* when
 318 mixed with A25 dinospores are shown in (a, d). Dynamics of dinospores, previously exposed to the different filtrates, when
 319 mixed with the compatible host *S. acuminata* ST147 are shown in (b, c and e). (c) is a zoom of (b) with dinospores densities
 320 for Am176 and CCMI1002. *S. acuminata* (ST147; blue), *A. minutum* (Da1257; yellow), *A. minutum* (Am176; red) and *A.*
 321 *minutum* (CCMI1002; dark red). In experiments with *S. donghaiensis* (Sc39; grey) filtrate, the graphs show results of the
 322 experiment at a dinospore: *S. acuminata* ratio of 1:1; results with other ratios can be found in Supporting Information Fig. S2.
 323 The arrow represents the sampling point for prevalence analysis. Lines represent the mean cell densities while the symbols
 324 represent the values of each replicate (N = 3).

325 Table 1 : Prevalence of *Amoebophrya* sp. (A25) in *S. acuminata* (ST147) during Experiment 2 after 47 hours of contact. Two
 326 controls are shown as the two experiments were performed during two different sets. Significant values between the control
 327 and the dinophyceae treatment (CCMI1002 or Sc39) are indicated as followed: "NS" non significant, "*" $0.05 > p\text{-value} > 0.01$,
 328 "***" $0.01 > p\text{-value} > 0.001$, "****" $p\text{-value} < 0.001$.

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	Control	CCMI1002 filtrate	P-value	Control	Sc39 filtrate	P-value
Prevalence Infected	61 ± 6	35 ± 17	NS	24 ± 16	44 ± 23	NS
(% of host cells)	Early stages	1 ± 2	NS	5 ± 9	12 ± 20	NS
	Advanced stages	60 ± 5	***	19 ± 7	33 ± 3	NS (0.07)
Progeny (dinospores/host)	105 ± 28	1 ± 0	**	43 ± 24	14 ± 3	NS

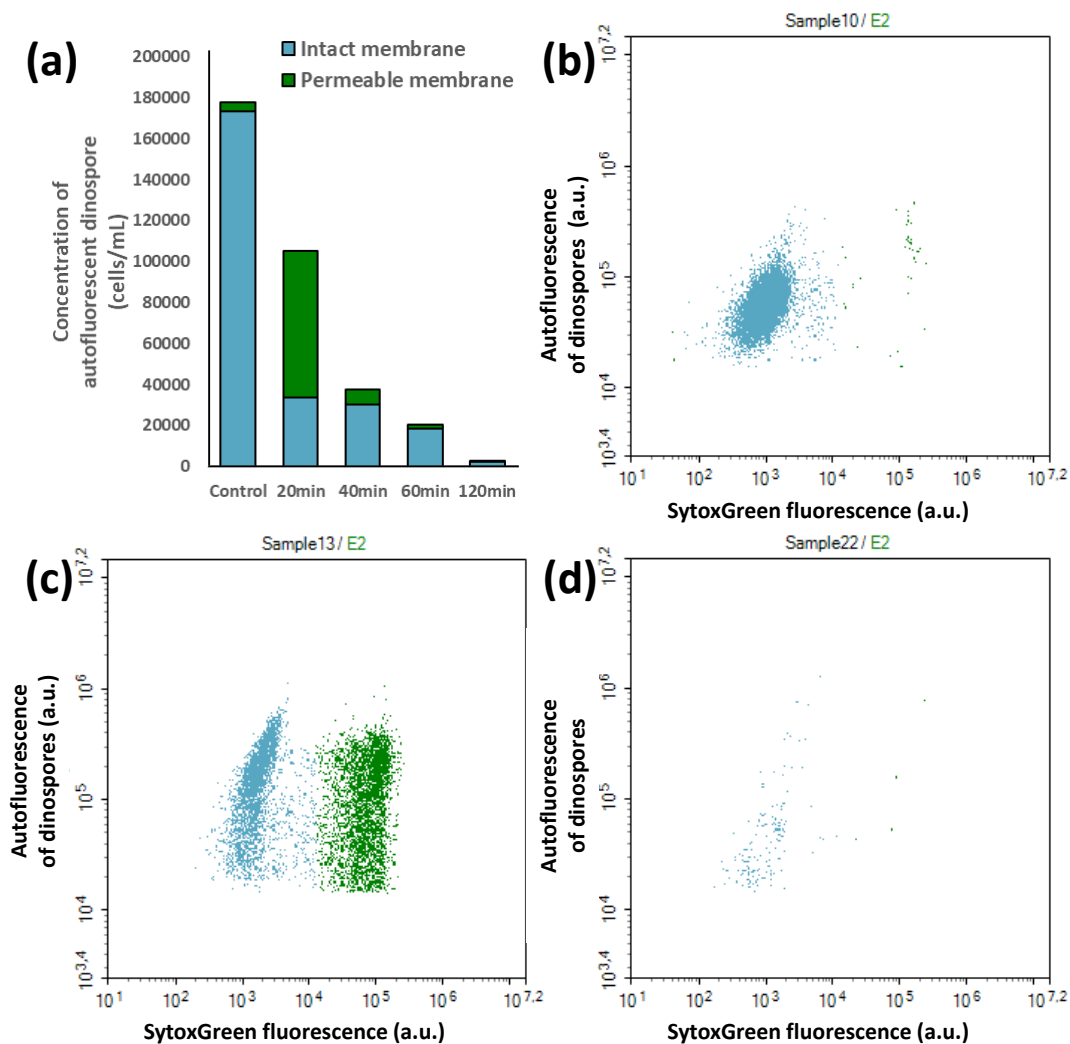
330

331

332 Exudates from *A. minutum* disrupted membranes of *Amoebophrya* sp.

333 In Experiment 3, it was tested whether the loss of autofluorescence from dinospores is concomitant
 334 to the loss of membrane integrity when exposed to *A. minutum* filtrate. The most potent strain *A.*
 335 *minutum* (CCMI1002) was used during this experiment. Following the exposure, a rapid decrease in
 336 the density of auto-fluorescent dinospores was observed, with a 40% decrease within 20 min of
 337 exposure and a 98% decrease after two hours (Fig. 4). This loss of auto-fluorescent dinospores was

338 preceded by the dinospore membrane permeabilization. After 20 min of exposure to the filtrate, 68%
 339 of the still auto-fluorescent dinospores were permeable to SytoxGreen.
 340



341
 342 Figure 4: Effects of *A. minutum* filtrate on the density of auto-fluorescent (Green from 405 nm laser) dinospores and on the
 343 green fluorescence (from 488 nm laser) of cells after SytoxGreen staining. SytoxGreen stain only enters cells with damaged
 344 permeable membranes. (a) means of the cumulative densities (cells mL⁻¹) of auto-fluorescent dinospores with impermeable
 345 (blue) and permeable (green) membranes to the stain. (b) dinospores in the control (stained but not exposed to *A. minutum*
 346 filtrate) and exposed to *A. minutum* filtrate for (c) 20 min, (d) 120 min.

347

348 Discussion

349 Co-culture experiments with *A. minutum* showed that co-occurring resistant dinoflagellate species
 350 could either decrease survival of the free-living stage of the parasite, or limit its infectivity during the
 351 second generation, or both. Cells and filtrates of *A. minutum* caused similar effects to the infection
 352 dynamic, demonstrating that Dinophyceae can remotely affect parasites through the exudation of
 353 Anti-Parasitic Compounds (APC). Once released, APC are rapidly diluted, highlighting the importance
 354 of cell concentration and ratios. One may expect a particularly efficient protection for cells in close
 355 contact with the APC producers. Formation of dense cell patches with concentrations orders of
 356 magnitude higher than background (Durham & Stocker, 2012; Breier *et al.*, 2018; Wheeler *et al.*, 2019;
 357 Basterretxea *et al.*, 2020) is likely more protective at micro-scales as this effect is density-dependent.

358 As effects were observed using filtrates from cultures non-exposed to *Amoebophrya* sp. or its chemical
359 cues, the release of APC is a passive defense mechanism. *A. minutum* exudates altered the integrity of
360 the membrane prior the loss of the natural autofluorescence of the free-living stages of *Amoebophrya*
361 sp. The loss of cell permeability might eventually lead to an osmotic cell lysis. The release of lytic APC
362 by *A. minutum* cells in the phycosphere (i.e. microenvironment surrounding the cells (Seymour *et al.*,
363 2017)) would act as a protective “shield” and must, at least partially, explain the resistance of *A.*
364 *minutum* against *Amoebophrya* sp. This strategy was evidently ruled out by some *Amoebophrya*
365 species, as it has already been reported that the genus *Alexandrium* could be infected by *Amoebophrya*
366 sp. (Chambouvet *et al.*, 2008; Lu *et al.*, 2016). This could be explained by two hypotheses; (i) either
367 *Amoebophrya* sp. only infects clones of *A. minutum* that do not release APC or, (ii) strategies to
368 counteract APC effects exist. The second hypothesis has already been proven with another genus,
369 *Amoebophrya* is able to acquire “antidotes” that enable it to avoid toxicity of *Karlodinium spp.* cells
370 (Place *et al.*, 2006), a potential host (Bai *et al.*, 2007). This genus produces hydrophobic membrane
371 permeabilizing compounds (Karlotoxins) with bioactivities and molecular targets that are similar to the
372 permeabilizing compounds from *Alexandrium* (Ma *et al.*, 2011 ; Long *et al.*, Under review in *Harmful*
373 *Algae*). The microalgal cells would be protected from their own toxins by their specific sterol
374 membrane composition (Deeds & Place, 2006), a hypothesis also made for the genus *Alexandrium* and
375 its allelochemicals (Ma *et al.*, 2011). While cells from *Amoebophrya* sp. do not have a specific sterol
376 signature (Leblond *et al.*, 2006; Place *et al.*, 2009), their sterol composition is rather related to sterol
377 content of the host. The parasite is able to retain host lipid content, including the antidote for toxins,
378 during the infection process. This strategy enables the parasite to avoid cell lysis and to infect its hostile
379 host.

380
381 However, not all potential hosts are hostiles, the APC potency was highly variable between *A. minutum*
382 strains. It was correlated with anti-microalgal (Long *et al.*, 2018) and ichthyotoxic (Borcier *et al.*, 2017;
383 Castrec *et al.*, 2018) activities. The mode of action of APC is very similar to the mode of action of anti-
384 microalgal allelochemicals described from the same strain (Long *et al.*, 2018; Long *et al.*, Under review
385 in *Harmful Algae*) and from *A. catenella* (formerly group I of the *A. tamarensis/fundyense/catenella*
386 species complex (Ma *et al.*, 2011)). Both allelochemicals disrupt cell membranes and eventually induce
387 cell lysis. It remains unclear whether APC are the same compounds than the ones described to have
388 anti-microalgal or ichthyotoxic effects. However, it is also known that *Alexandrium spp.* can modulate
389 its allelochemical potency against microalgae in response to changing physicochemical conditions
390 (Martens *et al.*, 2016; Long *et al.*, 2019) but also its toxicity in response to cues from dead microalgal
391 cells (Brown & Kubanek, 2020). It was also observed that genes associated with defensive responses
392 such as the production of reactive oxygen species were overexpressed in a non-allelopathic strain of
393 *A. fundyense* exposed to *Amoebophrya* sp. waterborne cues (Lu *et al.*, 2016). Thus, activation and
394 enhanced production of APC in the presence of a parasite cannot be excluded.

395
396 Similarly, *S. donghaiensis* passively releases APC in the surrounding environment but a potential active
397 defense remains to be investigated. In comparison with *A. minutum*, a different effect, potentially
398 mediated by different molecules, was observed in the presence of *S. donghaiensis*. The former species
399 did not affect the survival of the free-living stage of the parasite infecting *S. acuminata*, but decreased
400 its infectivity (i.e. ability to enter the cells) and/or progeny (i.e. ability to develop and produced the
401 next generation of dinospores). The production of extracellular bioactive compounds was yet reported
402 in *S. acuminata* (formerly identified as *S. trochoidea*; (Wang & Tang, 2008; Tang & Gobler, 2012) but

403 never tested in *S. donghaeinis*. APC might also indirectly act as a signaling system for *S. acuminata* that
404 could in turn modify its resistance against *Amoebophrya* sp., an interesting hypothesis that requires
405 more investigation. Importantly, these results remind us that chemical weapons are not limited to
406 harmful algal bloom species.

407

408 It was suggested that the presence of allelopathic genotypes could facilitate the proliferation of non-
409 allelopathic cells and therefore the whole population (John *et al.*, 2015; Felpeto *et al.*, 2018). Here, it
410 was additionally demonstrated that opportunistic (and competitive) species like *S. acuminata* could be
411 protected from parasitism and could benefit from a few anti-parasitic producers among *A. minutum*
412 and *S. donghaeinis* populations. The cumulative protective effect provided by resistant hosts likely
413 contributes to the survival of a sensitive dinoflagellate species in presence of its parasite, the private
414 good becoming a public good (Driscoll *et al.*, 2016). In cooperative associations, individuals that use a
415 common goods produced by others in the absence of feedback are called cheaters. This is the case for
416 non-allelopathic strains of *Prymnesium parvum* that benefits from the exclusion of diatom by another
417 allelopathic strain (Driscoll *et al.*, 2013). However, only the cheaters that are not or weakly sensitive
418 to APC will benefit from the “cure”. For some microalgal species, the APC “cure” might have strong
419 deleterious side effects. At least, a negative impact of *A. minutum* cells (but not of the filtrate) was
420 observed on the growth of *S. acuminata* in co-cultures. After all, our results highlight a potential
421 protective role of APC for the dinoflagellate but also suggest that the complexity of planktonic
422 community structure in environmental communities may lead to contrasting results.

423

424 APC producers never induced the complete loss of the parasite, as illustrated by the production of a
425 novel generation of dinospores, even in the presence of highly APC producers. These results suggest
426 that once inside their host, the parasites might be totally or partially protected from APC. Eventually,
427 such chemical defenses that moderate infections could contribute to the maintenance of the parasite
428 in time, whilst avoiding the collapse of its hosts. More generally, allelopathy prevents competitive
429 exclusion and promotes biodiversity in phytoplankton by favoring weaker competitors for nutrients
430 (Felpeto *et al.*, 2018). Similarly, APC promote biodiversity of parasites by favoring the most resistant
431 parasite that may not be the most virulent. Indeed, these results well explain the discrepancies
432 between the virulence of parasites that kill 100% of host cells within few days in the laboratory (this
433 study and others;(Chambouvet *et al.*, 2011a; Rodríguez & Figueroa, 2020)), and the coexistence of
434 hosts and parasites in ecological studies that does not always result in the complete collapse of the
435 sensitive population (Chambouvet *et al.*, 2011b; Cosgrove, 2014). It also supports the hypothesis that
436 the coexistence of different parasite cryptic species competing for the same host as reported by (Cai
437 *et al.*, 2020). All of these effects contribute to the explanation of the plankton paradox (Hutchinson,
438 1961). Chemical interactions between microorganisms tend to promote biodiversity (Czaran *et al.*,
439 2002; Felpeto *et al.*, 2018) and limit the effect of competitive exclusion for nutrients (or hosts for
440 parasites) within plankton.

441

442

443 **Conclusion**

444 Despite the ubiquity of genus *Amoebophrya* sp. in marine ecosystems, many opened questions remain
445 on parameters affecting the parasite dynamic. This study highlight that resistant dinoflagellates can
446 release exudates deleterious to the free-life stages of *Amoebophrya* sp. Chemical defenses must play
447 a role in the resistance of dinoflagellates to parasites and more largely a role in their competitiveness.

448 The exudation of anti-parasitic metabolites by resistant hosts in the surrounding environment provides
449 a novel mechanistic link between a host-parasite couple and the surrounding community without the
450 need of physical contact. The exudates not only protect the producer against parasitism but also have
451 the potential to affect the whole community by decreasing the propagation of the parasite. This study
452 revealed the importance of the composition of the plankton community during parasite infection as
453 the severity of the effect fluctuated depending on the species and the strains of the resistant partner,
454 their concentration and/or the ratio between the different partners. Another factor that has not been
455 assessed in this study but requires further consideration is the potential of chemosensing in these
456 interactions. Some parasites like the generalist parasite *Parvilucifera sinerae* can “sense” infochemicals
457 (e.g. DMSP) from potential hosts (Garcés *et al.*, 2013), even though they cannot actively select a
458 compatible host (Alacid *et al.*, 2016). Chemosensing of resistant host infochemicals by a parasite might
459 have significant consequences on the efficiency of anti-parasitic defenses and should be studied
460 through micro-scale studies. Overall, this study confirms that in the era of the -omic tools,
461 “reductionist” experiments are still required to disentangle interactomes (Fitzpatrick *et al.*, 2020). In
462 addition to the ecological relevance, the use of anti-parasitic compounds extracted from
463 dinoflagellates might be a mean to mitigate the parasites that can have devastating effects in algae
464 mass cultures (Carney & Lane, 2014).

465

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473

474 **Author contributions**

475 ML, LG, CJ, MS, MLG designed the study. ML, JS, DM, JT, CJ performed the experiments. ML, LG and CJ
476 performed the data analysis and wrote the manuscript that was discussed and revised by all co-
477 authors.

478

479 **Data availability**

480 The data that supports the findings of this study are available in the supplementary material of this
481 article.

482 **References**

- 483 **Alacid E, Park MG, Turon M, Petrou K, Garcés E. 2016.** A game of russian roulette for a generalist
484 dinoflagellate parasitoid: host susceptibility is the key to Success. *Frontiers in Microbiology* **7**.
- 485 **Alves-de-Souza C, Pecqueur D, Le Floc'h E, Mas S, Roques C, Mostajir B, Vidussi F, Velo-Suárez L,**
486 **Sourisseau M, Fouilland E, et al. 2015.** Significance of plankton community structure and nutrient
487 availability for the control of dinoflagellate blooms by parasites: A modeling approach (HG Dam, Ed.).
488 *PLOS ONE* **10**: e0127623.
- 489 **Anderson SR, Harvey EL. 2020.** Temporal variability and ecological interactions of parasitic marine
490 Syndiniales in coastal protist communities (BJ Campbell, Ed.). *mSphere* **5**: e00209-20,
491 /msphere/5/3/mSphere209-20.atom.
- 492 **Bai X, Adolf JE, Bachvaroff T, Place AR, Coats DW. 2007.** The interplay between host toxins and
493 parasitism by *Amoebophrya*. *Harmful Algae* **6**: 670–678.
- 494 **Basterretxea G, Font-Muñoz JS, Tuval I. 2020.** Phytoplankton orientation in a turbulent ocean: A
495 microscale perspective. *Frontiers in Marine Science* **7**: 185.
- 496 **Bjorbækmo MFM, Evenstad A, Røsæg LL, Krabberød AK, Logares R. 2020.** The planktonic protist
497 interactome: where do we stand after a century of research? *The ISME Journal* **14**: 544–559.
- 498 **Borcier E, Morvezen R, Boudry P, Miner P, Charrier G, Laroche J, Hegaret H. 2017.** Effects of
499 bioactive extracellular compounds and paralytic shellfish toxins produced by *Alexandrium minutum*
500 on growth and behaviour of juvenile great scallops *Pecten maximus*. *Aquatic Toxicology* **184**: 142–
501 154.
- 502 **Breier RE, Lalescu CC, Waas D, Wilczek M, Mazza MG. 2018.** Emergence of phytoplankton patchiness
503 at small scales in mild turbulence. *Proceedings of the National Academy of Sciences* **115**: 12112–
504 12117.
- 505 **Brown ER, Kubanek J. 2020.** Harmful alga trades off growth and toxicity in response to cues from
506 dead phytoplankton. *Limnology and Oceanography*.
- 507 **Brussaard CPD. 2004.** Viral Control of Phytoplankton Populations—a Review. *The Journal of Eukaryotic*
508 *Microbiology* **51**: 125–138.
- 509 **Cai R, Kayal E, Alves-de-Souza C, Bigeard E, Corre E, Jeanthon C, Marie D, Porcel BM, Siano R,**
510 **Szymczak J, et al. 2020.** Cryptic species in the parasitic *Amoebophrya* species complex revealed by a
511 polyphasic approach. *Scientific Reports* **10**: 2531.
- 512 **Carlson CJ, Burgio KR, Dougherty ER, Phillips AJ, Bueno VM, Clements CF, Castaldo G, Dallas TA,**
513 **Cizauskas CA, Cumming GS, et al. 2017.** Parasite biodiversity faces extinction and redistribution in a
514 changing climate. *Science Advances* **3**: e1602422.
- 515 **Carney LT, Lane TW. 2014.** Parasites in algae mass culture. *Frontiers in Microbiology* **5**.
- 516 **Castrec J, Soudant P, Payton L, Tran D, Miner P, Lambert C, Le Goïc N, Huvet A, Quillien V, Bouillot F,**
517 **et al. 2018.** Bioactive extracellular compounds produced by the dinoflagellate *Alexandrium minutum*
518 are highly detrimental for oysters. *Aquatic Toxicology* **199**: 188–198.

- 519 **Chambouvet A, Alves-de-Souza C, Cueff V, Marie D, Karpov S, Guillou L. 2011a.** Interplay between
520 the Parasite *Amoebophrya* sp. (Alveolata) and the Cyst Formation of the Red Tide Dinoflagellate
521 *Scrippsiella trochoidea*. *Protist* **162**: 637–649.
- 522 **Chambouvet A, Laabir M, Sengco M, Vaquer A, Guillou L. 2011b.** Genetic diversity of
523 *Amoebophryidae* (Syndiniales) during *Alexandrium catenella/tamarensis* (Dinophyceae) blooms in the
524 Thau lagoon (Mediterranean Sea, France). *Research in Microbiology* **162**: 959–968.
- 525 **Chambouvet A, Morin P, Marie D, Guillou L. 2008.** Control of toxic marine dinoflagellate blooms by
526 serial parasitic killers. *Science* **322**: 1254–1257.
- 527 **Chapelle A, Le Bec C, Amzil Z, Dreanno C, Klouch KZ, Labry C, Le Gac M, Pineau L, Siano R, Abernot
528 C, et al. 2014.** Etude sur la prolifération de la micro algue *Alexandrium minutum* en rade de Brest.
- 529 **Chapelle A, Le Gac M, Labry C, Siano R, Quere J, Caradec F, Le Bec E, Doner A, Gouriou J. 2015.** The
530 Bay of Brest (France), a new risky site for toxic *Alexandrium minutum* blooms and PSP shellfish
531 contamination.pdf. *Harmful algal news*: 4–5.
- 532 **Choi CJ, Brosnahan ML, Sehein TR, Anderson DM, Erdner DL. 2017.** Insights into the loss factors of
533 phytoplankton blooms: The role of cell mortality in the decline of two inshore *Alexandrium* blooms.
534 *Limnology and Oceanography* **62**: 1742–1753.
- 535 **Coats DW, Park MG. 2002.** Parasitism of photosynthetic dinoflagellates by three strains of
536 *Amoebophrya* (Dinophyta) : parasite survival, infectivity, generation time, and host specificity.
537 *Journal of Phycology* **38**: 520–528.
- 538 **Cosgrove S. 2014.** Monitoring methods and bloom dynamic studies of the toxic dinoflagellate genus
539 *Alexandrium*. PhD Thesis. National University of Ireland, Galway, Ireland.
- 540 **Czaran TL, Hoekstra RF, Pagie L. 2002.** Chemical warfare between microbes promotes biodiversity.
541 *Proceedings of the National Academy of Sciences* **99**: 786–790.
- 542 **Deeds J, Place A. 2006.** Sterol-specific membrane interactions with the toxins from *Karlodinium*
543 *micrum* (Dinophyceae) — a strategy for self-protection? *African Journal of Marine Science* **28**: 421–
544 425.
- 545 **Dougherty ER, Carlson CJ, Bueno VM, Burgio KR, Cizauskas CA, Clements CF, Seidel DP, Harris NC.
546 2016.** Paradigms for parasite conservation: Parasite Conservation. *Conservation Biology* **30**: 724–733.
- 547 **Driscoll WW, Espinosa NJ, Eldakar OT, Hackett JD. 2013.** Allelopathy as an emergent, exploitable
548 public good in the bloom-forming microalga *Prymnesium parvum*. *Evolution* **67**: 1582–1590.
- 549 **Driscoll WW, Hackett JD, Ferrière R. 2016.** Eco-evolutionary feedbacks between private and public
550 goods: evidence from toxic algal blooms (P Jeyasingh, Ed.). *Ecology Letters* **19**: 81–97.
- 551 **Durham WM, Stocker R. 2012.** Thin phytoplankton layers: characteristics, mechanisms, and
552 consequences. *Annual Review of Marine Science* **4**: 177–207.
- 553 **Farhat S, Le P, Kayal E, Noel B, Bigeard E, Corre E, Maumus F, Florent I, Alberti A, Aury J-M, et al.
554 2020.** Rapid protein evolution, organellar reductions, and invasive intronic elements in the marine
555 aerobic parasite dinoflagellate *Amoebophrya* spp.

- 556 **Felpeto AB, Roy S, Vasconcelos VM. 2018.** Allelopathy prevents competitive exclusion and promotes
557 phytoplankton biodiversity. *Oikos* **127**: 85–98.
- 558 **Fitzpatrick CR, Salas-González I, Conway JM, Finkel OM, Gilbert S, Russ D, Teixeira PJPL, Dangl JL.**
559 **2020.** The Plant Microbiome: From ecology to reductionism and beyond. *Annual Review of*
560 *Microbiology* **74**: annurev-micro-022620-014327.
- 561 **Garcés E, Alacid E, Reñé A, Petrou K, Simó R. 2013.** Host-released dimethylsulphide activates the
562 dinoflagellate parasitoid *Parvilucifera sinerae*. *The ISME Journal* **7**: 1065–1068.
- 563 **Granéli E, Hansen PJ. 2006.** Allelopathy in harmful algae: a mechanism to compete for resources? In:
564 Granéli E, Turner JT, eds. *Ecological Studies. Ecology of Harmful Algae*. Springer Berlin Heidelberg,
565 189–201.
- 566 **Guillou L, Viprey M, Chambouvet A, Welsh RM, Kirkham AR, Massana R, Scanlan DJ, Worden AZ.**
567 **2008.** Widespread occurrence and genetic diversity of marine parasitoids belonging to *Syndiniales* (
568 *Alveolata*). *Environmental Microbiology* **10**: 3349–3365.
- 569 **Hutchinson GE. 1961.** The Paradox of the plankton. *The American Naturalist* **95**: 137–145.
- 570 **John U, Tillmann U, Hülskötter J, Alpermann TJ, Wohlrab S, Van de Waal DB. 2015.** Intraspecific
571 facilitation by allelochemical mediated grazing protection within a toxigenic dinoflagellate
572 population. *Proceedings of the Royal Society B: Biological Sciences* **282**: 20141268.
- 573 **Johnson PTJ, Preston DL, Hoverman JT, LaFonte BE. 2013.** Host and parasite diversity jointly control
574 disease risk in complex communities. *Proceedings of the National Academy of Sciences* **110**: 16916–
575 16921.
- 576 **Kayal E, Alves-de-Souza C, Farhat S, Velo-Suarez L, Monjol J, Szymczak J, Bigeard E, Marie D, Noel B,**
577 **Porcel BM, et al. 2020.** Dinoflagellate host chloroplasts and mitochondria remain functional during
578 *Amoebophrya* Infection. *Frontiers in Microbiology* **11**: 600823.
- 579 **Kim S. 2006.** Patterns in host range for two strains of *Amoebophrya* (Dinophyta) infecting thecate
580 dinoflagellate : *Amoebophrya* spp. ex *Alexandrium affine* and ex *Gonyaulax polygramma*. *Journal of*
581 *Phycology* **42**: 1170–1173.
- 582 **Kim S, Gil Park M, Yih W, Coats DW. 2004.** Infection of the bloom-forming thecate dinoflagellates
583 *Alexandrium affine* and *Gonyaulax spinifera* by two strains of *Amoebophrya* (Dinophyta). *Journal of*
584 *Phycology* **40**: 815–822.
- 585 **Klouch KZ, Schmidt S, Andrieux-Loyer F, Le Gac M, Hervio-Heath D, Qui-Minet ZN, Quéré J, Bigeard**
586 **E, Guillou L, Siano R. 2016.** Historical records from dated sediment cores reveal the multidecadal
587 dynamic of the toxic dinoflagellate *Alexandrium minutum* in the Bay of Brest (France) (R Laanbroek,
588 Ed.). *FEMS Microbiology Ecology* **92**: fiw101.
- 589 **Leblond JD, Sengco MR, Sickman JO, Dahmen JL, Anderson DM. 2006.** Sterols of the Syndinian
590 dinoflagellate *Amoebophrya* sp., a parasite of the dinoflagellate *Alexandrium tamarense*
591 (Dinophyceae). *The Journal of Eukaryotic Microbiology* **53**: 211–216.
- 592 **Li C, Song S, Liu Y, Chen T. 2014.** Occurrence of *Amoebophrya* spp. infection in planktonic
593 dinoflagellates in Changjiang (Yangtze River) Estuary, China. *Harmful Algae* **37**: 117–124.

- 594 **Lima-Mendez G, Faust K, Henry N, Decelle J, Colin S, Carcillo F, Chaffron S, Ignacio-Espinosa JC,**
595 **Roux S, Vincent F, et al. 2015.** Determinants of community structure in the global plankton
596 interactome. *Science* **348**: 1262073–1262073.
- 597 **Long M, Peltekis A, González-Fernández C, Hégaret H, Bailleul B, (Under review).** Allelochemicals of
598 *Alexandrium minutum* : kinetics of membrane disruption and photosynthesis inhibition in a co-
599 occurring diatom. *Harmful Algae*.
- 600 **Long M, Holland A, Planquette H, González Santana D, Whitby H, Soudant P, Sarthou G, Hégaret H,**
601 **Jolley DF. 2019.** Effects of copper on the dinoflagellate *Alexandrium minutum* and its allelochemical
602 potency. *Aquatic Toxicology* **210**: 251–261.
- 603 **Long M, Tallec K, Soudant P, Lambert C, Le Grand F, Sarthou G, Jolley D, Hégaret H. 2018.** A rapid
604 quantitative fluorescence-based bioassay to study allelochemical interactions from *Alexandrium*
605 *minutum*. *Environmental Pollution* **242**: 1598–1605.
- 606 **Lu Y, Wohlrab S, Groth M, Glöckner G, Guillou L, John U. 2016.** Transcriptomic profiling of
607 *Alexandrium fundyense* during physical interaction with or exposure to chemical signals from the
608 parasite *Amoebophrya*. *Molecular Ecology* **25**: 1294–1307.
- 609 **Ma H, Krock B, Tillmann U, Bickmeyer U, Graeve M, Cembella A. 2011.** Mode of action of
610 membrane-disruptive lytic compounds from the marine dinoflagellate *Alexandrium tamarense*.
611 *Toxicon* **58**: 247–258.
- 612 **Martens H, Van de Waal DB, Brandenburg KM, Krock B, Tillmann U. 2016.** Salinity effects on growth
613 and toxin production in an *Alexandrium ostenfeldii* (Dinophyceae) isolate from The Netherlands.
614 *Journal of Plankton Research* **38**: 1302–1316.
- 615 **Park M, Cooney S, Yih W, Coats D. 2002.** Effects of two strains of the parasitic dinoflagellate
616 *Amoebophrya* on growth, photosynthesis, light absorption, and quantum yield of bloom-forming
617 dinoflagellates. *Marine Ecology Progress Series* **227**: 281–292.
- 618 **Paseka RE, White LA, Van de Waal DB, Strauss AT, González AL, Everett RA, Peace A, Seabloom EW,**
619 **Frenken T, Borer ET. 2020.** Disease-mediated ecosystem services: Pathogens, plants, and people.
620 *Trends in Ecology & Evolution* **35**: 731–743.
- 621 **Pelusi A, De Luca P, Manfellotto F, Thamatrakoln K, Bidle KD, Montresor M. 2020.** Virus-induced
622 spore formation as a defense mechanism in marine diatoms. *New Phytologist*: nph.16951.
- 623 **Place AR, Bai X, Kim S, Sengco MR, Wayne Coats D. 2009.** Dinoflagellate host-parasite sterol profiles
624 dictate karlotoxin sensitivity. *Journal of Phycology* **45**: 375–385.
- 625 **Place A, Harvey H, Bai X, Coats D. 2006.** Sneaking under the toxin surveillance radar: parasitism and
626 sterol content. *African Journal of Marine Science* **28**: 347–351.
- 627 **Pouneva ID. 2006.** Effect of abscisic acid and ontogenic phases of the host alga on the infection
628 process in the pathosystem *Scenedesmus acutus* — *Phlyctidium scenenedesmi*. *Acta Physiologiae*
629 *Plantarum* **28**: 395–400.
- 630 **Rodríguez F, Figueroa RI. 2020.** Confirmation of the wide host range of *Parvilucifera corolla*
631 (Alveolata, Perkinsozoa). *European Journal of Protistology* **74**: 125690.

- 632 **Rohrlack T, Christiansen G, Kurmayer R. 2013.** Putative antiparasite defensive system involving
633 ribosomal and nonribosomal oligopeptides in Cyanobacteria of the Genus *Planktothrix*. *Applied and*
634 *Environmental Microbiology* **79**: 2642–2647.
- 635 **Scholz B, Küpper F, Vyverman W, Ólafsson H, Karsten U. 2017.** Chytridiomycosis of marine
636 diatoms—The role of stress physiology and resistance in parasite-host recognition and accumulation
637 of defense molecules. *Marine Drugs* **15**: 26.
- 638 **Seymour JR, Amin SA, Raina J-B, Stocker R. 2017.** Zooming in on the phycosphere: the ecological
639 interface for phytoplankton–bacteria relationships. *Nature Microbiology* **2**: 17065.
- 640 **Siano R, Alves-de-Souza C, Foulon E, Bendif EM, Simon N, Guillou L, Not F. 2011.** Distribution and
641 host diversity of Amoebophryidae parasites across oligotrophic waters of the Mediterranean Sea.
642 *Biogeosciences* **8**: 267–278.
- 643 **Starr RC, Zeikus JA. 1993.** Utx—The culture collection of algae at the university of Texas at Austin
644 1993 List of cultures. *Journal of Phycology* **29**: 1–106.
- 645 **Tang YZ, Gobler CJ. 2012.** Lethal effects of Northwest Atlantic Ocean isolates of the dinoflagellate,
646 *Scrippsiella trochoidea*, on Eastern oyster (*Crassostrea virginica*) and Northern quahog (*Mercenaria*
647 *mercenaria*) larvae. *Marine Biology* **159**: 199–210.
- 648 **Vardi A, Van Mooy BAS, Fredricks HF, Popenorf KJ, Ossolinski JE, Haramaty L, Bidle KD. 2009.** Viral
649 glycosphingolipids induce lytic infection and cell death in marine phytoplankton. *Science* **326**: 861–
650 865.
- 651 **de Vargas C, Audic S, Henry N, Decelle J, Mahe F, Logares R, Lara E, Berney C, Le Bescot N, Probert I,
652 *et al.* 2015.** Eukaryotic plankton diversity in the sunlit ocean. *Science* **348**: 1261605–1261605.
- 653 **Velo-Suárez L, Brosnahan ML, Anderson DM, McGillicuddy DJ. 2013.** A quantitative assessment of
654 the role of the parasite *Amoebophrya* in the termination of *Alexandrium fundyense* blooms within a
655 small coastal embayment (AC Anil, Ed.). *PLoS ONE* **8**: e81150.
- 656 **Wang Y, Tang X. 2008.** Interactions between *Prorocentrum donghaiense* Lu and *Scrippsiella*
657 *trochoidea* (Stein) Loeblich III under laboratory culture. *Harmful Algae* **7**: 65–75.
- 658 **Wheeler JD, Secchi E, Rusconi R, Stocker R. 2019.** Not just going with the flow: the effects of fluid
659 flow on bacteria and plankton. *Annual Review of Cell and Developmental Biology* **35**: 213–237.
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662 **Supporting Information**

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664 Table S2: Details of microalgal and Syndiniales strains used in this study. “/” means that the data is unknown.

Strains IDs	Species	Isolation Date	Origin	Culture collection	Allelopathy	References
ST147 RCC1627	<i>Scripsiella acuminata</i> clade STR1	2005	Penzé, Bay of Morlaix, France	Roscoff Culture Collection	Unknown	Chambouvet et al., 2011; Farhat et al., 2018
Sc39 RCC4714	<i>S. donghaiensis</i>	2012	Bay of Brest, France	Roscoff Culture Collection	Unknown	Klouch et al., 2016
CCMI1002	<i>A. minutum</i>	2005	Eany, Co. Donegal, Ireland	Culture collection Marine Institute, Ireland	Yes	Long et al., 2018
Am176 AM89BM RCC749	<i>A. minutum</i>	1989	Penzé, Bay of Morlaix, France	Roscoff Culture Collection	Yes	Long et al., 2018
Da1257	<i>A. minutum</i>	/	Bay of Brest, France	Ifremer	Yes	Long et al., 2018
A25 RCC4383	<i>Amoebophrya</i> sp. Clade 1	2009	Penzé, Bay of Morlaix, France	Roscoff Culture Collection	/	Farhat et al., 2018

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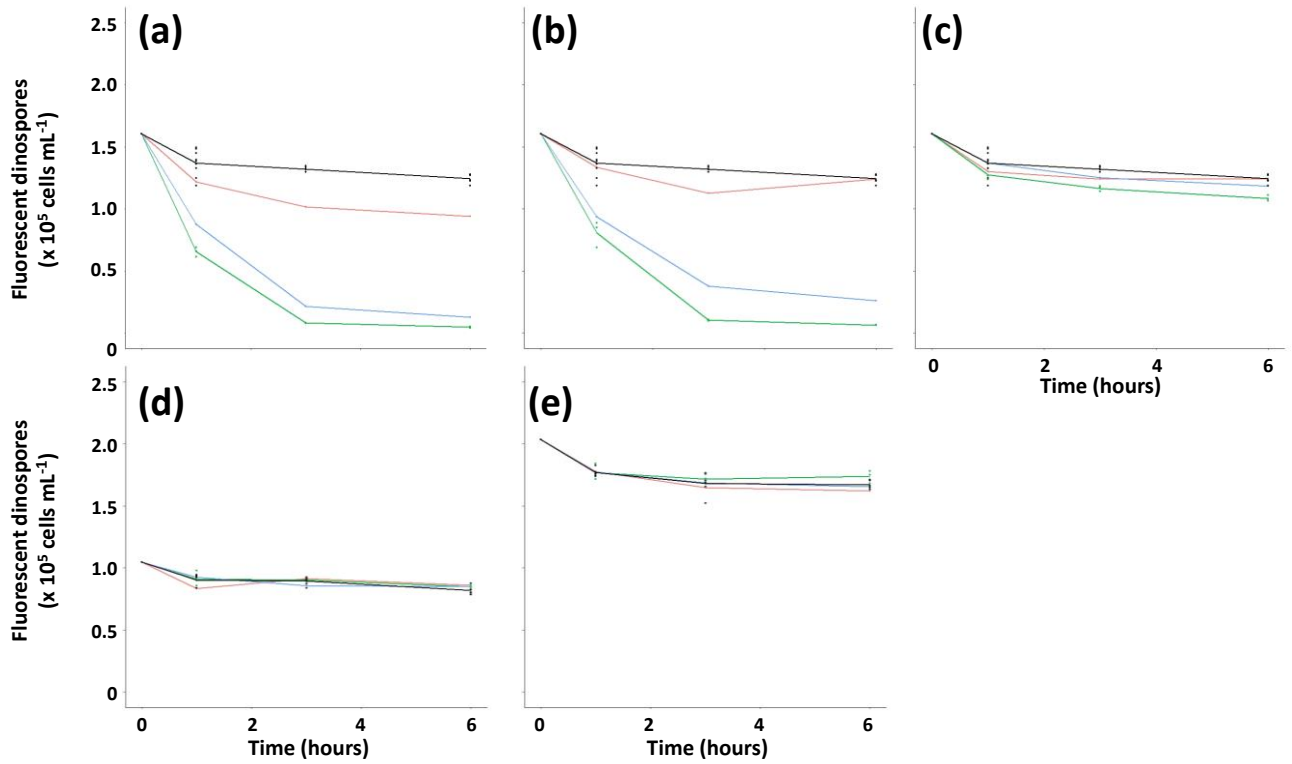
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667 Table S2: Effect of culture medium or filtrates on the mortality rate of autofluorescent dinospores of *Amoebophrya* sp. A25
668 over 24 hours. Loss rate was calculated according to Equation 1 in the manuscript. “NS” means that no significant difference
669 was observed.

	Initial density	24 hours density	Loss rate of autofluorescent dinospores	Significance
F/2 media	29500 ± 400	14000 ± 1000	0.031 ± 0.004	NS
ST147 filtrate	27000 ± 900	15000 ± 2000	0.026 ± 0.005	NS
A25-ST147 filtrate	29000 ± 1500	14000 ± 1000	0.030 ± 0.002	NS

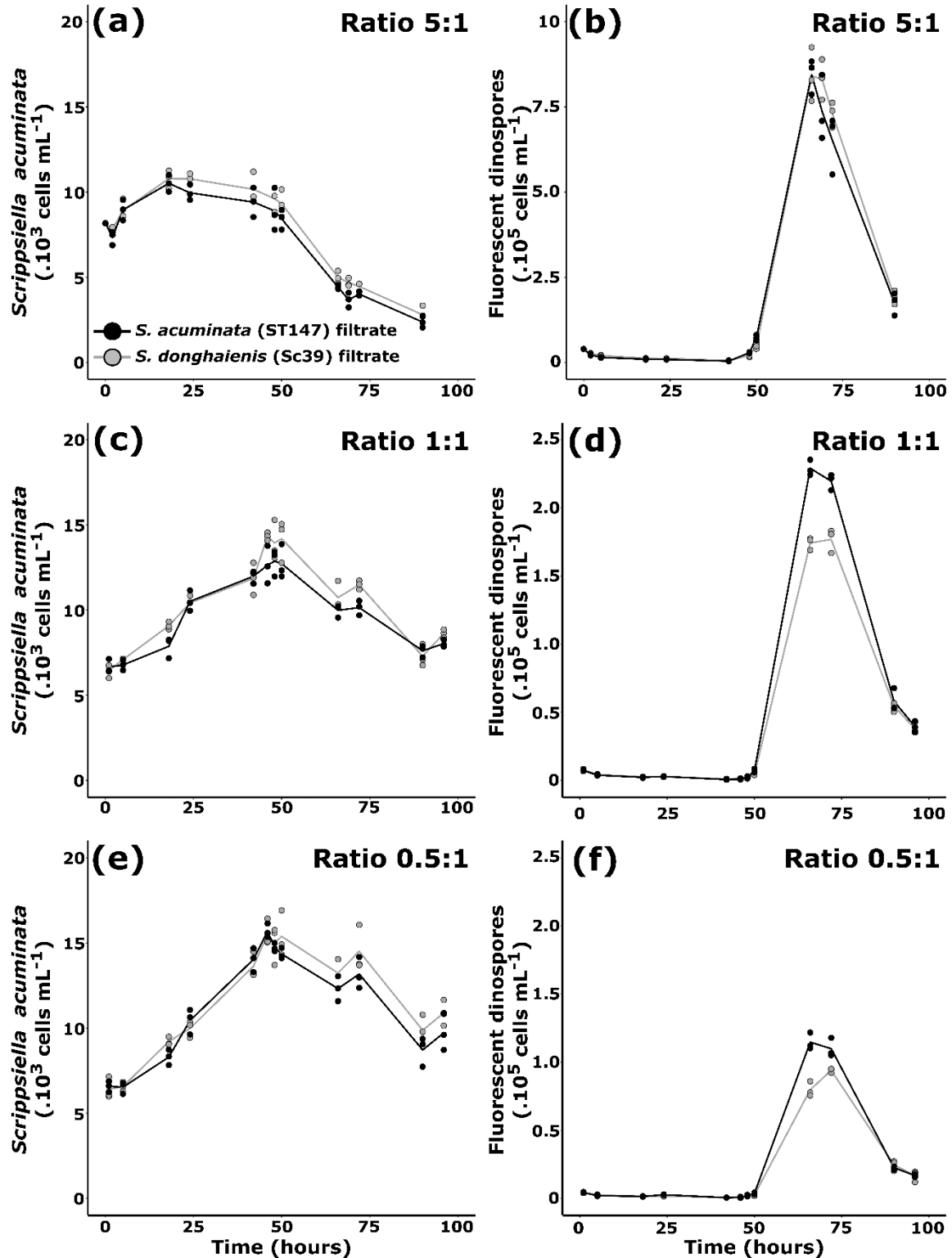
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673 Figure S1 : Concentration of fluorescent dinospores over 6 hours after exposure to (a) *A. minutum* CCM1002, (b) *A. minutum*
674 AM176, (c) *A. minutum* DA1257 and (d-e) *S. donghaiensis* Sc39. Dinospores were exposed to ST147 filtrate (control in black),
675 and filtrate at equivalent microalgal densities of 1000 (red lines), 5000 (blue lines) and the maximum concentration of (a, b,
676 c, e) 10000 cells mL⁻¹ and (d) 7000 cells mL⁻¹ (green lines).



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Figure S2: Effect of Sc39 *S. donghaiensis* filtrates (Theoretical cell concentration = 7000 cells mL^{-1} for (a) and (b), 10000 cells mL^{-1} for (c, d, e, f) on the infectivity of A25 dinospores to *S. acuminata* (ST147). Infections were performed at three different dinospores: *S. acuminata* ratios; 5:1(a, b),1:1 (C and D) and 0.5:1 (e, f). The densities of the host ST147 during the infection cycle are shown in graphs (a, c, e). The density of fluorescent dinospores are shown in graphs (b, d, f). The controls (Filtrate ST147) are shown in black while the conditions in presence of Sc39 filtrate are shown in grey.

