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Natural chemicals produced by marine microalgae as predator deterrents can be used to control ciliates contamination in microalgal cultures

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Abstract

Large-scale production of microalgae for biofuels is often hampered by contamination of cultures with predators that feed on microalgae. An important group of predators are ciliates. Some species of marine microalgae in natural ecosystems are known to produce chemicals that act as deterrents against predators. In this study, we tested whether these chemicals (trans,trans-2,4-decadienal, dimethyl sulfoniopropionate (DMSP), glycine betaine and proline) as well as a chemical analogue (methyl 3-(methylthio)propionate or MMP) can be used to control contamination of cultures of the microalga *Chlamydomonas* by the predatory ciliate *Sterkiella*. All chemicals were capable of rapidly eradicating the ciliates from a contaminated *Chlamydomonas* culture, but at a higher dose also had a negative effect on the microalga. For each chemical an optimal dose was determined at which ciliates were controlled and losses in microalgal biomass productivity were minimized (0.13 mM decadienal, 4.75 mM DMSP, 10 mM MMP, 250-300 mM proline and 250-

300 mM glycine betaine). In the case of DMSP, MMP and proline, biomass productivity was even the same as that of a non-contaminated culture. The chemicals were also effective against other ciliates (*Stylonychia notophora*, *Oxytricha* sp. and 2 different *Paramecium* species). These chemicals therefore have potential to be used as natural pesticides to control contamination of microalgal cultures by ciliates. Of all chemicals tested, DMSP and MMP are the most promising because they are effective at a relatively low dose and have a limited negative effect on microalgal productivity.

Key-words: microalgal productivity, contamination, ciliate control, natural pesticides, chemical analogues, dosage

1. Introduction

Microalgae have received significant interest in the past decade as a source of biomass for the production of biofuels [1–3]. In the past years, production of microalgae has moved from the laboratory to large-scale outdoor cultures. These large-scale production systems often experience large losses in productivity due to contamination of the culture by predators, parasites and competing microalgae [4–6]. Among these contaminants, ciliates that feed on microalgae are a particularly wicked problem. They achieve very high growth rates and as a result can cause a crash of the culture within a timeframe of only a few days [7].

Contamination of large-scale microalgal cultures by ciliates can be controlled by either preventive or curative measures. Prevention of contamination requires sterilisation of the culture medium as well as the air used to sparge the cultures because ciliates or their resting stages can be present in water as well as in air [8,9]. This is a challenge because production of microalgae requires large volumes of water (200 - 2000 L kg⁻¹ dry biomass) and cultures need to be continuously sparged with large volumes of air to remove excess oxygen. Even if the initial abundance of ciliates in the culture medium is as low as only 1 individual per m³ of culture, culture crashes may still occur within a couple of weeks [10]. Some curative measures have been proposed to control ciliates during a culture crash. Quinine sulphate at a concentration of 10- 20 mg L⁻¹ can be used to treat microalgal cultures that are infected by ciliates [11,12]. The supply of quinine, however, is limited as quinine has to be isolated from the bark of wild chinchona trees. Although copper sulfate reduce ciliate numbers in microalgal cultures, this chemical also has a negative impact on the microalgae themselves [12]. A recent study demonstrated that high concentrations of CO₂ (330-2000 mg L⁻¹, pH

<4.5) reduces numbers of the ciliate *Paramecium* in microalgal cultures [13]. Other predators of microalgae such as rotifers can be controlled by addition of the pesticide rotenone or by raising the free ammonia concentration (by addition of NH_4^+ in combination with an increase in pH), but these measures have no influence on ciliates [14,15].

Microalgae in natural ecosystems have evolved chemical defense mechanisms to fend off predators [16]. Several species of marine microalgae such as *Phaeocystis* spp. or *Emiliania huxleyi* produce dimethylsulfoniopropionate (DMSP) and this chemical acts as a deterrent to predators including ciliates [17]. Marine microalgae also accumulate the amino acid derivative glycine betaine, which is chemically similar to DMSP. Glycine betaine was shown to inhibit feeding of the predatory dinoflagellate *Amphidinium longum* on microalgae [17]. When marine diatoms are consumed by natural predators, polyunsaturated fatty acids present in cell membrane are oxidized to the aldehyde trans,trans-2,4-decadienal [18,19]. This was shown to reduce the feeding rate as well as the reproduction of marine copepods that feed on these diatoms [18,20]. Another study has shown that proline reduces feeding by ciliates on marine microalgae [21].

The aim of this study was to evaluate whether chemicals that are produced as feeding deterrents by marine microalgae in natural environments may be used to control ciliate contamination in freshwater microalgae cultures. We used the freshwater microalga *Chlamydomonas* and the hypotrich ciliate *Sterkiella* as model systems. In addition to three chemicals that are known to inhibit predators of marine microalgae (DMSP, glycine betaine, trans,trans-2,4-decadienal and proline), we also included methyl 3-(methylthio)propionate (MMP). Because MMP is a chemical analogue of DMSP [22,23], we hypothesized that it might have a similar activity. We evaluated whether these chemicals could eradicate

86 *Sterkiella* from *Chlamydomonas* cultures and were able to secure microalgal productivity.
87 In addition, we also tested the effect of the chemicals on four other ciliate species
88 (*Stylonychia notophora*, *Oxytricha* sp. and 2 different *Paramecium* species).

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2. Materials and methods

2.1. Cultivation of microalgae and ciliates

The chlorophyte microalga *Chlamydomonas reinhardtii* SAG 77.81 and the hypotrich ciliate *Sterkiella* sp. were used as model systems to evaluate the potential of five chemicals to control ciliate contamination in microalgal cultures. *Chlamydomonas reinhardtii* is a widely studied microalgal species. Hypotrich ciliates are a group of ciliates that commonly invade large-scale microalgal cultures ([11],[6]). *Chlamydomonas* cultures were maintained in 2 L batch cultures in Wright's Cryptophyte (WC) medium [24] in a temperature-controlled room (20 ± 2 °C) at a light intensity of $80 \mu\text{mole m}^{-2} \text{s}^{-1}$ and a light-dark cycle of 16:8. Growth of *Chlamydomonas* cells was monitored spectrophotometrically at optical density 750 nm (OD750) [25]. Optical density was calibrated against gravimetric dry weight measurements (using Whatman glass microfiber filters, grade GF/F) and against cell abundance (determined microscopically using a Bürker counting chamber).

The ciliate *Sterkiella* was isolated from a rainwater storage reservoir, Meulebeke, Belgium. Water from the reservoir was inoculated to a *Chlamydomonas* culture. After a few days a ciliate species emerged in the culture that consumed *Chlamydomonas*. The ciliate was isolated using a fine Pasteur pipette, washed several times in sterile WC medium and then transferred to a fresh *Chlamydomonas* culture. It was identified as *Sterkiella* based on morphological identification (Fig. 1) and partial 18S ribosomal DNA sequencing (GenBank accession number: MF375457). *Sterkiella* cultures were maintained in 6-well plates in 3 mL volume cultures. The cultures were transferred every 2 days to a fresh *Chlamydomonas* culture: 0.5 mL of the ciliate suspension was transferred to a well containing 2 mL of WC

medium and 0.5 mL of a stationary phase *Chlamydomonas* culture (5×10^6 *Chlamydomonas* cells mL^{-1}). Exponentially growing *Sterkiella* cultures with an abundance of about 500 cells mL^{-1} were used in all experiments. The ciliates were further concentrated by a 10 μm nylon mesh to obtain a density of 2000 cells mL^{-1} before addition to the experimental treatments.

Figure 1. Microscopic photograph of the hypotrich ciliate *Sterkiella* and the *Chlamydomonas* cells on which it feeds.

2.2. Evaluation of ciliate inhibition by natural chemicals

Five chemicals were tested for growth inhibition of ciliates. DMSP was obtained from Toronto Research Chemicals, Ontario, Canada while trans,trans-2,4-decadienal (hereafter decadienal), glycine betaine, MMP and proline were obtained from Sigma-Aldrich, Belgium. A stock solution of the chemicals was prepared on the day of the experiments. DMSP (1 M) was dissolved in distilled water. Glycine betaine (1 M) and proline (1 M) were added in relatively high volumes, so these chemicals were dissolved in WC medium. Due to their low solubility in water, decadienal (0.487 M) and MMP (1 M) were dissolved in methanol. This resulted in addition of a maximum of 0.06 % methanol for decadienal and 0.87 % methanol for MMP in the experimental treatments at the highest concentration tested.

Preliminary experiments had demonstrated that addition of up to 2% methanol to the cultures had no significant effect on either *Chlamydomonas* or *Sterkiella*.

Exploratory experiments were carried out to determine the concentration range at which the chemicals resulted in inhibition of the ciliate *Sterkiella*. These experiments showed that the chemicals not only eradicated ciliates but also had a negative effect on the microalga *Chlamydomonas*, at higher concentrations. Therefore, a range of concentrations was tested to find an optimal concentration at which *Sterkiella* was eradicated while *Chlamydomonas* was not affected. For each chemical, five different concentrations were tested in addition to a control treatment that received no chemicals (Table 1).

	Control	C 1	C 2	C 3	C 4	C 5
Decadienal (mM)	0	0.065	0.13	0.195	0.26	0.325
DMSP (mM)	0	4.5	4.75	5	5.25	5.5
MMP (mM)	0	1	5	10	15	20
Proline (mM)	0	200	250	300	350	400
Glycine betaine (mM)	0	200	250	300	350	400

Table 1. Concentration ranges used for each chemical in the experiments.

DMSP: dimethylsulfoniopropionate; MMP: methyl 3-(methylthio)propionate; C1, C2, C3, C4, C5: concentration 1, 2, 3, 4, 5.

Experiments were carried out in 100 mL volume cultures that were aerated with sterile-filtered air with gentle stirring (magnetic stirrer, 10 rpm). Each treatment was prepared in triplicate. These experiments were carried out in the same conditions (light, temperature) as for the cultivation of the *Chlamydomonas* stock culture (see above). The initial concentrations of microalgal prey and ciliate predator were 6.26×10^6 *Chlamydomonas* cells mL^{-1} (corresponding to OD750 of 0.3) and 25 *Sterkiella* cells mL^{-1} . Growth of

Chlamydomonas was monitored spectrophotometrically (OD750) while *Sterkiella* abundance was determined microscopically in a 1 mL subsample that was preserved with formaldehyde (5%). The cultures were monitored after 0.5, 24, 48 and 72 hours.

2.3. Assessing the impact of the chemicals on ciliates

Microscopic observations made clear that addition of the chemicals caused a change in the swimming behavior of the ciliate *Sterkiella*. This change in swimming behavior was quantified using video analysis. Healthy *Sterkiella* cells (25 cells mL⁻¹) suspended in WC medium were exposed to inhibitory concentrations of each chemical tested (0.13 mM decadienal, 4.75 mM DMSP, 10 mM MMP, 300 mM proline and 300 mM glycine betaine). After 30 min., the cell movement of the ciliates was recorded using a video camera attached to an Olympus BX51 microscope (200x magnification). At this magnification, each screenshot contained about 1 to 10 individual ciliates. Short videos of 180 sec. duration were recorded. The videos were analyzed using the free ImageJ software version 1.48 [26]. Ciliates were separated from the background in each frame and a sequence of frames was overlaid using the Z-projection to visualize the track of each ciliate. These tracks were manually recorded using the ‘free hand line’ tool. The shapes of the paths were classified as either a straight line, a curved line or a circle. The relative frequency of tracks from five videos were compared, corresponding to a minimum of 50 tracks per treatment.

The impact of the chemicals on other ciliate species was also investigated: *Stylonychia notophora*, *Oxytricha* sp. and 2 different *Paramecium* species. *Stylonychia notophora*, *Oxytricha* and one *Paramecium* species were isolated from an enrichment cultures prepared from pond water and another *Paramecium* species was isolated from a rainwater storage

reservoir. They were maintained in the lab in a similar way as the *Sterkiella* strain (fed with *Chlamydomonas*). Experiments were carried out in 10 mL volume cultures in 6-well plates. Microalgal prey with concentration of 6×10^6 *Chlamydomonas* cells mL⁻¹ were added as food for the ciliates. Suspension containing 25 ciliates mL⁻¹ were exposed to inhibitory concentrations of the five chemicals (0.13 mM decadienal, 4.75 mM DMSP, 10 mM MMP, 300 mM proline and 300 mM glycine betaine). Each treatment was prepared in triplicate. The number of surviving ciliates was determined microscopically after 0.5, 24 and 40 hours.

2.4. Statistical analysis

Two-way ANOVA was used to test for the independent and interacting effects of chemical concentration and ciliate presence/absence on the optical density of *Chlamydomonas* in the cultures after 72 hours. One-way ANOVA was used to evaluate the effect of chemical concentration on the abundance of ciliates for each. Duncan's Multiple Range Test was used for pairwise comparisons between samples. All statistical analyses were carried out using Sigmaplot 11.0 and MSTATC software.

3. Results and discussion

3.1. Control of ciliate contamination of microalgal cultures

In the control cultures without chemicals and without *Sterkiella*, *Chlamydomonas* biomass doubled within 72 hours from an initial OD750 of 0.3 to about 0.6 (Fig. 2, Table 2). When the culture was contaminated with 25 *Sterkiella* cells mL⁻¹ and did not receive chemicals, *Sterkiella* abundance increased from an initial abundance of 25 cells mL⁻¹ to 150

- 700 cells mL⁻¹ and *Chlamydomonas* biomass decreased below the initial OD750 of 0.3 within 72 hours. This illustrates the high population growth rate of the ciliate *Sterkiella* and its dramatic impact on a *Chlamydomonas* culture.

Addition of the chemicals had a negative impact on *Sterkiella* proliferation and this impact was concentration-dependent. For decadienal, DMSP and proline, the lowest concentration (corresponding to 0.06, 4.5, and 200 mM) resulted in significant reduction of *Sterkiella* abundance at 72 hours compared to the control. For MMP and glycine betaine, a significant reduction only occurred at the second lowest concentrations of 5 and 250 mM respectively. The three highest concentrations of all chemicals resulted in complete eradication of the ciliates after 72 hours. These results indicate that all chemicals can be used to completely eradicate ciliates from a microalgal culture. For most chemicals, these effects were readily visible after 0.5 hours (see Suppl. Fig. 1), indicating that they are fast acting.

Chemicals	<i>Chlamydomonas</i> Two-way ANOVA			<i>Sterkiella</i> One-way ANOVA
	S	C	SxC	P
Decadienal	<0.001	<0.001	0.001	0.0001
DMSP	<0.001	<0.001	<0.001	<0.001
MMP	<0.001	<0.001	<0.001	<0.001
Proline	<0.001	<0.001	<0.001	<0.001
Glycine betaine	0.0015	0.0031	<0.001	0.0059

Table 2. Statistical analysis of the experiment testing the effect of the chemicals on contamination of *Chlamydomonas* cultures by the ciliate *Sterkiella* after 72 hours. The independent and interacting effects of the chemicals (C) and of *Sterkiella* (S) on *Chlamydomonas* biomass (estimated from OD750) were tested using two-way ANOVA. The effect of the chemicals on *Sterkiella* abundance after 72 hours were tested using one-

way ANOVA. p-values for each effect are given. DMSP: dimethylsulfoniopropionate;
MMP: methyl 3-(methylthio)propionate.

All chemicals except for DMSP also had a negative effect on *Chlamydomonas* at the higher concentrations tested. DMSP did not have a negative effect in cultures without *Sterkiella*, but it seemed to have a negative effect in cultures that were contaminated with *Sterkiella*. However, even at the highest concentrations of the chemicals used, *Chlamydomonas* still grew, only at a lower rate than in the control. The negative impact of the chemicals on *Chlamydomonas* was much lower compared to the effect on the ciliate *Sterkiella*. The chemicals caused a significant reduction in the productivity of *Chlamydomonas* compared to the control culture at a dose of ≥ 200 mM glycine betaine, ≥ 0.13 mM decadienal, ≥ 300 mM proline and ≥ 5 mM MMP. To our knowledge, no previous studies have reported inhibitory effects of DMSP, glycine betaine, MMP or proline on microalgae. Casotti et al. [27] also reported growth inhibition of the marine diatom *Thalassiosira* by decadienal.

Figure 2. Influence of the chemicals decadienal, DMSP (dimethylsulfoniopropionate), MMP (methyl 3-(methylthio)propionate), proline and glycine betaine on growth of the microalga *Chlamydomonas* and the ciliate *Sterkiella*. Each chemical was tested at five different concentrations and compared with a control treatment (no addition of the chemical). The average of three biological replicates is given for each treatment. Error bars are $\pm$ standard deviation.

Left: Productivity of uninfected *Chlamydomonas* cultures ('*Chlamydomonas*') and *Chlamydomonas* cultures infected with the ciliate *Sterkiella* ('*Chlamydomonas* + *Sterkiella*')

estimated from the optical density at 750 nm (OD750) measured after 72 hours (the initial OD750 was 0.3). Two-way ANOVA was used to test for the effects of chemical concentration, ciliate presence and their interaction on OD750 of *Chlamydomonas* cultures; letter codes above the bars group treatments that are not significantly different at $p < 0.05$ (tested with Duncan's Multiple Range Test (post-hoc test)).

Right: Abundance of *Sterkiella* in the infected *Chlamydomonas* cultures after 0.5 and 72 hours (initial *Sterkiella* abundance was 25 cells mL⁻¹). Two one-way ANOVA tests were used to test for significant differences between chemical concentrations, at 0.5 and at 72 hours; letter codes above the bars group treatments that are not significantly different at $p < 0.05$ (tested with Duncan's Multiple Range Test (post-hoc test) for each ANOVA separately).

When the chemicals were added to a *Sterkiella*-contaminated *Chlamydomonas* culture, the productivity of the microalga was much higher than when no chemicals were added, indicating that these chemicals added were able to secure the productivity of a contaminated culture. When decadienal and glycine betaine were added to a contaminated *Chlamydomonas* culture, the productivity remained slightly lower than that of a control culture. When the other chemicals were added to a contaminated *Chlamydomonas* culture, the productivity was the same as that of a control culture at a concentration of 4.5 mM DMSP, 5 mM MMP and 250 mM proline (Fig. 2, Table 2). This demonstrates that these three chemicals are capable of restoring the productivity on a ciliate-infected culture to the level of a non-infected culture.

These results indicate that chemicals that are produced by various marine phytoplankton species as a defense mechanism against marine predators [16] can also protect the freshwater microalga *Chlamydomonas* against predation by the freshwater ciliate

Sterkiella. The concentrations at which the chemicals were effective against *Sterkiella* were generally higher than those reported in studies on marine ciliates. For instance, Strom et al. [21] reported that doses of 20 μ M of proline, DMSP and glycine betaine caused a significant reduction in the feeding rates of the ciliate *Coxiella* on the microalga *Rhodomonas*. Miralto et al. [18] demonstrated that decadienal reduced egg hatching rates of copepods at concentrations of 0.5 to 2.0 mg L⁻¹ (corresponding to 0.003 to 0.013 mM). The concentrations reported in these studies, however, correspond to the concentration at which a significant reduction in the feeding or reproduction rate of the predator occurred. In this study, on the contrary, the concentration was only considered to be effective if all ciliates were eradicated.

Four other ciliate species were exposed to the chemicals at a dose that was lethal to *Sterkiella* (0.13 mM decadienal, 4.75 mM DMSP, 10 mM MMP, 300 mM proline and 300 mM glycine betaine). DMSP, decadienal, proline and glycine betaine triggered death of all ciliates species after 30 min. The effect of MMP appeared to be slower: *Oxytricha* was affected only after 24 hours while *Stylonychia* and the two *Paramecium* species only after 40 hours (Fig. 3). These results suggest that these chemicals are effective against a wide range of ciliate species, but that the dose may be slightly different for each species.

Figure 3. Influence of an inhibitory concentration of the chemical MMP (10 mM methyl 3-(methylthio)propionate) on the abundance of four ciliate species compared with a control treatment receiving no chemicals after 0.5, 24 and 40 hours. The average of three biological replicates is given for each treatment. Error bars are $\pm$ standard deviation. R-*Paramecium* refers to *Paramecium* isolated from rain water P-*Paramecium* was isolated from pond water.

The impact of the chemicals was clearly visible in the swimming behavior of the ciliate *Sterkiella* within 30 min. of addition (Fig. 4). While healthy ciliates swam in straight or curved lines, affected ciliates only swam in curved lines or circles. All chemicals had the same effect on the swimming behavior of the ciliates (a representative example of the swimming behavior *Sterkiella* exposed to glycine betaine can be seen in the Fig. 5). After a while, ciliates stopped swimming altogether and died. Simple microscopic observation can thus be used to determine the lethal dose of a chemical to a ciliate species.

Figure 4. Influence of the addition of inhibitory concentrations of the chemicals decadienal (0.13 mM), DMSP (4.75 mM, dimethylsulfoniopropionate), MMP (10 mM, methyl 3-(methylthio)propionate), proline (300 mM) and glycine betaine (GB, glycine betaine) (300 mM) on the swimming behavior of the ciliate *Sterkiella* after 30 min., as determined by video analysis.

Figure 5. Comparison of the swimming behavior of the ciliate *Sterkiella* between a control treatment and exposure to an inhibitory concentration of the chemical glycine betaine

(300 mM) after 30 minutes. Images show the swimming path as determined by video analysis: circle (ci), curved (cu), straight (s).

3.2. Practical implications for the use of the chemicals as natural pesticides

These experiments show that chemicals that are naturally produced by marine microalgae, or the chemical analogue of one of these chemicals (MMP), have potential to be used as natural pesticides to control ciliate contamination in cultures of freshwater microalgae species. These chemicals are not toxic to humans. Two of them (decadienal, MMP) are approved for use as flavor compounds in foods [28,29]. Therefore, the use of these chemicals causes no direct health risk or risk of contamination of the microalgal biomass.

For proline and glycine betaine, the dose required to control the ciliate *Sterkiella* was very high (250-300 mM). This concentration is too high to be practical for application in large-scale cultures, where large volumes of culture medium need to be treated. The optimal dose of DMSP (4.75 mM), MMP (10 mM) and decadienal (0.13 mM) was much lower than the dose of proline and glycine betaine. Of these three chemicals, DMSP and MMP are preferred over decadienal as they don't have a negative impact on microalgal productivity when used at the optimal dose. The optimal dose of DMSP or MMP to eradicate ciliates is higher than the dose of quinine required to kill ciliates feeding on *Dunaliella* reported in an earlier study (0.03 mM; Moreno-Garrido et al. 2001). In this study, the dose was optimized to completely eradicate ciliates from contaminated cultures. Perhaps a lower dose may be effective to prevent contamination of cultures by ciliates. Further research on the preventive use of these chemicals is needed to confirm this.

A disadvantage of the chemicals MMP and decadienal is that they are volatile compounds. Consequently, the concentration of these chemicals might decrease over time after addition to the culture medium because they evaporate. Although we did not quantify the decrease in chemical concentration, the odor of decadienal (garlic-like odor) and MMP (pineapple odor) decreased rapidly over time and was no longer discernable after 2 days of incubation. An advantage of the volatility of the chemicals is that negative effects on microalgae will also disappear over time. In that way, the productivity of the microalgal culture can be secured as soon as ciliates have been eradicated.

Three of the chemicals investigated in this study are structurally very similar: DMSP, glycine betaine and MMP. This suggests that it may be rewarding to search for other structural analogues of these chemicals to control ciliate contamination in microalgal cultures. Perhaps, some of these structural analogues may be more effective at a lower dose. It would be rewarding to search for structural analogues that are non-volatile.

4. Conclusions

Our results show that chemicals that are produced as natural predator deterrents by marine microalgae (decadienal, DMSP, glycine betaine) or other chemicals (MMP, proline) can be used as natural pesticides to eradicate ciliates from microalgal cultures. These chemicals are non-toxic and therefore their use does not involve health risks. These chemicals act fast and kill ciliates within less than one hour. Although these chemicals have a negative effect on the microalga *Chlamydomonas* when used at high concentrations, they can recover the productivity of a contaminated culture when used at an appropriate dose. Glycine betaine and proline require a high dose to be effective, which makes them less attractive for use as a natural pesticide. DMSP, MMP and decadienal are effective in lower doses. The fact that several of these chemicals (DMSP, MMP, glycine betaine) are chemically similar offers possibilities to search for structural analogues of these chemicals that may be active against ciliate predators of microalgae.

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All the authors declare that there are no conflicts of interest.

Noinformed consent or human or animal rights are applicable.

Declaration of author's contributions

We confirm that the order of authors listed in the manuscript and author's contributions below has been approved by all authors. We confirm that Ms. Nguyen Thi Kim Hue is a corresponding author who has taken responsibility for the integrity of the work as a whole, from inception to finished article. We confirm the contributions of authors as the following:

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435 (3): Obtaining of funding; conception and design; statistical expertise; administrative, technical, or
436 logistic support; drafting of the article; critical revision of the article for important intellectual
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530 **Supplementary data**

Supplementary Figure 1. Influence of the chemicals decadienal, DMSP (dimethylsulfoniopropionate), MMP (methyl 3-(methylthio)propionate), proline and glycine betaine on growth of the microalgae *Chlamydomonas* and the ciliate *Sterkiella*. Each chemical was tested at five different concentrations and compared with a control treatment (no addition of the chemical). The average of three biological replicates is given for each treatment. Error bars are $\pm$ standard deviations.

Left and middle: Productivity of uninfected *Chlamydomonas* cultures ('*Chlamydomonas*') and *Chlamydomonas* cultures infected with the ciliate *Sterkiella* ('*Chlamydomonas* + *Sterkiella*') estimated from optical density at 750 nm (OD750) measured after 0.5, 24, 48 and 72 hours (the initial OD750 was 0.3). Two-way ANOVA was used to test for the effects of chemical concentration, ciliate presence and their interaction on OD750 of *Chlamydomonas* cultures, at 0.5, 24, 48 and at 72 hours.

Right: Abundance of *Sterkiella* in the infected *Chlamydomonas* cultures after 0.5, 24, 48 and 72 hours (initial *Sterkiella* abundance was 25 cells mL⁻¹). Five one-way ANOVA tests were used to test for significant differences between chemical concentrations, at 0.5, 24, 48 and at 72 hours.