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REVERSIBLE COLONY FORMATION AND THE ASSOCIATED COSTS IN *SCENEDESMUS OBLIQUUS*

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**ABSTRACT:**

Grazer-induced colony formation as a defence strategy in microalgae such as *Scenedesmus* species has been widely reported, but the associated costs and reversibility of the colonies are rarely studied. We experimentally showed that *S. obliquus* formed chained colonies in the presence of a predator, including predators separated from the algae by a membrane, but quickly reverted to single cells after the removal of the predator – a defining characteristic of an inducible defence. We detected the stress indicator astaxanthin esters in the algal populations in the presence of grazers, but not when grazers were absent. We found significant costs associated with *S. obliquus* colony formation in terms of lower population growth rate, lower photosystem II efficiency and lower cellular Chlorophyll *a* content. These results together show that colony formation as an inducible defence in *S. obliquus* against grazers comes at a substantial cost such that the defence must be switched off and the colonies revert to single cells when the predation risk disappears.
INTRODUCTION

In aquatic environments, morphological anti-predator traits are an important defence for microalgae to reduce predation risk, e.g., colony formation (Trainor, 1991; Lürling and Beekman, 1999; Jakobsen and Tang, 2002). The green microalga *Scenedesmus* sp., for instance, is usually present as single cells, but it can form chained colonies in the presence of grazers (Tollrian and Harvell, 1999). Colonies are the result of cell divisions without separation, the process of which is limited by cell multiplication and growth (Pickett-Heaps and Staehelin, 1975; Trainor et al., 1976). As most grazers are size selective, this increased-size defence reaction reduces the predation risk for the alga (Hessen and van Donk, 1993; Lürling and van Donk, 1996). Physical contact with the grazers is not required to elicit the response, as the predation risk can be communicated via chemical cues from the grazers (Hessen and Van Donk, 1993; Lampert et al., 1994; Lürling, 2000).

By definition, inducible defensive phenotypes should revert to the original phenotypes once the predation risk has disappeared (Tollrian and Harvell, 1999; Van Donk et al., 2011). Defensive colony formation by *Scenedesmus* spp. has been widely studied, but reversibility from the colonial to the unicellular form has rarely been tested (Verschoor, et al. 2009).

According to defence theory, the algae should pay some costs for their predator-induced defence response (Mole, 1994; Agrawal, 1998); otherwise the defensive (colonial) form would be the norm (Dodson, 1989). However, while the benefit of defensive traits for prey survival is clear, the costs are often unknown *a priori* and can be difficult to identify (Lürling and Van Donk, 2000). Thus far, evidence has suggested higher settling velocities for *Scenedesmus* colonies, thereby removing the algae from the euphotic zone (Lampert et al., 1994; Lürling and Van Donk, 2000).

Other possible costs can be considered for *Scenedesmus* colony formation as well: 1) reduced nutrient and light uptake due to the “package effect” (Kirk, 1994), resulting in lower growth rate; 2) decreased photosystem II (PSII) efficiency (Lürling and Van Donk, 2000); and 3) decreased Chlorophyll *a* content (Lürling and Van Donk, 2000; Yang et al., 2009).

Many green algae are known to accumulate secondary keto-carotenoids such as Astaxanthin (Ax) and its derivatives Astaxanthin-esters (Ax-E) as part of the xanthophyll cycle when exposed to stress such as high irradiance or UV (Figure 1). These stresses lead to the formation of reactive oxygen species (ROS), and Ax and Ax-E as antioxidants can protect the cells from ROS damages (Lemoine and Schoefs, 2010). As such, Ax and Ax-E accumulations have been used to indicate photo-oxidative stress in algae (Quin et al., 2008; Aburai et al., 2015), but there is no prior report that links their accumulation to predation-related stress.
In this study, we tested for colony formation in *Scenedesmus obliquus* induced by chemical cues from a grazer with different levels of feeding activity, and the associated costs in terms of population growth rate, photosystem II efficiency and cellular Chlorophyll *a* content. We hypothesised that direct or indirect grazing cues would induce colony formation, but non-feeding grazers would induce fewer colonies than actively feeding grazers. Furthermore, we tested the alga’s ability to revert to unicells and to recover the costs following the disappearance of the grazers. Lastly, we investigated, for the first time, the production of Astaxanthin (esters) by *S. obliquus* in response to grazing.

**METHODS**

To investigate how grazers affected colony formation and reversal in microalgae, and assess potential costs to the algae, we carried out a series of laboratory experiments.

**Organisms:**

The green alga *Scenedesmus obliquus* (Turpin) (recently renamed *Tetradesmus obliquus* (Turpin) Wynne (2016)) was bought from the Culture Collection of Algae and Protozoa (CCAP, strain number 276/6A), and grown in BG-11 medium (Sigma-Aldrich 73816 FLUKA) in batch culture in 250-mL flasks. *S. obliquus* is commonly found as single cells; however, it can form colonies in the presence of grazers (Lürling and van Donk, 2000; Zhu et al., 2015). The freshwater zooplankton *Daphnia magna* Straus, 1820 (Cladocera) was obtained from the Leibniz-Institute of Freshwater Ecology and Inland Fisheries (Germany). Genetically identical individuals originated from a single female were fed daily *ad libitum* with a mixture of two algae: *S. obliquus* and *Raphidocelis subcapitata* (Sphaeropleales), and kept in glass beakers with spring water (Evian mineral water: pH = 7.2; Ca²⁺ = 78 mg L⁻¹). *R. subcapitata* (CCAP, strain number 278/4) was cultivated in the same conditions as *S. obliquus*. Algae and zooplankton cultures were grown at a temperature of 21 ± 2°C and a light intensity of 80-90 µmol photons · m⁻² · s⁻¹ in an 18 hours light: 6 hours dark cycle.

Dialysis bags are semi-permeable membranes used in separation techniques for the removal or exchange of molecules based on different pore sizes. The dialysis bags used in this study (Medicell Membranes Ltd, London) and had a pore size of 12-14 kD. The bags allowed the passage of *Daphnia* infochemicals but prevented physical contact between *D. magna* and *S. obliquus*. The bags were washed following the manufacturer’s instructions before use.

**Colony induction experiment:**
The experimental set-up consisted of dialysis bags placed in 500 mL glass beakers (Figure 2), divided as follows: three replicates of control beakers each of: 1) single-celled *S. obliquus* inside (C1B) and outside (C1O) the dialysis bag to determine the background level of colony formation in the absence of grazers. The treatment beakers consisted of three replicates each of: 2) single-celled *S. obliquus* + nutrients inside the dialysis bag (T1B) and mineral water + nutrients + *D. magna* outside the dialysis bag (T1O) to assess colony formation induced by non-feeding *D. magna*; 3) single-celled *S. obliquus* + nutrients inside the dialysis bag (T2B) and single-celled *S. obliquus* with *D. magna* + nutrients outside the dialysis bag (T2O) to assess colony formation induced by actively grazing *D. magna*.

Before the start of the experiment, *S. obliquus* population was grown at a temperature of 21 ± 2°C under a cold light of 80-90 µmol photons·m·s⁻¹ and a photoperiod of 18 hours light: 6 hours dark. BG-11 medium was used as source of nutrients. An initial concentration of ca. 5 ×10⁵ cells mL⁻¹ of unicellular *S. obliquus* in exponential phase was added to the dialysis bags and relevant beakers. The total volume of the dialysis bag was 70 mL; each bag was sealed at both ends with clips. 10 genetically identical *D. magna* adults (4 ± 2 days old) were used in each predator treatment beaker. All the beakers were placed in front of a cold light of 80-90 µmol photons·m·s⁻¹ at a temperature of 21 ± 2°C and in a photoperiod of 18 hours light: 6 hours dark.

The beakers were manually shaken and their positions changed daily to ensure uniform exposure to light throughout the experiment. Moreover, the dialysis bags were gently shaken and inverted in the beaker twice a day to avoid sedimentation of algae and to mix the medium. The experiment lasted for 3 days. On Day 0 and Day 3, aliquots were collected for the following analyses: pigments, quantum yield (PSII efficiency) and cell (colony) counts. Additional samples for cell and colony counts were taken on Day 1.

**Colony reversibility experiment:**

A second experiment was conducted to test whether *S. obliquus* colonies were able to revert to single cells once the grazing risk had disappeared. For this experiment we used *S. obliquus* populations recovered from the *Colony induction experiment*.

At the end of the *Colony induction experiment*, aliquots were transferred from the different beakers into new beakers filled with 70 mL of deionized water and BG-11 nutrient medium, to create an initial inoculum of ca. 3 ×10⁵ cells mL⁻¹ of algal population dominated by single cells or colonies (Figure 3). The new beakers were exposed to the same light and photoperiod conditions as before, and were manually shaken and their positions changed daily to ensure uniform exposure to light throughout the experiment. The experiment lasted for 3 days. On Day 0 and Day 3, aliquots
were collected for the following analyses: pigments, quantum yield (for PSII efficiency) and cell (colony) counts. Additional samples for cell (colony) counts were taken on Day 1.

**Measurement of pigments**

The pigment contents of the algal populations were determined on Day 0 and Day 3, using the method described by Zapata et al. (2000) with HPLC (High Performance Liquid Chromatography). A 20-mL aliquot was transferred from each algal population with a sterile syringe into an Eppendorf tube and pellets were created from these aliquots after centrifugation. The samples were extracted with 90% HPLC grade acetone (Sigma-Aldrich) and sonicated with a sonicator probe (Fisher Scientific) for 1 minute at 40 Hz to lyse the cells. Once the pigments were extracted, each pellet was centrifuged at 6000 g for 5 minutes and 50 µL of the supernatants containing pigments were used for HPLC analysis. The HPLC system had a 150 × 4.6 mm column (Waters spherisorb ODS2, particle size diameter of 5 µm) with a flow rate of 1 mL min⁻¹ and the solvent gradient described in Zapata et al. (2000). Astaxanthin, its ester forms and other pigments (not reported) were identified by comparing our retention times and diode array spectra with those reported by Jeffrey et al. (1997). Specific pigment contents were expressed as percentages of peak area relative to the total peak area of all the pigments. Chlorophyll a was quantified against reference standard (Sigma-Aldrich, 96145).

**Quantum yield measurement (QY)**

PSII efficiency was measured in samples collected at the same time of day during the light period (ca. 80-90 µmol photons m⁻² s⁻¹) as effective quantum yield (QY) using an Aquapen (AP-C 100, Photon Systems Instruments). Fluorescence nomenclature and calculation was done according to the manufacturer’s instructions. At the end of the light period, a 2-mL aliquot was transferred from each algal population into a cuvette which was then inserted into the Aquapen. The variable fluorescent (Fᵥ) and the maximal fluorescence intensity (Fₘ) were measured with an excitation wavelength of 455 nm and emission wavelengths of 667-750 nm. The mean of three measurements was used to calculate the effective efficiency of PSII e⁻ -flow as Fᵥ/Fₘ. Afterward, the aliquot was recovered for cell counts (see below).

**Cell (colony) counts and measurement of algal growth rate**

Aliquots recovered from QY measurements were fixed with Lugol’s solution (Sigma-Aldrich 62650-1L-F) and stored in a dark refrigerator (~5 °C). Samples were counted within 15
days using a haemocytometer under a Leica inverted microscope (400× magnification). Numbers of single cells, number of colonies and number of cells per colony were recorded. The specific growth rates ($\mu$) were calculated for the periods of Day 0–Day 1 and Day 1–Day 3 using the equation: $\mu = \frac{\ln(X_2 / X_1)}{(t_2 - t_1)}$, where $X_1$ and $X_2$ are the counts of total cells at time $t_1$ and $t_2$, respectively.

**Statistical analysis**

Statistical analyses were performed using R studio software (v. 1.1.383). One-way ANOVA was used to test for differences between treatments in terms of (i) the change in astaxanthin %, QY values and pigment percentages; (ii) the number of cells in colonies; recorded over the course of the experiments. Differences between QY were calculated using the mean QY value of three replicates of each treatment on Day 0 and Day 3. These responses were considered during both colony formation and colony reversal. Model residuals were tested with a Shapiro-Wilks test, and no evidence was found suggesting deviation of residuals from a Normal distribution ($p > 0.05$ in all cases). Levene’s test showed no deviation from the assumption of homogeneity of variance across groups ($p > 0.05$ in all cases). Post-hoc Tukey tests tested for differences between colonies induced by non-feeding and actively feeding Daphnia. The level of statistical significance was set at $\alpha = 0.05$.

**RESULTS**

**Colony induction experiment:**

Astaxanthin esters were absent in all the treatments and controls on Day 0, but they appeared on Day 3 in the algal populations treated with D. magna, while they remained absent in the controls (Figure 4A). Differences in QY values across treatments were detected ($F_{4,10} = 284.5, p < 0.001$). S. obliquus populations exposed to D. magna showed a drop in QY; on the contrary, Daphnia-free populations of S. obliquus showed an increase in QY over time (Figure 4B).

All the replicates (controls and treatments) had the same initial concentration of Chlorophyll $a$ per cell (mean 0.139 ng cell$^{-1}$ ± 0.005 s.e.). On Day 3, cellular Chlorophyll $a$ content decreased drastically in all algal populations under the direct or indirect influence of D. magna (mean change: -0.13 ng cell$^{-1}$ ± 0.007 s.e.), whereas in the Controls the cellular Chlorophyll $a$ content increased significantly (mean change: 0.102 ng cell$^{-1}$ ± 0.001 s.e.; Figure 4C).

There was no evidence of any difference between direct contact with D. magna (T2O) and exposure to D. magna’s chemical cues (T1B, T2B) in the formation of S. obliquus colonies ($F_{2,6} =$
0.273, p = 0.77; Figure 5). Controls without *D. magna* had a low and stable amount of multi-celled clusters throughout the experiment. The number of cells per colony increased over time in the treatments exposed to *D. magna* stimuli, with an increase in 8-celled colonies by Day 3 in all replicates (Fig. 5, *F*<sub>4,10</sub> = 286.6, *p* < 0.001).

As expected, the algal populations in direct contact with *D. magna* decreased because they were being grazed. However, even the populations inside the dialysis bags showed lower growth rates (μ) in the presence of *D. magna* chemical cues: in the first period (Day 0–Day 1) algal population growth rates in the presence of *D. magna* chemical cues (μ = 0.02 and 0.01 d<sup>-1</sup> ± 0.004 s.e. for T1B and T2B, respectively) were much lower than that of the control populations (μ = 0.69 d<sup>-1</sup> ± 0.000 s.e for both C1B and C1O). In the second period (Day 1–Day 3), μ was still lower in algal populations exposed to *D. magna* chemical cues (μ = 0.03 and 0.04 d<sup>-1</sup> ± 0.004 s.e. for T1B and T2B, respectively) than in the control populations (μ = 1.13 and 1.07 d<sup>-1</sup> ± 0.02 s.e. for C1B and C1O, respectively).

**Colony reversibility experiment:**

Astaxanthin esters were present in all of the starting populations that were previously exposed to *D. magna*, and these pigments were no longer present on Day 3 following removal of the grazers (mean decrease: -5% ± 0.73 s.e., Fig. 6a). Astaxanthin esters were always absent in the control populations that were never exposed to *D. magna* (C1B in Fig. 3).

QY values increased between Day 0 and Day 3 in all populations with or without pre-exposure to *D. magna* (*F*<sub>3,8</sub> = 10.97, *p* < 0.005, Figure 6B). The Chlorophyll *a* level was low and similar for both control and pre-exposed populations on Day 0. At the end of the experiment, the amount of Chlorophyll *a* per cell increased in the control (from 0.3 to 0.8 ng cell<sup>-1</sup>) but less so in the pre-exposed populations (ca. +0.05 ng cell<sup>-1</sup>; Fig. 6C).

There was a significant decline in the number of cells per colony from Day 0 to Day 3 in the pre-exposed populations during the reversibility experiment, with the 8-celled colonies disappearing by Day 3 (Fig. 7, *F*<sub>3,8</sub> = 8.655, *p* = 0.006). The control population maintained a low and stable number of small colonies.

In the first day after removal from the grazer, the algal populations had a low growth rate (μ = 0.03, 0.03 and 0.07 d<sup>-1</sup>, for T2O, T1B and T2B respectively). As the experiment progressed...
(Day 1–Day 3), the growth rates of the pre-exposed populations increased and became comparable to that of the control population: $\mu = 1.40 \text{ d}^{-1}$ for C1B and T1B, $1.37 \text{ d}^{-1}$ for T2B and $1.39 \text{ d}^{-1}$ for T2O.

DISCUSSION

The ability to defend against predators is a major evolutionary driving force in organisms’ life histories; this is particularly the case for microalgae that lack mobility or physical refuge (Van Donk et al., 2011). *Scenedesmus* is a cosmopolitan freshwater algal genus with more than 1300 known species distributed globally (Coesel and Krienitz, 2008). Due to its ability to form easily recognisable chained colonies in response to grazing, it is widely used as a model organism in the study of morphological defence (e.g. Lürling and Van Donk, 1997; Lürling, 1999). While many reports describe *Scenedesmus* colony formation as an inducible defence response, test of reversibility of the colonies is rare in the earlier studies. Likewise, there is limited evidence of the costs associated with colony formation.

In our experiments, unicellular *S. obliquus* formed chained colonies in the presence of *D. magna* even without physical contact with the grazer, suggesting that the predation risk could be communicated via chemical cues. Moreover, the number of cells per colony increased over time, from 2-celled colonies on Day 0 to 8-celled colonies on Day 3 (Figure 5). These observations are consistent with other studies of defensive colony formation in microalgae against grazers (Lampert et al., 1994; Wiltshire and Lampert, 1999; Tang 2003).

Unlike terrestrial ecosystems where grazing is typically non-lethal to the plants, grazing in plankton often means death to the algal cells. *Daphnia* species are among the most dominant grazers in freshwater systems (Sterner, 1989), able to exert strong top-down control on algae leading to a ‘clear water phase’ in many lakes (Deneke, 1999). From the perspective of *S. obliquus*, it may be advantageous (or even necessary) for the algae to react to the mere presence of the grazer before grazing occurs. In contrast to previous work (e.g. Lampert et al., 1994) and our expectation, the extent of colony formation (in terms of the increase in number of colonial cells mL$^{-1}$ over time) was almost identical between the algal populations exposed to non-feeding *D. magna* and those exposed to actively grazing *D. magna*. In the earlier study (Lampert et al., 1994), the grazer was starved for 48 hours prior to the experiments, whereas in our study *D. magna* was starved only during the experiments. Our observations therefore suggest that the release of chemical cues does not depend on continuous, active feeding; rather, chemical cues resulting from recently fed grazers were sufficient to trigger colony formation.
The benefit of inducible defences is, by default, enhanced survival of the organism, but the associated cost(s) can be difficult to identify, and as a consequence there is very limited information on costs in the literature. Formation of colonies requires the production of special cellular structures and materials (Pickett-Heaps and Staehelin, 1975; Trainor, 1998), which may divert resources from other vital cellular functions. The enlarged volume-to-surface ratio of the colonies may also decrease the alga’s resource acquisition ability (Kirk, 1994). We showed that both PSII efficiency and cellular Chlorophyll \(a\) content decreased significantly during \(S. \) obliquus colony formation, either of which could lead to a reduced growth rate, as we also confirmed in our experiments. The observation that Astaxanthin esters increased in \(S. \) obliquus when the cells were exposed to grazer is also interesting. Astaxanthin esters are known to protect algal cells from photo-oxidative stress (Lemoine and Schoefs, 2010), but it is unclear what protective benefits they served against grazing. We may speculate that the chemical cues released by the grazer may have contained oxidative substances; as such, the pigments may have been a response to this grazer-associated oxidative stress rather than to grazing stress per se. Regardless, our observations suggest that Astaxanthin esters may be used to indicate a wider range of stress than previously known.

Interestingly, our results differ from Lürling and Van Donk (2000), who did not observe any change in QY in grazer-influenced cells. It is useful to point out that Lürling and Van Donk (2000) dark-adapted their samples and their measurements represented the maximum QY. In our study, we chose to measure effective QY without dark-adaptation, which was more indicative of the real-time photosynthetic activity of the cells (Murchie and Lawson, 2013). The omission of dark adaptation also minimised the possibility of the cells ‘recovering’ while away from the grazer’s influence (cf. Lürling and Van Donk, 2000). Moreover, effective QY is considered a good physiological indicator of how photosynthetic organisms respond to environmental stress (Rascher et al., 2000). Deviations of effective QY from the control usually indicate a reversible down-regulation of PSII photochemistry rather than irreversible damage to the photosynthetic apparatus (e.g. Demmig-Adams et al., 1996). This is in agreement with our second experiment where we saw rapid recovery of the effective QY values during colony reversal (Figure 6B). In our experiments we observed a lower growth rate than in the previous report; this difference may be partly attributed to the fact that we conducted our experiments under 18:6 light-dark cycle, rather than continuous light (cf. Lürling & van Donk, 2000).

Faced with the high costs associated with colony formation, \(S. \) obliquus reverted to unicells upon removal from the grazer, a prerequisite trait for colony formation to be described as an ‘inducible’ defence (Tollrian and Harvell, 1999). As expected, Astaxanthin esters also disappeared completely once the grazer was removed. Interestingly, but perhaps not surprisingly, colony
formation and reversal occurred at different rates: in the *Colony induction experiment*, unicells
came to colonial form at a rate of $71 \times 10^3$ cells mL$^{-1}$ d$^{-1}$. This was considerably higher than the
rate at which cells in colonial form changed back to unicells (*Reversibility experiment*: $28 \times 10^3$
cells mL$^{-1}$ d$^{-1}$). Colony formation protected the cells from certain death (grazing) whereas the
associated costs, albeit substantial, were not necessarily fatal to the cells. It is therefore reasonable
to argue that colony formation by *S. obliquus* under predation threat carried a much higher urgency
than the reverse process. At the end of the reversibility experiment (i.e., after 3 days without
predator cues), there were still about 5% cells in colonial form in the pre-exposed populations,
compared to only 0.3% in the control populations. Consistent with this observation, the costs were
also not fully recovered for the pre-exposed algal populations: While their growth rate and the PSII
efficiency recovered to being comparable to the control populations, their cellular Chlorophyll *a*
content still lagged behind that of the control populations. It therefore appears that the process of
Chlorophyll *a* synthesis may require a longer time to return to normal.

**CONCLUSIONS**

The algal genus *Scenedesmus* is a very useful model organism to study the ecology and
evolution of morphological defences against predators, but thus far the literature lacks detailed
information on the associated costs and colony reversibility. Here we not only showed that colony
formation by *S. obliquus* was reversible upon removal of the grazing threat; we also quantified the
costs associated with colony formation. This information will be useful for further cost-benefit
analysis of this defensive trait, especially when in combination of other environmental constraints.
The discovery of the production of Astaxanthin-esters, a commercially valuable antioxidant, under
predation-related stress is also interesting and deserves further investigation.

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References


Figure 1. Simplified schematic of a synthesis pathway of the pigments Astaxanthin and Astaxanthin-esters in *Scenedesmus* sp. (Lemoine and Schoefs, 2010). In the absence of stress, Zeaxanthin is disaggregated from energy dissipation and converted back to Violaxanthin (black line). When the cell is exposed to stress, Violaxanthin is transformed to Zeaxanthin, which is then converted to Astaxanthin and Astaxanthin-esters (grey dotted line).

Figure 2. Schematic of the experimental design for colony induction. Each beaker contained a dialysis bag with *Scenedesmus obliquus* single cells (grey shading). Treatment beakers also contained *Daphnia magna* (*Daphnia* figure) outside the dialysis bag, with (grey) or without (white)
S. obliquus as food. All beakers contained Evian water with nutrients (BG-11). T = treatment, C = control, B=bag, O= outside, 1 and 2 are to distinguish the first and second treatments respectively.

Figure 3. Schematic of the experimental design for colony reversibility. The starting algal populations were taken from the Colony induction experiment (see Fig. 2) and transferred into fresh medium.
Figure 4. Colony induction experiment. A) Changes in % Astaxanthin esters in *Scenedesmus obliquus* in the presence of *Daphnia magna*, between Day 0 and Day 3. Error bars represent the standard errors. B) Changes in the quantum yield (QY, indicating PSII efficiency) of *S. obliquus* between Day 0 and Day 3, in the presence or absence of *D. magna*. Error bars indicate standard errors; replicates sharing the same letters are not statistically different. C) Changes in Chlorophyll a (ng cell⁻¹) of *S. obliquus* between Day 0 and Day 3, in the presence or absence of *D. magna*. The error bars represent standard errors. T = treatment, C = control, B=bag, O= outside, 1 and 2 represent the first and second treatments, respectively.
Figure 5. Colony induction experiment. Proportion of cells in colonial form of *Scenedesmus obliquus* in the presence of *Daphnia magna* over time, for the different treatments. The error bars represent the standard errors. See Fig. 2 for the treatment group notations.
Figure 6. Colony reversibility experiment. A) Changes in % Astaxanthin esters in *Scenedesmus obliquus* in the presence of *Daphnia magna*, between Day 0 and Day 3. B) Changes in the quantum yield (QY, indicating PSII efficiency) of *S. obliquus* between Day 0 and Day 3, in the presence or absence of *D. magna*. The error bars represent the standard errors; replicates sharing the same letters are not statistically different. C) Changes in Chlorophyll *a* (ng cell$^{-1}$) of *S. obliquus* between Day 0 and Day 3, in the presence or absence of *D. magna*. The error bars represent standard errors. See Fig. 3 for the treatment group notations.
Figure 7. Proportion of cells in colonial form of *Scenedesmus obliquus* in the presence of *Daphnia magna* over time, for the different treatments. The error bars represent the standard errors. See Fig. 2 for the treatment group notations.