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# Influence of altered light conditions and grazers on *Scrippsiella trochoidea* (Dinophyceae) cyst formation

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ABSTRACT: We investigated whether or not the presence of copepods and different light conditions induced cyst formation in dinoflagellates. *Scrippsiella trochoidea* was exposed to *Acartia tonsa* directly and indirectly (grazer filtrate), in high light and low light conditions. The ingestion, faecal production and egg production of *A. tonsa* were compared between diets of *S. trochoidea* vegetative cells and temporary cysts. We found no effect of direct or indirect exposure to *A. tonsa* on *S. trochoidea* cyst formation in either high light or low light conditions. Controls and *A. tonsa* treatments kept in light displayed around 20% temporary cysts, whereas controls and *A. tonsa* treatments in low light were shown to have 50 to 80% temporary cysts. Thus, low light conditions had a strong effect on temporary cyst formation. No hypnocysts were observed in any experiment, which is probably related to the longer incubation times needed for their observation. Feeding on diets dominated by temporary cysts compared to vegetative cells decreased ingestion by a factor of 2.7, while faecal and egg production decreased by a factor of 2.2 and 2.9, respectively, suggesting that induction of temporary cysts in response to *A. tonsa* could be a survival strategy. However, *S. trochoidea* does not possess any grazer-induced defence in terms of temporary cyst formation, as it did not produce temporary cysts when exposed to *A. tonsa*. Rather, induction of temporary cysts seems to be controlled by decreased light intensity, which is a favorable trait for this species when driven to water depths where light is scarce.

KEY WORDS: *Scrippsiella trochoidea* · Cyst formation · Light intensity · Grazer density · Temporary cysts



## INTRODUCTION

Cyst formation is a common feature of many dinoflagellate species. Resting cysts or hypnocysts are surrounded by persistent cell walls and result from sexual processes. Temporary cysts are formed from vegetative cells by ecdysis, and are surrounded by a pellicle (Anderson & Wall 1978, Pfiester & Anderson 1987).

Hypnocyst formation is a response to a variety of detrimental environmental factors, such as nutrient limitation (Anderson et al. 1984, Imai 1989, Figueroa et al. 2006), changes in temperature (Hardeland 1994), salinity (Zonneveld & Susek 2007), day length (Sgrosso et al. 2001), light intensity (Imai 1989), iron stress (Doucette et al. 1989), and high vegetative cell densities (Garcés et al. 2004). After a certain maturation period, resting cysts could function as a 'seed bank' for initiating new blooms when conditions are favourable again (Anderson 1997, Rengefors & Anderson 1998).

Formation of temporary cysts has been regarded as a sampling (Garcés et al. 2002) or culture artefact, related to ageing of cultures (Jensen & Moestrup 1997), nutrient stress (Anderson & Wall 1978), bacterial attack (Nagasaki et al. 2000) or to changes in temperature (Grzebyk & Berland 1996) and unfavourable light conditions (Rintala et al. 2007). However, there have been some observations of temporary cyst formation *in situ* (Garcés et al. 1998, Xiao et al. 2001) or in large-scale mesocosm experiments (Olli 2004). Therefore, it has been suggested that temporary cysts are part of

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the natural life cycle of dinoflagellates (Garcés et al. 2002).

Although cyst formation in dinoflagellates is usually induced by adverse abiotic factors, cysts may also function as a defence mechanism against various biological threats, other than the bacterial attack mentioned above. For instance, Fistarol et al. (2004) have shown that *Scrippsiella trochoidea* significantly increased the production of temporary cysts in response to allelochemicals, which has also been confirmed in more recent studies (Tillmann et al. 2007, Tillmann & Hansen 2009). Furthermore, Toth et al. (2004) have shown that *Alexandrium ostenfeldii* shifted from motile cells to temporary cysts in the presence of chemicals released from a parasite. Hypnocysts have also been speculated to be a defence against predation because of their shape and chemical constitution (Sarjeant et al. 1987). The same authors also concluded that cysts could escape predation by planktonic grazers by sinking out of the water column. Alternatively, if cysts are eaten, they can survive gut passage and show a high germination rate after passing through the digestive system of certain copepod grazers (Montresor et al. 2003). Some studies have revealed that grazers play a role in life history alterations of phytoplankton, such as excystment of hypnocysts, and that this is a way to minimize predation losses. In the presence of high numbers of grazers or infochemicals released by them, the raphidophyte *Gonyostumum semen* and the dinoflagellate *Peridinium aciculiferum* reduced the recruitment rate of hypnocysts from the sediment. By adjusting the timing of recruitment from the 'seed-bank', these algae could avoid being grazed (Hansson 1996, Rengefors et al. 1998). Some phytoplankton species are also able to respond to infochemicals released by zooplankton by adjusting other defensive traits, such as morphology (Hessen & van Donk 1993, Tang 2003, Lundgren & Granéli 2010) and toxin production (Jang et al. 2003, Selander et al. 2006), and thus these species become less vulnerable to grazing (Hessen & van Donk 1993, Selander et al. 2006).

No studies have yet reported any effects of grazers or infochemicals released from grazing activities on encystment of temporary cysts or hypnocysts. Furthermore, little is known about the possibility of combined effects of grazers and abiotic factors, such as altered light conditions, on cyst formation in dinoflagellates. Light intensity in oceanic waters is subjected to great spatiotemporal variation depending on the position of the sun, the wavelength of light, cloud conditions, the optical properties of water, and the water depth (Hieronymi & Macke 2010). In addition, the effects of global warming are expected to cause an increase in aerosol concentrations in the atmosphere, thus leading to a reduced amount of light reaching the earth's surface (Kulmala et al. 2003). Such fluctuations in light intensity will certainly have an effect on the growth and photosynthesis of phytoplankton, but may also affect the cyst formation of dinoflagellates (Imai 1989, Rintala et al. 2007).

One aim of the present study was to examine the combined effects of both direct and indirect (infochemically driven) exposure to copepods and altered light conditions on dinoflagellate cyst formation. A second aim was to investigate ingestion, and faecal and egg production of copepods to evaluate the food quality and potential defensive ability of vegetative cells, as well as temporary cysts, of dinoflagellates.

## MATERIALS AND METHODS

We chose the dinoflagellate *Scrippsiella trochoidea* and the copepod *Acartia tonsa* as model species in this study. *S. trochoidea* is a cosmopolitan bloom forming species (Wang et al. 2007) and thus plays a significant role in ecosystem functioning across the world. Moreover, *S. trochoidea* was previously observed to form temporary cysts in response to allelochemicals in our laboratory (Fistarol et al. 2004) and we wanted to examine if such a mechanism would also occur in response to the presence of grazers (copepods) and/or low light conditions. *A. tonsa* was chosen due to culture availability at the time of the experiments. *A. tonsa* is also one of the most common copepod species in estuarine waters and can occur in areas such as the Chesapeake Bay (Purcell et al. 1994) where blooms of *S. trochoidea* have also been reported (Marshall & Egerton 2009).

This study consisted of 2 experiments: the *grazer exposure experiment* and the *grazing experiment*. The grazer exposure experiment investigated direct and indirect effects of increasing *Acartia tonsa* densities on *Scrippsiella trochoidea* cyst formation in both high light and low light conditions. In the grazing experiment we examined ingestion, and faecal and egg production of *A. tonsa* on vegetative cells and temporary cysts of *S. trochoidea*.

**Organisms and culture conditions**. The algal bank at the University of Gothenburg (GUMACC) provided the *Scrippsiella trochoidea* strain used in the present study (strain: GUMACC 110). *Rhodomonas salina*, used as food for copepod cultures, originated from Kalmar Algal Collection (KAC, Linnaeus University, Kalmar, Sweden, strain: KAC 30). Phytoplankton medium for growth and maintenance of *S. trochoidea* cultures were prepared by autoclaving filtered (munktell glass fiber filters, mesh size: 1.2 µm) natural seawater from the Swedish west coast, after the salinity

had been adjusted to 26 g $kg^{-1}$ by addition of Milli-Q water. Media for the *R. salina* culture were prepared in the same manner, except that the water originated from the Baltic Sea and that NaCl was added to obtain a salinity of 26 g $kg^{-1}$. *S. trochoidea* and *R. salina* had been growing at this salinity prior to the experiments presented here. Nitrogen (N) and phosphorus (P), as well as vitamins, were added at concentrations corresponding to F/2 medium, with N additions modified to yield an N:P ratio of 16:1 (Guillard 1975). Trace metals were added according to the L1 media recipe (Guillard & Hargraves 1993).

One set of *Scrippsiella trochoidea* cultures were established for the grazer exposure experiment and one set for the grazing experiment. For each experiment, the cultures were grown in duplicate in 2 l glass culture flasks at 16°C and a photon flux density of 100 µmol photons $m^{-2}$ $s^{-1}$, supplied at a light:dark cycle of 16:8 h. We chose this light:dark cycle because *S. trochoidea* occurs in Swedish waters during May to June when the day and night lengths match this cycle. Growth of these cultures was followed by daily measurements of chlorophyll *a* with a Turner Designs 10-AU fluorometer. *S. trochoidea* cultures were harvested in mid-exponential phase for both the grazer exposure experiment and the grazing experiment. The *Rhodomonas salina* culture was grown in a 10 l culture flask at the same temperature and light conditions as the *S. trochoidea* cultures, but was aerated with sterile air (Sartorius Minisart® filter, 0.2 µm) to facilitate mixing. This *R. salina* culture had been used as prey for copepod cultures prior to the experiments presented here, and had grown as a semi-continuous culture for 60 or 150 d depending on the experiment in which the culture was used. On a daily basis, 10% of the culture volume was removed, fed to copepod cultures, and replaced with F/2 medium.

All experiments were conducted with adult females of *Acartia tonsa*, which originated from cultures held at the Sven Lovén Centre for Marine Sciences, Gothenburg University. Water (10 l) from an *Acartia* culture was transported in a plastic container to our laboratory within 24 h. The copepods were then put in a 100 l glass aquarium with air bubbling at 16°C and under dim light. These copepods were subsequently fed daily with *Rhodomonas salina* at saturating concentrations. Eggs were collected after approximately 14 d and stored in a refrigerator at 2 to 3°C, until they were used to establish a new generation of *A. tonsa*, with adult females of known age. Eggs hatched within 48 h in filtered seawater at 16°C, the nauplii were then put in a 100 l aquarium, again fed on a daily diet of *R. salina* in dim light. After about 20 d, most nauplii had developed to adult individuals and the experiments could be started. A new generation of *A. tonsa* was established in the manner described above for the grazing experiment, which started approximately 3 mo later than the grazer exposure experiment.

**Grazer exposure experiment.** The experimental setup included 2 grazer treatments — low density treatment, LD, 6 *Acartia tonsa* $l^{-1}$, and high density treatment, HD, 30 *A. tonsa* $l^{-1}$ — and one grazer-free control, C, in both high light (100 µmol photons $m^{-2}$ $s^{-1}$, 16:8 h light:dark cycle) and low light (5 µmol photons $m^{-2}$ $s^{-1}$, 16:8 h light:dark cycle) conditions. Each treatment was replicated 3 times, i.e. a total of 18 glass culture flasks (Schott Duran, 0.5 l) were used (Fig. 1). The densities of *A. tonsa* used here, i.e. 6 to 30 $l^{-1}$, are within the range for this species *in situ* (Cervetto et al. 1993, Purcell et al. 1994).

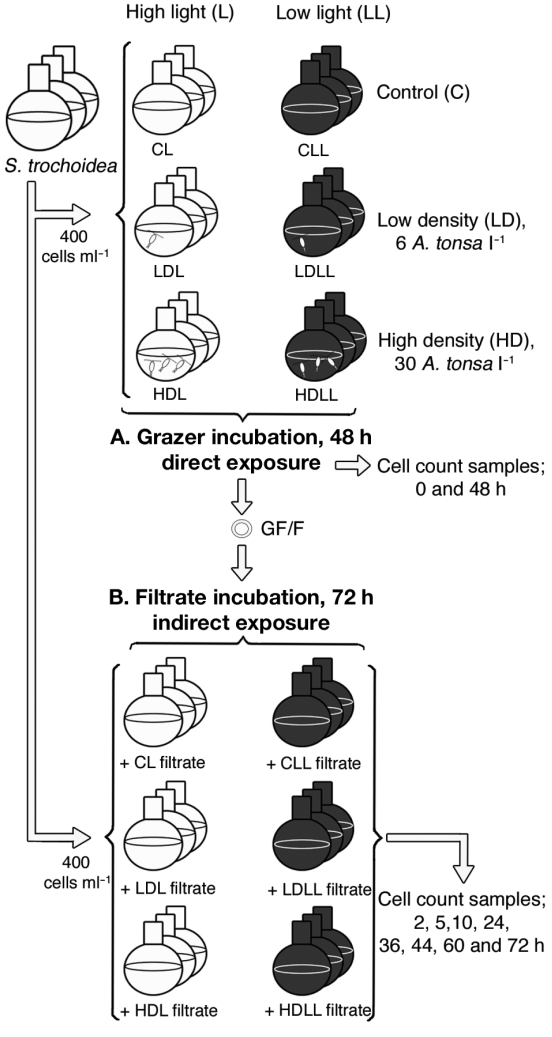


Fig. 1. Experimental design used to investigate effects of (A) direct and (B) indirect exposure to increasing densities of *Acartia tonsa* in high light (100 µmol photons $m^{-2}$ $s^{-1}$) and low light (5 µmol photons $m^{-2}$ $s^{-1}$) conditions on cyst formation by *Scrippsiella trochoidea*

Adult and healthy *Acartia tonsa* females were selected from the copepod culture under a stereomicroscope (Nikon SMZ–2T), and placed in 1000 ml filtered seawater for 12 h prior to the initiation of the experiment to facilitate gut clearance. *Scrippsiella trochoidea* cultures were harvested in mid-exponential phase and inoculated into each flask at a target concentration of 400 cells $ml^{-1}$, corresponding to a carbon (C) concentration of 560 µg C $l^{-1}$ (1357 pg C $cell^{-1}$, based on previous measurements of particulate organic carbon, POC). We chose this concentration because S. *trochoidea* cell concentrations lie around this value during the build-up of *S. trochoidea* blooms (Wang et al. 2007). At the corresponding carbon concentration, 560 µg C $l^{-1}$, *A. tonsa* ingestion rates are high but not completely saturated (Kiørboe et al. 1985). For treatments LD, 3 adult *A. tonsa* females were added to a total of 6 flasks, i.e. 3 flasks for treatment LD in high light conditions (LDL) and 3 flasks for treatment LD in low light conditions (LDLL). In the same manner, for treatments HD, 15 adult *A. tonsa* females were added to 6 flasks, 3 flasks for treatment HD in high light conditions (HDL) and 3 flasks for treatment HD in low light conditions (HDLL). A total of 6 flasks were left without grazers and used as controls; 3 flasks in high light (CL) and 3 flasks in low light conditions (CLL) (Fig. 1A). To facilitate mixing, all flasks were gently stirred by hand 5 times a day. Grazer and control incubations lasted for 48 h in order to allow the copepods time to adjust to the food. Samples for cell enumeration were taken initially and at the end of this incubation.

After 48 h the contents of each flask were filtered through GF/F glass fiber filters and the filtrates were used to inoculate new *Scrippsiella trochoidea* at a target concentration of 400 cells $ml^{-1}$ (Fig. 1B). This procedure allowed us to assess the indirect (i.e. infochemically driven) effect of increasing grazer density on *S. trochoidea* cyst formation in high light and in low light conditions. Filtrate incubation lasted for a total of 72 h, during which time samples for cell counts were taken from each flask in a time series as follows: 2, 5, 10, 24, 36, 44, 60 and 72 h. Previous experiments on the induction of temporary cysts in dinoflagellates in response to parasite-derived chemical signals have shown that such responses could occur quickly and subside within a few hours (Toth et al. 2004). This was the main reason why we sampled for cell counts in time intervals as short as 2 to 10 h, in addition to the longer intervals of 24 to 72 h.

**Grazing experiment.** In order to estimate the food quality of *Scrippsiella trochoidea* vegetative cells and temporary cysts, *Acartia tonsa* females were incubated with 3 different food suspensions as follows: (1) *S. trochoidea* dominated by vegetative cells (78% vegetative cells, 22% temporary cysts, hereafter referred to as the *vegetative cell treatment*), (2) a 50:50% mixture of vegetative cells and temporary cysts (hereafter referred to as the *mixture treatment*), and (3) *S. trochoidea* dominated by temporary cysts (83% temporary cysts, 17% vegetative cells, hereafter referred to as the *temporary cyst treatment*). Temporary cysts needed for the mixture treatment and the temporary cyst treatment were induced by placing duplicate culture flasks (1 l) with exponentially growing *S. trochoidea* (F/2 medium) in the dark for 72 h prior to the start of the experiment, after which 83% of the total number of vegetative cells and cysts consisted of temporary cysts. All food suspensions were prepared by diluting the vegetative cell cultures and the temporary cyst cultures with appropriate amounts of autoclaved filtered seawater (FSW) to obtain a saturating food concentration of 800 µg C $l^{-1}$. We had no measurement of the carbon content of temporary cysts and therefore initially aimed at setting the concentration of vegetative cells and temporary cysts in the mixture treatment to be of the same volume, so that the clearance rate of *A. tonsa* would not be affected. The volume of vegetative cells (7950 ± 1649 $\mu m^3$, mean ± SD, n = 10) and temporary cysts (6559 ± 1073 $\mu m^3$, n = 10) were measured, and no significant difference was found (Mann-Whitney *U* test, $p = 0.087$). Therefore, vegetative cells and temporary cysts were assumed to have the same carbon content and set at the same concentration (295 cells/cysts $ml^{-1}$) in the mixture treatment. Table 1 shows the target densities and real densities obtained in each treatment.

The grazing experiment lasted for 2 d and consisted of 2 subsequent 24 h incubations at 16°C and a light intensity of 100 µmol photons $m^{-2}$ $s^{-1}$ (light:dark cycle, 16:8 h). After each 24 h incubation period production of eggs and faecal pellets was assessed, while ingestion was only determined during the second 24 h incubation. *Acartia tonsa* females were chosen from the copepod culture in the same manner as described above for the grazer exposure experiment and acclimatized to the experimental diets for 24 h, before initiating the grazing experiment. The experimental containers were 250 ml Erhlenmeyer flasks, which were

Table 1. Target concentrations of prey (*Scrippsiella trochoidea*) cells as well as real initial cell concentrations (cells $ml^{-1}$) in the vegetative cell treatment, the mixture treatment, and the temporary cysts treatment (mean ± SD, n = 3)

| Treatment | Vegetative cells | | Temporary cysts | |
|---|---|---|---|---|
| | Target | Real | Target | Real |
| Vegetative cell | 462 | 440 ± 43.6 | 128 | 126 ± 40.8 |
| Mixture | 295 | 303 ± 15.3 | 295 | 290 ± 30.1 |
| Temporary cyst | 100 | 116 ± 24.0 | 490 | 551 ± 36.5 |

filled to a total volume of 100 ml with the different food suspensions detailed above. Three adult *A. tonsa* females were added to each flask, and the result was that the total copepod carbon corresponded to about 19% of the algal carbon in the experimental flasks. Each food suspension treatment was replicated 3 times, i.e. we used a total of 9 flasks. To calculate ingestion we also included triplicate control flasks, without addition of copepods, of the 3 different food suspensions during the second incubation, i.e. an additional 9 flasks were used during the second 24 h period. All experimental flasks were put on shaking tables to keep cells and cysts in suspension.

To calculate ingestion rates, we sampled for cell counts from all flasks initially on Day 1 and at the end of Day 2. After each 24 h incubation, the content of each flask was first filtered through a 200 µm net to collect copepods. The condition of the copepods was checked using a stereomicroscope before they were gently transferred to a new flask with a freshly prepared food suspension, in accordance with the procedures detailed above. The remaining filtrate was filtered onto a 40 µm mesh size nylon net in order to collect eggs and faecal pellets. Eggs and faecal pellets were rinsed with FSW into 50 ml centrifuge tubes and preserved with acidic Lugols solution (4% final concentration).

**Analytical procedures.** All samples for cell counts were preserved in a 0.5% final concentration of glutaraldehyde and stored in darkness and at 4°C before enumeration. Depending on varying concentrations of cysts and vegetative cells between samples, 2.65 or 10 ml Utermöhl chambers were used to count vegetative cells and cysts with an inverted microscope (Olympus CKX41) at 200× magnification. At least 100, but in 70% of all samples more than 300 cells (11% error), were counted for each cell type, except for the low light treatments towards the end of the grazer exposure experiment where the very low concentrations of the vegetative cells prevented us from counting more than 50 vegetative cells (28% error).

Production of eggs and faecal pellets from the various diets were assessed with the inverted microscope at 40× magnification using graded petri dishes. Faecal pellet volumes were estimated for all replicates in each treatment by measuring the length and width of 30 pellets per replicate. Previous measurements of POC and particulate organic nitrogen (PON) in our laboratory revealed an N:C ratio of approximately 0.2 for *Scrippsiella trochoidea*, a food N:C ratio at which *Acartia tonsa* contains 5.2 µg C ind.$^{-1}$ (Miller & Roman 2008). This carbon content of *A. tonsa* was used to estimate weight-specific ingestion rate and egg production.

For each treatment in the grazing experiment, we calculated the total weight-specific ingestion, i.e. ingestion on vegetative cells and temporary cysts summed, as well as weight-specific ingestion on vegetative cells and temporary cysts separately. All ingestion rates were calculated according to Frost (1972).

The carbon content of the eggs was estimated from an average egg diameter of 83.7 ± 2.5 µm (n = 10) and a carbon content of $0.14 \times 10^{-6}$ µg C µm$^{-3}$ (Huntley & Lopez 1992). Gross growth efficiency (GGE, % d$^{-1}$) of *Acartia tonsa* was determined by dividing the weight-specific egg production rate with the total weight-specific ingestion.

Samples for inorganic nutrients ($NO_3^-$, $PO_4^{3-}$, and $NH_4^+$) and pH were collected from each experimental flask after filtrate incubations in the grazer exposure experiment. $NO_3^-$ and $PO_4^{3-}$ samples were filtered through Whatman GF/C glass fiber filters and frozen until analysed photometrically (Hach Lange DR5000); samples for $NH_4^+$ were analysed immediately after collection, according to Valderrama (1995). Samples for pH were directly measured with a calibrated WTW inoLab pH meter.

**Statistical analyses.** All percentage data (i.e. percentage of cells as cysts and GGE) were arcsine transformed before statistical analysis. In the direct exposure experiment, data on percentages of cells as cysts were analysed for effects of increasing *Acartia tonsa* densities and altered light conditions on cyst formation of *Scrippsiella trochoidea* after 48 h with a 2-way ANOVA. In the indirect exposure experiment, a 3-way repeated measures ANOVA was used to examine how *S. trochoidea* cyst formation changed through the experiment. Mauchly's test was performed to test the assumption of sphericity, while Levene's tests were used to check all data for homogenous variances and Shapiro-Wilks' tests were used to check for normal distributions. In the indirect exposure experiment, data from the time points 5 h and 60 h had to be excluded from the statistical analyses as they did not fulfil the assumption of homogenous variances. Data from analyses of inorganic nutrients and pH were analyzed with a 1-way ANOVA. In the grazing experiment, 1-way ANOVA was used to examine differences in concentrations of vegetative cells and temporary cysts between control and grazing flasks at the end of incubation. Only if differences were statistically significant were ingestion rates calculated. Ingestion rates were tested against zero (insignificant ingestion), again using 1-way ANOVAs. The same test was also used to test whether the 3 different diets offered to *A. tonsa* resulted in significantly different ingestion rates, as well as in different faecal and egg productions. If the ANOVAs demonstrated statistically significant differences, they were followed by Tukey's post hoc tests. All statistical analyses were performed with SPSS 13.0. All data are presented as mean ± SD.

## RESULTS

### Grazer exposure experiment

#### Direct exposure

We observed no effect of increasing *Acartia tonsa* densities on percentages of cells as temporary cysts in either high light or low light conditions (Fig. 2A). There was, however, an effect of low light conditions. Grazer treatments and the control in the low light condition (CLL, LDLL and HDLL) all showed increasing percentages of cells as temporary cysts; from an initial average of 21.41 ± 1.43% to an average of 52.56 ± 2.20% after 48 h (Fig. 2A). At the same time, the grazer treatments and the control exposed to higher light intensities (CL, LDL and HDL) showed no such increase in temporary cyst densities as the average percentage of temporary cysts was initially 20.89 ± 1.39%, compared to 19.40 ± 0.85% after 48 h (Fig. 2A). Correspondingly, a 2-way ANOVA revealed a significant effect of light condition alone ($F_{1,12}$ = 195.37, $p < 0.001$), while no significant effect was detected concerning grazer density alone ($F_{2,12}$ = 0.38, $p = 0.692$). Similarly, no significant difference in terms of temporary cyst formation was found between the interaction of light condition and grazer density ($F_{2,12}$ = 0.25, $p = 0.782$). No hypnocysts were observed in any treatment of the direct exposure experiment in high light or low light conditions.

#### Indirect exposure

All grazer treatments and controls in both high light and low light conditions showed similar percentages of cells as temporary cysts during the first 10 h of the experiment, with an average of 21.26 ± 2.06%. After 10 h, the proportion of temporary cysts in controls and grazer treatments followed the same trend as for the direct exposure experiment. In fact, after 72 h of incubation with the grazer filtrates and in their controls, the percentages of cells as temporary cysts in the low light condition increased to an average of 82.47 ± 2.14% (CLL, LDLL and HDLL), whereas those in light (CL, LDL and HDL) remained at lower levels (19.49 ± 1.62%) (Fig. 2B). Three-way ANOVA revealed a significant effect of light condition over time ($F_{1,12}$ = 1578.71, $p < 0.001$), while no significant effects of increasing *Acartia tonsa* density filtrates could be discerned ($F_{2,12}$ = 0.06, $p = 0.946$). Moreover, there was no interaction effect of light condition and grazer density ($F_{2,12}$ = 1.96, $p = 0.184$). No hypnocysts were detected in any treatment in high light or low light conditions.

#### Inorganic nutrients and pH

After 72 h of filtrate incubation, nitrate and phosphate occurred at high concentrations in all controls and grazer treatments of the grazer exposure experiment. $NO_3^-$ concentrations varied from 452.67 ±

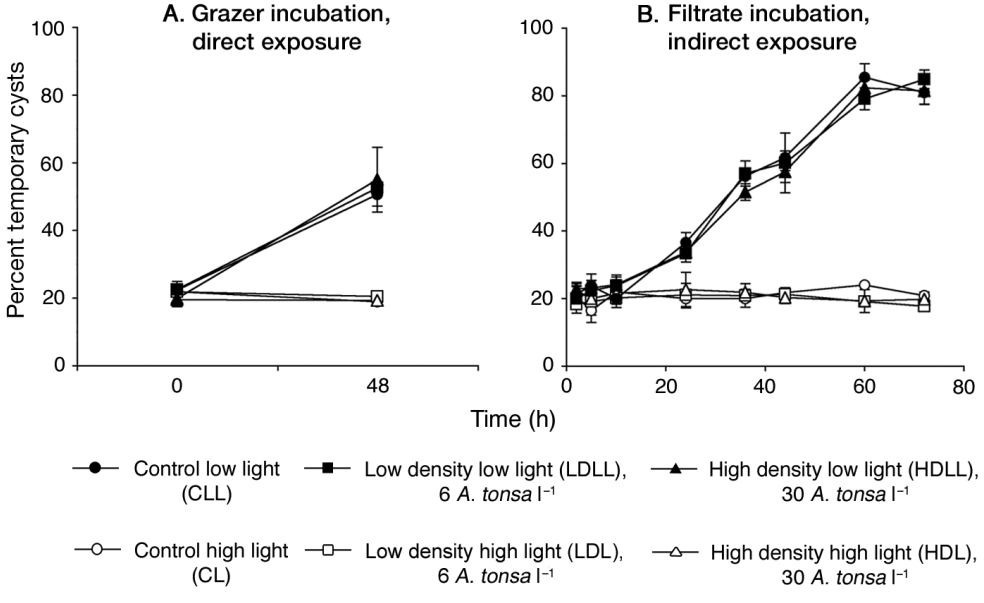


Fig. 2. *Scrippsiella trochoidea* and *Acartia tonsa*. Percentages of *S. trochoidea* cells as temporary cysts in the 2 tested light conditions, high light (100 µmol photons $m^{-2}$ $s^{-1}$) and low light (5 µmol photons $m^{-2}$ $s^{-1}$) after (A) direct exposure and (B) indirect exposure to either no copepods (controls), low (6 *A. tonsa* $l^{-1}$) or high (30 *A. tonsa* $l^{-1}$) densities of *A. tonsa* (mean ± SD; SD bars sometimes hidden behind symbol, n = 3)

33.12 µmol $l^{-1}$ in HDL to 533.68 ± 38.41 µmol $l^{-1}$ in CLL. $PO_4^{3-}$ concentrations ranged from 25.10 ± 1.45 µmol $l^{-1}$ in HDL to 30.63 ± 1.57 µmol $l^{-1}$ in CLL (Table 2). No significant differences in either $NO_3^-$ or $PO_4^{3-}$ concentrations were found between the control and the 2 grazer treatments within each tested light condition ($p > 0.05$). However, the control and the grazer treatments in the low light condition (CLL, LDLL and HDLL) all displayed significantly higher concentrations of $PO_4^{3-}$ compared to those in the high light (CL, LDL and HDL) ($F_{5,12} = 15.91$, $p < 0.050$). $NO_3^-$ concentrations were also higher in the control and in the grazer treatments exposed to lower light compared to the control and the grazer treatments in higher light, although the increase was not statistically significant ($F_{5,12} = 2.34$, $p = 0.106$) (Table 2).

At the end of the experiment, $NH_4^+$ concentrations were low and varied from 0.20 ± 0.05 µmol $l^{-1}$ in CLL to 0.46 ± 0.04 µmol $l^{-1}$ in HDLL (Table 2). The low- and the high-density grazer filtrate treatments (LDL, LDLL, HDL and HDLL) had significantly higher concentrations of $NH_4^+$ compared to the controls (CL and CLL). This was evident in both the high light ($F_{5,12} = 17.82$, $p = 0.005$ for LDL and $p = 0.001$ for HDL) and the low light condition ($p = 0.002$ for LDLL and $p = 0.014$ for HDLL) (Table 2).

Values of pH showed little variation between treatments, varying from 8.64 ± 0.11 in LDLL to 8.69 ± 0.05 in HDL (Table 2). No significant differences in pH could be detected between any grazer treatment and control in either light or low light conditions ($F_{5,12} = 0.21$, $p = 0.951$) (Table 2).

## Grazing experiment

### Ingestion

The total weight-specific ingestion rate, i.e. ingestion on vegetative cells and temporary cysts combined, of *Acartia tonsa* decreased with increasing concentrations of *Scrippsiella trochoidea* temporary cysts (Fig. 3). The observed total ingestion was 0.64 ± 0.03 µg C µg $C^{-1}$ $d^{-1}$ (64.45 ± 3.22% body C $d^{-1}$) in the vegetative cell treatment, which decreased significantly to 0.50 ± 0.07 µg C µg $C^{-1}$ $d^{-1}$ (49.39 ± 7.72% body C $d^{-1}$) in the mixture treatment ($F_{2,6} = 55.01$, $p = 0.019$), and to 0.24 ± 0.02 µg C µg $C^{-1}$ $d^{-1}$ (49.39 ± 7.72% body C $d^{-1}$) ($p < 0.001$) in the temporary cyst treatment. The ingestion on vegetative cells of *S. trochoidea* also decreased as the percentage of temporary cysts increased — from 0.60 ± 0.04 µg C µg $C^{-1}$ $d^{-1}$ (59.13 ± 2.87% body C $d^{-1}$) in the vegetative cell treatment to 0.41 ± 0.10 µg C µg $C^{-1}$ $d^{-1}$ (40.50 ± 9.70% body C $d^{-1}$) ($F_{2,6} = 29.46$, $p = 0.020$) in the mixture treatment, and to 0.21 ± 0.09 µg C µg $C^{-1}$ $d^{-1}$(21.26 ± 0.94% body C $d^{-1}$) ($p = 0.001$) in the temporary cyst treatment (Fig. 3). Ingestion rates on temporary cysts were not computed since their densities did not differ significantly at the end of the incubation.

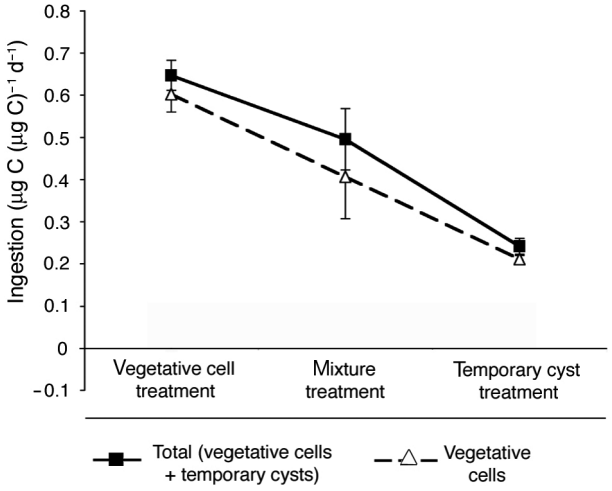


Fig. 3. *Acartia tonsa* feeding on *Scrippsiella trochoidea.* Weight-specific ingestion by *A. tonsa* in the vegetative cell treatment, the mixture treatment, and in the temporary cyst treatment. For each treatment, the total ingestion of both *S. trochoidea* vegetative cells and temporary cysts, as well as the separate ingestion of vegetative cells are shown (mean ± SD, n = 3)

Table 2. Inorganic nutrient concentrations (µmol $l^{-1}$) and pH values in the controls and grazer treatments of the 2 tested light conditions (high light and low light) after 3 d of filtrate incubation (indirect exposure experiment). Asterisks indicate significant differences from the control within each tested light condition; $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. *F* and p-values are also given (mean ± SD, n = 3).

| | High light (100 µmol photons $m^{-2}$ $s^{-1}$) | | | Low light (5 µmol photons $m^{-2}$ $s^{-1}$) | | | $F_{5,12}$ | p |
|---|---|---|---|---|---|---|---|---|
| | Control, CL | Low density, LDL | High density, HDL | Control, CLL | Low density, LDLL | High density, HDLL | | |
| $NO_3^-$ | 470.99 ± 36.68 | 467.23 ± 43.76 | 452.67 ± 33.12 | 533.68 ± 38.41 | 528.28 ± 37.51 | 497.75 ± 38.79 | 2.34 | 0.106 |
| $PO_4^{3-}$ | 26.22 ± 0.24 | 25.47 ± 1.39 | 25.10 ± 1.45 | 30.63 ± 1.57 | 29.99 ± 0.70 | 30.04 ± 0.66 | 15.9 | 0.000 |
| $NH_4^+$ | 0.20 ± 0.05 | 0.38 ± 0.06** | 0.41 ± 0.06*** | 0.20 ± 0.02 | 0.34 ± 0.01** | 0.46 ± 0.04* | 17.8 | 0.000 |
| pH | 8.67 ± 0.03 | 8.66 ± 0.08 | 8.69 ± 0.05 | 8.66 ± 0.07 | 8.64 ± 0.11 | 8.65 ± 0.04 | 0.21 | 0.951 |

### Egestion

*Acartia tonsa* faecal pellet production rates followed the same trend as ingestion, i.e. an increasing percentage of *Scrippsiella trochoidea* temporary cysts caused a decreased production of faecal pellets. This was apparent on both Day 1 and Day 2 of the experiment (Fig. 4). On Day 1, a significantly lower faecal pellet production rate, 13.89 ± 3.00 pellets female$^{-1}$ d$^{-1}$, was observed in the temporary cyst treatment compared with the vegetative cell treatment, which had a rate of 29.67 ± 3.21 pellets female$^{-1}$ d$^{-1}$ ($F_{2,6}$ = 22.51, p = 0.002), whereas in the mixture treatment the rate was 28.22 ± 3.24 pellets female$^{-1}$ d$^{-1}$ (p = 0.004). The same pattern was observed on Day 2 of the experiment; while the pellet production rate in the vegetative cell treatment was 31.78 ± 5.18 pellets female$^{-1}$ d$^{-1}$, the temporary cyst treatment had a significantly lower pellet production rate of 14.00 ± 1.53 pellets female$^{-1}$ d$^{-1}$ ($F_{2,6}$ = 14.93, p = 0.005). The pellet production rate in the temporary cyst treatment was also significantly lower than that observed in the mixture treatment which had a pellet production rate of 28.11 ± 5.01 pellets female$^{-1}$ d$^{-1}$ (p = 0.015).

Average faecal pellet sizes (mm$^3$ × 10$^{-4}$) varied from 1.16 ± 0.01 to 1.23 ± 0.05 and no significant differences could be detected on Day 1 ($F_{2,6}$ = 1.32, p = 0.330) or on Day 2 ($F_{2,6}$ = 1.21, p = 0.360).

### Reproduction

In accordance with ingestion rates and faecal pellet production rates, average egg production rates decreased with increasing concentrations of temporary cysts on both Day 1 and Day 2 (Fig. 5). On Day 1, the lowest egg production rate, 4.11 ± 0.84 eggs female$^{-1}$ d$^{-1}$, occurred in the temporary cyst treatment. Egg production rates increased significantly to 9.67 ± 2.08 eggs female$^{-1}$ d$^{-1}$ in the mixture treatment ($F_{2,6}$ = 14.9, p = 0.030) and to 12.67 ± 2.52 eggs female$^{-1}$ d$^{-1}$ in the vegetative cell treatment (p = 0.004). A similar trend occurred on Day 2, with an egg production rate of 4.88 ± 1.02 eggs female$^{-1}$ d$^{-1}$ in the temporary cyst treatment, which increased significantly to 10.33 ± 2.08 eggs female$^{-1}$ d$^{-1}$ in the mixture treatment ($F_{2,6}$ = 21.4, p = 0.014) and to 13.33 ± 1.53 eggs female$^{-1}$ d$^{-1}$ in the vegetative cell treatment (p = 0.002).

The gross growth efficiency (GGE) varied from 16.93 ± 4.83 to 17.11 ± 1.10 % (Fig. 5). There were no significant differences in GGE between treatments ($F_{2,6}$ = 0.011, p = 0.989).

## DISCUSSION

### Grazer exposure experiment

#### Effects of decreased light

Our results show that the factor that had induced *Scrippsiella trochoidea* temporary cyst formation was the low light condition. Temporary cyst formation was high under low light in both the direct and indirect exposure experiments (Fig. 2). Similarly, Rintala et al. (2007) have found that the dark survival strategy of the closely related *S. hangoei* includes reducing respiration rates to very low levels by increasing temporary cyst formation in response to darkness.

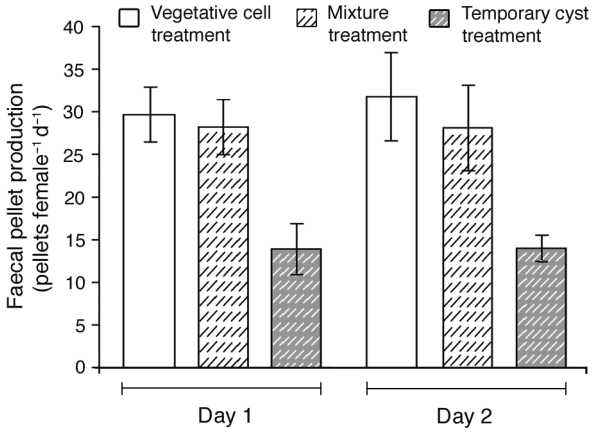


Fig. 4. *Acartia tonsa* feeding on *Scrippsiella trochoidea*. Faecal pellet production of *A. tonsa*, on Day 1 and Day 2 of the grazing experiment, feeding on *S. trochoidea* vegetative cells (vegetative cell treatment), a 50:50 % mixture of *S. trochoidea* vegetative cells and temporary cysts (mixture treatment), and temporary cysts (temporary cyst treatment) (mean ± SD, n = 3)

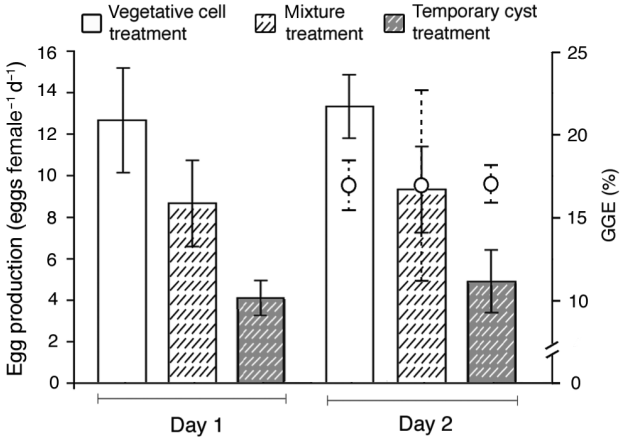


Fig. 5. *Acartia tonsa* feeding on *Scrippsiella trochoidea*. Egg production of *A. tonsa*, on Day 1 and Day 2 of the grazing experiment, in the vegetative cell treatment, the mixture treatment and in the temporary cyst treatment. On Day 2, gross growth efficiency (GGE,%) (white circles) in the different treatments is also presented (mean ± SD, n = 3)

As nitrate and phosphate concentrations were high and pH did not differ significantly between any treatments in either high light or low light conditions, we can assume that the increased formation of temporary cysts observed in response to decreased light intensity has nothing to do with either N or P deficiency or pH conditions. Although concentrations of ammonium were relatively low in all treatments (<0.5 µmol $l^{-1}$), they were significantly higher in all treatments where grazers were added, i.e. in treatments LDL, HDL, LDLL, and HDLL, compared to the controls, CL and CLL. This reflected ammonium excretion of copepods (Kiørboe et al. 1985). However, the higher levels of ammonium found in our grazer treatments had no effect on the formation of cysts, as the density of temporary cysts did not vary in the presence of *Acartia tonsa* and no hypnocysts were found in any of these treatments.

In the present study, high percentages of temporary cysts were observed in response to low light conditions, i.e. 50 to 80%. It is not common for all cells within a given population to form cysts in response to adverse conditions. Moreover, the proportion of cells forming cysts can vary a great deal between natural population surveys and laboratory experiments. In laboratory conditions, Rintala et al. (2007) reported that 50% of the *Scrippsiella hangoii* population consisted of temporary cysts after 17 d in darkness, whereas values of 22 to 50% temporary cysts were found for *Alexandrium ostenfeldii* when responding to parasite-derived infochemicals (Toth et al. 2004). The high percentages of temporary cysts reported here are in the range previously reported for *S. trochoidea* in response to allelochemicals in laboratory setups, i.e. up to 84% (Fistarol et al. 2004). Even higher percentages of temporary cysts were reported for *Prorocentrum minimum* in response to temperature changes, i.e. up to 100%, in a laboratory experiment by Grzebyk & Berland (1996). Values from artificial laboratory conditions are generally higher than those reported from field conditions, even though such field studies are scarce. For instance, we observed around 20% temporary cysts in our cultures, even without any stressor, which could be related to culturing effects. The fraction that formed temporary cysts in natural populations of *Heterocapsa triquetra*, without any specific stressor tested, was lower than 1% (Olli 2004). Garcés et al. (1998) observed 13 to 59% temporary cysts (within a 24 h period) when studying a natural population of *Alexandrium taylori*, with the highest values reported in the evening and at night when light intensities decrease. These values are in line with those reported here in response to low light intensities, i.e. 35% after 24 h (Fig. 2B), which suggests that results presented here could also be relevant outside laboratory conditions.

We suggest that temporary cyst formation in *Scrippsiella trochoidea* could be an effective mechanism for reducing population losses under conditions of low and fluctuating light intensities, which may be expected in future climate change scenarios (Kulmala et al. 2003). In such conditions, *S. trochoidea* may gain further advantages, as it would be able to overcome turbulence events, driving them to water depths where light is scarce.

### Effects of *Acartia tonsa* exposure

In the present study, no temporary cysts (or hypnocysts) were formed in response to increasing densities of *Acartia tonsa* in either the direct or the indirect grazer exposure experiment. Previous reports on *Scrippsiella trochoidea* temporary cyst induction in response to various detrimental chemical signals (Fistarol et al. 2004, Toth et al. 2004, Tillmann et al. 2007, Tillmann & Hansen 2009), indicate that *S. trochoidea* have receptors that respond to chemical signals. *Acartia* spp. have previously been reported to induce higher toxicity of *Alexandrium minutum* (Selander et al. 2006) and to induce morphological changes in *Phaeocystis globosa* (Long et al. 2007, Lundgren & Granéli 2010) via released infochemicals. Although the studies cited above imply that *S. trochoidea* could be able to respond to infochemicals released from *A. tonsa*, the present study showed that this was not the case. The role of infochemical degradation in this lack of response can be excluded since no response was detected in the direct exposure experiment, in which *A. tonsa* was present and able to produce infochemicals throughout the duration of the experiment. Similarly, we can exclude the possibility that *S. trochoidea* is only affected by higher concentrations of infochemicals than other species are. This is because we also tested very high densities of *A. tonsa* (200 $l^{-1}$) in a small direct exposure experiment (data not shown), which lasted for 5 d, but we did not observe any cyst induction in that experiment either.

Although findings on the occurrence of grazer-induced defence in phytoplankton are being increasingly reported in the international scientific literature, it is not a universal phenomenon, and evidently has not evolved for *Scrippsiella trochoidea.* Similarly, Van Donk et al. (1999) found prey specific responses to the cladoceran *Daphnia*; while some species reacted to infochemicals released from the grazer, others showed no response at all. This could depend on differences in the physiological state of different phytoplankton species (Van Donk et al. 1999).

### Defensive abilities of dinoflagellate cysts

The potential defensive abilities of dinoflagellate cysts warrants some attention, as such abilities should confer some protection against predation to justify

their utilisation by *Scrippsiella trochoidea* in response to grazer presence. Hypnocysts are rich in reserve material such as lipids and starch and therefore could constitute a good food source for potential grazers, provided that the grazers can break up, penetrate or dissolve the cyst wall (Persson 2000). However, hypnocysts have been shown to result in lower copepod ingestion rates and can even survive the gut passage of such grazers (Montresor et al. 2003). In the present study, hypnocysts were not observed as incubation only lasted 72 h. Since zooplankton densities can vary on even shorter time scales than this, the goal of the grazer exposure experiment was to test direct and indirect effects of *Acartia tonsa* on temporary cysts. Such cysts, in contrast to hypnocysts, can survive short-term environmental fluctuations and quickly re-establish a motile vegetative state when favourable conditions reoccur (Anderson 1998).

As far as we know, there are no reports of temporary cysts being able to withstand digestion and surviving gut passage. *Scrippsiella trochoidea* is, however, able to withstand the effect of certain allelochemicals by forming temporary cysts, thus indicating that there is some chemical resistance by these cysts (Fistarol et al. 2004). Hypnocysts and temporary cysts share the feature of immobility and therefore sink to the bottom, which could be another potential defence strategy against copepods inhabiting and feeding in the water column (Sarjeant et al. 1987). Due to the mucilage, which often surrounds temporary cysts, they often form clumps or aggregates (Olli 2004). However, Jenkinson & Biddanda (1995) suggest that adsorption of colloidal material, such as dissolved organic material (DOM), onto mucilaginous material could increase the availability of DOM to biological consumers. Seuront & Vincent (2008) demonstrated that high viscosity induced by increased amounts of mucilaginous material did not mechanically hamper zooplankton grazing. Nevertheless, given the larger size of temporary cyst aggregates and their position in the water column, we argue that aggregate formation could in fact make temporary cysts less accessible to pelagic predators.

### Grazing experiment

As shown in our results, the total ingestion rate of *Acartia tonsa* decreased as the percentage of temporary cysts in the diets increased. The highest ingestion reported, 0.65 ± 0.04 µg C µg $C^{-1}$ $d^{-1}$, occurred in the vegetative cell treatment. This value was still about half that reported for *A. granii* (da Costa et al. 2008), but comparable to the ingestion rates reported for *A. liljeborgii* and *A. clausi* feeding on *Scrippsiella trochoidea* at the same carbon concentration (800 µg C $l^{-1}$) (Band-Schmidt et al. 2008). When taking the separate ingestions of vegetative cells and temporary cysts into consideration, it is evident that the decreased total ingestion rates observed in the mixture treatment and the temporary cyst treatment were the result of decreased ingestion of vegetative cells as these cells became fewer while the amount of temporary cysts in the diet increased (Fig. 3). In contrast, the ingestions on temporary cysts were non-significant in all treatments, even when the temporary cysts occurred in high concentrations, i.e. in the mixture treatment and the temporary cyst treatment.

It is outside the scope of this study to determine which mechanisms are behind the lower ingestion rates observed on temporary cysts. However, they could be related to (1) lower capture rates, caused by decreased encounter rates (swimming vegetative cells versus non-swimming temporary cysts), (2) post-capture rejection, or (3) an active food selection against temporary cysts, as has been discussed for hypnocysts (Montresor et al. 2003). Lower capture rates seem less likely since we had high food densities and used shaking tables to keep cells and cysts in suspension. We cannot exclude either post-capture rejection or food selection, nor can we exclude the possibility of grazer deterrent substances being produced by the temporary cysts as reasons for this rejection or non-selection.

The total daily rations observed in the present study ranged from 64.45 ± 3.22% body C $d^{-1}$ on diets dominated by vegetative cells and 24.14 ± 2.22% on diets dominated by temporary cysts. The daily carbon requirement for the basic metabolism of copepods is very variable but thought to be in the range of about 30% of body C $d^{-1}$ (Woolridge 1999). This would indicate that a diet dominated by vegetative cells can sustain basic *Acartia tonsa* metabolic requirements, whereas a diet containing a high percentage of temporary cysts cannot. However, carbon requirements for optimal egg production could be as high as 180% of body C $d^{-1}$ (Kiørboe et al. 1985), which indicates that even the ingestion rates reported in the vegetative cell treatment are less than optimal for *A. tonsa*.

Egg production rates vary almost linearly with ingestion rates (Kiørboe et al. 1985). Therefore, the lower rates of egg production in the mixture treatment and the temporary cyst treatment are in line with the lower ingestion observed in these treatments. The highest egg production rates reported for the vegetative cell treatment in the present study were lower than the optimal egg production rates of *Acartia tonsa* (Kiørboe et al. 1985), and lower than the egg production rates observed for *A. clausii* and *A. liljeborgii* feeding on *Scrippsiella trochoidea* at comparable carbon concentrations (Band-Schmidt et al. 2008). However, as shown by Band-Schmidt et al. (2008), egg production

on the same diets can vary a great deal within species of *Acartia*. Furthermore, this does not affect our conclusion of decreased egg production in response to increased percentages of temporary cysts, as our treatments differed significantly from each other.

When zooplankton avoid feeding on phytoplankton with various properties, such as toxicity (Dutz 1998, Sopanen et al. 2006, Kozlowsky-Suzuki et al. 2009), non-preferred sizes (Berggreen et al. 1988), or low nutritional value (Jónasdóttir 1994), lower GGEs are often found, which demonstrates the advantage of allocating energy reserves to survival rather than reproduction when feeding on such phytoplankton (Sopanen et al. 2006). In the present study, GGE did not differ significantly between treatments. This is not surprising given that almost all of the ingested carbon in all treatments resulted from vegetative cells rather than temporary cysts. The GGEs observed here were lower than optimal values for *Acartia* on good food species, i.e. 28% (Dutz 1998) to 39% (Kiørboe et al. 1985) for *Rhodomonas baltica*, but still notably higher than those reported on toxic species such as *Prymnesium parvum*, i.e. 1.5 to 3.3% (Sopanen et al. 2006).

Similar to egg production rates, faecal pellet production was lower on diets of temporary cysts compared to vegetative cells. This again reflects the lower ingestion in treatments with temporary cysts.

Even though ingestion rates, egg production and GGEs were suboptimal in the diets dominated by *Scrippsiella trochoidea* vegetative cells, they were still relatively high. However, pronounced effects on the entire ecosystem may occur in scenarios of low light intensities, when the percentage of temporary cysts increases. This is because of the marked decreased ingestion and egg production observed on diets dominated by temporary cysts. Although starvation requirements of *A. tonsa* are low (Kiørboe et al. 1985), they would most likely switch to alternative carbon sources, such as other phytoplankton species or microzooplankton to ensure a high rate of reproduction. Once *S. trochoidea* has established dominance, the result could be decreased transfer of energy to higher trophic levels in these ecosystems. Thus, the ability to form temporary cysts could help ensure bloom development and maintenance of *S. trochoidea* blooms.

## CONCLUSIONS

As reflected in the lower ingestion, faecal pellet production, and egg production on diets dominated by temporary cysts compared to vegetative cells of *Scrippsiella trochoidea*, the grazing experiment indicated some defensive abilities of temporary cysts. Despite this, *S. trochoidea* does not seem to react with increased production of temporary cysts when exposed either directly or indirectly to increasing *A. tonsa* densities, at least not under the circumstances investigated here. Rather, induction of temporary cysts seems to be controlled by abiotic factors, such as decreased light intensity.

Although it is hard to extrapolate laboratory results to field conditions, we suggest that formation of temporary cysts is a useful mechanism to minimize population losses when *Scrippsiella trochoidea* is driven to water depths where light is scarce by, for instance, turbulence events. As reflected in the lower ingestion and egg production on diets dominated by temporary cysts, additional benefits, such as a shift in predation pressure towards other species, may arise in such situations. The occurrence of temporary cysts could contribute to the continued success and maintenance of *S. trochoidea* blooms, not only by reducing population losses, but also by providing an adaptive advantage over species unable to form temporary cysts.

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