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FEATURE ARTICLE

Plastid retention, use, and replacement in a kleptoplastidic ciliate

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ABSTRACT: The marine oligotrich ciliate *Strombidium rassoulzadegani* retains and utilizes the chloroplasts of its algal food. It does not appear to be able to induce its captured plastids to divide, and so the plastids must be replaced with new ones from recently ingested food. We measured the plastid replacement rate of *S. rassoulzadegani*, its growth and feeding rates, chlorophyll retention, and mortality when starved, and determined whether the ciliate showed differential grazing or plastid retention when presented with different algal foods. *S. rassoulzadegani* had similar mortality rates when starved following growth on either *Tetraselmis chui* or *Rhodomonas lens*. When presented with a source for new plastids, the ciliate can incorporate its first new plastid within 30 min and completely replace all of its plastids within 48 to 72 h. *S. rassoulzadegani* did not show a preference for either *Tetraselmis* or *Rhodomonas* when grazing. However, it did preferentially acquire the *Tetraselmis*-derived plastids. Our results contrast with those for other mixotrophic ciliates; for example, *Mesodinium rubrum* can maintain its plastids for extended periods of time (weeks), while *Strombidium capitatum* can quickly lose (40 h) and replace (9 h) its prey-derived plastids. *S. rassoulzadegani* also seems to be able to grow more efficiently when grazing on *Tetraselmis* than on *Rhodomonas*. *S. rassoulzadegani* may contribute to the primary production in the tide pools where it is found and by using autotrophic and heterotrophic nutritional strategies may be able to survive changes in food availability.

KEY WORDS: Kleptoplasty · Mixotrophy · Growth efficiency · Ciliate

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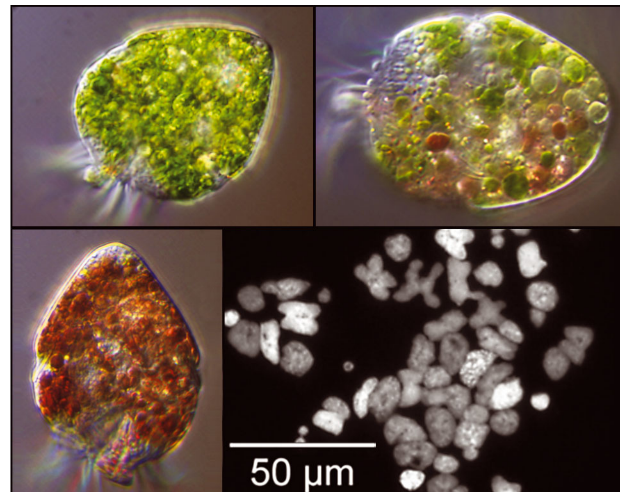

Strombidium rassoulzadegani fed *Tetraselmis chui* (top left), *Rhodomonas lens* for 15 h (top right), *R. lens* for 72 h (bottom left), and after 96 h of starvation (bottom right); fluorescence image of single squashed cell.

Photos: Donald M. Schoener

INTRODUCTION

Kleptoplasty, the retention and utilization of the plastids of ingested algal prey, is found in a wide range of protists, including foraminifera (Anderson & Bé 1976), dinoflagellates (Takishita et al. 2002), and ciliates (Stoecker et al. 1988, 1989), and it is known to occur in some metazoans as well (Teugels et al. 2008). Some mixotrophic protists consume algae, retaining the prey plastids, and sometimes other organelles, but digesting the rest of the algal cell (Johnson et al. 2007). Mixotrophs may be important primary producers and grazers within their ecosystems (Stoecker

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1999). *Mesodinium rubrum*, for example, can contribute up to 22% of planktonic primary production (Sanders 1995). Laybourn-Parry et al. (1997) demonstrated that *Stentor* spp. could contribute 69% of the total primary production in an Australian lake. Under certain circumstances, being a mixotroph may give an organism an advantage over those that are only heterotrophic or autotrophic. By grazing on algae, mixotrophs can reduce algal abundance and thus limit competing heterotrophic predators while at the same time reducing competition with algae for inorganic nutrients (Tittel et al. 2003).

Many plastid-retaining ciliates have been shown to be capable of photosynthesis. For example, *Laboea strobila* derives ~22% of its growth through photosynthesis (Putt 1990). An Antarctic strain of *Mesodinium rubrum* achieved the majority (~90%) of its growth through photosynthesis (Johnson & Stoecker 2005). Kleptoplastidic oligotrich ciliates such as *Strombidium capitatum, S. conicum, S, chlorophilum, S. acutum* and *S. rassoulzadegani* have all been shown to incorporate $^{14}CO_2$ (Stoecker et al. 1989, McManus et al. 2012).

Strombidium rassoulzadegani is a kleptoplastidic ciliate found in tide pools (McManus et al. 2010). It normally feeds on the reproductive unicells of green macroalgae and retains their chloroplasts (McManus et al. 2004, who called it *S. stylifer*). *S. rassoulzadegani* is able to retain plastids and grows *in vitro* on phylogenetically diverse algae (McManus et al. 2012). In the present study, we presented *S. rassoulzadegani* 2 different algal diets, the chlorophyte *Tetraselmis chui* and cryptophyte *Rhodomonas lens*. Chlorophytes have 2 membranes surrounding their chloroplasts and are thus thought to have arisen from an endosymbiotic event in which an organism without plastids incorporated a photosynthetic prokaryote in a primary endosymbiosis (Gould et al. 2008). *Rhodomonas* is a cryptophyte. These algae have 4 membranes surrounding their plastids. Furthermore, they often have a vestigial nucleus, called a nucleomorph, associated with their plastid (Cavalier-Smith 2002). Cryptophyte plastids are believed to have arisen from a secondary endosymbiotic event in which a photosynthetic eukaryote was incorporated into a non-photosynthetic organism (Gould et al. 2008). Like other kleptoplastidic oligotrichs, *S. rassoulzadegani* does not appear to retain any part of its prey's nucleus. In the present study, we measured plastid replacement rates, maximum retention time, starvation mortality, growth rates, grazing rates, and growth efficiency in this ciliate when it was presented with different algal diets and discuss its ecophysiology in comparison with other kleptoplastidic ciliates.

MATERIALS AND METHODS

Cultures

Strombidium rassoulzadegani was isolated from a tide pool on Long Island Sound and maintained in culture on the chlorophyte alga *Tetraselmis chui* (clone PLY429) at 19°C and a 12 h light:12 h dark cycle at 100 µmol photons m^{-2} s^{-1} in f/2 medium at a salinity of 33. The ciliate is identical in small subunit ribosomal gene sequence to Clade VII of Katz et al. (2005). The relationship of this species to other green tidepool oligotrichs is discussed by McManus et al. (2010). The food organisms *Tetraselmis chui* (hereinafter '*Tetraselmis*') and *Rhodomonas lens* (hereinafter '*Rhodomonas*') were maintained at a salinity of 20 in f/2 medium under the same light and temperature regime as the ciliates.

Ciliate and algal carbon contents were estimated using volume to carbon relationships developed by Menden-Deuer & Lessard (2000). Average *Strombidium rassoulzadegani* cell volume was calculated from measurements taken of 10 ciliates. The shape of the ciliate was assumed to be a cone topped with a half sphere. Algal volumes were also an average of 10 cells; algal cell shapes were assumed to be prolate spheroids.

To evaluate the ciliate's use of chloroplasts and other algal-derived material, we observed cultures using brightfield and differential interference contrast microscopy as well as fluorescence of specimens stained with the DNA intercalating fluorochrome 4′,6-diamidino-2-phenylindole (DAPI).

Plastid replacement

To measure the time needed for *Strombidium rassoulzadegani* to replace its plastids under food-replete conditions, we used algae with differently colored plastids. Ciliate cultures were initiated with either *Rhodomonas* (red chloroplasts) or *Tetraselmis* (green chloroplasts) as the sole food source. Individual *S. rassoulzadegani* that had been maintained on *Tetraselmis* were rinsed in 0.22 µm filtered sea water (FSW) and transferred into separate wells of 96-well plates that had fresh medium and growth-saturating concentrations of *Rhodomonas*; ciliates that had been maintained on *Rhodomonas* were transferred to *Tetraselmis* in the same manner. Between 10 and 12 individuals from wells that did not show growth were subsequently examined live under the microscope at

0 min, 1 min, 5 min, 15 min, 30 min, 1 h, 2 h, 4 h, 8 h, 12 h, 24 h, 48 h, and 72 h. Images were taken at the first appearance of new plastids positioned immediately under the ciliate's pellicle, when the ciliates had replaced approximately half their plastids, and when all plastids had been replaced.

Plastid retention during starvation

Strombidium rassoulzadegani was grown at saturating concentrations of *Tetraselmis* or *Rhodomonas*. Ciliates were transferred 3 times through FSW rinses and then placed into algae-free f/2 medium. Ciliates were then starved for 24, 48, 72 and 96 h under a 12 h light:12 h dark cycle at 100 µmol photons m^{-2} s^{-1}. At each sampling time, we picked 15 to 20 individuals and pressed them between a cover slip and a glass microscope slide. We made space for the ciliate between the cover slip and the slide by putting small amounts of silicone grease on the corners of the cover slip. Enough pressure was put on the cover slip so that the whole flattened cell could be viewed in one focal plane, but not so much that the cell's contents would be carried away by capillary action. Autofluorescence of these cell 'squashes' was examined using an Olympus BX50 microscope at 460–490 nm excitation and >520 nm emission wavelengths. Fluorescence micrographs were taken, and relative chlorophyll *a* content per cell was estimated as the total area of fluorescent plastids using the ImageJ software (Rasband 1997).

Ciliate mortality when starved

To measure starvation mortality of *Strombidium rassoulzadegani*, we first grew triplicate cultures of ciliates with either *Tetraselmis* or *Rhodomonas* as food. Once the ciliates achieved high abundance, they were separated from the algae by dilution with FSW followed by reverse filtration under a submerged 20 µm nylon mesh. Rounds of dilution and reverse filtration were continued until there was <1 algal cell ml^{-1}. After being separated from the algae, the ciliates were placed in new medium at a concentration of several hundred cells per ml. Ciliates were then incubated at 100 µmol photons m^{-2} s^{-1} on a 12 h light:12 h dark cycle. Samples (1 ml) were taken at t_0 and after every subsequent 24 h and fixed in acidic Lugol's iodine. Survivors were counted on an inverted microscope.

Growth and grazing rates

We measured numerical (growth) and functional (grazing) responses with respect to concentrations of either *Rhodomonas* or *Tetraselmis* and used these to estimate the gross growth efficiency (GGE) for *Strombidium rassoulzadegani* that had previously been grown on a diet of *Tetraselmis*. We also used these growth curves to determine if there was any difference in the maximum growth or grazing rate due to diet. Responses were calculated using the equations of Frost (1972), as modified by Heinbokel (1978) (Eqs. 1 to 4). These equations can be used to estimate grazing by comparing the growth rate of algae in the presence and absence of grazers.

$$I = F \times \overline{C} \tag{1}$$

$$F = \frac{V \times (k_c - g)}{\overline{S}} \tag{2}$$

$$\overline{S} = \frac{S_1 - S_0}{t \times k_s} \tag{3}$$

$$\overline{C} = \frac{C_1 - C_0}{t \times (k_s - g)} \tag{4}$$

where I is ingestion, F is the clearance rate, V is the volume of the experimental chamber, g is the growth rate of algae with ciliate predators present, k_c is the growth rate of algae without ciliates, $\overline{S}$ is the average ciliate concentration, S_t and S_0 are the ciliate concentrations at the end and the beginning of the experiment, respectively, t is the elapsed time of the experiment, k_s is the growth rate of the ciliate, $\overline{C}$ is the average algal concentration, and C_0 and C_t are algae concentrations at the beginning and end of the experiment, respectively.

Ciliates were first acclimated at each food concentration for 24 h prior to the experiment. For each food concentration, the ciliates were then placed in triplicate 10 ml wells within hanging cell culture inserts. There were equal initial concentrations of algae within the wells inside and outside of the inserts. The inserts had an 8 µm pore size filter on the bottom that allowed medium to pass through but not ciliates or algae. This allowed us to account for any increase in algal growth rates under grazing due to remineralization of nutrients by the ciliates. Preliminary experiments suggested that this effect could be significant. Initial algal samples were taken, and we then added 10 to 15 of the pre-acclimated *Strombidium rassoulzadegani* cells to each well. Algal prey concentrations ranged between 10^2 and 10^5 cells ml^{-1} and were chosen based on preliminary experiments.

After 3 d, the contents in the wells were fixed with Lugol's acidic iodine, and ciliates and algae were counted on an inverted microscope. We fit the response curves to a modified Michaelis-Menten equation that incorporates a threshold (the *x*-intercept is the food concentration at which growth or grazing is zero) (Montagnes 1996) using Sigma Plot 11 (Eq. 5). Ciliate and algal carbon contents were used to convert algae ingested per ciliate to a specific ingestion rate (IR, units of d^{-1}) so that we could compare ingestion rates with specific growth rates. An extra sum of squares *F*-test was performed to compare the curves and fit parameters for the 2 different foods (Motulsky & Ransnas 1987). This procedure was used to determine if one set of parameters could describe both data sets or if food type produced significantly different curves. The null hypothesis was that a single curve best fits both data sets. The modified equation is:

$$V = \frac{V\text{max} \times [C - T]}{K\text{m} + [C - T]} \tag{5}$$

where V is the ciliate growth or grazing rate, Vmax is maximum ciliate growth or grazing rate, $[C]$ is the algal concentration, and Km is the algal concentration at which the ciliate growth rate is half the maximum growth rate. T is the threshold or lowest food concentration at which the ciliates will grow or graze.

Gross growth efficiency

Ciliate GGEs were calculated for both foods as the specific growth rate divided by specific ingestion rate (μ IR^{-1}, dimensionless). GGE represents the ratio of new biomass to ingested carbon. For mixotrophs, GGE is not constrained to be <1, as it would be in a strict heterotroph, because growth can be supported by inorganic carbon obtained through photosynthesis.

Feeding selectivity

We determined whether *Strombidium rassoulzadegani* could discriminate between plastid sources through either selective grazing or plastid retention by presenting the ciliate with diets consisting of *Tetraselmis*, *Rhodomonas*, or a mixture of the 2 at saturating concentrations. Ciliates were first acclimated at saturating concentrations of only *Tetraselmis* for 24 h. Saturating concentrations of *Tetraselmis*, *Rhodomonas*, or a mixed diet of the two were prepared in triplicate 10 ml wells with hanging cell culture inserts. A total of 20 pre-acclimated ciliates were then placed into each well. Then, 3 to 6 cells were taken from each treatment diet at 0, 1, 15, and 72 h and prepared as live wet mounts. Micrographs were taken for image analysis by ImageJ (Rasband 1997) to determine the percent of red plastids in the ciliates over time. A time-zero blank was subtracted from subsequent time points to account for *S. rassoulzadegani's* red eyespot (McManus et al. 2004). The effect of diet and time on the fraction of red plastids was analyzed by 2-way analysis of variance followed by Tukey's post-hoc pairwise multiple comparison (Montgomery 2005). At 72 h, the remaining ciliates and algae were fixed, and ingestion rates were determined as in the previous grazing experiment. The percentage of *Rhodomonas* grazed in separate and mixed diets was calculated as 100 × *Rhodomonas* grazed ÷ (*Rhodomonas* grazed + *Tetraselmis* grazed). A *t*-test was used to determine if there was a significant difference between the percentage of *Rhodomonas* grazed by *S. rassoulzadegani* on separate diets vs. mixed diets. A logit transformation was applied to normalize proportional data (Warton & Hui 2011).

RESULTS

Cultures

Average *Strombidium rassoulzadegani* cell volume was 33 510 μm^3; carbon content was 6.5 ng $cell^{-1}$. *Tetraselmis* cell volume was 781 μm^3, and calculated carbon content was 0.112 ng $cell^{-1}$. Average cell volume for *Rhodomonas* was 451 μm^3, and calculated carbon content was 0.067 ng $cell^{-1}$.

We did not observe any nuclear material other than that of the ciliates when stained with DAPI.

Plastid replacement

When ciliates that were maintained on a diet of *Rhodomonas* (Fig. 1A) were switched to a diet of *Tetraselmis* (Fig. 1B), the first green *Tetraselmis* plastid was in place immediately under the pellicle of the ciliate within 30 min (Fig. 1C,D). At 24 h after being moved to the *Tetraselmis* diet, the ciliate had only a few red *Rhodomonas* plastids visible (Fig. 1E), and after 48 h, all of the ciliates examined contained only green plastids (Fig. 1F). Ciliates initially grown on the *Tetraselmis* (Fig. 2A) diet and then moved to a diet consisting of *Rhodomonas* (Fig. 2B) also showed

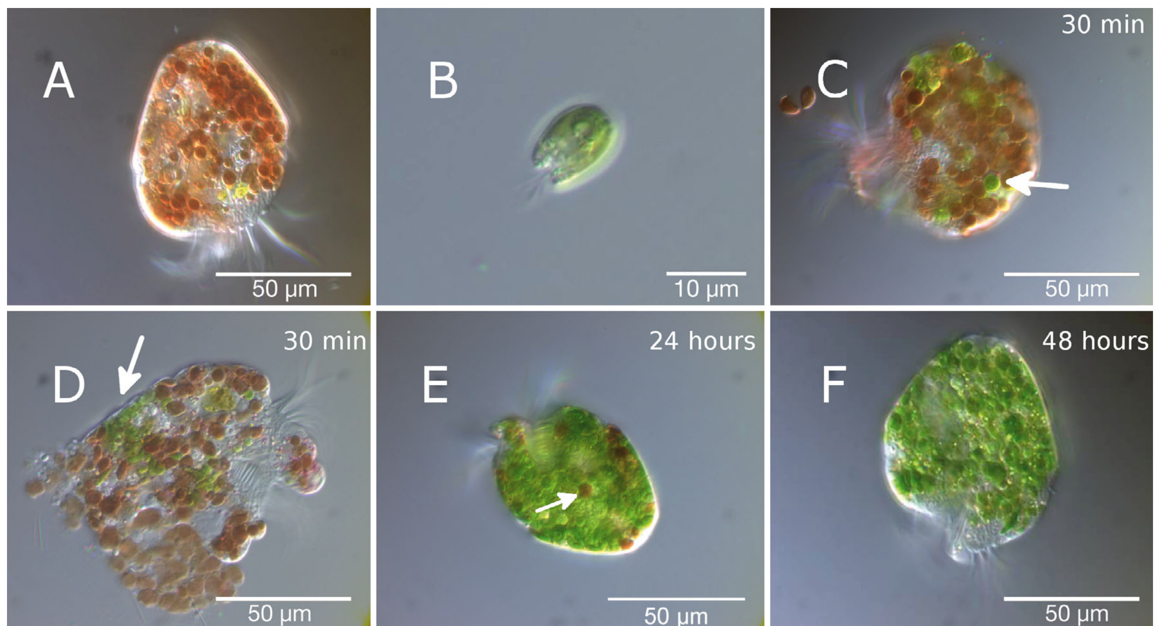

Fig. 1. *Strombidium rassoulzadegani* replaces *Rhodomonas*-derived plastids with *Tetraselmis*-derived plastids within 48 h of being switched from a diet of *Rhodomonas* to a diet of only *Tetraselmis*. (A) *S. rassoulzadegani* fed only *Rhodomonas*; (B) a single *Tetraselmis* cell; (C,D) *S. rassoulzadegani* that had been previously fed only *Rhodomonas* 30 min after being switched to a diet of *Tetraselmis.* Arrows indicate green *Tetraselmis*-derived plastids. *S. rassoulzadegani* (E) 24 h and (F) 48 h after being placed on the *Tetraselmis* diet

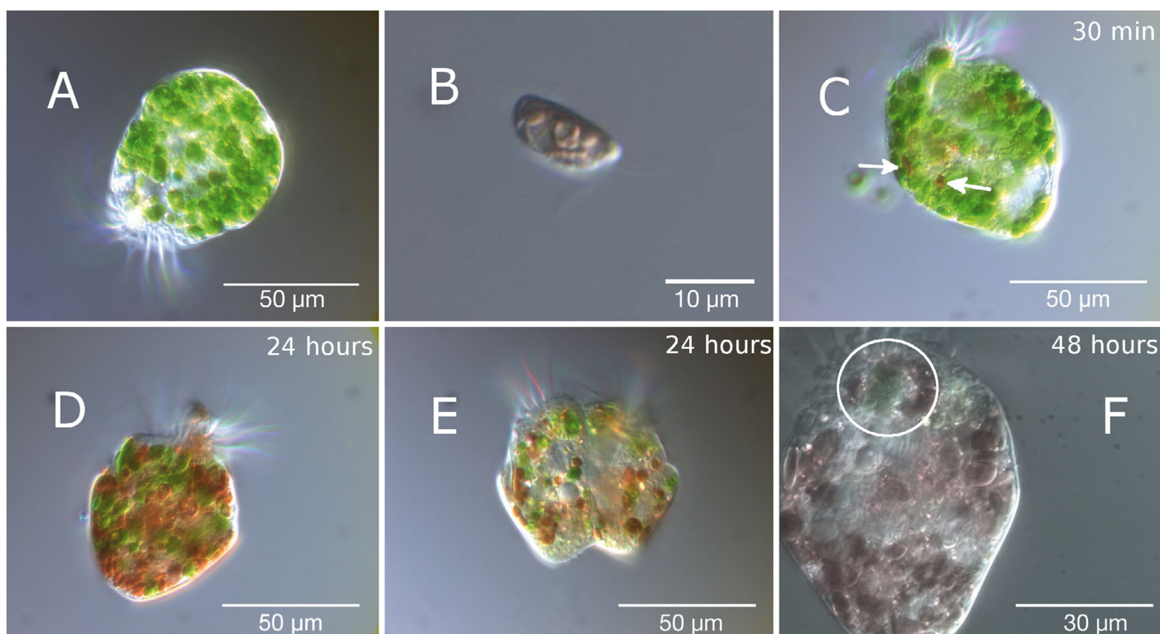

Fig. 2. *Strombidium rassoulzadegani* still retains some *Tetraselmis*-derived plastids 48 h after being switched from a diet consisting of *Tetraselmis* to one consisting of only *Rhodomonas*. (A) *S. rassoulzadegani* fed only *Tetraselmis*; (B) a single *Rhodomonas* cell; (C) *S. rassoulzadegani* that had been previously fed only *Tetraselmis*, 30 min after being switched to a diet of *Rhodomonas.* Arrows indicate the first appearance of *Rhodomonas*-derived plastids. *S. rassoulzadegani* (D,E) 24 h and (F) 48 h after being switched to a diet of *Rhodomonas*; a small number of *Tetraselmis*-derived plastids still remain at 48 h, indicated with a circle

the first new plastid within 30 min (Fig. 2C). However, at 24 h, only about half of the plastids were red (Fig. 2D,E), and after 48 h, there were still a few green *Tetraselmis* plastids remaining near the oral region of the ciliate (Fig. 2F).

We controlled for plastid dilution through growth by conducting the experiment in 96 well plates with one cell in each well and at each time point picking from wells that still had only one ciliate. Even if cells had been growing at a typical rate of one division per day, however, the rate of replacement of *Rhodomonas* chloroplasts with *Tetraselmis* ones was much faster than simple dilution could account for, indicating that the ciliates were actively switching from one plastid type to another.

Plastid retention during starvation

After acclimation on either *Tetraselmis* or *Rhodomonas*, individual *Strombidium rassoulzadegani* cells were moved into algae-free medium, cell squashes were examined and photographed for image analysis, and fluorescent area was measured at each time point as a proxy for chlorophyll content. Average fluorescent area was calculated from 10 to 20 cells (Table 1). Because the data were not normally distributed or homoscedastic, a generalized linear model (GLM) was used to assess the impact of starvation and diet on plastid retention measured as fluorescent area (Montgomery 2005). We found no significant effect of starvation ($p = 0.576$), diet ($p = 0.239$), or their interaction ($p = 0.624$) on fluorescent area.

When starved after being acclimated on a diet of *Tetraselmis*, *Strombidium rassoulzadegani* showed high variability from cell to cell in fluorescent area (Table 1). Ranges (difference between maximum and minimum values) for ciliates acclimated on *Tetraselmis* were 6004 μm^2 cell^{-1} at 24 h, 3823 μm^2 cell^{-1} at 48 h, 4725 μm^2 cell^{-1} at 72 h, and 4475 μm^2 cell^{-1} after 96 h. Some members of the population were able to retain plastids for up to 96 h after being separated from their algal food (up to 5454 μm^2 cell^{-1}). However, there were also members of the population that retained few plastids (at minimum 979 μm^2 cell^{-1}). The increase in fluorescence area after 48 h, though not statistically significant, may have been due to higher mortality rates in cells that started out with fewer plastids. Ciliates that were starved after growth on *Rhodomonas* showed a similar pattern to those grown on *Tetraselmis*. Variability was high, and there was no apparent decrease in fluorescence per cell with starvation (Table 1). There were not enough surviving *Rhodomonas*-acclimated cells after 72 h or *Tetraselmis*-acclimated cells after 96 h to prepare cell squashes for these time points.

Table 1. *Strombidium rassoulzadegani.* Fluorescence area (μm^2 cell^{-1}), a proxy for chlorophyll content, in cell squashes of ciliates when starved after being acclimated on a diet of either *Tetraselmis* or *Rhodomonas*. Standard deviations are in parentheses. There was no significant effect of time of starvation or diet on fluorescence area

Starvation time (h)	*Tetraselmis*	*Rhodomonas*
24	1629 (1438)	530 (325)
48	893 (814)	558 (436)
72	1598 (1420)	382 (733)
96	1953 (1146)	NA

Ciliate mortality when starved

For ciliates grown on *Tetraselmis,* the exponential rate of decline in ciliate abundance from 0 to 48 h was 0.26 d^{-1} ($R^2 = 0.49$, $p = 0.039$), and after 48 h, it increased sharply to 0.94 d^{-1} ($R^2 = 0.94$, $p < 0.0001$) (Fig. 3). When *Strombidium rassoulzadegani* was first grown on *Rhodomonas* and then starved, we found similar exponential mortality rates, but the sharp increase in mortality occurred after 24 h (0.86 d^{-1}, $R^2 = 0.97$, $p < 0.0001$) (Fig. 3).

While the mortality rates for the ciliates acclimated on either of the 2 diets were not very different, there

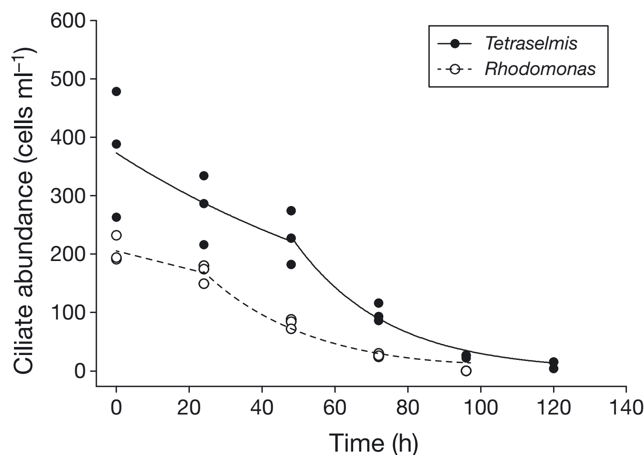

Fig. 3. Changes in abundance of *Strombidium rassoulzadegani* when starved after being fed a diet of either *Tetraselmis* or *Rhodomonas*. While there was a large reduction in ciliate abundance, some cells were still present at 120 h on the *Tetraselmis* diet, but none after 96 h on the *Rhodomonas* diet. Separate exponential decay fits were made to the data before and after 48 h for the *Tetraselmis* diet and 24 h for the *Rhodomonas* diet

does appear to be a longer lag time in the ciliates that were acclimated on *Tetraselmis*. The inflection point appeared to occur after 48 h in the ciliates fed *Tetraselmis* and 24 h for ciliates fed *Rhodomonas*. Algal growth during this experiment was not detectable.

Growth and grazing

To determine if there were any differences in ingestion or growth rates based on diet, we measured growth and grazing rates over a range of algal food concentrations for the 2 algal foods. When we compared numerical response curves (growth rate vs. algal food concentration), we found significant differences when the data were fit to single curve or separate curves for each diet treatment (probability that separate curves provide a better fit than a single curve for the 2 datasets: $p < 0.001$). Curve parameters were also significantly different. The maximum growth rate ($V\text{max}_\mu$) values were 1.25 d^{-1} for *Tetraselmis*, and 0.92 d^{-1} for *Rhodomonas* ($p = 0.003$). The half saturation concentrations ($K\text{m}_\mu$) were 522 ng C ml^{-1} for *Tetraselmis* and 282 ng C ml^{-1} for *Rhodomonas* ($p < 0.001$), and threshold food concentrations for growth (T_μ) were 0 ng C ml^{-1} for *Tetraselmis* and 133 ng C ml^{-1} for *Rhodomonas* ($p = 0.038$) (Fig. 4, Table 2). We also found a significant difference in the overall fit of the functional response curves (ingestion vs. food concentration) for the 2 data sets ($p = 0.007$). However, there were no significant differences in the separate parameters (Table 2).

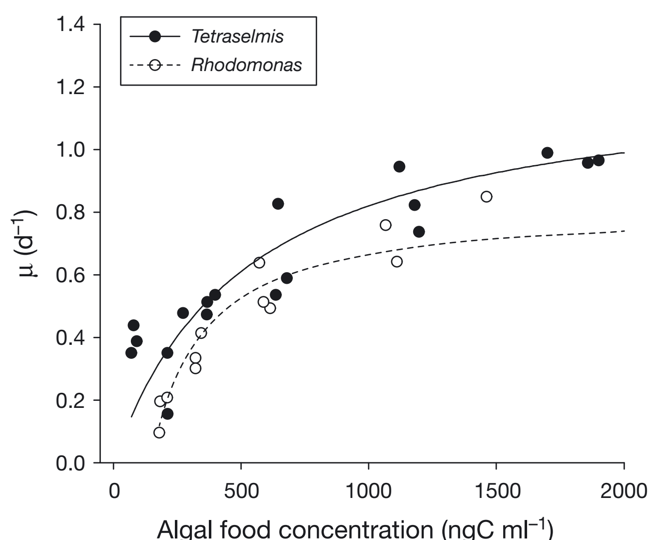

Fig. 4. *Strombidium rassoulzadegani*. Specific growth rate (μ) vs. algal food concentration. Data are best fit by separate curves

Table 2. Result of extra sum of squares *F*-test comparing growth and grazing rates of *Strombidium rassoulzadegani* on algal diets of *Tetraselmis* and *Rhodomonas*. The null hypothesis for the overall fit is that there is no difference between the 2 data sets fit to separate models or fit to the same model. *V*max: maximum ciliate growth (subscript μ) or grazing (subscript i) rate, T_μ: threshold or lowest food concentration at which the ciliates will grow or graze, *K*m: algal concentration at which the ciliate growth rate is half the maximum growth rate. *Significantly different from zero at α = 0.05. Standard errors for parameter estimates are in parentheses

	Tetraselmis	*Rhodomonas*	p
Growth			
Overall fit	–	–	<0.001
$V\text{max}_\mu$	1.25* (0.1)	0.92* (0.06)	0.003
T_μ (ng C ml^{-1})	0 (44.9)	133* (37.5)	0.038
$K\text{m}_\mu$ (ng C ml^{-1})	522* (164.8)	282* (110.4)	<0.001
Grazing			
Overall fit	–	–	0.007
$V\text{max}_i$	9.5* (1.6)	12.7* (1.4)	0.126
T_i (ng C ml^{-1})	10 (87.3)	0 (119)	0.905
$K\text{m}_i$ (ng C ml^{-1})	759* (367.6)	729* (334.7)	0.934

Gross growth efficiency

Strombidium rassoulzadegani fed *Tetraselmis* across a range of food concentrations had GGEs ranging from 0.08 to 0.56, with a marked increase at low food concentrations (Fig. 5). On a diet of *Rhodomonas,* it showed lower GGEs, ranging from 0.02 to 0.14, with no sharp increase at low food concentration (Fig. 5).

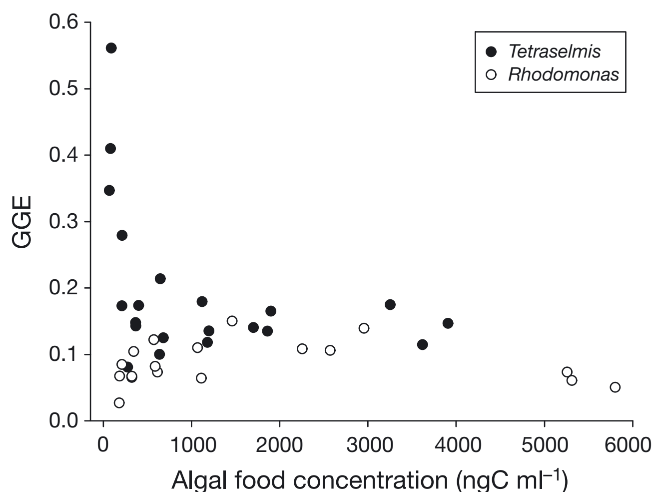

Fig. 5. *Strombidium rassoulzadegani.* Gross growth efficiencies (GGE) on a diet of either *Tetraselmis* or *Rhodomonas* vs. algal food concentration. Ciliates fed *Tetraselmis* have a GGE > 1 at low food concentrations, indicating a subsidy from retained chloroplasts. When the ciliate was fed *Rhodomonas,* GGE did not appear to vary with food concentration

Feeding selectivity

In the experiment comparing unialgal and mixed diets, we found no evidence for food selection. Ingestion rates were approximately the same on the 2 unialgal diets, and the 2 algae comprised nearly equal portions of the diet when offered together (43% *Rhodomonas* and 57% *Tetraselmis*). However, the proportion of red *Rhodomonas*-derived plastids to green *Tetraselmis*-derived plastids (~10%) remained low and constant on the mixed diet (Fig. 6). The proportion of red chloroplasts increased over time in the *Rhodomonas*-only diet as the ciliates replaced their green chloroplasts in ~72 h, consistent with the plastid turnover observations.

DISCUSSION

There seems to be a wide range in plastid retention and turnover rates in organelle-retaining ciliates. For example, *Mesodinium rubrum* retains plastids, nuclei, and mitochondria from its algal prey (Taylor et al. 1971, Hibberd 1977, Oakley & Taylor 1978); the algal genetic material is at least partially transcriptionally active within the ciliate (Johnson et al. 2007). An Antarctic strain of *Mesodinium rubrum* is able to maintain its plastids for up to 8 wk without replacing them under cold, low light (2.5 µmol photons m^{-2} s^{-1}) conditions that induced relatively low growth rates (~0.2 d^{-1}) (Johnson & Stoecker 2005). Furthermore, this *M. rubrum* isolate's plastids retain the ability to divide (Johnson et al. 2006). The Antarctic *Mesodinium* sp. was found to be photosynthetic throughout an 8 wk starvation period, and *M. rubrum* maintained under low light conditions were able to survive starvation longer than those exposed to high light. In contrast, when Stoecker & Silver (1990) grew *Strombidium capitatum* on a mixed diet of cryptophytes and prymnesiophytes and then starved them, the ciliates preferentially retained cryptophyte-derived plastids for up to 40 h. However, *S. capitatum* only retained the prymnesiophyte-derived plastids for 16 h when starved (Stoecker & Silver 1990). When cryptophytes were added to a diet of *S. capitatum* that already consisted of prymnesiophytes and chlorophytes, the ciliate was able to quickly (within 4 h) take up the cryptophyte plastids. Half of the ciliate's plastids were derived from the cryptophyte and half from the original diet (Stoecker & Silver 1990). When it was switched back to its original diet, *S. capitatum* replaced 84% of its cryptophyte plastids within 9 h. Another kleptoplastidic oligotrich, *Laboea strobila*, was grown by Stoecker et al. (1988) on a mixed diet of the prymnesiophyte *Isochrysis galbana* and the cryptophyte *Chroomonas salina.* They then starved the ciliate under a 12 h light:12 h dark cycle and in the dark. *L. strobila* was able to survive and retain plastids from either source under starvation for up to 6 d. While the ciliates exposed to the 12 h light:12 h dark cycle retained both types of plastids, they preferentially retained *Isochrysis*-derived plastids in the dark. In our study, *S. rassoulzadegani* survival when starved was most similar to that of *L. strobila. S. rassoulzadegani*, when acclimated on a diet of either *Tetraselmis* or *Rhodomonas* and then separated from algal prey, was able survive starvation for 120 h for ciliates grown on *Tetraselmis* and 96 h for ciliates grown on *Rhodomonas*. There was an initial short period of little mortality followed by an exponential decrease in ciliate abundances (Fig. 3). Mortality increased strongly after 48 h for ciliates acclimated on *Tetraselmis* and 24 h for ciliates acclimated on *Rhodomonas*. This was most likely due to dysfunction of the aging chloroplasts and starvation. In experiments designed to track the loss of plastids in *S. rassoulzadegani* over time, there was no significant effect of either time or diet on plastid retention. Furthermore, there was great variability in plastid content from cell to cell. Notably, we did not observe any aplastidic cells, and previous studies have indicated that kleptoplastidic oligotrichs only rarely digest their stolen plastids (Laval-Peuto & Febvre 1986).

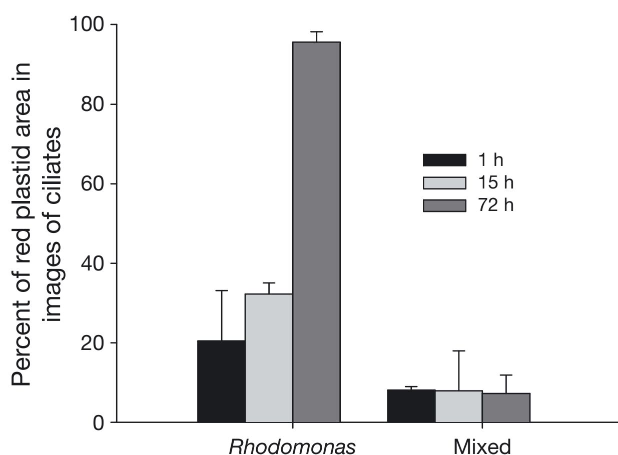

Fig. 6. *Strombidium rassoultadegani.* Proportion of *Rhodomonas*-derived plastids, measured as the percentage of the area of red plastids of the total plastid area. In multiple pairwise comparisons, we saw no difference within the mixed diet, but there was a significant difference between the 1 and 72 h time points in the *Rhodomonas* diet and a significant difference between diets

Oligotrich ciliates like *Laboea strobila, Strombidium capitatum*, and *S. rassoulzadegani* that retain chloroplasts apparently do not sequester the algal nucleus or have any regulatory control over their plastids. Therefore, they must replace their aging plastids with newly ingested ones. Within 30 min of being offered a new algal food source, *S. rassoulzadegani* had plastids from the new algal food positioned at the periphery of the cell (Figs. 1 & 2). Furthermore, all of the ciliate's plastids were replaced after 48 to 72 h (Figs. 1, 2 & 6), depending on the algal food source. While *S. rassoulzadegani* apparently cannot reproduce its plastids, it can replace them when there is a readily available source. This process is notably slower than that of *S. capitatum. S. rassoulzadegani* does not appear to strongly select *Tetraselmis* over *Rhodomonas* when grazing on mixed diets, but they do preferentially acquire the plastids from *Tetraselmis* over *Rhodomonas*-derived plastids when both are available. However, when offered no other option, *S. rassoulzadegani* will readily incorporate the plastids of *Rhodomonas* (Figs. 2 & 6). The observed difference in the transition time from *Tetraselmis*-derived plastids to *Rhodomonas*-derived plastids and *Rhodomonas*-derived plastids to *Tetraselmis*-derived plastids may be explained by the preferential sequestration of the *Tetraselmis*-derived plastids (Fig. 6).

Heterotrophic protist GGEs can range between 0.1 and 0.7 with an average of ~0.33, and these change very little with food concentration (Verity 1985). However, the mixotrophic ciliate *Stentor* spp. can have higher (0.64 to 0.82) GGEs because of the carbon subsidy it receives from photosynthesis of its algal endosymbiont (Laybourn 1976, reviewed by Caron & Goldman 1990). Approximately 22% of *Laboea strobila* photosynthate goes to subsidize the ciliate's growth (Putt 1990), suggesting a small boost to GGE. Johnson & Stoecker (2005) report that grazing only accounted for 8.8 to 10.8% of the growth of an Antarctic *Mesodinium* sp., depending on light conditions. This resulted in values of 9.26 to 11.36 by our definition of GGE (growth ÷ ingestion), underscoring the dominance of autotrophy in *M. rubrum* (Johnson & Stoecker 2005). As mentioned above, this was an Antarctic strain, and it is unclear how it might compare to more temperate *Mesodinium* spp.

Although *Strombidium rassoulzadegani* fed and grew on both diets, there were some evident differences across the range of algal food concentrations used. In general, the ciliate ate less and grew faster on *Tetraselmis* than on the *Rhodomonas* diet (Figs. 4 & 7). These differences in growth and grazing rates resulted in differences in GGE, especially at low food concentrations (Fig. 5). On *Tetraselmis*, growth efficiencies were high (0.56 maximum) when food was scarce (Fig. 5). In contrast, GGEs were very similar between the diets at high food concentrations. Thus, it appears that *S. rassoulzadegani* can make better use of *Tetraselmis* chloroplasts, growing more autotrophically at low food concentrations, but relies more on heterotrophic growth at high concentrations. *S. rassoulzadegani* cannot be maintained in the dark on *Tetraselmis* or *Rhodomonas* (McManus et al. 2012). This suggests that the ciliate is an obligate mixotroph when feeding on these 2 algae, and that when autotrophy is not possible, there is some cost associated with retaining plastids that outstrips the nutritional value of the rest of the cell.

The constant GGEs of the ciliate at different concentrations of *Rhodomonas* are similar to what has been seen in heterotrophic ciliates (Verity 1985). This suggests that *Strombidium rassoulzadegani* with *Rhodomonas*-derived plastids is autotrophic enough to overcome the cost of sequestering the cryptophyte plastids in the light but not to subsidize growth when there is little food available.

The sharp increase in GGE at low food concentration with the *Tetraselmis* diet suggests that the ciliate is mainly autotrophic at low algal food concentrations, feeding mainly to replace chloroplasts. At high food concentrations, the GGE on *Tetraselmis* is similar to that on *Rhodomonas*, suggesting either an increased importance of heterotrophy or perhaps 'luxury' consumption of *Tetraselmis* to maintain fresh plastids. Digestion of sequestered plastids has rarely been observed in oligotrichs

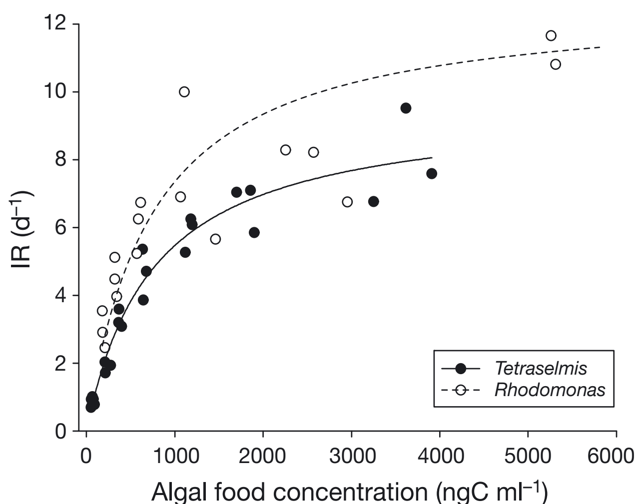

Fig. 7. Specific ingestion rate (IR) vs. algal food concentration. Data are best fit by separate curves

(Laval-Peuto & Febvre 1986), so the increased supply of chloroplasts at high feeding rates may require egestion of older plastids.

Except for the differences in GGE, growth and grazing were broadly similar for both foods. This is surprising considering the divergent phylogenetic origins of the 2 algae. Chlorophytes are thought to have resulted from a primary endosymbiosis with a photosynthetic prokaryote, while cryptophytes derived from a secondary symbiosis between eukaryotes (Gould et al. 2008). It should be expected that gene loss or transfer from green chloroplasts to host nuclei would not be the same as that from red chloroplasts (Dyall et al. 2004), and one might then predict that the 2 kinds of chloroplasts would not be equally independent or capable of 'enslavement' by the ciliate. To some extent, our data support this idea. *Tetraselmis* chloroplasts are clearly a primary source of energy to the ciliates, allowing rapid growth even at very low food levels, whereas feeding on *Rhodomonas* results in GGEs no different from heterotrophic growth. Observations on the uptake and cycling of inorganic carbon are necessary to fully resolve the roles of the 2 kinds of chloroplasts in ciliate metabolism and growth.

Mixotrophic ciliates like *Mesodinium* and *Stentor* can be important contributors to total primary production when they are found in high abundance (Smith & Barber 1979, Wilkerson & Grunseich 1990, Sanders 1995, Laybourn-Parry et al. 1997, Herfort 2012). *Strombidium rassoulzadegani* may contribute similarly in the tidepools where it is found. Switching between mostly autotrophic and heterotrophic growth may allow this ciliate to maintain high growth rates when faced with changes in the availability of algal food.

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