

ORIGINAL ARTICLE

**Preferential Plastid Retention by the Acquired Phototroph
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ABSTRACT

The ciliate genus *Mesodinium* contains species that rely to varying degrees on photosynthetic machinery stolen from cryptophyte algal prey. Prey specificity appears to scales inversely with this reliance: The predominantly phototrophic *M. major/rubrum* species complex exhibits high prey specificity, while the heterotrophic lineages *M. pulex* and *pupula* are generalists. Here, we test the hypothesis that the recently described mixotroph *M. chamaeleon*, which is phylogenetically intermediate between *M. major/rubrum* and *M. pulex/pupula*, exhibits intermediate prey preferences. Using a series of feeding and starvation experiments, we demonstrate that *M. chamaeleon* grazes and retains plastids at rates which often exceed those observed in *M. rubrum*, and retains plastids from at least five genera of cryptophyte algae. Despite this relative generality, *M. chamaeleon* exhibits distinct prey preferences, with higher plastid retention, mixotrophic growth rates and efficiencies, and starvation tolerance when offered *Stoeatula major*, a cryptophyte that *M. rubrum* does not appear to ingest. These results suggest that niche partitioning between the two acquired phototrophs may be mediated by prey identity. *M. chamaeleon* appears to represent an intermediate step in the transition to strict reliance on acquired phototrophy, indicating that prey specificity may evolve alongside degree of phototrophy.

ACQUIRED phototrophs, organisms that obtain their photosynthetic abilities either by hosting photosynthetic endosymbionts or by stealing organelles from their prey (Stoecker et al. 2009), provide a fascinating window into the evolutionary ecology of eukaryotic photosynthesis (Johnson 2011a). By harnessing the photosynthetic machinery of other organisms, these taxa extend their metabolic niche from strict heterotrophy to encompass primary production, recapitulating the first steps along the endosymbiosis pathway (Blackbourn et al. 1973; Falkowski et al. 2004; Sagan 1967) and fundamentally altering their ecological role (Moeller et al. 2016).

The *Mesodinium* genus, which contains ciliates that range from strict heterotrophy—the aplastidic *M. pulex*

and *M. pupula*—to strict phototrophy—the organelle stealing *M. rubrum* and *M. major* (Garcia-Cuetos et al. 2012; Hansen et al. 2013; Johnson 2011b), is a model system for this transition. Its best known representatives are members of the red tide-forming *M. major/rubrum* species complex (Johnson et al. 2016), which form blooms in estuaries (Crawford et al. 1997; Herfort et al. 2011, 2012), upwelling zones (Jimenez and Intriago 1987; Packard et al. 1978), and fjords (Lindholm 1985) around the world. Laboratory study of *M. rubrum* cultures has revealed that, in addition to retaining chloroplasts (kleptoplastids) and mitochondria from its red cryptophyte algal prey (Gustafson et al. 2000), *M. rubrum* also retains transcriptionally active prey nuclei (Johnson et al. 2007; Kim et al. 2016; Lasek-

Nesselquist et al. 2015) which enables the ciliate to photoacclimate (Moeller et al. 2011) and maintain photosynthetic growth over long periods of starvation (Hansen and Fenchel 2006; Johnson and Stoecker 2005; Smith and Hansen 2007). Measurements of photosynthetic and respiration rates suggest that *M. rubrum* obtains ~98% of its carbon from photosynthesis (Johnson and Stoecker 2005), and thus it is considered a strict acquired phototroph.

A new *Mesodinium* species, *M. chamaeleon*, has been described whose mixotrophic metabolic strategy places it intermediate between *M. pulex/pupula* and *M. major/rubrum* (Moestrup et al. 2012). Like *M. rubrum*, *M. chamaeleon* captures and ingests cryptophyte prey, and transiently retains organelles from these prey. Unlike *M. rubrum*, which separates prey nuclei from chloroplast-mitochondrion complexes (Hibberd 1977; Johnson et al. 2007), *M. chamaeleon* sequesters these organelles in “organelle complexes,” membraned food vacuoles that contain the remnants of whole ingested prey (Moestrup et al. 2012). These organelle complexes serve as short-term photosynthetic units, allowing *M. chamaeleon* to achieve modest photosynthetic rates up to 6.3 pg C/cell/h, and are eventually digested (Moestrup et al. 2012). Recently, similar ultrastructural descriptions have been published for an additional species, *M. coatsi* (Nam et al. 2014), which, like *M. chamaeleon*, contains blue-green plastids when collected, grows best when fed green cryptophytes from the genus *Chroomonas*, and exhibits a “benthic” swimming behavior in that it congregates on surfaces.

These intriguing findings invite a more detailed exploration of the ecophysiology of *M. chamaeleon*. In this study, we examine how *M. chamaeleon*'s growth, ingestion rates, and photosynthetic capacity depend upon prey identity and availability. We hypothesize that *M. chamaeleon*'s prey preferences fall intermediate between *M. pulex/pupula*, which are generalist heterotrophs (Jakobsen et al. 2006; Tarangkoon and Hansen 2011), and *M. major/rubrum*, which appear to specialize on cryptophytes from the *Teleaulax*, *Plagioselmis*, *Geminigera* (TPG) clade (Hansen et al. 2013; Johnson et al. 2016; Park et al. 2007; Peltomaa and Johnson 2017). We seek to answer three research questions: (i) Does *M. chamaeleon* exhibit differences in growth response when offered different cryptophyte prey species? (ii) If these differences exist, are they driven by phototrophic or heterotrophic mechanisms? (iii) How do these differences affect stress tolerance (i.e. starvation and changes in light environment)?

To address these three questions, we performed a series of experiments on a new isolate of *M. chamaeleon* collected from coastal Rhode Island, USA, the location of the first description of a ciliate resembling *M. chamaeleon* (Hargraves 1991). First, we assayed growth on five different cryptophyte species under starved and prey-replete conditions to infer the relative rates of phototrophic and mixotrophic growth by prey type. Second, we measured ingestion rates and plastid content to determine the mixotrophic yield and retention efficiency for different prey species. Third, we conditioned *M. chamaeleon* to two prey species on which it exhibited varied growth responses,

and assayed its ability to tolerate stress by measuring its response to starvation and large increases in light availability. Our results underscore *M. chamaeleon*'s intermediate position in the *Mesodinium* genus as a mixotroph that displays greater flexibility in prey ingestion and metabolism than its heterotrophic and predominantly phototrophic congeners.

METHODS AND MATERIALS

Cultures and growth conditions

We obtained a new culture of *Mesodinium chamaeleon* by performing single-cell isolations on a water sample collected from just above the oxycline in the Narrow River (Narragansett Bay, RI) in September 2015. *Mesodinium chamaeleon* stock cultures are maintained at 18°C in 0.2- μ m-filtered, autoclaved seawater (collected from Vineyard Sound, MA, PSU ~ 35ppt, pH ~ 8.0) at a light level of 4 μ mol quanta/m²/s. This light level was chosen based on the low light experienced by the *M. chamaeleon* population in situ at the oxycline (located at a depth of ~6 m in turbid water) in the Narrow River. *M. chamaeleon* is fed biweekly with the red cryptophyte *Storeatula major* (strain SM or g, isolated by Allen Lewitus from the Chopank River, Cambridge, MD, USA). This culture has now been maintained in these laboratory conditions for > 18 mo. Growth rates for *M. chamaeleon* did not differ across the media types assayed—K (Keller et al. 2007), L1 (Guillard and Hargraves 1993), f/2 (Guillard and Ryther 1962), or filtered seawater (data not shown)—so all experiments were conducted in filtered seawater.

Periodically during culture maintenance, and as described below for experimental setup and data collection during our study, *M. chamaeleon* must be separated from co-cultured prey and other debris. We accomplished this separation using the gravity filtration method described by Peltomaa and Johnson (2017). Briefly, we loaded cultures (or sample volumes) onto 8.0- μ m pore size Transwell filter inserts in 6-well plates (Corning, NY), allowing the *M. chamaeleon* to concentrate on the filter's surface while the smaller cryptophyte cells passed through. We then washed the concentrated *M. chamaeleon* with at least 100 ml of sterile, filtered seawater to remove any remaining prey. Prey removal was confirmed by microscopy at experimental setup, and periodically during experiments, by enumerating fixed cells at 100X magnification.

In this study, we conditioned *M. chamaeleon* to five different cryptophyte prey species representing five different genera: (i) *Chroomonas mesostigmatica* (CCMP 1168), obtained from the National Center for Marine Algae and Microbiota (NCMA, Bigelow Laboratory, East Boothbay, ME), (ii) *Guillardia theta* (CCMP 2712), obtained from the NCMA, (iii) *Hemiselmis cryptochromatica* (CCMP 1181), obtained from the NCMA, (iv) *S. major*, and (v) *Teleaulax amphioxeia* (GCEP01), isolated by Mengmeng Tong from Eel Pond (Falmouth, MA). We define “conditioning” as first washing the *M. chamaeleon* culture free of prey, and then offering *M. chamaeleon* exclusively the conditioning prey species for a period of approximately 2 wk (at least

three generations of *M. chamaeleon* cells, during which time prey to *M. chamaeleon* ratios were kept greater than 20:1). Note that, as we report below, conditioning *M. chamaeleon* to a prey species did not always result in complete replacement of its ingested plastids because of differences in grazing and organelle retention across prey species.

Experiment 1: Growth and grazing functional response

We designed a factorial experiment to measure growth and ingestion rates by first conditioning *M. chamaeleon* to one of the five prey species, washing it free of prey, and then offering it new prey of a single species in ratios of 0:1, 1:1, 5:1, 10:1, 25:1, and 50:1 prey:*M. chamaeleon*. Predator-free controls were also set up for each of the five initial prey concentrations; all treatments and controls were run in triplicate. At $T = 0, 24, 48,$ and 72 h, samples were preserved with acid Lugol's solution (1% final concentration), and *M. chamaeleon* and prey cells were enumerated on a Sedgewick-Rafter counting chamber at 100X magnification.

Statistical analyses for these and other experiments were carried out in R (R Core Team 2014). We calculated the growth rate (in units of per day) of *M. chamaeleon* over the full 72-h experiment by fitting a linear model to the log of population size plotted against time point (Base R function *lm*). Growth rates were also calculated for each 24-h interval. Ingestion rates (in units of prey cells per *M. chamaeleon* cell per day) were calculated using the method of Jeong and Latz (1994), which compares prey growth in predator-free controls with growth (or loss) in *M. chamaeleon*-containing treatments. We fit Holling Type II functional response curves (Holling 1959) to ingestion data of the form $\alpha * \text{Prey Concentration} / (\beta + \text{Prey Concentration})$, where α was the maximum grazing rate, and β was the half-saturating prey concentration (Base R function *nls*). Similarly, we fit a modified saturating functional response to growth data, using the form $\alpha * \text{Prey Concentration} / (\beta + \text{Prey Concentration}) + \gamma$. Here, γ is the phototrophic (i.e. starved) growth rate, which may be positive or negative depending on prey conditioning, α is the maximum increase (i.e. the additional growth achieved through mixotrophy), and β is, as above, the half-saturation constant. Mixotrophic yield was calculated as the ratio of growth to ingestion rates, which gives yield in units of *M. chamaeleon* cells produced per cryptophyte prey cells ingested. Because of methodological constraints, our design was not fully factorial. Specifically, the small size of *H. cryptochromatica* and the use of Lugol's fixative and Sedwick Rafter chambers (limited to 100X), made it difficult to accurately count prey in fixed samples, so no ingestion data are shown for this species. In addition, due to poor growth of *M. chamaeleon* on *G. theta* and *T. amphioxeia*, as well as our subsequent finding of incomplete organelle replacement, we were unable to collect complete datasets for *M. chamaeleon* conditioned to those two species.

Experiment 2: Plastid dynamics

We studied the dynamics of prey uptake and retention by developing a quantitative PCR (qPCR) assay to track the abundance and composition of *M. chamaeleon* plastids over time (Peltomaa and Johnson 2017). This assay counted ingested plastids (which, because they are embedded in organelle complexes) by targeting a fragment of the plastid-encoded large subunit of RuBisCO (*rbcL*) gene and *M. chamaeleon* cells by targeting a fragment of the nuclear small subunit (SSU) rRNA (18S) gene. Primers (synthesized by Eurofins MWG Operon LLC, Louisville, KY) were designed to specifically target each species based on known sequence mismatches, and annealing temperatures were optimized to prevent cross-amplification (Table 1). Because of substantial cross-amplification between primers designed for *C. mesostigmatica* and *H. cryptochromatica*, we were unable to perform qPCR studies involving combinations of these two species, and instead chose to focus on *C. mesostigmatica* because of divergence in *M. chamaeleon*'s growth response between it and *S. major*. Standard curves for each species were prepared by collecting triplicate dilution series containing 1, 10, 100, 1,000, and 10,000 cryptophyte cells (or 5, 50, 500, and 5,000 *M. chamaeleon* cells) on a 0.4- μm -pore size Isopore polycarbonate filter (HTTP02500, EMD Millipore, Darmstadt, Germany). We extracted DNA using the phenol/chloroform method of Gast et al. (2004), except that cells were washed off the filters by pipetting, and no beads were necessary to disrupt cell membranes. All qPCR assays were conducted using a CFX96 Real-Time PCR detection system (Bio-Rad, Hercules, CA) with SsoFast EvaGreen Supermix (Bio-Rad; reaction volume: 20 μl) with reaction conditions as described in Peltomaa and Johnson (2017).

Based on data from Experiment 1, we focused on *M. chamaeleon* conditioned on *C. mesostigmatica* or *S. major* and offered either *C. mesostigmatica*, *G. theta*, *S. major*, or *T. amphioxeia*. Experiments, performed in triplicate, lasted for 8 d: On day 0, washed *M. chamaeleon* were placed into co-culture with cryptophyte prey at a ratio of 10 cryptophytes per *M. chamaeleon* cell. At $T = 0, 24, 48,$ and 72 h, samples of these co-cultures were taken, *M. chamaeleon* were washed free of prey using the filtration method described above, and then *M. chamaeleon* cells were collected polycarbonate filters and stored at -20 °C for up to 4 wk prior to DNA extraction. On Day 4, the remainder of each culture was washed free of prey, live *M. chamaeleon* cells were photographed using light microscopy at 200X magnification, and then these cells placed back in the incubator for a further 4 d under prey-free (starved) conditions. On Day 8, DNA was extracted from these cells.

Copy number of *M. chamaeleon*, conditioning plastid type, and prey plastid type were determined for each extracted sample by comparing amplification cycle threshold (C_t) to the standard curves, and the number of plastids (i.e. organelle complexes) from each species per *M. chamaeleon* cell was computed as the ratio of plastid

Table 1. Primers used for quantitative PCR assays and their annealing temperatures

Primer	Sequence	Temp. (°C)
<i>Chroomonas mesostigmatica</i> CM_rbcL_F	5' GCTGCTGCTGCTGGTGAAG 3'	63.2
<i>Chroomonas mesostigmatica</i> CM_rbcL_R	5' TTTACGTGCCAGATACCCATAGA 3'	
<i>Guillardia theta</i> GT_rbcL_F	5' CGAAGGTGTAACCGTGCTG 3'	63.2
<i>Guillardia theta</i> GT_rbcL_R	5' AGATCGCCATACTTTGAATAGCAGTA 3'	
<i>Storeatula major</i> SM_rbcL_F2	5' GATTAAGGTCACACTCTTCAACATTACT 3'	60.0
<i>Storeatula major</i> SM_rbcL_R2	5' GTAAGATCATACTGTTCTTACGAGC 3'	
<i>Teleaulax amphioxeia</i> TAM_rbcL_F2	5' TGCATCTGCAGCTACAGGTGAAA 3'	58.2
<i>Teleaulax amphioxeia</i> TAM_rbcL_R2	5' CTGACGAGACCACTTAGCAATACT 3'	
<i>Mesodinium chamaeleon</i> MC_1640F	5' TGGAGGTGTCGGGTGCT 3'	63.0
<i>Mesodinium chamaeleon</i> MC_1830R	5' AGGGATGTTTTGGAGCGTGA 3'	

copy number to *M. chamaeleon* copy number. Plastid retention efficiency was calculated as the ratio of plastids retained (based on qPCR assay data) to prey ingested (based on grazing data) in the first 24 h of the experiment, to reduce the effects of differential digestion rates.

Experiment 3: Stress response

Finally, we contrasted tolerance of starvation and light stress in *M. chamaeleon* conditioned to *C. mesostigmatica* or *S. major*. We washed conditioned *M. chamaeleon* free of prey, and then set up triplicate experiments at two light levels: low light (normal culture conditions, 4 $\mu\text{mol quanta}/\text{m}^2/\text{s}$ with a 14-h:10-h light:dark cycle) and high light (97 $\mu\text{mol quanta}/\text{m}^2/\text{s}$ with 24 h of light). At $T = 0, 24,$ and 48 h, and every 48 h thereafter for a total of 14 d, we made a series of measurements to quantify cell physiology. First, we fixed and counted cells as described above. Second, we measured the quantum yield for photochemistry in PSII (F_v/F_m), a proxy for photosynthetic health, using a Fluorescence Induction and Relaxation (FIRe) system (Satlantic Inc., Halifax, Nova Scotia, Canada). Third, we measured chlorophyll-*a* content by filtering cells onto Whatman GF/F filters, extracting pigments overnight in 90% acetone (Parsons et al. 1984), and quantifying chl-*a* using a TD-700 fluorometer (Turner Designs, San Jose, CA). Fourth, we quantified plastid content over time using the qPCR assay described above.

RESULTS

Growth rates differ by prey type and availability

When conditioned to five different prey species, the acquired phototroph *Mesodinium chamaeleon* exhibited divergent growth responses (Fig. 1). When well-fed (i.e. when prey availability exceeded 50 prey cells per *M. chamaeleon* cell), *M. chamaeleon* exhibited mixotrophic growth rates in excess of 0.5 per day, with highest growth rates achieved when offered *Storeatula major* and *Hemiselmsis cryptochromatica*, and lower, but positive, growth rates on *Chroomonas mesostigmatica*, *Guillardia*

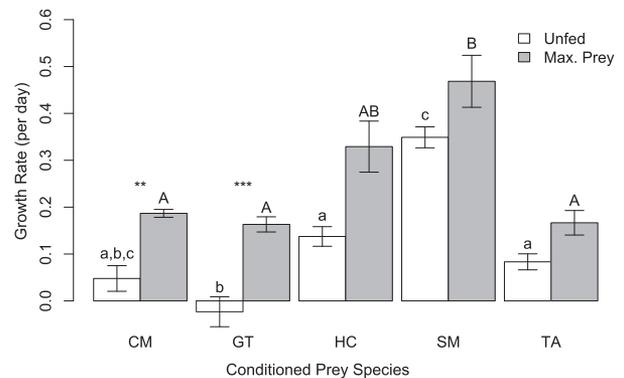


Figure 1 *Mesodinium chamaeleon* growth rate as a function of prey type and availability. *Mesodinium chamaeleon* cells were conditioned to (fed exclusively on) five different species of cryptophyte prey for at least three generations. These cells were then washed free of prey, and their growth rates were measured over a 72-h period either in the absence of prey (Unfed, white bars), or when offered prey at a ratio of 50 prey cells: 1 *M. chamaeleon* cell (Max. Prey, gray bars). *M. chamaeleon* exhibited the highest growth rate when conditioned to *Storeatula major*, or when fed either *S. major* or *Hemiselmsis cryptochromatica*. Lower case letters indicate significant differences between unfed treatments and upper case letters indicate significant differences between fed treatments at the $P < 0.05$ level (Tukey's HSD). Stars indicate differences between starved and well-fed prey conditions within prey conditioning treatments (** $P < 0.01$; *** $P < 0.001$; t-test).

theta, and *Teleaulax amphioxeia*. When starved of prey, *M. chamaeleon*'s capacity for phototrophic growth varied strongly among prey types, with *S. major*-conditioned cells achieving growth rates at least twice as high as cells conditioned to other species.

The beneficial growth effects of *S. major* were also apparent when prey conditioning changed over time (Fig. 2). In general, *M. chamaeleon* conditioned on other prey species exhibited growth rates that increased over time when offered *S. major* (Fig. 2, top row). In contrast, *M. chamaeleon* transferred from *S. major* to *G. theta* exhibited declining growth rates over time (Fig. 2, middle row). As a consequence, average growth rates over the full 72-h experiment varied by conditioning and prey

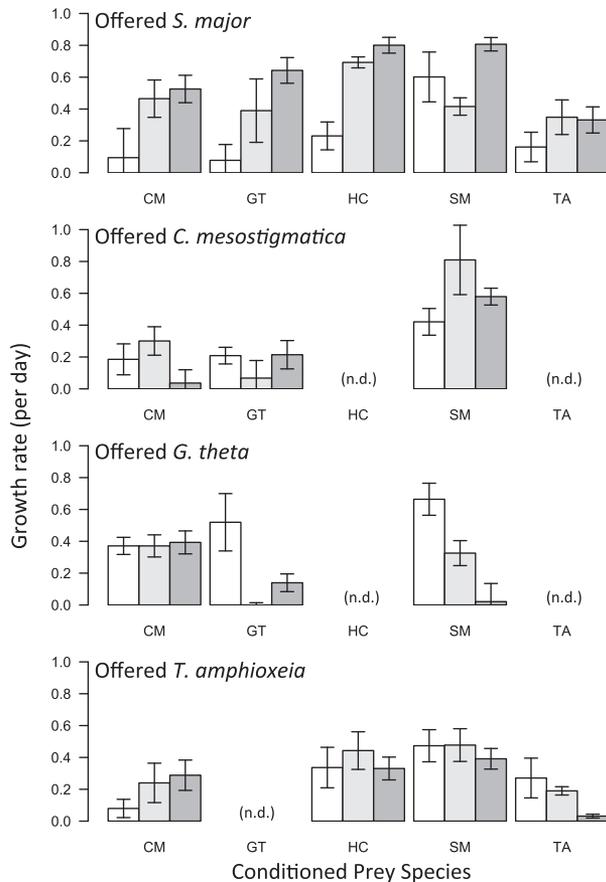


Figure 2 Growth rates for *Mesodinium chamaeleon* offered four different cryptophyte prey species—*Storeatula major* (top row), *Chroomonas mesostigmatica* (second row), *Guillardia theta* (third row), and *Teleaulax amphioxeia* (bottom row)—when conditioned on those species or *Hemiselmis cryptochromatica*; n.d. indicates missing data when a prey combination was not tested. Prey were offered at a ratio of 50 cryptophytes: 1 *M. chamaeleon* cell, and growth was measured for 72-h at 24-h intervals. White bars show growth rates for the first 24 h, light gray for the second 24 h, and dark gray for the third 24 h interval. Note that growth rates generally increase over time when *M. chamaeleon* are offered *S. major*, but may decrease in other cases, such as when *S. major*-conditioned cells are offered *G. theta*.

offering, but generally growth rates were highest for *M. chamaeleon* offered *S. major* or conditioned to *S. major* or *H. cryptochromatica* (Fig. S1).

Varied ingestion, plastid retention, and growth rates support divergent mixotrophic yields

Mesodinium chamaeleon exhibited a Holling Type II functional response, with ingestion rates saturating as prey availability increased (Fig. 3). The maximum ingestion rates achieved varied by prey type and conditioning (Fig. 3). With the exception of *M. chamaeleon* offered *G. theta*, ingestion rates were highest for cells conditioned to *S. major*. Maximum ingestion rates of ~25 cryptophytes per *M. chamaeleon* cell per day were achieved by *S. major*-

conditioned cells offered *C. mesostigmatica*. The lowest rates were observed for cells conditioned to *G. theta*.

In contrast to varied ingestion rates, plastid retention rates diverged strongly among prey types (Fig. 4A, B). Specifically, well-fed *M. chamaeleon* conditioned on *S. major* retained rough 25% more plastids than when conditioned on *C. mesostigmatica* (Fig. 4A). When offered different prey, *M. chamaeleon* cells increased their content of *S. major* and *C. mesostigmatica* plastids by 5–10 plastids per day, but retained only small amounts of *G. theta* plastids and negligible amounts of *T. amphioxeia* plastids (Fig. 4B). Consistent with these findings, an assay of plastid content in *G. theta*-conditioned cells revealed that *G. theta*-conditioned *M. chamaeleon* still had substantial amounts of *S. major* plastids, the prey on which they had been grown prior to conditioning (14.0 ± 2.8 *S. major* plastids per *M. chamaeleon* compared to 4.3 ± 0.4 *G. theta* plastids per *M. chamaeleon*; data are means \pm standard errors). As a consequence of these differences in plastid uptake, retention efficiencies were greatest for *S. major* and *C. mesostigmatica* plastids, marginally lower for *G. theta* plastids, and less than 0.25 for *T. amphioxeia* plastids (Fig. 4C).

Variation in retention efficiency was not well correlated with mean growth rate (compare Fig. 4C, D). In particular, *S. major*-conditioned *M. chamaeleon* maintained relatively high growth rates even when offered cryptophytes from which they did not readily retain plastids (compare top and bottom halves of Fig. 4D, see also Fig. 2 for how *S. major* can drive growth rate recovery over time). However, mixotrophic yield, which was higher for *S. major*-conditioned *M. chamaeleon* offered *S. major* prey than for any other treatment, is consistent with our findings of higher photosynthetic (starved) growth rates in *S. major*-conditioned *M. chamaeleon* and greater retention efficiency of *S. major* plastids in this species.

Stress tolerance is greatest for *Mesodinium chamaeleon* conditioned to preferred prey

When starved of prey at normal growth irradiances (compare filled points in Fig. 5), *M. chamaeleon* maintained its population size and photosynthetic efficiency for longer when conditioned to *S. major* than when conditioned to *C. mesostigmatica* (Fig. 5A, B), although over time its per-cell chlorophyll-*a* content and plastid counts converged (Fig. 5D, F). When exposed to an additional stress of high light (compare filled and open points in Fig. 5), *M. chamaeleon* exhibited a precipitous drop in PSII yield (Fig. 5B), and a slower but pronounced decline in chlorophyll-*a* and plastid content (Fig. 5D, F). However, *S. major*-conditioned *M. chamaeleon* cells actually recovered positive population growth rates from days 2 to 8, in contrast with *C. mesostigmatica*-conditioned cells, whose population sizes monotonically declined (Fig. 5A). Further underscoring differences between conditioning treatments, *M. chamaeleon* cultures containing *S. major* plastids exhibited increases in total chlorophyll-*a* over the first two days of the experiment (Fig. 5C).

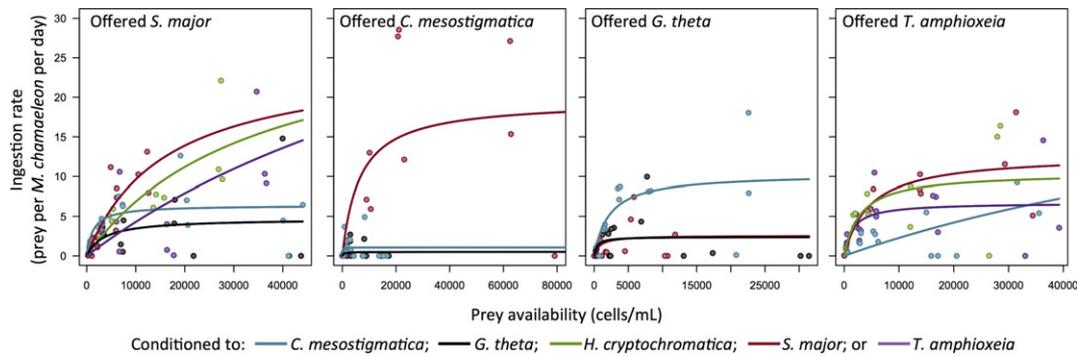


Figure 3 Ingestion rates (prey consumed per *Mesodinium chamaeleon* per unit time) were measured when offered four different species of cryptophyte prey. Data points represent 24-h ingestion rates calculated according to the method of Jeong and Latz (1994), and are presented for *M. chamaeleon* conditioned to between three and five other prey species: *Chroomonas mesostigmatica* (blue), *Guillardia theta* (black), *Hemiselmis cryptochromatica* (green), *Storeatula major* (red), and *Teleaulax amphioxeia* (purple). Lines show best fits for a Holling Type II functional response in which ingestion rates saturate with increasing prey availability. Although ingestion rates were generally highest for *M. chamaeleon* offered *S. major*, considerable variation exists depending upon prey conditioning.

DISCUSSION

Our data support *M. chamaeleon*'s ecological positioning as a mixotrophic intermediary between the predominantly phototrophic (> 95% of carbon from photosynthesis, Smith and Hansen 2007) *M. rubrum*/*M. major* species complex, and the heterotrophs *M. pulex* and *M. pupula* (Garcia-Cuetos et al. 2012). While *M. chamaeleon* can sustain photosynthetic growth for short periods of time depending on plastid conditioning (see Fig. 1, 5), its growth is enhanced when offered a continuous supply of prey (Fig. 1 and Fig. S1). This finding is consistent with previous observations of *M. chamaeleon*'s handling of ingested prey (Moestrup et al. 2012), which differs from *M. rubrum* in that prey cells appear to be left intact as they are slowly digested, and with the phylogeny of the Mesodiniidae, which places *M. chamaeleon* as an intermediate lineage between heterotrophic and phototrophic branches (Garcia-Cuetos et al. 2012).

Mixotrophic growth in *M. chamaeleon* is sustained by relatively high rates of ingestion and plastid retention from a relatively wide diversity of prey. We measured maximum ingestion rates of between 10 and 25 cryptophytes per day, compared with fewer than 10 cryptophytes per day measured in temperate *M. rubrum* (Hansen and Fenchel 2006; Peltomaa and Johnson 2017; Smith and Hansen 2007; Yih et al. 2004). More importantly, maximum *M. rubrum* growth rates are achieved at much lower ingestion rates (~1 prey cell/d; Yih et al. 2004; Hansen and Fenchel 2006; Smith and Hansen 2007) than found for *M. chamaeleon*. Our counts of 15–20 organelle complexes within a single *M. chamaeleon* cell were similar to measurements made on temperate cultures of *M. rubrum* (Hansen and Fenchel 2006; Kim et al. 2016, 2017; Peltomaa and Johnson 2017) (though slightly higher than polar cultures of *M. rubrum* with ~10 plastids per cell, Johnson et al. 2006): This suggests that digestion rates of prey plastids are much higher in *M. chamaeleon* than *rubrum*. Unlike *M. rubrum*, which can maintain growth for

weeks to months by replicating photosynthetic machinery (Johnson and Stoecker 2005; Johnson et al. 2007), *M. chamaeleon* growth rates become negative within a few days of the cessation of feeding. Thus, although well-fed *M. chamaeleon* can achieve short-term growth rates similar to those observed in temperate *M. rubrum* (Peltomaa and Johnson 2017), *M. chamaeleon* does not seem to have the same ability to replicate ingested photosynthetic machinery and is instead much more dependent upon the continual ingestion of prey cells.

The balance between photosynthesis and heterotrophy in mixotrophs is affected by light availability (Stoecker 1998). In this study, most experiments were conducted at relatively low light levels, in keeping with the natural environment from which our cultures were collected. While these low light levels still facilitated prey growth rates in excess of 0.6 d^{-1} for *S. major* and greater than 0.35 d^{-1} for other cryptophytes (Fig. S2; note that rates were measured in filtered seawater with no nutrient additions) and photosynthetic (starved) growth of *M. chamaeleon* of up to 0.35 d^{-1} (Fig. 1), it is likely that the relative contributions of phototrophy to *M. chamaeleon*'s carbon budget may increase as a function of light, producing similar light-dependent growth as observed in *M. rubrum* (Moeller et al. 2011; Smith and Hansen 2007). Even *M. rubrum*, considered predominantly phototrophic, may obtain up to 20% of its carbon from heterotrophy when light is limiting (Smith and Hansen 2007). However, increases in available light may also result in increases in photooxidative stress for microplankton (Strom 2001). This may explain reductions in plastid number, chlorophyll content, and initial mortality in our light stress experiment. Studies of heterotrophic *Mesodinium* lineages have also shown more rapid decreases in plastid number and elevated mortality at high light (Tarangkoon and Hansen 2011). Although we did not measure photosynthetic rates in our study, Moestrup et al. (2012) report *M. chamaeleon* carbon fixation rates of approximately 20–25% that of *M. rubrum*, consistent with a mixotrophic strategy.

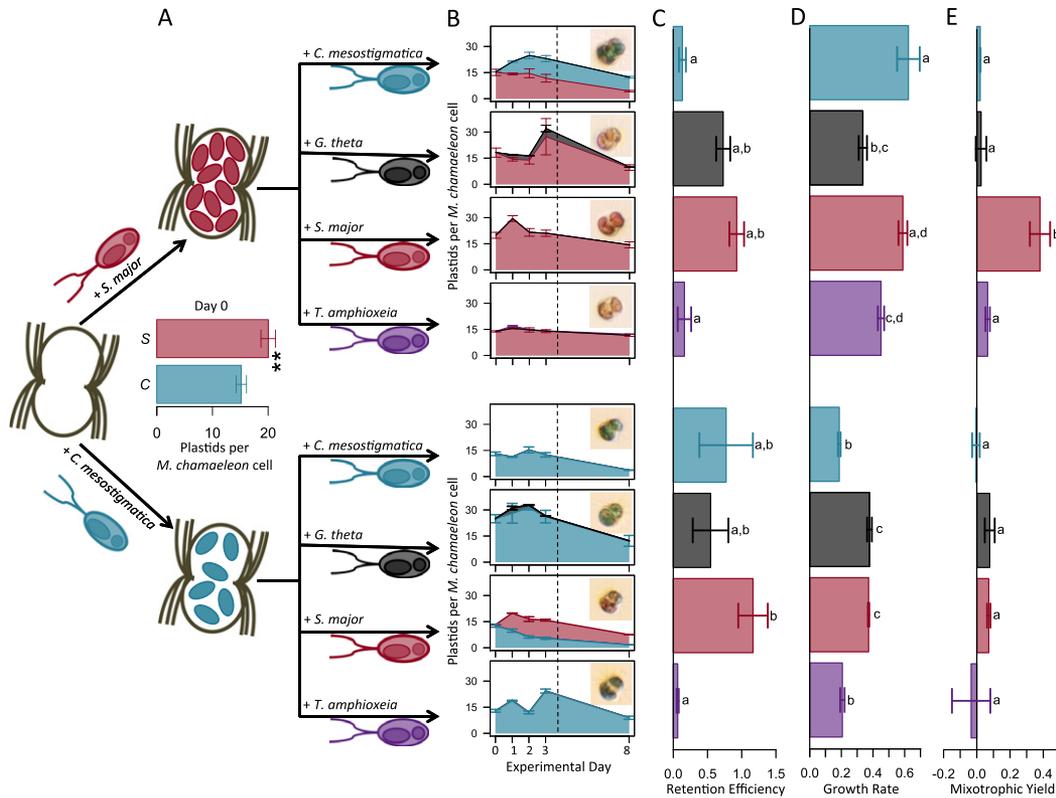


Figure 4 Effects of prey type on *Mesodinium chamaeleon* plastid content and growth efficiency. Column **A**: When conditioned on *Storeatula major*, *M. chamaeleon* contains a significantly higher concentration of prey plastids than when conditioned on *Chroomonas mesostigmatica* (*t*-test, $P < 0.01$). Conditioned *M. chamaeleon* was then washed free of prey cells, and offered four different types of cryptophyte prey at an initial ratio of 10 prey cells per *M. chamaeleon* cell. Column **B**: Stacked area plots show that over 3 d, *M. chamaeleon* preferentially retained plastids from *C. mesostigmatica* (blue area) and *S. major* (red area), but retained relatively few *Guillardia theta* (black) or *Teleaulex amphioxeia* (purple) plastids. These retention differences were apparent during visual observations of cells (inset images, photographed on Day 4). On Day 4 (vertical dashed line), *M. chamaeleon* cells were washed free of any remaining prey, and incubated for a further 4 d at which point (Day 8) a final measurement was taken to examine plastid retention during starvation. Column **C**: Consistent with these observations, plastid retention efficiency (the fraction of ingested cells from which a plastid was retained) was greatest when *M. chamaeleon* was offered *S. major*, but lowest when offered *T. amphioxeia*. Column **D**: These differences in plastid retention were not wholly responsible for differences in *M. chamaeleon* growth rates over 72-h. Column **E**: However, mixotrophic yield (the number of *M. chamaeleon* cells produced per prey cells ingested) was highest for *S. major*-conditioned cells offered *S. major*, suggesting a greater degree of phototrophy when *M. chamaeleon* has plastids from this species. Different letters on panels C–E indicate statistically significant differences at the $P < 0.05$ level (Tukey's HSD).

Mesodinium chamaeleon exhibits physiological plasticity depending upon prey type, exhibiting greater growth rates, organelle retention, retention efficiency, and mixotrophic yield on *S. major* than on other prey types. Because ingestion rates of *S. major* were not definitively higher than other cryptophytes, it seems that *M. chamaeleon*'s "preference" (i.e. its increased performance) is grounded in its physiology, rather than the behavior of it or its prey. Prey preference may result from either an active sequestration process or a delay in prey digestion when the prey is optimal. Although we did not quantify digestion rates in our study, the minuscule level of observed organelle retention for *G. theta* and *T. amphioxeia* prey, despite high ingestion rates, strongly suggests that certain cryptophyte prey are quickly digested.

Collectively, our data suggest that *M. chamaeleon* may achieve higher, sustained photosynthetic rates when it possesses *S. major* organelle complexes, though this

remains to be confirmed by measurements of photosynthetic rates. Intriguingly, we observed initial increases in total chlorophyll-*a* when *M. chamaeleon* cells were conditioned on *S. major* (Fig. 5), suggesting that some pigment biosynthesis may take place within *M. chamaeleon* cells. Because prey cells are retained as organelle complexes with plastids, nuclei, and all other cellular machinery contained in the same *M. chamaeleon* vacuole (Moestrup et al. 2012), it seems likely that any pigment synthesis or photoacclimation responses (Fig. 5) are mediated by continued functioning of prey machinery, rather than under the direct control of *M. chamaeleon*. In contrast, *M. rubrum*, which appears to maintain a single prey nucleus from which transcripts are targeted to numerous chloroplast-mitochondrion complexes (Johnson et al. 2007; Lasek-Nesnelquist et al. 2015), may have a relatively greater degree of control over its acquired photosynthetic machinery.

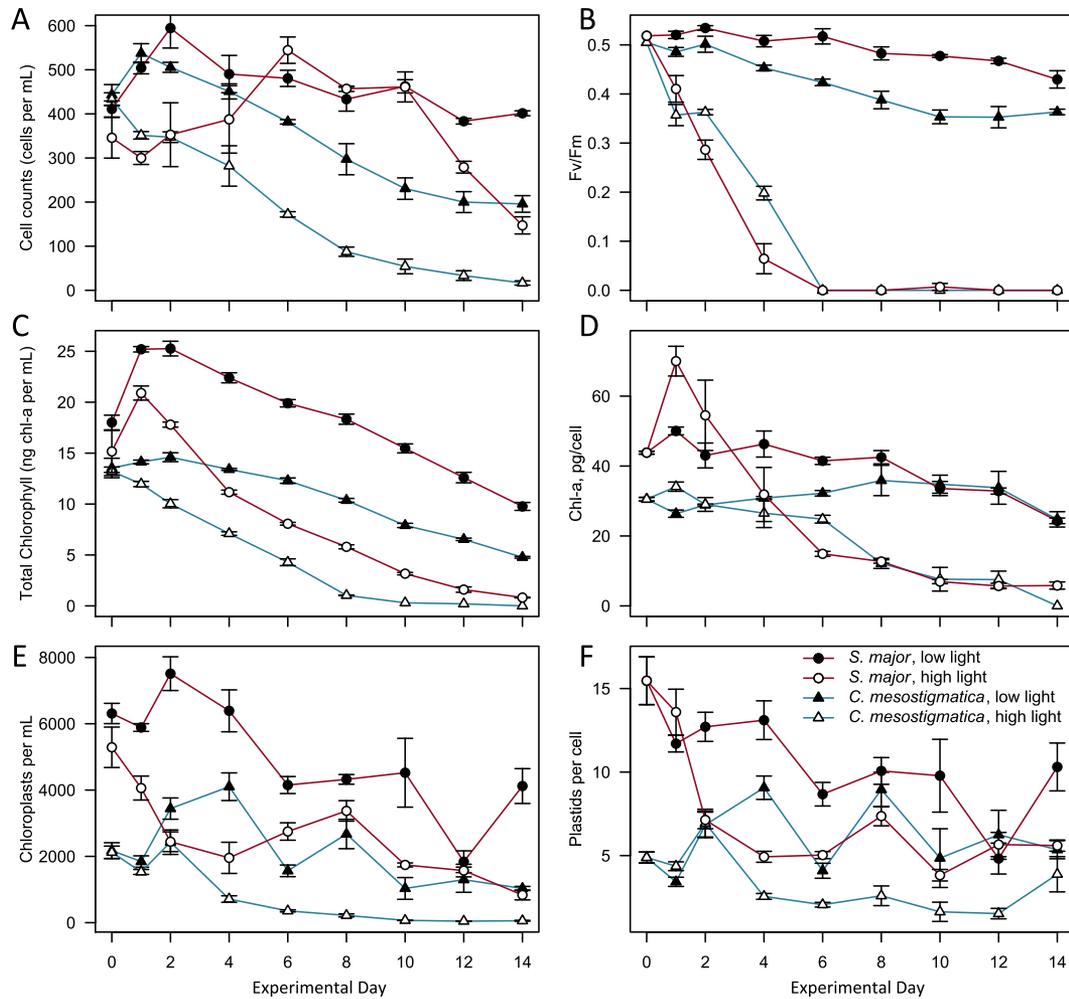


Figure 5 Growth and photophysiology of stressed *Mesodinium chamaeleon*. *M. chamaeleon* was conditioned to either *Chroomonas mesostigmatica* (triangular points, blue lines) or *Storeatula major* (circular points, red lines), and then starved for 14 d under either normal (filled symbols) or high (hollow symbols) light conditions. Cultures moved to high-light environments showed immediate signs of stress, with declining population sizes (panel **A**) and precipitous declines in photosynthetic efficiency (F_v/F_m , panel **B**). Over time, total chlorophyll-*a* (panel **C**) and, as a result, chlorophyll-*a* per cell (panel **D**) declined more rapidly for cultures moved to high light. Plastid retention over time was also lower in high-light conditions (panels **E** and **F**). Tolerance to starvation and light stress differed with prey conditioning, with *S. major*-conditioned cultures generally outperforming those conditioned to *C. mesostigmatica*. For example, *S. major*-conditioned *M. chamaeleon* grew for longer under normal light conditions, and recovered positive growth rates after 2 d at high light (panel **A**), perhaps due to relatively greater retention of prey plastids (panels **E** and **F**). Additionally, data from the first 48 h of the experiment suggest that some chlorophyll-*a* synthesis may have occurred in *S. major*-conditioned cultures (panel **C**).

Prey preference in *M. chamaeleon* stands in stark contrast to studies of *M. rubrum*. First, *M. chamaeleon* is relatively more general than *M. rubrum*, capable of achieving positive growth rates on all species offered in our study, whereas laboratory studies of *M. rubrum* suggest that its prey uptake is confined to a single genus (Hansen et al. 2012; Park et al. 2007; Peltomaa and Johnson 2017), and field studies show that blooms of *M. rubrum* are usually supported by a single plastid type (Johnson et al. 2016). Prey preferences suggest an opportunity for niche partitioning among these acquired phototrophs: *M. chamaeleon* shows weak growth and negligible plastid retention when offered *T. amphioxeia*, the prey species

preferred by *M. rubrum* (Johnson et al. 2016), and *M. rubrum* will not eat (and exhibits negative growth rates when exposed to) *S. major* (Peltomaa and Johnson 2017).

However viable in our laboratory setting, *S. major* is unlikely to be the most common prey of *M. chamaeleon* in the field because it is not known to be associated with oxycline environments where *M. chamaeleon* is found, and it appears to be rare in nature (Johnson et al. 2016 M. D. Johnson, D. Beaudoin, H. V. Moeller and P. E. Hargraves, in prep). Indeed, our original isolate from the Narrow River was blue-green in color, consistent with the observations of Moestrup et al. (2012) and Hargraves (1991), and of Nam et al. (2014) who described *M. coatsi*, a

Table 2. Comparison of *Mesodinium* species grouped by trophic strategy

Species functional group	Viable prey	Maximum ingestion rate (prey cells per <i>Mesodinium</i> per day)	Starvation tolerance	Organelles retained	Photosynthetic rate (pg C per cell per h)	C from photosynthesis	Trophic status
<i>M. pulex/pupula</i>	At least 3 cryptophytes (<i>Guillardia</i> , <i>Teleaulax</i> , and <i>Hemiselmis</i>), 2 dinoflagellates (<i>Gymnodinium</i> , <i>Heterocapsa</i>), and 1 ciliate (<i>Metanophrys</i>) ^{a,b}	20–30 ^a	2 wk, with 0 cell divisions ^a	Transiently, in food vacuoles	0.37–2.32 ^a	< 4% ^a	Heterotrophic
<i>M. chamaeleon/coatsi</i>	At least 5 cryptophyte genera (<i>Stoeatula</i> most preferred, <i>Teleaulax</i> least preferred) ^{c,d}	10–25 ^c	> 2 wk, with 0.5–1 cell division ^c	Slowly degrading complexes containing whole prey cells ^{d,e}	6.3 ^d	0–70% (depending on prey type) ^e	Mixotrophic
<i>M. rubrum/major</i>	1 cryptophyte genus (<i>Teleaulax</i> in temperate strains; <i>Geminigera</i> in polar strain) ^{f,g}	5–10 ^{f,h,i,j}	> 1 mo, with 4 cell divisions ^{i,k}	Prey chloroplast-mitochondrial complexes and, separately, prey nuclei ^{l,m,n}	13–88 ^o	> 95% ⁱ	Predominantly phototrophic

^aTarangkoon and Hansen (2011).^bJakobsen et al. (2006).^cThis study. See also Nam et al. (2014) for a table comparing ultrastructure and behavior.^dMoestrup et al. (2012).^eNam et al. (2014).^fPeltomaa and Johnson (2017).^gJohnson et al. (2016).^hYih et al. (2004).ⁱSmith and Hansen (2007).^jHansen and Fenchel (2006).^kJohnson and Stoecker (2005).^lKim et al. (2017).^mJohnson et al. (2007).ⁿGustafson et al. (2000).^oStoecker et al. (1991).

close relative of *M. chamaeleon*. In our case, collected *M. chamaeleon* contained plastids from a *Hemiselmis* species (M. D. Johnson, D. Beaudoin, H. V. Moeller and P. E. Hargraves, in prep). While methodological constraints prevented us from fully exploring *M. chamaeleon*'s growth response to *H. cryptochromatica* in this study, what data we did collect suggested robust growth rates when conditioned on that species. Previous authors have grown *M. chamaeleon* and *M. coatsi* on *Chroomonas* species (Moestrup et al. 2012; Nam et al. 2014). In our study, while *M. chamaeleon* did retain plastids from *C. mesostigmatica*, it did not achieve the same levels of robust growth as with *S. major*, which may explain differences in the ability to condition *M. chamaeleon* on a single prey species (Moestrup et al. 2012). Intriguingly, Moestrup et al. (2012) report uptake of red plastids from *T. amphioxieia*, which we observed only extremely minimally in our study, suggesting that *M. chamaeleon* of different origins may vary in their prey preferences.

As a mixotrophic acquired phototroph, *M. chamaeleon* occupies a unique ecological niche that may have allowed for its evolution and persistence in the *Mesodinium* genus (Table 2; See also Nam et al. (2014) for a comparison of *Mesodinium* ultrastructure and behavior). Unlike the more derived *M. rubrum* and *major* lineages, *M. chamaeleon* appears to have retained a higher degree of dietary flexibility, likely facilitating its coexistence with specialized predators. And, because it can supplement its energy needs through phototrophy, *M. chamaeleon* may be able to tolerate relatively lower prey environments than the heterotrophs *M. pupula* and *pulex*. Consistent with descriptions of other *M. chamaeleon* (Moestrup et al. 2012) and *M. coatsi* (Nam et al. 2014) cultures as "benthic," we observed a tendency for *M. chamaeleon* cells to "settle" on the bottoms of culture flasks and chambers. This likely also affects their rates of encounter with cryptophyte prey that differ in their swimming behavior: For example, *S. major* and *C. mesostigmatica* tended to aggregate near the bottoms of experimental flasks (M.D. Johnson, pers. observ.). Finally, like *M. rubrum*, *M. chamaeleon*'s acquired phototrophy allows it to give a "second life" to acquired organelles of certain cryptophyte species, contributing their photosynthetic contributions to community production even after predation of the original host. In addition to its contributions to primary production, *M. chamaeleon* may, like *M. rubrum* (Kim et al. 2012; Nielsen et al. 2012) and *M. coatsi* (Kim et al. 2015), serve as a source of plastids to other microplankton, and a channel for transfer of energy to higher trophic levels. Future work on the ecology and evolution of acquired phototrophs within the *Mesodinium* genus and beyond will shed further light on how these mixotrophs persist and contribute to microplanktonic communities in the world's coastal waters.

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AUTHOR CONTRIBUTIONS

MDJ conceived the research. MDJ and HVM designed and carried out the experiments, analyzed the data, and wrote the paper.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1. Growth kinetics of *Mesodinium chamaeleon*.

Figure S2. Growth rates of cryptophyte prey in the absence of *Mesodinium chamaeleon*.

Table S1. Estimates of parameters for Holling Type II Functional Response fits to ingestion rate data.

Table S2. Estimates of parameters for saturating growth rate response to prey availability.