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The balance of autotrophy and heterotrophy during mixotrophic growth of *Karlodinium micrum* (Dinophyceae)

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We studied autotrophic and heterotrophic C metabolism during mixotrophic growth of Karlodinium micrum (Leadbeter et Dodge) Larsen (Dinophyceae) on prey Storeatula major (Cryptophyceae). Our goal was to determine the balance of autotrophy and heterotrophy that supports mixotrophic growth in K. micrum. Assimilation of inorganic ^{14}C and ^{14}C-labeled prey was used to separate the quantity and quality (i.e., lipid, polysaccharide and protein) of C obtained by autotrophy and heterotrophy, respectively. Growth rates (μ) of mixotrophic K. micrum were 0.52–0.75 div.$\cdot$day^{-1}, equal to or greater than the maximum autotrophic growth rate (0.55 div.$\cdot$day^{-1}) of K. micrum. Autotrophy represented 27–69% of gross C uptake during mixotrophic growth. Cellular photosynthetic performance (PP^{cell}, pg C cell$^{-1}\cdot$day^{-1}) was 24–52% lower during mixotrophic growth than during autotrophic growth of K. micrum. Mixotrophic K. micrum assimilated 16% less photosynthate as protein compared to autotrophic K. micrum, while protein was the major net assimilation product (52%) from ingested prey C. Growth efficiency (%GE) of mixotrophic cultures, based on both autotrophic and heterotrophic C sources, averaged 36 ± 2.9%, slightly lower than the 40–50% GE typical of purely autotrophic K. micrum, but higher C gains associated with heterotrophic feeding more than compensated for the decrease in %GE in mixotrophic K. micrum. We conclude that mixotrophic growth of K. micrum is dominated by heterotrophic metabolism, although photosynthesis continues at a lowered rate. This is consistent with a shift toward secondary production in plankton assemblages dominated by mixotrophically growing K. micrum.

INTRODUCTION

Planktonic protists exhibit a variety of nutritional strategies, including strict autotrophy and strict heterotrophy, and strategies that combine these modes that are collectively referred to as mixotrophy (Jones, 1994). Mixotrophic organisms have been described in freshwater, estuarine and marine ecosystems where they play an important role in plankton dynamics (Stickney *et al.*, 2000; Tittel *et al.*, 2003). A broad range of mixotrophic behaviors have been described for dinoflagellates (Schnepf and Elbrächter, 1992; Stoecker, 1999), but the physiological bases of mixotrophic growth are speculative for most species (Hansen *et al.*, 2000; Skovgaard *et al.*, 2000). Here, we examined the balance of autotrophy and heterotrophy that supports mixotrophic growth of the dinoflagellate, *Karlodinium micrum*.

Stoecker (Stoecker, 1998) categorized mixotrophic nutrition using three conceptual models based on the balance of autotrophy and heterotrophy, functional responses of grazing to light and nutrients and possible benefits of mixotrophic nutrition to the organisms. An 'ideal' mixotroph (Model I) is equally adept as an autotroph or a heterotroph; phagotrophic 'algae' (Model II) are primarily photosynthetic but ingest prey under

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certain conditions; photosynthetic 'protozoa' (Model III) are primarily phagotrophic but photosynthesize in certain conditions using either their own plastids or 'kleptochloroplasts'. *Karlodinium micrum* (syn. *Gyrodinium galatheanum*) best fits Model II (Stoecker, 1998) in a group with other photosynthetic dinoflagellates, including *Prorocentrum minimum* (Stoecker *et al.*, 1997), *Heterocapsa triquetra* (Legrand *et al.*, 1998) and *Ceratium furca* (Bockstahler and Coats, 1993a, 1993b; Smalley and Coats, 2002). A major unresolved issue for mixotrophs categorized as Model II is the physiological role of feeding. Stoecker (Stoecker, 1998) suggested three categories: (i) feeding as a means to obtain C, (ii) feeding as a source of inorganic nutrients (N and P) and (iii) feeding to supply other growth factors. Experimental determinations of these physiological roles of feeding in Model II mixotrophs are lacking.

Karlodinium micrum is a nonthecate, photosynthetic dinoflagellate capable of ingesting a variety of small prey by phagocytosis (Li *et al.*, 1996, 1999). Mixotrophic growth of *K. micrum* on prey *S. major* (Cryptophyceae) yields growth rates that are 2- to 3-fold higher than the maximum autotrophic growth rate, although growth and feeding do not occur in the dark (Li *et al.*, 1999). Feeding is stimulated either by inorganic nutrient limitation or by high light (Li *et al.*, 2000a). These observations suggest that phagotrophy supplies nutrients to support autotrophic growth, although feeding in nutrient-saturated cultures indicates that phagotrophy may serve other purposes, such as the acquisition of C or essential micronutrients (Li *et al.*, 2000a). We know that the maximum growth rate of mixotrophic *K. micrum* exceeds that of autotrophic *K. micrum* (Li *et al.*, 1999), but the balance of autotrophy and heterotrophy during mixotrophic growth remains unknown.

The approach we used to determine the balance of autotrophy and heterotrophy in mixotrophic *K. micrum* was based on the following simplified expression of unicellular growth:

$$\text{Growth rate} = \frac{\overbrace{(\text{PP}^{\text{cell}} \times \%\text{GE})}^{\text{[autotrophy]}} + \overbrace{(\text{HP}^{\text{cell}} \times \%\text{GE})}^{\text{[hetrotrophy]}}}{\text{C cell}^{-1}} \quad (1)$$

where autotrophy is described as the product of photosynthetic performance (PP^{cell}, pg C·cell^{-1}·day^{-1}) and autotrophic gross growth efficiency (%GE), describing the percentage of fixed C that is incorporated into new biomass. Heterotrophy is described as the product of C ingested per predator [cellular heterotrophic performance (HP^{cell}), pg C·cell^{-1}·day^{-1}] and the percentage of ingested C that is incorporated into new biomass (%GE). Adolf *et al.* (Adolf *et al.*, 2003) showed that autotrophic growth of *K. micrum* consisted of relatively low rates of C-specific light absorption leading to low PP^{cell} and low %GE typical of dinoflagellates (Falkowski *et al.*, 1985; Tang, 1996). The basis of low growth rates in heterotrophic dinoflagellates (compared to ciliates) varies among species examined (Hansen, 1992; Strom and Morello, 1998). Framing our experiments within the context of equation (1) allows us to explicitly address issues of C acquisition versus growth efficiency in comparing autotrophic and mixotrophic growth of *K. micrum*.

Strict interpretation of equation (1) requires that the quality (i.e., % protein) of autotrophic and heterotrophic C assimilated by mixotrophic *K. micrum* be the same. If the quality of autotrophic and heterotrophic C differs, then the efficiency at which each C source contributes to growth may differ. In autotrophic organisms, for instance, accumulation of alternative photosynthetic end products, that is, lipid and polysaccharides, rather than protein that is essential for cellular growth (Morris, 1981), will result in lower growth rates for equivalent amounts of C fixation. Thus, evaluation of the qualitative and quantitative nature of C assimilated by the cell is important to address the balance of autotrophy and heterotrophy during mixotrophic growth (Putt, 1990).

The recent recognition that mixotrophic nutrition is common among dinoflagellates is important to biological oceanographers working in coastal areas, where dinoflagellates play important ecological roles both as primary producers (Kirk, 1994) and as causative agents of harmful algal blooms (HABs) (Hallegraeff, 1993). Heterotrophic and mixotrophic dinoflagellates can be a significant source of secondary production, and their grazing can impact the biomass of primary producers (Hansen, 1992; Bockstahler and Coats, 1993a, 1993b; Li *et al.*, 1996, 2000b; Stoecker *et al.*, 1997; Smalley and Coats, 2002; Johnson *et al.*, 2003; Jeong *et al.*, 2005). Mixotrophic nutritional strategies observed in autotrophic (plastid-bearing) and heterotrophic (nonplastidic) dinoflagellates raise the important ecological question, What is the trophic balance of carbon (C) flow through these populations? Here we address this question by examining the physiological bases of mixotrophic growth in *K. micrum* with goals to (i) quantify the effect of feeding on cellular photosynthetic rates, (ii) determine the major assimilation end products of photosynthesis during autotrophic and mixotrophic growth and the end products assimilated from ingested prey, and (iii) compare growth efficiency during autotrophic and mixotrophic growth.

METHODS

Algal cultures, biochemical analyses

Karlodinium micrum (strain GE, 10–15 μm) (Dinophyceae, CCMP 1974) was isolated as a clonal culture from the mesohaline portion of Chesapeake Bay in 1995 by Dr. Aishao Li (Li *et al.*, 1996). A recent study by Bergholtz *et al.* (Bergholtz *et al.*, 2005) has suggested that *K. micrum* is synonymous with *Gymnodinium veneficum* Ballantine (strain Plymouth 101), isolated in 1950 by M. Parke, and that both strains should be renamed *Karlodinium veneficum*. We will continue to use the name *K. micrum* here to be consistent with previous work on mixotrophic nutrition in this species. *Storeatula major* (strain g, 4–9 μm) (Cryptophyceae) was isolated from Chesapeake Bay in 1990 by Dr Alan Lewitus (National Oceanic and Atmospheric Administration). Identification of the cryptophyte was accomplished through the kind assistance of Dr P. Kugrens (Colorado State University). Cultures of both the dinoflagellate and the cryptophyte contained heterotrophic bacteria, although preliminary experiments showed no uptake of fluorescently labeled bacteria by *K. micrum* (J. E. Adolf, unpublished data). Further, autotrophic growth of bacterized and axenic *K. micrum* was similar, suggesting no contribution of bacteria to *K. micrum* nutrition (J. E. Adolf, unpublished data). Cell counts to determine growth rates and frequency of *K. micrum* cells containing ingested cryptophyte prey were routinely made by filtering 3–5 mL culture aliquots fixed in 1% glutaraldehyde onto 25-mm blackened membrane filters (0.8 μm pore size, Poretics) and by counting at 400× on an epifluorescence microscope (Nikon Eclipse E800) using filter set EF-4 B-2A (exciter filter 450–490 nm, dichromatic beam splitter 500 nm, barrier filter 515 nm). Ingested cryptophytes were counted as orange fluorescent inclusions (OFIs) (Li *et al.*, 1996) that were clearly visible inside the red fluorescent dinoflagellate cells. OFIs were enumerated in the first 100–150 cells encountered on a slide. Chlorophyll *a* (Chl *a*) was determined by high-performance liquid chromatography (HPLC) (see below) in all cultures, with the exception of MIXO 1 for which Chl *a* was measured fluorometrically (Turner Designs model 10-AU, Turner Designs, Sunnyvale, CA, USA) in 90% acetone extracts. Comparison of Chl *a* determined by HPLC and Turner Designs fluorometer produced the regression, Chl *a* (HPLC) = 0.418 + 1.001 × Chl *a* (Turner fluorometer), $n = 424$, $r^2 = 0.94$. Particulate C and N were measured on samples collected on pre-combusted Whatman GF/C filters with a Leeman Lab 440 HA elemental analyzer.

Semicontinuous batch culture of mixotrophic *K. micrum*

Commonly used abbreviations are listed in Table I, and experimental designations and descriptions are listed in Table II. Semicontinuous batch cultures (SBCs) were used (MIXO 1 and MIXO 3) to maintain steady-state feeding and growth rates over the course of experiments that lasted ~1 week. For all SBCs, growth irradiance (E_o) was 10.8 mol photons·m^{-2}·day^{-1} (250 μmol photons·m^{-2}·s^{-1}, 12 : 12 L : D), temperature was 20°C and salinity was 15. Duplicate SBCs of mixotrophic (MIXO 1 and MIXO 3) *K. micrum* and prey *S. major* were maintained in 2.8-L Fernbach flasks in f/2-Si (Guillard, 1975) medium with P at f/20 concentration (883 μM NO_3 and 3.6 μM PO_4). AUTO 1 (Table I) was maintained as an autotrophically grown SBC control for the MIXO 1 experiment. AUTO 1 was diluted 50% every 2 days, whereas MIXO 1 and prey *S. major* were diluted 40 and 60% daily, respectively. All dilutions took place immediately preceding 'lights on' in the incubation chamber. MIXO 1 was fed daily from the SBC of *S. major* at the same time that cultures were diluted. Growth rate for each SBC was calculated from cell counts made immediately following dilution and then 24 h later and was averaged over the 6-day period during which SBCs were maintained. The average prey : predator ratio at the time of feeding was 1.4 (±0.20) over the 6-day MIXO 1 experiment and 0.5 (±0.12) over the 4-day MIXO 3

Table I: Commonly used abbreviations in the text

Abbreviations	Definition
PP^{cell}	Photosynthetic performance (pg C·$cell^{-1}$·day^{-1})
HP^{cell}	Heterotrophic performance (pg C·$cell^{-1}$·day^{-1})
%GE	Growth efficiency [100 × (C production/C intake)]
OFI	Orange fluorescent inclusion
SBC	Semicontinuous batch culture
I^{cell}	Ingestion rate [prey (predator × day)$^{-1}$]
F	Clearance rate [μL (predator × h)$^{-1}$]
PE	Photosynthesis–irradiance
E_o	Incident irradiance [mol photons (m^2 × day)$^{-1}$]
PP	Photosynthetic pigment
NPP	Nonphotosynthetic pigment
P_m^{cell}	Maximum cellular photosynthesis rate from PE curve [pg C (cell × h)$^{-1}$]
α^{cell}	Cellular light-limited photosynthetic efficiency [pg C (cell × h)$^{-1}$] [μmol photons (m^2 × s)$^{-1}$]$^{-1}$
$\Phi_{C(max.)}$	Maximum quantum efficiency of photosynthesis (mol C fixed/mol photons absorbed)

Table II: Summary of experiments

Experiment	Notes
MIXO 1	Seven-day SBC of mixotrophic *Karlodinium micrum* fed *Storeatula major* once each day, f/2-Si (P/20)
MIXO 2	Mixotrophic *K. micrum* pulse-fed one time with *S. major*, f/2-Si (P/20)
MIXO 3	Four-day SBC of mixotrophic *K. micrum* fed *S. major* once each day, f/2-Si (P/20)
AUTO 1	SBC of autotrophic *K. micrum* run simultaneously with MIXO 1, f/2-Si (P/20)
AUTO 2	Autotrophic control culture run simultaneously with MIXO 2, f/2-Si (P/20)
AUTO	Nutrient-replete autotrophic *K. micrum* cultures, f/2-Si (Adolf *et al.*, 2003)
AUTO-N	Stationary phase N-deprived autotrophic *K. micrum*, f/2-Si (N/20)
AUTO-P	Stationary phase P-deprived autotrophic *K. micrum*, f/2-Si (P/20)

All experiments were conducted at E_o 250 μmol photons·m^{-2}·s^{-1} (12 : 12 L : D), salinity 15 and temperature 20°C. f/2-Si is the growth medium with indicated changes. SBC, semicontinuous batch culture. MIXO 1 and MIXO 3 were conducted separately and with different food supply rates. AUTO refers to nutrient-replete autotrophic cultures presented in Adolf *et al.* (Adolf *et al.*, 2003). Further details of the experiments are given in *Methods*.

experiment. We computed clearance (F) and grazing rate (I) using the equations of Frost (Frost, 1972) from changes in prey cell densities in MIXO 1 and in an ungrazed prey control grown in MIXO 1 filtrate (GF/F filtrate of MIXO 1 culture), during a 24-h period in which cell samples were taken at 4-h intervals throughout the light period when most grazing occurred (J. E. Adolf, personal observation). HP^{cell} (pg C·cell^{-1}·day^{-1}) was calculated according to

$$HP^{cell} = I^{cell} \times (\text{prey cell C quota}) \quad (2)$$

where I^{cell} has units prey (predator × day)$^{-1}$ and using an average mid-day 'prey cell C quota' value of 57 pg C·cell^{-1} measured for *S. major*. Photosynthesis–irradiance (PE) curve (see below) measurements and samples for bio-optical and biochemical analyses were taken within 2 h of 'lights on'.

^{14}C tracing of autotrophic and heterotrophic assimilation end products

A generalized diagram of the experimental setup used to measure the assimilation of inorganic ^{14}C and ^{14}C-labeled prey by mixotrophic *K. micrum* in MIXO 2 and MIXO 3 experiments is shown in Fig. 1. MIXO 2

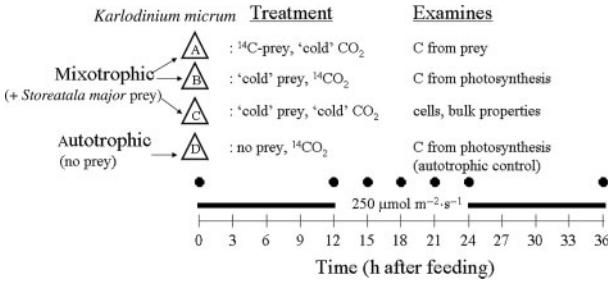

Fig. 1. Schematic representation of the experimental design used to separately measure autotrophic and heterotrophic C metabolism in mixotrophic *Karlodinium micrum* in the MIXO 2 (pulse-fed) and MIXO 3 (semicontinuous batch culture) experiments. Three parallel mixotrophic cultures and one autotrophic control were run. Mixotrophic cultures received either ^{14}C-labeled prey (A), $^{14}CO_2$ and unlabeled prey (B) or no radio-label (C). Autotrophic controls (D) received $^{14}CO_2$. Feeding was designed such that the prey introduced at the start of the dark phase was significantly reduced by ingestion within 12 h.

and MIXO 3 used duplicate cultures grown in medium consisting of f/2-Si with P at f/20 concentration. E_o was 10.8 mol photons·m^{-2}·day^{-1} (250 μmol photons·m^{-2}·s^{-1}, 12 : 12 L : D), temperature was 20°C and salinity was 15. MIXO 2 was a 'pulse-fed' experiment wherein physiological measurements were made over the first L : D cycle following feeding of an autotrophic (AUTO 2) culture. MIXO 3 was maintained as a mixotrophic SBC for 3 days before the experimental procedures outlined in Fig. 1 were started at t = 72 h. For each experiment, a late-exponential phase autotrophic culture of *K. micrum* was split into four aliquots at the beginning of the dark period (A–D in Fig. 1). One aliquot was not fed (D in Fig. 1; autotrophic control), and of the other three, one was immediately fed with rinsed, ^{14}C-labeled prey (see below) and used to measure heterotrophic C metabolism (A in Fig. 1), while the remaining two aliquots were fed with rinsed, unlabeled prey (see below). The three fed cultures received the same amount of prey. Of the two cultures fed with unlabeled prey, one culture (B in Fig. 1) was spiked with $NaH^{14}CO_3^-$ (ICN, Irvine, CA, USA) on the following morning and used to measure photosynthetic C uptake and metabolism over the subsequent 24 h encompassing one L : D cycle (see below). The other culture (C in Fig. 1) received no radioactive tracer and was used for cell samples and to determine PE curves ~2 h after 'lights on', corresponding to t = 14 h (Fig. 2B, MIXO 2) and t = 86 h (Fig. 2C, MIXO 3).

Rinsed ^{14}C-labeled prey and rinsed unlabeled prey were prepared from 900 mL *S. major* cultures that had been started from a small inoculum (1 : 100 vol.) 6 days prior to use. The ^{14}C-labeled prey culture was spiked with 0.5 mL of $NaH^{14}CO_3^-$ to give an activity of 8552 DPM mL^{-1} and grown next to the unlabeled prey to

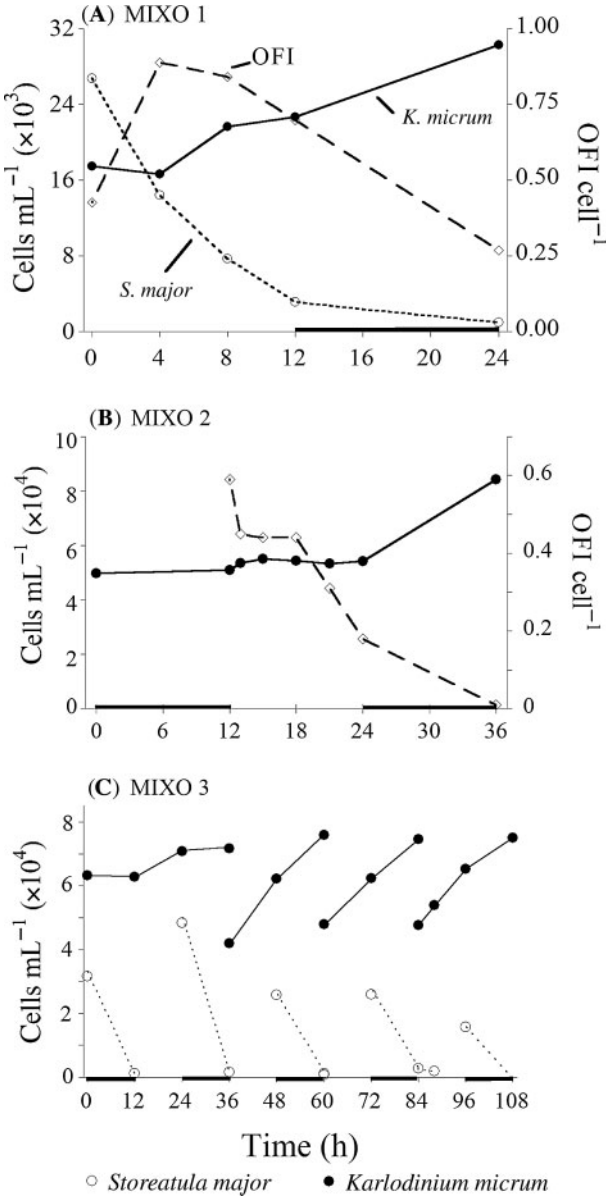

Fig. 2. Cell dynamics in the three mixotrophy experiments. (**A**) One representative day from the MIXO 1 experiment that was maintained as a semicontinuous batch culture for 1 week. (**B**) and (**C**): The full extent of experiments MIXO 2 and MIXO 3, respectively. OFI, orange fluorescent inclusion, indicative of ingested *Storeatula major* inside *Karlodinium micrum*. OFIs are omitted from panel **C** for clarity. The white diamonds in panel B refer to OFIs as for panel A.

check for adverse effects of prolonged growth in the presence of ^{14}C. The two cultures achieved similar biomass by the end of the growth period. Before feeding, each prey culture was centrifuged for 5–10 min at room temperature (IEC Clinical Centrifuge, 5000×*g*) in acid-rinsed 50-mL polypropylene tubes to remove nonparticulate ^{14}C. The supernatant was decanted from the pelleted cells, and the tube was refilled with culture medium to resuspend the cells. This procedure was repeated two more times to yield rinsed prey cells. *In vivo* fluorescence was used to check for equal recovery of cells between centrifuging and to assure that the biomasses in ^{14}C-labeled and unlabeled prey were equal following the rinsing procedure. An aliquot of the unlabeled prey was taken to determine prey cells mL^{-1}, and an aliquot of the labeled prey was used to determine DPM mL^{-1}. These two measurements were used to calculate DPM prey cell^{-1} that was converted to DPM prey C^{-1} using the value 68 pg C•prey cell^{-1} measured in the unlabeled prey culture at the time of feeding.

Several autotrophically grown *K. micrum* cultures were used as controls in this study. 'AUTO' cultures (Table I) refers to batch cultures (Adolf *et al.*, 2003) at the same growth irradiance used for mixotrophy experiments (250 μmol photons•m^{-2}•s^{-1}, 12 : 12 L : D). 'AUTO 1' was grown in SBC conditions in the same experiment as 'MIXO 1'. Duplicate P- or N-deprived cultures ('AUTO-P', 'AUTO-N') were produced by inoculating a small volume of exponentially growing cells into f/2-Si medium that had either N or P at f/20 concentration (i.e., one-tenth concentration of f/2). Cultures were monitored daily until stationary phase was reached. Measurements of cellular nutrient ratios (mol) in N-deprived (C : N = 8.2) and P-deprived (C : P = 203.5) cultures confirmed intracellular nutrient deficiency compared to nutrient-replete autotrophic cultures (C : N = 5.0, C : P = 37.9).

^{14}C measurements of photosynthesis

PE relationships were determined at 20°C in temperature-regulated 'photosynthetrons', essentially as described by Lewis and Smith (Lewis and Smith, 1983). Detailed methods are presented in Adolf *et al.* (Adolf *et al.*, 2003). To measure PP^{cell}, aliquots (~300 mL) of parent cultures were placed into 1-L screw-cap culture flasks and spiked with Na$H^{14}CO_3^-$ to achieve an activity of 1.6 × 10^5 DPM mL^{-1} (±10%) at the start of the light period. Spiked aliquots were incubated adjacent to parent cultures in growth chambers. Samples were taken both at the end of the light period and at the end of the dark period to represent gross and net C fixation, respectively. At each time point, duplicate samples were collected on Whatman GF/F filters, followed by a rinse of the filters with culture medium. One of each pair of filters was gently acidified with 0.01 N HCl and placed directly into a vial containing scintillation cocktail (Packard Ultima-Gold AP, Packard Bioscience, Meriden, CT, USA) to measure TOTAL ^{14}C uptake. The other filter was folded (sample on inside), placed into a 1.7-mL microcentrifuge tube with 1 mL dH_2O and stored frozen

(–20°C) until processing. We analyzed these samples for ^{14}C incorporation into photosynthetic end products using a serial extraction technique similar to that described by Morris *et al.* (Morris *et al.*, 1974). Specific solvents and extraction methods followed Lancelot (Lancelot, 1984), except that separation of soluble from insoluble material was accomplished by microcentrifugation instead of filtration. Briefly, frozen filters plus water in microcentrifuge tubes were completely lyophilized and then extracted with 1 mL $CHCl_3$ at 4°C for 1 h. Tubes were then centrifuged (Eppendorf microcentrifuge, 5 min, room temperature), and the supernatants were transferred to scintillation vials containing 4.5 mL Packard Ultima-Gold AP scintillation cocktail that accommodated the sample : cocktail ratios required by the method. Tubes were refilled with 1 mL $CHCl_3$, centrifuged and the supernatants transferred to the same scintillation vials. This procedure was repeated following 1-h incubation in hot (85–90°C) anhydrous ethanol (EtOH) and then in hot (85–90°C) 5% trichloracetic acid (TCA). A third rinse with TCA improved recovery of TCA-soluble material. The filter remaining in the tube after the final solvent extraction was placed into a vial containing scintillation cocktail. Summed DPM recovered using this method averaged 97% [coefficient of variation (CV) = 6%] of the DPM in the unfractionated controls. The fraction of ^{14}C in any biochemical class relative to the sum of ^{14}C counts collected in all fractions was calculated to improve the precision of the technique (Cuhel and Lean, 1987). Following Lancelot (Lancelot, 1984), $CHCl_3$-soluble material is referred to as LIPID, hot EtOH-soluble material as low-molecular-weight metabolites (LMW), hot 5% TCA-soluble material as polysaccharides (PSACCH) and hot 5% TCA-insoluble material as protein (PROT). $\%\Delta^{14}C$ (overnight) was calculated as

$$\%\Delta^{14}C = \frac{(DPM^{D} - DPM^{L})}{DPM^{L}} \quad (3)$$

where DPM^{L} and DPM^{D} Are the activities recovered on the unfractionated controls taken at the end of the light and dark periods, respectively.

HPLC and absorption reconstruction

Pigment analyses were performed using HPLC according to the methods of Van Heukelem and Thomas (Van Heukelem and Thomas, 2001) at the Analytical Services facility of Horn Point Laboratory (Cambridge, MD, USA). Samples for HPLC were collected on Whatman GF/F filters, folded with the sample on the inside, flash frozen in liquid N_2 and stored at –80°C until analysis. Identified pigments were classified as photosynthetic pigments [PPs: Chl *a*, Chl *c2*, Chl *c3*, fucoxanthin, 19′-butanoyloxy fucoxanthin (but. fucoxanthin), 19′-hexanoyloxy fucoxanthin (hex. fucoxanthin), gyroxanthin diester 1 and 2] and nonphotosynthetic pigments [NPPs: diadinoxanthin (DD), diatoxanthin (DT), carotenes, including the Chl *a* degradation products pheophorbide and chlorophyllide]. Total particulate absorption spectra of the cultures were determined from samples collected on Whatman GF/F filters according to the procedure of Kishino *et al.* (Kishino *et al.*, 1985). Absorbance spectra (m^{-1}) were reconstructed from HPLC pigment data according to Bidigare *et al.* (Bidigare *et al.*, 1990) to determine the ratio of absorbance with and without NPP ($a^{PPs} : a^{TOT}$). The equations of Dubinsky *et al.* (Dubinsky *et al.*, 1986) and Kirk (Kirk, 1994) were used to weight absorption spectra for the output of different light sources. Further details on these methods are given in Adolf *et al.* (Adolf *et al.*, 2003).

To determine the contribution of Chl *a* from uningested prey to total Chl *a* measured in mixotrophic cultures, prey Chl *a* was calculated based on microscopic cell counts of uningested prey and a value of 1.1 pg Chl *a*·prey $cell^{-1}$. This calculation resulted in determinations of uningested prey Chl *a* as 1.8, 0.2 and 3.8% of total Chl *a* in MIXO 1, MIXO 2 and MIXO 3, respectively.

RESULTS

Semicontinuous and pulse-feeding techniques proved effective to grow *K. micrum* for physiological experiments (Fig. 2A–C). Prey were rapidly ingested and visible as OFI inside predator cells of *K. micrum* in the feeding conditions we used. OFIs appeared rapidly as prey were grazed and disappeared due to digestion and dilution by cell division (Fig. 2A and B).

PP^{cell} ranged from 29.6 to 61.3 pg C·$cell^{-1}$·day^{-1} among autotrophic and mixotrophic *K. micrum*, with highest values in AUTO (Table III). Grazing cultures had clearance rates (*F*) between 2.7 and 8.9 μL·$predator^{-1}$·h^{-1}, with the lowest value corresponding to the lowest ingestion rate, *I*. Ingestion rates among mixotrophic cultures varied 6-fold and accounted for HP^{cell} of 21.5–96.3 pg C·$cell^{-1}$·day^{-1} (Table III). In the experiments presented here, ingestion rates simply reflected the food supply rate. Division rates of mixotrophic *K. micrum* were equal to or greater than those observed in the autotrophic *K. micrum* (Table III). Net carbon production (NET, Table III), calculated from observed division rates and cellular C quotas, was similar in AUTO, MIXO 2 and MIXO 3 treatments, despite differences of PP^{cell}. In contrast, the highest value of NET was observed for MIXO 1 where HP^{cell} was also

Table III: C-budget summaries for autotrophic (AUTO) and mixotrophic (MIXO 1–3) Karlodinium micrum cultures used in this study

	PP	F	I	HP	SUM	μ (div d^{-1})	NET	%GGE
AUTO	61.3 (0.73)	—	—	—	61.3 (0.73)	0.55 (0.021)	28.9 (1.21)	47 (2.1)
MIXO 1	29.6 (4.02)	7.5 (0.40)	1.7 (0.19)	96.3 (11.02)	125.9 (11.73)	0.75 (0.081)	42.3 (4.63)	34 (4.8)
MIXO 2	37.6 (3.07)	8.9 (1.26)	0.7 (0.02)	49.4 (1.05)	87.0 (3.25)	0.57 (0.150)	30.0 (1.26)	34 (1.9)
MIXO 3	46.4 (1.38)	2.7 (0.23)	0.3 (0.02)	21.5 (1.09)	67.9 (1.76)	0.52 (0.070)	26.8 (3.66)	39 (5.5)

PP, photosynthetic performance (pg C·cell^{-1}·day^{-1}); *F*, clearance rate (μL·predator^{-1}·h^{-1}); *I*, ingestion rate (prey predator^{-1}·day^{-1}); HP, heterotrophic performance (pg C ingested·predator^{-1}·day^{-1}); SUM = PP + HP; NET, net C production (pg C day^{-1}) based on the culture growth rate (μ, div. ·day^{-1}) and a C quota value of 62 pg cell^{-1}; %GE (growth efficiency) = 100 × (NET/SUM). Values in parentheses are SDs.

highest. %GE, based on the sum of autotrophic and heterotrophic C gain, was 34–47%, similar among the treatments we used despite their different nutritional status.

PP^{cell} and PE curve parameters corresponded to lower photosynthetic capacity of *K. micrum* in mixotrophic growth compared to nutrient-replete autotrophic growth, a reduction that reflected cellular Chl *a* content. PP^{cell} was negatively correlated with HP^{cell} in simple linear regression (Fig. 3). P_m^{cell} (Fig. 4A) and α^{cell} (Fig. 4B) were both lower in mixotrophic *K. micrum*, except for MIXO 2 wherein an autotrophic culture was fed cryptophyte prey and the PE relationship was measured <15 h later. Cellular PE parameters (Fig. 4A and B) in experiments MIXO 1 and MIXO 3 were approximately twice those in AUTO-P, a P-limited batch culture, although Chl *a* was similar. MIXO 1 and MIXO 3 had higher cellular PE parameters and Chl *a* than those measured in AUTO-N, a N-limited batch culture. P_m^{chl} and α^{chl} were less variable among treatments and were not significantly related to cellular Chl *a* content (data not shown). P_m^{chl} (g C·g Chl a^{-1}·h^{-1}) and α^{chl} (g C·g Chl a^{-1}·h^{-1}) (μmol photons·m^{-2}·s^{-1})$^{-1}$ averaged 3.5 (SD = 0.33) and 0.014 (SD = 0.0016), respectively, in all cultures excluding nutrient-deprived autotrophic cultures (AUTO-N and AUTO-P), where P_m^{chl} and α^{chl} averaged 2.2 (SD = 0.46) and 0.009 (SD = 0.0014).

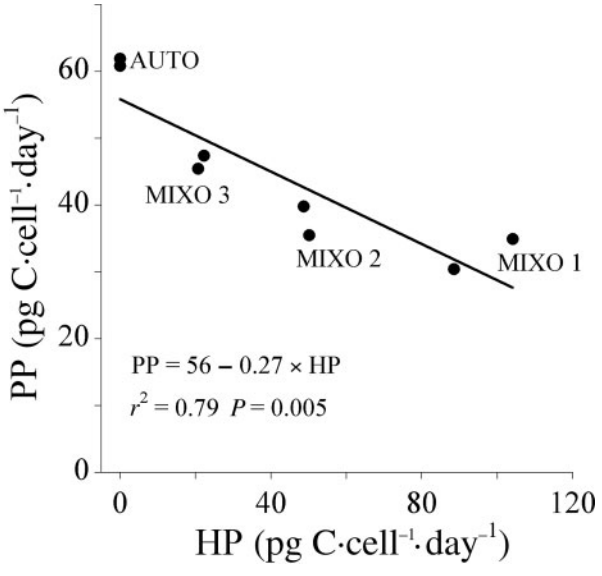

Fig. 3. The relationship between photosynthetic performance (PP^{cell} over the light period, pg C·cell^{-1}·day^{-1}) and heterotrophic performance (HP^{cell}, pg C·cell^{-1}·day^{-1}) in *Karlodinium micrum*.

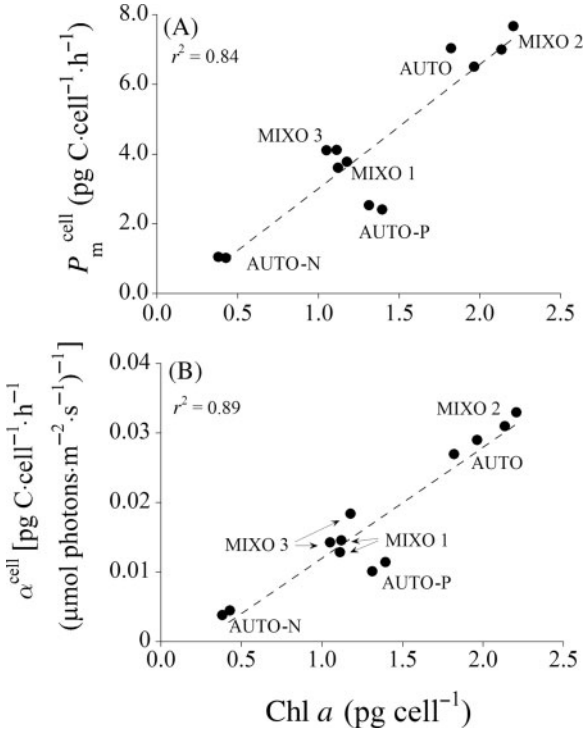

Fig. 4. The relationship between photosynthesis–irradiance (PE) parameters and Chl *a* cell^{-1} in autotrophic and mixotrophic *Karlodinium micrum*. (**A**) P_m^{cell} is the light-saturated photosynthetic rate normalized to cell number. (**B**) α^{cell} is the light-limited slope of the PE curve normalized to cell numbers.

Cellular photopigment quotas in mixotrophic and nutrient-deprived *K. micrum* differed from expected values established for nutrient-replete autotrophic *K. micrum* cultures (Fig. 5, Table IV). In autotrophic *K. micrum*, all photopigments, except DD + DT, but. fucoxanthin and hex. fucoxanthin, showed a significant negative relationship with growth irradiance (Fig. 5). Cellular photopigment quotas from mixotrophy and nutrient starvation experiments were compared to cellular nutrient quotas measured in autotrophic cultures grown at the same growth irradiance (10.8 mol photons$\cdot m^{-2}\cdot day^{-1}$, 12 : 12 L : D). All cellular photopigment quotas in mixotrophic cultures were significantly lower ($P < 0.05$) than those measured in autotrophic *K. micrum*. Cellular photopigment quotas, except hex. fucoxanthin, but. fucoxanthin and DD + DT, were significantly lower in nutrient-deprived autotrophic *K. micrum* than in nutrient-replete autotrophic *K. micrum* (Table IV).

Representative pie diagrams from MIXO 1 show an accumulation of NPP in mixotrophic *K. micrum* on % weight (Fig. 6A and B) and % absorption (Fig. 6C and D) bases. In all treatments, Chl *a* was the dominant pigment by weight and absorption. Likewise, fucoxanthin was the dominant accessory pigment. The increased proportion of NPP in mixotrophic *K. micrum* was the result of accumulated pheophorbide, alloxanthin and β-carotene (Fig. 6B and D). The effect of accumulated NPP on photosynthesis would be mediated through a decrease in the ratio of absorption by photosynthetically active pigments (PPs) to TOTAL pigments. Figure 7 shows a statistically significant, positive relationship between PPs : TOTAL absorption and $\Phi_{C(max.)}$ for *K. micrum* cultures spanning a range of nutritional conditions, suggesting that changes in composition of the pigment system of *K. micrum* accompany changes in the efficiency at which *K. micrum* uses absorbed light to fix C [$\Phi_{C(max.)}$].

Figure 8 shows the time course of C assimilation into macromolecular fractions in an autotrophic culture (AUTO 2) and MIXO 2, which had been started by feeding an aliquot of AUTO 2 at the beginning of the previous dark period (0 h, not shown on graph). Changes of photosynthetic C assimilation were observed within 24 h of introducing food to autotrophically grown *K. micrum* (Fig. 8). At the end of the light period, the most pronounced changes were reduced assimilation of polysaccharide C in mixotrophic cultures (24 h, Fig. 8C). By the end of the dark period, AUTO 2 and MIXO 2 had similar polysaccharide C levels (36 h, Fig. 8C), but MIXO 2 contained lower levels of protein C than AUTO 2 (36 h, Fig. 8D). Tracing of C assimilation from ingested prey in the same experiment (MIXO 2) is shown in Fig. 9. Comparison of ^{14}C-labeled organic material in the culture between $t = 0$ h (when ^{14}C was initially added as evenly labeled prey cells) and $t = 12$ h (when all prey cells had been ingested) indicated assimilation of ~67% of ingested C, most of which was assimilated into protein (Fig. 9). Combined results of ^{14}C tracing experiments for MIXO 2 and MIXO 3 showed that allocation of photosynthetically assimilated C to protein was significantly lower in mixotrophic *K. micrum* compared to autotrophic *K. micrum* (Table V). Reduced allocation of photosynthetic ^{14}C to protein in mixotrophic *K. micrum* was compensated by increased allocation to lipid and polysaccharide. Allocation of ^{14}C assimilated by heterotrophy, at a time point 36 h post-feeding, was predominantly protein and reflected the distribution of ^{14}C in the prey it was fed (Table V).

DISCUSSION

Previous studies of physiological adaptations accompanying mixotrophic nutrition in *K. micrum* focused on conditions that increased the propensity of autotrophic cultures to graze (Li *et al.*, 2000a) or on short-term responses of photosynthesis to grazing (Li *et al.*, 1999) but did not address the balance of autotrophy and heterotrophy during mixotrophic growth. Here, we showed that autotrophy contributed 27–69% to gross C uptake of *K. micrum* during mixotrophic growth with *S. major* as prey. ^{14}C assimilation patterns showed a decreased allocation of photosynthetic ^{14}C to protein in grazing compared to nongrazing *K. micrum* and a higher allocation of prey-derived ^{14}C to protein in mixotrophic cells. Growth efficiencies during mixotrophic growth of *K. micrum* were typical of dinoflagellates, changed little from autotrophic growth efficiencies and implicated elevated C acquisition from prey as the mechanism responsible for elevated growth rates in mixotrophic versus autotrophic *K. micrum*. These results contrast with the inability of *K. micrum* to grow as a strict heterotroph on the same prey (Li *et al.*, 1999) and underscore the importance of both heterotrophic and autotrophic C metabolism during mixotrophic growth of *K. micrum*.

Photosynthetic performance during mixotrophic growth of *K. micrum*

Photosynthetic regulation accompanying mixotrophic growth plays a critical role in the balance of autotrophy and heterotrophy in mixotrophic organisms. Whether photosynthesis is stimulated, unchanged or depressed during grazing is a fundamental question with clear ecological implications. Decreases in total PP^{cell} and autotrophic % protein during mixotrophic growth

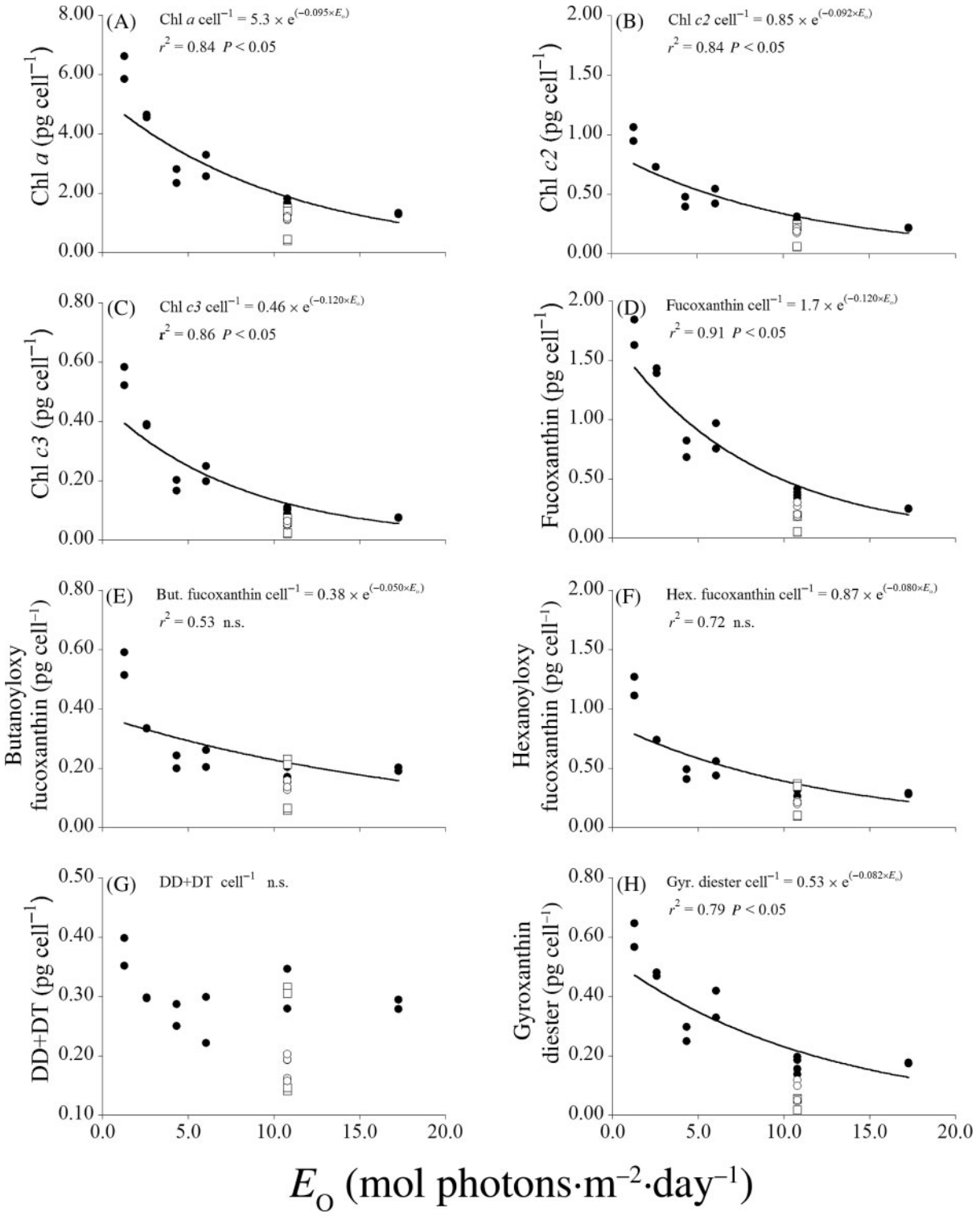

Fig. 5. Changes in photopigment cell^{-1} as a function of growth irradiance in nutrient-replete autotrophic *Karlodinium micrum* (black circles, regression line), mixotrophic *K. micrum* (white circles, MIXO 1 and MIXO 3) and nutrient-deprived autotrophic *K. micrum* (white squares, AUTO-N, AUTO-P).

Table IV: Comparisons of photopigment cell^{-1} between nutrient-replete autotrophic Karlodinium micrum and mixotrophic (MIXO) or nutrient-deprived autotrophic (AUTO-N and AUTO-P) K. micrum at the same growth irradiance (10.8 mol photons •m^{-2}•day^{-1})

	MIXO (n = 4)		Nutrient-deprived AUTO (n = 4)	
	% change	P	% change	P
Chl *a* cell^{-1}	−28.3	0.004	−42.1	0.072
Chl *c2* cell^{-1}	−30.0	0.005	−46.5	0.053
Chl *c3* cell^{-1}	−45.8	0.000	−55.4	0.006
Fucoxanthin cell^{-1}	−36.4	0.005	−67.7	0.001
Hexanoyloxy fucoxanthin cell^{-1}	−33.6	0.003	−28.6	0.287
Butanoyloxy fucoxanthin cell^{-1}	−29.7	0.006	−30.8	0.237
DD + DT cell^{-1}	−36.4	0.025	−19.4	0.384
Gyroxanthin diester cell^{-1}	−53.7	0.007	−78.8	0.000

'% change' refers to the treatment relative to nutrient-replete autotrophic cultures [100 × (treatment – AUTO)/AUTO]. DD, diadinoxanthin; DT, diatoxanthin. Statistical P is derived from a t-test where $P < 0.05$ was considered significant.

reflected a decreased role of autotrophic nutrition during mixotrophic growth of *K. micrum*. Changes of PP^{cell} that accompanied grazing in *K. micrum* were similar to observations of the effects of grazing on PP^{cell} for *Fragilidium subglobosum* (Hansen *et al.*, 2000; Skovgaard *et al.*, 2000). PP^{cell} of food-saturated *F. subglobosum* was decreased 48–69% compared to autotrophic cells (Skovgaard *et al.*, 2000), with the strongest effect of grazing on PP^{cell} associated with increased food concentrations (Hansen *et al.*, 2000). Our experiments with *K. micrum* were not food saturated, thus the best comparison of our data and those of Skovgaard *et al.* (Skovgaard *et al.*, 2000) is for MIXO 1 in which PP^{cell} was decreased 52% compared to AUTO, and the grazing rate was approximately half of the maximum values reported by Li *et al.* (Li *et al.*, 2000a). These observations support a conclusion that *K. micrum* and *F. subglobosum* regulate PP^{cell} similarly during grazing, with each species showing a decrease of PP^{cell} associated with increased heterotrophy.

Differences of P_m^{cell} between autotrophic and mixotrophic *K. micrum* are consistent with the conclusion that lower PP^{cell} reflects a significant reduction in cellular investment in photosynthesis. Decreases in P_m^{cell} observed in mixotrophic *K. micrum* suggest decreased cellular quotas or activities of Calvin cycle enzymes (Falkowski, 1992; Geider and MacIntyre, 2002). Parallel changes in P_m^{cell} and Chl *a* cell^{-1} in autotrophic (Adolf *et al.*, 2003) and mixotrophic (Fig. 4A) cultures of *K. micrum* suggest that acclimation of the photosynthetic apparatus primarily involves changes in photosynthetic unit (PSU) 'numbers' (cf. Prézelin, 1987). Reductions of PP^{cell} (day-long incubations) were accompanied by reductions of P_m^{cell} (30 min PE curve), except in MIXO 2 that showed PE curve parameters similar to autotrophic cultures, but PP^{cell} similar to other mixotrophic treatments. This discrepancy between P_m^{cell} and PP^{cell} in MIXO 2 most likely reflects a lag between prey ingestion and effects on cellular photosynthesis as, in the MIXO 2 experiment, PE curve measurements were made at a time when ^{14}C uptake rates of mixotrophic and autotrophic cultures had not yet diverged (Fig. 8). Thus, our PE data suggest that lower PP^{cell} in mixotrophic *K. micrum* results from a reduction of P_m^{cell} rather than from a reduction of cellular Chl *a*.

Mixotrophy and nutrient starvation significantly lowered cellular photopigment quotas from expected values established for nutrient-replete autotrophic *K. micrum* (Table IV). Quotas of Chl *a* and Chl *c3* were lower in mixotrophic cells despite potential contributions from ingested cryptophytes contained in food vacuoles. This pattern was consistent with reductions in P_m^{cell} suggested by PE curve data and further supports the conclusion that heterotrophic metabolism dominates when *K. micrum* uses a mixotrophic nutritional mode.

We measured the effects of pigment composition on $\Phi_{C(max.)}$ in autotrophic and mixotrophic *K. micrum* to examine potential effects of prey-derived and/or endogenously produced NPP on photosynthesis (Bidigare *et al.*, 1989). Pheophorbide constituted a significant fraction of NPP in these cultures, and its specific and spectral absorption properties, which are similar to those of Chl *a* (Jeffrey *et al.*, 1997), underlie its significant contribution to cellular light absorption

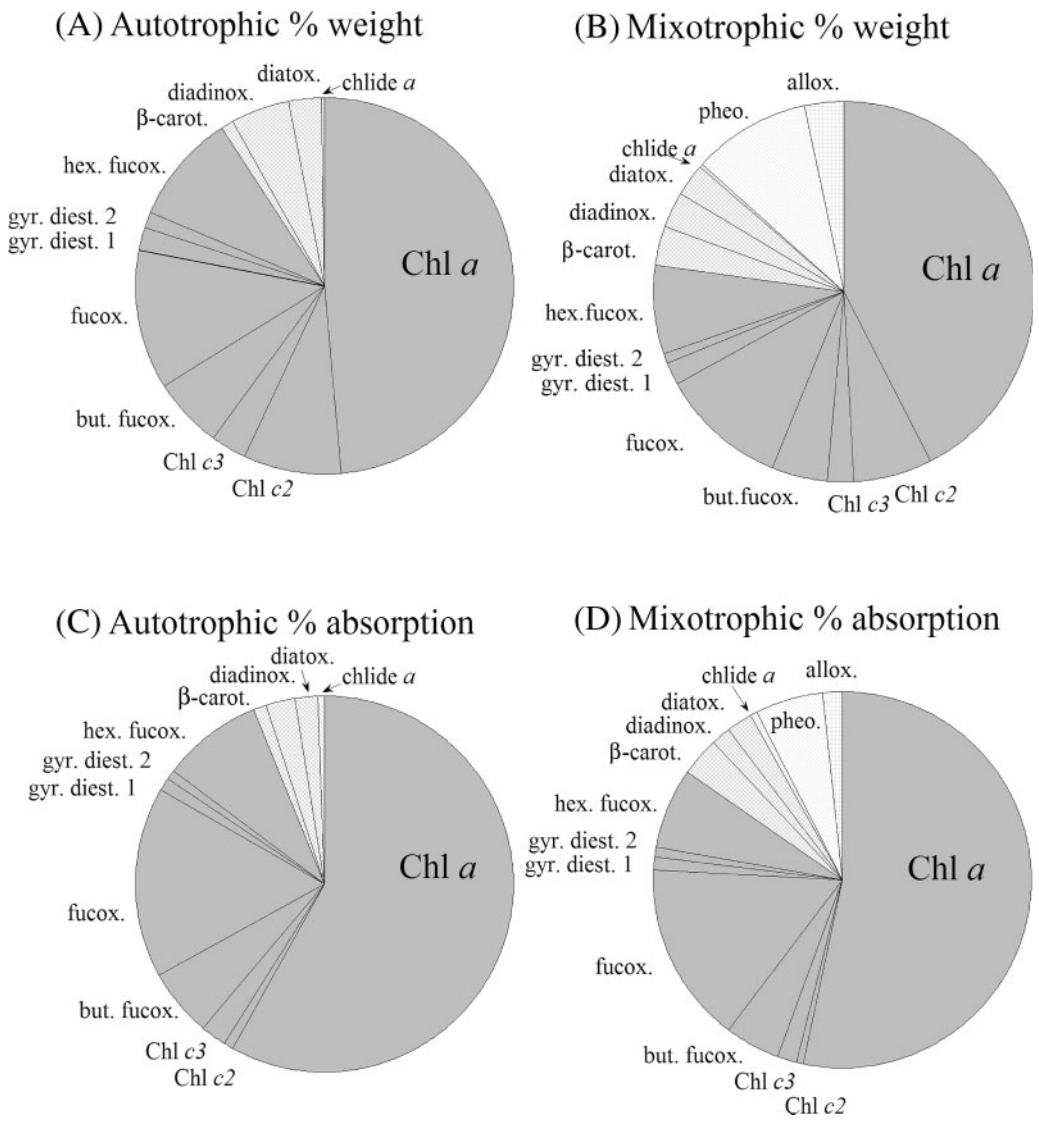

Fig. 6. The relative proportion of photopigments in autotrophic (AUTO 1) and mixotrophic (MIXO 1) *Karlodinium micrum*. Panels **A** and **B** show the relative pigment concentration by weight for AUTO 1 and MIXO 1, respectively. Panels **C** and **D** show the relative pigment concentration by absorption for AUTO 1 and MIXO 1, respectively. Cultures were sampled at the beginning of the light period immediately before re-feeding (MIXO 1), and dilution of these semicontinuous batch cultures occurred. Values from duplicate cultures for each treatment were pooled to make these graphs.

measured as a decline of PPs : TOTAL absorption. Herbivorous protists produce pheophorbides as chlorophyll digestion products (Strom, 1993), supporting a conclusion that the presence of this pigment in mixotrophic *K. micrum* resulted from digested prey. We observed ~77% decrease of $\Phi_{C(max.)}$ accompanied by ~11% decline of PPs : TOTAL pigment absorption (Figs 6 and 7), suggesting that absorption by NPP did not explain the decline of $\Phi_{C(max.)}$ we observed in *K. micrum* for a range of nutritional conditions. Mixotrophic chlorophytes show altered metabolic demands associated with heterotrophic C metabolism that may impact $\Phi_{C(max.)}$. Photoheterotrophic assimilation of acetate in *Pyrobotrus stellata* was associated with a shift in light harvesting and energy transfer toward PS I, a strategy favoring cyclic ATP production needed in acetate metabolism and disfavoring NADPH production and C fixation (Brandt and Wiessner, 1984; Boichenko *et al.*, 1992; Wolf *et al.*, 1996). A shift in the fate of absorbed light from C fixation to ATP production to support heterotrophic metabolism may be an important physiological mechanism in mixotrophic dinoflagellates deserving further study.

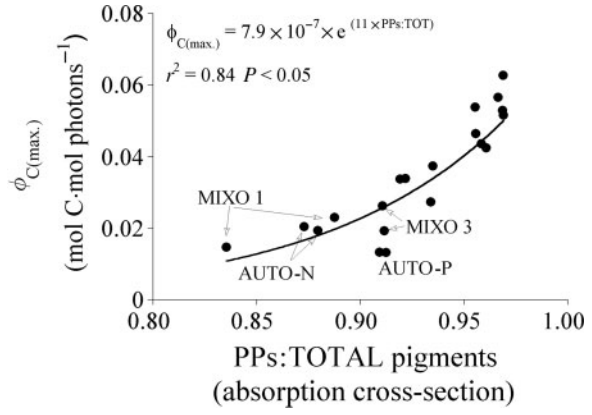

Fig. 7. The relationship between $\Phi_{C(max.)}$ (the maximum quantum efficiency of photosynthetic C fixation with unit mol C·mol photon^{-1}) and PPs : TOTAL (the absorption-based ratio of photosynthetic to total pigments) in autotrophic and mixotrophic *Karlodinium micrum*.

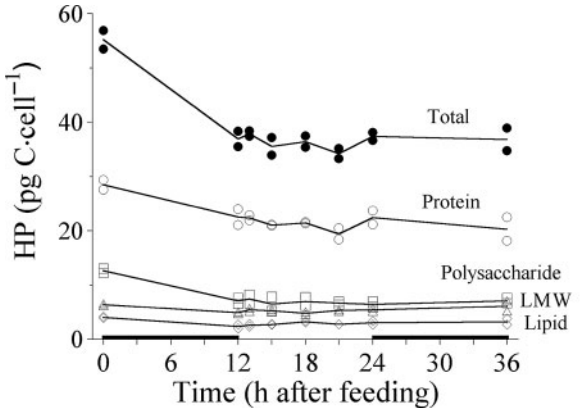

Fig. 9. Accumulation of metabolic end products in *Karlodinium micrum* after introduction of ^{14}C-labeled prey. HP, heterotrophic performance; LMW, low-molecular-weight metabolites.

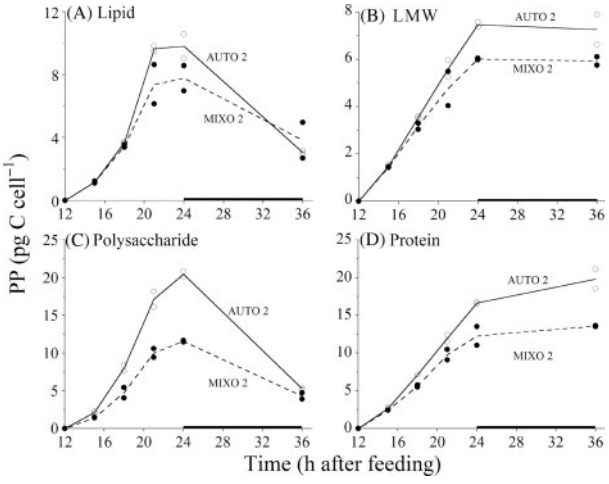

Fig. 8. Time course of photosynthetic (**A**) lipid, (**B**) low-molecular-weight (LMW) metabolites, (**C**) polysaccharide and (**D**) protein following introduction of prey to autotrophically grown *Karlodinium micrum*. AUTO 2 was maintained under autotrophic conditions for this experiment, while MIXO 2 was started by feeding an aliquot of AUTO 2 with *Storeatula major* prey.

^{14}C allocation patterns from photosynthesis and prey

Differences of ^{14}C allocation patterns between photosynthetically and prey-derived C suggest differences in the use of C from these two sources by *K. micrum* for growth. Reduced allocation of photosynthetic ^{14}C to protein, as we observed for mixotrophic *K. micrum*, has been shown for light- and nutrient-limited phytoplankton and is generally accompanied by a decrease of growth rate (Morris, 1981; Harding *et al.*, 1985; DiTullio and Laws, 1986; Harding and Jones, 1988; de Madariaga, 1992). In our study, N- and P-deprived, stationary phase, autotrophic *K. micrum* cultures allocated 31 ± 0.01% of fixed ^{14}C to protein, lower than in nutrient-replete exponential phase cultures (Table V). ^{14}C-labeling patterns for the 'kleptoplastidic' ciliate, *Laboea strobila* (Putt, 1990), are qualitatively similar to the pattern we found for *K. micrum*, showing reduced protein synthesis by plastids retained in the ciliate compared to the same plastids in free-living prey cells. At the same time, protein assimilation from prey ingested by *L. strobila* was high (Putt, 1990). The increased allocation of prey-derived ^{14}C to protein suggests that prey-derived C contributes more to cellular growth than photosynthetically derived C in mixotrophic *K. micrum*. This qualitative difference in C assimilation patterns of autotrophy and heterotrophy in mixotrophic *K. micrum* reinforces the quantitative differences and further underscores the importance of heterotrophic metabolism to mixotrophic growth.

Mixotrophic growth

Gross growth efficiency (%GE in Table III) of mixotrophic *K. micrum* was typical of dinoflagellates (Straile, 1997), varying little as a function of nutritional status, leading us to conclude that increased growth rate associated with mixotrophic nutrition in *K. micrum* was attributable to increased C gain by the cells. A similar conclusion can be drawn for *F. subglobosum* where a 3.8-fold increase of growth rate was accompanied by no change of GE (calculated as our %GE from Table IV in Hansen *et al.*, 2000). Although mixotrophic *F. subglobosum* respired less of its assimilated C than did autotrophic *F. subglobosum* (Hansen *et al.*, 2000), the magnitude of this difference did not explain the reported growth rate differences between autotrophic and mixotrophic cells in that study.

Table V: Summary statistics for proportions (%$C^{frac.}$) of C incorporation from photosynthesis and heterotrophy into end products in autotrophic and mixotrophic Karlodinium micrum

	% $C^{frac.}$ (photosynthesis)			% $C^{frac.}$ (heterotrophy)		
	AUTO (n = 4)	MIXO (n = 4)	P	MIXO (n = 4)	PREY (n = 6)	P
LIPID	8 (0.5)	12 (3.4)	n.s.	11 (2.4)	10 (1.5)	n.s.
LMW	20 (1.2)	18 (4.4)	n.s.	13 (4.7)	11 (1.0)	n.s.
PSACCH	14 (1.0)	22 (7.9)	n.s.	25 (0.1)	25 (1.4)	n.s.
PROT	57 (1.4)	48 (3.5)	0.003	52 (4.4)	54 (2.3)	n.s.

The column 'PREY' indicates the ^{14}C composition of evenly labeled prey at the time of feeding. Values in parentheses are SDs of the mean. LMW, low-molecular-weight metabolites; PSACCH, polysaccharide; PROT, protein; n.s., not significant.

These findings lead to the conclusion that potential C gain from prey is more important than growth efficiency in explaining growth rate changes arising from mixotrophic nutrition. The relatively large size of the prey compared to predator and constraints on maximum cellular photosynthesis (Adolf *et al.*, 2003) underlie the differences in potential C gain from photosynthesis and phagotrophy in *K. micrum*. At maximum grazing rates (Li *et al.*, 1999), C gain from phagotrophy would be 260–340 pg C•*K. micrum*$^{-1}$•day^{-1}. PE curve measurements on nutrient-replete autotrophic *K. micrum* indicated potential fixation of <100 pg C•*K. micrum*$^{-1}$•day^{-1} over a 12-h period in saturating light conditions. Thus, in terms of potential gross C gain, *K. micrum* has a 2- to 3-fold higher capacity associated with heterotrophy than with autotrophy. In a natural population, of course, the realized balance would depend on the relative availability of prey and light, which would be exaggerated by the decrease in illumination as both predator and prey biomass developed, driving cells in high biomass situations toward enhanced heterotrophy.

Our results, stressing the importance of heterotrophic C metabolism during mixotrophic growth of *K. micrum*, appear counterintuitive, considering previous observations that *K. micrum* is an obligate autotroph (Li *et al.*, 1999). One resolution may be that photosynthesis during mixotrophic growth provides essential micronutrients that *K. micrum* cannot obtain from prey, consistent with the lack of dark heterotrophic growth in *K. micrum*. In contrast to *K. micrum*, *F. subglobosum* (Skovgaard, 1996) and *Gyrodinium resplendens* (Skovgaard, 2000) can grow as strict heterotrophs, perhaps reflecting their ability to acquire essential nutrients from prey, *Ceratium* spp. The physiological basis of this difference in strict heterotrophic capabilities remains to be determined.

Evolutionary and ecological implications

The study of mixotrophs that combine phagotrophy and photosynthesis is relevant to protistan evolution, as the diversity of protistan autotrophs arose through uptake and retention of plastids (Raven, 1997; Okamoto and Inouye, 2005). Thus, the study of extant mixotrophic dinoflagellates can be regarded as a study of the ecological context within which these evolutionary events took place. A significant insight comes from the observation that reduced photosynthetic capacity accompanies grazing in *K. micrum*. In terms of the C flux through pelagic ecosystems where mixotrophic organisms predominate (Stickney *et al.*, 2000), our study suggests a shift toward heterotrophy during grazing due to both a reduction in the photosynthetic capacity of the mixotrophs and the removal of autotrophic prey. Important research that remains to be done includes expanding our knowledge of the simple balance between autotrophy and heterotrophy that operates in the variety of mixotrophic protists that are observed in pelagic ecosystems.

ACKNOWLEDGEMENTS

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