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Nutrient Acquisition and Population Growth of a Mixotrophic Alga in Axenic and Bacterized Cultures

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Abstract

Axenic growth of a mixotrophic alga, *Ochromonas* sp., was compared in several inorganic and organic media, and in the presence of live bacteria under nutrient-replete and low-nutrient conditions. Axenic growth in the light was negligible in inorganic media with or without the addition of glucose. Addition of vitamins increased growth rate, but average cell size declined, resulting in no net increase in biomass. Supplementing axenic cultures with a more complex organic substrate resulted in moderate growth and higher maximal abundance (and biomass) than in the inorganic media with added vitamins. The absence of light did not greatly affect population growth rate in the presence of complex dissolved organic compounds, although cell size was significantly greater in the light than in the dark. The highest growth rates for the alga (up to 2.6 d^{-1}) were measured in treatments containing live bacteria. Increases in cell number of *Ochromonas* sp. in the presence of bacterial prey were similar in the light and dark, although chloroplast and cell sizes differed. Bacterial abundance was reduced and dissolved phosphorus and ammonia were rapidly released in bacterized cultures in the light and dark, indicating high rates of bacterial ingestion and suggesting an inability of the alga to store or utilize N and P in excess of the quantities required for heterotrophic growth. Low-nutrient conditions in the presence of bacteria were promoted by adding glucose to stimulate bacterial growth and the uptake of N and P released by algal phagotrophy. Subsequent decreases in dissolved N and P following the addition of glucose corresponded to a second period of rapid growth of the alga in both light and dark. This result, combined with evidence for slow axenic growth of this strain, indicated that nutrient acquisition for this species in the presence of bacteria was accomplished primarily via ingestion of bacteria.

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Introduction

Phytoplankton assemblages are usually populated, and occasionally dominated, by species displaying a mixotrophic nutritional mode that combines photosynthesis with phagocytosis of particulate food. Mixotrophy has been observed for various algal groups, including chrysophytes, dinoflagellates, prymnesiophytes, and cryptophytes. Numerous studies have demonstrated that these mixotrophic populations can have a significant impact on their bacterial and algal prey [9–11, 15, 23].

The potential ecological advantages of phagotrophy for algal species remain largely speculative, but include support of growth in the dark, supplementation of photosynthetic carbon fixation, acquisition of macronutrients (nitrogen and phosphorus) for photosynthetic growth, and fulfillment of micronutrient requirements (e.g., acquisition of vitamins, essential fatty acids, iron) [13, 14, 17, 22]. It is likely that phagotrophy fulfills different requirements for different phytoplankton species along a gradient of mixotrophic behavior from nearly pure phototrophy to nearly pure heterotrophy [13, 24]. Furthermore, changes in environmental parameters such as light level and nutrient concentrations may cause shifts within a species in the relative importance of photosynthesis and phagotrophy, or in the specific role that phagotrophy performs [14, 17, 28, 29].

Species of the chrysophyte genus *Ochromonas* are commonly identified as mixotrophs in both freshwater and marine plankton. Numerous species are placed within the genus; these can be identified by two heterokont flagella (the longest with mastigonemes), one or two plate-like golden-brown plastids, and lack of a cell wall or siliceous scales [7]. Previous studies with *Ochromonas* spp. and closely related genera (e.g., *Poterioochromonas malhamensis*) have indicated that heterotrophy dominates the nutrition of these species. Most of these clones are primarily phagotrophic and grow equally well in the dark and light in the presence of particulate food [1, 3, 5, 12, 20]. However, it has been reported that the ingestion rates of some of these species are responsive to nutrient conditions [26], or that they can enhance their photosynthetic abilities by heterotrophic activity [20]. These behaviors may reflect strain/species differences, but they seem difficult to reconcile for mixotrophic species that appear to rely almost exclusively on phagotrophy for their nutrition.

The specific contribution of phagotrophy to the nutrition of chrysophyte algae is complicated by the presence of live bacteria in many of the cultures that have been employed to examine the behavior of these algae [1, 20]. In part, this situation exists because some mixotrophs appear to have rather rigid requirements for particulate food. The activity of live bacteria in these algal cultures makes it difficult to determine the relative importance of bacteria and algae in nutrient uptake, retention, and recycling, and thus the roles of mixotrophic species typically have been inferred rather than experimentally demonstrated. Experimental studies of axenic cultures of mixotrophic algae provide one mechanism for characterizing the role that bacteria play in the nutrition of phagotrophic algae [3, 4, 12, 24].

In this study, we examined mixotrophic behavior of an *Ochromonas* strain isolated from a freshwater pond and grown under axenic and bacterized culture conditions. Population growth was negligible (0–0.05 d^{-1}) for the axenic culture in an inorganic salts medium with or without glucose in the light or dark. Faster growth of the axenic alga was observed with the addition of vitamins in the light (0.16 d^{-1}) or in the presence of a complex dissolved carbon source in the light and dark (up to 0.41 d^{-1}). Axenic cultures with organic supplements in the light had significantly larger cell sizes than cultures incubated in continuous darkness. By far the fastest growth rates were obtained when live bacteria were present in the algal cultures (up to 2.6 d^{-1}). A comparison of algal growth in bacterized cultures under nutrient replete or low-nutrient conditions indicated that nutrient acquisition of this mixotroph was strongly mediated by phagotrophy.

Methods

Cultures

Ochromonas sp. strain BG-1 is a small (typically 5–8 μm), free-swimming member of the Chrysomonadida with a single chloroplast that becomes reduced in the dark and at high prey concentrations. It was isolated from a freshwater pond in Malaysia by organically enriching water samples in the dark. It was identified as potentially mixotrophic when cultures became pigmented when grown in the light (E. Lim, pers. comm.). Epifluoresence microscopy confirmed the presence of a chloroplast. Identification as a species of *Ochromonas* is consistent with the lack of siliceous scales and the presence of mastigonemes on the longer of two flagella as established by electron microscopy (Fig. 1). The strain was axenized by sequential transfers of single cells with a sterile, pulled-glass pipette through sterile medium and into an antibiotic mixture with heat-killed bacteria. The antibiotic mixture was prepared by combining 100 mg of penicillin and 50 mg of streptomycin in 10 ml of sterile distilled water with 20 mg of chloramphenicol dissolved in 95% ethanol. This mixture was filter-sterilized and a dilution series prepared. *Ochromonas* sp. grew in the presence of up to 4% of the

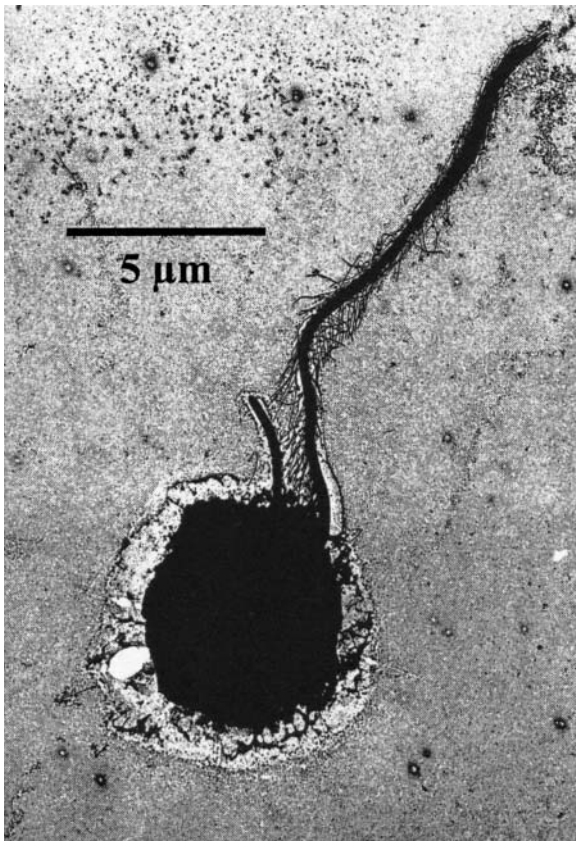

Fig. 1. Scanning electron micrograph of *Ochromonas* sp. (strain BG-1). Note flagellar hairs (mastigonemes) on the longer flagellum and the lack of scales or spines.

Table 1. Minimal salts and DY-IV algal media used for experiments[a]

Minimal salts media	Final concentration (mg L^{-1})
Nutrient-deplete (low nutrients)	
$NaHCO_3$	1680
$CaCl_2 \bullet 2H_2O$	20
$MgSO_4 \bullet 7H_2O$	7.4
H_3BO_3	2.5
$NaSiO_3 \bullet 9H_2O$	15
KCl	10
Trace metals	1 ml of stock (8)
Nutrient-replete (added to nutrient-deplete)	
NH_4Cl	47
NaH_2PO_4	5

DY-IV Media	Final concentration (mg L^{-1})
$CaCl_2 \bullet 2H_2O$	20
$MgSO_4 \bullet 7H_2O$	74
Na_2HPO_4	6.6
$NaNO_3$	20
$NaSiO_3 \bullet 9H_2O$	15
NH_4NO_3	10
KCl	10
MES buffer (pH 6.8)	250
Vitamin mix	1 ml of stock (16)
Trace metals	10 ml of stock (16)

[a] Trace metals (Fe, Cu, Zn, Co, Mn, Mo) in the minimal salts medium were added at the same concentrations used in f/2 [8]. The nutrient-replete medium was identical to the nutrient-deplete except NH_4Cl and NaH_2PO_4 were added as indicated. DY-IV is a modification of DY-III [16] in which sodium phosphate replaces glycerophosphate (C.D. Sandgren, pers. comm.) The vitamin mix includes B_{12} (cyanocobalamin), biotin, and thiamine. The pH of all media was brought to 6.8 with sterile HCl.

antibiotic mixture. Heat-killed bacteria were prepared as previously described [24].

Axenic cultures of *Ochromonas* sp. were maintained at 20°C on a 10:14 light:dark cycle in DY-IV medium that was originally designed for maintenance of *Dinobryon* species (Table 1). Maintenance light intensity was 350 µeinsteins m^{-2} s^{-1}. For experiments with added live bacterial prey, *Pasteurella* sp. was grown separately from the algae in 0.5% yeast extract. Bacteria were centrifuged and rinsed to remove medium and inorganic nutrients, and added to the *Ochromonas* sp. cultures at an initial concentration of 10^7 bacteria mL^{-1}.

Experimental Design and Conditions

For all experiments, stock cultures were centrifuged at 7000 rpm for 10 min, resuspended in the experimental medium to an initial concentration of *Ochromonas* sp. cells of 700–2500 cells mL^{-1}, and allowed to acclimate to experimental conditions 18–24 h prior to sampling (day 0). All treatments were run in duplicate and mixed slowly on a horizontal shaker. Light was supplied at 460 µeinsteins m^{-2} s^{-1} by 40-W cool white fluorescent lamps; a low light treatment (75 µeinsteins m^{-2} s^{-1}) was added in the experiment with live bacteria. Light intensity was monitored using a Biospherical Instruments model QSL-100 Quantum Irradiance meter. Treatments in continuous darkness were wrapped with aluminum foil. All experimental manipulations were run at 20°C in a walk-in environmental room. Several experiments were used to elucidate the importance of phagotrophy and its role in the nutrition of *Ochromonas* sp. Species of *Ochromonas* are known for their nutritional flexibility, including an ability to grow on dissolved organic matter. Two experiments addressed this question using an axenic *Ochromonas* sp. culture. In the first, we examined growth of the alga in a minimal salts and trace metal medium (Table 1). A second set of cultures was supplemented with 18 mg glucose L^{-1} at the beginning of the experiment to determine the effect of a simple organic carbon source on algal growth, and also as a “control” for subsequent experiments in which glucose was used to stimulate bacterial growth (see below). Duplicate sets of both types of cultures were incubated in a 12:12 light:dark cycle and continuous darkness. Dark cultures were employed to document purely heterotrophic growth of the alga under each experimental condition. Axenic cul-

tures acclimated in minimal salts medium with and without glucose were amended with sterilized nitrogen and phosphorus (360 μM N as NH_4Cl, and 20 μM P as NaH_2PO_4) on day 2. To test the possibility that vitamins limited phototrophic growth in the minimal salts medium, algal abundance was followed in the light in an additional set of cultures to which the DY-IV vitamin mix (Table 1) was added to the nutrient-replete minimal salts medium.

Growth of axenic *Ochromonas* sp. was also compared in DY-IV medium (Table 1) and a modified DY-IV formula that lacked the organic buffer 2-[*N*-morpholino]ethanesulfonic acid (MES). MES is 6.6% nitrogen and 15% sulfur by weight, and the contribution of MES to DY-IV medium is 1.17 mM nitrogen, 1.17 mM sulfur, and 7.03 mM carbon. To examine the possibility that MES uptake could contribute to the nutrition of *Ochromonas* sp., MES was withheld in a set of cultures. Duplicate sets of both types of cultures were incubated in a 12:12 light:dark cycle and continuous darkness. Both the DY-IV and the modified DY-IV formulas provide vitamins and trace metals.

The absence of bacterial contamination in these two experiments was confirmed at several time points by epifluorescence microscopy and by inoculation of aliquots from each replicate into 0.5% yeast extract; the yeast extract was then monitored for growth of bacteria.

The role of bacterivory in the nutrition of *Ochromonas* sp. was examined in treatments with live bacteria under nutrient replete minimal salts medium (Table 1) and in minimal salts medium without added N and P. In the minimal salts medium, it was expected that nutrient remineralization would occur as *Ochromonas* sp. ingested bacteria and released dissolved of N and P. To ensure that N or P were limiting in these treatments, glucose was added to the minimal salts medium. Bacteria were expected to outcompete the alga for dissolved nitrogen and phosphorus under these condidtions. An initial glucose addition of 1 mg L^{-1} was followed by subsequent additions of 2, 4, and 8 mg glucose L^{-1} (final concentration) on days 1, 2, and 3, respectively. The amount of glucose supplement was increased on each subsequent day to ensure that bacteria were not carbon limited when measured concentrations of dissolved ammonium and phosphate were seen to increase. Sets of both types of cultures (glucose amended and unamended) were incubated in 12:12 light:dark cycle and continuous darkness.

Cell Abundance and Volume, Ingestion Rates, Nutrient Concentration

At each time interval, cells were sampled using aseptic technique and preserved with 1% glutaraldehyde (final concentration). Changes in *Ochromonas* sp. abundance were determined microscopically using Palmer Maloney counting chambers at 400×; average cell diameters were determined from video images of 20–30 individual cells using Scion Imaging software. Biovolume was calculated assuming spherical cells, and total biomass (as biovolume) was calculated as average cell biovolume × average abundance. Bacterial abundance was determined from DAPI-stained samples using epifluorescence microscopy [19].

For the treatments with added bacteria, bacterivory by the algae was determined 24 h after the start of the experiments using fluorescently labeled bacteria (FLB) as tracers [25]. Soluble reactive phosphate (SRP) and ammonia concentrations were determined daily in the low-nutrient treatments with bacteria using standard spectrophotometric methods [18].

Statistical Analysis

Maximal growth rates of *Ochromonas* sp. were computed from slopes of regressions of natural log-transformed data during the exponential growth period and compared for statistical differences in slope; differences in biovolume between treatments were examined for natural log-transformed data by ANOVA with repeated measures [30].

Results

Changes in Ochromonas Abundance and Biovolume

Axenic *Ochromonas* sp. in the minimal salts medium exhibited no significant growth in the light or dark (Fig. 2A). Supplementing the minimal salts medium with 18 mg glucose L^{-1} had very little effect on algal growth in the light and dark relative to glucose-free medium ($\mu = 0.04–0.05\ d^{-1}$; Fig. 2B, Table 2). Motile cells were present in all of these treatments and cell volume remained relatively high (Fig. 2C, 2D), although there was a significant decrease ($P < 0.01$) in average biovolume after day 2 in cultures amended with glucose and incubated in the dark (Fig. 2D). Initial *Ochromonas* sp. abundance averaged 2.5×10^3 cells ml^{-1} across these treatments. By day 7, average abundances were approximately 4.6×10^3 and 3.0×10^3 cells ml^{-1} for treatments with and without glucose in the light, 3.6×10^3 cells ml^{-1} in the dark with glucose, and 1.6×10^3 cells ml^{-1} in the dark without glucose. The potential of phototrophic growth rate to be limited by trace growth factors was demonstrated by the moderate growth ($0.16\ d^{-1}$) when vitamins were added to the minimal salts medium (Table 2). However, cell size in the vitamin-added treatment decreased to 112 ± 19 μm^3 $cell^{-1}$ as population size increased. The cumulative result was no net gain in population biovolume in the treatments with added vitamins.

Axenic growth rates based on changes in cell abundances of *Ochromonas* sp. in complete DY-IV medium (Fig. 3) were similar in the light and dark, and greater than in the minimal salts medium (Fig. 2), even after vitamin addition (Table 2). Growth rates of the alga in complete DY-IV medium were 0.36 and 0.41 d^{-1} in the light and dark, respectively (Table

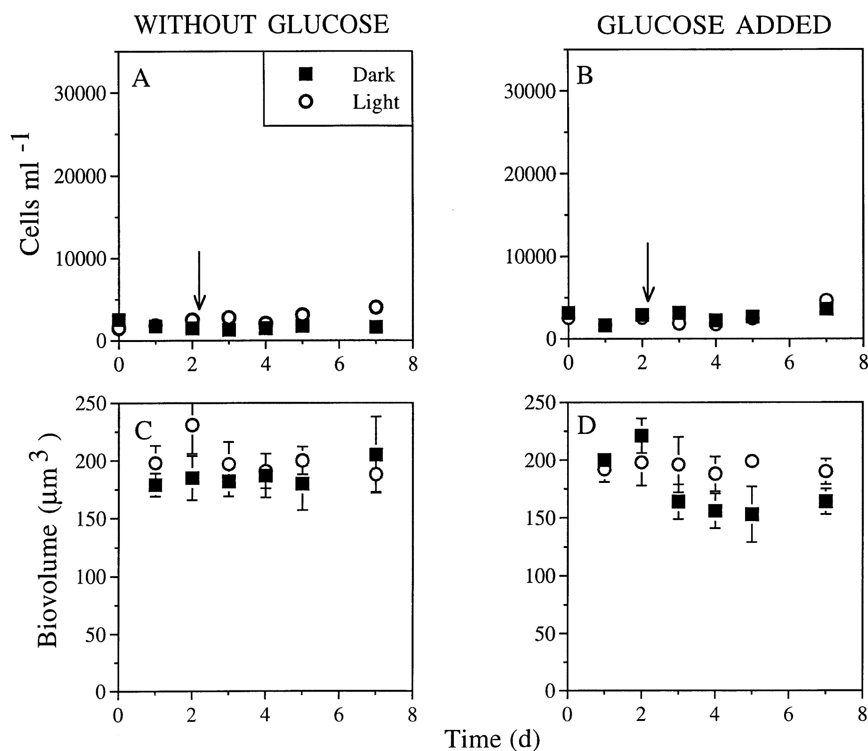

Fig. 2. Abundance and biovolume cell^{-1} of axenic *Ochromonas* sp. maintained in a minimal salts medium lacking glucose (A, C) and with glucose added (B, D). Filled symbols represent incubations in the dark; open symbols represent incubations in the light (460 μE m^{-2} s^{-1}). Arrows indicate addition of inorganic nitrogen and phosphorus to the basal medium (Table 1).

2), while maximum abundance of *Ochromonas* sp. averaged 3.1×10^4 and 2.8×10^4 cells ml^{-1} in the light and dark, respectively (Fig. 3A). However, biovolume was significantly less ($P < 0.01$) for cells in the dark compared to the light by day 2 (Fig. 3C). Thus, the rate of total biomass accumulation for the alga in the dark was less than that in cultures incubated in the light in the DY-IV medium (Fig. 3E).

Table 2. Specific growth rates (μ, d^{-1}) calculated during periods of maximum changes in abundance[a]

Treatment supplements	Light	Dark
1 Axenic, minimal salts	0	0
2 Axenic, minimal salts + glucose	0.05	0.04
3 Axenic, minimal salts + vitamins	0.16	No data
4 Modified DY-IV	0.27	0.24
5 DY-IV	0.36	0.41
6 Live bacteria, nutrient replete	1.3	1.1
7 (low light)	(1.3)	
8 Live bacteria, low nutrients + glucose	1.6	2.6
9 (low light)	(1.7)	

[a] Light treatments were on a 12h:12h light:dark cycle at an intensity of 460 μE m^{-2} s^{-1}, except for low light treatments (75 μE m^{-2} s^{-1}, in parens) in the experiments with live bacteria. The axenic minimal salts medium is equivalent to that in the live-bacteria, nutrient-replete treatment. Growth rates were significantly different ($P < 0.01$) between treatments 4 and 5, and 6 and 8 in the light and in the dark. The only significant difference in growth rate between light and dark treatments was in the live bacteria + glucose experiment (8 light vs 8 dark, $P < 0.01$). There were no significant differences between growth in high light and low light (6 vs 7, 8 vs 9).

Removal of MES from the DY-IV medium resulted in significantly reduced growth rates of *Ochromonas* sp. compared to the complete medium (Fig. 3B vs 3A, Table 2), although these rates were still greater than values obtained in the minimal salts medium (Fig. 2 vs. 3B). Growth rates based on increase in abundance were not significantly different in the light and dark when MES was withheld from the medium (0.24 and 0.27 d^{-1}). Cell abundances attained during the experimental period were 1.2×10^4 cells ml^{-1} and 0.9×10^4 cells ml^{-1} in the light and dark, respectively (Fig. 3A). Similar to the result in complete DY-IV medium, biovolume was significantly less for cells in the dark compared to the light by day 3 ($P < 0.01$). Overall for this experiment, average biovolume by day 9 ranged from 18 ± 3 μm^3 cell^{-1} in the dark without MES (Fig. 3D) to 203 ± 17 μm^3 cell^{-1} in the light with MES (Fig. 3C). Although total biovolume was much less when MES was withheld from the medium (Fig. 3F vs 3E), the larger individual biovolume cell^{-1} in the light was again reflected by the total biovolume (Fig. 3F).

The importance of phagotrophy to the nutrition of this species is highlighted by the rapid rate of increase and greater final algal abundances that occurred when bacteria were present (Fig. 4A, 4B; note different ranges for axes in

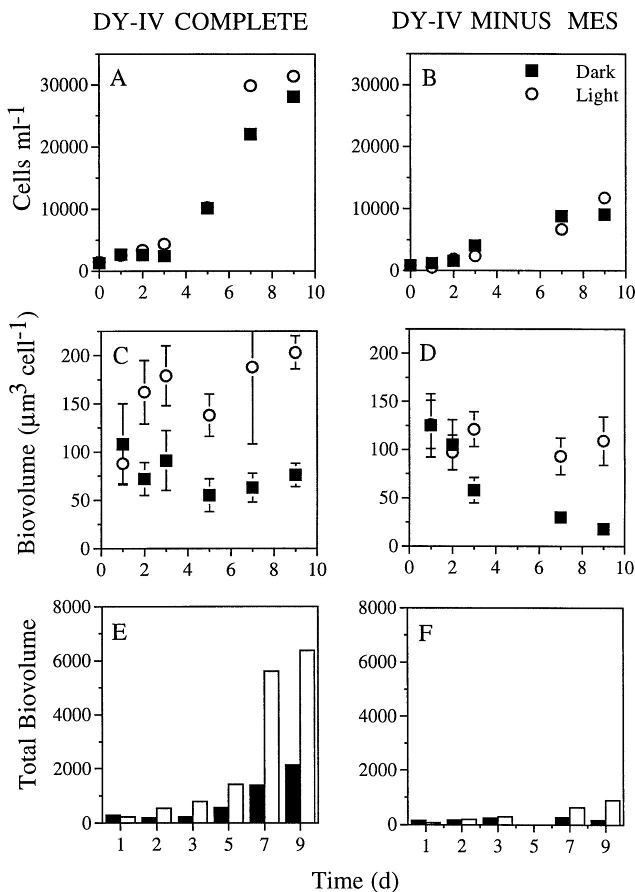

Fig. 3. Abundance and biovolume of axenic *Ochromonas* sp. maintained in complete DY-IV medium (A, C, E) and modified (minus MES) DY-IV algal medium (B, D, F). Total biovolume is plotted as $\mu m^3 \times 1000\ mL^{-1}$. MES is an organic buffer containing carbon, sulfur, and nitrogen.

Fig. 4 vs Figs. 2, 3). Changes in algal abundance in inorganic medium with bacteria and high concentrations of N and P, but without glucose, initially followed the same trend in the three light regimes (Fig. 4A). The highest specific growth rates occurred during the first 24 h and averaged 1.2 d^{-1} for the three light levels. After day 3, abundance remained relatively stable in the two treatments receiving light, but declined in the dark (Fig. 4A). In the low-nutrient treatment supplemented with glucose additions on days 1, 2, and 3, changes in abundance were generally the same in the light and dark until day 5 when the dark treatment had approximately 1.4 times as many algal cells as the treatments with light (Fig. 4B). The glucose supplemented treatment with bacteria also had the highest *Ochromonas* sp. growth rate observed in any experiment ($\mu = 2.6\ d^{-1}$). In all treatments with live bacteria, average *Ochromonas* sp. biovolume declined from >150 $\mu m^3\ cell^{-1}$ to 75 $\mu m^3\ cell^{-1}$ within 18 h (Figs. 4C, 4D) with no clear relationship to the light regime. The relative similarity of cell sizes resulted in abundance changes closely reflecting changes in total biomass in all experiments with bacteria.

Bacterial Abundance and Nutrient Concentrations

Bacterial abundance in all of the bacterized treatments declined by an order of magnitude during the first 18 h of the experiment and remained at approximately 10^6 bacteria ml^{-1} for several days (Fig. 5). Bacteria in the dark without glucose added increased between days 4 and 5 (Fig. 5A) following the decline in *Ochromonas* sp. abundance in that treatment (Fig. 4A). Bacteria increased in all of the glucose-supplemented treatments after the final and largest glucose addition on day 3 (Fig. 5B).

Nutrient concentrations were determined only for the glucose-supplemented treatments because inorganic nitrogen and phosphorus were added in high concentrations in the nutrient-replete treatments. In the glucose-supplemented treatments, dissolved ammonia and phosphate increased during the first day by a factor of 4 to 5 (Fig. 6) and subsequently declined, especially after the final glucose addition, to levels at or below the initial concentrations.

Bacterivory

The rate of ingestion of bacteria by *Ochromonas* sp., as determined using fluorescently labeled bacteria on day 1, varied little between treatments (Table 3). We had not expected the bacteria to be grazed to such low levels during the first 24 h, and the added FLB, intended to be at tracer levels, were actually more abundant than live bacteria (note that the FLB were added to subsamples only). Consequently, the ingestion rates based on FLB uptake (Table 3) were likely inflated for that time point because total bacterial abundance was increased by the addition of FLB. Nonetheless, the similarity of the grazing data in all treatments indicate that neither light level, dissolved nutrient level, nor glucose addition altered the ingestion rate of *Ochromonas* sp.

Bacterial ingestion rates in the bacterized cultures were also calculated from the rates of disappearance of bacteria and the geometric mean of algal abundances over the initial 24 h of the experiment (when bacterial abundance decreased significantly). These calculations also indicated no relationship between light regime or nutrient regime and ingestion rate, and yielded ingestion rates of 17–18 bacteria $alga^{-1}\ h^{-1}$

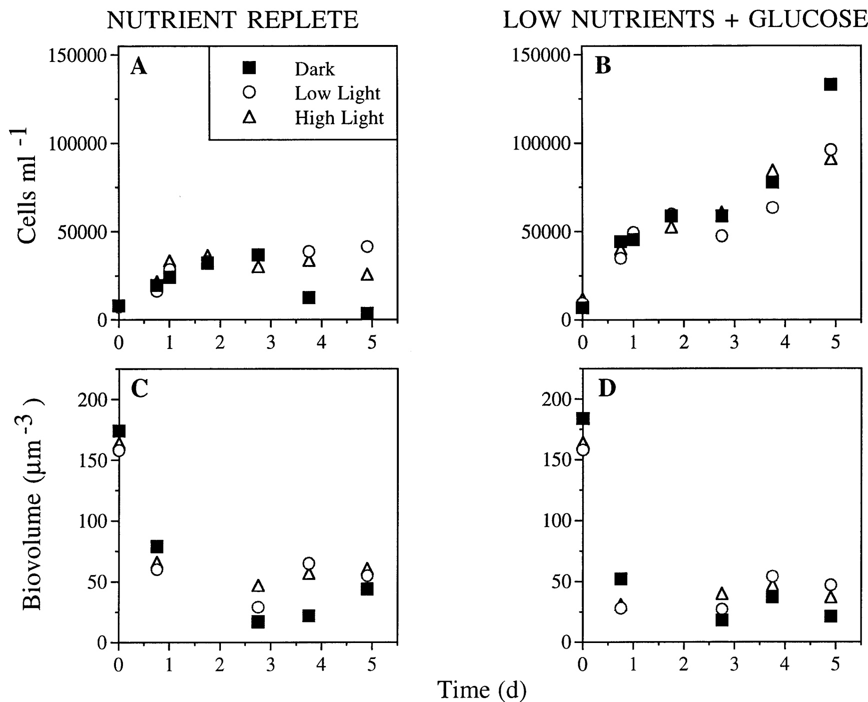

Fig. 4. Abundance and biomass of *Ochromonas* sp. in the presence of live bacteria. Treatments were either nutrient-replete inorganic medium (A, C) or minimal salts medium with glucose supplements (B, D). See Fig. 5 for timing of glucose additions. Open triangles are high light (460 µE m^{-2} s^{-1}), open circles are low light (75 µE m^{-2} s^{-1}), and filled squares are dark treatments. Note the difference in scale for cells ml^{-1} compared to Fig. 2 and 3.

in all treatments. These calculations did not take into account growth of bacteria in the treatments. If bacterial production was positive in any of the treatments, then the true ingestion rates in those treatments were underestimated by this method.

Bacterial abundance reached similar minimal values in all bacterized cultures regardless of glucose enrichment or predator abundance (Fig. 5). This result indicated a threshold concentration for bacteria of approximately 0.5 to 1 × 10^6 bacteria ml^{-1} for this strain of *Ochromonas,* below which bacterial grazing was much less effective.

Discussion

In a continuum of mixotrophic abilities ranging from predominantly heterotrophic to predominantly autotrophic [13, 24], the BG-1 strain of *Ochromonas* is clearly closer to the heterotrophic end of the spectrum. Although phototrophic growth occurs in the genus, most *Ochromonas* species that have studied in culture have relatively poor photosynthetic abilities and require high concentrations of bacteria to reach maximal growth rates [1, 6, 20]. The present study using axenic and bacterized cultures of *Ochromonas* strain BG-1 allowed a direct comparison of and growth in the presence of prey (*Pasteurella* sp.) and axenic growth on minimal media and media with dissolved organic compounds. This comparison has provided new insight into the interplay between photosynthesis, the uptake of dissolved inorganic nutrients, and heterotrophic growth via bacterivory or the uptake of organic compounds.

Axenic Growth in Minimal Medium and Organically Enriched Media

Axenic growth of *Ochromonas* sp. on the minimal salts medium was virtually nonexistent under light and dark conditions, and supplementation with glucose yielded only slight increases in algal abundance over the seven day experimental period (Table 2, Fig. 2). Thus, the availability of a labile organic carbon source, per se, did not substantially augment photosynthetic growth. However, many algae, including some species of *Ochromonas,* require a supplement of B vitamins for rapid growth in axenic culture [7], and our experimental manipulations with minimal salts medium lacked a vitamin supplement. An increase in population growth rate (0.16 d^{-1}) in the light was noted when the vitamin mix (Table 1) was added to the minimal salts medium. Total biomass of *Ochromonas* was static in the minimal salts plus vitamins since the increase in abundance was balanced by a decrease in the size of the cells. Hence it was not vitamins (cyanocobalamin, biotin, and thiamine) that limited biomass accumulation in the minimal salts medium.

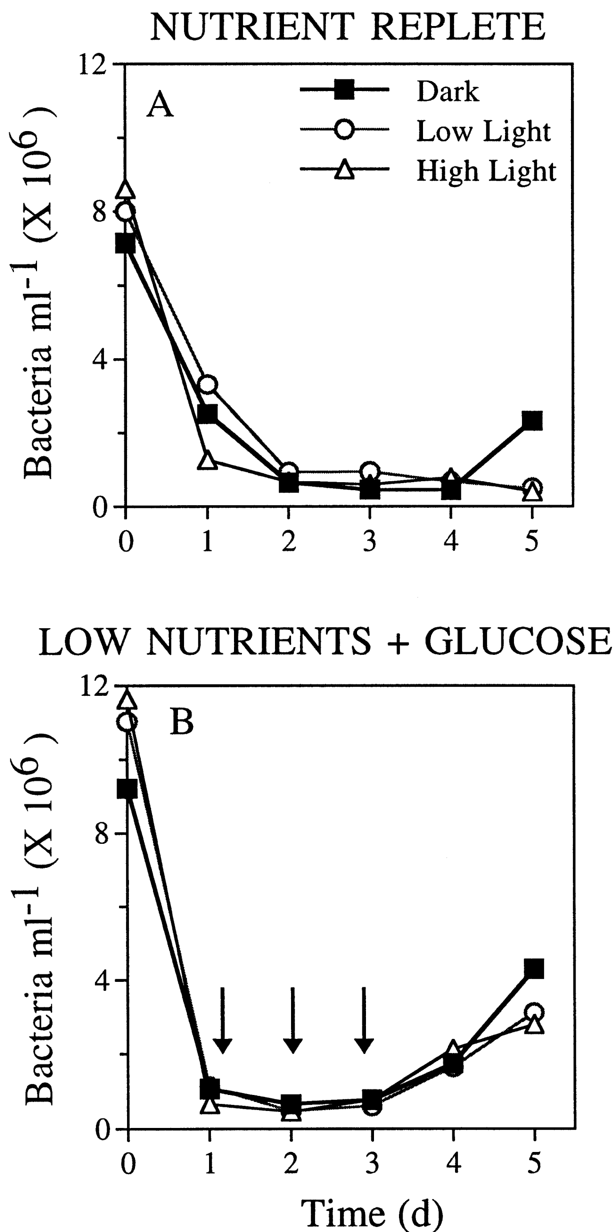

Fig. 5. Bacterial abundance in the experimental treatments with live bacteria and *Ochromonas* sp. Treatments were either nutrient-replete inorganic medium (A) or minimal salts medium with glucose supplements (B). High light intensity was 460 μE m^{-2} s^{-1}; low light intensity was 75 μE m^{-2} s^{-1}. Arrows indicate the time of glucose additions (see Methods).

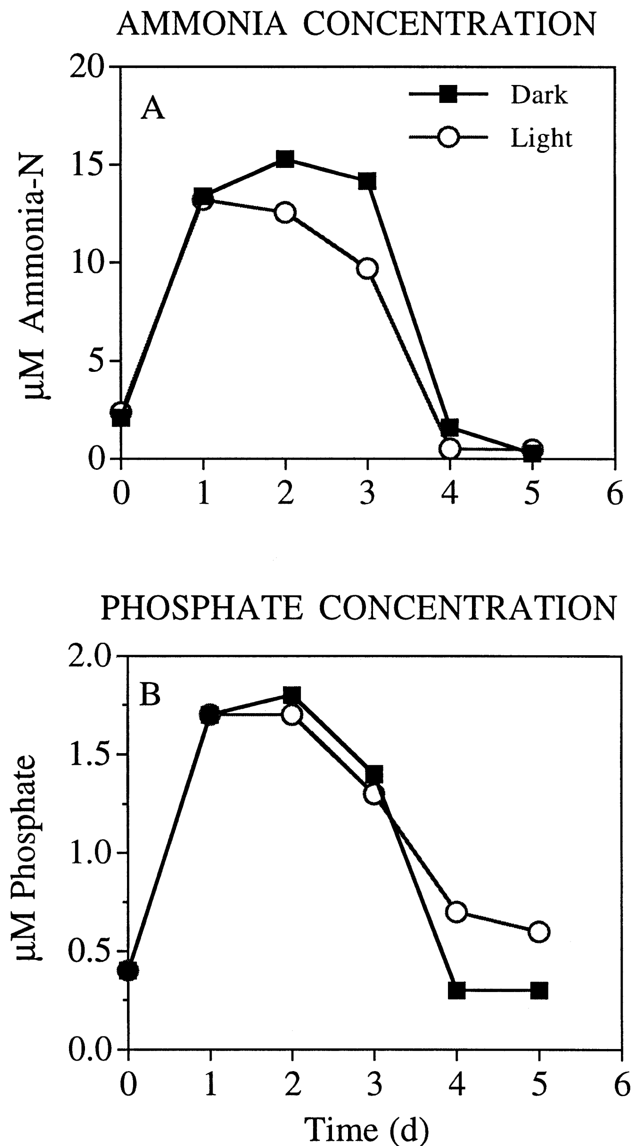

Fig. 6. Changes in concentration of dissolved ammonia (A) and phosphate (B) in the low-nutrient, glucose-supplemented treatments with live bacteria.

Axenic growth of *Ochromonas* sp. was significantly enhanced ($P<0.05$) in light and continuous darkness in the modified DY-IV media relative to the minimal salts medium with or without vitamins or glucose (Table 2, Fig. 3B vs Fig. 2A, 2B). However, cultures incubated in the light in the modified DY-IV showed a small increase in total biomass due to maintenance of larger cell size relative to the dark (Figs. 3D, 3F). The growth rates of *Ochromonas* sp. in complete DY-IV medium were significantly higher than rates in the modified DY-IV in both light and dark treatments ($P < 0.05$). As in the modified DY-IV medium, growth rate in complete DY-IV was not statistically different in the dark relative to the light (Table 2), but average cell size (Fig. 3C) was significantly larger in the light ($P<0.05$). In this case, the larger cell size resulted in an accumulation of algal biomass that was much greater in the light than in continuous darkness for the complete DY-IV treatments (Fig. 3E). These

Table 3. Ingestion rates (bacteria alga^{-1} h^{-1}) at t = 27h in nutrient-replete and nutrient-deplete (glucose added) treatments for experiments with non-axenic *Ochromonas*[a]

	Nutrient replete			Nutrient deplete		
	Dark	Low light	High light	Dark	Low light	High light
FLB	33	36	34	33	34	36
Disappearance	17	18	18	17	17	18

[a] FLB rates were calculated from ingestion of fluorescently labeled bacteria; disappearance rates were calculated from changes in bacterial abundance and the geometric mean abundance of the predator *Ochromonas* sp.. Calculations did not account for bacterial growth in the treatments. Low and high light intensities were 75 and 460 μE m^{-2} s^{-1}, respectively.

results indicate an interaction of photosynthesis with the osmotrophic nutrition of the alga that affected cell size but not the specific growth rate. The differences in cell size were, at least in part, a function of larger chloroplast size in cells incubated in the light.

The differences between algal populations grown in complete vs modified DY-IV suggest that dissolved organics can contribute more than photosynthesis to biomass accumulation in this strain of *Ochromonas.* Used as a buffer in the original DY-IV formulation, the addition of MES also resulted in a much higher concentration of dissolved organic material than was present in the modified DY-IV medium (i.e., without MES). Cultures in DY-IV, with and without MES, showed only minor changes in pH, so we infer that MES was used by the alga as a source of energy, carbon, nitrogen, sulfur, or some combination of these constituents via osmotrophy. Axenic cultures of the BG-1 strain of *Ochromonas* also grew well with and without light in other buffered and unbuffered organic media (R. Sanders, personal observation). These included 0.1% cerophyll (buffered), 0.5% yeast extract, and a complex organic mixture (1 gL^{-1} each of glucose, bacto-peptone, and yeast extract, plus trace metals). Growth on organic media is not unique to this strain of *Ochromonas.* Closely related species of mixotrophic algae, including *Ochromonas* sp., *O. danica, O. minuta,* and *Poterioochromonas malhamensis,* also can be cultured axenically in rich organic broths [27], although they often grow more rapidly when preying on bacteria.

Growth in Bacterized Media

Nutrient acquisition by *Ochromonas* sp. in the presence of bacteria was almost exclusively accomplished via phagotrophy. This conclusion is strongly supported by our comparison of axenic and bacterized cultures. The highest growth rates for the BG-1 strain of *Ochromonas* in the present study were observed in treatments with live bacteria (Table 2), which is consistent with laboratory investigations of some other *Ochromonas* strains that found the addition of particulate food significantly enhanced algal growth [2, 6, 12]. Growth rates of *Ochromonas* sp. (BG-1) in treatments with bacteria (range of μ = 1.1–2.6 d^{-1}) were significantly greater than those grown axenically in DY-IV medium with or without MES ($P < 0.01$), or in minimal media with or without the vitamin supplement (Table 2). Moreover, growth rates and overall increases in algal biomass in the glucose-supplemented treatments with live bacteria were greater than in the nutrient-replete cultures with bacteria (Fig. 4B vs 4A) even though dissolved inorganic nutrients were in much higher concentrations in the latter treatment. This suggests that phagotrophy could supply essentially all of the major nutrients (C, N, P). An alternative explanation is that the higher growth rate in bacterized cultures was attributable to production of some limiting element or metabolism of algal waste products by the bacteria. Our data cannot eliminate a potential contribution of trace compounds excreted by bacteria or recycling of algal waste by bacteria. However, the rapid growth of the alga in the dark when bacteria were the only source of carbon suggests that the major bacterial contribution to *Ochromonas* growth is as a prey item.

Changes in the biovolume of algal cells in the bacterized cultures also provided interesting comparisons in relation to changes observed in the axenic cultures. Algae grown axenically in the dark in the presence of organic compounds showed marked decreases in average cell size relative to treatments in the light (Figs. 2D, 3C, 3D). In contrast, all treatments with bacteria showed significant ($P < 0.05$) decreases in average cell size regardless of the light regime (Figs. 4C, 4D). This suggests that algae grown in the light with dissolved organic compounds may utilized their photosynthetic ability, whereas algae feeding actively on bacteria probably expended little energy on maintaining the photo-

synthetic apparatus. These results indicate the strong phagotrophic tendency of this species, and an interesting dichotomy in the physiology of the alga when growing heterotrophically on dissolved vs particulate organic material.

Comparison with Other Ochromonas Species

The most basic finding of this study, the strong heterotrophic tendency of *Ochromonas* strain BG-1, is consistent with previous studies with other species of *Ochromonas* and species in closely related genera [1, 3, 12, 20, 24]. The ingestion rates that we determined for *Ochromonas* strain BG-1 (17-36 bacteria alga^{-1} h^{-1}) are similar to the range (1–35 bacteria alga^{-1} h^{-1}) found for other mixotrophic flagellates of its size [3, 20, 24], although a maximum rate of 190 bacteria alga^{-1} h^{-1} was calculated from assumptions on cell yield for one strain of *Ochromonas* [12]. Other strains of *Ochromonas* ingest cyanobacteria and other algae, and even exhibit cannibalism [2, 5, 12]. Cannibalism was not observed in the BG-1 strain and other particulate foods were not tested.

Some aspects of our results and the interpretation of our data differ from previous reports on *Ochromonas.* The degree to which these differences truly represent unique nutritional strategies among strains/species vs the confounding influence of bacteria in nonaxenic cultures of these algae is not yet clear. For example, in studies with another freshwater *Ochromonas* species in bacterized cultures, Rothhaupt suggested that the alga released N and P when bacterial abundance was high, but took up dissolved nutrients in the light when bacterial abundance was low and photosynthetic growth prevailed [20, 21]. Rothhaupt [20] concluded that bacteria in his cultures were not responsible for significant uptake of dissolved phosphorus in the light, based on the observation that no uptake was detected in the dark. In contrast, a comparison of axenic and bacterized growth of the BG-1 strain in the present study showed little evidence that it could grow photosynthetically via the direct uptake of inorganic nutrients at a rate similar to the strain studied by Rothhaupt [20]. These results would appear to indicate species- or strain-specific differences in the photosynthetic abilities of these two species of *Ochromonas.*

However, reconciling differences between studies employing bacterized or axenic cultures as species-specific differences in mixotroph physiology can be problematic. Ingestion rates in the presence of bacterial abundance near the alga's grazing threshold could support algal growth rates comparable to rates we observed in the absence of bacterial prey. For example, an ingestion rate of 1 bacteria alga^{-1} h^{-1} would be sufficient to support algal growth rates comparable to rates observed in axenic cultures in DY-IV medium without MES (taking into account the carbon content of *Pasteurella* sp. [3] and the biovolume of *Ochromonas* sp., and assuming a carbon:volume ratio of 0.08 and a gross growth efficiency = 40%). Thus, algal biomass could be maintained, or increased slowly, by ingesting bacteria growing on dissolved substrates and remineralized nutrients. That is, it might not be necessary to invoke uptake of dissolved nutrients by the alga to explain the observed growth rates in the presence of bacteria.

It is also worth noting that direct utilization of dissolved organic compounds by *Ochromonas* in bacterized cultures cannot easily be differentiated from indirect utilization (i.e., uptake by bacteria and subsequent ingestion of bacteria by the alga). Dissolved organic compounds in the present study did support moderate growth rates of *Ochromonas* sp. in axenic cultures (Fig. 3). Therefore, some minor component of algal growth (or maintenance of population density) at very low bacterial abundances in bacterized cultures may be a consequence of heterotrophic activity of the alga that is not a consequence of phototrophy or phagotrophy. However, in order for this behavior to contribute significantly to the nutrition of the alga, it must be able to compete successfully with the bacteria for the uptake of organic substrates. This latter situation is very different from growing on high concentrations of dissolved organic compounds in axenic culture.

Conclusions

Overall, our data indicate a strong dependence on phagotrophy by the BG-1 strain of *Ochromonas* that is consistent with information on other species within this genus. Our studies with axenic and bacterized cultures of this species have indicated that photosynthesis by axenic cultures of the alga in the presence of dissolved organic compounds increased the rate of accumulation of algal biomass. Although similar rates of increases in cell number were observed in the light and dark, significantly larger cell sizes in the light resulted in higher biomass in the light during the same experimental period. When grown in the presence of bacterial prey, chloroplast size and cell size were reduced, and the alga excreted N and P. These data suggest that *Ochromonas* sp. did not store significant amounts of these nutrients for photosynthetic growth in excess of those required for heterotrophic growth.

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