Photoadaptation Alters the Ingestion Rate of *Paramecium bursaria*, a Mixotrophic Ciliate

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Bacteriovorous protozoa harboring symbiotic algae are abundant in aquatic ecosystems, yet despite a recent interest in protozoan bacterivory, the influence of light on their ingestion rates has not been investigated. In this study, *Paramecium bursaria* containing endosymbiotic *Chlorella* was tested for the effect of light on its ingestion rate. *P. bursaria* was grown for 4 to 6 days under five different light fluxes ranging from 1 to 90 microeinsteins s\(^{-1}\) m\(^{-2}\). Ingestion rates were determined by using 0.77-μm-diameter fluorescent microspheres. 4',6-Diamidino-2-phenylindole dihydrochloride-labeled *Enterobacter cloacae* was used in one experiment to confirm differences in uptake rates of bacteria by *P. bursaria*. Unlike phagotrophic photoflagellates, the ciliates demonstrated different ingestion rates in response to different light intensities. Although symbionts contribute carbon to their host via photosynthesis, the paramecia of the present study fed faster after exposure to higher light intensities, whereas their aposymbiotic counterparts (lacking endosymbionts) were unaffected. Light-induced changes in ingestion rates were not immediate, but corresponded to the period of time required for endosymbiont populations to change significantly. This strongly suggests that the symbionts, stimulated by higher light levels, may dictate the feeding rates of their hosts. Thus, light, apart from temperature, may influence the impact of certain protists on natural bacteria and may affect laboratory-based determinations of protistan feeding rates.

In nature many species of protozoa, particularly ciliates and amoebae, harbor algal endosymbionts. As many as 41 species of freshwater ciliates and 25 species of freshwater amoebae have been found to contain algal endosymbionts (6), and in certain freshwater ecosystems a single symbiont-bearing species can constitute an average of 64% of ciliate biomass (2). Nearly all large planktonic protozoa in the euphotic zone of the marine environment appear to have endosymbiotic algae or flagellates (6). *Mesodinium rubrum*, an extremely widespread marine ciliate species containing cryptomonad symbionts, is abundant in coastal waters from polar zones to the tropics (29), and in shallow waters of the tropics, benthic foraminifera belonging to 11 families have been found to harbor photosynthetic endosymbions (29). Interactions between host and symbiont include nutritional contributions of each member. The protozoan host may contribute nutrients as well as increased motility to the photosynthetic component. Ciliates containing *Chlorella* as endosymbionts display photoaccumulation, i.e., positive phototaxis (21). The major contribution of the algal symbiont is maltose, and up to 38% of photosynthetically fixed material from the *Paramecium bursaria-Chlorella* complex has been found in the ciliate fraction (22). Effects of light on growth and other aspects of the *P. bursaria-Chlorella* association have been well studied (11, 12, 15, 32, 33). The effect of light on the rate at which the hosts consume bacteria, however, has not been examined, and a recent resurgence of interest in protistan bacterivory (5, 20, 25-27) may have overlooked the effect of light on feeding rates of protozoa with algal endosymbionts. Since the diet of protozoan hosts can be augmented with products of symbiont photosynthesis (19, 21), we were interested in finding whether exposure of the protozoan hosts to various light regimens would alter their rate of particle uptake.

**MATERIALS AND METHODS**

**Cultures.** Cultures of *P. bursaria* used in the first sets of experiments described below were obtained from the Carolina Biological Supply Co., Burlington, N.C. Additional cultures for later experiments were obtained from Carl Johnson of Vanderbilt University. These included *P. bursaria* stock Mit B syngen 1, mating type I (green), and stock Mit-C\(_a\), syngen 1, mating type III (aposymbiotic, i.e., without symbiotic *Chlorella*). Paramecia were cultured and tested at 22 to 24°C in medium of lettuce juice (1.25%, wt/vol) in K-DS solution (0.6 mM KH\(_2\)PO\(_4\), 1.4 mM Na\(_2\)HPO\(_4\), 2 mM sodium citrate, 1.5 mM CaCl\(_2\), pH 7.0) (14). The medium was inoculated with 24-h-old *Klebsiella pneumoniae* 1 day prior to inoculation with *P. bursaria*. Stock cultures were grown at a light intensity of 37 microeinsteins s\(^{-1}\) m\(^{-2}\). Cultures of *K. pneumoniae* and *Enterobacter cloacae* were obtained from S. Goss of Tennessee Technological University and were grown on nutrient agar or in nutrient broth at 35°C.

**Feeding studies.** Light was provided by two 40-W cool-white fluorescent bulbs. Light flux was measured with an LI 190 SA Li-Cor quantum sensor (Li-Cor, Lincoln, Neb.) connected to a Grant 1201 data logger (Science/Electronics, Dayton, Ohio) which read in millivolts. The readings were converted to microeinsteins per second per square meter in the photosynthetically active radiation range (400 to 700 nm). Different light intensities were achieved by positioning the protozoa at various vertical distances from a light source. These experiments were conducted in an environmental chamber (Environmental Specialties, Inc., Raleigh, N.C.) with circulating air to maintain a constant temperature of 23 ± 1°C and 65% humidity at all light levels. To monitor

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temperature at the position of the protozoan suspensions, additional beakers containing water were placed at the various positions, and the water temperature was measured 8 h after the lights came on.

For assessment of the effect of different light intensities at a constant photoperiod, suspensions of P. bursaria were divided into 15-ml aliquots and placed in 50-ml beakers with plastic petri dish lids covering the top. They were fed once initially with washed suspensions of K. pneumoniae and then exposed for 4 to 6 days to light fluxes of 1, 7, 13, 37, and 90 microeinstein s\(^{-1}\) m\(^{-2}\) on a 16:8-h light-dark cycle. This experiment was first performed with only 1, 7, 37, and 90 microeinstein s\(^{-1}\) m\(^{-2}\).

Ingestion rates were determined by using fluorescent microspheres (beads) 0.77 \(\mu\)m in diameter (Polysciences, Inc., Warrington, Pa.) as a model for bacteria (3, 16). A suspension of beads made in a buffered salts solution (23) was added to the protozoan suspension to yield a final bead concentration of \(10^7\) beads per ml. Prior to each experiment, bead suspensions were examined microscopically to assure that beads were monodispersed. The paramecia were allowed to feed for 5 or 6 min at the light intensity at which they were maintained, after which 20-\(\mu\)l subsamples were transferred to a glass microscope slide, fixed with formalin, and examined immediately for uptake of fluorescent beads, using epifluorescence microscopy. Fluorescing beads within the protozoa were enumerated. The brief feeding period was required to feed for 5 or 6 min at the light intensity at which they were maintained, after which 20-\(\mu\)l subsamples were transferred to a glass microscope slide, fixed with formalin, and examined immediately for uptake of fluorescent beads, using epifluorescence microscopy. Fluorescing beads within the protozoa were enumerated. The brief feeding period was required to

![Graph](http://aem.asm.org/)

**FIG. 1.** Effect of light intensity on bead uptake by P. bursaria. Values are means \(\pm\) 1 standard error. The cells were maintained at the various light intensities 4 days prior to feeding. The three highest uptake rates were not significantly different from each other, but each was significantly higher than rates at the two lowest intensities. \(\mu\)E, microeinstein.

After this period, suspensions from the highest light intensity were split for determination of feeding at the two light intensities. One set was fed for 6 min at the highest intensity; the other set was fed for 6 min at the lowest intensity. Likewise, those acclimated to the lowest intensity were fed at both the lowest and the highest intensities.

Adaptation was examined further by monitoring bead uptake rates daily for P. bursaria exposed to four different light intensities. The additional strains, including the apobiosymbiotic controls, were also examined daily for effect of light on ingestion rates.

Light has been shown to increase the number of endosymbionts in P. bursaria (17, 22). Therefore, to find whether the adaptation time for altered ingestion rates of the present study corresponded to the time required for the endosymbiont populations to change significantly, the number of Chlorella per Paramecium was determined daily until significant differences in endosymbiont concentrations were observed for paramecia grown in the highest and lowest light intensities. Ten individuals from each light treatment were randomly selected and lysed by allowing them to burst under the coverslip of a microscope slide. Lysing paramecia released endosymbionts for more accurate enumeration. Since Chlorella within the paramecia from the highest light intensity were actively reproducing, they exhibited a range of sizes and shapes which were difficult to distinguish from other small vacuoles and organelles of their hosts. Therefore, epifluorescence microscopy was used to observe autofluorescence of the symbionts, facilitating enumeration.

**RESULTS AND DISCUSSION**

**Feeding rates.** Results of the first feeding studies (Fig. 1) show that paramecia from the three highest light intensities yielded higher ingestion rates than those from the two lowest light intensities. A linear relationship \((r = 0.84)\) between light intensity and ingestion rate was observed. The experiments that used only four light intensities yielded the same result; i.e., rates at the two highest light intensities were significantly \((P < 0.05)\) higher than those at the two lowest intensities.
Experiments with the new strains of green and apysymbiotic paramecia showed differences between the lowest and each of the three highest intensities for the green paramecia, whereas no differences in ingestion rates were observed between any two light intensities for the apysymbiotic paramecia (Fig. 2). Rates of the green paramecia increased linearly with increasing light intensity \((r = 0.83)\), whereas rates of apysymbiotic paramecia did not \((r = 0.39)\). Relationships between feeding and light intensity were nearly identical for the symbiont-containing paramecia obtained from the two different sources. 4',6-Diamidino-2-phenylindole dihydrochloride-labeled \(E.\ cloaca\) was consumed at different rates, consistent with the results of the bead studies. Paramecia containing symbionts ingested an average of \(23 \pm 3.8\) bacteria per individual in 6 min when grown in the highest light intensity compared with an average of \(9 \pm 1.7\) bacteria per individual when grown in the lowest light intensity. Sherr et al. (27) reported that larger ciliates showed no preference for bacteria over beads, although small flagellates did. The question of food selectivity was not examined in the present study.

Feeding rates were not normally distributed; i.e., the frequency distribution of the number of beads ingested per individual examined did not follow a normal curve. Therefore, nonparametric analyses were required. This phenomenon was also observed for marine ciliated protozoa feeding on beads and bacteria (27a).

Light temperature differences occurred between certain light levels; however, this can be ruled out as a factor in altering feeding rates. In one experiment, the temperature of water positioned at each of the light intensities ranged from 22 to 23°C, but this range could not account for the differences in feeding rates since equal temperatures at 7 and 13 microeinsteins \(s^{-1} \text{ m}^{-2}\) yielded different feeding rates; no differences in rates occurred between 1 and 7 microeinsteins \(s^{-1} \text{ m}^{-2}\) when the temperatures were 22.0 and 22.5°C, respectively. Moreover, in experiments with the apysymbiotic paramecia, no differences were observed over the entire range of light intensities and their corresponding temperatures.

There were no significant differences in numbers of bacteria in the test media; therefore, the bead/bacteria ratios were equal in all treatments. This rules out an apparent difference in feeding rates due to differences in numbers of bacteria present during feeding studies.

**Adaptation.** The light intensity at which the paramecia were fed for 6 min had no effect on feeding rates. Those grown for 4 days at the lowest intensity \((1 \text{ microeinstein s}^{-1} \text{ m}^{-2})\) had similar rates when fed at either the low or the high light intensity \((2.3 \pm 0.9 \text{ and } 1.2 \pm 0.3 \text{ beads per individual, respectively})\). Likewise, those grown at the high intensity \((90 \text{ microeinsteins s}^{-1} \text{ m}^{-2})\) exhibited no difference in feeding rates when fed at either the low or high intensity \((20.2 \pm 2.8 \text{ and } 27.8 \pm 6.2 \text{ beads per individual, respectively})\); yet the average rate of paramecia acclimated to the high-intensity light was significantly higher than the average rate of those acclimated to the low-intensity light, consistent with results of all other experiments of the present study. This indicates that an adaptation period of between 6 min and several days had occurred rather than an immediate response to a particular light intensity.

In general, 3 to 6 days of acclimation to different light intensities were required before differences in feeding rates were observed with endosymbiont-bearing populations. Daily monitoring of feeding rates revealed that significant differences in rates began to appear after 3 days for \(P.\ bursaria\) obtained from the Carolina Biological Supply Co., indicating an adaptation period after removal from the light conditions of the stock cultures. The green strains obtained from Vanderbilt University showed differences between the lowest and each of the three highest intensities between 4 and 6 days. Since apysymbiotic strains were unaffected by light intensity, daily monitoring of ingestion rates was not conducted for these paramecia.

Chlorella symbionts reproduce within \(P.\ bursaria\), and investigators have reported increased numbers of such symbionts in paramecia grown in light versus continuous darkness (22). In the present study, daily enumeration of Chlorella per Paramecium revealed that significant differences in "symbiont load" appeared at 4 days, with the greater number of symbionts within ciliates grown at the higher light intensities. The average number of Chlorella per Paramecium on the day the cultures were initially split was 467. By the fourth day, the average numbers were 568 and 381 for those in high and low light, respectively. Figure 3 shows the symbiont numbers of high- and low-light-adapted paramecia over time. The period of 4 days closely corresponds to the adaptation period required for altered feeding rates to occur, strongly suggesting that the symbiont load, stimulated by higher light intensities, dictates feeding rates of their host ciliate.

Results of the present study indicate that exposure to various light regimens may affect the feeding rates of certain protozoa harboring algal endosymbionts and thereby influence the protozoan impact on bacterial populations. Others have reported effects of light on nutrient uptake by symbiont-bearing hosts. Lee and Zucker (13) reported that light increased \(^{14}\text{C}\) uptake by foraminifera with algal symbionts, but no distinction could be made between direct uptake of labeled compounds and indirect uptake via ingestion of bacteria. Thorington and Margulis (31) found that light can influence the quantity and rate of nutrient transfer from hydra to their symbionts, and although food ingestion rates were not examined in this study, the twofold-greater total thymidine content in hydras grown in the light indicated that ingestion rates were stimulated by light.
In contrast, Stoecker et al. (28) examined obligate mixtropathy in ciliates which retain functional chloroplasts derived from ingested algae and found that ingestion of algal food was not significantly altered by exposure to three different light fluxes after 14 h of feeding. However, there may not have been sufficient time for adaptation to occur, and species differences among ciliates cannot be ruled out. Phagotrophic photoflagellates which can ingest bacteria have received much attention recently due to their potential impact on bacterial populations (3, 5, 20, 24). Studies have reported no effect of light on feeding rates of Ochromonas danica (1) and Dinobryon species (4). Data of Porter (20), however, show that ingestion rates of the photoflagellate Poterioochromonas malhamensis decreased at very high light levels (1,500 microeinstein s⁻¹ m⁻²) but were relatively constant at 0 to 400 microeinstein s⁻¹ m⁻².

The mechanism by which light increases the ingestion rate of P. bursaria is undetermined. The respiration rate of algae is very high compared with that of the host (18), and this suggests that the algae may consume large amounts of organic matter during respiration. Since nutrients for the algae must derive from host predation, increased algal activity stimulated by higher light intensities may cause the protozoan host to feed at a higher rate to meet the algal demand. This contrasts the suggestion of ter Kuile et al. (30) that feeding in a foraminiferan stimulates photosynthesis of its symbionts. However, Jorgensen et al. (10) found that photosynthesis of symbionts within a foraminiferan increased linearly with increasing light intensities up to 100 microeinstein s⁻¹ m⁻², and this supports our findings of linear increases in feeding with increasing light up to 90 microeinstein s⁻¹ m⁻², if, in fact, algal activity stimulates feeding. Such symbiotic interactions may account for the different feeding behaviors exhibited by P. bursaria and photoflagellates in response to light.

Natural environmental conditions which reduce light, such as long periods of overcast days or increased turbidity due to plankton blooms and runoff from storm events, may reduce the protozoan host predation rate in certain environments in which symbiont-bearing species may be abundant. Field investigations of protozoan bacteivory should take such conditions into consideration as well as assess the numerical significance of protozoan-algal symbiotic associations in natural assemblages.

In the laboratory, multiple samples or cultures should be maintained in equivalent light intensities and exposure periods. In our study, altered feeding rates resulted when the difference in distance from a light source was <0.33 m, emphasizing the importance of stock maintenance conditions. Further research regarding the proportion of protozoan-symbiont associations in natural populations is needed as well as a systematic screening of light effects on other protistan host species to assess the significance of light on natural bacterivory.

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REFERENCES


