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MIXOTROPHY AND NITROGEN UPTAKE BY *PFIESTERIA PISCICIDA* (DINOPHYCEAE)[1]

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The nutritional versatility of dinoflagellates is a complicating factor in identifying potential links between nutrient enrichment and the proliferation of harmful algal blooms. For example, although dinoflagellates associated with harmful algal blooms (e.g. red tides) are generally considered to be photo. trophic and we inorganic nutrients such as nitrate or phospbate, many of these species also have pronounced heterotrophic capabilities either as osmotrophs or phagotrophs. Recently, the widespread occurrence of the heterotrophic toxic dinoflagellate, *Pfiesteria piscicida* Steidinger et Burkholder, has been documented in turbid estuarine waters. *Pfiesteria piscicida* has a relatively proficient grazing ability, but also has an ability to function as a phototroph by acquiring chloroplasts from algal prey, a process termed kleptoplastidy. We tested the ability of kleptoplastidic *P. piscicida* to take up ^{15}N-labeled NH_4^+, NO_3^-, urea, or glutamate. The photosynthetic activity of these cultures was verified, in part, by use of the fluorochrome, primulin, which indicated a positive relationship between photosynthetic starch production and growth irradiance. All four N substrates were taken up by *P. piscicida*, and the highest uptake rates were in the range cited for phytoplankton and were similar to N uptake estimates for phagotrophic *P. piscicida*. The demonstration of direct nutrient acquisition by kleptoplastidic *P. piscicida* suggests that the response of the dinoflagellate to nutrient enrichment is complex, and that the specific pathway of nutrient stimulation (e.g. indirect stimulation through enhancement of phytoplankton prey abundance vs. direct stimulation by saprotrophic nutrient uptake) may depend on *P. piscicida*'s nutritional state (phagotrophy vs. phototrophy).

Key index words: **cryptophytes; fish kills; harmful algal bloom; kleptoplastidy; nutrient loading; *Pfiesteria piscicida*; primulin; starch; toxic dinoflagellates**

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Pfiesteria piscicida Steidinger et Burkhotder (Dinophyceae) is one of several myzocytotically feeding "heterotrophic" dinoflagellates with a reported capability for kleptoplastidy, the process by which functional chloroplasts are retained from algal prey (Larsen 1992, Laval-Peuto 1992, Schnepf and Elbrächter 1992, Lewitus et al. 1999). Although this form of mixotrophy has been acknowledged in dinoflagellates for a number of years, studies examining the role and regulation of phototrophic and phagotrophic nutrition in kleptoplastidic species are rue (e.g. Fields and Rhodes 1991, Skovgaard 1998), at least partly a consequence of the difficulty in recognizing and culturing kleptoplastidic dinoflagellates (Schnepf et al. 1989, Schnepf and Elbrächter 1992, Lewitus et al. 1999). Schnepf and Elbrächter (1992) and Skovgaard (1998) suggested that dinoflagellates with an inconsistent chloroplast number per cell may be kleptoplastidic, and further speculated that the ability is more common in dinoflagellates than previously considered. It is also possible that problems associated with maintaining seemingly "phototrophic" dinoflagellates in a laboratory culture may be related, in some cases, to the unrecognized need to replenish prey supply. Awareness of tbe prevalence of these and other mixotrophic protists has grown dramatically in recent years (Stoecker 1998), stressing the need to understand their physiological ecology and role in microbial food web function.

Stoecker's (1998) classification scheme for mixotrophic protists presented a conceptual model for kleptoplastidic "protozoa" (e.g. some ciliates, sarcodines, and dinoflagellates) based on their relative dependency on phototrophic vs. phagotrophic nutrition, and the response of these processes to light,

dissolved inorganic (nutrients, and particulate food availability. In this model: (a) carbon, nitrogen, and phosphorus are acquired primarily through phagotrophy; (b) growth ivery slow or ceases in the prolonged absence of prey; and (c) photosynthesis is thought to be used to supplement carbon nutrition by covering respiratory demands during periods when prey arc scarce. The data to support the model are based primarily on information from studies with sarcodines or ciliates. Recently, however, Skovgaard (1998) suggested that photosynthesis in kleptoplastidic *Gymnodinium 'gracilentum'* is used primarily to enhance survival under food limitation, based on the short (-2 day) turnover time of kleptochloroplasts.

Kleptoplastidy also has been demonstrated in *Pfiesteria piscicida* (Lewitus et al. 1999), an ichthyotoxic dinoflagellate that, over the last decade, has been implicated as a causative factor of fish kills in North Carolina estuaries and the Chesapeake Bay (Burkholder et al. 1992, 1995, Burkholder and Glasgow 1997, Maryland Department of Natural Resources 1997, University of Maryland Center for Environmental Science 1997). Unlike *G. gracilentum*, a longer kleptochloroplast retention time (at least 9 days) was observed in *P. piscicida*, but Lewitus et al. (1999) considered this and the low photosynthetic rates (0.54 pg C·cell^{-1}·h^{-1}) underestimates of photosynthetic capacity because of the limiting growth irradiance used in their experiment. Even at that low irradiance, however, a population doubling was measured, suggesting that phagotrophy was not essential for cell division. The growth, or even maintenance, of photosynthetically active *P. piscicida* populations implies that nutrients were taken up saprotrophically in support of photoautotrophy. The potential ability of *P. piscicida* to acquire nutrients directly has implications toward the hypothesized role of nutrient loading in promoting the dinoflagellate's growth and toxic activity (Burkholder et al. 1995, 1997, 1998, Burkholder and Glasgow 1997, Lewitus et al. 1999). That is, recognition that *P. piscicida* can function as a phytoplankter, and not just as a heterotrophic protist, stresses consideration of nutrient stimulatory mechanisms analogous to those that affect phytoplankton. In this study, we tested the hypothesis that kleptoplastidic *P. piscicida* can take up N nutrients directly.

MATERIALS AND METHODS

The *Pfiesteria piscicida* isolate was obtained from the Neuse River estuary, North Carolina, and its identity was confirmed by thecal plate configuration (Steidinger et al. 1996), using scanning electron microscopy (Glasgow, Jr., unpubl.). The nontoxic zoospores used in this experiment were derived from toxic zoospore cultures maintained in fish aquariums. Before beginning the experiment, toxic zoospores were transferred to media (f/2-enriched filtered seawater; Guillard 1975 but without Si) containing *Rhodomonas* sp. Karsten CCMP757 (Cryptophyceae), an 8-μm diameter cryptophyte previously shown to be a preferred prey species and source of kleptochloroplasts for *P. piscicida* (Glasgow et al. 1998, Lewitus et al. 1999). Thii culture was maintained at 25° C, 15 ppt, and a light:dark cycle of 12:12 h (80 μE·m^{-2}·s^{-1}), and inspected microscopically several times a day for *P. piscicida* feeding behavior.

After 5 days of maintenance, *P. piscicida* was observed to shift from a lag phase, when grazing was not apparent, to an active feeding phase when the majority of the population was observed to rapidly ingest cryptophytes (referred to as a "swarming response" or "feeding event"; Glasgow et al. 1998, Lewitus et al. 1999). This feeding event was followed by a reduction in grazing activity, decreased cryptophyte abundance, and increased *P. piscicida* abundance. Five hours after the feeding event, aliquots (73 mL) from this culture were gently transferred to 18 125-ml flasks. Six of these were sampled immediately (designated "initial" treatment), and 12 were placed in the dark. After being kept for 15 h in the dark, the 12 flasks were incubated at a relatively low irradiance (70 μE·m^{-2}·s^{-1}) for 3 b, after which six flask, were sampled (designated "low light" treatment). and the remaining sir placed under a relatively high irradiance (360 μE·m^{-2}·s^{-1}) and sampled 3 h *later* (designated "high light" treatment). The principle of this experimental design was to allow comparison of starch content and N uptake in: (a) "initial" J? *piscicida* populations (containing recently ingested chloroplasts); (b) "low light" populations (kleptoplastidic cultures incubated for a short rime under a relatively low irradiance); and (c) "high light" populations (kleptoplastidic cultures examined after a shift-up in growth irradiance). These three populations were considered to be kleptoplastidic based on microscopic insspections indicating that grazing on cryptophytes did not occur after the initial feeding event, and examination of DAPI-stained samples indicating that plastid-containing *P. piscicida* did not contain cryptophyte nuclei (Lewitus et al. 1999).

The above cultures were sampled for *P. piscicida* and *Rhodomonas* cell counts, particulate nitrogen, dissolved nutrients (NO_3^-, NO_2^-, dissolved free amino acids (DFAA), urea, and NH_4^+), uptake of ^{15}N-labeled NH_4^+, NO_3^-, urea, or glutamate, and dinoflagellate and cryptophyte cell, chloroplast, and starch areas. Cell counts were measured on acid-Lugol's-fixed samples, using a Nageotte Bright Line hemacytometer (depth 0.5 mm). For particulate nitrogen, water was filtered onto precombusted Whatman GF/C filters, frozen, and later measured using a Control Equipment CHN analyzer (Parsons et al. 1984). Dissolved nutrients were measured in the filtrate from sterile 0.45-μm polycarbonate membrane filters. NO_3^- and NO_2^- concentrations were determined colorimetrically using a Technicon autoanalyzer, and urea was measured by the urease method (Parsons et al. 1984). NH_4^+ concentration was determined by the phenol/hypochlorite technique (Solarzano 1969). and DFAA determined by reverse-phase high-performance liquid chromatography (Lindroth and Mopper 1979). Nutrient uptake rates were determined using ^{15}N tracer techniques, following Glibert and Capone (1993). Labeled substrates were added at an initial concentration of 2 μg at N L^{-1}, and samples were incubated for 30 min. Isotopic analyses were conducted using a Finnigan MAT 251 mass spectrometer, coupled with a Europa ANCA sample inlet system.

Cell morphological parameters were determined using image analysis (Optronics image analysis system with Flashpoint software) on 4',6-diamidino-2-phenylindole (DAPI)- or primulin-stained samples. Samples were fixed with 0.5% $NiSO_4$ (Sieracki et al. 1987) and then 2% hexamethylenetetramine-buffered formaldehyde (Throndsen 1978. Smecker et al. 1989) incubated with a fluorochrome (DAPI or primulin), filtered, and the filters rapidly frozen on a liquid nitrogen-cooled steel Mock (Lewitus et al. 1999). The DAPI-staining method followed Porter and Feig (1980) and was used to determine the percentage of plastid-containing zoospores that also contained ingested cryptophyte nuclei (stained by DAPI). As mentioned. DAPI fluorescence indicative of the presence of cryptophyte nuclei inside zoospores was not observed, and therefore these data are not presented. The primulin method followed Caron (1988). Like DAPI, primulin has an excitation maximum in the ultraviolet, but, whereas DAPI stains DNA, primulih reacts with plasmalemma, thecal plates, and starch (Sterling 1964. Revilla et al. 1986, Klut et al. 1989). We used primulin fluorescence to estimate the starch area within

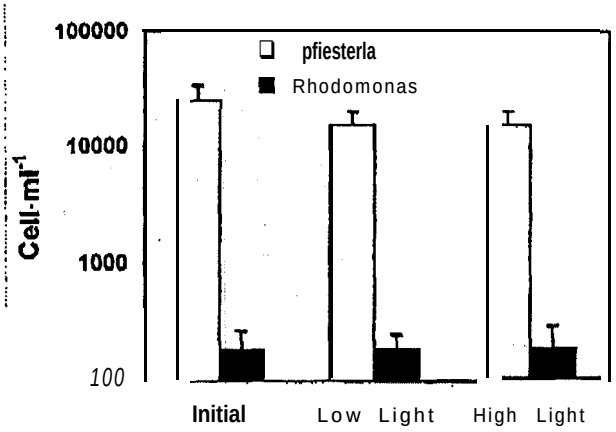


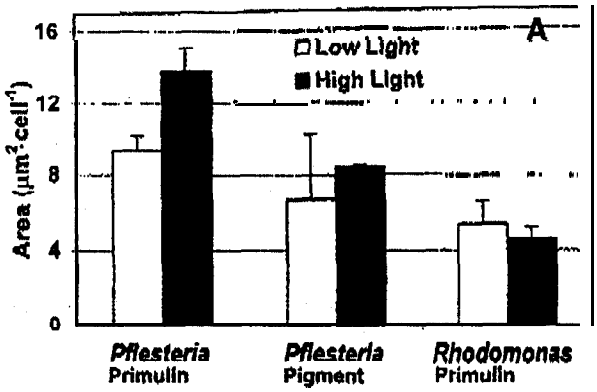
FIG. 1. The mean and standard deviation of *Pfiesteria piscicida* white) or *Rhodomonas* sp. (black) abundance in the initial, low ght, or high light cultures.

free-living cryptophyte chloroplasts and zoospore kleptochloroplasts. Starch is a photosynthetic product of cryptophytes that is produced within the periplastidal space (Santore 1985), and a previous study demonstrated the accumulation of starch within *P. piscicida* kleptochloroplasts (Lewitus et al. 1999).

In another experiment, we tested the relationship between the primulin-fluorescent area within *Cryptomonas* sp. HP9001 (Cryptophyceae) cells and the cellular nonstructural carbohydrate in batch cultures grown at three irradiances (211, 104, or 8 $\mu E \cdot m^{-2} \cdot s^{-1}$) and sampled from exponential growth phase. Samples were harvested by centrifugation, resuspended in a phosphate buffer, pH 6.8 (Lewitus and Caron 1990), and cell material extracted by cell disruption, using a Biospecs Products Mini-Beadbeater-8™ (two 45-s runs on "Homogenize" setting using 0.5-mm diameter zirconia/silica beads). After centrifugation (microcentrifuge, 16,000 × *g*), the supernatant and Pellet were analyzed for total nonstructural carbohydrates, using 30% perchloric acid extraction and a colorimetric assay based on the phenol-sulfuric acid reaction (Burke et al. 1992). Absorbance was converted to concentration in milligram glucose equivalents Per milliliter using a standard curve (r = 0.9932).

A *t*-test (significance level of 0.05) was used in all comparisons of means referred to below.

FIG. 2. Epifluorescence micrograph of primulin-stained *Pfiesteria piscicida* zoospore excited by ultraviolet light, showing the kleptochloroplast inside an epithecal vacuole (left). The pigmented area of the kleptochloroplast is reddish orange (phycoerythrin autofluorescence), and the primulin stain shows up as yellowish white fluorescence surrounding the pigmented area. (scale bar, 4 pm).



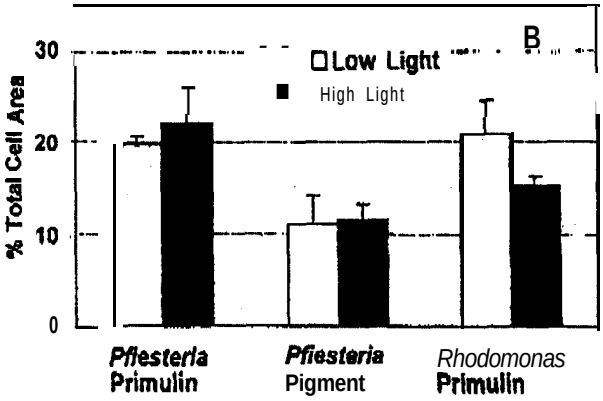


FIG. 3. The mean and standard deviation of (A) area per cell or (B) % total cell area of *Pfiesteria piscicida* primulin fluorescence, *Pfiesteria piscicida* pigment fluorescence, or *Rhodomonas* sp. primulin fluorescence of low light (white) or high light (black) cultures.

RESULTS

Mean *P. piscicida* population abundance in "initial" cultures (those sampled 5 h after the feeding event) was nearly 100-fold greater than that of *Rhodomonas* (Fig. 1). Cryptophyte abundance did not change significantly during the experiment. remaining below 200 cell·mL^{-1}. Cultures incubated under "low" or "high" light contained ca. 40% lower zoospore abundances than initial cultures, but cell numbers did not vary with light treatment.

Primulin-stained samples were analyzed for three variables (Figs. 2, 3): (1) "*Pfiesteria* primulin," the area within the plastid-containing zoosporc vacuole characterized by primulin fluorescence (yellow to white area of epithcca in Fig. 2), (2) "*Pfiesteria* pigment," the area within the plastid-containing zoospore vacuole characterized by pigment (phycoerythrin) autofluorescence (reddish orange area in Fig.

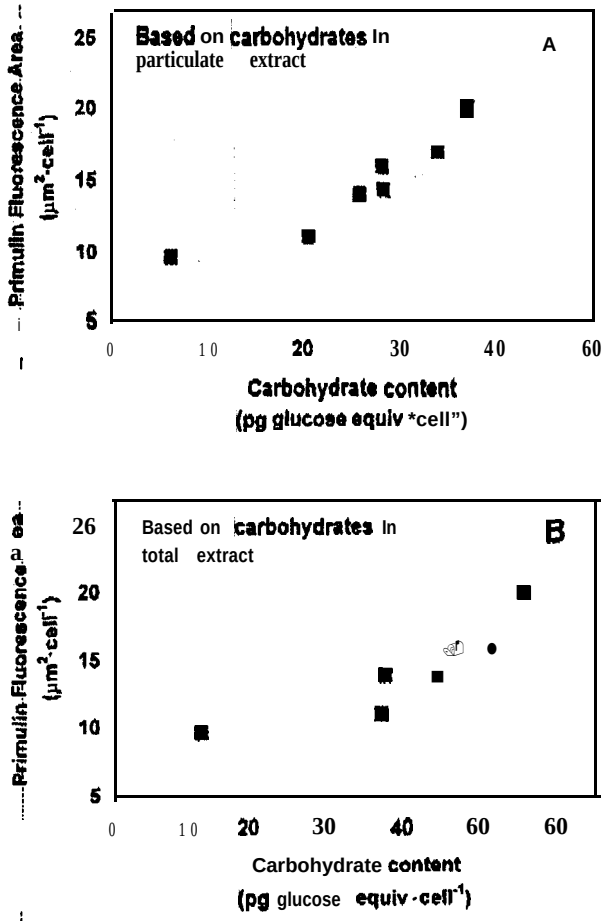


FIG. 4. The relationship between the primulin fluorescence area per cell and nonstructural carbohydrate content per cell in *Cryptomonas* sp. HP9001 cultures. After total nonstructural carbohydrate extraction, carbohydrate concentrations were measured in both the dissolved (supernatant) and particulate (pellet) fractions. The carbohydrate content is plotted, based strictly on the particulate extract (A) or based on the sum of particulate and dissolved extracts (B).

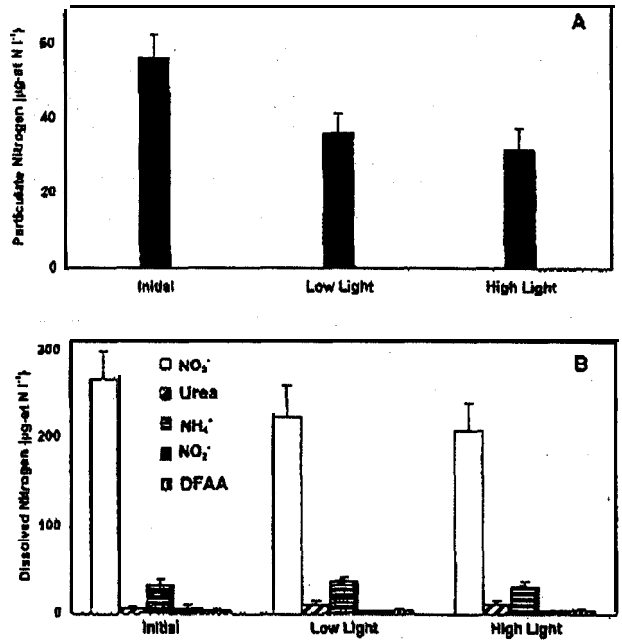


FIG. 5. Mean and standard deviations of (A) particulate nitrogen concentrations and (B) dissolved nitrogen concentrations of initial, low light, or high light cultures. DFAA indicates diilvcd free amino acids.

2), and (3) "*Rhodomonas* primulin," the area within the free-living cryptophyte chloroplast characterized by primulin fluorescence (not included in Fig, 2). In comparing light treatments, the "*Pfiesteria* primulin" area per zoospore was nearly 50% greater in high-light than in low-light cultures, while the amount of "*Pfiesteria* pigment" or "*Rhodomonas* primulin" area did not vary with treatment (Fig. 3A). The treatment effect on *Pfiesteria* primulin fluorescence and not *Rhodomonas* primulin fluorescence is evidence that the positive relationship between growth irradiance and primulin-reactive material (presumably starch) is not a function of prey ingestion. Although the primulin fluorescent area per zoospore was significantly greater in cultures grown at high light, the relative proportion of this area to total cell area did not vary significantly with growth irradiance (Fig. 3B). The average area of high- and low-light cells was 54 and 47 μm^2, respectively (data not shown). The relatively greater absolute, but not proportional, *Pfiesteria* primulin area per cell in high vs. low light cultures suggests that the increase in cell size was related to an increase in primulin-stained cell material. Our assumption that this material was starch is supported by the strong correlation between primulin fluorescence area and nonstructural carbohydratc content in cells of the cryptophyte, *Cryptomonas* sp. (Pig. 4). The correlation coefficient between the primulin-stained area per cell and the carbohydrate content was 0.92 or 0.87 when carbohydrates were measured in the pelleted or total extract, respectively.

Particulate nitrogen decreased after incubation at low or high light by an amount (64% or 55%, respectively; Fig. 5A) that corresponded roughly to the decrease in zoospore cell abundance (61% or 59%, respectively; Fig. 1). The dissolved nitrogen pool. was predominantly composed of NO_3^- throughout the experiment (Fig. 5B), but the mean ratio of NH_4^+, urea, and DFAA to NO; concentration increased over time by 41%, 164%, and 62%, respectively, at low light, and 40%, 183%, and 90%, respectively, at high light. These patterns suggest the net biological removal of NO_3^- and regeneration of NH_4^+, urea, and DFAAs over the course of the experiment.

Uptake of all four ^{15}N-labeled substrates was detected (Table 1). Not surprisingly, given the relatively high NO_3^- concentrations in the dissolved N pool, NO; was taken up at the greatest rate, followed by glutamate, NH_4^+, and urea. Ceils grown at high light took up NH_4^+ or urea at significantly greater rates than low-light-grown cells, but NO_3^- or glu-

TABLE 1. ^{15}N uptake rates (mean ± standard deviation) of cultures grown at low or high irradiance, expressed as absolute label uptake or uptake normalized to the cell abundance of *Pfiesteria piscicida*.

Treatment	Absolute uptake rate (μg at N·L^{-1}·h^{-1})				Uptake per *Pfiesteria piscicida* cell (fmole N cell^{-1} h^{-1})			
	NO_3^-	NH_4^+	Urea	Glutamate	NO_3^-	NH_4^+	Urea	Glutamate
Low light	2.9 ± 0.2	0.13 ± 0.03	0.0020 ± 0.0009	1.00 ± 0.37	200 ± 70	5.4 ± 1.0	0.16 ± 0.01	65 ± 9
High light	1.5 ± 0.4	0.13 ± 0.04	0.0079 ± 0.0029	0.95 ± 0.40	120 ± 50	12 ± 3	0.54 ± 0.21	72 ± 38

tamate uptake did not increase with growth irradiance. Because these were mixed cultures (*P. piscicida* and *Rhodomonas*), it is impossible to determine absolute uptake rates in either group. However, given the relatively low abundance of cryptophytes (ca. 1% of zoospore abundance), the contribution of *Rhodomonas* to overall uptake rates was likely to be very minor. In fact, applying Stolte and Riegman's (1996) maximum NO; or NH_4^+ uptake rate per cell surface area of 8.5 × 10^{-11} μmol·μm^{-2}·h^{-1}, estimates of *Rhodomonas* population (mean area = 45 μm^2) uptake were approximately 0.7 ng at N L^{-1}·h^{-1}, or roughly 0.02% to 0.04% (for NO_3^-) or 0.5% (for NH_4^+) of the measured absolute uptake rates (Table 1). Even in the case of urea, where relatively low uptake rates were measured, theoretical estimates of *Rhodomonas* population uptake (using a maximum uptake rate of 3 fmol N·cell^{-1}·h^{-1}; Syrett et al. 1986, Price and Harrison 1988) only accounted for 28% or 8% of low or high light estimates, respectively. Therefore, the measured N uptake rates can be accounted for predominantly by *P. piscicida* uptake.

DISCUSSION

In Stoecker's (1998) review of mixotrophic models, kleptoplastidic protists were considered to acquire nutrients predominantly through phagotrophic means, and photosynthesis was thought to be important as a survival mechanism for maintaining respiratory requirements during prey limitation. An inference of this model is that photosynthetically driven cell growth (and therefore associated saprotrophic nutrient uptake) is slow relative to that supported by phagotrophy. The potential contribution of photosynthesis to *P. piscicida*'s growth was not addressed in this study, and remains to bc determined as a critical test of the model. However, the present findings suggest that saprotrophic uptake can play an important role in *P. piscicida*'s nutrition. The results not only establish that the dinoflagellate can acquire N-nutrients directly when kleptoplastidic, but, based on the following arguments, suggest that nutrient uptake rates were comparable to those estimated for phytoplankton, and rivaled N uptake through phagotrophy in *P. piscicida*.

When normalized per cell (based on the entire *P. piscicida* population; i.e. with and without ingested plastids present), uptake rates of NO;, NH_4^+, and glutamate (Table 1) were within the range of those reported for phytoplankton (Syrett et al. 1986, Antia et al. 1991, Lomas and Clibcrt 1999A). Hypothetically, direct nutrient uptake may have been predominated by, or even confined to, kleptoplastidic *P. piscicida*. Lewitus et al. (1999) presented evidence for greater survival of kleptoplastidic cells over apoplastidic cells in photosynthetically active *P. piscicida* cultures. The speculation that a fraction of the dinoflagellate population was responsible for the bulk of nutrient uptake would suggest that cellular uptake rates can be potentially higher than those reported here.

Based on previous experiments on *P. piscicida* grazing properties (Glasgow et al. 1998, unpubl. data), comparisons can be made between potential nutrient acquisition via phagotrophy and kleptoplastidy. Ingestion rates by *P. piscicida* zoospores on *Cryptomonas* sp. LP757, a cryptophyte resembling *Rhodomonas* in size and morphology, averaged 1.5 cryptophyte cells per zoospore per day over a 16-h period. Using tbia estimate for ingestion of cryptophytes, assuming that all of the prey contents were ingested per encounter (a false assumption, given the myzocytotic mode of feeding), and using an estimate of cryptophyte N content of 1 pmol·cell^{-1} (Lewitus and Caron 1990), an N uptake rate can be estimated at 54 fmol N per *P. piscicida* zoospore per hour. Based on a population density equivalent to those measured in this study, phagotrophic N uptake would be approximately 0.8 μg at N L^{-1}·h^{-1}. Though rough, this estimate suggests that rates of N acquisition by kleptoplastidic *P. piscicida* (Table 1) may approach or even exceed that obtained through grazing.

The disproportional distribution of ambient nutrients in experimental cultures precludes meaningful comparisons of substratespecific uptake rates. It is likely that the relatively high NO_3^- uptake rates were related to high ambient NO_3^- concentrations (Lomas and Clibert 1999, and references therein). Also, it is possible that the relatively low urea uptake rates were a function of catabolic enzyme inhibition (e.g. urease) by the relatively high ambient NH_4^+ concentrations (Flynn and Butler 1986, Antia et al, 1991, Berg et al. 1997). Perhaps a more relevant comparison is the effect of growth irradiance on uptake rates for specific substrates. Only urea (absolute and cellular rates) or NH_4^+ (cellular rates) were taken up at greater rates at the higher irradiance level. Research is needed to determine whether this effect of growth h-radiance reflects N substrate preference in kleptoplastidic *P. piscicida*.

From microscopic observations of natural popu-

lations, Glasgow (unpubl. data) has observed chloroplast inclusions in Neuse River, North Carolina "presumptive *Pfiesteria*" populations for days after its initial bloom period. In light of the association between prey depletion and kleptoplastidy in laboratory batch cultures of *P. piscicida* (Lewitus et al. 1999), it is possible that such chloroplast inclusions in field populations are indicative of kleptoplastidy. However, the differentiation between kleptoplastidic and actively grazing *Pfiesteria* remains problematic, and may depend on the development of specific barkers of photosynthetic activity. In this regard, the use of primulin fluorescence as an indicator (and/or measure) of photosynthetic starch production may be useful for assessing the ecological relevance (occurrence, role, and regulation) of kleptoplastidy in *P. piscicida*.

As pointed out, the physiological ecology of klcptoplastidic *P. piscicida* may bear importantly on the mechanism by which nutrients may regulate their growth. In laboratory experiments (Burkholder et al. 1995, 1998, Burkholder and Glasgow 1997. Glasgow et al. 1998), nutrient (N or P) stimulation of *P. piscicida*'s growth has been demonstrated, either in response to elevated phytoplankton prey supply (the so-called "indirect stimulation" by nutrients) or through direct acquisition of substrates, as shown with T-labeled protein hydrolysate ("direct stimulation"). Based on those laboratoty results and direct correlations between "presumptive. *Pfiesteria*" abundance, phytoplankton abundance, and nutrient concentrations in natural waters (Burkholder et al. 1997, Burkholder and Glasgow 1997), a working hypothesis depicting seasonal changes in mechanisms of nutrient stimulation in a temporate estuary such as the Neuse River is presented in Figure 6, Most of the fish kills involving *P. piscicida* occur in summer. However, a presumably important factor determining the extent and magnitude of toxic activity is the abundance of nontoxic zoospores, the direct precursors of toxic zoospores. Nontoxic zoospore abundance in the Neuse River has been shown to co-vary with chlorophyll during spring phytoplankton bloom periods (Burkholder and Glasigow 1997), with the implication that nutrient regulation of phytoplankton biomass also will indirectly control nontoxic *P. piscicida* abundance ("indirect /nutrient stimulation"). Also, bloom parameters (magnitude, composition) may affect the proportion of nontoxic zoospores that become kleptoplastidic. Wc hypothesize that the maintenance (i.e. as zoospores rather than other cell forms such as cysts or amoebae) and/or growth of these kleptoplastidic populations are dependent on the quantity and quality of available nutrients ("direct stimulation"). In this respect, *P. piscicida*'s potential to cause fish Mb could depend on the supply of nutrients available to support seed populations of nontoxic kleptoplastidic zoospores that "fuel" toxic outbreaks.

Whether or how nutrients stimulate *P. piscicida* is a critical issue in predicting the dinoflagellate's potential impact on estuarine fish populations. The organism is widespread (from Delaware Bay to Mobile Bay, Alabama), but toxic outbreaks have been documented in a relatively narrow range of its latitudinal distribution; that is, in various North Carolina estuaries from 1991-1998, and in the Pocomoke River in summer, 1997 (Burkholder et al. 1995, Lewitus et al. 1995, Burl&older and Glasgow 1997, Maryland Department of Natural Resources 1997, University of Maryland Center for Environmental Science 1997). On comparing regions affected by *P. piscicida* toxicity with those where *P. piscicida* is found but not known to cause problems, certain general distinctions in estuarine properties are suggestcd, including tidal flushing characteristics and fish population dynamics, and also nutrient concentrations (Burkholder and Glasgow 1997). For example, using fish mortality bioassays and scanning electron microscopic confirmation, *P. piscicida was* recently discovered in the pristine North Inlet estuary, South Carolina (Lewitus, Willis, Glasgow, and Burkholder, unpubl. data). In contrast to sites of known *P. piscicida* toxic events, North Inlet is characterized not only by higher flushing rates, but also by a lack of anthropogenic influence and relatively low inorganic nutrient concentrations (e.g. seasonal maxima in dissolved inorganic nitrogen at some sites rarely exceed 5 μM; Lewitus et al. 1998). Although the North Inlet I? *piscicida* populations became toxic in fish aquariums, this potential toxicity, to our knowledge, has not been exhibited in a natural habitat. The hy-

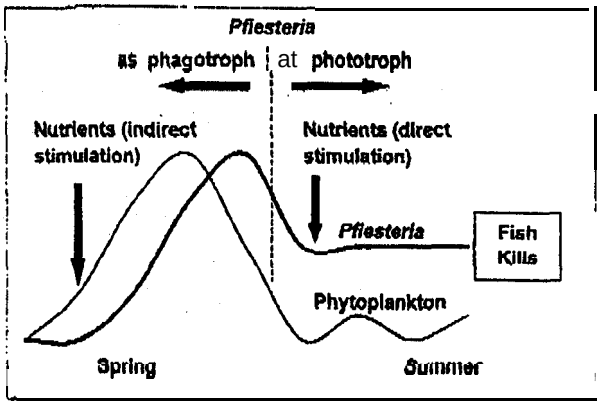


FIG. 6. Model showing hypothesized seasonal changes in mechanisms of nutrient stimulation of *Pfiesteria piscicida*'s growth. In the spring, nutrient acquisition by phagotrophic *P. piscicida* zoospores is derived from phytoplankton prey, and therefore nutrient enrichment may stimulate *P. piscicida*'s growth by increasing the magnitude of phytoplankton blooms ("indirect nutrient stimulation"). After the bloom diiipates, nutrients are acquired directly by phototrophic (kleptoplastidic) zoospores, and *P. piscicida*'s growth, or at least maintenance of zoospore populations, may be controlled by the quantity and quality of ambient nutrients ("direct nutrient stimulation"). Both forms of nutrient stimulation would lead to higher abundances of nontoxic zoospores, the direct precursors for toxic zoospores, and therefore increase the potential for toxic outbreaks (e.g. "Fish Kills").

pothesized link between high nutrient concentrations and *P. piscicida* toxic activity suggests that continued coastal eutrophication may lead to an increase in the magnitude and geographical range of *P. piscicida* toxic events.

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