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Infection of *Gymnodinium sanguineum* by the Dinoflagellate *Amoebophrya* sp.: Effect of Nutrient Environment on Parasite Generation Time, Reproduction, and Infectivity

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ABSTRACT. Preliminary attempts to culture *Amoebophrya* sp., a parasite of *Gymnodinium sanguineum* from Chesapeake Bay, indicated that success may be influenced by water quality. To explore that possibility, we determined development time, reproductive output, and infectivity of progeny (i.e. dinospores) for *Amoebophrya* sp. maintained on *G. sanguineum* grown in four different culture media. The duration of the parasite's intracellular growth phase showed no significant difference among treatments; however, the time required for completion of multiple parasite generations did, with elapsed time to the middle of the third generation being shorter in nutrient-replete media. Parasites of hosts grown in nutrient-replete medium also produced three to four times more dinospores than those infecting hosts under low-nutrient conditions, with mean values of 380 and 130 dinospores/host, respectively. Dinospore production relative to host biovolume also differed, with peak values of 7.4 per 1,000 μm^3 host for nutrient-replete medium and 4.8 per 1,000 μm^3 host for nutrient-limited medium. Furthermore, dinospores produced by "high-nutrient" parasites had a higher success rate than those formed by "low-nutrient" parasites. Results suggest that *Amoebophrya* sp. is well adapted to exploit *G. sanguineum* populations in nutrient-enriched environments.

Key Words. Algal blooms, infection level, parasitism, phytoplankton.

GROWING concern over the global increase of harmful algal blooms (HABs) (Anderson 1997; Hallegraeff 1993), along with the widely recognized importance of microbial processes in planktonic ecosystems (Azam et al. 1983), has prompted recent interest in the role of microbes as regulators of phytoplankton bloom dynamics. Viruses or virus-like particles have been reported from more than 50 species representing 12 of the 14 classes of eukaryotic algae (Nagasaki and Yamaguchi 1997), and some have been linked to declines of phytoplankton blooms (Bratbak, Egge, and Heldal 1993; Bratbak et al. 1995; Nagasaki et al. 1994a, b). Gliding bacteria (e.g. *Cytophaga* sp.) are known to attack and kill a variety of marine microalgae, including harmful taxa (Imai, Ishida, and Hata 1993). Other bacteria secrete algicidal substances (Doucette 1995; Fukami et al. 1991, 1992; Lovejoy, Bowman, and Hallegraeff 1998) or metabolites that inhibit growth and sexuality of specific phytoplankton species (Ishio et al. 1989; Sawayama et al. 1991; Sawayama, Sako, and Ishida 1993). Protistan grazers, including ciliates, heterotrophic dinoflagellates, and mixotrophic dinoflagellates, are also viewed as important consumers of phytoplankton production and have been implicated in the top-down control of HAB species (Jeong et al. 1999a, b; Kamiyama 1997; Matsuyama, Miyamoto, and Kotani 1999; Nakamura, Suzuki, and Hiromi 1995). Top-down control of some phytoplankton species may even arise from the actions of eukaryotic parasites including fungi and certain flagellates (Bruning, Lingeman, and Ringelberg 1992; Coats 1999; Norén, Moestrup, and Rehnstam-Holm 1999).

Marine dinoflagellates are infected by several endoparasites belonging to three dinoflagellate genera (*Amoebophrya, Coccodinium,* and *Duboscquella*), the most thoroughly studied species being *Amoebophrya ceratii* (Cachon 1964; Chatton and Biecheler 1934, 1935). Hosts infected by *A. ceratii* are unable to reproduce and are ultimately killed as the parasite completes its life cycle (Cachon and Cachon 1987; Elbrächter 1973). These attributes, along with occasionally high infection levels, have long been accepted as evidence that *A. ceratii* can contribute to the decline of dinoflagellate blooms (Cachon 1964). This parasite has even been suggested as a possible agent for the biological control of toxic red tides (Taylor and Pollinger 1987).

Reports of dinoflagellates infected by *A. ceratii* are available for several coastal and estuarine systems, where epizootic outbreaks usually follow the accumulation of host organisms to bloom densities (Coats et al. 1996; Elbrächter 1973; Fritz and Nass 1992; Nishatani et al. 1984). While high host abundance appears to be a prerequisite for epizootic outbreaks of parasitic dinoflagellates, other factors including nutrient concentrations, mixing, and depth of the water column may also be important (Coats et al. 1996). For example, Nishatani et al. (1984) noted decreased levels of parasitism in *Alexandrium catenella* during periods of low inorganic phosphate concentrations, but were unable to clearly differentiate between the effects of nutrient availability and host density on parasite success. Such issues have been difficult to address due to the sporadic and unpredictable occurrence of parasites in field populations and the absence of appropriate cultures for experimental studies.

Until recently, all *Amoebophrya* infections of free-living dinoflagellates were attributed to either *A. ceratii* or *Amoebophrya leptodisci,* the latter being specific to the heterotrophic dinoflagellate *Pratjetella medusoides.* Consequently, *A. ceratii* is widely believed to lack host specificity, with infections reported for over 20 dinoflagellate species, some of which are toxic (Coats and Bockstahler 1994; Drebes 1984). However, recent work suggests that *A. ceratii* is probably a species complex composed of several more or less host specific parasites (Coats et al. 1996; Coats 1999; Gunderson, Goss, and Coats 2000). To avoid confusion, we continue to use *A. ceratii* in reference to works of previous authors, but think it prudent to refer to the parasite under study here as *Amoebophrya* sp. This is the same parasite previously referred to as *A. ceratii* (Coats and Bockstahler 1994), *A. ceratii* ex *Gymnodinium sanguineum* (Coats et al. 1996), and *Amoebophrya* sp. ex *Gymnodinium sanguineum* (Gunderson et al. 1999). One of the objectives of our current research is to assess the influence of host quality on the success of *Amoebophrya* sp. Experiments reported here were designed to test the hypothesis that nutrient enrichment of host environment results in shorter parasite generation time, higher reproductive output, and increased infectivity of dispersal life history stages (dinospores).

MATERIALS AND METHODS

Laboratory cultures. Chesapeake Bay isolates of *Gymnodinium sanguineum* and its parasites *Amoebophrya* sp. were maintained as stock cultures in f/2-Si medium (Guillard and Ryther 1962) formulated using 16 ppt Bay water (= CBf/2 me-

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dium). Parasites were propagated by sequentially transferring aliquots of infected *G. sanguineum* culture into uninfected host culture at ca. 2-d intervals. For experiments, stock *G. sanguineum* in exponential growth were concentrated by gravity filtration using 12-μm Nucleopore filters and transferred to each of four media: 1) Gulf Stream water diluted to 16 ppt (GS), 2) GS with f/2-Si enrichment (GSf/2), 3) 16 ppt Chesapeake Bay water (CB), and 4) CBf/2. The CBf/2 and GSf/2 media contained macronutrients (except silicate), trace metals, vitamins, etc. according to standard formulation (Guillard and Ryther 1962). Cell concentrates were washed repeatedly with target medium, diluted to ca. 1,000 cells/ml, and acclimated for > 10 d prior to experiments. All stock and experimental cultures were maintained at 20 °C under a 14:10 light:dark cycle of 200 $\mu E\ m^{-2}\ s^{-1}$ cool-white fluorescent light. Inorganic nitrogen (N) and phosphorous (P) concentrations were 32 μM and 0.6 μM, respectively, in Chesapeake Bay water, and 4 μM and 0.2 μM, respectively, in Gulf Stream water. Nutrient additions for f/2 media increased inorganic N and P concentrations by 882 μM and 36 μM, respectively.

Parasite generation time. Parasite development times were determined by following the synchronous spread of infections in GS, GSf/2, CB, and CBf/2 host cultures over three generations. To synchronously infect cultures, recently formed (≤ 6-h old) dinospores of *Amoebophrya* sp. were harvested by gravity filtration of stock host-parasite cultures using a 12-μm Nucleopore filter. Dinospore abundance was determined for glutaraldehyde-fixed (1.2% final concentration) subsamples of the filtrate using a hemocytometer and a Zeiss Axioscope (×400) equipped with epifluorescence microscopy (450–490 nm excitation; 520 nm barrier filter) for distinguishing the green autofluorescence of the parasite (Coats and Bockstahler 1994). Aliquots (0.1 ml) of harvested dinospores (2,200 ± 400 per ml) were added to triplicate 300-ml vol. of host cultures to produce final cell densities of about 0.66 dinospores/ml and about 1,000 *G. sanguineum*/ml. This dinospore:host ratio was expected to yield an initial parasite prevalence of 0.01%, assuming a dinospore success rate of 20% as previously reported (Coats et al. 1996). For controls, harvested dinospores were removed by filtration using glass fiber filters (Whatman GF/F), and the resulting filtrate was added to target cultures to yield triplicate 300-ml vol. at 1,000 *G. sanguineum*/ml for each medium. The volume of filtrate added to controls was equivalent to that of harvested dinospores added to infected treatments.

Treatments and controls were gently swirled to evenly distribute cells and sampled at approximately 12-h intervals (range 9–18 h) over the following 11 d (246 h). After mixing, a 10-ml aliquot was removed from each culture and preserved with modified Bouin's solution (Coats and Heinbokel 1982) for determination of host abundance, host cell volume, parasite prevalence, and parasite developmental stage. Estimates of host abundance were obtained by enumerating cells present in microscope transects (×100) of triplicate Sedgwick-Rafter chambers. For each chamber, successive transects were examined until 100 cells had been counted or five transects (= half the chamber area) had been scanned. To approximate host cell volume, cross-section profiles (×400) of 30 randomly selected cells were digitally captured using a Sony CCD video camera interfaced with a personal computer. Images were analyzed using Image Pro Plus software to obtain cell cross-sectional area, and cell volume was then calculated assuming spherical geometry [i.e. vol. = $4/3 \cdot \pi^{-1/2} \cdot \text{area}^{3/2}$]. Parasite prevalence was determined for specimens processed by the quantitative protargol staining (QPS) technique (Montagnes and Lynn 1993). Stained preparations were examined at ×1,250, with ≥ 100 cells for each sample scored as either uninfected, early infections, or late infections, following established criteria (Coats and Bockstahler 1994). Duration of the intracellular phase of the parasite's life cycle and midpoints of successive parasite generations were estimated by analyzing temporal oscillations in abundance of early and late infections (Coats and Bockstahler 1994).

Spore production. Duplicate 400-ml cultures (~ 1,000 cells/ml) of *G. sanguineum* adapted to CBf/2 or GS media were inoculated with CBf/2-grown dinospores at a final density of 1,250 cells/ml. The resulting dinospore:host ratio was expected to yield a parasite prevalence of ca. 25%, assuming a 20% success rate as above (Coats et al. 1996). Inoculated cultures were subsampled at 4-h intervals over 4 d, with 5-ml aliquots preserved in 1.25% glutaraldehyde (final concentration) for tracking dinospore abundance and percent hosts containing green-fluorescing trophonts of *Amoebophrya* sp. Using fluorescence microscopy to detect *Amoebophrya* sp. provides good estimates for dinospores and late stage infections, but can underestimate the prevalence of early infections (Coats and Bockstahler 1994). Also, samples must be processed immediately, as the green fluorescence of the parasites fades markedly within a few hours following fixation. Thus, a separate set of 10-ml aliquots was fixed with modified Bouin's solution for assessing host abundance, host cell volume, and parasite developmental stage, as above. When dinospores from the ensuing generation of parasites reached peak densities, 20-ml samples were taken from each culture and used to assess the ability of dinospores to infect new hosts (see below).

Dinospore success. CBf/2 and GS cultures of *Gymnodinium sanguineum* were diluted with the corresponding medium to yield five different host densities ranging from 100 to 2,500 cells/ml and then distributed to scintillation vials as triplicate 10-ml aliquots for each host density and medium formulation. Young dinospores were harvested from CBf/2 and GS treatments of the "spore production" experiment described above and added to scintillation vials of host dilutions of complementary medium to give a ratio of 2.5 dinospores/host cell. This ratio was expected to yield a parasite prevalence of 50%, assuming 20% success rate of dinospores, as above (Coats et al. 1996). Treatments were incubated for 36 h and then fixed with modified Bouin's solution to determine host abundance and parasite prevalence.

Statistics. Data are reported in the text as mean ± standard error of the mean (SE). One-way analysis of variance (ANOVA) was conducted using SigmaStat (Jandel Scientific Software), with a posteriori comparisons by Tukey pairwise test. Analysis of covariance (ANCOVA) was performed on data from the spore success experiment using SAS/GLM (Statistical Analysis System/General Linear Model). For the ANCOVA, the proportion of dinospores successfully infecting new hosts was the dependent variable, while medium type and host density were independent variables. The dependent variable was arcsine square-root transformed prior to the analysis, medium type was treated as a categorical variable, and host density was treated as a covariate.

RESULTS

Parasite generation time. *Gymnodinium sanguineum* adapted to GS, CB, GSf/2, and CBf/2 medium differed significantly in cell size ($p < 0.001$; ANOVA), with mean values of 17,700 ± 1010, 20,400 ± 570, 23,500 ± 640, and 25,300 ± 680 μm^3, respectively. Tukey pairwise comparison indicated that cells grown in CBf/2 medium were significantly larger ($p < 0.05$) than those in unenriched Gulf Stream and Chesapeake Bay water (GS and CB media). Cells grown in GSf/2 medium were

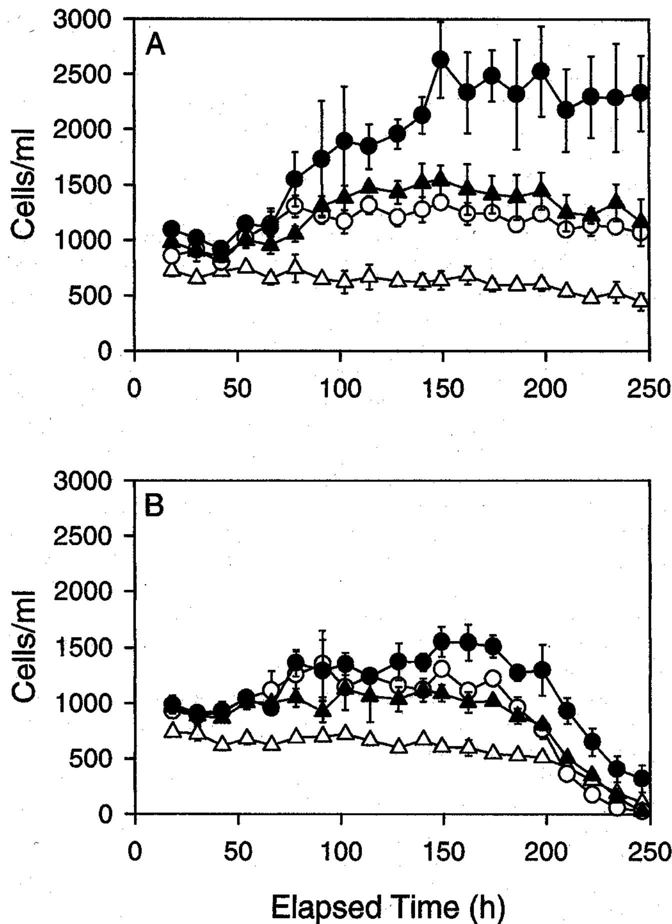

Fig. 1. Temporal changes in the abundance of the dinoflagellate *Gymnodinium sanguineum* maintained in GS (△), CB (▲), GSf/2 (○), and CBf/2 (●) media. Uninfected controls (**A**); cultures inoculated with the parasitic dinoflagellate *Amoebophrya* sp. at a density of 0.66 dinospores/ml (**B**). Infected cultures showed reduced host abundance relative to uninfected controls, especially toward the end of the 11-d incubation. Error bars indicate standard error of the mean.

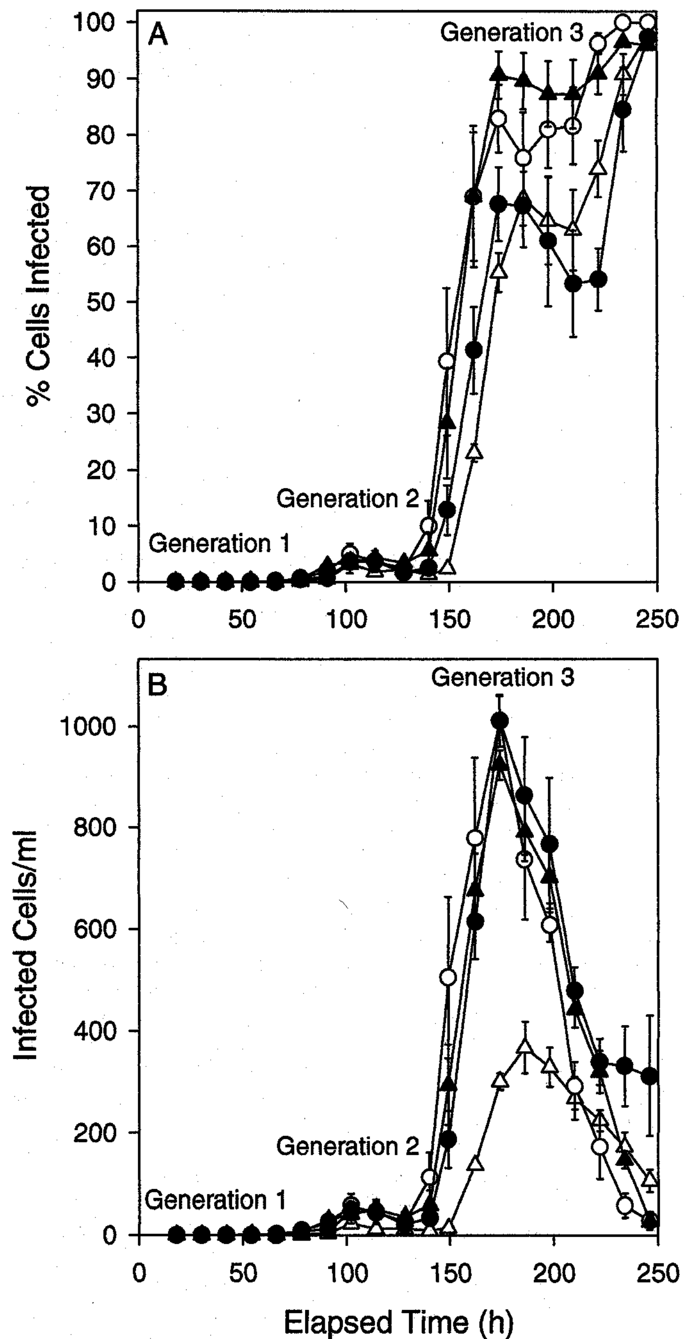

Fig. 2. Prevalence of *Amoebophrya* sp. infecting *Gymnodinium sanguineum*(**A**) and the abundance of infected hosts (**B**) for infected *G. sanguineum* cultures shown in Fig. 1; GS (△), CB (▲), GSf/2 (○), and CBf/2 (●) media. Data show sequential spread of infections through successive parasite generations, with peak abundance of infected hosts occurring in the third generation. Error bars indicate standard error of the mean.

only larger ($p \leq 0.05$) than those maintained in unenriched Gulf Stream water.

Uninfected *G. sanguineum* maintained in GS medium declined gradually from 730 ± 50 to 450 ± 80 cells/ml during the 11-d incubation (Fig. 1A). By contrast, uninfected CB and GSf/2 cultures increased slightly to 1,300 ± 80 and 1,500 ± 40 cells/ml by 115 h, respectively, while uninfected CBf/2 *G. sanguineum* showed steady growth to 150 h, with mean abundance reaching 2600 ± 350 cells/ml by stationary phase. Growth of infected GS, CB, and GSf/2 cultures exhibited trends similar to those of uninfected controls over the first 175 h of the experiment; however, cell yields for CB & GSf/2 treatments were somewhat lower than controls (Fig. 1B). Infected CBf/2 cultures increased more slowly and had a lower maximum abundance (1,550 ± 130 cells/ml) than uninfected controls. After 175 h, *G. sanguineum* abundance declined dramatically in all four infected treatments.

Parasite prevalence was at or below detection levels (≤ 0.17%) in all cultures throughout the first generation of *Amoebophrya* sp. During the second generation, 3–5% of host cells were infected (Fig. 2A, peak at 102 h), with no discernible difference among treatments. Infection levels increased abruptly in the third parasite generation, with peak values of 69 ± 4.8% at 186 h for GS cultures and 83 ± 6.0%, 91 ± 4.2%, and 68 ± 6.7% at 174 h for CB, GSf/2, and CBf/2 treatments, respectively. Parasite prevalence approached 100% in all cultures as *Amoebophrya* sp. progressed into the fourth generation. Estimates for infected *Gymnodinium sanguineum* per ml (i.e. parasite prevalence multiplied by host density; Fig. 2B) indicated that the peak number of infections produced in the third gen-

Table 1. Duration of the intracellular phase of *Amoebophrya* sp. infecting *Gymnodinium sanguineum* and time required to reach the middle of the third generation for cultures grown in different media. Mean ± SE at 20°C.

Medium	Duration of intracellular phase (h)	Middle of third generation (h)	Duration of intracellular phase (h) host cell volume (1,000 μm³)
GS	53 ± 4.0	197 ± 2.4[a]	3.0 ± 0.19[a]
CB	47 ± 2.0	179 ± 1.6[b]	2.3 ± 0.12[ab]
GSf/2	54 ± 4.6	188 ± 1.0[c]	2.3 ± 0.25[ab]
CBf/2	49 ± 3.6	187 ± 2.3[bc]	2.0 ± 0.10[b]

[abc] Different letters within columns indicate significant difference at $P < 0.05$ by Tukey pairwise test.

eration of *Amoebophrya* sp. did not differ for CB, GSf/2, and CBf/2 treatments (range 920 ± 30 to 1,010 ± 50), but was significantly lower (370 ± 50) for GS cultures ($p < 0.001$; ANOVA).

Analysis of parasite life-history stages during the third generation (i.e. early vs. late infections; see Coats and Bockstahler 1994 for details) showed no difference in the duration of the intracellular phase of *Amoebophrya* sp., but did reveal significant differences in the time required to reach the mid-point of the third generation (Table 1). These data, which do not account for differences in cell volume, indicate faster development in unenriched vs. enriched Chesapeake Bay water, but slower development in unenriched vs. enriched Gulf Stream water. When normalized to host cell volume, the duration of the parasite's intracellular phase decreased from low to high nutrient conditions, with values for cells maintained in GS medium differing significantly from those grown in CBf/2 medium (Table 1).

Spore production. Inoculation of CBf/2 and GS cultures at a ratio of 1.25 dinospores/host generated a parasite prevalence of 23 ± 0.4% and 19 ± 1.2%, respectively, for samples taken 41–49 h ($n = 3$) after addition of dinospores. Host abundance during that period was 1,180 ± 50 cells/ml for CBf/2 cultures and 860 ± 10 cells/ml for GS cultures. As a result, the abundance of infected *G. sanguineum* prior to the production of dinospores stabilized at 270 ± 10 and 160 ± 10 cells/ml for CBf/2 and GS treatments, respectively (Fig. 3A). Dinospores were formed over the following 15–20 h, with peak occurrence (102,000 ± 5,500 cells/ml) in CBf/2 medium being four to five times higher than that in GS medium (24,000 ± 500 cells/ml). The number of dinospores produced per infected host was three to four times higher for CBf/2 cultures (380 ± 34 and 130 ± 12 for CBf/2 and GS treatments, respectively); however, host cell volume was 27% greater for CBf/2 grown *G. sanguineum* (52,200 ± 4600 μm³ for CBf/2 and 32,400 ± 1,400 μm³ for GS cultures). Relative to host biovolume, peak production of dinospores by *Amoebophrya* sp. was roughly two times higher when infecting *G. sanguineum* in CBf/2 medium than when infecting hosts grown in GS medium (Fig. 3B; 7.4 vs. 4.8 dinospores per 1,000 μm³ host). The rapid decrease in dinospore abundance after peak occurrence in CBf/2 cultures was associated with infection of new host cells by numerous dinospores. Examination of protargol-stained preparations revealed an average of 17 ± 1.2 very early stage parasites in the cytoplasm of each newly infected *G. sanguineum* in CBf/2 cultures at 73 h, compared to 2 ± 0.4 new infections/host in GS cultures.

Spore success. Dinospores harvested during the preceding study of spore production (Fig. 3, arrows), exhibited different success rates when inoculated into uninfected cultures of *G.*

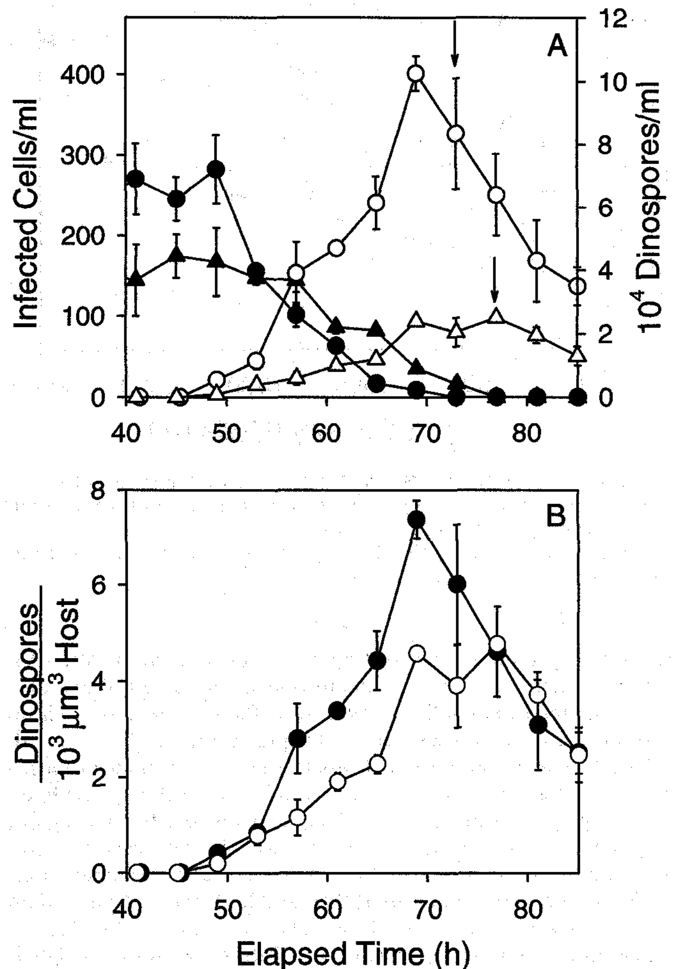

Fig. 3. Production of dinospores by *Amoebophrya* sp. when infecting *Gymnodinium sanguineum*. **A**. Abundance of infected hosts (closed symbols) and dinospores (open symbols) for infected *G. sanguineum* cultures maintained in CBf/2 medium (circles) and GS medium (triangles). **B**. Number of dinospores produced normalized to host biovolume for CBf/2 (●) and GS (○) treatments. *Amoebophrya* sp. infecting *G. sanguineum* grown in nutrient replete media (CBf/2) produced more dinospores than those infecting nutrient limited (GS) hosts. Error bars represent standard error of the mean. Arrows indicate time when dinospores were harvested for the experiment shown in Fig. 4.

sanguineum grown in the corresponding medium (Fig. 4). The percent of CBf/2 dinospores that infected new host cells averaged 27 ± 2.7% over host densities of 130–2,300 cells/ml compared to 19 ± 2.3% for GS dinospores over host densities of 75–1,600. ANCOVA showed an insignificant interaction term ($p = 0.30$) for host density and medium type, indicating that the slope of success rate vs. host density was the same for both media. Success rate increased significantly with host density ($p = 0.0015$), and mean values adjusted for host density indicated significantly higher success in CBf/2 medium than in GS medium ($p = 0.0001$).

DISCUSSION

Environmental stressors, including chemical pollution, habitat alteration, and eutrophication, can have a variety of effects on host-parasite systems, with outcomes ranging from disproportionately negative impacts on host species to significantly reduced rates of parasitism (Lafferty and Kuris 1999). Higher

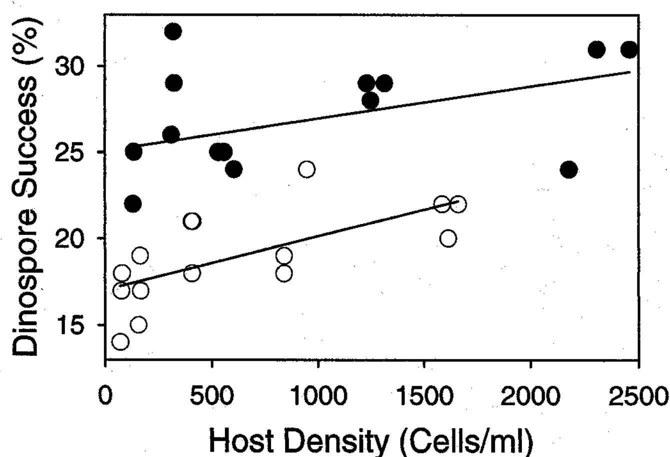

Fig. 4. Percent CBf/2 (●) and GS (○) grown dinospores of *Amoebophrya* sp. that successfully infected *Gymnodinium sanguineum* cultures in corresponding media. Dinospore success rate increased with host density and was consistently higher for nutrient replete (CBf/2) host cultures.

ecosystem productivity resulting from eutrophication often favors elevated levels of parasitism due to increased abundance of host or intermediate host organisms. Parasite diversity within host taxa can also vary with eutrophication (Valtonen et al. 1997). Nutrient loading that leads to the development of harmful or non-harmful phytoplankton blooms may thus provide an ideal setting for transmission of algal parasites. In this regard, fungal infections are known to increase during seasonal phytoplankton blooms in eutrophic freshwater lakes (Heaney et al. 1988; Kudoh and Takahashi 1990), and high rates of parasitism by *Amoebophrya* spp. are often linked to elevated host abundance during red tides (Coats et al. 1996; Nishitani et al. 1984; Taylor 1968).

Factors other than host density (e.g. parasite fecundity and host susceptibility) are also fundamental to parasite success and may vary with environmental conditions. For example, light-limited growth of the diatom *Asterionella formosa* substantially reduced zoospore production by the fungal parasite *Rhizophydium planktonicum* and rendered hosts cells less susceptible to infection (Bruning 1991a). At very low irradiances, zoospores of *R. planktonicum* failed to infect potential host cells (Bruning 1991b). Macronutrient concentrations and ratios are known to affect the size and chemical composition of algal cells (Antia et al. 1963; Goldman 1980; Goldman, McCarthy, and Peavey 1979) and may thereby influence parasite biology. For example, phosphorous limitation of *A. formosa* depressed zoospore production by *R. planktonicum* (Bruning and Ringelberg 1987).

Our data indicate that host nutrient environment can influence the success of *Amoebophrya* sp. by altering parasite reproductive output and infectivity of progeny. *Amoebophrya* sp. produced far more dinospores when cultured with *Gymnodinium sanguineum* grown in nutrient-replete medium (CBf/2) than when grown in unenriched Gulf Stream water (GS medium). Host cell volume also differed between these treatments, but was not sufficient to account for observed differences in dinospore production, suggesting that *Amoebophrya* sp. is more efficient at utilizing host biomass under nutrient-replete conditions. Cachon (1964) reported that dinospores of *A. ceratii* are ca. 8 μm long and slightly narrower when free-swimming, but become spherical and measure 5 μm in diameter as they invade a new host. We did not measure dinospore size during our experiments, however, dinospores formed in stock cultures have dimensions similar to those reported by Cachon (1964) (DWC, pers. observ.). Assuming a dinospore diameter of 5 μm (= 65 μm^3) , our estimates for dinospore production (7.4 dinospores per 1,000 μm^3 host biovolume in CBf/2 medium and 4.8 dinospores per 1,000 μm^3 host biovolume for GS medium) indicate that *Amoebophrya* sp. infecting *G. sanguineum* converts host biomass to parasite biomass with gross efficiencies ranging from 48% in nutrient-replete medium to 31% under nutrient-limited conditions.

Dinospores of *Amoebophrya* sp. produced from *Gymnodinium sanguineum* grown in enriched medium (CBf/2) were more successful in establishing new infections than those resulting from "nutrient-limited" hosts (GS medium), with a 42% higher yield of new infections over a broad range of host densities. Whether reduced success of dinospores in nutrient-limited media was due to differences in parasite fitness (e.g. dinospore survival time, swimming speed, etc.) or changes in host properties that reduce susceptibility remains to be determined. For this experiment, dinospore inoculums were adjusted to give similar dinospore:host ratios at all host densities, thus increasing encounter rate between host cells and dinospores as host density increased. The observed increase in dinospore success rate with host density most likely reflects changes in encounter probability.

The influence of nutrient environment on development time of *Amoebophrya* sp. from *G. sanguineum* was not clear cut. No difference was evident in the duration of the parasite's intracellular growth phase; however, the time required to reach the middle of the third parasite generation differed significantly among treatments, suggesting some cumulative effect of nutrient environment over successive generations. Host biovolume also differed significantly among treatments, with cell size increasing with nutrient enrichment. Thus, *Amoebophrya* sp. that infected small, "nutrient-limited" hosts reached maturity in about the same length of time as those infecting large, "nutrient-replete" hosts. When normalized to host cell volume, the duration of the parasite's intracellular phase differed between hosts grown in unenriched Gulf Stream water (GS medium) and those grown in enriched Chesapeake Bay water (CBf/2). This situation could result from either (1) a predetermined intracellular development time that is independent of host volume, or (2) a variable intracellular development time that is dependent on host volume, but that occurred coincidentally in the same amount of time, due to differences in parasite growth rate. At present, we are unable to conclusively demonstrate which of these alternatives is correct. However, higher production of dinospores in CBf/2 than in GS medium, as discussed above, indicates that parasites infecting large, "nutrient-replete" hosts achieve a larger biomass before reaching maturity. Since the duration of the parasite's intracellular phase did not differ among treatments, it seems reasonable to conclude that parasites infecting "nutrient-replete" hosts have a faster growth rate.

During our experiment to determine parasite generation time, the abundance of *Gymnodinium sanguineum* in nutrient-replete medium (CBf/2) inoculated with *Amoebophrya* sp. failed to increase to levels achieved by uninfected controls. This deviation in host abundance was clearly evident during the second generation of *Amoebophrya* sp., even though parasite prevalence did not reach high levels until the third generation. Reduced host abundance relative to controls during the first two parasite generations was also evident, but less conspicuous, in the slower growing CB and GSf/2 cultures. Given that parasite-induced mortality would have removed only a small percentage of *G. sanguineum* during the first and second generations of *Amoebophrya* sp. (ca. 5%), other factors probably contributed to the

observed trends. One possibility is that compounds present in the dinospore inoculum may have inhibited growth of *G. sanguineum*; however the inoculum was very small (0.1 ml) relative to culture volume (300 ml). Furthermore, all controls received an equal volume of dinospore filtrate. Other alternatives include (1) *Amoebophrya* present in infected treatments may have produced metabolites that inhibited *G. sanguineum* growth and (2) bacteria present in the dinospore inoculum, but not in the dinospore filtrate, may have influenced growth of host cells.

While parasite prevalence and total number of infected cells varied among treatments during the generation time experiment, we do not interpret these results as indicating direct effects of nutrient environment on parasite success. Unlike parasite generation time, these parameters are strongly dependent on host abundance and associated host-parasite encounter rates, which were not the same in all treatments.

Our studies of *Amoebophrya* sp. from *Gymnodinium sanguineum* clearly demonstrate a relationship between host nutrient environment and parasite success. Differences in dinospore production and infectivity probably reflect differences in host quality, as well as host size. While we do not have data on the chemical composition of host cells, it is likely that C, N, and P content and perhaps C:N:P ratios differed for host cells across treatments. That our nutrient-replete Chesapeake Bay medium (CBf/2) was probably rich in terrigenous compounds and received additions of minor nutrients, including vitamins and trace metals, while unenriched Gulf Stream medium (GS) did not, may also have influenced host quality and observed patterns in dinospore production and infectivity. Defining whether macronutrients or micronutrients alone have strong effects on parasite success will require additional study. Nonetheless, *Amoebophrya* sp. from *G. sanguineum* appears well adapted to exploit host populations in enriched environments that promote bloom formation.

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