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Predicting grazing mortality of an estuarine dinoflagellate, *Pfiesteria piscicida*

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ABSTRACT: Grazing can prevent or substantially decrease net growth of phytoplankton populations if grazing coefficients are similar to or greater than growth coefficients of the phytoplankter. Potential grazing by microzooplankton on small dinoflagellates (<20 µm), such as *Pfiesteria piscicida*, can be high, but extremely variable in estuaries. Blooms should only be possible during 'windows of low grazing pressure'. To better understand this variability, microzooplankton assemblages were collected at stations with low (~5 psu), medium (~10 psu) and high (~15 psu) salinity on the Pocomoke River, MD, and their grazing on cultured, stained non-toxic *P. piscicida* zoospores was measured. Grazing coefficients varied from 0 to 7.6 d^{-1}. On most dates average grazing coefficients were >2 d^{-1}, indicating that microzooplankton grazing had the potential to regulate densities of non-toxic zoospores. Potential grazing was positively correlated with salinity, abundance of photosynthetic and heterotrophic dinoflagellates and with abundance of <20 µm planktonic ciliates. Grazing on zoospores was negatively associated with abundance of cryptophytes. Approximately 26% of the variation in grazing coefficient could be predicted from salinity, whereas 67% of the variation could be predicted from abundance of cryptophytes, dinoflagellates and small ciliates. Net growth of non-toxic zoospore populations is most likely in low-salinity waters with abundant cryptophytes but with low concentrations of microzooplankton.

KEY WORDS: Harmful algal blooms · Biological control · Dinoflagellates · Microzooplankton grazing · *Pfiesteria piscicida* · CMFDA · Chesapeake Bay · Pocomoke River

INTRODUCTION

Pfiesteria piscicida (Dinamoebiales: Pyrrhophyta) has been linked to toxic events and fish kills along the Atlantic coast of the USA (Burkholder et al. 1992, 1995, 2001, Steidinger et al. 1996). In the Chesapeake Bay region, *Pfiesteria*-like dinoflagellates were linked to fish kills or fish lesions in 1997 in the lower Pocomoke River, the Manokin River, and Chicamacomico River, all subestuaries on the Eastern Shore of Maryland, USA (Maryland Department of Natural Resources 1998, Burkholder et al. 2001).

Pfiesteria piscicida zoospores vary in their toxicity to fishes and palatability to grazers (Burkholder et al. 1992, 2001, Marshall et al. 2000, Stoecker et al. 2001). Zoospores are classified as TOX-A (actively toxic), TOX-B (temporarily non-toxic in the absence of live fishes, but capable of toxicity) and NON-IND (undetectable toxicity in the presence or absence of fishes) (Burkholder et al. 2001). The distribution and abundance of TOX-A, TOX-B and NON-IND zoospores in nature is not known. It is usually assumed that populations of TOX-B zoospores grow in the plankton as mixotrophs eating small algae and that toxicity is induced (e.g. TOX-B transformed to TOX-A) in the presence of dense schools of fishes (Burkholder & Glasgow 1997a,b). Toxic zoospores may also be recruited from amoebae and resting cysts in the presence of dense schools of fish (Burkholder et al. 1992, Burkholder & Glasgow 1997b).

For a net increase in a population, the growth rate has to be higher than the mortality rate, unless recruitment

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is supplying new individuals. In culture, the maximum growth rates for *Pfiesteria piscicida* non-toxic zoospores are between 1 and 2 d^{-1}, with most reported rates closer to 1 (Glasgow et al. 1998, Stoecker et al. 2000, Burkholder et al. 2001). Both protistan and metazoan grazers have been observed to consume zoospores (Burkholder & Glasgow 1995, Mallin et al. 1995, Stoecker et al. 2000). In an investigation conducted in the summer of 1999, we found that potential grazing coefficients on NON-IND zoospores in the Chicamacomico River were usually >2 d^{-1} (Stoecker et al. 2000). These data indicate that microzooplankton grazing may be important in preventing net growth of non-toxic (i.e. TOX-B and NON-IND) populations of zoospores *in situ.*

Although *Pfiesteria piscicida* can grow over a wide salinity range, the optimum for growth in culture is ~ 15 psu (Burkholder & Glasgow 1997a). It is interesting that toxic outbreaks have often occurred at a salinity lower than optimum for growth of *P. piscicida*. For example, in 1997, the toxic area on the Pocomoke River was between 7 and 11 psu, on the Manokin (Kings Creek branch) between 0.5 and 10 psu, and in the Chicamacomico River <10 psu (Maryland Department of Natural Resources 1998). It is possible that net growth of *P. piscicida* zoospores was higher at low salinity because mortality due to grazing was lower, algal prey was more abundant, fish populations that induced toxicity were more abundant, or a combination of these factors.

Our objectives were to (1) determine if microzooplankton grazing has the potential to regulate net growth of *Pfiesteria piscicida* non-toxic zoospores in the Pocomoke River; (2) compare potential grazing at high-, medium- and low-salinity stations; and (3) determine if potential microzooplankton grazing on zoospores was correlated with abundance of potential grazers and prey of *P. piscicida*.

Information on grazing pressure on *Pfiesteria piscicida* and the factors that regulate it may be useful in predicting the antecedent conditions necessary for toxic outbreaks in the presence of fishes because blooms of TOX-B (not actively toxic) zoospores probably precede toxic events. Several other species of small algae have been implicated in harmful algal blooms (Hallegraeff 1993, Glibert et al. 2001), and it is possible that their populations are regulated by microzooplankton grazing as well (Kamiyama 2000). Understanding the factors associated with high or low grazing is important in predicting blooms of harmful algae.

MATERIALS AND METHODS

Culture and staining of *Pfiesteria piscicida*. Cultures of *P. piscicida* (Strain MDFDEPMR23) had been isolated from a stored sample from the Chicamacomico River, MD, grown on an algal enrichment, and was provided to us by Dr. K. A. Steidinger on 10 December 1998. It's identification as *P. piscicida* had been confirmed using scanning electron miscrocopy (SEM) (Steidinger pers. comm.). Strain MDFDEPMR23 has not been shown to be toxic in bioassays with fish and is thus considered 'non-inducible' with regard to toxicity (J. M. Burkholder pers. comm.).

Cultures were maintained in seawater media at 20°C with added cryptophyte (*Storeatula major*) prey as described in Stoecker et al. (2000), except that cultures were grown at 5, 10 and 15 psu. Dense cultures (10^3 to 10^4 cells ml^{-1}) of *Pfiesteria piscicida* with low concentrations of algal prey (<50 cells ml^{-1}) were stained for 1 h with 1 µM CMFDA (5-chloromethyfluorescein diacetate, Molecular Probes). Stained cells have green fluorescence but remain motile and capable of division (Li et al. 1996, Stoecker et al. 2000).

Enumeration of *Pfiesteria piscicida* stained with CMFDA. To enumerate cells in stained cultures, 3 to 5 ml fixed with glutaraldehyde (1%, final concentration) samples were gently filtered (<15 mm Hg pressure) onto 2 µm pore-size black-membrane filters and then mounted on glass slides with immersion oil (Resolve) under a cover slip. Slides were enumerated with epifluorescence microscopy at 200× with a Nikon Eclipse standard microscope (Nikon filter set EF-4 B-2A; exciter filter 450 to 490 nm, dichromatic beam splitter 500 nm, barrier filter 515 nm). The procedure for enumerating stained *P. piscicida* in samples from the grazing experiments was similar, except that 3 ml samples were filtered and slides were stored frozen at –20°C until counted.

Enumeration of phytoplankton and microzooplankton in water samples. Samples were fixed with cold glutaraldehyde (1% final conc.) and stored at 4°C in the dark for enumeration of phytoplankton and heterotrophic dinoflagellates. Aliquots (3 to 5 ml) of the glutaraldehyde-fixed samples were stained with proflavin (final concentration 5 µg ml^{-1}), gently filtered (<15 mm Hg pressure) onto 2 µm pore-size black-membrane filters, and then mounted on glass slides with immersion oil (Resolve) under a cover slip (Sieracki et al. 1993). The dominant micro- and nanophytoplankton (photosynthetic dinoflagellates, cryptophytes and centric diatoms) and <20 µm heterotrophic dinoflagellates were enumerated using epifluorescence microscopy (Nikon filter set EF-4 B-2A; exciter filter 450 to 490 nm, dichromatic beam splitter 500 nm, barrier filter 515 nm) at 200×. In addition, *Pfiesteria*-like dinoflagellates (defined here as ~10 to 15 µm gymnodinioid dinoflagellates without their own plastids) were also enumerated separately.

Samples from the water used in the experimental incubations were also fixed with acid Lugol's solution

(5% final conc.) for enumeration of planktonic ciliates, >20 µm heterotrophic dinoflagellates and metazoan microzooplankton. 25 ml aliquots were concentrated by sedimentation in Utermöhl chambers and examined on a Nikon Eclipse inverted microscope.

Determination of potential grazing on *Pfiesteria piscicida*. Surface water samples were collected from a small boat at 3 stations in the Pocomoke River and nearby Sound on 2 occasions in May, 3 in August, 1 in September and 1 in October 2000 (Fig. 1). Stations were chosen on each date so that water of high (~ 15 psu), medium (~10 psu) and low (~5 psu) salinity could be obtained. Thus, the position of the stations depended on freshwater flow and tides and varied from date to date (Fig. 1). Samples were passed through a 200 µm mesh size to remove larger zooplankton as the water was gently poured into a 1 l capacity wide-mouth polycarbonate bottle. At the time of sample collection, water temperature and salinity were measured using a YSI 30 salinity-conductivity-temperature meter.

The water samples were transported in a cooler to Horn Point Laboratory and the grazing experiments initiated within 4 h of sample collection. All incubation bottles were made of polycarbonate and had been acid-washed, rinsed with distilled water, and sterilized before use. For each water sample there were 2 treatments, with duplicate incubation bottles for each treatment. The <200 µm treatment contained the natural assemblage of bacteria, algae, protozoa and other microzooplankton. The <1.2 µm treatment was the same, except that the water was gently filtered through a GF/C glass-fiber filter to remove algae, protozoa and other microzooplankton. Thus, the <1.2 µm treatment is a control for net changes in zoospores concentration caused by cell losses due to other factors than grazing and due to division.

150 ml of the <200 µm-filtered or 100 ml of the <1.2 µm-filtered water was added to each 250 ml polycarbonate bottle. At time $t = 0$, an aliquot of the stained *Pfiesteria piscicida* culture grown at the appropriate salinity was added to each replicate to achieve a calculated density of 500 cells ml^{-1}. The incubation bottles were gently mixed by inverting them several times and then samples were immediately withdrawn and fixed for determination of initial concentrations of stained *P. piscicida*. The bottles were then incubated in the dark at 22 to 24°C and sampled after 0.5, 1, 1.5, 2, 3, 4 and 5 h.

Community growth or grazing coefficient for each replicate was calculated using data on free-stained *Pfiesteria piscicida* zoospore concentrations from all time points (example shown in Fig. 2) using the approach developed by Frost (1972) for measuring zooplankton grazing. For each replicate, the slope of a regression of the natural log (ln) of zoospores ml^{-1} versus time was calculated. The slope of the <200 µm treatment is an estimate of net growth rate, K, of zoospores in the presence of the natural microzooplankton assemblage. The slope for the <1.2 µm treatment is an estimate of the rate of increase or decrease, µ, of zoospores in the absence of grazing. The estimate of 'µ' is a minimum estimate since the 1.2 µm filtration removed potential

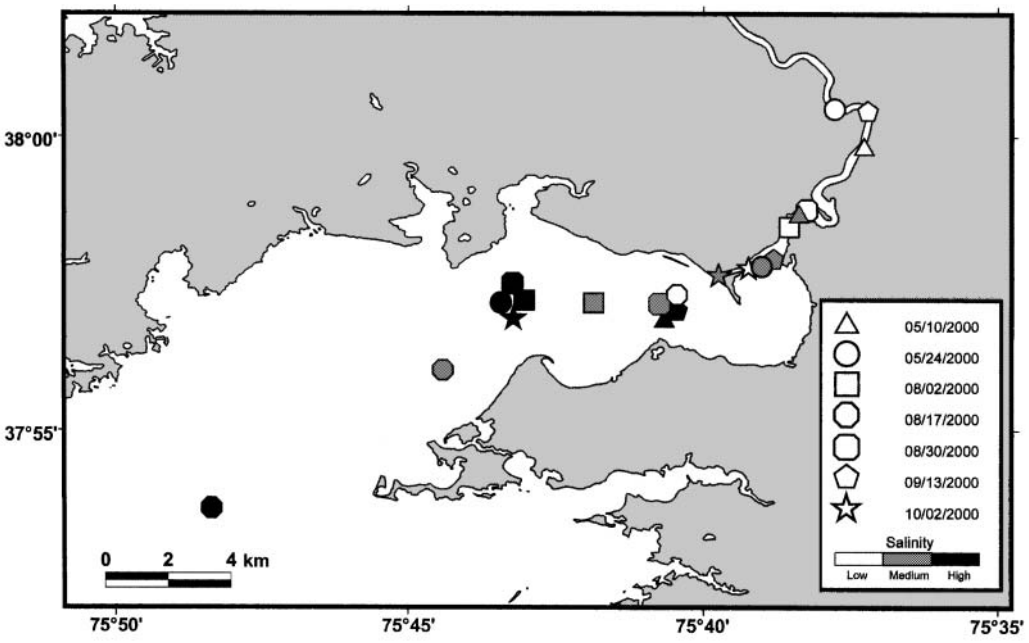

Fig. 1. Sampling stations on the Pocomoke River for collection of high (H), medium (M) and low (L) salinity surface water

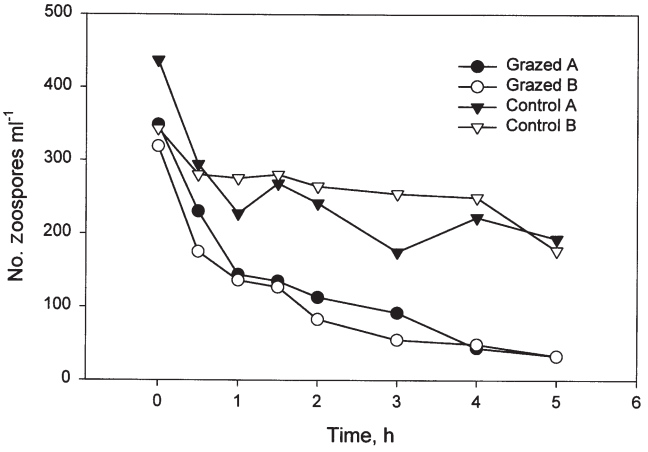

Fig. 2. *Pfiesteria piscicida.* Changes in concentration of added fluorescent-labeled zoospores during the grazing experiment conducted on 10 May 2000. Data are shown for incubations with surface water from the 'high' salinity station (see Table 1). In the duplicate grazed bottles, zoospores were added to surface water that had been screened through a 200 µm mesh and thus contained microzooplankton but not mesozooplankton. In the duplicate control bottles, zoospores were added to surface water that had been filtered through a GF/C glass-fiber filter (mesozooplankton and microzooplankton removed)

prey for *P. piscicida* as well as its potential predators. A minimum estimate of the grazing coefficient, *g*, for each replicate of the <200 µm treatment was calculated as µ-*K*; the average 'µ' for the <1.2 µm treatment was used in the calculation.

Statistical analysis. All statistics were done using Jandel SigmaStat Version 2.0.

RESULTS

Environmental conditions

The positions in the Pocomoke sub-estuary, where it was possible to collect high (H)-, medium (M)- and low (L) salinity surface water varied with sampling date. On 17 August 2000, which was after a rainy period, stations were displaced seaward, with all stations displaced to Pocomoke Sound. In contrast, on 10 May and 9 September, the L and M stations were located in the river proper (Fig.1). Surface water matching the target salinity was not always obtained. The H stations varied between 11.4 and 15.6 psu, the M stations between 7.8 and 11.2 psu, and the L stations between 2.0 and 5.9 psu (Table 1).

Temperature varied among dates, with the lowest water temperatures (19 to 20°C) on 24 May and 2 October and the highest water temperatures (26 to 27°C) on 2 August. There was little difference in water temperature among H, M and L stations on any 1 sampling date (Table 1).

The phytoplankton assemblages varied with date and station (Table 1). Centric diatoms were observed in all samples, and consistent patterns in their abundance were not observed. Cryptophytes were usually less abundant in the H sample than at in the M or L samples. Photosynthetic dinoflagellates were more abundant in the summer samples than in the spring or fall samples (Table 1), and common species were *Karlodinium micrum* (= *Gyrodinium galatheanum*) and *Prorocentrum minimum*.

Microzooplankton also varied with sampling date and salinity (Table 1). The photosynthetic ciliate *Mesodinium rubrum* (= *Myrionecta rubra*) occurred at densities >100 ml^{-1} in 3 of the samples with a salinity <8 psu (Table 1). Small (≤20 µm) ciliates included choreotrichs, strombidiids, cf. *Mesodinium pulex* and the bacterivorous scuticociliates. Large ciliates (>20 µm) included strombidiids and tintinnids. Both small and large ciliates tended to be low in abundance in samples with a salinity <7 psu (Table 1). Small ciliates declined in abundance between May and October (Table 1). Heterotrophic dinoflagellates were mostly small, non-thecate types and included *Gyrodinium* spp. and *Oxyhrris* sp. Except on 17 August, heterotrophic dinoflagellates were relatively low in abundance at the low-salinity station (Table 1). *Pfiesteria*-like cells were always low in abundance (Table 1). Metazoan microzooplankton, including rotifers, copepod nauplii and benthic invertebrate larvae occurred at densities of <2 ml^{-1} (data not shown).

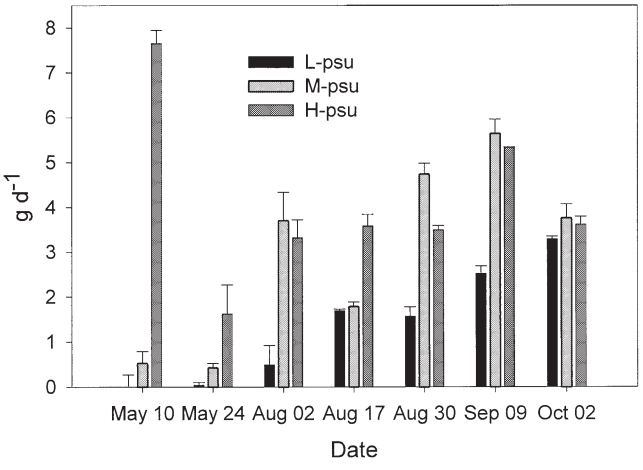

Fig. 3. Potential grazing coefficients of natural assemblages of microzooplankton on *Pfiesteria piscicida* zoospores in high-, medium- and low-salinity surface waters, Pocomoke River and Sound, 2000. Means ± SD

Table 1. Salinity, *in situ* water temperature, and composition of microplankton assemblages (cells ml^{-1}) at high (H), medium (M) and low-salinity stations, Pocomoke River, 2000. Ciliates ≤20 and >20 µm choreotrichs and strombidiids and *Mesodinium rubrum* (*Meso*); Het: heterotrophic dinoflagellates; Photo: autotrophic and mixotrophic dinoflagellates; Cryp: cryptophytes; Diat: centric diatoms; *P*-like: *Pfiesteria*-like dinoflagellates (also included in heterotrophic dinoflagellates); ns: no sample

Date Site	Salinity (psu)	Temp. (°C)	Ciliates ≤20 µm	Ciliates >20 µm	Dinoflagellates *Meso*	Dinoflagellates Het	Dinoflagellates Photo	Other Cryp	Other Diat	Other *P*-like
10 May										
H	11.4	23.3	199	9	16	50	6	12	47	3
M	7.8	23.4	50	17	360	12	6	48	17	1
L	4.9	23.6	0	0	765	6	0	96	17	4
24 May										
H	15.3	19.6	63	6	6	82	6	6	22	9
M	10.7	19.8	23	23	13	13	6	13	17	8
L	5.0	20.6	16	6	31	16	0	26	25	≤2
02 Aug										
H	12.7	26.8	53	31	9	100	6	9	97	6
M	9.2	26.9	37	37	7	107	43	7	197	3
L	2.0	26.7	13	0	0	9	47	9	63	3
17 Aug										
H	15.6	24.6	16	19	0	41	53	12	110	2
M	10.6	24.4	23	10	10	67	53	24	73	3
L	3.8	25.1	22	0	6	44	12	12	50	≤2
30 Aug										
H	14.6	25.4	13	6	0	104	16	6	138	8
M	11.2	25.5	13	13	0	137	80	13	110	19
L	5.9	25.5	0	9	6	13	135	6	41	2
13 Sep										
H	14.4	25.2	6	9	6	163	56	6	72	ns
M	10.0	25.4	0	7	0	33	814	12	0	ns
L	4.3	25.9	0	0	491	9	205	48	16	ns
02 Oct										
H	15.3	18.7	2	16	0	110	9	6	69	≤2
M	10.0	19.9	7	33	10	14	43	13	30	≤2
L	5.9	19.3	0	9	25	0	31	12	25	≤2

Potential grazing by microzooplankton on *Pfiesteria piscicida*

Potential grazing on non-toxic zoospores of *Pfiesteria piscicida* was high, with an average grazing coefficient, *g*, of 2.8 d^{-1} (Fig. 3). Variation among dates was significant, with the lowest average *g* for the H, M and L salinity samples (0.6 d^{-1}) occurring on 24 May (Fig. 3, Table 2). Grazing on zoospores was always lowest in the L sample (Fig. 3), with salinity range having a significant effect on the grazing coefficient (Table 3). Salinity accounted for about 26% of the variability in the grazing coefficient (Fig. 4). The potential grazing coefficient could also be predicted from the species composition of the microplankton assemblage, with a combination of cryptophyte abundance, photosynthetic dinoflagellate abundance and small ciliate abundance accounting for about 67% of the variability in *g* (Table 4). Inclusion of the abundance of *Mesodinium rubrum*, >20 µm ciliates, and diatoms and salinity did not significantly improve the fit of the regression model (Table 4). Grazing was positively associated with dinoflagellate and ciliate abundance but negatively with cryptophyte abundance.

Table 2. Comparison among dates of potential microzooplankton grazing (g d^{-1}) on NON-IND *Pfiesteria piscicida* zoospores, Pocomoke River, 2000

Date	Mean	SE
10 May	2.95	1.498
24 May	0.64	0.338
02 Aug	2.52	0.689
17 Aug	2.36	0.399
30 Aug	3.26	0.588
13 Sep	4.49	0.636
02 Oct	3.53	0.128

One-way ANOVA

Source of variation	df	MS	*F*	p
Among dates	6	0.0149	2.663	0.03
Residual	35	0.0056		
Total	41			

Table 3. Comparison among high-, medium- and low-salinity stations of potential microzooplankton grazing (g d^{-1}) on NON-IND *Pfiesteria piscicida* zoospores, Pocomoke River, 2000. ns: not significant

Station	Mean		SE	
High	4.08		0.502	
Medium	2.93		0.540	
Low	1.46		0.305	
One-way ANOVA				
Source of variation	df	MS	*F*	p
Among stations	2	0.0421	8.172	0.001
Residual	39	0.0052		
Total	41			
Pairwise multiple comparison (Tukey test)				
Comparison			p	
High vs Low			<0.05	
High vs Medium			ns	
Medium vs Low			ns	

DISCUSSION

During 2000, the average potential microzooplankton grazing coefficient in the Pocomoke River and Sound on non-toxic zoospores of *Pfiesteria piscicida* was >2 d^{-1} at stations with a surface salinity ≥6 psu (Tables 1 & 3, Fig. 3). Since the maximum specific growth rate, μ, of non-toxic zoospores is 1 to 2 d^{-1} (Glasgow et al. 1998, Stoecker et al. 2000, Burkholder et al. 2001), it should not have been possible for zoospore populations to increase in the more saline waters. An exception was in May, when low grazing coefficients were observed at salinities >6 psu (Fig. 3). At the low-salinity stations (2 to 6 psu) the average potential grazing was 1.46 d^{-1}, with coefficients of <1 d^{-1} occurring in May and in early August. Thus, windows with low grazing pressure occurred in which net growth of non-toxic zoospores might have been possible. These windows were most common in low-salinity water in late spring. In contrast, in 12 to 16 psu surface waters of the Chicamacomico River in 1999, mean grazing coefficients were also usually high, but windows of low grazing were observed sporadically during mid summer. In both cases *Pfiesteria*-like dinoflagellates were not abundant and toxic outbreaks did not occur.

In laboratory experiments, grazing by microzooplankton on TOX-A is lower than on TOX-B and NON-IND zoospores (Stoecker et al. 2001). Grazing control of zoospores may be low during toxic events when TOX-A zoospores are dominant.

In the study conducted in the Chicamacomico River in 1999, the most commonly observed micrograzers with ingested zoospores were tintinnid ciliates and *Strombidium* spp. (Stoecker et al. 2000). In the experiments with Pocomoke River samples, we commonly observed small non-thecate heterotrophic dinoflagellates (*Oxyhrris* spp. and *Gyrodinium* spp.) with ingested labelled zoospores. Some mixotrophic dinoflagellates can also consume *Pfiesteria piscicida* non-toxic zoospores (Stoecker et al. 2000). Potential microzooplankton grazing on *P. piscicida* was positively associated with densities of small ciliates and dinoflagellates (Table 4). It is interesting that grazing was negatively associated with cryptophyte abundance. Cryptophytes are a particularly good food for growth of zoospores, and are sources of kleptochloroplasts (stolen chloroplasts) that can provide photosynthate to *P. piscicida* (Burkholder & Glasgow 1995, Glasgow et al. 1998, Lewitus et al. 1999). Estuarine waters rich in cryptophytes should be favorable to net growth of *P. piscicida* populations because of relatively high growth rates coupled to low grazing mortality.

Mesozooplankton, including copepods and rotifers, also graze on *Pfiesteria piscicida* zoospores (Burk-

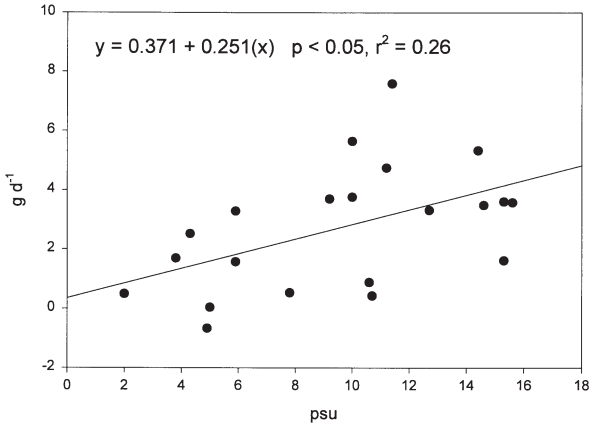

Fig. 4. Potential microzooplankton grazing on *Pfiesteria piscicida* zoospores as a function of salinity

Table 4. ANOVA of prediction of microzooplankton grazing coefficient (g d^{-1}) on NON-IND *Pfiesteria piscicida* zoospores from protist abundance (Table 1). Variables chosen with a 'best subsets regression' with adjusted R^2 as best criterion. g d^{-1} = 1.187 – (0.0215 × cryp) + (0.00556 × pdin) + (0.0182 × hdin) + (0.0217 × ≤ 20cil); N = 21; R^2 = 0.667; adjusted R^2 = 0.584; SE of estimate = 1.343, where cryp = cryptophytes ml^{-1}; pdin = autotrophic or mixotrophic dinoflagellates ml^{-1}; hdin = heterotrophic dinoflagellates ml^{-1}

Source of variation	df	MS	*F*	p
Regression	4	14.463	8.017	<0.001
Residual	16	1.804		
Total	20	4.336		

holder & Glasgow 1995, Mallin et al. 1995). However, grazing by microzooplankton is probably a more important mortality factor for zoospores than grazing by mesozooplankton. During the late spring and summer, the dominant copepod in mesohaline waters of Chesapeake Bay is *Acartia tonsa*. Mean adult and copepodite densities are about 6 adults and 8 copepodites l^{-1} (Chesapeake Bay Program, Stn ET5.2, May to Oct 1997 to 1999: www.chesapeakebay.net). In mesohaline waters of Chesapeake Bay, mean clearance rates of *A. tonsa* adults and copepodites is about 7 and 3 ml ind.$^{-1}$ d^{-1}, respectively (White & Roman 1992). Assuming that similar values apply to the Pocomoke and Chicamacomico Rivers, this would result in average grazing coefficients of copepods on the order of 0.1 d^{-1}, which is much lower than the measured grazing coefficients of microzooplankton assemblages (Stoecker et al. 2000 and data presented herein). Copepod grazing may indirectly have a negative effect on the total grazing pressure on zoospores because copepods have higher clearance rates for ciliates than they do for small flagellates such as *P. piscicida* zoospores (Stoecker & Sanders 1985, Gifford & Dagg 1988, Hansen et al. 1994). Microzooplankton populations, particularly planktonic ciliates, respond quickly to changes in mesozooplankton abundance, and, in turn, microzooplankton strongly control <15 µm flagellates (Miller et al. 1995, Adrian et al. 2001).

Differences in community structure and productivity along salinity gradients (Marshall & Nesius 1993, Sin et al. 2000) may influence the net growth of non-toxic zoospores both by controlling availability of prey for zoospores and by affecting grazing pressure. Cryptophytes are ubiquitous in oligohaline and mesohaline regions of tidal estuaries in the lower Chesapeake Bay (Marshall & Nesius 1993). This should favor net growth of *Pfiesteria piscicida* zoospores. Dinoflagellates, which are associated with high grazing coefficients on zoospores, are less abundant in the upper, oligohaline sections of tidal rivers than in the middle and lower, more saline, sections (Marshall & Nesius 1993). Higher nutrient levels in the upper, less saline, sections of tidal rivers, such as the Pocomoke (Maryland Department of Natural Resources 1998), may be associated with reduced grazing pressure. Eutrophication of tidal rivers is correlated with a decrease in total micro- and mesozooplankton biomass and specifically with a decrease in tintinnid biomass at mesohaline stations (Park & Marshall 2000). Presumably, eutrophication would result in decreased grazing pressure on small dinoflagellates such as *P. piscicida*. Freshets that reduce salinity as well as increase nutrient loading in estuaries may provide windows of opportunity for net growth of zoospore populations due to increased availability of prey (Glibert et al. 2001) and reduction in grazing mortality. The relationships among these factors and their relative importance as antecedent conditions for toxic *P.* outbreaks remain to be investigated.

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