Spring 2005

Feeding Behavior and Conditioning in Two Heterotrophic Dinoflagellates

Todd A. Egerton
Old Dominion University

FEEDING BEHAVIOR AND CONDITIONING

IN TWO HETEROTROPHIC DINOFLAGELLATES

by

Todd A. Egerton
B.S. June 2001, Susquehanna University

A Thesis Submitted to the Faculty of
Old Dominion University in Partial Fulfillment of the
Requirement for the Degree of

MASTER OF SCIENCE

BIOLOGY

OLD DOMINION UNIVERSITY
May 2005

Approved by:

Harold G. Marshall (Director)

William Resetarits (Member)

David Seaborn (Member)

ABSTRACT

FEEDING BEHAVIOR AND CONDITONING IN TWO HETEROTROPHIC DINOFLAGELLATES

Todd A. Egerton
Old Dominion University, 2005
Director: Dr. Harold G. Marshall

Growth and abundance of heterotrophic dinoflagellate predators are generally attributed to the availability of algal prey. Several species of dinoflagellates feed on a wide variety of food types including fish. However the actual feeding preferences of dinoflagellates have been much less studied. In the few studies that have been carried out on dinoflagellate feeding preference, none have looked at possible factors that may affect preference. I conducted three experiments on the toxic dinoflagellate *Pfiesteria piscicida* and the related unnamed species Cryptoperidiniopsis brodyi which, (1) calculated the feeding preferences between algal *Rhodomonas* prey and fish blood cells and identified factors that effect the preferences, (2) looked for an effect of prior diet conditioning on the grazing rates of both species, and (3) compared the growth rates of both species from two different feeding histories. Data from these three experiments demonstrate that *P. piscicida* and C. brodyi feed on both algal and fish prey and have the ability to feed preferentially. Both species of dinoflagellates showed a strong preference for fish blood cells over *Rhodomonas.* Furthermore, the feeding preferences were influenced by total prey abundance, but not by prior diet conditioning.

ACKNOWLEDGMENTS

I would like to thank my committee members Dr. Harold Marshall, Dr. William Resetarits and Dr. Dave Seaborn for their help during this research. I am especially grateful to my major advisor, Harold Marshall for continued financial support while studying at Old Dominion University. I would also like to thank Todd Stem for his inspiration and mentoring, without which these experiments would never have taken place. I extend a warm thanks to Dr. Sławomir Cerbin who helped with culturing techniques and provided immeasurable assistance in writing and editing.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Theoretical background

Understanding the mechanisms governing community composition has long been a focus of ecological research (eg. Hutchinson 1941, Udvardy 1959, MacArthur 1960). A number of factors, both stochastic (Sousa 1979), and density dependent, such as competition (Elton 1946, Crombie 1947, Dayton 1971, Tilman 1977) and predation (Paine 1971, Menge 1976) have been recognized as significantly affecting community structure. Some of the most studied processes are those allowing coexistence between multiple species competing for a limited resource (Dugdale 1967, Kilham 1971, Stewart and Levin 1973, Patrick 1976, Tilman 1981).

Competition for a limited resource was first modeled by Lotka (1924) and Volterra (1931), tested by Gause (1934) and led to the principle of competitive exclusion (Hardin 1960). This theory states that when a community is in equilibrium, competition allows for only one species to fill a specific niche. Many examples of competitive exclusion have been identified in numerous systems. These include studies of flour beetles, frogs, cra[1]yfish, protists and zooplankton (Gause 1934, Park 1948, Inger and Greenberg 1966, Bovbjerg 1970, Smith et al. 1975).

However, the diversity of competitors observed in some systems appears to contradict the predicted outcomes of competition theory (Connel 1978). One such group of organisms, as recognized by Hutchinson (1961), is phytoplankton. The 'paradox of

---

The model journal used in this thesis is the *Journal of Phycology*

the plankton' is that a high species richness of phytoplankton species can coexist in seemingly the same niche. Hutchinson proposed that this is due to the lack of equilibrium in aquatic systems at the scale of the plankton. An examination of any body of water suggests that resource conditions may be highly dynamic due to turbulence, predation, micro-nutrient zones and other factors, thus preventing environmental conditions from persisting long enough for one species to become the competitive dominant. Hutchinson's (1961) paper stimulated a substantial body of work refining models to further explain the paradox of the plankton (Petersen 1975, Tilman 1980, Tilman et al. 1982, Siegel 1998, Yamamoto et al. 2002). Several other non-equilibrium as well as equilibrium mechanisms that allow for coexistence have been identified as a result.

Partitioning of resources is one example of an equilibrium mechanism that can allow for higher species diversity. Multiple competitors may coexist by specializing on narrower niches within a given range of resources (Morin 1999). Partitioning of resources may take the form of spatial or temporal species separation (eg. Fox 1981, Pleasants 1980) or by exploiting different resources (Tilman 1982). Differential resource usage may create functionally different niches to occupy and reduce the degree of competition between multiple species. Tilman (1977) reported coexistence mediated by differential resource use by two diatom species. Each diatom species had a unique set of nutrient requirements and uptake rates that allowed the two to coexist within a certain range of nutrient ratios. Differential resource use in the form of preferential feeding is a mechanism that has been identified in several aquatic systems (Wood 1968, Bryan and Larkin 1972, Vadas 1977, Scott and Murdoch 1983).

Classical optimal foraging theory assumes that a predator will preferentially consume the resource that will maximize its fitness through maximum net energetic intake (MacArthur and Pianka 1966, McNamara and Houston 1985). The specific foraging behavior that produces optimal results will be different depending on the conditions of the situation. Different levels of resources may favor generalists, specialists, or facultative strategists. In cases where a predator encounters changing levels of prey, a facultative strategy should provide maximal energy uptake (Glasser 1984). In this situation a predator would specialize on the most profitable prey item when it is abundant, and expand its diet to less profitable ones when resources are scarce.

Optimal foraging theory requires that the organism in question to be able to differentiate between available food sources and feed preferentially. This behavior has been demonstrated with several aquatic metazoan predators (Frost 1972,Vadas 1977, Scott and Murdoch 1983, Steinberg 1985). These preferences may be based on chemical cues, prey size, nutritional content, or other identifying characteristics. The majority of preferences observed in mollusks involve an optimal prey size, although some predators show preferences between similar sized particles (Heinbokel 1978). To a lesser extent, selective predation has been identified in the protist kingdom (Stoecker et al. 1981, Johnson and Anderson 1986, Simek et al. 1995).

Feeding preferences are often not static, and may be altered depending on the prior and current conditions. Preferences between two available food types may change as the ratio of one type to the other changes (Murdoch et al. 1975). In this situation, called switching, a predator will feed disproportionately on the most abundant food type, and show less preference when equal amounts of both food types are present. Predator feeding selectivity can also be effected by overall food abundance due to hunger, with

preferential selection being reduced at low food levels (Akre and Johnson 1979, Pastorok 1980). Prey preferences can vary on an individual-by-individual basis as well through conditioning. Avila (1998) demonstrated the conditioning of nudibranch mollusks using different diet histories. The study showed the ability of the nudibranchs to detect different prey items was influenced by the diet the animals had been previously fed. A similar experiment reported that blue crab feeding preference was significantly effected by the size of the food used in prior conditioning (Micheli 1995). While these are examples of learned behavior in metazoans, other studies suggest conditioning can also occur with cultures of single cell organisms (Anderson 1980).

Phytoplankton are strongly influenced by environmental conditions, with each species having an optimal range for development along several biotic and abiotic gradients (Hulburt 1982, Higashi and Seki 2000). In addition, many species are capable of acclimating to different levels of nutrients, temperature, and light (Bannister 1979, Hulburt 1985). Dinoflagellates in particular, have complex life histories that vary with the environment, with different life stages in response to different situations (Prezelin and Matlick 1983). Dinoflagellates are capable of detecting and reacting to many stimuli including light, temperature, gravity, chemical, and mechanical cues (Levandowsky and Kaneta 1987, Cancellari et al. 2001).

Study system

About half of all dinoflagellate species are obligate heterotrophs lacking chloroplasts with the vast majority of species that do contain chloroplasts requiring some additional uptake of organic substances (Gaines and Elbrachter 1987). Heterotrophic dinoflagellates have been recognized as significant components in marine and estuarine

communities (Tislelius and Kylensteirna 1996, Jeong 1999), and have been shown to reduce both algal and fish populations through grazing and toxic activity (Hansen 1991, Burkholder 1998). Predation by heterotrophic dinoflagellates can be a significant limiting factor in some toxic algal blooms (Matsuyama et al. 1999).

Due to their toxin production and associated fish kills, *Pfiesteria piscicida, Pfiesteria shumwayae,* and *Pfiesteria*-like dinoflagellates have been studied extensively since their identification in 1992 (e.g. Burkholder et al. 1992, Kane et al. 1998, Gordon et al. 2002, Vogelbein et al. 2002). These species have been identified in estuarine waters throughout the eastern United States, as well as in Northern Europe and New Zealand (Rublee et al. 1999, Burkholder et al. 2001). These dinoflagellates are grouped together based on morphological, ecological, and genetic similarities (Marshall 1999, Parrow and Burkholder 2003). The relationship of *Pfiesteria* and several *Pfiesteria*-like species compared to other dinoflagellates is shown using a phylogram (Fig. 1) constructed from 18s RNA sequences taken from GenBank (National Center for Biotechnology Information http://www.ncbi.nlm.nih.gov). One group of *Pfiesteria*-related species are the cryptoperidinopsoids. These dinoflagellates have not been formally defined although the term cryptoperiniopsoid or Cryptoperidiniopsis has been used in previous studies (Seaborn et al. 1999, Burkholder et al. 2001, Seaborn et al 2002, Parrow and Burkholder 2003). The informally adopted name Cryptoperidiniopsis brodyi is used to identify the species addressed in the present study. Electron micrographs of the two dinoflagellates studied are shown in Fig. 2.

Pfiesteria species are capable of feeding on a diverse assemblage of algal species (Seaborn et al. 1999), bacteria (Parrow and Burkholder 2003), finfish, shellfish, and mammalian red blood cells (Burkholder and Glasgow 1995, Glasgow et al. 2001). The

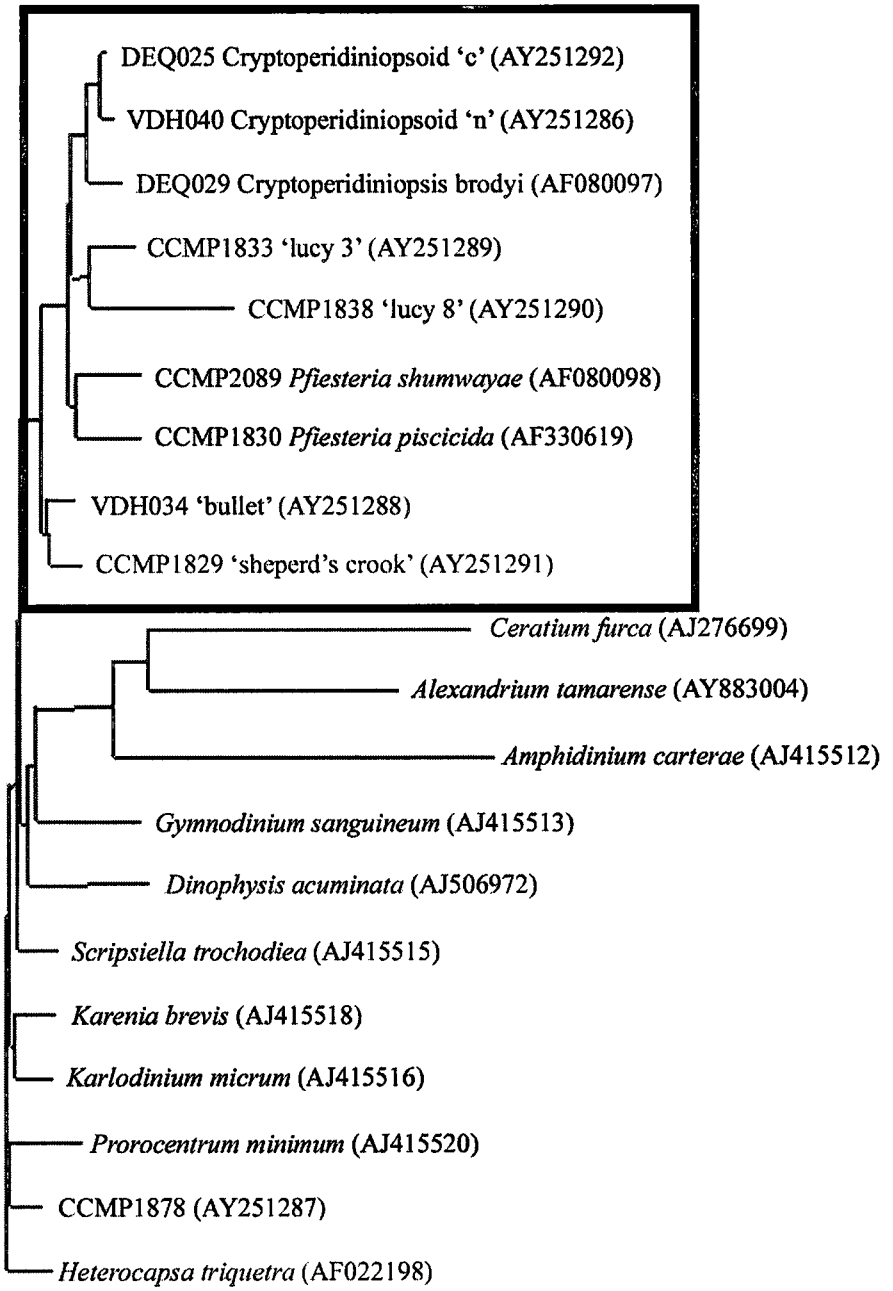

FIG. 1. Cladogram showing the relationship between 20 dinoflagellate species. The group of *Pfiesteria*-like dinoflagellates within the box are found to be closely related to each other based on neighbor joining analysis; values greater than 50 shown. These relationships are based on published 18s RNA sequences (accession numbers in parenthesis) available at the National Center for Biotechnology Information http://www.ncbi.nlm.nih.gov. Cladistic analysis was done using Clustal X and TreeView software.

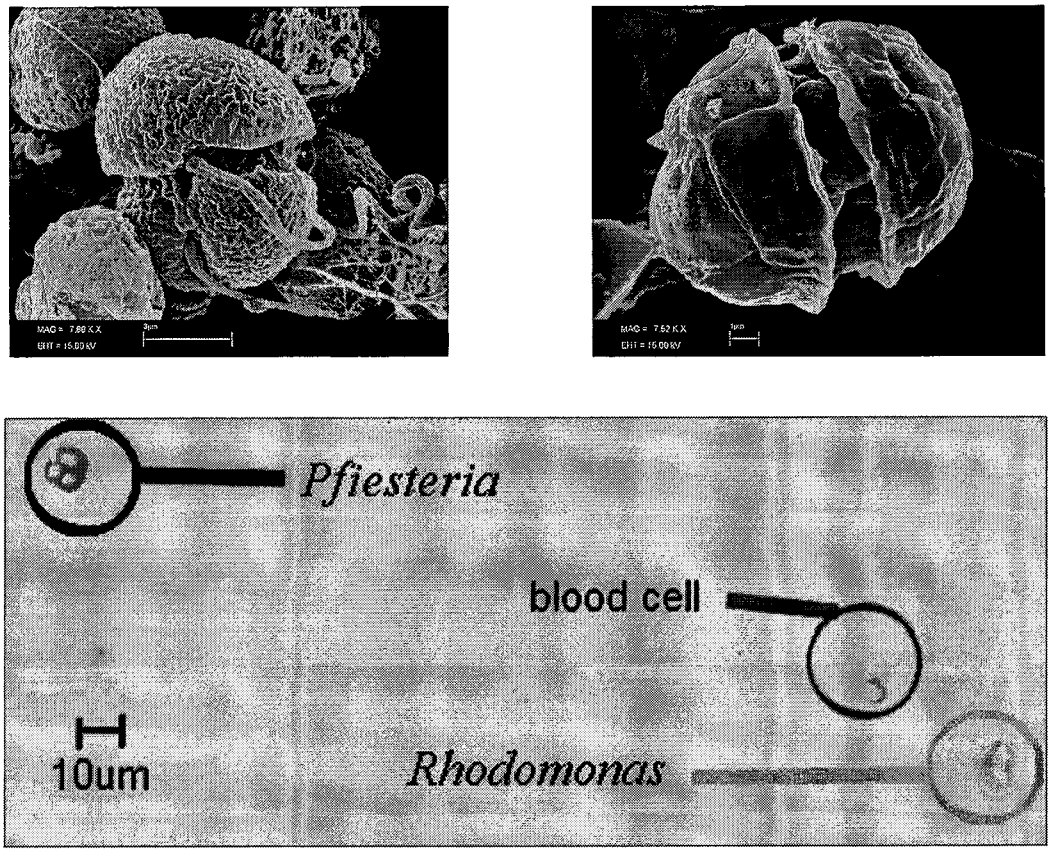

FIG. 2. Scanning electron micrographs of *Pfiesteria piscicida* (left) and Cryptoperidiniopsis brodyi (right). Light micrograph (below) showing size relationship between the algal prey *Rhodomonas* sp., fish blood cell, and *Pfiesteria piscicida*.

current understanding of the natural history of PLOs is that they are common inhabitants of estuaries and remain benign until they are triggered by a biotic or abiotic factor, causing the population to rapidly reproduce and form potentially toxic blooms (Rublee et al. 1999). The presence of a toxin has been the topic of debate in recent literature (Vogelbein et al. 2002, Burkholder and Glasgow 2003). One possible explanation for the disparity in results reported by different researchers may be due to physiological or behavioral differences between different strains of the same species (Gordon 2002). Alternatively, differences in feeding behavior may arise from being exposed to different prior conditions.

Purpose

In complex systems that include multiple omnivorous predators and multiple prey items, there is a strong chance that competing predators show differing preferences both to maximize energy intake and avoid competitive exclusion. Due to their wide diet, this is likely the scenario with heterotrophic dinoflagellates and in particular *Pfiesteria*-like dinoflagellates. Prior studies to identify the optimal food source for these dinoflagellates relied on comparing growth curves (Seaborn et al. 1999), and did not actually provide the dinoflagellates with a food preference when given multiple food types. This study will investigate whether or not such a preference is shown between algal and fish prey.

Additionally, PLOs appear to have different life histories depending on the environment they are subjected too, specifically whether or not they have been in the presence of fish. The toxin producing activity of *P. piscicida* and *P. shumwayae* has been shown to be dependent on their proximity to fish (Marshall et al. 2000). When fish are removed from cultures for extended periods of time, the ability to produce toxin is

reduced or lost entirely (Burkholder et al. 2001). Since toxin production is directly related to feeding, this suggests that their feeding behavior may be influenced by the presence of specific prey. This research examines whether *P. piscicida* and C. brodyi possess intrinsic feeding preferences, and whether these preferences may be influenced by present and prior conditions.

Hypotheses

Experiment 1)- Feeding preference

a) Determine if different PLOs feed preferentially, given the choice between the alga *Rhodomonas* and the Atlantic Croaker.

H_o: all PLOs feed unpreferentially, ie. All species feed on prey solely based on its availability.

H_A: one or more species of PLOs feed preferentially on either algae or fish

b) Determine whether PLO feeding preference is influenced by ratio of food offered (N_1/N_2).

H_0: PLO feeding preference will remain constant regardless of N_1/N_2.

H_A: feeding preferences will increase or decrease as a result of differences in N_1/N_2.

c) Determine whether PLO feeding preference is influenced by total food abundance.

H_0: PLO feeding preference will remain constant regardless of total abundance

H_A: feeding preference will increase or decrease as a result of differences in total abundance

Experiment 2)- Conditioning and grazing rate

Determine if PLO grazing rate on each food type is affected by prior feeding history.

H_o: no difference in grazing rate on algae and fish between feeding histories.

H_A: one or more species of PLO will have a higher grazing rate on the prey type they have been conditioned on.

Experiment 3)- Conditioning and growth rate

Determine if PLO growth rate on each food type is affected by feeding history.

H_o: no difference between growth rate of PLOs between feeding histories.

H_A: one or more species of PLO will have a higher growth rate when feeding on the prey type they have been conditioned on.

CHAPTER II

MATERIALS AND METHODS

Culturing

Cultures of *Pfiesteria piscicida* were originally obtained from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP1834), West Boothbay Harbor, ME. The dinoflagellate informally named 'cryptoperediniopsis brodyi' (01DEQ039) was established from water and sediment samples taken from Virginia waters during routine sampling. Dinoflagellate species were identified using scanning electron microscope analysis utilizing the suture swelling technique (Burkholder and Glasgow 1995). Real-time PCR analysis was used to verify species identification and test for cross contamination (Bowers et al. 2000).

Unialgal cultures were established from single cell isolations by serial dilutions. A unialgal culture refers to a clonal culture of a single dinoflagellate species, along with its obligate food source. The algal food source for all dinoflagellate cultures was the cryptophyte *Rhodomonas* sp (CCMP 768). Algal fed cultures were grown in 200 ml Falcon tissue flasks using a F/2-Si nutrient media (Guillard 1975) and stored in the dark at room temperature (26°C). The media was made using 0.2 μm filtered Atlantic Ocean seawater. The salinity was diluted to 15 ppt for all media used in this study. A 1 ml aliquot of *Rhodomonas* (~14,000 cells/ml) was added to each flask as needed, on average 2-3 times per week, media changes were made monthly.

Prey types and preparation

Algal prey used in the experiment was the same strain used in maintenance of the dinoflagellates. Fish prey used during conditioning was prepared using frozen Atlantic

Menhaden (*Brevoortia tyranus*) from Chesapeake Bay, purchased fresh at The Dockside Inn, Virginia Beach, VA and fresh Atlantic croaker (*Micropogonias undulatus*), purchased at George's Seafood, Norfolk, VA. The three-month fish conditioning diet consisted of a combination of fish tissue (primarily from *Brevoortia* for the first two months) and fish blood cells (primarily from *Micropogonias* for the third month).

Fish tissue used in the conditioning experiments was prepared using the following technique. The fish were thawed and rinsed of excess mucous. Filets, including skin and scales, were cut from the body of the fish with care made not to penetrate the body cavity. Filets were placed in a standard food processor along with 150 ml of F/2 nutrient media at 15 ppt. The blender was set to the highest setting for one minute. The contents were first strained through a 500 μm mesh to remove any scales and large pieces of tissue, followed by passage through a 100 μm aperture net, and finally a 10 μm aperture mesh. The solution was stored in 50 ml centrifuge tubes and frozen or used as needed. Particle densities were recorded using a hemocytometer following a 1:1000 dilution.

Fish blood cells were processed by dissecting fresh *Micropogonias* along the ventral section of the body and removing the internal organs. The dorsal aorta was exposed and cut using a scalpel. Blood was taken from the aorta with a syringe and filtered through a 100 μm aperture net. The blood was mixed with F/2 nutrient media at 9 ppt and diluted as necessary. Samples of *Rhodomonas*, *Brevoortia*, and *Micropogonias* were incubated and checked for parasites and other contamination.

Experiment 1: Feeding preference

Dinoflagellate densities were measured and reduced as needed by dilution to obtain equal initial cell densities between all treatments. All dinoflagellates used had been raised on algal prey.

Seven food treatments were made up of varying ratios of *Rhodomonas* cells and *Micropogonias* blood cells with a total abundance 3 times that of the dinoflagellate density. Initial food levels are shown in Fig. 3.

Two food treatments were compared to address the effect of total food abundance. A high treatment indicated a 3:1 prey : dinoflagellate ratio, while a low treatment indicated a 1:1 ratio. The noted 50% *Rhodomonas* 50% blood treatment (Treatment 4 in Fig. 3) was compared with the 50% *Rhodomonas* 50% blood treatment shown as Treatment 8 in Fig. 3.

Dinoflagellates and food treatments were added to a 96 well Nunclon© tissue culture plate using micropipettes. The volume of each well was brought up to 150 µl using nutrient media. There were three replicate treatments for each species plus three controls that contained no dinoflagellates. After the application of the food treatments, the well plate was sealed with tape and kept in the dark for 6 hours. After 6 hours each well was fixed with 2 µl 25% gluteraldehyde. Additionally, three replicates for each treatment were added to a separate identical well plate and fixed immediately. Each well was analyzed using an inverted light microscope at 200x magnification. All dinoflagellate and prey cells were identified and counted in each well.

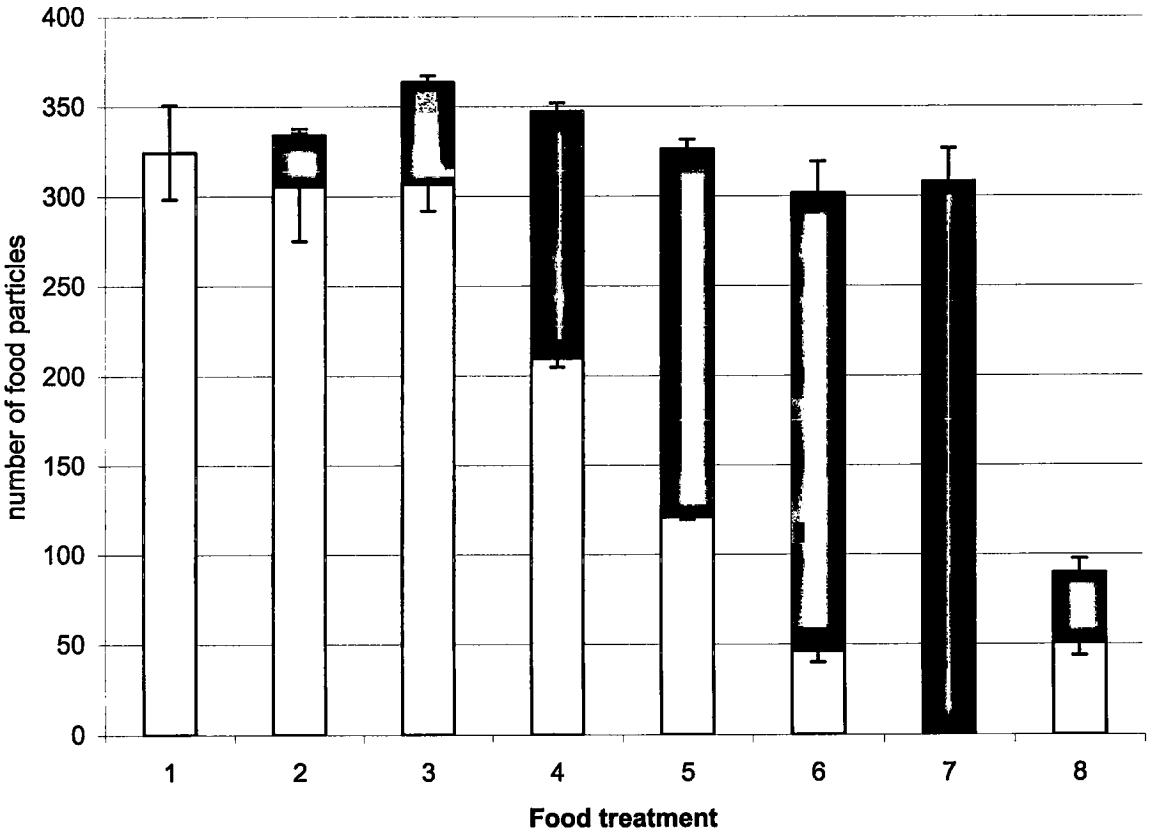

FIG. 3. Mean initial levels of *Rhodomonas* (black) and fish blood cells (white) for each treatment. Error bars shown represent variability in initial abundance within 3 replicates.

The index of preference (C) was calculated using the following equation first described by Murdoch (1969), where N^1_e, N^2_e are the numbers of prey types I and II eaten; N^1, N^2 are the numbers of prey types I and II initially.

$$\frac{N_e^1}{N_e^2} = C\frac{N^1}{N^2}$$

Analysis of variance (ANOVA) was used to compare the index of preference. To address the effect of food ratio on preference, the index of preference was compared between species and food ratio treatments. A Ryan-Einot-Gabriel-Welsch F *post-hoc* test was performed to separate the effect between the different food treatments. A separate ANOVA was used to compare the index of preference between species and the level of overall abundance. SPSS for Windows 10.0 was used for all analyses.

Experiment 2: Conditioning and grazing rate

Cultures of *P. piscicida* and C. brodyi were maintained on separate diets for three months prior to the grazing experiment. The dinoflagellates were fed either an algal or fish diet as described above. Care was taken so that both food treatments received roughly equal amount of food during the conditioning. Dinoflagellates of each species from both feeding histories and *Rhodomonas* were added to a 96 well plate as above at a 3:1 *Rhodomonas*: dinoflagellate ratio. There were three replicate treatments for each species plus a control with no dinoflagellates. The well plate was placed in the dark for 6 hours. At the conclusion, each well was fixed with gluteraldehyde. All dinoflagellates and *Rhodomonas* cells were identified and counted as above. The grazing rates were compared between species and feeding history with ANOVA.

Experiment 3: Conditioning and growth rate

Cultures of *P. piscicida* and C. brodyi were maintained on separate diets for three months prior to the growth rate experiment. The dinoflagellates were fed either an algal or fish diet as described above. Care was taken so that both food treatments received roughly equal amount of food during the conditioning. The flasks used in the experiment were identical to those used in the routine maintenance. Three replicate flasks for each treatment were used. Food levels were held constant between treatments, initially at ~100 times that of the dinoflagellates present, and were prevented from becoming limiting during the course of the study by adding more food if necessary. Single 1 ml aliquots were taken every 2-3 days for 26 days and preserved in Lugol's solution. Dinoflagellate cell densities were calculated using Palmer-Maloney slides at 100x.

The maximal growth rates (μ) for each flask were calculated using the equation

$$\mu = \frac{\ln\left(\frac{N_t}{N_0}\right)}{t}$$

where N_0=number of cells at the beginning of the slope and N_t=number of cells at the end; t= incubation time (h). An ANOVA was used to compare the maximum growth rates between species and feeding history.

CHAPTER III

RESULTS

Grazing rates

*Pfiesteria piscicida,*and Cryptoperidiniopsis brodyi fed on both the *Rhodomonas* algal prey and the fish blood cells during the experiment. In all treatments, food levels of both types were reduced by both species of dinoflagellates compared to the no-dinoflagellate controls as shown in Fig. 4. Both dinoflagellates species demonstrated a functional feeding response to levels of fish blood cells (Fig. 5) and *Rhodomonas* cells (Fig. 6). The grazing rate of *P. piscicida* on blood cells was significantly higher than that of C. brodyi. *P. piscicida* also had a significantly higher grazing rate on *Rhodomonas* than C. brodyi as presented in Table 1.

Results of ANOVA show that the grazing rates of both species were also significantly greater on fish blood cells than on *Rhodomonas*. *P. piscicida* had a maximum grazing rate of 3.49 blood cells x dinoflagellate^{-1} over the course of 6 hours, while C. brodyi's maximum grazing rate was 2.00 blood cells x dinoflagellate^{-1}x 6 hours^{-1}. Maximum grazing rates on R*hodomonas* were significantly lower. *P. piscicida* grazed as many as 1.88 *Rhodomonas* x dinoflagellate^{-1}x 6 hours^{-1} with C. brodyi's maximum only 0.747 *Rhodomonas* x dinoflagellate^{-1}x 6 hours^{-1}.

Preference experiments

The feeding preferences of *P. piscicida* and C. brodyi were calculated by comparing relative predation on the two food types compared to their initial abundance. The preferred food type for each species was fish blood cells in all treatments as shown in Fig. 7. Results from ANOVA (Table 2) indicate no significant difference in feeding

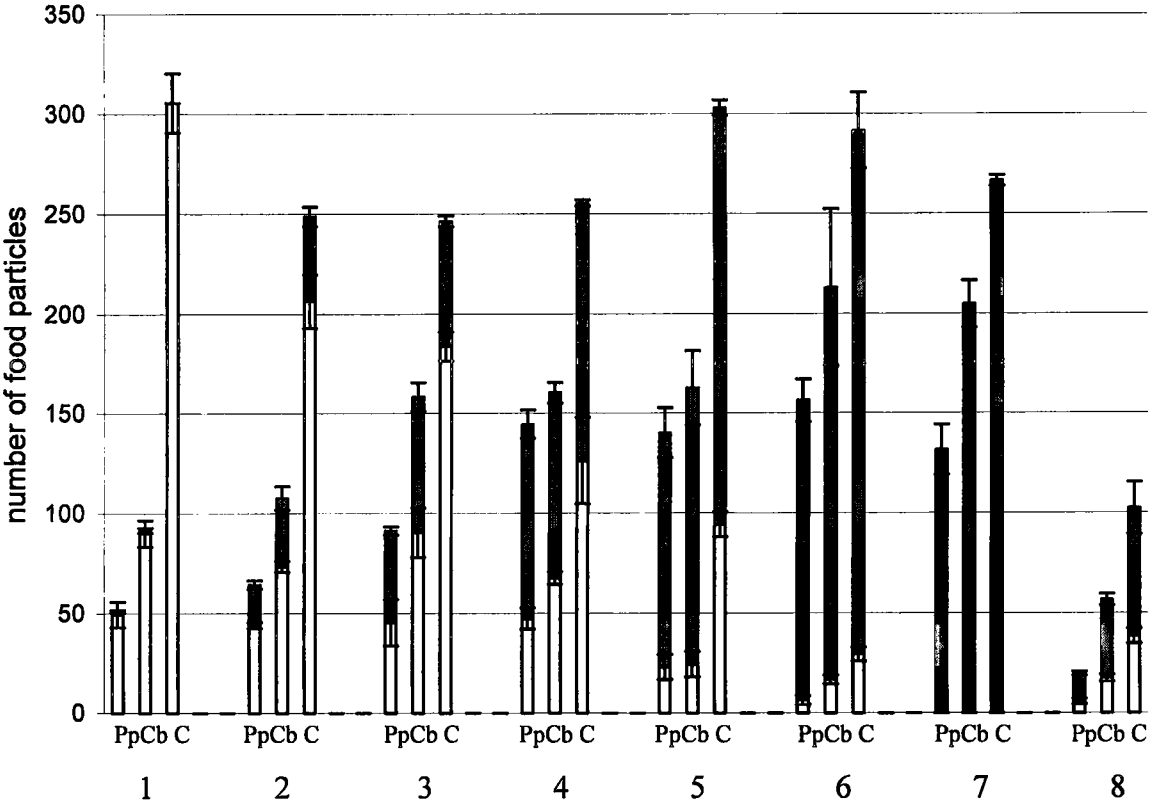

FIG. 4. Remaining levels of *Rhodomonas* (black) and fish blood cells (white) after 6 hours of grazing by *Pfiesteria piscicida* (Pp) and Cryptoperidiniopsis brodyi (Cb). At each food treatment (1-8) both food types were significantly reduced by both species compared to the control (C), which contained no dinoflagellates.

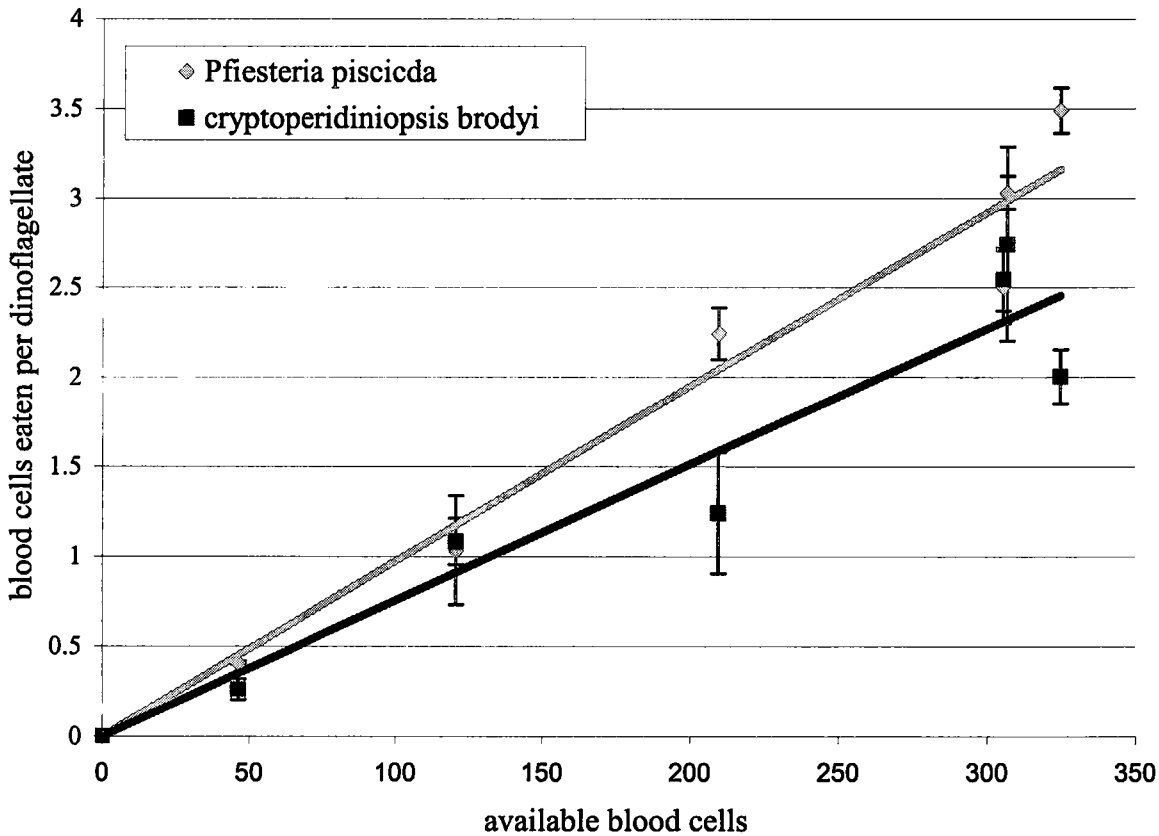

FIG. 5. Grazing rates of *Pfiesteria piscicida* and Cryptoperidiniopsis brodyi on fish blood cells at different prey abundances. Both species demonstrated a linear Type-1 functional response, with increased grazing activity at increased prey levels. *P. piscicida* had a maximum grazing rate of 3.49 blood cells x $dino^{-1}$ x 6 hr^{-1}, and C. brodyi's maximum grazing rate was 2 blood cells x $dino^{-1}$x 6 hr^{-1}.

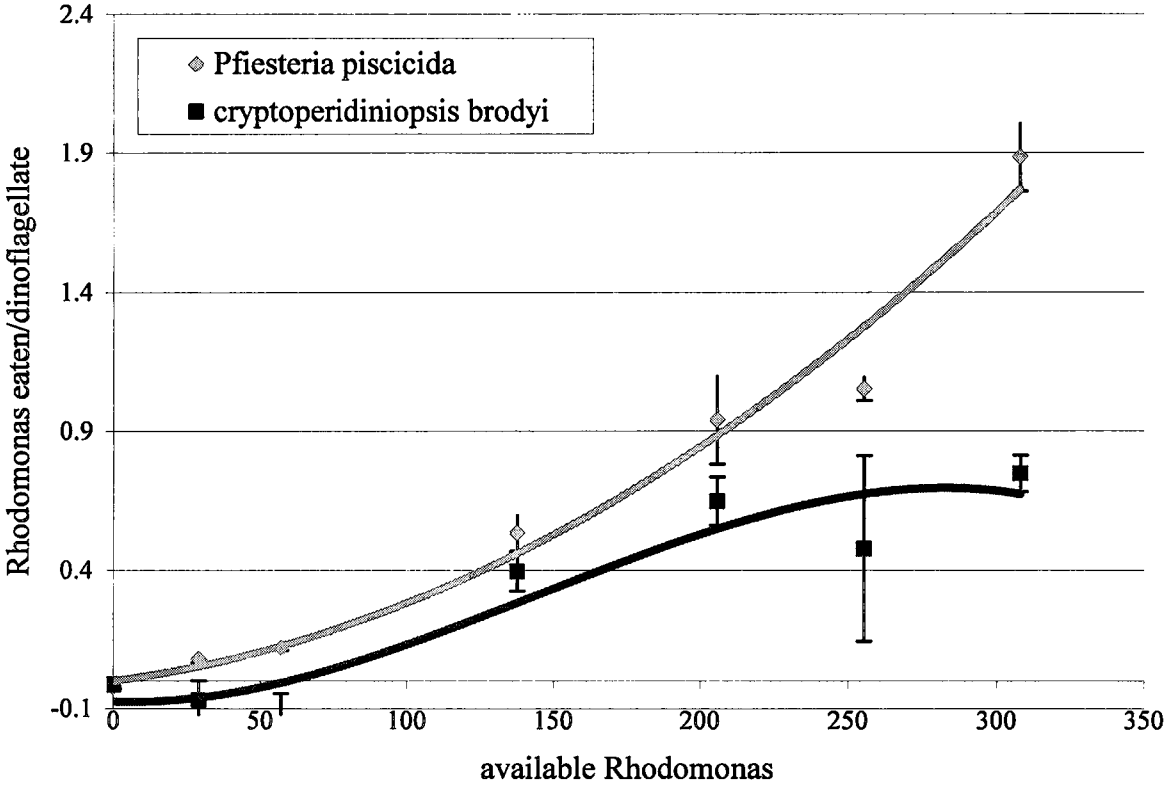

FIG. 6. Grazing rates of *Pfiesteria piscicida* and Cryptoperidiniopsis brodyi on *Rhodomonas* at different prey abundances. *P .piscicida* demonstrated a Type–1 functional response to increasing *Rhodomonas* levels with a maximum rate of 1.88 *Rhodomonas* x dino^{-1}x 6 hr^{-1}. C. brodyi demonstrated a Type-3 response, with grazing rates leveling off at a maximum of 0.747 *Rhodomonas* x dino^{-1}x 6 hr^{-1}.

TABLE 1. ANOVA table comparing grazing rates between two dinoflagellate species (*Pfiesteria piscicida* and Cryptoperidniopsis brodyi) crossed with two food types (*Rhodomonas* sp. and fish blood cells).

Source	df	SS	MS	F	P
Species	1	5.172	5.172	119.373	<0.001
Food type	1	6.150	6.150	141.938	<0.001
Species x food type	1	9.343×10^{-2}	9.343×10^{-2}	2.156	0.180
Error	8	0.347	4.333×10^{-2}		
Total	12	61.280			

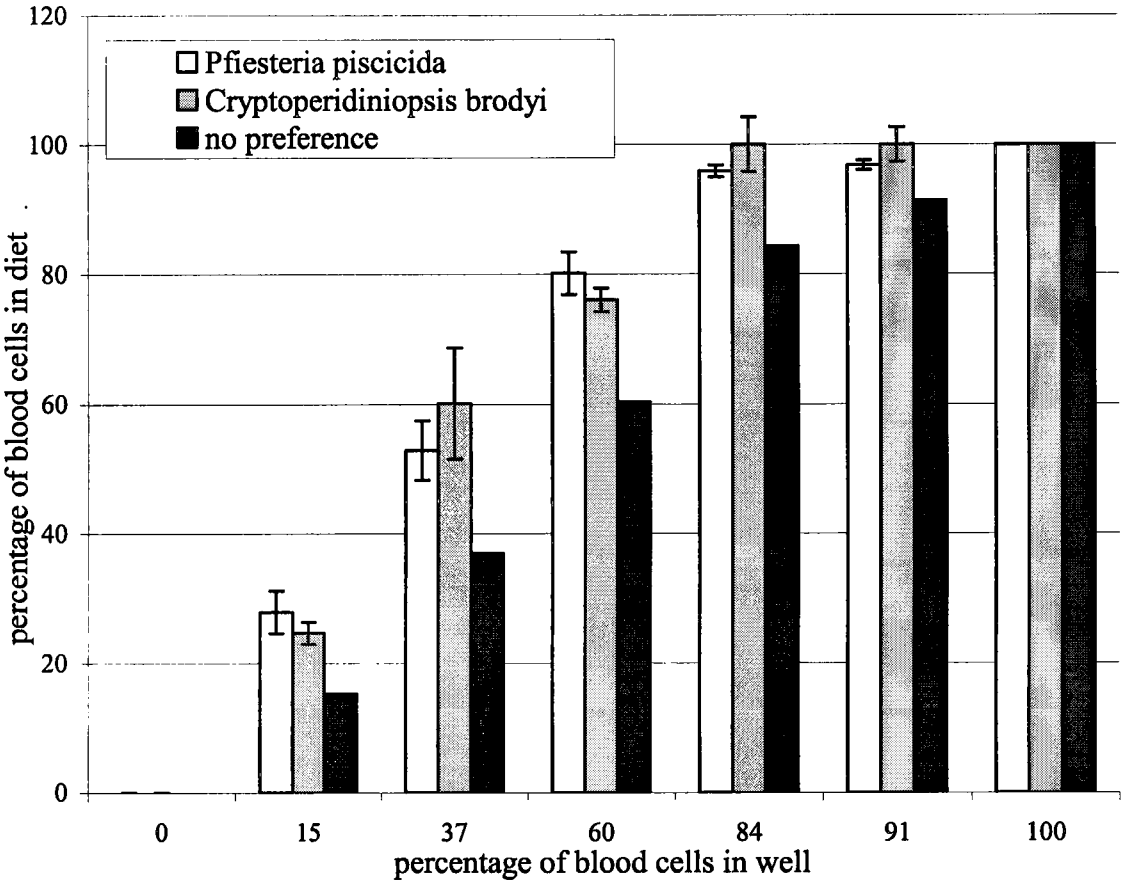

FIG. 7. Percentages of blood cells in the diets of *Pfiesteria piscicida* (white) and Cryptoperidiniopsis brodyi (gray) compared to the percentage of blood cells available in each well (black). At each food ratio, both species have a significantly higher percentage of blood in their diet than in their surroundings signifying a preference for blood cells.

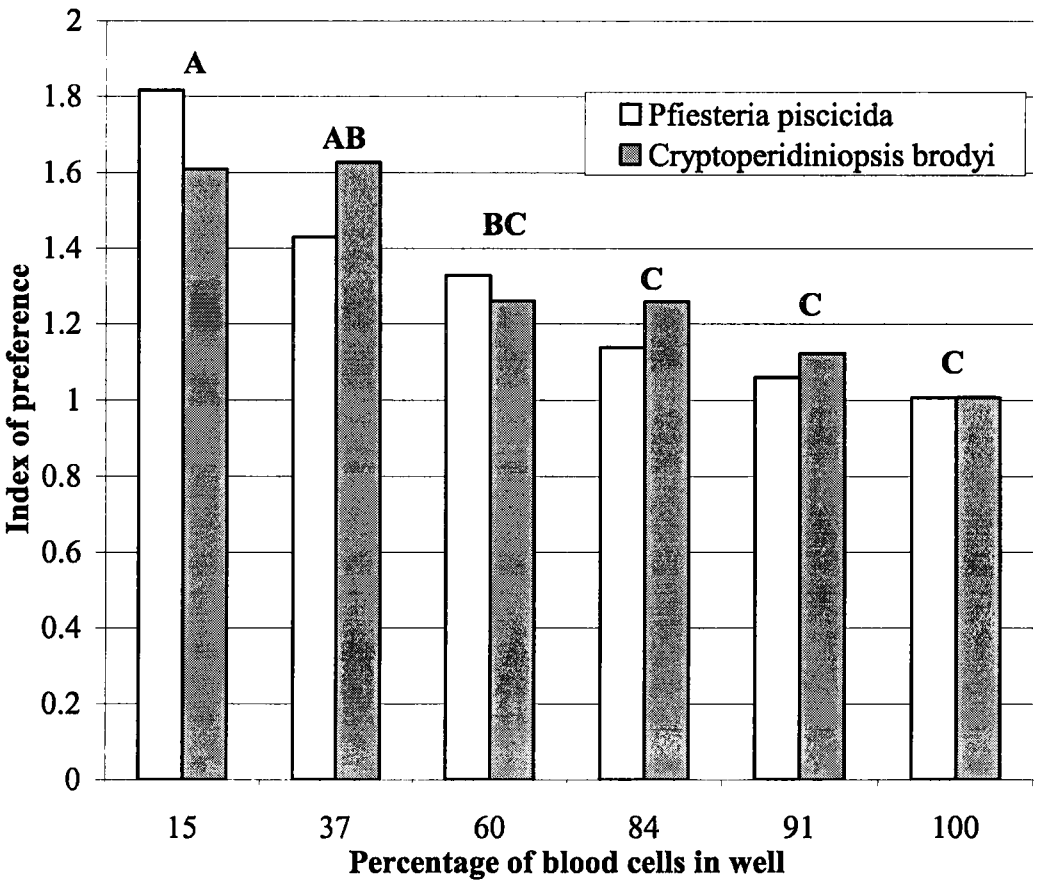

FIG. 8. Indexes of preference for fish blood cells over *Rhodomonas* shown by *Pfiesteria piscicida* (white) and Cryptoperidiniopsis brodyi (gray) at different initial percentages of blood cells. There was no significant differences between species. The results of the REGWF *post hoc* test are shown above the bars, with significant differences in the index of preference between food ratio treatments shown by dissimilar letters.

TABLE 2. ANOVA table comparing the index of preference (C) between two dinoflagellate species (*Pfiesteria piscicida* and Cryptoperidniopsis brodyi) crossed with six different ratios of food.

Source	df	SS	MS	F	P
Species	1	2.639×10^{-3}	2.639×10^{-3}	0.079	0.782
Ratio	5	1.967	0.393	11.715	<0.001
Species x ratio	5	0.139	2.786×10^{-2}	0.830	0.542
Error	23	0.772	3.358×10^{-2}		
Total	35	61.824			

TABLE 3. ANOVA table comparing the index of preference (C) between two dinoflagellate species (*Pfiesteria piscicida* and Cryptoperidniopsis brodyi) crossed with two different levels of total abundance.

Source	df	SS	MS	F	P
Species	1	0.286	0.286	11.768	0.009
Abundance	1	0.104	0.104	4.274	0.073
Species x abundance	1	0.425	0.425	17.469	0.003
Error	8	0.772	2.433×10^{-2}		
Total	12	24.100			

from both species from both feeding histories were able to grow on an algal diet. Data from the *Rhodomonas* treatments are shown in Fig. 9. The maximum period of growth for all strains was observed between day 15 and 18. *P. piscicida* exhibited a mean maximum growth rate of 0.360 divisions/day over the course of the 26 day study, while C. brodyi's mean maximum growth rate was 0.325 divisions/day. Results of an ANOVA (Table 5) show no significant maximum growth rate response to species or feeding history.

TABLE 4. ANOVA table comparing the grazing rates on *Rhodomonas sp.* between two dinoflagellate species (*Pfiesteria piscicida* and Cryptoperidniopsis brodyi) crossed with two different feeding histories (algal fed and fish fed).

Source	df	SS	MS	F	P
Species	1	1.564×10^{-2}	1.564×10^{-2}	0.009	0.928
History	1	4.885	4.885	2.697	0.139
Species x history	1	2.170	2.170	1.198	0.306
Error	8	14.488	1.811		
Total	12	86.311			

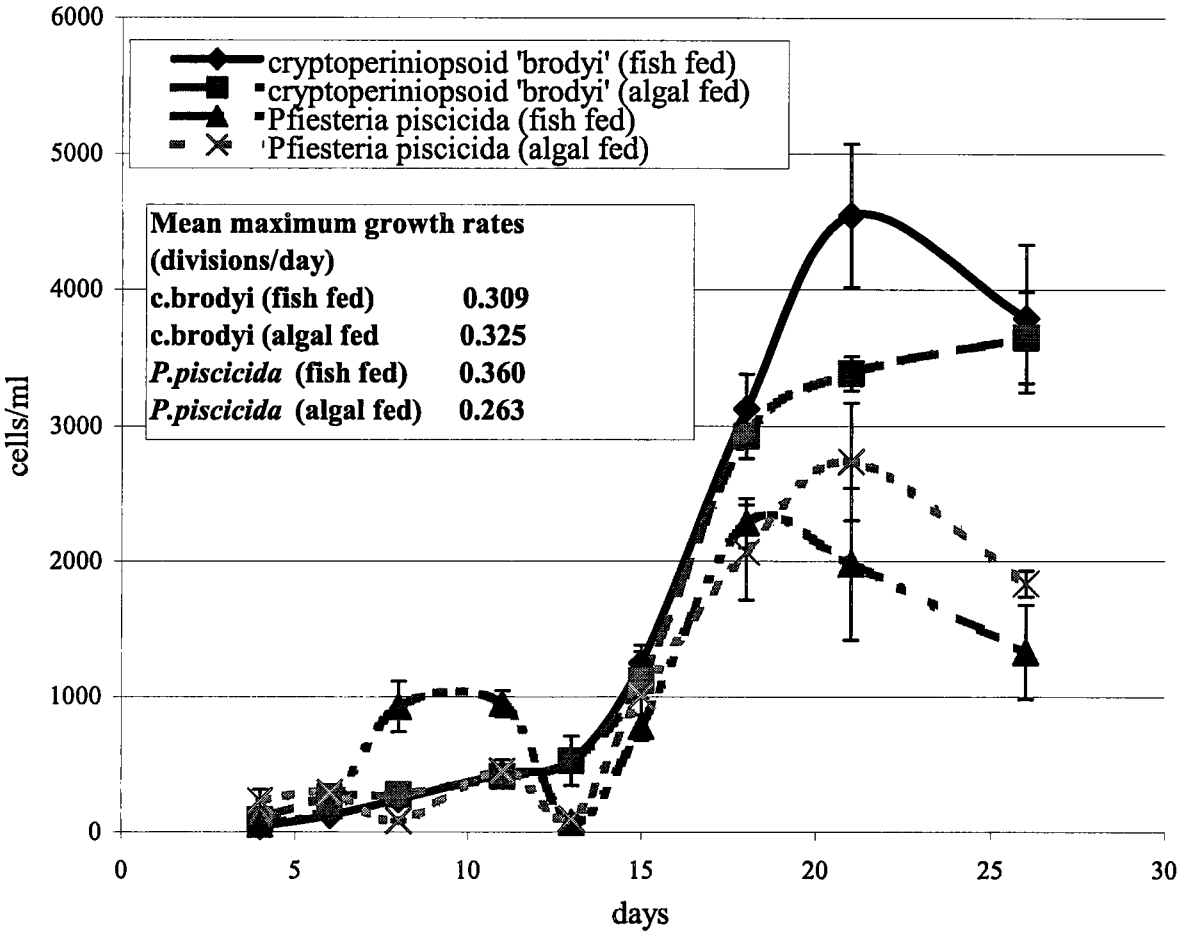

FIG. 9. Growth rates of *Pfiesteria piscicida* and Cryptoperidiniopsis from different feeding histories; either algal fed or fish fed over 26 days. There were no significant effect of species or feeding history on the maximum rate of growth.

TABLE 5. ANOVA table comparing the maximum growth rates between two dinoflagellate species (*Pfiesteria piscicida* and Cryptoperidniopsis brodyi) crossed with two different feeding histories (algal fed and fish fed).

Source	df	SS	MS	F	P
Species	1	8.797×10^{-5}	8.797×10^{-5}	0.013	0.913
History	1	4.910×10^{-3}	4.910×10^{-3}	0.705	0.426
Species x history	1	9.639×10^{-3}	9.639×10^{-3}	1.384	0.273
Error	8	5.571×10^{-2}	6.964×10^{-3}		
Total	12	1.253			

CHAPTER IV

DISCUSSION

Changes in feeding behavior can strongly affect the ability of a predator to survive in situations with competitors and multiple prey types. Two similar species of predators that are found in similar habitats might be expected to have differing feeding behavior as a mode of coexistence. The aim of this study was to determine whether two closely related heterotrophic dinoflagellates feed preferentially when presented with varying levels of two food types and whether these preferences differ. Grazing data demonstrated that both *Pfiesteria piscicida* and Cryptoperidiniopsis brodyi have the ability to feed preferentially. This study is the first to demonstrate feeding preferences in *Pfiesteria*-like dinoflagellates and that these preferences may be influenced by total food abundance.

Feeding preference

Multiple studies of heterotrophic dinoflagellate diet have been carried out (eg. Jacobson and Anderson 1986, Jacobson and Anderson 1996, Nacschall 1998) including studies on the diet of *Pfiesteria* and related species (Seaborn et al. 1999). These studies have demonstrated that dinoflagellates have the ability to feed on numerous types of food sources and some species may select prey based primarily on size (Jakobsen and Hansen 1997). Dinoflagellates are rather unique among aquatic predators because often their preferred prey is equal in size or slightly larger than themselves (Hansen 1992). Fish red blood cells and *Rhodomonas* were chosen as food types because of their near uniform size and shape which are both similar in size to the dinoflagellates. Numerous other experiments with animal predators have looked more thoroughly at the nature of feeding

preferences and demonstrated that they may be affected by different factors including food availability and prior conditioning (eg. Vadas 1977, Steinberg 1985, Avila 1998). This degree of experimental work looking at feeding preferences has not been carried out with protistan predators. My data shows that dinoflagellates are capable of preferential feeding and begins to explain what influences these preferences. For all the ratios of blood to *Rhodomonas* presented, both species of dinoflagellates exhibited a feeding preference for fish blood cells over algal prey. This preference was strongly exhibited even at low ratios of the preferred food type. Furthermore, the strength of the preference was affected by the total amount of food available.

In terms of resource use there are three major approaches a consumer may exercise. Generalist species indiscriminately consume available resources based only on their abundance. A generalist diet plotted against the proportion of a specific food type would be equal to the proportion of that food type in its environment, as indicated by the black 'no preference' columns in Fig. 6. Species which are specialists have the ability to differentiate between food types and feed to a greater extent on a preferred food type. A specialist diet as plotted on the same figure would have a value either less than or greater than 1 for the preferred food as is shown by both *P. piscicida* and C. brodyi. A third mechanism of resource consumption is known as facultative consumption or switching (Murdoch 1969). Consumers utilizing this strategy have the ability to feed on a variety of prey and feed more heavily on the most abundant species. When this prey becomes rare, the predator switches preferences for the more abundant food type. The plot of a diet utilizing this strategy would result in a decrease in the strength of preference at low ratios of the preferred food type. The apparent decrease in feeding preference shown in Fig. 7 as the ratio of the preferred food type increases is expected, and is an artifact of the

design. A preference cannot exceed 100%, and therefore must decrease as it approaches this level.

The grazing data show that *P. piscicida* and C. brodyi are not simple generalist predators, but can specialize on a preferred food type. The strength of the preference (C) is considered weak, always near 1.0, and less than 3 (as defined by Murdoch et al. 1975). Switching from one food type to another is only possible with predators that exhibit weak feeding preferences. However the preference is strong enough that even when the preferred food type becomes scarce, the degree of preference is not reduced. Switching does not occur, and blood remains the preferred food type at all food ratios. This means that both species are actively searching for their preferred food type, even when they are inundated in high abundances of alternative prey.

Optimal foraging theory assumes that time spent on a specific aspect of an organism's feeding behavior should be increased as long as the gain in energy exceeds the loss (MacArthur and Pianka 1966). This idea is extended to assume that when there are plentiful prey items, a predator can 'afford' to specialize its diet to only include the most profitable type of prey. However when there are low levels of prey, a specialized feeding strategy uses more energy in searching for specific prey than is gained. In this case the predator must include a wider variety of prey in its diet to survive. This hypothesis was tested by comparing treatments 4 (abundant prey) and 8 (scarce prey) (Table 3). *Pfiesteria piscicida* exhibited classical optimal foraging behavior. The degree of preference was higher at higher prey than at lower prey availability. This is consistent with what would be expected by an organism that can specialize on a prey type, but is still capable of feeding on multiple prey. Cryptoperidiniopsis brodyi however did not display this behavior, and in fact demonstrated the opposite result.

This paradoxical behavior is difficult to explain. At high prey abundance, an 'optimal' predator will spend more time sorting through all available prey for its preferred type. At low prey abundance, a predator's grazing rate should be primarily limited by the time spent finding any available prey. One possible answer may be a difference in the ability of the two species to differentiate between the experimental high and low treatments. The data in Fig. 4 show that *P. piscicida*'s grazing rate continues to increase as available *Rhodomonas* increases. C. brodyi's grazing rate however reaches a maximum level that does not increase considerably as *Rhodomonas* levels increase over 200 cells. The grazing of C. brodyi both at the high and low treatments might not be limited by either searching or sorting time, but by another factor such as satiation or digestion time.

Conditioning

Growth rate and grazing rate experiments are commonly used to attempt to determine optimal conditions or preferred food types of organisms such as dinoflagellates. Often, experiments measure the growth rate of organisms on different food sources in order to determine which treatment leads to the highest growth rate, which is then deemed the preferred food type. However, an organisms preferred food, and the one that produces the highest growth rates are not necessarily one in the same. Although fish blood cells were found to be the preferred food type, and were grazed at a higher rate than *Rhodomonas*, this food type failed to support either species during the 26-day growth rate study. Food levels were monitored and supplemental blood cells and *Rhodomonas* were added on an 'as needed' basis, ruling out starvation as a cause of mortality. There could be confounding factors leading to the death of the blood cell fed

cultures including possibly higher levels of bacteria or another variable, however water quality tests were compared between the two treatments following the study with no conclusive differences.

Low growth, or in this case no growth of non-toxic *P. piscicida* and C. brodyi fed on a fish diet is consistent with results of Burkholder et al. (2001). The authors demonstrated that non-toxic *P. piscicida's* growth rate was much lower on fish than the toxic strain. The cryptoperidiniopsoids only demonstrated negligible growth unless their diet was supplemented with algal prey (Burkholder et al. 2001). While both species showed no growth on fish blood cells during the growth rate experiment in the present study, I did maintain both species on a diet of fish tissue and blood cells for a period of three months. This result may be due to a lack of an unknown requirement in a blood-only diet. While both species may prefer to feed on blood, it may not contain all that is needed to support them. The scenario of a fish blood-only diet is highly artificial due to the ubiquitous nature of phytoplankton in a natural environment. In natural conditions both species would constantly be in the presence of algal prey, and fish prey would be less available.

Even without the benefit of a dataset that includes growth rates on blood cells, it is possible to test for an effect of prior diet conditioning on both species feeding on *Rhodomonas*. Results of ANOVA reveal no significant difference in the grazing or growth rates of either species between the algal fed or fish fed treatments. These results indicate that prior diet conditioning is not a significant factor in these aspects of either species' feeding behavior. In the majority of studies dealing with conditioning, behavior that is modified by experience is usually referred to as a learned behavior (ex. Hughes 1979, McNamara and Houston 1985, Micheli 1995). In all cases, the study organisms are

animals, with some degree of central nervous system and the capability of at least some degree of memory. It appears that, under these circumstances, *P. piscicida* and C. brodyi lack the ability to learn, and modify their behavior based on prior experience.

Pfiesteria research implications

Both *Pfiesteria piscicida* and Cryptoperidiniopsis brodyi fed on both *Rhodomonas* and fish blood cells during the experiment. This result was expected for *P. piscicida* and is consistent with previous studies showing fish predation and mortality caused by this species (Burkholder and Glasgow 1997, Gordon et al. 2002). The vast majority of heterotrophic dinoflagellates, including all *Pfiesteria*-like species have been shown to feed on other algal species. However it has been thought that the ability to kill and feed on fish was unique to members of the genus *Pfiesteria* (Marshall et al. 2000). During the course of this study, *P. piscicida* and C. brodyi were observed to feed on fish blood cells and muscle tissue from three species of fish common to the Chesapeake Bay; Atlantic Menhaden (*Brevoortia tyranus*), Atlantic Croaker (*Micropogonias undulates*) and Spot (*Leiostomus xanthurus*).

This study demonstrates predation on, as well as preference for, fish blood cells and tissue by Cryptoperiniopsis brodyi. Experimental work with C. brodyi has not demonstrated toxin production. (Seaborn et al. 2002). Burkholder et al. 2001, reported that although cryptoperidiniopsoids were observed to attack larval fish, their attacks did not cause the rapid mortality associated with toxic *P. piscicida*. There are several other unnamed species of heterotrophic dinoflagellates that are closely related to *Pfiesteria* and Cryptoperidinopsis, based on morphological and genetic similarities (Rublee et al. 2001,

Steidinger et al. 2001). My results suggest that more work is needed in on feeding behavior and the possible threat to fish of similar species.

Recently the degree to which *Pfiesteria* uses toxin versus micropredation has been a topic of contention among researchers (Vogelbein et al. 2002, Gordon et al. 2002). It appears that even strains of *Pfiesteria* that do not produce toxin are capable of killing fish when in direct contact. Also the degree of toxicity appears to change over time even within strains (Burkholder et al. 2001). This suggests two hypotheses. Either toxic strains are genetically different than non-toxic strains, or the predatory activity of the dinoflagellates may be influenced in some way, such as their prior exposure to fish.

However, a study of the ITS regions of 16 strains of *P. piscicida* including Tox-A, Tox-B, and non-inducible types revealed no genetic difference (Torstein et al. 2003). While this result only indicates that a genetic difference between toxic and non-toxic strains does not exist within the regions tested in the study, it does suggest that perhaps toxin production is influenced by an external factor. Bioassay studies suggest that the extent to which *Pfiesteria* species kill fish is related to the amount of time the dinoflagellates have been exposed to live fish (Burkholder et al. 2001). Strains that are actively killing fish may lose this ability after being excluded from contact with fish for extended periods of time (Burkholder and Glasgow 1997, Gordon et al 2002). Furthermore, even species that are actively killing fish do so at a much greater rate when actually in physical contact with the fish (Gordon and Dyer 2004). This suggests that the ability to kill fish is highly variable, even within strains, and influenced by external factors.

In addition, detection and attraction to fish excreta and mucus also appears to be influenced by the prior history of the dinoflagellates. Cancellieri et al. (2001)

demonstrated using a microcapillary assay, that *P. piscicida, P. shumwayae* and cryptoperidiniopsoid zoospores actively swam in the direction of finfish extracts. The study also compared the level of attraction between actively toxic strains and non-toxic strains. They found that the toxic *Pfiesteria* strains were attracted to fish at a much higher rate than the non-toxic *Pfiesteria*. Furthermore, the level of attraction of the toxic strains decreased with time removed from fish. Interestingly, the cryptoperidiniopsoids also showed an intermediate attraction towards fish.

These changes in the ability to detect and kill fish are aspects of the feeding behavior of the organism. Therefore I expected to observe other changes in response to prior conditioning. However after three months of exposure to fish prey there were no effects on either grazing rate of *Rhodomonas* or growth rate when feeding on *Rhodomonas* for either species. While these data do not support the hypothesis that prior conditioning affects these specific aspects of the dinoflagellates' ecology, other data reveal that the organisms preference changes in response to prey density.

Population level implications

Both species of dinoflagellate displayed feeding behavior that is consistent with simple predator-prey models. Each species' per capita grazing rate on both *Rhodomonas* and fish blood cells increased with increased levels of prey density (Figs. 3 and 4). This behavior fits the functional response model described by Holling (1965). When feeding on blood cells, both species display a linear, Type 1 functional response with high r^2 values. However, the grazing rates on *Rhodomonas*, presented on Fig. 4, fit a Type 3 functional response, also with high r^2 values. In both cases *P. piscicida* exhibits a higher grazing rate. The *Rhodomonas* grazing data for C. brodyi shows that this species has a

maximum per capita grazing rate in the vicinity of 0.7 cells per 6 hours. Increased levels of *Rhodomonas* failed to induce higher grazing rates. This suggests that above this level, prey abundance is no longer the limiting factor in the feeding behavior of this species.

One possible factor that would limit the grazing rate of a species at high prey abundances is the size of the food storage organ; in this case a food vacuole. Predators with larger capacities to store captured prey will be able to feed on more prey than predators with small capacities. The similarity in size between *P. piscicida* and C. brodyi however suggests similar sized food vacuoles. Another possible factor may be differences in the time needed by each dinoflagellate to process its prey. *P. piscicida* may breakdown the *Rhodomonas* faster than C. brodyi, allowing it to obtain higher grazing rates.

A third possible factor may be the nature in which the two predators attack their prey. Swarming behavior, in which several dinoflagellates feed on a single food item was observed by both species on both prey types, but to a greater degree on *Rhodomonas*. C. brodyi however was more often observed exhibiting this behavior than *P. piscicida*. Often, multiple individuals apparently ignored other *Rhodomonas* and instead joind other dinoflagellates in feeding on a specific *Rhodomonas* cell. This behavior in cryptoperidiniopsoids has also been documented by Parrow and Burkholder (2003). Often between 3 and 7 dinoflagellates were observed to be attached to a single prey item at once, although some events included upwards of 20 dinoflagellates. A multiple organism feeding strategy would explain why the maximum grazing rate observed is less than 1 *Rhodomonas* per dinoflagellate. This suggests that dinoflagellates' ability to detect prey may depend on the presence of an injured/ damaged prey cell.

Heterotrophic dinoflagellates such as these species utilize a special feeding organelle called a peduncle. The peduncle extends from the cell and functions as a hollow harpoon to penetrate and attach the dinoflagellate to its prey. The contents of the prey is then taken up through the peduncle and subsequently ingested by the dinoflagellate. It can be assumed that this process is 'leaky' and results in a release of some of the prey's cell contents into the water column. Dinoflagellates are known to have the ability to detect several environmental factors, including chemical stimuli (Levadondowsky and Kaneta 1987). *P. piscicida* has been shown to detect and swim towards a gradient of fish tissue and excreta (Cancellieri 2001). This may indicate that these dinoflagellates either prefer to feed on 'leaky' cells or fail to detect intact cells as potential prey. If this is the case, a higher level of undamaged *Rhodomonas* cells would not lead to an increased level of feeding. This would indicate that intraspecific competition for food may be limiting the grazing rates of these species, especially C. brodyi, at an even higher rate than would be expected for the amount of available prey. This may also explain the difference in preference response to total abundance shown in Table 3. If C. brodyi was only cueing in on a small subset of the prey, the 'leaky' cells could be limiting in both the high and low abundances that were tested. This would indicate that C. brodyi may not have recognized a higher level of *Rhodomonas* because there was still a limiting number of injured cells.

Community level implications

While this experiment only studied the feeding behavior of the dinoflagellates when they were the only predator present, some discussion may be made regarding the implications of the research in relation to community level interactions. Both *P.*

piscicida and C. brodyi are members of the phytoplankton assemblage of the Chesapeake Bay and other surrounding estuaries. Within these environments they interact with hundreds of phytoplankton and zooplankton taxa. Being obligate heterotrophic species, the most important group of planktars to these dinoflagellates are potential prey. *P. piscicida* and C. brodyi exhibited the ability to feed on algal and fish prey. Previous studies also show that both species can feed on a variety of algal types as well (Seaborn et al. 1999, Seaborn et al. 2002).

A wide diet would be favored in systems with variable resources, such as changes in estuarine phytoplankton composition. Species such as these would have the advantage over other predators that relied solely on one food source. One potential group of competitors in the Chesapeake Bay of *Pfiesteria* like dinoflagellates are tintinnid ciliates. This is an interesting example of one potential competitor that may actually eat the other, as ciliates commonly graze on dinoflagellates. In fact, at least one species of tintinnids shows a strong preference for dinoflagellates over other types of algal prey (Stoecker et al. 1981). The difference between the feeding preference of this ciliate and the dinoflagellates in the present study is that the ciliate didn't graze, or only grazed at a low level, on other food types. A strong preference along with a narrow diet makes a predator depend on a single type of prey. This situation is more likely to become more easily disrupted, as it only requires a small number (as low as one) of prey species to be removed in order to deplete the predator's population. Conversely, if a predator can feed on multiple types of prey its population may be more stable, as a loss of one food type (even the preferred type) can be replaced with another alternative prey. A wide diet, such as that of these dinoflagellates, would have an advantage over other predators that relied

solely on one food source, especially in environments such as temperate cosmopolitan estuaries that have changes in temperature, salinity, and nutrient levels.

Natural selection suggests that a preferred food type will be one that would result in the highest fitness. If this were the case, the ability to differentiate between food types and recognize the most profitable one would be a competitive advantage over those that fed indiscriminately. *Pfiesteria piscicida* demonstrated a higher feeding preference when the overall abundance was higher. This ability to not only feed preferentially, but to alter a feeding preference based on the surrounding environment would help favor it over competitors lacking these traits. This advantage would include competition between *P. piscicida* and C. brodyi. While C. brodyi's *Rhodomonas* grazing rate reached a maximum of 0.74 cells during the study, *P. piscicida*'s rate was not only higher, but continued to increase with increased abundance of *Rhodomonas*. The higher grazing rates of *P. piscicida* suggest that especially at higher levels of algal prey, *P. piscicida* might have a competitive advantage over C. brodyi.

The possibility that this lower per capita grazing rate might be due to C. brodyi's recognition of only wounded cells as potential prey is an interesting hypothesis that deserves further investigation. Depending on the resource use of *P. piscicida*, multiple scenarios regarding their potential competition are possible. If for instance *P. piscicida* shares this behavior with C. brodyi, and only feeds on injured cells, they will compete for a smaller portion of available prey, and the degree of competition will be greater. If however, *P. piscicida* recognizes all *Rhodomonas* cells as prey items it will attack more cells than C. brodyi would if it were the sole predator. This increases the total amount of resources available and would appear to decrease the level of competition, therefore providing the possibility of coexistence. However, in attacking the cells, they will

become leaky and more attractive to competing C. brodyi. If this scenario is correct, the population of C. brodyi would be benefited by the action of *P. piscicida*. This relationship would also apply to fish prey as well. If both species of dinoflagellates have a preference for fish blood cells, but only *P. piscicida* has the ability to kill fish, C. brodyi would need to depend on fish kills from some other means. This would include fish killed by *P. piscicida*. Again, C. brodyi would benefit from the predation of *P. piscicida* much the way a scavenger benefits from a predator. This scenario might also explain a diversity of species that may be found at a fish kill, but the inability of certain species to kill fish.

CHAPTER V

CONCLUSIONS

Pfiesteria piscicida and Cryptoperidiniopsis brodyi have the ability to graze on algal and fish prey, and showed functional responses to increased food abundance. Furthermore, these dinoflagellates feed preferentially, with both species preferring fish blood cells over *Rhodomonas*. These preferences are strong enough to be observed at all food ratios, including when the preferred food type was scarce. Prior feeding history in these species does not influence the grazing rate or growth rate of either species. The strength of the preference appears to be influenced by the level of overall food abundance, consistent with the optimal foraging theory. Possible differences in feeding behavior, including swarming may also influence their grazing rates and preferences.

REFERENCES

Anderson, D. M. 1980. Effects of temperature conditioning on development and germination of *Gonyaulax tamarensis* (Dinophyceae) hypnozygotes. *J. Phycol.* 16: 166-72.

Bannister, T. 1979. Quantitative description of steady state, nutrient-saturated algal growth, including adaptation. *Limnol. Oceanogr.* 24: 76-96.

Bowers, H., Tengs, T., Glasgow, H., Burkholder, J., Rublee, P. & Oldach D. 2000. Development of real-time PCR assays for rapid detection of *Pfiesteria piscicida* and related dinoflagellates. *Appl. Environ. Microbiol.* 66: 4641-8.

Bovbjerg, R. V. 1970. Ecological isolation and competitive exclusion in two crayfish. *Ecology.* 51: 225-36.

Burkholder, J. M. & Glasgow, H. 1997. *Pfiesteria piscicida* and other *Pfiesteria*-like dinoflagellates: behavior, impacts, and environmental controls. *Limnol. Oceanogr.* 42: 1052-75.

Burkholder, J. M. 1998. Implications of harmful microalgae and heterotrophic dinoflagellates in management of sustainable marine fisheries. *Ecol. Appl.* 8: S37-S62.

Burkholder, J. M., Glasgow, H. & Deamer-Melia, N. 2001. Overview and present status of the toxic *Pfiesteria* complex (Dinophyceae). *Phycologia.* 40: 186-214.

Cancellieria, P., Burkholder, J. M., Deamer-Melia, N., & Glasgow, H. 2001. Chemosensory attraction of zoospores of the estuarine dinoflagellates, *Pfiesteria piscicida* and *P. shumwayae*, to finfish mucus and excreta. *J. Exp. Mar. Biol. Ecol.* 264: 29-45.

Dayton, P. K. 1971. Competition, disturbance, and community organization: the provision and subsequent utilization of space in a rocky intertidal community. *Ecol. Monogr.* 41: 351-89.

Dugdale, R. C. 1967. Nutrient limitation in the sea; dynamics, identification, and significance. *Limnol. Oceanogr.*. 12: 685-95.

Elton, C. 1946. Competition and the structure of ecological communities. *J. Anim. Ecol.* 15: 54-68.

Feinstein, T. N., Traslavina, R., Sun, M., & Lin, S. 2002. Effects of light on photosynthesis, grazing, and population dynamics of the heterotrophic dinoflagellate *Pfiesteria piscicida* (Dinophyceae). *J. Phycol.* 38: 659-69.

Fox, B. J. 1981. Niche partitioning and species richness. *Ecology*. 62. 1415-25.

Gaines, G. & Elbrachter, M. 1989. Heterotrophic nutrition. *In* F. Taylor (Ed.) *The Biology of the Dinoflagellates*. pp 224-68. Blackwell, Oxford.

Glasgow, H. B., Burkholder, J. M., Morton, S. L., & Springer, J. 2001. A second species of ichtyotoxic *Pfiesteria* (Dinamoebales, Dinophyceae). *Phycologia*. 40: 234-45.

Glasser, J. 1984. Evolution of efficiencies and strategies of resource exploitation. *Ecology*. 65: 1570-78.

Gordon, A, Dyer, B., Seaborn, D., & Marshall, H.G. 2002. Comparative toxicity of *Pfiesteria* spp., prolonging toxicity of *P. piscicida* in culture and evaluation of toxin(s) stability. *Harmful Algae*. 1: 1-10.

Hansen, P. 1991. Quantitative importance and trophic role of heterotrophic dinoflagellates in a coastal pelagial food web. *Mar. Ecol. Prog. Ser.* 73: 253-61.

Hansen, P. 1992. Prey size selection, feeding rates and growth dynamics of heterotrophic dinoflagellates with special emphasis on *Gyrodinium spirale*. *Mar. Biol.* 114: 327-34.

Hardin, G. 1960. The competitive exclusion principle. *Science*. 131: 1292-8.

Higashi, Y. & Seki, H. 2000. Ecological adaptation and acclimatization of natural freshwater phytoplankters with a nutrient gradient. *Environ. Pollut.* 109: 311-20.

Hulburt, E. 1982. The adaptation of marine phytoplankton species to nutrient and temperature. *Ocean Sci. Eng.* 7: 187-228.

Hulburt, E. 1985. Adaptation and niche breadth of phytoplankton species along a nutrient gradient in the ocean. *J. Plankton Res.*. 7: 581-94.

Hutchinson, G. E. 1941. Ecological aspects of succession in natural populations. *Am. Nat.* 75: 406-18.

Hutchinson, G. E. 1961. The paradox of the plankton. *Am. Nat.* 95:137-45.

Inger, R. F. & Greenberg, B. 1966. Ecological and competitive relations among three species of frogs. *Ecology* 47: 746-59.

Jacobson, D. & Anderson, D. 1986. Thecate heterotrophic dinoflagellates: feeding behavior and mechanisms. *J. Phycol.* 22: 249-58.

Jeong, H. J. 1999. The ecological role of heterotrophic dinoflagellates in marine planktonic community. *J. Eukaryot. Microbiol.* 46: 390-6.

Kane, A. S., Oldach, D., & Resimschuessel, R. 1998. Fish lesions in the Chesapeake Bay: *Pfiesteria*-like dinoflagellates and other etiologies. *Maryland Med. J.* 47: 106-12.

Kilham, P. 1971. A hypothesis concerning silica and the freshwater planktonic diatoms. *Limnol. Oceanogr.*. 16: 10-18.

Levandowsky, M. & Kaneta, P. 1989. Behaviour in Dinoflagellates. *In* F. Taylor (Ed.) *The Biology of the Dinoflagellates*. pp 360-97. Blackwell, Oxford.

Lotka, A. J. 1924. *Elements of physical biology*. Williams & Wilkins, Baltimore.

MacArthur. R. H. 1960. On the relative abundance of species. *Am. Nat.* 94: 25-36.

MacArthur, R. H. and Pianka, E. R. 1966. On optimal use of a patchy environment. *Am. Nat.* 100: 603-9.

Mallin, M. 1994. Phytoplankton ecology of North Carolina estuaries. *Estuaries*. 17: 561-74.

Marshall, H. 1999. *Pfiesteria piscicida* and dinoflagellates similar to *Pfiesteria. Va. J. Sci.* 50: 281-6.

Marshall, H. G., Gordon, A. S., Seaborn, D. W., Dyer, B., Dunstan, W. M., & Seaborn, A. M.2000. Comparative culture and toxicity studies between the toxic dinoflagellate *Pfiesteria piscicida* and a morphologically similar cryptoperidiniopsoid dinoflagellate. *J. Exp. Mar. Biol. Ecol.*. 255: 51-74.

McNamara, J. & Houston, A.. 1985. Optimal foraging and learning. 1985. *J. Theor. Biol.* 117: 231-49.

Menge, B. A. 1976. Organization of the New England Rocky intertidal community: role of predation, competition, and environmental heterogeneity. *Ecol. Mongr.* 46: 355-93.

Morin, P. 1999. *Community Ecology*. Blackwell, New Brunswick.

Murdoch, W. 1969. Switching in general predators: experiments on predator specificity and stability of prey populations. *Ecol. Mongr.* 39: 335-54.

Oaten, A. & Murdoch, W. 1975. Functional response and stability in predator-prey systems. *Am. Nat.* 109: 289-98.

Paine, R. T. 1971. A short-term experimental investigation of resource partitioning in a New Zealand rocky intertidal habitat. *Ecology* 52: 1096-106.

Park, T. 1948. Experimental studies on interspecies competition. I. Competition between populations of the flour beetles *Trifolium confusum* (Duval) and *Tribolium canteneneum* (Herbst). *Ecol. Mongr.* 18: 265-307.

Parrow, M. & Burkholder, J. 2003. Reproduction and sexuality in *Pfiesteria shumwayae* (Dinophyceae). *J. Phycol.* 39: 697-711.

Parrow, M. & Burkholder, J. 2003. Estuarine heterotrophic cryptoperiniopsoids (Dinophyceae): life cycle and culture studies. *J. Phycol.* 39: 678-96.

Patrick, R. 1976. The formation and maintenance of benthic diatom communities. *Pro. Am. Philos. Soc.* 120: 475-83.

Petersen, R. 1975. The paradox of the plankton: an equilibrium hypothesis. *Am. Nat.* 109: 35-49.

Pinckney, J. L., Paerl, H. W., Haugen, E., & Tester, P. A. 2000. Responses of phytoplankton and *Pfiesteria*-like dinoflagellate zoospores to nutrient enrichment in the Neuse River Estuary, North Carolina, USA. *Mar. Ecol. Prog. Ser.* 192: 65-78.

Pleasants, J. M. 1980. Competition for bumblebee pollinators in Rocky Mountain plant communities. *Ecology*. 61: 1446-59.

Prezelin, B. & Matlick, H.. 1983. Nutrient-dependent low-light adaptation in the dinoflagellate *Gonayaulax polyedra. Mar. Biol.* 74: 141-50.

Rublee, P. A., Kempton, J., Schaefer, E., Burkholder, J. M., Glasgow, H. B., &. Oldach, D.1999. PCR and FISH detection extend the range of *Pfiesteria piscicida* in estuarine waters. *Va. J. Sci.* 50: 326-35.

Scott, M. & Murdoch, W. 1983. Selective predation by the backswimmer, *Notonecta. Limnol. Oceanogr.* 28: 352-66.

Seaborn, D. W., Seaborn, A. M,. Dunstan, W. M., & Marshall, H. G. 1999. Growth and feeding studies on the algal feeding stage of a *Pfiesteria*-like dinoflagellate. *Va. J. Sci.* 50: 337-44.

Seaborn, D., Dunstan, W., Marshall, H.G., Gordon, A., &. Seaborn, A M. 2002. Growth and toxicity studies of the dinoflagellates *Cryptoperiniopsis* sp., *Gyrodinium galatheanum,* and *Pfiesteria piscicida. In* G. Hallegraeff (Ed). *Harmful Algal Blooms, Proceedings of the Ninth International Conference on Harmful Algal Blooms*. 92-6. IOC UNESCO, Paris.

Siegel, D. A. 1998. Resource competition in a discrete environment: why are plankton distributions paradoxical? *Limnol. Oceanogr.* 43: 1133-46.

Simek, K, Bobkova, J., Macek, M., Nedoma, J., & Psenner, R. 1995. Ciliate grazing on picoplankton in a eutrophic reservoir during the summer phytoplankton maximum: a study at the species and community level. *Limnol. Oceanog.* 40: 1077-90.

Smith, O. L., Shugart, H. H., O'Neill, R. V., Booth, R. S., & McNaught, D. C. 1975. Resource competition and an analytical model of zooplankton feeding on phytoplankton. *Am. Nat.* 109: 571-91.

Sousa, W. P. 1979. Disturbance in marine intertidal boulder fields: the nonequilibrium maintenance of species diversity. *Ecology.* 60: 1225-39.

Steinberg, P. 1985. Feeding preferences of *Tegula funebralis* and chemical defenses of marine brown algae. *Ecol. Monogr.* 55: 333-49.

Sullivan, B. E. & Andersen, R. A. 2001. Salinity tolerances of 62 strains of *Pfiesteria* and *Pfiesteria*-like heterotrophic flagellates (Dinophyceae). *Phycol. Res.* 49: 207-14.

Thomas, W. T. 1966. Surface nitrogenous nutrients and phytoplankton in the northeastern tropical Pacific ocean. *Limnol. Oceanogr.* 11: 393-400.

Tilman, D. 1977. Resource competition between planktonic algae: an experimental and theoretical approach. *Ecology.* 58: 338-48.

Tilman, D. 1980. Resources: a graphical-mechanistic approach to competition and predation. *Am. Nat.* 116: 362-93.

Tilman, D. 1981. Tests of resource competition theory using four species of Lake Michigan algae. *Ecology* 62: 802-15.

Tilman, D. 1982. *Resource Competition and Community Structure.* Princeton UP, Princeton.

Tilman, D., Kilham, S. S., & Kilham, P. 1982. Phytoplankton community ecology: the role of limiting nutrients. *Annu. Rev. Ecol. Syst.* 13: 349-72.

Tiselius, P. & Kuylenstierna, M. 1996. Growth and decline of a diatom spring bloom: Phytoplankton species composition, formation of marine snow and the role of heterotrophic dinoflagellates. *J. Plankton Res.* 18: 133-55.

Udvardy, M. F. 1959. Notes on the ecological concepts of habitat, biotope and niche. *Ecology* 40: 725-8.

Vadas, R. 1977. Preferential feeding: an optimization strategy in sea urchins. *Ecol. Monogr.* 47: 337-71.

Volterra, V. 1931. Variation and fluctuations of the number of individuals in animal species living together. *In* R. N. Chapman, (Ed.) *Animal Ecology*. McGraw-Hill, New York.

Yamamoto, T., Seike, T., Hashimoto, T., & Tarutani, K. 2002. Modelling the population dynamics of the toxic dinoflagellate *Alexandrium tamarense* in Hiroshima Bay, Japan. *J. Plankton Res.* 24: 33-47.

VITA

Todd A. Egerton
Department of Biological Sciences
Old Dominion University
Norfolk VA, 23529

Education:

Susquehanna University, B.S. Biology (Cum Laude), 2001.
Thesis: Comparison of species composition of epiphytic algae on emergent macrophytes from three lakes of different productivities.
Dr. Jack R. Holt, advisor.

Old Dominion University, M.S. Biology, 2005.
Thesis: Feeding behavior and conditioning in two heterotrophic dinoflagellates.Dr. Harold Marshall, advisor.

Professional Positions:

Research Associate, Old Dominion University Research Foundation, June 2004- present
Research Assistant, Department of Biology, Old Dominion University, Aug 2001-Apr 2004
Aquarist Assistant, National Aquarium in Baltimore, 2000.
Teaching Assistant, Susquehanna University, Aug 1999- May 2001.

Abstracts and presentations:

Egerton, T.A. and H.G. Marshall. 2004. Feeding behavior and conditioning in three *Pfiesteria*-like dinoflagellates. 58th Annual meeting of the Phycological Society of America.

Egerton, T.A., S. Cerbin, and H.G. Marshall. 2003. Observations and Historical Trends of a Potentially Harmful Dinoflagellate Bloom in the Lafayette River, Norfolk, Virginia. Virginia Journal of Science **54** (2).

Stem, T.W., T.A. Egerton and H.G. Marshall. 2003. An examination of excystment in two heterotrophic dinoflagellates with complex life cycles: *Pfiesteria piscicida* and an unnamed cryptoperidiniopsoid. Virginia Journal of Science. **54** (2).

Marshall, H., T. Egerton, T. Stem, M. Kokocinski, and J. Hicks. 2002. Increased abundance of *Dinophysis acuminata* in Chesapeake Bay, USA. International Symposium, Phycological section of the Polish Botanical Society.