1999

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David W. Seaborn
Old Dominion University

A. Michelle Seaborn
Old Dominion University

William M. Dunstan
Old Dominion University

Harold G. Marshall
Old Dominion University, hmarshal@odu.edu

Original Publication Citation

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. *Virginia Journal of Science, 50*(4), 337-343.

Virginia Journal of Science
Volume 50, Number 4
Winter 1999

Growth and Feeding Studies on the Algal Feeding Stage of a *Pfiesteria*-like Dinoflagellate

David W. Seaborn[1], **A. Michelle Seaborn**[2], **William M. Dunstan**[2], and **Harold G. Marshall**[1]

ABSTRACT

The dinoflagellate *Cryptoperidiniopsis* sp. was isolated from sediment samples taken from Virginia estuaries, and established in culture for subsequent growth and feeding studies. The maximum abundance, or yield, of *Cryptoperidiniopsis* is exponentially related to the concentration of algal prey and is saturated at about 4.00×10^5 mL^{-1}. Salinity from 10-20 ppt and temperature between 15-25 C have no effect on the yield of this form of *Cryptoperidiniopsis.* Light intensity has a secondary effect in that the algal prey reproduces more quickly in higher light as they are being grazed. Growth rates of *Cryptoperidiniopsis* were highest with a cryptophyte, *Cryptomonas,* as food, but growth was also demonstrated utilizing both diatoms and chlorophytes. *Cryptoperidiniopsis* sp. is similar to *Pfiesteria* in that it feeds myzocytotically with a peduncle, is similar in size and shape, has a complex life cycle, and is distinguished only by plates hidden under membranes.

INTRODUCTION

Over the past decade, the importance of heterotrophic dinoflagellates in coastal waters has received increased attention. Studies have found they are abundant (Lessard 1991; Jeong, 1999) and they capture a variety of prey by several different means (Hansen and Calabo, 1999; Schnepf and Elbrachter, 1992). Some feed on other dinoflagellates (Hansen and Nielsen, 1997; Jeong et al., 1997) as well as zooplankton (Jeong 1994). One of the most extreme examples is *Pfiesteria piscicida,* which can feed heterotrophically, but also has the capability to survive through photosynthesis by ingesting and using the chloroplasts of other algae (Lewitus et al., 1999). This dinoflagellate has been documented to possess a widespread distribution in turbid estuaries (Burkholder et al., 1995; Burkholder and Glasgow, 1997; Steidinger et al., 1996).

Prior to this study we isolated several dinoflagellates from sediments coming from Virginia estuaries (Marshall et al., 1998; Marshall et al., 1999) and these included a strain of *Cryptoperidiniopsis* (identification confirmed by Drs. Karen Steidinger through SEM analysis and Parke Rublee with a genetic probe) which was common in our samples (Figure 1). Dr. Steidinger indicated this strain is morphologically distinct from the one known species, *C. brodyii*, first found in Florida, which differed morphologically by a slight variation in its apical plates. The objectives of this study were to:

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1 Department of Biological Sciences, Old Dominion University, Norfolk, Virginia 23529

2 Department of Ocean, Earth, and Atmospheric Sciences, Old Dominion University, Norfolk, Virginia 23529

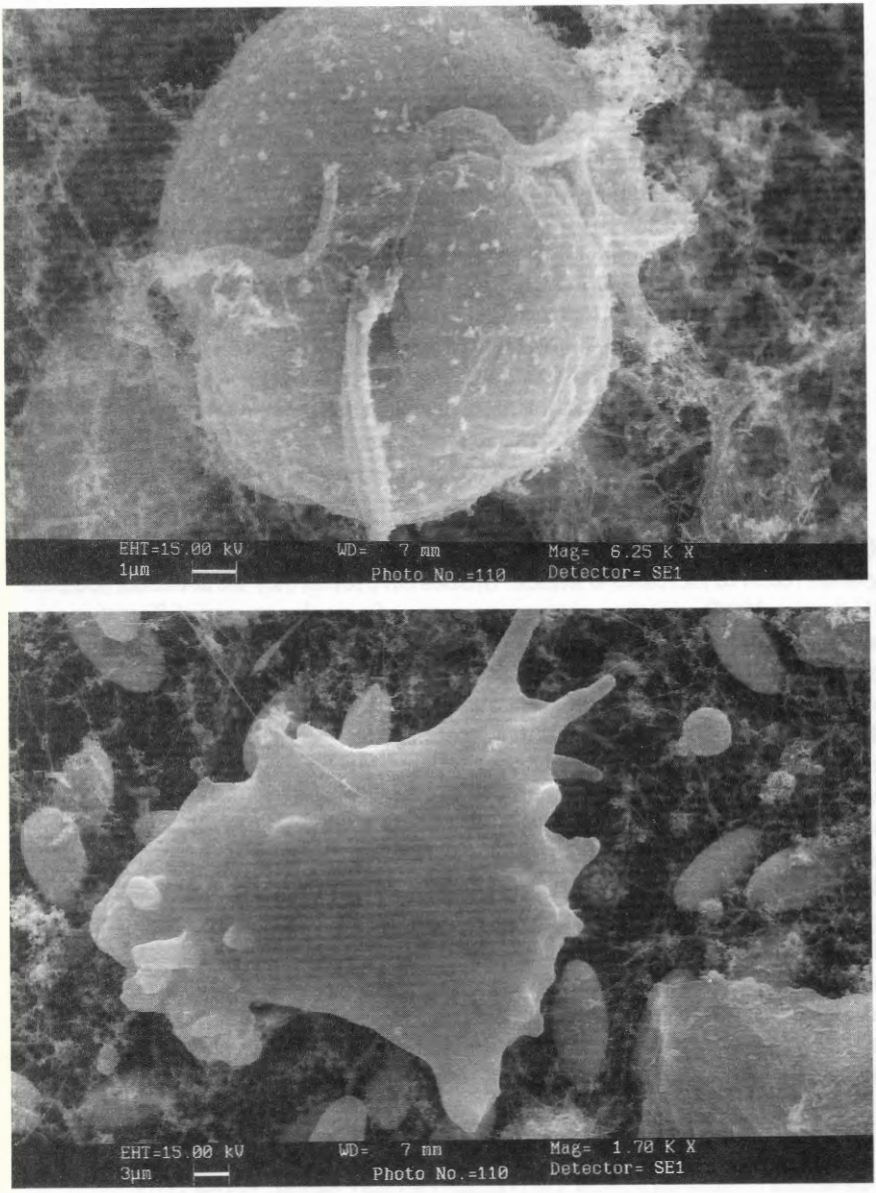

FIGURE 1. *Cryptoperidiniopsis* sp. A. (Upper picture) Ventral view of the motile vegetative stage; B. (Lower picture) Lobose amoeboid stage.

1. To determine the relationship of *Cryptoperidiniopsis* in our cultures to several environmental factors. These included light intensity, prey concentration, salinity, and temperature;
2. To determine the growth rate of these cells; and
3. To identify any prey preferences this species may have.

METHODS

A series of sediment samples (250 mL) were taken with a petite ponar grab in 1998 by personnel from the Virginia Department of Health's Shellfish Sanitation Division and the Virginia Department of Environmental Quality as part of the *Pfiesteria* Monitoring Program in Virginia. Sub-samples from these sediment samples were incubated with f/2-Si medium in a 50 mL Falcon tissue culture flask. To each sediment incubation, 5 mL of the food source *Cryptomonas* (CCMP 767 Provesali-Guillard) were added. Dinoflagellates were subsequently isolated from these incubations and identified.

Cultures of *Cryptoperidiniopsis* (strain DEQ-002) were tested for their growth response to four factors: light, concentration of prey, salinity, and temperature. Triplicate 250 mL Falcon tissue culture flasks were used for both the experimental (dinoflagellates and prey) and control (only prey). All experiments were conducted in incubators, in order to achieve constant temperature and fixed light conditions. To each flask, 5 mL of dinoflagellates and 10 mL of *Cryptomonas* were added. The *Cryptomonas* inoculum has a concentration of 1.891×10^6 mL^{-1} while the dinoflagellates were at 1.338×10^4 mL^{-1}. Flasks were filled to 100 mL with f/2-Si medium at 15 ppt (except for those adjusted for the salinity experiment). The medium was created by diluting water from the mouth Chesapeake Bay with double de-ionized water and passing it through a 0.2 um glass filter. The initial concentrations in the flasks were 1.89×10^5 mL^{-1} for the prey and 6.69×10^2 mL^{-1} for the dinoflagellates. All studies included triplicate culture sets.

Concentration of prey was investigated on three levels. These were 2X (1.89×10^5 mL^{-1}), 1X (0.95×10^5 mL^{-1}), and 8X (7.55×10^5 mL^{-1}). The growth of *Cryptoperidiniopsis* was observed under three different set temperatures: 15C, 20C, and 25C. Light concentration was also varied on three levels. This was achieved by leaving one set of triplicates over time in a direct line with the incubator lights while another set was wrapped once with mesh screening. A third set of triplicates was wrapped twice in mesh screening to prevent more light from reaching the cells.

RESULTS

The *Cryptoperidiniopsis* in our study is very similar to *Pfiesteria piscicida*. It feeds myzocytotically with a peduncle, possesses similar size and shape, has a complex life cycle which includes both cyst and amoeboid stages, and has its distinguishing plates hidden under layers of membranes (Seaborn and Marshall, 1998). This species was found in 27 of 51 tested sediment samples from the Virginia portion of Chesapeake Bay. Scanning electron microscopy reveals the Virginia strain is slightly, yet consistently, different from the one know species *C. brodyii*.

The initial concentration of *Cryptomonas* prey had a significant effect on the maximum *Cryptoperidiniopsis* abundance (Figure 2). This response was similar where light and temperature were controlled. The intensity of light had a significant effect

on the maximum dinoflagellate yield (Figure 3). The growth rate of *Cryptoperidiniopsis* (1.43) is high when compared to other dinoflagellates such as *Pfiesteria piscicida* (Figure 4). Peak dinoflagellate abundances and growth rates did not differ for temperatures between 15-25C. Maximum dinoflagellate abundances and growth rates did not significantly differ for salinities from 10-20 ppt. *Cryptoperidiniopsis* grew at a much high rate when feeding on the cryptophyte, *Cryptomonas*, as opposed to other algal prey (Figure 5).

CONCLUSIONS

The incubated sediment samples yielded a strain of *Cryptoperidiniopsis* which is common from Virginia tidal waters. Preliminary data of food preferences suggest this dinoflagellate has a higher growth rate when feeding on cryptophytes as opposed to other algal prey. This result is similar to the cryptophyte feeding preferences shown by *Pfiesteria piscicida* (Glasgow et al., 1998). Both dinoflagellates feed in a similar fashion through the use of a peduncle and have complex life cycles including the formation of amoeboid stages. The concentration of cryptophytes is an important factor which determines the abundance and duration of *Cryptoperidiniopsis* in the water column. The dinoflagellate exhibits robust growth in salinities from 10-20 ppt and in temperatures between 15-25C.

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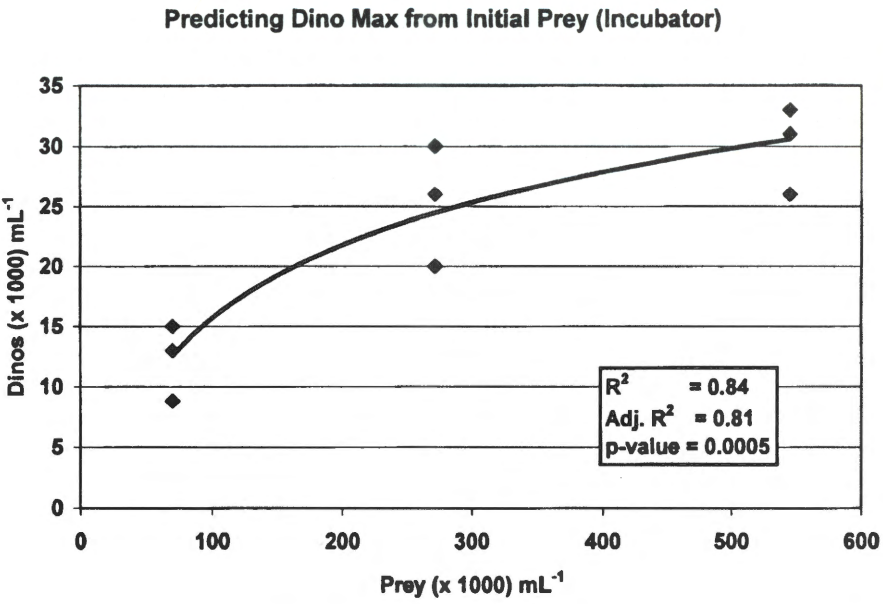

FIGURE 2. Regression model of the relationship between initial abundance of *Cryptomonas* and yield of *Cryptoperidiniopsis*.

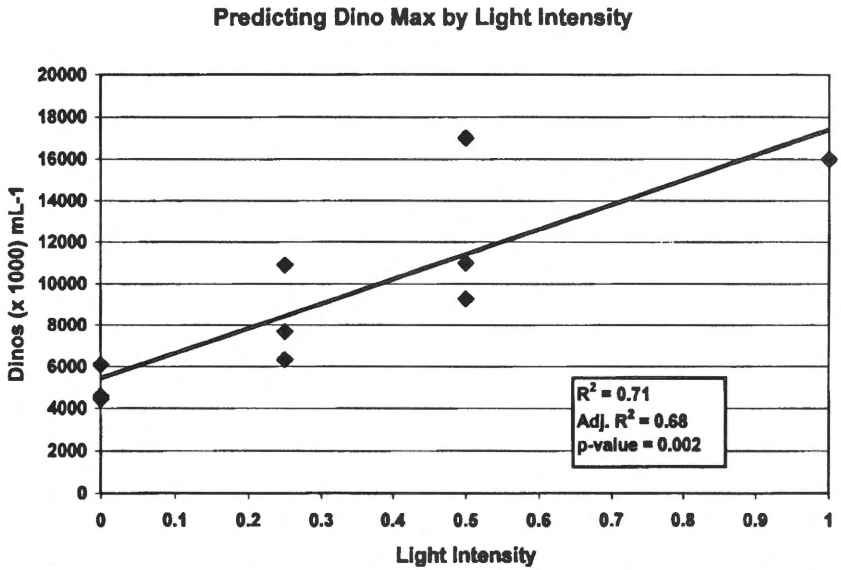

FIGURE 3. Linear regression of peak *Cryptoperidiniopsis* concentrations in relation to light intensity.

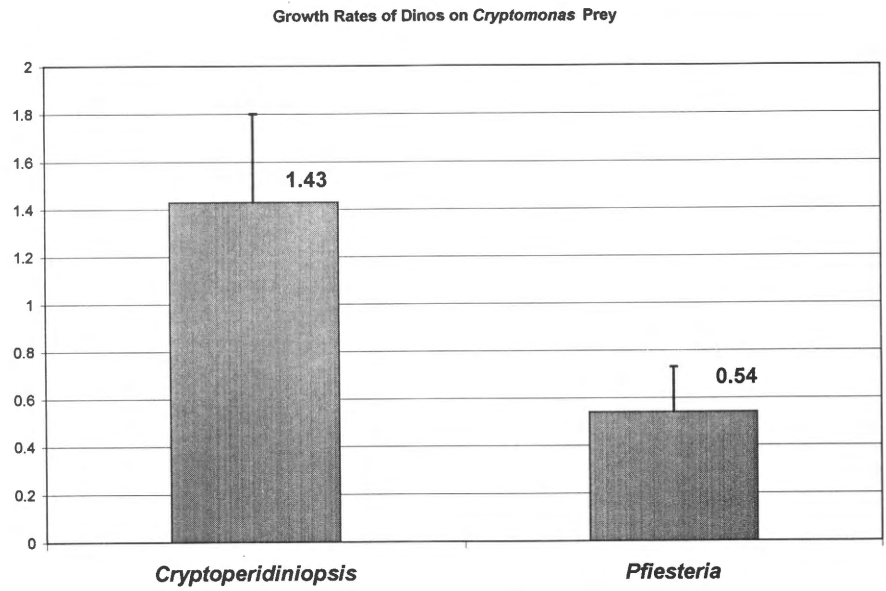

FIGURE 4. Comparison of growth rates for *Cryptoperidiniopsis* and *Pfiesteria* feeding on *Cryptomonas* prey. Error bars represent two standard deviations from the mean.

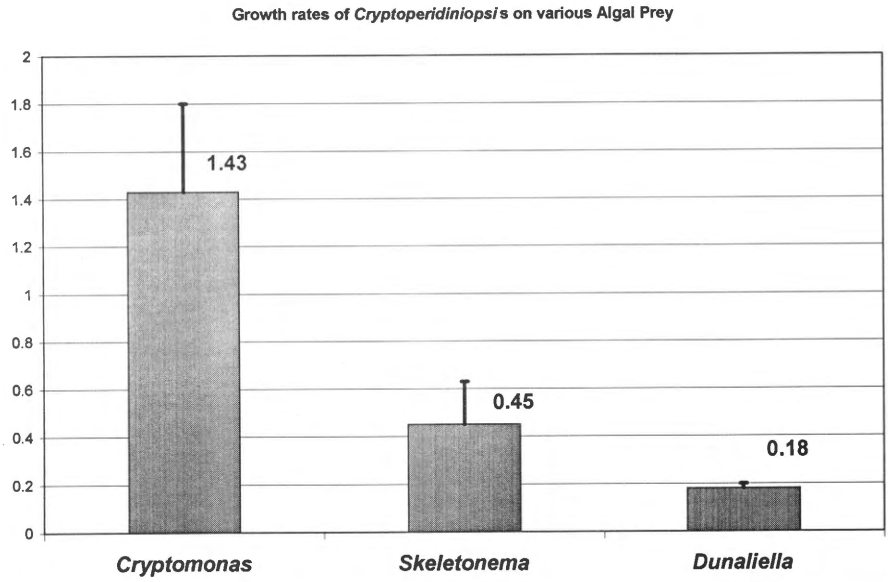

FIGURE 5. Comparison of *Cryptoperidiniopsis* growth rates from three different algal food sources. Error bars represent two standard deviations from the mean.

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