

**GROWTH DYNAMICS OF A YORK RIVER ESTUARY HETEROTROPHIC  
DINOFLLAGELLATE GRAZING *KATODINIUM ROTUNDATUM***

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Master of Arts

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by

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APPROVAL SHEET

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## ABSTRACT

A 10 X 13  $\mu\text{m}$  thecate heterotrophic dinoflagellate (HDINO) was isolated from a natural water sample from the lower York River estuary (VA) during mid-September 1993. HDINO was fed *Katodinium rotundatum*, an 8 X 10  $\mu\text{m}$  autotrophic red tide dinoflagellate. In an effort to characterize the autecology of this isolate, a series of experiments were performed to determine the effects of temperature, salinity, and prey concentration on HDINO. To determine the effect of temperature on the growth rate ( $\mu$ ) of HDINO, 4 temperatures (10, 15, 20, and 25  $^{\circ}\text{C}$ ) were tested. The highest growth rate (mean  $\mu=0.47 \text{ d}^{-1} \pm 0.05 \text{ SE}$ ;  $r^2=0.93$ ) for HDINO was observed at 25  $^{\circ}\text{C}$ . To examine the effects of salinity on HDINO growth rate, HDINO + *K. rotundatum* were kept at room temperature (19-25  $^{\circ}\text{C}$ ) at 5 salinities (10, 15, 20, 25, and 30 psu). The highest observed HDINO growth rate occurred at 20 psu (mean  $\mu=1.25 \text{ d}^{-1} \pm 0.18 \text{ SE}$ ;  $r^2=0.85$ ).

HDINO was then grown at varying concentrations of *K. rotundatum* in two separate experiments designed to examine the effects of prey concentration on HDINO growth rate. In the first experiment, 3 concentrations of *K. rotundatum* ( $25 \times 10^3$ ,  $60 \times 10^3$ , and  $1000 \times 10^3$  cells  $\text{ml}^{-1}$ ) were tested, and the highest observed growth rate occurred at  $60 \times 10^3$  cells  $\text{ml}^{-1}$  (mean  $\mu=0.63 \text{ d}^{-1} \pm 0.07 \text{ SE}$ ;  $r^2=0.71$ ). Four concentrations ( $40 \times 10^3$ ,  $50 \times 10^3$ ,  $90 \times 10^3$ , and  $200 \times 10^3$  cells  $\text{ml}^{-1}$ ) were tested in the second experiment. The highest HDINO growth rate, mean  $\mu=0.79 \text{ d}^{-1} \pm 0.14 \text{ SE}$  ( $r^2=0.90$ ), was observed at  $40 \times 10^3$  *K. rotundatum*  $\text{ml}^{-1}$ . Ingestion and clearance rates, measured for HDINO in the second prey concentration effects experiment, ranged from -15 to 200 *K. rotundatum* HDINO $^{-1} \text{ d}^{-1}$  and  $-8.85 \times 10^{-5}$  to  $1.40 \times 10^{-3}$  ml HDINO $^{-1} \text{ d}^{-1}$ .

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## INTRODUCTION

### Microbial food webs

When considering predator-prey dynamics, it seems reasonable to restrict the concept to "big organism eats little organism." However, there is little adherence to size constraints between predator and prey in the marine planktonic community. In fact, it is difficult to classify protozoans based on whether or not they are solely photosynthetic. Turner and Roff (1993) suggest that Lindeman's (1942) trophic-level concept and particle-size spectra are ineffective generalizations for describing the marine planktonic community and that a new paradigm, trophospecies, which takes into account both an organism's feeding and its predators, would better characterize the dynamics of marine plankton. Recent evidence has shown that dinoflagellates once believed to be solely phototrophic are actually mixotrophic, autotrophic species capable of heterotrophy as environmental conditions dictate (Bockstahler and Coats 1993a). There also are functionally autotrophic protozoans, heterotrophs which either possess photosynthetic endosymbionts or are capable of retaining the chloroplasts of ingested prey cytoplasm (Stoecker and Silver 1987, Turner and Roff 1993).

Recent studies have shown that predator-prey relationships in the microbial food chain are probably more complicated than the suggested 10:1 predator-prey size ratio

(Fenchel 1987). For example, Goldman and Dennett (1990) have shown that the flagellate *Paraphysomonas imperforata*, a raptorial grazer, is able to ingest prey with a diameter two times greater than itself. *Tiarina fusus*, a ciliate, is capable of ingesting members of the genus *Dinophysis*, which are about the same size as the ciliate; however, in the presence of heterotrophic species of *Dinophysis*, *T. fusus* becomes the prey (Hansen 1991b).

There has been increased interest in the role of heterotrophic and mixotrophic dinoflagellates in the marine food web. Data from several field investigations suggest that heterotrophic dinoflagellates annually attained biomass levels comparable to levels reached by ciliates (Smetacek 1981, Lessard 1991). In an attempt to explain the trophic role of heterotrophic dinoflagellates in a marine environment, Hansen (1991a) sampled from a permanent station in Kattegat, Denmark and found that the most common genera were *Protooperidinium* and *Diplosalis*. Heterotrophic dinoflagellates were found to be abundant in the North Atlantic (Shapiro et al. 1989), as well as on a seasonal basis in the subarctic Pacific Ocean (Strom and Welschmeyer 1991).

In the Chesapeake Bay, located in the Mid-Atlantic region of the eastern U.S. coast, Lessard (1991) noted that heterotrophic dinoflagellates comprised most of the protozoan biomass, reaching a maximum following the decline in spring bloom diatom populations and occurring later than ciliates in

the region. Studies by Bockstahler and Coats (1993a and 1993b), investigating mixotrophy in Chesapeake Bay dinoflagellates, demonstrated that the dinoflagellate, *Gymnodinium sanguineum*, once thought to be solely autotrophic, was actually mixotrophic and an active grazer of ciliate populations. In the lower York River (VA), a tributary of the Chesapeake Bay, Mackiernan (1968) observed that *Oblea rotunda*, a heterotrophic dinoflagellate then referred to as *Diplosalis rotundata*, was numerous during red tides and actively fed on other dinoflagellates.

For the present research, an unidentified heterotrophic dinoflagellate (HDINO) was isolated from the York River and its general morphology and behavior described. Its growth responses to environmental conditions (i.e., temperature, salinity, and prey concentration effects on growth and grazing) also were examined by feeding it an autotrophic red tide dinoflagellate, *Katodinium rotundatum*, in a series of one-factor experiments. In addition to examining the autecology of HDINO, the secondary focus of this study was to investigate the predator-prey interactions that may be responsible for the demise of red tides.

#### **Red tides in the York River: Field observations**

Mackiernan (1968) characterized the seasonal dinoflagellate flora of the lower York River estuary, and emphasized the species composition of red tides, their

duration, and environmental factors affecting the events. For red tides occurring in the York River in 1967, there were several species of dinoflagellates, both autotrophic and heterotrophic, present: *Glenodinium* sp., *Peridinium trochoideum*, *Gymnodinium splendens*, *Polykrikos kofoidii* (heterotrophic), *Gyrodinium pinque* (heterotrophic), *Cochlodinium heterolobatum*, *Prorocentrum micans*, *Glenodinium foliaceum*, *Ceratium furca*, *Gonyaulax diegensis-digitale*, and *Gonyaulax monilata*.

The following year, "red water" was observed from the Virginia Institute of Marine Science to the Naval Weapons Station, dominated by *P. micans* and *C. furca* (Simmonds 1968). It was the first time that *P. micans* was documented as the dominant dinoflagellate responsible for a red tide in the lower river. In another York River investigation (Stofan 1973), the dominant dinoflagellate species *Prorocentrum minimum* and *P. micans* (autotrophic) and *Oblea rotunda* (heterotrophic), were found in >25% of river mouth samples. For the York River mouth region, the dominant species were the same except that the autotroph *Heterocapsa triquetra* was present in 25% or more of the samples. Zubkoff et al. (1979) examined mesoscale features of York River dinoflagellate blooms from 1975 to 1977, noting that during 1975 and 1976 *G. splendens* was the dominant species under normal ranges of salinity and temperature. However, in 1977 when river temperature was 3-4°C above the normal seasonal temperature

and salinity 3-5 psu above normal seasonal ranges, *C. heterolobatum* was the dominant species.

#### **Factors contributing to red tide formation**

Costas et al. (1993) stated that the literature suggests that there are three primary factors controlling dinoflagellate blooms: 1) abiotic, or physical factors, such as water column stratification, wind stress, or tides; 2) increased nutrient input via eutrophication or pollution; and 3) biological factors such as biosynthetic rates or endogenous control of mitosis. There is interest in analyzing the relationship between red tide organisms and environmental conditions because dinoflagellates have lower growth rates in comparison with other marine flagellates (Costas et al. 1993).

Studies also have documented the importance of cysts in the formation of red tides (Anderson and Wall 1978, Imai and Itoh 1987). The dinoflagellate cysts serve as a seed population that can withstand unfavorable environmental conditions until the optimal combination of physical and nutrient factors are present. Anderson (1989) stressed that some red tide species form resting stages, or cysts, that can remain fully viable under harsh conditions; however, many of the organisms responsible for "red water" in the Chesapeake Bay have no identifiable cysts. Possibly, red tide cells may be advected out of a region by currents, "spatially dispersed horizontally [as well as vertically]," or meteorologically

disrupted (Zubkoff et al. 1979).

In spring 1988, the development of 3 major blooms were monitored in Port River (South Australia) (Cannon 1990). The blooms formed in the bottom layers of the river and moved with tidal flow, and increases in cell numbers of the bloom organisms occurred at the surface when there was minimum water movement. Destratification was responsible for the breakup of the blooms. Natural sediment samples used in experiments produced cultures of the dinoflagellates *Alexandrium minutum* and *Prorocentrum micans*. Blooms of these particular species seem to be initiated by cysts (Cannon 1990). Nehring (1995) emphasized that cyst surveys served as an important means of evaluating areas with potential toxicity problems. Resting cysts of the neritic *Gymnodinium catenatum*, a potentially toxic, chain-forming naked dinoflagellate, were found in German coastal sediments. Previously, this dinoflagellate was not known to inhabit Northern European waters. The dinoflagellate was germinated successfully in experiments with natural seawater, and under favorable conditions, it is likely that the vegetative form of *G. catenatum* will appear in northern Europe (Nehring 1995).

In a two-year study that began in 1975, Tyler and Seliger (1978) traced the yearly subsurface transport of *P. minimum* var. *mariae-lebouriae* from the mouth of the Chesapeake Bay to the upper bay and reported the physical mechanisms (i.e., water circulation patterns, density gradients, wind mixing)

contributing to the dinoflagellate's transport. The *P. minimum* that re-entered the Chesapeake Bay system were descendants of cells which had previously bloomed mid-bay and were "not of oceanic origin" (Tyler and Seliger 1978).

#### **Toxic dinoflagellate "blooms"**

To date, most red tides in the Chesapeake Bay have not been considered toxic. In one of the few cases in which toxicity was noted, Luckenbach et al. (1993) found that *P. minimum*, at bloom concentration and at concentrations reduced to 33% of bloom levels, caused mortality in juvenile Eastern oysters *Crassostrea virginica*. *C. heterolobatum* has also caused oyster larvae mortality (Ho and Zubkoff 1979).

Toxic blooms have plagued Florida and the New England fisheries for many years leading to the establishment of toxin monitoring programs. In 1987 the toxic dinoflagellate *Gymnodinium breve*, which had previously impacted only Florida's finfish fishery, was transported in a Gulf Stream meander to North Carolina's coastal waters for the first time (Tester et al. 1991). The intrusion shut down the 1987-1988 shellfish fishery, resulting in losses exceeding \$25 million (Tester and Fowler 1990).

This algae, as well as other toxic algae, and low oxygen levels due to eutrophication were once believed to be the main cause of fish kills. However, Burkholder et al. (1992) linked *P. piscicida*, which has several life stages, to fish kills in

North Carolina's estuaries. The cell paralyzes fish with a toxin and feeds on stunned prey (Burkholder et al. 1995a, 1995b). Lewitus et al. (1995) documented the presence of *Pfiesteria piscicida*, the "phantom dinoflagellate", in northern Chesapeake Bay waters.

#### **"Red water" and the impact of zooplankton grazing**

Little is known about the mechanisms which lead to the disappearance of red tides (Sellner and Brownlee 1990, Sellner et al. 1991). Grazing pressure by zooplankton could lead to the disappearance of "red water." Most studies have examined the impact of macrozooplankton (i.e., copepods and rotifers) grazing on blooms of autotrophic dinoflagellates (Sellner and Olson 1985, Uye 1986, Buskey and Stoecker 1988, Sellner and Brownlee 1990, Nielsen 1991, Sellner et al. 1991). In a study of copepod grazing (Huntley et al. 1986), 13 species of dinoflagellates were offered to the copepods *Calanus pacificus* and *Paracalanus parvus*; 5 species were rejected. Starved copepods could not be induced to feed on one of the species, and at bloom concentrations, *Calanus pacificus* experienced high mortality and reproduction terminated. In another study, Uye and Takamatsu (1990) studied the feeding interactions between *Pseudodiaptomus marinus* and *Acartia omorii*, inshore marine copepods, and 15 red-tide rhabdophycean flagellates and dinoflagellates. As above, they concluded that one of the important factors in monospecific red tide development

involves chemically-mediated rejection by copepods (see Huntley et al. 1986). Sellner and Brownlee (1990) noted that the ciliate *Favella* sp., "identified [by Stoecker et al. (1981)] as a major grazer" in other coastal systems, exerted little grazing pressure in summer bay blooms. *Favella* sp., at densities of 213 and 104 individuals  $l^{-1}$  and clearing 2.06 and 1.77  $\mu l$  individual $^{-1}$   $h^{-1}$ , respectively, removed only minor fractions of "available phytoplankton biomass in dinoflagellate-rich and poor assemblages," and therefore had minimal herbivorous control of dinoflagellate blooms in the Chesapeake Bay region (Sellner and Brownlee 1990).

In a study of winter dinoflagellate-microflagellate blooms, Sellner et al. (1991), investigating the vertical distribution of *K. rotundatum*, found that the highest chlorophyll levels were in the near-surface waters of the Patuxent River Estuary (Maryland), and there was evidence that *K. rotundatum* aggregated diurnally in surface lighted depths. Assuming that all copepods (*Eurytemora affinis* and *Acartia tonsa*) were feeding on the bloom and that copepodites fed at 80% of adult rates, estimated zooplankton ingestion suggested that grazing played a major role in *Katodinium* bloom dissipation (Sellner et al. 1991). However, these findings were not consistent with zooplankton grazing estimates for blooms caused by other Chesapeake Bay bloom-forming dinoflagellates (i.e., *Gymnodinium* sp., *Gyrodinium* sp., *Ceratium lineatum*, and *P. minimum* var. *mariae-lebouriae*) where

blooms are not greatly impacted by copepod grazing (Sellner et al. 1991).

In Japan, Uye (1986) investigated the effect of copepod grazing on *Chattonella antiqua*, a raphidophycean red tide flagellate responsible for several fish kills. Though this study showed that copepods did feed on the raphidophycean under simulated red tide conditions, the percentage of *C. antiqua* removed by grazing copepods each day decreased as the cell concentration of the *C. antiqua* bloom increased. Thus, copepod grazing was probably more important during the initial stages of red tide development.

Of most relevance to the present study, Nakamura et al. (1992) examined the impact of *Gyrodinium dominans*, a herbivorous dinoflagellate, on red tides comprised of *C. antiqua*. Through examination of laboratory batch cultures of *G. dominans* and *C. antiqua*, it appeared as if *G. dominans* might be a contributing factor to the disappearance of *C. antiqua* red tides. Over a period of eight days, cell concentrations of *G. dominans* increased logarithmically from about  $10^{1.3}$  cells to  $>10^{3.5}$  cells  $\text{ml}^{-1}$ . Cell concentrations of *C. antiqua* increased slowly from over  $10^{2.5}$  cells to only about  $10^{3.5}$  cells  $\text{ml}^{-1}$  until Day 6, and then decreased before Day 8 to well below  $10^1$  cells  $\text{ml}^{-1}$ . This decrease suggested that *G. dominans* was feeding heavily on *C. antiqua*, and consequently, the authors suggested *G. dominans* was likely responsible for the disappearance of *C. antiqua in situ* (Nakamura et al.

1992). Because heterotrophic dinoflagellates are present in the Chesapeake Bay region in such large numbers (e.g., Lessard 1991), they may be, like *G. dominans* in Japanese red tides, grazers of "red water" in the main bay and its tributary estuaries.

#### **Feeding behaviors of heterotrophic dinoflagellates**

Dinoflagellates exhibit raptorial (which means "adapted for seizing prey") feeding behaviors (Verity 1991). Elbrachter (1991) divided the mechanisms of dinoflagellate food uptake into three categories: 1) phagotrophy *sensu stricto*, 2) pallium feeding, and 3) myzocytosis. Phagotrophy, the uptake of whole food particles, has been documented for the dinoflagellate *Noctiluca*, which also utilizes mucoid filtration as a feeding mechanism (Elbrachter 1991). Pallium feeding occurs when a veil or pseudopod is extruded by the feeding dinoflagellate and used as a net to trap prey (Mackiernan 1968, Jacobson and Anderson 1986). Digestion occurs extracellularly. Myzocytosis refers to the process by which cell fluids are extracted and transferred from prey to predator (Elbrachter 1991, Verity 1991). This transfer can occur through the use of a peduncle, a "highly extensible structure" that "protrudes during feeding" (Spero 1982). For *Protoperidinium* and *Diplosalis*, Hansen (1991a) reported that the most common mechanisms of prey capture included engulfment and use of a peduncle or a pallium to extract the prey's

contents. Wilcox and Wedemayer (1992) refer to the feeding tube of *Amphidinium* as a "phagopod," a hollow cylinder that is inserted into prey.

There have been several investigations of the feeding behavior of heterotrophic dinoflagellates. Jacobson and Anderson (1986) observed the feeding behavior of eighteen species of thecate heterotrophic dinoflagellates, involving the genera *Protoperidinium*, *Oblea* (*Diplosalis*) and *Zygabikodinium*, on prasinophytes, other dinoflagellates, and diatoms. These species of heterotrophic dinoflagellates fed by means of a pallium that was extruded from the flagellar pore, enabling them to capture up to 58 diatom cells in a chain (Jacobson and Anderson 1986). In another study (Hiroaki et al. 1993), the dinoflagellate *Oxyphysis oxytoxoides* circled its prey several times prior to attacking it. A hollow tube was then inserted into the prey through which a small amount of fluid was injected; *O. oxytoxoides* then sucked out the prey's cytoplasm. Food vacuoles formed, and the dinoflagellate expanded. Before feeding was complete, *O. oxytoxoides* usually began swimming, dragging the shrunken prey along with it (Hiroaki et al. 1993). In a similar study, Mackiernan (1968) noted that *Oblea rotunda* used its flagella to attach itself to the prey item, towing the cell as it swam, and extruded a protoplasmic lobe from its flagellar pore that engulfed the prey cell. Apparently, digestion of the prey occurred outside of the cell but within the protoplasmic

lobe.

### **Summary of objectives**

The observations above suggest that macrozooplankton play minor roles in bloom dissipation; therefore, heterotrophic dinoflagellates, given their high biomass, might be important predators in Chesapeake Bay "red water." There generally are two approaches that can be taken to examine the impact that grazing may have on an algal bloom: 1) observations of predator and prey abundance and co-occurrence in the field and 2) laboratory studies of the autecology of a specific predator on a particular algal species, from which inferences about the natural environment may be made. To investigate the impact of heterotrophic dinoflagellate grazing on red tides, an actual red tide event could be rigorously sampled, and the abundances of the heterotrophic dinoflagellates with respect to the red tide organism could be documented over the course of the event. Such a study can be conducted only if the red tide occurs, and there are many other factors, in addition to grazing, impacting the distribution of red tides in the natural environment. Furthermore, there is little known about heterotrophic dinoflagellates. For these reasons, grazing of a red tide dinoflagellate by a heterotrophic dinoflagellate was examined in the more controlled environment of a laboratory.

The major hypothesis is that heterotrophic

dinoflagellates can, through active grazing, act as controls for red tides of autotrophic dinoflagellates. In this study, the functional responses of a heterotrophic dinoflagellate isolate (HDINO) were examined through a series of one-factor experiments in order to: 1) determine how differences in temperature, salinity, and prey concentration affected predator growth rate and 2) calculate rates of ingestion and clearance and relate these rates to prey concentration. In order to address these concerns, working null hypotheses were as follows: 1) temperature has no affect on HDINO growth rate, 2) salinity has no affect on HDINO growth rate, and 3) prey concentration has no affect on HDINO growth rate. The results of this study will be used to infer the impact of HDINO grazing in situ and assess the possible ecological role of HDINO in the natural environment.

## MATERIALS AND METHODS

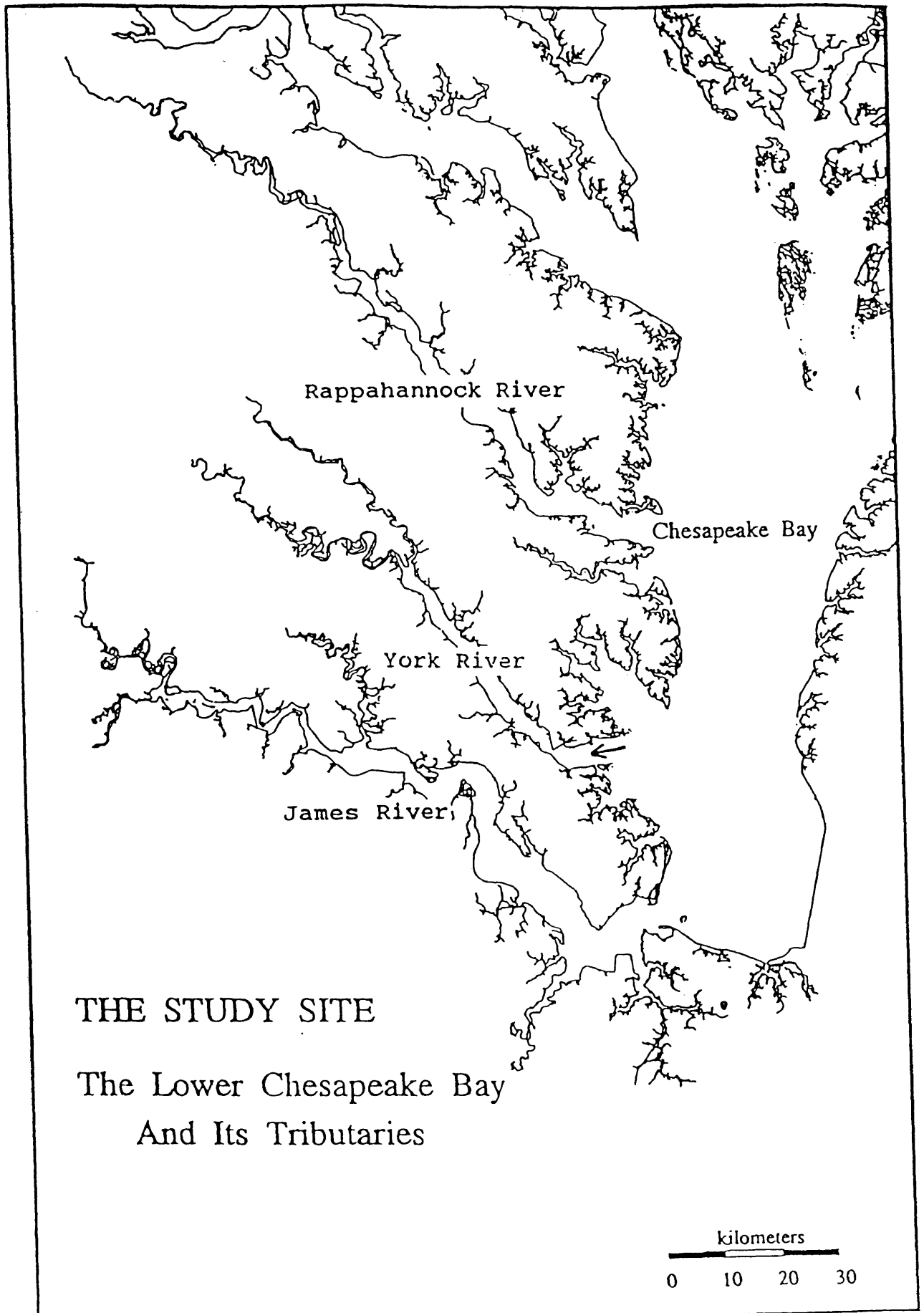
### Study area

The York River (Figure 1) extends 50 km from the fresh water Pamunkey and Mattaponi Rivers to the lower Chesapeake Bay (Haas 1975, Haas 1977, Zubkoff et al. 1979). The average tidal range at the river's mouth is 0.7 m (Haas 1977). The lower York River, which has a mean width of about 2.5 km, possesses a channel 16-18 m deep. Water columns of the lower Chesapeake Bay tributaries, including the York River, "oscillate between conditions of vertical stratification and homogeneity on a cycle" that is closely connected to the neap and spring tides (Haas 1975). The lower York River is polyhaline (15-25 psu) and has a wide annual temperature range (2-28 °C) (Mackiernan 1968, Haas 1975, Munday and Zubkoff 1981).

### Prey culture acquisition and maintenance

Several species of algae were maintained in the laboratory to serve as potential prey for heterotrophic dinoflagellates collected and isolated from natural samples taken from the York River during August and September 1993. Potential food sources included the red tide dinoflagellate, *P. minimum* (isolated from the lower York River, VA by A. D. Smith), *P. micans* (purchased from Carolina Biological Supply Company), *Gyrodinium* sp. (obtained from D. Wayne Coats,

Figure 1. The Chesapeake Bay and its tributaries. The lower York River is designated by the arrow.



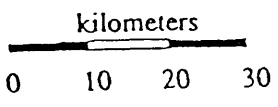
Rappahannock River

Chesapeake Bay

York River

James River

THE STUDY SITE  
The Lower Chesapeake Bay  
And Its Tributaries



Environmental Research Center, Edgewater, MD), and *K. rotundatum* (purchased from the Provasoli-Guillard Center for Culture of Marine Phytoplankton, Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, ME). In addition, a small flagellated chlorophyte, *Dunaliella tertiolecta* (obtained from D. Wayne Coats), *Isochrysis galbana* (provided by P. Tester), and *Rhodomonas* sp. (provided by E. Haugen) also were cultured. Prey cultures were maintained in York River water collected from the Virginia Institute of Marine Science (VIMS) ferry pier with a plastic bucket. The water was filtered (0.2  $\mu$ m Metrical filter), distributed to glass test tubes (10 ml) or cloth-stoppered Erlenmeyer flasks of various volumes, and autoclaved. Nutrients were added to the sterilized water (200  $\mu$ l Alga-Gro concentrated medium per 10 ml water). The tubes and flasks were inoculated with media from an established culture of the respective algae. The cultures of *K. rotundatum* were incubated at approximately 15 °C and the cultures of *D. tertiolecta*, *P. micans*, *Gyrodinium* sp., *I. galbana*, *Rhodomonas* sp. and *P. minimum* at 20 °C (later increased to 25 °C) on a 12:12 light-dark cycle. To maintain exponential growth, the cultures were transferred to fresh media at least every two weeks.

#### **Isolation of heterotrophic dinoflagellate**

York River surface water (YRSW) was collected from the VIMS ferry pier (Gloucester Point, VA) on September 15, 1993

and distributed to several 14-ml, sterile polystyrene tubes. A few drops from a *D. tertiolecta* culture were added to the tubes, which subsequently were placed in an 20 °C incubator. Within two days, a variety of heterotrophic dinoflagellates were observed in the samples.

During late-September and throughout October, attempts were made to isolate two potential grazers, an unidentified *Gymnodinium*-like dinoflagellate (HDINO) and another *Gyrodinium*-like dinoflagellate, believed to be athecate and heterotrophic, by micropipetting cells from samples in a petri dish (Baxter S/P diSPo) and washing the cells several times with 0.2 or 0.45 µm syringe-filtered YRSW. The washed cells were then placed into 1-2 ml of media containing *D. tertiolecta* and incubated at 20 °C. Attempts to culture the *Gyrodinium*-like dinoflagellate were unsuccessful, but HDINO cultures persisted. After *K. rotundatum* batch cultures were established in December 1993, the HDINO was added to dark tubes containing *K. rotundatum* media. Light was excluded from the tubes so that the autotrophic cells would not photosynthesize, and hence, not grow and divide. Transfers into fresh cultures of *K. rotundatum* were made regularly until it was the sole eukaryotic prey for the HDINO. Thus, the predator-prey unit of HDINO and *K. rotundatum* was established.

**Temperature effects**

Twenty-five ml of *K. rotundatum* (ca. 5 days old) batch cultures with initial concentrations approximating  $1 \times 10^5$  cells  $\text{ml}^{-1}$  were added to each of sixteen 50-ml sterile flasks and divided into groups of four. Flasks were wrapped in electrical tape to exclude light. Three flasks in each group were inoculated with 1 ml of media from a HDINO culture believed to be in log phase (initial concentrations ranging from about  $11\text{-}15 \times 10^4$  cells  $\text{ml}^{-1}$ ) that had been maintained at 15 °C, and the remaining flask served as a *K. rotundatum*-only control. Groups of flasks were incubated at each of 4 temperatures (10, 15, 20, and 25 °C) and sampled (1-2 ml) daily for cell counts.

**Salinity effects**

Sargasso Sea water (35 psu), collected in August 1990, was diluted with distilled water to obtain salinities of 10, 15, 20, 25, and 30 psu. *K. rotundatum* from a batch culture grown at 15 °C was concentrated by centrifugation (about  $5 \times 10^5$  cells  $\text{ml}^{-1}$ ) and aliquots added to twenty 50-ml sterile flasks wrapped in electrical tape and containing 25 ml of Alga-Gro enriched media at the designated salinities. Within each salinity group (four flasks per group), three flasks were inoculated with HDINO, grown at 20 °C and at initial concentrations of an estimated  $12.5 \times 10^3$  HDINO cells, and the remaining flask served as a *K. rotundatum*-only control. The

flasks were incubated at room temperature, ranging from 19-25 °C. Samples (1-2 ml) were drawn from each flask daily, and slides were made for cell counts. None of the t=0 slides could be counted due to extensive clouding by a faulty Calcofluor stain. Slides for Days 1 through 4 were stained with Proflavin only.

#### **Prey concentration effects I**

An initial attempt to examine prey concentration effects of *K. rotundatum* on the HDINO at 25 °C and 20 psu was unsuccessful due to the prey's inability to withstand temperatures >15 °C. A second experiment was designed to account for the apparent low temperature preference of *K. rotundatum*.

Five prey concentrations ( $2.5 \times 10^3$ ,  $5 \times 10^3$ ,  $25 \times 10^3$ ,  $60 \times 10^3$ , and  $1 \times 10^6$  cells ml<sup>-1</sup>) were established by dispensing 20 psu batch cultures into sterile 50-ml flasks containing sufficient 20 psu water required to produce the desired prey concentration. There were four flasks per concentration group, triplicate experimental flasks and one prey-only control. One milliliter from a HDINO culture (<200 cells ml<sup>-1</sup>) was added to each of the flasks, except the controls. The flasks were sampled (t=0) and incubated at 15 °C and ca. 20  $\mu\text{E m}^{-2} \text{ s}^{-1}$  of light on a 12:12 light-dark cycle. Samples (1-2 ml) were drawn daily for cell counts which were made using both epifluorescence microscopy (see below) and a hemacytometer.

**Prey concentration effects II**

Four prey concentrations ( $40 \times 10^3$ ,  $50 \times 10^3$ ,  $100 \times 10^3$ , and  $200 \times 10^3$  cells  $\text{ml}^{-1}$ ) were established by diluting a 20 psu *K. rotundatum* batch culture in nutrient-enriched ( $200 \mu\text{l}$  Alga-Gro\* $10 \text{ ml}^{-1}$ ) 25 ml sterile 20 psu water in 50-ml flasks. There were four flasks in each concentration group, triplicate experimental flasks and one control containing only prey. One hundred microliters from a HDINO culture ( $<100$  cells  $\text{ml}^{-1}$ ) was added to each of the flasks, except the controls. An additional three flasks containing only 25 ml of media were inoculated, and these flasks provided  $t=0$  estimates of HDINO abundance. All flasks were incubated at  $15^\circ\text{C}$  in dim light ( $3.4 \mu\text{E m}^{-2} \text{ s}^{-1}$ ) on a 12:12 light-dark cycle. Samples were drawn daily. Cell counts were made using epifluorescence microscopy (see below).

**Cell counts**

Epifluorescence microscopy was used to enumerate autotrophic and heterotrophic dinoflagellates in the samples (Haas 1982, Bjornsen and Kuparinen 1991, Strom 1991). Subsamples (1-3 ml) from cultures were transferred by pipet to a filtration tower, and glutaraldehyde 6% (at  $50 \mu\text{l ml}^{-1}$ ), proflavin hemisulfate 0.033% (at  $<50 \mu\text{l ml}^{-1}$ ), and calcofluor (working stock: 10 mg in 100 ml distilled water,  $50 \mu\text{l ml}^{-1}$ ) were added. Each subsample was filtered under a gentle vacuum through either a pre-stained 1- $\mu\text{m}$  membrane filter (Poretics)

or a 1- $\mu\text{m}$  membrane filter (Nuclepore) stained with Irgalin black (Haas 1982, Fritz and Triemer 1985, Strom 1991).

All cell counts were made using a Zeiss epifluorescent microscope equipped with an ocular micrometer. Attempts were made to count at least either 25 cells or 200 grids. Under blue light excitation (filter block, ca. 450 nm), the Proflavin stain causes cells to fluoresce bright green. The chlorophyll of autotrophic cells autofluoresces red, and this feature was used to distinguish autotrophic dinoflagellates from the heterotrophic dinoflagellates as well as the food vacuoles of the HDINO. Under UV illumination, the Calcofluor stain causes the thecal plates of armored dinoflagellates to fluoresce bright blue-white (Fritz and Triemer 1985, Strom and Buskey 1993). HDINO was also videotaped using a color camera (Hitachi KP-C550) attached to an inverted microscope (ausJENA).

#### **Data Analyses**

In all experiments, HDINO growth rates ( $\mu$ ;  $\text{d}^{-1}$ ) were determined from changes in HDINO abundance ( $\text{cells ml}^{-1}$ ) in each replicate through time and calculating the slope of the resulting line (Strom and Buskey 1993). This calculation is based on the standard growth rate equation:

$$\mu = \frac{(\ln N_f - \ln N_i)}{(t_f - t_i)}$$

where  $N_f$ =final number of cells,  $N_i$ =initial number of cells,

$t_f$ =final time, and  $t_i$ =initial time. By calculating HDINO  $\mu$  as the slope of each replicate for each treatment in each experiment, cell concentrations that fall between the experiment's start and finish are incorporated into the growth rate determination.

The design of the experiment made it difficult to find a suitable method to test significance differences among mean HDINO growth rates. In each experiment, subsamples for cell counts were drawn from the same replicate flasks over time, rather than randomly from different flasks. This method of sampling produces false replication and nullifies the use of one-way analysis of variance (ANOVA) and a posteriori tests, such as Tukey or Scheffe, to test any significant difference between mean HDINO growth rates. Therefore, regression analyses were performed on the data from each experiment. The  $r^2$  values served as the only indication of significant growth ( $r^2 > 0.60$ ).

HDINO clearance ( $F$ ;  $\mu l \text{ pred}^{-1} t^{-1}$ ) and ingestion ( $I$ ;  $\text{prey } \text{pred}^{-1} t^{-1}$ ) rates were determined using a Quattro Pro program written by Chunzi Guo based on the Frost (1972) equations as modified by Heinbokel (1978). Clearance rate ( $F$ ) was calculated for each of the experimental flasks using the equation:

$$F = Vg/H_{\text{mean}}$$

where  $V$ =initial volume of experimental culture,  $g$ =grazing

coefficient of HDINO on its prey, and  $H_{\text{mean}}$ =the mean HDINO concentration over the course of the experiment (Heinbokel 1978). The grazing coefficient ( $g$ ) was calculated using the equation:

$$C_f' = C_i' e^{(k-g)(t_f-t_i)}$$

where  $C_i'$  and  $C_f'$  are the concentration of *K. rotundatum* prey in the flasks with HDINO at  $t_i$  and  $t_f$ , respectively. To account for changes in prey abundance independent of grazing, a growth constant ( $k$ ) was calculated for *K. rotundatum* using the equation:

$$C_f = C_i e^{k(t_f-t_i)}$$

where  $C_i$  and  $C_f$  represent the concentration of *K. rotundatum* in the prey-only control flasks at  $t_i$  and  $t_f$ , respectively. If concentrations of prey decrease in the controls, then  $k$  is negative. The mean HDINO concentration, used to account for the growth of the heterotroph during the experiment, was calculated from the equation:

$$H_{\text{mean}} = \frac{H_f - H_i}{\ln H_f - \ln H_i}$$

where  $H_f$ =final concentration of HDINO and  $H_i$ =initial concentration of HDINO (Heinbokel 1978).

Ingestion rate ( $I$ ) was calculated as:

$$I = FK_i$$

where  $F$ =clearance rate and  $K_i$ =the initial number of *K.*

*rotundatum.*

## RESULTS

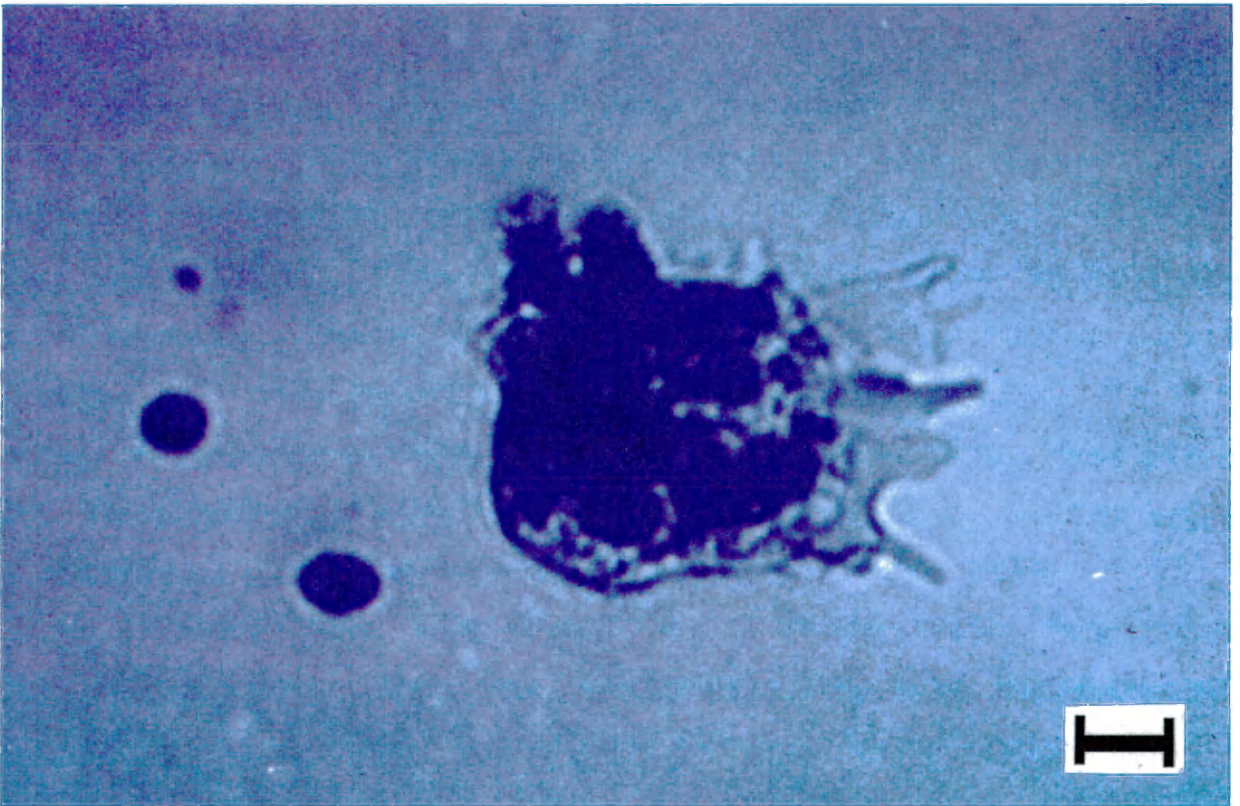
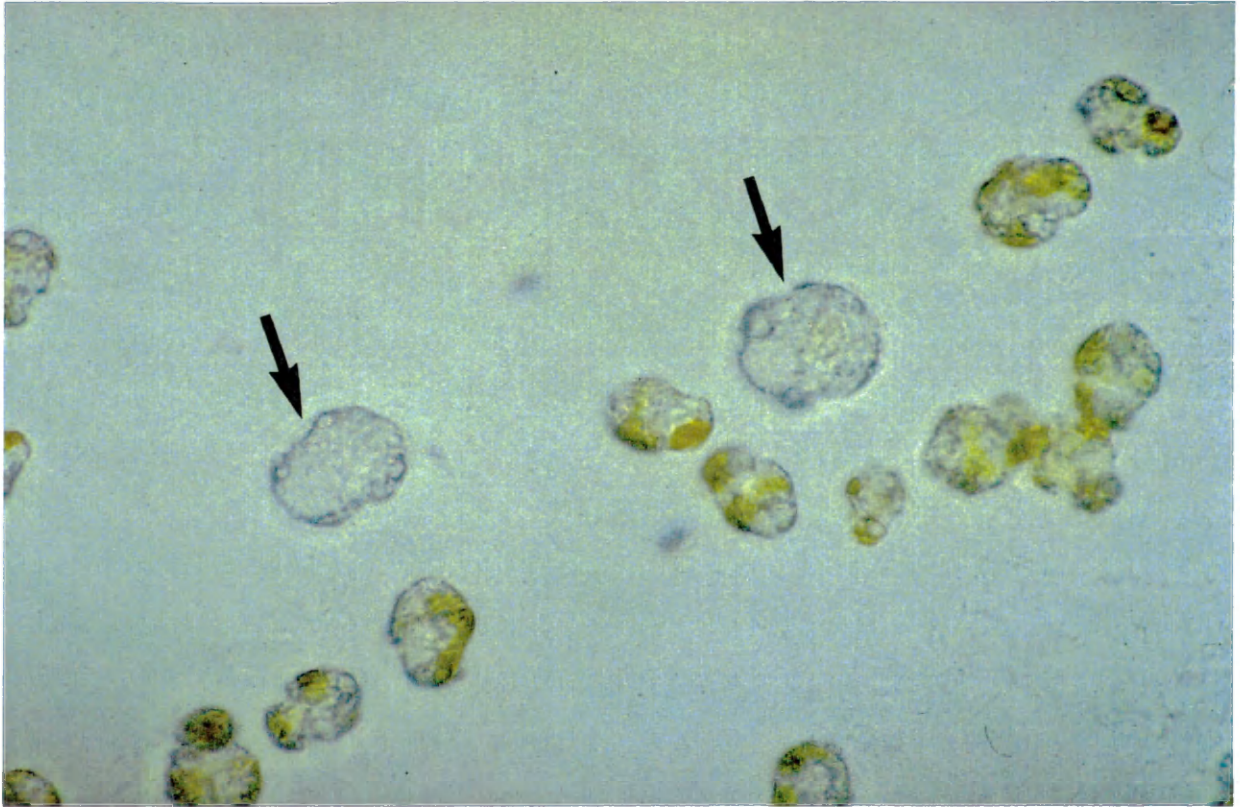
### **Light microscope observations of HDINO morphology and behavior**

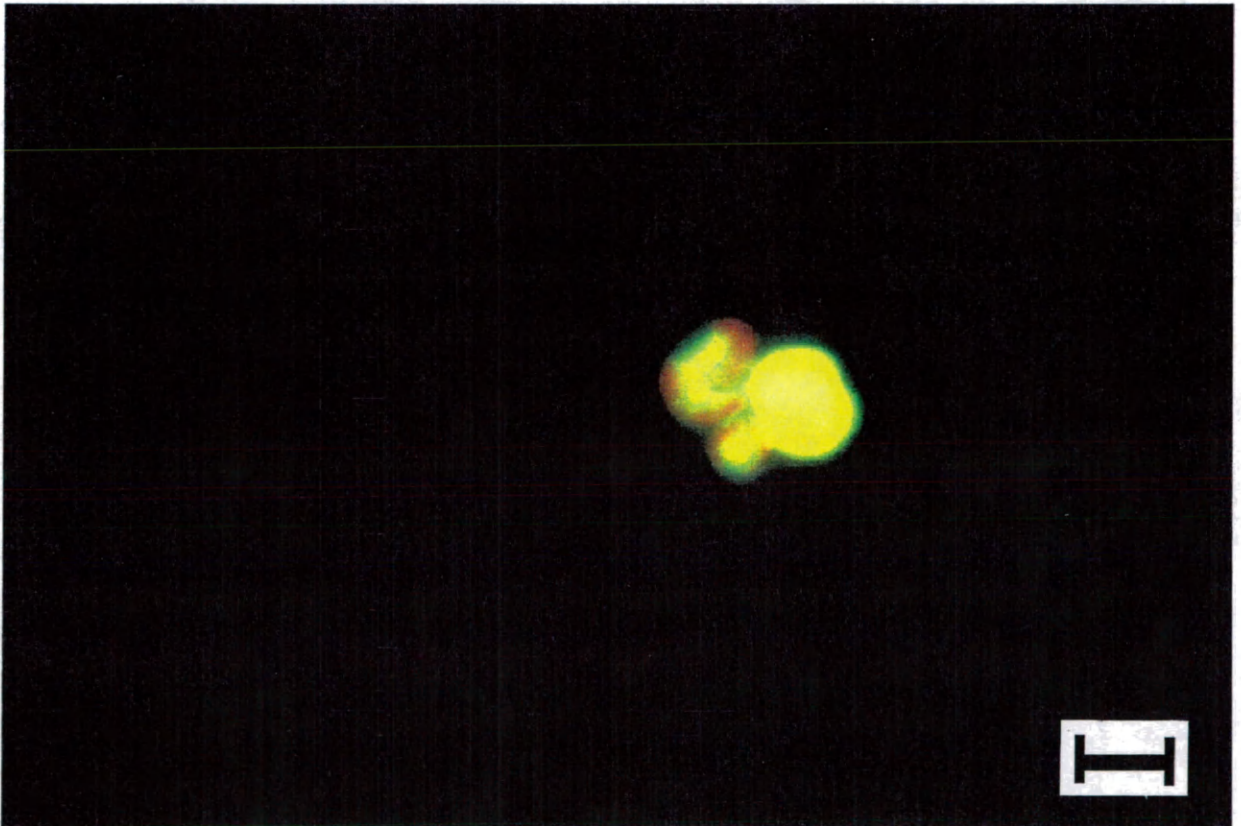
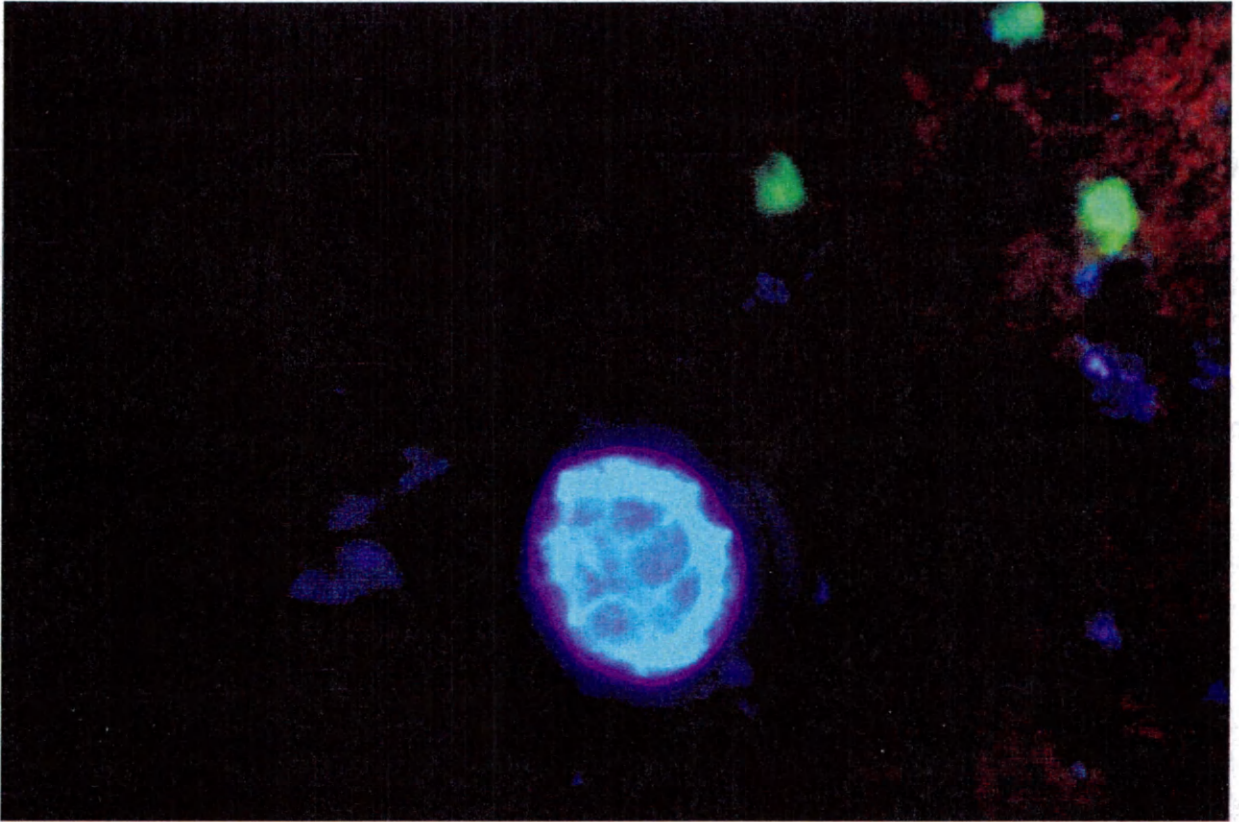
The thecate HDINO (Figure 2A-D) is ca. 10 X 13  $\mu\text{m}$ , and it resembles the zoospore life stage of *Pfiesteria piscicida*, the toxic dinoflagellate reported by Burkholder et al. (1992), as well as *Gymnodinium fungiforme* whose feeding behavior was reported by Spero (1982; 1985). Star-shaped amoeboid forms also were observed in some cultures of HDINO for a period of ca. 3 weeks; HDINO was not observed in these cultures for that period. Amoeba are characteristic a life stage documented for *P. piscicida* by Burkholder et al. (1992) and Steidinger et al. (1995). The cues initiating amoeba formation (see Figure 2B) in HDINO cultures are not known.

Thecate dinoflagellates are identified by a plate numbering system. Using the calcofluor stain, the thin thecal plates of the HDINO were seen under UV illumination, and the detail was sufficient to resolve sutures of each of the individual plates; however, no definitive determination of identity has been made by electron microscopy. Observations by epifluorescence microscopy also indicate that the HDINO contains no chlorophyll of its own. It is uncertain whether or not the HDINO possesses the capacity to retain and utilize its prey's chlorophyll.

The HDINO was a rapid swimmer that swarmed around its desired prey and was observed dragging prey items. However,

Figure 2. (A) Two representatives of HDINO, denoted by the arrows, shown with *K. rotundatum*, (B) One of the amoeboid forms (ca. 50  $\mu\text{m}$ ) observed, (C) HDINO stained with Calcofluor to display its thecal plates, and (D) HDINO (on the right) with two *K. rotundatum* cells. All scale bars = 10  $\mu\text{m}$ .



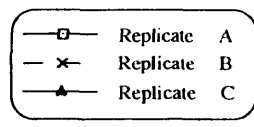
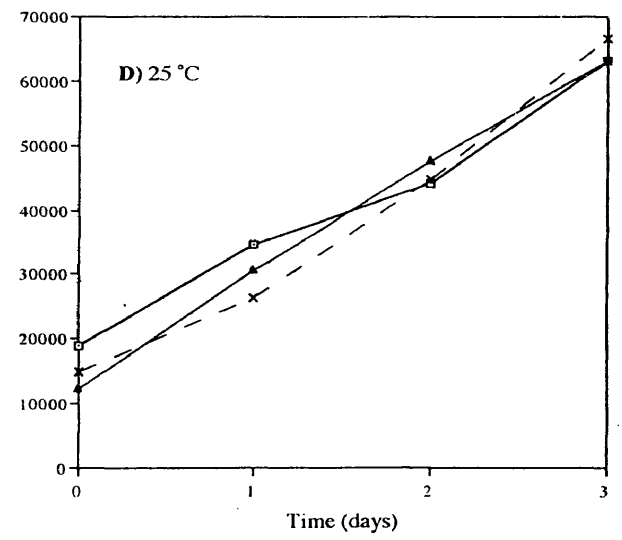
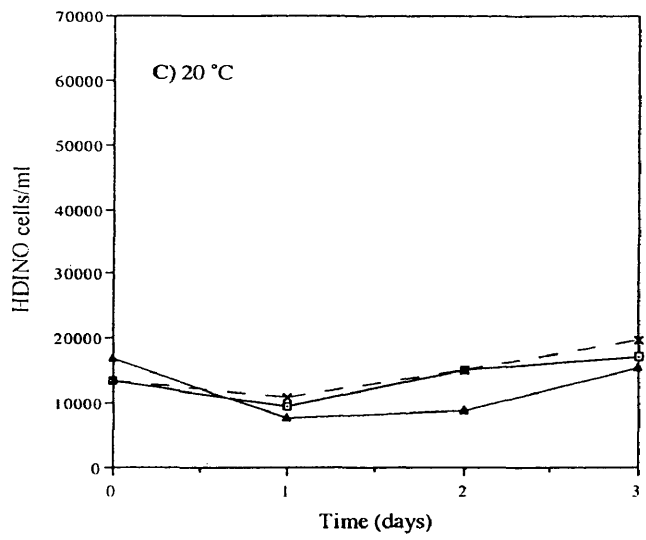
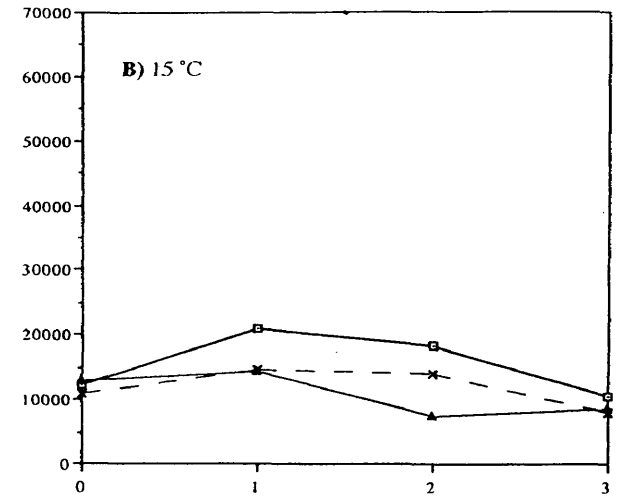
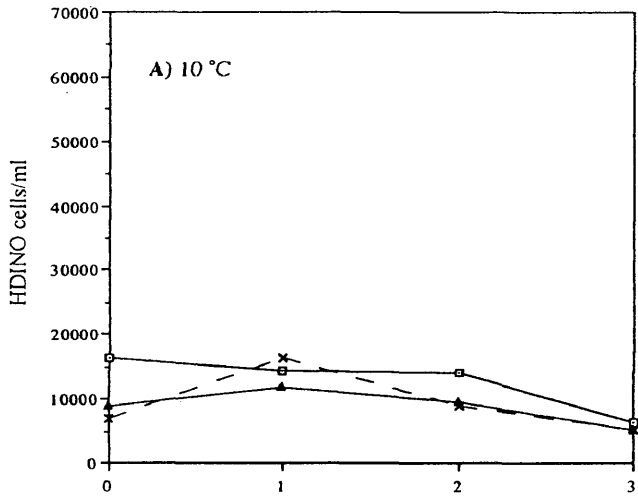


no pallium or tow thread was observed. Based on epifluorescence microscope observations and video recordings, the HDINO pierced its prey with a feeding tube, extracting cytoplasm into food vacuoles, and remained relatively motionless while extracting the prey's contents. Gradually, the prey cell shrunk, and after a few seconds (ca. 20-30 sec), all the prey's contents were drawn into a food vacuole in the HDINO. HDINO cell size varied based on how much prey was ingested. Predator cells that lacked food vacuoles were ca. 7 x 8  $\mu\text{m}$ , while well fed cells were as large as 17 x 18  $\mu\text{m}$ . In the second prey concentration effects experiment, the mean HDINO diameter at the start of the experiment was 11.5  $\mu\text{m}$ , but 3 days later its mean diameter had decreased to only 6.53  $\mu\text{m}$ . To date, the HDINO has been successfully cultured only on *D. tertiolecta* (a chlorophyte), *K. rotundatum*, *Isochrysis galbana* (a prymnesiophyte), *Rhodomonas* sp. (a cryptophyte), and *P. minimum*.

#### **Temperature experiment**

HDINO abundance varied for each replicate over time at each of the four temperatures tested (Figure 3). For temperatures 10 and 15  $^{\circ}\text{C}$ , the general trend was a decrease in HDINO cell numbers over the course of the experiment. At 20  $^{\circ}\text{C}$ , there was a decrease in HDINO abundance from days 0 to 1; however, after day 1, cell numbers increased gradually. Therefore, for each replicate, HDINO  $\mu$  was determined by

Figure 3. The changes in HDINO abundance over a 3-day period for (A) 10 °C, (B) 15 °C, (C) 20 °C, and (D) 25 °C.



calculating the slopes of the data for days 1 to 3. The growth rates for each of the replicates in each temperature treatment were averaged, and the mean growth rate for each temperature calculated (Table 1, Figure 4). HDINO grew at both 20 and 25 °C, and the largest increase in HDINO cell numbers occurred at 25°C yielding the highest calculated mean HDINO  $\mu$  (0.47 d<sup>-1</sup>; r<sup>2</sup>=0.93).

### **Salinity experiment**

As a result of the faulty Calcofluor stain, day 0 slides were clouded and could not be counted. Most of the *K. rotundatum* cells in this experiment appeared to have died before day 1 samples were taken because there were no indications of any *K. rotundatum* cells present for days 1 to 4. The prey cells might have died as a result of stress induced by a combination of the high temperature (25 °C), no light, and centrifugation. HDINO abundance is shown for each replicate of each salinity treatment (Figure 5). Because the numbers of HDINO cells present were so low and variable for salinities 10, 25, and 30 psu over the course of the experiment, the only quantifiable growth occurred at 15 and 20 psu. The mean growth rates were 0.93 and 1.25 d<sup>-1</sup>, respectively (Table 2, Figure 6).

Table 1. HDINO growth rates ( $\mu$ ) in the temperature effects on HDINO growth rate experiment.

°C	Replicate	$\mu$ (d <sup>-1</sup> )	Mean $\mu$ (d <sup>-1</sup> )
10	A	-0.280	
	B	-0.148	-0.205 $\pm$ 0.039 SE
	C	-0.187	
15	A	-0.059	
	B	-0.091	-0.113 $\pm$ 0.040 SE
	C	-0.190	
20	A	0.300	
	B	0.297	0.315 $\pm$ 0.016 SE
	C	0.347	
25	A	0.385	
	B	0.501	0.474 $\pm$ 0.046 SE
	C	0.537	

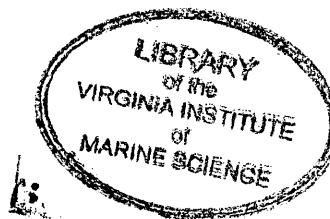


Figure 4. Mean HDINO growth rates ( $\mu$ ) calculated for each of the treatments in the temperature effects on growth rate experiment.

# Temperature Effects on HDINO Growth

Mean Growth Rates and Standard Errors

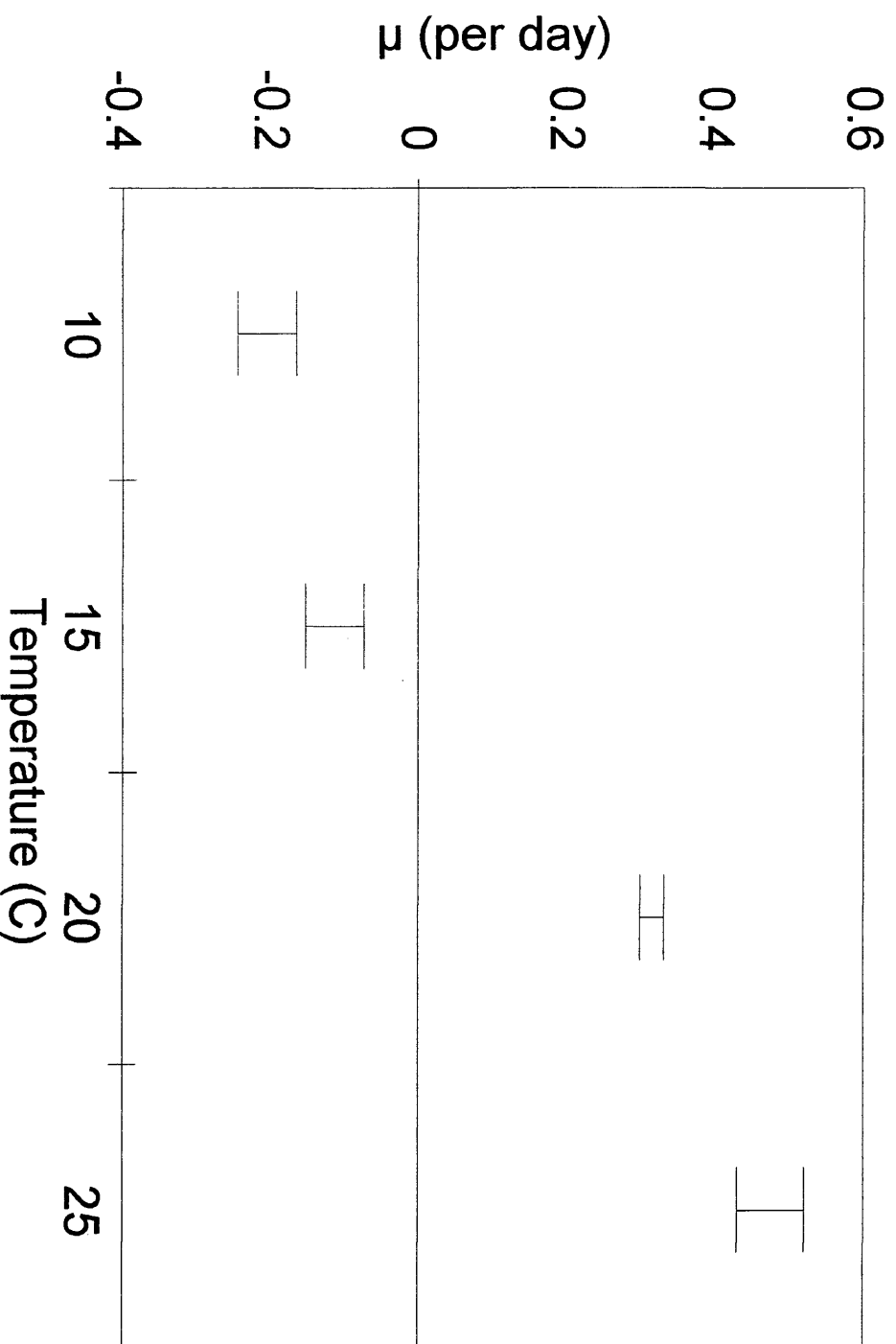


Figure 5. The changes in HDINO abundance over a 3-day period for (A) 10 psu, (B) 15 psu, (C) 20 psu, (D) 25 psu, and (E) 30 psu.

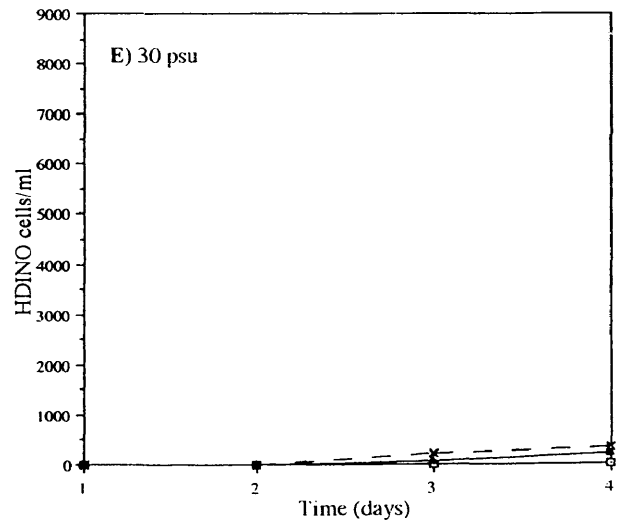
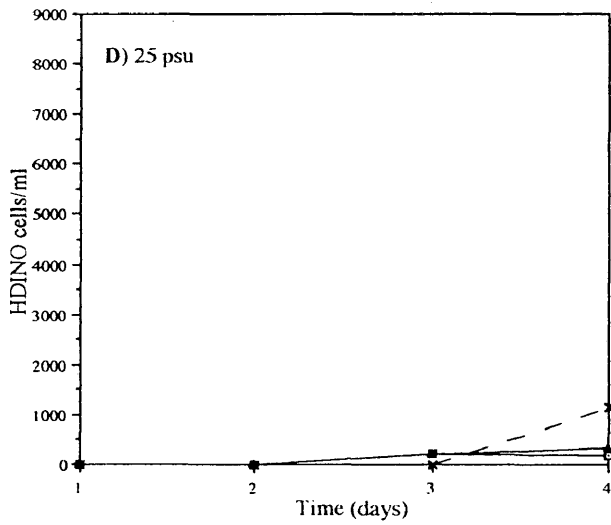
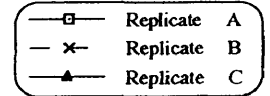
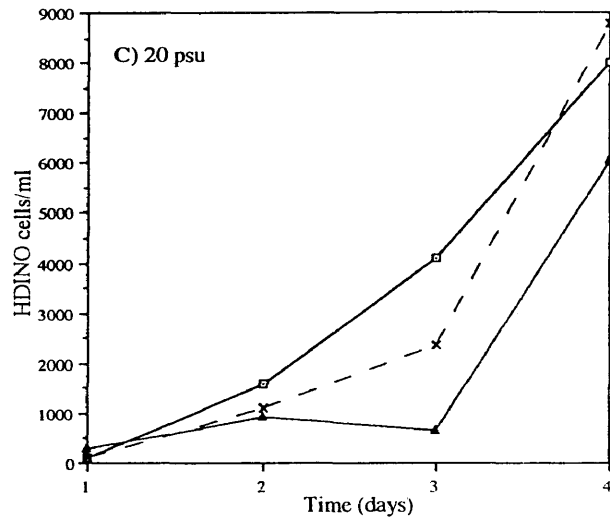
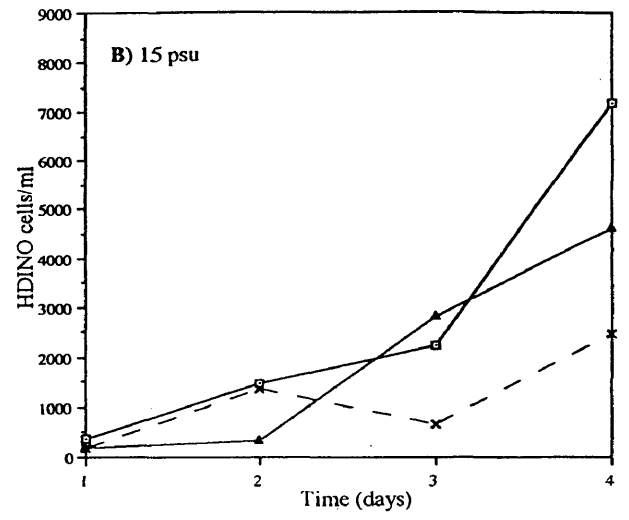
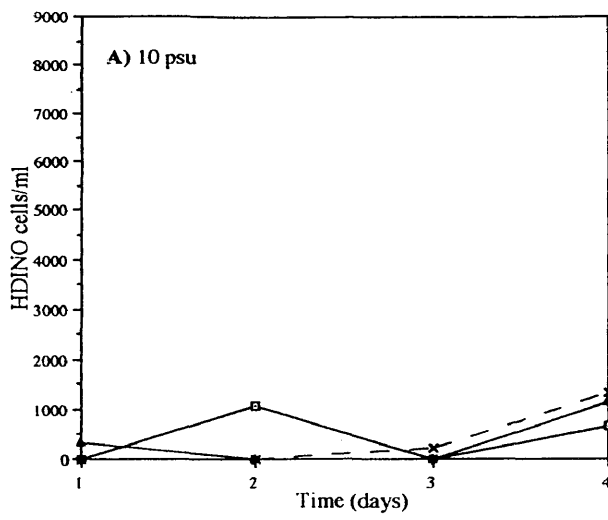


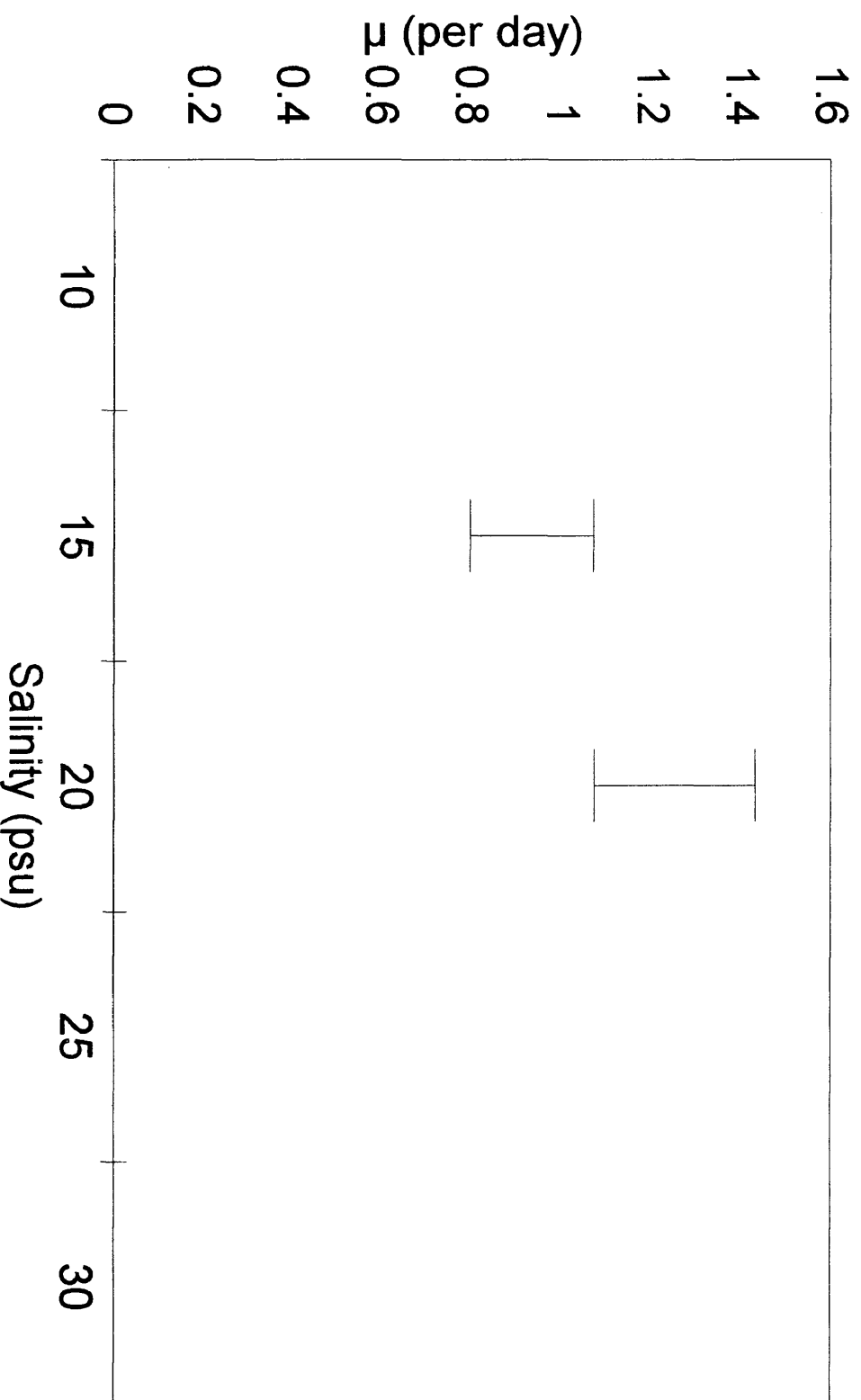
Table 2. HDINO growth rates ( $\mu$ ) in the salinity effects on HDINO growth rate experiment.

Psu	Replicate	$\mu$ ( $d^{-1}$ )	Mean $\mu$ ( $d^{-1}$ )
15	A	0.926	
	B	0.700	$0.932 \pm 0.136$ SE
	C	1.17	
20	A	1.43	
	B	1.44	$1.25 \pm 0.181$ SE
	C	0.889	

Figure 6. Mean HDINO growth rates ( $\mu$ ) calculated for each of the treatments in the salinity effects on growth rate experiment.

# Salinity Effects on HDINO Growth

Mean Growth Rates and Standard Errors



### Prey concentration experiments I and II

In experiment I, HDINO cells  $\text{ml}^{-1}$  were plotted over time for each replicate of prey concentration treatment (Figure 7). The prey levels  $2.5 \times 10^3$  *K. rotundatum*  $\text{ml}^{-1}$  and  $5.0 \times 10^3$  *K. rotundatum*  $\text{ml}^{-1}$  produced no HDINO growth. There was no clear trend demonstrated in the replicates for  $25 \times 10^3$  and  $1000 \times 10^3$  *K. rotundatum*  $\text{ml}^{-1}$  treatments. At  $60 \times 10^3$  *K. rotundatum*  $\text{ml}^{-1}$ , HDINO abundance increased with time. The growth rates for the replicates in each treatment were determined, and the mean HDINO  $\mu$  (Table 3) for each of the prey concentrations were graphed (Figure 8) as a function of prey concentration. The resulting curve resembled a Type I hyperbolic response curve. Rates increased from  $0.26 \text{ d}^{-1}$  at  $25 \times 10^3$  *K. rotundatum*  $\text{ml}^{-1}$  to  $0.63 \text{ d}^{-1}$  at  $60 \times 10^3$  *K. rotundatum*  $\text{ml}^{-1}$ , then declined to  $<0.14 \text{ d}^{-1}$  at the highest prey density ( $1000 \times 10^3$  *K. rotundatum*  $\text{ml}^{-1}$ ). The highest mean  $\mu$  ( $0.63 \text{ d}^{-1}$ ) occurred at  $60 \times 10^3$  *K. rotundatum*  $\text{ml}^{-1}$  ( $r^2=0.71$ ).

For experiment II, HDINO abundance was plotted in the same manner as in experiment I (Figure 9). HDINO growth occurred at all four of the tested prey concentrations ( $40 \times 10^3$ ,  $50 \times 10^3$ ,  $100 \times 10^3$ , and  $200 \times 10^3$  cells  $\text{ml}^{-1}$ ). The mean growth rates ranged from  $0.66$  to  $0.79 \text{ d}^{-1}$  (Table 4, Figure 10). The highest mean HDINO  $\mu$  ( $0.79 \text{ d}^{-1}$ ;  $r^2=0.90$ ) was measured at  $40 \times 10^3$  *K. rotundatum*  $\text{ml}^{-1}$ . The  $r^2$  values for the other four prey concentrations ranged from  $0.66$  to  $0.76$ . The results for experiments I and II were combined to produce the

Figure 7. The changes in HDINO abundance over a 3-day period for prey concentrations of (A)  $25 \times 10^3$  *K. rotundatum* ml<sup>-1</sup>, (B)  $60 \times 10^3$  *K. rotundatum* ml<sup>-1</sup>, and (C)  $1000 \times 10^3$  *K. rotundatum* ml<sup>-1</sup>.

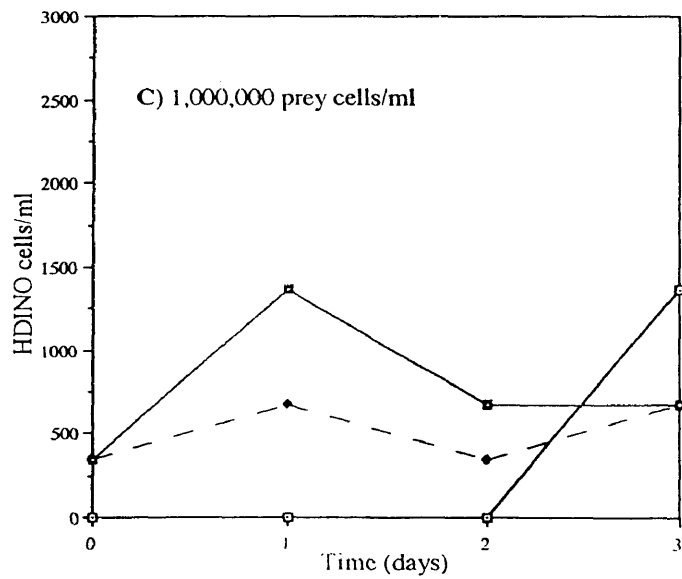
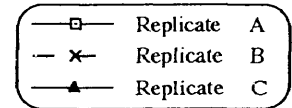
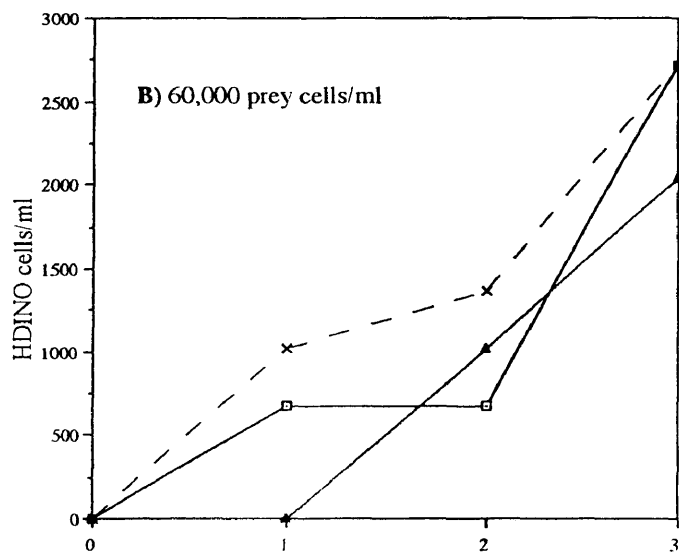
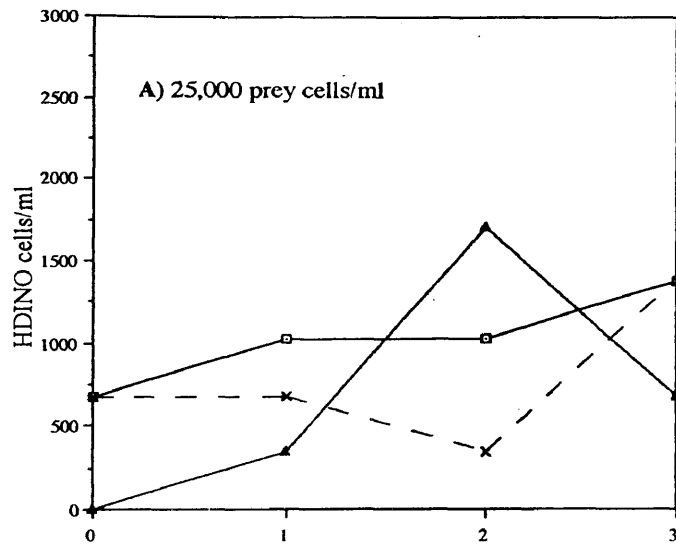


Table 3. HDINO growth rates ( $\mu$ ) for the prey concentration effects on HDINO growth rate experiment I.

Prey Conc.	Replicate	$\mu$ ( $d^{-1}$ )	Mean $\mu$ ( $d^{-1}$ )
25x10 <sup>3</sup>	A	0.208	
	B	0.139	0.231 $\pm$ 0.061 SE
	C	0.346	
60x10 <sup>3</sup>	A	0.692	
	B	0.490	0.625 $\pm$ 0.068 SE
	C	0.693	
1x10 <sup>6</sup>	A	0	
	B	0.138	0.092 $\pm$ 0.046 SE
	C	0.138	

Figure 8. Mean HDINO growth rates ( $\mu$ ) calculated for each of the treatments in the prey concentration effects on growth rate experiment I.

# Prey Conc. Effects on HDINO Growth-I

Mean Growth Rates and Standard Errors

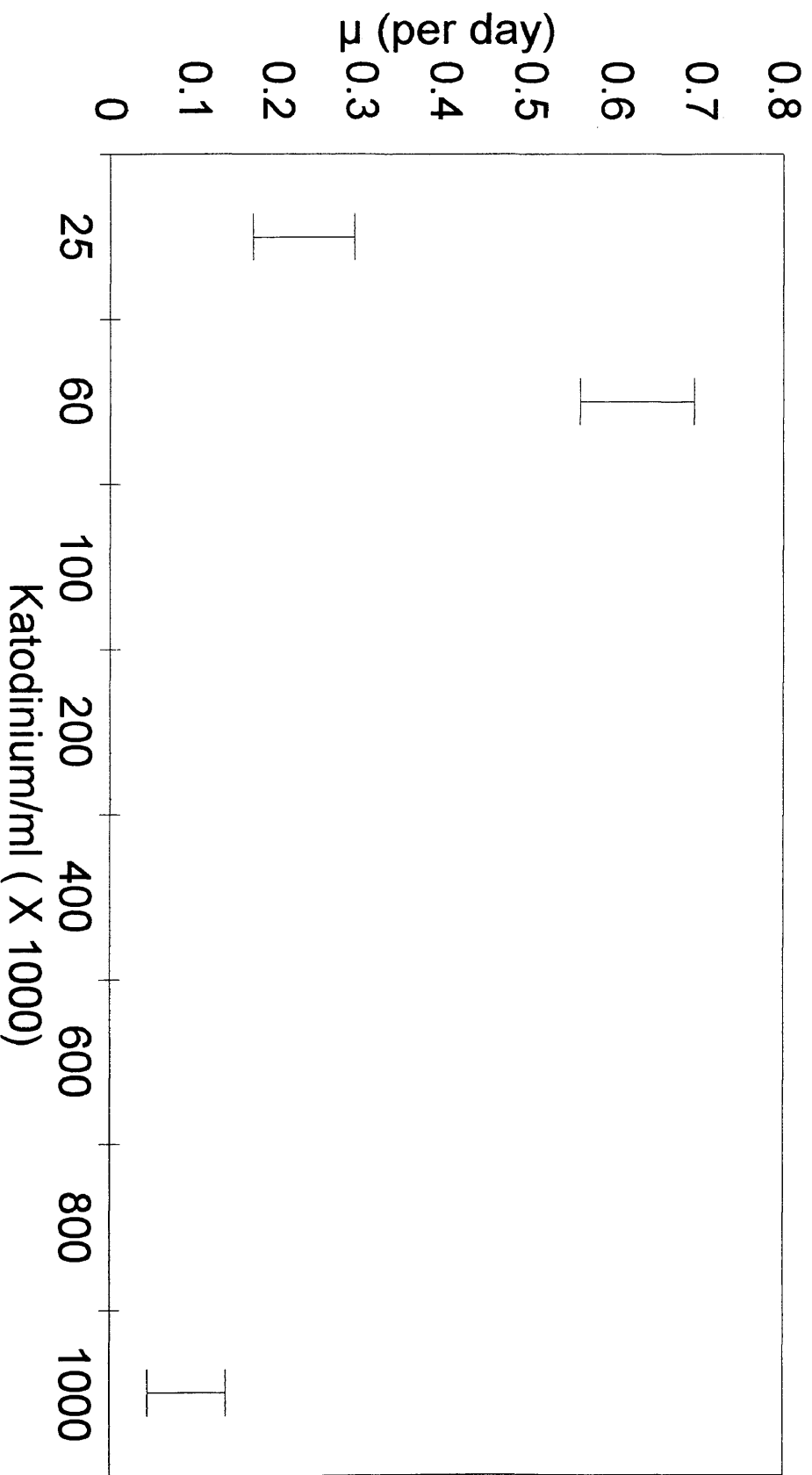


Figure 9. The changes in HDINO abundance over a 3-day period for prey concentrations of (A)  $40 \times 10^3$  *K. rotundatum* ml<sup>-1</sup>, (B)  $50 \times 10^3$  *K. rotundatum* ml<sup>-1</sup>, (C)  $90 \times 10^3$  *K. rotundatum* ml<sup>-1</sup>, and (D)  $200 \times 10^3$  *K. rotundatum* ml<sup>-1</sup>.

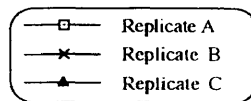
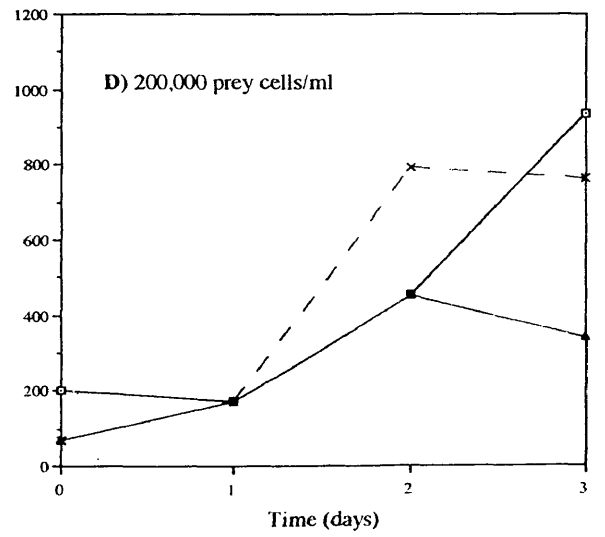
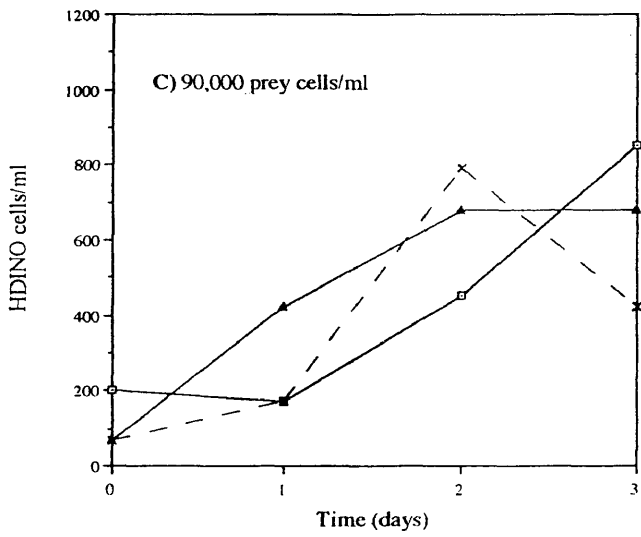
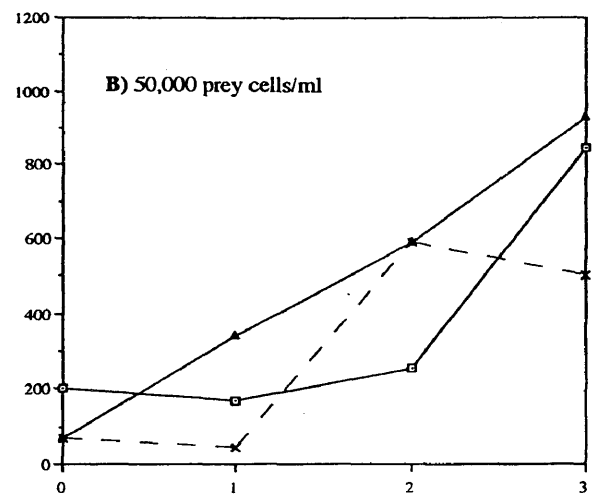
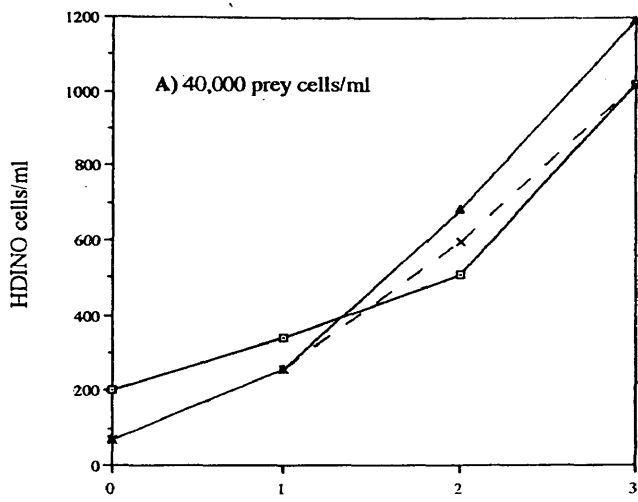


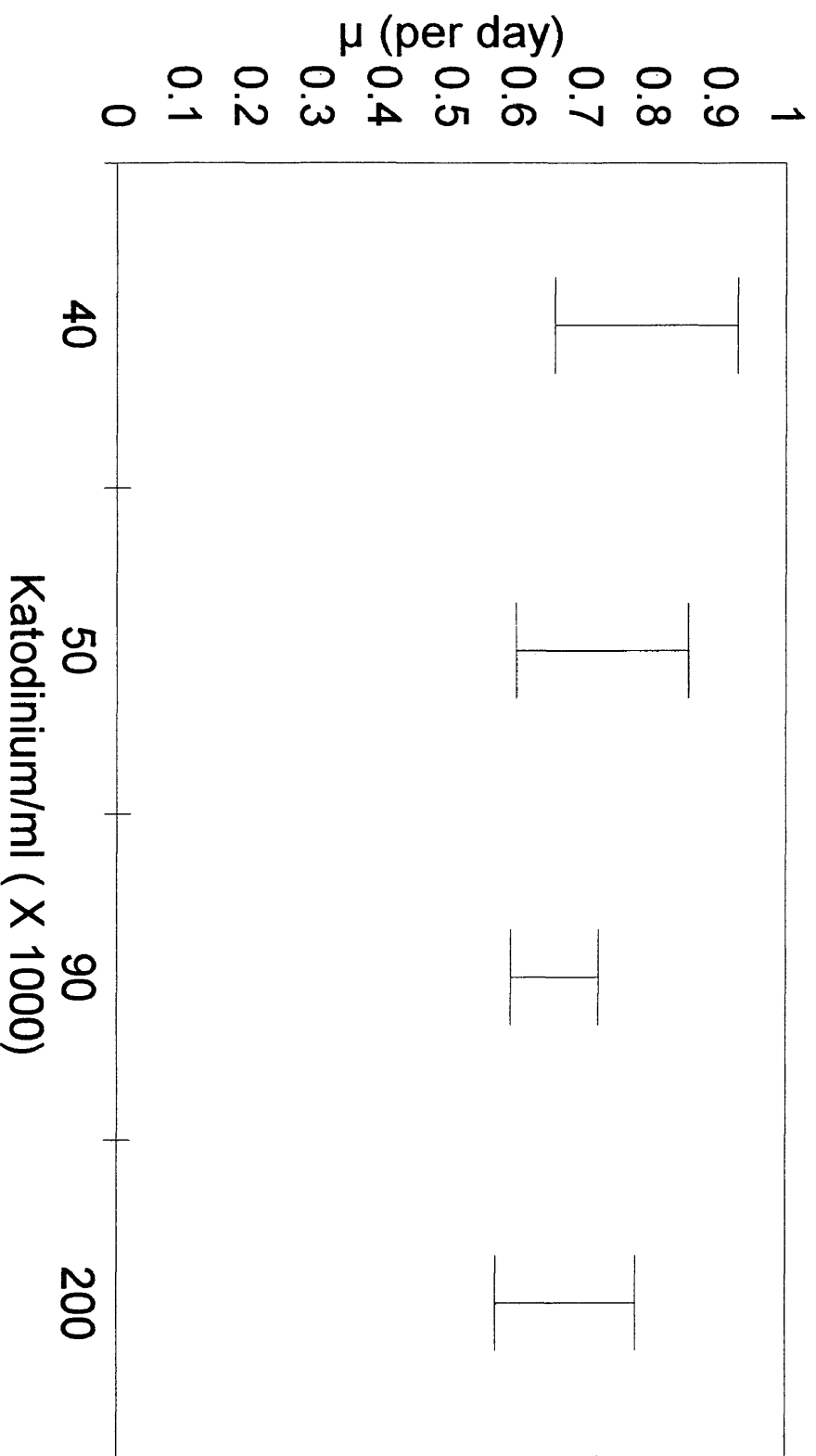
Table 4. HDINO growth rates ( $\mu$ ) for the prey concentration effects on HDINO growth rate experiment II.

Prey Conc.	Replicate	$\mu$ ( $d^{-1}$ )	Mean $\mu$ ( $d^{-1}$ )
40x10 <sup>3</sup>	A	0.523	
	B	0.897	0.792 $\pm$ 0.136 SE
	C	0.957	
50x10 <sup>3</sup>	A	0.471	
	B	0.869	0.727 $\pm$ 0.128 SE
	C	0.842	
90x10 <sup>3</sup>	A	0.526	
	B	0.704	0.656 $\pm$ 0.066 SE
	C	0.737	
200x10 <sup>3</sup>	A	0.554	
	B	0.880	0.672 $\pm$ 0.104 SE
	C	0.581	

Figure 10. Mean HDINO growth rates ( $\mu$ ) calculated for each of the treatments in the prey concentration effects on growth rate experiment II.

# Prey Conc. Effects on HDINO Growth-II

Mean Growth Rates and Standard Errors



curve (Figure 11), which suggests that HDINO growth rate increases with increasing prey concentration until reaching a level of saturation where the growth rate may begin to decrease. Figure 11. Combined mean HDINO growth rate ( $\mu$ ) results from prey concentration effects experiments I and II.

Ingestion and clearance rates were calculated using data for the first 2 days of the prey concentration effects experiment II (Table 5, Figures 12 and 13). Both ingestion and clearance, with the exception of one point, appeared to be constant over all prey levels.

Figure 11. Combined mean HDINO growth rates ( $\mu$ ) calculated from prey concentration effects on growth rate experiments I and II.

# Combined Prey Conc. Effects (I and II)

Mean HDINO Growth Rates and SE

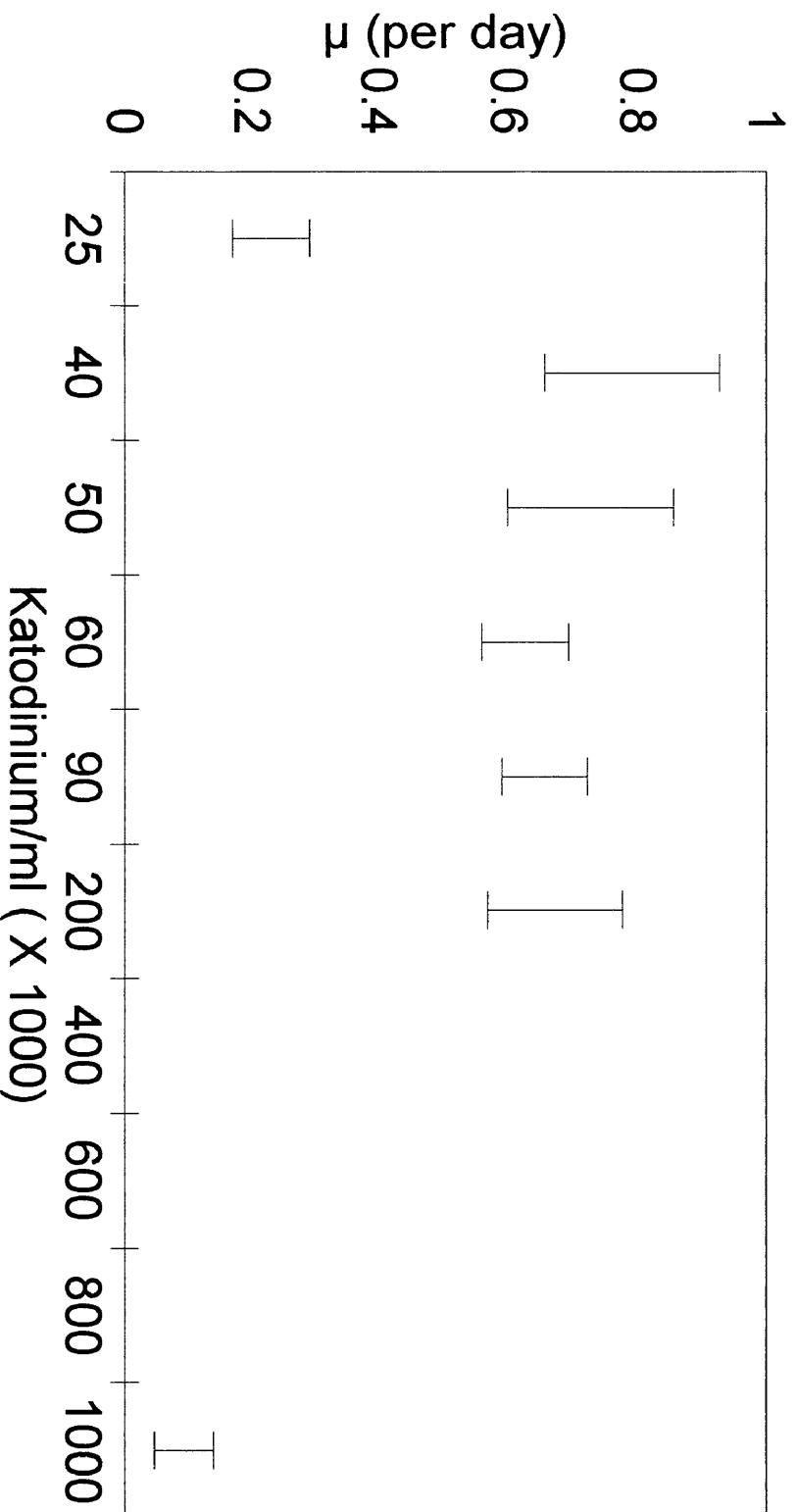


Table 5. HDINO ingestion (I) and clearance (F) rates for the prey concentration effects on HDINO growth rate experiment II.

Initial <i>K. rotundatum</i> ml <sup>-1</sup>	I (cells HDINO <sup>-1</sup> d <sup>-1</sup> )	F (ml HDINO <sup>-1</sup> d <sup>-1</sup> )
34.0 x 10 <sup>3</sup>	-1.88	-8.85 x 10 <sup>-5</sup>
38.0 x 10 <sup>3</sup>	-13.7	-5.26 x 10 <sup>-4</sup>
40.8 x 10 <sup>3</sup>	19.2	9.19 x 10 <sup>-4</sup>
43.5 x 10 <sup>3</sup>	-15.0	-4.60 x 10 <sup>-4</sup>
51.6 x 10 <sup>3</sup>	10.2	3.07 x 10 <sup>-4</sup>
58.4 x 10 <sup>3</sup>	6.64	1.72 x 10 <sup>-4</sup>
78.8 x 10 <sup>3</sup>	-18.9	-2.49 x 10 <sup>-4</sup>
97.8 x 10 <sup>3</sup>	52.3	7.35 x 10 <sup>-4</sup>
105 x 10 <sup>3</sup>	11.0	1.20 x 10 <sup>-4</sup>
190 x 10 <sup>3</sup>	30.8	1.88 x 10 <sup>-4</sup>
199 x 10 <sup>3</sup>	13.8	7.84 x 10 <sup>-5</sup>
204 x 10 <sup>3</sup>	200	1.40 x 10 <sup>-3</sup>

Figure 12. HDINO ingestion rates (I) from prey concentration effects on growth rate experiment II.

# HDINO INGESTION

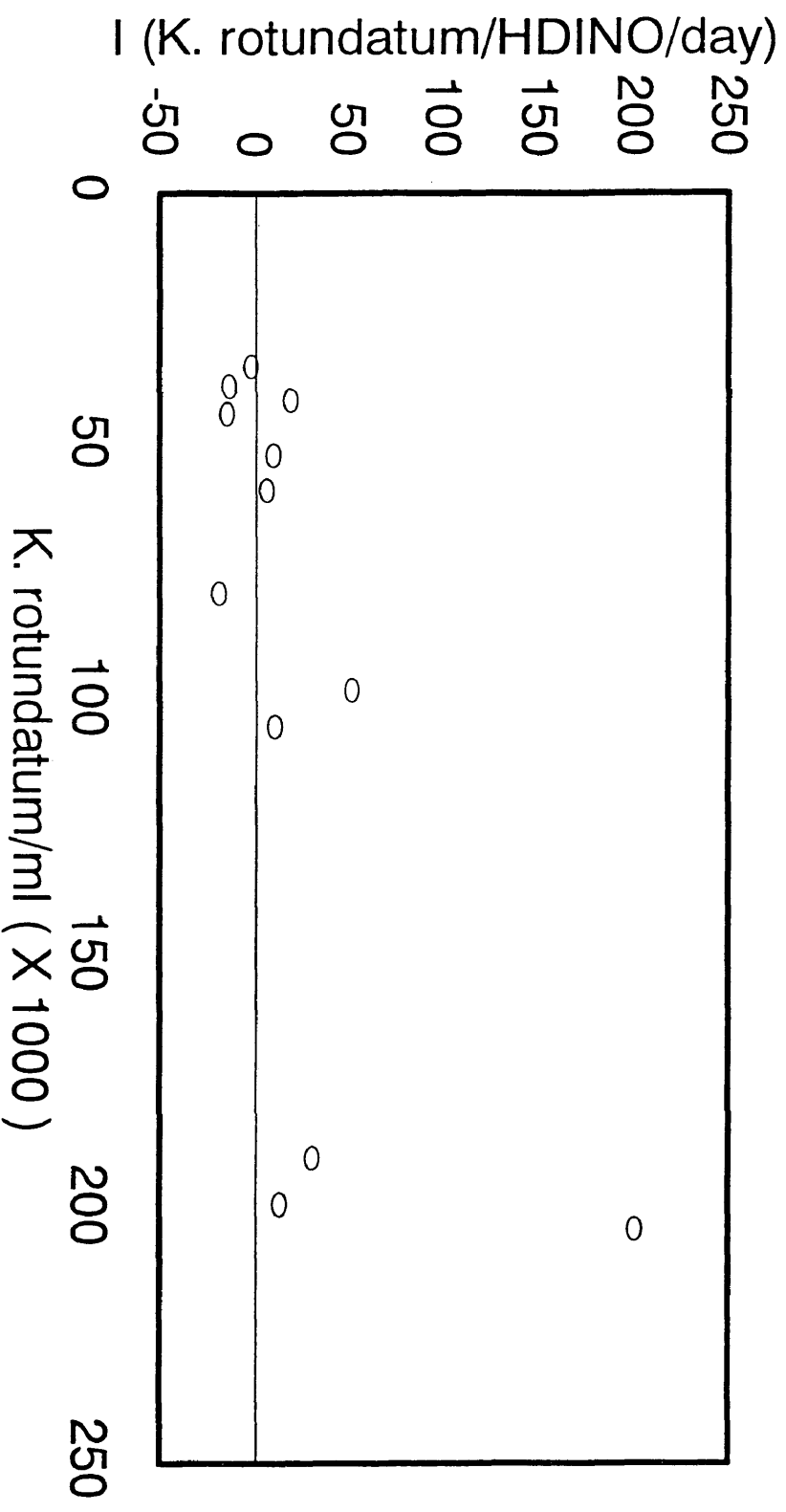
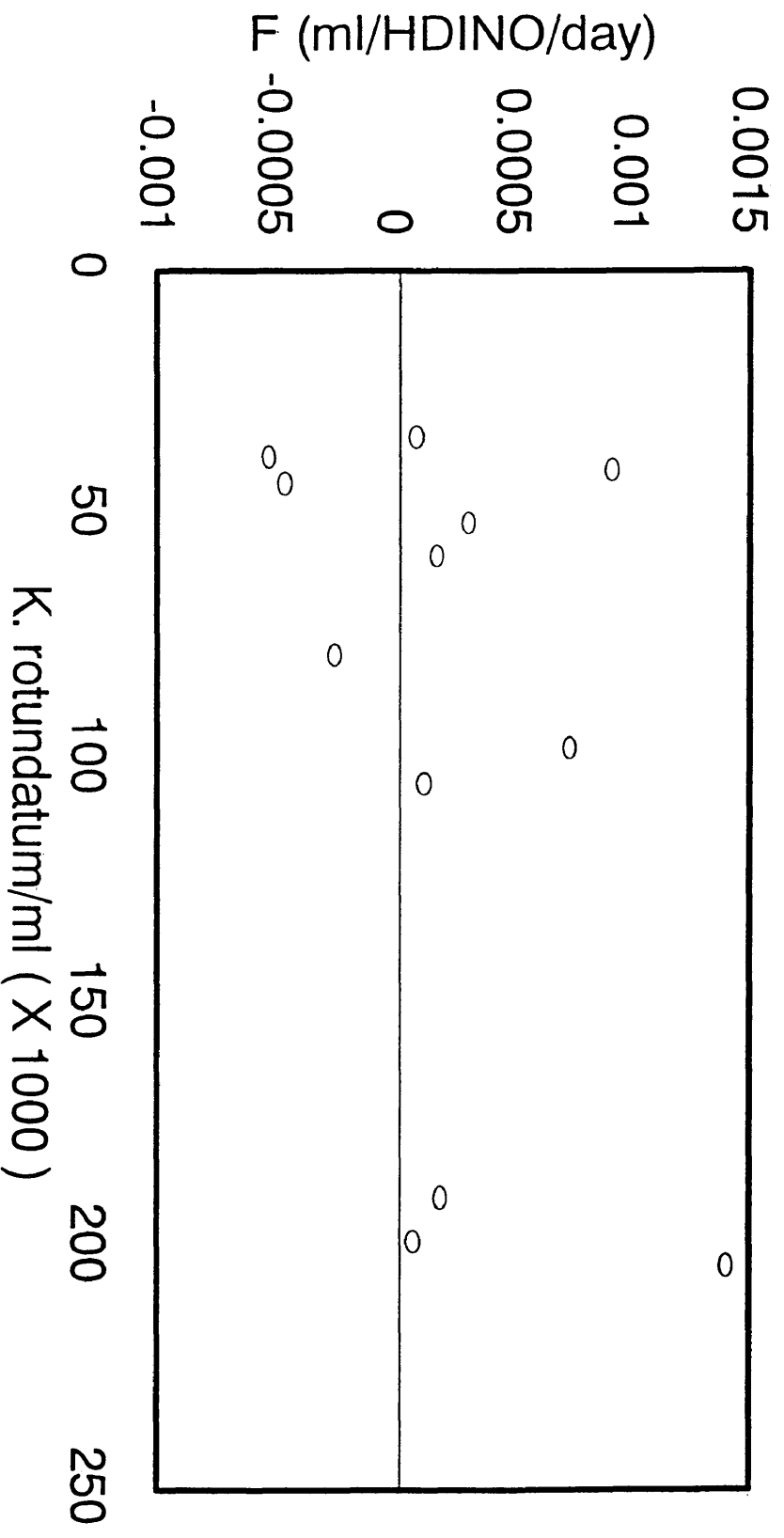


Figure 13. HDINO clearance rates (F) from prey concentration effects on growth rate experiment II.

# HDINO CLEARANCE



## DISCUSSION

This study of heterotrophic dinoflagellate functional responses was not dramatically different from previous work in terms of methodology (e.g. Strom 1991, Nakamura et al. 1992, Strom and Buskey 1993, Tester et al., in prep.). However, studies with the exception of one point, appear to be constant over all prey levels. Investigations of grazer dinoflagellate physiology, specifically those in the nanoplankton size range, in the Chesapeake Bay/York River estuarine system are few. Studies by Lessard and Swift (1985), Lessard et al. (1988), Lessard (1991), and Bockstahler and Coats (1993a, 1993b) represent sources of dinoflagellate grazing data for the Chesapeake Bay.

The main purpose of this study was to examine the effects of various environmental factors on the growth rate of a heterotrophic dinoflagellate isolated from the lower York River. The results of these experiments were used to examine HDINO as a potential grazer of red tides. Previous York River investigations were descriptive studies focusing on dinoflagellates which were present in the plankton. This study was a laboratory examination of the autecology of HDINO and of its potential to graze a red tide species of dinoflagellate, in this case, *K. rotundatum*.

**HDINO growth**

Based on the results of the temperature effects experiment, it appears that the highest HDINO growth rate was observed at 25 °C. The range of temperatures tested (10-25 °C) was not sufficient to provide an optimal temperature; however, 25 °C yielded the highest HDINO growth rate (0.47 d<sup>-1</sup>). The growth rates observed in this experiment were lower than those observed in subsequent experiments probably because HDINO experienced growth inhibition at high prey concentrations used in the temperature experiment. For example, the initial *K. rotundatum* concentration in the temperature effects experiment ranged between 89-119 x 10<sup>3</sup> cells ml<sup>-1</sup>. The temperature experiment was the first experiment in the one-factor experiment series, and there was no prior knowledge that HDINO growth might be inhibited at a concentration of >40 x 10<sup>3</sup> *K. rotundatum* ml<sup>-1</sup>, as demonstrated in the subsequent prey concentration experiments (see Figure 11).

Based on the results of the temperature effects experiment, the salinity effects experiment was conducted at room temperature over a range of 19-25 °C. HDINO exhibited growth at two salinities (15 and 20 psu), and there was no measurable HDINO growth for 10, 25, and 30 psu. The optimal salinity range for HDINO growth was 15 to 20 psu (mean  $\mu$ =0.93 and 1.25 d<sup>-1</sup>, respectively). It is not surprising that an estuarine protozoan, such as HDINO, grew well at this salinity. The April-September salinity range for the York

River is 12 to 28 psu (Munday and Zubkoff 1981). HDINO was isolated in September from lower York River surface water that was ca. 18 psu. Though no quantifiable growth occurred at 10, 25, and 30 psu, HDINO was able to survive at those salinities. If the experiment were allowed to progress beyond the fourth day and more *K. rotundatum* added to replenish HDINO's food source, the HDINO might have adapted to those salinities. Even so, 20 psu supported the highest mean HDINO growth over the course of this experiment.

Three out of the five prey concentrations ( $25 \times 10^3$ ,  $60 \times 10^3$ , and  $1 \times 10^6$  *K. rotundatum* ml<sup>-1</sup>) in the first prey concentration effects experiment and all four prey concentrations in the second experiment produced HDINO growth. The highest mean HDINO growth rate ( $0.79 \text{ d}^{-1}$ ) occurred at  $40 \times 10^3$  *K. rotundatum* ml<sup>-1</sup>. When the mean growth rates for the two experiments are combined, the resulting curve suggests that HDINO growth rate is sensitive to prey concentration. HDINO mean growth rate increased sharply between  $25 \times 10^3$  and  $40 \times 10^3$  *K. rotundatum* ml<sup>-1</sup> and experienced growth inhibition, between  $40 \times 10^3$  and  $1 \times 10^6$  *K. rotundatum* cells ml<sup>-1</sup>.

The reason for the decline in HDINO growth is not clear. Some phytoplankton, in dense cultures, are thought to produce autoinhibitors, compounds which may inhibit the phytoplankton's own growth (Curl and McLeod 1961, Blanchemain et al. 1994). Blanchemain et al. (1994) found that *S.*

*costatum* might alter its own culture media, reducing growth. If a phytoplankton species can inhibit its own growth in dense cultures, its growth inhibitor might also affect its predator's growth. In a study by Gentien and Arzul (1990), *Gyrodinium cf. aureolum*, an autotrophic dinoflagellate, was found to produce ectocrines that inhibited growth of the diatom *Chaetoceros gracile*. In a directly related study, Buskey (1995) noted that the unidentified chrysophyte responsible for Texas brown tides might have inhibitory effects on the growth of *Noctiluca scintillans*, a heterotrophic dinoflagellate. Future work should examine the production of substances, such as those that might be produced by prey cells, which could reduce HDINO growth rate.

The maximum specific growth rate ( $0.79 \text{ d}^{-1}$ ) for HDINO, in the prey concentration effects experiment, compared favorably with growth rates calculated for other heterotrophic dinoflagellates in the literature. Growth rates calculated for dinoflagellates are typically lower than rates of growth for other algae. Strom (1991) observed a maximum observed growth rate of  $0.75 \text{ d}^{-1}$  for a heterotrophic dinoflagellate, *Gymnodinium* sp., isolated from the subarctic Pacific Ocean. Lessard and Falkenhayn (1994) isolated an athecate heterotrophic dinoflagellate from Georges Bank and found that, at its optimal temperature ( $18 \text{ }^{\circ}\text{C}$ ), growth rate ranged from  $0.2\text{-}0.7 \text{ d}^{-1}$ , and was hyperbolically related to food concentration. The range of growth rates measured for the

athecate Georges Bank heterotrophic dinoflagellate is not very different from the range of rates calculated in the HDINO prey concentration experiments with *K. rotundatum* as prey. Jacobson and Anderson (1993) examined the growth and grazing dynamics of *Protoperidinium hirobis* and found that its growth rate plateaued at  $1.23 \text{ d}^{-1}$ . Another species of *Protoperidinium*, *P. huberi* was found to have a specific growth rate, measured as a function of prey concentration, ranging from  $0.04\text{-}0.72 \text{ d}^{-1}$  (Buskey et al. 1994).

The temperature effects experiment results suggested that HDINO preferred  $25 \text{ }^{\circ}\text{C}$ , but both HDINO prey concentration experiments were performed at  $15 \text{ }^{\circ}\text{C}$  to ensure *K. rotundatum*'s survival. Temperature is an important environmental factor affecting autotrophic dinoflagellate growth. Morton and Norris (1990) noted that temperatures between  $21$  and  $31 \text{ }^{\circ}\text{C}$  limited the division rate of *Prorocentrum lima*, a toxic autotrophic dinoflagellate, even though growth was possible from  $16$  to  $33 \text{ }^{\circ}\text{C}$ . Jochem (1990) reported the temperature ranges of several nanoflagellate species, including *K. rotundatum*, for the Kiel Bight and Kiel Fjord. *K. rotundatum* occurred where temperatures ranged from just above freezing to ca.  $13 \text{ }^{\circ}\text{C}$ . From December to February 1989, a *K. rotundatum* red tide occurred in the Patuxent River, a northern tributary of the Chesapeake Bay. Sellner et al. (1991) reported that water temperature during the bloom was ca.  $10 \text{ }^{\circ}\text{C}$ . *K. rotundatum* also appeared to be very sensitive to light

deprivation, and the lack of high light might have been responsible for a cessation of cell division or even cell death.

It is reported that dinoflagellates have an average light intensity optimum of approximately 10% full sunlight (Guillard and Keller 1984, Morton et al. 1992). In the first prey concentration effects experiment, *K. rotundatum* was grown under light intensities of ca.  $20 \mu\text{E m}^{-2} \text{s}^{-1}$  while, in the second experiment, it was grown in ca.  $3.4 \mu\text{E m}^{-2} \text{s}^{-1}$  light. Both light intensities are well below 10% full sunlight. HDINO, being heterotrophic, did not have any light requirements itself; however, prey quality factors, not taken into account in the experimental design of this study, may have affected HDINO growth rate and possibly contributed to the high variability that occurred among some of the experimental replicates.

An objective of this study was to examine the potential of HDINO to graze a red tide population by extrapolating the laboratory results to the natural environment. Based on the results of the temperature and salinity preference of HDINO, there was an ecological predator-prey mismatch in these experiments. An autotrophic dinoflagellate, which commonly blooms in the winter-early spring and is accustomed to temperatures between 0 and 15 °C, was used as prey for a HDINO isolated in the late summer (HDINO grew best at 25 °C). HDINO is probably more abundant during the late spring-early fall

months, and therefore, does not occur concurrently with *K. rotundatum* red tides. The optimal salinity for HDINO was 20 psu, which indicates that HDINO is more likely to be present in the tributaries of the lower Chesapeake Bay. *K. rotundatum* red tides commonly occur in the northern bay tributaries. In the winter 1989 Patuxent River *K. rotundatum* red tide, Sellner et al. (1991) reported that the salinities in the region of the red tide ranged from 5 to 13 psu, less than optimal for HDINO growth (see Figure 6), and with ambient temperatures  $<10^{\circ}\text{C}$ , conditions were far from optimum for HDINO growth for either parameter. Further, bloom levels of *K. rotundatum* were not conducive to HDINO growth. Cell densities reached ca.  $1.0 \times 10^5$  *K. rotundatum*  $\text{ml}^{-1}$  and contributed as much as 1900  $\mu\text{g C l}^{-1}$  in near-surface waters (Sellner et al. 1991). Based on the results of the single factor culture experiments with *K. rotundatum* as prey, HDINO would experience growth inhibition at such high field concentrations of *K. rotundatum*. The growth rates observed in the temperature effects experiment, with initial *K. rotundatum* concentrations ranging between 89-119  $\times 10^3$  cells  $\text{ml}^{-1}$ , were lower those observed in subsequent experiments probably because HDINO experienced growth inhibition at high prey concentrations used. In the prey concentration effects experiments, the highest HDINO growth rate measured occurred at *in situ* concentrations of about 40  $\times 10^3$  *K. rotundatum*  $\text{ml}^{-1}$  and declined at the higher prey concentrations. This growth inhibition of HDINO at high prey

concentrations suggests that HDINO is not an effective grazer of *K. rotundatum* red tides in the natural environment.

HDINO appeared to vary in size based on prey availability. Heterotrophic dinoflagellates generally vary in cell size based on feeding conditions. The biovolume of the athecate heterotrophic dinoflagellate from Georges Bank isolated by Lessard and Falkenhayn (1994), dependent on prey size and concentration, varied from 60-1000  $\mu\text{m}^3$ . Strom (1991) found that the cell volume of *Gymnodinium* sp. varied from 600 to 1200  $\mu\text{m}^3$  and was hyperbolically related to food concentration. *G. dominans*, in the study by Nakamura et al. (1992), was ca. 50 to 60  $\mu\text{m}$  long and 30  $\mu\text{m}$  wide ( $2.0-3.0 \times 10^4 \mu\text{m}^3$  volume) at the time of isolation. By the sixth day, of an experiment examining the changes in *C. antiqua*-fed *G. dominans* cell numbers over time, *G. dominans* were described as "small, starving cells" (ca. 30  $\mu\text{m}$  long and 15  $\mu\text{m}$  wide) without food vacuoles (Nakamura et al. 1992). *Protoperidinium* cf. *divergens*, a heterotrophic dinoflagellate used in grazing study by Jeong and Latz (1994), initially had an estimated spherical diameter (ESD) ranging from 55.4 to 73.6  $\mu\text{m}$  (median ESD=64.1), but after 12 days without added prey, the ESD for *P. cf. divergens* ranged from 11.2 to 75.4  $\mu\text{m}$  (median ESD=14.93). Jacobson and Anderson (1993) found that the mean size of *P. huberi* decreased with a decrease in food concentration. The diameter of food replete-*P. huberi* was ca. 22  $\mu\text{m}$  while the diameter of food deplete-*P. huberi* was ca. 18

$\mu\text{m}$ . This represents a 45% decrease in cell volume (Jacobson and Anderson 1993). For HDINO the mean diameter, ca. 11  $\mu\text{m}$  ( $697 \mu\text{m}^3$ ), decreased to ca. 7  $\mu\text{m}$  ( $180 \mu\text{m}^3$ ), ca. a 74% reduction in cell volume, with *K. rotundatum* depletion.

#### **HDINO ingestion and clearance**

At first glance the ingestion and clearance curves that were produced from the second prey concentration effects experiment did not resemble typical representatives of such curves (Figure 14 A-B, from Buskey et al. 1994). These graphs present food concentration measured in carbon units ( $\mu\text{gC l}^{-1}$ ). Although an attempt was made to use an ecologically relevant range of *K. rotundatum* in the prey concentration experiments, the amount of *K. rotundatum* carbon was not considered. When *K. rotundatum* concentrations were converted from cells  $\text{ml}^{-1}$  to  $\mu\text{gC l}^{-1}$  (Table 6), using a conversion value of 35 pg carbon per cell (Edler 1979), it became obvious that this study examined prey concentrations that were well into the upper range ( $>1.50 \times 10^3 \mu\text{gC l}^{-1}$ ) of carbon concentrations used by Buskey et al. (1994) (see Figure 14 A-B). Thus, the HDINO ingestion and clearance rates for this study resembled the high prey concentration region of a representative ingestion or clearance curve, and there was no observable difference in the rates over the concentrations used (see Figures 12-13).

*Protoperidinium huberi*, the heterotrophic dinoflagellate studied by Buskey et al. (1994), fed with pallium and was ca.

three times (at 42  $\mu\text{m}$ ) the diameter of HDINO. With *Ditylum brightwelli*, a diatom, as a food source, the maximum ingestion ( $I_{\text{max}}$ ) of *P. huberi* was reached at ca. 600  $\mu\text{gC l}^{-1}$  ( $I_{\text{max}}$  ca. 0.7  $\mu\text{gC ind}^{-1} \text{h}^{-1}$ ). The clearance rates of *P. huberi* decreased asymptotically as food concentration increased, and the maximum clearance rate ( $F_{\text{max}}$ ), measured at low food concentration ( $<300 \mu\text{gC l}^{-1}$ ), was ca. 23  $\mu\text{l ind}^{-1} \text{h}^{-1}$  (Buskey et al. 1994).

HDINO was offered *K. rotundatum* concentrations which ranged from  $1.19 \times 10^3$  to  $7.14 \times 10^3 \mu\text{gC l}^{-1}$  (Table 6). The highest ingestion and clearance rates for HDINO were  $2.91 \times 10^{-4} \mu\text{gC HDINO}^{-1} \text{h}^{-1}$  and  $0.058 \mu\text{l HDINO}^{-1} \text{h}^{-1}$ , respectively. These rates are much lower than the rates measured for *P. huberi* likely reflecting HDINO's smaller size. However, in a study by Strom (1991), a 12  $\mu\text{m}$  *Gymnodinium* sp., when fed an *Isochysis galbana*+*Synechococcus* sp. mixture (0 to 300  $\mu\text{gC l}^{-1}$ ), had ingestion rates ranging from 1.00 to 51.0  $\text{pgC ind}^{-1} \text{h}^{-1}$  and clearance rates ranging from 0.19 to 1.64  $\mu\text{l ind}^{-1} \text{h}^{-1}$ . When the *Gymnodinium* sp. was fed only *I. galbana*, its  $I_{\text{max}}$  was 12.1  $\text{pgC ind}^{-1} \text{h}^{-1}$ . Its minimum clearance rate was 0.1  $\mu\text{l ind}^{-1} \text{h}^{-1}$ . The half saturation constant for *Gymnodinium* sp. was 19.2  $\mu\text{gC l}^{-1}$  (Strom 1991). HDINO ingestion rates ranged from -27.5 to 291  $\text{pgC HDINO h}^{-1}$ . HDINO clearance rates ranged from -0.022 to 0.058  $\mu\text{l HDINO}^{-1} \text{h}^{-1}$ . These rates were all lower than

Figure 14. (A) Clearance and (B) ingestion rates for *Protoperidinium huberi* grazing *Ditylum brightwelli* (Buskey et al. 1994).

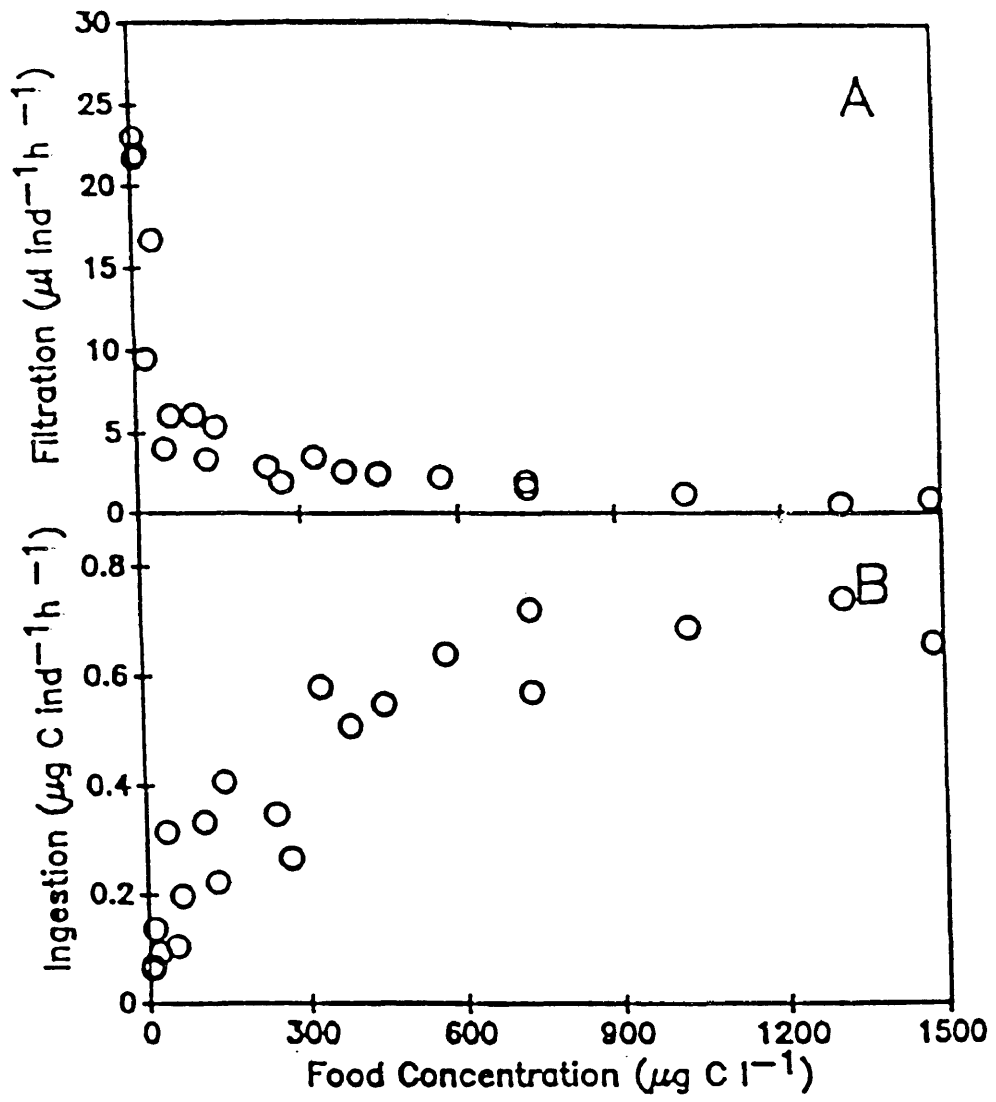


Table 6. Conversion of *K. rotundatum* cells ml<sup>-1</sup> (in parentheses) in the prey concentration effect experiment to µgC l<sup>-1</sup>. The ingestion (I) and clearance (F) rates for HDINO are also expressed in terms of carbon for comparison with other literature values.

µgC l <sup>-1</sup>	I (µgC HDINO <sup>-1</sup> h <sup>-1</sup> )	I (pgC HDINO <sup>-1</sup> h <sup>-1</sup> )	F (µl HDINO <sup>-1</sup> h <sup>-1</sup> )
1.19x10 <sup>3</sup> (34,000)	-2.74x10 <sup>-6</sup>	-2.74	0.004
1.33x10 <sup>3</sup> (38,000)	-2.00x10 <sup>-5</sup>	-20.0	-0.022
1.43x10 <sup>3</sup> (40,800)	2.80x10 <sup>-5</sup>	28.0	0.038
1.52x10 <sup>3</sup> (43,500)	-2.19x10 <sup>-5</sup>	-21.9	-0.019
1.81x10 <sup>3</sup> (51,600)	1.48x10 <sup>-5</sup>	14.8	0.013
2.04x10 <sup>3</sup> (58,400)	9.69x10 <sup>-6</sup>	9.69	0.007
2.76x10 <sup>3</sup> (78,800)	-2.76x10 <sup>-5</sup>	-27.5	-0.010
3.42x10 <sup>3</sup> (97,800)	7.63x10 <sup>-5</sup>	76.3	0.031
3.68x10 <sup>3</sup> (105,000)	1.60x10 <sup>-5</sup>	16.0	0.005
6.65x10 <sup>3</sup> (190,000)	4.49x10 <sup>-5</sup>	44.9	0.008
6.97x10 <sup>3</sup> (199,000)	2.02x10 <sup>-5</sup>	20.2	0.003
7.14x10 <sup>3</sup> (204,000)	2.91x10 <sup>-4</sup>	291	0.058

those calculated for the *Gymnodinium* sp. possibly because HDINO was exposed to such high amounts of *K. rotundatum* carbon that it had surpassed the level of prey carbon to meet its cellular requirements.

Jeong and Latz (1994) measured the grazing rates for the heterotrophic dinoflagellates, *P.* cf. *divergens* and *Protooperidinium crassipes*, fed *Gonyaulax polyedra*, a thecate autotrophic dinoflagellate known to form red tides. The maximum ingestion and rates of *P.* cf. *divergens* and *Protooperidinium crassipes* were 0.20 and 0.08 prey *Protooperidinium*<sup>-1</sup> h<sup>-1</sup>, and their maximum clearance rates were 0.67 and 0.47  $\mu\text{l Protooperidinium}^{-1} \text{ h}^{-1}$ , respectively. *P.* cf. *divergens* reached its critical concentration at  $\geq 1.1 \times 10^3$  *G. polyedra* cells ml<sup>-1</sup> ( $3.68 \times 10^3 \mu\text{gC l}^{-1}$ ), and *P. crassipes* reached its critical concentration at  $> 700$  *G. polyedra* cells ml<sup>-1</sup> ( $2.34 \times 10^3 \mu\text{gC l}^{-1}$ ) (Jeong and Latz 1994). If the ingestion rates of these heterotrophic dinoflagellates, which are over 3 times larger than HDINO, were saturated at prey concentrations of  $2.34 \times 10^3$  and  $3.68 \times 10^3 \mu\text{gC l}^{-1}$ , it is likely that, at *K. rotundatum* concentrations ranging from  $1.19 \times 10^3$  to  $7.14 \times 10^3 \mu\text{gC l}^{-1}$ , critical concentrations for ingestion by HDINO were well in excess in the second prey concentration effects experiment.

In many instances the magnitude of the decline of prey in the controls was substantial, and nearly as great as observed in the experimental treatments. No explanation of this

decline is immediately apparent, but it is likely a result of this algae's need for exacting culture conditions for survival and growth which were apparently not met in this experiment. Considering the magnitude of the prey's decline in the controls and the resulting negative growth constants, there was some reluctance to include these data in the calculations of ingestion rate. However, having atypical data is not justification for that data's exclusion, and including the controls in the grazing calculations provided reasonable rates based on alternative estimates of ingestion.

An estimate of HDINO ingestion can be calculated based on HDINO growth rate and efficiency, and prey size (Webb, personal communication). Assuming a growth efficiency of 0.5 (i.e., half of assimilated carbon is used for growth), and a 1:1 ratio of predator:prey size and carbon content, HDINO would need to ingest 2 prey items per day to sustain a growth rate of 1 doubling per day ( $dd^{-1}$ ). This rate is roughly equivalent to the ingestion rates observed in the present study and support the decision to include the loss of prey observed in the controls in the calculation of the ingestion rates. Observed rates in excess of 2 prey items ingested per HDINO per day suggest either that *K rotundatum* is not a good source of carbon or most of the carbon is excreted or respired and not used for growth. Perhaps, a GGE=0.5 is high for HDINO and perhaps it is closer to 0.25, or the prey contained less carbon than the predator. The growth rate may be  $>1 dd^{-1}$ .

### **Trophic considerations**

While trophic level members are thought to "do the same thing and only one thing; those of a trophospecies may do the same suite of several things" (Turner and Roff 1993). There is a theory (the endosymbiont theory, proposed by Lynn Margulis) which states that eukaryotic cells which have organelles containing DNA obtained those organelles phylogenetically from free-living prokaryotes. The theory that many algae, particularly the euglenoids and dinoflagellates, originated from "intertaxonic, endocytic combinations of protozoic with protophytic symbionts" is supported by algal cells which have heterotrophic modes (Sitte 1990).

Though observations suggested that HDINO is probably an obligate heterotroph, whether or not HDINO actually possesses the ability to retain and use ingested chloroplasts from prey was not explored. If HDINO does feed in a similar manner (see description on pg. 20) as documented for *O. oxytoxoides* (injecting a small amount of fluid into the prey cell), the chloroplasts would be broken down into the cytoplasm as they were sucked into the HDINO, and rendered useless. Chlorophyll, which is very labile, appeared in the food vacuoles, glowing bright red under epifluorescent blue light. There were vacuoles that were pale orange, representing chlorophyll decomposition in the vacuole.

Hansen et al. (1994) re-examined predator-prey size ratio

assumptions based on data now available in the literature, covering pelagic predators in the nano-, micro-, and mesozooplanktonic size classes. For the only heterotrophic dinoflagellate reported in the study, the optimal size ratio (predator:prey) was 1:1 (Hansen 1992, Hansen et al. 1994). Based on this finding, *K. rotundatum* and *D. tertiolecta*, as well as *P. minimum*, were suitable prey size, and the increased difference in size ratio may explain why the HDINO was unable to graze *P. micans* and *Gyrodinium* sp. (approximate predator:prey size ratio 1:2). Also, the thickness of the cell wall of *P. micans* might have made it more difficult for HDINO to graze. Though cannibalism was not observed, it was likely that it did occur. On-going research by E. Haugen and P. Tester documented (via videotape) a *Pfiesteria*-like heterotrophic dinoflagellate feeding on itself (E. Haugen, personal communication). Buskey et al. (1994) observed *P. huberi* cannibalism. Davidson et al. (1995) included a factor to account for cannibalism in a model of ingestion by *Oxyrrhis marina*, a heterotrophic dinoflagellate, as a function of prey density. Jeong and Latz (1994) observed cannibalism in cultures which contained high abundances of the two *Protoperidinium* spp. in their study and suggested that it might be a mechanism to survive prolonged starvation.

#### **Future considerations**

According to Hallegraeff (1993), there are three types of

harmful algal blooms: 1) species which are harmless and discolor water but indirectly cause fish kills by growing into dense accumulations that deplete water column oxygen, 2) species which produce toxins that can gain entrance into the food chain, causing illness in humans, and 3) species that are non-toxic to humans but can damage or clog the gills of fish and invertebrates. There have been reports concerned with species composition of York River red tides, but no work investigating whether or not these blooms are truly harmless. With the Luckenbach et al. (1993) and Ho and Zubkoff (1979) investigations suggesting that Chesapeake Bay red tide organisms may have a negative effect on juvenile oysters, the impact of toxic dinoflagellate blooms in this estuarine system warrants further examination.

Though these experiments represent measurements in a controlled environment, the results suggest that there are <20  $\mu\text{m}$  heterotrophic dinoflagellates present in the York River estuary capable of grazing autotrophic dinoflagellates that are of the same size. More research needs to be done to determine the niche of these heterotrophic nano-dinoflagellates in the York River system. There are biological factors that are responsible, in combination with physical and nutrient factors, for the decimation of red tide blooms. It is uncertain which, if any, planktonic organisms are able to most effectively graze the blooms. Previous work suggests that extensive grazing of red tide organisms by

copepods is questionable, and as presented in this investigation, dinoflagellates may be more effective "red water" grazers. However, this HDINO isolate does not seem capable of grazing autotrophic cells present at bloom concentrations.

A comprehensive investigation of species identification and seasonal composition changes, not unlike Mackiernan's 1968 study, would help determine abundance of heterotrophic dinoflagellates in this system as well as determine how their distribution changes from year to year. In addition, another laboratory experiment with a temporally consistent predator-prey seasonal combination could provide results which better reflect grazing and associated feeding behaviors exhibited by heterotrophic dinoflagellates in the natural environment. In general, more attention needs to be given to the life history of dinoflagellates. Recent work has shown that some dinoflagellates change modes of nutrition based on what stage they are in their life cycle. If the HDINO in this study is a *P. piscicida*-like species, its potential involvement, directly or indirectly, in any future York River/Chesapeake Bay fish kills may warrant consideration.

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APPENDIX A

HDINO cell counts for temperature, salinity, and prey  
concentration experiments

Table A1. Heterotrophic dinoflagellate (HDINO) cells ml<sup>-1</sup> for replicates in the temperature effects experiment.

Time	Temperature	A	B	C	Average
0	10 °C	16.3x10 <sup>3</sup>	6.79x10 <sup>3</sup>	8.83x10 <sup>3</sup>	10.6x10 <sup>3</sup>
	15 °C	12.2x10 <sup>3</sup>	10.9x10 <sup>3</sup>	12.9x10 <sup>3</sup>	12.0x10 <sup>3</sup>
	20 °C	13.5x10 <sup>3</sup>	13.5x10 <sup>3</sup>	17.0x10 <sup>3</sup>	14.7x10 <sup>3</sup>
	25 °C	19.0x10 <sup>3</sup>	14.9x10 <sup>3</sup>	12.2x10 <sup>3</sup>	13.4x10 <sup>3</sup>
1	10 °C	14.3x10 <sup>3</sup>	16.3x10 <sup>3</sup>	11.9x10 <sup>3</sup>	14.2x10 <sup>3</sup>
	15 °C	21.1x10 <sup>3</sup>	14.6x10 <sup>3</sup>	14.3x10 <sup>3</sup>	16.6x10 <sup>3</sup>
	20 °C	9.51x10 <sup>3</sup>	10.9x10 <sup>3</sup>	7.81x10 <sup>3</sup>	9.36x10 <sup>3</sup>
	25 °C	34.6x10 <sup>3</sup>	26.5x10 <sup>3</sup>	30.6x10 <sup>3</sup>	30.6x10 <sup>3</sup>
2	10 °C	13.9x10 <sup>3</sup>	8.83x10 <sup>3</sup>	9.51x10 <sup>3</sup>	10.8x10 <sup>3</sup>
	15 °C	18.3x10 <sup>3</sup>	13.9x10 <sup>3</sup>	7.47x10 <sup>3</sup>	13.2x10 <sup>3</sup>
	20 °C	15.3x10 <sup>3</sup>	15.3x10 <sup>3</sup>	8.83x10 <sup>3</sup>	13.1x10 <sup>3</sup>
	25 °C	44.2x10 <sup>3</sup>	44.8x10 <sup>3</sup>	47.5x10 <sup>3</sup>	45.5x10 <sup>3</sup>
3	10 °C	6.45x10 <sup>3</sup>	5.09x10 <sup>3</sup>	5.09x10 <sup>3</sup>	5.55x10 <sup>3</sup>
	15 °C	10.5x10 <sup>3</sup>	8.15x10 <sup>3</sup>	8.49x10 <sup>3</sup>	9.06x10 <sup>3</sup>
	20 °C	17.3x10 <sup>3</sup>	19.7x10 <sup>3</sup>	15.6x10 <sup>3</sup>	17.5x10 <sup>3</sup>
	25 °C	63.2x10 <sup>3</sup>	66.6x10 <sup>3</sup>	63.2x10 <sup>3</sup>	64.3x10 <sup>3</sup>
4	10 °C	8.49x10 <sup>3</sup>	8.51x10 <sup>3</sup>	6.45x10 <sup>3</sup>	7.70x10 <sup>3</sup>
	15 °C	12.6x10 <sup>3</sup>	7.13x10 <sup>3</sup>	7.13x10 <sup>3</sup>	8.94x10 <sup>3</sup>
	20 °C	22.8x10 <sup>3</sup>	19.7x10 <sup>3</sup>	18.3x10 <sup>3</sup>	20.3x10 <sup>3</sup>
	25 °C	38.7x10 <sup>3</sup>	29.2x10 <sup>3</sup>	24.5x10 <sup>3</sup>	30.8x10 <sup>3</sup>

Table A2. Heterotrophic dinoflagellate (HDINO) cells ml<sup>-1</sup> for replicates in the salinity effects experiment.

Time	Salinity (psu)	A	B	C	Average
1	10			3.77x10 <sup>2</sup>	3.77x10 <sup>2</sup>
	15	3.77x10 <sup>2</sup>	1.89x10 <sup>2</sup>	1.89x10 <sup>2</sup>	2.52x10 <sup>2</sup>
	20	0.94x10 <sup>2</sup>	0.94x10 <sup>2</sup>	2.83x10 <sup>2</sup>	1.57x10 <sup>2</sup>
	25	0	0	0	0
	30	0	0	0	0
2	10	10.6x10 <sup>2</sup>	0	0	3.54x10 <sup>2</sup>
	15	14.7x10 <sup>2</sup>	13.6x10 <sup>2</sup>	3.40x10 <sup>2</sup>	10.6x10 <sup>2</sup>
	20	15.8x10 <sup>2</sup>	11.2x10 <sup>2</sup>	9.06x10 <sup>2</sup>	12.1x10 <sup>2</sup>
	25	0	0	0	0
	30	0	0	0	0
3	10	0	2.12x10 <sup>2</sup>	0	0.70x10 <sup>2</sup>
	15	22.6x10 <sup>2</sup>	6.79x10 <sup>2</sup>	28.3x10 <sup>2</sup>	19.2x10 <sup>2</sup>
	20	40.8x10 <sup>2</sup>	23.8x10 <sup>2</sup>	6.79x10 <sup>2</sup>	23.8x10 <sup>2</sup>
	25	2.12x10 <sup>2</sup>	0	2.12x10 <sup>2</sup>	1.42x10 <sup>2</sup>
	30	0	2.12x10 <sup>2</sup>	0	0.70x10 <sup>2</sup>
4	10	6.60x10 <sup>2</sup>	13.2x10 <sup>2</sup>	11.3x10 <sup>2</sup>	10.4x10 <sup>2</sup>
	15	71.7x10 <sup>2</sup>	24.5x10 <sup>2</sup>	46.2x10 <sup>2</sup>	47.5x10 <sup>2</sup>
	20	80.2x10 <sup>2</sup>	87.7x10 <sup>2</sup>	60.4x10 <sup>2</sup>	76.1x10 <sup>2</sup>
	25	1.89x10 <sup>2</sup>	11.3x10 <sup>2</sup>	1.89x10 <sup>2</sup>	5.03x10 <sup>2</sup>
	30	0	3.40x10 <sup>2</sup>	3.40x10 <sup>2</sup>	2.26x10 <sup>2</sup>

Table A3. Heterotrophic dinoflagellate (HDINO) cells ml<sup>-1</sup> for replicates in the prey concentration effects I.

Time	Prey (cells ml <sup>-1</sup> )	A	B	C	Average
0	2.5x10 <sup>3</sup>	3.40x10 <sup>2</sup>	0	0	1.13x10 <sup>2</sup>
	5x10 <sup>3</sup>	3.40x10 <sup>2</sup>	0	0	1.13x10 <sup>2</sup>
	25x10 <sup>3</sup>	6.79x10 <sup>2</sup>	6.79x10 <sup>2</sup>	0	4.53x10 <sup>2</sup>
	50x10 <sup>3</sup>	0	0	0	0
	1x10 <sup>6</sup>	0	3.40x10 <sup>2</sup>	3.40x10 <sup>2</sup>	2.27x10 <sup>2</sup>
1	2.5x10 <sup>3</sup>	0	0	0	0
	5x10 <sup>3</sup>	3.40x10 <sup>2</sup>	0	3.40x10 <sup>2</sup>	2.27x10 <sup>2</sup>
	25x10 <sup>3</sup>	10.2x10 <sup>2</sup>	6.79x10 <sup>2</sup>	3.40x10 <sup>2</sup>	6.79x10 <sup>2</sup>
	50x10 <sup>3</sup>	6.79x10 <sup>2</sup>	10.2x10 <sup>2</sup>	0	5.66x10 <sup>2</sup>
	1x10 <sup>6</sup>	0	6.79x10 <sup>2</sup>	13.6x10 <sup>2</sup>	6.79x10 <sup>2</sup>
2	2.5x10 <sup>3</sup>	3.40x10 <sup>2</sup>	0	3.40x10 <sup>2</sup>	2.27x10 <sup>2</sup>
	5x10 <sup>3</sup>	0	0	0	0
	25x10 <sup>3</sup>	10.2x10 <sup>2</sup>	3.40x10 <sup>2</sup>	17.0x10 <sup>2</sup>	10.2x10 <sup>2</sup>
	50x10 <sup>3</sup>	6.79x10 <sup>2</sup>	13.6x10 <sup>2</sup>	10.2x10 <sup>2</sup>	10.2x10 <sup>2</sup>
	1x10 <sup>6</sup>	0	3.40x10 <sup>2</sup>	6.79x10 <sup>2</sup>	3.40x10 <sup>2</sup>

Table A3. (Continued)

Time	Prey (cells ml <sup>-1</sup> )	A	B	C	Average
3	2.5x10 <sup>3</sup>	3.40x10 <sup>2</sup>	0	3.40x10 <sup>2</sup>	2.27x10 <sup>2</sup>
	5x10 <sup>3</sup>	3.40x10 <sup>2</sup>	3.40x10 <sup>2</sup>	10.2x10 <sup>2</sup>	5.66x10 <sup>2</sup>
	25x10 <sup>3</sup>	13.6x10 <sup>2</sup>	13.6x10 <sup>2</sup>	6.79x10 <sup>2</sup>	11.3x10 <sup>2</sup>
	50x10 <sup>3</sup>	27.1x10 <sup>2</sup>	27.2x10 <sup>2</sup>	20.4x10 <sup>2</sup>	24.9x10 <sup>2</sup>
	1x10 <sup>6</sup>	13.6x10 <sup>2</sup>	6.79x10 <sup>2</sup>	6.79x10 <sup>2</sup>	9.06x10 <sup>2</sup>
4	2.5x10 <sup>3</sup>	0	0	10.2x10 <sup>2</sup>	3.40x10 <sup>2</sup>
	5x10 <sup>3</sup>	10.2x10 <sup>2</sup>	3.40x10 <sup>2</sup>	3.40x10 <sup>2</sup>	5.66x10 <sup>2</sup>
	25x10 <sup>3</sup>	27.2x10 <sup>2</sup>	17.0x10 <sup>2</sup>	27.2x10 <sup>2</sup>	23.8x10 <sup>2</sup>
	50x10 <sup>3</sup>	47.6x10 <sup>2</sup>	17.0x10 <sup>2</sup>	27.2x10 <sup>2</sup>	30.6x10 <sup>2</sup>
	1x10 <sup>6</sup>	0	0	0	0
5	2.5x10 <sup>3</sup>	0	0	0	0
	5x10 <sup>3</sup>	0	0	3.40x10 <sup>2</sup>	1.13x10 <sup>2</sup>
	25x10 <sup>3</sup>	10.2x10 <sup>2</sup>	13.6x10 <sup>2</sup>	6.79x10 <sup>2</sup>	10.2x10 <sup>2</sup>
	50x10 <sup>3</sup>	37.4x10 <sup>2</sup>	20.4x10 <sup>2</sup>	37.4x10 <sup>2</sup>	31.7x10 <sup>2</sup>
	1x10 <sup>6</sup>	0	0	0	0

Table A4. Heterotrophic dinoflagellate (HDINO) cells ml<sup>-1</sup> for replicates in the prey concentration effects II.

Time	Prey (cells ml <sup>-1</sup> )	A	B	C	Average
0	***	2.04x10 <sup>2</sup>	0.68x10 <sup>2</sup>	0.68x10 <sup>2</sup>	1.13x10 <sup>2</sup>
1	40x10 <sup>3</sup>	3.40x10 <sup>2</sup>	2.55x10 <sup>2</sup>	2.55x10 <sup>2</sup>	2.83x10 <sup>2</sup>
	50x10 <sup>3</sup>	1.66x10 <sup>2</sup>	0.42x10 <sup>2</sup>	3.40x10 <sup>2</sup>	1.83x10 <sup>3</sup>
	90x10 <sup>3</sup>	1.70x10 <sup>2</sup>	1.70x10 <sup>2</sup>	4.25x10 <sup>2</sup>	2.55x10 <sup>3</sup>
	200x10 <sup>3</sup>	1.70x10 <sup>2</sup>	1.70x10 <sup>2</sup>	1.70x10 <sup>2</sup>	1.70x10 <sup>2</sup>
2	40x10 <sup>3</sup>	5.09x10 <sup>2</sup>	5.94x10 <sup>2</sup>	6.79x10 <sup>2</sup>	5.94x10 <sup>2</sup>
	50x10 <sup>3</sup>	2.55x10 <sup>2</sup>	5.94x10 <sup>2</sup>	5.94x10 <sup>2</sup>	4.81x10 <sup>2</sup>
	90x10 <sup>3</sup>	4.53x10 <sup>2</sup>	7.92x10 <sup>2</sup>	6.79x10 <sup>2</sup>	6.41x10 <sup>2</sup>
	200x10 <sup>3</sup>	4.53x10 <sup>2</sup>	7.92x10 <sup>2</sup>	4.53x10 <sup>2</sup>	5.66x10 <sup>2</sup>
3	40x10 <sup>3</sup>	10.2x10 <sup>2</sup>	10.2x10 <sup>2</sup>	11.9x10 <sup>2</sup>	10.8x10 <sup>2</sup>
	50x10 <sup>3</sup>	8.49x10 <sup>2</sup>	5.09x10 <sup>2</sup>	9.34x10 <sup>2</sup>	7.64x10 <sup>2</sup>
	90x10 <sup>3</sup>	8.49x10 <sup>2</sup>	4.25x10 <sup>2</sup>	6.79x10 <sup>2</sup>	6.51x10 <sup>2</sup>
	200x10 <sup>3</sup>	9.34x10 <sup>2</sup>	7.64x10 <sup>2</sup>	3.40x10 <sup>2</sup>	6.79x10 <sup>2</sup>

Table A4. (Continued)

Time	Prey (cells ml <sup>-1</sup> )	A	B	C	Average
4	40x10 <sup>3</sup>	7.92x10 <sup>2</sup>	14.7x10 <sup>2</sup>	11.3x10 <sup>2</sup>	11.3x10 <sup>2</sup>
	50x10 <sup>3</sup>	5.94x10 <sup>2</sup>	11.9x10 <sup>2</sup>	11.0x10 <sup>2</sup>	9.62x10 <sup>2</sup>
	90x10 <sup>3</sup>	8.49x10 <sup>2</sup>	11.9x10 <sup>2</sup>	8.49x10 <sup>2</sup>	9.62x10 <sup>2</sup>
	200x10 <sup>3</sup>	5.09x10 <sup>2</sup>	7.64x10 <sup>2</sup>	8.49x10 <sup>2</sup>	7.07x10 <sup>2</sup>

\*\*\* Values represent estimates for HDINOs present at time 0 for all prey concentration groups.

## APPENDIX B

*K. rotundatum* cell counts for temperature and prey  
concentration experiments

Table B1. *K. rotundatum* cells ml<sup>-1</sup> for replicates in the temperature effects on HDINO growth rate experiment.

Time	Temperature	A	B	C	Average
0	10 °C	119x10 <sup>3</sup>	111x10 <sup>3</sup>	104x10 <sup>3</sup>	111x10 <sup>3</sup>
	15 °C	89.0x10 <sup>3</sup>	94.4x10 <sup>3</sup>	95.1x10 <sup>3</sup>	92.8x10 <sup>3</sup>
	20 °C	89.0x10 <sup>3</sup>	105x10 <sup>3</sup>	109x10 <sup>3</sup>	101x10 <sup>3</sup>
	25 °C	99.2x10 <sup>3</sup>	112x10 <sup>3</sup>	93.1x10 <sup>3</sup>	101x10 <sup>3</sup>
1	10 °C	128x10 <sup>3</sup>	132x10 <sup>3</sup>	132x10 <sup>3</sup>	131x10 <sup>3</sup>
	15 °C	0.679x10 <sup>3</sup>	127x10 <sup>3</sup>	123x10 <sup>3</sup>	83.5x10 <sup>3</sup>
	20 °C	104x10 <sup>3</sup>	102x10 <sup>3</sup>	117x10 <sup>3</sup>	108x10 <sup>3</sup>
	25 °C	141x10 <sup>3</sup>	142x10 <sup>3</sup>	169x10 <sup>3</sup>	151x10 <sup>3</sup>
2	10 °C	98.8x10 <sup>3</sup>	102x10 <sup>3</sup>	106x10 <sup>3</sup>	102x10 <sup>3</sup>
	15 °C	0	104x10 <sup>3</sup>	93.4x10 <sup>3</sup>	65.8x10 <sup>3</sup>
	20 °C	99.2x10 <sup>3</sup>	81.9x10 <sup>3</sup>	62.5x10 <sup>3</sup>	81.2x10 <sup>3</sup>
	25 °C	0	0	0	0
3	10 °C	90.3x10 <sup>3</sup>	81.9x10 <sup>3</sup>	104x10 <sup>3</sup>	91.9x10 <sup>3</sup>
	15 °C	0	77.8x10 <sup>3</sup>	75.1x10 <sup>3</sup>	50.9x10 <sup>3</sup>
	20 °C	2.38x10 <sup>3</sup>	5.43x10 <sup>3</sup>	7.13x10 <sup>3</sup>	4.98x10 <sup>3</sup>
	25 °C	0	0	0	0
4	10 °C	86.3x10 <sup>3</sup>	85.2x10 <sup>3</sup>	86.9x10 <sup>3</sup>	86.2x10 <sup>3</sup>
	15 °C	0	30.6x10 <sup>3</sup>	61.5x10 <sup>3</sup>	30.7x10 <sup>3</sup>
	20 °C	0	0	0	0
	25 °C	0	0	0	0

Table B2. *K. rotundatum* cells ml<sup>-1</sup> for replicates in the prey concentration effects experiment I.

Time	Rep-A	Rep-B	Rep-C	Average
0	1107x10 <sup>3</sup>	1162x10 <sup>3</sup>	1032x10 <sup>3</sup>	1048x10 <sup>3</sup>
	59.4x10 <sup>3</sup>	55.0x10 <sup>3</sup>	57.7x10 <sup>3</sup>	56.9x10 <sup>3</sup>
	28.5x10 <sup>3</sup>	27.9x10 <sup>3</sup>	25.5x10 <sup>3</sup>	23.2x10 <sup>3</sup>
	5.09x10 <sup>3</sup>	5.09x10 <sup>3</sup>	8.15x10 <sup>3</sup>	5.52x10 <sup>3</sup>
	2.04x10 <sup>3</sup>	5.43x10 <sup>3</sup>	2.72x10 <sup>3</sup>	3.23x10 <sup>3</sup>
1	646x10 <sup>3</sup>	690x10 <sup>3</sup>	390x10 <sup>3</sup>	574x10 <sup>3</sup>
	73.9x10 <sup>3</sup>	52.7x10 <sup>3</sup>	84.0x10 <sup>3</sup>	70.2x10 <sup>3</sup>
	28.3x10 <sup>3</sup>	42.2x10 <sup>3</sup>	11.2x10 <sup>3</sup>	27.2x10 <sup>3</sup>
	2.72x10 <sup>3</sup>	9.17x10 <sup>3</sup>	6.11x10 <sup>3</sup>	6.00x10 <sup>3</sup>
	2.38x10 <sup>3</sup>	5.43x10 <sup>3</sup>	2.72x10 <sup>3</sup>	3.74x10 <sup>3</sup>
2	487x10 <sup>3</sup>	481x10 <sup>3</sup>	455x10 <sup>3</sup>	475x10 <sup>3</sup>
	61.6x10 <sup>3</sup>	98.9x10 <sup>3</sup>	91.1x10 <sup>3</sup>	83.9x10 <sup>3</sup>
	51.6x10 <sup>3</sup>	33.8x10 <sup>3</sup>	43.9x10 <sup>3</sup>	43.1x10 <sup>3</sup>
	2.04x10 <sup>3</sup>	10.2x10 <sup>3</sup>	6.45x10 <sup>3</sup>	18.7x10 <sup>3</sup>
	2.38x10 <sup>3</sup>	5.43x10 <sup>3</sup>	2.72x10 <sup>3</sup>	3.51x10 <sup>3</sup>
3	391x10 <sup>3</sup>	318x10 <sup>3</sup>	339x10 <sup>3</sup>	349x10 <sup>3</sup>
	65.0x10 <sup>3</sup>	71.7x10 <sup>3</sup>	86.1x10 <sup>3</sup>	74.3x10 <sup>3</sup>
	24.4x10 <sup>3</sup>	23.6x10 <sup>3</sup>	57.2x10 <sup>3</sup>	35.1x10 <sup>3</sup>
	1.36x10 <sup>3</sup>	4.76x10 <sup>3</sup>	8.83x10 <sup>3</sup>	4.98x10 <sup>3</sup>
	1.36x10 <sup>3</sup>	5.09x10 <sup>3</sup>	2.38x10 <sup>3</sup>	2.94x10 <sup>3</sup>

Table B3. (continued)

Time	Rep-A	Rep-B	Rep-C	Average
4	451x10 <sup>3</sup>	367x10 <sup>3</sup>	268x10 <sup>3</sup>	362x10 <sup>3</sup>
	93.9x10 <sup>3</sup>	124x10 <sup>3</sup>	122x10 <sup>3</sup>	113x10 <sup>3</sup>
	59.4x10 <sup>3</sup>	51.1x10 <sup>3</sup>	69.4x10 <sup>3</sup>	60.0x10 <sup>3</sup>
	8.15x10 <sup>3</sup>	5.43x10 <sup>3</sup>	10.9x10 <sup>3</sup>	8.15x10 <sup>3</sup>
	2.38x10 <sup>3</sup>	5.09x10 <sup>3</sup>	4.08x10 <sup>3</sup>	3.85x10 <sup>3</sup>
5	207x10 <sup>3</sup>	198x10 <sup>3</sup>	94.5x10 <sup>3</sup>	166x10 <sup>3</sup>
	63.9x10 <sup>3</sup>	38.9x10 <sup>3</sup>	60.0x10 <sup>3</sup>	54.3x10 <sup>3</sup>
	18.9x10 <sup>3</sup>	11.2x10 <sup>3</sup>	25.0x10 <sup>3</sup>	18.4x10 <sup>3</sup>
	1.70x10 <sup>3</sup>	1.70x10 <sup>3</sup>	3.74x10 <sup>3</sup>	7.13x10 <sup>3</sup>
	1.02x10 <sup>3</sup>	1.36x10 <sup>3</sup>	0.34x10 <sup>3</sup>	0.91x10 <sup>3</sup>

Table B4. *K. rotundatum* cells ml<sup>-1</sup> for replicates in the prey concentration effects experiment II.

Time	Rep-A	Rep-B	Rep-C	Average
0	199x10 <sup>3</sup>	190x10 <sup>3</sup>	204x10 <sup>3</sup>	198x10 <sup>3</sup>
	97.8x10 <sup>3</sup>	105x10 <sup>3</sup>	78.8x10 <sup>3</sup>	93.7x10 <sup>3</sup>
	43.5x10 <sup>3</sup>	51.6x10 <sup>3</sup>	58.4x10 <sup>3</sup>	51.2x10 <sup>3</sup>
	34x10 <sup>3</sup>	40.8x10 <sup>3</sup>	38x10 <sup>3</sup>	37.6x10 <sup>3</sup>
1	160x10 <sup>3</sup>	150x10 <sup>3</sup>	120x10 <sup>3</sup>	143x10 <sup>3</sup>
	71.3x10 <sup>3</sup>	48.9x10 <sup>3</sup>	65.9x10 <sup>3</sup>	62x10 <sup>3</sup>
	28.5x10 <sup>3</sup>	20.4x10 <sup>3</sup>	25.1x10 <sup>3</sup>	24.7x10 <sup>3</sup>
	21.7x10 <sup>3</sup>	13.6x10 <sup>3</sup>	17x10 <sup>3</sup>	17.4x10 <sup>3</sup>
2	156x10 <sup>3</sup>	140x10 <sup>3</sup>	95.1x10 <sup>3</sup>	130x10 <sup>3</sup>
	49.8x10 <sup>3</sup>	78.8x10 <sup>3</sup>	72.5x10 <sup>3</sup>	67x10 <sup>3</sup>
	23.8x10 <sup>3</sup>	19.7x10 <sup>3</sup>	23.8x10 <sup>3</sup>	22.4x10 <sup>3</sup>
	12.2x10 <sup>3</sup>	8.83x10 <sup>3</sup>	17x10 <sup>3</sup>	12.7x10 <sup>3</sup>
3	149x10 <sup>3</sup>	89x10 <sup>3</sup>	45.5x10 <sup>3</sup>	94.4x10 <sup>3</sup>
	46.9x10 <sup>3</sup>	53.7x10 <sup>3</sup>	47.5x10 <sup>3</sup>	49.4x10 <sup>3</sup>
	17.7x10 <sup>3</sup>	14.3x10 <sup>3</sup>	23.8x10 <sup>3</sup>	18.6x10 <sup>3</sup>
	10.2x10 <sup>3</sup>	17.7x10 <sup>3</sup>	12.2x10 <sup>3</sup>	13.4x10 <sup>3</sup>
4	103x10 <sup>3</sup>	70x10 <sup>3</sup>	11.5x10 <sup>3</sup>	61.4x10 <sup>3</sup>
	44.2x10 <sup>3</sup>	25.8x10 <sup>3</sup>	23.1x10 <sup>3</sup>	31x10 <sup>3</sup>
	9.51x10 <sup>3</sup>	13.6x10 <sup>3</sup>	35.3x10 <sup>3</sup>	19.5x10 <sup>3</sup>
	8.83x10 <sup>3</sup>	8.83x10 <sup>3</sup>	8.83x10 <sup>3</sup>	8.83x10 <sup>3</sup>

**VITA**

Angela Denise Smith was born on May 25, 1969 in Danville, VA. After graduating from Bluestone Senior High School, Skipwith, VA in 1987, she entered Norfolk State University (Norfolk, VA) as a Dozoretz National Institute student. She received her B.S. in biology with pre-medicine emphasis in May 1991. In the fall of 1991, she entered the Virginia Institute of Marine Science, and began her thesis work under the auspices of the Patricia Roberts Harris fellowship in the biological sciences department. She is currently employed as a student trainee with the NOAA/NMFS, Southeast Fisheries Science Center in Beaufort, NC.