MICROZOOPLANKTON TROPHIC INTERACTIONS AND THEIR IMPACT ON PHYTOPLANKTON PRODUCTION AND COMMUNITY STRUCTURE IN THE SOUTH SLOUGH ARM OF COOS BAY, OREGON

by

RICHARD JOSEPH COWLISHAW

A DISSERTATION

Presented to the Department of Biology
and the Graduate School of the University of Oregon
in partial fulfillment of the requirements
for the degree of
Doctor of Philosophy

June 2002

"Microzooplankton Trophic Interactions and their Impact on Phytoplankton Production and Community Structure in the South Slough Arm of Coos Bay, Oregon," a dissertation prepared by Richard J. Cowlishaw in partial fulfillment of the requirements for the Doctor of Philosophy degree in the Department of Biology. This dissertation has been approved and accepted by:

Richard Emlet

Dr. Richard Emlet, Chair of the Advisory/Examination Committee

5/31/02

Date

Committee in charge: Dr. Richard Emlet, Chair
Dr. Lynda Shapiro, Adviser
Dr. Richard Castenholz
Dr. Steve Rumrill
Dr. Patricia McDowell

Accepted by:

Dean of the Graduate School

An Abstract of the Dissertation of

Richard Joseph Cowlishaw for the degree of Doctor of Philosophy

in the Department of Biology to be taken June 2002

Title: MICROZOOPLANKTON TROPHIC INTERACTIONS AND THEIR IMPACT ON PHYTOPLANKTON PRODUCTION AND COMMUNITY STRUCTURE IN THE SOUTH SLOUGH ARM OF COOS BAY, OREGON.

Approved: _____
Dr. Lynda Shapiro

The role of microzooplankton as consumers of phytoplankton production was investigated in the South Slough Arm of Coos Bay, Oregon, a shallow estuarine system with a direct exchange with the coastal waters that are subject to seasonal coastal upwelling (April-September). Ciliates and heterotrophic nanoflagellates (Hflag) were observed to track <5-µm phytoplankton biomass (which contributed on average 42% to the annual total biomass) during the spring, summer and early fall when diatoms were a dominant component to >5-µm phytoplankton. Ciliates were positively correlated with both <5-µm and total phytoplankton biomass between October-March when nanoflagellates dominated the >5-µm phytoplankton assemblage. Seasonal dilution-method grazing experiments showed that microzooplankton utilized 48 to 92% of primary

production in the South Slough. Grazing impacts reflected the trophic relationships intimated by the field results between ciliate and phytoplankton biomass, except when heterotrophic dinoflagellates contributed significantly to the microzooplankton and imposed significant grazing impacts on diatom production and biomass. In one dilution experiment, the suppression of autotrophic picoplankton (Apico) net-growth in low dilution treatments was observed to correspond with enhanced net-growth of Hflag. Reduction of larger protozoans (i.e., ciliates) by fractionation in a subsequent experiment eliminated these responses in comparison to unfractionated controls. The findings support the hypothesis that omnivory and trophic interactions among microzooplankton can foster non-linear relationships between apparent prey growth and dilution factor that are often reported and attributed to a functional feeding response by a single grazer group. In a second experiment, the net-growth of ciliate biomass and Hflag abundance significantly decreased and increased, respectively, with increasing copepod abundance, but the net-growth of Apico remained unchanged. These findings support the interpretation of the dilution experiment results and provide an explanation for the numerical constancy of natural picoplankton populations and the failure of mesozooplankton manipulations to promote trophic cascade effects on picoplankton abundance in field experiments. It is concluded that microzooplankton are significant grazers of phytoplankton production in the South Slough and likely play a key role in energy transfer and the stabilization of food availability for larger organisms in this ecosystem.

CURRICULUM VITA

NAME OF AUTHOR: Richard Joseph Cowlishaw

PLACE OF BIRTH: La Mesa, California

DATE OF BIRTH: July 15, 1965

GRADUATE AND UNDERGRADUATE SCHOOLS ATTENDED:

University of Oregon
Oregon State University

DEGREES AWARDED:

Doctor of Philosophy in Biology, 2002, University of Oregon
Bachelor of Science in Biology, *magna cum laude*, 1989, Oregon State University

AREAS OF SPECIAL INTEREST:

Microzooplankton Ecology
Planktonic Trophic Dynamics
Planktonic Microscale Heterogeneity

PROFESSIONAL EXPERIENCE:

Graduate Research Fellow, South Slough National Estuarine Research Reserve, 1998-2001

Teaching Assistant, Department of Biology, University of Oregon, Eugene, 1996-98

Fisheries Biologist/Technician, National Marine Fisheries Service. John Day and Bonneville Dams, Columbia River, Oregon, 1994-96

Agroforestry Extension Agent, US Peace Corps Volunteer, Guatemala, 1992-94

AWARDS AND HONORS:

Best Graduate Student Presentation, Pacific Estuarine Research Society, 1999

GRANTS:

Graduate Research Fellowship, National Estuarine Research Reserve, National Oceanic and Atmospheric Administration, 1999-2001

ACKNOWLEDGEMENTS

I express sincere thanks and appreciation to my advisor Professor Shapiro and the members of my advisory committee for their assistance in the preparation of this manuscript. This body of work could not have been completed without the incredible patience, support and understanding of my wife, Nancy, the inspiration from my son, Evan, and the love of my parents, Joseph and Roseann Cowlishaw. The research was supported in part by a National Estuarine Research Reserve Graduate Research Fellowship, Number NA870R0270, and by a grant from Oregon Sea Grant, Number NA650L-01, to Dr. Lynda Shapiro at the University of Oregon.

DEDICATION

I dedicate this manuscript to my wife, Nancy, for her love, understanding and unwavering support during the course of my academic career.

TABLE OF CONTENTS

CHAPTER PAGE

LIST OF TABLES

TABLE PAGE

TABLE PAGE

TABLE PAGE

LIST OF FIGURES

FIGURE PAGE

FIGURE PAGE

CHAPTER I

GENERAL INTRODUCTION

Over the past thirty years our understanding of plankton ecology and the trophic connectivity between primary production and metazoan consumers has undergone a profound transformation as a result of technological advances in how we collect and analyze plankton samples. Earlier knowledge of plankton communities was based on the contents of plankton net collections. Because only large and robust phytoplankton species such as diatoms were sampled by such means, early models of secondary production in the ocean were based on linear food chains composed of relatively few links between primary producers and higher consumers (Ryther 1969).

The revelation that the most of the respiration in marine water could be attributed to microorganisms led Pomeroy (1974) to advance a new model in which protozoa play pivotal roles in the transfer and recycling of carbon and nutrients in ocean food web. Developments in microscopy and the analysis of whole water revealed the existence of an abundant group of phytoplankton 0.2-5 μm in size, the ultraphytoplankton (Murphy and Haugen 1985), composed of phototrophic cyanobacteria about 1 μm in size (Waterbury et al. 1979; Iturriaga and Mitchell 1986) and larger eukaryotes (Shapiro and Guillard 1986). Other

findings suggesting that much of the marine primary production was generated by ultraphytoplankton (Li et al. 1983; Stockner and Antia 1988), coupled with the fact that most metazoan grazers cannot feed effectively on cells of this size (Nival and Nival 1976; Paffenhöfer 1984; Shumway et al. 1985; Riisgard 1988) prompted a repositioning of protozoans such as flagellates and ciliates from microbial loop bacterivores (Azam et al. 1983) to the chief grazers of the sea. As single cell organisms, protozoans have growth and metabolic rates comparable to their unicellular prey (Fenchel 1968; Finlay 1977; Banse 1982; Stoecker and Guillard 1982; Montagnes 1996) and, thereby, can maintain a tighter trophic coupling with the growth of phytoplankton populations (Miller et al. 1991; Sherr and Sherr 1994; Landry et al. 1997).

The postulated transfer of small phytoplankton production to metazoan consumers via protistan intermediate consumers is substantiated by numerous findings that protozoans make a substantial, perhaps essential contribution to the nutrition and reproduction of copepods (Kleppel 1993; Verity and Paffenhöfer 1996; Ohman and Runge 1994). Other results suggest that protozoans are regular prey for rotifers (Arnt 1993) invertebrate and fish larvae (Stoecker and Govoni 1984; Stoecker et al. 1987; Lair et al. 1994), and benthic suspension feeders (Le Gall et al. 1997; Dupuy et al. 1999). It is thought that protozoan food can offer higher nutritional value and the provision of essential compounds not attainable by phytoplankton food to metazoan consumers (Stoecker and Capuzzo 1990).

Investigations from a diversity of marine environments provide convincing evidence that microzooplankton, heterotrophs <200 µm composed largely of protozoans, are chief consumers of phytoplankton production in marine systems (Beers et al. 1980; Smetacek 1981; Paranjape 1987, 1990; Sherr and Sherr 1994; Landry et al. 1997; Levinsen et al. 1999; Strom et al. 2001). Feeding by ciliates and small flagellates on phytoplankton <20 µm (Jonsson 1986; Sherr et al. 1989) and the ability of heterotrophic dinoflagellates to consume large, chain-forming diatoms (Jacobsen and Anderson 1986) denotes that microzooplankton can potentially impart significant grazing losses on the production of the total phytoplankton assemblage.

A variety of techniques have been developed to directly quantify the impact of microzooplankton grazing (i.e., size-fractionation, metabolic inhibitors, tracers of ingestion) but the seawater dilution method described by Landry and Hassett (1982) has achieved near standard status among investigators. There are three principal reasons for its preference: 1) it is quantitative; 2) it requires minimal manipulation of the natural assemblage; and 3) it allows simultaneous estimation of the rates of prey growth and mortality due to grazing. The application of this technique across trophic gradients has made it apparent that microzooplankton impart significant grazing impacts on marine primary production and efficiently assimilate this biomass into food particles accessible to larger planktonic and benthic consumers.

My dissertation research focused on the role of microzooplankton as grazers of phytoplankton production in the South Slough Arm of Coos Bay, Oregon. The South Slough is a shallow estuarine environment that undergoes significant hydrographic changes during the year. Most of the precipitation occurs between October and May, which establishes a strong, longitudinal salinity gradient in the South Slough (Rumrill, in review). During the months of low precipitation, the South Slough is a marine-dominated system (Rumrill, in review). Findings suggests that the South Slough waters can be influenced by coastal processes such as upwelling that typically occurs between April and September (Croegner and Shanks 2000; Huyer 1983). These seasonal changes of hydrography affect the composition of phytoplankton assemblage (Hughes 1997). During the period of higher precipitation and coincidental shorter photoperiod, phytoplankton are generally <20 μm in size and large diatoms are rare or absent. During the spring and summer months, however, longer photoperiod and the possible influence of coastal processes give rise to a large contribution of chain-forming centric and pennate diatoms to the phytoplankton assemblage.

Because of this seasonal variation in phytoplankton composition, the South Slough presents an interesting environment to study the trophic role of microzooplankton and quantify their grazing impact on primary production. The importance of microzooplankton herbivory in the coastal waters along the west coast of North America has been documented (Beers and Stewart 1967; Beers et

al. 1980; Landry and Hassett 1982; Neuer and Cowles 1994; Strom et al. 2001); however, their role in the small estuaries and embayments along the Oregon coast has yet to be investigated.

Chapter II presents a descriptive study of the seasonal relationships between ciliate abundance and biomass and different size-fractions of phytoplankton biomass in the South Slough. Preliminary sampling suggested that ciliates were a chief component of the microzooplankton assemblage throughout the year. The purpose of this study was to establish an annual, semi-continuous profile of key heterotrophic and autotrophic microplankton components so that the results of the discrete grazing experiments described in Chapter III could be interpreted within a larger biological context in this system. Microzooplankton grazing impacts were quantified using the dilution method (Landry and Hassett 1982). An assumption of this method is that ingestion is a linear function of grazer density and its violation is regularly attributed to a functional feeding response by microzooplankton (i.e., Gallegos 1989). In one experiment, however, the suppression of the apparent growth of picophytoplankton (cells 0.2-2.0 μm) in low dilution treatments was observed to correspond with the enhanced apparent growth of heterotrophic nanoflagellates 2-10 μm. It was hypothesized that the low dilution treatment releases of heterotrophic nanoflagellates from predation by larger microzooplankton (i.e., ciliates) and their increased apparent growth and corresponding grazing potential caused the suppression of the apparent growth of picophytoplankton in those treatments. Chapter IV describes

an experimental test of this hypothesis and an interpretation of its results within the context of the general application of the dilution method. As a test of this theory in undiluted environments, I conducted an additional experiment in which ciliate abundance was attenuated by copepod predation and the dynamics of the apparent growth of heterotrophic nanoflagellates and picophytoplankton were measured along a copepod density gradient. The results of this experiment are also described in Chapter IV. Chapter V presents an overall summary of findings and conclusions for my dissertation.

In order to increase the clarity of argument and save space, I have made use of acronyms for biological terms throughout my dissertation, particularly in Chapter I. To benefit the reader I have catalogued these acronyms and their definitions by chapter in Table 1. It is anticipated that the table will allow the reader to quickly reference a particular acronyms if and when the need arises.

Table 1. Acronyms and definitions of biological terms.

Acronym	Definition	Chapter(s)
AGR	Apparent growth rate	III, IV
CILC	Ciliate carbon	II
EPICO	Eukaryotic Picophytoplankton	III, IV
HFLAG	Heterotrophic nanoflagellates 2-10 µm in size	III, IV
< and >5CHL	< and >5-µm chlorophyll	II, III
< and >5CHLC	< and >5-µm chlorophyll carbon	II
NCIL	Naked ciliates	II
NCILC	Naked ciliate carbon	II
PP	Potential Production	II
PPICO	Prokaryotic Picophytoplankton	III, IV
PSS	Prey standing stock	III
TCHL	Total chlorophyll	II, III
TCHLC	Total chlorophyll carbon	II

CHAPTER II

SEASONAL RELATIONSHIPS BETWEEN PLANKTONIC CILIATES AND PHYTOPLANKTON BIOMASS IN THE SOUTH SLOUGH OF COOS BAY, OREGON.

Abstract

The standing stocks of ciliates and phytoplankton were measured on a biweekly basis from March 1999 to March 2000 in the marine-dominated region of the South Slough, the southern arm of the Coos Bay Estuary (Oregon). Total and <5-µm chlorophyll ranged 0.3-6.4 µg l^{-1} (avg. 2.1) and <0.1-3.0 µm l^{-1} (avg. 0.93), respectively. The contribution of <5-µm chlorophyll to total chlorophyll ranged 14-83% (avg. 42%) throughout the year. Naked ciliates dominated the ciliate assemblage, ranging 2.8-91 cells ml^{-1} (avg. 20). Tintinnid abundance ranged 0.2-3.5 cells ml^{-1} (avg. 1.2). Ciliate biomass was determined from June 1999 - March 2000 and ranged 4.7-80 µg C l^{-1} (avg. 21). The abundance and biomass of naked ciliate were significantly correlated with <5-µm phytoplankton (= ultraphytoplankton) biomass throughout the sample year. This was most apparent during the months of April-September when diatoms dominated the >5-µm phytoplankton biomass. Between October and March, naked ciliates were positively correlated with total phytoplankton biomass, likely due to the

dominance of >5-µm phytoplankton by nanoflagellates during this period. These appear to have been accepted as food by naked ciliates since the carbon ratio of ciliate:ultraphytoplankton suggested that ultraphytoplankton were insufficient to support the ciliate biomass observed during this seasonal period. The robust numerical relationships between ciliates and small phytoplankton standing stocks reported here imply that ciliates play an important trophic role in the South Slough.

Introduction

The advent of epifluorescence microscopy, whole water sampling techniques and other advances has brought about a new paradigm regarding the dynamics of planktonic food webs (Pomeroy 1974; Azam et al. 1983). The revelation that much of the marine primary production was generated by phytoplankton cells <5 µm in size (Li et al. 1983; Platt et al. 1983; Murphy and Haugen 1985; Stockner and Antia 1988) repositioned protozoans from "microbial loop" bacterivores (Azam et al. 1983) to principal grazers of the sea. As single cell organisms, protozoans have growth and metabolic rates more comparable to their protistan prey than most metazoan consumers (Fenchel 1968; Finlay 1977; Banse 1982; Stoecker and Guillard 1982; Montagnes 1996) and, thereby, can potentially maintain a tighter trophic coupling with the growth of phytoplankton populations (Miller et al. 1991; Sherr and Sherr 1994; Landry et al. 1997). Among the numerous types of protozoa in the plankton, ciliates stand out as an

abundant and ubiquitous group now recognized as significant consumers of primary production in planktonic systems (Pierce and Turner 1992).

Planktonic ciliates can be categorized as either tintinnids (lorica-bearing) of the Suborder Tintinnina, or naked ciliates (aloricate), most of which belong to the Order Oligotrichida and the Suborder Strobilidiina (Lynn and Small 1985). Much more was known about the abundance and distribution of tintinnids than naked ciliates prior to the introduction of whole-water sample analysis. This is principally due to the conspicuous and durable test (lorica) that can withstand collection (empty or with organism retained) with plankton nets (Kimor and Golanksky 1977) and size-fractionation (Beers and Stewart 1967; Beers et al. 1980). More recent analysis based on whole water counts suggest that naked ciliates are typically more abundant than tintinnids by factors of 2 to 10 (Pierce and Turner 1992), although at varying temporal and spatial scales, and in certain systems, tintinnids have been observed to dominate ciliate abundance and standing stocks (Revelante and Gilmartin 1983; Stoecker et al. 1984; Verity 1987 Leakey et al. 1993).

Ciliates are generally categorized as microzooplankton (cells 20-200 μm), but ciliates <20 μm are frequently observed and can numerically dominate the nanoheterotroph assemblage (Sherr et al. 1986). On a predator-prey size basis alone, they are potential consumers of a wide range of microplankton prey: bacteria (Sherr and Sherr 1987; Sherr et al. 1989), pico- and nano-phytoplankton (Ferrier-Pages and Rassoulzadegan 1994b; Jonsson 1986; Christaki et al. 1998;

Christaki et al. 1999), heterotrophic nanoflagellates (Ohman and Snyder 1991; Verity 1991; Jürgens et al. 1996), dinoflagellates (Stoecker et al. 1981; Stoecker et al. 1984), and small diatoms (Smetacek 1981; Sime-Ngando et al. 1995; Montagnes 1996;).

Ciliates make a significant ecological impact as herbivores in most marine environments (Pierce, and Turner 1992). Field grazing experiments with ciliate-dominated microzooplankton communities intimate that these protozoans are important consumers of phytoplankton production in a diversity of marine systems, frequently grazing much or all of the daily phytoplankton production (Burkill 1982; Gifford 1988; Froneman and McQuaid 1997; Tamigneaux et al. 1997). Grazing and egestion by ciliates is also considered to be an important mechanism for retaining nutrients within the euphotic zone for reutilization by phytoplankton (Johannes 1965; Ferrier-Pages and Rassoulzadegan 1994a;), in contrast to the fecal-pellet formation by metazooplankton that can enhance nutrient export from the surface waters (Sieburth et al. 1978; Paffenhöfer and Knowles 1979; although see Smetacek 1981).

A growing amount of evidence suggests that ciliates, among other protozoans, are common prey for many metazoan consumers such as copepods (Kleppel 1993; Verity and Paffenhöfer 1996), rotifers (Arnt 1993), and invertebrate and fish larvae (Stoecker and Govoni 1984; Stoecker et al. 1987; Lair et al. 1994). The inclusion of ciliates in the diet of certain copepod species has been observed to enhance the reproductive output of copepods (Stoecker

and Egloff 1987) and sustain their fecundity when phytoplankton food is in reduced supply (Ohman and Runge 1994).

The estuaries along the Atlantic Coast of North America (Revelante and Gilmartin 1987; Verity 1987; Dolan and Coats 1990) and in Europe (Leakey et al. 1992; Sorokin and Sorokin 1996) have been the sites of most estuarine studies of protozoan and microplankton dynamics. Although a few such studies have been done in the larger estuaries along the West Coast of North America (San Francisco Bay: Ambler et al. 1985; Murrell and Hollibaugh 1998; Columbia River estuary: Baross et al. 1994; Small and Morgan 1994), the smaller drowned-river valley estuaries that dominate the coastline of Oregon, Washington, and British Columbia (Emmett et al. 2000) are largely unexplored.

Characteristic of the Pacific Northwest estuarine systems is the large annual variation in the physical and biological attributes of estuarine water due largely to two factors: 1) A pronounced season of precipitation and freshwater runoff between October and May (Emmett et al. 2000), and 2) Episodic coastal upwelling that typically occurs between April and September (Huyer 1983). During summer months prevailing northwest winds blowing along and with the California Current draw surface waters offshore to be replaced by colder, saltier, nutrient-rich water from depth that can consequently stimulate phytoplankton production (primarily diatoms) seaward of the upwelled waters (Small and Menzies 1981). Although upwelling is a coastal phenomenon, relaxation of this wind forcing, along with tidal exchange can result in cross-shelf transport of

nutrient rich water and material towards the shore and into estuarine environments (Roegner and Shanks 2001). Reversal to southwest winds between October and March sets up a near shore, northward counter-current (Davidson Current) and a general onshore flow that promotes downwelling circulation of surface waters over the coastal shelf. The effect of this, together with the seasonal precipitation and shorter day length that coincide, is a dramatic shift in the composition of the phytoplankton assemblage (Hughes 1997).

The primary objective of this study was to quantify the temporal distribution of ciliate abundance and biomass in relation to annual and seasonal fluctuations of size-fractionated chlorophyll biomass and specific physical variables in the South Slough of Coos Bay, Oregon. The findings of this study shed light on the quantitative and relative importance of ciliates as potential food for larger consumers in the planktonic food web of the South Slough and systems like it. In addition, the information reported here indirectly infers which trophic interactions are key to the planktonic transfer of phytoplankton-derived carbon in this habitat.

Materials and Methods

Study Site

The South Slough is the southern arm of Coos Bay located on the south-central coast of Oregon, USA (Fig. 1). The geomorphic typology of Coos Bay is a tidally-dominated drowned river mouth (Rumrill in review) common to west coast

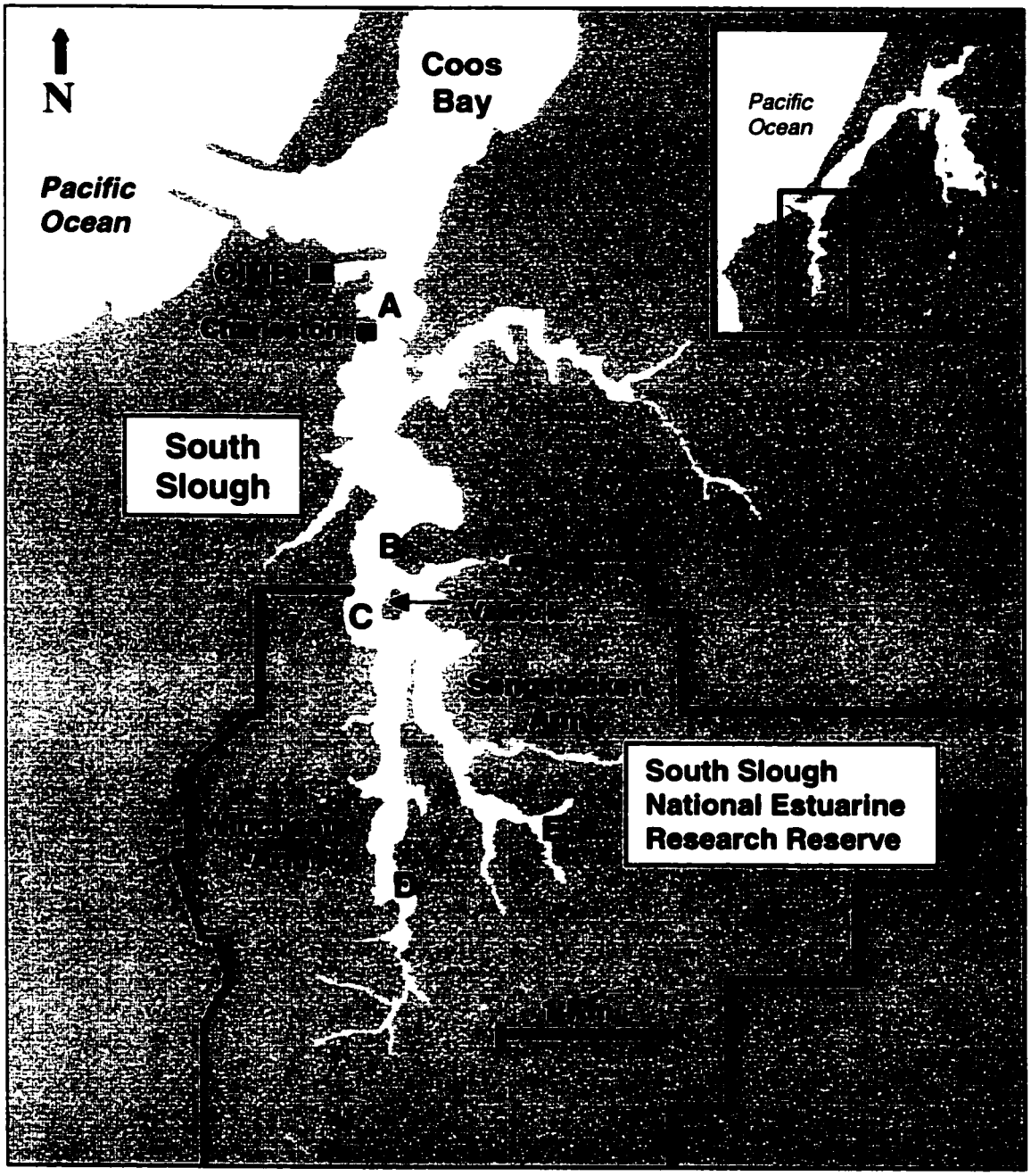

Fig. 1. Study site map. Insert shows the South Slough in relation to Coos Bay. The boundary line delineates the area of the South Slough within the South Slough National Estuarine Research Reserve. A, Charleston Coast Guard data collection station; B, Valino Is. SWMP (System-Wide Monitoring Program) station; C, sampling station; D, Winchester SWMP station E, Sengstacken SWMP station; and OIMB, Oregon Institute of Marine Biology.

of North America (Emmett et al. 2000). The watershed surface area of Coos Bay covers 1,576 km^2, of which the South Slough comprises 193 km^2, or 12% (Rumrill in review). A substantial part of the South Slough watershed (24%) resides within the boundary of the South Slough National Estuarine Research Reserve (SSNERR). The South Slough extends north to south for approximately 7.62 km and is divided into two arms, the Winchester and the Sengstacken, that converge at Valino Island. The average total rainfall between October and May is 140 cm but declines to <10 cm from June to September. River input parallels precipitation and varies from 155.6 $m^3 s^{-1}$ in the winter to 2.5 $m^3 s^{-1}$ in the summer. The tides are mixed semidiurnal with a mean-tidal amplitude of 2.3 m. The South Slough is a shallow system (avg. depth, 2 m) and has extensive mud flats, rich in biota, relative to its size (Rumrill, in review). Sampling for this study was conducted in the marine-dominated reach of this system.

There are three permanent water quality meter (YSI, Inc.) stations in the SSNERR as part of a NERR System-Wide Monitoring Program (SWMP) (Fig. 1). The meters are mounted onto pilings approximately 50 cm from the bottom. Physical parameters (i.e., temperature, conductivity, dissolved oxygen, and turbidity) are sampled every 30 minutes and data are routinely uploaded into a database approximately every 30 days. Archival data for the SSNERR SWMP sites and those of other NERRs can be accessed at the central database management website (http://inlet.geol.sc.edu/cdmohome.html).

Field Sampling

Sampling was conducted between 6 March 1999 and 22 March 2000 during which physical measurements and biological samples were taken approximately every two weeks. Whole water grab samples were collected with a weighted 20-l bucket from a depth of ~1 m at high tide in the main channel of the South Slough near Valino Is. (Fig. 1). Samples for ciliate enumeration (200 ml) and chlorophyll-*a* analysis (200 ml) were taken from three replicate bucket collections per sample day.

Sample site surface measurements of temperature (° Celsius) and salinity (PPT) were taken with a handheld meter (YSI, Model 85). Temperature and salinity data from the Winchester SWMP site and from the Charleston Coast Guard station (www.co-ops.nos.noaa.gov) were downloaded for comparisons of temperature and density upstream and seaward of the sample site (see Fig. 1). Density (ρ, kg m^{-3}) was calculated using the equation defined by Millero and Poisson (1981) and converted to sigma-t (σ-t=ρ-1000). Daily rainfall data at North Bend Municipal Airport were obtained from the meteorological records archived at the facility. The airport is approximately 11 linear km from lower reach of South Slough. The rainfall metric for each sample day was the accumulated rainfall (cm) of the last five days. Predictions of the upwelling index (units of m^3 s^{-1} 100 m $coastline^{-1}$) at 42°N 125°W (about 80 nautical miles south from Charleston) were acquired from the Pacific Fisheries Environmental Laboratory website (http://www.pfeg.noaa.gov). As indicators of the direction and magnitude of

coastal upwelling (offshore transport; positive values) or downwelling (onshore transport; negative values), the indices are calculated in terms of Ekman mass-transport due to wind stress whereby the net movement of water is 90° to the right of the wind direction in the Northern Hemisphere. Cumulative hourly averages for 1 to 7 days prior to each sample date were generated and associations with these statistics and other physical/biological variables were analyzed.

Ciliate samples were immediately fixed with acid Lugol's solution (final concentration = 5%). Aliquots of 60-100 ml of the fixed samples were allowed to settle (>18 hrs) in columns onto glass viewing plates. Ciliates were enumerated with an inverted microscope (Leica DMIL) until at least 200 cells of the most abundant taxon/morpho-type were counted. Individual cells were identified to genus when possible with the aid of several taxonomic references (Lynn and Small 1985; Maeda 1986; Maeda and Carey 1985); however, classification of ciliates beyond the level of genus was often difficult due to distortive effects of the fixative. Ciliates were categorized as either naked or tintinnids. Naked ciliates (NCIL) were further delineated into two size-classes of < and >20 µm (length). Determination of the mixotrophic-status of NCIL was hindered by the fixation method. For samples between 23 June 1999 and 22 March 2000, the length and width measurements of 30 or more individual ciliates of each taxon/morpho-type were measured at x320 magnification with a calibrated ocular micrometer. Assuming standard geometric shapes, biovolume measurements were made for

each taxon/morpho-type and standing stock was measured with the conversion factor 0.19 pg C μm^3 (Putt and Stoecker 1989).

Samples for determination of chlorophyll *a* concentration were filtered onto 0.2-µm and 5.0-µm polycarbonate membranes. These membranes were then placed into centrifuge tubes containing 10 ml of 90%-acetone (10% distilled water) to extract chlorophyll *a* from intact phytoplankton cells concentrated on the membranes. After 24 h in the dark at 4° C, the acetone extractions were centrifuged at ~3500 rpm for 4-5 min and decanted into appropriate glass tubes. Each extraction was analyzed with at Turner 10-AU fluorometer (Parsons et al. 1984) that was routinely calibrated with a pure chlorophyll-*a* standard (Sigma Chemical Co.) to measure sample chlorophyll concentration from fluorescence. Readings were taken before and after the addition two drops of 5% HCL (v/v), the difference providing a correction for the contribution of phaeo-pigments to the initial concentration (Parsons et al. 1984). The concentration of <5 µm chlorophyll in each sample replicate was determined by the difference of total chlorophyll (0.2 µm membrane) and >5 µm chlorophyll (5.0 µm membrane) concentration. Qualitative assessment of the phytoplankton >10 µm was made from the settled Lugols-fixed samples. Phytoplankton carbon standing stock was estimated with the carbon:chlorophyll conversion of 45:1, a compromise of the range 27:1 to 67:1 suggested by Riemann et al. (1989) based on results of natural phytoplankton populations in eutrophic waters. As with most carbon:chlorophyll

conversions, it is likely that this multiplier underestimates the contribution of cyanobacteria to phytoplankton carbon (Joint and Pomroy 1986).

Data Analysis

Correlation analyses (Pearsons product-moment) of untransformed data were performed to evaluate the relationships between ciliate standing stocks and size-fraction chlorophyll concentration. Functional relationships between biological (ciliate and phytoplankton biomass and abundance) and individual physical parameters (temperature, salinity and upwelling index) were determined with geometric mean regression analysis (Model II), as both independent and dependent variables were subject to error (Ricker 1973; Laws and Archie 1981). Regressions of log-transformed chlorophyll data on multiple physical variables were calculated for biological and physical variable comparisons yielding significant correlation coefficients. All statistical tests were conducted with Statistica (5.0) software program at the significance level $\alpha = 0.05$. Error bars about the sample date means represent ±1 standard deviation (SD).

Results

Physical Data

Surface water temperature and salinity at the sample site ranged 8.8-16.9 °C and 18.4-33.9 PPT, respectively (Fig. 2A; Table 2). Winter rainfall in 1999 diminished as spring progressed into early summer, whereas temperature, salinity and density steadily increased, though temperature at a lesser rate (Fig. 2B; Table 2). Temperature climbed to the highest recording of the sample year

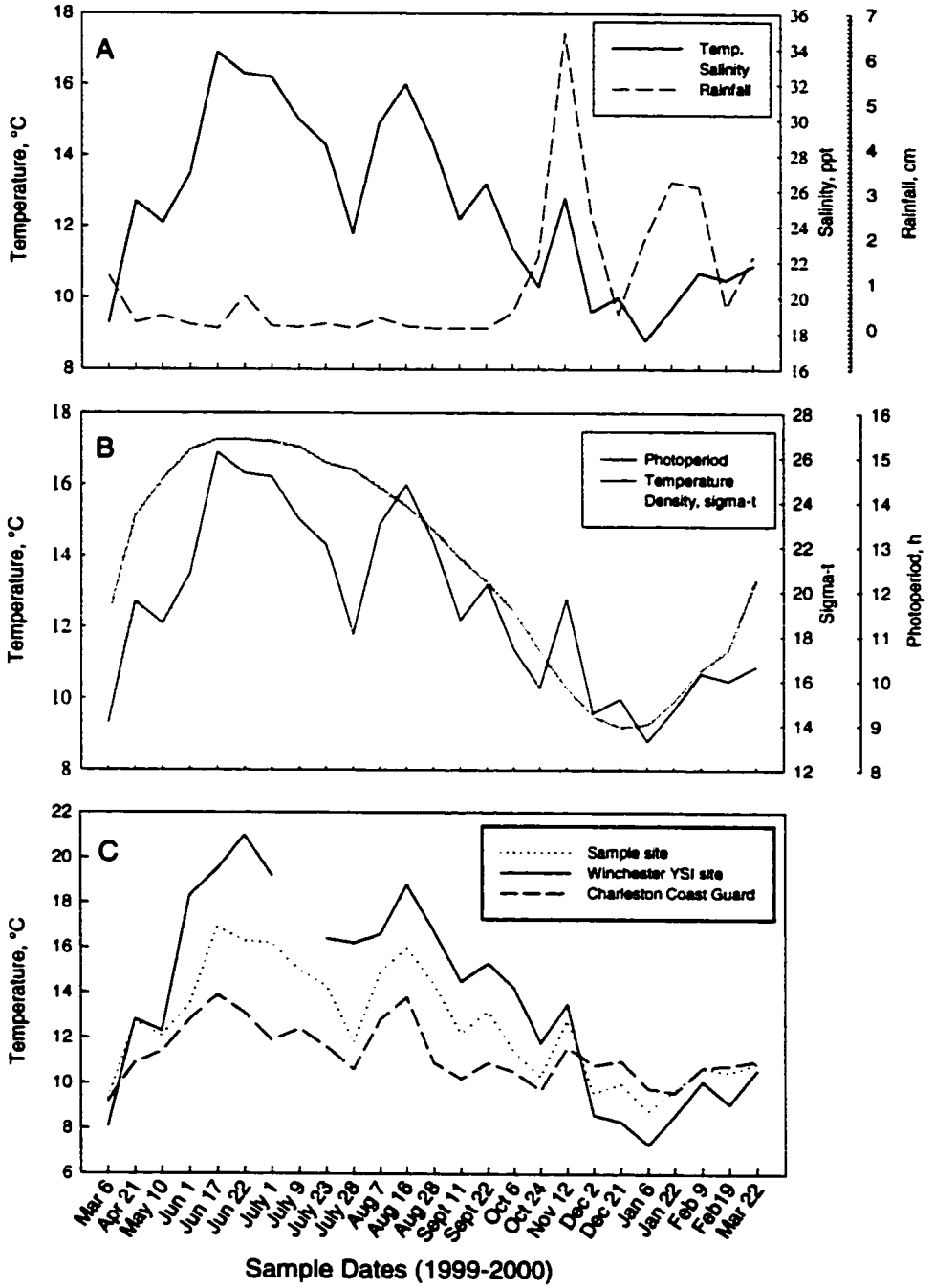

Fig. 2. Temporal profiles of physical variables. A, Sample site surface water temperature and salinity, and accumulated rainfall for the previous 5 days; B, sample site water temperature, density (sigma-t) and photoperiod; C, high tide water temperature at Charleston Coast Guard Station, the sample site and at the Winchester SWMP site.

Table 2. Annual and seasonal sample date statistics for the physical variables of temperature, salinity, and density (sigma-t) at the sample site [average ± 1 SD (range)]. Annual, n = 25; April-September, n = 14; October-March, n = 11

Parameters	Annual (1999 - 2000)	April - September	October - March
Temperature, °Celsius	12.6 ± 2.5 (8.8 - 16.9)	14.3 ± 1.7 (11.8 - 16.9)	10.4 ± 1.1 (8.8 - 12.8)
Salinity, ppt	29.2 ± 4.3 (18.4 - 33.9)	31.2 ± 3.0 (23.5 - 33.7)	26.6 ± 4.4 (18.4 - 33.9)
Density, sigma-t	21.7 ± 3.0 (14.1 - 25.6)	22.9 ± 2.2 (17.5 - 25.2)	20.1 ± 3.2 (14.1 - 25.6)

by 17 June. Compared to salinity and density, which showed relatively little fluctuation during the summer following the spring rise, water temperature oscillated markedly. Between 17 June and 28 July, it fell steadily over 5 degrees to 11.8 °C and then increased over the next two sample dates to 16.0 °C (16 Aug.). Following this second summer peak, water temperature decreased through early autumn to 10.3 °C (24 Oct.). With the initiation of the rainy season during late October and early November, density dropped dramatically while water temperature rose more than 2 °C. During the fall and winter, temperature varied positively with rainfall, whereas salinity/density showed a general negative relationship with precipitation. Despite the temporal variability in water temperature, this variable was significantly correlated with photoperiod (hours between sunrise and sunset for each sample day) during the sample year ($r = 0.83$, $n = 25$, $p<0.001$; Fig. 2B)

A comparison of high tide water temperature at the Coast Guard station (range: 9.2-13.9°C), the Winchester SWMP site (range: 7.3-21.0°C; no datum recorded for 9 July) sample site temperature (Fig. 2C) revealed the formation of a distinct temperature gradient along the South Slough between April and November. The gradient developed quickly to a maximum by late June when the 21°C temperature at the Winchester SWMP site was nearly 5°C and 8°C higher than the surface water at the sample site and the Coast Guard station, respectively. The gradient decreased with the progression of summer to fall and

by December had switched its polarity with cooler temperatures at the head of the slough.

The average hourly upwelling indices for 1, 3 and 5 days prior to each sample date are plotted in Fig. 3. The degree of oscillation between sample dates dampened with the day step-increase of computed averages. In general, indices during the spring, summer and early fall were positive with several peaks in magnitude and suggest that this was a period of fluctuations between episodes of intense, weak and relaxed upwelling (Small and Menzies 1981). Between October and February, indices were generally negative and imply that this was period of downwelling circulation over the coastal shelf.

Chlorophyll Dynamics

Total chlorophyll concentration (TCHL) for the sample year ranged 0.3-6.4 $\mu g\ l^{-1}$ (avg. 2.1; Fig. 4, Table 3); <5-µm chlorophyll concentration (<5CHL) ranged <0.1-3.0 $\mu g\ l^{-1}$ (avg. 0.93). The percent contribution of <5CHL to TCHL for the sample year averaged 42% and ranged 14-83%. Between April and September (spring/summer period), TCHL was >1.5 $\mu g\ l^{-1}$ on all sample dates. Two distinct modes during this period were observed in early June and early August (3.5 and 6.4 $\mu g\ l^{-1}$). An analogous bimodal distribution was less clear for <5CHL, although comparable high values for this fraction were determined in late June and mid August (2.9 and 3.0 $\mu g\ l^{-1}$). A prevalence of several genera of chain-forming centric (*Chaetoceros*, *Thalassiosira*, *Asterionella*) and pennate (*Navicula* sp., *Pseudo-nitzchia* sp.) diatoms was observed during this period, and

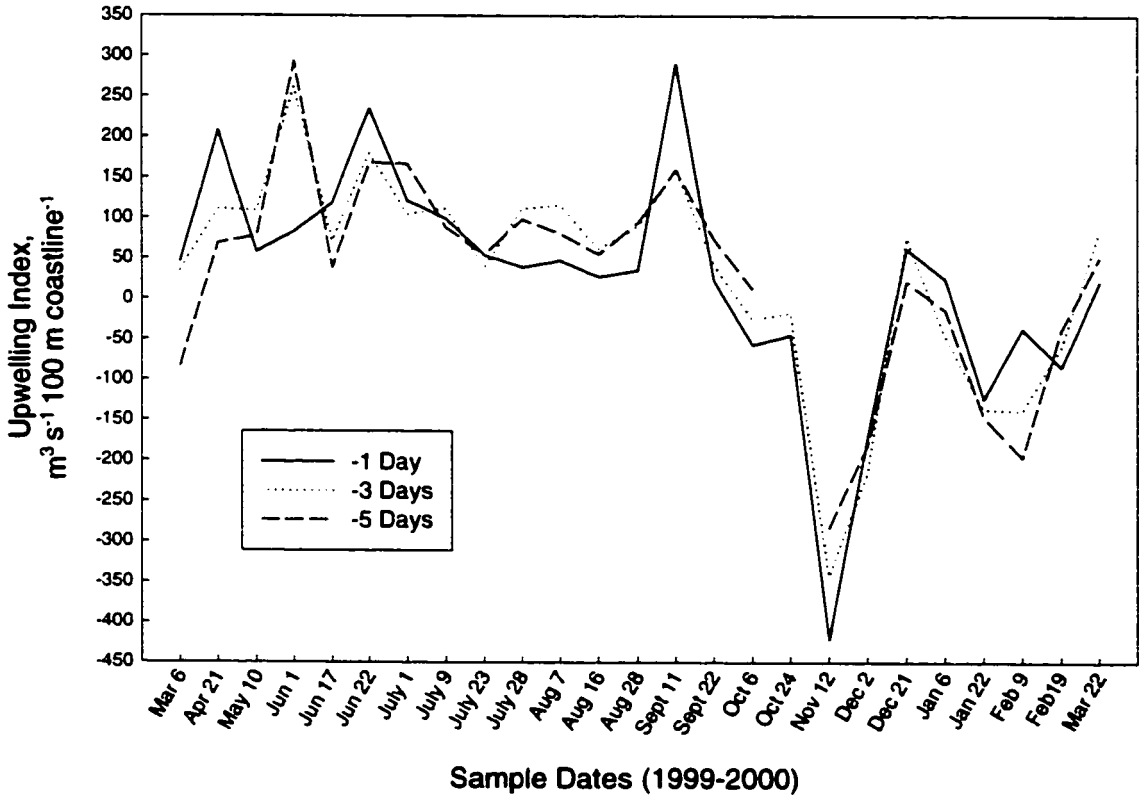

Fig. 3. Average hourly upwelling indices of 1, 3, and 5 days prior to each sample date. Positive values indicate offshore flow (upwelling) and negative values indicate onshore flow. No data was available on October 8 and 24 for the -5 day calculation.

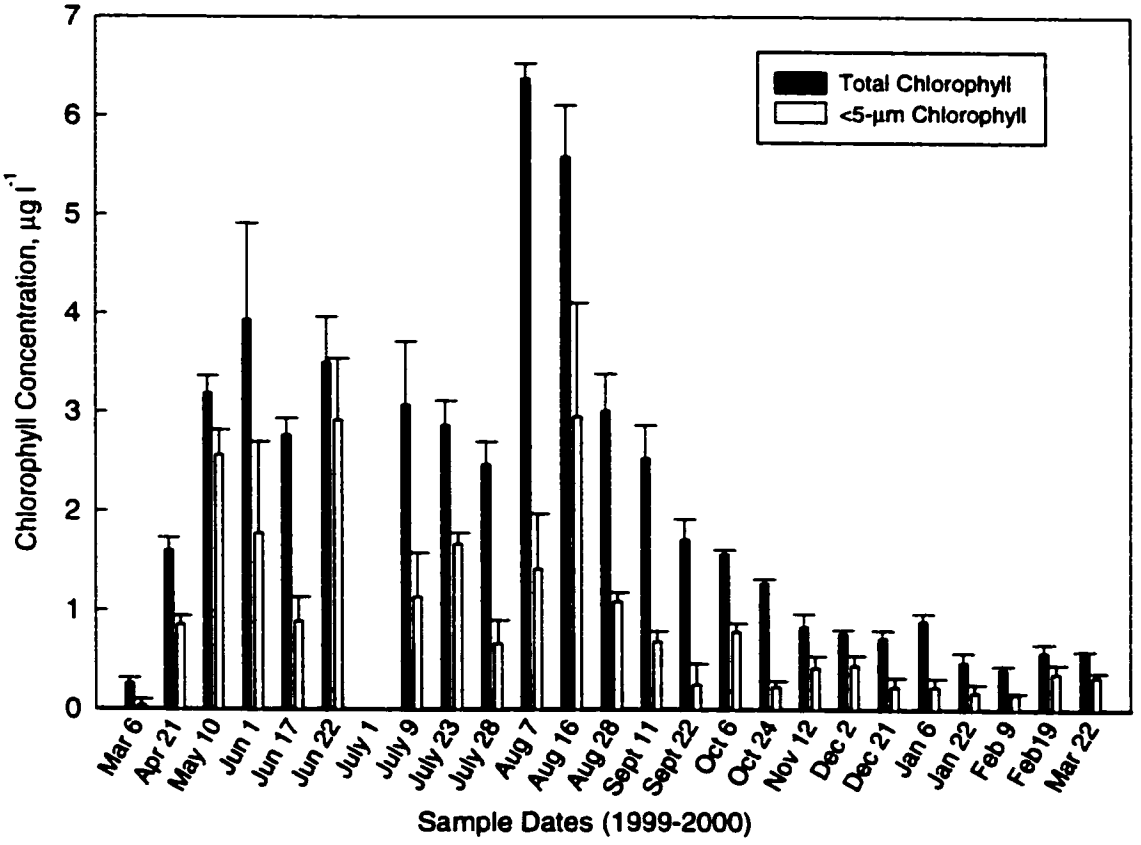

Fig. 4. Total and <5-µm chlorophyll concentrations at the sample site. No samples were collected on July 1. N = 3 and error bars represent ±1 SD.

Table 3. Annual and seasonal sample date statistics for biological variables at the sample site [mean-average ± 1 SE (range)]. Chlorophyll: annual, n = 24; April-September, n = 13; October-March, n = 11. Ciliate abundance: annual, n = 25; April-September, n = 14; October-March, n = 11. Ciliate carbon: annual, n = 20; April-September, n = 10; October-March, n = 10.

Parameters	Annual (1999-2000)	April-September	October-March
Total Chlorophyll, $\mu g\ l^{-1}$	2.1 ± 0.3 (0.3 - 6.4)	3.3 ± 0.4 (1.6 - 6.4)	0.8 ± 0.1 (0.3 - 1.6)
>5 µm Chlorophyll, $\mu g\ l^{-1}$	1.2 ± 0.2 (0.2 - 5.0)	1.8 ± 0.3 (0.6 - 5.0)	0.5 ± 0.1 (0.2 - 1.0)
<5 µm Chlorophyll, $\mu g\ l^{-1}$	0.9 ± 0.2 (0.04 - 3.0)	1.5 ± 0.3 (0.3 - 3.0)	0.3 ± 0.1 (0.04 - 0.8)
Total Naked Ciliates, cells ml^{-1}	19.5 ± 3.7 (2.5 - 90.5)	27.1 ± 5.8 (8.0 - 90.5)	9.9 ± 1.4 (2.5 - 17.9)
Total Naked Ciliates, $\mu g\ C\ l^{-1}$	19.5 ± 3.9 (4.6 - 77.6)	28.6 ± 6.6 (6.2 - 77.6)	10.3 ± 1.4 (4.6 - 20.0)
>20 µm Naked Ciliates, cells ml^{-1}	14.8 ± 3.3 (1.9 - 80.5)	21.1 ± 5.3 (6.4 - 80.5)	6.7 ± 1.0 (1.9 - 12.7)
>20 µm Naked Ciliates, $\mu g\ C\ l^{-1}$	18.5 ± 3.8 (4.5 - 75.1)	27.4 ± 6.4 (5.9 - 75.1)	9.7 ± 1.3 (4.5 - 18.6)
<20 µm Naked Ciliates, cells ml^{-1}	4.7 ± 0.7 (0.6 - 11.8)	6.0 ± 1.0 (1.5 - 11.8)	3.0 ± 0.6 (0.6 - 7.1)
<20 µm Naked Ciliates, $\mu g\ C\ l^{-1}$	1.0 ± 0.2 (0.06 - 2.5)	1.2 ± 0.2 (0.4 - 2.5)	0.7 ± 0.2 (0.06 - 1.4)
Tintinnids, cells ml^{-1}	1.2 ± 0.2 (0.02 - 3.5)	1.7 ± 0.2 (0.5 - 3.5)	0.6 ± 0.3 (0.02 - 2.0)
Tintinnids, $\mu g\ C\ l^{-1}$	1.4 ± 0.2 (0.1 - 3.9)	1.9 ± 0.4 (0.5 - 3.9)	0.9 ± 0.3 (0.1 - 2.6)

diatoms were particularly prominent at the TCHL peaks. Between October and March (fall/winter period), TCHL was generally <1.5 μg l^{-1} except for one sample date at the transition between spring/summer and fall/winter periods of the year (6 October). Diatoms were generally absent and the phytoplankton shifted to a dominance of nanoplankton (<20 μm). Cryptomonads, spanning a size range of ~6 to 18 μm, were a prominent and abundant component of the assemblage during the late fall and winter months (also see Hughes 1997). The contribution of <5CHL to TCHL during the two seasonal periods was comparable: April-September, 43%, and October-March, 40%.

Photoperiod explained 37 and 43% of the variation of log-transformed values of < and >5CHL (simple linear regressions; Table 4). Geometric-mean regressions revealed that the fluctuations of sample-site water temperature could account for 49 and 37% of the variation of < and >5CHL data, respectively. Density (sigma-t) explained 33% of the >5CHL variation but had no significant influence on <5CHL data distribution. Oscillation of the average hourly upwelling index for the previous 6 days of each sample date could explain 44% and 24% of the variation in < and >5CHL data distribution (Table 4). Multiple regressions of log-transformed chlorophyll data vs. different combinations of physical variables (i.e., photoperiod, sample-site temperature and density, and cumulative averages of hourly upwelling indices for the previous 7 days) were performed to discern whether multiple physical variables could explain more of the variation in the size-fraction chlorophyll data than single independent variables alone. Because

Table 4. Regression analysis results of size-fraction chlorophyll vs. temperature and density (sigma-t). N = 24 for each analysis.

Independent Variable	Regression Model	Dependent Variable	F (1, 22)	Slope	R^2
Photoperiod	I	>5-µm Chl.	16.31	0.65***	0.43
		<5-µm Chl.	13.11	0.61**	0.37
Temperature	II	>5-µm Chl.	13.11	0.61**	0.37
		<5-µm Chl.	21.33	0.70***	0.49
Sigma-t	II	>5-µm Chl.	10.99	0.58**	0.33
		<5-µm Chl.	1.46	0.25	0.06
Upwelling Index -1D hr avg.	I	>5-µm Chl.	4.33	0.41*	0.17
		<5-µm Chl.	1.09	0.22	0.05
Upwelling Index -2D hr avg.	I	>5-µm Chl.	8.48	0.53**	0.28
		<5-µm Chl.	3.00	0.35	0.12
Upwelling Index -3D hr avg.	I	>5-µm Chl.	9.94	0.56**	0.31
		<5-µm Chl.	3.00	0.37	0.12
Upwelling Index -4D hr avg.	I	>5-µm Chl.	14.27	0.63**	0.39
		<5-µm Chl.	7.00	0.49*	0.24
Upwelling Index -5D hr avg.	I	>5-µm Chl.	14.95	0.65***	0.42
		<5-µm Chl.	6.62	0.49*	0.24
Upwelling Index -6D hr avg.	I	>5-µm Chl.	16.44	0.66***	0.44
		<5-µm Chl.	6.61	0.49*	0.24
Upwelling Index -7D hr avg.	I	>5-µm Chl.	0.179	0.09	0.01
		<5-µm Chl.	2.49	0.32	0.10

*p<0.05, **p<0.01, ***p<0.001

several of the physical variables were multicollinear, consideration was given only to those combinations of variables whose addition and deletion did not lead to unreasonable changes in the magnitude or sign of the partial regression coefficients nor to large standard errors of these coefficients (Zar 1996). Analyses with physical variables that yielded significant partial regression coefficients could not explain more variation in the <5CHL data than temperature alone (Table 5). Temperature, density and the average upwelling index of the previous 3 days accounted for 74% of the variation of the >5CHL data set. Of the three physical variables, a comparison of the standardized partial regression coefficients in all regression computations revealed density to have the highest degree of influence on >5CHL variability.

Ciliate Dynamics

Abundance of naked ciliates (NCIL) and tintinnids are plotted in Fig. 5A. Mean abundance of NCIL ranged from a low of 2.8 cells ml^{-1} on 22 March 2000 to a high of 91 cells ml^{-1} on 22 June (avg. 20; Table 3). They were generally an order of magnitude more abundant than tintinnids, which ranged 0.2-3.5 cells ml^{-1} (avg. 1.2 cells ml^{-1}; Table 3). Two notable abundance peaks of NCIL coincided with the two maxima of <5CHL during this period. The percent contribution of < and >20-µm NCIL to the total NCIL abundance over the sample year (Fig. 5b) averaged 26% and 74% respectively (Table 3). The pattern of tintinnid abundance was well correlated with the distribution of <20-µm NCIL ($r = 0.70$, $p<0.001$). A distinct minimum of tintinnid abundance was observed on 28

Table 5. Multiple regression results of log-transformed < and >5-µm chlorophyll vs. physical variables (UI=upwelling index). For all regressions n = 24. Regressions of two independent variables, df = 2, 21; and of three independent variables, df = 3, 20)

Independent Variables	Dependent Variable	F	Multiple R	Std. Partial Regression Coefficients	R^2
Temperature Sigma-t	Log [<5-µm Chlorophyll]	10.67	0.71***	0.63** 0.15	0.50
Photoperiod Sigma-t	Log [<5-µm Chlorophyll]	7.88	0.66***	0.51** 0.26	0.43
Temperature Sigma-t	Log [>5-µm Chlorophyll]	21.79	0.82***	0.43** .052**	0.67
Photoperiod Sigma-t	Log [>5-µm Chlorophyll]	23.68	0.83***	0.43*** 0.56**	0.69
Temperature Sigma-t UI (-1D hrly avg.)	Log [>5-µm Chlorophyll]	16.20	0.84***	0.36* 0.53** 0.20	0.71
Temperature Sigma-t UI (-2D hr avg.)	Log [>5-µm Chlorophyll]	18.30	0.86***	0.31* 0.52*** 0.27*	0.73
Temperature Sigma-t UI (-3D hr avg.)	Log [>5-µm Chlorophyll]	18.78	0.86***	0.31* 0.50** 0.28*	0.74
Temperature Sigma-t UI (-4D hr avg.)	Log [>5-µm Chlorophyll]	18.00	0.85***	0.32* 0.46** 0.28	0.73
Photoperiod Sigma-t UI (-1D hr avg.)	Log [>5-µm Chlorophyll]	15.47	0.84***	0.37* 0.57*** 0.10	0.70
Photoperiod Sigma-t UI (-2D hr avg.)	Log [>5-µm Chlorophyll]	16.40	0.84***	0.29 0.58*** 0.13	0.71
Photoperiod Sigma-t UI (-3D hr avg.)	Log [>5-µm Chlorophyll]	16.29	0.84***	0.29 0.57*** 0.19	0.71
Photoperiod Sigma-t UI (-4D hr avg.)	Log [>5-µm Chlorophyll]	15.97	0.84***	0.31 0.54*** 0.18	0.71

*p<.05; **p<.01; ***p<.001

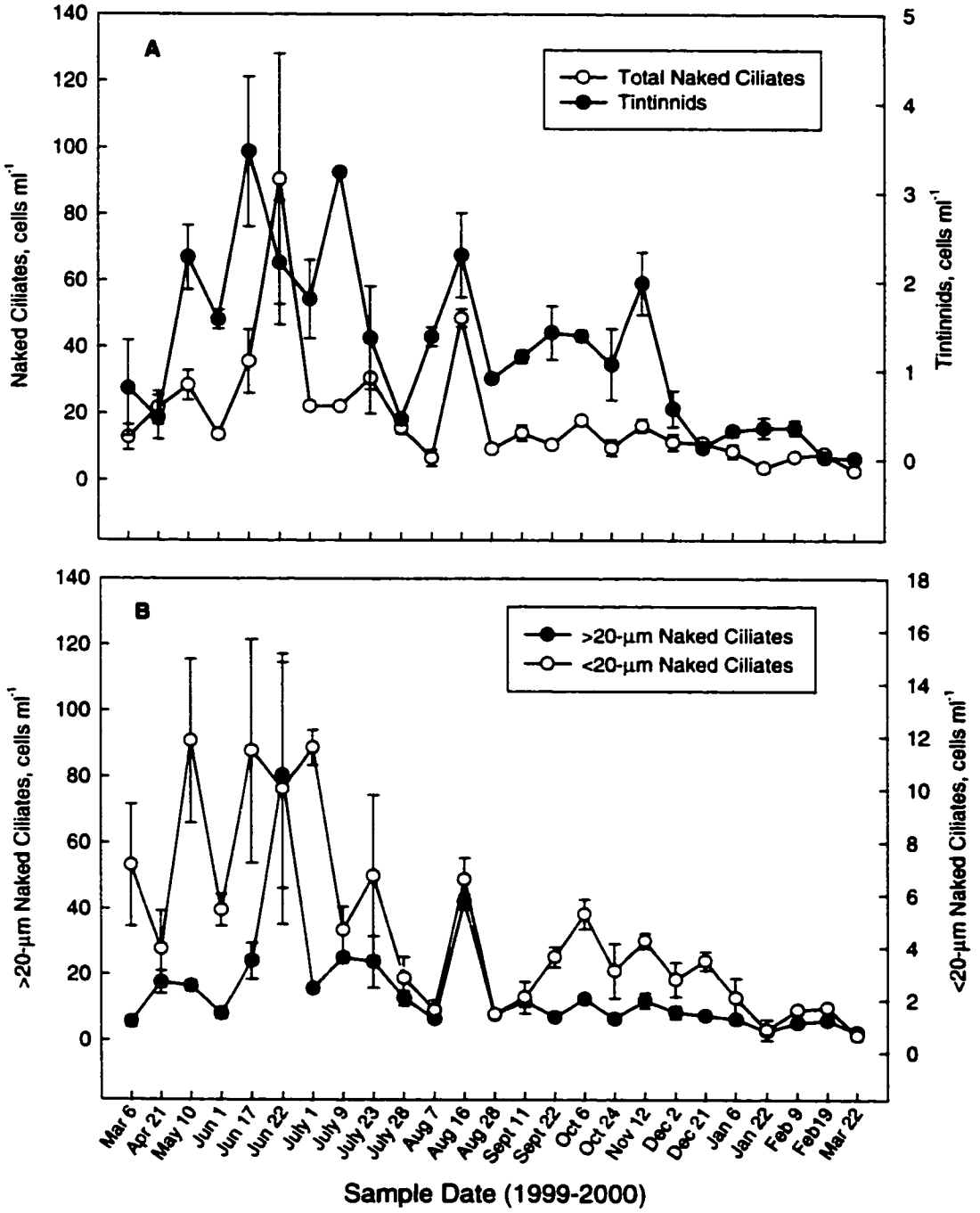

Fig. 5. Ciliate abundance. A, total naked ciliate and tintinnid abundance; B, < and >20-µm naked ciliate abundance. Note the differences in scale along the y-axis. N = 3, except for Aug. 28 when n = 1. Error bars represent ±1 SD.

July, coinciding with the mid-summer drop in water temperature at the sample site.

Ciliate Composition

The >20-µm fraction of NCIL was dominated by many *Strombidium*-like ciliates throughout the year. The genera *Strobilidium*, *Laboea*, and *Tontonia* were observed mostly in the spring and summer months. The great majority of the >20-µm NCIL were in the size range of 20-40 µm. Ciliates >40 µm, particularly *Strombidinopsis* sp., were observed periodically at low abundance in the spring and summer. *Halteria, Lohmaniella* and *Balanion* spp. were major contributors to <20-µm NCIL assemblage. Low numbers (<100 cells l^{-1}) of 'predatory' ciliates of the genera *Didinium* and *Tiarina* were observed during the sample year, although *Tiarina* reached concentrations of 9 cells ml^{-1} on 1 July. The tintinnid assemblage was comprised mostly of species in the genera *Tintinnopsis, Stenosemella, Eutintinnus, Favella* and *Heliostomella*, with a greater diversity observed in the spring and early summer.

Ciliate and Phytoplankton Biomass

Total ciliate carbon (CILC) for the dates between 22 June 99 and 22 March 00 (n=19) is plotted with total and <5-µm chlorophyll carbon (and <5CHLC) in Fig. 6A. TCHLC ranged 18.4-287 µg C l^{-1} (avg. 18.4); <5CHLC ranged 7.1-133 µg C l^{-1} (avg. 38.3). Total CILC ranged 4.7-80 µg C l^{-1} (avg. 21) during this period. The maximum and minimum ciliate carbon amounts occurred on the first and last dates of the sample year, respectively. NCil made up >80%

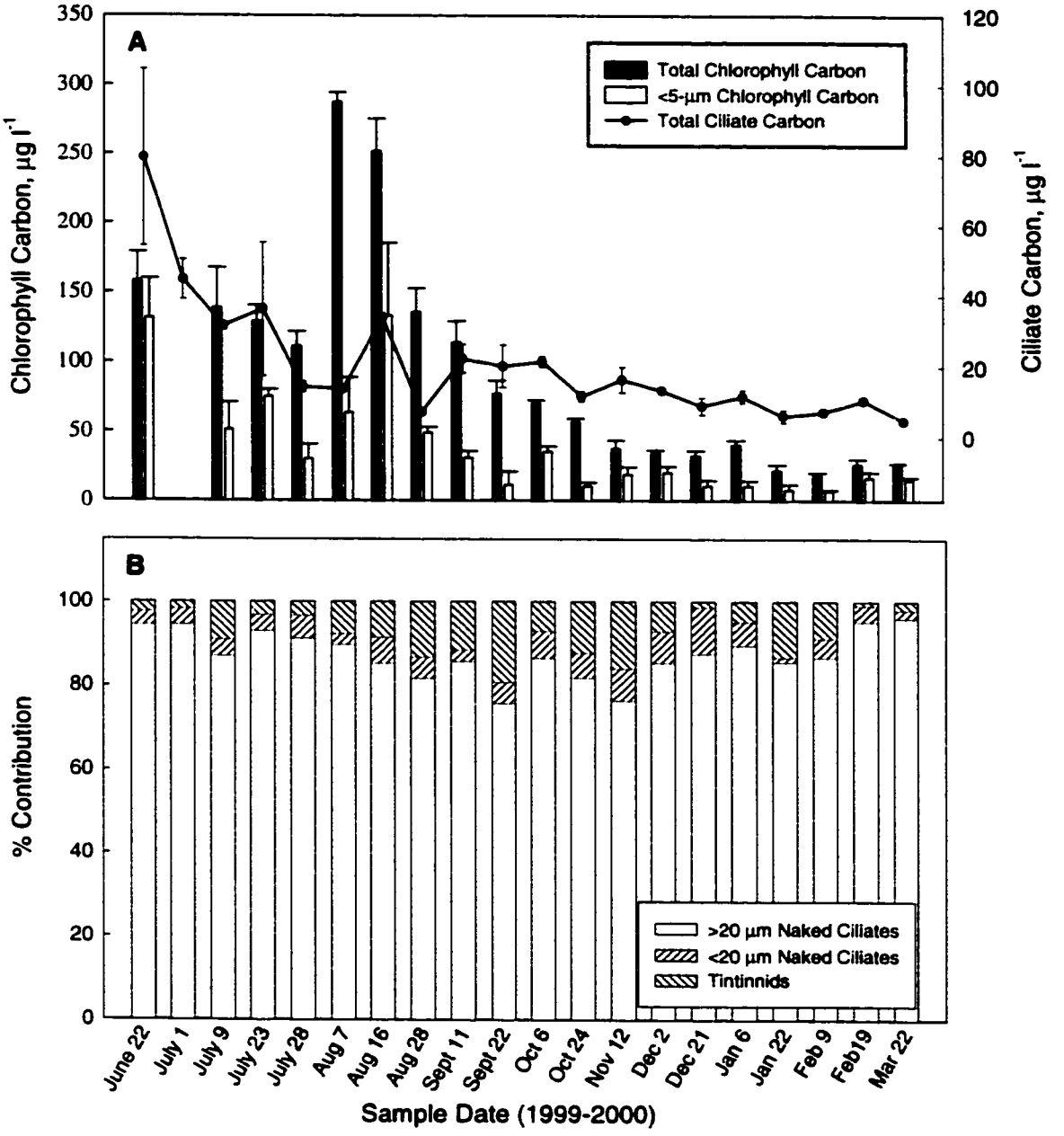

Fig. 6. Ciliate and Chlorophyll Carbon. A, total ciliate and chlorophyll carbon concentrations. N = 3 except for Aug. 28 when n = 1 for ciliates; error bars represent ±1 SD. No chlorophyll samples were taken on July 1. Carbon:chlorophyll ratio = 45; B, contributions of < and >20 µm naked ciliates and tintinnids to total ciliate carbon.

(avg. 92%) of CILC during the sample year (Fig. 6B; Table 3); of this, 75-90% (avg. 87%) was made up by >20-µm NCIL and 1-11% (avg. 5%) by <20-µm NCIL. Tintinnids contributed 1-19% (avg: 8%) to CILC (Table 3).

The ratio CILC:TCHLC ranged 0.04-0.50 (avg. 0.26; Fig. 7). The ratio CILC:<5CHLC ranged 0.15-1.8 (avg. 0.70). Comparison of these ratios with regards to spring/summer (April-September; n = 9) and fall/winter (October-March; n = 10) periods of the year generally revealed higher ratios during the latter period.

Correlation Analyses

Correlation coefficients of ciliate abundance and carbon vs. chlorophyll standing stocks are shown in Table 6. Statistically significant but weak correlations were determined for both NCIL and >20-µm NCIL vs. TCHL ($r = 0.46$ and $r = 0.45$). The correlation between tintinnid abundance and TCHL was comparably stronger ($r = 0.57$). No correlation of statistical significance was determined between <20-µm NCIL and TCHL. Relatively strong correlations were calculated between cell numbers of each ciliate group/size class and <5CHL. The relationships for NCIL and >20-µm NCIL vs. <5CHL were more robust ($r = 0.77$ and $r = 0.75$) than for <20-µm NCIL and tintinnids vs. <5CHL ($r = 0.62$ and $r = 0.60$).

In terms of carbon biomass, no significant correlations were determined between the carbon of any ciliate group/size-fraction and TCHLC. Strong positive correlations were found for naked ciliate carbon (NCILC) and each NCILC size

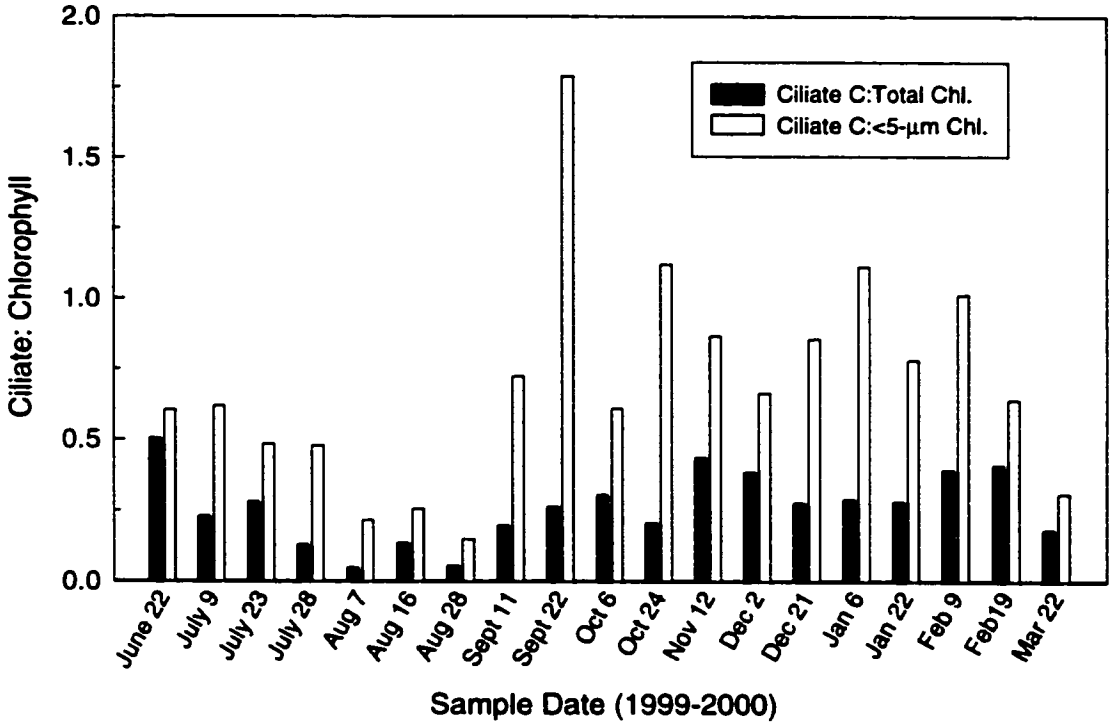

Fig. 7. Ratios of total ciliate-carbon:chlorophyll (total and <5 µm) carbon for the sample year.

Table 6. Correlations between abundance (n=24) and carbon (n=19) of ciliates and chlorophyll.

	Total Chlorophyll		>5-µm Chlorophyll		<5-µm Chlorophyll	
	Abundance	Carbon	Abundance	Carbon	Abundance	Carbon
Total NCIL	.46*	.44	.07	.40	.77***	.81***
>20-µm NCIL	.45*	.44	.07	.41	.75***	.81***
<20-µm NCIL	.32	.40	-.01	.34	.62**	.76***
Tintinnids	.57**	.37	.39	.24	.60**	.34

*p<.05; **p<.01; ***p<.001

group vs. <5CHLC, but no significant correlation was calculated between tintinnid carbon and this chlorophyll carbon fraction. Overall, no significant correlations were determined between the abundance/carbon of any group/size class of ciliates and >5CHL standing stocks.

Correlation analyses were performed on data subsets delineated in terms of spring/summer and fall/winter periods of the sample year (Table 7). During the spring/summer period, the abundance and carbon of NCIL and each NCIL size class were positively correlated with <5-μm chlorophyll; no such relationships were determined for tintinnids. Provided the omission of the NCIL abundance vs. TCHL and <5CHL correlates for one sample date (6 March 1999), which were outside the 95% confidence interval for both plots (Fig. 8), the abundance and carbon of NCIL and each NCIL size class were positively correlated with both TCHL and <5CHL during the fall/winter period. No significant correlations were determined between the abundance and biomass of any ciliate group/size class and >5CHL.

Abundance and carbon of NCIL and tintinnids were positively correlated with temperature for the sample year. Model II linear regressions were conducted to determine the influence of temperature on the variability of the ciliate abundance and carbon. Temperature explained 40 and 60% of the variation of total NCIL and tintinnid abundance, and 49 and 23% of the variation of NCIL and tintinnid carbon (Table 8).

Table 7. Seasonal correlations of ciliates and chlorophyll standing stocks. April-September: abundance, n=13; carbon, n=9. October-March: abundance, n=11; carbon, n=10. Data for 6 March 1999 were not included in the analysis.

April-September	Total Chlorophyll		>5-µm Chl.		<5-µm Chl.	
	Abund.	Carbon	Abund.	Carbon	Abund.	Carbon
Total NCIL	.13	.04	-.39	-.51	.70**	.73*
>20-µm NCIL	.15	.04	-.35	-.51	.67*	.72*
<20-µm NCIL	-.02	.11	-.47	-.49	.58*	.80***
Tintinnids	.22	-.18	-.004	-.19	.35	-.04
October-March	**Total Chlorophyll**		**>5-µm Chl.**		**<5-µm Chl.**	
	Abund.	Carbon	Abund.	Carbon	Abund.	Carbon
Total NCIL	.71*	.81**	.45	.51	.72*	.85***
>20-µm NCIL	.67*	.81**	.39	.49	.73*	.85***
<20-µm NCIL	.78**	.70*	.57	.46	.66*	.68*
Tintinnids	.62	.50	.47	.40	.51	.39

*p<.05; **p<.01; ***p<.001

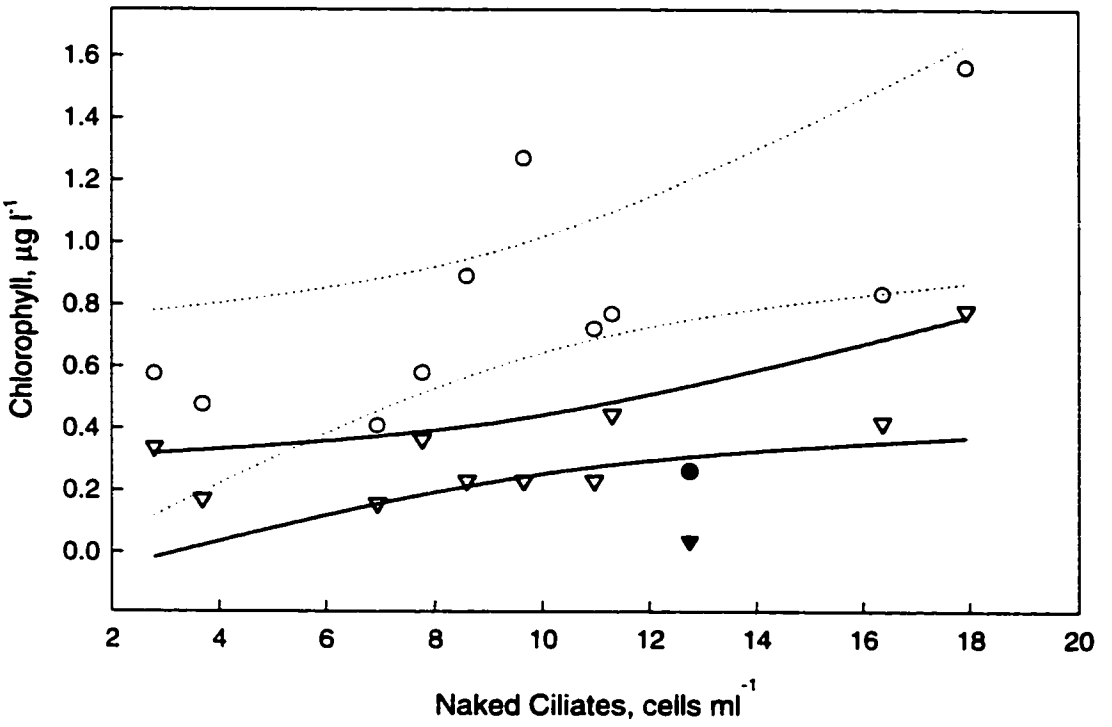

Fig. 8. Total (circles) and <5-µm (inverted triangles) chlorophyll concentration vs. naked ciliate abundance for sample dates in October-March. Curved lines are the 95% confidence intervals for each plot. Filled points are data correlates for 6 March 1999, which were omitted from the correlation analysis.

Table 8. Results of geometric mean regressions of ciliate abundance (n=25) and carbon (n=19) vs. sample site temperature. NCIL, nake ciliates.

Y, X	Ciliate Abundance			Ciliate Carbon		
	F (1, 23)	Slope	r^2	F (1, 17)	Slope	r^2
NCIL, Temp	15.61	0.64***	0.40	25.03	0.70***	0.49
>20-µm NCIL, Temp	14.29	0.62***	0.38	27.89	0.70***	0.46
<20-µm NCIL, Temp	7.95	0.51**	0.26	5.30	0.63**	0.39
Tin, Temp	34.04	0.77***	0.60	2.71	0.48*	0.23

*p<.05; **p<.01; ***p<.001

Discussion

Measurements of ciliate standing stocks presented here suggest that ciliates are an abundant and productive component of the South Slough planktonic ecosystem. In comparison with the findings of other studies in a diversity of marine and estuarine environments, the abundance average and range of naked ciliates in the South Slough are very high, whereas these statistics for tintinnids fall within the middle of the estimates (Table 9). Carbon concentrations of South Slough ciliates are within the range of data for other estuarine and nearshore regions (Table 10) but overall suggest that the ciliate standing stocks in the South Slough are also high. The contribution of tintinnids to total ciliate abundance and carbon in this study averaged 6% and 8% respectively. Relatively low contributions of tintinnids to ciliate standing stocks were also observed by Fessenden & Cowles (1994) in coastal waters off Oregon and by Strom et al. (1993) in the subarctic Pacific. Although it is frequently the case that naked ciliates dominate the ciliate assemblage and standing stock in marine systems (Smetecek 1981; Revelante and Gilmartin 1983; Gifford 1988; Vaqué et al. 1997), higher contributions of tintinnids have been reported. Beers et al. (1980) reported a tintinnid contribution of 13% and 26% to total ciliate abundance and carbon in Southern California nearshore waters. The annual dominance of the ciliate assemblage by tintinnids was observed and quantified in Narragansett Bay (Verity 1987) and in Long Island Sound (Capriulo and Carpenter 1983).

Table 9. Abundance of ciliated protozoans in different oceanographic regions. Mean (range), cells l^{-1}.

Location	Naked Ciliates	Tintinnids	Author(s)
Coastal			
Limfjiord, Denmark	(1400-126,000)	(max: 4200)	Andersen & Sørensen 1986
Peru, upwelling region	(2200-16700)	170-1300	Beers et al. 1971
Celtic Sea	(2488-4000)		Burkill et al. 1987
Coastal Oregon USA	4100 (1200-7700)	<100	Fessenden & Cowles 1994
Baltic Sea	(7300-230000)		Kuuppo-Leinikki 1990
Coastal Washington USA	(2700-27000)	(360-2000)	Landry & Hassett 1982
Grand Banks	(2700-3870)	(170-990)	Paranjape 1990
Northern Adriatic	(18-39280)	(1-16880)	Revelante & Gilmartin 1983
Saanich Inlet, British Columbia winter conditions	(120-2100)		Takahashi & Hoskins 1978
Coastal NW Mediterranean Sea	1884 (0-11202)	629 (0-7436)	Vaqué et al. 1997
Estuarine/Bay			
Kariega Estuary, South Africa	(450-1950)	<100	Froneman & McQuaid 1997
Halifax Harbour, Canada	(2680-11360)	(0-1440)	Gifford 1988
Schelde Estuary, Belgium	14200 (max: 120000)	1800 (max: 2900)	Muylaert et al. 2000
South Slough, Oregon USA	19500 (2500-90500)	1200 (20-3500)	This study
Narragansett Bay, USA	530 (5-2803)	1500 (90-8181)	Verity 1986
Open Ocean			
North Atlantic	(1900-17200)	(60-220)	Gifford et al. 1995
North Pacific	(500-28000)	<150	Strom et al. 1993

Table 10. Standing stock of ciliates in different coastal and estuarine regions.

Location	Carbon biomass, mean (range) µg l^{-1} Naked Ciliates	Tintinnids	Total Ciliates	Author(s)
Coastal				
Peru, upwelling region			1.9 (0.7 - 4.8)	Beers et al. 1971
English Channel			4.7 (1.2-19.7)	Linley et al. 1983
Oregon USA, upwelling coast			11 (3 - 32)	Fessenden & Cowles 1994
Southampton Water, UK			(2.7 - 219)	Leakey et al. 1992
North Adriatic Sea Mixed Stratified	(0.04 - 3.6) (0.10 - 42)	(0.0-6.1) (0.02 - 46)	(0.06 - 7.8) (0.38 - 88)	Revelante & Gilmartin 1983*
Saanich Inlet, British Columbia winter conditions			0.6 (0.3 - 1.2)	Takahashi & Hoskins 1978
Estuarine				
The Solent , UK	0.5 (0.04 -2.9)	1.46 (0.6-3.2)	1.7 (0.6 - 6.1)	Burkill 1982
Chesapeake Bay, USA (surface mixed layer)			(8.4 - 16)	Dolan & Coats 1990
Schelde Estuary, Belgium	22 (max: 180)	2.1 (max: 180)	161 (max: 2300)	Muylaert et al. 2000
Damaricotta Estuary, Gulf of Maine USA	6.5 (0.02 - 30.4)	10.5 (0.04 - 47.7)	16.9 (1.0-23.0)	Revelante & Gilmartin 1987*
Lower St. Lawrence Estuary, Canada			11.2 (0.23 - 51.6)	Sime-Ngando et al. 1995
South Slough, Oregon USA	19.5 (4.6 - 77.6)	1.4 (.09-3.9)	20.8 (4.7 - 79.5)	This study

*Values converted from biovolume to carbon with the carbon:biovolume conversion factor of .19 (Choi & Stoecker, 1989)

The high standing stocks of ciliates in the South Slough, their general preference for food particles <10-µm in size (Rassoulzadegan 1982; Jonsson 1986), and the high and sustained contribution of phytoplankton <5-µm (= ultra-phytoplankton) to total phytoplankton standing stocks (~40%) throughout the sample year provide a basis of understanding for the observations that were made between ciliates and ultraphytoplankton in this study. The positive correlations between ciliate standing stocks and total chlorophyll between October and March reflect the downscaling of cell-size among phytoplankton >5-µm from large diatom-dominance in the spring/summer period to a prevalence of nanoflagellates (cells <20 µm) and picoplankton species. Revelante and Gilmartin (1983) and Burkill (1982) reported similar correlations between ciliate and nanophytoplankton populations over annual cycles. Verity (1986) demonstrated functional relationships between the grazing rates of a tintinnid-dominated microzooplankton assemblage and both <5- and <10-µm chlorophyll concentrations in Narragansett Bay. In contrast, ciliate biomass was positively correlated with >8-µm chlorophyll but not the <8-µm fraction in the Gulf of Alaska, in spite of significant positive relationships between microzooplankton grazing rates and both chlorophyll fractions (Strom et al. 2001).

The statistically significant and positive relationships between ciliates and chlorophyll described here suggest that a robust trophic interaction between these protistan grazers and small cell phytoplankton is operating in the South Slough planktonic community. The fact that significant positive and not negative

relationships were determined between ciliates and phytoplankton may appear to run contrary to what is theoretically considered a tightly coupled predator-prey system. That is, we may expect the periods of predator and prey density cycles to oscillate out of phase in terms of classical Lotka-Volterra predation model. Models such as this, however, typically operate in the absence of consumers of the predator species and with the assumption that the predators do not feed on several different prey species. Ciliates, however, are important prey for a diversity of metazooplankton species (Stoecker and Capuzzo 1990; Sanders and Wickam 1993) and are often preferred over phytoplankton food by many different copepod species (Kleppel 1993; Verity and Paffenhöfer 1996). In addition, it is now recognized that protozoan food is nutritionally important to benthic suspension feeders (Dupuy et al. 1999).

Ciliates utilize alternate food sources and nutritional modes that likely enable them to persist in the plankton through periods of low phytoplankton production and standing stocks. High rates of bacterivory by natural assemblages of ciliates have been documented (Sherr and Sherr 1987). Many ciliate species are omnivorous, utilizing both autotrophic and heterotrophic bacteria and flagellates as food (Rassoulzadegan et al. 1988; Ohman and Snyder 1991; Jürgens et al. 1996). Plastid retention and function by phagotrophic ciliates, particularly among members of the family Strombidiidae, is frequently observed (Jonsson 1987; Stoecker et al. 1989). Though not directly ascertained in this study, mixotrophy may explain in part the prevalence and relative

importance of many different *Strombidium*-like ciliates that were observed throughout the annual cycle in this study. Thus, it can be argued that the coupled fluctuations between ciliates and nano- and ultra-phytoplankton described here are a result of both community level interactions and diverse nutritional strategies of ciliates.

The interpretation of the correlation analysis made to this point is further supported by studying the relationship between ratio CILC:<5CHLC and <5CHLC during the different seasonal periods (Fig. 9). With the exception of one datum at the transition point between spring/summer and fall/winter periods (1.8; 22 September 1999), CILC:<5CHLC showed a relatively stable relationship over a wide range of <5CHLC during spring/summer period, suggesting that increases in <5CHLC were paralleled by increases in CILC. During the fall/winter period, CILC:<5CHLC showed a general increase with decreasing <5CHLC to approach 1.0 and exceed this value on three sample dates. Together, this suggests that, during the spring/summer period, ultraphytoplankton biomass was sufficient to support the observed CILC and these two components were tightly coupled. During the fall/winter period, the ultraphytoplankton biomass was not adequate to support the observed CILC and, consequently, ciliates ingested larger phytoplankton and/or heterotrophic food (i.e., bacteria, flagellates) in addition to ultraphytoplankton.

The positive coupling between ciliates and small phytoplankton was likely accentuated by a similar response to temperature variation observed in this

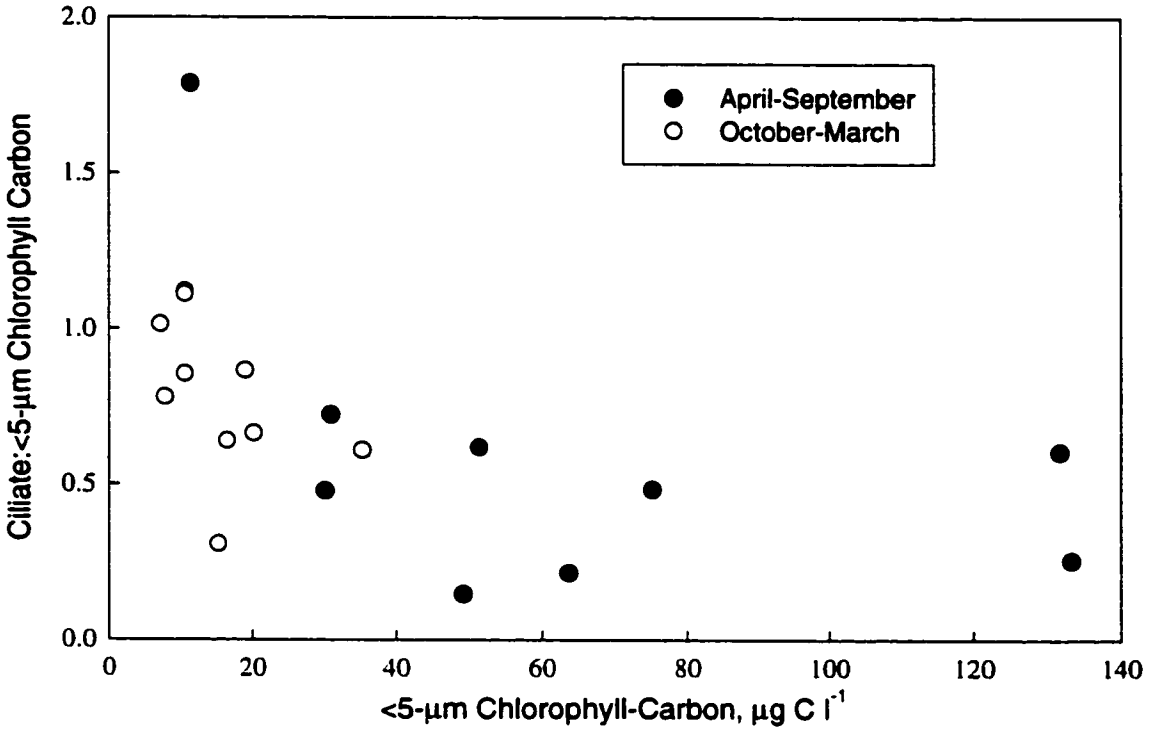

Fig. 9. Scatter plot of the ratio ciliate-carbon:<5-µm chlorophyll carbon vs. <5-µm chlorophyll carbon.

study. Temperature alone accounted for nearly half of the variation in ultraphytoplankton (<5CHL), and addition of density and upwelling index variables to the analysis could not both increase the power and yield significant standardized partial coefficients for these variables. The shallow bathymetry of the South Slough provides conditions for rapid warming of tidally advected coastal water (Fig. 2c) and a potentially enhanced thermal environment for phytoplankton growth. Verity (1986) described a strong, positive thermal effect on the growth of phytoplankton <10 μm over an annual cycle in Narragansett Bay. Laboratory and field studies have demonstrated the positive influence of temperature on picophytoplankton (cells 0.2-<2.0 μm), particularly cyanobacteria (Murphy and Haugen 1985; Andersson et al. 1994; Iriarte and Purdie 1994; Agawin et al. 1998). In a comprehensive survey of the published data from oceanic, coastal and estuarine areas, the results of Agawin et al. (2000) show that the contribution of picoplankton production to total primary production increases with increasing temperature.

Standing stocks of ciliates were also determined to be linear functions of temperature in this study. Previous studies have demonstrated temperature-enhanced growth (Finlay 1977; Muller and Geller 1993; Nielsen and Kiorboe 1994; Montagnes 1996) and feeding (Rassoulzadegan 1982; Aelion and Chisholm 1985; Sherr et al. 1988; but see Stoecker and Guillard 1982) among many ciliate taxa. My results imply that much of the ciliate production is fed in large part by ultraphytoplankton production. In addition, NCIL carbon and

ultraphytoplankton showed a statistically similar response to temperature across seasons (Tables 3 and 7). A comparable thermal response in growth by phytoplankton and ciliates may facilitate a rapid assimilation of phytoplankton production into secondary production in the South Slough (Verity 1986; Nielsen and Kiørboe 1994).

In contrast, the results of single and multiple independent variable regressions suggest that fluctuations of water density had more influence on the distribution of phytoplankton >5 μm (>5CHL) than temperature during the sample year. This likely reflects the seasonal contribution of diatoms to standing stocks of phytoplankton and their association with coastal upwelling processes. Croegner & Shanks (2001) studied the chlorophyll dynamics over several tidal cycles during spring and summer seasons from an anchor station midway between this study's sample site and the Coast Guard sample station (Fig. 1). They reported that density accounted for 40% (range: <1-79%) of weekly-scale variation in chlorophyll concentrations during the summer of 1996, although their measurements were based on total chlorophyll fluorescence in the water column. Our results suggest that the dynamics of >5-μm phytoplankton were driven less by the thermal dynamics associated with the South Slough than by processes associated with the near-shore environment.

Contribution of Protozoan Food

Assuming that most metazooplankton and bivalves in the South Slough cannot efficiently feed on particles <5-μm (Nival and Nival 1976; Paffenhöfer

1984; Shumway et al. 1985; Riisgard 1988), the contribution of protozoan food in terms of ciliate carbon alone contributed a projected annual average of 32% (range: 6-75%) of the available planktonic food (protozoan + phytoplankton) to these consumers. In terms of seasonal differences in this contribution, ciliate carbon provided an average of 26% (range: 6-75%) to the available carbon between April and September, and 36% (range: 20-52%) between October and March. Consideration of other heterotrophic food not quantified in this study (i.e., dinoflagellates and nanoflagelattes) would increase the overall protozoan contribution. The consumption and the preference of ciliates and other protozoan food by copepods have been well documented (Kleppel et al. 1988; Fessenden and Cowles 1994; Verity and Paffenhöfer 1996). The higher nutritional value of protozoan food over algal food has been proposed as the underlying motivation for this selective feeding behavior (see Stoecker and Capuzzo 1990, and references therein). Baldwin and Newell (1991) demonstrated the ingestion of heterotrophic food by the meroplankton larvae of the oyster *Crassostrea virginica* and proposed that a diet of heterotrophic and autotrophic food is necessary to attain a full complement of nutrients and energy for larval development. Ciliates and other protozoan food have also been shown to contribute a significantly to the diets of the adult oyster *Crassostrea gigas* (Le Gall et al. 1997; Dupuy et al. 1999). This could be particularly important in the lower reach of South Slough where its relatively extensive tide flats support a diverse community of

suspension-feeding fauna (cockles and soft-shell clams) and the commercial cultivation of oysters (Rumrill, pers. comm.).

Conceptual Model

The key interactions inferred from the numerical data in this study are presented as a conceptual model in Fig. 10. The robust relationship between ciliates and ultraphytoplankton across seasons, together with the sustained and relatively high contribution of this algal fraction to the total phytoplankton crop, suggest that this an important node of energy transfer in the South Slough. The seasonal relationship between ciliates and total phytoplankton standing stock reflected the composition and dominance shift in phytoplankton >5 μm standing stock from diatoms in the spring/summer period to nanoflagellates in the fall/winter period. The absence of a significant relationship between ciliates and phytoplankton >5 μm throughout the year and the relative increase in the representation of ciliate carbon in the fall/winter period suggests that ciliates are supplementing their principal prey of ultraphytoplankton with larger phytoplankton and/or heterotrophic food. Overall, these data suggest that ciliates represent a productive component in the South Slough planktonic environment and may provide a sustaining carbon source to larger consumers during the seasons of reduced phytoplankton growth and biomass.

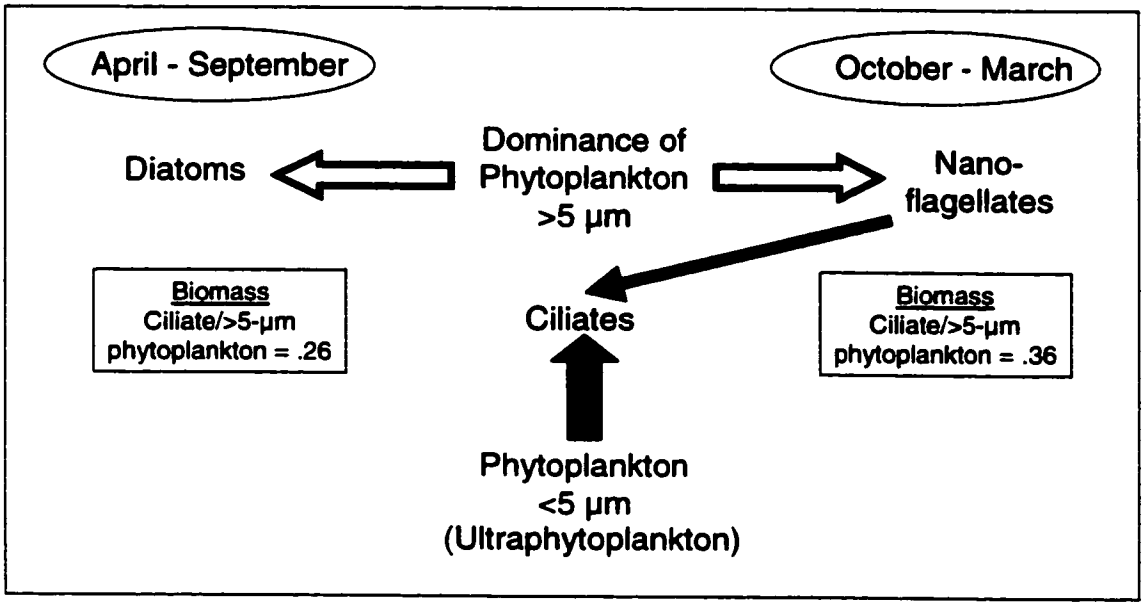

Fig. 10. Conceptual model presenting the inferred seasonal ciliate-phytoplankton interactions in the South Slough. Ciliates and ultra-phytoplankton were strongly coupled throughout the sample year (1999-2000). Between October-March ciliates ingested >5-µm phytoplankton in addition to ultraphytoplankton. The ratio of biomass for ciliates and >5-µm phytoplankton is a metric of the relative contribution of ciliate biomass to potential food for higher consumers in the South Slough. Thickness of the filled arrows indicates the relative importance of the interaction.

CHAPTER III

MICROZOOPLANKTON AS GRAZERS OF PHYTOPLANKTON PRODUCTION IN THE SOUTH SLOUGH OF COOS BAY, OREGON

The annual and seasonal relationships between the standing stocks of ciliate and size-fractionated phytoplankton presented in Chapter II suggest that a robust trophic interaction between ciliates and small phytoplankton operates in the South Slough. The impact that ciliates impart as grazers on phytoplankton production, however, cannot be ascertained by correlations of field data. Direct measurement of this rate parameter can only be achieved by controlled experiments using natural assemblages.

In Chapter III, I present the findings of grazing experiments conducted at specific seasonal periods in the South Slough. I employed the seawater dilution method, a preferred technique to directly quantify microzooplankton grazing because it offers: (1) the simultaneous measurement of the specific phytoplankton growth rate and the rate of phytoplankton mortality due to grazing, and (2) minimal handling of the plankton assemblage. The temporal profile of key heterotrophic and autotrophic microplankton described in Chapter II provides a basis to discuss the results of the discrete, but appropriately timed experiments within a larger biological context.

Abstract

The role of microzooplankton as grazers of phytoplankton production was investigated in the South Slough arm of Coos Bay, Oregon. The results of dilution method experiments conducted during the different seasonal periods showed that microzooplankton grazed 48 to 96% of the daily phytoplankton production. Microzooplankton imposed significant grazing impacts on the total phytoplankton assemblage when nanoflagellates dominated the phytoplankton >5 µm. The grazing impact determined when diatoms dominated the >5-µm phytoplankton fraction was dependent on the composition of the microzooplankton. When heterotrophic dinoflagellates were absent or rare, microzooplankton grazed at significantly high rates on the production of <5-µm phytoplankton only. Higher numbers of heterotrophic dinoflagellates resulted in in high grazing impacts on the production of total phytoplankton and components of the <5-µm phytoplankton fraction. These findings suggest that the effects of grazing strongly depend on the coincidental compositions of the microzooplankton and phytoplankton assemblages. The enhanced and suppressed net-growth response of heterotrophic nanoflagellates and picophytoplankton, respectively, to low dilutions observed in one experiment imply that trophic interactions within the microzooplankton guild can challenge the ability of the dilution linear model to accurately assess grazing on picophytoplankton production.

Introduction

Descriptive and experimental studies from a diversity of marine environments provide compelling evidence that microzooplankton (< 200 µm) (Sieburth et al. 1978), specifically the protistan or protozooplankton component, are the chief consumers of phytoplankton production in marine systems (Beers et al. 1980; Smetacek 1981; Paranjape 1987, 1990; Sherr and Sherr 1994; Landry et al. 1997; Levinsen et al. 1999; Strom et al. 2001). The major contribution of ultraphytoplankton (cells 0.2-5.0 µm; Murphy and Haugen 1985) to annual primary production in marine systems (Li et al. 1983; Platt et al. 1983; Murphy and Haugen 1985) and the inefficiency of most metazooplankton grazers in consuming this small-cell biomass (Nival and Nival 1976; Paffenhöfer 1984) position the microzooplankton as important trophic nodes between the microbial and metazoan marine food-web components (Sherr et al. 1986). Considerable support for this argument stems from field and laboratory findings that demonstrate the importance of protozoan food to copepod nutrition and reproduction across trophic gradients (Kleppel et al. 1988; Kleppel 1993; Fessenden and Cowles 1994; Nielsen and Kiorboe 1994; Landry et al. 1997).

The coincidental composition of the microzooplankton and phytoplankton assemblages factor importantly into what grazing interactions and impacts will occur (Peters 1994). Ciliate-dominated microzooplankton assemblages generally have a greater grazing impact on nanophytoplankton (cells <20 µm) (Burkill 1982; Burkill et al. 1987; Gifford 1988; Strom and Welschmeyer 1991; but see

Paranjape 1990), which is consistent with laboratory feeding-preference observations (Rassoulzadegan and Etienne 1981; Gifford 1985; Jonsson 1986) and cytostome aperture constraints (Fenchel 1986). This is not to say that ciliate grazing on diatoms is inconsequential, since large ciliates are often observed with diatoms in their food vacuoles (Smetacek 1981; Sime-Ngando et al. 1995) and high standing stocks of ciliates are observed to correlate with high microzooplankton grazing rates (Strom et al. 2001).

Size-based models of trophic structure are challenged, however, by the feeding capabilities of another, often abundant group of microzooplankton, heterotrophic dinoflagellates, whose predator:prey ratio can exceed 1:1 (Jacobson and Anderson 1986; Hansen et al. 1994). Microzooplankton assemblages with large representation of heterotrophic dinoflagellates regularly exert significant grazing pressure on diatom production (Neuer and Cowles 1994; Archer et al. 1996; Froneman and McQuaid 1997; Strom et al. 2001). Strom et al. (2001) reported high biomass of heterotrophic dinoflagellates coincidental with high rates of microzooplankton grazing on diatom blooms in the Gulf of Alaska.

The small estuaries and embayments along the coast of Oregon provide an interesting opportunity to examine the impact of microzooplankton grazing on phytoplankton production. The phytoplankton assemblage undergoes substantive seasonal changes of size and composition (Hughes 1997). Whether a result of autochthonous production, advection from the coastal environment or both,

chain-forming centric and pennate diatoms are abundant in the estuary during the spring and summer months and frequently dominate the phytoplankton biomass. During this period precipitation is seasonally low and a relatively longer daily photoperiod prevails (Emmett et al. 2000). This leads to higher water temperatures within the estuary that may stimulate autochthonous phytoplankton growth (Eppley 1972; Verity 1986; Andersson et al. 1994). Coastal upwelling typically occurs from April to September along the Oregon coast (Huyer 1983). During this time northwest winds episodically draw surface waters offshore to be replaced by colder, saltier, nutrient-rich water from depth that consequently stimulates phytoplankton production, particularly that of diatoms, seaward of the upwelled waters (Small and Menzies 1981). Periodic relaxation of this wind forcing, along with tidal exchange can result in cross-shelf transport of accumulated algal biomass towards the shore and into estuarine environments (Roegner and Shanks 2001). A shortened daily photoperiod, increased precipitation and cooler temperatures characterize the months of October to March. Southwest winds prevail during this period, which draws coastal surface waters shoreward and establishes downwelling circulation along the coastline (Huyer 1983). Diatoms are generally rare during this time and smaller phytoplankton such as flagellates <20 μm and picoplankton (cells 0.2-2 μm) dominate the assemblage (Hughes 1997).

The importance of microzooplankton herbivory in the coastal waters along the west coast of North America has been documented (Beers and Stewart 1967;

Beers et al. 1980; Landry and Hassett 1982; Neuer and Cowles 1994; Strom et al. 2001); however, their role in the small estuaries and embayments along the Oregon coast has yet to be investigated. This report describes the results of seasonal dilution-method (Landry and Hassett 1982) experiments conducted in this habitat. It was anticipated that a temporal profile of key heterotrophic and autotrophic microplankton components described in Chapter II would provide a basis to discuss the results the discrete, but appropriately timed, experiments within a larger biological context.

Materials and Methods

Study Site

The reader is referred to Chapter II for a detailed description and map (Fig. 1) of the South Slough. Briefly, the South Slough is the southern arm of Coos Bay located on the south-central coast of Oregon, USA. Coos Bay is best characterized as a tidally-dominated, drowned river-mouth, common to the Oregon coast (Emmett et al. 2000). A substantial part of the South Slough watershed resides within the boundary of the South Slough National Estuarine Research Reserve (SSNERR). The tides are mixed-semidiurnal with a mean-tidal amplitude of 2.3 m, which gives rise to an average depth of approximately 2 m (Rumrill in review). The Oregon Institute of Marine Biology (OIMB) of the University of Oregon is located within a distance of a 30 min boat ride from the sample site. Experiments were conducted both at OIMB and in situ at the sample site (Chapter II). All data analysis was performed at OIMB.

Dilution Grazing Experiments

Microzooplankton grazing was quantified with the dilution method (Landry and Hassett 1982). This technique is routinely applied in both marine and freshwater systems for estimating herbivory and bacterivory by microzooplankton (Rivkin et al. 1999, and references therein). It is preferred over other direct methods of grazing assessment (i.e., size-fractionation, metabolic inhibitors, tracers of ingestion; see Gifford 1988 for review) because 1) it is quantitative; 2) it minimizes handling of natural assemblage; and 3) it allows simultaneous estimation of both growth (k) and mortality (g) of prey in a natural plankton assemblage. The dilution technique expresses change in phytoplankton growth with time as:

$$P_t = P_0 e^{(k-g)} \text{ or } (1/t)\ln(P_t/P_0) = k - g$$

where P_0 and P_t are prey density estimates (chlorophyll concentration or cell abundance) at the beginning and the end of the experiment; k is the instantaneous growth rate of prey; g is the mortality rate of prey due to grazing; and, t equals the time of incubation. Values of k and g are calculated from changes in prey density following incubations at different dilutions of seawater containing the natural microplankton assemblage. The term $(1/t)\ln(P_t/P_0)$ is the apparent growth rate (AGR) of the prey at specific dilution fractions (Fig. 11). The linear slope of this relationship is the grazing rate, g, and the ordinal-intercept is

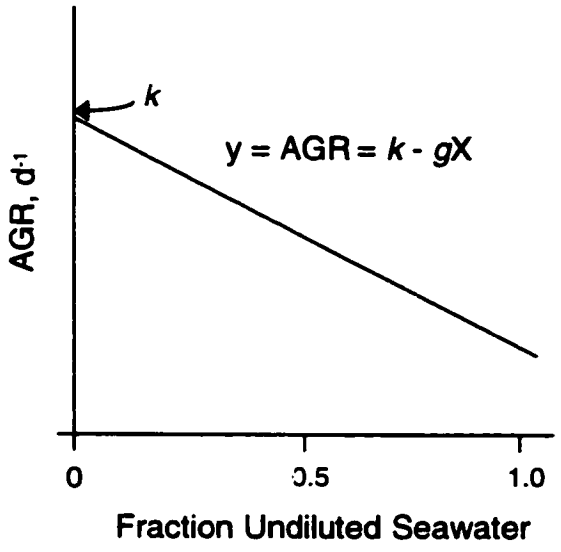

Fig. 11. The dilution model. AGR, apparent growth rate of prey; *k*, specific growth rate of prey (ordinal intercept); *g*, mortality of prey due to grazing (slope); and X, the fraction of undiluted seawater.

the growth rate of prey, k, in the theoretical absence of grazers. Because grazers are diluted with their food, the observed rate of change in prey density is linearly related to the dilution factor, X_i,

$$AGR = k - gX_i.$$

The method requires three assumptions: 1) phytoplankton growth rates are not density-dependent; 2) ingestion is a linear function of consumer density; 3) the growth equation adequately describes phytoplankton growth and changes in the exponential growth rate of prey along the dilution gradient are attributed solely grazing (i.e., nutrient limitation and/or differential nutrient remineralization along the dilution gradient are not factors) (Landry and Hassett 1982; Gifford 1988). The validity of the second assumption is challenged by possible functional feeding responses of grazers that can give rise to deviations from linearity (i.e., feeding thresholds/cessation at high dilutions/low food levels; feeding saturation at low dilutions/high food levels) (Gifford 1988; Tremaine and Mills 1987; Gallegos 1989; Evans and Paranjape 1992), or possible by trophic interactions within the among the microzooplankton (see Discussion: Non-linear Apparent Growth of Prey).

Experiments were conducted on 15 March 1999 (MAR99), 28 August 1999 (AUG99), 5 March 2000 (MAR00), and 28 May 2001 (MAY01) in order to measure microzooplankton grazing impact during spring/summer period (May and August) and replicate measurements during the winter period (early- to mid-March). For each experiment, high tide collections of seawater were made with a

weighted bucket from 1-m depth at the South Slough sample site. Water was then siphoned through a submerged 200-µm-mesh screen into 25-liter polyethylene carboys. Particle-free diluent was prepared back at the Oregon Institute of Marine Biology (OIMB) laboratory by sequentially filtering collected water through 0.45 µm and 0.2 µm membranes (MAR99 and AUG99) or Gelman capsule filters (MAR00 and MAY01) under low positive pressure (<5 in Hg). Appropriate volumes of diluent were added to <200-µm size-fractionated water collected at the same site to achieve target dilution factors that varied in number among experiments although in each case were at least increments of 0.25 (MAR99). Higher dilutions were added to the AUG99 (0.05), MAR00 and MAY01 (0.10 and 0.05) experiments. Low concentrations of macronutrients (nitrate, 10 $\mu g\text{-}l^{-1}$; phosphate, 1 $\mu g\text{-}l^{-1}$; (Landry and Hassett 1982)) were added to each mixture to minimize potential nutrient limitation along the dilution gradient. Additional undiluted treatments without nutrient additions were incubated in each experiment. Following sufficient time for homogenization of the dilution mixtures (>1 hr), three (MAY01) or four (MAR99, AUG99 and MAR00) replicate volumes of each dilution treatment were gently siphoned into acid-washed bottles (0.5-liter, MAR99; 1.0-liter, AUG99, MAR00) or Whirl-Paks (0.7-liter, MAY01). Twenty-four (24) hr incubations of treatment vessels were carried out either at the collection site in a plexiglass carriage suspended at ~1.5 m depth from the davit of the R/V Squid (MAR99 and 2000 experiments), or in an outdoor Nalgene tank with circulating water from the OIMB seawater system (AUG99 and MAY01). In

the latter case, the tank was covered with neutral-density screening to simulate ambient light at the collection depth. All equipment in direct contact with collected and dilution treatment water (i.e., buckets, carboys, containers, tubing) was acid-washed (10% hydrochloric acid) and copiously rinsed with deionized water prior to use. Non-latex (Nitryl) gloves were worn throughout the experimental set-up.

Initial samples of 200 ml were taken in triplicate from each dilution-fraction mixture for analysis of chlorophyll concentration. Aliquots of 75-100 ml each sample were filtered onto 0.2 and 5.0 µm (AUG99 and MAR00 experiments only) polycarbonate membranes (Poretics), which were subsequently transferred into 90% acetone and kept in darkness at 4º C for at least 24 hr (Parsons et al. 1984). Chlorophyll concentration was determined with a Turner 10-AU fluorometer that was routinely calibrated with a pure chlorophyll-*a* standard (Sigma) to measure chlorophyll concentration from fluorescence. Readings were taken before and after the addition two drops of 5% hydrochloric acid (v/v) to correct for the contribution of phaeo-pigments to the initial concentration (Parsons et al. 1984). The concentration of <5-µm chlorophyll (<5CHL) was determined by the difference of total chlorophyll (0.2 µm membrane; TCHL) and >5-µm chlorophyll (5.0 µm membrane; >5CHL) concentration in each sample. Qualitative assessment of phytoplankton >10 µm was made from the settled Lugols-fixed samples (see below). Size-fraction measurements were attempted for MAY01 but clogging on the 5-µm membrane due to the relatively very high chlorophyll standing stocks (38 µg l^{-1}) precluded this step. The actual dilution factors in each

experiment was determined as the fraction of the averaged initial chlorophyll concentration or cell counts (see below) at each target dilution to that in the undiluted treatment. Equal volumes were taken from each treatment vessel at the end of the incubation and processed in an identical manner.

For AUG99 and MAY01, triplicate samples of 50-100 ml were taken from each dilution-fraction mixture at each dilution factor for initial density measurements of prokaryotic and eukaryotic picophytoplankton (PPICO and EPICO, cells 1-2 μm in size;) and heterotrophic nanoflagellates 2 - 10 μm (HFLAG) along the dilution factor gradient. Samples were immediately fixed with ice-cold glutaraldehyde (0.25% final conc.). For picophytoplankton enumeration, 10 to 50 ml aliquots (higher volumes for higher dilutions) were filtered under low positive pressure (<5 in. Hg) onto black 0.2-μm membrane filters (Poretics) and viewed with epifluorescence microscopy (PPICO, green light filter set; EPICO, blue light filter set). Initial density of HFLAG was determined in the undiluted, 0.75, 0.50 and 0.25 target dilution fraction mixtures in each experiment. Subsamples (15 - 60 ml) from each dilution-fraction were incubated with DAPI (4',6-diamidino-2-phenylindole dihydrochloride; final volume 0.01 μg ml^{-1}) for 10-15 min in the dark and then concentrated onto black 0.8 μm or 1.0 μm polycarbonate membrane filters (Poretics) under gentle vacuum (<5 in. Hg; Sherr et al. 1993). Filters were mounted onto slides and inspected with epifluorescence microscopy under UV light. Picophytoplankton and HFLAG cells were counted in random fields or diametric transects until at least 200 cells of each group were

enumerated (Murphy and Haugen 1985). Final samples were taken from two (AUG99) or three (MAY01) of the dilution treatment replicates and processed in the same way.

Initial and final samples (200 ml) were taken from the undiluted mixture and two undiluted treatment replicates, respectively, and fixed with acid Lugols (final conc., 5%) for microzooplankton counts. Aliquots of 75-100 ml of the fixed samples were settled (>18 hrs) in columns onto glass viewing plates. The entire plate was scanned with an inverted microscope and all ciliates and conspicuous heterotrophic dinoflagellates (i.e, *Protoperidinium* spp. and *Gyrodinium* spp. were enumerated.

Phytoplankton carbon biomass was estimated with the carbon:chlorophyll conversion of 45:1, a compromise of the range 27:1 to 67:1 suggested by Riemann et al. (1989). Initial carbon concentration in undiluted treatments for each picophytoplankton group was determined differently. Accurate cell-diameter measurements of PPICO for biovolume estimation were hindered by very bright autofluorescence that extended beyond the cell wall; therefore, the conversion factor of 0.25 pg C $cell^{-1}$ was applied (Li et al. 1992). EPICO carbon was determined with carbon:biovolume conversion factor 0.36 pg C um^{-3} (Verity et al. 1992). Biovolume was determined from the average cell diameter of 40 randomly selected cells measured with an ocular micrometer at 1000X, assuming spherical cell shape.

Data Analysis

Dilution experimental data were analyzed by simple linear regression of AGR of prey (chlorophyll or cell counts) vs. dilution factor. In cases when non-linear curves were visibly apparent in the relationship between AGR and dilution factor, decomposition of the dilution plots into two regions resulted in more statistically robust regressions (Gifford 1988; Gallegos 1989; Rivkin et al. 1999). The instantaneous growth rate of prey, k, was estimated from the ordinal-intercept of the least-squares line for the regression of region 1 data subset (higher dilution region). The grazing rate, g, was estimated as the difference between k calculated for region 1 and the arithmetic mean of the AGR estimates for the lower dilution region (Rivkin et al. 1999). All statistical analyses were performed with Statistica (version 5.0) software at the significance level α=0.05. Error bars about the means represent ±1 standard deviation.

Estimations of the grazing impact on each chlorophyll fraction or picophytoplankton group were calculated as the percentage of potential production grazed d^{-1} with the computation (Gifford 1988),

$$[(P_0e^{k} - P_0e^{(k-g)})/ P_0 e^{k}] \times 100$$

where P_0 is the initial prey density in the undiluted treatment, k is the specific growth rate of prey (day^{-1}), and g is the grazing rate (day^{-1}). Grazing impact on the prey standing stock was defined as the percentage of the initial standing stock plus potential production ingested d^{-1} (Gifford 1988),

$$[(P_0e^{k} - P_0e^{(k-g)})/(P_0 + P_0e^{k})] \times 100.$$

Time-averaged prey density (P_{avg}) in undiluted treatments were calculated according to Frost (1972),

$$P_{avg} = P_0(e^{(k-g)} - 1)/(k-g)$$

Community ingestion rates (I, µg chl or cells l^{-1} d^{-1}) of microzooplankton were then calculated as (Strom et al. 2001),

$$I = gP_{avg}.$$

Chlorophyll and prey cells ingested were converted to carbon equivalents with the conversion equations given above.

Results

A comparison of the initial biological measurements for each experiment with the seasonal averages of field data described in Chapter II (Table 11) suggests that conditions were seasonally representative for the South Slough. Total and <5-µm chlorophyll concentrations (TCHL and <5CHL) reflected the seasonal differences and for the most part fell within the seasonally ranges. TCHL for the MAY01 experiment (38 $\mu g l^{-1}$) was clearly outside the range for April-September period due a bloom of the chain-forming diatom *Thalassiosira* spp., yet followed the trend of higher TCHL for this seasonal period. Ciliate abundance in the undiluted treatments of each experiment reflected the relative seasonal trends observed in the field data (Table 11). Abundance for each experiment was observed to be low compared to the field data statistics. This may have been the result of incidental mortality due to the 200-µm prescreening

Table 11. Initial conditions in undiluted treatments for dilution experiments in comparison with the seasonal averages (range) of these parameters described in Chapter II. TCHL, total chlorophyll concentration; <5CHL, <5-µm chlorophyll concentration; and DINO, heterotrophic dinoflagellate abundance. DINO abundance for the seasonal averages is for *Gyrodinium spirale* only (Appendix II). For the dilution experiments, values for CHL are averages of n samples (±1 SD) and values for microzooplankton represent total counts of 100 ml samples for each experiment. ND, not determined.

Experiment	Temp., °C	Salinity, PPT	TCHL, µg l^{-1}	<5CHL, µg l^{-1}	Dominant Phytoplankton Taxa >5 µm	Microzooplankton, cells ml^{-1}	
						Ciliates	DINO
15 March 99 (n = 4)	9.4	25.4	1.4 (0.1)	ND	Nanoflagellates (Cryptomonads)	2.4	0
22 March 00 (n = 3)	10.9	26.5	0.9 (0.04)	0.2 (0.05)	Nanoflagellates (Cryptomonads)	2.7	0.2
October-March (1999-2000)	10.4	26.6	0.8 (0.3 - 1.6)	0.3 (0.04 - 0.8)	Nanoflagellates	10 (2.5 - 18)	0.3 (0 - 1.3)
28 August 99 (n = 3)	14.4	32.7	3.0 (0.4)	1.1 (0.1)	Chain-forming diatoms *Chaetoceros* spp	10.2	<0.1
28 May 01 (n = 3)	13.4	29.2	38 (2.3)	ND	Chain-forming diatoms *Thalassiosira* spp.	6.6	1.4
April-September (1999)	14.3	31.2	3.3 (1.6 - 6.4	1.5 (0.3 - 3.0)	Chain-forming diatoms	27 (8.0 - 91)	2.8 (<0.1 - 15.8)

to remove or reduce the metazooplankton component in the experimental water (Gifford 1985). If so, underestimates of grazing rates would be determined. The abundance of heterotrophic dinoflagellates was generally low for each experiment except for the MAY01 when relatively high numbers of heterotrophic thecate (*Protoperidinium* spp.) and athecate (*Gyrodinium spirale*) dinoflagellates were observed (Table 11). In terms of field data, very low numbers of *Gyrodinium spirale* were observed during the October-March period compared to the April-September period (Appendix A). Although it is not apparent from Table 11, higher numbers of *G. spirale* were observed in the early summer of sample year, and the abundances for August were comparable to the October-March period (Appendix A).

The initial abundance of HFLAG was 650 cells ml^{-1} for the AUG99 experiment and 380 cells ml^{-1} (SD, 10 cells ml^{-1}) in MAY01 experiment (Table 12). The densities of PPICO and EPICO were 16 x 10^3 and 15 x 10^3 cells ml^{-1} for the AUG99 experiment, and 4.2 x 10^3 and 28 x 10^3 cells ml^{-1} for the MAY01 experiment (Table 12). Although the contribution of each picoplankton group varied between experiments, the overall picoplankton abundance (PPICO + EPICO) for each experiment was very similar.

Daily ingestion rates and grazing impacts on the daily prey standing stock (PSS) and daily potential production (PP) varied with the time of year (Table 13). Similar apparent growth rates in undiluted TCHL treatments with and without the addition of nutrients for each experiment suggests nutrient limitation was not a

Table 12. Initial concentrations of prokaryotic (PPICO) and eukaryotic (EPICO) picophytoplankton and heterotrophic nanoflagellates (HFLAG) for the AUG99 and MAY01 dilution experiments.

Experiment	Picophytoplankton, x 10^3 cells ml^{-1}		HFLAG, 10^2 cells ml^{-1}
	PPICO	EPICO	
28 August 99 (n = 3)	16 (1.9)	15 (3.0)	6.5 (0.4)
28 May 01 (n = 3)	4.2 (0.2)	28 (1.3)	3.8 (0.1)

Table 13. Phytoplankton growth and microzooplankton grazing rates (± 95% CL) for each chlorophyll fraction and picophytoplankton group. Ratios of grazing:growth rates (*g*:*k*), estimations of ingestion rates and percent daily losses of prey standing stock (PSS) and potential production (PP) are given. TCHL, total chlorophyll; < and >5CHL, < and >5-µm chlorophyll; PICO and EPICO, prokaryotic and eukaryotic picophytoplankton; PR, piecewise regression NS, not significant.

Experiment	Size/Cell Fraction	*n*	r^2	*k*, d^{-1}	*g*, d^{-1}	*g*:*k*	Ingestion µg C L^{-1} d^{-1}	PSS grazed d^{-1} (%)	PP grazed d^{-1} (%)
15 March 99	TCHL	24	0.45[b]	0.59 (0.09)	0.29 (0.14)	0.49	21.2	25	57
28 August 99	TCHL	20	NS	0.58 (0.14)	-0.21 (0.23)	---	0.00	0.00	0.00
	>5CHL	20	NS	0.25 (0.14)	-0.79 (0.24)	---	0.00	0.00	0.00
	<5CHL	20	0.75[c]	1.5 (0.24)	1.4 (0.40)	0.92	73.2	76	96
	PPICO	10	0.84[b]	0.38 (0.05)	0.24 (0.09)	0.64	1.0	22	67
	EPICO	10	0.68[b]	1.1 (0.11)	0.31 (0.17)	0.28	4.7	26	40
5 March 00	TCHL	23	0.55[c]	0.66 (0.13)	0.53 (0.22)	0.81	23.3	41	85
	>5CHL	24	0.22[a]	0.42 (0.15)	0.33 (0.28)	0.81	12.0	29	84
	<5CHL	24	NS	0.49 (0.62)	0.13 (1.1)	---	0.00	0.00	0.00
28 May 01	TCHL	18	0.87[c]	0.57 (0.06)	0.50 (0.10)	0.89	898	40	92
	PPICO (PR)	15	0.83[c]	0.40 (0.06)	0.37	0.92	0.4	31	93
	EPICO. (PR)	15	0.89[c]	1.5 (0.10)	1.2	0.82	25.9	70	90

[a]$p<0.05$, [b]$p<0.01$ [c]$p<0.001$

factor in this study. For MAR99 (Fig. 12), the specific growth rate for TCHL was 0.59 d^{-1} and the grazing rate was 0.29 d^{-1} (Table 13). The grazing impact equaled 25 and 57% of the daily PSS and PP, and the ingestion rate was 21 µg C l^{-1} d^{-1}. Significant growth and grazing rates were determined for TCHL and >5CHL in the MAR00 experiment (Fig. 13). The growth rate for TCHL was over 30% greater than the rate for >5CHL (0.66 vs. 0.42 d^{-1}). The grazing rate on TCHL also was nearly 30% greater than the rate of grazing mortality for >5 Chl (0.53 and 0.33 d^{-1}). Ingestion rate of TCHL carbon was 23 µg C l^{-1} d^{-1}, equivalent to 41 and 85% of the PSS and PP. Ingestion rate of >5CHL carbon (12 µg C l^{-1} d^{-1}) was considerably less than of TCHL carbon, but the impact on the daily PSS and PP (29 and 84%) was similar due to equal *g*:*k* ratios (0.81). The regression for <5CHL was not significantly different from zero; however, it is likely that some grazing on this phytoplankton fraction occurred, accounting for the substantial difference of ingestion rates on TCHL and >5CHL.

For the AUG99 experiment, significant regressions were determined for <5CHL (Fig. 14) and the PPICO and EPICO components of this fraction (Fig. 15). The growth rate of <5CHL (1.5 d^{-1}) was 75% greater than the estimate for PPICO (0.38 d^{-1}) and about 25% greater than the growth rate of EPICO (1.1 d^{-1}) (Table 13). The grazing rate on <5CHL (1.4 d^{-1}) was substantially higher (83 and 78%) than the rates on PPICO and EPICO (0.24 and 0.31 d^{-1}). The ingestion rate of <5CHL carbon was 73 µg C l^{-1} d^{-1}, which accounted for 76 and 96% of the daily PSS and PP for this chlorophyll fraction. The ingestion rate of PPICO carbon was

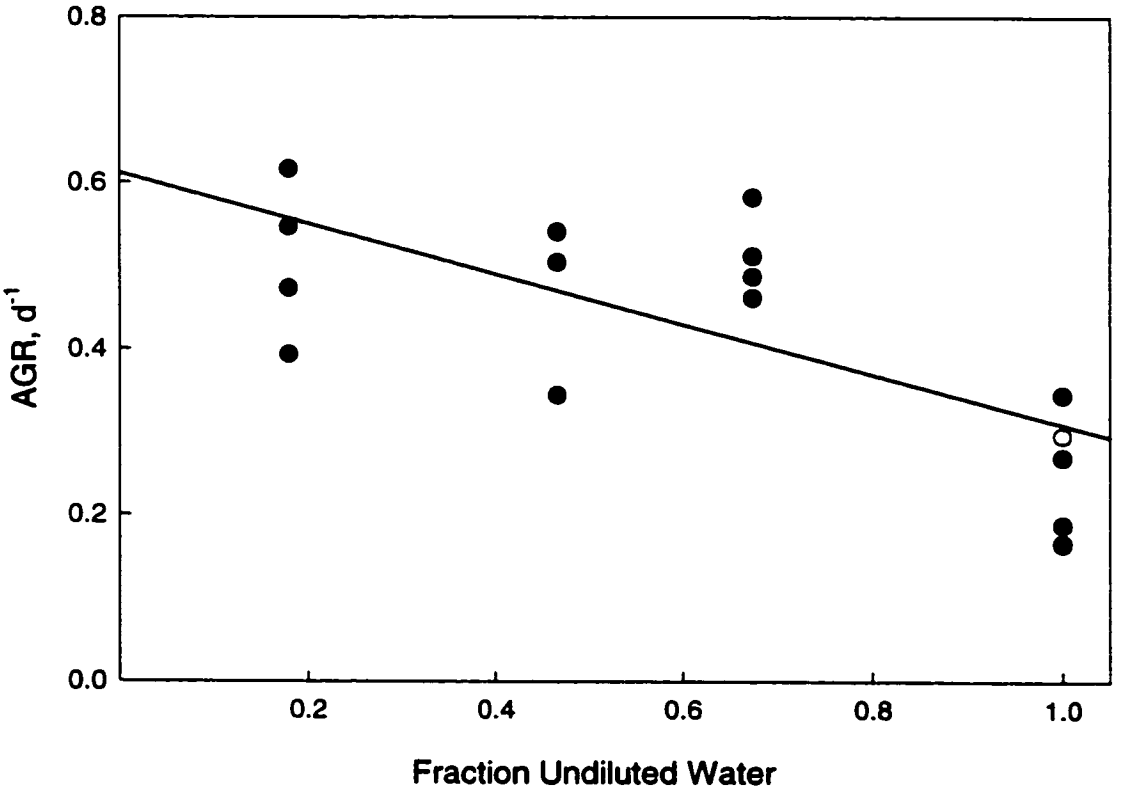

Fig. 12. Apparent growth rate (AGR) of total chlorophyll vs. dilution factor for the 15 March 1999 experiment. Open circles represent undiluted replicates without nutrients added.

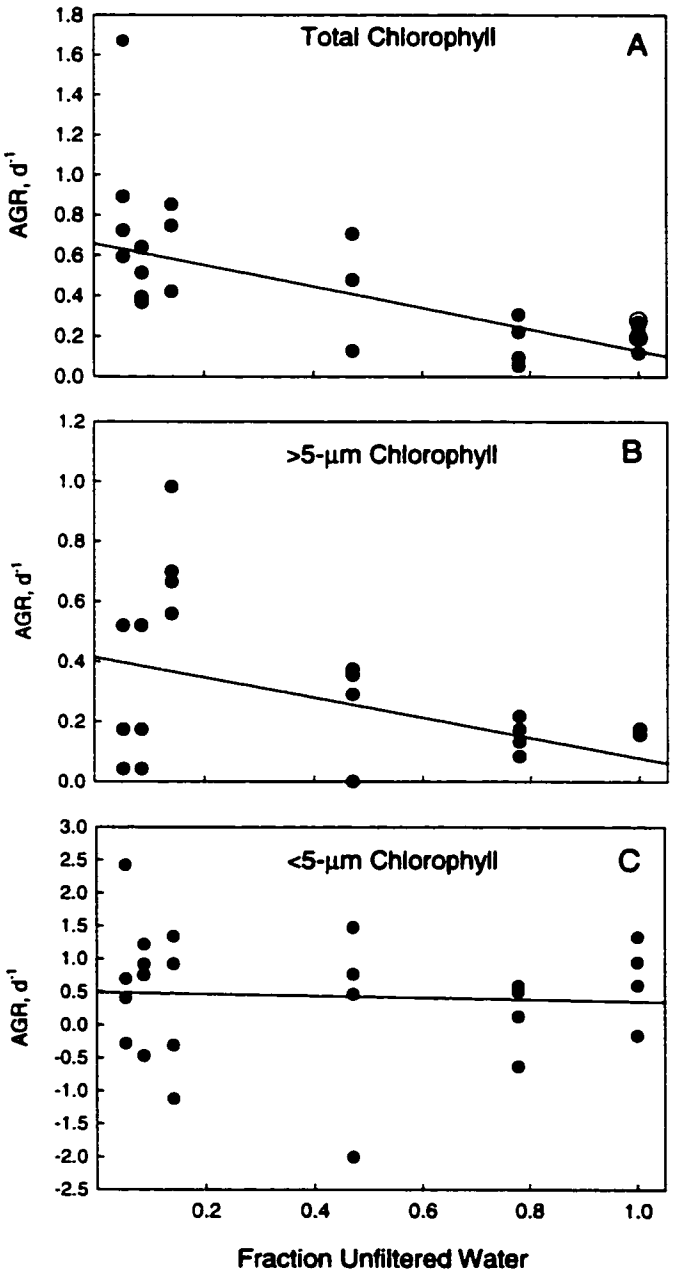

Fig. 13. Apparent growth rate (AGR) vs. dilution factor for the 22 March 2000 experiment. A, total chlorophyll; open circles represent undiluted replicates without nutrients added; outlier in the dilution treatment not included in regression; B, >5-µm chlorophyll; C, <5-µm chlorophyll. N = 4 for each dilution fraction.

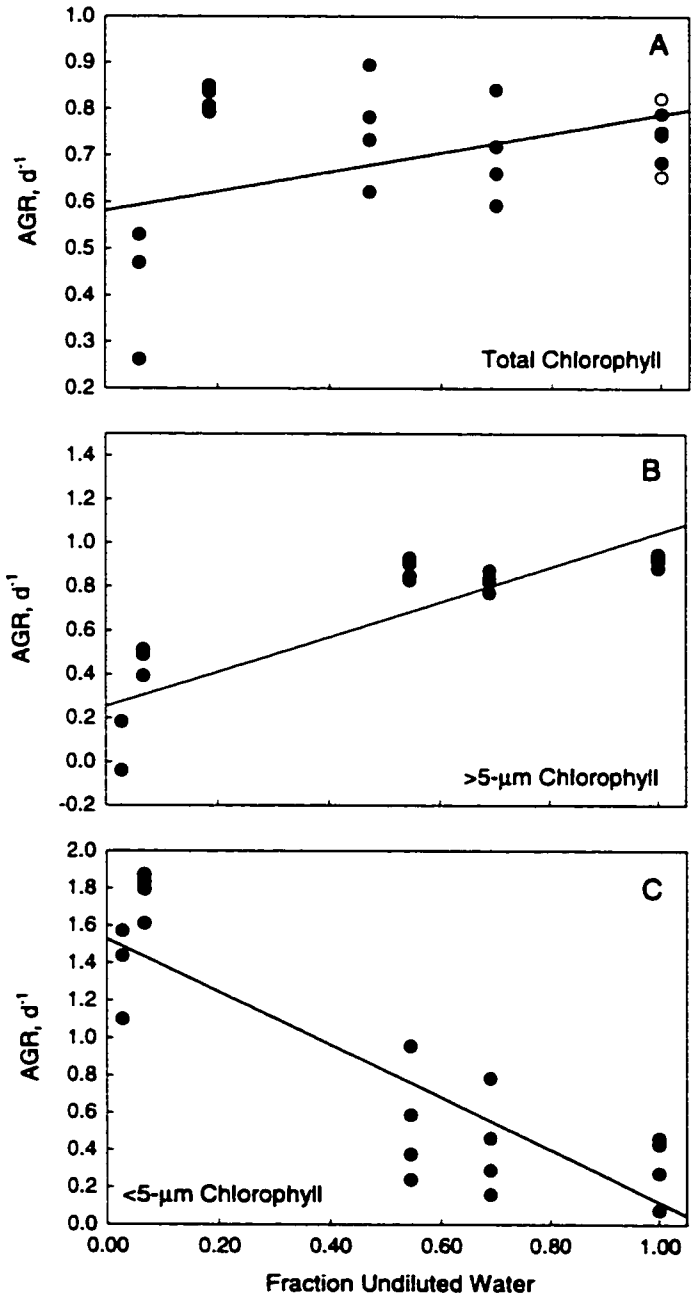

Fig. 14. Apparent growth rate (AGR) vs. dilution factor for the 28 August 1999 experiment. A, total chlorophyll; open circles represent undiluted replicates without nutrients added; B, >5-µm chlorophyll; C, <5-µm chlorophyll.

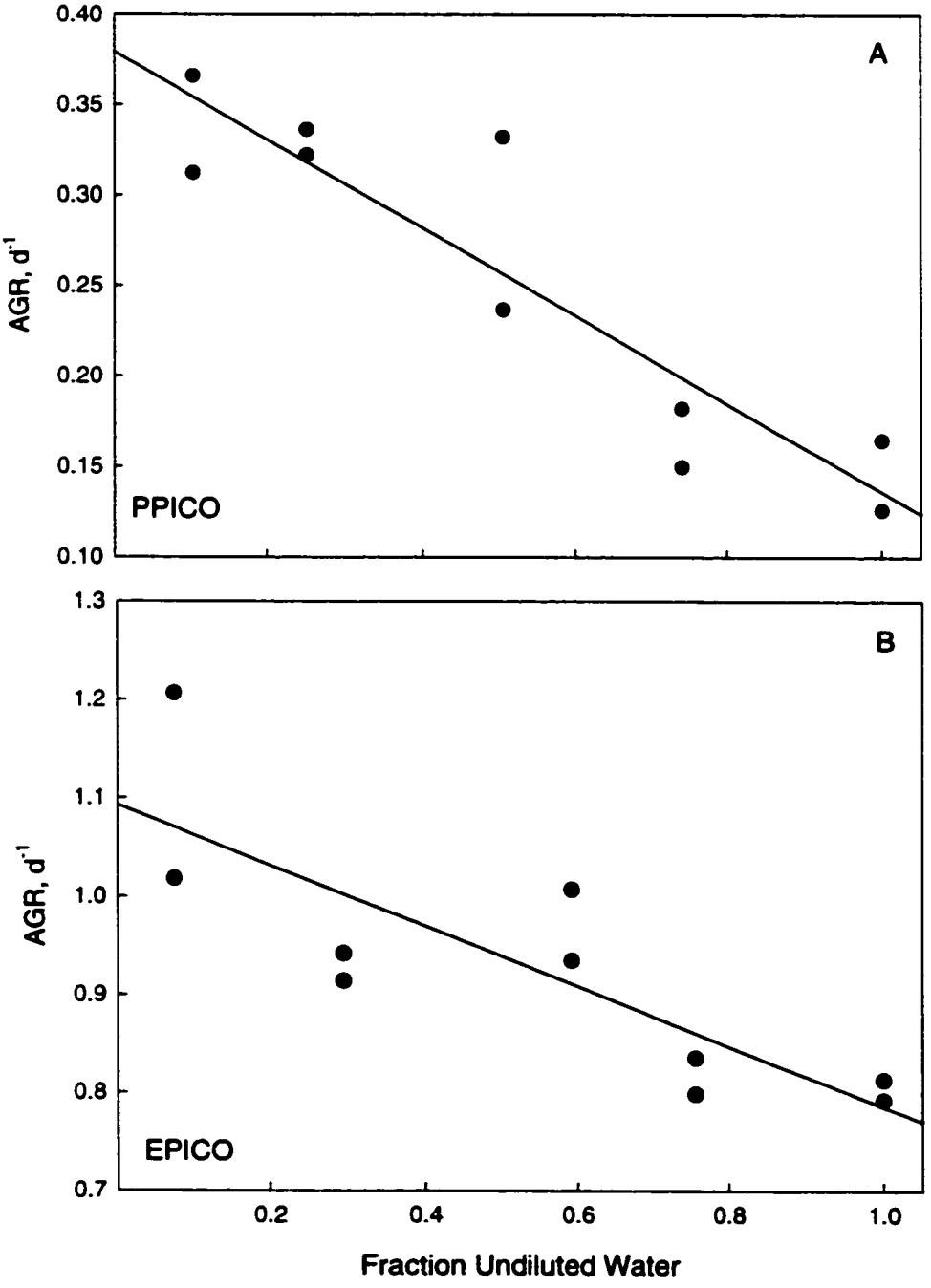

Fig. 15. Plots of apparent growth rate (AGR) of picophytoplankton vs. dilution factor for 28 August 1999 experiment. A, PPICO, prokayotic picophytoplankton; B, EPICO, eukaryotic picophytoplankton.

1.0 µg C l^{-1} d^{-1}, <1% of the ingestion rate for <5CHL carbon. This amounted to 22 and 67% of the daily PSS and PP for PPICO (Table 13). The ingestion rate of EPICO was 4.7 µg C l^{-1} d^{-1}, or 6% of the ingestion rate of <5CHL carbon. Grazing accounted for 16 and 40% of the daily PSS and PP for EPICO.

The near parity between the growth and grazing rates (0.50 d^{-1} and 0.57 d^{-1}; Fig. 16; Table 13) of TCHL determined in the MAY01 experiment suggest there was a strong coupling between microzooplankton grazing and the production of chain-forming *Thalassiosira* sp. diatoms. Thecate *Protoperidinium*-like dinoflagellates were observed attached to whole chains of these diatoms via a pallial-feeding veil (Jacobson and Anderson 1986). Other athecate *Gyrodinium*-like dinoflagellates were observed with food vacuoles filled with diatom cells, as seen by others (Strom and Strom 1996). No ciliates were observed with diatoms in food vacuoles. Ingestion rate of TCHL carbon was 898 µg C l^{-1} d^{-1} and the grazing impact equaled 40 and 92% of the daily PSS and PP.

Although direct measurements of grazing on the production of the <5CHL fraction in this experiment were not possible, piecewise regressions for PPICO and EPICO generated significant growth and grazing rates for both groups (growth: 0.40 d^{-1} and 1.5 d^{-1}; grazing: 0.37 and 1.2 d^{-1}; Fig. 16; Table 13). Ingestion rates of PPICO and EPICO carbon were 0.4 µg C l^{-1} d^{-1} and 26 µg C l^{-1} d^{-1}. Grazing impacts on daily PSS and PP equaled 31 and 93% for PPICO, and 70 and 90% for EPICO.

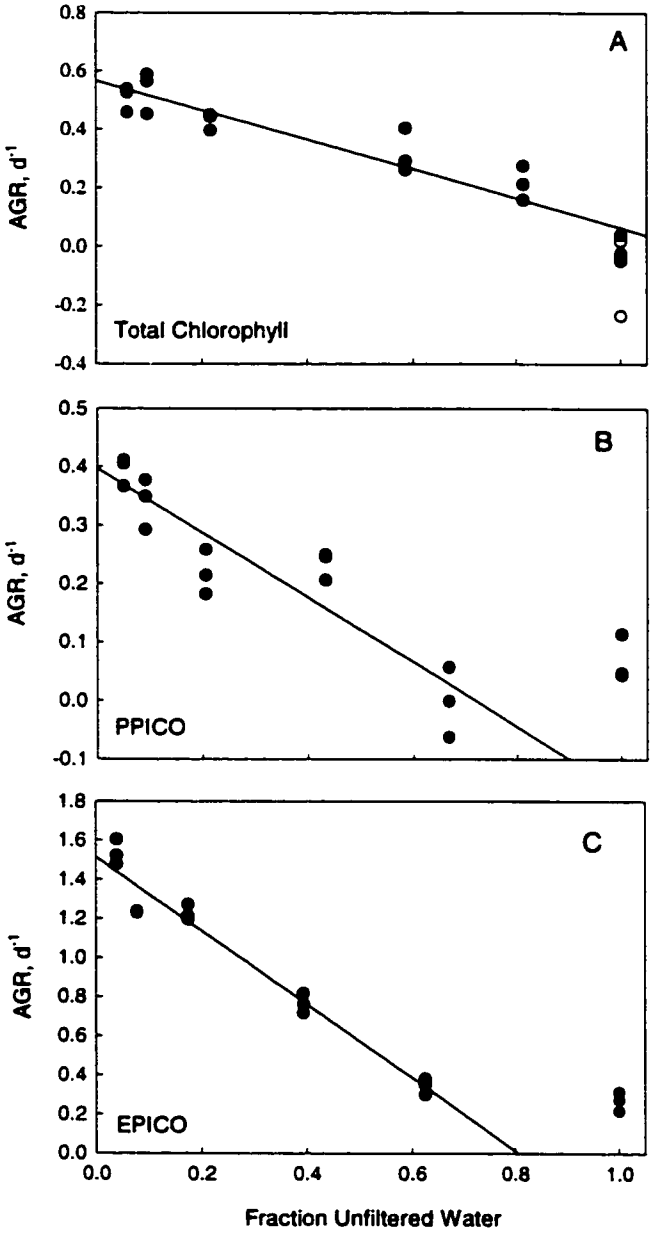

Fig. 16. Apparent growth rate (AGR) vs. dilution factor for the 28 May 2001 experiment. A, total chlorophyll; open circles represent undiluted replicates without nutrients added; B, prokaryotic picophytoplankton, PPICO; C, eukaryotic picophytoplankton, EPICO. Undiluted treatments were not included in the regression analysis for picoplankton groups.

The effect of dilution on the apparent growth rate (AGR) of HFLAG in AUG99 and MAY01 experiments is illustrated in Fig. 17. AGR increased significantly with an approximate 25% dilution in each experiment (one-way ANOVA; AUG99, $F_{1,2} = 23.8$, $p<0.05$; MAY00, $F_{1,4} = 81.2$, $p<0.001$). Positive growth was maintained into the 0.50 target dilution treatments in the AUG99 experiment and into the 0.25 target dilution treatments in the MAY00 experiment.

Discussion

Dilution Experiments

The impact of microzooplankton grazing in the South Slough varied seasonally and in response to variation in the both the microzooplankton and phytoplankton assemblages. For the March experiments, when the phytoplankton assemblage was predominantly composed of nanoplankton species, grazing accounted for significant daily losses of total phytoplankton production (TCHL). Phytoplankton growth and microzooplankton grazing rates were both higher in the MAR00 experiment. The dissimilarity in the *g:k* ratios (Table 13) between the two experiments (MAR99=0.49 vs. MAR00=0.87) was apparently due more to differences in grazing rates (0.29 d^{-1} vs. 0.65 d^{-1}) than to disparity in the phytoplankton growth rates (0.59 d^{-1} vs. 0.75 d^{-1}). Initial density of ciliates was comparable for the two experiments but the water temperature was ~1.5 degrees C higher (Table 11). What effect this had on the difference in grazing impacts estimated for these two experiments is speculative; however, it

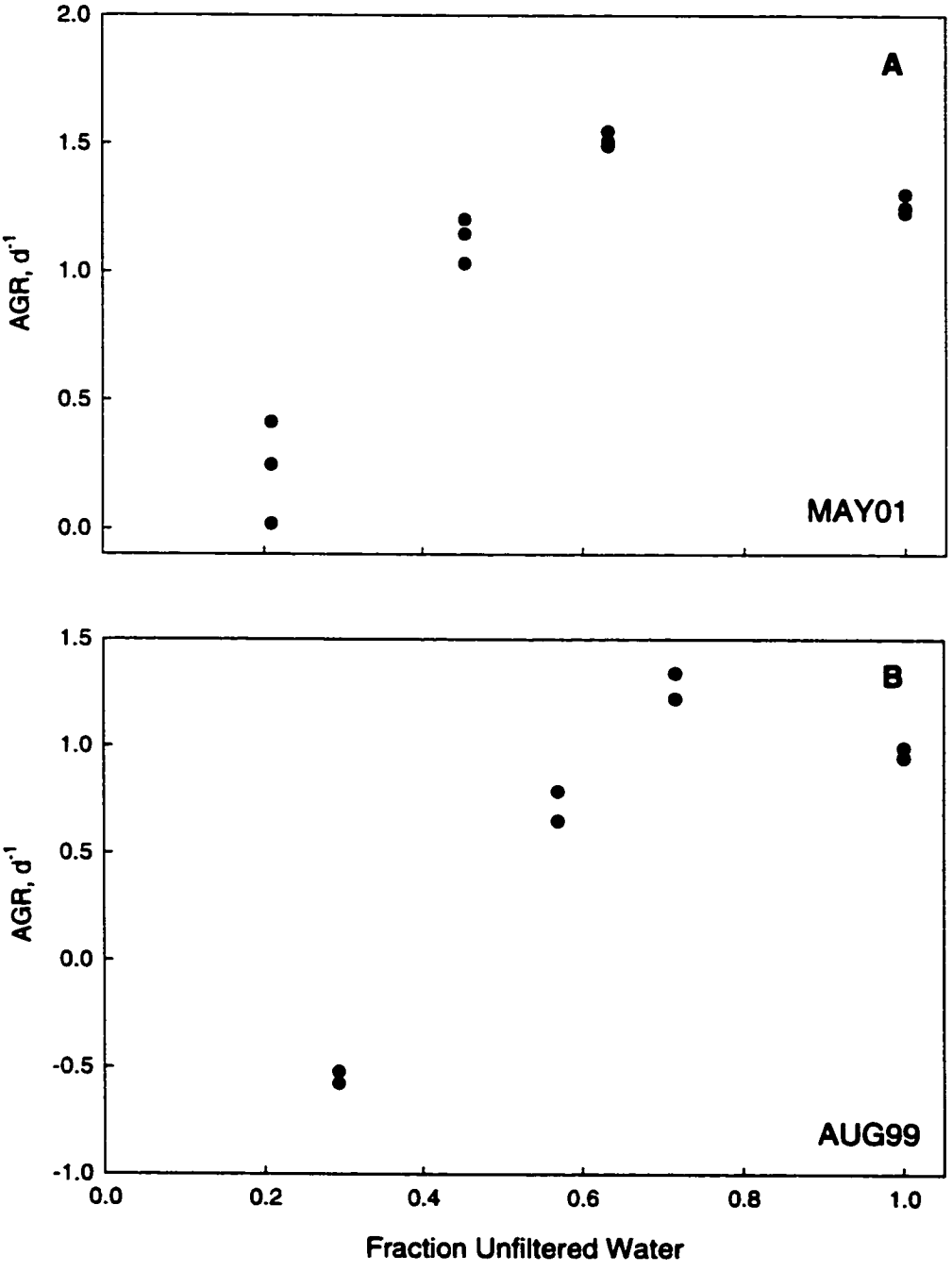

Fig. 17. Apparent growth rate (AGR) vs. dilution fraction for heterotrophic nanoflagellates. A, MAYO1 experiment, n = 3 for each treatment ; B, AUG99 experiment, n = 2 for each treatment.

has been shown that ciliate growth and grazing rates correlate positively with temperature (Finlay 1977; Banse 1982; Rassoulzadegan 1982; Sherr et al. 1988; Muller and Geller 1993). Nielsen and Kiorboe (1994) reported strong functional relationships between temperature and growth rates of field populations of ciliates in the southern Kattegat (Denmark). Rates of phytoplankton growth and microzooplankton grazing were positively correlated with temperature in a series of dilution experiments conducted in Northern Puget Sound (WA) (Strom et al. 2001). In Chapter II, I described significant positive correlations between temperature and standing stocks of naked ciliates and phytoplankton over period of nine months (June 1999 - March 2000) in the South Slough. A comparable thermal response in growth by phytoplankton and ciliates may facilitate a rapid assimilation of phytoplankton production into secondary production in the South Slough (Verity 1986; Nielsen and Kiørboe 1994).

The high grazing impacts of microzooplankton on total phytoplankton production determined for the MAR99 and MAR00 experiments was likely due to the composition of the >5-µm phytoplankton assemblage observed during the fall/winter period in the South Slough. Diatoms are generally rare or absent and nanoflagellates <20 µm dominate this phytoplankton fraction. Although ciliates show a preference for particles 2-10 µm in size (Jonsson 1986; Rassoulzadegan et al. 1988), trophic relationships between ciliate grazers and larger nanoflagellates have been observed and inferred in the laboratory and field. Gifford (1985) demonstrated relatively high growth rates for oligotrich ciliates

when fed two dinoflagellate strains with cell lengths 15 and 26 µm and a cryptomonad strain with a cell length 14 µm. In a Chesapeake Bay study, Stoecker et al. (1984) observed positive correlations between patches of phototrophic dinoflagellates and the horizontal distribution of the tintinnid *Favella* and the two naked ciliates *Balanion* and *Strombilidium*. Revelante and Gilmartin (1983) in the Northern Adriatic Sea, and Burkill (1982) in the Solent (UK), reported similar correlations between ciliate and nanophytoplankton standing stocks over annual cycles.

In light of the results for the March dilution experiments, greater inference can be made regarding the significantly positive correlations between ciliate and total phytoplankton standing stocks during the October-March period (1999-2000) in the South Slough (Chapter II). The high grazing impacts described in this chapter suggest that ciliates do not simply track the numerical growth response of phytoplankton but are instrumental in controlling the net production of total phytoplankton during this period. In Chapter II, I demonstrated that the ultraphytoplankton biomass was not sufficient to support the observed ciliate biomass during the October-March period, suggesting that ciliates were likely feeding on phytoplankton >5 µm and/or heterotrophic food (i.e., bacteria, flagellates) in addition to ultraphytoplankton. These experimental data suggest that ciliates indeed consume larger phytoplankton during this seasonal period and do so very efficiently.

The results of the AUG99 and MAY01 dilution experiments provide an interesting comparison in terms of the grazing impact on phytoplankton production and the composition of the microzooplankton assemblage. For the AUG99 experiment the microzooplankton community grazed nearly 100% of the daily potential production of ultraphytoplankton (cells 0.2-5 µm) (Table 13). No significant grazing impact was determined on larger (>5 µm) phytoplankton production. The standing stock of this larger phytoplankton fraction was dominated by 150-200 µm-diameter "ball"-like formations of the chain-forming diatom *Chaetoceros socialis*. The AGR of the undiluted treatments for >5CHL was 0.92 d^{-1} (SD = 0.03, n = 4), equivalent to 1.3 doublings d^{-1} for this phytoplankton fraction, and suggests that microzooplankton grazing was likely not a significant loss process for phytoplankton >5 µm. Ciliates dominated the >10 µm microzooplankton assemblage at this time, composed predominantly of *Strombidium*-like cells 20-40 µm in length; heterotrophic dinoflagellates were rare. Although large naked ciliates and tintinnids can feed at high rates on diatoms (Smetacek 1981; Sime-Ngando et al. 1995), the ciliates in this experiment appeared to graze specifically and very efficiently on ultraphytoplankton.

The close correspondence between high rates of microzooplankton grazing and ultraphytoplankton growth (g:k = 1.4:1.5 = 0.92; Table 13) connotes both a substantial channeling of ultraphytoplankton production into grazer biomass and a sizeable loss to primary production standing stock. Assuming no

grazing loss for phytoplankton >5 µm growing at a rate of 0.92 d^{-1}, the effect of intense grazing on fast growing ultraphytoplankton would result in a 48% daily loss of total primary production in this experiment. It must be noted that flexible algal cells >5 um can pass through the pore openings of membranes (Murphy and Haugen 1985) and the chlorophyll concentration measured probably represents a greater fraction of the optimal size range of algal food particles for oligotrich ciliates as demonstrated by Jonsson (1986) and Fenchel (1980).

The relatively large contribution of the heterotrophic dinoflagellates *Protoperidinium* spp. and *Gyrodinium spirale* to the microzooplankton assemblage at the time of MAY01 experiment is a likely explanation for the high grazing rate on TCHL during a chain-forming diatom bloom (Tables 11 and 13). The 1:1 linear size ratio of between dinoflagellates and their prey (Jacobson and Anderson 1986; Hansen et al. 1994) is a consequence of their ability to handle and ingest relatively large prey in a diversity of ways. Thecate dinoflagellates can engulf and digest their prey in an extracellular feeding veil ("pallium"; Jacobson and Anderson 1986) or, in the case of athecate dinoflagellates, can compact large cells or chains of cells into food vacuoles (Buck and Newton 1995; Strom and Strom 1996). Both types of feeding strategies were observed microscopically in this experiment.

The tight coupling between microzooplankton grazing and phytoplankton production, together with a high algal biomass, resulted in a community ingestion rate of almost 900 µg C l^{-1} d^{-1} (Table 13). Neuer and Cowles (1994) found similar

high ingestion rates (612-1762 μg C l^{-1} d^{-1}) during bloom periods off the Oregon coast. They observed a close relationship between the abundance of gymnodinoid dinoflagellates and both community grazing rates and the abundance of large phytoplankton (>20 μm). Strom et al. (2001) reported enhanced grazing rates with blooms of diatoms compared to lower algal biomass periods in the Gulf of Alaska. They attributed this response in large part to the relatively large contribution of heterotrophic dinoflagellates to microzooplankton biomass.

The differences in the grazing impact on prokaryotic and eukaryotic picophytoplankton (PPICO and EPICO) production in the AUG99 experiment were more a consequence of differing growth rates (0.38 d^{-1} vs. 1.1 d^{-1}) than varying grazing rates (0.24 d^{-1} vs. 0.31 d^{-1}; Table 13). Overall, daily losses attributed to grazing for each picophytoplankton group were comparatively less than the impact on the ultraphytoplankton fraction, suggesting that picophytoplankton did not form a large part of the microzooplankton prey field in this experiment in terms of ingested biomass. Daily losses of PPICO and EPICO production to grazing were greater in the MAY01 experiment. Growth rates for both groups (PPICO, 0.40 d^{-1}; EPICO, 1.5 d^{-1}) were similar to those estimated in AUG99 (Table 13). The substantial difference in grazing impact on picophytoplankton production between the two experiments was attributed to higher grazing rates in the MAY01 experiment (PPICO, 0.37 d^{-1}; EPICO, 1.2 d^{-1}). Growth rates for PPICO determined here fall within the range 0.1-1.0 d^{-1} typically

observed for field and laboratory populations of *Synechococcus* (Campbell and Carpenter 1986a; Liu et al. 1995; Liu et al. 1999). The grazing impact estimates on PPICO production in this study were substantial (AUG99, 67%; MAY01, 93%) and within the range of findings from other studies in which the dilution method was applied (14-99%, NW Atlantic, Campbell and Carpenter 1986b; 45-91%, NE Pacific, Strom and Welschmeyer 1991; 30-164%, Chesapeake Bay, McManus and Cantrell 1992). The higher grazing rates on the faster growing EPICO, however, equated to an appreciably higher carbon transfer via microzooplankton ingestion than for PPICO. Although grazing impact on ultraphytoplankton production was not quantified directly in the MAY01 experiment, the very high trophic coupling observed between grazers and picophytoplankton components does imply that significant grazing on ultraphytoplankton by microzooplankton occurred in this experiment.

In Chapter II, I described significantly positive correlations between naked ciliate and ultraphytoplankton standing stocks in the South Slough between April-September (1999). In contrast, no numerical relationships were determined between ciliates and total or >5-µm phytoplankton standing stocks. Large, chain-forming diatoms frequently dominate the >5-µm phytoplankton assemblage during this seasonal period, and were observed to do so in the AUG99 and MAY01 experiments. Microzooplankton imparted very high grazing impacts on ultraphytoplankton production in the AUG99 experiment and on the production of the picoplankton components of the ultraphytoplankton fraction in the MAY01

experiment. In context with the field relationships, these results suggest that the strong numerical coupling between ciliates and ultraphytoplankton observed between April-September (Chapter II) is comparable to trophic coupling, and ciliate grazing is a key factor in controlling the net production of ultraphytoplankton in the South Slough throughout this seasonal period.

The high grazing impacts on total phytoplankton production in the MAY01 experiment was paralleled by a relatively high contribution of heterotrophic dinoflagellates to the microzooplankton assemblage. Such an outcome would not have been predicted by the seasonal correlations between ciliate and total phytoplankton standing stocks (Chapter II). This experimental outcome suggests that the nature of the grazing impact that can be imposed by microzooplankton depends on the coincidental composition of the microzooplankton and phytoplankton assemblages (Peters 1994). These data suggest that a microzooplankton assemblage composed of relatively high numbers of dinoflagellates, ciliates, and heterotrophic nanoflagellates can collectively give rise to high grazing impacts on the entire phytoplankton assemblage during this productive seasonal period in the South Slough.

Non-linear Apparent Growth of Prey

Due to a non-linear relationship between AGR and dilution factor along the dilution gradient, piece-wise linear regressions were applied for both picophytoplankton groups in the MAYO1 experiment. A suppression of apparent growth in the lowest dilution treatments referenced to the least-squares lines of

the regressions is visually apparent in dilution plots for each picophytoplankton group (Fig. 16). Although not as readily apparent, similar relationships for these groups were observed in the AUG99 experiment (Fig. 15). Non-linear relationships such as these are generally attributed to saturating feeding response by the microzooplankton community (Gallegos 1989; Evans and Paranjape 1992), which minimizes or negates the treatment effect of dilution. Observations of enhanced AGR of HFLAG that coincided with the apparent suppressed growth of picophytoplankton in this study (Fig. 17), however, may pose a challenge to both the categorization of microzooplankton as a single guild of grazers and the application of a linear trophic model. An interpretation of these results is that HFLAG were both grazers of picophytoplankton and prey for larger protozoans (i.e., ciliates) in these experiments. The uncoupling of the 'intraguild' predation (ciliates-HFLAG) (Polis and Holt 1992) by the dilution treatment resulted in the increased net-growth of HFLAG and the ensuing enhanced grazing pressure on picophytoplankton, which offset the dilution effect on ciliate herbivory.

The interactions proposed here are substantiated by data in the literature. Although ciliates are known to consume picophytoplankton at high rates (Rassoulzadegan et al. 1988; Christaki et al. 1998), there is evidence suggesting that heterotrophic nanoflagellates are important, if not the principal consumers of both autotrophic and heterotrophic picoplankton in most environments (Campbell and Carpenter 1986b; Kuosa 1991; Sanders et al. 1992; Sherr and Sherr 1994). I

monitored HFLAG abundance in the South Slough over a seven-month period that seasonally overlapped the experimental sample dates (Appendix A). Significantly positive correlations were determined between HFLAG abundance and ultraphytoplankton biomass. Laboratory data and field observations suggest that oligotrich ciliates can have a high predation impact on HFLAG (Dolan and Coats 1991; Verity 1991; Jürgens et al. 1996) and can achieve the highest growth rates when HFLAG are included as prey (Ohman and Snyder 1991). Sanders et al. (1992) speculated that the high ratios of bacteria:HFLAG in eutrophic environments are a conquence of intense predation of larger organisms on HFLAG.

Ciliate predation on HFLAG is likely to have important ecological and methodological consequences that may be easily overlooked (Jürgens et al, 1991). As in this case, for example, herbivory by HFLAG on picophytoplankton and omnivory by ciliates on HFLAG and picophytoplankton may constitute a direct challenge to the ability of a linear model to accurately assess microzooplankton grazing on picophytoplankton production (Polis and Strong 1996). This will be examined further in Chapter IV.

Conclusion

The results of the seasonal grazing experiments within the context of the field data (Chapter II) suggest that microzooplankton are major players in the algal-based energy flow in the South Slough. Microzooplankton grazing utilized from 48% to nearly 100% of the daily primary production in the South Slough.

These values are similar to others for microzooplankton grazing in upwelling waters and in temperate coastal and estuarine environments (Table 4). The experimental results verify the importance of the compositional makeup of the microzooplankton and phytoplankton assemblages in predicting the nature of the grazing effect. The enhanced and suppressed net-growth response of heterotrophic nanoflagellates 2-10 µm and picophytoplankton, respectively, to low dilutions of natural microplankton assemblages imply that trophic interactions within the microzooplankton guild may challenge the ability of the dilution linear model to accurately assess grazing on picophytoplankton production. These results of this study substantiate the role of microzooplankton as important consumers of phytoplankton production in the South Slough.

Table 14. Estimates of community-level grazing impact of microzooplankton on phytoplankton production in other upwellling, coastal and estuarine environments. All estimates determined with the dilution method.

Author	Location	% Potential Phytoplankton Grazed (d^{-1})
Landry and Hasset (1982)	Washington Coast	17 - 52
Neuer and Cowles (1994)	Oregon Coast (upwelling)	16 - 121
Edwards et al. (1999)	Arabian Sea (monsoon, upwelling)	4 - 60
Gifford (1998)	Halifax Harbour, Nova Scotia	40 - 100
McManus and Cantrell (1992)	Chesapeake Bay	50 - 60
Froneman and McQuaid (1997)	Kariega Estuary, South Africa	55 - 151
Present study	South Slough, Coos Bay	48 - 96

CHAPTER IV

MICROZOOPLANKTON TROPHIC INTERACTIONS AND THEIR IMPLICATIONS FOR LINEAR TROPHIC MODELS

The experimental findings presented in Chapter III strongly suggest that microzooplankton are important and efficient grazers of phytoplankton production in the South Slough. Measurements of grazing were ascertained with the dilution method, a technique with the basic assumption that ingestion is a linear function of grazer density. Violation of this assumption is regularly explained in terms of a functional feeding response of the microzooplankton guild. The diverse feeding strategies demonstrated by microzooplankton members and the ramifications these are likely to have on the operation of a linear model are generally not considered.

In one experiment described in Chapter III, the net growth dynamics of autotrophic picophytoplankton and heterotrophic nanoflagellates along the dilution gradient led me to hypothesize that trophic interactions among microzooplankton members can give rise to non-linear relationships between the net growth of phytoplankton and dilution factor. In Chapter IV, I describe the results of an experimental test that supports this hypothesis. In addition, I present the findings of another experiment that suggest complex feeding interactions

among microzooplankton members can diffuse the cascade of top-down trophic effects and may explain the frequently reported constancy of natural picoplankton populations.

Abstract

Based on previous dilution-method (Landry and Hassett 1982) experimental results in Chapter III, it was hypothesized (H_1) that the suppression of the apparent growth of picophytoplankton in low dilution treatments can result from the dilution-mediated release of heterotrophic nanoflagellates from predation by larger microzooplankton (i.e., ciliates). That is, increased herbivory by nanoflagellates offsets the positive effect that dilution would have on picophytoplankton growth. To test this hypothesis, microzooplankton >20-µm were initially reduced by fractionation for one dilution series and the apparent growth of prey vs. dilution factor was compared with an unfractionated dilution series. The fractionation treatment eliminated both the suppression (picophytoplankton) and enhancement (heterotrophic nanoflagellates) of apparent growth in low dilution treatments, which were observed in an unfractionated dilution series. Based on these results, it was hypothesized (H_2) that the reduction of ciliate abundance by top-down predation in undiluted environments can give rise to a similar predation release of heterotrophic nanoflagellates and corresponding suppression of picophytoplankton. As a test, a density gradient of *Acartia tonsa* calanoid copepods was prepared and the apparent growth of ciliate biomass, heterotrophic nanoflagellates and

picophytoplankton across the gradient during a 24 h incubation was measured. The apparent growth of ciliate biomass and heterotrophic nanoflagellate abundance significantly increased and decreased, respectively, with decreasing copepod abundance, but the apparent growth of picophytoplankton remained unchanged. The results provide a theoretical explanation for the annual and seasonal constancy of picoplankton populations that are routinely reported. The outcomes of both experiments suggest that linear models do not accurately and consistently assess microzooplankton grazing on picoplankton production, and predict the large- to small-cell trophic interactions within the microplankton assemblage.

Introduction

The discovery of an abundant and ubiquitous picophytoplankton (cells 0.2-2.0 µm; Waterbury et al. 1979; Johnson and Sieburth 1979) and their substantial contribution to autotrophic production in marine systems (Li et al. 1983; Platt et al. 1983; Murphy and Haugen 1985; Joint and Pomroy 1986) prompted substantive changes in our understanding of the trophic connectivity in planktonic communities (Pomeroy 1974; Azam et al. 1983; Sherr and Sherr 1988). The relative constancy of autotrophic and heterotrophic picoplankton populations (Davis et al. 1985; Stockner and Antia 1988) in temperate environments during the growing season and in tropical systems year round has implied a predation control mechanism (Johnson et al. 1982; Azam et al. 1983; Iturriaga and Mitchell 1986). The sustained constancy of picoplankton standing stocks following direct

and indirect removal of predators (Landry et al. 1993; Calbet and Landry 1999) suggests, however, that the actual mechanism of control may be more complex than predicted by a simple predator-prey linear model.

Due to the numerical dominance of smaller size, the primary trophic role attributed to heterotrophic nanoflagellates (2-20 µm) has been as bacteriovores (Fenchel 1982; Azam et al. 1983; Wright and Coffin 1984). Although it is apparent that small flagellates (<5 µm) do apparently ingest bacteria-size particles (Wikner and Hagström 1988; Sherr et al. 1991), larger flagellates derive most of their energy from larger prey cells (Sherr and Sherr 1991). Numerical relationships of field populations (Linley et al. 1983; Davis et al. 1985), together with field and laboratory experimental results (Johnson et al. 1982; Campbell and Carpenter 1986; Hagström et al. 1988; Kuosa 1991; Sherr et al. 1991; Caron and Goldman 1993), provide convincing evidence that nanoflagellates are important consumers of in marine systems. The utilization of both heterotrophic and autotrophic food may offer flagellates greater ecological flexibility to persist in heterogeneous food environments (Goldman and Caron 1985) and capitalize on the possible asynchronous fluctuations in each prey population.

Small naked ciliates (<20 µm) are also known to consume picophytoplankton at high rates (Rassoulzadegan et al. 1988; Sherr et al. 1991; Christaki et al. 1998) although larger ciliates generally have higher clearance rates for particles of slightly larger size (2-10 µm; Rassoulzadegan 1982; Jonsson 1986; Sherr et al. 1991). Laboratory data and field observations suggest

that oligotrich ciliates can have a high predation impact on heterotrophic nanoflagellates (Dolan and Coats 1991; Verity 1991; Jürgens et al. 1996) and can achieve higher growth rates when heterotrophic nanoflagellates are included as prey (Ohman and Snyder 1991; Verity 1991). Sanders et al. (Sanders et al. 1992) speculated that the high ratios of bacteria: heterotrophic nanoflagellates in eutrophic environments are a consequence of intense predation of larger organisms on nanoflagellates. This arguably holds equally true for picophytoplankton (Sherr and Sherr 1991), most of which are bacteria.

Ciliate predation on heterotrophic nanoflagellates is likely to have important ecological and methodological consequences that are at present overlooked. For example, it has been proposed that omnivory (i.e., feeding on multiple trophic levels) can have proximate stabilizing effects on aquatic community structure that may offset direct and indirect effects of cascading trophic interactions (Sprules and Bowerman 1988; Polis and Strong 1996). Secondly, such trophic complexity may not be appropriately addressed with a linear model formulated to quantify the interaction and energy flow between the microzooplankton grazer guild and their prey.

Since its inception, the seawater dilution method (Landry and Hassett 1982) has become the standard technique for assessing grazing rates and impacts on phytoplankton production. It is preferred over other quantitative methods (i.e., size-fractionation, metabolic inhibitors, tracers of ingestion) because it can derive simultaneous rate estimates for algal growth (k) and

mortality due to grazing (g) with minimal direct handling of the natural microplankton assemblage. The model is chiefly based on the assumption that changes in the apparent growth rate (AGR) of prey in different known dilutions are linearly related to the dilution of grazer-prey encounter rate using the formulation

$$(1/t)\ \ln(P_t/P_0) = k - g(X)$$

where P_0 and P_t are the prey densities in each dilution-fraction treatment at the beginning and end of the incubation; $\ln(P_t/P_0)$ is equivalent to the prey-AGR in each dilution-fraction treatment during the incubation time period, t; and X is the fraction of undiluted water. The negative slope of this relationship is an estimate of the rate of prey mortality due to grazing, g. The ordinal-intercept of the least-squares regression line is an estimate of the intrinsic prey growth rate, k, in the theoretical absence of grazers (100% dilution).

It follows that a linear increase in the prey-AGR with increased dilution is indirectly attributed to a linear reduction of the grazer community ingestion rate. Non-linear relationships (Fig. 18) between prey-AGR and the dilution factor are generally explained as the result of a modified feeding response by the collective group of grazers, the microzooplankton (Gifford 1988; Gallegos 1989; Rivkin et al. 1999; Strom et al. 2001). No significant change in prey-AGR at high dilutions is typically attributed to a cessation of feeding by grazers at a low prey-density

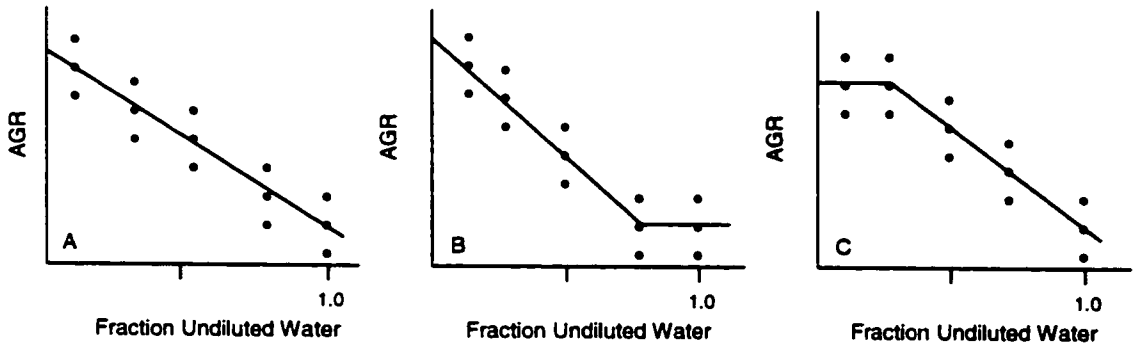

Fig. 18. Graphic examples of non-linear relationships between apparent growth rate (AGR) of prey and dilution factor. A, linear relationship, no feeding response; B, feeding saturation or trophic complexity at low dilutions; C, threshold feeding response at high dilutions.

threshold. No significant change in prey-AGR at low dilutions (high prey density) is regularly attributed to feeding saturation by microzooplankton. This is a reasonable argument if a single predator-prey relationship is being considered. It may prove to be lacking, however, if larger grazers are actually omnivores and consume both smaller grazers and an algal food source accessible to both consumer groups (Polis and Strong 1996), as may be the case for ciliates, heterotrophic nanoflagellates and picophytoplankton.

Here I present experimental findings that support an alternative explanation to the observed suppression of prey growth at low dilutions in the context of trophic interactions among microzooplankton members. In a previous dilution experiment (Chapter III), a significant increase in the apparent growth of heterotrophic nanoflagellates 2-10 μm (HFLAG, to distinguish from all nanoflagellates) was observed to coincide with the suppression of the apparent growth of picophytoplankton in a low dilution treatment (Fig 19). I hypothesize that (1) the apparent growth of picophytoplankton in the undiluted treatments is controlled by the combined grazing activity of consumers at two trophic levels: ciliates and HFLAG, and (2) the apparent growth of HFLAG is suppressed by ciliate predation in the undiluted environment but not the low dilution environment. The potential positive effect of this dilution on the net growth of picophytoplankton is offset by the numerical growth and grazing of HFLAG in the low dilution environment.

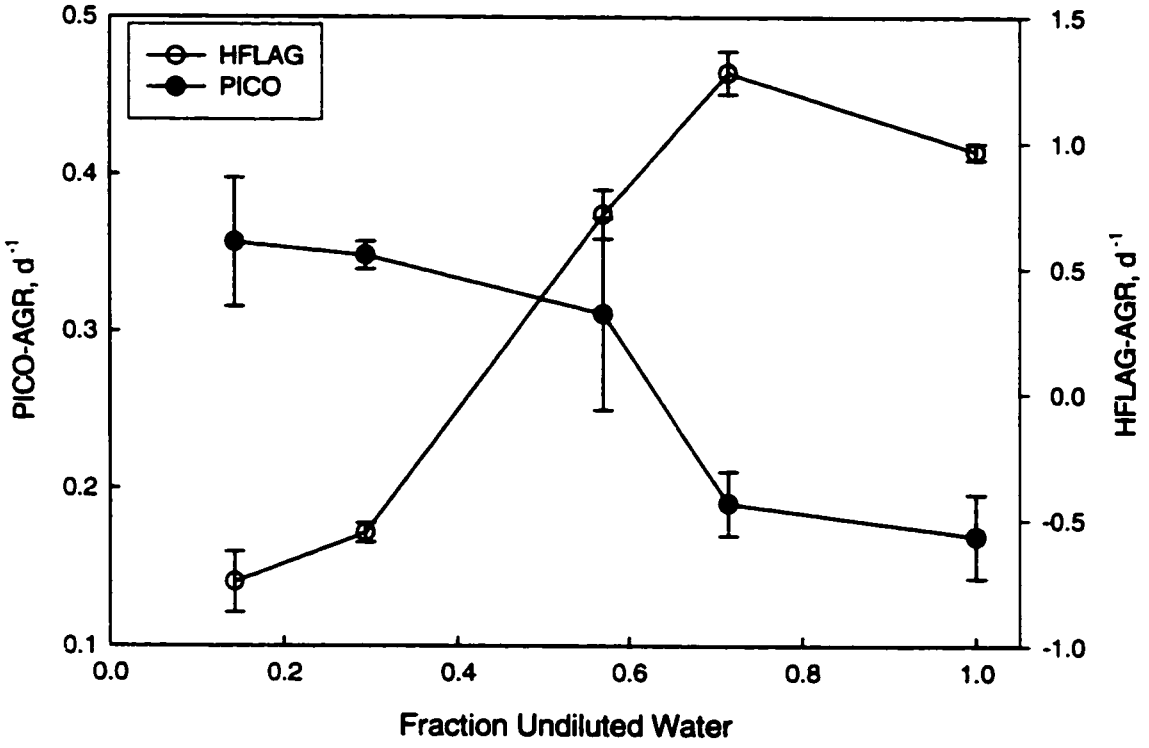

Fig. 19. Apparent growth rate (AGR) of picophytoplankton (PICO) heterotrophic nanoflagellates (HFLAG) vs. dilution factor for an experiment conducted on 28 August 1999. Error bars represent ±1 SD of two replicates for each dilution factor.

To test these hypotheses, two experiments were conducted and the results are reported here. In the first, ciliate predation on HFLAG was initially reduced with a 20-μm mesh fractionation treatment and the AGR of picophytoplankton and HFLAG in the fractionated dilution-series and unfractionated dilution-series were measured. I predicted that the fractionation treatment would eliminate (1) the suppression of picophytoplankton-AGR and (2) the enhanced HFLAG-AGR, in the low dilution treatments of the fractionated dilution series, relative to outcomes in the unfractionated dilution series. Assuming (1) HFLAG are not food limited in the low dilution treatment and that the enhanced net-growth and (2) herbivory of HFLAG are the primary causes of the suppressed picophytoplankton-AGR in the low dilution treatment, it can be shown that the partition of overall grazing impact on picophytoplankton by HFLAG and ciliates and the rate of ciliate predation on HFLAG can be estimated.

In the second experiment, I tested the prediction that the attenuation of ciliate predation on HFLAG in treatments with increasing numbers of copepods will foster comparable increases in the net-growth of HFLAG and suppressed net-growth of picophytoplankton as observed in low dilution treatments. This experiment provided an independent means of testing the interpretation of the dilution experiment. The findings of each experiment and their interpretations presented here can offer an alternative explanation for the relative numerical constancy of picoplankton populations in nature and the lack of predation-

mediated indirect effects on picoplankton growth in field experiments (Landry et al. 1993; Calbet and Landry 1999; Calbet et al. 2001).

Materials and Methods

Dilution Experiment

Estuarine water was collected at high tide with a weighted plastic bucket from the primary tidal channel of the South Slough of Coos Bay, Oregon. Cell-free diluent was prepared by serial filtration through 0.45-µm and 0.2-µm filter capsules (Gelman). Additional water was collected and gently siphoned (silicone tubing) into carboys through a submerged 200-µm mesh screen to minimize the macrozooplankton component in the assemblage. This water was mixed with the diluent in 10-l containers to achieve the following target fractions of unfiltered water: 0.05, 0.10, 0.25, 0.50, 0.75 and 1.00. Low concentrations of macronutrients (nitrate, 10 $\mu g\text{-}l^{-1}$; phosphate, 1 $\mu g\text{-}l^{-1}$; Landry and Hassett 1982) were added to each mixture to minimize potential nutrient limitation at all dilution levels. Triplicate volumes (700 ml) of each dilution treatment were gently siphoned into acid-washed Whirl-Paks. A second dilution series of equal dilution fractions was prepared by gently siphoning water from each mixture into Whirl-Paks through a submerged 20-µm pore-diameter mesh. The <20 µm and <200 µm dilution series will be referred to as the nanoplankton and microplankton series, respectively, in the following sections of this paper. Treatments were incubated for 24 h in an outdoor Nalgene tank with circulating water from the OIMB seawater system. The tank was covered with sufficient neutral density

screening to simulate the ambient light at the collection depth. All equipment in direct contact with experimental water (i.e., buckets, carboys, containers, tubing) was acid-washed (10% HCL) and copiously rinsed with deionized water prior to use. Non-latex gloves (Nytril) were worn throughout the experimental set-up.

Triplicate initial samples (50-100 ml, depending on the dilution) were gently siphoned from each dilution mixture of the microplankton-series to estimate the abundance of prokaryotic picophytoplankton (PPICO), eukaryotic picophytoplankton (EPICO) and heterotrophic nanoflagellates 2-10 µm (HFLAG). Comparable sample volumes were taken from each treatment vessel of both dilution series for final measurements of these parameters. It was assumed that the 20-µm fractionation of the microplankton assemblage imparted no reductions in the numbers of picophytoplankton and HFLAG and the initial density of these groups were equal in each dilution series. The picophytoplankton and HFLAG samples were fixed with 0.50% ice-cold glutaraldehyde and stored in darkness at 4º C until sample slide preparation (usually within 48-72 hr). Initial and final samples (200 ml) were taken from the undiluted mixture and treatment bottles (n = 2) and fixed with acid Lugols (final conc. 5%) to estimate ciliate abundance. For picophytoplankton enumeration, 10-50 ml aliquots (depending on dilution) were filtered under low positive pressure (<5 in Hg) onto black 0.2-µm membranes (Poretics) with backing filters. Cell counts of HFLAG were made from the samples taken from the target dilution fraction-mixtures of 0.25 - 1.00. Fifteen to 60 ml samples were incubated with DAPI (4',6-diamidino-2-phenylindole

dihydrochloride; final volume 0.01 μg ml^{-1}) for 10-15 min in the dark and then concentrated onto black 0.8 μm membranes with backing filters under gentle vacuum (<5 in Hg). Membranes were then mounted onto slides with immersion oil and viewed with epifluorescence microscopy (Leica; PPICO, green light; EPICO, blue light; HFLAG, UV light) (Sherr et al. 1993). Cells were counted in random fields or diametric transects until at least 200 cells (Murphy and Haugen 1985; Booth 1987) of each group were enumerated in each sample. HFLAG counts were delineated into size classes of 2-5 μm and 6-10 μm.

Carbon concentration for each picoplankton group was determined differently. Accurate cell-diameter measurements of PPICO for biovolume estimation were hindered by very bright autofluorescence that extended beyond the cell wall; therefore, the conversion factor of 0.25 pg C $cell^{-1}$ for *Synechococcus* was applied (Li et al. 1992). Cell-biovolume of EPICO was determined from the average cell diameter of 40 randomly selected cells measured with an ocular micrometer at 1000X, assuming spherical cell shape. Carbon was determined with the conversion factor 0.36 pg C um^{-3} (Verity et al. 1992).

For ciliate cell counts, 100-ml of fixed sample were settled (>18 hrs) in columns onto glass viewing plates and all ciliates >10 μm were enumerated with an inverted microscope at 320X. Ciliates (naked and tintinnids combined) were delineated into < and >20 μm size fractions. The ciliate abundance estimates included heterotrophic and mixotrophic ciliates alike, as the nutritional status of

each ciliate morphotype/group could not be ascertained due the masking effects of the Lugols fixative on chlorophyll autofluorescence.

Copepod Predation Experiment

Copepods were collected at high tide in the South Slough sample site (Chapter II) with a 0.5-m diameter plankton net (330 µm mesh) equipped with a closed cod-end and gently poured into 22-l carboy containing in situ water. The carboys were transported back to the lab where similar-sized, undamaged adults of the calanoid copepod *Acartia tonsa* Dana were sorted into beakers containing water from the collection site and allowed to acclimatize at ambient temperature for 24 hours. The following day, additional water was collected from ~1 m depth with a weighted bucket at the same site and gently siphoned through silicone tubing into 22-l carboys through a submerged 200-µm screen to remove the macrozooplankton assemblage. Dissolved nitrate and phosphate (10 $\mu g\text{-}l^{-1}$ and 1 $\mu g\text{-}l^{-1}$ respectively) were added to the carboys in the lab to control for differential nutrient remineralization and limitation along the experimental predation gradient. Water was then gently siphoned into 20 clear, polycarbonate flasks to a volume of 575 ml. No copepod additions were made to eight of these flasks, four of which were harvested at time zero to establish the initial densities of the parameters of interest, and the other four served as copepod-free controls. To the remaining eight flasks were added 5 and 10 copepods, four replicates each. The flasks were sealed with silicone stoppers, allowing no air space. Treatments were incubated for 24 h in an outdoor experimental tank as described above.

Flasks were gently shaken every 4-6 hr to minimize sedimentation. All equipment in direct contact with the experimental water (i.e., buckets, carboys, containers, tubing) were acid-washed (10% hydrochloric acid) and copiously rinsed with deionized water prior to use. Non-latex gloves (Nitryl) were worn throughout the experimental set-up.

Initial and final samples for the enumeration of ciliates >10 µm, HFLAG, PPICO, and EPICO were taken from each treatment replicate and processed as described above. The biomass of ciliates was determined with biovolume estimates from ocular measurements of up to 50 individual cells (when possible) of each morpho-species group, assuming standard geometric shapes. Ciliate cell-carbon quota was estimated from cell-volume using the conversion factor 0.19 pg C um^{-3} (Putt and Stoecker 1989).

Data Analysis

The grazing rate (*g*) and the instantaneous growth rate of prey (*k*) were estimated from the respective slope and ordinal-intercept of statistically significant regressions of the apparent growth rate (AGR) of picophytoplankton vs. dilution factor for each dilution series. The AGR of PPICO and EPICO in the low dilution treatments of the microplankton-series plot were observed to not significantly differ from the AGR in the undiluted treatments. The data sets were reexamined by decomposition of the dilution plots into two regions: region 1 (higher dilution region), which had a significant and negative linear slope; and region 2 (lower dilution region), whose slope was statistically zero (Gifford 1988;

Gallegos 1989; Rivkin et al. 1999). The instantaneous growth rate of prey, k, was estimated from the ordinal-intercept of the least-squares line for the regression using region 1 data. The rate of prey mortality due to grazing, g, was estimated as the difference between k calculated for region 1 and the mean-AGR in the undiluted treatments (Gallegos 1989).

The deviation of the mean-AGR for each picophytoplankton group in the low dilution treatment from the AGR predicted by the line equation of $k - gX$ (Fig. 20) was attributed to the enhanced growth and grazing rates of HFLAG. It is proportional to the HFLAG grazing rate in the undiluted treatments with the following assumptions: 1) HFLAG were predation-limited and not food-limited in the dilute environment; and 2) no functional grazing response of ciliates. The HFLAG grazing rate in the undiluted treatments (g_{HFLAG}) was calculated as

$$g_{HFLAG} = g_{DIL} \times [(1 - P) / (1 - P_{DIL})]$$

where g_{DIl} is the grazing rate in the low dilution treatments; P and P_{DIL} are the ciliate predation rates on HFLAG in the undiluted and low dilution treatments as calculated below. The ciliate grazing rate (g_{CIL}) on picophytoplankton in the undiluted treatment was measured by the difference of composite grazing rate, g, as defined above (see Fig. 20) and g_{HFLAG}.

Clearance rates, F (= volume cleared of prey grazer^{-1} time^{-1}), of each picophytoplankton group by HFLAG and ciliates were calculated using the equation by Frost (1972),

$$F = (V \times g^*) / \langle D \rangle$$

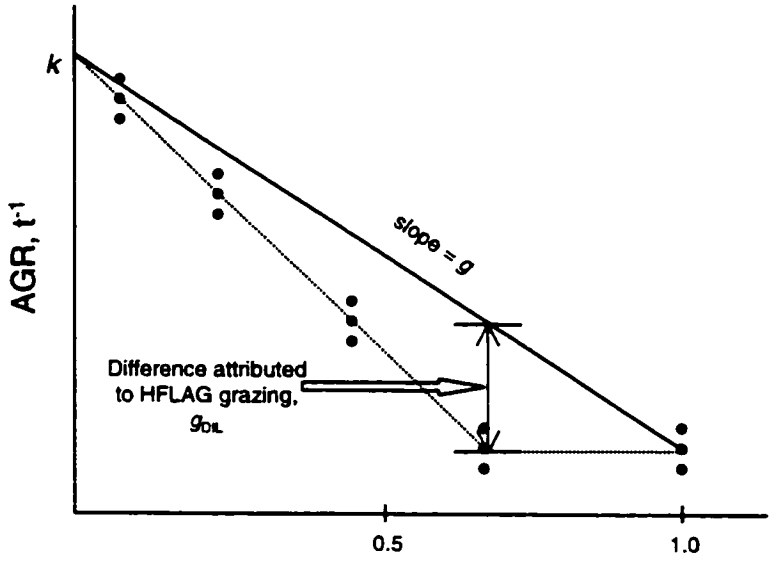

Fig 20. Determination of the grazing rate for heterotrophic nanoflagellates (HFLAG). The difference between the mean apparent growth rate (AGR) of the low dilution treatment replicates and the AGR predicted by the least-squares regression line is attributed to the enhanced HFLAG grazing rate (g_{DIL})in these treatments. The HFLAG grazing rate in the undiluted treatments (g_{HFLAG}) was measured by multiplying g_{DIL} by ratio of ciliate predation rates in each treatment as described in the text.

where V is the volume of treatment water; g^* is the respective grazing rate for HFLAG (g_{HFLAG}) or ciliates (g_{CIL}); and <D> is the time-averaged density of HFLAG or ciliates in the undiluted treatments determined as follows,

$$<D> = (D_t - D_0) / (\ln D_t/D_0)$$

where D_t and D_0 are the initial and final mean densities of HFLAG or ciliates in the undiluted treatments. Ingestion rates of each picophytoplankton group (I, cells l^{-1} d^{-1}) by HFLAG and ciliates, combined and independently, were calculated as (Strom et al. 2001)

$$I = g^* \times C_{AVG}$$

where g^* is the respective grazing rate for HFLAG (g_{HFLAG}) or ciliates (g_{CIL}), and C_{AVG} is the time-averaged concentration of PPICO or EPICO in undiluted treatments determined according to Frost (1972),

$$C_{AVG} = C_0(e^{(k-g)} - 1) / (k-g)$$

where C_0 is the initial prey concentration in the undiluted treatments and k and g are the prey-growth and grazing rate coefficients for each picophytoplankton group. The carbon equivalents of the prey cells ingested were determined with the conversion equations given above.

The ciliate predation rate on HFLAG (P) was calculated as

$$P = (AGR_{UNDIL} - AGR_{LDIL}) / (1 - X)$$

where AGR_{UNDIL} and AGR_{LDIL} are the mean-apparent growth rates of HFLAG in the undiluted and low dilution treatments; and X is the actual dilution factor of the low dilution treatments as determined by the fraction of initial HFLAG densities in

the low dilution and undiluted treatments. A more accurate rate measurement would have been attained with X being the fraction of the time-average density of ciliates in the low dilution and undiluted treatments; however, ciliate abundance was not determined in the low dilution treatments.

Differences in the AGR of picophytoplankton and HFLAG within and between dilution series were tested with two-sample *t*-tests. One-way ANOVA was employed to test for significant differences in the AGRs of picophytoplankton, HFLAG and ciliate biomass between treatments along the copepod density gradient. Linear regressions were performed to determine the slopes of the AGRs of ciliate biomass and HFLAG vs. copepod density. All statistical analyses were performed with Statistica (version 5.0) software at the significance level $\alpha = 0.05$. Error bars about the means represent ± 1 standard deviation.

Results

Dilution Experiment

Initial estimates of specific physical and biological parameters for each dilution experiment are given in Table 15. The abundance of eukaryotic picophytoplankton (EPICO) was almost seven times the density of prokaryotic picophytoplankton (PPICO) (4.2×10^3 vs. 28×10^3 cells ml^{-1}). The overall abundance of heterotrophic nanoflagellates 2-10 µm in size (HFLAG) was 380 cells ml^{-1}. HFLAG 2-5 µm averaged 303 cells ml^{-1} and the mean abundance of HFLAG 6-10 µm was 71 cells ml^{-1}.

Table 15. Initial estimates of physical and specific biological parameters for the dilution experiment. PPICO, prokaryotic, and EPICO, eukaryotic picophytoplankton; HFLAG, heterotrophic nanoflagellates: mean (± 1 S.D.) of three replicate samples. Ciliate abundance represents one total count of a 100-ml sample.

		Picophytoplankton x 10^3 cells ml^{-1}		HFLAG, cells ml^{-1}		Ciliates, cells ml^{-1}	
Temp. ºC	Salinity, ppt	PPICO	EPICO	2-5 µm	6-10 µm	<20 µm	>20 µm
13.4	29.2	4.2 (0.2)	28 (1.3)	303 (20)	71 (9.6)	1.8	4.8

The initial total-ciliate abundance was 6.6 cells ml^{-1}. The abundance of ciliates < and >20 µm was 1.8 cells ml^{-1} and 4.8 cells ml^{-1}. The assemblage was dominated by a prolate-spheroid shaped, *Strombidium* sp. ciliate with the length-width dimensions 20 x 25 µm. The 20-µm fractionation of the microplankton assemblage resulted in a 36% reduction of ciliates >20 µm (Table 16). The reduction of ciliates <20 µm was <5%. The time-average densities of total ciliates and each size fraction in the undiluted treatments of each dilution series are given in Table 17. The time-average densities of ciliates <and >20 µm in the nanoplankton-series were 44% and 6% lower than in the microplankton-series.

The apparent growth rates (AGRs) of PPICO and EPICO vs. dilution fraction for each dilution series are plotted in Fig. 21. For the microplankton series, the mean-AGRs of PPICO and EPICO were 0.07 d^{-1} (± 0.04) and 0.27 d^{-1} (± 0.05) in the undiluted treatments, and -0.002 d^{-1} (± 0.06) and 0.34 d^{-1} in the low dilution treatments. In the nanoplankton series, the mean-AGRs of PPICO and EPICO were 0.0 (± 0.09) and 0.52 d^{-1} (± 0.04) in the undiluted treatments, and 0.15 d^{-1} (± 0.06) and 0.92 d^{-1} (± 0.02) in the low dilution treatments. One-tailed *t*-tests were conducted to test the null hypothesis that the mean-AGR for each picophytoplankton group in the low dilution was less than or equal to the mean-AGR in the undiluted treatments (Table 18). The mean-AGR in the low dilution treatments for each picophytoplankton group were not significantly greater than in the undiluted treatments in microplankton-series (null retained), whereas a significantly greater mean-AGR in the low dilution treatment of the

Table 16. Initial abundance (cells ml^{-1}) of ciliates < and >20 µm before and after fractionation with a 20-µm mesh screen.

	Ciliates >20-µm	Ciliates <20-µm
Pre-fractionation	4.8	1.8
Post-fractionation	3.1	1.7
% Difference	36	4.8

Table 17. Time-averaged densities (cells ml^{-1}) of total ciliates and each ciliate size-fraction (cells < and >20 µm) in the undiluted treatments in the microplankton and nanoplankton dilution series

Dilution Series	Total Ciliates	Ciliates >20 µm	Ciliates <20 µm
Microplankton	8.2	5.1	3.0
Nanoplankton	5.8	2.9	2.8
% Difference	29	44	6.3

Table 18. Results of one-sided *t*-tests of the mean (±1 S.D) apparent growth rate (AGR) of prokaryotic (PPICO) and eukaryotic (EPICO) picophytoplankton in the low dilution and undiluted treatments in the microplankton- and nanoplankton-series.

Dilution Series	AGR in Low Dilution Treatments	AGR in Undiluted Treatments	Statistical Results
Microplankton			
PPICO	0.00 (0.06)	0.07 (0.04)	$t = -1.71 < t_{0.05(1),4} = 2.13$, $p > 0.05$
EPICO	0.34 (0.04)	0.27 (0.05)	$t = 2.06 < t_{0.05(1),4} = 2.13$, $p > 0.05$
Nanoplankton			
PPICO	0.15 (0.06)	0.00 (0.09)	$t = 2.47 > t_{0.05(1),4} = 2.13$, $p < 0.05$
EPICO	0.92 (0.02)	0.52 (0.04)	$t = 15.3 > t_{0.05(1),4} = 2.13$, $p < 0.001$

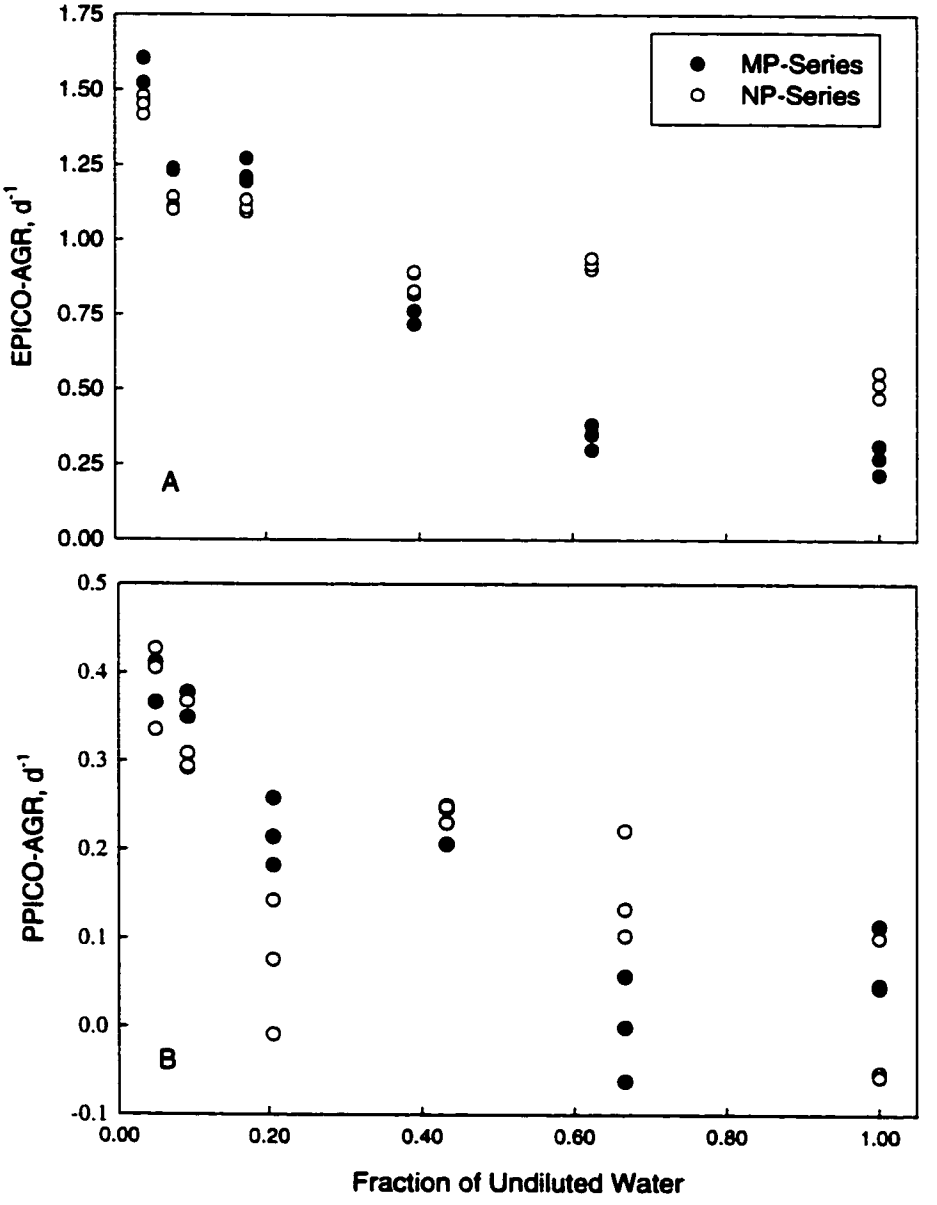

Fig. 21. Apparent growth rate (AGR) of (A) eukaryotic (EPICO) and (B) prokaryotic (PPICO) picophytoplankton vs. dilution factor for the microplankton (MP) and nanoplankton (NP) series.

nanoplankton-series was determined for each picophytoplankton group (null rejected).

The fractionation treatment imparted a linear relationship between prey-AGR and the dilution factor in the nanoplankton series that was not observed in the microplankton series. This was made apparent by a dual regression approach for each picophytoplankton group in each dilution series: one in which data for all dilution fractions were included and a second in which data for the undiluted fractions were omitted from the analysis. The omission of the AGR data for the undiluted treatments in the microplankton series amounted to an increase in the power of the analysis for each picophytoplankton group and a significant change in the slope for each regression line (Table 19). In contrast, the omission of these data in the nanoplankton series reduced the power of the analysis for both picoplankton groups but did not induce significant changes in the slopes of each plot.

The AGR of HFLAG vs. dilution factor for each dilution series is plotted in Fig. 22. In the microplankton series, the mean-AGR of HFLAG was 1.3 d^{-1} (± 0.06) in the undiluted treatments and 1.5 d^{-1} (± 0.06) in the low dilution treatments. In the nanoplankton series, the mean-AGR was 1.4 (± 0.07) in the undiluted treatments and 1.3 d^{-1} (± 0.07) in the low dilution treatments. One-sided *t*-tests were employed to test the null hypothesis that the mean-AGR in the low dilution treatments was less than or equal to the mean-AGR in the undiluted treatments within each dilution series (Table 20). In the microplankton series, the

Table 19. Linear regression results of apparent growth rate (AGR) of prokaryotic and eukaryotic picophytoplankton vs. dilution factor (DF) for the microplankton and nanoplankton dilution series.

Dilution Series/ Picophytoplankton Group	All dilution fractions	Undiluted fraction omitted	Slopes Significantly Different?
Microplankton			
Prokaryotic Picophytoplankton	AGR = 0.36 - 0.36(DF) $F(1, 16) = 41.30$, $p<0.0001$ $r^2 = 0.72$	AGR = 0.40 - 0.60(DF) $F(1, 13) = 62.0$, $p<0.0001$ $r^2 = 0.83$	$\|t\| = 2.18 \geq t_{0.05(2),29} = 2.05$, Yes
Eukaryotic Picophytoplankton	AGR = 1.4 - 1.3(DF) $F(1, 16) = 127.1$, $p<0.0001$ $r^2 = 0.89$	AGR = 1.5 - 1.9(DF) $F(1, 13) = 316.2$, $p<0.0001$ $r^2 = 0.96$	$\|t\| = 3.19 \geq t_{0.05(2),29} = 2.05$, Yes
Nanoplankton			
Prokaryotic Picophytoplankton	AGR = 0.34 - 0.33(DF) $F(1, 16) = 31.94$, $p<0.0001$ $r^2 = 0.67$	AGR = 0.33 - 0.29(DF) $F(1, 13) = 9.35$, $p<0.01$ $r^2 = 0.42$	$\|t\| = 0.38 \leq t_{0.05(2),29} = 2.05$, No
Eukaryotic Picophytoplankton	AGR = 1.3 - 0.77(DF) $F(1, 16) = 81.74$, $p<0.0001$ $r^2 = 0.84$	AGR = 1.3 - 0.74(DF) $F(1, 13) = 21.82$, $p<0.001$ $r^2 = 0.63$	$\|t\| = 0.16 \leq t_{0.05(2),29} = 2.05$, No

Table 20. Statistical analysis results of the mean apparent growth rates of heterotrophic nanoflagellates 2-10 µm (HFLAG-AGR) within and between dilution series. Null hypothesis within each dilution series: AGR (mean ± 1SD) in the low dilution (target dilution factor 0.75) treatments ≤ AGR in undiluted treatments within each dilution series (one-sided *t*-test). Null hypothesis between the dilution series: AGR in the nanoplankton series ≤ to the AGR in the microplankton series. No difference in AGRs in undiluted treatments between the dilution series (two-sided *t*-test).

Dilution Series	HFLAG-AGR Low dilution treatments	HFLAG-AGR Undiluted treatments	Test Results
Microplankton	1.5 ± 0.03	1.3 ± 0.04	$t = 9.39 > t_{0.05(1),4} = 2.13$, reject null $p < 0.0001$
Nanoplankton	1.3 ± 0.07	1.4 ± 0.07	$t = -2.28 < t_{0.05(1),4} = 2.13$, retain null
		Between AGR in Undiluted treatments	$t = 3.85 > t_{0.05(1),4} = 2.13$, reject null $p < 0.01$

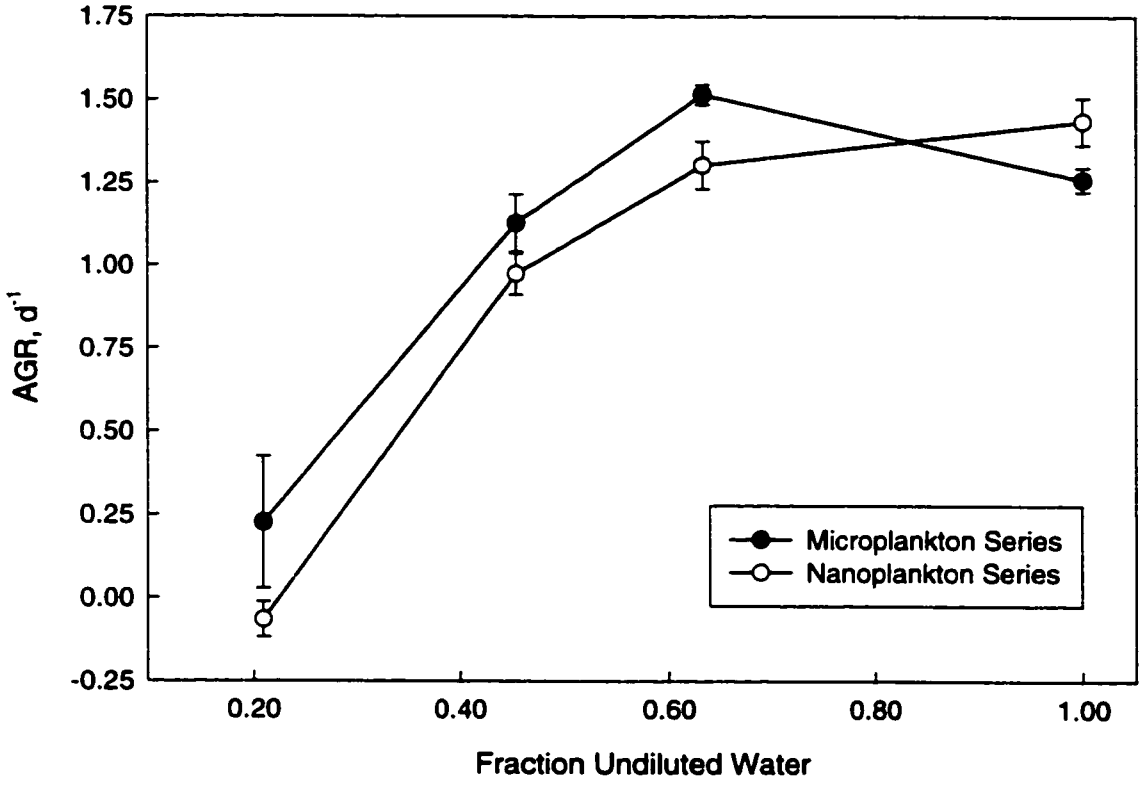

Fig. 22. Apparent growth rate (AGR) of heterotrophic nanoflagellates 2-10 μm vs. dilution factor in the microplankton and nanoplankton dilution series. Error bars represent ±1 SD for 3 replicates for each dilution factor.

mean-AGR in the low dilution treatments was significantly greater than in the undiluted treatments (null rejected). In contrary, the mean-AGR in the low dilution treatments of the nanoplankton series was not significantly greater than in the undiluted treatments (null retained). A one-sided *t*-test was performed to test the null hypothesis that the mean-AGR of the undiluted treatments in the nanoplankton series was less than or equal to the mean-AGR of those treatments in the microplankton series. This was rejected and the mean-AGR in the undiluted treatments of the nanoplankton series was determined to be significantly greater than in the microplankton series.

The community (HFLAG + ciliates) grazing rates on PPICO and EPICO were 0.33 and 1.24 d^{-1} (Table 21). HFLAG grazing rates on PPICO and EPICO were comparable, 0.18 and 0.21 d^{-1}, as likewise were the per-cell clearance rates, 0.018 and 0.022 µl $cell^{-1}$ hr^{-1}. In contrast, ciliate grazing rates on PPICO and EPICO differed markedly, 0.14 and 1.0 d^{-1}, as did per-cell clearance rates,1.22 and 8.45 µl $cell^{-1}$ hr^{-1}.

The ciliate predation rate on HFLAG 2 - 10 µm in the undiluted treatments was 0.69 d^{-1} and the per-cell clearance rate was 3.55 µl $cell^{-1}$ hr^{-1} (Table 22). The predation rate on the HFLAG size classes of 2-5 µm and 6-10 µm was 0.68 d^{-1} and 0.76 d^{-1}, corresponding to the respective clearance rates of 3.48 and 3.91 µl $ciliate^{-1}$ hr^{-1}. The similar time-average densities of ciliates <20 µm in the undiluted treatments of each of the dilution series (Table 17) suggest that little if any predation on smaller ciliates by larger ciliates had occurred.

Table 21. Grazing and clearance rates of heterotrophic nanoflagellates 2-10 µm (HFLAG) and ciliates for prokaryotic and eukaryotic picophytoplankton.

Picophytoplankton Group	Grazing rates, d^{-1}			Clearance rates, µl hr^{-1}	
	HFLAG + Ciliates	HFLAG	Ciliates	HFLAG	Ciliates
Prokaryotic	0.32	0.18	0.14	0.018	1.22
Eukaryotic	1.2	0.21	1.0	0.021	8.44

Table 22. Predation and clearance rates of ciliates on heterotrophic nanoflagellates 2-10 µm (HFLAG).

Predation rates, d^{-1}			Clearance rates, µl hr^{-1}		
HFLAG 2 - 15 µm	HFLAG 2 - 5 µm	HFLAG 6 - 15 µm	HFLAG 2 - 15 µm	HFLAG 2 - 5 µm	HFLAG 6 - 15 µm
0.69	0.68	.77	3.55	3.48	3.91

Copepod Predation Gradient Experiment

Initial estimates of physical and biological parameters for the copepod predation gradient experiment are provided in Table 23. The mean abundance of PPICO (73 x 10^3 cells ml^{-1}) was markedly greater than the mean abundance of EPICO (43 x 10^3 cells ml^{-1}). The mean abundance of HFLAG was 670 cells ml^{-1}. Although the mean abundance of ciliates <20 µm was greater than the abundance of larger ciliates (8.3 cells ml^{-1} vs. 5.2 cells ml^{-1}), the initial biomass of the larger ciliate fraction was substantially greater (1.6 µg C l^{-1} vs. 9.0 µg C l^{-1}).

The AGRs of HFLAG and ciliate biomass showed a marked increase and decrease, respectively, with increasing copepod-density (Fig. 23). These relationships were found to be statistically significant (Table 24). ANOVA analysis for each ciliate biomass fraction (<and >20 µm) vs. copepod density found results similar to the effect of treatment on the AGR of total ciliate biomass. In contrast, the AGRs of PPICO and EPICO abundance showed no apparent change across the copepod-density treatments (Fig. 24). The null hypothesis of zero change in mean-AGR across treatments could not be rejected by one-way ANOVA analysis for either picoplankton group (Table 24).

The AGRs of ciliate biomass and HFLAG were linearly related to copepod density (Table 25). Ciliate biomass-AGR decreased from 0.03 d^{-1} (± 0.09) in the controls (0 copepods) to -0.54 d^{-1} (± 0.11) in the 10-copepod treatments. The HFLAG-AGR increased from 1.35 d^{-1} (± 0.03) in the controls to 1.58 d^{-1} (± 0.04) in the 10-copepod treatments. Ciliate predation rate on HFLAG, calculated by

Table 23. Initial estimates of physical and specific biological parameters for the copepod predation experiment. Mean (± 1 S.D.) of four replicate samples. PPICO, prokaryotic picophytoplankton; EPICO, eukaryotic picophytoplankton; HFLAG, heterotrophic nanoflagellates 2-10 µm. Units for ciliate biomass are µg carbon l^{-1}.

Temp. °C	Salinity, ppt	Picophyto-plankton x10^3 cells ml^{-1}		HFLAG, x10^2 cells ml^{-1}	Ciliates <20 µm		Ciliates >20 µm	
		PPICO	EPICO		cells ml^{-1}	µg C l^{-1}	cells ml^{-1}	µg C l^{-1}
14.7	33.3	73 (3.8)	43 (2.8)	6.7 (0.3)	8.3 (1.2)	1.6 (0.2)	5.2 (1.2)	9.0 (1.8)

Table 24. Summary of one-way ANOVA comparing the effect of copepod density on the growth of prokaryotic and eukaryotic picophytoplankton, heterotrophic nanoflagellates 2-10 µm, and ciliate biomass.

Dependent Variable	Source of Variation	*DF*	*SS*	*MS*	*F*	p
Prokaryotic Picophytoplankton	Copepod Density	2	0.1040	0.0520	3.861	0.062
	Error	9	0.1212	0.0135		
	Total	11	0.2252			
Eukaryotic Picophytoplankton	Copepod Density	2	0.0068	0.0034	0.665	0.540
	Error	9	0.0458	0.0051		
	Total	11	0.0524			
Heterotrophic Nanoflagellates	Copepod Density	2	0.1043	0.0521	59.04	<0.0000
	Error	9	0.0078	0.0009		
	Total	11	0.1121			
Ciliate Biomass	Copepod Density	2	0.6741	0.3371	29.05	0.0001
	Error	9	0.1044	0.0116		
	Total	11	0.7785			
<20 µm Ciliate Biomass	Copepod Density	2	0.2749	0.1375	52.59	<0.0000
	Error	9	0.0235	0.0026		
	Total	11	0.2984			
>20 µm Ciliate Biomass	Copepod Density	2	0.8541	0.4270	25.51	0.0002
	Error	9	0.1506	0.0167		
	Total	11	1.0047			

Table 25. Linear regression results for the apparent growth rates (AGRs) of heterotrophic nanoflagellates 2-10 µm (HFLAG) and ciliate biomass vs. copepod density.

Dependent and Independent Variables	Regression Results
HFLAG-AGR vs. Copepod Density	AGR = 1.35 + 0.02(CD) $F_{1, 10} = 120$, $p<0.0000$ $r^2 = 0.92$
Ciliate Biomass-AGR vs. Copepod Density	AGR = 0.02 - 0.06(CD) $F_{1, 10} = 63.1$, $p<0.0000$ $r^2 = 0.86$

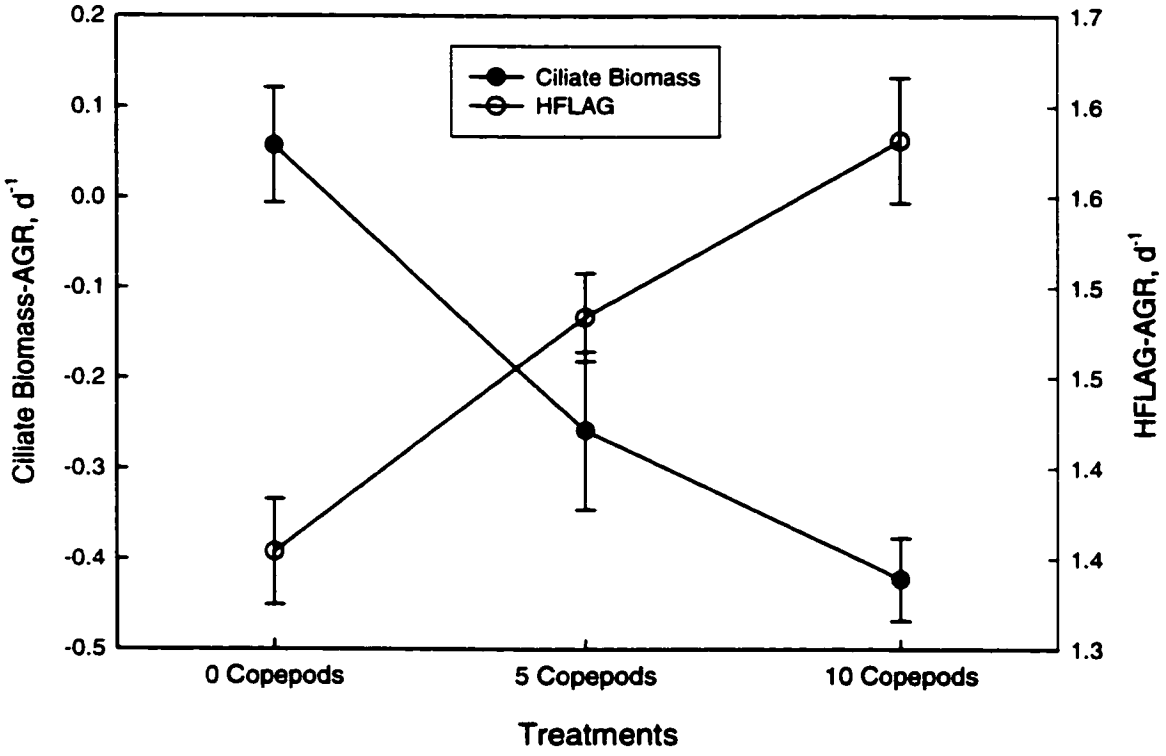

Fig. 23. Apparent growth rate (AGR) of heterotrophic nanoflagellates 2-10 µm (HFLAG) and ciliate biomass vs. copepod density. N = 4 for each independent variable across treatments; error bars represent ±1 standard deviation.

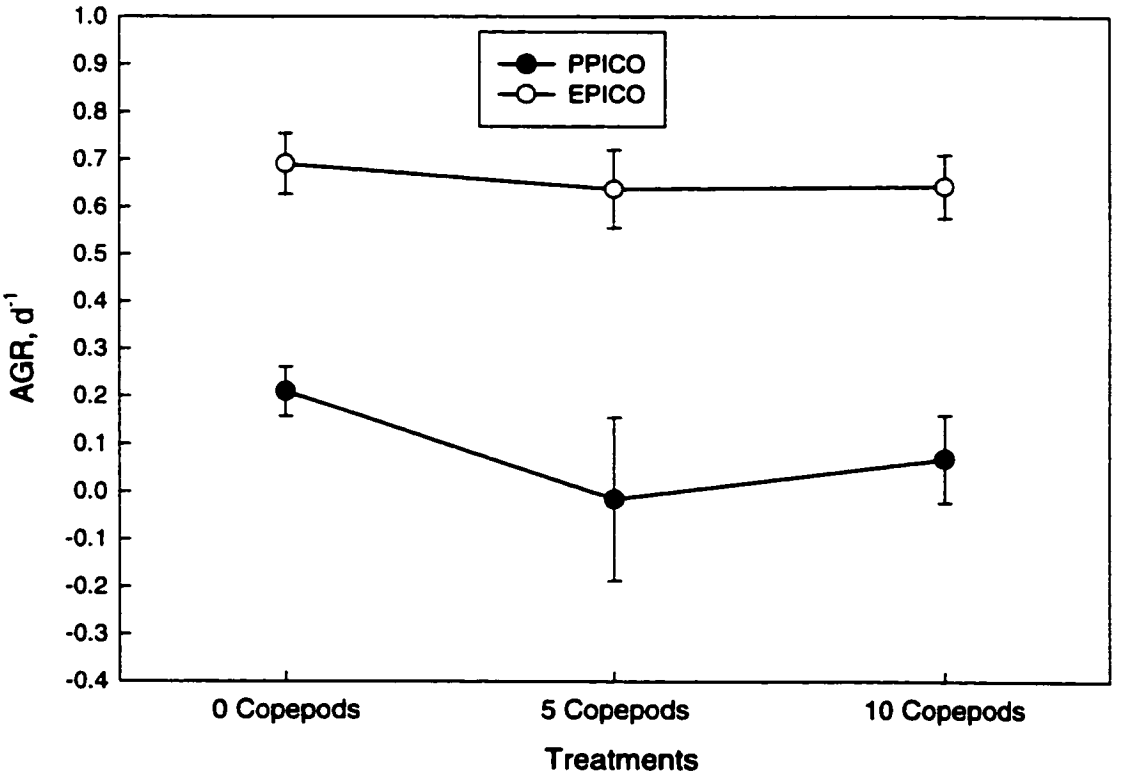

Fig. 24. Apparent growth rate (AGR) of prokaryotic (PPICO) and eukaryotic (EPICO) picophytoplankton vs. copepod density. N = 4 for each independent variable across treatments; error bars represent ±1 standard deviation.

dividing the slope of the least-squares line of the HFLAG-AGR by the slope of the least-squares line of ciliate biomass, was 0.39 d^{-1}. The clearance rate of HFLAG by ciliates was 1.03 µl $ciliate^{-1}$ hr^{-1}.

Discussion

The utility of the dilution method (Landry and Hassett 1982) and its widespread application has produced a large and growing dataset of microzooplankton grazing rates and phytoplankton/bacterial growth rates, quantified in a diversity of estuarine, coastal and oceanic environments (Rivkin et al. 1999 and references therein). It has become the standard tool for biologists in the assessment of microplankton carbon transfer in both marine and lake environments. However, its chief theoretical assumption that microzooplankton ingestion is a linear function of microzooplankton density, along with explanations proposed for its violation (Gallegos 1989), promotes the compartmentalization of the microplankton into guilds of grazers and prey that belies the known complexity of the microbial food web (Sherr and Sherr 1988). Often overlooked are the possible effects that intraguild trophic interactions such as ciliate predation on heterotrophic nanoflagellates (Polis and Holt 1992) and ciliate omnimory (Lawler and Morin 1993) can have on the biomass, production and population dynamics of picoplankton and its transfer to higher food web members (Polis and Strong 1996). Such strategies have been suggested by laboratory experimental data (Goldman and Caron 1985; Ohman and Snyder 1991) but

have not been given adequate consideration in the interpretations of empirical results and observations as they apply to natural planktonic populations.

Reported here is an alternative explanation for the nonlinear relationships between prey apparent growth rate (AGR) and the dilution factor frequently observed at low dilutions in dilution method experiments, which heretofore has been interpreted in terms of the functional feeding-saturation response of a single grazer guild to a diluted prey field (Gallegos 1989; Evans and Paranjape 1992). These results suggest that a trophic-linkage triangle between omnivorous ciliates, heterotrophic nanoflagellates 2-10 μm in size (HFLAG) and picophytoplankton (Fig. 25), and the decoupling of the ciliate-HFLAG interaction by dilution can offer a different explanation for the suppression of apparent picophytoplankton growth at low dilutions of natural microplankton assemblages.

Numerical relationships between field populations suggest that ciliate predation on heterotrophic nanoflagellates is an important top-down control mechanism in planktonic systems (Dolan and Coats 1991; Weisse 1991; Sanders et al. 1992). The results of laboratory feeding experiments not only confirm the ciliate-heterotrophic nanoflagellate feeding link (Jürgens et al. 1996) but also imply that much of the picoplankton apparently consumed by microzooplankton is likely channeled to larger consumers such as ciliates through heterotrophic nanoflagellates (Hagström et al. 1988; Bernard and Rassoulzadegan 1990). Although ciliates, mostly cells <20 μm, can ingest picoplankton-size particles at high rates (Sherr et al. 1989; Christaki et al. 1999),

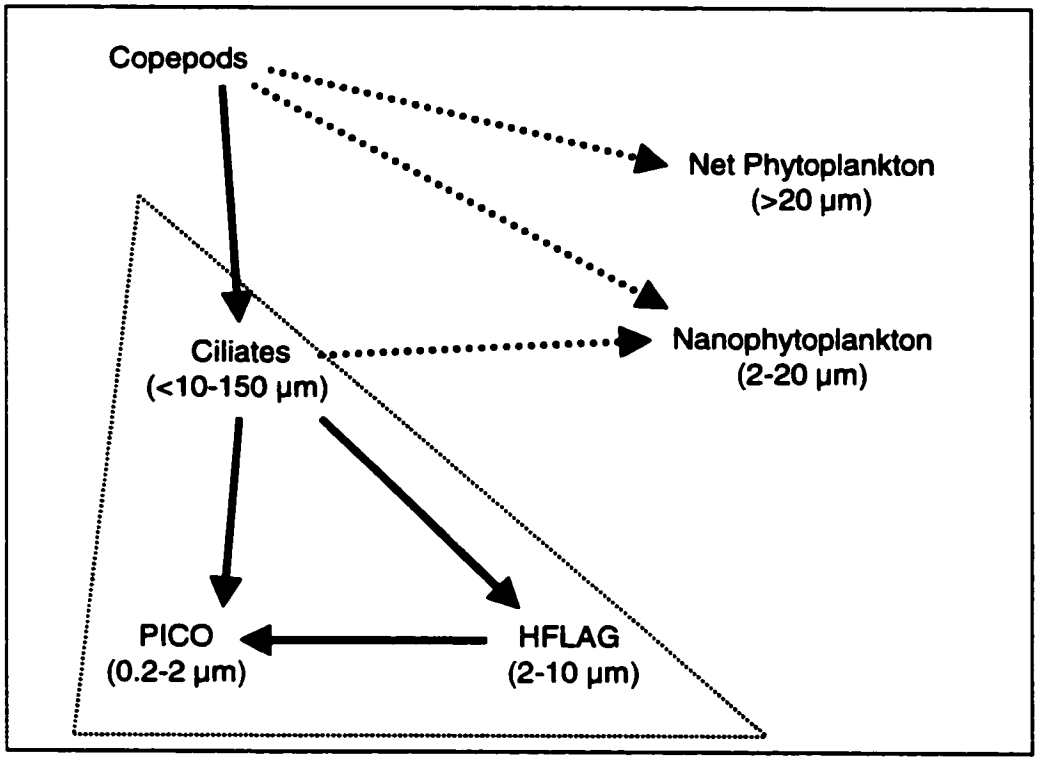

Fig. 25. Trophic connectivity model highlighting the key interactions investigated in this study, principally the ciliate-heterotrophic nano-flagellate (HFLAG)-picophytoplankton (PICO) triangle.

it is generally thought that these particles are at the low end of the particle-retention range for larger ciliates (Jonsson 1986; Rassoulzadegan et al. 1988; Sherr et al. 1991). As a result, heterotrophic nanoflagellates can attain significant importance as consumers of picoplankton, forming a principal link between them and higher consumers of the food web (Haas and Webb 1979; Johnson et al. 1982; Hagström et al. 1988; Wikner and Hagström 1988; Sherr et al. 1991). The importance of heterotrophic nanoflagellates as consumers of <5-μm phytoplankton in the South Slough was intimated by the significant correlation between HFLAG and <5-μm chlorophyll during spring and summer seasons (Chapter III).

It has been shown here that the non-linear relationship between picophytoplankton-AGR and dilution factor can result from ciliate predation on HFLAG and the resultant release of HFLAG from predation in low dilutions of seawater. Attributing the suppression of picophytoplankton-AGR in the low dilutions solely to the enhanced growth and grazing of HFLAG would neglect the possibility of functional feeding response of ciliates, which may have also occurred. To simplify, I have assumed that the deviation of picophytoplankton-AGR in the low dilution treatments from their expected values is a result of enhanced HFLAG grazing and is in linear proportion to the decrease in the ciliate predation rate on HFLAG in these treatments.

The results are substantiated by the agreement of the calculated clearance rates with those obtained by other investigators from different aquatic

environments and laboratory cultures (Table 26). The clearance rates by ciliates were at the high end of the reported value range. This likely reflects the dominance of the ciliate assemblage by a *Strombidium* sp. 20 µm x 25 µm in size and the significant numerical contribution (38%) of ciliates <20 µm in this experiment; ciliates of this size have been shown to consume picoplankton at relatively high rates (Sherr et al. 1989). The clearance rates of HFLAG prey by ciliates also compare well with published measurements (Table 27). If the assumption that HFLAG were not food-limited was violated, it is possible that the predation rates of ciliates on HFLAG were underestimated. This would also correspond to an underestimation of the HFLAG grazing rate on picophytoplankton. Regardless, it is apparent that ciliate omnivory and the ciliate-HFLAG trophic interaction can play instrumental roles in the suppression of picophytoplankton-AGR at low dilutions. These findings strongly suggest that investigations implementing the dilution method to assess microzooplankton grazing and its impacts on picoplankton production should include the analysis of HFLAG growth along the dilution gradient. A significant enhanced growth response of HFLAG coincidental with the suppressed prey-AGR in low dilution treatments can be an indication of multiple trophic links, challenging the view of a single-step, linear transfer of picoplankton production to microzooplankton consumers.

The outcome of the copepod predation experiment empirically furthers the argument that the suppression of picoplankton growth in dilution method

Table 26. Clearance rates of algal prey by heterotrophic nanoflagellates and ciliates from various studies using living phytoplankton or fluorescently labeled algae.

Protozoan	Prey Density, cells ml^{-1}	Clearance rate $\mu l\ cell^{-1}\ h^{-1}$	Source
Nanoflagellates			
Size unspecified, Baltic Sea <2-20 µm chysomonads,	10^3 - 10^6	<0.0001 - 0.011	Kuosa 1991
North Atlantic	10^6	0.048 - 0.175	Weisse and Scheffel-Moeser 1991
5-10 µm flagellates, Georgia estuary	10^4	0.02 - 0.15	Sherr et al. 1991
2-4 µm *Pseudobodo* sp.	~10^6	0.001 - 0.002	Parslow et al. 1986
7-12 µm, *Paraphysomonas imperforata*	~10^6	0.005 - 0.013	Goldman and Caron 1985
2-10 µm flagellates, Oregon estuary	10^3 - 10^4	0.018 and 0.021	This study
Ciliates			
Lohmaniella spiralis	10^4 - 10^5	1.6 - 13.4	Jonsson 1986
Strombidium reticulatum	10^4 - 10^5	1.1 - 3.1	Jonsson 1986
Stromdidium vestitum	10^4 - 10^5	0.1 - 0.5	Jonsson 1986
15-60 µm ciliates, Georgia estuary	10^3 - 10^4	0.2 - 8.3	Sherr et al. 1991
Tintinnopsis sp.	10^3 - 10^4	0.1 - 7.5	Verity 1985
10-35 µm ciliates, Oregon Estuary	10^3 - 10^4	1.22 and 8.44	This study

Table 27. Clearance rates of heterotrophic nanoflagellates by ciliates from various field and laboratory studies.

Ciliate	Clearance rate, $\mu l\ Cil^{-1}\ h^{-1}$	Source
Community	1 - 2	Weisse 1991
Strobilidium spiralis	1 - 4	Verity 1991
Tintinnopsis dadayi	2 - 13	Verity 1991
Halteria grandinella	2.5 (± 0.05)	Jurgens et al. 1996
Strobilidium velox	2.2 (± 0.01)	Jurgens et al. 1996
Strobilidium sp.	3 - 15	Cleven 1996
Halteria sp.	3 - 39	Cleven 1996
Codonella sp./*Tintinnidium* sp.	3 -7	Cleven 1996
Community	3.5 and 3.9	This study

experiments can be a consequence of the relaxation of ciliate predation on HFLAG and the resultant numerical response of HFLAG. It was predicted that the attenuation of ciliate abundance by copepod predation would result in the increase of HFLAG abundance and their grazing rate on picophytoplankton, thereby compensating for the reduction in grazing by ciliates and suppressing any positive indirect effect on the net-growth of picophytoplankton that a linear trophic model (i.e., (Carpenter et al. 1985) would predict. Such top-down trophic effects are probable since many common calanoid copepods, particularly *Acartia* spp., readily consume and often prefer protozoan food (Wiadnyana and Rassoulzadegan 1989; Kleppel 1993), and directly and indirectly control ciliate field populations (Fessenden and Cowles 1994; Nielsen and Kiorboe 1994). Theoretically, ciliates are only one or two trophic steps away from picoplankton, and within the construct of a linear trophic model it is remains intriguing that the metazooplankton-ciliate interaction fails to manifest cascading direct or indirect effects on picoplankton abundance. Increasing numbers of calanoid copepods in microcosm experiments conducted in the subarctic Pacific resulted in the significant decrease of ciliate net-growth but brought about no obvious change in the growth of chlorophyll <3 μm (Landry et al. 1993). They attributed this to the possible chloroplast enrichment of this small fraction by the lysing of larger cells upon size-fractionation. In similar experiments carried out in the oligotrophic Pacific, Calbet and Landry (1999) reported little or no increase in the net growth rate of chlorophyll <2 μm in treatments with increasing zooplankton biomass,

whereas relatively large decreases in the net growth of heterotrophs >5 μm and the overall enhancement in the net-growth of heterotrophic flagellates 2-5 μm were observed. In a study of the trophic interactions between mesozooplankton and the microbial web in a mesotrophic lake, Adrian et al. (Adrian et al. 2001) observed that ciliates responded strongest to mesozooplankton removal and heterotrophic flagellates to a lesser extent, but bacterial abundance showed no significant change. The failure of changes in mesozooplankton abundance to cascade to bacterial level was attributed to the many direct and indirect pressures on bacteria, whereby no apparent change is observed.

The adherence to the linear trophic model in aquatic systems is surprising given the overwhelming evidence that natural communities are reticulate food webs rife with omnivores (Polis and Strong 1996) and the microplankton are no exception (Goldman and Caron 1985; Verity 1991). In the study reported here, a linear decrease in the net-growth of ciliate biomass was mirrored by the increase in the net-growth of HFLAG along the copepod density gradient; the net growth of picophytoplankton, however, remained unchanged. It is difficult to explain these observations in a way other than in terms of a ciliate-HFLAG-picophytoplankton trophic triangle as modeled in Fig. 25. Although the direct and indirect effects of copepod predation on ciliates and HFLAG were apparent, the trophic cascade to the picoplankton level was prevented by a trophic compensation mechanism fostered by the attenuation of ciliate predation on fast growing HFLAG; HFLAG numbers increase and impart a compensating grazing

effect on picophytoplankton. The ciliate predation rate on HFLAG (0.39 d^{-1}) should be considered an underestimate since HFLAG were also likely preyed upon by *A. tonsa* copepods (Gifford and Dagg 1988). An extrapolation of the line of least-squares for HFLAG-AGR vs. the time-averaged densities of ciliates in each treatment to the y-axis gives a y-intercept of 2.3 and is an approximation of the specific growth rate, k_{HFLAG}, (d^{-1}) of HFLAG, equal to 3.3 doublings d^{-1}. HFLAG are capable of growth rates up to 5 doublings d^{-1} (Goldman and Caron 1985), so this is a reasonable estimate of this parameter in this experiment. Applying the exponential growth formula,

$$\ln (P_t/P_0) = k_{HFLAG} - P$$

where P_t and P_0 are the final and initial densities of HFLAG in the control (copepod-free) vessels, k_{HFLAG} is the specific growth rate of HFLAG and P is the ciliate predation rate on HFLAG), ciliate consumption of HFLAG was equal to ~33% of the HFLAG daily production. A similar operation and calculation can be employed for the dilution experiment. The y-intercept of the line connecting the mean HFLAG-AGRs in the low and undiluted treatments is 1.9, the specific growth rate of HFLAG equal to 2.7 doublings d^{-1}. Applying the ciliate predation rate (0.69 d^{-1}) and the growth equation above, ciliates consumed ~50% of the HFLAG daily production in this experiment. Even as approximations it is clear that ciliate predation was instrumental in limiting the potential impact of HFLAG as grazers of picophytoplankton in both experiments. If ciliates were not also significant grazers of picophytoplankton, an attenuation of ciliate net-growth or

abundance by either top-down copepod predation or dilution would, according to the linear trophic model, promote observable decreases, and not stasis, in the net-growth of picophytoplankton due to the predation-released growth and grazing response of HFLAG. Such a decrease was visually observed in the AGR of prokaryotic picophytoplankton (PPICO) for two of the three treatments in the microplankton series of the dilution experiment (Fig. 21) and may reflect the relatively lower grazing rate of ciliates on PPICO.

The comparable results in each of the experiments strongly suggest that the trophic dynamic within the ciliate-HFLAG-picophytoplankton triangle (Fig. 25) is robust across concentration gradients. It can diffuse the direct and indirect effects of trophic cascades as envisioned in theory (Polis and Holt 1992; Polis and Strong 1996) and offer an explanation for the relative numerical constancy of natural picoplankton populations (Davis et al. 1985). Furthermore, ignoring the HFLAG contribution to grazing on picophytoplankton together with the ciliate-HFLAG trophic interaction could lead to significant over-estimations of energy transfer efficiency through the microplankton. Assuming similar gross growth efficiencies for HFLAG and ciliates (Caron and Goldman 1990) and including the ciliate predation impact factor of 0.50, an omission of this kind in the dilution experiment would have over-estimated the transfer efficiency by ~42% in the case for PPICO and ~15% for eukaryotic picophytoplankton.

Omnivory is more the rule than the exception in terrestrial and aquatic systems (Sprules and Bowerman 1988; Polis and Strong 1996), and its

prevalence challenges the application of linear trophic models in most natural systems. The theoretical basis of the dilution method oversimplifies the microplankton community by imposing linear relationships between microzooplankton and prey where and when a reticulate food web prevails. Thus, more caution must be exercised in fitting data to the model when results contrary to linear expectations arise. Before adopting the assumption that significant deviations from the least-square line at low dilutions of seawater are due to a non-linear feeding response by a single grazer group, the findings of this study strongly suggest that release from predation pressure of intermediate grazers should be considered in order to rule out the contribution of complex trophic interactions among the microzooplankton to this phenomena.

CHAPTER V

GENERAL CONCLUSION

The field and experimental data presented in this dissertation strongly suggest that microzooplankton play an important trophic role in the planktonic food web of the South Slough. Comparable growth and metabolic rates between protozoans and their protistan prey (Fenchel 1968; Finlay 1977; Banse 1982; Stoecker and Guillard 1982; Montagnes 1996) provide a physiological basis for tight trophic coupling between these two components. The robust numerical relationships between ciliates and phytoplankton biomass described in Chapter II substantiate this prediction. Ciliate abundance and biomass were highly correlated with <5-µm phytoplankton (ultraphytoplankton) biomass throughout the sample year (6 March 1999 - 22 March 2000). Between October and March of the sample year, ciliates were correlated with total phytoplankton biomass, likely due to the dominance of >5-µm phytoplankton by nanoflagellates during this period. These appear to have been accepted as food by naked ciliates since the carbon ratio of ciliate:ultraphytoplankton suggested that ultraphytoplankton were insufficient to support the ciliate biomass observed during this seasonal period. A comparable response by ciliates and ultraphytoplankton biomass to temperature observed in this study likely facilitates a rapid assimilation of

phytoplankton biomass into secondary production in the South Slough (Verity 1986; Nielsen and Kiørboe 1994).

The findings of the dilution-method grazing experiments (Landry and Hassett 1982) described in Chapter III ascribe a function to the numerical relationships between ciliates and phytoplankton biomass presented in Chapter II. Microzooplankton grazing accounted for 48-96% of the daily primary production across seasons in the South Slough. Microzooplankton imposed significant grazing impacts on the total phytoplankton assemblage when nanoflagellates dominated the phytoplankton >5 μm. The grazing impact determined when diatoms dominated the >5-μm phytoplankton fraction was dependent on the composition of the microzooplankton. When heterotrophic dinoflagellates were absent or rare, microzooplankton grazed at significantly high rates on the production of <5-μm phytoplankton only. Higher numbers of heterotrophic dinoflagellates resulted in in high grazing impacts on the production of total phytoplankton and components of the <5-μm phytoplankton fraction. These findings suggest that the effects of grazing strongly depend on the coincidental compositions of the microzooplankton and phytoplankton assemblages.

Chapter IV presented experimental data that provide an alternate explanation for non-linear relationships between the apparent growth rate of prey vs. dilution factor that are often observed in low dilution treatments of dilution method experiments. Based on the results of a dilution experiment, in which the

suppression of picophytoplankton apparent growth in low dilution treatments corresponded with the enhanced apparent growth of heterotrophic nanoflagellates, I proposed and tested the following hypothesis in Chapter IV: the suppression of the apparent growth of picophytoplankton in low dilution treatments can result from the dilution-mediated release of heterotrophic nanoflagellates from predation by larger microzooplankton (i.e., ciliates). That is, increased herbivory by nanoflagellates offsets the positive effect that dilution would have on picophytoplankton growth. By initially reducing the abundance of larger microzooplankton with gentle fractionation in one dilution series, I eliminated both the enhanced apparent growth of heterotrophic nanoflagellates and the suppressed apparent growth of picophytoplankton observed in a control dilution series. In short, I made linear the relationship between the apparent growth of picophytoplankton and the dilution factor by uncoupling the ciliate-heterotrophic nanoflagellate interaction in the undiluted treatments. Non-linear relationships between the apparent growth rate of prey and dilution factor are regularly explained in terms of functional feeding responses of a microzooplankton guild. Specifically for this case, grazing saturation in low dilution treatments suppresses the apparent growth of picophytoplankton in these treatments. The results of this experiment suggest that complex trophic interactions among microzooplankton members can challenge the ability of the dilution method to accurately assess the carbon flow picophytoplankton producers to terminal microzooplankton consumers.

Based on this outcome, I hypothesized that the reduction of ciliate abundance by top-down predation in undiluted environments can give rise to a similar predation release for heterotrophic nanoflagellates and the corresponding suppression of picophytoplankton. As a test, a density gradient of *Acartia tonsa* calanoid copepods was prepared and the apparent growth of ciliate biomass, heterotrophic nanoflagellates and picophytoplankton was measured across the gradient following a 24 h incubation (Chapter IV). The apparent growth of ciliate biomass and heterotrophic nanoflagellate abundance significantly increased and decreased, respectively, with decreasing copepod abundance, but the apparent growth of picophytoplankton remained unchanged. The suppression, and not reduction of picophytoplankton apparent growth across the copepod density gradient suggests that ciliates were feeding on both heterotrophic nanoflagellates and picophytoplankton. If ciliates were not omnivores, then increases in heterotrophic nanoflagellates with increasing copepod density would be paralleled by decreases in picophytoplankton. These findings suggest that complex trophic interactions by and among microzooplankton members can challenge the ability of linear models to predict the effects of top-down trophic interactions on small phytoplankton producers. Furthermore, this experimental outcome and its interpretation provides an possible explanation for the annual and seasonal constancy of natural picoplankton populations that is often reported (Davis et al. 1985; Stockner and Antia 1988) and the sustained constancy of

picoplankton standing stocks following direct and indirect experimental removal of metazooplankton (Landry et al. 1993; Calbet and Landry 1999).

APPENDIX A

ADDITIONAL DATA FROM THE 6 MARCH 1999 - 22 MARCH 2000 FIELD SAMPLING STUDY IN THE SOUTH SLOUGH

Heterotrophic Nanoflagellates

Between 6 March and 6 October 1999, biweekly samples of heterotrophic nanoflagellates were collected at high tide from the South Slough sample site in coordination with the sampling program described in Chapter II. Samples were immediately fixed with cold glutaraldehyde (0.25% final concentration). Aliquots of 15-20 ml from each sample were incubated with DAPI (4',6-diamidino-2-phenylindole dihydrochloride; final volume 0.01 µg ml^{-1}) for 10-15 min in the dark and then concentrated onto black 0.8 µm or 1.0 µm polycarbonate membrane filters (Poretics) under gentle vacuum (<5 in. Hg; Sherr et al. 1993). Filters were mounted onto slides and inspected with epifluorescence microscopy under UV light. Fluorescing cells were counted in random fields or diametric transects until at least 200 cells were enumerated (Booth 1987; Murphy and Haugen 1985). The abundance of heterotrophic nanoflagellates ranged 3.7×10^2 - 11×10^2 cells ml^{-1} (average, 7.0×10^2 cells ml^{-1}; SE, 65 cells ml^{-1}). Cell size of nanoflagellates ranged 2-10 µm. Sample date abundance of nanoflagellates is plotted with <5-µm chlorophyll concentration (Chapter II) in Fig. 26. Two abundance peaks of

nanoflagellates coincided with the two concentration maxima of <5-µm chlorophyll concentration (Chapter II). Nanoflagellate abundance was significantly correlated with <5-µm chlorophyll concentration ($r = 0.61$, $p<0.05$) during the sample period. No relationship was determined between nanoflagellate abundance and total or >5-µm chlorophyll concentration. Although possibly due to intercorrelation of variables, numbers of nanoflagellates and naked ciliates (Chapter II) were positively correlated ($r = 0.54$, $p<0.05$).

The Heterotrophic Dinoflagellate *Gyrodinium spirale*

The abundance of the heterotrophic dinoflagellate *Gyrodinium spirale* in the South Slough was measured biweekly between March 1999 and March 2000 in conjunction with the sampling program described in Chapter II. Abundance averaged 1.7 cells ml^{-1} (SE, 0.8) and ranged 0.0-15.8 cells ml^{-1}. Cell abundance showed two pronounced peaks in June and early July (15.8 and 13.3 cells ml^{-1}), after which their numbers were generally reduced (<0.5 cells ml^{-1}) (Fig. 27).

The Autotrophic Ciliate *Myrionecta rubra*

The abundance of the obligate autotrophic ciliate *Myrionecta rubra* (= *Mesodinium rubrum*; Order Haptorida) averaged 1.8 cells ml^{-1} (SE, 0.64) and ranged 0-14 cells ml^{-1} (Fig. 28). Its numbers climbed steadily during the spring (with the exception of 17 June) to a maximum on 22 June, coincidental with the late spring chlorophyll maximum (Chapter II). Its abundance then dropped precipitously by the next sample date and, except for a two small peaks in August, remained low or at zero for the rest of the sample year.

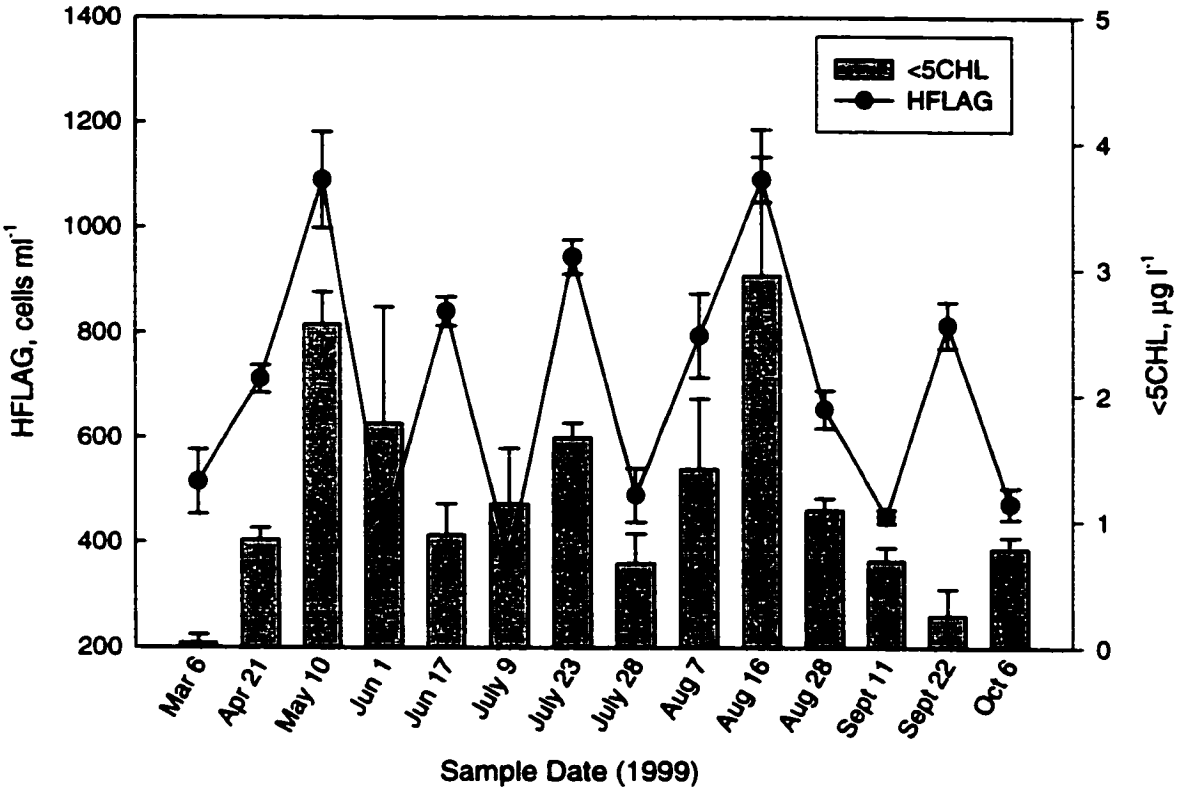

Fig. 26. Abundance of heterotrophic nanoflagellates 2-10 µm (HFLAG) and concentration of < 5 µm chlorophyll (<5CHL) between 6 March - 6 October 1999. N = 3 for each variable on all dates; error bars represent ±1 SD.

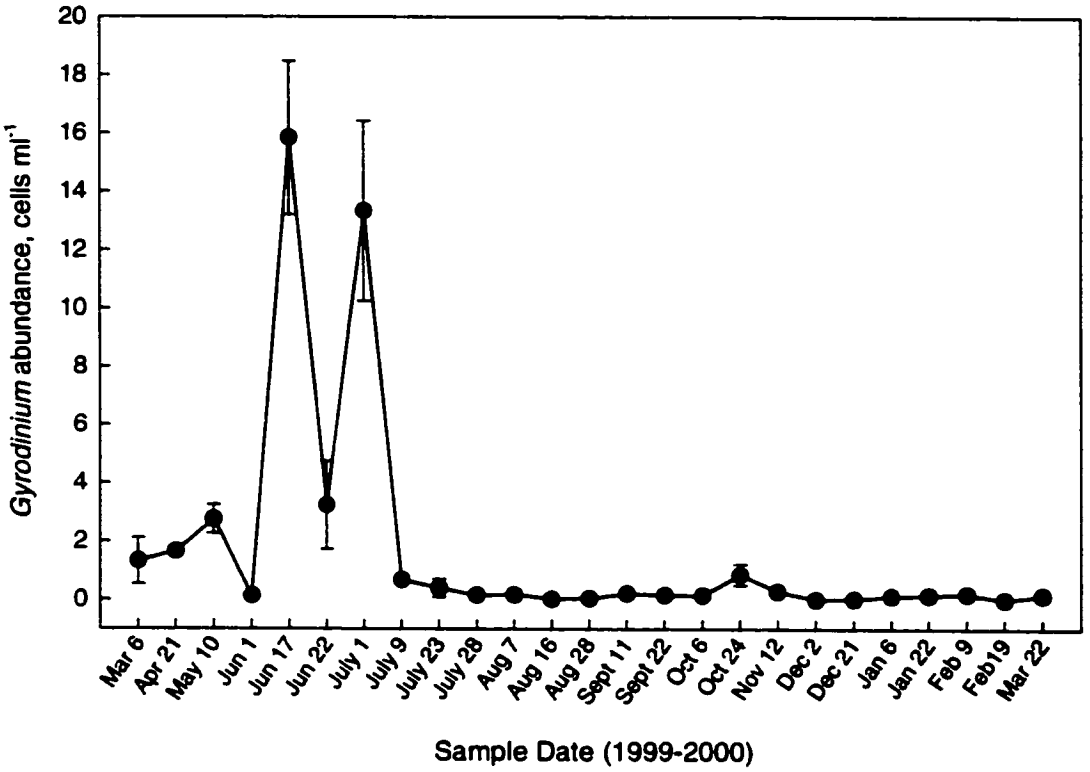

Fig. 27. *Gyrodinium spirale* dinoflagellate abundance (± 1 SD) between 6 March 1999 and 22 March 2000 in the South Slough. N = 3 for each date except for 28 August when n = 1.

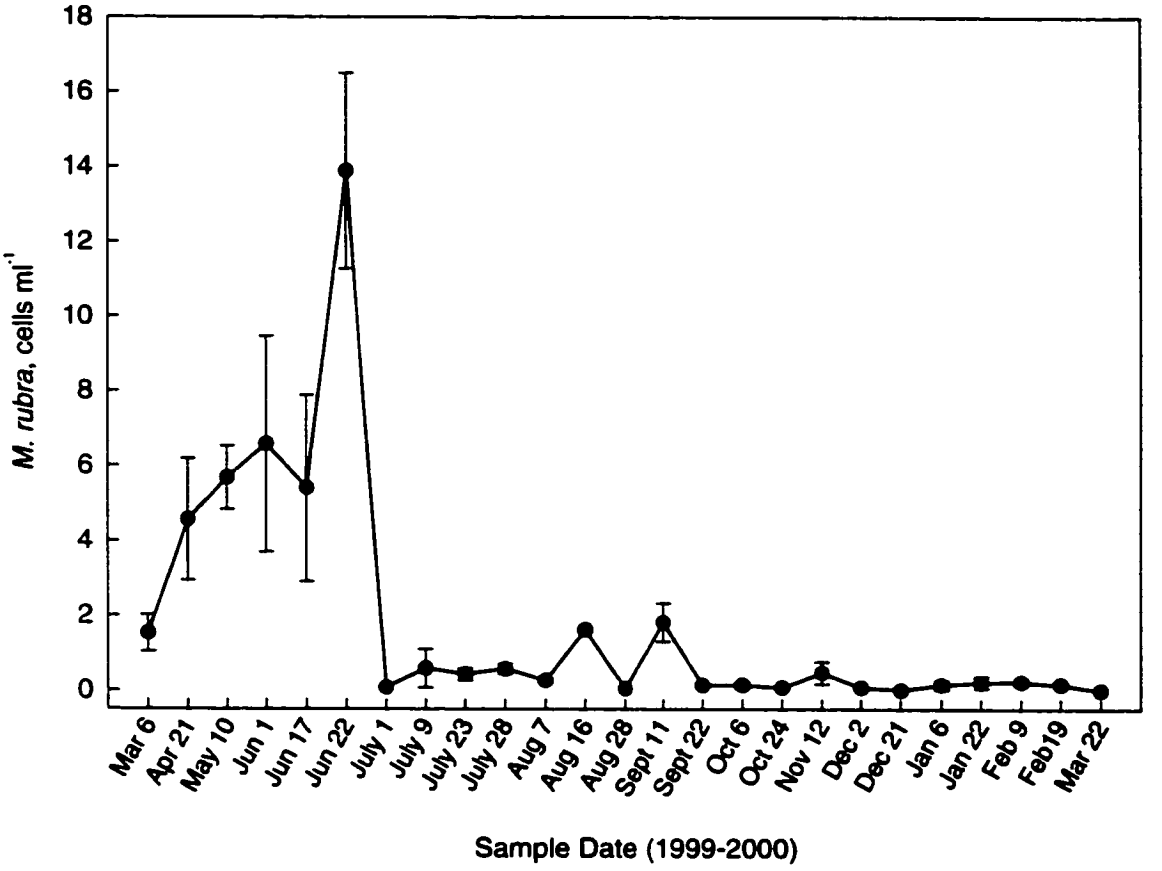

Fig. 28. Abundance of the autotrophic ciliate *Myrionecta rubra*. Error bars represent ±1 SD of 3 replicates per sample date, except for 28 Aug. when n=1.

LITERATURE CITED

Chapter I

Arnt, H. 1993. Rotifers as predators on components of the microbial web (bacteria, heterotrophic flagellates, ciliates) - a review. Hydrobiologia 255/256: 231-246.

Azam, F., Fenchel, T., Field, J. G., Gray, J. S., Meyer-Reil, L. A., and Thingstad, F. 1983. The ecological role of water-column microbes in the sea. Mar. Ecol. Prog. Ser. 10: 257-263.

Banse, K. 1982. Cell volumes, maximal growth rates of unicellular algae and ciliates, and the role of ciliates in the marine pelagial. Limnol. Oceanogr. 27: 1059-1071.

Beers, J. R., Reid, F. M. H., and Stewart, G. L. 1980. Microplankton population structure in Southern California nearshore waters in late spring. Mar. Biol. 60: 209-226.

Beers, J. R., and Stewart, G. L. 1967. Micro-zooplankton in the euphotic zone at five locations across the California Current. J. Fish. Res. Board Can. 24: 2053-2068.

Croegner, G. C., and Shanks, A. L. 2001. Import of coastally-derived chlorophyll a to South Slough, Oregon. Estuaries 24: 244-256.

Dupuy, C., Le Gall, S., Hartmann, H. J., and Breret, M. 1999. Retention of ciliates and flagellates by the oyster Crassostrea gigas in French Atlantic coastal ponds: Protists as a trophic link between bacterioplankton and benthic suspension-feeders. Mar. Ecol. Prog. Ser. 177: 165-175.

Fenchel, T. 1968. The ecology of marine microbenthos. III. The reproductive potential of ciliates. Ophelia 5: 123-136.

Finlay, B. J. 1977. The dependence of reproductive rate on cell size and temperature in freshwater ciliated protozoa. Oecologia 30: 75-81.

Gallegos, C. L. 1989. Microzooplankton grazing on phytoplankton in the Rhode River, Maryland: nonlinear feeding kinetics. Mar. Ecol. Prog. Ser. 57: 23-33.

Hughes, M. P. 1997. Temporal and spatial variability of phytoplankton in coastal and estuarine habitats in Coos Bay, Oregon. Masters thesis, University of Oregon.

Jacobson, D. M., and Anderson, D. M. 1986. Thecate heterotrophic dinoflagellates: Feeding behavior and mechanisms. J. Phycol. 22: 249-258.

Jonsson, P. R. 1986. Particle size selection, feeding rates and growth dynamics of marine planktonic oligotrichous ciliates (Ciliophora: Oligotrichina). Mar. Ecol. Prog. Ser. 33: 265-277.

Kleppel, G. S. 1993. On the diets of calanoid copepods. Mar. Ecol. Prog. Ser. 99: 183-195.

Lair, N., LeVeille, J.-C., Reyes-Marchant, P. and Taleb, H. 1994. The feeding of a larval fish, *Lebistes reticulatus*, on ciliates and rotifers. Mar. Microb. Food Webs 8: 337-346.

Landry, M. R., Barber, R. T., Bidigare, R. R., Chai, F., Coale, K. H., Dam, H. G., Lewis, M. R. et al. 1997. Iron and grazing constraints on primary production in the central equatorial Pacific: An EqPac synthesis. Limnol. Oceanogr. 42: 405-418.

Landry, M. R., and Hassett, R. P. 1982. Estimating the grazing impact of marine micro-zooplankton. Mar. Biol. 67: 283-288.

Le Gall, S., Bel Hassen, M., and Le Gall, P. 1997. Ingestion of a bacterivorous ciliate by the oyster Crassostrea gigas: protozoa as a trophic link between picoplankton and benthic suspension-feeders. Mar. Ecol. Prog. Ser. 152: 301-306.

Levinsen, H., Nielsen, T. G., and Hansen, B. W. 1999. Plankton community structure and carbon cycling on the western coast of Greenland during the stratified summer situation. II. Heterotrophic dinoflagellates and ciliates. Aquat. Microb. Ecol. 16: 217-232.

Li, W. K. W., Subba Rao, D. V., Harrison, W. G., Smith, J. C., Cullen, J. J., Irwin, B., and Platt, T. 1983. Autotrophic picoplankton in the tropical ocean. Science 219: 292-295.

Miller, C. B., Frost, B. W., Wheeler, P. A., Landry, M. R., Welschmeyer, N., and Powell, T. M. 1991. Ecological dynamics in the subarctic Pacific, a possibly iron-limited ecosystem. Limnol. Oceanogr. 36: 1600-1615.

Montagnes, D. J. S. 1996. Growth responses of planktonic ciliates in the genera Strobilidium and Strombidium. Mar. Ecol. Prog. Ser. 130: 241-254.

Murphy, L. S., and Haugen, E. M. 1985. The distribution and abundance of phototrophic ultraplankton in the North Atlantic. Limnol. Oceanogr. 30: 47-58.

Neuer, S., and Cowles, T. J. 1994. Protist herbivory in the Oregon upwelling system. Mar. Ecol. Prog. Ser. 113: 147-162.

Nival, P., and Nival, S. 1976. Particle retention efficiencies of an herbivorous copepod, Acatia clausi (adult and copepodite stages): effects on grazing. Limnol. Oceanogr. 21: 24-38.

Ohman, M. D., and Runge, J. A. 1994. Sustained fecundity when phytoplankton resources are in short supply: Omnivory by Calanus finmarchicus in the Gulf of St. Lawrence. Limnol. Oceanogr. 39: 21-36.

Paffenhöfer, G.-A. 1984. Food ingestion by the marine copepod Paracalanus in relation to abundance and size distribution of food. Mar. Biol. 80: 323-333.

Paranjape, M. A. 1987. Grazing by microzooplankton in the eastern Canadian Arctic in summer 1983. Mar. Ecol. Prog. Ser. 40: 239-246.

—. 1990. Microzooplankton herbivory on the Grand Bank (Newfoundland, Canada): A seasonal study. Mar. Biol. 107: 321-328.

Pomeroy, L. 1974. The ocean's food web, a changing paradigm. Bioscience 24: 499-504.

Riisgard, H. U. 1988. Efficiency of particle retention and filtration rate in 6 species of North East American bivalves. Mar. Ecol. Prog. Ser. 45: 217-223.

Ryther, J. H. 1969. Photosynthesis and fish production in the sea. Science 166: 72-76.

Sherr, E. B., and Sherr, B. F. 1994. Bacterivory and herbivory: key roles of phagotrophic protists in pelagic food webs. Microb. Ecol. 28: 223-235.

Sherr, E. B., Sherr, B. F., and McDaniel, J. 1991. Clearance rates of < 6 mu m fluorescently labeled algae (FLA) by estuarine protozoa: Potential grazing impact of flagellates and ciliates. Mar. Ecol. Prog. Ser. 69: 81-92.

Shumway, S. E., Cucci, T. L., Newell, R. C., and Yentsch, C. M. 1985. Particle selection, ingestion, and absorption in filter-feeding bivalves. J. Exp. Mar. Biol. Ecol. 91: 77-92.

Smetacek, V. 1981. The annual cycle of protozooplankton in the Kiel Bight. Mar. Biol. 63: 1-11.

Stockner, J. G., and Antia, N. J. 1988. Algal picoplankton from marine and freshwater exosystems: a multidisciplinary perspective. Can. J. Fish. Aquat. Sci. 43: 2472-2503.

Stoecker, D., and Guillard, R. R. L. 1982, Effects of temperature and light on the feeding rate of Favella sp. (ciliated Protozoa, suborder Tintinnina). Annls. Inst. Oceanogr., Paris 58 (suppl): 309-318.

Stoecker, D. K., and Capuzzo, J. M. 1990. Predation on protozoa: its importance to zooplankton. J. Plankton Res. 12: 891-908.

Stoecker, D. K., and Govoni, J. J. 1984. Food selection by young larval Gulf menhaden (Brevoortia patronus). Mar. Biol. 80: 299-306.

Stoecker, D. K., Verity, P. G., Michaels, A. E., and Davis, L. H. 1987. Feeding by larval and post-larval ctenophores on microzooplankton. J. Plankton Res. 9: 667-683.

Strom, S. L., Brainard, M. A., Holmes, J. L., and Olson, M. B. 2001. Phytoplankton blooms are strongly impacted by microzooplankton grazing in coastal North Pacific waters. Mar. Biol. 138: 355-368.

Verity, P. G., and Paffenhöfer, G. A. 1996. On assessment of prey ingestion by copepods. J. Plankton Res. 18: 1767-1779.

Waterbury, J. B., Watson, S. W., Guillard, R. R. L., and Brand, L. E. 1979. Widespread occurrence of a unicellular, marine, planktonic, cyanobacterium. Nature 277: 293-294.

Chapter II

Aelion, C. M. and Chisholm, S. W. 1985. Effect of temperature on growth and ingestion rates of *Favella* sp. Journal of Plankton Research 7: 821-830.

Agawin, N. S. R., Duarte, C. M. and Agusti, S. 1998. Growth and abundance of *Synechococcus* sp. in a Mediterranean Bay: Seasonality and relationship with temperature. Marine Ecology Progress Series 170: 45-53.

Agawin, N. S. R., Duarte, C. M. and Agusti, S. 2000. Nutrient and temperature control of the contribution of picoplankton to phytoplankton biomass and production. Limnology and Oceanography 45: 591-600.

Ambler, J. W., Cloern, J. E. and Hutchinson, A. 1985. Seasonal cycles of zooplankton from San Francisco Bay. Hydrobiologia 129: 177-197.

Andersson, A., Haecky, P. and Hagström, A. 1994. Effect of temperature and light on the growth of micro- nano- and pico-plankton: Impact on algal succession. Marine Biology 120: 511-520.

Arnt, H. 1993. Rotifers as predators on components of the microbial web (bacteria, heterotrophic flagellates, ciliates) - a review. Hydrobiologia 255/256: 231-246.

Azam, F., Fenchel, T., Field, J. G., Gray, J. S., Meyer-Reil, L. A. and Thingstad, F. 1983. The ecological role of water-column microbes in the sea. Marine Ecology Progress Series 10: 257-263.

Baldwin, B. S. and Newell, R. I. E. 1991. Omnivorous feeding by planktotrophic larvae of the eastern oyster *Crassostrea virginica*. Marine Ecology Progress Series 78: 285-301.

Banse, K. 1982. Cell volumes, maximal growth rates of unicellular algae and ciliates, and the role of ciliates in the marine pelagial. Limnology and Oceanography 27: 1059-1071.

Baross, J. A., Crump, B. and Simenstad, C. A. 1994. Elevated 'microbial loop' activities in the Columbia River estuarine turbidity maximum, p. 459-464. In: K. R. Dyer and R. J. Orth (eds., Changes in fluxes in estuaries: Implications from science to management. Fredensborg (Denmark), Olsen & Olsen.

Beers, J. R., Reid, F. M. H. and Stewart, G. L. 1980. Microplankton population structure in Southern California nearshore waters in late spring. Marine Biology 60: 209-226.

Beers, J. R. and Stewart, G. L. 1967. Micro-zooplankton in the euphotic zone at five locations across the California Current. Journal of the Fisheries Research Board of Canada 24: 2053-2068.

Beers, J. R. and Stewart, G. L. 1969. Micro-zooplankton and its abundance relative to the larger zooplankton and other seston components. Marine Biology 4: 182-189.

Burkill, P. H. 1982. Ciliates and other microplankton components of a nearshore food-web: standing stocks and production processes. Annales. Institut Oceanographique (Paris) 58: 335-350.

Christaki, U., Dolan, J. R., Pelegri, S. and Rassoulzadegan, F. 1998. Consumption of picoplankton-size particles by marine ciliates: Effects of physiological state of the ciliate and particle quality. Limnology and Oceanography 43: 458-464.

Christaki, U., Jacquet, S., Dolan, J. R., Vaulot, D. and Rassoulzadegan, F. 1999. Growth and grazing on *Prochlorococcus* and *Synechococcus* by two marine ciliates. Limnology and Oceanography 44: 52-61.

Croegner, G. C. and Shanks, A. L. 2001. Import of coastally-derived chlorophyll a to South Slough, Oregon. Estuaries 24: 244-256.

Dolan, J. R. and Coats, D. W. 1990. Seasonal abundances of planktonic ciliates and microflagellates in mesohaline Chesapeake Bay waters. Estuarine, Coastal and Shelf Science 31: 157-175.

Dupuy, C., Le Gall, S., Hartmann, H. J. and Breret, M. 1999. Retention of ciliates and flagellates by the oyster *Crassostrea gigas* in French Atlantic coastal ponds: Protists as a trophic link between bacterioplankton and benthic suspension-feeders. Marine Ecology Progress Series 177: 165-175.

Fenchel, T. 1968. The ecology of marine microbenthos. III. The reproductive potential of ciliates. Ophelia 5: 123-136.

Ferrier-Pages, C. and Rassoulzadegan, F. 1994a. N remineralization in planktonic protozoa. Limnology and Oceanography 39: 411-419.

Ferrier-Pages, C. and Rassoulzadegan, F. 1994b. Seasonal impact of the microzooplankton on pico- and nanoplankton growth rates in the Northwest Mediterranean Sea. Marine Ecology Progress Series 108: 283-294.

Fessenden, L. and Cowles, T. J. 1994. Copepod predation on phagotrophic ciliates in Oregon coastal waters. Marine Ecology Progress Series 107: 103-111.

Finlay, B. J. 1977. The dependence of reproductive rate on cell size and temperature in freshwater ciliated protozoa. Oecologia 30: 75-81.

Froneman, P. W. and McQuaid, C. D. 1997. Preliminary investigation of the ecological role of microzooplankton in the Kariega Estuary, South Africa. Estuarine, Coastal and Shelf Science 45: 689-695.

Gifford, D. J. 1988. Impact of grazing by microzooplankton in the Northwest Arm of Halifax Harbor, Nova Scotia. Marine Ecology Progress Series 47: 249-258.

Heinbokel, J. F. 1978. Studies on the functional role of tintinnids in the Southern California Bight. I. Grazing and growth rates in laboratory cultures. Marine Biology 47: 177-189.

Hughes, M. P. 1977. Temporal and spatial variability of phytoplankton in coastal and estuarine habitats in Coos Bay, Oregon. M. S. Thesis. University of Oregon.

Huyer, A. 1983. Coastal upwelling in the California Current system. Progress in Oceanography 12: 259-284.

Iriarte, A. and Purdie, D. A. 1994. Size distribution of chlorophyll a biomass and primary production in a temperate estuary (Southampton Water): The contribution of photosynthetic picoplankton. Marine Ecology Progress Series 115: 283-297.

Johannes, R. E. 1965. Influence of marine protozoa on nutrient regeneration. Limnology and Oceanography 10: 434-442.

Joint, I. R. and Pomroy, A. J. 1986. Photosynthetic characteristics of nanoplankton and picoplankton from the surface mixed layer. Marine Biology 92: 465-474.

Jonsson, P. R. 1986. Particle size selection, feeding rates and growth dynamics of marine planktonic oligotrichous ciliates (Ciliophora: Oligotrichina). Marine Ecology Progress Series 33: 265-277.

Jonsson, P. R. 1987. Photosynthetic assimilation of inorganic carbon in marine oligotrich ciliates (Ciliophora, Oligotrichina). Marine Microbial Food Webs 2: 55-68.

Jurgens, K., Wickham, S. A., Rothhaupt, K. O. and Santer, B. 1996. Feeding rates of macro- and microzooplankton on heterotrophic nanoflagellates. Limnology and Oceanography 41: 1833-1839.

Kimor, B. and Golanksky, B. 1977. Microplankton of the Gulf of Elat: Aspects of seasonal and bathymetric distribution. Marine Biology 42: 55-67.

Kleppel, G. S. (1993). On the diets of calanoid copepods. Marine Ecology Progress Series 99: 183-195.

Kleppel, G. S., Frazel, D., Pieper, R. E. and Holliday, D. V. 1988. Natural diets of zooplankton off southern California. Marine Ecology Progress Series 49: 231-241.

Lair, N., LeVeille, J.-C., Reyes-Marchant, P. and Taleb, H. 1994. The feeding of a larval fish, *Lebistes reticulatus*, on ciliates and rotifers. Marine Microbial Food Webs 8: 337-346.

Landry, M. R., Barber, R. T., Bidigare, R. R., Chai, F., Coale, K. H., Dam, H. G., Lewis, M. R., Lindley, S. T., McCarthy, J. J., Roman, M. R. et al. 1997. Iron and grazing constraints on primary production in the central equatorial Pacific: An EqPac synthesis. Limnology and Oceanography 42: 405-418.

Laws, E. A. and Archie, J. W. 1981. Appropriate use of regression analysis in marine biology. Marine Biology 65: 13-16.

Le Gall, S., Bel Hassen, M. and Le Gall, P. 1997. Ingestion of a bacterivorous ciliate by the oyster *Crassostrea gigas*: Protozoa as a trophic link between picoplankton and benthic suspension-feeders. Marine Ecology Progress Series 152: 301-306.

Leakey, R. J. G., Burkill, P. H. and Sleigh, M. A. 1992. Planktonic ciliates in Southampton Water: abundance, biomass, production, and role in pelagic carbon flow. Marine Biology 114: 67-83.

Leakey, R. J. G., Burkill, P. H. and Sleigh, M. A. 1993. Planktonic ciliates in Southampton water: quantitative taxonomic studies. Journal of the Marine Biology Association, U.K. 73: 579-594.

Li, W. K. W., Subba Rao, D. V., Harrison, W. G., Smith, J. C., Cullen, J. J., Irwin, B. and Platt, T. 1983. Autotrophic picoplankton in the tropical ocean. Science 19: 292-295.

Lynn, D. H. and Small, E. B. 1985. Phylum Ciliophora, p. 339-575. In: J. J. Lee, S. H. Hunter and E. G. Bovee (eds), Illustrated guide to the Protozoa. Lawrence, KS, Society of Protozoologists.

Maeda, M. 1986. An illustrated guide to the species of the families Halteriidae and Strobilidiidae (Oligotrichida, Ciliophora), free swimming protozoa common to the aquatic environment. Bulletin of the Ocean Research Institute, University of Tokyo 21: 1-67.

Maeda, M. and Carey, P. G. 1985. An illustrated guide to the species of the family Strombidiidae (Oligotrichida, Ciliophora), free swimming protozoa common in the aquatic environment. Bulletin of the Ocean Research Institute, University of Tokyo 19: 1-68.

Miller, C. B., Frost, B. W., Wheeler, P. A., Landry, M. R., Welschmeyer, N. and Powell, T. M. 1991. Ecological dynamics in the subarctic Pacific, a possibly iron-limited ecosystem. Limnology and Oceanography 36: 1600-1615.

Millero, F. J. and Poisson, A. 1981. International one-atmosphere equation of state of seawater. Deep-Sea Research 28: 625-629.

Montagnes, D. J. S. 1996. Growth responses of planktonic ciliates in the genera *Strobilidium* and *Strombidium*. Marine Ecology Progress Series 130: 241-254.

Muller, H. and Geller, W. 1993. Maximum growth rates of aquatic ciilated protozoa: the dependence on body size and termperature considered. Archiv fuer Hydrobiologie 126-: 315-327.

Murphy, L. S. and Haugen, E. M. 1985. The distribution and abundance of phototrophic ultraplankton in the North Atlantic. Limnology and Oceanography 30: 47-58.

Murrell, M. C. and Hollibaugh, J. T. 1998. Microzooplankton grazing in northern San Francisco Bay measured by the dilution method. Aquatic Microbial Ecology 15: 53-63.

Nielsen, T. G. and Kiorboe, T. 1994. Regulation of zooplankton biomass and production in a temperate, coastal ecosystem. 2. Ciliates. Limnology and Oceanography 39: 508-519.

Nival, P. and Nival, S. 1976. Particle retention efficiencies of an herbivorous copepod, *Acatia clausi* (adult and copepodite stages): effects on grazing. Limnology and Oceanography 21: 24-38.

Ohman, M. D. and Runge, J. A. 1994. Sustained fecundity when phytoplankton resources are in short supply: Omnivory by *Calanus finmarchicus* in the Gulf of St. Lawrence. Limnology and Oceanography 39: 21-36.

Ohman, M. D. and Snyder, R. A. 1991. Growth kinetics of the omnivorous oligotrich ciliate *Strombidium* sp. Limnology and Oceanography 36: 922-935.

Parsons, T. R., Maita, Y. and Lalli, C. M. 1984. Manual of chemical and biological methods for seawater analysis. New York: Pergamon Press.

Pierce, R. W. and Turner, J. T. 1992. Ecology of planktonic ciliates in marine food webs. Review of Aquatic Science 6: 139-181.

Platt, T., Rao, D. V. S. and Irwin, B. 1983. Photosynthesis of picoplankton in the oligotrophic ocean. Nature 301: 702-704.

Pomeroy, L. 1974. The ocean's food web, a changing paradigm. Bioscience 24: 499-504.

Putt, M. and Stoecker, D. K. 1989. An experimentally determined carbon: Volume ratio for marine "oligotrichous" ciliates from estuarine and coastal waters. Limnology and Oceanography 34: 1097-1103.

Rassoulzadegan, F. 1982. Dependence of grazing rate, gross growth efficiency and food size range on temperature in a pelagic oligotrichous ciliate *Lohmanniella spiralis* Leeg., fed on naturally occurring particulate matter. Annales. Institut Oceanographique (Paris) 58: 177-184.

Rassoulzadegan, F., Laval-Peuto, M. and Sheldon, R. W. 1988. Partitioning of the food ration of marine ciliates between pico- and nanoplankton. Hydrobiologia 159: 75-88.

Revelante, N. and Gilmartin, M. 1983. Microzooplankton distribution in the Northern Adriatic Sea with emphasis on the relative abundance of ciliated protozoans. Oceanologica Acta 6: 407-415.

Ricker, W. E. 1973. Linear regressions in fishery research. Journal of the Fisheries Research Board of Canada 30: 409-434.

Riemann, B., Simonsen, P. and Stensgaard, L. 1989. The carbon and chlorophyll content of phytoplankton from various nutrient regimes. Journal of Plankton Research 11: 1037-1045.

Riisgard, H. U. 1988. Efficiency of particle retention and filtration rate in 6 species of North East American bivalves. Marine Ecology Progress Series 45: 217-223.

Roegner, G. C. and Shanks, A. L. 2001. Import of Coastally-Derived Chlorophyll a to South Slough, Oregon. Estuaries 24: 244-256.

Rumrill, S. In review. Site profile of the South Slough National Estuarine Research Reserve.

Sherr, B. F., Sherr, E. B. and Rassoulzadegan, F. 1988. Rates of digestion of bacteria by marine phagotrophic protozoa: Temperature dependence. Applied and Environmental Microbiology 54: 1091-1095.

Sherr, E. B., Rassoulzadegan, F. and Sherr, B. F. 1989. Bacterivory by pelagic choreotrichous ciliates in coastal waters of the NW Mediterranean Sea. Marine Ecology Progress Series 55: 235-240.

Sherr, E. B. and Sherr, B. F. 1987. High rates of consumption of bacteria by pelagic ciliates. Nature 325: 710-711.

Sherr, E. B. and Sherr, B. F. 1994. Bacterivory and herbivory: key roles of phagotrophic protists in pelagic food webs. Microbial Ecology 28: 223-235.

Sherr, E. B., Sherr, B. F., Fallon, R. D. and Newell, S. Y. 1986. Small, aloricate ciliates as a major component of the marine heterotrophic nanoplankton. Limnology and Oceanography 31: 177-183.

Shumway, S. E., Cucci, T. L., Newell, R. C. and Yentsch, C. M. 1985. Particle selection, ingestion, and absorption in filter-feeding bivalves. Journal of Experimemtal Marine Biology Ecology 91: 77-92.

Sieburth, J. M., Smetacek, V. and Lenz, J. 1978. Pelagic ecosystem structure: Heterotrophic compartments of the plankton and their relationship to plankton size fractions. Limnology and Oceanography 23: 1256-1263.

Sime-Ngando, T., Gosselin, M., Roy, S. and Chanut, J.-P. 1995. Significance of planktonic ciliated protozoa in the Lower St. Lawrence Estuary: comparison with bacterial, phytoplankton and particulate organic carbon. Aquatic Microbial Ecology 9: 243-258.

Small, L. F. and Menzies, D. W. 1981. Patterns of primary productivity and biomass in a coastal upwelling region. Deep-Sea Research 28: 123-149.

Small, L. F. and Morgan, S. R. 1994. Phytoplankton attributes in the turbidity maximum of the Columbia River estuary, USA, p. 465-472. In: K. R. Dyer and R. J. Orth [eds.], Changes in fluxes in estuaries: Implications from science to management. Fredensborg (Denmark), Olsen & Olsen.

Smetacek, V. 1981. The Annual Cycle of Protozooplankton in the Kiel Bight. Marine Biology 63: 1-11.

Smetacek, V. 1984. Growth dynamics of a common Baltic protozooplankter: the ciliate genus *Lohmanniella*. Limnologica 15: 371-376.

Sorokin, Y. I. and Sorokin, P. Y. 1996. Plankton and primary production in the Lena River Estuary and in the south-eastern Laptev Sea. Estuarine, Coastal and Shelf Science 43: 399-418.

Stockner, J. G. and Antia, N. J. 1988. Algal picoplankton from marine and freshwater exosystems: a multidisciplinary perspective. Canadian Journal of Fisheries and Aquatic Sciences 43: 2472-2503.

Stoecker, D. and Guillard, R. R. L. 1982. Effects of temperature and light on the feeding rate of *Favella* sp. (ciliated Protozoa, suborder Tintinnina). Annales. Institut Oceanographique (Paris) 58 (suppl): 309-318.

Stoecker, D., Guillard, R. R. L. and Kavee, R. M. 1981. Selective predation by *Favella ehrenbergii* (Tintinnia) on and among dinoflagellates. Biological Bulletin 160: 136-145.

Stoecker, D. K., Davis, L. H. and Anderson, D. M. 1984. Fine scale spatial correlations between planktonic ciliates and dinoflagellates. Journal of Plankton Research 6: 829-842.

Stoecker, D. K. and Egloff, D. A. 1987. Predation by *Acartia tonsa* Dana on planktonic ciliates and rotifers. Journal of Experimental Marine Biology and Ecology 110: 53-68.

Stoecker, D. K. and Govoni, J. J. 1984. Food selection by young larval Gulf menhaden (*Brevoortia patronus*). Marine Biology 80: 299-306.

Stoecker, D. K. and Silver, M. W. 1990. Replacement and aging of chloroplasts in *Strombidium capitatum* (Ciliophora: Oligotrichida). Marine Biology 107: 491-502.

Stoecker, D. K., Silver, M. W., Michaels, A. E. and Davis, L. H. 1989. Enslavement of algal chloroplasts by four *Strombidium* spp. (Ciliophora, Oligotrichida). Marine Microbial Food Webs 3: 79-100.

Stoecker, D. K., Verity, P. G., Michaels, A. E. and Davis, L. H. 1987. Feeding by larval and post-larval ctenophores on microzooplankton. Journal Plankton Research 9: 667-683.

Stoecker, D. K. and Capuzzo, J. M. 1990. Predation on Protozoa: Its importance to zooplankton. Journal of Plankton Research 12: 891-908.

Strom, S. L., Postel, J. R. and Booth, B. C. 1993. Abundance, variability, and potential grazing impact of planktonic ciliates in the open subarctic Pacific Ocean. Progress in Oceanography 32: 185-203.

Tamigneaux, E., Mingelbier, M., Klein, B. and Legendre, L. 1997. Grazing by protists and seasonal changes in the size structure of protozooplankton and phytoplankton in a temperate nearshore environment (western Gulf of St. Lawrence, Canada). Marine Ecology Progress Series 146: 231-247.

Tumantseva, N. I. and Kopylov, A. I. 1985. Reproduction and production rates of planktic infusoria in coastal waters of Peru. Oceanology 25: 390-394.

Utermohl, H. 1958. Zur vervollkommnung der quantitativen phytoplankton-methodik. Mitteilungen. Internationale Vereinigung fuer Theortische und Angewandte Limnologie 9: 1-38.

Verity, P. G. 1987. Abundance, community composition, size distribution, and production rates of tintinnids in Narragansett Bay, Rhode Island. Estuarine, Coastal and Shelf Science 24: 671-690.

—. 1986. Grazing of phototrophic nanoplankton by microzooplankton in Narragansett Bay. Marine Ecology Progress Series 29:105-115.

Verity, P. G. 1991. Measurement and simulation of prey uptake by marine planktonic ciliates fed plastidic and aplastidic nanoplankton. Limnology and Oceanography 36: 729-750.

Verity, P. G. and Paffenhöfer, G. A. 1996. On assessment of prey ingestion by copepods. Journal of Plankton Research 18: 1767-1779.

Zar, J. 1996. Biostatistical analysis. Upper Saddle River, Prentice Hall.

Chapter III

Archer, S. D., R. J. G. Leakey, P. H. Burkill, and M. A. Sleigh. 1996. Microbial dynamics in coastal waters of East Antarctica: herbivory by heterotrophic dinoflagellates. Mar. Ecol. Prog. Ser. 139: 239-255.

Beers, J. R., F. M. H. Reid, and G. L. Stewart. 1980. Microplankton population structure in Southern California nearshore waters in late spring. Mar. Biol. 60: 209-226.

Beers, J. R., and G. L. Stewart. 1967. Micro-zooplankton in the euphotic zone at five locations across the California Current. J. Fish. Res. Board Can. 24: 2053-2068.

Booth, B. C. 1987. The use of autofluorescence for analyzing oceanic phytoplankton communities. Bot. Mar. 30: 101-108.

Buck, K. R., and J. Newton. 1995. Fecal pellet flux in Dabob Bay during a diatom bloom: contribution of microzooplankton. Limnol. Oceanogr. 40: 306-315.

Burkill, P. H. 1982. Ciliates and other microplankton components of a nearshore food-web: standing stocks and production processes. Annl. Instit. Océanogr., Paris 58: 335-350.

Burkill, P. H., R. F. C. Mantoura, C. A. Llewellyn, and N. J. P. Owens. 1987. Microzooplankton grazing and selectivity of phytoplankton in coastal waters. Mar. Biol. 93: 581-590.

Campbell, L., and E. J. Carpenter. 1986a. Diel patterns of cell division in marine *Synechococcus* spp. (Cyanobacteria): use of the frequency of dividing cells technique to measure growth rate. Mar. Ecol. Prog. Ser. 32: 139-148

—. 1986b. Estimating the grazing pressure of heterotrophic nanoplankton on *Synechococcus* spp. using the sea water dilution and selective inhibitor techniques. Mar. Ecol. Prog. Ser. 33: 121-129.

Edwards, E. S., Burkill, P. H., and Stefox, C. E. Zooplankton herbivory in the Arabian Sea during and after the SW monsoon, 1994. Deep-Sea Res. II 46: 843-863.

Emmett, R., R. Lianso, J. Newton, R. Thom, M. Hornberger, C. Morgan, C. Levings et al. 2000. Geographic Signatures of North American West Coast Estuaries. Estuaries 23: 765-792.

Evans, G. T., and M. A. Paranjape. 1992. Precision of estimates of phytoplankton growth and microzooplankton grazing when the functional response of grazers may be nonlinear. Mar. Ecol. Prog. Ser. 80: 285-290.

Fenchel, T. 1980. Suspension feeding in ciliated protozoa: feeding rates and their ecological significance. Microb. Ecol. 6: 13-25.

Fessenden, L., and T. J. Cowles. 1994. Copepod predation on phagotrophic ciliates in Oregon coastal waters. Mar. Ecol. Prog. Ser. 107: 103-111.

Froneman, P. W., and C. D. McQuaid. 1997. Preliminary investigation of the ecological role of microzooplankton in the Kariega Estuary, South Africa. Estuarine, Coastal and Shelf Sci. 45: 689-695.

Frost, B. W. 1972. Effects of size and concentration of food particles on the feeding behaviour of the marine planktonic copepods *Calanus pacificus*. Limnol. Oceanogr. 17: 805-815.

Gallegos, C. L. 1989. Microzooplankton grazing on phytoplankton in the Rhode River, Maryland: nonlinear feeding kinetics. Mar. Ecol. Prog. Ser. 57: 23-33.

Gifford, D. J. 1985. Laboratory culture of marine planktonic oligotrichs (Ciliophora, Oligotrichida). Mar. Ecol. Prog. Ser. 23: 257-267.

—. 1988. Impact of grazing by microzooplankton in the Northwest Arm of Halifax Harbor, Nova Scotia. Mar. Ecol. Prog. Ser. 47: 249-258.

Hansen, B., P. K. Bjoernsen, and P. J. Hansen. 1994. The size ratio between planktonic predators and their prey. Limnol. Oceanogr. 39: 395-403.

Hughes, M. P. 1977. Temporal and spatial variability of phytoplankton in coastal and estuarine habitats in Coos Bay, Oregon, p. 98. M. S. Thesis. University of Oregon.

Huyer, A. 1983. Coastal upwelling in the California Current system. Prog. Oceanogr. 12: 259-284.

Jacobson, D. M., and D. M. Anderson. 1986. Thecate heterotrophic dinoflagellates: feeding behavior and mechanisms. J. Phycol. 22: 249-258.

Jonsson, P. R. 1986. Particle size selection, feeding rates and growth dynamics of marine planktonic oligotrichous ciliates (Ciliophora: Oligotrichina). Mar. Ecol. Prog. Ser. 33: 265-277.

Jürgens, K., S. A. Wickham, K. O. Rothhaupt, and B. Santer. 1996. Feeding rates of macro- and microzooplankton on heterotrophic nanoflagellates. Limnol. Oceanogr. 41: 1833-1839.

Kleppel, G. S. 1993. On the diets of calanoid copepods. Mar. Ecol. Prog. Ser. 99: 183-195.

Kleppel, G. S., D. Frazel, R. E. Pieper, and D. V. Holliday. 1988. Natural diets of zooplankton off southern California. Mar. Ecol. Prog. Ser. 49: 231-241.

Kuosa, H. 1991. Picoplanktonic algae in the northern Baltic Sea: seasonal dynamics and flagellate grazing. Mar. Ecol. Prog. Ser. 73: 269-276.

Landry, M. R., and R. P. Hassett. 1982. Estimating the grazing impact of marine micro-zooplankton. Mar. Biol. 67: 283-288.

Levinsen, H., T. G. Nielsen, and B. W. Hansen. 1999. Plankton community structure and carbon cycling on the western coast of Greenland during the stratified summer situation. II. Heterotrophic dinoflagellates and ciliates. Aquat. Microb. Ecol. 16: 217-232.

Li, W. K. W., P. M. Dickie, B. D. Irwin, and A. M. Wood. 1992. Biomass of bacteria, cyanobacteria, prochlorophytes and photosynthetic eukaryotes in the Sargasso Sea. Deep-Sea Res. 39: 501-519.

Li, W. K. W., D. V. Subba Rao, W. G. Harrison, J. C. Smith, J. J. Cullen, B. Irwin, and T. Platt. 1983. Autotrophic picoplankton in the tropical ocean. Science 219: 292-295.

Liu, H., R. R. Bidigare, E. A. Laws, M. R. Landry, and L. Campbell. 1999. Cell cycle and physiological characteristics of *Synechococcus* (WH7803) in chemostat culture. Mar. Ecol. Prog. Ser. 189: 17-25.

Liu, H., L. Campbell, and M. R. Landry. 1995. Growth and mortality rates of *Prochlorococcus* and *Synechococcus* measured with a selective inhibitor technique. Mar. Ecol. Prog. Ser. 116: 277-287.

McManus, G. B., and M. C. Ederington-Cantrell. 1992. Phytoplankton pigments and growth rates, and microzooplankton grazing in a large temperate estuary. Mar. Ecol. Prog. Ser. 87: 77-85.

Murphy, L. S., and E. M. Haugen. 1985. The distribution and abundance of phototrophic ultraplankton in the North Atlantic. Limnol. Oceanogr. 30: 47-58.

Neuer, S., and T. J. Cowles. 1994. Protist herbivory in the Oregon upwelling system. Mar. Ecol. Prog. Ser. 113: 147-162.

Nielsen, T. G., and T. Kiorboe. 1994. Regulation of zooplankton biomass and production in a temperate, coastal ecosystem. 2. Ciliates. Limnol. Oceanogr. 39: 508-519.

Nival, P., and S. Nival. 1976. Particle retention efficiencies of an herbivorous copepod, *Acatia clausi* (adult and copepodite stages): effects on grazing. Limnol. Oceanogr. 21: 24-38.

Ohman, M. D., and R. A. Snyder. 1991. Growth kinetics of the omnivorous oligotrich ciliate *Strombidium* sp. Limnol. Oceanogr. 36: 922-935.

Paffenhöfer, G.-A. 1984. Food ingestion by the marine copepod Paracalanus in relation to abundance and size distribution of food. Mar. Biol. 80: 323-333.

Paranjape, M. A. 1987. Grazing by microzooplankton in the eastern Canadian Arctic in summer 1983. Mar. Ecol. Prog. Ser. 40: 239-246.

—. 1990. Microzooplankton herbivory on the Grand Bank (Newfoundland, Canada): A seasonal study. Mar. Biol. 107: 321-328.

Parsons, T. R., Y. Maita, and C. M. Lalli. 1984, Manual of chemical and biological methods for seawater analysis. New York, Pergamon Press.

Peters, F. 1994. Prediction of planktonic protistan grazing rates. Limnol. Oceanogr. 39: 195-206.

Platt, T., D. V. S. Rao, and B. Irwin. 1983. Photosynthesis of picoplankton in the oligotrophic ocean. Nature 301: 702-704.

Polis, G. A., and Holt, R. D. 1992. Intraguild predation: the dynamics of complex trophic interactions. Trends Ecol. Evol. 7: 151-154.

Polis, G. A., and D. R. Strong. 1996. Food web complexity and community dynamics. Amer. Nat. 147: 813-846.

Rassoulzadegan, F. 1982. Dependence of grazing rate, gross growth efficiency and food size range on temperature in a pelagic oligotrichous ciliate *Lohmanniella spiralis* Leeg., fed on naturally occurring particulate matter. Annal.. Instit. Océanogr., Paris 58: 177-184.

Rassoulzadegan, F., and M. Etienne. 1981. Grazing Rate of the Tintinnid *Stenosemella ventricosa* (Clap. & Lachm.) Joerg. on the spectrum of the naturally occurring particulate matter from a Mediterranean neritic area. Limnol. Oceanogr. 26: 258-270.

Rassoulzadegan, F., M. Laval-Peuto, and R. W. Sheldon. 1988. Partitioning of the food ration of marine ciliates between pico- and nanoplankton. Hydrobiologia 159: 75-88.

Revelante, N., and M. Gilmartin. 1983. Microzooplankton distribution in the Northern Adriatic Sea with emphasis on the relative abundance of ciliated protozoans. Oceanol. Acta 6: 407-415.

Riemann, B., P. Simonsen, and L. Stensgaard. 1989. The carbon and chlorophyll content of phytoplankton from various nutrient regimes. J. Plankton Res. 11: 1037-1045.

Rivkin, R. B., J. N. Putland, M. R. Anderson, and D. Deibel. 1999. Microzooplankton bacterivory and herbivory in the NE subarctic Pacific. Deep-Sea Res. 46: 2579-2618.

Roegner, G. C. and Shanks, A. L. 2001. Import of coastally-derived chlorophyll a to South Slough, Oregon. Estuaries 24: 244-256.

Rumrill, S. In review. Site profile of the South Slough National Estuarine Research Reserve.

Sanders, R. W., D. A. Caron, and U. G. Berninger. 1992. Relationships between bacteria and heterotrophic nanoplankton in marine and fresh waters: an inter-ecosystem comparison. Mar. Ecol. Prog. Ser. 86: 1-14.

Sherr, E. B., D. A. Caron, and B. F. Sherr. 1993. Staining of heterotrophic protists for visualization via epifluorescence microscopy, pp. 213-228. *In*: P. F. Kemp, B. F. Sherr, E. B. Sherr, and J. J. Cole, [eds.], Handbook of methods in aquatic microbial ecology. Boca Raton, Lewis Publishers.

Sherr, E. B. and Sherr, B. F. 1994. Bacterivory and herbivory: key roles of phagotrophic protists in pelagic food webs. Microbial Ecology 28: 223-235.

Sherr, E. B., B. F. Sherr, and G. A. Paffenhoefer. 1986. Phagotrophic protozoa as food for metazoans: a "missing" trophic link in marine pelagic food webs? Mar. Microb. Food Webs 1: 61-80.

Sieburth, J. M., V. Smetacek, and J. Lenz. 1978. Pelagic ecosystem structure: heterotrophic compartments of the plankton and their relationship to plankton size fractions. Limnol. Oceanogr. 23: 1256-1263.

Sime-Ngando, T., M. Gosselin, S. Roy, and J.-P. Chanut. 1995. Significance of planktonic ciliated protozoa in the Lower S.t Lawrence Estuary: comparison with bacterial, phytoplankton and particulate organic carbon. Aquat. Microb. Ecol. 9: 243-258.

Small, L. F., and D. W. Menzies. 1981. Patterns of primary productivity and biomass in a coastal upwelling region. Deep-Sea Res. 28: 123-149.

Smetacek, V. 1981. The annual Cycle of protozooplankton in the Kiel Bight. Mar. Biol. 63: 1-11.

Stoecker, D. K., L. H. Davis, and D. M. Anderson. 1984. Fine scale spatial correlations between planktonic ciliates and dinoflagellates. J. Plankton Res. 6: 829-842.

Strom, S. L., M. A. Brainard, J. L. Holmes, and M. B. Olson. 2001. Phytoplankton blooms are strongly impacted by microzooplankton grazing in coastal North Pacific waters. Mar. Biol. 138: 355-368.

Strom, S. L., and M. W. Strom. 1996. Microplankton growth, grazing, and community structure in the northern Gulf of Mexico. Mar. Ecol. Prog. Ser. 130: 229-240.

Strom, S. L., and N. Welschmeyer. 1991. Pigment-specific rates of phytoplankton growth and microzooplankton grazing in the open subarctic Pacific Ocean. Limnol. Oceanogr. 36: 50-63.

Tremaine, S. C., and A. L. Mills. 1987. Tests of the critical assumptions of the dilution method for estimating bacterivory by microeucaryotes. Appl. Environ. Microbiol. 53: 2914-2921.

Verity, P. G. 1986. Grazing of phototrophic nanoplankton by microzooplankton in Narragansett Bay. Mar. Ecol. Prog. Ser. 29: 105-115.

—. 1991. Measurement and simulation of prey uptake by marine planktonic ciliates fed plastidic and aplastidic nanoplankton. Limnol. Oceanogr. 36: 729-750.

Verity, P. G., Robertson, C. Y., Tronzo, C. R., Andrews, M. G., Nelson, J. R., and Sieracki, M. E. 1992. Relationships between cell volume and the carbon and nitrogen content of marine photosynthetic nanoplankton. Limnol. Oceanogr. 37: 1434-1446.

Chapter IV

Adrian, R., Wickham, S. A., and Butler, N. M. 2001. Trophic interactions between zooplankton and the microbial community in contrasting food webs: the epilimnion and deep chlorophyll maximum of a mesotrophic lake. Aquat. Microb. Ecol. 24: 83-97.

Azam, F., Fenchel, T., Field, J. G., Gray, J. S., Meyer-Reil, L. A., and Thingstad, F. 1983. The ecological role of water-column microbes in the sea. Mar. Ecol. Prog. Ser. 10: 257-263.

Bernard, C., and Rassoulzadegan, F. 1990. Bacteria or microflagellates as a major food source for marine ciliates: possible implications for microzooplankton. Mar. Ecol. Prog. Ser. 64: 147-155.

Booth, B. C. 1987. The use of autofluorescence for analyzing oceanic phytoplankton communities. Bot. Mar. 30: 101-108.

Calbet, A., and Landry, M. R. 1999. Mesozooplankton influences on the microbial food web: Direct and indirect trophic interactions in the oligotrophic open ocean. Limnol. Oceanogr. 44: 1370-1380.

Calbet, A., Landry, M. R., and Nunnery, S. 2001. Bacteria-flagellate interactions in the microbial food web of the oligotrophic subtropical North Pacific. Aquat. Microb. Ecol. 23: 283-292.

Campbell, L., and Carpenter, E. J. 1986. Estimating the grazing pressure of heterotrophic nanoplankton on *Synechococcus* spp. using the sea water dilution and selective inhibitor techniques. Mar. Ecol. Prog. Ser. 33: 121-129.

Caron, D. A., and Goldman, J. C. 1993. Predicting excretion rates of protozoa: Reply to the comment by Landry. Limnol. Oceanogr. 38: 472-474.

Carpenter, S. R., Kitchell, J. F., and Hodgson, J. R. 1985. Cascading trophic interactions and lake productivity. Bioscience 35: 634-639.

Christaki, U., Dolan, J. R., Pelegri, S., and Rassoulzadegan, F. 1998. Consumption of picoplankton-size particles by marine ciliates: Effects of physiological state of the ciliate and particle quality. Limnol. Oceanogr. 43: 458-464.

Christaki, U., Jacquet, S., Dolan, J. R., Vaulot, D., and Rassoulzadegan, F. 1999. Growth and grazing on *Prochlorococcus* and *Synechococcus* by two marine ciliates. Limnol. Oceanogr. 44: 52-61.

Cleven, E. J. 1996. Indirectly fluorescently labelled flagellates (IFLF): A tool to estimate the predation on free-living heterotrophic flagellates. J. Plankton Res. 18: 429-442.

Davis, P. G., Caron, D. A., Johnson, P. W., and Sieburth, J. M. 1985. Phototrophic and apochlorotic components of picoplankton and nanoplankton in the North Atlantic: geographic, vertical, seasonal and diel distributions. Mar. Ecol. Prog. Ser. 21: 15-26.

Dolan, J. R., and Coats, D. W. 1991. Seasonal abundances of planktonic ciliates and microflagellates in mesohaline Chesapeake Bay waters. Est. Coast. Shelf Sci. 31:157-175.

Evans, G. T., and Paranjape, M. A. 1992. Precision of estimates of phytoplankton growth and microzooplankton grazing when the functional response of grazers may be nonlinear. Mar. Ecol. Prog. Ser. 80: 285-290.

Fenchel, T. 1982. Ecology of Heterotrophic Microflagellates. IV. Quantitative Occurrence and Importance as Bacterial Consumers. Mar. Ecol. Prog. Ser. 9: 35-42.

Fessenden, L., and Cowles, T. J. 1994. Copepod predation on phagotrophic ciliates in Oregon coastal waters. Mar. Ecol. Prog. Ser. 107: 103-111.

Frost, B. W. 1972. Effects of size and concentration of food particles on the feeding behaviour of the marine planktonic copepods *Calanus pacificus*. Limnol. Oceanogr. 17: 805-815.

Gallegos, C. L. 1989. Microzooplankton grazing on phytoplankton in the Rhode River, Maryland: Nonlinear feeding kinetics. Mar. Ecol. Prog. Ser. 57: 23-33.

Gifford, D. J. 1988. Impact of grazing by microzooplankton in the northwest arm of Halifax Harbor, Nova Scotia. Mar. Ecol. Prog. Ser. 47: 249-258.

Gifford, D. J., and Dagg, M. J. 1988. Feeding of the estuarine copepod *Acartia tonsa* Dana: carnivory vs. herbivory in natural microplankton assemblages. Bull. Mar. Sci. 43: 458-468.

Goldman, J. C., and Caron, D. A. 1985. Experimental studies on an omnivorous microflagellate: implications for grazing and nutrient regeneration in the marine microbial food chain. Deep-Sea Res. 32: 899-915.

Haas, L. W., and Webb, K. L. 1979. Nutritional mode of several non-pigmented microflagellates from the York River estuary, Virginia. J. Exp. Mar. Biol. Ecol. 39: 125-134.

Hagström, A., Azam, F., Andersson, A., Wikner, J., and Rassoulzadegan, F. 1988. Microbial loop in an oligotrophic pelagic marine ecosystem: possible roles of cyanobacteria and nanoflagellates in the organic fluxes. Mar. Ecol. Prog. Ser. 49: 171-178.

Iturriaga, R., and Mitchell, B. G. 1986. Chroococcoid cyanobacteria: a significant component in the food web dynamics of the open ocean. Mar. Ecol. Prog. Ser. 28: 291-297.

Johnson, P. W., and Sieburth, J. M. (1979). Chroococcoid cyanobacteria in the sea: a ubiquitous and diverse phototrophic biomass. Limnol. Oceanogr.

Johnson, P. W., Huai-Shu, X., and Sieburth, J. M. 1982. The utilization of chroococcoid cyanobacteria by marine protozooplankters but not by calanoid copepods. Annls. Inst. Oceanogr., Paris 58: 297-308.

Joint, I. R., and Pomroy, A. J. 1986. Photosynthetic characteristics of nanoplankton and picoplankton from the surface mixed layer. Mar. Biol. 92: 465-474.

Jonsson, P. R. 1986. Particle size selection, feeding rates and growth dynamics of marine planktonic oligotrichous ciliates (Ciliophora: Oligotrichina). Mar. Ecol. Prog. Ser. 33: 265-277.

Jürgens, K., Wickham, S. A., Rothhaupt, K. O., and Santer, B. 1996. Feeding rates of macro- and microzooplankton on heterotrophic nanoflagellates. Limnol. Oceanogr. 41: 1833-1839.

Kleppel, G. S. 1993. On the diets of calanoid copepods. Mar. Ecol. Prog. Ser. 99: 183-195.

Kuosa, H. 1991. Picoplanktonic algae in the northern Baltic Sea: seasonal dynamics and flagellate grazing. Mar. Ecol. Prog. Ser. 73: 269-276.

Landry, M. R., Gifford, D. J., Kirchman, D. L., Wheeler, P. A., and Monger, B. C. 1993. Direct and indirect effects of grazing by *Neocalanus plumchrus* on plankton community dynamics in the Subarctic Pacific. Prog. Oceanogr. 32: 239-258.

Landry, M. R., and Hassett, R. P. 1982. Estimating the grazing impact of marine micro-zooplankton. Mar. Biol. 67: 283-288.

Lawler, S. P., and Morin, P. J. 1993. Food web architecture and population dynamics in laboratory microcosms of protists. Amer. Nat. 141: 675-686.

Li, W. K. W., Dickie, P. M., Irwin, B. D., and Wood, A. M. 1992. Biomass of bacteria, cyanobacteria, prochlorophytes and photosynthetic eukaryotes in the Sargasso Sea. Deep-Sea Res. 39: 501-519.

Li, W. K. W., Subba Rao, D. V., Harrison, W. G., Smith, J. C., Cullen, J. J., Irwin, B., and Platt, T. 1983. Autotrophic picoplankton in the tropical ocean. Science 219: 292-295.

Linley, E. A. S., Newell, R. C., and Lucas, M. I. 1983. Quantitative relationships between phytoplankton, bacteria and heterotrophic microflagellates in shelf waters. Mar. Ecol. Prog. Ser. 12: 77-89.

Murphy, L. S., and Haugen, E. M. 1985. The distribution and abundance of phototrophic ultraplankton in the North Atlantic. Limnol. Oceanogr. 30: 47-58.

Nielsen, T. G., and Kiorboe, T. 1994. Regulation of zooplankton biomass and production in a temperate, coastal ecosystem. 2. Ciliates. Limnol. Oceanogr. 39: 508-519.

Ohman, M. D., and Snyder, R. A. 1991. Growth kinetics of the omnivorous oligotrich ciliate *Strombidium* sp. Limnol. Oceanogr. 36: 922-935.

Platt, T., Rao, D. V. S., and Irwin, B. 1983. Photosynthesis of picoplankton in the oligotrophic ocean. Nature 301: 702-704.

Polis, G. A., and Holt, R. D. 1992. Intraguild predation: the dynamics of complex trophic interactions. Trends Ecol. Evol. 7: 151-154.

Polis, G. A., and Strong, D. R. 1996. Food web complexity and community dynamics. American Naturalist 147: 813-846.

Pomeroy, L. 1974. The ocean's food web, a changing paradigm. Bioscience 24: 499-504.

Putt, M., and Stoecker, D. K. 1989. An experimentally determined carbon: Volume ratio for marine "oligotrichous" ciliates from estuarine and coastal waters. Limnol. Oceanogr. 34: 1097-1103.

Rassoulzadegan, F. 1982. Dependence of grazing rate, gross growth efficiency and food size range on temperature in a pelagic oligotrichous ciliate *Lohmanniella spiralis* Leeg., fed on naturally occurring particulate matter. Annls. Inst. Oceanogr., Paris 58: 177-184.

Rassoulzadegan, F., Laval-Peuto, M., and Sheldon, R. W. 1988. Partitioning of the food ration of marine ciliates between pico- and nanoplankton. Hydrobiologia 159: 75-88.

Rivkin, R. B., Putland, J. N., Anderson, M. R., and Deibel, D. 1999. Microzooplankton bacterivory and herbivory in the NE subarctic Pacific. Deep-Sea Res. II 46: 2579-2618.

Sanders, R. W., Caron, D. A., and Berninger, U. G. 1992. Relationships between bacteria and heterotrophic nanoplankton in marine and fresh waters: An inter-ecosystem comparison. Mar. Ecol. Prog. Ser. 86: 1-14.

Sherr, B. F., and Sherr, E. B. 1991. Proportional distribution of total numbers, biovolume, and bacterivory among size classes of 2-20 mm nonpigmented marine flagellates. Mar. Microb. Food Webs 5: 227-237.

Sherr, E., and Sherr, B. 1988. Role of microbes in pelagic food webs: a revised concept. Limnol. Oceanogr. 33: 1225-1226.

Sherr, E. B., Caron, D. A., and Sherr, B. F. 1993. Staining of heterotrophic protists for visualization via epifluorescence microscopy, pp. 213-228. *In*: P. F. Kemp, B. F. Sherr, E. B. Sherr, and J. J. Cole [eds.], Handbook of methods in aquatic microbial ecology. Boca Raton, Lewis Publishers.

Sherr, E. B., Rassoulzadegan, F., and Sherr, B. F. 1989. Bacterivory by pelagic choreotrichous ciliates in coastal waters of the NW Mediterranean Sea. Mar. Ecol. Prog. Ser. 55: 235-240.

Sherr, E. B., Sherr, B. F., and McDaniel, J. 1991. Clearance rates of < 6 mu m fluorescently labeled algae (FLA) by estuarine protozoa: Potential grazing impact of flagellates and ciliates. Mar. Ecol. Prog. Ser. 69: 81-92.

Sprules, W. G., and Bowerman, J. E. 1988. Omnivory and food chain length in zooplankton food webs. Ecology 69: 418-426.

Stockner, J. G., and Antia, N. J. 1988. Algal picoplankton from marine and freshwater ecosystems: a multidisciplinary perspective. Can. J. Fish. Aquat. Sci. 43: 2472-2503.

Strom, S. L., Brainard, M. A., Holmes, J. L., and Olson, M. B. 2001. Phytoplankton blooms are strongly impacted by microzooplankton grazing in coastal North Pacific waters. Mar. Biol. 138: 355-368.

Verity, P. G. 1985. Grazing, respiration, excretion, and growth rates of tintinnids. Limnol. Oceanogr. 30: 1268-1282.

—. 1991. Measurement and simulation of prey uptake by marine planktonic ciliates fed plastidic and aplastidic nanoplankton. Limnol. Oceanogr. 36: 729-750.

Verity, P. G., Robertson, C. Y., Tronzo, C. R., Andrews, M. G., Nelson, J. R., and Sieracki, M. E. 1992. Relationships between cell volume and the carbon and nitrogen content of marine photosynthetic nanoplankton. Limnol. Oceanogr. 37: 1434-1446.

Waterbury, J. B., Watson, S. W., Guillard, R. R. L., and Brand, L. E. 1979. Widespread occurrence of a unicellular, marine, planktonic, cyanobacterium. Nature 277: 293-294.

Weisse, T. 1991. The annual cycle of heterotrophic freshwater nanoflagellates: role of bottom-up versus top-down control. J. Plankton Res. 13: 167-185.

Weisse, T., and Scheffel-Moeser, U. 1991. Uncoupling the microbial loop: Growth and grazing loss rates of bacteria and heterotrophic nanoflagellates in the North Atlantic. Mar. Ecol. Prog. Ser. 71: 195-205.

Wiadnyana, N. N., and Rassoulzadegan, F. 1989. Selective feeding of *Acartia clausi* and *Centropages typicus* on microzooplankton. Mar. Ecol. Prog. Ser. 53: 37-45.

Wikner, J., and Hagström, A. 1988. Evidence for a tightly coupled nanoplanktonic predator-prey link regulating the bacterivores in the marine environment. Mar. Ecol. Prog. Ser. 50: 137-145.

Wright, R. T., and Coffin, R. B. 1984. Measuring microzooplankton grazing on planktonic marine bacteria by its impact on bacterial production. Microb. Ecol. 10: 137-149.

Chapter V

Banse, K. 1982. Cell volumes, maximal growth rates of unicellular algae and ciliates, and the role of ciliates in the marine pelagial. Limnol. Oceanogr. 27: 1059-1071.

Calbet, A., and Landry, M. R. 1999. Mesozooplankton influences on the microbial food web: Direct and indirect trophic interactions in the oligotrophic open ocean. Limnol. Oceanogr. 44: 1370-1380.

Davis, P. G., Caron, D. A., Johnson, P. W., and Sieburth, J. M. 1985. Phototrophic and apochlorotic components of picoplankton and nanoplankton in the North Atlantic: geographic, vertical, seasonal and diel distributions. Mar. Ecol. Prog. Ser. 21: 15-26.

Fenchel, T. 1968. The ecology of marine microbenthos. III. The reproductive potential of ciliates. Ophelia 5: 123-136.

Finlay, B. J. 1977. The dependence of reproductive rate on cell size and temperature in freshwater ciliated protozoa. Oecologia 30: 75-81.

Landry, M. R., and R. P. Hassett. 1982. Estimating the grazing impact of marine micro-zooplankton. Mar. Biol. 67: 283-288.

Landry, M. R., Gifford, D. J., Kirchman, D. L., Wheeler, P. A., and Monger, B. C. 1993. Direct and indirect effects of grazing by *Neocalanus plumchrus* on plankton community dynamics in the Subarctic Pacific. Prog. Oceanogr. 32: 239-258.

Montagnes, D. J. S. 1996. Growth responses of planktonic ciliates in the genera *Strobilidium* and *Strombidium*. Mar. Ecol. Prog. Ser. 130: 241-254.

Nielsen, T. G. and Kiorboe, T. 1994. Regulation of zooplankton biomass and production in a temperate, coastal ecosystem. 2. Ciliates. Limnol. Oceanogr. 39: 508-519.

Stockner, J. G., and Antia, N. J. 1988. Algal picoplankton from marine and freshwater ecosystems: a multidisciplinary perspective. Can. J. Fish. Aquat. Sci. 43: 2472-2503.

Stoecker, D., and Guillard, R. R. L. 1982, Effects of temperature and light on the feeding rate of *Favella* sp. (ciliated Protozoa, suborder Tintinnina). Annls. Inst. Oceanogr., Paris 58 (suppl): 309-318.

Verity, P. 1986. Grazing of phototrophic nanoplankton by microzooplankton in Narragansett Bay. Mar. Ecol. Prog. Ser. 29:105-115.

Appendix A

Booth, B. C. 1987. The use of autofluorescence for analyzing oceanic phytoplankton communities. Bot. Mar. 30: 101-108.

Murphy, L. S., and E. M. Haugen. 1985. The distribution and abundance of phototrophic ultraplankton in the North Atlantic. Limnol. Oceanogr. 30: 47-58.

Sherr, E. B., D. A. Caron, and B. F. Sherr. 1993. Staining of heterotrophic protists for visualization via epifluorescence microscopy, pp. 213-228. *In*: P. F. Kemp, B. F. Sherr, E. B. Sherr, and J. J. Cole, [eds.], Handbook of methods in aquatic microbial ecology. Boca Raton, Lewis Publishers.