ADDRESSING PREY SELECTION AND PREDATOR FITNESS: INGESTION BY, AND GROWTH OF, *OCHROMONAS DANICA* ON MULTIPLE BACTERIAL PREY

by

Briony L. L. Foster

Presented to the Faculty of the Graduate School of

The University of Texas at Arlington in Partial Fulfillment

of the Requirements

for the Degree of

MASTER OF SCIENCE IN BIOLOGY

THE UNIVERSITY OF TEXAS AT ARLINGTON

August 2011

ACKNOWLEDGEMENTS

There have been a great number of people who have helped me through this degree. A few have been particularly supportive of me throughout this process. I would like to thank my parents, all three of them, for their support and incredible patience while I worked on this degree. I would like to acknowledge Sealy Hambright, Christi Hull and Blaine Thompson who all continue to be sounding boards for those late night “brilliant” science ideas, reliable sources for bench protocols and good friends. Thank you to my committee members, Woo-Suk Chang, Jim Grover and Jorge Rodrigues for pushing me when I needed it and contributing to the progress of my education. Most of all I would like to thank my mentor and friend Thomas Chrzanowski. My education would have stopped at the bachelor’s degree if he had not encouraged me to want more for myself. Not only has he constantly supported me educationally, he and his wife Jan have supported me personally and made me one of their own. I now have a second family and the best pumpkin pie recipe ever created.

I would like to dedicate this completed document to two women who were never afraid to pick up a red editing pen. To Mum and Maga

July 15, 2011

ABSTRACT

ADDRESSING PREY SELECTION AND PREDATOR FITNESS:

INGESTION BY, AND GROWTH OF, *OCHROMONAS*

DANICA ON MULTIPLE BACTERIAL

PREY

Briony L. L. Foster, M.S.

The University of Texas at Arlington, 2011

Supervising Professor: Thomas H. Chrzanowski

Nanoflagellates provide an important link in aquatic food webs by consuming bacterial biomass and subsequently providing nutrients to higher trophic levels by becoming prey themselves and regenerating nutrients consumed in excess of metabolic needs. Nanoflagellate predation has typically been addressed by examining one or two aspects of a seemingly complex prey identification process, and rarely has the subsequent fitness of the predator been quantified. In this work we examine how bacterial prey size, growth state, growth rate, nutritional composition, and phylogenetic class effect ingestion rate by, and growth rate of, the mixotrophic flagellate *Ochromonas danica*. A phylogenetically diverse group of fifteen bacterial species were offered to *O. danica* in single prey feeding experiments. Bacterial cells were harvested from batch cultures grown in R2A broth under identical environmental conditions. *O. danica* was harvested from a chemostat culture prior to each feeding experiment to ensure physiologically

similar predatory cells. Four different bacterial mortality curves were observed from the feeding experiments indicating differential recognition by O. danica of different prey. Ingestion rates were affected more by prey growth state and prey class than by any other metric. Growth rate of the predator was affected by prey nutritional quality (carbon:element), prey growth rate, and prey class. Results suggest that *Ochromonas danica* is a relatively indiscriminant consumer of bacterial prey but subsequent growth rates vary substantially for different types of prey. We conclude that *O. danica* may adjust ingestion rates to meet its nutritional demands, though it is largely unable to discriminate among prey prior to phagosome formation.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

Figure Page

LIST OF TABLES

CHAPTER 1

AN INTRODUCTION TO PREY SELECTION

Nutrients supporting aquatic food webs are made available to higher trophic levels by metabolic activities and interactions among members of the lowest tropic level. Bacteria and phytoplankton are the only groups of organisms within aquatic food webs capable of incorporating significant amounts of dissolved nutrients into biomass. Bacteria are particularly important in this process since these organisms are distributed throughout the water column, they are abundant, they have fast growth rates, and they have high assimilation efficiencies (Pomeroy 1974). Nutrients contained within bacterial biomass would be effectively removed from trophic dynamics in the absence of significant levels of predation. In such a case, bacteria would become a sink for nutrients, limiting their flow into higher trophic levels. Consequently, predation upon bacteria is one of the most important interactions driving nutrient dynamics (Fenchel 1982; Azam *et al* 1983; Anderson and Sørensen 1986). The major predators of bacteria in aquatic systems are the heterotrophic and mixotrophic nanoflagellates. These predators can consume as much as 100% percent of bacterial secondary production (Chrzanowski and Šimek 1993). Since the recognition of this important predator-prey interaction, many aquatic nutrient dynamics studies have focused on understanding factors that affect predation upon bacteria with particular attention given to the process of prey identification and selection.

Selection of prey has been described using a number of indices addressing both physical and chemical properties of the prey. Prey surface hydrophobicity and motility have been identified as potential factors that may influence selection. Particle-particle contact, similar to nanoflagellate-bacteria contact may be influenced by hydrophobic interactions. Although the

attractive molecular force between flagellate predators and their prey is small, it is sufficient to increase interception rates above what would be considered chance (Monger *et al.* 1999). However, a subsequent study (Matz and Jürgens 2001) revealed that differences in prey surface hydrophobicity could not fully explain differential feeding rates by three nanoflagellate predators. Similarly, motility may influence contact rates and interaction between predator and prey. Motile prey are more likely to encounter a predator then are non-motile prey (Gonźalez *et al.* 1993). Nevertheless, highly motile prey (>25 μm s^{-1}) are not ingested as readily as are their slower counterparts, despite encountering predators at a higher rate (Matz and Jürgens 2005).

Prey size has also been identified as a factor influencing selection by flagellate predators. It has been suggested that predators are physically able to ingest prey falling within a limited size range and it is prey within this range that are cropped (Pernthaler 2005). Certainly exceptions exist and in some cases predators are capable of ingesting prey as large as themselves (Boenigk and Arndt 2000); however, laboratory and field studies have demonstrated that there is a preferred prey size for predators (Andersson *et al.* 1986; Chrzanowski and Šimek 1990, Gonzales *et al.* 1990; Šimek and Chrzanowski 1992, Boenigk and Arndt 2000). It has also been suggested that prey that are large relative to the size of all potential prey within the prey pool, may be 'preferentially' selected simply due to an increased chance of interception (Hammer *et al.* 1999; Boenigk and Arndt 2000)

There is an interesting, if not fascinating, relationship among bacterial cell size, growth rate and nutrient content that may link prey selection to predator fitness and aquatic nutrient dynamics. This relationship revolves around two aspects of bacterial physiology, 1) cell size is linked to growth rate, and 2) growth rate is linked to cell quota (Q, concentration of a nutrient element per cell or per cell size), particularly for phosphorus. Phosphorus (P) is the element that most often limits productivity in aquatic systems, thus aspects of bacterial growth link both nutrient dynamics and prey selection (Elser and Hassett 1994 *and references therein)*.

Cell element quotas change as bacteria shift growth rate. Rapidly growing cells have lower carbon (C):P and nitrogen (N):P ratios than slowly growing cells. Elser *et al*. (2000) showed P quotas increased at a faster rate than C and N quotas as cell growth rate increased. This can be attributed to the increased level of P-rich translational machinery present in growing cells (Elser *et al*. 2000b; Chrzanowski and Grover 2008). Ultimately, the fastest growing cells would have the lowest C:P and N:P ratios and would possibly make the most nutritious prey (Elser *et al.* 2000a), that is, they would supply a predator with the largest pulse of the most limiting element. If growing cells are more nutritious than non-growing prey and predators are able to distinguish between the two, then rapidly growing cells should be preferentially cropped.

Consider a predator able to select prey according to nutritional quality. Such a predator should experience enhanced fitness by reducing energy expended capturing and processing prey of poor nutritional quality. Clearly some level of prey selection based on nutritional value occurs. Microflagellates preferentially select algal prey with a low C:N ratio when presented with mixed assemblages containing similarly sized cells (John and Davidson 2001). A similar observation was made for the nanoflagellate *Ochromonas danica* feeding on bacterial prey. Shannon *et al*. (2006) demonstrated that prey selection was dependent on nutritional quality (as C:N:P) to a greater extent than cell size. Further, the fitness of *O. danica*, measured as ingestion and growth rate, varies with the nutritional quality of its prey (Grover and Chrzanowski 2009). However, Gruber *et al.* (2009) suggest a different strategy. They separately offered two ciliates, *Eulotes vannus* and *Cyclidium glaucoma*, equal portions of rapidly (exponentially) growing and quiescent cells (in stationary phase) bacteria (*Escherichia coli)* to determine if prey selection occurs when a choice is given. In accordance with theory, quiescent cells were determined to have a high C:P and N:P ratios while exponential cells had low C:P and N:P ratios. However, stationary cells were selectively cropped to 22% (*C. glaucoma*) and 33% (*E. vannus*) of their original concentrations, while rapidly growing bacteria were not significantly diminished.

It is evident from the forgoing review of single-prey studies that some level of prey selection likely occurs in the flagellate-bacteria predator-prey system and while this selection may be influenced by prey physiology, bacteria in aquatic environments represent a tremendous phylogenetic diversity. Some have started to examine feeding behaviors of flagellates when presented prey representing different classes of bacteria. For example, members of the Betaproteobacteria appear to be more susceptible to predation then members of the Alphaproteobacteria when supplied to predators in mixed assemblages (Salcher *et al.* 2005). Selection of Betaproteobacteria was also demonstrated by Jezbera *et al.* (2005), who observed that two different heterotrophic flagellates preferentially selected a member of the Betaproteobacteria over a member of the Gammaproteobacteria when given a choice. In this case, *Aeromonas hydrophila* was ingested three times as readily as *Pseudomonas fluorescens*.

Further, it seems likely that nutrient demands of a predator will change during periods of growth and senescence. In a series of predator-prey studies, Flynn *et al.* (1996) not only observed different mortality rates for different prey by a single predator (*Oxyrrhis*), they also observed different feeding behaviors when prey were presented at different concentrations and/or simultaneously with other prey. They concluded that prey selection was dependent on the nutritional requirements of the predator as (C:N), which changed during the course of a feeding experiment leading to different nutritional demands (selection or rejection of certain prey).

Protozoa must be able to not only distinguish among various types of bacteria in their environment they also must be able to adjust the choice of prey as demands for nutrients change (Flynn *et al.* 1996). A considerable literature exists describing carbohydrate-binding lectin-type receptors as the means by which protozoa identify prey. Working in aquatic systems, Wooton *et al.* (2007) demonstrated that certain prey surface carbohydrates (in this case mannose) correspond with predator recognition receptors that directly affect the ingestion mechanism. When these receptors were blocked by washing the predator with the specific

carbohydrate, ingestion rates decreased. Wildshutte *et al.* (2004) demonstrated that the amoebas *Naegleria gruberi* and *Acanthamoeba* could differentiate between strains of *Salmonella enterica* having different O-antigens, but could not differentiate when the strains had identical O-antigens. Additionally, ingestion rates of the same strain differed between the two predators, suggesting different prey may be better suited for specific types of predators.

Bacterial surface characteristics are known to vary among species and with changes in growth condition (Firon *et al*. 1984; McEldowney and Fletcher 1986; Brown *et al*. 1990). So it seems that prey selection is likely a complex process. It appears as a constantly changing interplay between the changing internal/external chemical composition of potential prey, the changing nutrient demands of predators, and the ability of the predator to adjust its receptors to capture suitable prey. The characteristics change with extant conditions, yet must change in concert if a predator is to persist in a given habitat.

Here I use the cosmopolitan mixtrophic bacterivore *Ochromonas danica* as a model protozoan to investigate feeding behavior of a flagellate when it is supplied actively growing or quiescent bacteria. I address the following questions: Does the growth state of prey influence ingestion rate by a protozoa? Do protozoan differentially ingest different types of bacteria and does this affect protozoan fitness?

CHAPTER 2

MATERIALS AND METHODS

2.1 Experimental Overview

Bacteria in different growth states and of varying nutritional quality (as C:N:P) were fed to the bacterivorous flagellate *O. danica*. The bacteria used as prey include 2 genera within the Firmicutes, 9 genera spanning 3 classes within the Proteobacteria, and 3 organisms of unknown affiliation isolated from a freshwater habitat. The rate at which each type of bacteria was ingested and the subsequent growth rate of *O. danica* while preying on a given type of bacterium were used to assess the nutritional quality of the various types of bacteria. For prey strains used refer to Table 3.1.

2.2 Experimental Methods

2.2.1 Maintenance of Predator and Prey and Isolation of Lake Bacteria

2.2.1.1 Maintenance of Predator and Prey

Ochromonas danica (UTEX 1298) was maintained on semi-solid *Ochromonas* medium (OM; Starr 1978) at room temperature (RT, ~23°C). Cultures were transferred to fresh medium at approximately six week intervals.

Bacteria were maintained at RT on R2A (Difco) agar and transferred to fresh medium at various intervals.

2.2.1.2 Isolation of bacteria from freshwater

Water (0.1 L) was collected in August 2010 by grab sample from a depth of ~ 0.5 m from the near shore area of Lake Arlington, TX (32°42'30" N, 97°12'30"W). Aliquots of the sample were spread across R2A agar plates within one hour of collection. Plates were

incubated at RT for 5 days after which colonies were selected based on morphology and transferred to R2A agar plates. Cultures were re-streaked repeatedly to confirm purity.

2.2.2 Predator-Prey experiments

Each predator-prey experiment had three components: growth and preparation of *O. danica*, preparation of the bacteria in different growth states and assessment of their nutritional quality, and determination of ingestion rates and subsequent growth rates of *O. danica*.

2.2.2.1 Growth and preparation of *O. danica*

Ochromonas danica was grown in chemostat culture. A continuously stirred and aerated 800 mL chemostat (Applikon) was operated in the dark at 25°C with a dilution rate of 0.037 h^{-1}. Chemostats were assumed to be in steady state after a three volume turnover (Simonds *et al.* 2010). Chemostat outflow was collected aseptically in 1 or 2 L bottles (Nalgene). Cells harvested from chemostats were washed 3X with sterile Standard Mineral Base medium (White and Hageman 1998) lacking any source of C, N or P (hereafter referred to as SMB buffer), concentrated by centrifugation (Sorvall RT6000B, 2000 rpm, 10°C, 7 min), and resuspended in 10 mL of SMB buffer. Concentrated cells were enumerated by direct count using epifluorescence microscopy after staining with acridine orange (Hobbie *et al*. 1977). Triplicate samples of washed *O. danica* were filtered onto precombusted (475°C, 2 h) glass-fiber filters (Whatman GF/F) for later determination of C, N and P content. Additionally, triplicate aliquots of washed cells were preserved in glutaraldehyde (4% final concentration) and later used to determine cell size.

2.2.2.2 Preparation of the bacteria in different growth states and assessment of their nutritional quality

Growth curves for each bacterium were determined by measuring optical density (OD_{600}) of cells growing in R2A broth. An overnight culture (25°C, 100 opm) was used to inoculate triplicate 50 mL Erlenemyer flasks containing 30mL of R2A broth to an OD of ~ 0.01. Cultures were incubated as above and OD recorded at regular time intervals until the cultures showed no further change.

Growth curves were used to establish the OD corresponding to cells in mid exponential phase growth and corresponding to cells in stationary phase growth. The mid-exponential phase of growth was determined by taking the elapsed time from inoculation (the start of the growth) to the time of transition into stationary phase and dividing by two. Optical density corresponding to mid-exponential phase was then determined from a straight line fitted to the linear portion of the growth curve. Late-stationary phase was defined as four times the time it took the culture to go from mid-exponential phase to transition phase, with time counted from mid-exponential phase.

Batch cultures of each bacterium (100 mL, R2A broth) were inoculated and grown as above until mid-exponential phase or late-stationary phase cells were obtained. Triplicate samples of culture were filtered onto precombusted glass-fiber filters (Whatman GF/F) for later determination of C, N and P content. The remaining cells were washed 3X with sterile SMB buffer, concentrated by centrifugation (Sorvall RT6000B, 2000 rpm, 10°C, 15 min), and resuspended in 10 mL of SMB buffer. Washed cells were enumerated by direct count using epifluorescence microscopy after staining with acridine orange (Hobbie *et al.* 1977). Triplicate aliquots of washed cells were preserved in glutaraldehyde (4% final concentration) and later used to determine cell size.

2.2.2.3 Determination of Ingestion Rates and Subsequent Growth Rates of *O. danica*

Feeding experiments were conducted in triplicate flasks containing 20 mL of SMB buffer. *Ochromonas danica* was added to target a final concentration of $4x10^5$ cells mL^{-1} and bacteria were added to target a final concentration of $3x10^7$ cells mL^{-1}. This concentration of bacteria is sufficient to saturate the ingestion and growth kinetics of *O. danica* (Grover and Chrzanowski 2009). Controls (duplicates) consisted of cultures of *O. danica* without bacteria and of cultures of bacteria without *O. danica*. Cultures and controls were gently shaken (60 opm) in the dark at 25°C. Samples were taken one hour after inoculation and then every 45

minutes for a total elapsed time of 4.75 h. Samples were preserved in ice cold glutaraldehyde (4% final concentration) and stored at 4°C until processed by flow cytometry.

2.2.3 Analysis

Analysis consisted of three components: determination of cell concentrations for each sample by flow cytometry, determination of cell volume and chemical composition of prey and calculation of ingestion rates by, and growth rates of, *O. danica*.

2.2.3.1 Flow Cytometry

A BD LSRII flow cytometer fitted with a 488 nm argon laser was used to quantify *O. danica* and bacteria. Preserved samples were stained with 10 µL of 1000X SYBR Safe DNA gel stain (Invitrogen, Carlsbad CA) and incubated for a minimum of 30 minutes before analysis. The cytometer was consistently set to the same flow rate and the following settings: SSC 231V, Threshold 8000V; FSC 484, Threshold 8000V. All samples were processed using BD FACSFlow sheath. Data were collected for 60 s following an initial 20 s of operation during which no data were collected. Flow cytometer data was stored and analyzed by BD FACSDiva Software v6.1. Each population (protozoan and bacterial) was separated empirically by gating natural breaks in the FSC data on a histogram plotting total events by FSC. Conversions of cytometer data describing each population to cell concentrations were achieved using regressions fitting known concentrations of cells to cytometer events. A range of known concentrations of *O. danica* and each type of bacterium at each phase of growth (mid-exponential or late-stationary phase) were determined by direct microscope count. Cells were preserved, stained, as processed for flow cytometry as above.

2.2.3.2 Cell volume and chemical composition

Bacterial volumes were determined by direct epifluorescent microscopy (Olympus BH2) at a magnification of 1250x using SYBR green as the fluorochrome. Cell volume (Vb) of bacteria was determined from length and width of at least 60 cells according to the formula for a cylinder capped by two hemispheres:

$$Vb = 1.33\pi(W/2)^3 + \pi(W/2)^3(L-W)$$

where (L) is length and (W) is width. Length and width of individual cells were determined from digital images (Olympus DP70 camera) and imaging software (Simple PCI, Compix).

The element content *O. danica* and bacteria was determined from cells collected on precombusted (475°C, 2 hours) glass-fiber filters (Whatman GF/F). The C and N content was determined using a CHN analyzer (Perkin-Elmer series 2200 CHN analyzer). The P content was determined by persulfate digestion (Menzel and Corwin 1965; Strickland and Parsons 1972). Carbon, N and P concentrations were normalized to cell abundance (Qx $cell^{-1}$) and to cell volume (Qx μm^{-3}).

2.2.3.3 Calculating ingestion rate and growth rate

The growth rate of *O. danica* was calculated as the slope of a line determined by regressing the natural logarithm of abundance against time. The average growth rate of *O. danica* in two control cultures (lacking bacteria) was subtracted from the growth rate in experimental cultures (with bacteria) to obtain the growth rate supported by ingestion of bacteria.

Bacterial mortality was calculated as the slope of a line determined by regressing the natural logarithm of abundance against time. The average growth rate of bacteria in two control cultures (lacking *O. danica*) was subtracted from the mortality rate in experimental cultures (with *O. danica*) to obtain the mortality rate due to *O. danica* predation.

The rate at which bacteria were ingested by *O. danica* was determined by dividing the average bacterial concentration from T1 to T4 by the average *O. danica* concentration from T1 to T4 and then dividing by the elapsed time. Average cell concentrations were calculated by the following formula:

$$N_{T1}\ (\text{cell mL}^{-1}) \left(\frac{\{[e\text{^}(GR_{Trial} - GR_{Control})(T4)] - [e\text{^}(GR_{Trial} - GR_{Control})(T1)]\}}{[e\text{^}(GR_{Trial} - GR_{Control})(T4-T1)]} \right)$$

Where GR_{Trial} is the growth/mortality rate (h^{-1}) of each experimental trial, $GR_{Control}$ is the average growth/mortality rate (h^{-1}) of the duplicate control cultures corresponding to each experiment and TX is the time at sampling point X.

2.2.3.4 Calculating element imbalance

Both predator and prey are characterized by element ratios (C:P for example) and an imbalance, or the difference between the element ratio of the predator and that of its prey ($C:P_{predator} – C:P_{prey}$) would indicate disproportionate recycling of one nutrient (e.g., $C:P_{predator} > C:P_{prey}$ implies P excretion).

To determine the element imbalance between *O. danica* and the bacterium on which it was preying, the element ratio of a given bacterium (as C:N, C:P or N:P) in either exponential or late-stationary phase growth, was subtracted from the element ratio of *O. danica* (as C:N, C:P or N:P). A positive value predicts N or P regeneration and a negative value predicts C or N regeneration. Mean element ratios of *O. danica* were determined at the start of each feeding experiment from a total of 12 replicates, triplicate samples for each of the four feeding experiment sets preformed

2.2.4 Statistical analysis

Graphical and statistical analyses were performed using Sigma Plot (v11). T-tests were performed for comparison of ingestion rates by *O. danica* for each prey species harvested at exponential and stationary phases of growth. Correlation analyses were performed to identify prey traits (Size, element content, element ratios and element ratio imbalance between predator and prey) that may influence ingestion rates and growth rate of *O. danica*.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Experimental background

Physical and chemical (C:N:P) characteristics of bacteria vary with environmental conditions (such as temperature, growth rate, and nutrient availability) (Elser *et al*. 2000a; Chrzanowski and Grover 2008). Similarly, protozoa undergo changes in nutrient content and size as functions of temperature and growth rate (Simonds *et al*. 2010). Since prey selection by nanoflagellates is likely due to more than one physical or chemical characteristic of bacteria serving as prey, and perhaps, due to changing nutrient demands of the predator, every attempt was made to minimize variability among prey types due to environmental conditions. All bacteria for feeding trials were grown in the same medium and under the same environmental conditions and the predator was grown in chemostat culture at constant temperature and dilution rate. Similarly, for each feeding trial, predator and prey were combined in similar proportions: *Ochromonas danica* was targeted at final concentration of 4.0×10^5 cells mL^{-1} and bacteria were targeted to a final concentration of 3.0×10^7 cells mL^{-1}. Actual predator and prey concentrations deviated slightly from targeted values: The concentrations of bacteria at the start of each feeding trail ranged from 9.2×10^6 to 5.82×10^7 cells mL^{-1} with a mean of 2.92×10^7 cells mL^{-1} while the concentration of *O. danica* ranged from 3.35×10^5 to 4.31×10^5 cells mL^{-1} with a mean of 3.95×10^5 cells mL^{-1}.

3.2 Prey Characteristics

The bacteria used in these studies represent at least 2 phyla and 4 classes and their sizes, growth rates, and nutrient quotas varied substantially (Table 3.1). There was a considerable difference in mean cell size between cells harvested from mid-exponential phase growth and those harvested from late-stationary phase growth. Generally, cells harvested from the mid-exponential phase of growth were larger than cells isolated from the late-stationary phase of growth. Considering all data, there was a 20 fold difference between the smallest (0.0708 $\mu m^{-3} \pm 0.0360$) and largest (1.3546 $\mu m^{-3} \pm 0.7058$) mean prey sizes offered to *O. danica*.

Element quotas also varied among cell types and between growth states (Table 3.1). The large disparities in quotas between cells growing in exponential phase and cells growing in stationary-phase were, in part, due to differences in cell size. Carbon quotas ranged from 0.325±0.111 fmol μm^{-3} (Lake Isolate #5-Stationary) to 234.74±45.917 fmol μm^{-3} (Lake Isolate #10-Exponential), Q_N ranged from 0.063 ± 0.016 fmol μm^{-3} (Lake Isolate #5-Stationary) to 47.228±29.779 fmol μm^{-3} (*Herbaspirillum seropedicae*-Exponential) and Q_P ranged from 0.005 ± 0.001 fmol μm^{-3} (Lake Isolate #5-Stationary) to 3.182 ± 0.506 fmol μm^{-3} (Lake Isolate #10-Exponential).

Element quotas were used to determine the element stoichiometry of each cell type (as C:N, C:P, and N:P). Element ratios are thought to be a better indicator of nutritional quality of prey than cell element quotas alones (Elser *et al.* 2000b; John and Davidson 2001; Shannon *et a*l. 2007 Chrzanowski and Grover 2008). The C:N ratio was the least variable of the stoichiometric indices, varying twofold. The C:N ratio ranged from 4.29 *(Aquaspirillum* sp.-Stationary) to 9.45 (*E. coli*-Exponential). There was greater variability in the C:P and N:P ratios. This variability is expected and likely reflects the RNA content in cells during different phases of growth. The C:P ratios ranged from 29.22 (*Pseudomonas fluorescens*-Stationary) to 120.87

Table 3.1 Characteristics of bacteria used for feeding studies

Organism	ATCC #	Class	Growth state	Starting cell density (cells mL^{-1})	Starting cell volume (μm^{-3} ± SD) (n)	Growth rate (hr^{-1})
Rhodospirillum sp	*n/a*	α-proteobacteria	Exponential	$2.65x10^7$	1.0238 ± 0.4235 (193)	0.816
			Stationary	$2.75x10^7$	0.1035 ± 0.0700 (342)	
Sphingomonas paucimobilis	*n/a*	α-proteobacteria	Exponential	$5.82x10^7$	0.1784 ± 0.1123 (212)	0.364
			Stationary	$2.95x10^7$	0.0708 ± 0.0360 (515)	
Aquaspirillum sp	*49643*	β-proteobacteria	Exponential	$3.63x10^7$	0.1429 ± 0.0736 (353)	0.141
			Stationary	$3.08x10^7$	0.0796 ± 0.0401 (420)	
Herbaspirillum seropedicae	*39852*	β-proteobacteria	Exponential	$4.48x10^7$	0.4068 ± 0.4923 (182)	0.449
			Stationary	$2.77x10^7$	0.1119 ± 0.0573 (436)	
Ralstonia pickettii	*n/a*	β-proteobacteria	Exponential	$2.76x10^7$	0.2567 ± 0.1631 (154)	0.414
			Stationary	$2.84x10^7$	0.1355 ± 0.0687 (302)	
*Escherichia coli (*K-12)	*n/a*	γ-proteobacteria	Exponential	$2.84x10^7$	0.9487 ± 0.4810 (171)	0.405
			Stationary	$2.38x10^7$	0.7749 ± 0.3985 (67)	
Pasteurella sp.	*n/a*	γ-proteobacteria	Exponential	$1.16x10^7$	0.1237 ± 0.0607 (420)	0.314
			Stationary	$9.20x10^6$	0.3086 ± 0.1877 (181)	
Pseudomonas aeruginosa (PA01)	*n/a*	γ-proteobacteria	Exponential	$1.73x10^7$	0.2443 ± 0.1335 (178)	0.581
			Stationary	$2.56x10^7$	0.0942 ± 0.0477 (378)	
Pseudomonas fluorescens	*17386*	γ-proteobacteria	Exponential	$1.44x10^7$	0.6932 ± 0.3329 (207)	0.577
			Stationary	$2.96x10^7$	0.1582 ± 0.0727 (509)	
Salmonella typhimurium	*14028*	γ-proteobacteria	Exponential	$5.4x10^7$	0.9617 ± 0.3888 (123)	0.558
			Stationary	$1.81x10^7$	0.2200 ± 0.1015 (261)	
Listeria monocytogenes	*7646*	Bacilli	Exponential	$1.89x10^7$	0.2138 ± 0.1680 (276)	0.427
			Stationary	$3.76x10^7$	0.1337 ± 0.0620 (324)	
Staphylococcus aureus	*6538*	Bacilli	Exponential	$4.05x10^7$	0.3269 ± 0.1873 (119)	0.556
			Stationary	$3.49x10^7$	0.2544 ± 0.1315 (83)	
Lake Isolate # 4	n/a	n/a	Exponential	$1.65x10^7$	1.3546 ± 0.7058 (98)	0.604
			Stationary	$3.17x10^7$	0.2772 ± 0.1833 (167)	
Lake Isolate # 5	n/a	n/a	Exponential	$3.6x10^7$	0.4787 ± 0.1761 (141)	0.500
			Stationary	$3.62x10^7$	0.1026 ± 0.0463 (443)	
Lake Isolate # 10	n/a	n/a	Exponential	$1.41x10^7$	0.7779 ± 0.3286 (143)	0.644
			Stationary	$3.95x10^7$	0.3608 ± 0.1665 (157)	

Table 3.1 – *Continued*

Organism	Growth state	Q_C (fmol C cell^{-1} ± SD)	Q_N (fmol N cell^{-1} ± SD)	Q_P (fmol P cell^{-1} ± SD)	C:N	C:P	N:P
Rhodospirillum sp	Exponential	215.150 ± 75.900	30.600 ± 9.082	2.158 ± 0.401	7.03	99.71	14.18
	Stationary	1.068 ± 0.550	0.213 ± 0.109	0.021 ± 0.010	5.01	51.51	10.29
Sphingomonas paucimobilis	Exponential	110.542 ± 20.511	18.144 ± 8.153	1.556 ± 0.443	6.09	71.03	11.66
	Stationary	4.579 ± 1.045	0.896 ± 0.202	0.080 ± 0.027	5.11	56.91	11.13
Aquaspirillum sp	Exponential	35.602 ± 2.281	6.898 ± 0.202	0.430 ± 0.057	5.16	82.72	16.03
	Stationary	2.413 ± 1.003	0.562 ± 0.274	0.044 ± 0.030	4.29	54.43	12.68
Herbaspirillum seropedicae	Exponential	217.610 ± 147.440	47.228 ± 29.779	1.988 ± 0.645	4.61	109.47	23.76
	Stationary	2.716 ± 0.563	0.624 ± 0.139	0.035 ± 0.011	4.35	77.14	17.73
Ralstonia pickettii	Exponential	128.053 ± 45.983	28.199 ± 9.404	1.258 ± 0.411	4.54	101.81	22.42
	Stationary	1.585 ± 0.058	0.361 ± 0.011	0.025 ± 0.004	4.39	63.12	14.38
Escherichia coli	Exponential	164.967 ± 20.139	17.460 ± 2.314	1.800 ± 0.257	9.45	91.65	9.70
	Stationary	12.729 ± 9.133	2.426 ± 1.746	0.190 ± 0.110	5.25	66.96	12.76
Pasteurella sp.	Exponential	22.502 ± 1.666	4.302 ± 0.272	0.378 ± 0.002	5.23	59.59	11.39
	Stationary	6.925 ± 1.735	1.305 ± 0.336	0.153 ± 0.040	5.31	45.23	8.52
Pseudomonas aeruginosa	Exponential	90.971 ± 18.182	16.691 ± 3.767	1.804 ± 0.157	5.45	50.44	9.25
	Stationary	2.370 ± 0.729	0.480 ± 0.145	0.054 ± 0.017	4.93	44.05	8.93
Pseudomonas fluorescens	Exponential	77.342 ± 41.695	12.978 ± 6.625	1.805 ± 1.451	5.96	42.84	7.19
	Stationary	4.290 ± 1.353	0.823 ± 0.280	0.147 ± 0.061	5.21	29.22	5.61
Salmonella typhimurium	Exponential	143.422 ± 93.210	15.807 ± 9.846	1.974 ± 1.567	9.07	72.64	8.01
	Stationary	11.913 ± 4.2390	2.489 ± 0.895	0.208 ± 0.069	4.79	57.26	11.97
Listeria monocytogenes	Exponential	88.409 ± 9.301	14.962 ± 3.027	0.731 ± 0.078	5.91	120.87	20.46
	Stationary	na	na	na	na	na	na
Staphylococcus aureus	Exponential	56.066 ± 12.010	7.142 ± 1.139	1.227 ± 0.185	7.85	45.68	5.82
	Stationary	16.269 ± 0.809	3.753 ± 0.706	0.346 ± 0.088	4.33	46.99	10.84
Lake Isolate # 4	Exponential	121.073 ± 17.090	17.216 ± 0.443	2.391 ± 0.689	7.03	50.63	7.20
	Stationary	9.203 ± 4.055	1.819 ± 0.804	0.243 ± 0.100	5.06	37.81	7.47
Lake Isolate # 5	Exponential	119.026 ± 43.807	24.813 ± 8.845	1.600 ± 0.456	4.80	74.40	15.51
	Stationary	0.325 ± 0.111	0.063 ± 0.016	0.005 ± 0.001	5.15	59.84	11.62
Lake Isolate # 10	Exponential	234.74 ± 45.917	46.101 ± 15.254	3.182 ± 0.506	5.09	73.78	14.49
	Stationary	41.530 ± 8.683	9.500 ± 1.823	0.823 ± 0.162	4.37	50.44	11.54

(*Listeria monocytogenes*-Exponential) while N:P ratios ranged from 5.61 (*P. fluorescens*-Stationary) to 23.76 (*H. seropedicae*-Exponential). Interestingly, the lowest C:P and C:N ratios, were found in cells harvested from late-stationary phase growth. Low numeric value of these ratios indicates that P and N content of these cells was high relative to C content. Thus, cells with low ratios would be expected to be high-quality food for a predator potentially limited by these elements.

3.3 Feeding Experiments

Feeding experiments were performed in groups; four different feeding trials, each with a single type of bacterium, running simultaneously. Consequently, *O. danica* was harvested from the chemostat once for each group resulting in a total of four sets of duplicate *O. danica* controls (no prey), one set of duplicates for each of the four groups. We observed a consistent pattern in the growth kinetics of *O. danica* in the absence of bacterial prey. *Ochromonas danica* increased in abundance for approximately three hours then ceased to grow. Growth in the absence of prey was likely due to nutrients stored within cells. The shift in growth kinetics complicates analysis of predation rates on bacteria since there were few data points collected following the shift in *O. danica* growth. Consequently, the data from all controls were pooled and only data collected during the first 3.25 h of feeding and controls were used to estimate ingestion rates and *O. danica* growth rates.

Examples of each feeding experiment are shown in figures 3.1-3.15. The upper panels of each figure depict changes in *O. danica* abundance for each replicate when *O. danica* was preying on bacteria harvested from mid-exponential phase growth or from late-stationary phase growth. The lower panels depict the corresponding changes in prey abundance. *Ochromonas danica* preying on bacteria harvested from mid-exponential phase growth grew at rates in excess of controls in 12 of the15 feeding trails. While preying on bacteria harvested from late-stationary phase growth, *O. danica* grew at rates in excess of controls in 10 of the 15 feeding

trails. Generally, *O. danica* grew more rapidly when preying on cells harvested from exponential growth than when preying on cells harvested from stationary phase growth.

Comparison of the bacterial mortality data (Figures 3.1-3.15, panels B and D) reveals interesting feeding patterns by *O. danica*, not only when preying on a single prey species in different phases of growth, but also among the various species of prey. Four distinct ingestion efficiency patterns (IEP) emerged from the thirty feeding experiments (Table 3.2). Assuming predator growth and prey mortality are exponential, then we would expect, when data are converted to the natural logs, mortality should be linear. This feeding pattern was characterized as Type I IEP. This pattern was often observed in feeding trials (see for example: Figure 3.5, Panels B and D – Prey: *Ralstonia pickettii*). Some prey were not consumed and this lack of feeding was characterized as Type IV IEP (see for example Figure 3.3, Panel D - Prey: *Aquaspirillum* sp). Some bacteria were not ingested readily when first introduced to *O. danica*, but after a lag period, *O. danica* began ingesting prey at a constant rate. This produced bacterial mortality plots with a delayed start but linear decline once ingestion commenced. This type of feeding pattern is characterized as Type II IEP and is best depicted by data shown in Figure 3.9 Panel D (Prey: *P. fluorescens*). The final pattern describing mortality of the bacteria is characterized as Type III IEP. This particularly fascinating pattern is characterized by delayed ingestion of prey followed by a steady increase in the rate of bacterial mortality. Seemingly, *O. danica* consuming Type III IEP prey demonstrate a continued improvement in ability to recognize and ingest these prey and predation efficiency increases. This feeding pattern is best depicted in Figure 3.10 Panel B when *O. danica* was preying upon *Salmonella typhimurium*, and only seen for species harvested from mid-exponential phase growth.

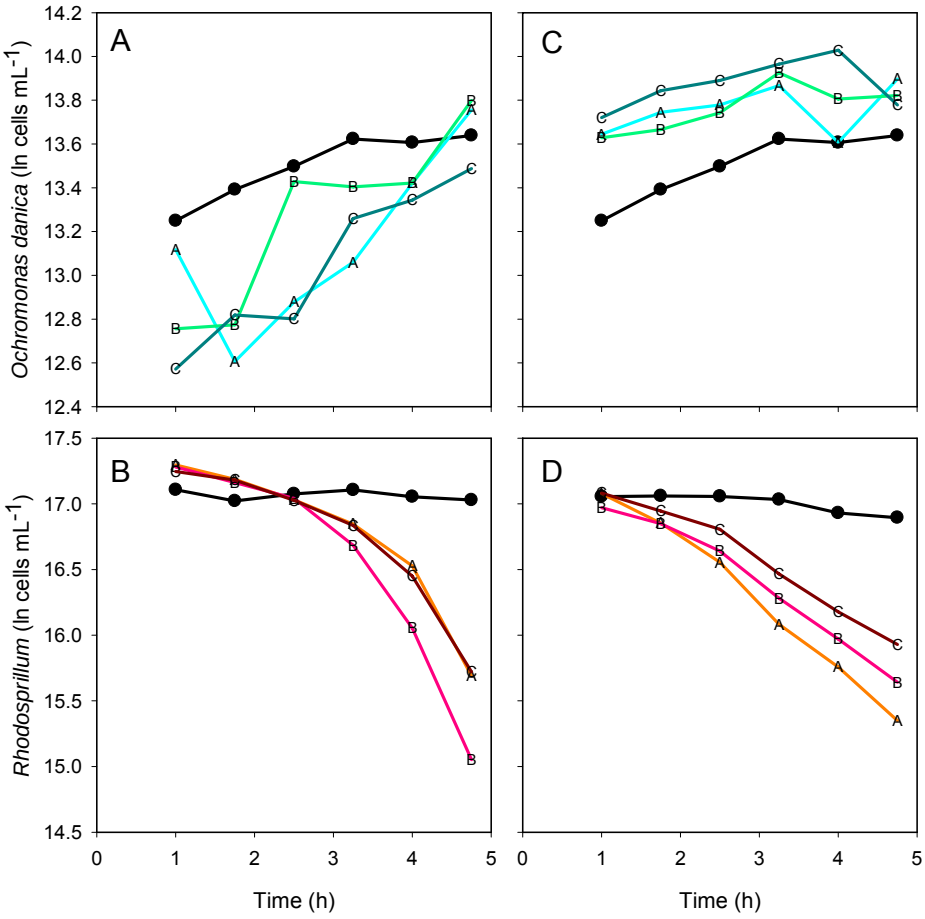

Figure 3.1 Changes in abundance of the flagellate *Ochromonas danica* (Panels A & C) and the bacterium *Rhodospirillum* sp. (Panels B & D) during a short-term feeding experiment. Panel A depicts changes in abundance of *O. danica* while preying upon the bacterium harvested from mid-exponential phase growth. Panel B depicts the corresponding mortality of the bacterium. Panel C depicts changes in *O. danica* abundance while feeding upon the bacterium harvested from late-stationary phase growth. Panel D depicts the corresponding mortality of the bacterium. In panels A & C circles represent the average of all *O. danica* controls (no prey) and in panels B&D circles represent the average of duplicate controls (no predator). Letters represent and match changes occurring in individual replicates.

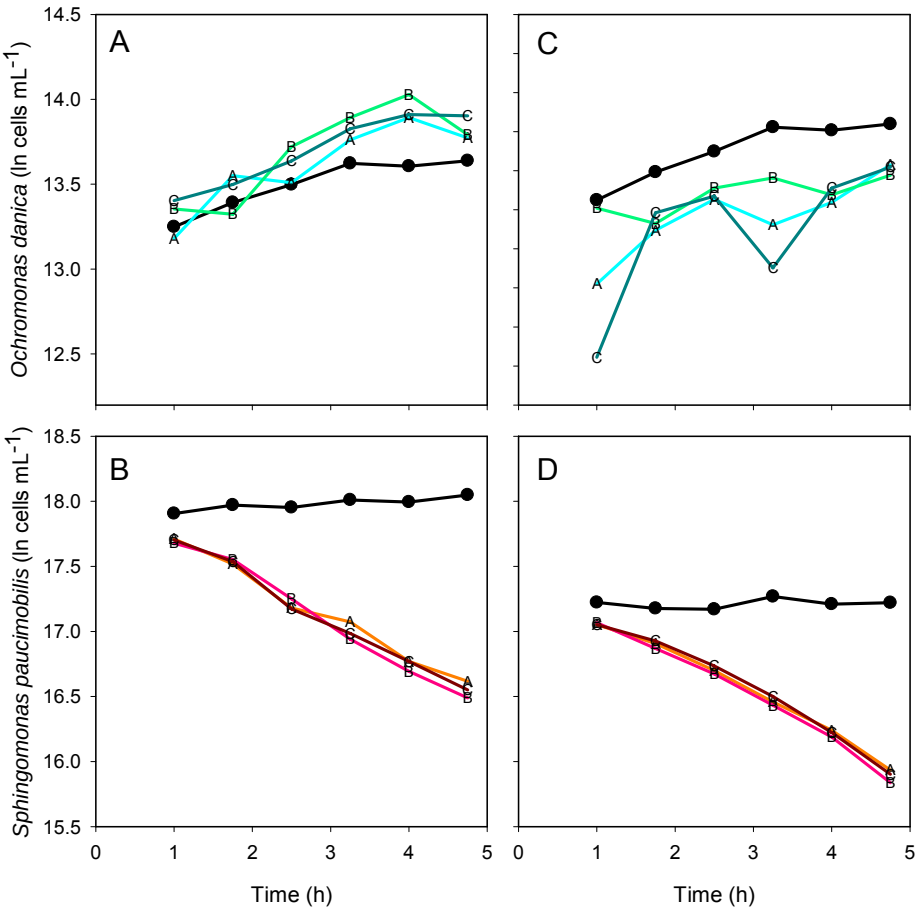

Figure 3.2 Changes in abundance of the flagellate *Ochromonas danica* (Panels A & C) and the bacterium *Sphingomonas paucimobilis* (Panels B & D) during a short-term feeding experiment. For full figure description refer to Figure 3.1.

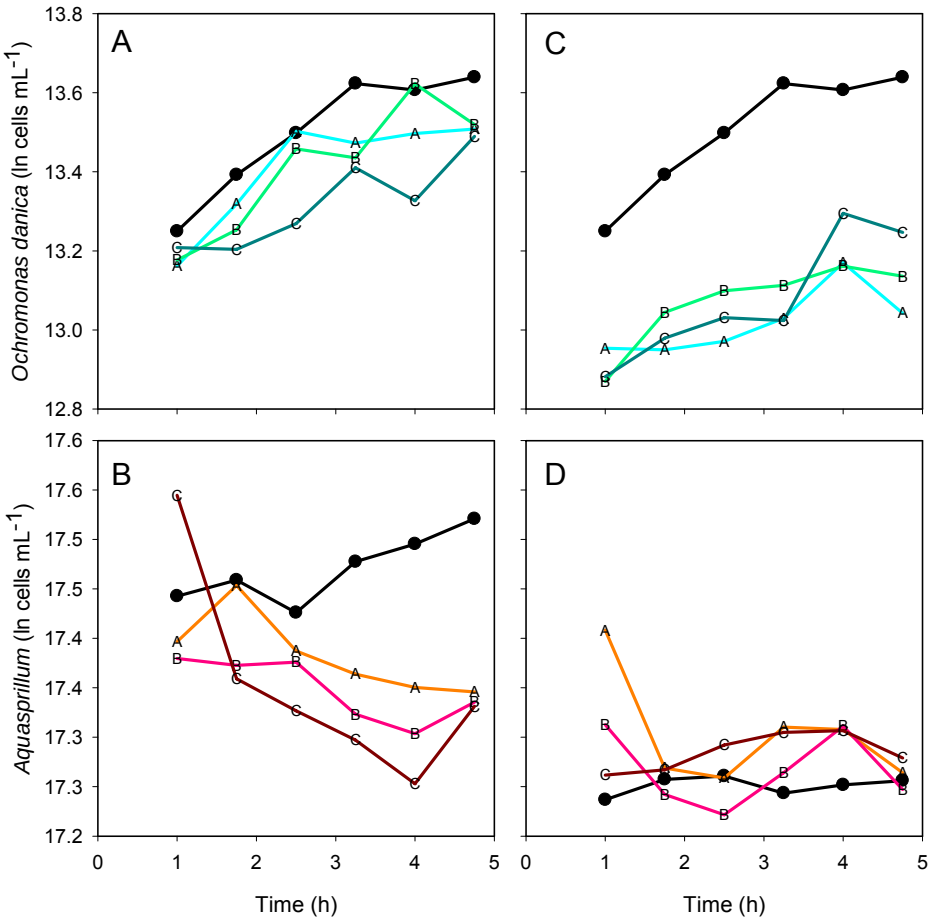

Figure 3.3 Changes in abundance of the flagellate *Ochromonas danica* (Panels A & C) and the bacterium *Aquaspirillum* sp. (Panels B & D) during a short-term feeding experiment. For full figure description refer to Figure 3.1.

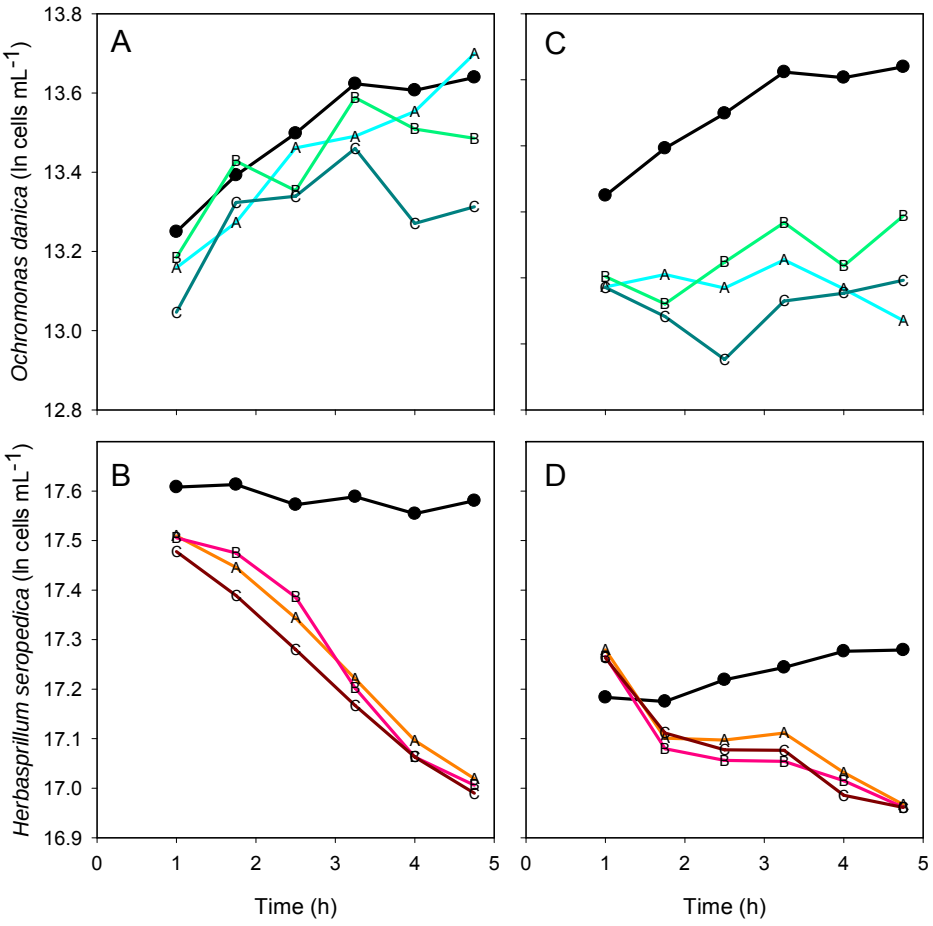

Figure 3.4 Changes in abundance of the flagellate *Ochromonas danica* (Panels A & C) and the bacterium *Herbaspirillum seropedica* (Panels B & D) during a short-term feeding experiment. For full figure description refer to Figure 3.1.

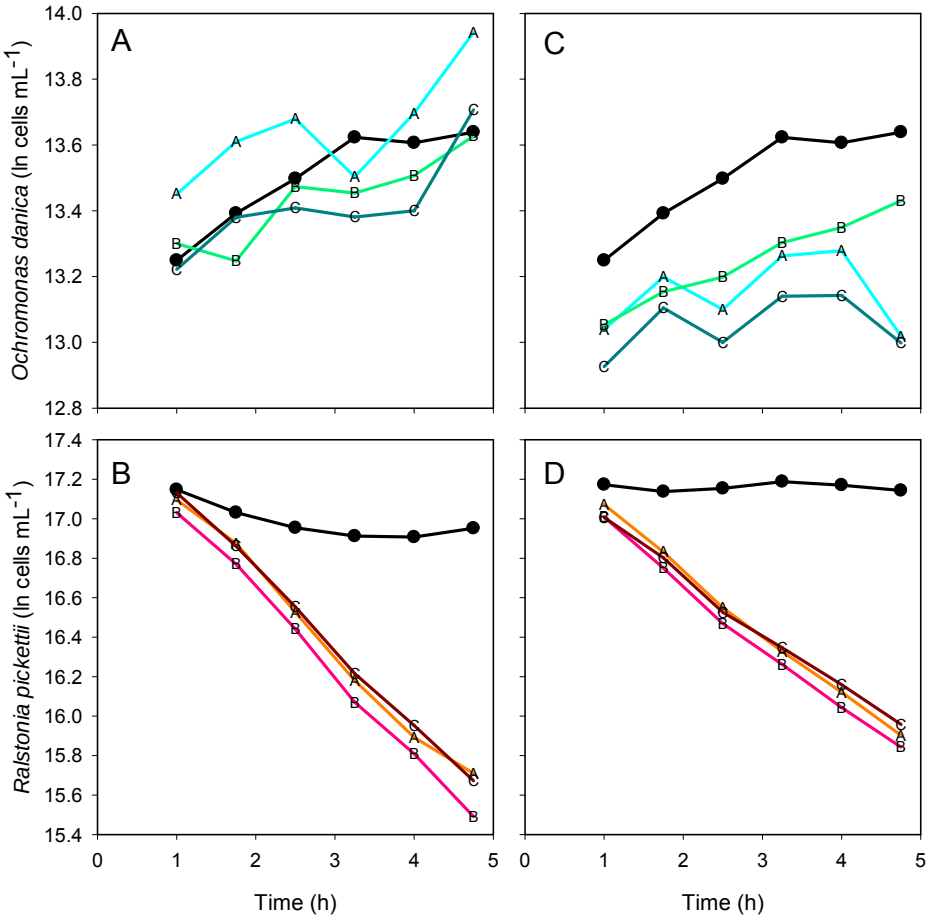

Figure 3.5 Changes in abundance of the flagellate *Ochromonas danica* (Panels A & C) and the bacterium *Ralstonia pickettii* (Panels B & D) during a short-term feeding experiment. For full figure description refer to Figure 3.1.

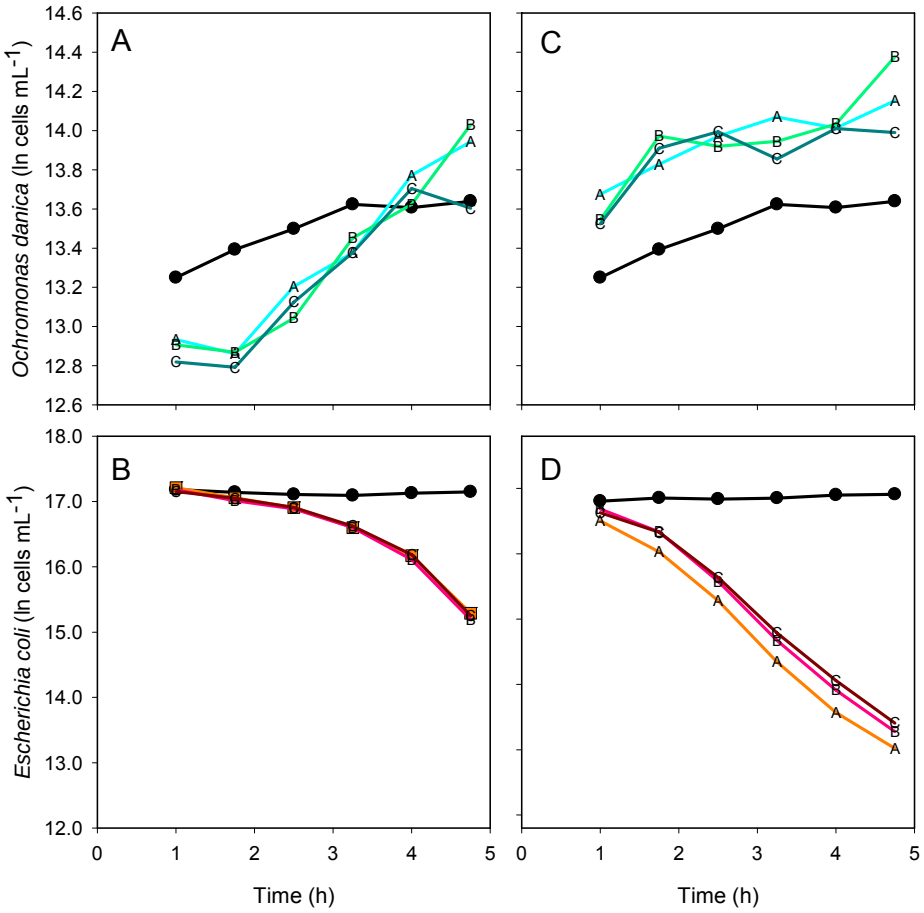

Figure 3.6 Changes in abundance of the flagellate *Ochromonas danica* (Panels A & C) and the bacterium *Escherichia coli* (Panels B & D) during a short-term feeding experiment. For full figure description refer to Figure 3.1.

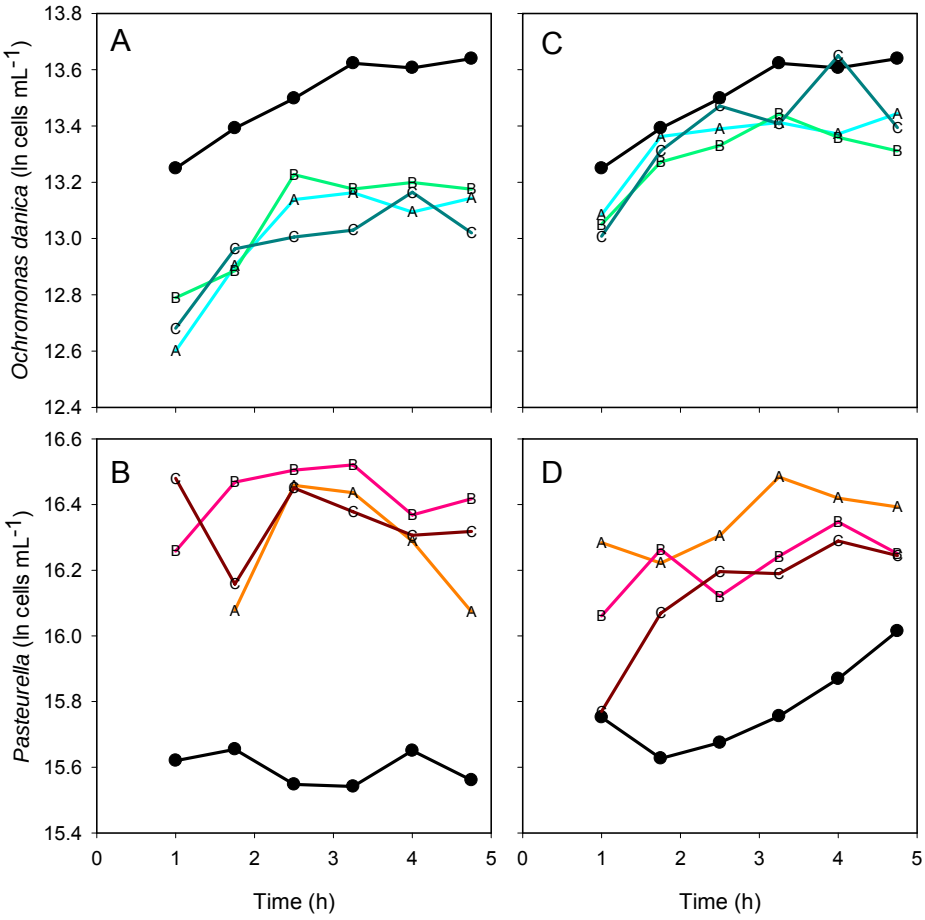

Figure 3.7 Changes in abundance of the flagellate *Ochromonas danica* (Panels A & C) and the bacterium *Pasteurella* sp. (Panels B & D) during a short-term feeding experiment. For full figure description refer to Figure 3.1.

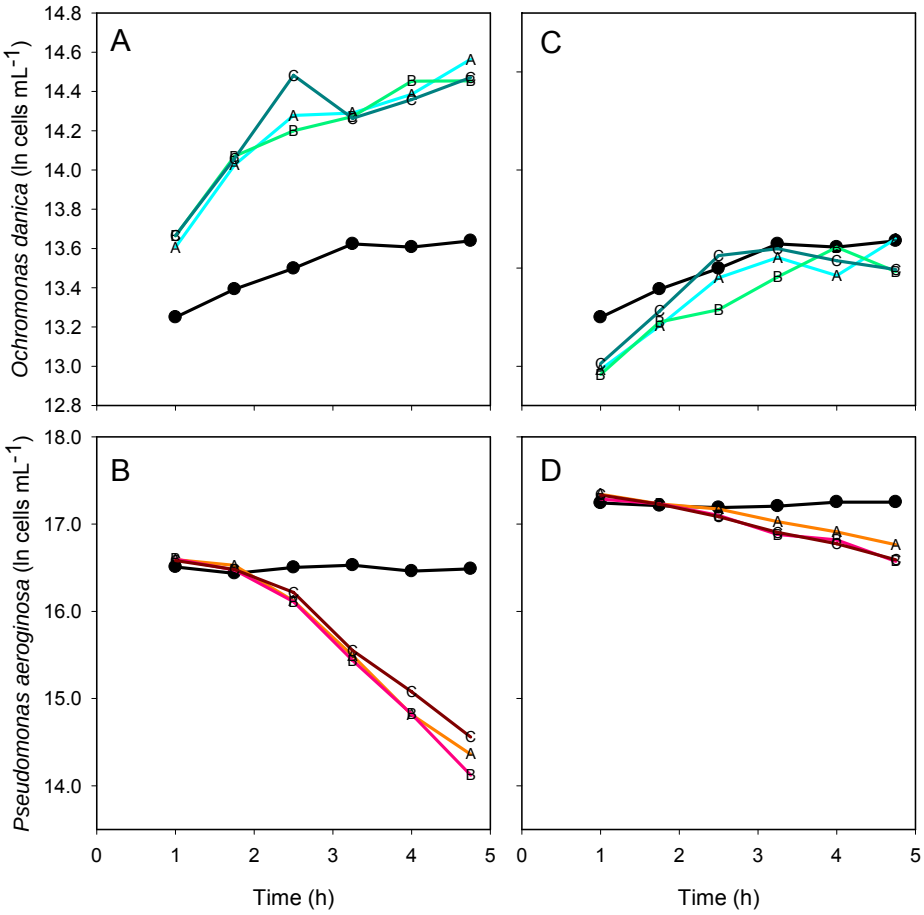

Figure 3.8 Changes in abundance of the flagellate *Ochromonas danica* (Panels A & C) and the bacterium *Pseudomonas aeruginosa* (Panels B & D) during a short-term feeding experiment. For full figure description refer to Figure 3.1.

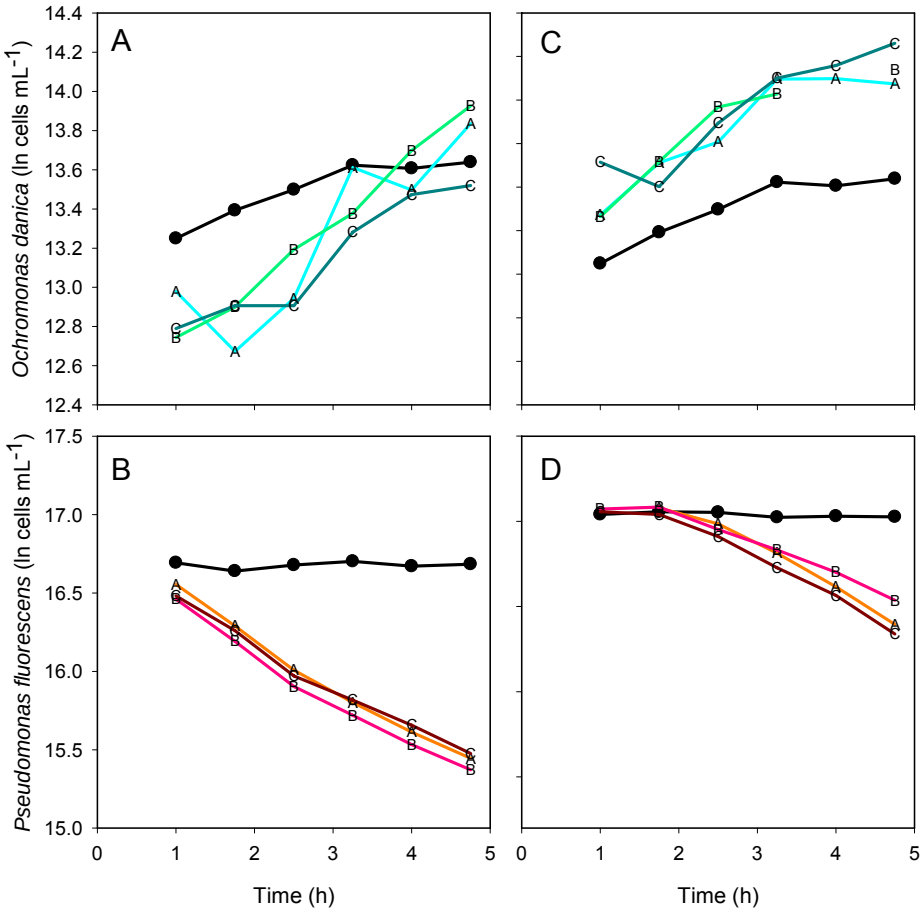

Figure 3.9 Changes in abundance of the flagellate *Ochromonas danica* (Panels A & C) and the bacterium *Pseudomonas fluorescens* (Panels B & D) during a short-term feeding experiment. For full figure description refer to Figure 3.1.

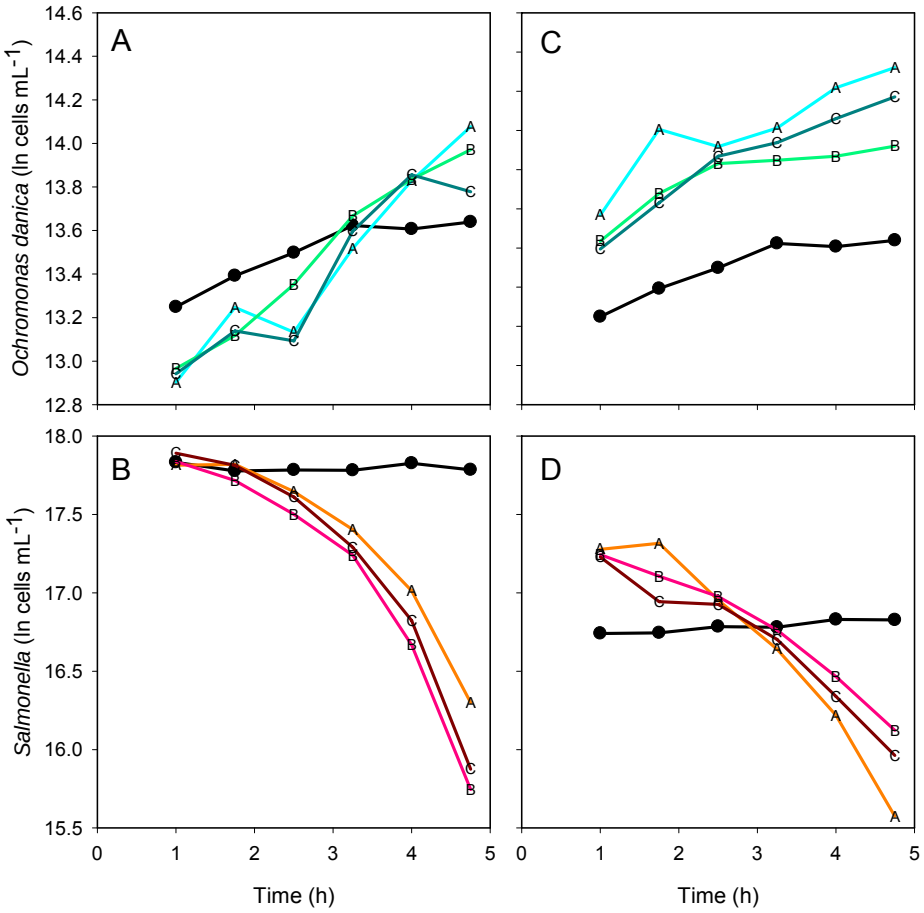

Figure 3.10 Changes in abundance of the flagellate *Ochromonas danica* (Panels A & C) and the bacterium *Salmonella typhimurium* (Panels B & D) during a short-term feeding experiment. For full figure description refer to Figure 3.1.

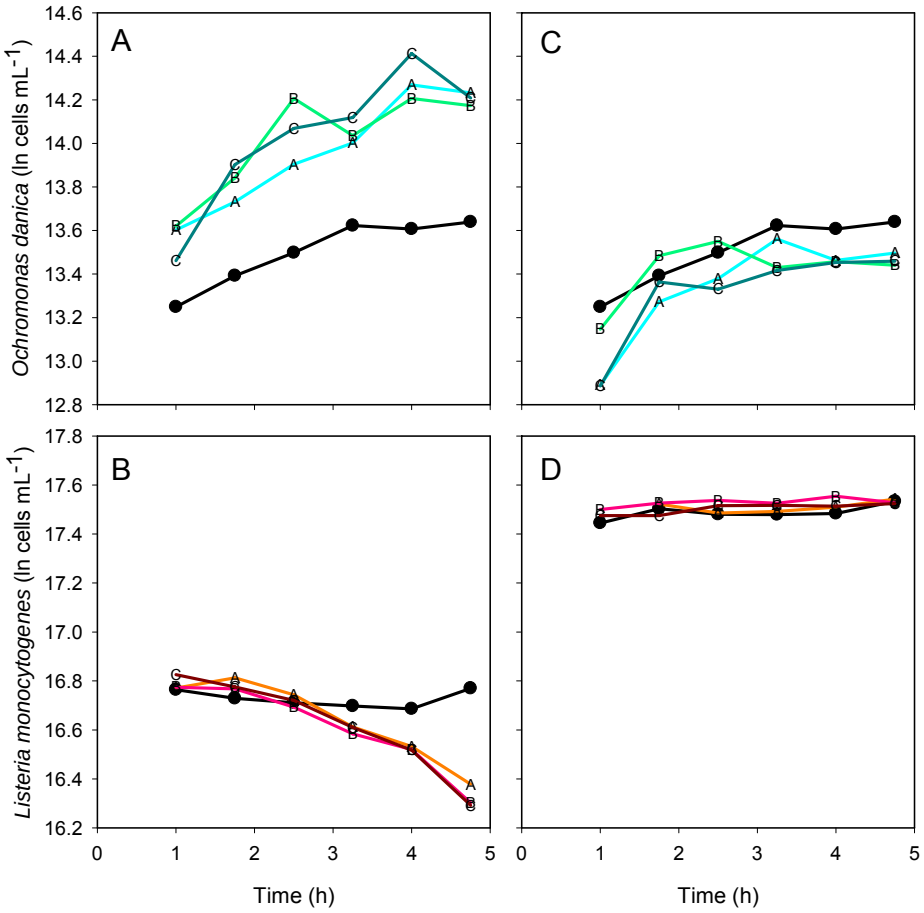

Figure 3.11 Changes in abundance of the flagellate *Ochromonas danica* (Panels A & C) and the bacterium *Listeria monocytogenes* (Panels B & D) during a short-term feeding experiment. For full figure description refer to Figure 3.1.

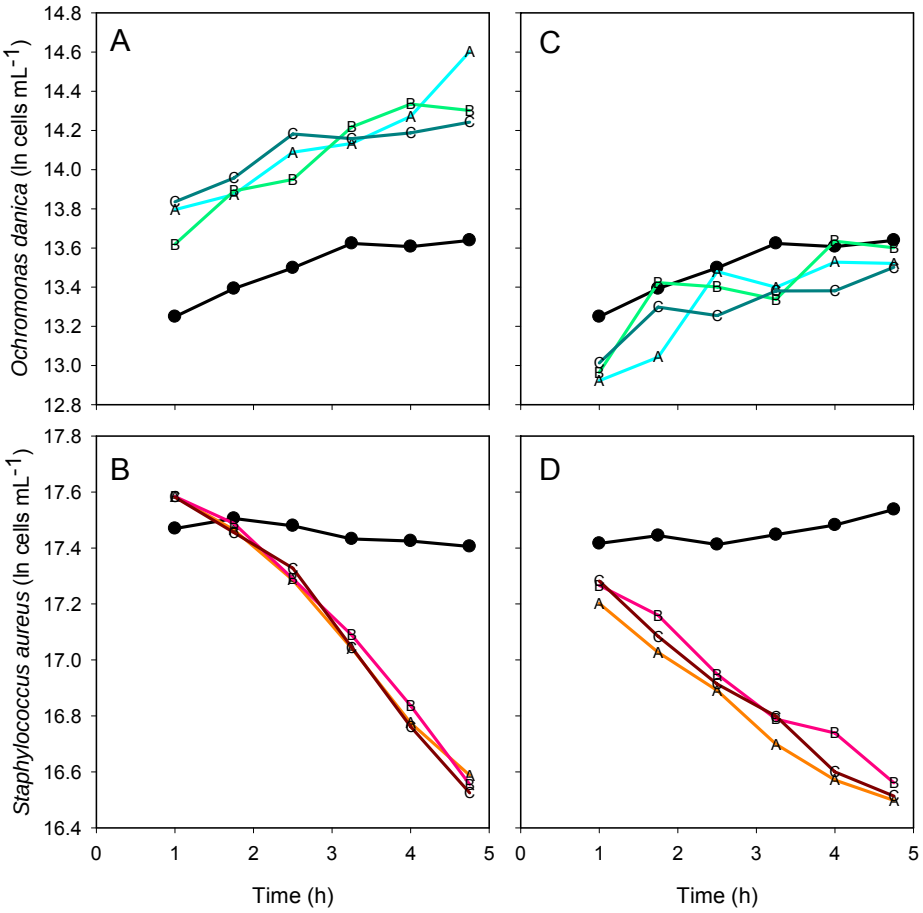

Figure 3.12 Changes in abundance of the flagellate *Ochromonas danica* (Panels A & C) and the bacterium *Staphylococcus aureus* (Panels B & D) during a short-term feeding experiment. For full figure description refer to Figure 3.1

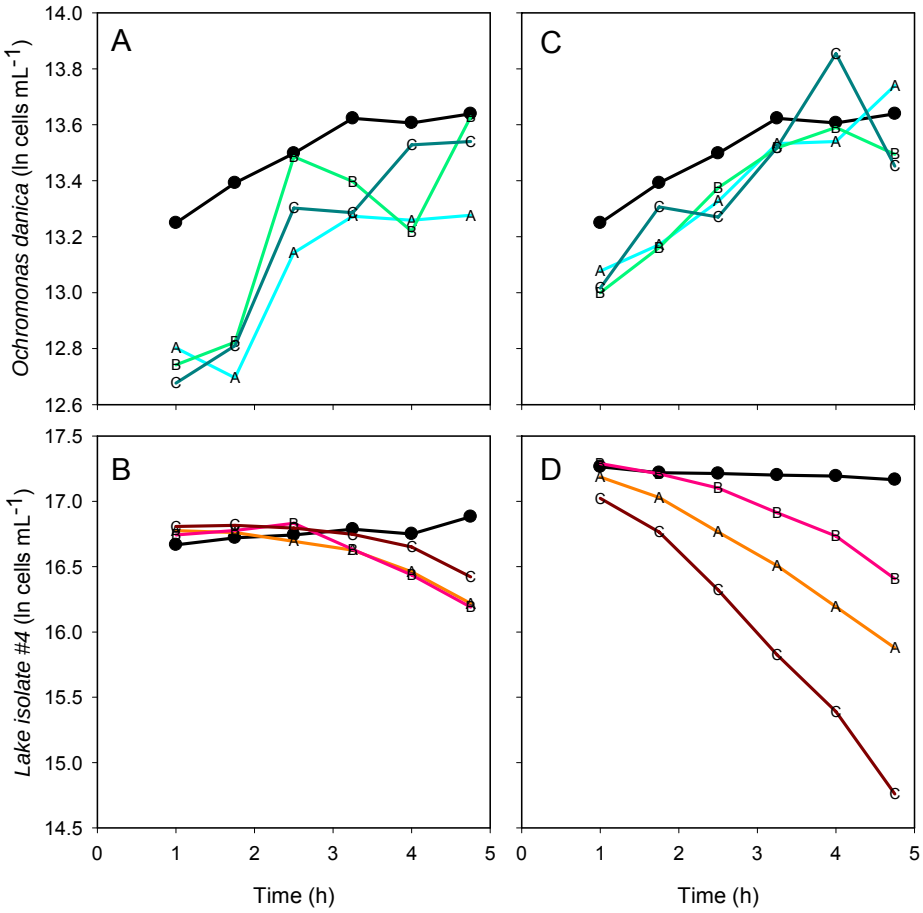

Figure 3.13 Changes in abundance of the flagellate *Ochromonas danica* (Panels A & C) and the bacterium Lake Isolate #4 (Panels B & D) during a short-term feeding experiment. For full figure description refer to Figure 3.1.

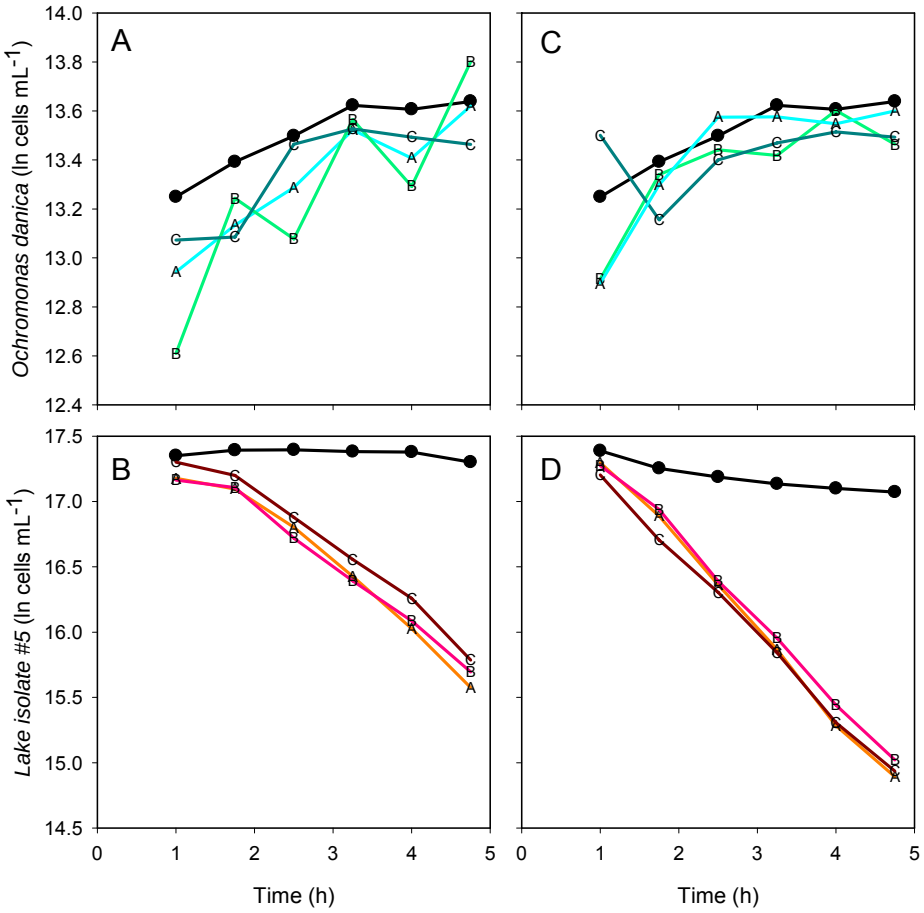

Figure 3.14 Changes in abundance of the flagellate *Ochromonas danica* (Panels A & C) and the bacterium Lake Isolate #5 (Panels B & D) during a short-term feeding experiment. For full figure description refer to Figure 3.1.

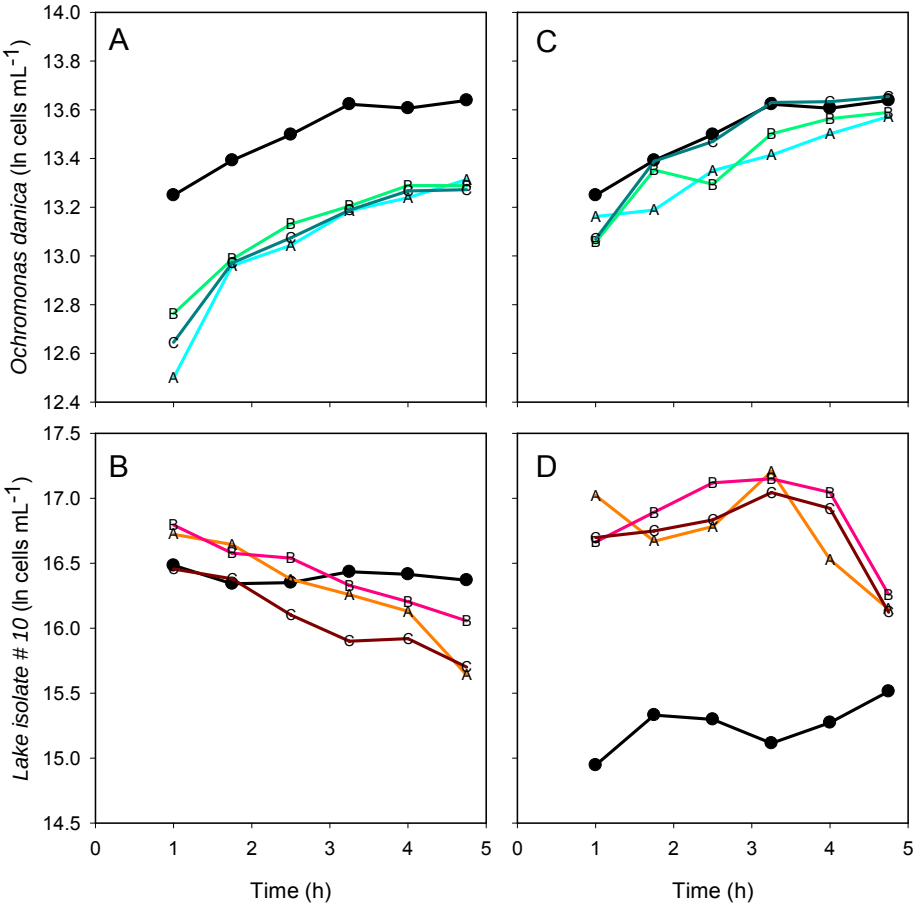

Figure 3.15 Changes in abundance of the flagellate *Ochromonas danica* (Panels A & C) and the bacterium Lake Isolate #10 (Panels B & D) during a short-term feeding experiment. For full figure description refer to Figure 3.1.

Table 3.2 Ingestion Efficiency Patterns (IEP) of *Ochromonas danica* grown on varied prey

Prey type	Growth state	Ingestion Efficiency Pattern
Rhodospirillum sp	Exponential	Type III
	Stationary	Type II
Sphingomonas paucimobilis	Exponential	Type I
	Stationary	Type I
Aquaspirillum sp	Exponential	Type I
	Stationary	Type IV
Herbaspirillum seropedicae	Exponential	Type I
	Stationary	Type I
Ralstonia pickettii	Exponential	Type I
	Stationary	Type I
Escherichia coli	Exponential	Type III
	Stationary	Type I
Pasteurella sp.	Exponential	Type IV
	Stationary	Type IV
Pseudomonas aeruginosa	Exponential	Type II
	Stationary	Type I
Pseudomonas fluorescens	Exponential	Type I
	Stationary	Type II
Salmonella typhimurium	Exponential	Type III
	Stationary	Type I
Listeria monocytogenes	Exponential	Type II
	Stationary	Type IV
Staphylococcus aureus	Exponential	Type I
	Stationary	Type I
Lake Isolate # 4	Exponential	Type II
	Stationary	Type I
Lake Isolate # 5	Exponential	Type II
	Stationary	Type I
Lake Isolate # 10	Exponential	Type I
	Stationary	Type IV

The IEPs described here may help explain data derived from previous feeding studies. Gruber *et al*. (2008) observed that the ciliate predators *E. vannus* and *C. glaucoma* preferentially selected of *E. coli* in stationary phase growth over *E. coli* in exponential phase growth. They concluded that selection for cells was likely due to the higher C:N and C:P ratios associated with the slower growing cells. *Ochromonas,* preying on *E. coli* in exponential phase growth, does not initially recognize this bacterium as prey (Type II IEP, Figure 3.6 Panel B).

Thus, in short term feeding trials, as conducted by Gruber *et al*. (2009), ingestion rates may underestimate the true feeding rate.

3.4 Factors affecting growth and ingestion

3.4.1 Prey growth state

When all experiments were considered and the ingestion rate of bacteria in exponential-phase growth was compared to the ingestion rate of cells in stationary-phase growth, there was a significant difference (t- test, $p < 0.05$) between ingestion rates for 11 of 15 paired experiments (Table 3.3, Figure 3.16, Figure 3.17). In eight experiments higher ingestion rates were found when *O. danica* preyed upon cells harvested from mid-exponential phase growth. Of these eight, three were significant because cells harvested from stationary phase were not ingested at all. With the exception of *Pasteurella*, which was not ingested in either growth phase, *O. danica* always ingested bacteria that were in exponential growth phase.

3.4.2 Prey size

Neither ingestion rate (Figure 3.18) nor growth rate (Figure 3.19) of *O. danica* was influenced by prey size. This was true when all data were considered (top panel) as a whole or when data were considered by prey growth state. *Ochromonas danica* has been previously shown to be a size-selective predator (Chrzanowski and Šimek 1990; Šimek and Chrzanowski 1992). The lack of correlation between ingestion rate or growth rate and mean prey size suggests that the size distribution of each bacterium was broad enough to allow for predation on at least a segment of the bacterial population presented as prey.

3.4.3 Growth rate and Prey element content

The rate at which *O. danica* ingested prey was not affected by growth rate of the prey, yet increasing prey growth rate was positively correlated with predator growth rate ($r=0.56$, $p=0.001$, $n=45$; Figure 3.20). It has been hypothesized, and subsequently observed, that rapidly growing bacteria are richer in P than slower growing cells (Elser *et al*. 2000a; Chrzanowski and Grover 2008) and bacteria with low C:element ratios should produce higher growth rates for a

Table 3.3 Ingestion rate and growth rate of *Ochromonas danica* grown on various prey

Prey type	Growth state	Ingestion rate (cells hr^{-1})	Growth rate (cells hr^{-1})
Rhodospirillum sp	Exponential	28.74 ± 7.03	0.144 ± 0.037
	Stationary	9.44 ± 1.05	-0.055 ± 0.018
Sphingomonas paucimobilis	Exponential	17.42 ± 1.44	0.064 ± 0.040
	Stationary	10.46 ± 3.09	0.197 ± 0.166
Aquaspirillum sp	Exponential	35.36 ± 1.38	-0.041 ± 0.007
	Stationary	Not ingested	-0.097 ± 0.036
Herbaspirillum seropedicae	Exponential	32.35 ± 0.45	-0.005 ± 0.008
	Stationary	39.08 ± 5.60	-0.138 ± 0.058
Ralstonia pickettii	Exponential	12.13 ±2.06	-0.004 ± 0.048
	Stationary	16.97 ± 2.08	-0.079 ± 0.019
Escherichia coli	Exponential	24.82 ± 0.60	0.080 ± 0.023
	Stationary	2.38 ± 0.57	-0.005 ± 0.017
Pasteurella sp.	Exponential	Not ingested	0.178 ± 0.044
	Stationary	Not ingested	0.118 ± 0.106
Pseudomonas aeruginosa	Exponential	2.80 ± 0.01	0.124 ± 0.024
	Stationary	24.54 ± 2.63	0.083 ± 0.035
Pseudomonas fluorescens	Exponential	9.14 ± 1.28	0.096 ± 0.055
	Stationary	12.31 ± 0.81	0.121 ± 0.042
Salmonella typhimurium	Exponential	39.67 ± 6.50	0.103 ± 0.041
	Stationary	8.87 ± 0.44	0.075 ± 0.032
Listeria monocytogenes	Exponential	10.30 ± 1.22	0.064 ± 0.052
	Stationary	Not ingested	0.119 ± 0.014
Staphylococcus aureus	Exponential	13.74 ± 1.81	0.061 ± 0.026
	Stationary	16.43 ± 5.22	0.104 ± 0.117
Lake Isolate # 4	Exponential	18.36 ± 1.91	0.118 ± 0.069
	Stationary	17.90 ± 7.87	0.213 ± 0.067
Lake Isolate # 5	Exponential	15.61 ± 0.99	0.074 ± 0.045
	Stationary	8.09 ± 1.08	0.012 ± 0.056
Lake Isolate # 10	Exponential	15.97 ± 3.17	0.139 ± 0.052
	Stationary	Not ingested	0.048 ± 0.021

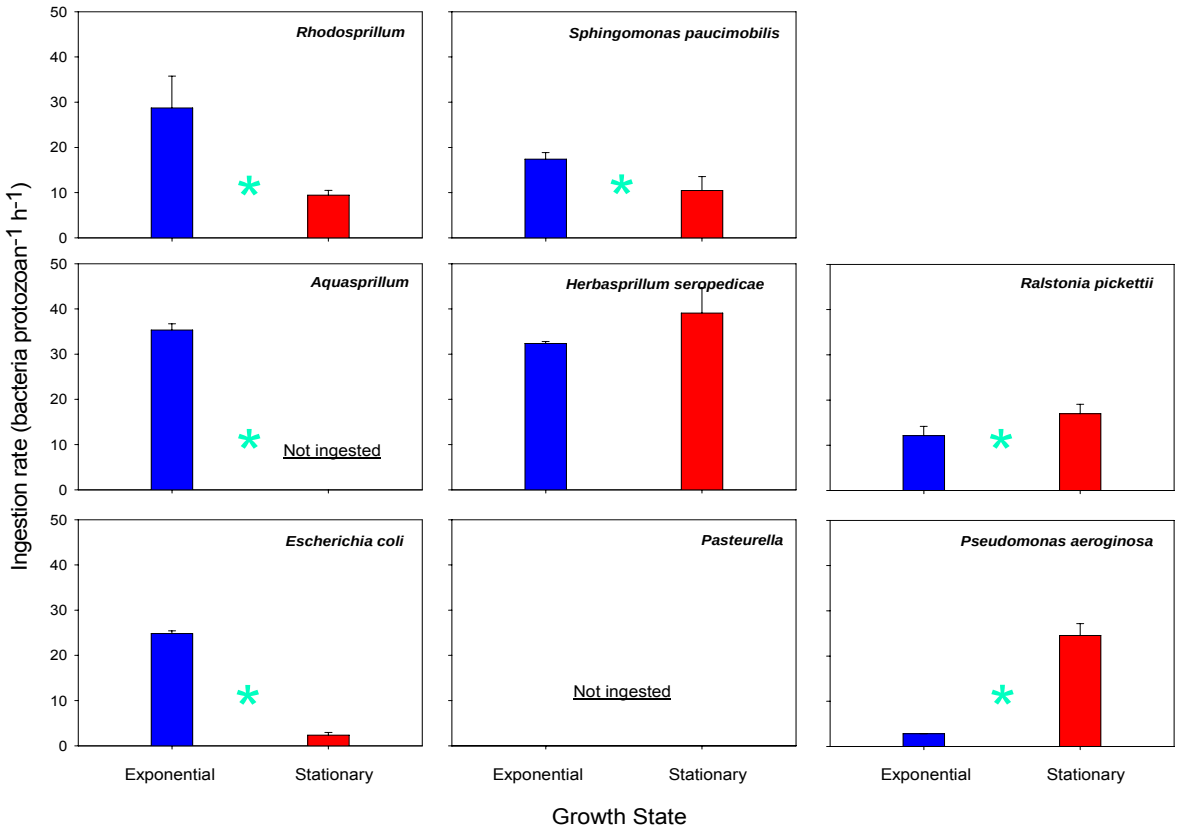

Figure 3.16 Ingestion of bacteria by the flagellate *Ochromonas danica*. Blue bars indicate ingestion of bacteria harvested from mid-exponential phase growth and red bars indicate ingestion of bacteria harvested from late-stationary phase growth. Stars indicate a significant difference ($p < 0.05$) between ingestion rates. Error bars indicated standard deviation where n=3. Not ingested indicates that the bacteria was not consumed by the predator.

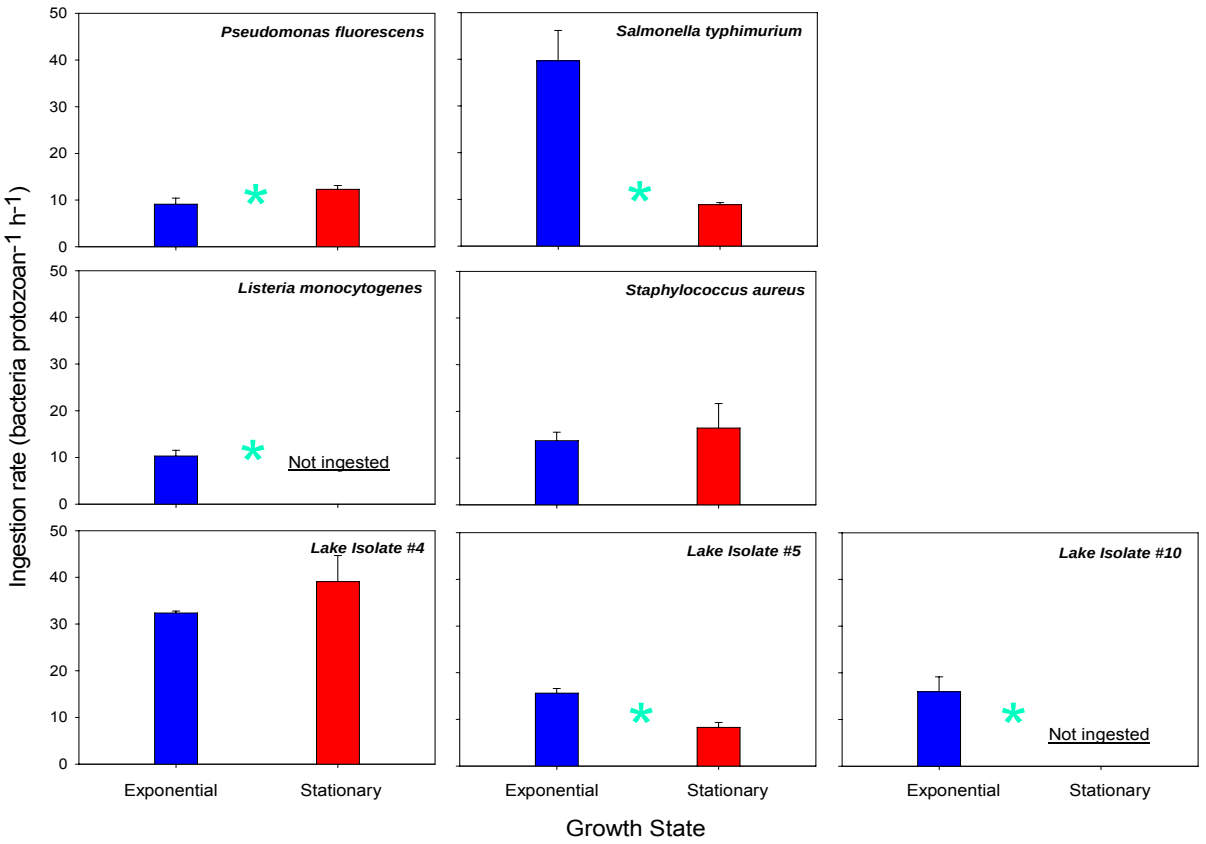

Figure 3.17 Ingestion of bacteria by the flagellate *Ochromonas danica* continued. Blue bars indicate ingestion of bacteria harvested from mid-exponential phase growth and red bars indicate ingestion of bacteria harvested from late-stationary phase growth. Stars indicate a significant difference ($p < 0.05$) between ingestion rates. Error bars indicated standard deviation where n=3. Not ingested indicates that the bacteria was not consumed by the predator.

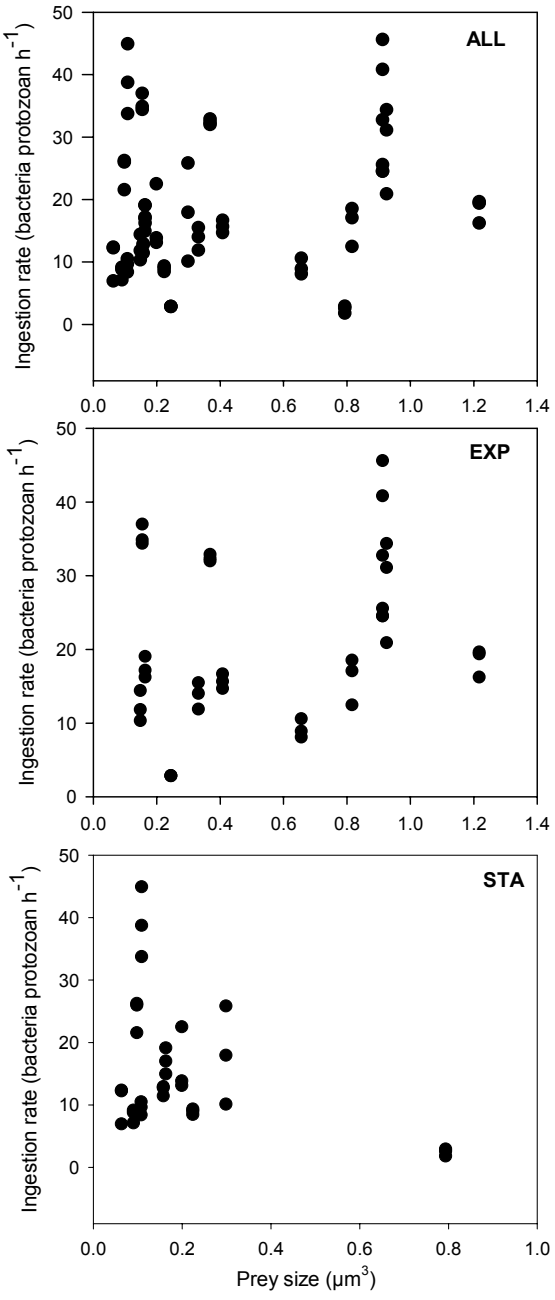

Figure 3.18 Effects of prey size on ingestion rate of *Ochromonas danica* for all prey (Top) exponential prey (Middle) and stationary prey (Bottom).

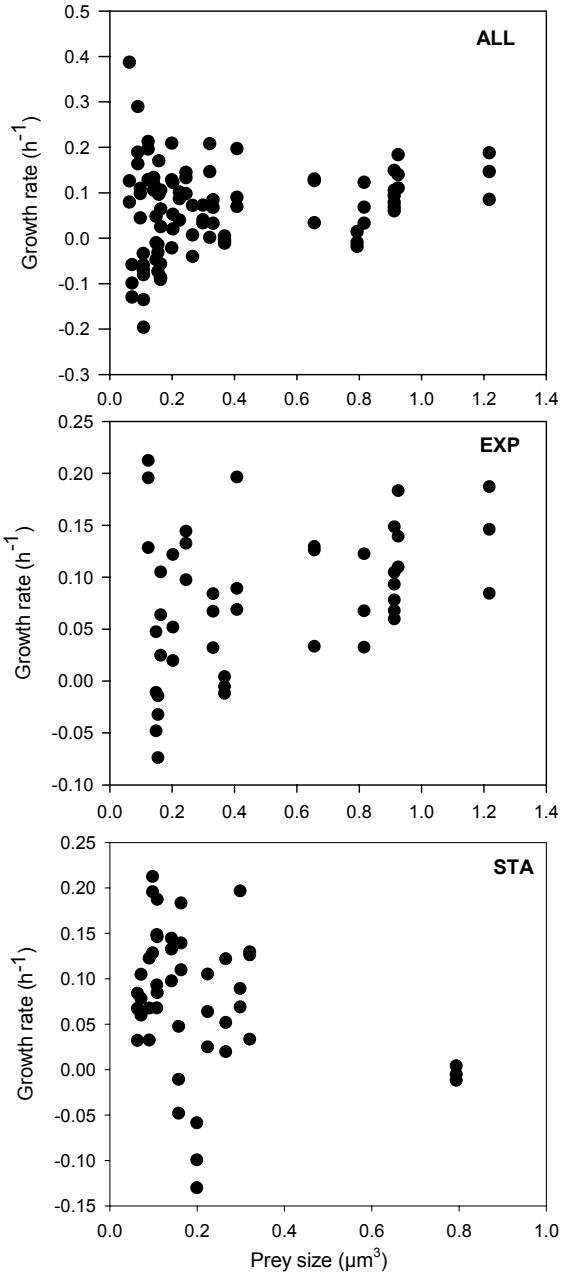

Figure 3.19 Effects of prey size on growth rate of *Ochromonas danica* for all prey (Top) exponential prey (Middle) and stationary prey (Bottom).

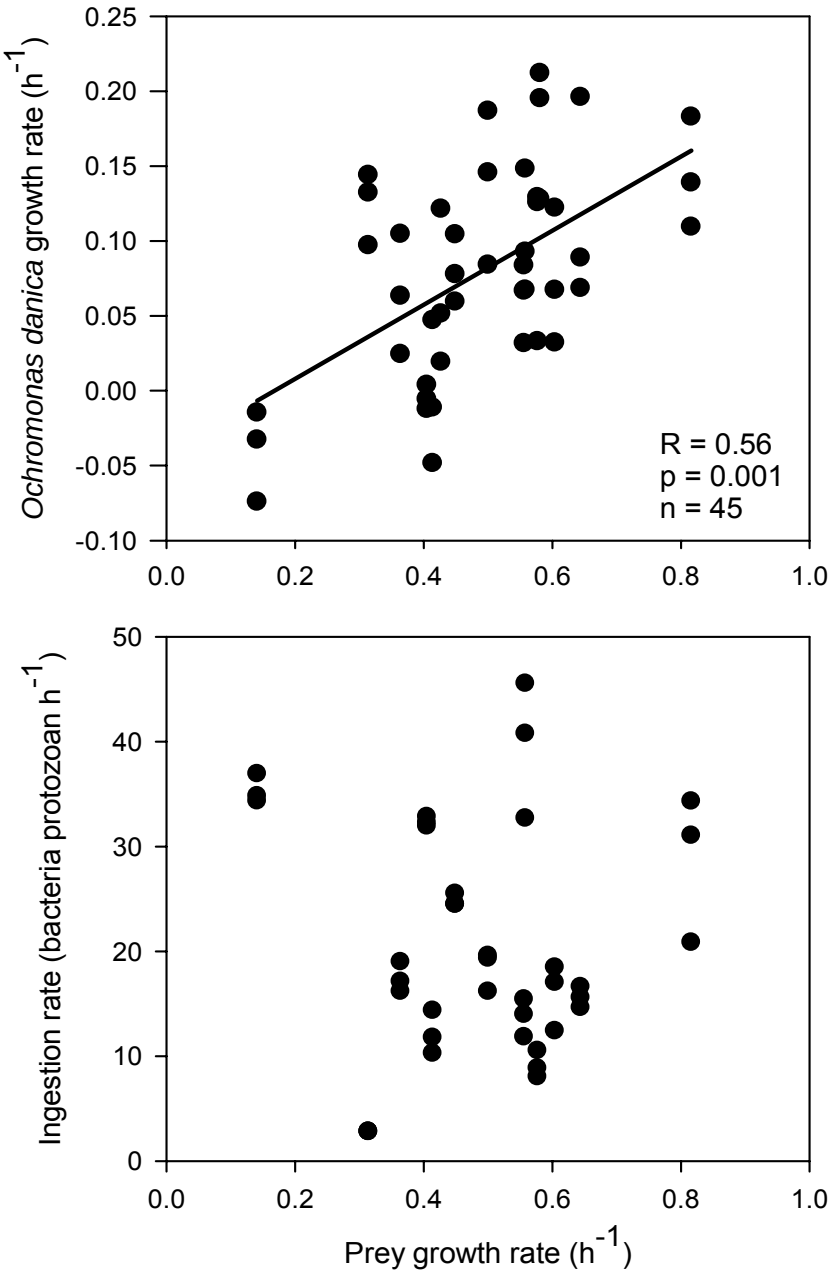

Figure 3.20 Relationship between growth rate of bacteria fed to *Ochromonas danica* and the subsequent growth rate (Top) and ingestion rate (Bottom) of the predator. Each point represents an individual feeing trial.

predator (Elser *et al*. 2000b; John and Davidson 2001; Shannon *et al*. 2007). When absolute prey nutrient content as C, N, and P (fmol cell^{-1}) and its effect on predator growth rate was considered, we found no correlation between absolute element content of prey and predator growth rate when all data were pooled or when only cells harvested from mid-exponential phase growth were considered (Figures 3.21-3.23). However, there was a positive correlation (C: r=0.323, p=0.045, n=39; N: r=0.345, p=0.032 n=39; P: r=0.337 p=0.036 n=39) between absolute element content of prey and growth rate for prey cells harvested from late-stationary phase growth. Careful examination of these data revealed that these correlations were driven by three data points all belonging to the same prey (Lake Isolate #10). Thus, is seems reasonable to conclude that there is not a robust relationship between absolute element content of prey and predator growth rate.

3.4.4 Growth rate and element ratios

Figures 3.24, 3.25, and 3.26 depict the relationship between *O. danica* growth rate and the C:N, C:P and N:P ratios of prey (respectively). There was a positive correlation between predator growth rate and C:N ratio when data from exponentially growing bacteria and stationary phase bacteria were pooled (r=0.29, p=0.006, n=87). This relationship did not hold when data were considered by growth phase. No relationship was found between *O. danica* growth rate and C:N ratio of cells harvested from mid-exponential phase growth (Figure 3.24). However, there was a significant positive correlation between *O. danica* growth rate and cells harvested from late-stationary phase growth (r=0.61, p=0.001, n=43). The C:N ratio of cells harvested from late-stationary phase spanned a very narrow range compared to that of cells harvested from mid-exponential phase growth so interpretation of these data are difficult. Nevertheless, a positive correlation between *O .danica* growth rate and the prey C:N ratio is unexpected. Such a correlation implies that *O. danica* improves its fitness when preying upon what would normally be considered 'poor' food quality.

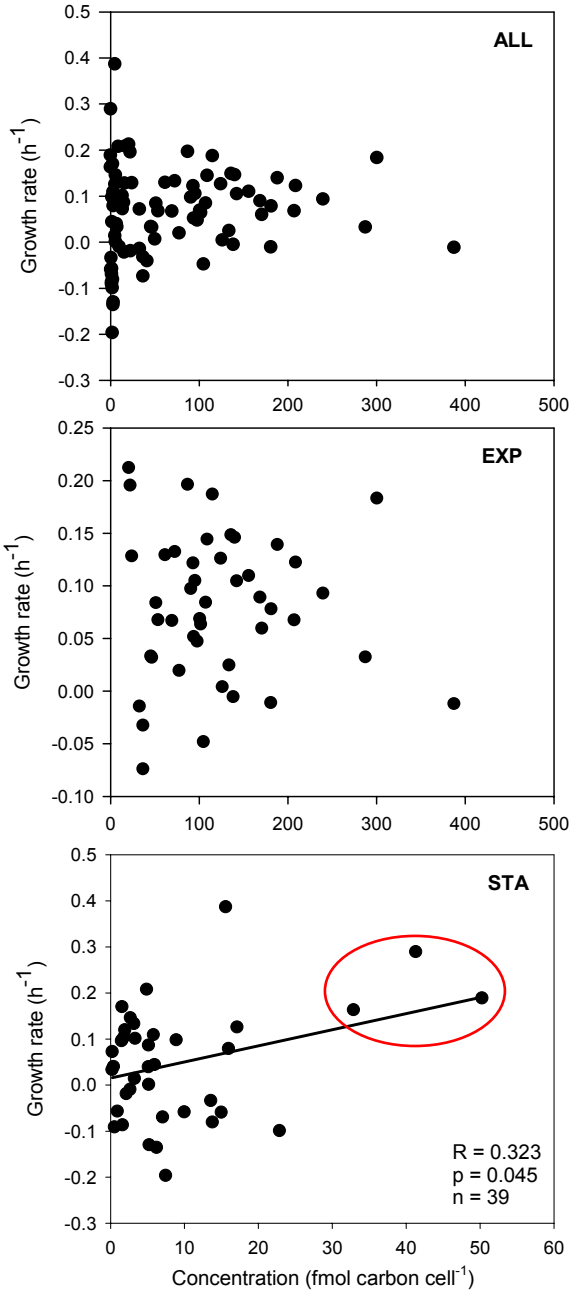

Figure 3.21 Effects of prey carbon quota on growth rate of *Ochromonas danica* for all prey (Top) exponential prey (Middle) and stationary prey (Bottom). The significant correlation observed for stationary prey is driven by three data points all belonging to the same bacterial prey (Lake Isolate #10–Red circle)

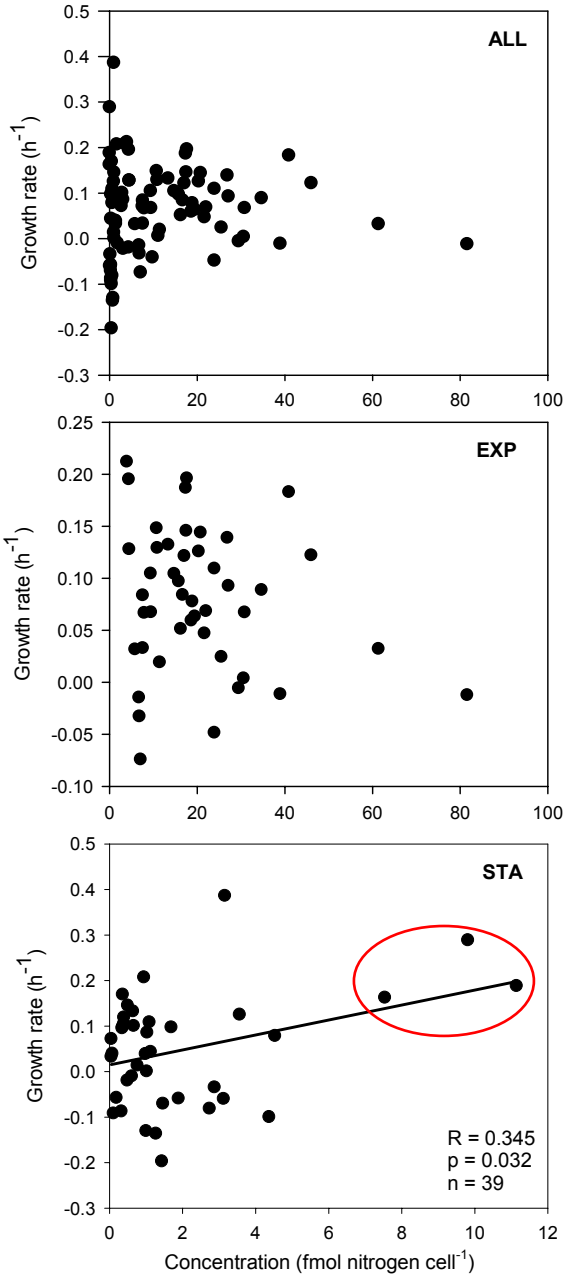

Figure 3.22 Effects of prey nitrogen quota on growth rate of *Ochromonas danica* for all prey (Top) exponential prey (Middle) and stationary prey (Bottom). The significant correlation observed for stationary prey is driven by three data points all belonging to the same bacterial prey (Lake Isolate #10–Red circle)

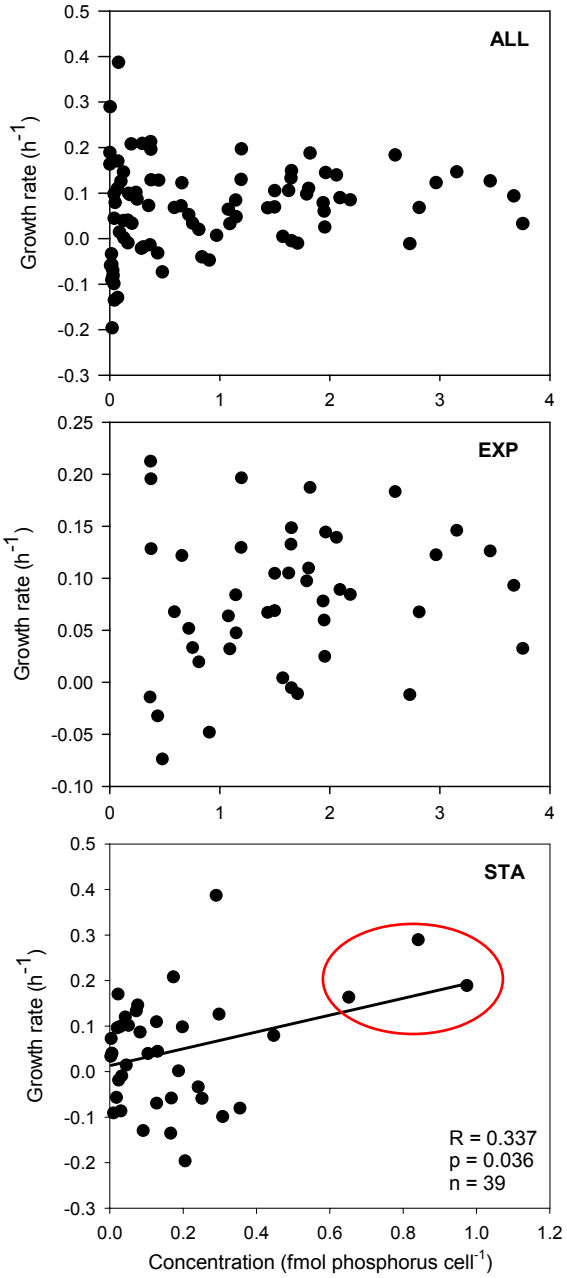

Figure 3.23 Effects of prey phosphorus quota on growth rate of *Ochromonas danica* for all prey (Top) exponential prey (Middle) and stationary prey (Bottom). The significant correlation observed for stationary prey is driven by three data points all belonging to the same bacterial prey (Lake Isolate #10–Red circle)

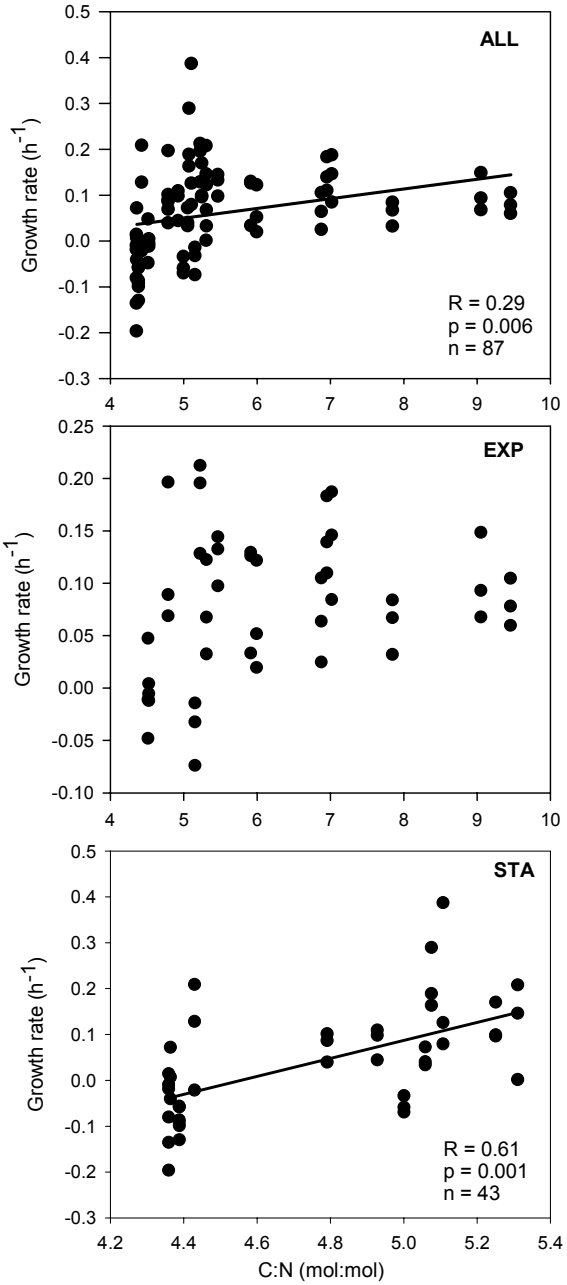

Figure 3.24 Effects of prey C:N ratio on growth rate of *Ochromonas danica* for all prey (Top) exponential prey (Middle) and stationary prey (Bottom).

There were negative correlations between growth rate and C:P (Figure 3.25) or N:P (Figure 3.26) when all data were considered (C:P: r=0.44, p=0.001, n=87; N:P: r=0.50, p=0.001, n=87). These relationships remained robust when data were split by prey growth phase. Negative correlations between element ratios and growth rate of *O. danica* suggest that this predator increases growth rate as the proportion of P in prey (relative to C and N) increases.

3.4.5 Ingestion rate and prey element content and element ratio

When all data were considered, there was a significant correlation between the rate at which *O. danica* ingested prey and the absolute C content (fmol C $cell^{-1}$) of prey (r=0.26, p=0.02, n=72); however, this relationship could not be demonstrated when prey were separated by growth phase (Figure 3.27). The rate at which *O. danica* ingested prey was not correlated to the absolute N (Figure 3.28) or P (Figure 3.29) content of prey.

Figures 3.30, 3.31, 3.32 depict the relationships between the rate at which *O. danica* ingested prey and the nutrient-element ratios of prey. When all data were considered, only the ratio of C:N could be related to ingestion rate (r=0.288, p=0.014, n=72). Similarly, when the data describing the nutrient-element ratios of prey were separated by growth phase of the prey, only the C:P ratio associated with exponentially growth prey was found to be associated with ingestion rate (r=0.57, p=0.001, n=39).

Seemingly, C content of prey drives the rate at which *O. danica* ingests prey whether C is considered as absolute C concentration (fmol C $cell^{-1}$) or as an index of food quality (C:N or C:P ratios). Yet, growth rate (metabolism) is seemingly influenced by P content of prey, particularly when increasing prey C:P and N:P ratios are associated with decreasing predator growth rate. (Summarized in Table 3.4) These finding suggest that nutrient 'poor' prey produce slower predator growth rates but nutrient poor prey are consumed at a higher rate than are nutrient rich prey. At first, it would appear that *O. danica* compensates for the lack of nutritional quality of some prey by increasing consumption of those prey types; however, increased consumption of poor quality prey does not necessarily translate into improved growth rate.

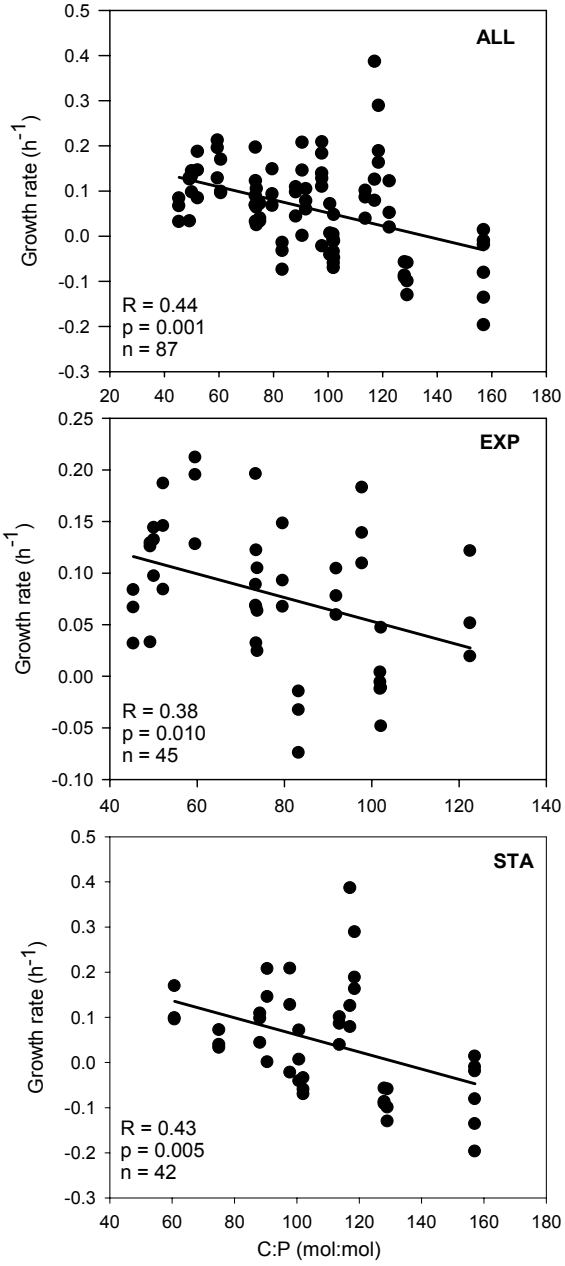

Figure 3.25 Effects of prey C:P ratio on growth rate of *Ochromonas danica* for all prey (Top) exponential prey (Middle) and stationary prey (Bottom).

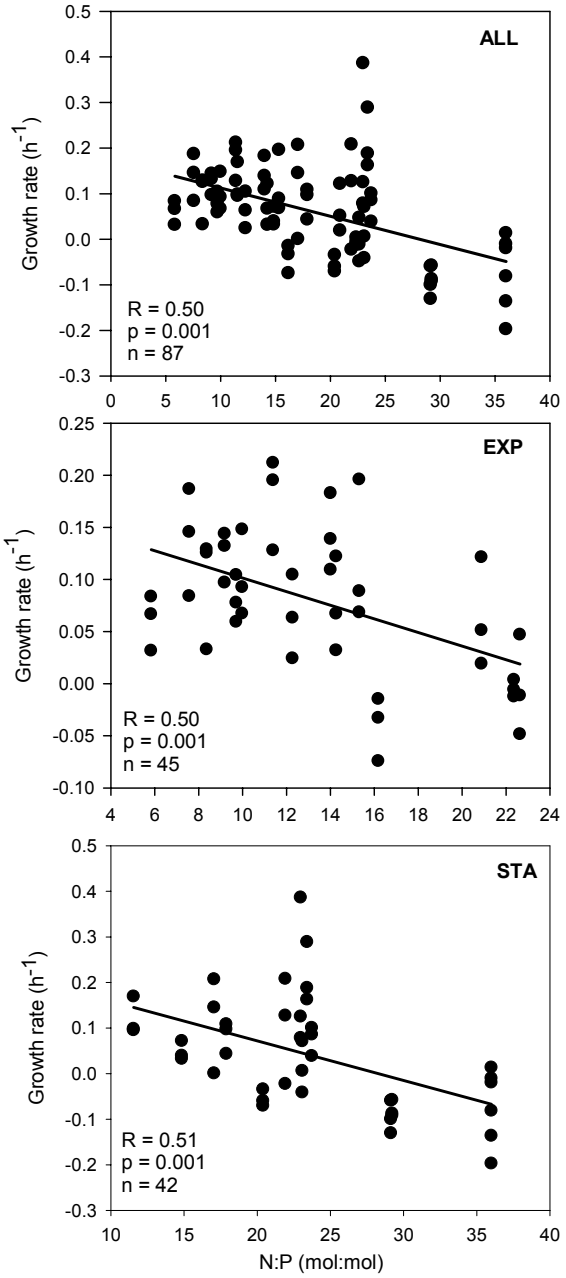

Figure 3.26 Effects of prey N:P ratio on growth rate of *Ochromonas danica* for all prey (Top) exponential prey (Middle) and stationary prey (Bottom).

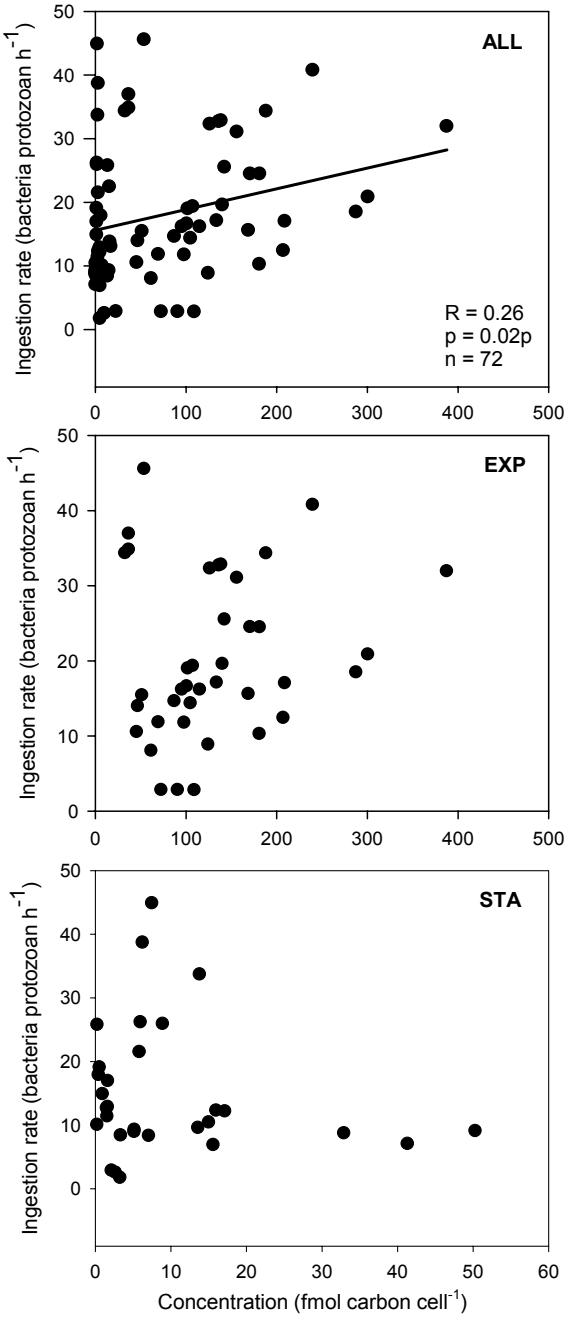

Figure 3.27 Effects of prey carbon quota on ingestion rate by *Ochromonas danica* for all prey (Top) exponential prey (Middle) and stationary prey (Bottom).

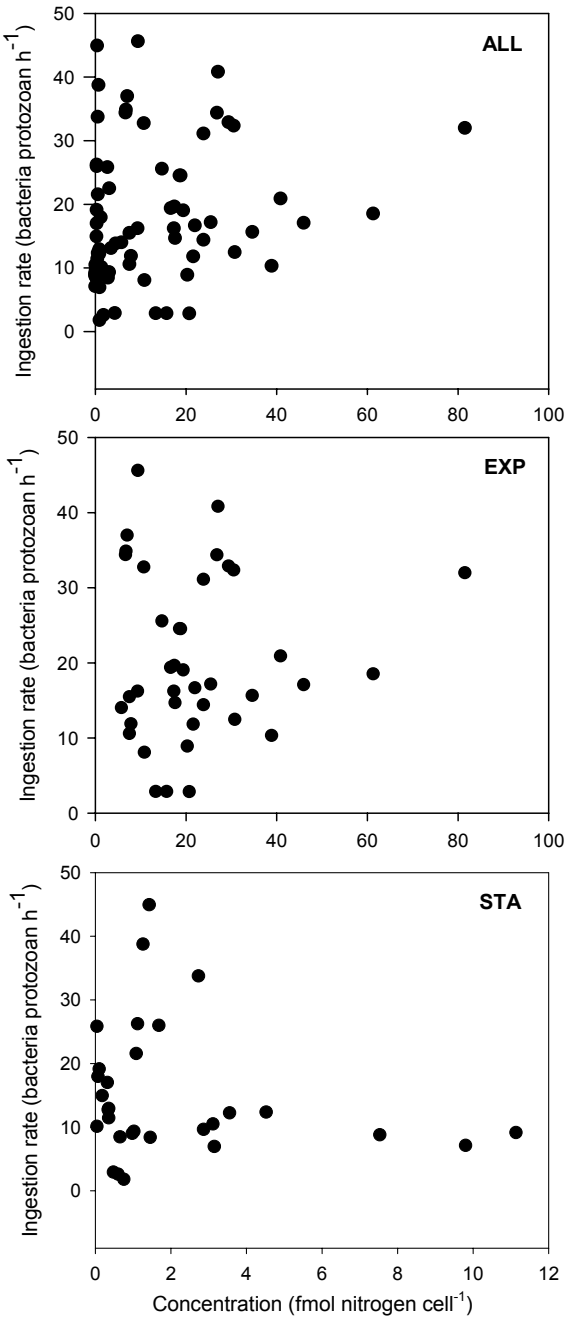

Figure 3.28 Effects of prey nitrogen quota on ingestion rate by *Ochromonas danica* for all prey (Top) exponential prey (Middle) and stationary prey (Bottom).

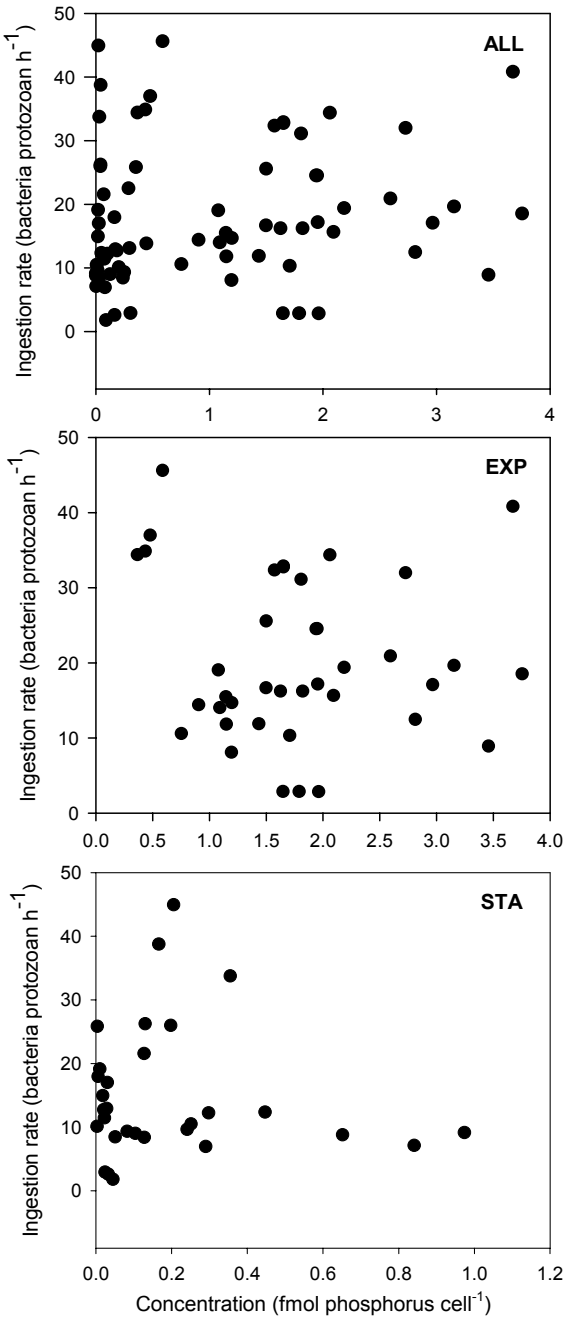

Figure 3.29 Effects of prey phosphorus quota on ingestion rate by *Ochromonas danica* for all prey (Top) exponential prey (Middle) and stationary prey (Bottom).

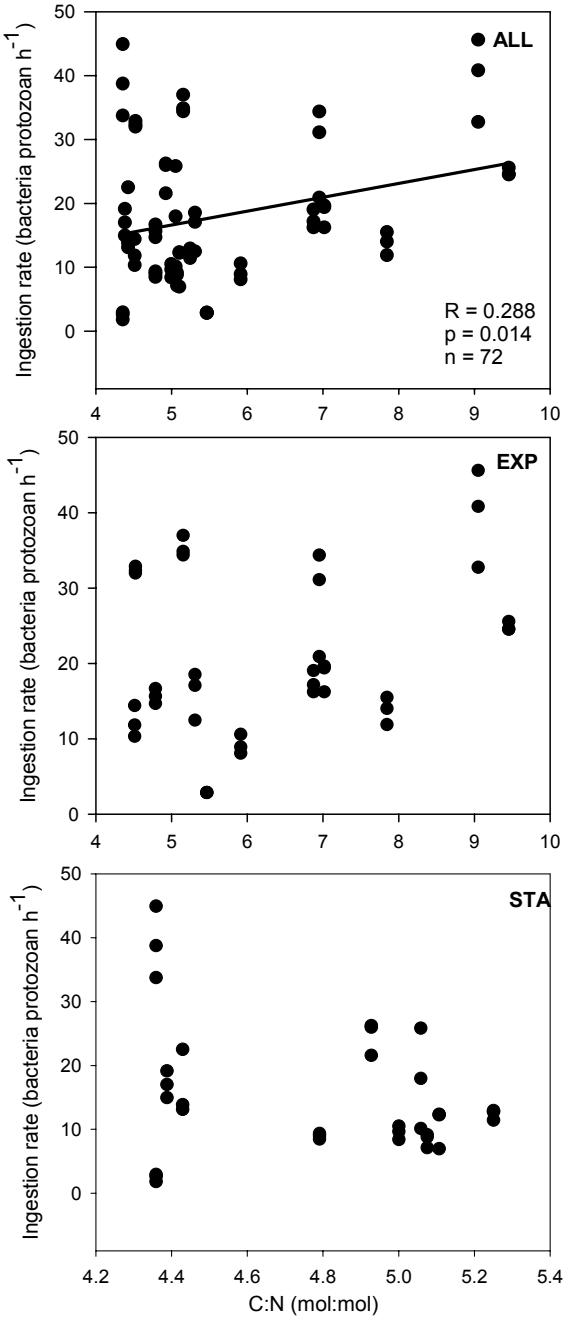

Figure 3.30 Effects of prey C:N on ingestion rate by *Ochromonas danica* for all prey (Top) exponential prey (Middle) and stationary prey (Bottom).

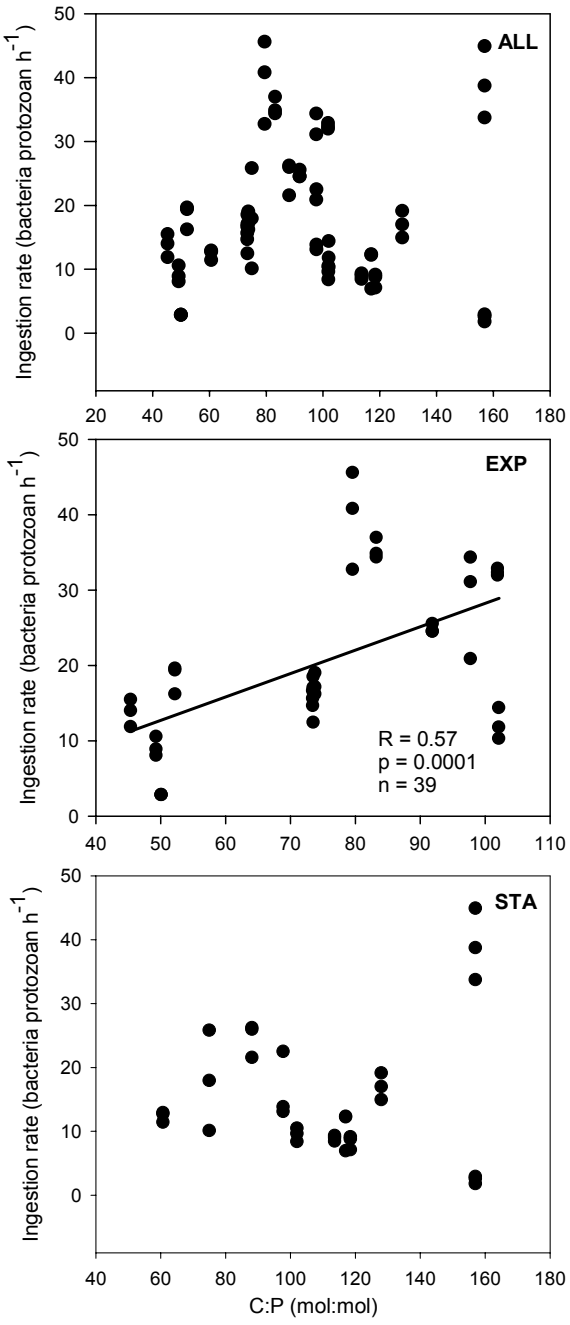

Figure 3.31 Effects of prey C:P on ingestion rate by *Ochromonas danica* for all prey (Top) exponential prey (Middle) and stationary prey (Bottom).

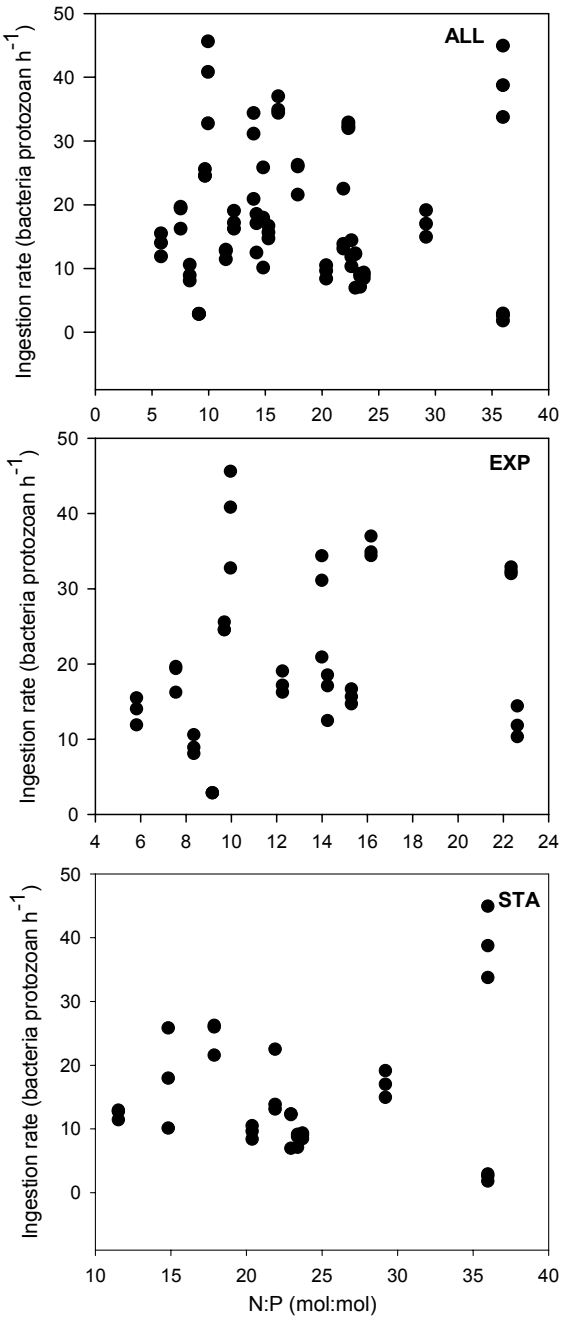

Figure 3.32 Effects of prey N:P on ingestion rate by *Ochromonas danica* for all prey (Top) exponential prey (Middle) and stationary prey (Bottom).

Table 3.4 –Summary of outcomes of correlation analyses between various prey characteristics and ingestion, and growth rate of *Ochromonas danica* (figures 3.18—3.32). Data are considered collectively (All), and by prey growth phase (Exponential & Stationary).NS; no significant correlation. ✓; significant correlation where $p < 0.05$. For r and n values see corresponding figures above.

Prey trait	Data considered	Growth rate (cells hr^{-1})	Ingestion rate (cells hr^{-1})
Size (μm^{-3})	All	NS	NS
	Exponential	NS	NS
	Stationary	NS	NS
QC (fmol $cell^{-1}$)	All	NS	✓
	Exponential	NS	NS
	Stationary	NS	NS
QN (fmol $cell^{-1}$)	All	NS	NS
	Exponential	NS	NS
	Stationary	NS	NS
QP (fmol $cell^{-1}$)	All	NS	NS
	Exponential	NS	NS
	Stationary	NS	NS
C:N (mol:mol)	All	✓	✓
	Exponential	NS	NS
	Stationary	✓	NS
C:P (mol:mol)	All	✓	NS
	Exponential	✓	✓
	Stationary	✓	NS
N:P (mol:mol)	All	✓	NS
	Exponential	✓	NS
	Stationary	✓	NS

When all data were considered, a negative correlation was found between growth rate of *Ochromonas* and prey ingestion rate ($r=0.335$, $p=0.004$, $n=72$). A similar correlation was found when only prey of stationary phase growth were considered. ($r=0.471$, $p=0.006$, $n=33$). There was no correlation between ingestion rate and growth rate of *O. danica* for prey cells harvested from exponential phase growth (Figure 3.33) It has been suggested or implied,

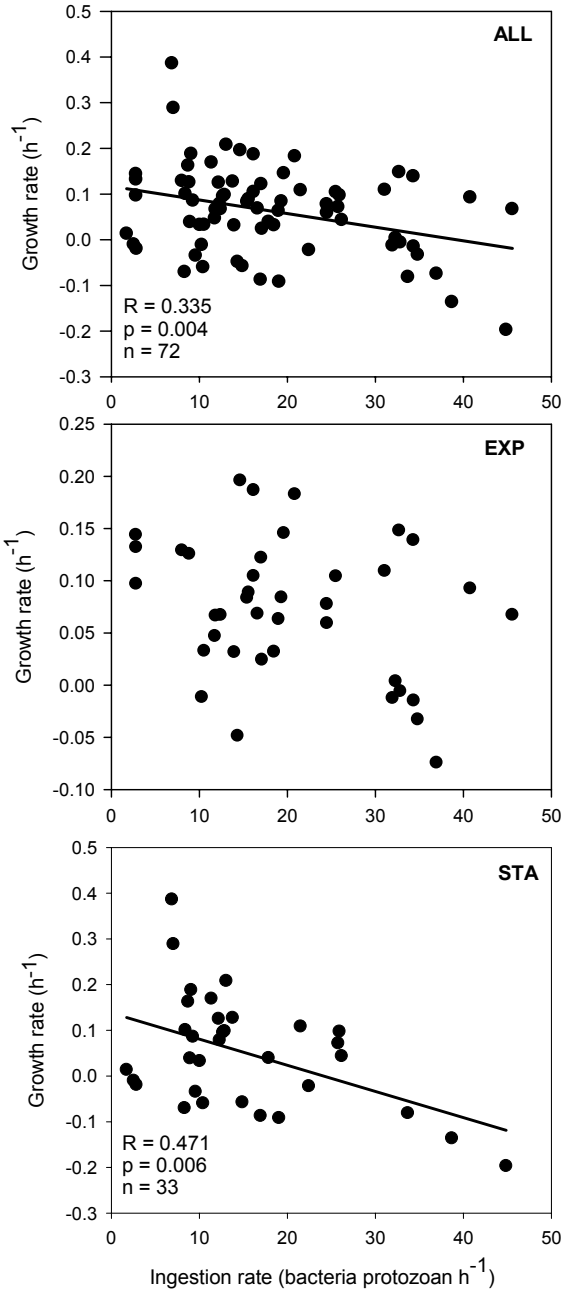

Figure 3.33 Effects of ingestion rate on growth rate of *Ochromonas danica* for all prey (Top) exponential prey (Middle) and stationary prey (Bottom).

assuming prey are digestible and non-toxic, that predator growth rate would increase with increasing ingestion rate (Jezbera *et al*. 2005; Salcher *et al*. 2006; Grover and Chrzanowski 2009; Gruber *et al*. 2009). Intuitively, this seems logical, but there appears to be a cost to the predator to form phagosomes and/or to ingest certain prey types. Members of the Betaproteobacteria were, in general, ingested at higher rates than members of the bacilli, Alphaproteobacteria and Gammaproteobacteria . This is consistent with the findings of Salcher *et al*. (2005) and Jezbera *et al*. (2005) who observed that members of the Betaproteobacteria are selected by flagellates at higher rates than members of other classes of bacteria. In this work, the Betaproteobacteria were, on average, ingested at a higher rate than members of other classes of bacteria, but *O. danica* failed to grow when consuming Betaproteobacteria in all feeding trials indicating that members of the Betaproteobacteria are unable to be effectively converted into flagellate biomass. When data from members of the Betaproteobacteria are removed from the data series, the correlation between *O. danica* growth rate and ingestion rate disappears ($r=0.005$, $p=0.97$, $n=66$): there is no relationship between the ability to ingest prey and the ability to grow. This leaves still unanswered the question: What is the link between ingestion rate of prey and growth rate of the predator.

It has been suggested that flagellates maintain a weak stoichiometric homeostasis (Simonds *et al*. 2010). Thus, flagellates should adjust ingestion rates to satisfy nutritional requirements to maintain some level of element homeostasis. If there is an imbalance between the nutritional requirements (C:N, C:P, N:P) of the predator and what their prey provide (C:N, C:P, N:P) then ingestion rate should increase or decrease as the nutrient disparity between the consumer and its prey increases. To examine this, the prey nutrient ratio (as C:N, C:P or N:P) was subtracted from its matching ratio in *O. danica* yielding positive or negative values dependent on the imbalance. Figure 3.34 depicts the relationship between ingestion rate of prey and the nutrient imbalance between predator and prey. It is apparent from these analyses that

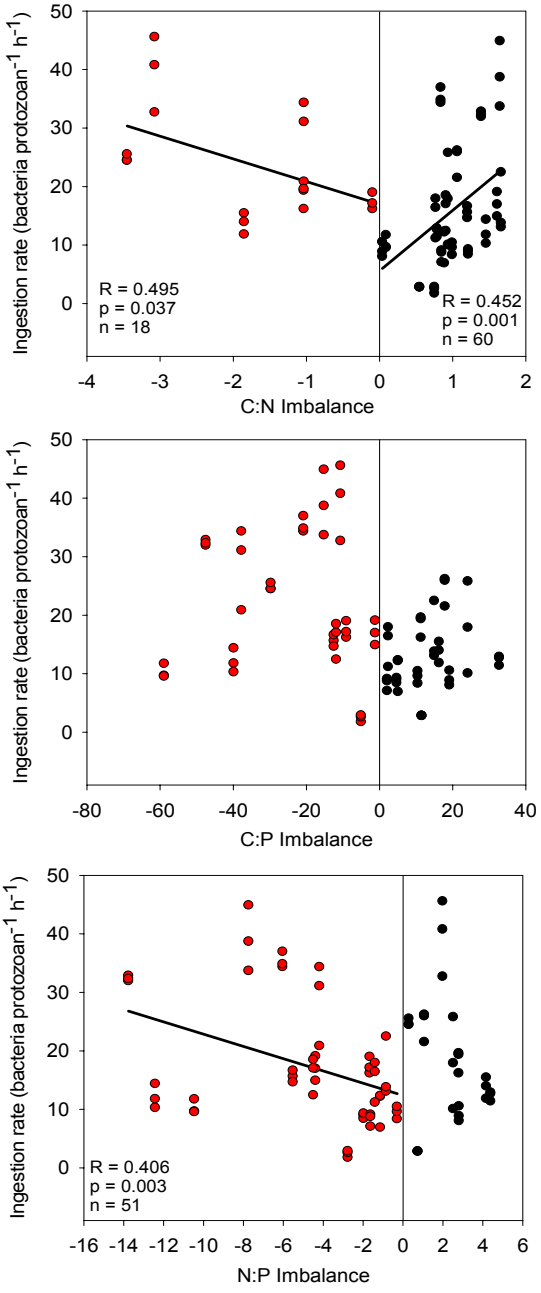

Figure 3.34 Ingestion of prey by *Ochromonas danica* as a function of element imbalance between *Ochromonas danica* and prey. C:N imbalance (Top), C:P imbalance (Middle) and N:P imbalance (Bottom).

changes in ingestion rate relative to nutrient imbalance only becomes important when N is involved. When C was imbalanced relative to N, *O. danica* increased levels of predation, and this was true whether the imbalance was the result of too much C ($r=0.495$, $p=0.037$, $n=18$; Red,) or too much N ($r=0.452$, $p=0.001$, $n=60$; Black). Similarly when N was in short supply relative to P, ingestion rates increased (bottom panel). Ingestion rates were not significantly different when P was in excess relative to N.

The biological significance of this is difficult to interpret. Though element ratios may serve as a means to examine element imbalances between predator and prey, they do not account for quantity of any element consumed by a predator. Ultimately, element availability, quantity (as well a quality) should drive phagotrophic metabolism as it does for osmotrophic metabolism. If metabolic needs of the predator drive ingestion rates then positive correlations between ingestion rate and element concentrations should be expected. Yet, overall such correlations were not observed for growth rate or ingestion rate.

This predator-prey interaction is complex. *Ochromonas danica* is able to consume a wide range of bacterial prey with little to suggest a discriminate feeding behavior. To some degree, prey quality may be a predictor of ingestion rate, but this ingestion rate cannot be translated to predator growth rate. The reverse is also true, predator growth rate does not reflect the rate at which it consumes prey.

Several recent studies have demonstrated that aquatic protozoa capture prey by a receptor-mediated process. If phagocytosis in *O. danica* is receptor mediated, as it is in almost all (if not all) characterized systems (Bossaro et al. 2008; Cosson and Soldati 2008), then *O. danica* has either several receptors capable of recognizing several different surface characteristics on bacteria or it has a receptor capable of recognizing a single, yet extremely common bacterial surface characteristic. Type III IEP indicates it is likely that *O. danica* expresses multiple receptors. If there is a single receptor, increasingly expressed over the

duration of feeding experiment, all prey types having the binding site for the receptor would be expected to have a similar mortality pattern (i.e. IEP).

Despite the initial recognition and phagocytosis of prey by surface characteristics, there is seemingly a mechanism to maintain or repress ingestion rates dependent on the predator's metabolic requirements (at least for N, see above). In general cell metabolism is regulated by feedback loops that reduce excess energy expenditure on metabolic pathways not required for growth (i.e. Reduce fiteness). It may be that *O. danica* slows ingestion rates of "good quality" prey to avoid expending energy obtaining and digesting prey in excess of its metabolic needs. This is fascinating since this level of regulation takes place only after energy has been expended to capture prey and it clearly does not always result in improved fitness of the predator.

REFERENCES

Andersen, P. and H.M. Sørensen. 1986. Population dynamics and trophic coupling in pelagic microorganisms in eutrophic coastal waters. Marine Ecology Progress Series 33:99-109.

Azam, F., T. Fenchel, J.G. Field, J.S. Grey, L.A. Meyer-Reil and F. Thingstad. 1983. The ecological role of water-column microbes in the sea. Marine Ecology Progress Series 31: 47-55.

Boenigk, J. and H. Arndt. 2000. Comparative studies on the feeding behavior of two heterotrophic nanoflagellates: the filter-feeding choanoflagellate *Monosiga ovata* and the raptorial-feeding kinetoplastid *Rhynchomonas nasuta*. Aquatic Microbial Ecology 22: 243-249.

Bossaro, S., C. Boakers and M. Sternert. 2008. Phagocytosis and host-pathogen interactions in *Dictyostelium* with a look at macrophages. International Review of Cell and Molecular Biology 271:253-300.

Brown, M.R.W., P.J. Collier and P. Gilbert. 1990. Influence of growth rate on susceptibility to antimicrobial agents: Modification of the cell envelope and batch and continuous culture studies. Antimicrobial Agents and Chemotherapy 34:1623-1628.

Chrzanowski, T.H. and J.P. Grover. 2008. Element content of *Pseudomonas fluorescens* varies with growth rate and temperature: a replicated chemostat study addressing ecological stoichiometry. Limnology and Oceanography 53:1242-1251.

Chrzanowski, T.H. and K. Šimek. 1990. Prey-size selection by freshwater flagellated protozoa. Limnology and Oceanography 35:1429-1436.

Chrzanowski, T. H. and K. Simek. 1993. Bacterial growth and losses due to bacterivory in a mesotrophic lake. Journal of Plankton Reaseach 15:771-785.

Cosson, P. and Soldati, T. 2008. Eat, kill or die: when amoeba meets bacteria. Current Opinion in Microbiology 11:271-276.

Difco Manuel. 1984. Dehydrated culture media and reagents for microbiology. Tenth Edition. Detroit. pp 1155.

Elser, J.J. and R.P. Hassett. 1994. A stoichiometric analysis of the zooplankton-phytoplankton interaction in marine and freshwater ecosystems. Nature 370:211-213.

Elser, J.J., W.J. O'Brien, D.R. Dobberfuhl and T.E. Dowling. 2000a. The evolution of ecosystem processes: growth rate and elemental stoichiometry of a key herbivore in temperate and arctic habitats. Journal of Evolutionary Biology 13:845-853.

Elser, J.J., R.W, Sterner, E. Gorokhova, W.F. Fagen, T.A. Markow, J.B. Cotner, J.F. Harrison, S.E. Hobbie, G.M. Odell and L.J. Weider. 2000b. Biological stoichiometry from genes to ecosystems. Ecology Letters 3: 540-550.

Fenchel, T. 1982. Ecology of heterotrophic microflagellates. I. Some Important forms and their functional morphology. Marine Ecology Progress Series 8:211-223.

Firon, N., I. Ofek and N. Sharon. 1984. Carbohydrate-binding sites of the mannose-specific fimbrial lectins of Enterobacteria. Infection and Immunity 43:1088-1090.

Flynn, K.J., K. Davidson and A. Cunningham. 1996. Prey selection and rejection by a microflagellate; implications for the study and operation of microbial food web. Journal of Experimental Marine Biology and Ecology 196:357-372.

Gonzalez, J.M., E.B. Sherr and B.F. Sherr. 1990. Size-selective grazing on bacteria by natural assemblages of estuarine flagellates and ciliates. Applied and Environmental Microbiology 56: 583-589.

Gonzalez, J.M,, E.B. Sherr and B.F. Sherr. 1993. Differential feeding by marine flagellates on growing versus starving, and on motile versus nonmotile, bacterial prey. Marine Ecology Progress Series 102:257-267.

Grover, J.P. and T.H. Chrzanowski. 2009. Dynamics and nutritional ecology of a nanoflagellate preying upon bacteria. Microbial Ecology 58: 231-243.

Gruber, D.F., S. Tuorto and G.L. Taghon. 2009. Growth phase and element stoichiometry of bacterial prey influence ciliate grazing selectivity. Journal of Eukaryotic Microbiology doi:10.1111/j.1550-7408.2009.00428.x.

Hammer, A., C. Grüttner and R. Schumann. 1999. The effect of electrostatic charge of food particles on capture efficiency by *Oxyrrhis marina* Dujardin (Dinoflagellate). Protist 150:375-382

Hobbie, J.E., R.J. Daley and S. Jasper. 1977. Use of nuclepore filters for counting bacteria by fluorescence microscopy. Applied and Environmental Microbiology 33:1225-1228.

Jezbera, J., K. Horňák and K. Šimek. 2005. Food selection by bacterivorous protests: insight from the analysis of the food vacuole content by means of fluorescence *in situ* hybridization. FEMS Microbiology Ecology 52:351-363.

John, E.H. and K. Davidson. 2001. Prey selectivity and the influence of prey carbon:nitrogen ratio on microflagellate grazing. Journal Experimental Marine Biology and Ecology 260:93-111.

McEldowney, S. and M. Fletcher. 1986. Effect of growth conditions and surface characteristic of aquatic bacteria and their attachment to solid surfaces. Journal of General Microbiology 132:513-523.

Menzel, D.W. and N. Corwin. 1965. The measurement of total phosphorus in seawater based on the liberation of organically bound fractions by persulfate oxidation. Limnology and Oceanography 10:280-282.

Monger B.C., M.R. Landry and S.L. Brown. 1999. Feeding selection of heterotrophic marine nanoflagellates based on the surface hydrophobicity of their picoplankton prey. Limnology and Oceanography 44:1917-1927.

Pernthaler, J. 2005. Predation on prokaryotes in the water column and its ecological implications. Nature Reviews Microbiology 3:537-546.

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

Salcher, M. M., J. Hofer, K. Horñák, J. Jezbera, B. Sonntag, J. Vrba, K. Šimek and T. Posch. 2006. Modulation of microbial predator-prey dynamics by phosphorus availability: Growth patterns and survival strategies of bacterial phylogenetic clades. FEMS Microbiology Ecology 60:40-50.

Shannon, S.P., T.H. Chrzanowski and J.P. Grover. 2007. Prey food quality affects flagellate ingestion rates. Microbial Ecology 56:66-73.

Šimek, K. and T.H. Chrzanowski. 1992. Direct and indirect evidence of size-selective grazing on pelagic bacteria by freshwater nanoflagellates. Applied and Environmental Microbiology 58:3715-3720.

Simonds, S., J.P. Grover, and T.H. Chrzanowski. 2010. Element content of *Ochromonas danica*: A replicated chemostat study controlling growth rate and temperature. FEMS Microbiology Ecology 74: 346-352.

Starr R. C. 1978. The culture collection of algae at the University of Texas at Austin. Journal of Phycology 14: 47-100.

Stricland, J.D. and T.R. Parsons. 1972. A practical handbook of seawater analysis. Second Edition. Bulletin of the Fisheries and Research Board of Canada 167:1-310

White, D. and G.D. Hegeman. 1998. Microbial physiology and biochemistry laboratory: A quantitative approach. Oxford university press. pp.147

Wildschutte, H., D.M. Wolfe, A. Tamewitz and J.G. Lawrence. 2004. Protozoan predation, diversifying selection, and the evolution of antigenic diversity in *Salmonella*. Proceedings of the National Academy of Sciences 101:10644-10649.

Wootton, E.C., M.V. Zubkov, D.H. Jones, R.H. Jones, C.M. Martel, C.A. Thornton and E.C. Roberts. 2007. Biochemical prey recognition by planktonic protozoa. Environmental Microbiology 9:216-222.

BIOGRAPHICAL INFORMATION

Briony Foster completed her bachelor's degree at the University of Texas Arlington in December 2008 with a major in microbiology and a minor in chemistry. She began her thesis work in January 2009 with Thomas H. Chrzanowski. It was working with Thomas where she found her passion for understanding microbial processes occurring aquatic systems. After completing her thesis she plans to work as a technician in a microbiology lab for a year before pursuing her PhD.