The mixotrophic protist *Ochromonas danica* is an indiscriminant predator whose fitness is influenced by prey type

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ABSTRACT: Microbial predator–prey interactions are one of the primary trophic interactions linking biogeochemical cycles to ecosystem dynamics. The mixotrophic flagellate *Ochromonas danica* was used as a model predator to investigate feeding trends when supplied with actively growing and non-growing bacteria representing a variety of phylogenetic groups. The rate at which bacteria were ingested and the subsequent growth rate (ecological fitness) of *O. danica* were determined for each type of prey in each growth state. *O. danica* preferred bacteria between 0.6 and 1.0 μm^3 . It was, in general, an indiscriminate predator in that it readily ingested growing and non-growing bacteria spanning a range of phylogenetic groups, with some notable exceptions. *Bacilli/Actinobacteria* were ingested at significantly lower rates than *Alpha-* and *Gamma-proteobacteria*. There were no significant differences among rates at which *Proteobacteria* (*Alpha-*, *Beta-*, and *Gamma-*) were ingested. The ecological fitness of *O. danica* could not be predicted from ingestion rates. Predation on *Bacilli/Actinobacteria* resulted in growth rates significantly lower than when the flagellate preyed upon *Gammaproteobacteria*. *Betaproteobacteria* were readily ingested but always resulted in a significant decline in predator fitness. The data reveal that different types of bacteria have different nutritional value to a consumer. If other flagellates respond similarly, then it would seem that trophic transfer efficiency and nutrient regeneration depend on the diet-breadth of predators.

KEY WORDS: Bacterivory · Trophic interactions · Predator–prey

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INTRODUCTION

Nutrients supporting aquatic food webs are made available to higher trophic levels by the metabolic activities and interactions among members of the lowest trophic level. Bacteria and phytoplankton are the only groups of organisms within aquatic food webs that are capable of incorporating significant amounts of dissolved nutrients into biomass. Bacteria are particularly important in this process since they 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, in effect, be removed from trophic dynamics in the absence of significant levels of predation. Consequently, predation upon bacteria is one of the most important interactions driving nutrient dynamics (Fenchel 1982, Azam et al. 1983, Andersen & Sørensen 1986). The major predators of bacteria in aquatic systems are heterotrophic and mixotrophic nanoflagellates. Since the recognition of this important predator–prey interaction, many aquatic nutrient dynamics studies have focused on understanding factors that affect predation on bacteria, with particular attention given to the process of prey identi-

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fication and selection (Pernthaler 2005, Anderson et al. 2011).

Selection of prey has been described using a number of indices addressing both physical and chemical properties of 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, Matz & Jürgens (2001) found that differences in prey surface hydrophobicity could not fully explain differential feeding rates by 3 nanoflagellate predators. Similarly, motility may influence contact rates and interaction between predators and prey. Motile prey are more likely to encounter a predator than are non-motile prey (Gonzalez et al. 1993). Nevertheless, highly motile prey ($>25 \mu\text{m s}^{-1}$) are not ingested as readily as their slower counterparts, despite encountering predators at a higher rate (Matz & Jürgens 2005).

Prey size has also been identified as a factor influencing selection by flagellate predators. It has been suggested that predators are best able to ingest prey falling within a particular 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 & Arndt 2000); however, laboratory and field studies have demonstrated that there is a preferred prey size for predators (Andersson et al. 1986, Chrzanowski & Šimek 1990, Gonzalez et al. 1990, Šimek & Chrzanowski 1992, Boenigk & Arndt 2000). It has also been suggested that large prey, relative to the size of all potential prey, may be ‘preferentially’ selected simply due to an increased chance of interception (Hammer et al. 1999, Boenigk & Arndt 2000).

There is an interesting relationship among bacterial cell size, growth, and nutrient content that may link prey selection to predator fitness and aquatic nutrient dynamics. This relationship revolves around 2 aspects of bacterial physiology: cell size is linked to growth rate, and growth rate is linked to nutrient quota (the concentration of a nutrient element per cell or per cell-size), particularly for phosphorus (P). P is the element that most often limits productivity in freshwater systems; thus, aspects of bacterial growth have links to both prey selection and nutrient dynamics (Elser & Hassett 1994 and references therein).

Cell element quotas change as bacteria shift growth rates. Elser et al. (2000a) showed that P quotas increased at a faster rate than carbon (C) and nitrogen (N) quotas as growth rate increased. Consequently, rapidly growing cells have lower C:P and N:P ratios than slowly growing cells. This difference can be attributed to the increased level of P-rich translational machinery present in growing cells (Elser et al. 2000b, Chrzanowski & Grover 2008). Ultimately, the fastest-growing cells should have low C:P and N:P ratios and should be more nutritious prey than non-growing or starved cells; i.e. they should supply a predator with the largest pulse of the element that is most likely limiting.

A predator feeding on growing bacteria should experience enhanced fitness by reducing the energy expended capturing and processing small prey of poor nutritional quality. Some level of prey selection based on nutritional value seems to occur. Microflagellates select algal prey with a low C:N ratio when presented a mixed assemblage containing similarly sized cells (John & Davidson 2001). A similar observation was made for the nanoflagellate *Ochromonas danica* feeding on a single type of bacterium (Shannon et al. 2007). However, recent work suggests that alternative strategies may exist. Gruber et al. (2009) separately offered 2 ciliates equal portions of rapidly (exponentially) growing and non-growing (stationary phase) bacteria to determine if prey selection occurs when a choice is given. In accordance with theory, the non-growing bacteria were of lower nutritional quality than the growing bacteria. However, contrary to expectations, the non-growing bacteria were selectively cropped, while the rapidly growing bacteria were not significantly diminished. Anderson et al. (2011) recently suggested that protists may have different responses to growth state of their prey and that these responses may depend on prey species.

It is evident from the foregoing material 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, there is a tremendous phylogenetic diversity among bacteria in aquatic environments. Some researchers have started to examine feeding behaviors of flagellates when presented prey representing different classes of bacteria. For example, *Actinobacteria* (high-GC Gram-positive bacteria) do not appear to be readily grazed by heterotrophic nanoflagellates (Jezbera et al. 2006). Members of the *Betaproteobacteria* appear to be more susceptible to ingestion than members of the *Alphaproteobac-*

teria when supplied to predators in mixed assemblages (Salcher et al. 2007). Selection of *Betaproteobacteria* was also demonstrated by Jezbera et al. (2005), who observed that 2 different heterotrophic flagellates preferentially selected a member of the *Betaproteobacteria* over a member of the *Gamma-proteobacteria* when given a choice.

In the present study, aspects of some of the work described above are combined. The mixotrophic bacterivore *Ochromonas danica* was used as a model predator to investigate feeding trends when supplied growing and non-growing bacteria representing a variety of phylogenetic groups. The bacteria used as prey (see Table 1) included 4 genera of Gram-positive bacteria, 3 within the Bacilli and 1 within the Actinobacteria, and 11 genera of Gram-negative bacteria spanning 3 classes within the *Proteobacteria*. We considered how ingestion varied as a function of prey type and growth state and how prey type and growth state influenced predator fitness.

MATERIALS AND METHODS

Organisms

Ochromonas danica (UTEX 1298) was maintained bacteria-free in *Ochromonas* medium (OM; Starr 1978) at room temperature (RT, ~23°C). Bacteria were maintained at RT on R2A agar (Difco). Many of the bacteria used as prey were existing laboratory strains (LS) whose origins are unknown. The identity of these strains was verified by sequencing the 16S rRNA gene (see 'Verification of bacterial identity'). Additional bacteria were obtained from the American Type Culture Collection (ATCC) (www.atcc.org) or were isolated from freshwater (lake isolate, LI) and identified from 16S rRNA gene sequencing.

Isolation of bacteria from freshwater

Water (0.1 l) was collected by grab sample from a depth of ~0.5 m from the near-shore area of Lake Arlington, Texas, USA (32° 42' 30" N, 97° 12' 30" W). Aliquots (100 µl) were spread onto R2A agar plates within 1 h of collection. Plates were incubated at RT for 5 d, after which colonies were selected based on morphology and transferred to fresh R2A agar plates. Cultures were re-streaked repeatedly to confirm purity.

Verification of bacterial identity

Genomic DNA was extracted using a QIAmp DNA Mini kit (Qiagen) according to manufacturer guidelines. The 16S rRNA gene was amplified using eubacterial primers 27f (5'-AGA GGT TTG ATC CTG GCT C) and 1492r (5'-GGT TAC CTT GTT ACG ACT T). PCR products were treated with ExoSAP-It (USB) and sequenced using an ABI3130xl sequencer at the Genome Core Facility at the University of Texas at Arlington. Forward and reverse sequences were aligned using Sequencher V 5.0 (GeneCodes) and matched to known sequences in the Ribosomal Database Project (<http://rdp.cme.msu.edu/>). Bacteria were assigned to genus based on sequence identity.

Predator-prey experiments

Each predator-prey experiment had 3 components: growth and preparation of *Ochromonas danica*, preparation of bacteria in different growth states, and determination of prey ingestion rates and subsequent growth rates of *O. danica*.

Growth and preparation of *Ochromonas danica*

Ochromonas danica was grown in OM in a continuously stirred and aerated 800 ml chemostat (Applikon) operated in the dark at 25°C at a dilution rate of 0.037 h⁻¹. The chemostat was assumed to be in steady state after 3 volume turnovers (Simonds et al. 2010). Chemostat outflow was collected aseptically in 1 or 2 l bottles (Nalgene). Cells harvested from chemostats were washed 3 times with sterile Standard Mineral Base medium (White & Hageman 1998) lacking any source of C, N, or P (hereafter referred to as SMB buffer), concentrated by centrifugation (Sorvall RT6000B, 720 × g, 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). Aliquots of washed cells were preserved in glutaraldehyde (2% final concentration) and later used to determine cell size.

Preparation of bacteria in different growth states

Growth curves for each species were determined by measuring the optical density (OD₆₀₀) of cells

growing in R2A broth (Teknova). An overnight culture (25°C, 100 rpm, New Brunswick Scientific G10 Gyrotory shaker) was used to inoculate triplicate 50 ml Erlenmeyer flasks containing 30 ml of R2A broth to an OD₆₀₀ of ~0.01. Cultures were incubated as above, and the OD recorded at regular time intervals until cultures showed no further change.

Growth curves were used to establish the OD corresponding to mid-exponential phase growth (growing bacteria) and to late-stationary phase growth (non-growing bacteria). The mid-exponential phase of growth was established as the elapsed time from inoculation (start of growth) to the time of transition into stationary phase divided by 2. OD corresponding to mid-exponential phase was determined from a straight line fitted to the linear portion of the data. Late-stationary phase was defined as 4 times the time it took a culture to go from mid-exponential phase to transition phase.

For feeding trials, 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 washed, concentrated, and enumerated as for *Ochromonas danica*. Aliquots of washed cells were preserved in glutaraldehyde (2% final concentration) and later used to determine cell size.

Determination of ingestion rates and subsequent growth rate of *Ochromonas danica*

Feeding experiments were conducted in triplicate flasks containing 20 ml of SMB buffer. Washed *Ochromonas danica* and washed bacteria, both suspended in SMB buffer, were combined to a final concentration of 4×10^5 and 3×10^7 cells ml⁻¹, respectively. Controls (duplicates) consisted of cultures of *O. danica* without bacteria and of cultures of bacteria without *O. danica*. Predator–prey cultures and controls were gently shaken (60 rpm) in the dark at 25°C. Samples were taken 1 h after predator and prey were combined and then every 45 min for a total elapsed time of 3.25 h. Samples were preserved in ice-cold glutaraldehyde (2% final concentration) and stored at 4°C until processed by flow cytometry.

Flow cytometry

A BD LSRII flow cytometer fitted with a 488 nm argon laser was used to quantify *Ochromonas dan-*

ica and bacteria. Preserved samples (2 ml) were stained with 10 µl of 1000× SYBR Safe DNA gel stain (Invitrogen) and incubated for a minimum of 30 min before analysis. The cytometer was consistently set to the same flow rate and the following settings: SSC 231, threshold 8000V; FSC 484, threshold 8000V. All samples were processed using BD FACS-Flow sheath. Data were collected for 60 s following an initial 20 s of operation during which no data were collected. Flow cytometer data were analyzed using BD FACSDiva Software v6.1. Each population (predator and prey) was separated empirically by gating natural breaks in FSC data on a histogram plotting total events by FSC. Conversions of cytometer data describing each population (protozoa and growing and non-growing bacteria) to cell concentrations were achieved using linear regressions fitting known concentrations of cells to cytometer events.

Cell volume

Bacterial volumes were determined by direct epifluorescent microscopy (Olympus BH2) at a magnification of 1250× using SYBR green (Invitrogen) as the fluorochrome. Cell volume of bacteria was determined from at least 67 cells (range 67 to 515; see Table 1) according to the formula for a sphere or for a cylinder capped by 2 half spheres (Chrzanowski & Grover 2008). Dimensions of individual cells were determined from digital images (Olympus DP70 camera) and imaging software (Simple PCI, Compix).

Calculating growth and ingestion rate

The growth rate of *Ochromonas 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 2 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.

The rate at which bacteria were ingested by *Ochromonas danica* was determined by dividing the number of bacteria consumed (corrected for controls) by the average *O. danica* concentration during exponential growth (corrected for controls) and dividing by the time of a feeding experiment (Heinbokel 1978).

Statistical analysis

Graphical and statistical analyses were performed using Sigma Plot (v11).

RESULTS AND DISCUSSION

Characteristics of bacteria vary with the conditions under which they are grown, such as temperature, growth rate, and nutrient availability (Elser et al. 2000a, Chrzanowski & 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 is likely due to more than one physical or chemical characteristic of bacteria serving as prey, every attempt was made to minimize variability among prey types due to environmental conditions. All bacteria were grown in the same medium and under the same conditions. The predator was grown in chemostat culture at a constant temperature and dilution rate. Similarly, for each feeding trial, predator and prey were combined in similar proportions. The concentration of bacteria was chosen to saturate the ingestion and growth kinetics of *Ochromonas danica* (Grover & Chrzanowski 2009), while the concentration of *O. danica* was sufficient to bring about rapid changes in bacterial abundance. Actual predator and prey concentra-

tions deviated from targeted values: concentrations of bacteria at the start of each feeding trial ranged from 9.20×10^6 to 5.82×10^7 cells ml^{-1} , while the concentration of *O. danica* ranged from 3.35×10^5 to 4.31×10^5 cells ml^{-1} .

Prey characteristics

The 15 bacterial species used as prey (Table 1) included 4 genera of Gram-positive bacteria, 3 within the *Bacilli* and 1 within the *Actinobacteria*, and 11 genera of Gram-negative bacteria spanning 3 classes within the *Proteobacteria* (1 *Alphaproteobacteria*, 3 *Betaproteobacteria*, and 7 *Gammaproteobacteria*). Growth rates and sizes varied substantially among the types of bacteria. The slowest growing bacterium, *Aquaspirillum*, grew with a doubling time of 4.9 h, while the most rapidly growing bacterium, *Citrobacter*, grew with a doubling time of 0.85 h. There was a statistically significant difference (*t*-test, $p < 0.05$) between sizes of growing and non-growing cells, and with the exception of *Microbacterium*, growing cells were larger than non-growing cells. Considering all data, there was a 20-fold difference between the smallest (non-growing *Sphingomonas*, mean $\pm$ standard deviation [SD]: $0.071 \pm 0.036 \mu\text{m}^3 \text{ cell}^{-1}$) and largest (growing *Aeromonas*, $1.355 \pm 0.706 \mu\text{m}^3 \text{ cell}^{-1}$) bacteria offered to *Ochromonas danica*.

Table 1. Differences in cell sizes of growing and non-growing bacteria. Growth rate is for growing cells. Each value is the mean $\pm$ SD of (n) measures. In all cases, there was a statistical difference between the sizes of growing and non-growing bacteria (*t*-test, $p < 0.05$). LS: lab strain, LI: lake isolate, ATCC: American Type Culture Collection

Class	Genus	Source	Growth rate (h^{-1})	Cell size ($\mu\text{m}^3 \text{ cell}^{-1}$)	
				Growing	Non-growing
<i>Alphaproteobacteria</i>	<i>Sphingomonas</i>	LS	0.364	0.178 ± 0.112 (212)	0.071 ± 0.036 (515)
<i>Betaproteobacteria</i>	<i>Aquaspirillum</i>	ATCC 49643	0.141	0.143 ± 0.074 (353)	0.079 ± 0.041 (420)
	<i>Herbaspirillum</i> ^a	ATCC 35892	0.449	0.407 ± 0.492 (182)	0.112 ± 0.057 (436)
	<i>Ralstonia</i>	LS	0.414	0.257 ± 0.163 (154)	0.135 ± 0.069 (302)
<i>Gammaproteobacteria</i>	<i>Aeromonas</i>	LI	0.604	1.355 ± 0.706 (98)	0.277 ± 0.183 (167)
	<i>Citrobacter</i>	LS	0.816	1.024 ± 0.424 (193)	0.104 ± 0.070 (342)
	<i>Escherichia</i>	LS	0.405	0.949 ± 0.481 (171)	0.775 ± 0.399 (67)
	<i>Pseudomonas</i>	LS	0.581	0.244 ± 0.133 (178)	0.094 ± 0.047 (378)
	<i>Pseudomonas</i> ^a	ATCC 17386	0.577	0.693 ± 0.333 (207)	0.158 ± 0.073 (509)
	<i>Salmonella</i>	LS	0.558	0.962 ± 0.220 (123)	0.220 ± 0.101 (261)
	<i>Stenotrophomonas</i>	LI	0.500	0.479 ± 0.176 (141)	0.103 ± 0.046 (441)
<i>Bacilli/Actinobacteria</i>	<i>Bacillus</i>	LI	0.644	0.778 ± 0.329 (143)	0.361 ± 0.166 (157)
	<i>Listeria</i>	LS	0.427	0.214 ± 0.107 (276)	0.134 ± 0.062 (324)
	<i>Microbacterium</i>	LS	0.314	0.124 ± 0.061 (420)	0.309 ± 0.188 (181)
	<i>Staphylococcus</i>	LS	0.556	0.327 ± 0.187 (119)	0.254 ± 0.131 (83)

^a*Herbaspirillum seropedicae* and *Pseudomonas fluorescens* were the known genus and species of this group

Factors affecting growth and ingestion

Ingestion as a function of prey growth state and size

Ochromonas danica ingested 14 of 15 types of growing bacteria and 12 of 15 types of non-growing bacteria. *Microbacterium* was the only bacterium that the flagellate did not consume. Of the growing bacteria that were consumed, ingestion rates ranged between 1.3 ± 0.1 (*Listeria*) and 24.3 ± 4.4 (*Salmonella*) bacteria protozoan⁻¹ h⁻¹. Non-growing *Aquaspirillum* and *Bacillus* were not consumed, while ingestion rates of other non-growing bacteria ranged between 0.9 ± 1.5 (*Listeria*) and 17.6 ± 1.2 (*Ralstonia*) bacteria protozoan⁻¹ h⁻¹.

When the rate at which a growing bacterium was ingested was compared to the rate at which the same but non-growing bacterium was ingested, there was a significant difference (*t*-test, $p < 0.05$) between ingestion rates for 11 of 15 paired experiments (Table 2). In 8 experiments, higher ingestion rates were found when *Ochromonas danica* preyed upon growing cells, while in 3 experiments, higher ingestion rates were found when *O. danica* preyed upon non-growing cells. Of the 8 experiments in which higher ingestion rates were found for growing cells, 2 were significant because non-growing cells were not ingested at all. Given the distribution of the data, there was no significant difference (*z*-test, $p > 0.05$) between rates at which *O. danica* ingested growing or non-growing bacteria. Furthermore, it appears

that *O. danica* is an indiscriminate predator: 93 % of bacteria presented as potential prey were ingested, either as growing or non-growing, and these bacteria, with the exception of the single member of the *Actinobacteria*, span the phylogenetic diversity of bacteria offered as prey.

Ingestion rate was a function of cell size (Fig. 1). Non-growing bacteria were characterized by a narrow range in size (most $< 0.4 \mu\text{m}$), while sizes of growing bacteria were more broadly distributed. The ingestion rate of all bacteria could be described by a non-linear regression model that revealed higher rates of ingestion of bacteria having volumes ranging between 0.6 and $1.0 \mu\text{m}^3$ (quadratic model; $r = 0.35$, $n = 90$, $p < 0.01$, where b , the coefficient describing curvature, was significant at $p < 0.05$). This pattern was clearly driven by the size distribution of growing bacteria (quadratic model; $r = 0.55$, $n = 45$, $p < 0.001$, where b was significant at $p < 0.001$).

Predator fitness as a function of prey growth state and size

Even though most bacteria, growing or not, were ingested by *Ochromonas danica*, there was no relationship between ingestion rate and subsequent growth rate of *O. danica*. This was true if data were considered as pairs of growing and non-growing bacteria (Table 2) or collectively (Fig. 2). When the

Table 2. *Ochromonas danica*. Rate at which growing and non-growing bacteria were ingested by *O. danica* and its subsequent growth rate on each type of prey. Each value is the mean $\pm$ SD of triplicate feeding trials. *: significant difference (*t*-test, $p < 0.05$) in the ingestion rate or growth rate when preying on each type of bacterium

Class	Genus	Ingestion rate (bacteria protozoan ⁻¹ h ⁻¹)		Growth rate (h ⁻¹)	
		Growing	Non-growing	Growing	Non-growing
Alphaproteobacteria	<i>Sphingomonas</i>	$20.4 \pm 0.6^*$	8.9 ± 3.1	0.064 ± 0.040	0.078 ± 0.047
Betaproteobacteria	<i>Aquaspirillum</i>	$3.2 \pm 1.1^*$	0	-0.041 ± 0.030	-0.097 ± 0.036
	<i>Herbaspirillum</i>	9.9 ± 0.4	12.1 ± 1.6	-0.005 ± 0.009	$-0.138 \pm 0.059^*$
	<i>Ralstonia</i>	11.7 ± 2.5	$17.6 \pm 1.2^*$	-0.004 ± 0.048	-0.079 ± 0.019
Gammaproteobacteria	<i>Aeromonas</i>	4.3 ± 1.3	$14.5 \pm 5.0^*$	$0.139 \pm 0.052^*$	0.048 ± 0.021
	<i>Citrobacter</i>	$17.3 \pm 2.1^*$	9.5 ± 2.5	$0.144 \pm 0.037^*$	-0.055 ± 0.018
	<i>Escherichia</i>	14.2 ± 1.1	12.1 ± 1.6	$0.081 \pm 0.023^*$	-0.005 ± 0.017
	<i>Pseudomonas</i>	5.3 ± 0.5	$9.6 \pm 1.8^*$	0.124 ± 0.024	0.083 ± 0.035
	<i>Pseudomonas</i>	$9.6 \pm 0.7^*$	3.9 ± 0.5	0.114 ± 0.024	0.121 ± 0.042
	<i>Salmonella</i>	$24.3 \pm 4.4^*$	6.9 ± 1.9	0.103 ± 0.041	0.075 ± 0.032
	<i>Stenotrophomonas</i>	$18.1 \pm 0.6^*$	15.5 ± 1.0	0.118 ± 0.069	0.213 ± 0.067
Bacilli/Actinobacteria	<i>Bacillus</i>	$8.9 \pm 0.6^*$	0	0.074 ± 0.045	0.012 ± 0.056
	<i>Listeria</i>	1.3 ± 0.1	0.9 ± 1.5	0.064 ± 0.052	0.119 ± 0.014
	<i>Microbacterium</i>	0	0	0	0
	<i>Staphylococcus</i>	9.1 ± 0.5	$13.5 \pm 1.0^*$	0.026 ± 0.050	0.015 ± 0.061

Fig. 1. Ingestion rate of bacteria as a function of bacterial size. Relationship when (a) all bacteria are considered, (b) only those bacteria that were growing, and (c) only those bacteria that were non-growing. Attributes describing non-linear regression models are given in each panel where appropriate

growth rate achieved by *O. danica* when preying on a growing bacterium was compared to the growth rate achieved when preying on the same

Fig. 2. *Ochromonas danica*. Growth rate as a function of the rate at which bacteria were ingested. See Fig. 1 for panel descriptions

non-growing bacterium, there were no significant differences (*t*-test, $p < 0.05$) between growth rates for 11 of 15 paired experiments (Table 2). In 3 of the remaining experiments, higher growth rates were found when *O. danica* preyed upon growing

Fig. 3. *Ochromonas danica*. Growth rate as a function of bacterial size. See Fig. 1 for panel descriptions. Attributes describing linear regression models are given in each panel where appropriate

bacteria, while in 1 experiment, higher growth rates were found when *O. danica* preyed upon a non-growing bacterium. Similarly, no correlations could be found between ingestion rate and growth rate when all data were considered collectively

(Fig. 2; All) or when considered by prey growth state (Fig. 2; Growing or Non-growing). Thus, prey growth state does not contribute consistently to the fitness of *O. danica*.

While *Ochromonas danica* appears to preferentially ingest bacteria ranging in size from 0.6 to 1.0 μm^3 , bacteria from all size categories were ingested, and there was no relationship between the rate at which bacteria were ingested and the subsequent growth rate of *O. danica*. However, the growth rate of *O. danica* correlated with the size of prey ingested: larger prey produced more rapid growth (Fig. 3; All, $r = 0.37$, $n = 90$, $p < 0.01$). This relationship was driven by the range in sizes of growing bacteria (Fig. 3; Growing, $r = 0.58$, $n = 45$, $p < 0.001$), as was the relationship between prey size and ingestion rate, and suggests that *O. danica* may more efficiently utilize nutrients captured from large, growing prey than from small prey.

Ochromonas danica responds rapidly to prey nutritional quality by varying both ingestion and growth rate (Grover & Chrzanowski 2009). Low growth rates were found for *O. danica* when it preyed upon bacteria grown under P-limitation. Rapidly growing bacteria are richer in P than slower-growing bacteria (Elser et al. 2000a, Chrzanowski & Grover 2008); thus, we speculate that large, rapidly growing prey may supply *O. danica* with sufficient P to support rapid growth. Further studies addressing the element content of growing and non-growing prey will be necessary to address this hypothesis.

Ingestion and growth as a function of prey type

It has been implied, assuming that prey are digestible and non-toxic, that predator growth rate increases as a function of ingestion rate (Jezbera et al. 2005, Salcher et al. 2007, Grover & Chrzanowski 2009, Gruber et al. 2009). Intuitively, this seems logical, but there appears to be a cost to a predator to form phagosomes and/or to capture and ingest certain prey types (Pernthaler 2005). Fig. 4 depicts the rate at which *Ochromonas danica* ingested bacteria as a function of prey type and its subsequent growth on those prey. In these analyses, Gram-positive bacteria were grouped to create a cluster of 4 species (3 *Bacilli* and 1 *Actinobacteria*). The *Alphaproteobacteria* contained a single species, the *Betaproteobacteria* contained 3 species, and the *Gammaproteobacteria* contained 7 species. There were significant differences among rates at which types of bacteria were ingested (ANOVA, $p < 0.001$ for All, Growing and

Fig. 4. *Ochromonas danica*. Rate of ingestion of bacteria of different phylogenetic groups and its subsequent growth on those prey types. Alpha: *Alphaproteobacteria*, Beta: *Betaproteobacteria*, Gam: *Gammaproteobacteria*, Bac-Act: *Bacilli* and *Actinobacteria*. Bars represent the mean ingestion or growth rate, and error bars are the standard deviation. Minimum n = 3

Non-growing) and among the subsequent rates of growth (ANOVA, $p < 0.001$ for All, Growing and Non-growing). Similar trends were found when data were grouped or considered by growth state.

When data were considered collectively, ingestion of members of the *Bacilli/Actinobacteria* group occurred at rates significantly lower than the rates at which members of the *Alpha*- and *Gammaproteobacteria* were ingested (Tukey test, $p < 0.05$). There were no significant differences among the rates at which members of the *Proteobacteria* were ingested (Tukey test, $p > 0.05$). The ecological fitness of *Ochromonas danica* could not be predicted from ingestion rates. Predation on members of the *Bacilli/Actinobacteria* group resulted in growth rates significantly lower (Tukey test, $p < 0.05$) than those obtained when the flagellate preyed upon members of the *Gammaproteobacteria*. Members of the *Betaproteobacteria* were readily ingested but always resulted in a significant decline in predator fitness (Tukey test, $p < 0.05$). The mortality of *O. danica* while preying upon *Betaproteobacteria* did not result from egestion of bacteria from food vacuoles. Flow cytometer scatter plots clearly revealed a decline in the abundance of bacteria during feeding trials, indicating that bacteria were ingested and processed by *O. danica*. This was not the case with some bacteria that were clearly not ingested (e.g. *Microbacterium*).

There were only minor differences in the patterns describing ingestion and growth rates when data grouped by growth state were compared to all data (compare all panels in Fig. 4). The most apparent difference is that the predator's ecological fitness was generally lower when preying on non-growing bacteria than when preying upon growing bacteria (but see earlier discussion).

There are 5 major phyla of bacteria common to freshwater systems: *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, *Cyanobacteria*, and *Verrucomicrobia* (Newton et al. 2011). Members of the *Proteobacteria* (classes *Alphaproteobacteria*, *Betaproteobacteria*, and *Gammaproteobacteria*) are of interest due to their cosmopolitan distribution and their interactions with flagellate predators. In the absence of predators, the *Alphaproteobacteria* and *Betaproteobacteria* rapidly increase in abundance, while other members of the community do not (e.g. *Actinobacteria*) (Šimek et al. 2003, Salcher et al. 2007, Tarao et al. 2009). In the presence of predators, the *Alphaproteobacteria* and *Betaproteobacteria* do not undergo dramatic changes in abundance, suggesting that mortality balances production. *Betaproteobacteria* in general appear to be very susceptible to predation and readily consumed (Jezbera et al. 2005, Salcher et al. 2007).

It seems logical to conclude that *Betaproteobacteria*, and other bacteria ingested at high rates, are important links in the trophic structure and support fit-

ness of their predators. However, *Ochromonas danica* failed to grow or grew poorly when preying upon *Betaproteobacteria* even though these bacteria were readily consumed. The 3 types of *Betaproteobacteria* used in the present study were a small selection of this class, but given their typical habitats (*Aquaspirillum*, aquatic; *Herbaspirillum*, plants and soil; *Ralstonia*, broad distribution), the results suggest that some feature of the *Betaproteobacteria* makes them poor quality food for flagellate predators.

Experimental approaches (as in the present study) addressing predator choice of prey cannot capture the complexity of natural systems. Aquatic bacterial assemblages are not stable over time; community structure shifts in response to changes in nutrient availability and to environmental drivers (Yannarell et al. 2003, Kent et al. 2004, 2006, Yannarell & Triplett 2004). A given flagellate confronts a huge and changing diversity of prey (both in growth state and phylogenetically). The data presented here revealed that different types of bacteria have different nutritional value to a consumer. If other flagellate predators of bacteria respond to specific bacteria in a similar manner as *Ochromonas danica* responded to *Betaproteobacteria*, then it would seem that trophic transfer efficiency and nutrient regeneration depend on the diet-breadth of predators, particularly when the bacterial community shifts to one dominated by organisms that do not support predator fitness.

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