Effect of Bacterial Quality and Density on Growth and Whole Body Stoichiometry of *Culex quinquefasciatus* and *Culex tarsalis* (Diptera: Culicidae)

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ABSTRACT Growth characteristics and whole body carbon (C), nitrogen (N), and phosphorus (P) concentrations were examined for the southern house mosquito, *Culex quinquefasciatus* Say, and *Culex tarsalis* Coquillett, reared on chemostat-grown bacteria, *Pseudomonas aeruginosa*. Whole body percentage of C, N, and P of *Cx. quinquefasciatus* larvae did not differ significantly across three bacterial concentrations (1, 5, and 10 mg of dry mass/liter) and two bacterial quality treatments (culture medium containing 5 μM P versus 50 μM P); whereas the P content of *Cx. tarsalis* larvae differed between the bacterial quality treatments. Low concentrations of high or low P bacteria decreased mass-specific growth rate (MGR), whereas intermediate and high bacterial concentrations affected MGR asymmetrically, depending on species. High concentrations of P-rich bacteria enhanced the growth rates of *Cx. quinquefasciatus* larvae relative to growth on the low P diets. *Cx. tarsalis* larvae reared on low P bacteria grew $\approx$3- to 4 times faster than larvae reared on high P bacteria. The observed asymmetric response in MGR may have been because of differential tolerance in larvae to putative toxins present in *P. aeruginosa* and may provide one reason why *Cx. tarsalis* larvae are not found in hypereutrophic aquatic habitats.

KEY WORDS *Culex quinquefasciatus*, *Culex tarsalis*, ecological stoichiometry, larval mosquito nutritional physiology

DISPARITIES IN ELEMENTAL COMPOSITION between animals and their food can affect individual consumer growth, consumer population dynamics, and community structure (Elser et al. 2000a). The growth rate hypothesis (GRH) proposes that rapid growth and whole body phosphorus content of many organisms are highly correlated (Elser et al. 2000b, c). A key concept of ecological stoichiometry that relates the consumer GRH to resource carbon:nitrogen:phosphorus (C:N:P) stoichiometry is consumer elemental homeostasis (Kooijman 1995). Homeostasis is observable as the unchanging pattern of elemental composition of heterotrophic consumers relative to variation in elemental composition of their food resources.

Homeostatic ability varies greatly among organisms and may range from strong (or strict) homeostasis in bacteria (Goldman et al. 1987a, Makino et al. 2003) and zooplankton (Urabe and Wantanabe 1992) to extremely weak (nonstrict) in algae (Rhee 1978). The degree of organismal elemental homeostasis varies depending on the elements in question. Although bacterial C:N seems to vary only slightly independent of the condition of growth (Nagata 1986, Lee and Fuhrman 1987, Goldman et al. 1987a), C:P and N:P may be extremely variable depending on the bacterial strain and C, N, and P content of the bacterial nutrient solution. N:P ranged from 10 to 27 for *Pseudomonas fluorescens* (Chrzanowski and Kyle 1996) and from 16 to 25 for a bacterial assemblage isolated from a eutrophic lake in Japan (Nakano 1994).

Theoretical models and empirical studies encompassing the elemental composition and nutrient requirements of bacterioplankton and their predators have not advanced to the same levels as for the phytoplankton–zooplankton trophic link because the elemental requirements of the bacterioplankton are not as well known (Nagata 1986, Lee and Fuhrman 1987, Martinussen and Thingstad 1987, Goldman et al. 1987b, Vadstein and Olsen 1989, Tezuka 1990, Nakano 1994). Early studies addressing the ecological stoichiometry of bacterial predators (bacteriovores) focused on P (Anderson et al. 1985, Bloem et al. 1988, Jurgens and Gude 1990) or N (Sherr et al. 1983, Anderson et al. 1985, Sherr et al. 1988) cycling (i.e., nutrient regeneration) through food webs. Goldman et al. (1987b) noted that N and P regeneration by a flagellate protozoan was low when it was feeding on N- and P-limited algae. Jurgens and Gude (1990) reported that P regeneration by bactivorous protozoans was lowered when the bacterial prey were cultured under P-limited conditions. Thus, nutrient regeneration by

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bactivorous protozoans may be related to the C:N:P ratio of their prey and the bactivore's stoichiometric nutrient requirements.

The associations of whole body C:N:P, whole body growth rate, and food C:N:P have been documented in planktonic crustaceans (Elser et al. 2000a, Sterner and Elser 2002) but remain largely unexplored in insects. Stoichiometric relationships and their effect on growth rate have been explored recently for algae and a boreal mayfly (Frost and Elser 2002) and plant resource and a desert weevil (Schade et al. 2003). Although growth rate was not examined, Markow et al. (1999) studied the elemental stoichiometry of several *Drosophila* species, along with their natural foods. There are no published accounts of larval mosquito whole body stoichiometry nor of larval mosquito food stoichiometry and its effect on mosquito growth rate.

We investigated the ecological stoichiometry of two mosquito species that tend to use larval habitats in southern California differing in resource abundance and P availability. The southern house mosquito, *Culex quinquefasciatus* Say, is often found in highly enriched habitats containing high levels of comparatively P-rich seston, whereas *Culex tarsalis* Coquillett is frequently found in less enriched habitats containing comparatively low P seston (Bohart and Washino 1978, Peck and Walton 2005). Stoichiometric theory predicts that for a particular sufficient food density or concentration such that C and N are not limiting and food of relatively high P content, consumers with comparatively high P content in their bodies will grow faster than organisms with low P content (Main et al. 1997, Elser et al. 2003). Stoichiometric theory also predicts that given sufficient food such that C and N are not limiting, consumers grown on low P foods will have reduced growth rates compared with growth on high P foods. We tested these predictions by comparing the larval whole body stoichiometry (C, N, and P content) of two *Culex* species grown on bacteria of low and high P content under three bacterial concentrations (dry mass [DM] of bacteria per liter) and by comparing larval mosquito growth at two P levels under controlled conditions in the laboratory. To our knowledge, this is the first study to examine the effects of changing bacterial C:N:P and bacterial density on a comparatively large bacteriovore mosquito larvae.

Materials and Methods

Bacteria. Although larval mosquito diets are a complex mix of microorganisms and detritus (Merritt et al. 1992), our aim to manipulate the stoichiometry of larval food was best achieved by using a single organism common in larval habitats. Because bacteria are considered an important larval food source (see references in Merritt et al. 1992), we used pure cultures of an aquatic bacterium that is commonly found in *Culex* larval habitats (Hoadley 1977) as our exclusive larval food.

Pseudomonas aeruginosa (ATCC #9027) was obtained in freeze-dried pellets and served as the exclusive food of mosquito larvae. *P. aeruginosa* was rehydrated on nutrient agar plates, incubated at 25°C until colonies were visible, and held for up to 1 mo at 4°C. Colonies were recultured axenically onto fresh agar plates monthly.

Bacterial Culturing. To provide food of two qualities, *P. aeruginosa* was grown in semicontinuous chemostats by using two types of modified Tezuka medium (MTM) (Chrzanowski and Kyle 1996) with P as NaH_2PO_4 added to produce conditions of abundant P (50 μM P) or limited P (5 μM P) with trace minerals added (chemical, concentration in milligrams per liter; Na Fe-EDTA, 7.0; $CuSO_4$, 0.4; $MnSO_4$, 1.4; and $ZnSO_4$, 1.4). All MTM ingredients were mixed with double-distilled water and sterilized in an autoclave for 30 min (120°C at 16 psi). Inoculum of agar-plated *P. aeruginosa* and sterile MTM was introduced into 1-liter (high P treatment) or 4-liter (low P treatment) Erlenmeyer flasks that served as semicontinuous chemostats. The 4-liter volume was needed because low P MTM produced one-fourth the number of bacterial cells compared with high P MTM. Each day 90% of the chemostat volume was drained and reserved, and the chemostat was refilled to its initial volume with sterile medium. Chemostats were held in Percival growth chambers (23 ± 1°C [mean ± SD], photoperiod of 16:8 [L:D] h). Bacterial cultures were stirred by constant aeration with 0.2-μm-pore filtered room air and vented through a 0.2-μm pore filter.

Chemical Composition of Bacteria. Every other day while larvae were being fed pure bacterial cultures, subsamples of *P. aeruginosa* cells were collected from chemostat product onto precombusted (450°C for 30 min) glass-fiber filters (Whatman GF/F, nominal retention 0.7 μm) and dried at 50°C. C and N content for dry bacteria on filters was determined using a CHN analyzer (model CE-440, Exeter Analytical, North Chelmsford, MA). For P analysis, subsamples of *P. aeruginosa* were collected on prewashed (24 h in double-distilled water at one filter per 100 ml) glass-fiber filters (Whatman GF/F, nominal retention 0.7 μm) and dried at 50°C. Phosphorus content for dry bacteria on filters was determined using the molybdenum-ascorbate method (APHA 1995). Elements are reported as percentage (mass/mass) or as ratios (mole:mole).

Mosquitoes. Egg rafts of *Cx. quinquefasciatus* were collected at the University of California Aquatic Research Facility (Riverside, CA) during spring 2003 by using 2-gal plastic tubs filled with a solution of Brewer's yeast and alfalfa pellets (1:1 ratio; ≈5 g/liter). Egg rafts of *Cx. tarsalis* were collected from gravid females caught in CO_2-baited suction traps placed near the Multipurpose Wetlands Research and Demonstration Project at Eastern Municipal Water District's (EMWD) Hemet/San Jacinto Regional Wastewater Reclamation Facility in San Jacinto, CA, during spring 2003. Larvae from initial egg rafts were raised to adults under standard laboratory conditions; the larval mosquito laboratory diet was a solution of 3 parts ground rat chow to 1 part Brewer's yeast (vol:vol) applied to 2-liter rearing pans at 2 ml/d. Adult females were allowed to blood feed on restrained chicks (the pro-

cedures applicable to chickens used as hosts were carried out under Protocol A-M 0104040 approved by the Institutional Animal Care and Use Committee of the University of California, Riverside) and then oviposit under insectary conditions (27°C and a photoperiod of 16:8 [L:D] h with 1 h of dawn/dusk, adult males and females given sugar water and wet raisins). Both species were reared through two generations before beginning the experiment.

Growth Experiments. Each day during growth experiments, *P. aeruginosa* cells were collected from the chemostat between 0800 and 1000 hours, centrifuged (25 min at 5,000 rpm at 4°C), and resuspended in basal MTM prepared with KCl, $MgSO_4(7H_2O)$, and $CaCl_2(2H_2O)$ at their normal concentration, and NaCl at 37 mg/liter. Subsamples of this resuspended culture were collected on preweighed polyproplyene membrane filters (Supor-200 membrane filter, diameter 47 mm, pore size 0.2 μm, Gelman Science, Ann Arbor, MI), dried at 50°C for 2 h, and weighed to ±0.1 mg. Dry masses of bacteria per liter (milligrams of DM per liter) in resuspended culture medium were calculated daily from two replicate loaded filters. Appropriate dilutions of resuspended culture with basal MTM then yielded the desired concentrations for growth experiments. Mosquito larvae were reared in three *P. aeruginosa* concentrations: 1, 5, and 10 mg DM/liter. Thus there were six treatment groups for each mosquito species: two bacterial quality levels (high and low P) and three bacterial concentrations.

Mosquito larvae were reared to the second instar in three 2-liter enamel rearing pans seeded with three egg rafts per pan, and fed the larval mosquito lab diet described above. Three-day-old *Cx. quinquefasciatus* or 4-d-old *Cx. tarsalis* larvae were transferred singly from rearing pans into plastic cups holding 20 ml of the bacterial treatment of interest. This was done to have approximately uniform initial weights and individuals of sufficient size for determining those weights. Cups with larvae were stored in plastic boxes in a Percival growth chamber (23°C and a photoperiod of 16:8 [L:D] h). Plastic boxes were shifted daily to control for temperature or light gradients within the growth chamber. Cups within a treatment group were replicated 60 times.

Larval mortality was noted daily and surviving larvae were transferred to cups containing fresh medium. Mortality was defined as total lack of movement of a larva after prodding. Growth experiments were terminated when mortality levels in any treatment group reached 50% (9 d posthatch for *Cx. quinquefasciatus* and 8 d posthatch for *Cx. tarsalis*). We chose 50% mortality as a stopping point so there would be sufficient larvae for elemental analysis. Larvae were usually in third instar when rearing was terminated; no pupation occurred.

A mass-specific growth rate (MGR) [$\log_e$(final mass/initial mass) d^{-1}] was calculated for mosquito larvae in the 12 treatment groups. Initial instar dry mass (±1 μg) was determined as the mean of a subsample (n = 63–67; 3 d posthatch for *Cx quinquefasciatus* and 4 d posthatch for *Cx. tarsalis*) of larvae used for the growth experiments. Dry mass (±1 μg) was determined for each surviving larva (9 d posthatch for *Cx. quinquefasciatus* and 8 d posthatch for *Cx. tarsalis*). All larvae were dried to constant mass in a 55°C oven for 24 h and weighed to the nearest microgram by using an electronic microbalance (model M2P, Sartorius Corp., Edgewood, NY).

Dried larvae were pooled within each treatment group and crushed into a fine powder for elemental analyses. C and N content of two subsamples from ground larvae in each treatment was determined using a CHN analyzer (model CE-440, Exeter Analytical). Phosphorus content for larvae was determined using the molybdenum-ascorbate method (APHA 1995). Elements are reported as percentages (mass/mass).

Statistical Analysis. Multivariate analysis of variance (MANOVA) (Number Cruncher Statistical Systems, Hintze 2001) was used to compare percentage of elemental concentrations of bacteria and mosquito larvae among bacterial treatments. Before analysis, elemental percentages were converted to proportions (p) and arcsine square-root transformed. Pillai's trace was used to test for statistically significant differences in elemental composition of bacteria or mosquito larvae among treatments (Tabachnick and Fidell 1996). Model terms significant in MANOVA were investigated further using analysis of variance (ANOVA). A sequential Bonferroni technique (Rice 1989) was used to control type I error rate among comparisons for treatments in the ANOVA.

Table 1. Elemental composition of *P. aeruginosa* grown at two phosphorus concentrations

P treatment	%C		%N		%P	
	Mean	SD	Mean	SD	Mean	SD
High	43.4	1.3	10.2	0.4	2.58	0.23
Low	44.2	4.3	10.1	0.6	1.36	0.31

Data are percentage of elemental composition on a mass basis, n = 10.

Results

Bacterial Stoichiometry. Mean percentage of elemental C, N, and P of *P. aeruginosa* reared in high or low P MTM is displayed in Table 1. Percentage of C, N, and P of *P. aeruginosa* differed significantly between P treatments (Pillai's trace = 0.868; $F_{3,\ 16}$ = 35.28; $P < 0.0005$); however, only P content of *P. aeruginosa* was significantly different between P regimes (ANOVA: $F_{1,\ 18}$ = 98.18; $P < 0.0005$). Mean cellular percentage of P decreased 47% when bacteria were grown on low P medium.

***Culex* Stoichiometry.** The elemental composition (percentage of C, N, and P) of *Cx. quinquefasciatus* and *Cx. tarsalis* differed significantly and differed between P treatments but jointly did not differ significantly among food concentrations or among higher order interactions between main effects (Table 2; full-model MANOVA). Percentage of C, N, and P of *Cx. quinquefasciatus* did not differ significantly between P

Table 2. MANOVA and ANOVA of *Culex* stoichiometry

Analysis	Model	Source	df	Pillai's trace	MS × 10^{-5}	*F*	*P*
MANOVA	Full model	P	3, 10	0.647		6.10	**0.013**
		DM	6, 22	0.629		1.68	0.172
		SP	3, 10	0.902		30.77	**<0.001**
		P × DM	6, 22	0.546		1.38	0.267
		DM × SP	6, 22	0.752		2.21	0.081
		P × SP	3, 10	0.417		2.38	0.131
		P × DM × SP	6, 22	0.659		1.80	0.145
	Cx. quinquefasciatus	P	3, 4	0.713		3.31	0.139
		DM	6, 10	1.08		1.94	0.168
		P × DM	6, 10	1.15		2.27	0.121
	Cx. tarsalis	P	3, 4	0.848		7.42	**0.041**
		DM	6, 10	0.865		1.27	0.351
		P × DM	6, 10	0.629		0.76	0.614
ANOVA	*Cx. tarsalis* %C	P	1		59.57	3.14	0.127
		DM	2		19.31	1.02	0.416
		P × DM	2		1.13	0.06	0.943
		Error	6		18.96		
	Cx. tarsalis %N	P	1		7.00	5.85	0.527
		DM	2		2.58	2.16	0.286
		P × DM	2		3.83	3.20	0.402
		Error	6		1.20		
	Cx. tarsalis %P	P	1		13.55	16.48	**0.007**
		DM	2		1.84	2.24	0.188
		P × DM	2		0.166	0.20	0.822
		Error	6		0.823		

Model factors are SP, species; P, P treatment; DM, dry mass treatment.
Significant *P* values at $\alpha = 0.05$ for MANOVA, and $\alpha' = 0.017$ by sequential Bonferroni technique for ANOVAs are in bold.

treatments, among food concentrations, or for the P treatment by food concentration interaction (Table 2; reduced model MANOVA for *Cx. quinquefasciatus*). P treatment significantly affected the elemental composition of *Cx. tarsalis* (Table 2; reduced-model MANOVA for *Cx. tarsalis*). Percentage of C and N of *Cx. tarsalis* did not differ significantly between P treatments and among food concentrations, but P content of larvae differed significantly between seston P treatments (Table 2; ANOVAs).

Mean N content of *Cx. quinquefasciatus* and *Cx. tarsalis* larvae ranged from 10.0 to 12.4%; mean carbon content ranged from 40.5 to 45.0%, and mean P content of larvae ranged from 1.20 to 1.50% across the 12 treatment groups (Table 3).

MGR. The growth rate responses of *Cx. tarsalis* and *Cx. quinquefasciatus* larvae to changes in bacterial quality and quantity differed appreciably (Fig. 1). Growth was comparatively suppressed for both species regardless of bacterial quality when bacteria were in low supply (1 mg DM/liter). *Cx. tarsalis* grown on a low concentration of low P bacteria grew 4 times faster than when grown on high P bacteria at the same concentration. In contrast to the growth rates of *Cx. quinquefasciatus* larvae reared on high P bacteria that were 1.5 to 3 times higher than for larvae reared on low P bacteria, *Cx. tarsalis* mass-specific growth on low P bacteria was nearly 3 times greater than when larvae were reared on high P bacteria at the intermediate and high concentrations.

All MGR ANOVA model terms were highly statistically significant (Table 4) and are indicative of different responses of the two mosquito species to changes in bacterial quality and bacterial quantity. *Cx. quinquefasciatus* larvae reared on high P bacteria grew faster than did larvae reared on low P bacteria at intermediate and high concentrations, and whereas mass-specific growth of larvae reared on low P bacteria leveled off at 5 and 10 mg of DM *P. aeruginosa* per liter, growth rates of larvae reared on high P food increased directly with bacterial abundance (Fig. 1). *Cx. tarsalis* reared on low P *P. aeruginosa* grew faster than did larvae reared on high P bacteria, regardless of bacterial concentration and bacterial concentrations of 1 mg DW/liter significantly suppressed growth relative to that at the two higher food levels.

Discussion

Although *Culex* larvae exhibited some variation in percentage of C, N, and P between species, between P regime and across bacterial concentrations, the general trend revealed in the stoichiometric analysis of the two *Culex* species is the homeostatic action of general physiological constraints within *Culex* larvae. The result of these constraining mechanisms was seen in the statistical equivalence of whole body percentage of C, N, and P within *Cx. quinquefasciatus* and C and N in *Cx. tarsalis*, regardless of changing bacterial concentration and bacterial P content. Significant differences in percentage of P for *Cx. tarsalis* show that this species is less homeostatic in its P content. Low bacterial density depressed larval growth regardless of bacterial P content or mosquito species, whereas intermediate and high bacterial density had an asymmetrical impact on the specific growth rate of *Culex* larvae, depending on the P treatment. Differential response to putative bacterial toxins may be one explanation for the asymmetric response in *Culex* MGR.

Table 3. *Culex* elemental stoichiometry

P treatment mg DM/liter	*Cx. quinquefasciatus*						*Cx. tarsalis*					
	High			Low			High			Low		
	1	5	10	1	5	10	1	5	10	1	5	10
%C	41.0 (0.34)	41.2 (0.40)	43.1 (0.44)	40.5 (0.04)	41.0 (0.96)	41.7 (0.66)	42.6 (0.88)	43.8 (0.88)	43.7 (1.65)	44.0 (1.10)	44.9 (0.34)	45.5 (0.01)
%N	11.7 (0.19)	10.8 (0.32)	10.0 (0.07)	11.0 (0.24)	11.2 (0.36)	11.5 (0.39)	11.5 (0.07)	11.6 (0.09)	11.9 (0.07)	11.8 (0.09)	12.4 (0.29)	11.9 (0.21)
%P	1.34 (0.035)	1.25 (0.040)	1.27 (0.072)	1.26 (0.028)	1.25 (0.005)	1.20 (0.018)	1.44 (0.094)	1.50 (0.054)	1.41 (0.001)	1.24 (0.012)	1.35 (0.034)	1.27 (0.029)
C:N	4.10 (0.997)	4.44 (0.317)	5.03 (0.082)	4.30 (0.557)	4.26 (0.201)	4.24 (0.347)	4.31 (0.676)	4.40 (0.961)	4.28 (0.275)	4.34 (1.03)	4.24 (0.439)	4.47 (0.389)
C:P	79.1 (18.45)	85.1 (5.86)	87.5 (1.42)	82.7 (10.60)	84.6 (3.94)	89.8 (7.18)	77.0 (9.92)	75.7 (15.3)	80.1 (5.33)	91.1 (21.7)	85.5 (8.96)	92.5 (10.7)
N:P	19.3 (4.72)	19.2 (2.39)	17.4 (0.22)	19.3 (1.93)	19.8 (1.17)	21.2 (2.25)	17.8 (1.85)	17.2 (3.09)	18.7 (0.57)	21.0 (4.74)	20.2 (2.32)	20.7 (2.19)

Data are derived from two subsamples of a homogenate of at least 34 instars from each treatment group (range 34–57) and are displayed as percentage of elemental composition on a mass basis (mean [SE, $n = 2$]) and elemental ratios (E:E) on an atomic basis (mole/mole).

P. aeruginosa percentage of P, C:P, and N:P were greatly influenced by growth medium P content. Cells grown on low P MTM had 48% less P than cells grown on high P MTM. Chrzanowski and Kyle (1996) investigated the P balance in *P. fluorescens* by varying the N:P ratio of the nutrient solution. They determined that *P. fluorescens* as well as several other bacteria from pure and mixed cultures of fresh and marine systems vary widely in their P content, depending on the P concentration of their environment. In our study, *P. aeruginosa* was not homeostatic in P when faced with variation in the P content of its nutrient supply and may consume more P than it needs when P is abundant.

Although this is the first study to examine the C, N, and P stoichiometry of larval mosquitoes, Peck and Walton (2005) recently investigated the C, N, and P stoichiometry of adult *Cx. quinquefasciatus* and *Cx. tarsalis* that had been raised in seston assemblages from natural developmental sites. Percentage of N of adults differed comparatively little from the N composition of larvae in this study; however, there were comparatively large ontogenetic differences in percentage of C and P. Adult *Cx. quinquefasciatus* and *Cx. tarsalis* had on average 5% more C and 1% more P than the larvae reported here. Ontogenetic shifts in N and P stoichiometry have been reported for *Daphnia magna* Straus (McKee and Knowles 1987) and *Drosophilia melanogaster* Meigen (Church and Robertson 1966). Our results for percentage of N follow closely those for *D. magna.* Percentage of P changed little between early instars and adults of *D. melanogaster* (Church and Robertson 1966) and for the two mosquito species in the current study (Peck and Walton 2005, this study). These comparisons should be viewed with caution, because our studies were not explicitly designed to examine variation in *Culex* stoichiometry during ontogeny. Mass-specific growth rates of both species in the same environment in nature are not known.

Comparative studies across taxa (Main et al. 1997, Sterner and Elser 2002) and across growth stages within taxa (Church and Robertson 1966, McKee and Knowles 1987) suggest that whole body N:P ratio changes with the elemental ratios of the food and is negatively correlated with MGR. Wild-caught fruit flies fed laboratory diets with higher P than that found in natural foods responded by increasing their whole body percent P compared with flies reared on laboratory diets without extra P (Markow et al. 1999). This increase in *Drosophila* whole body percent P with increasing P in the food depended on species and ranged from 3% (increase in percent P from 0.91 to 0.94 for *Drosophila nigrospiracula* Patterson and Wheeler) to 19% (increase in percent P from 1.05 to 1.30 for *Drosophila arizonae* Ruiz and Wasserman). When given bacteria with elevated P content, *Cx. tarsalis* larvae responded by increasing their P content. Compared with *Drosophila* spp. investigated by Markow et al. (1999), the larval mosquitoes in this study had greater whole body percent N and percent P, suggesting that the overall N and P needs of larval

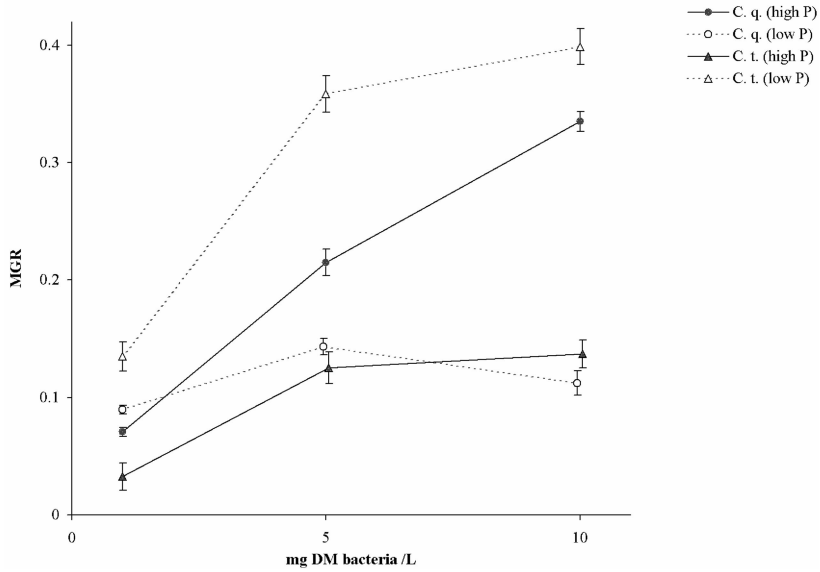

Fig. 1. Mass-specific growth rates for larvae of two *Culex* (C. q., *Cx. quinquefasciatus*; C. t., *Cx. tarsalis*) species grown at different food quality (low versus high P content) and quantity (dry mass bacteria per liter). Error bars are ±1 SE; *n* ranged from 33 to 57. Means are offset to facilitate illustration.

mosquitoes were greater than for *Drosophila* spp. even though N:P ratios in both genera were roughly similar.

The patterns of MGR observed here show that both bacterial density and the P content of bacteria interact to produce complex responses in larval growth. MGR at low bacterial densities was suppressed for both species regardless of the P content of bacteria, whereas MGR increased with increasing bacterial concentration, depending on species and P treatment of larval bacteria (Fig. 1). Growth rate responses similar to those observed in this study have been reported for zooplankton (Sterner et al. 1993) and the general effect of food density on larval mosquito growth has been documented previously for the *Culex pipiens* L. complex (Agnew et al. 2000, Mpho et al. 2000, Wynn and Paradise 2001) and *Cx. tarsalis* (Reisen et al. 1984, 1989, 1997; Smith et al. 1995). Although none of these studies measured larval MGR directly, they show that immature developmental rates decreased with decreasing food density. Although *Cx. quinquefasciatus* and *Cx. tarsalis* larvae sometimes overlap in distribution, *Cx. tarsalis* seems to prefer newly created or perturbated surface pools or irrigated crops, whereas *Cx. quinquefasciatus* exploits a wide range of breeding sites, including peridomestic containers, storm drain systems, and dairy lagoons (Reisen and Reeves 1990). These observed habitat preferences may reflect an inability for *Cx. tarsalis* to tolerate the high levels of bacteria (including *P. aeruginosa*) in sites preferred by *Cx. quinquefasciatus*.

Table 4. Three-way ANOVA of *Culex* mass-specific growth rate

Source	df	MS	*F*	*P*[a]
P	1	0.373	79.96	<0.001
DM	2	1.285	275.77	<0.001
SP	1	0.176	37.85	<0.001
P × DM	2	0.0422	9.06	<0.001
DM × SP	2	0.0396	8.50	<0.001
P × SP	1	2.743	588.48	<0.001
P × DM × SP	2	0.436	93.53	<0.001
Error	522	0.00466		

P, high or low P bacteria; DM, milligrams of dry mass bacteria per liter; and SP, mosquito species.

[a] Significant *P* values at $\alpha' = 0.007$ by sequential Bonferroni technique for ANOVAs.

The contrasting growth responses of larval *Cx. quinquefasciatus* on low P versus high P bacteria suggest that sufficient P is critical to maximization of MGR in this species. Previous work on other arthropods found a similar dependency of MGR on food P. Frost and Elser (2002) studied the growth response of benthic mayflies (*Caenis* sp.) by varying the density and P content of natural assemblages of Canadian lake periphyton and laboratory-grown algae. Mayfly growth was negative for all low-density food treatments, whereas it was positive for the high-density food treatments, and, within high-density food treatments, growth rate was a increasing function of periphyton and algal P content. Schade et al. (2003) studied the interaction between rainfall patterns, available soil P, plant [velvet mesquite, *Prosopsis velutina* (Woot.) Britt. & Rose] resource C:P, and percent P of a desert weevil (*Sabinia* sp.). *Sabinia* abundance was negatively correlated with *P. velutina* leaf C:P, and *Sabinia* was more abundant when soil P was high during a season of favorable rainfall. A review by Elser et al. (2003) of growth rate–stoichiometry relationships of insects, zooplankton, a swimming crab, a copepod, and various bacteria found that organism P content and growth rate were tightly coupled, implying that

growth of a diverse assemblage of organisms is positively correlated with the availability of P in their foods.

The large positive MGR response of *Cx. tarsalis* to low P bacteria at densities of 5 and 10 mg DM/liter (Fig. 1) was opposite to that observed for *Cx. quinquefasciatus* and cannot be explained by relating P content of bacteria and bacterial density to growth rate. The flat MGR response in *Cx. tarsalis* to high P bacteria may have been because of the presence of an antagonistic metabolite of *P. aeruginosa* grown on high P MTM. Jarrell and Kropinski (1982) found that a *P. aeruginosa* strain deficient in cell wall lipopolysaccharides was less virulent to a variety of insect orders than a wild-type strain by a factor of 10^4, suggesting that the lipopolysaccharides play an important role in the pathogenesis of *P. aeruginosa* in insects. When grown under P limitation, *P. fluorescens* switched from production of P-rich lipopolysaccharides to production of ornithine amide lipids lacking P (Minnikin and Abdolrahimzadeh 1974). *P. aeruginosa* cells growing in our P-limited chemostats may have switched from production of lipopolysaccharide to ornithine amide lipids, decreasing their pathogenic effect on *Cx. tarsalis* larvae.

Natural food assemblages of larval mosquitoes are extremely diverse biochemically, and these differences certainly play a role in the growth and development of larval mosquitoes (Merritt et al. 1992). *Cx. quinquefasciatus* is found in enriched habitats (Rutz et al. 1980, Reisen et al. 1991) that have high concentrations of *P. aeruginosa* (Hoadley 1977) and may have evolved a natural resistance to the pathogenic effects of bacteria such as *P. aeruginosa.* In contrast to this, *Cx. tarsalis* is found in comparatively less enriched habitats (Bohart and Washino 1978, Walton et al. 1998) with lower concentrations of *P. aeruginosa* and is less resistant to its growth-suppressing effect. Because the natural assemblage of microorganisms and their biochemical profile is undoubtedly different between larval habitats for these two larval mosquitoes, the differential tolerance to toxic or antagonistic compounds may be correlated with the larval habitat segregation seen in these two species. *Cx. tarsalis* mortality was two-fold greater for larvae reared in undiluted dairy water compared with constructed wetland outlet marsh water (Peck and Walton 2005). Ovipositional preference of *Culex* adults for waters with high concentrations of *P. aeruginosa* (Ikeshoji et al. 1975) may be another factor determining the observed larval distributions.

This study explored the effect of changing bacterial quantity and bacterial quality on early larval stages of *Cx. quinquefasciatus* and *Cx. tarsalis.* The observed asymmetric response in MGR may have been because of differential tolerance in larvae to *P. aeruginosa* cellular lipopolysaccharides. The extent that differential tolerance to putative bacterial toxins influences the spatial segregation of developmental sites for these species remains to be determined. This asymmetry may be resolved in further experiments using other species of bacteria lacking growth-suppressing compounds. Pure cultures of larval foods allowing growth to adulthood and tests of differences in fecundity because of changing food quantity and food quality will offer insight into the effect of larval habitat on larval life history parameters. The impact of variation in larval food quantity and quality on larval growth and development, and its corresponding effect on adult fecundity and survivorship, will ultimately impact such important metrics as vector capacity (Reisen 1989). Further experiments using pure cultures of larval foods should provide useful inferences relating variation in larval food quantity and quality to the probability of disease transmission.

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