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Sex and size matter: ontogenetic patterns of nutrient content of aquatic insects

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Abstract. C, N, and P content were measured across the ontogeny of lotic aquatic insects representing a diversity of life-history characteristics. The relationship between individual mass and nutrient content was used to show ontogenetic patterns of nutrient content by species. Species analyzed for C and N content exhibited a quasihomeostatic pattern across ontogeny. Percent C and %N varied among taxa irrespective of ontogeny, with %C ranging from 47.4 to 56.2% and %N ranging from 9.6 to 11.6%. P content also varied by species but declined nonlinearly across ontogeny and was best represented by a power function. Percent P varied from >7% in 1st-instar *Tabanus* larvae to only 0.34% in adult male *Ambrysus circumcinctus*. Females had more P per unit mass than males in 6 of the 10 species that could be sexed. In the leptophlebiid mayflies, %P increased in mature female nymphs relative to the penultimate developmental class, whereas %P content of males continued to decline to eclosion. Maximum terminal mass by species was the main factor driving the magnitude of change in %P through their ontogeny. Small-bodied, rapidly growing species exhibited the sharpest decline in P content. Nonhomeostatic patterns in %P across ontogeny and between sexes has important implications for population- and community-level dynamics and ecosystem processes. First, small-bodied, high-%P taxa have faster growth rates than larger individuals, which supports one of the predictions of the growth-rate hypothesis (GRH). Second, elemental imbalance between consumers and their food changes across ontogeny, and therefore, nutrient recycling rate by a species changes with population age structure. Last, community structure may reflect nutrient availability in food such that enriched environments are more likely to be dominated by taxa with high growth rates and, thus, relatively high P demand.

Key words: ecological stoichiometry, elemental composition, ontogeny, homeostasis, phosphorus, nutrient patterns, aquatic insects.

Ecological stoichiometry is the study of constraints and consequences of elemental imbalances between consumers and their food (Sterner and Elser 2002). Nutrient imbalances arise because the bodies of herbivores and detritivores are typically more enriched in N and P than the plants and detritus they eat (Sterner and Hessen 1994, Fagan et al. 2002, Cross et al. 2003). The C content of plants and detritus usually is similar except that aquatic plants and algae have lower C content than detritus (Kahlert 1998) and terrestrial plants (Sterner and Elser 2002, Shurin et al. 2006). In contrast, predators are more similar in elemental content to their prey (Sterner and Elser 2002). Patterns of CNP content of consumers and their food provides a basis for understanding the effects of elemental imbalances on growth rates of consumers

(Elser et al. 2003), nutrient recycling (Vanni et al. 2002, Evans-White and Lamberti 2006, Rothlisberger et al. 2008), population dynamics (Andersen et al. 2004, Moe et al. 2005), foodweb organization (Vrede et al. 2004), and community structure and function (Elser et al. 2000, Cross et al. 2005).

The CNP content of lotic macroinvertebrates and their food resources has recently received attention (Cross et al. 2003, Bowman et al. 2005, Evans-White et al. 2005, Liess and Hillebrand 2005, Back et al. 2008, Small and Pringle 2010). Organisms accomplish homeostasis by modifying the quality or quantity of organic matter ingested to maintain adequate supply relative to demand of essential elements required for metabolism, growth, and reproduction (Sterner and Elser 2002). Investigators have implicitly assumed that aquatic macroinvertebrates maintain an approximately constant, or homeostatic, elemental composition across life stages or sizes within species.

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However, the limited number of studies on CNP content of aquatic and terrestrial invertebrates spanning different developmental stages has revealed nonhomeostatic patterns across the ontogeny of species (zooplankton: Andersen and Hessen 1991, Hessen and Lynch 1991, Main et al. 1997, Villar-Argaiz et al. 2002; *Drosophila*: Elser et al. 2006; mayflies: Frost and Elser 2002, Back et al. 2008, Veldboom and Haro 2011). Specifically for insects, P content of 5 species of *Drosophila* larvae decreased with increasing larval development, and each species had differing P content (Elser et al. 2006). P content decreased across the early developmental stages of *Ephemerella* sp. mayflies (Frost and Elser 2002). P content decreased in early developmental stages of *Caenis* sp. and increased in later developmental stages, whereas C and N content were nearly constant across ontogeny (Back et al. 2008). P content also increased in late-stage larvae, pupae, and adults of the caddisfly *Brachycentrus occidentalis* (Veldboom and Haro 2011). However, these investigators compared, at most, the elemental content of several size classes and did not examine individuals across the continuum of ontogeny.

Food elemental content and species development are intimately linked by changing elemental needs across ontogeny. Because elemental content may change across the ontogeny of a species, the severity of elemental imbalance also may change (increase or decrease) assuming constant C, N, and P content of food. This linkage may affect survivorship of specific life-history stages as demonstrated for the copepod *Diatomus clavipes* (Villar-Argaiz and Sterner 2002).

Understanding how P content varies across ontogeny is of particular importance because P deficiency limits growth of invertebrates (Urabe et al. 1997, Sterner and Elser 2002). Furthermore, growth rates are positively correlated with body %P (Elser et al. 1996, 2003, Frost and Elser 2002, Weider et al. 2005) but negatively correlated with adult invertebrate body size (Woods et al. 2004). Phylogeny also may constrain P (Woods et al. 2004) and N (Fagan et al. 2002) content of invertebrates, independent of ontogeny.

Life-history traits, such as reproductive strategy, probably have a strong bearing on P content during ontogeny. P content of invertebrates that produce a single (usually large) batch of eggs (semelparous taxa) may increase as larvae mature. Percent P of somatic tissue is less than that of gametes (especially eggs) in the few taxa examined (Andersen and Hessen 1991, Markow et al. 1999, 2001, but see Færøvig and Hessen 2003). P content of iteroparous taxa (reproduce multiple times, fewer eggs) probably declines as larvae grow because adults can feed and supply

nutrients necessary for maintenance and reproduction. Differences in patterns of P content between iteroparous and semelparous taxa may be because semelparous insect taxa do not feed as adults. Therefore, the burden of P (and C, N, and other elements) acquisition rests entirely on larval feeding. Collectively, these previous studies suggest that sex, reproductive strategy, adult feeding status, trophic level, life-cycle completion time, and metamorphosis type may interact to produce varying patterns in P content across invertebrate ontogeny.

The objectives of our study were to: 1) describe the pattern of %CNP content of aquatic macroinvertebrates across their ontogeny, from egg or 1st instar to mature larva or adult, 2) assess whether nutrient content differs between sexes, and 3) identify consistent patterns (if any exist) in nutrient content related to life-history traits.

Methods

Study area

All insects were collected from 3 to 24 June 2009 from Cowhouse Creek in Coryell County, Texas, USA (lat 31.286122°N, long 97.883994°W). Cowhouse Creek is a tributary of the Brazos River in the Cross Timbers Level III Ecoregion (Griffith et al. 2004). Land cover in the 1180-km² catchment consists of shrubland (43%), grassland (34%), and forest (19%) (King et al. 2009). Stream habitats sampled were riffles, runs, and their margins. Riffles and runs consisted of large areas of shallow gravel and cobble substrates overlying limestone bedrock. Insects were collected using D-nets, kick screens, and by hand-picking insects from rocks. *Corydalus cornutus* eggs were collected from ash tree (*Fraxinus*) leaves overhanging the creek and *Tabanus* sp. eggs were collected from exposed rocks in riffles. The goal was to collect the entire size range of numerically dominant species. Insects were transported to the laboratory and sorted live under a stereomicroscope.

Chemical analyses

Intact whole insects were dried in Al weighing pans at 50°C for ≥48 h and then stored in a desiccator. The bottoms of the weighing pans were covered with paper towel to: 1) prevent insects from sticking to the pan while drying, and 2) provide evidence of fluid leakage from an insect's body that would lead to its exclusion from chemical analysis. Before chemical analyses, insects were redried for ≥24 h at 50°C. Individual insects were weighed on a microbalance (Mettler Toledo XP-26; Mettler-Toledo AG, Greifensee, Switzerland) to the nearest µg. No method was

available to measure simultaneously the C, N, and P content of a single individual. Therefore, C and N content were measured on one set of individuals, and P content was measured on a different set of individuals for each species. Enough material was available to measure C, N, and P content on only 8 taxa, whereas P content alone was measured on 10 additional taxa (18 total).

C and N content were measured simultaneously with a Thermo-Finnegan Flash 1200 elemental analyzer (ThermoQuest, Milan, Italy). Individual insects of each taxon were analyzed when possible. In a few cases, small individuals were combined to achieve the minimum 200 μg of dry mass needed for %C and %N analysis. Whole insects were placed in Sn capsules, gently crushed with a metal spatula, and sealed in the capsule. Standards of L-cystine (30% C and 11.67% N) and an internal standard of *Anax junius* dragonfly nymphs (49.9% C and 10.8% N) were run with samples for quality assurance/quality control (QA/QC). Mean (SD) % recovery of C and N from L-cystine standards ($n = 26$) were 101.5% (1.30) for C and 95.2% (0.63) for N and from *A. junius* standards ($n = 22$) were 101.1% (1.93) for C and 98.5% (0.45) for N.

For P measurement, individual insects were weighed as above, placed in 22-mL glass scintillation vials, pulverized with a metal spatula, capped with a lid containing a Teflon septum, and chemically digested in an autoclave for 1 h at 120°C by the method of Færøvig and Hessen (2003). The minimum mass required for P measurement was 10 μg (assuming 1% P content). For sample masses ≤ 2000 μg , 15 mL deionized (DI) water and 1.8 mL of digestion solution was used. For every 2000- μg increment, an additional 1.8 mL of digestion solution was used in place of 1.8 mL of DI water, up to a 22,000- μg maximum mass digested (19.8 mL digestion solution, no DI water). Individuals that weighed $>22,000$ μg were broken into subunits, digested in multiple vials, and composited after digestion. All samples with masses >2000 μg were diluted back to the ratio of 15 mL DI:1.8 mL digestion solution with DI water. P content was estimated via colorimetry by the ascorbic acid–molybdate method on a Lachat 8500 flow-injection autoanalyzer with an ASX-520 autosampler (Hach Co., Loveland, Colorado). Tissue standards of tomato leaf (SRM 1573a, 0.216% P), and bovine liver (SRM 1577c, 1.175% P) and dissolved inorganic standards were run for QA/QC. To ensure no bias among the 11 runs, a wide range of masses for each taxon was analyzed in ≥ 2 separate runs. The mean (SD, n) % recovery for P was 103.9% (14.3, 45) for tomato leaf, 92.7% (6.1, 26) for bovine liver, and 103.6 (6.5, 176) for inorganic P standards.

Data analyses

Patterns of C, N, and P content.—Percent C, N, and P were plotted as functions of dry mass for each taxon separately. C, N, and P curves were evaluated as linear, exponential, logarithmic, and power functions. The goodness of fit of each curve type was assessed using the r^2 value. Linear regressions were tested for slopes equal to 0 in R (version 2.13.1; R Core Development Team, Vienna, Austria) using the CAR library (Fox and Weisberg 2011). A slope equal to 0 is evidence of elemental homeostasis across ontogeny. Mass and % element data were $\log(x)$ -transformed prior to analysis to meet assumptions of linear models.

Sexual differences in nutrient content.—Mayfly nymphs were grouped into 5 development classes (DC) based on wing-pad development (Taylor and Kennedy 2006) and sexed based on eye development. Other immature insect taxa and earlier DC mayflies could not be sexed. DCs 3, 4, and 5 were defined by wing pads that reached abdominal segment 1, 2, and 3+, respectively (Fig. 1A–C). Females have simple eyes, and males have turbinate eyes (Fig. 1B, C). Adult Coleoptera (*Stenelmis*) and Hemiptera (*Ambrysus*, *Rheumatobates*, and *Rhagovelia*) also were sexed and %P was compared between sexes.

Analysis of covariance (ANCOVA) was used to assess whether change in %P with mass differed between males and females. Mass was the covariate and sex was the categorical factor in each ANCOVA model. Our analysis followed the framework outlined by Engqvist (2005). If the interaction term was significant, then the rate of change of %P differed between males and females (i.e., slopes not equal). The data were analyzed again without the interaction term and the main effects, sex and mass, were examined. The 2 models (significant interaction vs nonsignificant interaction) were then compared with analysis of variance (ANOVA), and a nonsignificant result indicated the simplest model was most appropriate (Engqvist 2005). If the main effects and interaction (sex, mass) were nonsignificant, we concluded that the sexes had the same %P and the regression line had a slope of 0, and thus, %P did not vary with mass. If only mass was significant, the slope was not equal to 0, and %P varied with mass but the sexes had the same %P. If only sex was significant, the sexes had the same slope, but one sex had higher %P than the other and the slope of both regression lines was 0. If both sex and mass were significant, then %P changed with mass and the sexes differ in %P, but the slopes of males and females were equal.

Nutrient content and life-history traits.—To test the hypothesis that smaller taxa had sharper declines in %P

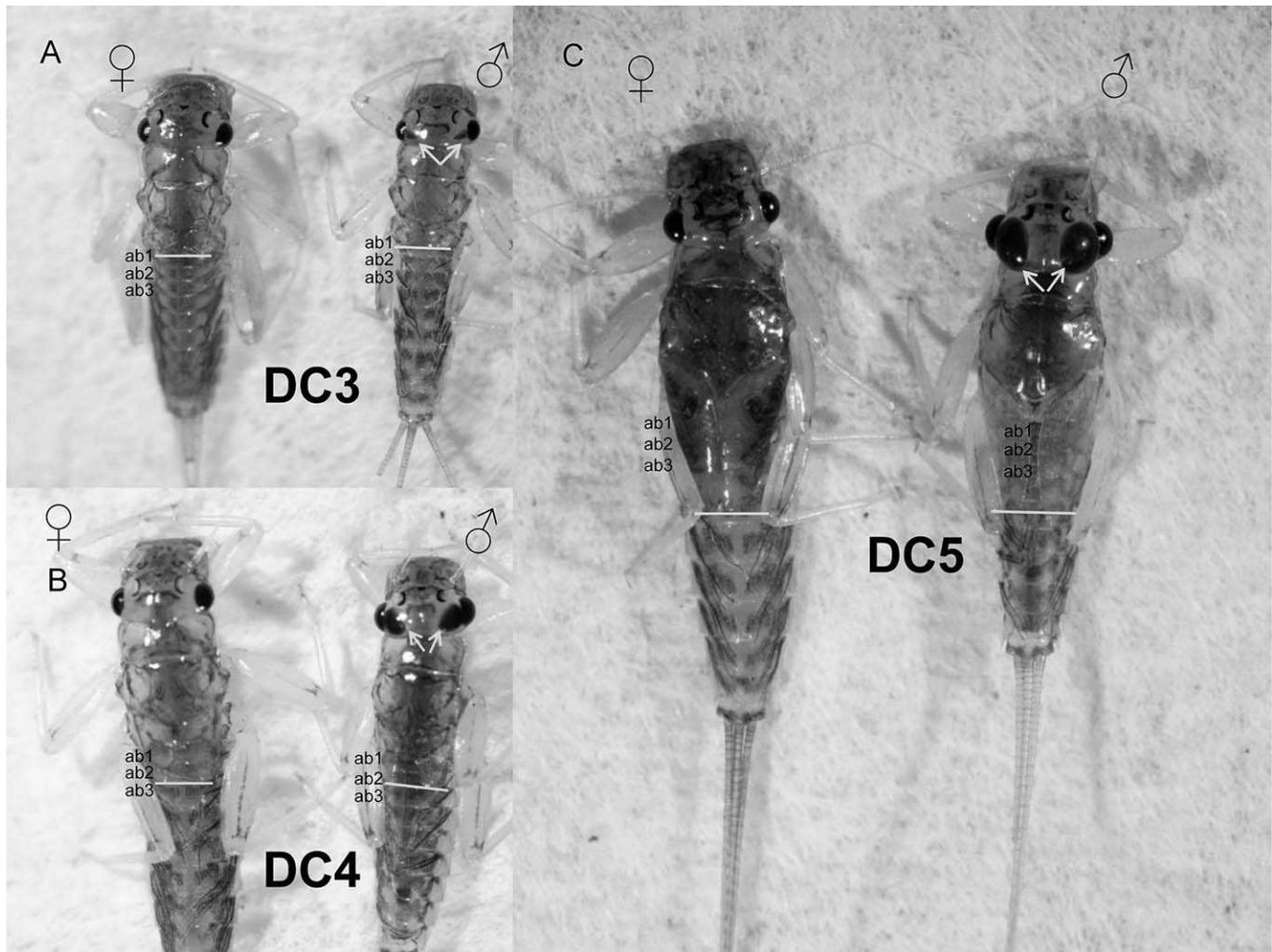


FIG. 1. Early (A), intermediate (B), and late (C) development classes (DC) for the mayfly *Neochoroterpes nanita*. Turbinate eyes of males are indicated by arrows. Horizontal line is the furthest extent of the wing pads. The first 3 abdominal segments are denoted as ab1, ab2, and ab3.

with increasing size, we plotted the maximum size of each taxon against the P decay rate to assess whether life-history traits influenced patterns in %P. Maximum size was a surrogate for life-cycle completion time. Based on the generation-time law (Bonner 1965, Peters 1983), we predicted smaller maximum-sized taxa would have a faster life-cycle completion time than larger maximum-sized organisms. Reproductive strategy, metamorphosis type, functional feeding group (FFG), and taxonomy were coded into the plot to detect whether patterns emerged based on these life-history traits.

Results

Patterns of %CNP content

Linear regression analysis revealed that %C and %N were invariant (i.e., slopes = 0) across the

ontogeny of 6 of the 8 taxa examined. *Ambrysus circumcinctus* had slopes < 0 for %C and N, whereas *Baetodes inermis* had slopes < 0 for %C and *Stenelmis* sp. had slopes < 0 for %N. Mean %C ranged from 47.4 to 56.2%, and no trends relative to phylogeny or FFG were evident (Table 1). Mean %N ranged from 9.6 to 11.6%. Trends within FFG showed qualitatively that predators had the highest %N content, followed by filter feeders, collector-gatherers, and grazers with the lowest %N content (Table 1). The C:N ratio ranged from 4.9 to 6.4 and predators had the lowest C:N ratios (Table 1). *Tabanus* sp. eggs were 10.9% N and 50.8% C.

The relationship between %P and mass was best represented by a power function across all 18 taxa (Fig. 2A–R). Without exception, small individuals had higher %P than larger individuals within species. Variability in larval %P declined as mass increased.

TABLE 1. Results of analysis of covariance (ANCOVA) of slopes of regressions for C and N content of aquatic insects across their ontogeny. ¹ = predator, ² = filter feeder, ³ = collector-gatherer, ⁴ = grazer, SE = standard error, CI = confidence interval.

Taxon	<i>n</i>	Mass range (µg)	Nutrient	Mean % (SE)	Slope	<i>r</i> ²	<i>p</i>	Slope CI (5%, 95%)	C:N
<i>Ambrysus circumcinctus</i> ¹	24	50–15,110	C	50.0 (0.50)	–0.0003	0.3305	0.004	–0.0004, –0.0001	5.0
			N	11.6 (0.12)	–0.00003	0.0935	0.05	–0.000089, -6×10^{-8}	
<i>Rhagovelia choreutes</i> ¹	14	200–1680	C	51.1 (0.60)	0.0009	0.0369	0.5108	–0.002, 0.004	5.2
			N	11.5 (0.15)	0.00003	0.0008	0.9255	–0.0007, 0.0008	
<i>Tabanus sp.</i> ¹	21	740–21,750	C	47.9 (0.50)	–0.0001	0.1744	0.0596	–0.0003, 0.000006	4.9
			N	11.3 (0.10)	–0.00001	0.0225	0.5164	–0.00004, 0.00002	
<i>Chimarra sp.</i> ²	31	170–3710	C	50.0 (0.66)	–0.0007	0.0188	0.4625	–0.002, 0.001	5.3
			N	11.0 (0.17)	–0.0003	0.0609	0.1809	–0.0008, 0.0001	
<i>Stenelmis sp.</i> ³	17	31–750	C	54.0 (1.06)	–0.0022	0.0236	0.5552	–0.01, 0.005	5.7
			N	11.0 (0.29)	–0.002	0.2534	0.0393	–0.004, –0.0001	
<i>Neochoroterpes nanita</i> ³	35	53–3390	C	47.4 (0.55)	0.0004	0.0126	0.2724	–0.0008, 0.002	5.3
			N	10.5 (0.13)	–0.0002	0.0364	0.5209	–0.0004, 0.00013	
<i>Baetodes inermis</i> ³	31	140–1560	C	56.2 (0.61)	–0.0057	0.3328	0.0005	–0.0075, –0.0023	6.4
			N	10.2 (0.13)	–0.0001	0.005	0.99	–0.0006, 0.0006	
<i>Psephenus texanus</i> ⁴	30	370–5070	C	52.8 (0.63)	0.0005	0.026	0.1838	–0.0006, 0.0006	6.4
			N	9.6 (0.13)	–0.0001	0.0648	0.1748	–0.0003, 0.00007	

Eggs (not shown in Fig. 2) were available for analysis for 2 species. *Corydalus cornutus* eggs had a mean %P of 1.84% (*n* = 21, range 0.82–3.99%), which was near the largest observed larval %P value (1.86%) for that species. *Tabanus sp.* eggs had a mean %P of 4.67% (*n* = 13, range 1.90–8.19%). First-instar *Tabanus sp.* larvae reared from eggs had a mean %P of 6.11% (*n* = 4, range 4.67–7.27%).

Sexual differences in nutrient content

Among the mayfly species, %P of all leptophlebiids (*Neochoroterpes nanita*, *Thraulodes gonzalesi*, and *Traverella presidiana*) differed significantly between the sexes (Table 2). However, *T. gonzalesi* and *T. presidiana* differences were mass dependent. Females were more P-enriched, and %P was higher in mature DC 5 nymphs than in DC 4 nymphs (Table 3). In contrast, %P of all swimmer mayfly taxa (*Baetis sp.*, *B. inermis*, and *Isonychia sicca*) did not differ between the sexes and %P declined as mass increased for all these taxa (Table 3). The slope of %P differed between males and females of the hemipterans *Rheumatobates hungerfordi* and *Rhagovelia choreutes*, with %P in females declining less per unit mass than males. Percent P of adult *A. circumcinctus* differed between sexes, and females were P-enriched compared to males. The rate of decline in %P with DC was similar for males and females. Adult *Stenelmis sp.* sexes had the same slope and did not differ in %P content (Table 3).

Nutrient content and life-history traits

No clear patterns in %P were revealed based on life-history traits (Fig. 3A–C). However, a strong

statistically significant linear relationship ($r^2 = 0.60$, $p < 0.001$) between taxon maximum size and P decay rate (i.e., the slope of the curve for each taxon in Fig. 2A–R) was evident. Species maximum size was the main factor driving the magnitude of changes in %P across ontogeny, with small-bodied, rapidly growing species exhibiting the sharpest decline in P content across ontogeny.

Discussion

Patterns of C, N, and P content

The degree to which aquatic macroinvertebrate species change their elemental content across their ontogeny is largely unknown. Our findings show that %C and %N are homeostatic across the ontogeny of 6 of the 8 aquatic insect species examined, and in those with significant variation, the changes were small relative to the changes in %P across the ontogeny of all taxa investigated. Both %C and %N were nonhomeostatic in *A. circumcinctus*, whereas only %C was nonhomeostatic in *B. inermis* and %N in *Stenelmis sp.* (Table 1). Even though the slopes were negative, it is unclear to us if they are biologically significant. Overall, the consistency of the %C and %N pattern suggests that these elements are quasi-homeostatic across the ontogeny of aquatic insects in general. In *Caenis sp.* mayflies, %C increased slightly across size classes, and %N decreased slightly across size classes at 2 sites, results suggesting that %C and %N were more-or-less homeostatic (Back et al. 2008). Percent C increased greatly across the ontogeny of a caddisfly, whereas %N declined gradually

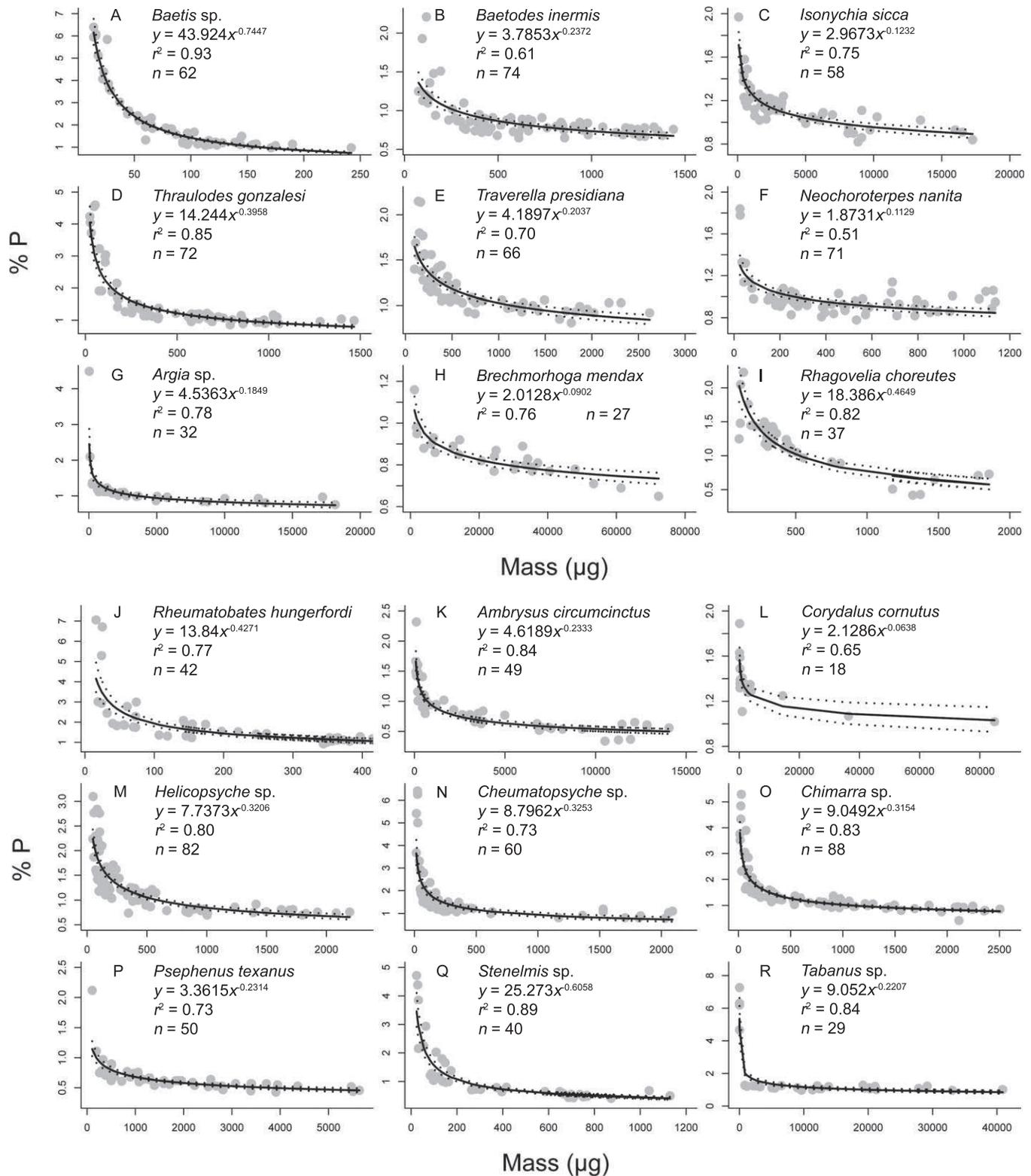


FIG. 2. Relationship between individual mass and %P for *Baetis* sp. (A), *Baetodes inermis* (B), *Isonychia sicca* (C), *Thraulodes gonzalesi* (D), *Traverella presidiana* (E), *Neochoroterpes nanita* (F), *Argia* sp. (G), *Brechmorhoga mendax* (H), *Rhagovelia choreutes* (I), *Rheumatobates hungerfordi* (J), *Ambrysus circumcinctus* (K), *Corydalus cornutus* (L), *Helicopsyche* sp. (M), *Cheumatopsyche* sp. (N), *Chimarra* sp. (O), *Psephenus texanus* (P), *Stenelmis* sp. (Q), and *Tabanus* sp. (R). Raw data were plotted to illustrate the nonlinear pattern of data. Solid line is a regression line, and dotted lines are 95% confidence intervals.

TABLE 2. Results of analysis of covariance (ANCOVA) comparing the relationship between body mass and %P of individuals with respect to sex. ns = not significant, # = $0.1 > p \geq 0.5$, * = $0.5 > p \geq 0.01$, ** = $0.01 > p \geq 0.001$, *** $p < 0.001$.

Taxon	df	Mass	Sex	Mass \times sex
<i>Neochoroterpes nanita</i>	$F_{1,55}$	2.38 ns	4.859*	5.191*
<i>Thraulodes gonzalesi</i>	$F_{1,51}$	125.136***	1.859 ns	28.908***
<i>Traverella presidiana</i>	$F_{1,53}$	138.922***	1.110 ns	7.276**
<i>Baetis</i> sp.	$F_{1,53}$	74.082***	3.475 ns	0.854 ns
<i>Baetodes inermis</i>	$F_{1,53}$	12.928***	2.516 ns	0.059 ns
<i>Isonychia sicca</i>	$F_{1,38}$	59.326***	2.931 ns	1.468 ns
<i>Rheumatobates hungerfordi</i>	$F_{1,12}$	11.285**	8.155*	3.491#
<i>Rhagovelia choreutes</i>	$F_{1,4}$	54.281**	52.584**	13.963*
<i>Ambrysus circumcinctus</i>	$F_{1,6}$	6.162*	30.565***	0.861 ns
<i>Stenelmis</i> sp.	$F_{1,6}$	0.399 ns	0.383 ns	1.075 ns

(Veldboom and Haro 2011). The increase in %C in the study by Veldboom and Haro (2011) is probably related to the fact that *B. occidentalis* is univoltine, overwinters as mature larvae, and pupates in early spring. Therefore, fat reserves are needed to fuel metamorphosis during pupation and respiration of the adult in the pupal chamber.

Percent C, %N, and C:N changed relatively little across the ontogeny of species in our study. Thus, our results were comparable to values from studies in which only terminal or homogenized life stages were examined (Frost et al. 2003, Evans-White et al. 2005, Liess and Hillebrand 2005, Lauridsen et al. 2012). In our study, the only pattern in %C was that taxa with

sclerotized or armored integuments had greater mean %C than taxa with membranous integuments (Table 1). The slightly higher %N and low C:N of predators relative to other FFGs in our study also agrees with results of other studies (Fagan et al. 2002, Evans-White et al. 2005, Hambäck et al. 2009). The %C and %N of *Tabanus* sp. eggs were similar to %C and %N of larvae, further suggesting ontogenetic homeostasis with respect to C:N.

In contrast, P clearly was not homeostatic across ontogeny. Thus, comparison of our %P data with literature values is difficult. However, %P of late-DC individuals in our study was similar to values reported for mature individuals in other studies

TABLE 3. Mean mass (n) and %P (SE) of male and female mayfly nymphs and adult Hemiptera and Coleoptera.

Taxon	Development class	Mass (μ g)		%P	
		Males	Females	Males	Females
<i>Neochoroterpes nanita</i>	3	235 (13)	308 (13)	0.96 (0.02)	0.96 (0.02)
	4	412 (4)	469 (8)	0.87 (0.02)	0.91 (0.03)
	5	669 (6)	900 (15)	0.87 (0.03)	0.97 (0.02)
<i>Thraulodes gonzalesi</i>	3	294 (10)	374 (8)	1.40 (0.06)	1.19 (0.04)
	4	635 (5)	713 (8)	1.08 (0.05)	1.05 (0.03)
	5	831 (12)	1009 (12)	0.99 (0.03)	1.10 (0.04)
<i>Traverella presidiana</i>	3	310 (17)	402 (10)	1.32 (0.05)	1.18 (0.04)
	4	560 (5)	948 (8)	1.14 (0.05)	0.97 (0.03)
	5	1492 (10)	2019 (7)	0.96 (0.02)	0.99 (0.02)
<i>Baetis</i> sp.	3	81 (4)	76 (11)	1.92 (0.20)	1.93 (0.09)
	4	108 (4)	101 (12)	1.16 (0.02)	1.29 (0.06)
	5	153 (9)	135 (7)	1.10 (0.03)	1.15 (0.02)
<i>Baetodes inermis</i>	3	456 (10)	504 (10)	0.78 (0.03)	0.83 (0.02)
	4	700 (6)	783 (10)	0.81 (0.04)	0.81 (0.02)
	5	1175 (10)	1244 (10)	0.74 (0.02)	0.75 (0.02)
<i>Isonychia sicca</i>	3	2175 (12)	2509 (8)	1.15 (0.02)	1.15 (0.02)
	4	5500 (5)	7668 (5)	1.03 (0.05)	1.00 (0.05)
	5	6802 (5)	13,768 (6)	0.99 (0.07)	0.98 (0.04)
<i>Rhagovelia choreutes</i>	Adult	346 (4)	571 (4)	0.62 (0.17)	0.84 (0.14)
<i>Rheumatobates hungerfordi</i>	Adult	309 (5)	381 (11)	1.15 (0.03)	1.18 (0.10)
<i>Ambrysus circumcinctus</i>	Adult	10747 (4)	12,674 (5)	0.40 (0.05)	0.60 (0.02)
<i>Stenelmis</i> sp.	Adult	660 (4)	847 (5)	0.57 (0.07)	0.59 (0.09)

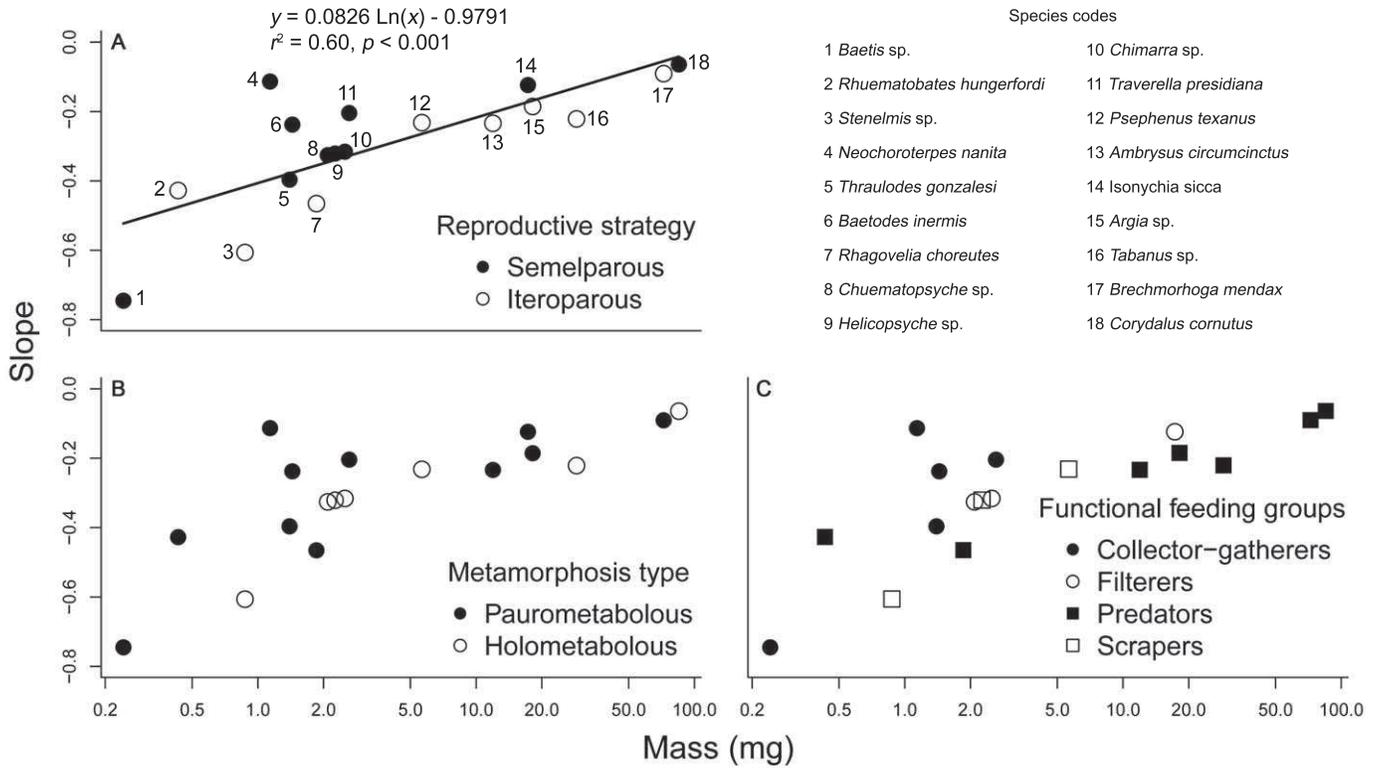


FIG. 3. Relationship between the largest mass observed for a taxon and the slope of the power function (from Fig. 2) that describes the decrease in %P (slope) across ontogeny by reproductive strategy (A), type of metamorphosis (B), and functional feeding group (C). Lines were fitted by least squares regression.

(e.g., Evans-White et al. 2005, Hambäck et al. 2009). The pattern of %P was nonlinear (power function) and declined with increasing size for all 18 taxa examined (Fig. 2A–R). Mean %P of eggs was equal to (*C. cornutus*) or less than (*Tabanus* sp.) that of 1st-instar larvae, but the range of P content in eggs was large (see Results). The highest egg %P exceeded the %P of the smallest larvae for both species. The large variation in egg %P suggests that not all eggs are created equal. Part of this variation could be because some eggs analyzed were not fertilized. Nutrients derived from males through mating represent a prezygotic investment in reproduction (Zeh and Smith 1985, Boggs 1990) and can influence the number and quality of eggs produced. Markow et al. (2001) demonstrated that males do contribute to the P content of *Drosophila* eggs during mating. Male contributions to the nutrient content of eggs also have been shown in butterflies (Boggs and Gilbert 1979, Boggs 1990) and beetles (Rooney and Lewis 1999). The fate of low-%P eggs is not known, but perhaps they fail to develop, or they hatch and larvae soon die. The fate of high-%P eggs also is not known, but larvae that hatch probably have an increased somatic growth rate and possibly have higher

survivorship resulting from faster growth (Arendt 1997).

We surmise that aquatic insects provision eggs with large amounts of P to promote rapid growth of early instars. However, this strategy is not shared by all insects. The fertilized eggs of the mammalian blood-feeding hemipteran *Rhodnius prolixus* (Reduviidae) contained only 0.61% P (calculated from table 1 in Ramos et al. 2011). Mammalian blood has a high P content (20–85 mg/L P; Rapoport and Guest 1941). After hatching, all sizes of *R. prolixus* feed on mammalian blood, thus a high-P diet may preclude the need for high-P eggs in blood-feeding insects.

The pattern of declining %P across ontogeny has been shown for *Ephemerella* sp. (Frost and Elser 2002) and *Caenis* sp. (Back et al. 2008) mayflies, brachycentrid caddisflies (Veldboom and Haro 2011), and zooplankton (Main et al. 1997). However Back et al. (2008) found greater %P in the largest *Caenis* sp. size class relative to intermediate developmental classes, as did Veldboom and Haro (2011) in *Brachycentrus* pupae relative to mature larvae. Back et al. (2008) speculated that the trend in *Caenis* sp. could have been a consequence of a large proportion of females carrying high-%P eggs in the sample (but they did not sex individuals in the

sample). In our study, all 3 leptophlebiid mayflies had increasing %P in the largest female individuals, but males did not. Declining %P with increasing size fits the predictions of the GRH (Elser et al. 1996, 2006, Main et al. 1997, Sterner and Elser 2002). Small individuals grow faster than large ones of the same species. Thus, growth slows as organisms age (Peters 1983). P is necessary to fuel rapid growth because of the high P content of ribosomes, messenger ribosomal ribonucleic acid (mRNA), and especially ribosomal RNA (rRNA). Unlike deoxyribonucleic acid (DNA), quantities of ribosomes, mRNA, and rRNA are not fixed in cells, and changes in these constituents can alter the amount of cellular P (Sterner and Elser 2002). Transcription rates and protein synthesis are positively correlated with P supply (Acharya et al. 2004, Vrede et al. 2004, Weider et al. 2005).

Nutrient ratios also are important because growth requires different relative amounts of C, N, and P, and other elements (Elser et al. 1996, Sterner and Elser 2002). Furthermore, somatic growth may have different elemental requirements than gamete production, especially with reference to P (Vrede et al. 1999, Færøvig and Hessen 2003). Because %C and %N did not vary markedly across ontogeny, the C:N ratio also did not vary much for the 8 taxa examined in our study. However the C:P and N:P ratio obviously increases with declining %P as organism size increases. Because the N:P ratio increases across ontogeny and growth rate slows with increasing size, N must not be limiting for growth when organism P is in high supply (i.e., when organisms are small). Thus, an optimal N:P (or C:P) may not exist for organisms undergoing rapid somatic tissue growth because only %P changes across ontogeny in a significant way.

Sexual differences in nutrient content

In all cases where a difference was detected, female insects were more enriched in P than males. Reproductive strategy does not seem to influence sexual patterns in %P content. Among semelparous mayflies, some taxa have females that are enriched in P relative to males and others have females with the same %P as males. Iteroparous (Hemiptera and Coleoptera) taxa also show both patterns (Table 2). Veldboom and Haro (2011) found 2 populations of *Brachycentrus* in which male pupae were more enriched in P than female pupae, and 2 populations where the %P was equal between pupae of both sexes. Morehouse et al. (2010) suggested that sexual differences in nutrient content should be expected based on difference in biochemical demand and composition of gametes and other sexually specific structures.

Nutrient content and life-history traits

Phylogeny may be an important secondary determinant of %P content in mayfly nymphs across their ontogeny. Mayflies showed 2 distinct patterns in %P that apparently were constrained by phylogeny. Percent P of *Baetis* sp., *B. inermis*, and *I. sicca* did not differ between males and females. However, females of all 3 leptophlebiid taxa (*N. nanita*, *T. gonzalesi*, and *T. presidiana*) were more enriched in P than males. This pattern is consistent with the pattern of increased P in late-instar *Caenis* nymphs documented by Back et al. (2008). Phylogenetic analysis indicates that *Caenis* (Caenidae) is more closely related to Leptophlebiidae (suborder Furcatergalia) and Baetidae is more closely related to Isonychiidae (suborder Pisciforma) than Caenidae and Baetidae are related to each other (Ogden et al. 2009).

Mature *Baetis* and *Caenis* nymphs are both small and can complete their life cycles and reproduce in ≤ 2 wk (Edmunds et al. 1976, Brittain 1982, Taylor and Kennedy 2006), yet they differ in ontogenetic patterns in %P. The %P of the largest *Caenis* sp. size classes increased in a manner similar to its slower-growing larger relatives, the leptophlebiids, whereas %P of *Baetis* sp. did not increase with size. In contrast, *B. inermis* and its close relative *Baetis* sp. share the same ontogenetic %P pattern despite *B. inermis* differing in morphology and behavior. *Baetodes inermis* has spines and tubercles on its legs and abdomen and does not swim actively (Edmunds et al. 1976). The causal factors responsible for the difference in %P between the suborders are not known, but warrant further study.

Reproductive strategy, type of metamorphosis, and FFG did not appear to be associated with the pattern of decreasing %P with increasing maximum size (Fig. 3A–C). Patterns did not differ between semelparous and iteroparous taxa, between pauro- or holometabolous taxa, or among FFGs. Moreover, the expected pattern that %P of FFGs would reflect the quality of food sources (i.e., increasing %P in collector-gatherers, filterers, scrapers, and predators) was not observed. Thus, the maximum size of a taxon drove the rate at which %P declined (Fig. 3A–C). The smallest taxa had the greatest decline in %P and the fastest life-cycle-completion times. The only exception was *Stenelmis* sp., which can take from 6 mo to 2 y to complete its life cycle (White 1978, Brown 1987).

Nonhomeostatic patterns in %P across ontogeny have important implications for population- and community-level processes and will affect predictions about consumer-driven nutrient recycling. The difference between the nutrient content of food and the nutrient demand of a species determines recycling

rates (Elser and Urabe 1999, Evans-White and Lamberti 2006, Rothlisberger et al. 2008). For a given species, small individuals may be a sink and hoard nutrients (especially P) whereas larger individuals become a source so that organisms advance along a sink–source continuum as they grow. The high %P of small individuals may profoundly influence their perceived quality to predators. Small individuals may not contain as much P (by mass) as larger individuals, but shorter prey-handling times may benefit predators that target small prey. Thus, predatory taxa may preferentially target small taxa and decrease P-excretion rates to conserve P for egg production. Nonpredatory taxa may hoard P across ontogeny to help meet P demands for reproduction. Although the actual mechanisms used by organisms to meet P demands for growth and reproduction are not clear, some combination of altered P-excretion rates, P uptake, and switching to higher-quality food or increased food ingestion rates probably are involved.

The effect of nutrient subsidies on patterns of ontogenetic nutrient content should be investigated. Nutrient subsidies may increase insect nutrient content across ontogeny or might result in larger individual size, egg size, or fecundity without altering %P. Subsidies can be passed up the food chain, thereby influencing community structure and function. Furthermore, high %P content of small taxa means high P demand, a possible mechanism for the proliferation of small, rapidly growing taxa in nutrient-enriched ecosystems (Miltner and Rankin 1998, King and Richardson 2007, Wagenhoff et al. 2011).

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