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Lipid content of terrestrial arthropods in relation to body size, phylogeny, ontogeny and sex

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Abstract. Energy storage in arthropods has important implications for survival and reproduction. The lipid content of 276 species of adult arthropods with wet mass in the range 0.2–6.13 g is determined to assess how lipid mass scales with body mass. The relative contribution of lipids to total body mass is investigated with respect to phylogeny, ontogeny and sex. The lipid content of adult insects, arachnids, and arthropods in general shows an isometric scaling relationship with respect to body mass (M) ($M_{\text{arthropod lipid}} = -1.09 \times M_{\text{dry}}{}^{1.01}$ and $M_{\text{arthropod lipid}} = -1.00 \times M_{\text{lean}}{}^{0.98}$). However, lipid allocation varies between arthropod taxa, as well as with sex and developmental stage within arthropod taxa. Female insects and arachnids generally have higher lipid contents than males, and larval holometabolous insects and juvenile arachnids have higher lipid contents than adults.

Key words. Allometry, arthropods, body size, energy storage, insects, lipid, scaling.

Introduction

The size of the energy reserves of an animal has potentially important implications for its ability to survive, grow and reproduce. Lipids serve a variety of important roles in both plants and animals (Hadley, 1985). They are essential to cell membranes, important for cell maintenance and can be extracted from almost all insect tissues (Downer & Matthews, 1976). Energy storage is another important role of lipids. Lipids can provide arthropods with the energy for many basic biological activities (e.g. locomotion and reproduction). Although the primary energy sources that fuel arthropod activity vary, and may include proteins and carbohydrates (Beenakkers *et al*., 1985; O'Brien, 1999), lipids are an energy dense and rapidly mobilized fuel (Beenakkers *et al*., 1985; Canavoso *et al*., 2001) that can be stored in anhydrous form, provide more calories per unit mass than glycogen and yield more metabolic water than glycogen upon oxidation (Downer & Matthews, 1976; Arrese & Soulages, 2010). These characteristics provide insects with ready reserves for temporal pulses of energetic demand to fuel processes such as metamorphosis, migration, and diapause (Mills, 1981; Hadley, 1985; Hahn & Denlinger, 2007). Lipids vary greatly in their structure and function, and serve additionally as integumental waterproofing; thermal insulation; buoyancy, communication (via pheromones) and cell membrane components; as well as in the regulation of body surface properties (via lubricants and surfactants; Hadley, 1985).

The total lipid content of an insect can range from 1% to >50% of wet mass and the major contributor to total lipid content in insects is triacylglycerol (>90%; Gilbert & Chino, 1974; Beenakkers *et al*., 1985). In arthropods, most triacylglycerol (also known as triacylglyceride or triglyceride) is located in fat bodies, which are loosely arranged structures containing lipids, protein, glycogen and free carbohydrates that serve as energy storage reservoirs, and have been described as analogous to the liver and adipose tissue of vertebrates (Gilbert & Chino, 1974; Beenakkers *et al*., 1985; Arrese & Soulages, 2010). Whole-body nutrient reserve levels are not only stored, but also sensed by the fat body; the fat body uses this information to orchestrate the storage and utilization of insect energy reserves for the coordination of insect growth, metamorphosis and reproduction (Arrese & Soulages, 2010). Lipids are also found in insect haemolymph [usually comprising ≤3% of the total lipid fraction (Beenakkers *et al*., 1985), although 5.1% is also reported (Punzo, 1990)], cell membrane constituents, cuticle (primarily in the epicuticle; Brown, 1975; Downer & Matthews, 1976) and ovaries and eggs (approximately 15% of lipid weight; Grapes *et al*., 1989). Lipid content and composition in these areas varies with body region and physiological condition, and the forms found include triacylglyceride, diglyceride, monoglycerides, free fatty acids, phospholipids, hydrocarbons, carotenoids, ketone bodies and hormones (Downer & Matthews, 1976).

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Many morphological, physiological and ecological characteristics of animals are directly related to body size (McMahon & Bonner, 1983; Peters, 1983; Calder, 1984; Schmidt-Nielsen, 1984; Brown & West, 2000). Body size relationships are often categorized as 'allometric scaling', and have the form $Y = aM^b$ (Y is a dependent variable comprising the morphological, physiological, or ecological characteristic of interest, a is a normalization constant, M is body mass and b is the scaling exponent). For linear scaling or isometry ($b = 1$), Y increases at the same rate as body mass (i.e. changes in mass do not lead to changes in Y); for positive allometry ($b > 1$), Y increases faster than body mass; and for negative allometry ($b < 1$), body mass increases faster than Y. Thus, an allometric approach allows the determination of whether and how an individual trait (e.g. lipid content) changes in relative proportion to body mass.

Allometric scaling exponents can vary with different body components. For example, mammalian kidneys, brains and liver show a negative allometry (i.e. the relative mass of these organs decreases with increasing animal size; Davidson, 1958), and mammalian hearts, lungs and muscles show isometric scaling (i.e. the relative mass of these organs remains the same, regardless of animal size; Calder, 1984). The existing literature on the scaling relationships of storage lipids in animals indicates that they tend to show linear (isometric) or hypermetric scaling with mass. For example, energy reserves vary in relation to size with an exponent of approximately 1 or greater than one in some vertebrates (Calder, 1984; Millar & Hickling, 1990; Hurst & Conover, 2003), although studies also suggest that there is considerable variation in the quantity of adipose tissue across species, and across individuals within species (Pond, 1978; Pond & Mattacks, 1989). Stored energy reserves also appear to show isometric scaling ($b = 1$) in some invertebrates (Foellmer & Fairbairn, 2005). It was therefore expected that lipid content would have an isometric scaling relationship with body size across arthropods.

Lipid content is also known to vary with physiological state and life-history stage. Sex and developmental stage are two important life-history variables that potentially affect arthropod lipid. Female arthropods often store proportionally more lipids than males (Zhou *et al*., 1995) because they use lipid to provision eggs. Ovarian and hemolymph lipid content in female *Labidura riparia* (Dermaptera) rises and falls in synchrony with cycles of vitellogenesis (Sayah, 2008), and increased lipid content of females compared with males is observed in *Coccinella septempunctata* (Coleoptera) over a range of geographic locations (Zhou *et al*., 1995) and in *Ceratitis capitata* (Diptera) over a range of diets (Nestel *et al*., 2005). It is thus expected that there would be sexual dimorphism in insect lipid content. However, other research shows that newly-emerged adults of *Adalia bipunctata* (Coleoptera) have no differences in lipid content between the sexes (Yasuda & Dixon, 2002).

Current data also suggest that juvenile arthropods will have a proportionally higher lipid content than adults, as a reflection of the high metabolic requirements of growth and development (Downer & Matthews, 1976) and because fat stores that are accumulated during larval stages can be used to fuel the physiological processes of adults (Beenakkers *et al*., 1985). Data on fat body stores in *Gryllus bimaculatus* (Orthoptera) show age-dependent changes in female fat body composition as lipids in fat body energy stores are re-allocated to oocyte growth as animals get older (Lorenz & Anand, 2004). Males do not have costs associated with egg synthesis; however, adult male arthropods can also accrue costs associated with reproduction such as mate competition (Crnokrak & Roff, 1995), and hence also need stored energy reserves. Larval energy stores may thus offset the energetic needs of adult arthropods of both sexes. Adult arthropods that forego feeding (e.g. some Saturniid moths) are an extreme, although not uncommon example of this pattern.

Thus, body size, taxonomy, physiological state, life-history stage and sex may all play important roles in determining the size of the lipid stores that are maintained by arthropods. However, although reports of lipid content exist within some arthropod groups (Zhou *et al*., 1995; Clarke *et al*., 1996; Lorenz & Anand, 2004; Foellmer & Fairbairn, 2005; Hahn, 2006), to date, no broad-scale comparative study of arthropod allocation to lipid has been undertaken. In the present study, the total lipid content of 312 terrestrial arthropod species (five classes, primarily insects) across a range of sizes and orders is measured to assess how investment in energy storage varies in arthropods. These data are used specifically to determine the scaling of lipid content with dry and lean body masses, as well as to investigate comparative lipid content with respect to phylogeny, ontogeny and sex.

Materials and methods

Arthropods were captured by net, by hand and using pit-fall traps, and stored at -15 °C until they could be identified. Animals were identified at the Museum of Southwestern Biology (University of New Mexico, Albuquerque, New Mexico). The capture location, date, phylogenetic determination and, where possible, developmental status and sex, were recorded for each individual.

Masses were determined to ± 0.00001 g using a Mettler AX205 balance (Mettler-Toledo, Inc., Columbus, Ohio) and animals were then dried to a constant mass at 60 °C in a VWR Scientific Mechanical Convection Oven Model 1390FM (VWR, Cleveland, Ohio). Dry masses were then recorded. Animals were crushed using a probe and/or tweezers and placed in vials containing petroleum ether (more than 3 volumes of ether to each estimated dry volume of animal) for 6–8 days to extract ether-soluble lipids (Mills, 1981; Zhou *et al*., 1995; Yasuda & Dixon, 2002). To increase lipid solubilization, ether was replaced every 2–3 days during the extraction period. After the final ether rinse (determined when rinsing with ether no longer yielded visibly extracted lipid or discoloured supernatant, and when animals had been subjected to ether extraction for $\geq$6 days), the ether was removed by pippetting, and the animals were left $\geq$24 h in a fume hood to allow evaporative removal of the remaining ether. Animals were then placed into an oven at 60 °C for $\geq$3 h for additional drying. Lipid-free dry mass was then measured, and lipid mass was estimated by subtracting lipid-free dry mass from total dry mass.

Ordinary least squares (OLS) and standardized major axis (SMA) regression analysis (RMA, version 1.17; Bohonak & Van der Linde, 2004), SIGMAPLOT (SPSS Inc., Chicago, Illinois), SMATR, version 2.0 (Falster *et al*., 2006), analysis of variance and covariance (ANOVA and ANCOVA) and Student's *t*-tests (statistical resource in EXCEL, Microsoft Corp., Redmond, Washington) were used to analyze the data in three subsets: Adults of all species from all classes were used to analyze the allometry of lipid content; adults of all species from classes Insecta and Arachnida were used to analyze sex differences in lipid content; and all developmental stages of all species from the classes Insecta and Arachnida were used to analyze developmental differences in lipid content. Dry masses were used for all data analyses because there was higher variance in wet mass than dry mass in the data. The impact of individual variation in lipid content (caused by differences in physiological state at time of capture) was reduced by the use of species averages to generate scaling relationships.

Data were additionally corrected for phylogenetic relatedness. Independent contrasts on lipid mass and dry mass were conducted using the PDAP module in MESQUITE, version 1.12, to make phylogenetic corrections at the level of family (Maddison & Maddison, 2006). Phylogenetic tree topology was constructed using published trees (Grimaldi & Engel, 2005), as described previously (Lease & Wolf, 2010).

Results

Arthropods from 312 species were sampled (713 individuals) and 276 of these species included adult representatives (592 individuals). Classes represented were Insecta, Arachnida, Chilopoda, Diplopoda, and Malacostraca. The dry body mass of the arthropods that were in the sample ranged from 0.1 mg to 2.41 g (0.2 mg to 6.13 g estimated wet mass; Table 1). Overall, lipid mass showed an isometric scaling relationship ($b = 1$) with body mass for all arthropods included in the present study. This was true regardless of whether lipid mass was analyzed with respect to dry body mass ($M_{\text{arthropod lipid}} = -1.09 \times M_{\text{dry}}{}^{1.01}$; Fig. 1A) or with respect to lean dry body mass ($M_{\text{arthropod lipid}} = -1.00 \times M_{\text{lean}}{}^{0.98}$; lean dry body mass is defined as dry body mass minus lipid mass; Fig. 1B.

The scaling of lipid content in arthropods is thus represented by the equation $M_{\text{arthropod lipid}} = \text{a } M^{\sim 1}$, although the scaling relationship exhibited a slight positive allometry when standardized major axis regression analysis was used to analyze the data ($M_{\text{arthropod lipid}} = -1.25\ M^{1.11}$, SMA versus $M_{\text{arthropod lipid}} = -1.09\ M^{1.01}$, OLS). This was also true for the scaling exponents derived using class-level taxonomic analyses. Lipid mass in arachnids showed a scaling relationship of $M_{\text{arachnid lipid}} = -1.13\ M^{1.01}$ (OLS; but $M_{\text{arachnid lipid}} = -1.24\ M^{1.08}$, SMA), and insect lipid mass showed a scaling relationship of $M_{\text{insect lipid}} = -1.09\ M^{1.01}$ (OLS; but $M_{\text{insect lipid}} = -1.25\ M^{1.11}$, SMA), although percentage lipid content of insects was not significantly higher than that of arachnids at the level of order, family or species ($P > 0.05$; two-tailed two-sample *t*-test). The small sample size and small size range of the other arthropod classes precluded within-class allometric analysis. Lipid scaling equations that were generated using independent contrasts were only slightly different from results obtained using SMA and OLS analysis, and confidence intervals of the scaling exponents for phylogenetically corrected lipid mass also overlapped $b = 1$ (95% confidence interval = 0.939–1.099). The slope of the scaling equation was only slightly different when branch lengths were varied, indicating low sensitivity of the data to phylogenetic adjustment.

Lipid content varied with class and order. On average, lipids made up 11% of dry body mass across all classes of arthropods, with lipids averaging 8.5% of arachnid body mass, 18.4% of chilopod body mass, 4.2% of diplopod body mass, 11.8% of insect body mass and 2.9% of malacostracan body mass. It is possible that low sample sizes for chilopods, diplopods and malacostracans may have caused individual animal variation as a result of physiological condition to lead to bias (i.e. an increase or decrease) of the class averages of lipid content. Taxonomic variation in lipid content is seen even more clearly when comparisons of lipid content are made between arthropod orders (Table 2) and families (Lease, 2008). In addition, allometric equations for arthropod taxa were determined for arthropod orders where three or more species were represented and three or more orders of magnitude were encompassed.

Table 1. Numbers, phylogenetic distribution, mean dry mass, dry mass range and mean percentage lipid content of adult terrestrial arthropods included in the sample.

	Phylum Arthropoda	Class Arachnida	Class Chilopoda	Class Diplopoda	Class Insecta	Class Malacostraca
Individuals (*n*)	592	52	9	7	519	5
Species (*n*)	276	27	3	2	243	1
Families (*n*)	107	15	2	2	87	1
Orders (*n*)	25	5	2	2	15	1
Classes (*n*)	5	NR	NR	NR	NR	NR
Mean dry mass[a] (g)	0.2012	0.2349	0.2429	0.9645	0.1340	0.0132
Min dry mass (g)	0.0001	0.0005	0.0612	0.3552	0.0001	0.0021
Max dry mass (g)	2.4106	1.5180	1.2065	2.3647	2.4106	0.0209
Mean lipid content[b] (%)	11.04	8.52	18.35	4.15	12.37	2.89

[a] Dry body mass of adult arthropods ranged from 0.1 mg to 2.41 g dry mass (0.2 mg to 6.13 g wet mass).
[b] Mean lipid content is presented as a proportion of dry mass.

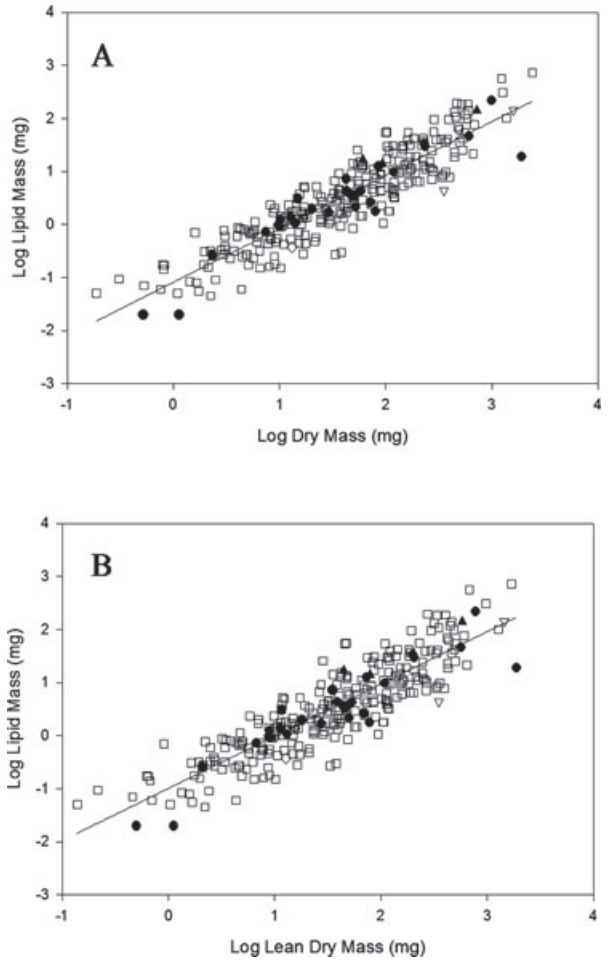

Fig. 1. The relationship between lipid mass and body mass for arthropods. Each data point represents the average adult lipid mass and average adult dry mass (A), or the average adult lipid mass and average adult lean dry mass (B), for an individual species. Solid circle, arachnid; hollow square, insect; solid (up) triangle, chilopod; hollow (down) triangle, diplopod; hollow diamond, Malacostraca). The scaling of lipid mass with respect to body size is generally isometric and is represented by the equations: $M_{\text{arthropod lipid}} = -1.09 \times M_{\text{dry}}^{1.01}$, and $M_{\text{arthropod lipid}} = -1.00 \times M_{\text{lean}}^{0.98}$.

Lipid scaling exponents for arthropod orders ranged from $b = 0.7$ (hymenopterans) to $b = 1.4$ (scorpions), and normalization constants ranged from $y = -0.89$ (cockroaches) to $y = -1.68$ (scorpions; Lease, 2008). Although the exponents for all groups averaged $b = 1.01$, an almost ten-fold variation in lipid content was observed within any specific body size (Fig. 1). Because of this taxonomic imprint on the data, sex and developmental analyses were performed on nested averages of phylogenetic groups.

Differences in lipid content were found between adult males and females when species were separated by sex (Table 3). Of the 312 arthropod species sampled, 77 species included known adult female representatives, and 69 species included known adult male representatives. With the exception of Odonata (dragonflies), female insects and arachnids had higher lipid content than males at the level of order. Higher lipid content in females was also generally reflected at the level of family, although there were several exceptions other than the odonates (e.g. Apidae and Halictidae). Gender differences in percentage of lipid content were significant at the level of order ($P = 0.04$; one-tailed paired t-test), family ($P = 0.03$; one-tailed paired t-test) and species ($P = 0.02$; one-tailed two-sample t-test). Scaling relationships for the species averages of dry mass and lipid mass also reflected the generally higher lipid content of females compared with males, although scaling exponents (i.e. slopes) did not differ between the sexes ($M_{\text{lipid}} = -0.91\ M^{0.93}$, $r^2 = 0.82$ for females; $M_{\text{lipid}} = -1.04\ M^{0.92}$, $r^2 = 0.76$ for males).

Lipid content also varied according to developmental stage. Of the entire arthropod sample, 42 species included known juvenile representatives, and 276 species included known adult representatives. At the level of class, juvenile arachnids and chilopods had higher average lipid contents than adults (Table 4). This relationship was also true for larvae of holometabolous insects compared with adults. By contrast, immature hemimetabolous insects had lower average lipid contents compared with adults (Table 4). Differences between adult and juvenile arthropod lipid contents, however, were either marginally significant or not significant at the level of order ($P = 0.054$), family ($P = 0.047$) and species ($P = 0.045$; one-tailed two-sample t-tests with immature arachnids, immature hemimetabolous insects and larval holometabolous insects combined for 'juvenile' category; Tables 2 and 5). The low statistical significance was possibly a consequence of the inverse relationship between juveniles and adults for hemimetabolous versus holometabolous insects, although, when tested separately (i.e. separate juvenile versus adult t-tests for arachnids, for holometabolous insects and for hemimetabolous insects), only arachnids showed a significant difference between adult and juvenile lipid content at the level of order ($P = 0.03$) and family ($P = 0.02$; one tailed paired t-test), and only holometabolous insects showed a significant difference between adult and juvenile lipid content at the level of species ($P = 0.03$; one-tailed two-sample t-test). In addition, the lipid contents of holometabolous larvae and hemimetabolous immature insect species were significantly different from one another ($P = 0.03$; one-tailed two-sample t-test). Finally, teneral insects (freshly moulted) had lower lipid contents than sclerotized adults, although this difference was not significant ($P > 0.05$; one-tailed paired t-test).

Discussion

General patterns in arthropod lipid content

In the present study, four different sources of variation in arthropod lipid content are considered: body size, taxonomy, sex and developmental stage. Arthropod lipid content is shown to vary isometrically ($b = 1$) with body mass. This isometric pattern holds generally true across approximately four orders of magnitude of body mass for insects, arachnids,

Table 2. Mean ± SE lipid mass and percentage lipid content in relation to dry body mass for adults of different orders of arthropods included in the study.

Class	Order	Families[a] (*n*)	Species (*n*)	Dry mass (mg)	Lipid mass (mg)	% Lipid content[b]
Arachnida						
	Araneae	10	19	217.8 ± 185.9	4.8 ± 1.7	9.27 ± 1.46
	Opiliones	1	1	14.0	1.1	7.63
	Scorpiones	2	5	264.2 ± 32.8	47.2 ± 13.2	13.82 ± 1.08
	Solifugae	1	1	71.7	2.6	3.92
	Uropygi	1	1	606.6	46.3	7.96
Chilopoda						
	Scolopendromorpha	1	2	392.7	78.8	21.81
	Scutigeromorpha	1	1	93.1	13.9	14.88
Diplopoda						
	Polydesmida	1	1	355.2	4.3	1.21
	Spirostreptida	1	1	1590.7	142.2	7.08
Insecta						
	Blattodea	3	4	90.8 ± 61.8	6.7 ± 4.1	8.16 ± 0.92
	Coleoptera	21	76	156.0 ± 44.9	27.9 ± 11.2	14.01 ± 1.52
	Diptera	11	20	17.9 ± 5.3	2.6 ± 1.3	9.82 ± 1.96
	Hemiptera	11	13	37.9 ± 18.8	3.0 ± 1.2	13.93 ± 2.84
	Hymenoptera	15	54	51.5 ± 21.6	1.8 ± 0.5	6.44 ± 1.09
	Isoptera	1	1	0.8	0.1	16.50
	Lepidoptera	9	19	103.8 ± 76.7	37.7 ± 33.2	12.57 ± 3.41
	Mantodea	1	3	68.1	6.7	8.31
	Megaloptera	1	2	202.3	4.1	7.28
	Neuroptera	1	1	33.0	6.7	20.26
	Odonata	4	11	158.0 ± 81.8	29.7 ± 21.7	13.45 ± 3.59
	Orthoptera	6	35	449.7 ± 199.2	58.1 ± 19.4	17.76 ± 7.73
	Phasmatodea	1	2	68.4	2.4	6.02
	Raphidioptera	1	1	2.8	0.3	11.74
	Zygentoma	1	1	5.8	1.1	19.32
Malacostraca						
	Isopoda	1	1	13.2	0.4	2.89

[a]Number of families indicates the number of family averages that were used to determine order averages (shown here) of dry mass and lipid mass; family averages themselves were determined using species averages for all adult arthropods included in the sample.
[b]Mean lipid content is presented as a proportion of dry mass.

and all arthropod groups sampled. Taxonomy, gender and life stage also have significant effects on arthropod lipid content. These sources of variation within and among insect taxa affect the overall arthropod lipid scaling relationship primarily as differences in normalization constants, and contribution to scatter around the isometric scaling relationship. Lipid contents vary with phylogeny but, on average, are generally higher in adult insects than adult arachnids, higher in female arthropods compared with male arthropods, higher in larval holometabolous insects and juvenile arachnids compared with adult holometabolous insects and adult arachnids, and higher in adult hemimetabolous insects compared with immature hemimetabolous insects.

Physiological and ecological consequences of the isometry of arthropod lipid content

Lipids serve as an important energy reservoir in insects (Hadley, 1985), and lipid content reflects an insect's available resources. Insects have finite resources with which to grow and reproduce. As a result, life-history strategies require trade-offs in resource allocation to growth, storage, maintenance and reproduction. Allocation patterns are affected by many environmental variables, including humidity, photoperiod, water and food availability, as well as temperature (Chown & Nicolson, 2004). Despite this, lipid content shows linear variation across all arthropods, indicating that total lipid is the same fraction of body mass, on average, across all body sizes of arthropods. The present data show that, although there is some variation in the absolute proportion of lipid content, the relationship between lipid content and body size across different taxonomic groups of arthropods is generally stable.

The isometric scaling observed in the current analysis has important physiological consequences. For example, because the metabolic rate of insects shows scaling of $M^{0.75}$ (Chown *et al*., 2007) and energy reserves show scaling of M^{1} (present study), it follows that fasting endurance will be greater for larger insects, and show scaling of $M^{0.25}$. The isometry of lipid scaling may also have important ecological consequences for foraging and nutritional models. For example, models of generalist insectivore energetics can incorporate the information yielded in the present study to treat the

Table 3. Comparison of female and male percentage lipid content (mean ± SE) for adult arachnids and insects of different orders and families.

		Females[a] (*n*)	Males[a] (*n*)	% Lipid content of females[b]	% Lipid content of males[b]	Lipid ratio of females to males[c]
Class			Orders			
Arachnida		2	3	13.91 ± 1.59[d]	9.33 ± 3.03[d]	1.49
Insecta		9	9	11.80 ± 2.32	9.30 ± 1.44	1.27
Order			Families			
Araneae		5	3	12.32 ± 1.48	9.69 ± 2.05	1.27
Blattodea		2	2	9.41 ± 0.48	6.75 ± 0.73	1.39
Coleoptera		5	6	17.15 ± 4.88	12.66 ± 4.71	1.35
Diptera		3	4	19.93 ± 4.93	15.79 ± 4.49	1.26
Hemiptera		3	4	23.53 ± 7.64	11.06 ± 4.77	2.13
Hymenoptera		10	8	6.51 ± 1.95	6.17 ± 1.52	1.06
Mantodea		1	1	9.30	5.18	1.80
Odonata		3	4	7.72 ± 1.32	14.95 ± 3.04	0.52
Orthoptera		3	4	10.75 ± 3.25	5.12 ± 0.45	2.10
Scorpiones		2	1	15.50 ± 4.86	14.39	1.08
Family	*(Order)*		Species			
Acrididae	Orthoptera	17	10	7.78 ± 1.50	6.35 ± 1.63	1.23
Andrenidae	Hymenoptera	1	2	3.65	2.25 ± 0.14	1.62
Apidae	Hymenoptera	2	2	3.28 ± 0.63	5.14 ± 1.53	0.64
Asilidae	Diptera	2	4	16.83 ± 6.31	12.33 ± 4.15	1.36
Bradynobaenidae	Hymenoptera	1	2	13.08	5.67 ± 0.81	2.31
Buprestidae	Coleoptera	1	1	7.36	8.72	0.84
Cantheridae	Coleoptera	1	1	35.82	9.38	3.82
Cerambycidae	Coleoptera	3	2	15.11 ± 5.44	7.57 ± 3.53	2.00
Coenagrionidae	Odonata	1	2	9.69	12.39 ± 2.98	0.78
Coreidae	Hemiptera	1	1	8.25	8.46	0.97
Gomphidae	Odonata	1	1	5.21	11.47	0.45
Halictidae	Hymenoptera	2	2	4.64 ± 2.36	14.33 ± 8.72	0.32
Libellulidae	Odonata	2	3	8.27 ± 2.88	11.89 ± 4.29	0.70
Lycosidae	Araneae	1	3	16.47	12.42 ± 10.50	1.33
Mantidae	Mantodea	1	1	9.30	5.18	1.80
Megachilidae	Hymenoptera	1	1	2.45	1.99	1.23
Meloidae	Coleoptera	2	1	15.09 ± 2.52	3.13	4.83
Mutillidae	Hymenoptera	2	1	11.93 ± 2.34	2.79	4.27
Mydidae	Diptera	1	1	29.59	20.44	1.45
Polyphagidae	Blattodea	1	1	8.93	7.48	1.19
Rhaphidophoridae	Orthoptera	2	1	17.23 ± 3.00	4.31	4.00
Rhopalidae	Hemiptera	1	1	30.95	25.12	1.23
Romaleidae	Orthoptera	1	1	7.23	5.14	1.41
Tenebrionidae	Coleoptera	3	4	12.35 ± 3.91	11.65 ± 1.31	1.06
Therevidae	Diptera	1	1	13.36	25.37	0.53
Vaejovidae	Scorpiones	2	1	20.37 ± 4.74	14.39	1.42

[a] Sample sizes reflect the number of species used to determine percentage of lipid content for each sex at the level of family; families used to determine lipid content for each sex at the level of order; and orders used to determine lipid content for each sex at the level of class. Only taxonomic groups where lipid content of both sexes was determined are shown.

[b] Mean lipid content is presented as a proportion of dry mass.

[c] Ratios >1 indicate higher female lipid content; ratios <1 indicate higher male lipid content.

[d] Gender differences in percentage of lipid content were significant at the level of order ($P = 0.04$), family ($P = 0.03$) and species ($P = 0.02$; for details, see text).

lipid per gram of prey mass as a constant when assessing energy content of prey, although caution will be necessary when dealing with dietary specialists as a result of interspecific variation in fat content (Barker *et al*., 1998; present study).

The scaling of lipid storage is also subject to seasonal variation as environmental conditions and life-history stages change. Rates of lipid accumulation and lipid content may increase or decrease as animals prepare for migration, reproduction, overwintering or diapause. For example, postfeeding diapause larvae can be 15–45% heavier and can accumulate 50–200% greater lipid reserves than nondiapause individuals (Hahn & Denlinger, 2007), and many ant species are shown to accumulate fat during high resource peaks that coincide with periods directly before sexual production and with extended overwintering (Hahn, 2006). These types of temporal variation in lipid content probably cause seasonal shifts in the allometry of lipid content in arthropods.

Table 4. The effect of developmental stage on lipid mass and percentage lipid content (mean ± SE) in relation to dry body mass for different groups of arachnids, chilopods and insects.

Class	Developmental stage	Orders[a] (*n*)	Families[a] (*n*)	Species[a] (*n*)	Dry mass (mg)	Lipid mass (mg)	% Lipid content[b]
Arachnida							
	Adult	5	15	30	234.9 ± 103.6	20.4 ± 10.8	8.52 ± 1.60
	Immature	4	9	11	85.7 ± 54.6	21.1 ± 16.3	16.84 ± 3.98
Chilopoda							
	Adult	2	2	3	242.9 ± 149.8	46.3 ± 32.5	18.35 ± 3.46
	Immature	1	1	2	166.6	39.6	23.96
Insecta							
Ametabola	Adult	1	1	1	5.8	1.1	19.32
Hemimetabola	Adult	7	28	69	125.3 ± 57.0	15.3 ± 8.1	12.02 ± 1.71
	Immature	5	8	23	66.3 ± 35.5	5.7 ± 2.7	10.17 ± 2.50
	Teneral adult	2	3	3	115.9 ± 59.1	7.7 ± 2.6	7.19 ± 1.27
Holometabola	Adult	7	59	173	82.0 ± 28.9	11.8 ± 5.7	11.73 ± 1.76
	Alate	1	1	6	18.7	6.3	26.56
	Egg	1	1	2	0.3	0.1	24.55
	Larva	4	13	19	156.0 ± 68.5	59.2 ± 39.9	17.67 ± 4.40

[a] Sample sizes reflect the number of species, families and orders used to determine percentage of lipid content for each developmental stage.
[b] Mean lipid content is presented as a proportion of dry mass.

Table 5. Mean ± SE lipid mass and percentage lipid content in relation to dry body mass for immature stages of various arthropod orders.

Class/Order	Developmental stage[a]	Families (*n*)	Species (*n*)	Dry mass (mg)	Lipid mass (mg)	% Lipid content[b]
Class Arachnida						
Araneae	Immature	6	8	49.7 ± 21.1	9.6 ± 6.7	15.70 ± 4.35
Opiliones	Immature	1	1	7.4	0.7	9.81
Scorpiones	Immature	1	1	247.5	69.8	28.20
Solifugae	Immature	1	1	38.4	4.2	13.65
Class Chilopoda						
Scolopendromorpha	Immature	1	2	166.6	39.6	23.96
Class Insecta						
Holometabola						
Coleoptera	Larva	5	5	131.9 ± 77.4	31.6 ± 24.6	16.31 ± 4.43
Diptera	Larva	2	2	338.7 ± 329.3	177.0 ± 176.3	30.19 ± 22.71
Hymenoptera	Alate	1	6	18.7	6.3	26.56
	Egg	1	2	0.3	0.1	24.55
Lepidoptera	Larva	5	11	146.5 ± 108.7	27.3 ± 22.3	14.41 ± 1.94
Neuroptera	Larva	1	1	6.7	0.7	9.75
Hemimetabola						
Blattodea	Immature	1	1	34.3	0.8	2.42
Hemiptera	Immature	2	4	14.4 ± 9.8	2.9 ± 2.1	17.95 ± 1.81
Mantodea	Immature	1	1	9.0	1.0	8.96
Odonata	Immature	1	2	72.6	9.4	11.89
	Teneral adult	2	2	56.8 ± 7.5	5.1 ± 2.0	8.45 ± 2.05
Orthoptera	Immature	3	15	201.1 ± 126.5	14.3 ± 6.6	9.62 ± 2.26
	Teneral adult	1	1	175.0	10.4	5.92

[a] Only taxonomic groups where lipid content for multiple developmental stages was determined are shown.
[b] Mean lipid content is presented as a proportion of dry mass.

Physiological and ecological consequences of variation in arthropod lipid content

Arthropod lipid content varies with respect to phylogeny, sex and life stage. Some of the taxonomic differences in lipid content observed in the current data could be attributable to differences in fatty acid composition that are known to occur between arthropod groups (Fast, 1966). For example, ethanolomine phosphoglycerides comprise approximately 25% of total phospholipid in most insects but make up 50% of total phospholipid in others, and insect fatty acid chain-length is found to be associated with ethanolomine phosphoglyceride content (Fast, 1966); such trends could underlie differences in insect lipid content. Most of the taxonomic variation in lipid content that is found in the present study, however, is likely to be attributable to the wide variation in life histories of the arthropods in the sample group. For example, the energetic cost of locomotion (Harrison & Roberts, 2000), environmental

temperature (Chown *et al*., 2002), ageing (Casas *et al*., 2005; Nestel *et al*., 2005) and development (Nestel *et al*., 2003) can vary significantly within and between arthropod taxa. In addition, larval nutrition can affect adult lipid storage (Hahn, 2005). These factors can affect short-term and long-term metabolic demands (Bennett *et al*., 1999), and thus alter relative lipid stores via effects on both lipid accumulation and use.

Female insects and arachnids tend to have higher lipid contents than males in the present study. Oogenesis in insects is nutrient limited, and insufficient energy reserves can inhibit egg development (Wheeler, 1996). In addition, annual total egg mass is correlated with body size for some ectotherms, such as fish (Charnov *et al*., 2001) and lizards (Warne & Charnov, 2008), as is egg size and fecundity for some arthropods, such as Lepidoptera (Garcia-Barros, 2000; Jimenez-Perez & Wang, 2004). This may be the result of a correlation between female body size and the amount of material available for production of egg yolk (Garcia-Barros, 2000). The sexual dimorphism in lipid content observed in most arthropod groups in the present study is thus probably attributable to egg production costs, and implies that fitness in female arthropods may depend more on energetic 'capital' (i.e. stored resources) than fitness of males. Finally, the reverse sexual dimorphism observed in the lipid content of several taxonomic groups may also reflect direct links between lipid content and fitness. For example, fat content affects the outcome of aerial contests for mating territories in male *Calopteryx maculata* (Odonata) more than do body size or flight ability (88% of winners have higher fat content than losers; Marden & Rollins, 1994). Coincidentally, three of the seven families of arthropods that exhibit higher lipid content for males over females (this study) are odonates.

Differences are found in the lipid content of arthropods at different developmental stages in the present study, and the nature of these differences varies across differing life-history strategies. Allocation patterns in arthropods often shift during ontogeny; this is because larval allocation of resources to storage, growth and reproduction can be different from adult allocation of resources to the same factors. For example, metabolic costs can vary with insect age (Greenlee & Harrison, 2004) and stage of development (Nestel *et al*., 2003), and costs of physiological processes such as overwintering can vary between adults and larvae (Bel Venner *et al*., 2009). In the present study, both holometabolous insects and arachnids show the predicted pattern of early-stage animals having greater investment in lipid stores than adults. If maintained to maturity, these energy stores could offset some or all of the energy required for the adult life stages in some species (e.g. Odonata). Hemimetabolous insects, by contrast, show the opposite pattern; early-stage animals have proportionally lower lipid contents than adults. The reasons for these differences are unclear, but in holometabolous insects, there is a defined morphological shift between the periods of growth and reproduction ('larvae are … quite unlike the adult'; Chapman, 1998). In hemimetabolous insects discrete growth intervals also occur, but overlap with the shift from an immature insect to an adult insect ('larvae … essentially resemble adults'; Chapman, 1998). This overlap may contribute to the differences in immature versus adult lipid content that are observed between holometabolous and hemimetabolous insects. For example, overlap in periods of growth and development of reproductive parts in hemimetabola may cause overlap in pulses of accumulation and use of energy, and thus affect energy storage. On the other hand, metamorphosis in the holometabola includes a nonfeeding stage of breakdown of larval tissue and formation of adult tissue that is fueled completely by energy reserves, which accumulate steadily during larval development (Downer & Matthews, 1976). This abrupt transition between energy accumulation (as a larva) and energy use (as a pupa, preparing to be an adult) might underlie the higher relative lipid content of holometabolous larvae compared with hemimetabolous larvae. Trade-offs between intake and stored resources in fuelling growth, metamorphosis and reproduction may also contribute to the differences in relative fat stores within and among arthropod taxa.

Limitations of the data set

The current method (petroleum ether extraction) does not discriminate between the different pools of lipid within the arthropod body (Mills, 1981; Zhou *et al*., 1995; Yasuda & Dixon, 2002). The allocation to storage lipids versus structural lipids or to somatic versus gonadal reserves is not differentiated, nor is the proportion of lipid that is removed from the fat body determined. However, it can be assumed that the cuticular lipid component is minimal compared with the stored lipid component because cuticular lipids typically account for only approximately 0.1% of the total mass of insects (Gibbs, 1998).

An additional limitation of the data set is limited replication within different taxonomic groups of arthropods. It is acknowledged that the existence of an enormous range of morphological, physiological and biochemical diversity in arthropods necessitates that caution be utilized when extrapolating general characteristics to the group (Downer & Matthews, 1976). The aim of the present study is to investigate total lipid content across a broad array of body sizes and taxonomic groups of arthropods. The trade-off in the data set is low robustness within specific arthropod taxonomic groups. The research does not span the entire range of life-history/ecological possibilities of arthropods, nor does it establish whether the high degree of phylogenetic variation in lipid contents recorded in the study is attributable to variation in lipid content at the individual, species or higher taxonomic levels.

Conclusions

Analysis of the data indicates that lipid content scales isometrically with respect to body mass across four orders of magnitude of body mass (0.0002–6.13 g) in arthropods. Factors other than size are also found to affect arthropod lipid content. The results demonstrate that female insects and arachnids have higher lipid contents than males, and larval holometabolous insects and juvenile arachnids have higher lipid contents than adults. Physiological and life-history variation exists throughout the arthropod world, and it is recognized that many ecological factors not investigated in the

present study probably also contribute to the observed variation in arthropod lipid content. For example, aquatic insects, migratory insects, high altitude insects and insects that over-winter all have different metabolic needs, and thus are likely to have differential tightening or relaxing of the constraints that affect their allocation to lipid storage. It is expected that some of this ecological variation affects the scaling of lipid content for these groups. Although not comprehensive, these data provide an important empirical foundation for future studies of how arthropods vary investment in energy storage and use.

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