The relation of energy and organic carbon in aquatic invertebrates[1]

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Abstract

The physical and nutritional energy values of aquatic invertebrates were studied in relation to organic carbon content. The ratio of energy content to the weight of organic carbon increased with increasing carbon. The dependence is described in regression equations which permit prediction of the energy content with an accuracy of about ±5% ($P > 0.95$). Even the use of the mean conversion factor of 46 kJ g^{-1} organic carbon yields considerably more accurate results than the use of a general conversion factor for ash-free dry weight.

Accurate energy values of animals are essential for studies of animal energetics. Determination of the energy content of animals with the aid of bomb calorimeters now used requires samples relatively large in comparison with the small size of most freshwater invertebrates. Most scientists use dry weight or ash-free dry weight to estimate biomass. As the energy content of dry or ash-free dry biomass varies considerably from species to species (e.g. Cummins and Wuycheck 1971), and within one species from season to season (e.g. Schindler et al. 1971; Wissing and Hasler 1971; Snow 1972), the use of general energy equivalents for dry or ash-free dry weight does not yield very accurate values.

As noted earlier by Platt and Irwin (1973), the energy equivalent for biomass expressed in terms of organic carbon should theoretically vary less than the energy equivalent for biomass expressed in terms of dry or ash-free dry weight. The use of 41.9 kJ g^{-1} C ($= 10$ kcal g^{-1} C) as an approximate conversion factor has been recommended by Winberg (1971*a*). However, new methods have made it possible to determine organic carbon rapidly and accurately even from submicrogram samples unsuitable for calorimetry. To obtain more accurate general energy equivalents for biomass expressed in terms of organic carbon, we have studied the relation between energy content and organic carbon in freshwater invertebrates from various taxonomic groups.

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Materials and methods

Material was collected in November and December 1974 from the mesohumic, oligotrophic lake Pääjärvi and the meromictic, eutrophic lake Lovojärvi (*Daphnia*, *Cordulia*, and *Chaoborus*) near Lammi Biological Station in southern Finland. *Chironomus* larvae were collected from a sewage pond. Planktonic species were collected with a plankton net (70-μm mesh). Different species were separated by size fractionation, and the resulting fractions were examined under a low-power stereoscopic microscope. *Erpobdella* was collected by hand from the underside of littoral stones. Other species, collected with various nets and dredges, were picked from the sieving residues individually with forceps or pipettes. The resulting samples were thus monospecific with a minimal amount of detritus. In the *Daphnia* sample, *Bosmina* sp. was present, but its share of the total biomass of the sample was less than 1%. Sorting was completed within a few hours after collection. The *Mysis*, *Gammaracanthus*, and *Pallasea* samples were frozen at –20°C before further treatment.

All the samples were oven-dried for 24 h at 60°C, and homogenized in small mortars. The percentages of ash, total carbon, inorganic carbon, and nitrogen as well as

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the energy content were then determined for each sample from five replicate portions weighed on an electronic microbalance with a stated precision of ±1 μg. The percentages of organic carbon were calculated by difference from the percentages of total and inorganic carbon.

For the determination of ash, portions of from 1.4 to 20 mg of the homogenized material were incinerated in a muffle furnace at 500°C, low enough to avoid decomposition of $CaCO_3$ (Paine 1964). Traces of organic carbon were still present in the samples after 3 h, so they were kept in the furnace for more than 12 h. Loss of weight from $CaCO_3$ controls during incineration was less than 0.5%.

Total carbon was determined from 100–400-μg portions of the oven-dry material and inorganic carbon ($CaCO_3$-C) from 100–1,500-μg portions of ash by combustion at 950°C in an oxygen flow in the presence of a cupric oxide catalyst. The resulting carbon dioxide was measured with a nondispersive infrared gas analyzer (Hartmann and Braun, Uras 2). The combustion peak areas on the recorder chart were measured with a planimeter. The carbon analyzer was calibrated against oxalic acid and $CaCO_3$. Both gave equivalent calibration coefficients, so the oxidation of oxalic acid was probably complete. The 95% confidence limits for the linear calibration were ±0.7% (the statistical treatment and terminology follow Snedecor and Cochran 1967).

Nitrogen was determined from 0.6–3.1-mg portions of the homogenized material, digested with a solution of 5 ml of concentrated H_2SO_4, 2 ml of H_2O_2, and 0.2 g of Dewarda's alloy. Ammonia was then distilled with a Büchi 1500 distillation apparatus. Thereafter 2 ml of $NaKC_4H_4O_6$ solution and 2 ml of Nessler's reagent were added, and the absorption was determined at 420 nm in a spectrophotometer. Replicate calibrations at different levels of nitrogen resulted in 95% confidence intervals of from ±1.8 to 8.1% ($n = 4$–6) of the mean.

The energy contents were determined with a Phillipson microbomb calorimeter calibrated with benzoic acid. The pellet size varied from about 5–15 mg dry wt. Because an oily substance was seen to leave the *Limnocalanus* pellets during pressing, which led to a decrease of 4.2 kJ g^{-1} AFDW in the energy content, most pellets were made by packing the homogenized material into preweighed platinum cups. The 95% confidence limits for the calibration were ±1% of the mean ($n = 14$), similar to the precisions reported by Phillipson (1964), Schindler et al. (1971), Bröckel (1973), and Driver et al. (1974).

The possible effect of oven-drying on the carbon content was investigated. The total carbon content of living *Macrocyclops albidus* females was 23.0 ± 0.8 μg C per individual (mean ±95% confidence interval). Analyses after routine drying for 24 h at 60°C resulted, similarly, in an average of 23.0 ± 0.8 μg C per individual ($n = 22$). The respective values for *Eudiaptomus gracilis* adults were 4.9 ± 0.9 for living ($n = 11$) and 5.7 ± 1.0 for oven-dried material ($n = 6$). Portions of a freeze-dried sample of *Mysis relicta* dried for 24 h at 60°C lost 3.0 ± 0.6% of their weight ($n = 5$). However percentages of carbon before and after oven-drying calculated from the freeze-dry weight did not differ from each other (46.0 ± 0.8%, $n = 7$; 46.2 ± 0.6%, $n = 5$), suggesting that the weight loss was due to a decrease in water level. Thus no carbon loss was apparent from samples dried at 60°C as compared to living material, in agreement with the finding of Schindler et al. (1971) that heat-drying at 110°C and freeze-drying gave similar energetic values for freshwater zooplankton.

The effect of ash on the determination of energy content by calorimetry was studied by adding various amounts (0–80%) of $CaCO_3$ to benzoic acid. The resulting decrease in the energy content was 0.69 kJ g^{-1} $CaCO_3$ ($n = 22$), fairly close to the value (0.58 kJ g^{-1} $CaCO_3$) obtained by Paine (1966). We corrected our results for the observed decrease, assuming that all the measured inorganic carbon was derived from the decomposition of $CaCO_3$.

Table 1. Ash, carbon, and nitrogen levels, energy contents, and energy-to-carbon ratios, with 95% confidence limits, in 19 species of freshwater invertebrates. AFDW—ash-free dry weight.

species	ash % dry wt	total C % dry wt	inorganic C % dry wt	organic C % AFDW	nitrogen % AFDW	kJ / g AFDW	kJ_N / g AFDW	kJ / g org. C	kJ_N / g org. C
Hirudinea									
Erpobdella octoculata	4.47 ± 0.08	48.3 ± 2.3	0.0034 ± 0.0010	50.6 ± 2.4	9.4 ± 0.6	22.1 ± 0.6	19.8 ± 0.6	43.9 ± 2.5	40.8 ± 2.8
Cladocera									
Daphnia cristata	8.07 ± 0.03	50.7 ± 0.8	0.0665 ± 0.0038	55.2 ± 0.9	7.4 ± 1.2	25.3 ± 0.6	23.4 ± 0.6	45.8 ± 0.4	42.4 ± 0.7
Copepoda									
Limnocalanus macrurus	2.92 ± 0.05	62.1 ± 0.6	0.0044 ± 0.0008	64.0 ± 0.6	6.2 ± 0.3	31.2 ± 0.4	29.7 ± 0.3	48.8 ± 0.4	46.4 ± 0.5
Eudiaptomus gracilis	5.16 ± 0.06	49.8 ± 0.4	0.0044 ± 0.0004	52.6 ± 0.4	10.1 ± 0.4	24.7 ± 0.3	22.2 ± 0.2	47.3 ± 2.1	42.2 ± 0.7
Macrocyclops albidus	4.66 ± 0.02	48.2 ± 0.5	0.0042 ± 0.0009	50.7 ± 0.6	10.2 ± 1.0	22.8 ± 0.3	20.3 ± 0.4	45.0 ± 0.4	39.9 ± 0.4
Mysidacea									
Mysis relicta	8.61 ± 0.07	50.0 ± 1.0	0.0441 ± 0.0062	54.6 ± 1.1	10.0 ± 0.4	25.7 ± 0.1	23.3 ± 0.2	47.3 ± 1.3	42.5 ± 1.0
Isopoda									
Asellus aquaticus	28.62 ± 0.22	34.3 ± 0.9	2.3800 ± 0.0600	44.7 ± 1.1	9.7 ± 1.4	19.9 ± 0.6	17.6 ± 0.6	44.6 ± 2.5	39.3 ± 1.9
Amphipoda									
Pallasea quadrispinosa	30.37 ± 0.76	35.4 ± 1.2	2.6200 ± 0.1000	47.0 ± 1.7	9.4 ± 0.7	21.3 ± 0.4	19.0 ± 0.4	45.3 ± 2.1	40.4 ± 1.8
Gammaracanthus lacustris	20.69 ± 0.21	41.7 ± 0.8	1.1600 ± 0.0700	51.1 ± 0.9	9.6 ± 0.7	24.1 ± 0.2	21.7 ± 0.2	47.1 ± 0.8	42.5 ± 1.1
Ephemeroptera									
Leptophlebia vespertina	6.24 ± 0.07	49.3 ± 1.2	0.0121 ± 0.0031	52.6 ± 1.3	10.1 ± 0.8	24.4 ± 0.8	21.9 ± 0.8	46.4 ± 1.3	41.6 ± 1.4
Centroptilum luteolum	8.14 ± 0.17	49.7 ± 1.8	0.0106 ± 0.0031	54.1 ± 1.9	9.9 ± 1.2	26.1 ± 0.4	23.7 ± 0.5	48.4 ± 1.7	43.8 ± 2.1
Heptagenia fuscogrisea	5.39 ± 0.13	52.2 ± 1.2	0.0125 ± 0.0019	55.1 ± 1.2	8.8 ± 0.8	26.2 ± 0.6	24.1 ± 0.5	47.5 ± 1.7	43.6 ± 1.5
Odonata									
Cordulia aenea	4.52 ± 0.07	47.4 ± 0.8	0.0016 ± 0.0014	49.6 ± 0.8	9.0 ± 0.7	22.4 ± 0.8	20.2 ± 0.9	45.2 ± 2.1	40.7 ± 2.4
Megaloptera									
Sialis sp.	4.28 ± 0.04	49.2 ± 0.6	0.0058 ± 0.0011	51.1 ± 0.6	9.3 ± 1.1	23.5 ± 0.3	21.2 ± 0.4	46.0 ± 0.8	41.5 ± 1.0
Trichoptera									
Agrypnia obsoleta	4.72 ± 0.02	47.3 ± 1.4	0.0313 ± 0.0058	49.7 ± 1.3	8.0 ± 1.9	21.5 ± 0.4	19.6 ± 1.1	43.3 ± 1.3	39.2 ± 2.9
Limnephilidae sp.	9.02 ± 0.12	46.4 ± 0.7	0.0100 ± 0.0015	51.0 ± 0.8	6.2 ± 1.0	22.0 ± 0.3	20.5 ± 0.3	43.2 ± 0.4	40.2 ± 0.5
Diptera									
Chaoborus flavicans	6.93 ± 0.04	47.3 ± 0.7	0.0016 ± 0.0006	50.8 ± 0.7	9.5 ± 0.4	22.7 ± 0.3	20.4 ± 0.4	44.7 ± 0.8	40.1 ± 0.6
Chironomus plumosus	5.16 ± 0.11	45.1 ± 0.8	0.0124 ± 0.0014	47.5 ± 0.9	8.7 ± 1.4	20.8 ± 2.2	18.6 ± 2.0	43.7 ± 4.6	39.1 ± 4.1
Heteroptera									
Notonecta glauca	3.86 ± 0.08	50.0 ± 0.9	0.0048 ± 0.0006	52.0 ± 1.0	10.3 ± 0.6	23.8 ± 0.4	21.3 ± 0.5	45.9 ± 1.7	41.0 ± 1.6
Mean				51.8	9.0	23.7	21.5	45.8	41.4
Standard deviation				4.0	1.3	2.6	2.7	1.7	1.9

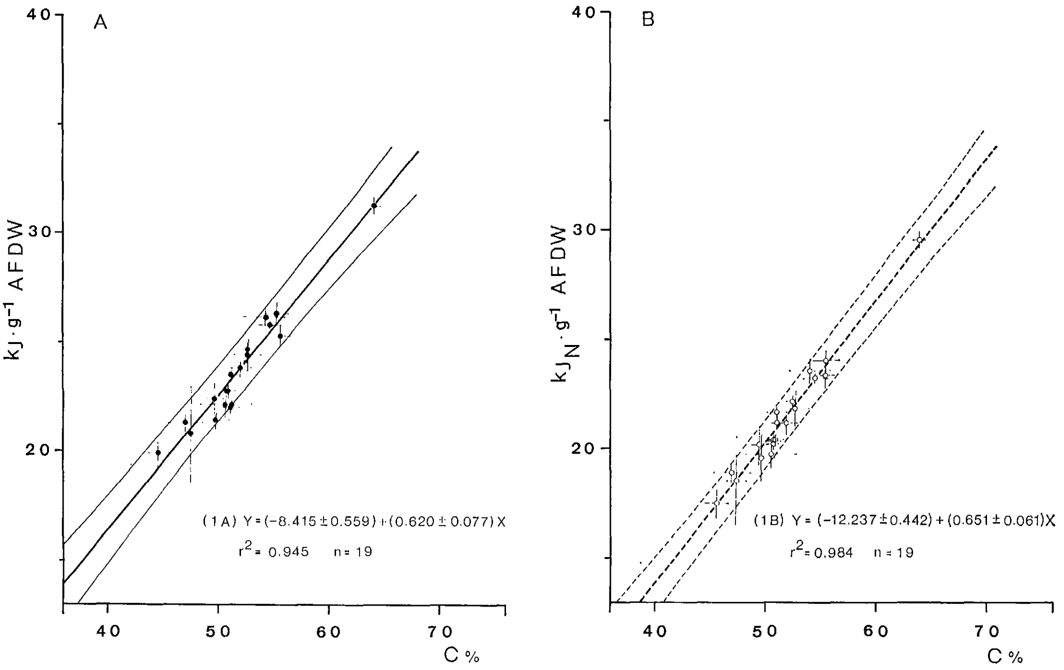

Fig. 1. Relationship between energy content and organic carbon level in freshwater invertebrates. Each circle represents one species, vertical and horizontal bars show 95% confidence intervals. Regression equations are given with 95% confidence limits for the parameters. Regression lines are shown with 95% confidence belts for prediction. A—Uncorrected. B—Energy values corrected for nitrogen. AFDW—Ash-free dry weight; C—percentage of organic carbon in AFDW.

Our material contained little ash, so this correction was at most only 1.5%.

The nitrogen correction (–24.7 kJ g^{-1} N) proposed by Kersting (1972) for energy values determined by bomb calorimetry was used to estimate "physiological" energy values (cf. Winberg 1971*b*).

Results and discussion

The main results of the analyses are shown in Table 1. Most of the variation in the energy content in terms of ash-free dry weight was accounted for by variations in the percentage of organic carbon of the ash-free dry weight. This relation could be described by a linear regression equation (Fig. 1A) with a very high coefficient of determination. The nitrogen correction for the energy values reduced the unexplained variation from 5.5 to 1.6% of the total variation (Fig. 1B).

The variation in energy content per organic carbon was much less than that per dry weight or ash-free dry weight (Table 2). The mean energy equivalent (with 95% confidence limits of the mean) for

Table 2. Coefficient of variation and the maximum deviations from the mean, expressed as percentages of the energy content in terms of dry weight (DW), ash-free dry weight (AFDW), and organic carbon (OC). Standard deviation and maximum deviations from the regressions 1A and 1B (*see text*) between the energy content and the organic carbon level are shown for comparison.

	$\frac{J}{DW}$	$\frac{J}{AFDW}$	$\frac{J}{OC}$	Reg. 1A	Reg. 1B
C.V.	16.1	10.9	3.6	2.6*	2.3*
+ Max dev.	39.9	31.6	6.7	5.4†	4.4†
- Max dev.	34.2	15.9	5.6	3.7†	3.2†

*Standard deviation from regression at the mean carbon level.

†Maximum deviations from the regression line.

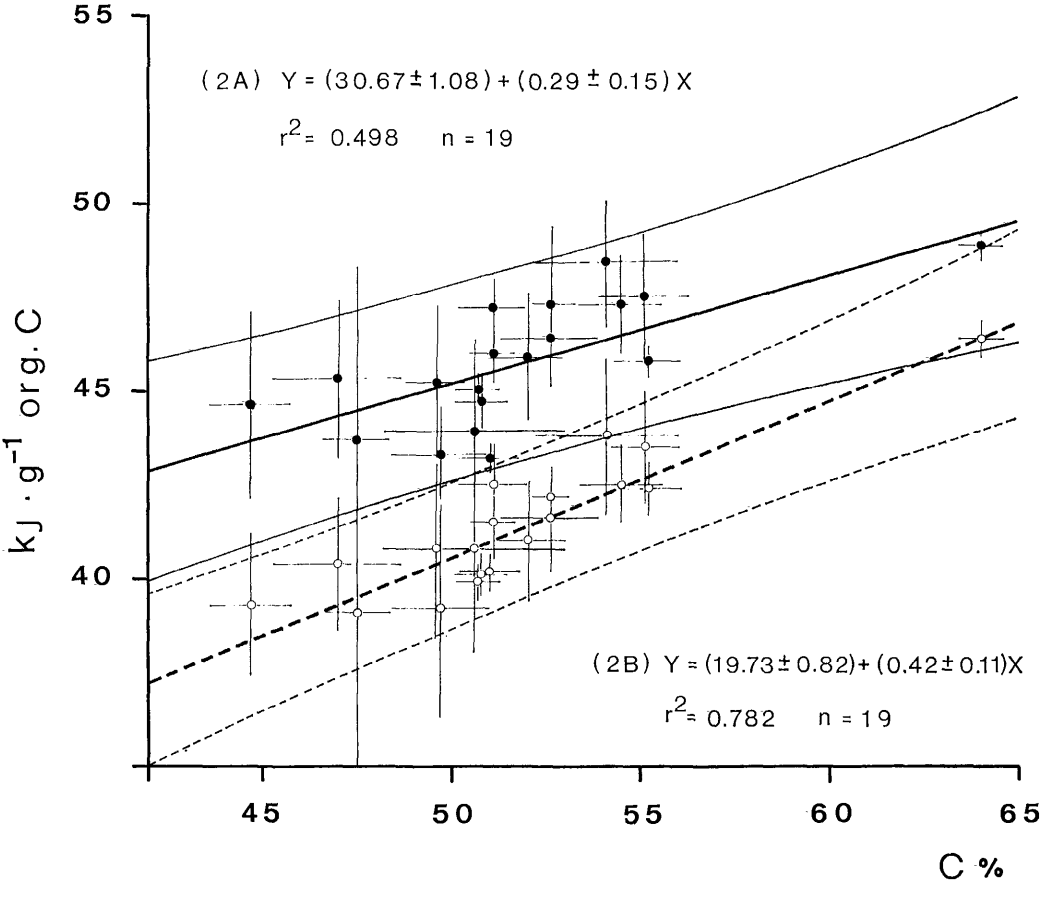

Fig. 2. Relationship between energy-to-carbon ratio and organic carbon level in freshwater invertebrates. Uncorrected—closed circles; corrected for nitrogen—open circles.

organic carbon was 45.7 ± 0.8 kJ g^{-1} C (= 10.9 kcal g^{-1} C) or, when the nitrogen correction was taken into account, 41.4 ± 0.9 kJ g^{-1} C (= 9.9 kcal g^{-1} C). Closer inspection of the data revealed that the ratio of energy content to organic carbon was also dependent on the percentage of organic carbon (Fig. 2). Here, also, the nitrogen correction improved the coefficient of determination considerably. In regressions 2A and 2B (Fig. 2), the coefficients of determination were lower than in regressions 1A and 1B (Fig. 1) because the variation explained by the mean energy equivalent for carbon was already excluded from the former regressions.

We also tried multiple linear equations which included nitrogen level or C : N ratio as additional independent variables, but the coefficients of determination were then lower than in regressions 1B and 2B.

The accuracy of energy values predicted from our regressions on the basis of carbon values is almost comparable to the accuracy of direct calorimetric measurements. At the mean carbon level, the 95% confidence limits for the predicted energy value of an individual sample consisting of five replicates are ±1.3 kJ or ±5.6%, based on regression 1A, and ±1.1 kJ or ±4.9%, based on regression 1B. From regressions 2A and 2B, the confidence limits for prediction are ±1.4 kJ or ±5.7%, and ±1.0 kJ or ±4.8%. In our direct calorimetric measurements the 95% confidence limits for energy content of a sample consisting of five replicates varied between ±0.9 and ±3.5% in 90% of the measurements (total range ±0.5 to ±10.7%).

The nitrogen correction is based on the fact that the main end product of nitrogen metabolism in many aquatic animals is NH_3, whereas the end product of oxidation in the bomb calorimeters is primarily N_2 (Kersting 1972). The correction when urea, uric acid, or other nitrogen-containing compounds are excreted is still higher. The nitrogen corrected energy values for food provide a simple way of taking into account the energy losses with nitrogenous excretory products. According to our material an approximate nitrogen correction of -10% for the animal food of animals that excrete ammonia might be useful. However, in some cases the wet combustion methods may underestimate the nitrogen level (Giese 1967) and therefore this correction may be too low. It should also be noted that since NH_3 can be oxidized further by microorganisms, the nitrogen corrected energy values should not be used in constructing ecosystem energy budgets.

Platt et al. (1969) presented equations for converting the carbon content of zooplankton to energy values. For a given carbon level their equations give much higher values than our regressions. However, they determined carbon by a wet combustion method which may underestimate (cf. Platt and Irwin 1973) so that their energy values, expressed in terms of carbon, were probably too high.

The general conversion factor proposed by Platt and Irwin (1973) for phytoplankton was 47.7 ± 3.3 kJ g^{-1} C, or, when the nitrogen correction is taken into account, 44.9 ± 3.9 kJ g^{-1} C (mean ±95% confidence limits, $n = 10$; recalculated from the original data). These conversion factors are higher than ours, but because of the greater variation in the results of Platt and Irwin, the differences were not statistically significant.

Lipids contain more energy per unit weight than proteins or carbohydrates; the generally accepted average physical energetic equivalents for lipids are 39.6, for proteins 23.7, and for carbohydrates 17.2 kJ g^{-1} (Winberg 1971*b*). Lipids also contain more carbon per unit weight (70–80%) than carbohydrates (40–44%) or proteins (50–54%) (Winberg 1971*a*). The energy content per unit carbon of lipids is thus on the average 1.4 times higher than that of carbohydrates and 1.2 times higher than that of proteins. As high lipid levels are reflected in high carbon percentages, the ratio of energy to carbon should theoretically increase with increasing carbon level. In accordance with these theoretical considerations, there was a statistically significant positive correlation between the ratio of energy to carbon and the percentage of carbon in our material (Fig. 2).

The analogous correlation calculated from the data of Platt and Irwin (1973) was also significant, but unlike that from our results, its sign was negative. In their combined data, however, we found a highly significant positive correlation between lipid level and measured energy content in terms of ash-free dry weight, showing the importance of lipids for energy values. Altogether, energy values calculated from chemical composition agreed well with the measured energy values, as they noted. We also estimated carbon levels from chemical compositions reported by Platt and Irwin. In the samples with energy-to-carbon ratios differing from theoretical expectations the calculated carbon levels were up to about 40% higher than the measured carbon levels, although in the other samples the agreement was good. It is therefore likely that the underestimation of carbon has led to unexpectedly high energy-to-carbon ratios in some of Platt and Irwin's samples. This assumption is also supported by comparison of nitrogen values calculated from protein levels with the nitrogen values that they measured in the CHN analyzer along with carbon.

Theoretically the C : N ratio should also reflect the proximate chemical composition of organic matter. As lipids and carbohydrates lack nitrogen, the amount of carbohydrates must be negligible to allow a prediction of energy content per ash-free dry weight from C : N ratios. We did not find the C : N ratio useful for prediction of energy values, at least partly because of the much lower precision of our determinations of nitrogen than those of carbon.

The monospecific samples used in our study probably represent the total range of energy-to-carbon ratios encountered in nature for freshwater invertebrates better than do composite samples. The distribution of the energy equivalents of ash-free dry weight from our study (range 19.9–31.2 kJ g^{-1}, mean 23.7 kJ g^{-1}) agrees with earlier ones based on a greater range of material (Prus 1970; Cummins and Wuycheck 1971; Driver et al. 1974; Caspers 1975). The general conversion factor suggested above is thus likely to be representative of the majority of aquatic invertebrates.

Biomass expressed as organic carbon can be converted to energy units with reasonable accuracy by using the mean conversion factor of 46 kJ g^{-1} organic carbon. The carbon level then need not be known, and thus weighing samples is unnecessary. As the determination of carbon is possible from smaller samples than are needed for calorimetry or even for weighing, the use of carbon as a measure of biomass is particularly advantageous in studying the energetics of small animals. If the carbon level (per ash-free dry weight) can be determined, or at least estimated, on the basis of previous knowledge, fat level, etc., a somewhat more accurate energy equivalent for organic carbon or ash-free dry weight is obtained from the presented regression equations.

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