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Efficiencies of ingestion and assimilation by an invertebrate predator using C and P dual isotope labeling

Abstract—*Daphnia rosea* was uniformly labeled with both ^{14}C and ^{33}P, then offered as prey to *Bythotrephes cederstroemi* Schoedler, a nonindigenous predatory cladoceran which has become established in the Laurentian Great Lakes. Both C and P from the prey were ingested with equal efficiency (58–59%, SE = 2%) by *Bythotrephes* and retained with equal efficiency over the next 12 h. Feeding behavior by the predator involves a fastidious process of shredding and discard of exoskeletal material and selective ingestion of soft tissue. Most of the ingested matter is consequently digested; assimilation efficiency is 85% (SE = 2%).

Investigations of the effects of an introduced, nonindigenous invertebrate predator, *Bythotrephes cederstroemi* (Cladocera: Cercopagidae), on Great Lakes food webs (Lehman 1988, 1991) raised important questions about the nutritional physiology of the organism. In particular, estimates of in situ prey mortality (Lehman and Cáceres 1993) are sensitive to efficiencies of ingestion and assimilation. The predator discards much debris, mainly exoskeletal refuse, while feeding on its plankton prey. In terms of mass balance, the discarded fractions are analogous to losses during "sloppy feeding" (Dagg 1974; Lampert 1978). Observation suggests that soft prey tissues alone are consumed, which may mean that discrimination practiced during the initial handling time provides a highly assimilable diet. It is possible, therefore, that assimilation efficiencies achieved by *Bythotrephes* are high compared with values commonly cited for crustacean zooplankton in general (e.g. Conover 1966; Dagg 1976).

Stoichiometries of C:P for herbivorous freshwater zooplankton have been found to vary among taxa (Andersen and Hessen 1991). Body tissue stoichiometries of the animals may differ from those of their algal food (Hessen 1990; Sterner 1990), which means that rates of ingestion, assimilation, or metabolism must become uncoupled on an element-specific ba-

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D. K. Branstrator prepared the labeled *Daphnia* for these experiments.

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sis. These observations for herbivores had not been well examined or extended to invertebrate planktivores like *Bythotrephes*. Consequently, it seemed worthwhile to develop a method to establish the feeding economies of C and P simultaneously for the organism.

Bythotrephes was collected from offshore Lake Michigan by 1-m-diameter nets of 300-μm-mesh aperture fitted with 2-liter nonfiltering PVC cod ends. Animals were isolated individually in filtered lake water (FLW) and transported to Ann Arbor for use in experiments after 24 h acclimation at 16°C. Feeding trials were conducted with adult (3-barb), parthenogenic females bearing embryos in early stages of development (no eye pigment visible).

Prey (*Daphnia rosea*) were drawn from cultures labeled with both $^{33}PO_4$ and $Na^{14}CO_3$ in glass-stoppered 125-ml bottles for 4 d, a sufficient time to achieve uniform labeling (Lehman and Naumoski 1985; Branstrator in press). Prior to feeding trials, prey were isolated from culture and rinsed copiously in FLW, then grasped by their tail spines with watchmaker forceps and offered to individual *Bythotrephes* in 2.5-ml FLW in tissue culture wells. *Bythotrephes* typically grasped prey within seconds. The ingestion process was monitored microscopically until completed and uningested prey debris was discarded, within 15 min. The predator was then transferred to FLW for times ranging from 0 to 12 h and the feeding-well contents, including uningested debris, were transferred to scintillation vials and frozen over dry ice. I added neonatal *Bythotrephes* to the postingestion chambers to provide additional, unlabeled food for the predators. After incubation, *Bythotrephes* and the contents of the incubation chambers were transferred individually to scintillation vials and frozen over dry ice. I freeze-dried all frozen samples, pulverized *Bythotrephes* with glass pestles, and added scintillation cocktail (Biosafe II). Samples were subjected immediately to liquid scintillation counting ($t = 0$) and again 52 d later ($t = 52$) after two half-lives of the ^{33}P radioisotope, in order to discriminate between the isotopes, as explained below.

Background-corrected count rates in a spectrum that included all β-particle emissions were apportioned between ^{14}C and ^{33}P according to the simultaneous linear equations

$$\text{cpm}(0) = C^* + P^* \quad (1)$$

$$\text{cpm}(t) = C^* + P^* \cdot \exp[t/25.4/\ln(2)]. \quad (2)$$

C^* and P^* are counts attributable to ^{14}C and ^{33}P on day 0; the half-life of ^{33}P is 25.4 d. Emission energies of ^{14}C and ^{33}P particles are almost identical, and internal channels ratios indicated that all samples were counted at nearly identical efficiencies.

Total isotope inventories C^*_{tot} and P^*_{tot} were defined as

$$C^*_{tot} = C^*_{feed} + C^*_{chase} + C^*_{pred} \quad (3)$$

and

$$P^*_{tot} = P^*_{feed} + P^*_{chase} + P^*_{pred} \quad (4)$$

where the subscript "feed" denotes radiotracer present in the feeding chamber (including prey debris), "chase" denotes isotope in the postfeeding incubation chamber, and "pred" denotes tracer retained by *Bythotrephes* after the incubation period.

Statistical analyses were performed with SYSTAT version 5.0.

Ingestion efficiency, IE, can be calculated from the fraction of total radiotracer in the prey that was ingested by the predator. Some of the ingested tracer was subsequently released to the postfeeding chamber during incubation.

Dynamics of tracers for C and P must be considered individually because, whereas the experimental design constituted a closed system for P which has no gaseous phase, C could be lost when converted to CO_2 during metabolism by *Bythotrephes* in the postfeeding chamber, which was open to the atmosphere. Control trials revealed that 85% of added [^{14}C] bicarbonate was eliminated by the procedures. Loss of CO_2 in the experiments was evidenced by the fact that linear regression of the sum of $C^*_{chase} + C^*_{pred}$ vs. time of postfeeding incubation had a significantly negative slope (2,020 cpm h^{-1}; $P = 0.011$), equal to a loss of 5% h^{-1}, whereas regression of $P^*_{chase} + P^*$ pred vs. time was not significantly different from zero ($P = 0.461$). As a result, ingested ^{14}C (C^*_{ing}) in these experiments could be reconstructed from

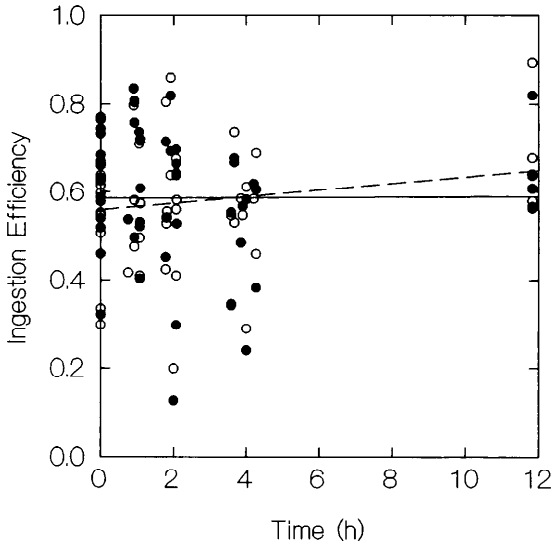

Fig. 1. Ingestion efficiencies by *Bythotrephes* for C (●, solid line) and P (○, broken line) calculated from Eq. 7 and 8.

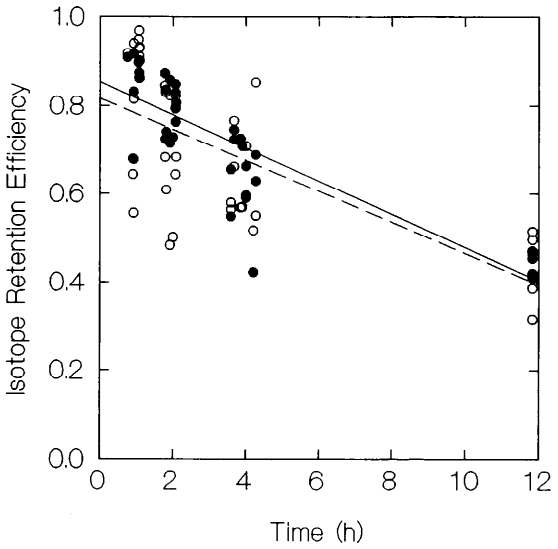

Fig. 2. Retention efficiencies for C (●, solid line) and P (○, broken line) by *Bythotrephes* during postfeeding incubations calculated from Eq. 11 and 12.

$$C^*_{ing} = (C^*_{chase} + C^*_{pred}) \times 1.05', \quad (5)$$

whereas ingested ^{33}P could be calculated simply as

$$P^*_{ing} = P^*_{chase} + P^*_{pred}. \quad (6)$$

Element-specific ingestion efficiencies can thus be calculated from

$$IE_C = C^*_{ing}/(C^*_{ing} + C^*_{feed}) \quad (7)$$

and

$$IE_P = P^*_{ing}/(P^*_{ing} + P^*_{feed}). \quad (8)$$

Analysis of covariance could detect no significant effects of tracer ($P = 0.798$) or of incubation time ($P = 0.374$) on simultaneous determinations of IE for C and P (Fig. 1); IE_C = 0.586 (SD = 0.15, SE = 0.021, $n = 52$), IE_P = 0.578 (SD = 0.14, SE = 0.020, $n = 52$). Variabilities of IE appeared to be idiosyncrasies of the handling and refuse discard process.

Retention efficiency (RE) of ingested tracer by *Bythotrephes* during postfeeding incubation was defined as

$$RE_C = C^*_{pred}/C^*_{ing} \quad (9)$$

and

$$RE_P = P^*_{pred}/P^*_{ing}. \quad (10)$$

Analysis of covariance (data in Fig. 2) detected no significant differences between elements ($P = 0.242$) but did detect a strong decline in retention over time ($P < 0.0005$), which is the result of gut release, respiration, and excretion. Linear regressions revealed very similar trends for each isotope:

$$RE_C = 0.854 \text{ (SE} = 0.020) - 0.038 \text{ (SE} = 0.004) \times t, \quad (11)$$

and

$$RE_R = 0.817 \text{ (SE} = 0.030) - 0.035 \text{ (SE} = 0.006) \times t. \quad (12)$$

Moreover, linear regression of $C^*_{pred} : P^*_{pred}$ vs. time shows no trend (slope = 0.000, SE = 0.007, $P = 0.981$). Paired t-test of $C^*_{pred} : P^*_{pred}$ with $C^*_{feed} : P^*_{feed}$ could detect no differences ($P = 0.322$), implying that C and P are ingested and retained essentially in the same stoichiometries that they occur in the *Daphnia* prey.

Accuracy of estimates for assimilation efficiency (AE) depends on the extent to which it is possible to distinguish egested material from excreted material released into the incubation chamber during the postfeeding period. Egested matter should be discounted from ingestion:

$$\text{Assimilation} = \text{Ingestion} - \text{Egestion}, \quad (13)$$

whereas excreted material has participated in metabolism and should be counted in addition to isotope retained in the predator tissue:

$$\text{Assimilation} = \text{Retention} + \text{Excretion}. \quad (14)$$

For P, it is impractical to discriminate isotope that is released into the postfeeding chamber, designated P^*_{chase}, as to whether it originated as feces or as a metabolite. It seems logical that the contribution of egested material to the measured pool would be greatest in the first hours of incubation and would decline to zero after an appropriate gut passage time. Visual observations revealed that isolated *Bythotrephes* completely purge their guts 12 h after isolation from prey, but quantitative assessment of mass changes is not feasible by visual observation alone.

For C, however, unbiased estimation of AE is possible because metabolized ^{14}C would be released as CO_2 and thus lost from solution. The ^{14}C accumulating in the postfeeding incubation chamber represents nonvolatile organic compounds released mainly in egestion, plus some excreted organic materials that were not assimilated and fully metabolized. Thus,

$$C^*(t) = (C^*_{ing} - C^*_{chase})/C^*_{ing} \quad (15)$$

represents the time-course of ^{14}C in the predator's tissue, including isotope that was assimilated and then lost. True assimilation efficiency is the asymptote of $C^*(t)$ vs. time:

$$C^*(t) = (1 - AE)\exp(-kt) + AE. \quad (16)$$

Figure 3 shows results computed by Eq. 15. AE = 0.846 (SE = 0.022) was estimated by nonlinear regression (Eq. 16).

Data for ingestion efficiencies demonstrate that although substantial quantities of prey biomass are discarded during feeding, whatever discrimination is practiced by the predator does not result in differential relative ingestion rates based on C or P mass balance. Mean ingestion efficiency for C of 59% (SE = 2%) and for P of 58% (SE = 2%), obtained here by the dual isotope method, are indistinguishable from the independent estimate of 55–59% for P found by Burkhardt and Lehman (in prep.), based on direct chemical measurements.

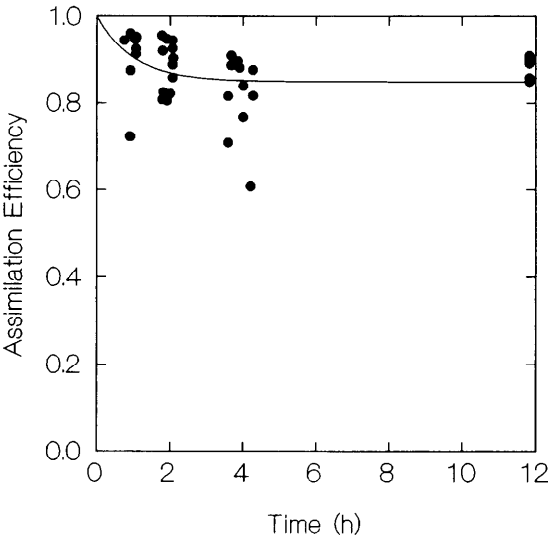

Fig. 3. Assimilation efficiency for prey C calculated as an asymptote (Eq. 16).

Assimilation efficiency of 85% (SE = 2%) for C is near the high extreme of values reported for crustacean zooplankton (e.g. Dagg 1976) and is probably the consequence of a fastidious feeding behavior that results in discard of poorly digestible tissue before ingestion.

The observation that *Bythotrephes* seems to ingest and retain C and P from its *Daphnia* prey in fixed proportions may reflect the roughly similar stoichiometries of these two cladocerans. Andersen and Hessen (1991) have reported that ratios of P to DW by mass for *Daphnia* species average 1.43 (SD = 0.27), whereas Burkhardt and Lehman (in prep.) found that P content of *Bythotrephes* ranges from 1.42 (P/DW) for juveniles to 1.07 for adults. Similarity of the stoichiometries of predator and prey, coupled with constancy in proportions of C and P ingested and retained by *Bythotrephes,* suggests that *Daphnia* provides a balanced diet for this invertebrate predator.

This dual isotope method for measuring efficiencies of element ingestion, retention, and assimilation was developed to study an exotic predator in the Nearctic, but it should prove useful in studies of elemental economies of other plankton species. It should be particularly useful in comparing the feeding efficiencies of different predators and in ranking the

profitability (gain per unit handling time; e.g. Branstrator in press) of their prey. Most importantly, it provides a direct way to investigate the mechanisms by which predators may adjust ingestion, retention, and assimilation efficiencies when feeding on prey of different elemental stoichiometry.

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Effect of dissolved organic carbon on ^{59}Fe scavenging

Abstract—The scavenging rates of tracer ^{59}Fe via sedimentation and loss rates of dissolved organic C (DOC) from limnocorrals containing different proportions of DOC-rich stream water and DOC-poor lake water were measured. ^{59}Fe scavenging rates were inversely correlated with concentration of DOC, decreasing by a factor of four as DOC increased from 550 to 1,550 μmol liter^{-1}. In contrast to Fe, the loss rates for DOC increased with increasing DOC concentration; thus the mechanisms for loss of Fe and DOC appeared to be different. The effect of DOC on Fe scavenging was consistent with competition between organic ligands and particles for aqueous Fe and with DOC-enhanced colloid stability. Fe scavenging was poorly related to particle concentration and settling velocity.

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During the last decade, processes that remove dissolved trace and minor metals from water have been studied intensively to understand and predict the fluxes and concentrations of metals in natural waters (Santschi 1988; Honeyman and Santschi 1988). Iron has received special attention, because the biogeochemical redox cycles of Fe are interconnected to the cycles of nutrients and other elements (e.g. Sholkovitz and Copland 1982).

Filtration operationally separates trace metals into suspended and dissolved pools. Suspended trace metals are removed from surface water as the particles sink at velocities from