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Diet composition from allozyme analysis in the predatory cladoceran *Bythotrephes cederstroemi*

Abstract—Investigating diet composition with visual analyses of gut contents requires that the predator consume recognizable hard parts. Soft tissue remains of prey in the gut of the predatory cladoceran *Bythotrephes cederstroemi* were identified with cellulose acetate electrophoresis. We found that *B. cederstroemi* collected from an offshore station in Lake Michigan had fed on cyclopoid and calanoid copepods and *Daphnia galeata*. Many individuals had eaten more than one prey type. Allozyme electrophoresis is a relatively simple way to analyze trophic relationships in freshwater systems, especially when traditional analyses are not feasible.

Gut content analysis and experimental observation are typical means of investigating predator diet composition, but the feeding habits of some predators make conventional application of these techniques almost impossible. Many planktonic organisms are difficult to maintain in artificial enclosures used in predation experiments; even when the animals survive the study conditions, questions can arise concerning the applicability of results to nature. Gut content analysis that depends on morphological identification of pieces of ingested prey also may be problematic when the predator does not consume recognizable hard parts.

Dramatic declines of several species of *Daphnia* followed the invasion of *Bythotrephes cederstroemi* in Lake Michigan (Lehman 1988). This invertebrate predator was implicated in these changes (Lehman 1991), although direct measurement of predation proved difficult. The autecology of *B. cederstroemi* was largely unknown before its invasion of North America, and its life cycle was completely documented only recently (Yurista 1992). Some of its laboratory feeding rates had been reported (Mordukhai-Boltovskaya 1958), but its feeding preferences were not investigated in detail.

Investigations of prey selection by *B. cederstroemi* typify the difficulties of determining diet breadth. *B. cederstroemi* is cannibalistic when held in experimental containers (pers. obs.), and this may have complicated several recent studies that attempted to quantify predation (Sprules et al. 1990; Vanderploeg et al. 1993). *B. cederstroemi* was reported to consume a variety of prey items under experimental conditions (Mordukhai-Boltovskaya 1958; Vanderploeg et al. 1993) and may be a generalist predator. However, diet breadth determined from enclosure experiments may be incomplete, because the reported predation rates are energetically inconsistent when compared with consumption rates determined from mass-balance models of *B. cederstroemi* (Burkhardt 1991; Yurista and Schulz 1995). Furthermore, it is not possible to recognize prey remains in the gut of *B. cederstroemi* because these predators shred their prey, and examination of gut contents of preserved specimens and individuals fed known prey revealed no identifiable hard parts of prey.

We used cellulose acetate allozyme electrophoresis and scanning laser densitometry to identify soft tissue remains of prey from *B. cederstroemi* (syn. *B. cederstroemii*) Schödler (Crustacea: Cladocera), a recently introduced predator in the Laurentian Great Lakes (Bur et al. 1986; Lange and Cap 1986; Lehman 1987; Cullis and Johnson 1988). Multilocus phenotypes for each taxon of potential prey in the lake plankton assemblage were obtained from species-specific migration rates for multiple enzymes. Characteristic banding patterns for these reference specimens were matched with bands from gut material of an individual *B. cederstroemi*, and the types of prey consumed were identified.

Five enzymes found to be monomorphic for many zooplankton species (Boileau and Hebert 1988*a*,*b*; Weider 1991) were evaluated for prey identification: *Ao* (aldehyde oxidase, AO, EC 1.2.3.1), *Fum* (fumarate hydratase, FUM, EC 4.2.1.2), *Mdh* (malate dehydrogenase, MDH, EC 1.1.1.37), *Pgm* (phosphoglucomutase, PGM, EC 2.7.5.1), and *Pgi* (phosphoglucose isomerase, PGI, EC 5.3.1.9). Monomorphic enzymes were preferred; however, even species with polymorphisms could be uniquely identified on the basis of their banding patterns. All five enzymes could be assayed with material from a single *B. cederstroemi*.

Electrophoresis was performed on cellulose acetate gels (Helena Scientific) using a Tris-glycine buffer, with staining according to Hebert and Beaton (1989). The gels were run at 200 V for 25 min at 16°C. After staining, the gels were rinsed with deionized water, visually scored, and scanned with an Ultroscan XL laser densitometer. This device measured both the absorbance and migration distance of the allozyme bands. GelScan XL Software (model 2400 for the IBM XT, LKB Produkter AB) allowed accurate quantification of migration distance for each band. Most (84.3%) visually observed peaks were present in the densitometer scan; occasionally, faint peaks were visible on the gel, but not discernible from background variation on the densitometer scan (classified as <0.007 absorbance units, compared with an average of 0.070 absorbance units for *B. cederstroemi* peaks). To avoid errors in scoring, only peaks that met three criteria were included: they were visible on the gel, they were present above background noise on the densitometer scan, and they had a well-defined peak shape on the scan. Sixty-five percent of total potential peaks fulfilled these criteria. These criteria may have reduced the total number of identifiable prey, but they ensured that no scratches on the gel or other artifacts were included as peaks. A digitizer

Table 1. Number of replicate standard runs for each potential prey species for each enzyme used to construct the index for comparing standards to unknown species. Copepodite stage—C1–C6.

Prey type	*Ao*	*Fum*	*Mdh*	*Pgm*	*Pgi*
Cyclopoid species (C1–C6)	15	14	15	8	12
Daphnia galeata	15	14	15	13	11
Diaptomus sp. (C1–C6)	9	12	12	5	23
Epischura sp. (C1–C6)	3	3	3	3	3

may be preferable to the densitometer for rapid direct measurement of migration distances and for eliminating the need to cross-check visually observed bands with scanned peaks.

Plankton samples were collected from an offshore reference station at 43°N, 86°40′W (36 km offshore; depth, 100 m) in Lake Michigan on 12 August 1991. *B. cederstroemi* was taken with a 1-m-diameter 300-μm-mesh net from a 0–20-m vertical tow, rinsed in filtered lake water, and immediately frozen on Teflon over dry ice. The crustacean zooplankton assemblage was sampled with a 0.5-m-diameter 63-μm mesh Puget Sound closing net from a 0–20-m vertical tow. The material from this tow was poured through a 63-μm Nitex sieve until a thin layer of plankton had collected on the sieve. Replicate samples were placed in plastic Petri dishes on dry ice. In the laboratory, frozen specimens were stored at −80°C. An additional 30–0-m net tow was collected with the Puget Sound closing net, and subsamples were enumerated to quantify the abundance of crustaceans.

Machine standards were run to assess the consistency of migration distances. A homogenate of neonate *B. cederstroemi* was applied to 7–10 lanes on each of three different gels for each enzyme. From these runs, average within- and between-gel variances for the same sample were determined. The variance in migration distances within gels was generally lower than that between gels, probably due to slight differences in voltage and the time voltage was applied between runs. Between-gel standard errors of migration distances ranged from 37.62±0.05 (standard densitometer distances, about equivalent to mm) for *Pgm* to 37.09±1.44 for *Ao*, whereas within-gel standard errors ranged from 29.24±0.05 for *Mdh* to 34.65±0.37 for *Ao*.

In a preliminary test, several putative prey species had species-specific migration rates for the five enzymes assayed in this study. Allozyme banding patterns of the prey were distinct from those of *B. cederstroemi*. Known individual prey were offered with forceps to *B. cederstroemi*, and a clear allozyme signal from the ingested prey item was found when a whole extract of the predator was assayed an hour later. All equipment was cleaned thoroughly after each run. No residual bands from previous samples were observed on subsequent gels. Neonates that had never eaten served as standards for *B. cederstroemi* allozyme migration distances. There were no apparent complications from age-specific shifts in allozyme phenotypes. Comparisons of neonate and adult banding patterns for four of the enzymes (*Ao, Fum, Mdh,* and *Pgm*) revealed no statistically significant differences in migration rate (Mann-Whitney *U*-test; $P > 0.22$ in all cases; Wilkinson 1990).

An allozyme survey of potential prey was conducted by thawing some of the frozen zooplankton and identifying organisms to species or genus. Animals were ground with forceps, probes, and glass rods; the volume of homogenate was adjusted to ~5 μl with Tris-glycine (TG) buffer (Hebert and Beaton 1989). Samples were assayed for all five enzymes. *B. cederstroemi* neonates again were used as migration distance standards. Material from many individuals (~10 for daphnid species and *Epischura lacustris,* and 30–40 for copepod taxa other than *E. lacustris*) was homogenized for each replicate run to obtain an accurate assessment of population-level variation. The number of replicate runs for each potential prey item for each enzyme is shown in Table 1. The migration distance for each enzyme was converted into a percentage of the *B. cederstroemi* enzyme migration distance, and an index of the relative migration distances of these standard peaks was created. Each taxon was characterized by one or several (in the case of multiple bands) migration distance(s) in this standard index. Standard deviations from the replicate standard runs were used to establish migration distance tolerance limits containing each taxon. Ranges of both 1 and 2 SD about the mean migration distance were entered into the standard index. These ranges theoretically should encompass 68 and 95% of the total range of peak values for each taxon.

Many taxa had multiple peaks for a particular enzyme. These multiple peaks did not necessarily limit the ability to identify each taxon, but they complicated the procedure. Sometimes each of the multiple bands had a migration distance distinct from bands of all other taxa in the assemblage, which still allowed unambiguous prey identification. When bands of several organisms did overlap for one enzyme, we made a few simplifying assumptions to identify the prey. From the standard runs, the multiple peaks for each taxon were ranked according to their relative frequency of occurrence. Often a particular taxon had a peak that was always present in the standard runs. If this peak was not present in the unknown, the corresponding taxon was assumed to be absent. If an unknown peak was identified as being either the secondary or tertiary peak of a first taxon or the primary peak of a second taxon, it was considered most likely to be that of the second taxon, unless other peaks indicated that the first taxon also had been consumed. Similarly, if an unknown peak was within 1 SD of the standard peak for a first taxon, but only within 2 SD of the standard peak for a second taxon, it was considered most likely to be that of the first taxon, barring additional evidence from other peaks.

Given this information, prey assemblages must be characterized for every sample location. Site-specific characterization is essential to encompass both the local genetic variability and different species composition a predator may encounter. Reduced numbers of zooplankton species inhabit offshore Lake Michigan (Balcer et al.

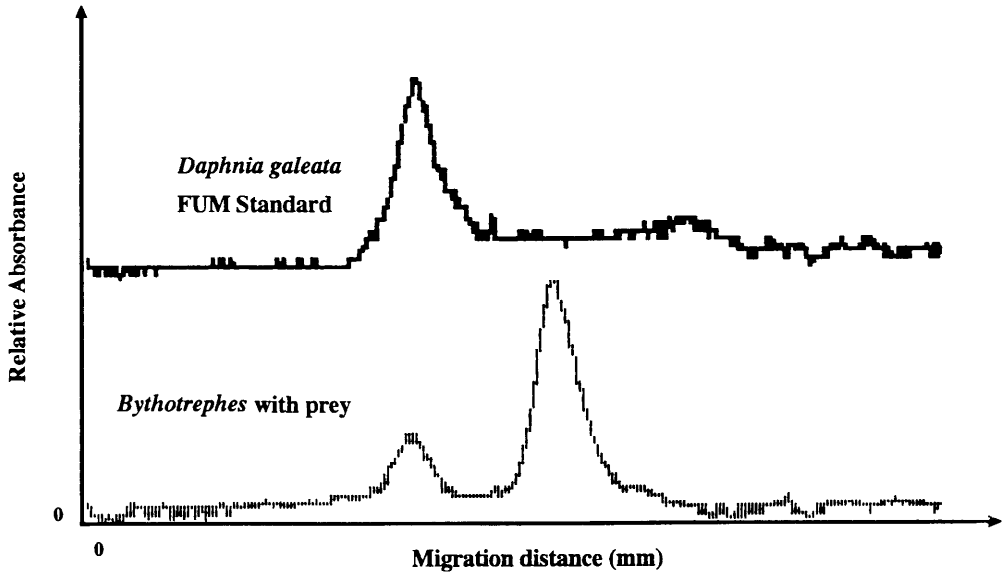

Fig. 1. Identification of the prey *D. galeata* using a single enzyme (*Fum*). The upper laser densitometer absorbance spectrum is typical of *D. galeata* from the offshore station sampled in Lake Michigan. The lower spectrum is of a *Bythotrephes cederstroemi* from this same station. The large, faster migrating peak is the characteristic monomeric peak for *B. cederstroemi,* and the smaller, slower migrating peak is the putative prey item *D. galeata.* No other potential prey present at this station on 12 August 1991 had a similar migration distance for *Fum.*

1984), limiting the number of decision rules necessary to identify banding patterns. Nonetheless, constructing standard peak indices for potential prey is a laborious process, requiring a large sample size of organisms and a great deal of analysis.

Thirty adult *B. cederstroemi* were used to determine predator diets; they were thawed and homogenized after first removing their tail spines. It was not necessary to dissect out the gut to obtain clear prey signals. Gels were stained for ~15–30 min, until the fainter prey bands had time to develop fully. Migration distances of the peaks from these gels were scored. Peaks were assigned to taxa by comparison with the standard peak index for the entire zooplankton assemblage.

Pgi was not a particularly useful enzyme for distinguishing among prey items from the community we sampled. It is, however, very useful for distinguishing *Bosmina longirostris* and *Eubosmina coregoni,* which were not present in this sample. For several of the enzymes reported to be monomorphic in *B. cederstroemi* (Weider 1991), multiple bands were sometimes present (*Fum* and *Mdh*). This difference in the level of enzyme polymorphism may be due to differences among the *B. cederstroemi* populations in Lakes Erie, Huron, and Michigan, temporal sampling differences, or to an increase in the level of genetic variation in populations of *B. cederstroemi* in the Laurentian Great Lakes.

Prey taxa from 13 of the 25 *B. cederstroemi* collected in the field that had allozymes from prey in their guts were identified unambiguously based on their enzyme peak migration distances. For some taxa, the results of a single enzyme were sufficient to identify a prey item (Fig. 1), but in others, the absorbance profiles from several enzymes were required for confirmation (Fig. 2). Peaks of prey taxa from 10 additional *B. cederstroemi* could be identified with good probability based on the simplifying assumptions described above. This left only two individuals for which prey could not be identified because it was equally probable that several of the taxa had been consumed. Four peaks (out of 110) were found that did not match peaks from the standard index. We were unable to assay rotifer allozymes, and it is possible that these peaks (two of which appear to be the same) belong to rotifers such as *Asplanchna* spp., which were abundant, or to another prey species that was present in low numbers. These peaks also may have belonged to prey we had surveyed, but were uncommon and not found in our standard runs.

Of the 30 *B. cederstroemi* tested, five did not exhibit any bands in addition to their own. Fifty percent of the *B. cederstroemi* contained a single prey taxon, and 32% contained several prey taxa. This estimate of the number of different prey types consumed is conservative, because it does not include the few unknown prey or the cases where another prey item may have been present but could not be conclusively identified. The number of *B. cederstroemi* containing each prey taxon was calculated and compared with the abundance of that taxon in the field (Table 2).

These results show that *B. cederstroemi* from this sample had been feeding on cyclopoid copepods, *Daphnia galeata,* and several species of calanoid copepods. Many

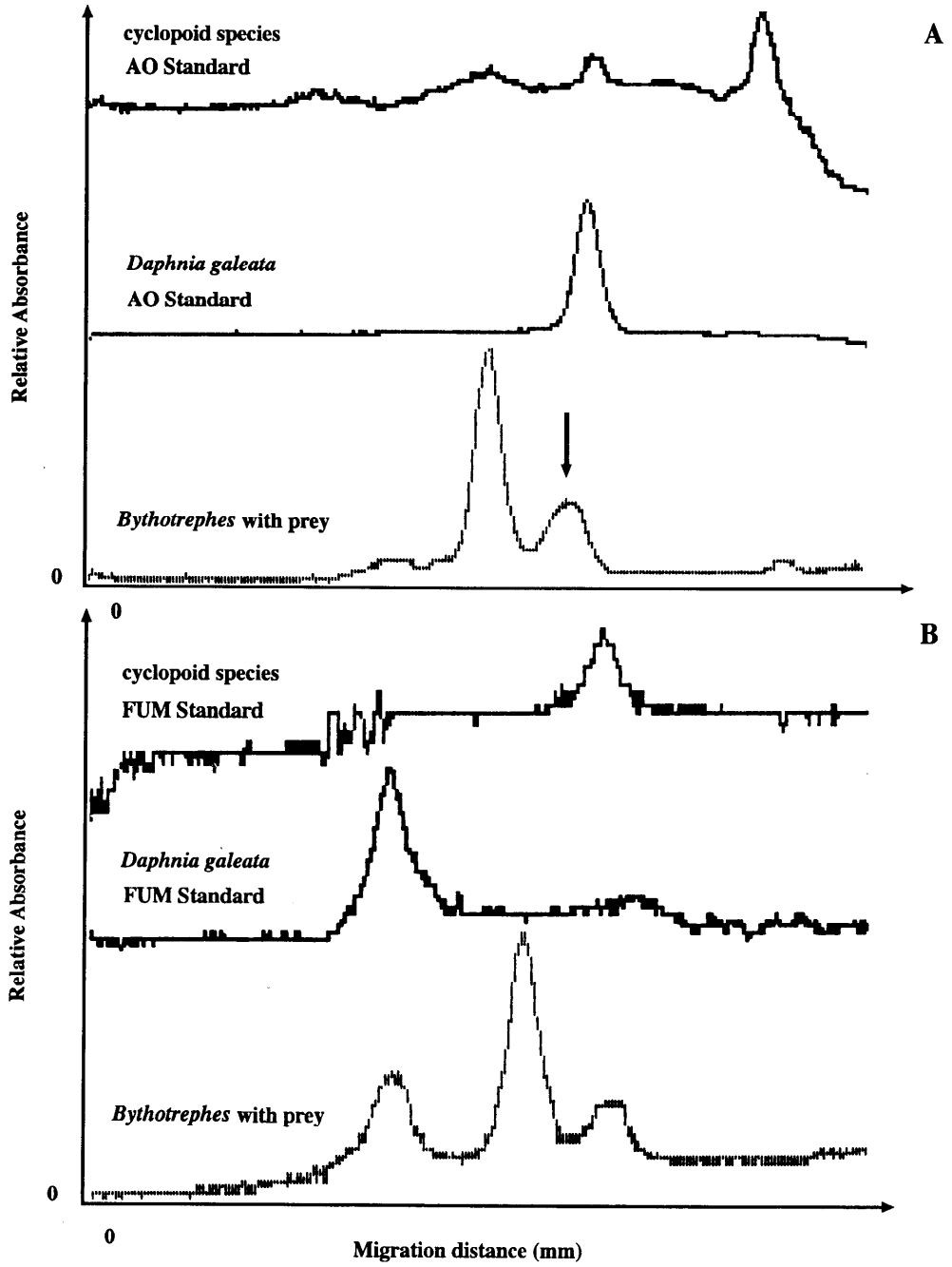

Fig. 2. Identification of prey using multiple enzymes. A. Three migration spectra for the enzyme *Ao*: cyclopoid species standard (upper), *D. galeata* standard (middle), and *Bythotrephes cederstroemi* with prey (lower). Migration distances of potential prey overlap for the middle peak (arrow). B. Same standard taxa and same *B. cederstroemi* analyzed for *Fum.* Using the information from both of these enzymes we determined that this *B. cederstroemi* had consumed both *D. galeata* and cyclopoid species.

individuals had eaten more than one type of prey, suggesting that *B. cederstroemi* may be a generalist predator. The implied ability of *B. cederstroemi* to capture copepods, especially cyclopoids, was surprising because in several of our laboratory predation experiments *B. cederstroemi* did not consume a significant number of either calanoid or cyclopoid copepods. Some preliminary results did suggest that *B. cederstroemi* may feed on naupliar or early copepodid stages, and Vanderploeg et al. (1993) also reported that *B. cederstroemi* fed on nauplii in Lake Huron. This allozyme technique can distinguish species but not developmental stages. A seasonal analysis of cohort-

structured populations may resolve the question of whether particular groups are consumed primarily when their juvenile stages are present.

Allozyme electrophoresis to examine prey remains provides a qualitative measure of the species eaten, but cannot provide an estimate of the amount of prey consumed. The allozymes revealed by this technique almost certainly are from recently eaten prey. The length of time prey enzymes remain detectable in the gut of *B. cederstroemi* after ingestion is unknown, although potentially confounding effects from partially digested enzymes were not observed. Migration distances of ingested prey allozymes were similar for periods of 1–3 h of digestion. Even limited digestion likely resulted in sufficient denaturation of the allozymes to prevent the staining reactions, which depend on enzyme activity.

The amount of allozyme found in the gut of *B. cederstroemi* is likely to be a function of the mass of prey rather than number of prey eaten, so it is unlikely that this technique could be made more quantitative. The relationships between mass of prey, absorbance of the allozyme band, and gut residence time would need to be known, and the time-course of digestion and ambient temperature would still complicate interpretation.

Allozyme electrophoresis is a relatively simple way of analyzing trophic relationships, especially when compared to the intricacies of immunological assays (Feller et al. 1979; Calver 1984; Theilacker et al. 1986). The bands of prey allozymes are clearly present on the gels of predator homogenate, but require slightly longer to stain. That they have not been exploited more frequently (*but see* Young 1983) may result from advice in a standard allozyme reference manual to stain for as short a time as possible to avoid "complicated banding patterns" (Richardson et al. 1986). The fact that we observed additional bands only from *B. cederstroemi* that had consumed prey (and never in neonates) indicates that this complication may sometimes hold useful information. Allozyme electrophoresis has been used successfully on large samples of cultured algae (Murphy and Guillard 1976; Murphy 1978; Gallagher 1980, 1982; Brand et al. 1981) and there have been attempts to perfect it for small quantities of algae as well (Hebert and Beaton 1989). Technique modifications may open the way to new methods of analysis for herbivore diet choice as well.

The major requirement for application of this method is that all potential diet items be analyzed for their allozyme banding patterns, and that the migration distances be distinct enough to allow unambiguous identifications. In our study this was not prohibitively complicated, and it may be true of many pelagic freshwater systems. Because the technique is fairly laborious, it is most useful for distinguishing a few prey species in a simple system or for identifying particular prey with a unique banding pattern. Finding clearcut differences in prey allozymes might be more challenging in marine systems, where the number of potential prey species may be much greater and where many enzymes are highly polymorphic.

Monomorphic enzymes are often of little value for intrapopulation studies precisely because they exhibit no intraspecific variability. They are exceptionally useful, however, for distinguishing zooplankton taxa and can be used to identify the soft tissue present in the gut of some predators. This qualitative measure of the types of prey ingested should complement existing methods for assessing predator diet composition. In situations where more conventional investigations are difficult or impossible, allozyme analysis should be a useful companion to other tracers of trophic interactions.

Table 2. Abundances of Lake Michigan crustacean zooplankton estimated from subsamples of a single 30–0-m net tow collected concurrently (12 August 1991) with *Bythrotrephes cederstroemi* compared with their frequency of detection in the diets of 30 *B. cederstroemi*. Five individuals contained no identifiable prey, 15 contained a single prey taxon, and 10 had consumed more than one taxon. Copepodite stage—C1–C6.

	Areal abundance (m^{-2}) (30 m to surface)	Frequency detected
Cyclopoid species (C1–C6)	60,993	
Daphnia galeata	83,149	18
Diaptomus sp. (C1–C6)	175,447	4
Epischura sp. (C1–C6)	1,910	8
Nauplii	77,547	5

Kimberly L. Schulz
Peder M. Yurista

Department of Biology
Natural Science Building
University of Michigan
Ann Arbor 48109-1048

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