FEEDING HABITS OF IMMATURE STAGES OF *ISOPERLA NANA* (INSECTA: PLECOPTERA: PERLODIDAE) IN JORDAN CREEK (VERMILION COUNTY, ILLINOIS)

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INTRODUCTION

Isoperla nana (Walsh) 1862 (Plecoptera: Perlodidae) is an important component of the stream food webs of Jordan Creek (Vermilion County, Illinois) because of its occurrence in headwater reaches, which are often affected by agricultural practices. Food habits of naiads of the genus *Isoperla* differ among species. Some species facultatively consume food (Feminella and Stewart 1986) but others show ontogenic (Lancaster et al. 2005, Cereghino 2006) or seasonal (Malmqvist et al. 1991) shifts of food.

Little is known about the feeding ecology of the species, especially the naiads (Stewart and Stark, 2002). *Isoperla nana* (Figure 1) has been classified as herbivorous, as no evidence of animal matter was seen (Frison 1935). Yet its mouthparts (Figure 2) are those of a carnivore (Claassen 1931).

Two complementary techniques were used to investigate the feeding ecology of *I. nana* naiads. Gut content analysis assesses the types of food an animal consumes (e.g., Malas and Wallace 1977). Carbon (C) and nitrogen (N) stable isotope analyses (SIA) inform about assimilated food by determining the nature of diet (Van Der Klift and Ponsard 2003).

We hypothesize that the trophic level and food habits of *I. nana* vary across developmental instars and seasons.

Figure 1. A large late instar of *Isoperla nana* collected from Jordan Creek, Vermilion County, Illinois.

Figure 2. Labium of a late instar *Isoperla nana* with glossae (g) unequal to paraglossae (pg), not as in herbivorous stoneflies. (lp) labial palp.

MATERIALS AND METHODS

Qualitative sampling of Isoperla nana naiads, using a dip net

- For gut analyses, *I. nana* naiads were collected in April and May 2006.
- For SIA, *I. nana* naiads and other invertebrates were collected on December 13 2005, March 20, April 12 and May 05, 2006. Algae, roots of aquatic plants, dead branches and leaves, and biofilms were also collected.

Gut content analyses

Guts of *I. nana* naiads were analyzed using methods described by Cummins (1973), with slight modification:

Figure 3. Materials needed to collect the *Isoperla nana* gut contents onto a membrane filter.

- Slides were examined under stereomicroscope with a digital camera attached.
- Image were processed with Image-Pro® Express, version 5.1.
- Random fields of view (15) were digitized.
- Food types were divided into categories and their proportional areas were calculated.
- Small (head capsule width 0.4-1.0mm) and large (head capsule width 1.1-1.4 mm) size *I. nana* were created to pool the limited number of individuals.

Stable isotope analyses

- Samples were thawed, oven dried (65˚C for 72h), homogenized, and weighed
- then loaded into tin capsules and combusted in an elemental analyzer coupled to an isotope ratio mass spectrometer (Thermo Finnigan MAT 252) for N and C composition analyses at the Isotope Geochemistry laboratory.
- Results from SIA were analyzed by plotting $\delta^{13}C$ against $\delta^{15}N$ values.
- Trophic levels can be assessed due to enrichment in $\delta^{15}N$ in the bodies of consumers relative to their food (Peterson and Fry 1987, Lancaster et al. 2005).

- To facilitate interpretation of the SIA results, we grouped taxa according to their trophic level as reported in the literature (Merritt and Cummins 1996, Thorp and Covich 2001).

Statistical analyses

- We used SPSS version 12 (SPSS, Inc., Chicago, IL) for all statistical tests with a significant difference at $P < 0.05$. Nonparametric tests were used due to small sample size.

RESULTS

Types of food consumed by *I. nana* naiads

- 34 gut samples of *I. nana* (April-May 06) were analyzed, with17 of them empty.
- Diatoms (0.43, SD 0.23), and amorphous detritus (0.31, SD 0.14) were highly consumed (Figure 4). They were in equivalent amount in the guts of *I. nana* naiads (Friedman test, n = 17, χ^2 = 0.059, df = 1, P = 0.81) but showed significant differences in proportions while compared with the rest of the food types (Friedman test, n = 17, χ^2 = 39.491, df = 4, P = 0).
- Animal material (0.13, SD 0.16), plant fragments (0.05, SD 0.05) and algae (0.04, SD 0.03), in equivalent amount in the gut (Friedman test, n = 17, χ^2 = 1.284, df = 2, P = 0.53), contributed much less to the diet of *I. nana* naiads (Figures 5,6).

Figure 4. Selected food types in the gut of *Isoperla nana* collected from Jordan Creek, Vermilion County, Illinois.

Figure 6. the bifid chaeta of an aquatic oligochaete (indicated by the arrow) in the gut of *Isoperla nana*.

Figure 5. A probable chironomid larva found in the gut of large *Isoperla nana*.

- Proportions of amorphous detritus (Mann-Whitney test, n_1=8, n_2 = 9, U =19, P = 0.11), diatoms (n_1=8, n_2 = 9, U =33, P = 0.80) , animal remains (n_1=8, n_2 = 9, U = 36, P = 1.00) or algae (n_1=8, n_2 = 9, U = 31.5, P = 0.69) ingested were not different between small and large classes of naiads.
- The proportion of plant fragments (Figure 7) was significantly greater in later spring (large size naiads) than in earlier spring (small size naiads) in the year (n_1=8, n_2 = 9, U = 8, P = 0.005).

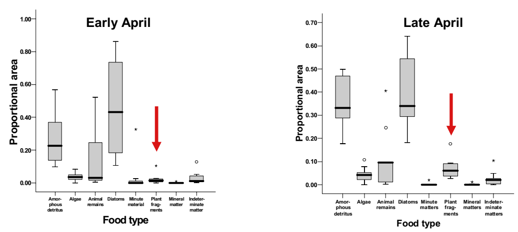


Figure 7. Boxplots of proportional areas of food particles in gut contents of small size *Isoperla nana* naiads in early April and large size ones in later April. Red arrows show significant difference of food eaten between the two categories

Trophic levels of *I. nana* naiads

- A total of 34 animal taxa (119 samples) were analyzed for C and N isotopes (Figure 8).
- The mean $\delta^{15}N$ values (1.70 ‰) for algae and dead leaves were the lowest of all samples. Values for grasses were found to be close to those for herbivorous insects.
- The $\delta^{13}C$ values of animal samples ranged from -33.79 to -23.93 ‰ and the $\delta^{15}N$ values from 2.25 to 11.33 ‰.
- The Heptageniidae (19, 28, 30; Ephemeroptera) are primary consumers in Jordan Creek, functioning as herbivores. Plecoptera, Trichoptera, Coleoptera, and Annelida are a mixture of carnivores, omnivores or herbivores. Sialidae (26, Megaloptera), Tabanidae (31, Diptera) and all the families of Odonata (5, 8, 15, 14, 22) are entirely carnivorous (Figure 8).

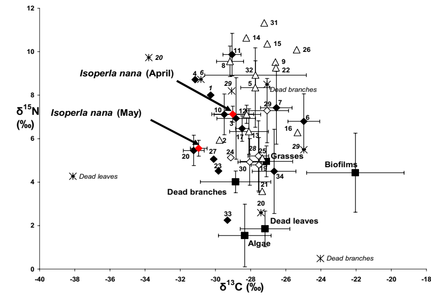
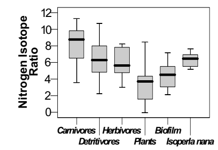


Figure 8. Means and standard deviation (SD) for $\delta^{15}N$ and $\delta^{13}C$ isotope values for all taxa sampled at Jordan Creek in December 2005, and March, April, and May 2006. Outlier value (asterisk), SD (vertical and horizontal lines), carnivore (empty triangle), herbivore (empty diamond), detritivore (black diamond), *Isoperla nana* (red diamond), dead plants grasses and biofilms (black square).

- Comparison of $\delta^{15}N$ and $\delta^{13}C$ values of *I. nana* by size (small and large) and by dates of collection indicated that *I. nana* naiads (Figure 8) collected in April 2006 possess significantly greater $\delta^{15}N$ and $\delta^{13}C$ values than those collected in May (Mann-Whitney test, n_{April} = 4, n_{May} = 4, U_N = 0, U_C = 0, P = 0.03). No significant difference was observed in terms of size class (n_{Small} = n_{Large} = 4; U_N = 7, P_N = 0.89; U_C = 5, P_C = 0.49), probably because of the small sample size.
- $\delta^{15}N$ values of carnivores (Figure 9) were different from those of detritivores (Mann-Whitney test, n_1 = 20, n_2 = 42, U =226, P = 0.003) and herbivores (n_1 = 20, n_3 = 14, U = 61, P = 0.005). No difference was observed between the detritivores and the herbivores (n_2= 42, n_3 = 14, U = 268.5, P = 0.629).

Figure 9. Means and standard deviation (SD) of nitrogen isotope ratios for all trophic groups (according to Merritt & Cummins 1996, Thorp & Covich 2001) compared to Isoperla nana isotopic values.



- $\delta^{15}N$ of *I. nana* and the carnivores were significantly different (Mann-Whitney test, $n_{I.\ nana}$ = 8, n_1 = 20, U = 38, P = 0.033). There were no difference of $\delta^{15}N$ between *I. nana* and the detritivores ($n_{I.\ nana}$ = 8, n_2= 42, U = 166, P = 0.969), and the herbivores ($n_{I.\ nana}$ = 8, n_3= 14, U = 49, P = 0.664).
- $\delta^{13}C$ of the three trophic groups were not significantly different (Kruskal-Wallis test, n_1 = 20, n_2 = 42, n_3 = 14, χ^2 = 10.628, df = 2, P = 0.177). Comparison between *I. nana* and the carnivores (Mann-Whitney test, $n_{I.\ nana}$ = 8, n_1 = 20, U =16, P = 0.001), and the detritivores (Mann-Whitney test, $n_{I.\ nana}$ = 8, n_2 = 42, U = 94.5, P = 0.037), then the herbivores ($n_{I.\ nana}$ = 8, n_3 = 14, U = 7, P = 0) showed significant differences of $\delta^{13}C$.

DISCUSSION

Isoperla nana naiads ingest animals as well as plant material, including diatoms which were assimilated, so these animals can be classified as omnivores. More woody particles ingested and isotope depletion in later dates of collection could suggest that more plant food sources were available in later spring. This is consistent with results from a study of other species of *Isoperla* consuming more diatoms that were abundant in spring (Malmqvist et al. 1991). Scarcity of prey in streams with unstable food resources also may lead to algivory for stoneflies with flexible-diet (Feminella and Stewart 1986).

Although there is a shift in food utilization between sample periods, possible ontogenetic changes in food used could not be disentangled from seasonal changes in food availability, because of the small sample size of *I. nana* available for analysis. Having replicate streams would be informative but would need tremendous time for gut content analyses and funding for SIA expenses. Laboratory rearing could also provide more details about the food preferences of *I. nana.* Further studies of invertebrates in small streams will help land managers develop strategies for conservation of remaining natural aquatic habitats threatened by land use practices in Illinois.

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