Diel periodicity of a small Midwestern stream riffle fish assemblage, with comments on electroshocking and kick seining comparisons

Jeremy S. Tiemann and Bernadette L. Tiemann

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607 East Peabody Drive Champaign, Illinois 61820

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Illinois Department of Natural Resources One Natural Resources Way Springfield, Illinois 62702

TABLE OF CONTENTS

TOPIC	PAGE
LIST OF TABLES	iii
ABSTRACT	2
Introduction	2
MATERIAL AND METHODS	4
Sampling	4
Statistical analyses	6
RESULTS	7
Diel periodicity	7
Sampling gear comparisons	. 9
Discussion	9
Diel periodicity	9
Sampling gear comparisons	11
ACKNOWLEDGMENTS	15
LITERATURE CITED	16

LIST OF FIGURES

TABLE	<u>Page</u>
TABLE 1. – Mean fish species abundance per 10 m ² (standard	
deviation) and repeated measure analysis of variance results [F (P-	
values)] among time periods in Jordan Creek, Vermilion County,	
Illinois from April to November 2003.	8
TABLE 2. – Mean fish species abundance per 10 m ² (standard	
deviation) and repeated measure analysis of variance results [F (P-	
values)] between sampling gears in Jordan Creek, Vermilion	
County, Illinois from April to November 2003	10

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Jeremy S. Tiemann^{1*} and Bernadette L. Tiemann²

¹Illinois Natural History Survey - Center for Biodiversity 607 East Peabody Drive Champaign, Illinois 61820

> ²First School 1403 East Oak Street Mahomet, Illinois 61853

Note: The proposal for this project submitted to the Illinois Wildlife Preservation Fund called for the examination of diel and longitudinal differences of stream riffle fish assemblages in the Sangamon River and Jordan Creek, with emphasis on *Noturus* species. However, due to high water levels in the Sangamon River for most of the study period, adequate sample sizes from the Sangamon River could not be obtained and the data consequently were discarded. Because of this act, and to the small riffle sizes in Jordan Creek, longitudinal differences in riffle fish assemblages were not compared. The only data analyzed and discussed were the diel differences of stream riffle fish assemblages in Jordan Creek. To add to this study, comparisons of electroshocking and kick seining were included.

ABSTRACT. - Diel periodicity (four time periods: morning crepuscular, diumal, evening crepuscular, and nocturnal) and comparison of sampling gears (two sampling gears: backpack electroshocking and kick seining) were examined in a small eastern Illinois stream riffle fish assemblage from April - November 2003. Using a sequential Bonferroni correction of a standard $\alpha = 0.05$, repeated measure multivariate analysis of variance (MANOVA) indicated that the riffle fish assemblage significantly varied among time periods. More species and higher abundances of Semotilus atromaculatus, Ambloplites rupestris, Micropterus dolomieu, and Etheostoma spectabile were collected during day than night. Perhaps these results can be attributed to light intensity and/or resource partitioning. Repeated measure MANOVA also indicated that the riffle fish assemblage significantly varied between sampling gears. Kick seining collected more species and more cyprinids (Luxilus chrysocephalus, Lythrurus umbratilis, Notropis stramineus, Pimephales notatus, and S. atromaculatus) than electroshocking, whereas electroshocking collected more percids (Etheostoma blennioides and E. caeruleum) than kick seining. When used together, these two gears collected a better representative of the riffle fish assemblage than one gear would have collected when used alone.

INTRODUCTION

Temperate, warmwater stream fish assemblages have been shown to vary spatially (e.g., Fuselier and Edds, 1996; Taylor et al., 1996) and temporally (e.g., Gelwick, 1990; Gillette et al., in press) in habitat use, with significant differences in abundances and richness. Temperate, warmwater stream fish assemblages also have been shown to exhibit diel periodicity (e.g., Sanders, 1992), mainly in relationship to feeding (e.g., Dewey, 1988; Kwak et al., 1992); these studies, however, have conflicting results. Diel periodicity of fish assemblages is influenced by interactions between environmental conditions, availability of food and habitats, and susceptibility of predation, with fish assemblages altering patters of activity and habitat selection accordingly (David and Closs, 2003). Reebs et al. (1995) suggested that diel periodicity of temperate, warmwater stream fish assemblages is still poorly understood. Diel periodicity can have significant

implications for stream assemblage structure (David and Closs, 2003), and determining the appropriate level of sampling effort to characterize stream fish assemblages can be difficult (Meador et al., 2003). Fisheries biologists, under a variety of constraints (e.g., time, costs, and personnel), are required to accurately determine fish assemblages so appropriate management decisions can be made (Dumont and Dennis, 1997), especially when dealing with threatened and endangered fishes. Therefore, guidelines for creating sampling procedures are needed when designing surveys to assure that reliable data proficiently are collected, and that new and/or improved sampling methods are developed (Patton et al., 2000). One such way is multiple procedures (e.g., combination of electroshocking and seining), which can provide more accurate and unbiased results than single procedures (Hall and Durham, 1979).

Two kinds of methods, electroshocking (e.g., Meador et al., 2003; Reynolds et al., 2003) and kick seining (e.g., Wildhaber et al., 2000; Tiemann et al., in press), commonly have been used when sampling riffle fish assemblages. Both methods have positive and negative aspects (Onorato et al., 1998). Compared to seines, electroshockers require less manpower, are not as affected by stream habitat (e.g., irregular substrate types and swift flow conditions), and are less selective when sampling; however, seines are not restricted by water quality (e.g., turbidity), kill relatively few fish, and are less expensive, easier to fix, and safer to use. Onorato et al. (1998) stated the importance of knowing the selectivity of different sampling gears when designing a field experiment, realizing that capture efficiency among sampling gears can vary by species and habitat.

In accordance with the strong nocturnal peak activity in macroinvertebrate abundance (Brown and Basinger-Brown, 1984; Merritt and Cummins, 1996), we sampled

diel periodicity of a small east-central Illinois stream riffle fish assemblage. Our objectives were to 1) determine if diel periodicity of riffle fish assemblage variables occur, and whether there are correlations between the macroinvertebrate drift and these variables; and 2) compare effectiveness between electroshocking and kick seining in terms of riffle fish assemblage variables. Specifically, our *a priori* hypotheses were that 1) because of the strong nocturnal peak activity in macroinvertebrate abundance, the riffle fish assemblage variables would be highly correlated with macroinvertebrate drift, and would be higher during dusk and dawn than day and night; and 2) because of repeatedly disturbing the substrate when kick seining, the riffle fish assemblage variables would be lower at kick seining sites than electroshocking sites.

MATERIAL AND METHODS

Sampling. – We took monthly samples from March to November 2003 at four riffles on Jordan Creek, Vermilion County, Illinois (March data were discarded due to no fish being collected). Jordan Creek is a 2nd order tributary of the Salk Fork Vermillion River of the Wabash River drainage. Jordan Creek is 17 km long, drains a glaciated basin of approximately 30 km², and has a morphology consisting of pebble riffles, gravel runs, and sandy pool bottoms (Angermeier, 1985). The basin topography consists of gentle rolling hills with wide, flat valleys; the watershed is primarily agricultural with the principal crops being corn and soybeans, but also contains riparian habitats of oak-maple-ash forests (Angermeier, 1985). Most of the abundant fish species are widely distributed in the Midwest, and therefore might indicate typical patters for Midwestern stream fish assemblages (Angermeier, 1985).

We collected fishes and macroinvertebrates during a morning crepuscular period (dawn), a diurnal period (mid-day), an evening crepuscular period (dusk), and a nocturnal period (approximately midnight - headlamps were used during this period). Starting time periods for each month were randomly chosen, and samples were collected during all moon phases. For fishes, we evenly spaced three transects along the length of each riffle and placed two 10 m² (5 m x 2 m) sampling quadrants along each transect before sampling at a site. Riffles were paired in terms of sampling, meaning the order of sampling from downstream to upstream was either seining, seining, electroshocking, electroshocking, or vice versa. We flipped a coin to determine the method of sampling for the downstream riffle pair. To minimize disturbance, we sampled riffles and transects within riffles from downstream to upstream and quadrants within transects from near shore to far shore. For electroshocking, we collected fishes by a single downstream-toupstream zigzag pass using a Model 15-D POW Electrofisher (Smith-Root, Inc.; Vancouver, WA) backpack electroshocker; power was standardized to reduce both variability of survey data and injury to fishes (Miranda and Dolan, 2003). Single-pass electroshocking has been shown to be an effective sampling technique for small (1st and 2nd order), warmwater stream fish assemblages (Edwards et al, 2003). For kick seining, we collected fishes by disturbing the substrate upstream from a stationary 2 m long, 3 mm mesh seine while proceeding downstream to the seine. We identified fishes after completion of a time period and released them into the quadrant from which they came; we standardized abundance to number/10 m² and counted the number of species present (richness).

During each sampling period, we collected macroinvertebrates from three 500 μ drift nets that were staggered 10 m apart upstream from the fished area, and were placed 1 h before and pulled 1 h after completion of fish sampling. The purpose of this act was to provide relative availability of prey that co-occur with fishes since macroinvertebrates comprise a critical fish food resource in Jordan Creek (Angermeier, 1982). We preserved macroinvertebrates in 70% ethanol and identified them to family in the laboratory; we standardized the data to number/hour.

Statistical analyses. - Because multiple procedures (e.g., combination of both electroshocking and seining) provide more accurate and unbiased results than single procedures (Hall and Durham, 1979), we combined all data within a time period to examine riffle fish assemblage variables among time periods. We evaluated the data for normality using the Shapiro-Wilk test (Zar, 1999) and homogeneity of variance using the Levene's test (Milliken and Johnson, 1984); to improve normality, we transformed the data where necessary using $log_{10}(x+1)$ (Zar, 1999). All fishes occurred in more than 5% of the 128 samples (4 time periods per riffle x 4 riffles per month x 8 months), and therefore did not have to be eliminated from abundance analyses following Gauch (1982). We used repeated measure multivariate analysis of variance (MANOVA) (Maceina et al., 1994) to assess diel periodicity for riffle fish assemblage variables (abundance and richness); in addition, we calculated Pearson's correlation coefficient to examine potential relationships between the macroinvertebrate drift and the riffle fish assemblage variables. We also used repeated measure MANOVA to compare electroshocking and kick seining data among time periods. We used the Statistical Analysis System, Version 8.1 (SAS Institute, Inc.; Cary, NC) to conduct all tests.

Because of multiple tests, we applied sequential Bonferroni correction of a standard $\alpha = 0.05$ where appropriate to help control overall experimental Type I error rate (Rice, 1989). We used Tukey's studentized range test for comparisons among time periods and between sampling gears.

RESULTS

We collected 3084 fishes representing 16 species, 13 genera, and five families (Table 1). Campostoma anomalum was the most abundant species collected (1258 individuals or 40.8% of total), followed by Etheostoma caeruleum (398 individuals or 2.9% of total) and Pimephales notatus (243 individuals or 7.9% of total).

Diel periodicity. – The riffle fish assemblage significantly varied among time periods (MANOVA, degrees of freedom = 17, 44; λ = 0.01; F = 186.31; P < 0.0001). Individual analysis of variances demonstrated that four species (Semotilus atromaculatus, Ambloplites rupestris, Micropterus dolomieu, and Etheostoma spectabile), in addition to richness, significantly varied among time periods at sequential Bonferroni adjusted alphas (Table 1). Four other species (C. anomalum, Lythrurus umbratilis, Noturus flavus, and Lepomis macrochirus) had moderate differences (0.05 > α > sequential Bonferroni adjusted alphas) (Table 1). Tukey's test indicated that S. atromaculatus had higher abundances during day than night but neither time period differed from dawn or dusk, whereas A. rupestris, M. dolomieu, and E. spectabile had higher abundances during day than either dawn, dusk, or night (Table 1). Tukey's test showed that both C. anomalum and L. umbratilis had slightly higher abundances during day than night, whereas N. flavus was the opposite with higher abundances during night than day; L. macrochirus had

TABLE 1. – Mean fish species abundance per 10 m² (standard deviation) and repeated measure analysis of variance results [F (P-values)] among time periods (32 samples per time period) in Jordan Creek, Vermilion County, Illinois from April to November 2003. Superscript letters (a, b) indicate significant Tukey's grouping, and asterisks (*) indicate significant sequential Bonferroni-adjusted P-values

Fishes	Morning	Diurnal	Evening	Nocturnal	Time period
	crepuscular		crepuscular		df _{3,59}
Family Cyprinidae					
Campostoma anomalum	1.54 (0.11)	1.18 (0.01)	1.82 (0.23)	1.20 (0.01)	3.04 (0.04)
Luxilus chrysocephalus	0.34 (0.37)	0.30 (0.37)	0.21 (0.17)	0.32 (0.40)	0.74 (0.53)
Lythrurus umbratilis	0.06 (0.09)	0.13 (0.15)	0.13 (0.18)	0.03 (0.01)	3.24 (0.03)
Nocomis biguttatus	0.02 (0.01)	0.02 (0.01)	0.02 (0.01)	0.03 (0.02)	0.15 (0.93)
Notropis stramineus	0.04 (0.04)	0.08 (0.07)	0.07 (0.08)	0.04 (0.05)	0.93 (0.43)
Pimephales notatus	0.34 (0.44)	0.41 (0.35)	0.32 (0.39)	0.19 (0.11)	1.53 (0.22)
Semotilus atromaculatus	0.17 (0.19) ab	0.28 (0.12) a	0.16 (0.08) ab	0.05 (0.04) b	5.80 (0.002)*
Family Catostomidae				,	(/
Hypentelium nigricans	0.01 (0.01)	0.02 (0.00)	0.01 (0.01)	0.00 (0.00)	1.94 (0.13)
Family Ictaluridae			, ,	` ,	-50 (41.00)
Noturus flavus	0.18 (0.00)	0.16 (0.20)	0.23 (0.03)	0.31 (0.01)	3.86 (0.01)
Family Centrarchidae				•	. ,
Ambloplites rupestris	0.00 (0.00) b	0.07 (0.01) a	0.01 (0.01) ^b	0.01 (0.01) b	14.31 (< 0.0001)*
Lepomis macrochirus	0.05 (0.01)	0.02 (0.01)	0.04 (0.01)	0.00 (0.00)	4.51 (0.006)
Micropterus dolomieu	0.00 (0.00) b	0.04 (0.04) a	0.00 (0.00) ^b	0.01 (0.01) b	5.06 (0.004)*
Family Percidae				,	` ,
Etheostoma blennioides	0.22 (0.05)	0.26 (0.11)	0.27 (0.08)	0.22 (0.13)	0.47 (0.70)
Etheostoma caeruleum	0.51 (0.06)	0.53 (0.15)	0.55 (0.15)	0.42 (0.19)	0.89 (0.45)
Etheostoma flabellare	0.32 (0.02)	0.31 (0.13)	0.32 (0.07)	0.26 (0.11)	0.56 (0.64)
Etheostoma spectabile	0.04 (0.02) b	0.12 (0.02) a	0.05 (0.01) ^b	0.04 (0.03) b	5.90 (0.001)*
Abundance	3.84 (1.14)	4.61 (0.91)	4.19 (0.41)	3.11 (0.18)	1.89 (0.14)
Richness	9.31 (2.04) ab	10.81 (1.46) a	9.56 (1.90) ab	8.19 (1.53) b	6.02 (0.001)*

somewhat higher abundances during dawn than night. Tukey's test also indicated that richness was higher during day than night but neither time period differed from dawn or dusk (Table 1). Neither fish abundance (Pearson's correlation, r = -0.12, P = 0.34) nor richness (Pearson's correlation, r = -0.25, P = 0.06) were correlated with macroinvertebrate abundance.

Sampling gear comparisons. – The riffle fish assemblage also significantly varied between sampling gears (MANOVA, degrees of freedom = 17, 46; λ = 0.01; F = 247.14; P < 0.0001). Individual analysis of variances demonstrated that seven species (Luxilus chrysocephalus, L. umbratilis, Notropis stramineus, P. notatus, S. atromaculatus, Etheostoma blennioides, and E. caeruleum), in addition to richness, significantly varied between sampling gears at sequential Bonferroni adjusted alphas (Table 2). Two other species (N. flavus and Etheostoma flabellare) had moderate differences (Table 2). Tukey's test indicated that the five cyprinids had higher abundances when kick seining than electroshocking, whereas the two percids had higher abundances when electroshocking than kick seining (Table 2). Tukey's test showed that N. flavus had slightly higher abundances when kick seining than electroshocking, whereas E. flabellare had somewhat higher abundances when electroshocking than kick seining. Tukey's test also indicated that richness was higher when kick seining than electroshocking (Table 2).

DISCUSSION

Diel periodicity. - Our results showed that the riffle fish assemblage differed among time periods. Four species (S. atromaculatus, A. rupestris, M. dolomieu, and E.

TABLE 2. – Mean fish species abundance per 10 m² (standard deviation) and repeated measure analysis of variance results [F (P-values)] between sampling gears (64 samples per time period) in Jordan Creek, Vermilion County, Illinois from April to November 2003. Superscript letters (a, b) indicate significant Tukey's grouping, and asterisks (*) indicate significant sequential Bonferroni-adjusted P-values

Fishes	Electroshocking	Seining	Time period	
			$\mathbf{df}_{1,59}$	
Family Cyprinidae				
Campostoma anomalum	1.63 (0.37)	1.59 (0.27)	0.41 (0.53)	
Luxilus chrysocephalus	0.06 (0.03) b	0.53 (0.13) a	45.25 (< 0.0001)*	
Lythrurus umbratilis	0.01 (0.01) b	0.16 (0.10) a	26.15 (< 0.0001)*	
Nocomis biguttatus	0.01 (0.01)	0.03 (0.01)	2.13 (0.15)	
Notropis stramineus	0.01 (0.01) b	0.10 (0.03) a	14.76 (0.0003)*	
Pimephales notatus	0.09 (0.06) b	0.54 (0.18) a	39.00 (< 0.0001)*	
Semotilus atromaculatus	0.09 (0.08) b	0.24 (0.12) a	13.99 (0.0004)*	
Family Catostomidae			,	
Hypentelium nigricans	0.01 (0.01)	0.01 (0.01)	1.50 (0.23)	
Family Ictaluridae			. ,	
Noturus flavus	0.18 (0.12)	0.26 (0.06)	4.32 (0.04)	
Family Centrarchidae				
Ambloplites rupestris	0.02 (0.04)	0.02 (0.03)	0.00 (1.00)	
Lepomis macrochirus	0.03 (0.03)	0.02 (0.02)	0.22 (0.64)	
Micropterus dolomieu	0.00 (0.01)	0.02 (0.03)	4.00 (0.05)	
Family Percidae			,	
Etheostoma blennioides	0.31 (0.04) a	0.17 (0.04) b	22.19 (< 0.0001)*	
Etheostoma caeruleum	0.60 (0.05) a	0.40 (0.08) b	9.30 (0.003)*	
Etheostoma flabellare	0.36 (0.03)	0.24 (0.06)	5.42 (0.02)	
Etheostoma spectabile	0.06 (0.03)	0.06 (0.05)	0.12 (0.74)	
Abundance	3.47 (0.54)	4.40 (0.84)	2.79 (0.10)	
Richness	8.28 (0.93) b	10.66 (1.31)*	29.26 (< 0.0001)*	

species (C. anomalum, L. umbratilis, and L. macrochirus) had moderately higher abundances either during dawn or day than night. Only N. flavus had a slightly higher abundance during night than day. All of the above eight species, except N. flavus, are visual predators (Pflieger, 1997), which could account for their higher abundances either during dawn or day; N. flavus is a nocturnal predator that mainly feeds by sensory barbels (Pflieger, 1997).

Light intensity has been shown to regulate diel periodicity (Reebs et al., 1995). High light intensity allows for early detection of predators; therefore, diurnality might not be a disadvantage for cyprinids (Reebs et al., 1995). This idea, in addition to cyprinids migrating to stream margins with decreasing light intensity (Garner, 1996), might account for higher cyprinid abundances during day. Twilight, on the other hand, increases predation pressure due to decreased predator detection from lower light intensities (Garner, 1996), which might explain the moderately higher *L. macrochirus* abundance during dawn than night.

Predator-prey interactions, in addition to competition, are other factors related to diel periodicity of stream fish assemblages (Kwak et al., 1992). Riffle fish assemblages with naturally broad variation in fish and prey size might allow for effective food resource partitioning among species (Dewey, 1988). Syntopical fish species in Jordan Creek extensively overlap in prey taxa they consume, and generally do not specialize on a given prey taxa (Angermeier, 1982). For example, Noturus species are dominant food consumers during night, whereas other fishes (e.g., cyprinids, centrarchids, and percids) are dominant food consumers during day (Burr and Stoeckel, 1999); one such reason for

this shift in dominance is to avoid direct competition for food resources. Riffle fish assemblages in Jordan Creek might weakly segregate food resources by consuming different proportions of prey items and more strongly partition food resources by utilizing prey at different times (Angermeier, 1982). Therefore, resource partitioning, in addition to light intensity, might lead to two distinctly different fish assemblages inhabiting riffles with transitional periods during dawn and dusk, and could account for the difference in richness among time periods.

Interpretation of our macroinvertebrate results was complicated by weather and small sample size. Macroinvertebrates in Jordan Creek, as in most temperate streams, undergo unpredictable diel and seasonal fluctuations (Angermeier, 1982). One factor responsible for seasonal fluctuations was heavy rains during late spring and early summer that caused re-occurring periods of elevated discharge. In Jordan Creek, where high flows rarely exceed the banks, high discharges result in scouring of macroinvertebrates (Angermeier, 1985). Therefore, we acknowledge the inadequacies in our macroinvertebrate data and recommend caution in interpretation. Although the data were approaching significance in terms of a correlation between macroinvertebrate abundance and fish richness, failure to detect significant trends might not lead to definitive conclusions (Kwak et al., 1992). Future studies on diel periodicity of stream fish assemblages could benefit from increasing the number of sampling periods, increasing the number of streams sampled, and within those streams, increasing the number of areas sampled to avoid pseudoreplication. Future studies also could address how seasonal patterns (e.g., temperatures), moon phases, light intensities, and microhabitat segregation affect diel periodicity.

Sampling gear comparisons. – Our results also showed that the riffle fish assemblage differed between sampling gears. Five cyprinids (*L. chrysocephalus*, *L. umbratilis*, *N. stramineus*, *P. notatus*, and *S. atromaculatus*) had significantly higher abundances when kick seining than electroshocking, whereas two percids (*E. blennioides*, and *E. caeruleum*) had higher abundances when electroshocking than kick seining. Two other species (*N. flavus* and *E. flabellare*) had moderate differences between sampling gears; *N. flavus* had slightly higher abundances when kick seining than electroshocking, whereas *E. flabellare* had somewhat higher abundances when electroshocking than kick seining. Richness also was higher when kick seining than electroshocking. The cyprinids might not have been affected by kick seining as much as the percids because the cyprinids were not substrate oriented fishes like the percids were (Pflieger, 1997); however, this idea does not appear to be the case with *N. flavus* (see below).

The results of *N. flavus* were surprising; we expected *N. flavus* to have higher abundance by electroshocking than kick seining for the same reasons as the percids. From a study on Jordan Creek, Larimore (1954) stated that seining is efficient under ideal conditions (*e.g.*, "smooth, even bottom with no snags, large boulders, ledges, or undercut banks"); however, Jordan Creek is not like this description, and electroshocking therefore was expected to be the better method in terms of higher abundance and richness. Electroshocking has been shown to be a better method than seining for producing more consistent results (in terms of abundance and richness) when sampling over areas with strong current and/or irregular substrate (Dauble and Gray, 1980; Wiley and Tsai, 1983). Electroshocking also has been shown to require less effort than seining to estimate richness (Patton *et al.*, 2000). Physical obstacles do not limit the electroshocker;

therefore electroshocking can more completely cover the entire stream, which should make it a more effective sampling method than seining (Larimore, 1954). We expected repeated habitat disruptions from kick seining to cause fishes to leave quadrants, and skew the riffle fish assemblage data, but this hypothesis did not occur. One thing that was not done was multiple-pass electroshocking; Meador *et al.* (2003) suggested that multiple-pass electroshocking might be necessary to make meaningful data interpretations.

On the contrary to the above, seines have been shown to collect more cyprinids than electroshockers (Onorato et al., 1998), whereas larger fish tend to be more susceptible to capture with electrical gears (Peterson and Rabeni, 2001). Smaller fish are more difficult to capture with electrical gears due to the lower voltage differential that runs across them, and larger fish are more successful at avoiding capture by seining (Peterson and Rabeni, 2001). In addition, physical habitat characteristics can affect sampling efficiencies by providing refuges for substrate-oriented fishes (Peterson and Rabeni, 2001). These fishes can become lodged under these refuges when electroshocking and therefore might be overlooked, thus skewing the riffle fish assemblage data (Wiley and Tsai, 1983). The premises mentioned above might explain the higher N. flavus abundance by kick seining than electroshocking; they also might explain the higher richness by kick seining than electroshocking since most fishes in our study were typical small, riffle-dwelling fishes. Future studies on comparisons of sampling gears in stream fish assemblages could benefit from multi-pass electroshocking, adding to the types of sampling gears (e.g., electric-seine, tow barge), altering the voltage of the electroshocker, and sampling all available habitats (e.g., riffles, runs, and pools).

In conclusion, the riffle fish assemblage appeared to exhibit diel periodicity, with significant differences in richness and abundance of several species. Perhaps this act can be attributed to light intensity and/or resource partitioning. Also, electroshocking and kick seining produced significantly different results in the riffle fish assemblage (e.g., richness and abundances of several species). The seine appeared better at collecting water column fishes (e.g., cyprinids) than electroshocking, whereas the electroshocker appeared better at collecting substrate oriented fishes (e.g., percids) than kick seining. The two gears complimented each other when used together, and collected a better representation of the present riffle fish assemblage than one gear would have collected when used alone.

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