

DIEL VARIATION IN SUBSTRATE PREFERENCE  
OF CYPRINID FISHES  
A THESIS  
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BY  
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**ABSTRACT****THESIS PROJECT:** Diel Variation in Substrate Preference of Cyprinid Fishes**STUDENT:** Luke Etchison**DEGREE:** Master of Science**COLLEGE:** Sciences and Humanities**DATE:** May, 2012**PAGES:** 17

Diel substrate preferences were tested in artificial stream experiments for six cyprinids: redbfin shiner (*Lythrurus umbratilis*), silver shiner (*Notropis photogenis*), spotfin shiner (*Cyprinella spiloptera*), central stoneroller (*Campostoma anomalum*), striped shiner (*Luxilus chrysocephalus*), and bluntnose minnow (*Pimephales notatus*). We used experimental pools containing two of three substrate types (sand, cobble, and gravel) per trial to test if habitat preferences differed in day and night observations. Over 85% of specimens were observed over cobble substrate during the day, and all species switched substrate preferences at night for the tanks containing cobble. Our results suggest that day-night differences in habitat use may occur in other small-bodied species. Interpretations for habitat use of these species in the majority of studies that are based on daytime observations may need to be modified. Interpretations for habitat use by fish assemblages in the majority of studies that are based on daytime observations may need to be modified.

## INTRODUCTION

Stream ecosystems and their associated habitat structure can vary from day to night with differences in light regime, pH, dissolved oxygen, or other variables that result in influences on the distributional and abundance patterns of fish use of habitat (Neu et al. 1974; Campbell & Neuner, 1985; Shuler et al. 1994). Animals commonly choose habitats that maximize foraging profitability while minimizing predation risks (Gilliam & Fraser, 1987). However, as habitat and predator activity vary over 24-hr periods, habitat suitability and foraging profitability subsequently vary.

Catch differences over 24-hr periods in the same location exist for multiple taxa in multiple studies (Hobson, 1965; Kronfeld-Schor & Dayan, 2003; Helfman, 1981; Hubbs, 1984; Matthews, 1986; Adams et al. 1988) but details of this diel variation is generally lacking. Diel movements of animals have been evaluated in terrestrial (Young, 1999; Sorrell, 2009) and aquatic ecosystems (Holomuzki, 1986; Clark & Levy, 1988; Laurila et al. 1997; Greenwood & Metcalfe, 1998; Yu & Peters, 2002). The current adaptive explanations for such diel movements are that behaviors provide predator avoidance, bioenergetic, and resource utilization advantages (Clark & Levy, 1988). However, qualitative movement information for a wide range of aquatic taxa does not currently exist. With the exception of game fish, diel movements are rarely examined in aquatic taxa (Yu & Peters, 2002). Studies of diel movement patterns focus primarily on changes in assemblages at a single location over a 24-hr period (Hobson, 1965; Helfman, 1981; Hubbs, 1984; Matthews, 1986).

Several fishes have preferences for specific substrates when a broad range of substrata are available (McKee, 1982; Power, 1983), or preferences can change with day or night use. Davey et al. (2011) used a semi-natural artificial stream to evaluate juvenile creek chub (*Semotilus atromaculatus*) diel activity as a response to predator presence and habitat structural complexity. Creek chub preferred structurally complex habitats during the day and moved to the less complex habitat at night. Creek chub utilized diel shifts with and without a predator. One drawback to the study of Davey et al. (2011) is that individuals were exposed to predator chemical cues that can potentially confound fish behavior (Wisenden, 2000; Vanderpham et al. 2012). Chemical alarm pheromones released with tissue damage occurs in a wide variety of taxa (Mathis et al. 1995), and these alarm pheromones attract predators (Chivers et al. 1995). Chemical releases that occur external to habitat preference experiments with the design of Davey et al. (2011) may affect behavior of observation fish. Some fish taxa can recognize alarm pheromones of other species and will avoid areas of release even after the pheromone has dissipated (Wisenden et al. 1994). Consequently, fish in these experiments may be exposed to behavior altering chemicals and could be avoiding areas that are perceived as high risk areas.

An experiment that eliminates extraneous variables and is restricted to only substrate variation choice will allow us to distinguish if fish have specific preferences for substrate characteristics. If substrate preferences in such an experiment change from day to night this may imply an innate behavioral response. A potential mechanism for such an innate behavioral response is the behavior is the product of selection for predator avoidance.

The current study is a test for diel variation in substrate preferences of small-bodied cyprinid species with control of confounding variables that have the potential to influence habitat preferences (olfactory cues, equal depth, flow variation, competitors and predators). We hypothesize that substrate size preferences will change in night and day trials.

## METHODS

Fishes were collected by seine over predominantly finer substrates (2-m high, 10-m long, 5-mm mesh) or backpack electrofisher in the West Fork White River drainage, Indiana. We selected six species that occurred in the highest abundance at our sites for trials. These species are also the overall highest abundant cyprinids in the watershed: bluntnose minnow (*Pimephales notatus*), spotfin shiner (*Cyprinella spiloptera*), central stoneroller (*Campostoma anomalum*), sand shiner (*Notropis stramineus*), silver shiner (*Notropis photogenis*), and redbfin shiner (*Lythrurus umbratilis*). Fish used for experimental trials were 80 to 130 mm. Fish were transported from the collecting sites to acclimation tanks at Ball State University, and subsequently to a wet lab containing three experimental tanks (800-l plastic wading pools) using treated tap water. Three substrate types for experimental trials were selected that are abundant in the White River: sand (<2mm), gravel (2-32mm), and cobble (32-128mm). Substrates were collected from White River. Substrates were allowed to completely dry to control for presence of forage organisms for focal individuals. Each experimental tank consisted of two substrate types as in Figure 1. The ambient light schedule for the facility was 12:12 light:dark. The acclimation tanks did not contain substrate and fish were never fed. Two air diffusers were placed on the

outer edge of each tank where the two substrates met. Each air diffuser was placed on opposite sides of the tank to control for unequal distribution of dissolved oxygen.

However, dissolved oxygen was not considered to be a factor influencing behavior because of the shallow depth (~15-20 cm).

Experimental procedure. Fish were acclimated for 24-hrs in a holding tank prior to trials. After acclimation ten individuals of the same species were randomly added to the center of each experimental tank. Fish were acclimated for 24-hrs in the experimental tanks before observations were recorded. Observations for habitat preferences were recorded twice daily at 1100 and 2300 hrs for three days and then averaged for that trial. One of the authors (LE) observed fish locations from behind a plywood barrier to minimize visual disturbance to fishes. The observations consisted of the number of fish above each substrate. For night observations an overhead red incandescent light was used to observe fish locations to minimize fish response to light. Fishes were euthanized after all trials were completed. A Chi-square goodness of fit test (Minitab version 16.0) was used to compare species substrate preferences during day or night trials with a 50:50 expected null distribution. In other words, we expected no preference for a substrate (5:5 distribution of observational individuals over each substrate). Combined trial averages were used to compare day and night observations for each species using a paired t-test (Minitab version 16.0). Paired t-test p-values were converted into a q-statistic using the QValue method (Storey, 2002) for the R statistical package (Ihaka & Gentleman, 1996) to control for Type I error. Q-values are used to create a minimum estimate of false

discovery across multiple comparisons. Q-values are interpreted as p-values (significance  $\leq 0.05$ ). However, q-values are not as conservative as traditional Bonferonni corrections.

## **RESULTS**

We completed three trials (3 day and 3 night observations with 10 individuals per trial) per experimental unit, and we found a significant diel change in substrate preferences in all species. There were no observations of schooling during any trials. All species preferred cobble substrates during the day, and there were no significant differences in substrate preferences for cobble and sand, or cobble and gravel for night observations. When given the choice of sand or gravel during the day, only silver shiner and central stoneroller preferred gravel. At night all species preferred gravel (Figures 1-3). The results of paired t-tests indicated that all species had significantly different habitat preferences in day and night comparisons for the experimental tanks containing cobble substrate (Table 1).

## **DISCUSSION**

One goal of fish ecology research is to identify the habitat used by fishes to allow prediction. We identified distinct changes in substrate preferences in day and night observations. Other studies that test for habitat preferences based on collecting typically use only day or night collections (Ross et al. 1985), resulting in a simple habitat preference for each species that may be incomplete, based on our results. Our results suggest that night and day habitat use will likely differ in other small-bodied cyprinids.

We expected daytime cobble preferences for these six species because small-bodied fishes typically use spaces among cobble substrates for shelter, foraging, and reproduction (Albanese et al. 2004). Gravel substrate use at night rather than sand, may be due to other explanations, for example, individuals of these species could be actively foraging for algae or invertebrate prey items in the gravel substrate. These forage items typically have lower abundances in smaller, homogeneous substrates such as sand.

Competition shapes assemblage structure through resource partitioning of co-occurring species. In addition, the presence of predators can drive patterns of substrate preference. Preference patterns may be an innate response or are developed with predator presence in an ecosystem. Regardless of the evolutionary origin, small-bodied cyprinid species in our study utilized a behavioral substrate preference that appears to minimize predation risk (Savino & Stein, 1989). Common predator interactions for these small-bodied species include game fishes (e.g., smallmouth bass, channel catfish) and crayfish. In small streams, the abundances of smallmouth bass and crayfish are high compared to benthic predators such as channel catfish. In stream ecosystems, juveniles of all taxa and adults of small-bodied darters and minnows often use similar habitat to avoid predation (Schlosser, 1987). Exposure to diurnal feeding predators likely is a mechanism to drive small-bodied fish into substrate for cover even when crayfish occur in substrates (Rahel & Stein, 1988). Crayfish are typically less active during the day, which may reduce crayfish as a daytime threat to small-bodied fish.

Our results suggest that further studies of habitat preferences at a fine temporal scale may be informative. All of our experimental species shifted habitat preference between day and night. However, fish assemblages and their associated habitats are typically compared across seasonal temporal scales (Gorman & Karr 1978). We expect that fish assemblage studies that incorporate diel habitat use may produce incomplete results. For example, Eros et al. (2003) used daytime depletion fish surveys based on physical habitat patches and found that most fishes in the study did not vary macrohabitat patch use seasonally. However, they did find that juvenile stone loach utilized coarser substrates in riffles compared to adults that occurred over fine substrates in pool habitats. Additionally, they found that the pool assemblages were dominated by adults (greater than or equal to two years age). Eros et al. (2003) concluded that their stream assemblages were stable among and within patches. Our study suggests that patch use may change from day to night, with a potential of different results from Eros et al. (2003). An alternative explanation is that larger-bodied fishes occur in open pools with fine substrates (minimal cover) because of decreased risk of predation with their size class (Schlosser, 1987). Large-bodied individuals may not be pool specialists, but escaped risk of predation with increased body size.

This is the first study to our knowledge to test for substrate preference variation in small-bodied cyprinids in a 24-hr period. Further investigations of substrate preference variation across size classes and life stages and with incorporation of diel variation will provide improved understanding of species specific habitat requirements which can lead to better understanding of stream assemblage structure. Additionally, we recommend

testing for diel variation as a response to intraspecific and interspecific competition.

Understanding diel substrate preference variation can give insight on complete habitat requirements of fishes which can potentially aid in restoration of suitable habitat for a multitude of fish species.

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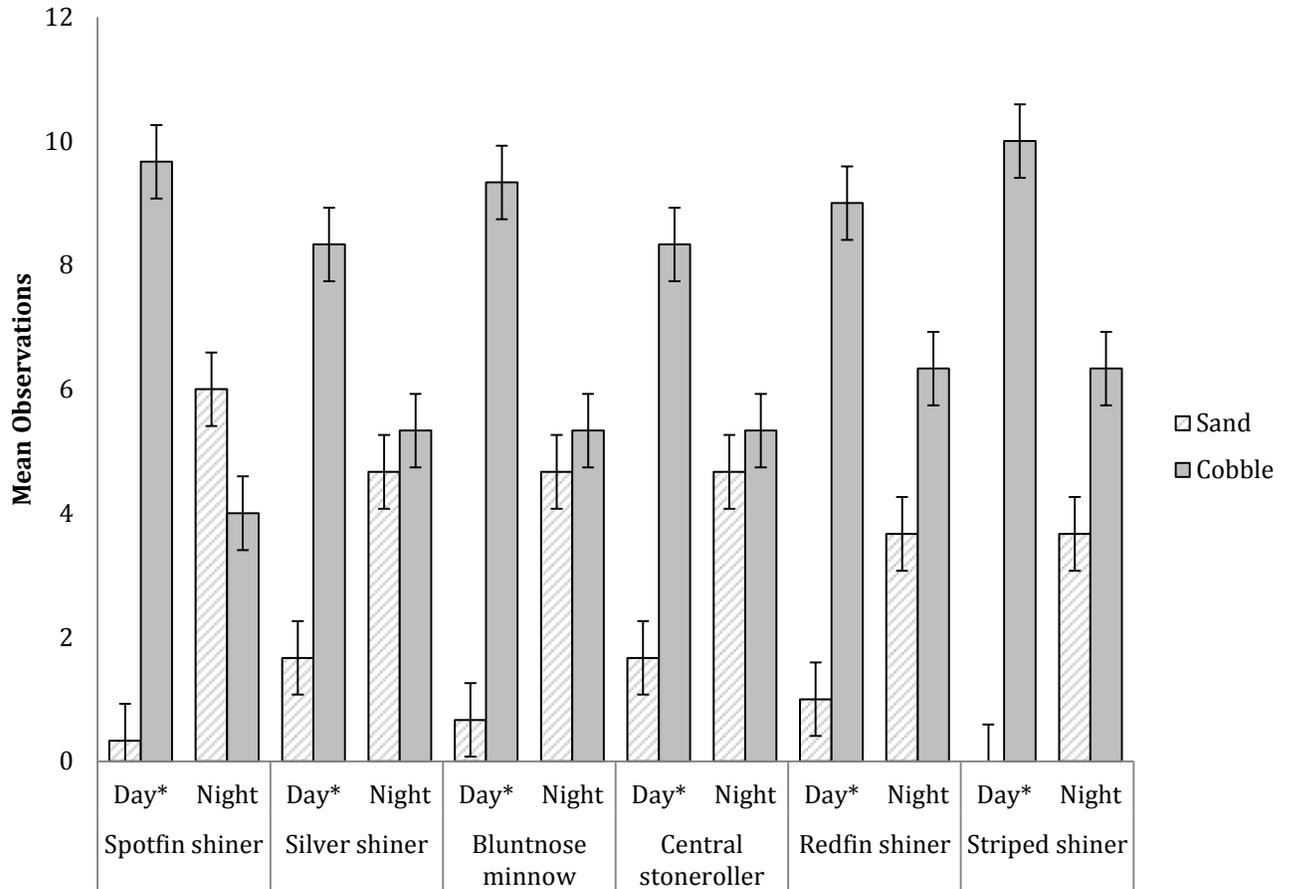
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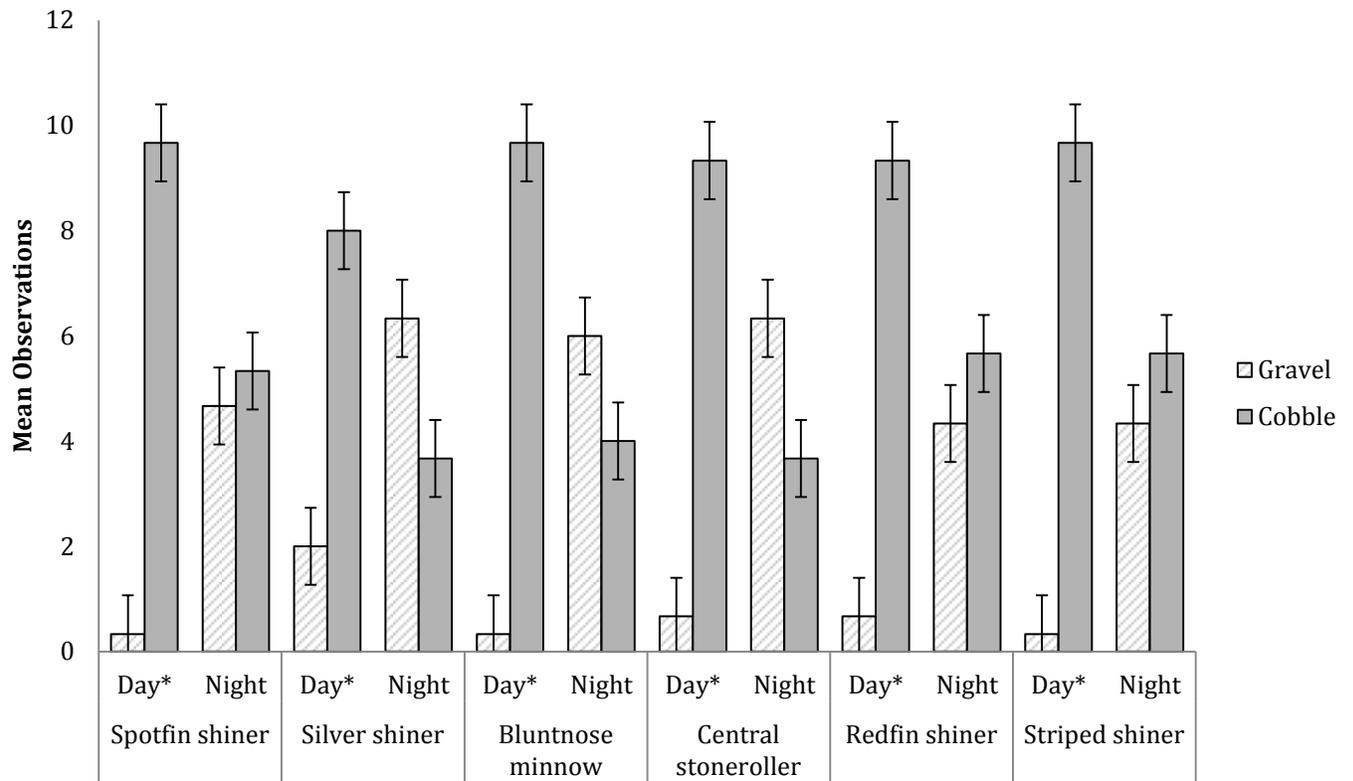
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**Table 1. Significance for paired t-tests by species and treatment. Bold values indicate significant q-values.**

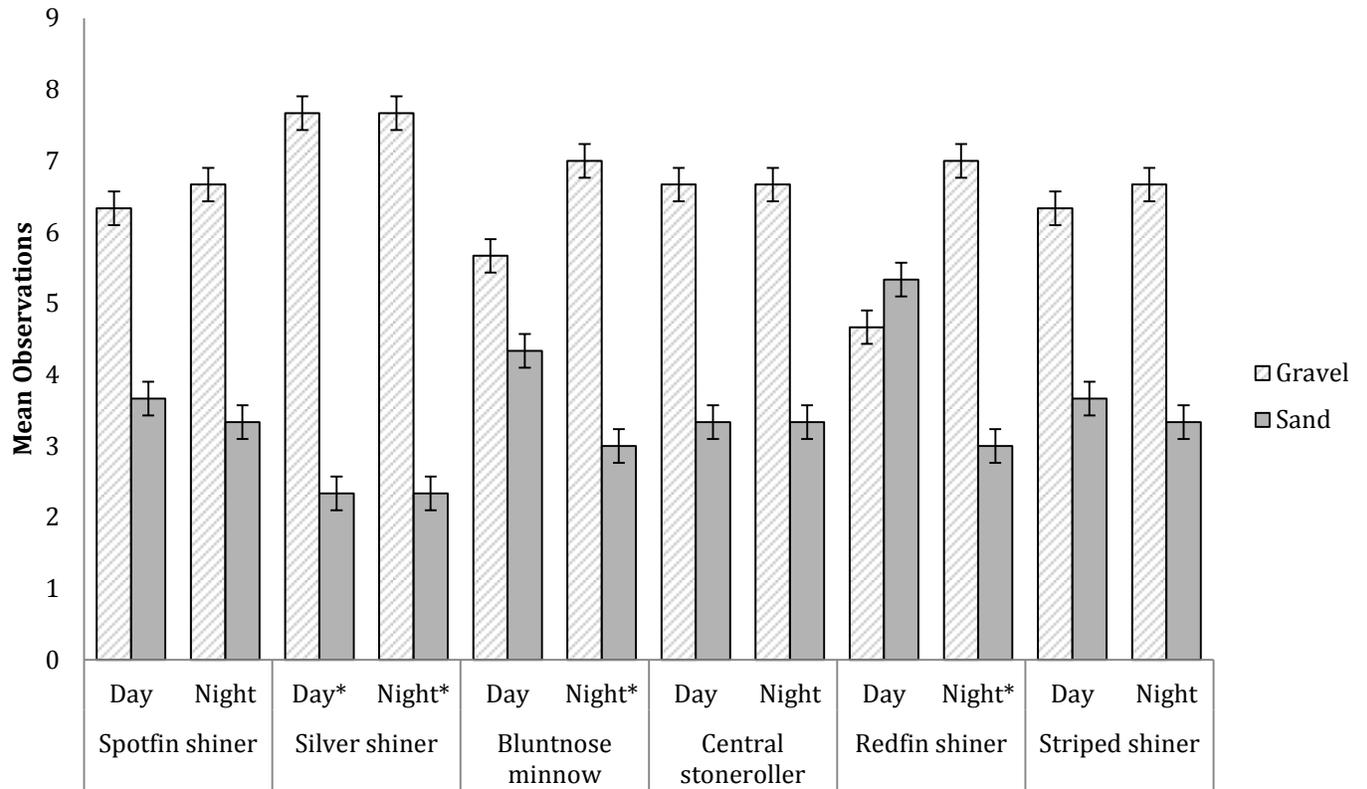
<b>Treatment</b>	<b>Species</b>	<b>q-value</b>
Cobble/Gravel	Spotfin Shiner	<b>0.016</b>
	Silver Shiner	<b>0.013</b>
	Bluntnose Minnow	<b>0.013</b>
	Central Stoneroller	<b>0.014</b>
	Redfin Shiner	<b>0.016</b>
	Striped Shiner	<b>0.014</b>
Cobble/Sand	Spotfin Shiner	<b>0.013</b>
	Silver Shiner	<b>0.029</b>
	Bluntnose Minnow	<b>0.024</b>
	Central Stoneroller	<b>0.016</b>
	Redfin Shiner	<b>0.021</b>
	Striped Shiner	<b>0.013</b>
Gravel/Sand	Spotfin Shiner	0.096
	Silver Shiner	0.202
	Bluntnose Minnow	0.050
	Central Stoneroller	0.202
	Redfin Shiner	0.065
	Striped Shiner	0.096



**Figure 1. Mean (+/- SE) observations of individuals occurrences over substrate types in trials using sand and cobble, by species. Significance for preference differences among substrates is indicated with \*.**



**Figure 2. Mean (+/- SE) observations of individuals occurrences over substrate types in trials using gravel and cobble, by species. Significance for preference differences among substrates is indicated with \*.**



**Figure 3. Mean (+/- SE) observations of individual occurrences over substrate types in trials using gravel and sand, by species. Significance for preference differences among substrates is indicated with \*.**