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May 9, 1991

Mr. Carl Becker Division of Natural Heritage Illinois Department of Conservation 524 S. Second St. Springfield, IL 62706

Dear Carl:

Enclosed are 20 copies of our report on the crayfishes and mussels of two Illinois streams which was funded by the Illinois Nongame Wildlife Conservation Fund and the U.S. Forest Service (Shawnee National Forest). <u>Orconectes illinoiensis</u> was the only open-stream inhabiting crayfish in Big Grande Pierre Creek and Lusk Creek. Representatives of 11 species of mussels were found (nine from Big Grande Pierre, seven from Lusk Creek) scattered throughout both streams. The most abundant species in Big Grande Pierre was <u>Lampsilis siloquoidea</u>; the most abundant species in Lusk Creek was the introduced species, <u>Corbicula fluminea</u>.

The streams provide outstanding habitat for <u>O</u>. <u>illinoiensis</u> which is endemic to southern Illinois and, given the sizes of the streams, support a relatively diverse mussel fauna. Based on our findings and observations, we feel that these two streams should be provided the maximum protection possible under current state law and that efforts should be begun to include them in the National Wild and Scenic River System in order to preserve these outstanding aquatic habitats.

We thoroughly enjoyed working on this project and would like to thank the Nongame Wildlife Commission and the Division for providing the support which made it possible.

Sincerely,

Joyce M. Carmody

Joyce M. Carmody, M.S. Graduate Assistant

M. Ann Phillippi, Ph.D. Assistant Professor



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## HABITAT USE BY CRAYFISH (<u>ORCONECTES</u> <u>ILLINOIENSIS</u>) AND MUSSELS IN TWO ILLINOIS STREAMS

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## ABSTRACT

Big Grande Pierre and Lusk Creek are two southern Illinois streams in Pope County proposed for inclusion in the National Wild and Scenic River System. Twenty-one stations on each stream were sampled for crayfishes and mussels between November 1989 and October 1990 to elucidate habitat use. Air and water temperature, turbidity, stream width, dissolved oxygen, alkalinity, and pH were recorded at each station. In addition, water depth, substrate composition, and percentage of cover (leaf litter and live vegetation) were recorded in each microhabitat sampled for crayfishes and mussels; stream position (upper riffle, middle riffle, lower riffle, middle pool, edge of pool, or isolated pool) and water velocity also were recorded in microhabitats sampled for crayfish. For all individual crayfish, carapace length was determined, and each was categorized according to sex and/or morphological class (sex/class): form I male, form II male, female, undifferentiated juvenile.

<u>Orconectes illinoiensis</u> was the only open-stream inhabiting crayfish in both streams. Conspecific competition likely accounts for the resource partitioning demonstrated by sex/classes. In general, <u>O</u>. <u>illinoiensis</u> displayed the greatest frequencies in depths < 40 cm, in substrates of coarse grain (i.e., boulder and/or cobble) or

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substrate mixtures which included coarse and/or medium sized (i.e., pebble and/or granule) particles, and in upper and lower riffles or edges of pools. It was least abundant at depths > 50 cm, in homogeneous medium- or fine-grained (i.e., sand and/or silt) substrates, and in middle portions of riffles. The crayfish was associated with leaf litter and > 25% live vegetative cover, when available.

Representatives of 11 species of mussels were found (nine from Big Grande Pierre, seven from Lusk Creek) scattered throughout both streams. The most abundant species in Big Grande Pierre was <u>Lampsilis siloquoidea</u>; the most abundant species in Lusk Creek was an introduced species, <u>Corbicula fluminea</u>.

The streams provide outstanding habitat for <u>O</u>. <u>illinoiensis</u> which is endemic to southern Illinois and, given the sizes of the streams, support a relatively diverse mussel fauna. Therefore, Big Grande Pierre and Lusk Creek should be included in the National Wild and Scenic River System in order to preserve this outstanding habitat.

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#### INTRODUCTION

Six streams in southern Illinois (Figure 1) were included in the Nationwide Rivers Inventory (1982) as having outstandingly remarkable scenic and/or recreational values. and are being considered for Federal protection as Wild, Scenic, or Recreational Rivers (Federal Register, 1982; Shawnee National Forest, 1989). Little is known about the biota of most of these streams, and more information is needed in order to evaluate them for protection. Because of time constraints, I elected to study aspects of only two of the streams. Some biotic information exists for Big Creek (Smith, 1971; Page, 1974 and 1985; Evers and Page, 1977); the Big Muddy River is too wide and deep for my sampling methods; the lower two thirds of the nominated section of Bay Creek is mostly privately owned land which is heavily used for farming (Shawnee National Forest, 1989); and, finally, Hutchins Creek, which I initially included in the study, was excluded early on due to excessive habitat destruction within the stream bed caused by off-road vehicles and gravel removal. Consequently, I selected Big Grande Pierre and Lusk creeks, which are little studied and flow through Forest Service land, for study.

Big Grande Pierre is a fifth-order stream, approximately 32 km long, which flows south through Pope County, Illinois, into the Ohio River. Thirty-nine percent

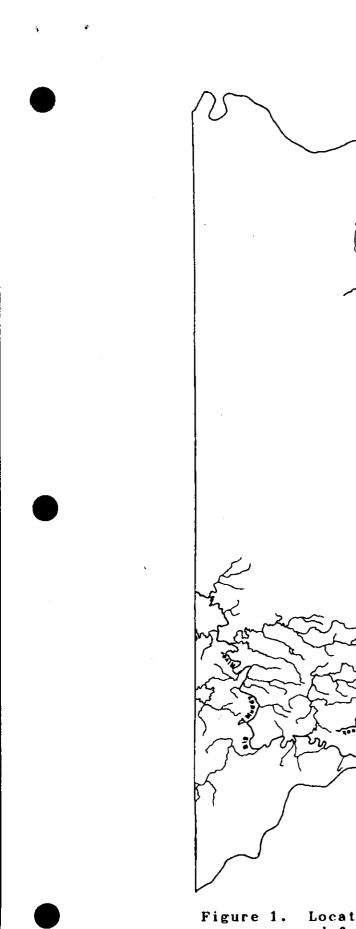




Figure 1. Location of six southern Illinois streams proposed for Federal protection.

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of the stream flows through National Forest land, which amounts to 631 ha within a corridor approximately 0.8 km wide (0.4 km on each side) throughout the length of the stream (Shawnee National Forest, 1989). Private landowners within the corridor cultivate crops and/or raise cattle, horses, and other livestock. Over most of its length, the stream is clear and of moderate gradient, consisting of pools and riffles with substrates of sand, gravel, and bedrock (Shawnee National Forest, 1989). In the lower portions, the stream is more turbid and sluggish, with a sand and silt bottom, and high earthen banks.

Lusk Creek is a fourth-order stream, approximately 45 km long, located in Pope County, Illinois. Lusk Creek flows south into the Ohio River at Golconda, Illinois. Page et al. (1989) considered Lusk Creek to be one of the top 20 outstanding aquatic ecosystems in Illinois based upon distributional data of fishes, mussels, and crayfishes. Approximately 55% of the stream flows through 1994 ha of National Forest land within the 0.8 km corridor (Shawnee National Forest, 1989); some of the remaining land within the corridor is farmed or used as pasture. The stream has a moderate gradient with riffles and some minor rapids (Shawnee National Forest, 1989). Lusk Creek flows through Lusk Creek Gorge where large expanses of exposed rock form vertical walls up to 30.5 m high.

This research was designed to survey and examine

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habitat use by two important groups of stream-dwelling macroinvertebrates, crayfishes and mussels, in Big Grande Pierre and Lusk creeks. Twenty-three species of crayfish are known from Illinois; they are among the largest aquatic invertebrates and are often present in large populations (Page, 1985). As successful, opportunistic omnivores, crayfishes are energetically important because, when abundant, they may control or determine the trophic dynamic structure of a lake or stream (Lorman and Magnuson, 1978; Corey, 1988; Hanson et al., 1990). Knowledge of crayfish and their interactions with other components of the ecosystem is necessary in order to predict long-term results of management strategies related to crayfishes. Likewise, mussels have been recognized as valuable in documenting changes in water quality due to water pollution (Warren et al., 1984). Interest in the mussel fauna of small to medium-sized streams within the middle and upper Ohio River valley has increased in recent years due to their extirpation from many areas. The freshwater mussel fauna of southern Illinois is poorly known, but efforts are underway to document population locations (Cummings pers. comm., Animal groups are associated with measurable 1989a). ranges of environmental conditions. In particular, habitat studies are of primary importance in determining animal distributions in aquatic ecosystems, assessing environmental impacts, and managing for desired species (Cummins, 1962;

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Brower and Zar, 1977). Studies indicate the importance of substrate, water velocity, depth, cover, competition, and food availability to crayfish (Rhoudes, 1962; Black, 1963; Lee et al., 1977; Boyd and Page, 1978; Daniels, 1980; Maude and Williams, 1983; Flynn and Hobbs, 1984; Rabeni, 1985; Berrill et al., 1985; Gore and Bryant, 1990), various mussels (Shoup, 1943; Harman, 1972; Tevesz and McCall, 1979; Strayer, 1981; Sterns, 1982; Warren et al., 1984; Belanger et al., 1985), as well as for fish and other vertebrates (Rankin, 1986; Meffe and Sheldon, 1987; Todd and Rabeni, 1989; Heggenes et al., 1990).

Prior to this study, the Illinois Natural History Survey (INHS) had records of two species of crayfish from Big Grande Pierre (<u>Orconectes illinoiensis</u> and <u>Cambarus</u> <u>diogenes</u>) and no mussels. Recently, nine species of mussels were recorded from Big Grande Pierre: <u>Actinonaias</u> <u>ligamentina carinata</u>, <u>Amblema plicata</u>, <u>Anodonta grandis</u>, <u>Lampsilis radiata luteola</u>, <u>L. ventricosa</u>, <u>Leptodea fragilis</u>, <u>Potamilus alatus</u>, <u>Quadrula guadrula</u>, and <u>Strophitus</u> <u>undulatus</u> (Hunter/ESE, 1989). The INHS had records of four species of crayfish (<u>Orconectes illinoiensis</u>, <u>Cambarus</u> <u>diogenes</u>, <u>Cambarus laevis</u>, and <u>Fallicambarus fodiens</u>), and four species of mussels (<u>Lampsilis siliquoidea</u>, <u>Leptodea</u> <u>fragilis</u>, <u>Strophitus undulatus</u>, and <u>Toxolasma parvus</u>) from Lusk Creek. Additionally, Hunter/ESE (1989) reported the following from Lusk Creek: <u>Anodonta grandis</u>, <u>A. imbecilis</u>,

Lampsilis r. luteola, L.teres teres, Potamilus alatus, P. ohiensis, and Quadrula quadrula.

Documenting the crayfishes, mussels, and their habitat relationships within these two streams was the impetus of this study. Specific objectives of this research were to survey the crayfishes and mussels of the streams; measure selected chemical and physical characteristics of each stream; and examine the effects of substrate, water depth, water velocity, stream position, and cover on the distributional patterns of different sizes and sex/classes of the organisms.

#### **METHODS**

Three different stations (30 m each) on each stream were sampled during seven sample periods (Nov 89, Apr-Oct 90), resulting in 21 stations sampled per stream (Figures 2 and 3). All stations were on the mainstem of the streams, within the corridor (0.8 km) established by the Forest Service.

## Physical and Chemical Measurements

Air and water temperatures, turbidity, stream width, dissolved oxygen, alkalinity, and pH were measured at each station. All measurements were made in the field with the exception of turbidity (JTU) which was measured in the lab using a LaMotte turbidity kit. Average stream width was determined from five equidistant widths within each station. Dissolved oxygen (mg/L) and alkalinity (mg/L) were measured with Hach field kits. An Extech pH meter or a Beckman Electromate pH meter was used to determine pH. Percentage of vegetative cover along the stream bank was estimated to determine bank stability using the following categories: 0, 1-25, 26-50, 51-75, 76-100%.

Average depth, water velocity, substrate composition, stream position, and percentage of cover (leaf litter or live vegetative) were determined for each within-station position selected for crayfish sampling. For each seine

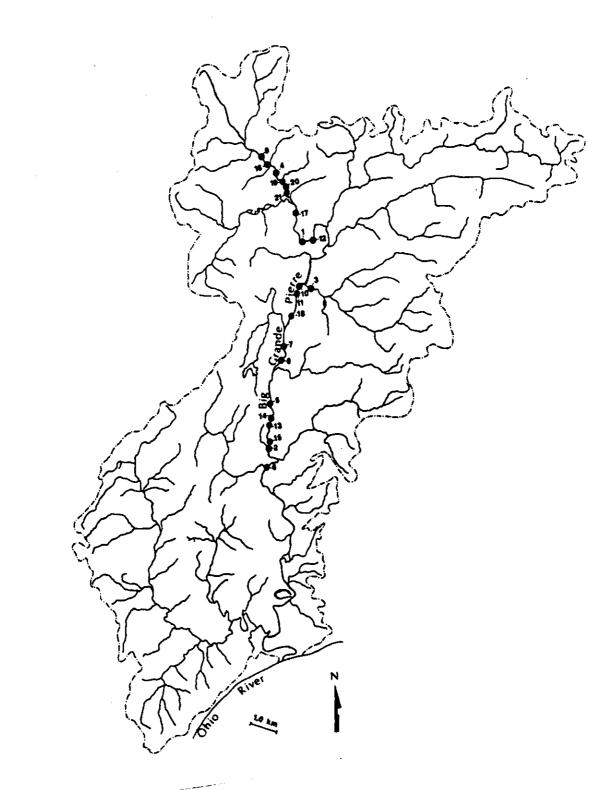


Figure 2. Location of 21 stations sampled within the watershed of Big Grande Pierre Creek, Pope County, Illinois, November 1989-October 1990.

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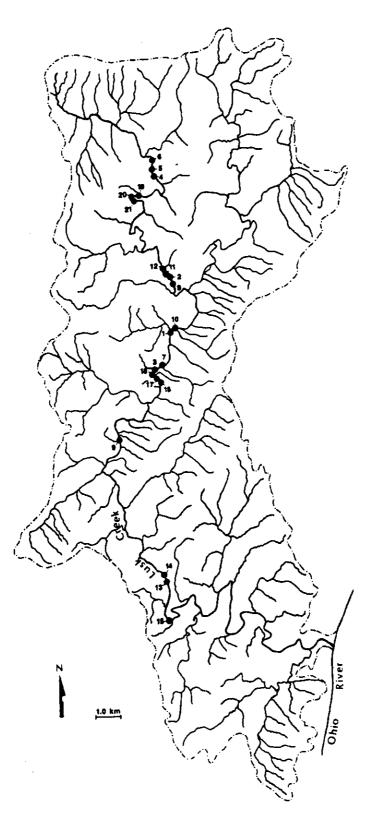


Figure 3. Location of 21 stations sampled within the watershed of Lusk Creek, Pope County, Illinois, November 1989-October 1990.

haul, four depth measurements were taken. Average velocity was determined repeatedly by timing a lemon over a known distance (N = 3). Percentage of substrate types, according to size (Table 1; modified from Cummins, 1962), within each area seined was determined visually and/or by touch. For analysis, substrate types were categorized into eight groups according to grain class (Table 1): predominantly coarse (i.e., boulder and/or cobble); predominantly medium (i.e., pebble and/or granule); predominantly fine (i.e., medium sand and/or silt); solid (i.e., bedrock); coarse/medium mixture (i.e., boulder and/or cobble mixed with pebble); medium/fine mixture (i.e., pebble or granule mixed with medium sand or silt); coarse/medium/fine mixture (i.e., cobble and pebble mixed with medium sand and/or silt); or other mixtures of 3 or more (i.e., a mixture of two types of substrate with bedrock, or other conglomerates of 4 or more substrate types). Stream position was classified as: upper riffle, middle riffle, lower riffle, middle of pool, edge of pool, or isolated pool. The percentage (0, 1-25, 26-50, 51-75, 76-100%) and type of cover (leaf litter; live vegetation; other, such as tires, cans, etc.) was also determined within each area seined (except for Nov 89). Average depth, substrate composition, and percentage of cover also were determined in each position sampled for mussels.

Substrate type	Grain class	Size (mm)
bedrock	solid	solið
silt	fine	< 0.25
medium sand	fine	0.25 - 2.0
granule	med i um	2.0 - 4.0
pebble	med i um	4.0 - 64.0
cobble	coarse	64.0 - 256
boulder	coarse	> 256

Table 1. Classification of substrates according to size (modified from Cummins, 1962).

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Crayfish Sampling

Crayfish populations were sampled with a 2.5 X 2.1 m (3 mm mesh) seine working from downstream to upstream in order to reduce disturbance. Six seine hauls (five in Nov 89) each covering a three square meter area were taken within each station. This was accomplished by having a helper face downstream holding the seine at arms width, while also standing on the lead line. A pre-measured area upstream from the seine was disturbed by kicking, forcing crayfish into the net.

Captured crayfishes were fixed in 80% alcohol, identified using Page (1985) and Hobbs (1976), weighed (wet), and measured from the tip of the rostrum to the posterior edge of the carapace (carapace length, mm). Individuals were assigned to sex and/or morphological categories (sex/class) as follows: form I male, form II male, female, or undifferentiated juvenile. Morphologically, there are two distinct types of males in Cambarinae. Juvenile males (form II) molt into first form males (form I) during their first breeding season; their first pleopods become corneous, hard, and distinctly sculptured at the tip (Hobbs and Jass, 1988; Pennak, 1989). After the first breeding season, these form I males may undergo another molt into a juvenile morphology (form II); their pleopods are indistinguishable from a true juvenile I classified them as form I or form II according to male.

their first pleopods. Those crayfish which were too small to determine sex were classified as undifferentiated.

## <u>Mussel Sampling</u>

Transects were established across each station. A total of twenty 0.5 square meter plots (frame made of polyvinyl chloride) were searched by hand for mussels along the transects (modified from Cummings pers. comm., 1989a). At each station, other portions of the stream and the stream banks were searched for mussel shells. Live mussels were placed on ice and stored in a freezer. After a few days, the mussels were allowed to thaw (their shells opened) and were transferred to 10% formalin. Mussels were identified by Kevin Cummings (INHS).

## <u>Data Analysis</u>

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Means of variables were compared with t-tests, except for means of proportional data (percentages) which were compared with Wilcoxon Rank Sum Tests (Brower and Zar, 1977; SAS Institute, 1985). Goodness of fit, chi-square  $(X^2)$ tests were used to analyze contingency tables and to determine whether observed frequencies of distributions deviated significantly from expected frequencies (Brower and Zar, 1977). The relationships between carapace length and habitat variables were examined using one-way analyses of variance (ANOVA) (Cody and Smith, 1987). A post-hoc

Duncan's test was used to compare mean carapace lengths when habitat effects were significant. Carapace length was regressed on water velocity and depth.

A principal components analysis (PCA) was performed on a correlation matrix of habitat variables. The first four principal components were extracted and pairwise plots of individuals by sex/class and by presence/absence of crayfish were examined. Details of PCA analysis are provided in Manly (1986) and SAS Institute (1985). All tests were conducted at the 0.05 level of significance.

#### RESULTS

## Stream Characteristics

Means of habitat variables are compared in Table 2. Percentages of pebble and granule were significantly higher, and percentage of medium sand was significantly less in Big Grande Pierre than in Lusk Creek. Dissolved oxygen and total alkalinity were significantly higher, and stream depth and air temperature were significantly lower in Big Grande Pierre than in Lusk Creek. Other habitat variables were not statistically different between the two streams.

The velocity of Big Grande Pierre ranged from 0 to 85.5 cm/s ( $\overline{X}$  = 18.2 cm/s). Turbidity ranged from 0 to 20 JTU; pH ranged from 6.10 to 9.96. By station, the area of the stream bank which was covered with vegetation ranged from 0 to 75%.

Lusk Creek flowed at a mean rate of 11.5 cm/s, with a range from 0 to 125.0 cm/s. Turbidity ranged from 0 to 60 JTU; pH ranged from 6.5 to 9.0. Percent of vegetation covering the banks ranged from 0 to 100%.

## Crayfish Capture Success

A total of 241 seine hauls yielded 551 crayfish, including 548 <u>Orconectes illinoiensis</u> and three <u>Cambarus</u> <u>diogenes</u> (Table 3). I deleted <u>C. diogenes</u> from all analyses. Crayfish were found at every station on Big

Table 2. Means  $(\overline{X})$  and standard deviations (SD) of habitat variables found in samples from Big Grande Pierre (N = 21) and Lusk (N = 21) creeks, Nov 1989-Oct 1990.

	Big Gran	<u>de Pierre</u>		<u>usk</u>	
variable <sup>a</sup>	<u> </u>	SD	x	SD	Р
boulder (%)	5.4	14.5	8.9	18.5	0.170
cobble (%)	30.2	26.7	32.2	27.9	0.592
pebble (%)	40.5	29.9	26.4	26.1	0.000*
granule (%)	6.4	15.2	1.9	6.5	0.020*
med. sand (%)	5.5	16.4	18.6	34.6	0.000*
silt (%)	8.8	20.0	5.0	13.3	0.052
bedrock (%)	3.4	13.4	7.1	22.7	0.442
	100%		100%		
depth (cm)	25.5	15.4	32.4	16.6	0.001*
velocity (cm/s)	) 18.2	10.1	11.5	5.3	0.119
air temp (C)	21.9	7.4	24.1	6.6	0.015*
water temp (C)	18.5	6.6	18.6	7.0	0.914
stream width (m)	11.3	8.7	10.9	4.4	0.642
total alkalini: (mg/L CaCO <sub>3</sub> )	ty 14.1	4.0	7.1	2.5	0.000*
dissolved oxygen (mg/L)	9.2	2.9	8.5	2.4	0.033*

<sup>4</sup> The first seven variables are mean percentages of substrates and were compared with Wilcoxon Rank Sum Tests; all other variables were compared with t-tests.

\* Statistically significant at P = 0.05.

Table 3.	Summary of numbers and size ranges of crayfishes
	from Big Grande Pierre (BGP) and Lusk creeks, Nov
	1989-Oct 1990.

Stream	Species	Total number	Size range (mm CL <sup>a</sup> )
BGP	<u>Orconectes</u> illinoiens	<u>is</u> 333	5.6 - 40.9
	<u>Cambarus diogenes</u>	2	13.1 - 14.0
Lusk	<u>O. illinoiensis</u>	215	7.3 - 36.1
	<u>C. diogenes</u>	1	14.4

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<sup>a</sup> CL = carapace length

Grande Pierre and all Lusk Creek stations except station 13. The largest number of individuals captured (78 and 94) occurred during October in Big Grande Pierre (Table 4). In Lusk Creek, the largest number of individuals (47) were captured during August. Undifferentiated juveniles were only found from June through August, 1990. Form I males were captured in each sampling period except June and August. Form II males and females were found during all sample periods.

Means of habitat variables were compared between seine hauls yielding crayfish and those not yielding crayfish (Table 5). Mean stream width and air and water temperatures were significantly less, and percent cobble was significantly greater in areas where <u>O</u>. <u>illinoiensis</u> was captured. Other habitat variables were not statistically different between hauls yielding and not yielding <u>O</u>. <u>illinoiensis</u>.

### Habitat Use By Crayfish

Frequencies, by sex/class of crayfishes captured within seven depth intervals (10 cm each) are presented in Table 6. Contingency table chi-square analysis revealed that sex/classes of <u>O</u>. <u>illinoiensis</u> do not distribute themselves in uniform proportions throughout the seven depth intervals  $(X^2 = 33.1; df = 18; P < 0.025)$ . Individual chi-square analysis, by sex/class, showed that frequency of occurrence

Date	Male I	Male II	Female	Undiffer- entiated	Total captures
		BIG GRANDE	PIERRE C	REEK	
18Nov89	14	3	21	0	38
26Apr90	2	17	8	0	27
16Jun90	0	20	1 2	14	46
14Ju190	3	12	6	3	24
18Aug90	0	11	13	2	26
010ct90	23	20	35	0	78
210ct90	30	19	45	0	94
<u>total</u>	72	102	140	19	333
		LUSI	<u> CREEK</u>		
19Nov89	8	1	21	0	30
29Apr90	2	12	14	0	28
09Jun90	0	7	7	0	14
18Ju190	0	6	6	14	26
20Aug90	0	28	18	1	47
22Sep90	3	17	18	0	38
200ct90	8	6	18	0	32
<u>TOTAL</u>	21	77	102	15	215

Table 4. Numbers of <u>Orconectes illinoiensis</u> captured from Big Grande Pierre and Lusk creeks, Nov 1989-Oct 1990.

Pierre and Lusk creeks, Nov 1989-Oct 1990.							
	Ine	dividual s	eine hauls				
	wi O. illi	th noiensis		hout noiensis			
variable <sup>a</sup>	X	SD	x	SD	Р		
boulder (%)	7.5	16.6	6.5	7.0	0.429		
cobble (%)	35.1	27.6	24.5	25.4	0.004*		
pebble (%)	32.7	28.8	34.8	29.1	0.470		
granule (%)	3.9	12.0	4.8	11.7	0.300		
med. sand (%)	9.4	23.6	16.8	33.7	0.120		
silt (%)	6.7	14.7	7.1	20.6	0.300		
bedrock (%)	5.0	18.2	5.6	19.6	0.870		
	100%		100%				
depth (cm)	27.2	13.6	31.9	20.0	0.054		
velocity (cm/s)	16.4	6.9	15.4	5.9	0.224		
air temp (C)	21.6	7.5	25.6	5.5	0.000*		
water temp (C)	17.2	7.2	20.9	5.2	0.024*		
stream width (m)	10.3	6.0	12.5	8.1	0.024*		
total alkalinity (mg/L CaC0 <sub>3</sub> )	10.7	4.8	10.5	5.0	0.828		
dissolved oxygen (mg/L)	9.0	2.8	8.5	2.3	0.123		

<sup>4</sup> The first seven variables are mean percentages of substrates and were compared with Wilcoxon Rank Sum Tests; all other variables compared with t-tests.

\* Statistically significant at P = 0.05.

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Table 5.

Means  $(\overline{X})$  and standard deviations (SD) of habitat

variables associated with successful (N = 151) and unsuccessful (N = 90) seine hauls from Big Grande

Derth	<b>B</b>		Frequency of captures <sup>a</sup> (%)			
Depth interval	Frequency sampled (%	) MI	MII	F	U	Total
(cm)	N = 241	N = 93	N = 179	N = 242	N = 34	N = 548
0-9.9	14 (5.8)	4 (4.3)	28 (15.6)	19 (7.9)	1 (2.9)	52 (9.5)
10-19.9	74 (30.7)	27 (29.0)	44 (24.6)	74 (30.6)	12 (35.3)	157 (28.6)
20-29.9	58 (24.1)	26 (28.0)	51 (28.5)	56 (23.1)	10 (29.4)	143 (26.1)
30-39.9	48 (19.9)	24 (25.8)	32 (17.9)	64 (26.4)	7 (20.6)	127 (23.2)
40-49.9	20 (8.3)	8 (8.6)	14 (7.8)	16 (6.6)	3 (8.8)	41 (7.5)
50-59.9	15 (6.2)	2 (2.2)	10 (5.6)	6 (2.5)	1 (3.0)	19 (3.5)
60 +	12 (5.0)	2 (2.1)	0 (0)	7 (2.9)	0 (0)	9 (1.6)

Table 6. Observed frequencies of sex/classes of Orconectes illinoiensiscaptured in seven depth intervals.

\* MI = form I male; MII = form II male; F = female; U = undifferentiated juvenile of form I males (MI) and undifferentiated juveniles (U) was independent of depth interval (MI  $X^2 = 6.6$ , U  $X^2 = 3.4$ ; df = 6; P > 0.10). However, the distribution of form II males (MII) and females (F) was not independent of depth interval (MII  $X^2 = 43.0$ , F  $X^2 = 15.4$ ; df = 6, P < 0.025). Form II males exhibited most preference for depth intervals of 0-9.9 and 20-29.9 cm and least preference for depths greater than 60 cm; females seemed to prefer depths from 30-39.9 cm, and showed least preference for depths greater than 50 cm.

Frequencies of occurrence for each sex/class in the eight substrate groups was determined (Table 7). Contingency table chi-square analysis indicated that the distribution of the sex/classes differed from the expected distribution among the substrate groupings ( $x^2$  = 74.5; df = 21; P < 0.01). Individual chi-square analyses indicated that all sex/classes show partiality for specific substrate groupings (MI  $X^2$  = 39.2, MII  $X^2$  = 67.8, F  $X^2$  = 24.2, U  $X^2$  = 21.3; df = 7; P < 0.01). Occurrence of form I males was disproportionately high in predominantly coarse substrates and mixtures of coarse, medium, and fine substrates, or medium and fine mixtures and was lower than expected in all other groups. Form II males favored mixtures of coarse, medium, and fine substrates and seemed to show least preference for predominantly medium, fine, or solid substrates. Occurrence of females was unexpectedly high in areas with coarse, medium, and fine mixtures and was low in

	Frequency of captures <sup>4</sup> (%)					
Substrate group	Frequency sampled <sup>b</sup> (%) MI	MII	MII F		Total	
<u> </u>	N = 240 N = 8	)3 N = 179	N = 242	N = 34	<u>N</u> = 548	
coarse (cs)	44 (18.3) 35 (37	.6) 35 (19.6)	57 (23.6)	5 (14.7)	132 (24.1)	
medium (md)	37 (15.4) 7 (7.	5) 15 (8.4)	35 (14.5)	2 (5.9)	59 (10.8)	
fine (fn)	26 (10.8) 4 (4	.3) 6 (3.3)	14 (5.8)	1 (2.9)	25 (4.6)	
solid	10 (4.2) 1 (1	.1) 1 (0.6)	8 (3.3)	2 (5.9)	12 (2.2)	
cs/md mix	43 (18.0) 10 (10	.8) 38 (21.1)	40 (16.5)	13 (38.2)	101 (18.4)	
md/fn mix	11 (4.6) 8 (8	.6) 3 (1.7)	10 (4.1)	0 (0)	21 (3.8)	
cs/md/fn mix	49 (20.4) 25 (26	.9) 74 (41.3)	69 (28.5)	4 (11.8)	172 (31.4)	
other mix of 3 or more	20 (8.3) 3 (3	.2) 7 (3.9)	9 (3.7)	7 (20.6)	26 (4.7)	

Table 7. Observed frequencies of sex/classes of <u>Orconectes illinoiensis</u> captured in eight substrate groups.

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<sup>a</sup> MI = form I male; MII = form II male; F = female; U = undifferentiated juvenile

<sup>b</sup> One sample (solid/fn) with no captures, was excluded.

predominantly fine substrates or other mixtures of three or more. Undifferentiated juveniles seemed to prefer mixtures of coarse and medium substrates or other mixtures of three or more, and seemed to avoid most other substrate groupings.

Frequencies for crayfish captured in different stream positions are presented in Table 8. Contingency table chisquare analysis indicated that sex/classes of <u>O</u>. <u>illinoiensis</u> were not distributed in expected proportions among these areas  $(X^2 = 37.6; df = 15; P < 0.01)$ . Form I males and females showed no preference for stream position  $(MI X^2 = 11.0, F X^2 = 4.88; df = 5; P > 0.05)$ . However, form II males and undifferentiated juveniles did display an affinity for stream positions (MII  $X^2 = 23.9, U X^2 = 11.6;$ df = 5; P < 0.05). Occurrence of form II males was higher than expected in upper riffles and edges of pools and was lower in middle of pools. Juveniles favored edges of pools, and lower riffles and were absent from upper and middle portions of riffles.

Frequencies of captures in varying amounts of leaf litter and live vegetative cover are presented in Tables 9  $\cdot$ and 10, respectively. Contingency table chi-square analysis revealed that distribution of sex/classes of <u>O</u>. <u>illinoiensis</u> in varying amounts of leaf litter differed from expected distributions ( $X^2 = 67.9$ ; df = 12; P < 0.10). Form II males were distributed uniformly with respect to litter abundance ( $X^2 = 4.0$ ; df = 4; P > 0.10), but distributions of the other

Stream	Fna			Frequency of captures <sup>a</sup> (%)									
position	Frequency sampled <sup>b</sup> (%)		)	MI		MII		F		U		Total	
	N	= 229	]	N = 93	N	= 172	N	= 236	]	<u>N = 34</u>	N	= 535	
upper riffle	16	(7.0)	3	(3.2)	27	(15.7)	17	(7.2)	0	(0)	47	(8.8)	
mid riffle	31	(13.5)	8	(8.6)	19	(11.0)	23	(9.7)	0	(0)	50	(9.3)	
lower riffle	19	(8.3)	4	(4.3)	12	(7.0)	22	(9.3)	6	(17.6)	44	(8.2)	
middle pool	122	(53.3)	65	(69.9)	79	(46.0)	127	(53.8)	17	(50.0)	288	(53.8)	
edge of pool	33	(14.4)	11	(11.8)	31	(18.0)	41	(17.4)	9	(26.5)	92	(17.2)	
isolated pool	l 8	(3.5)	2	(2.2)	4	(2.3)	6	(2.5)	2	(5.9)	14	(2.6)	

Table 8. Observed frequencies of sex/classes of Orconectes illinoiensiscaptured in six stream positions.

<sup>4</sup> MI = form I male; MII = form II male; F = female; U = undifferentiated juveniles

<sup>b</sup> Positions in stream were not consistently recorded in November, 1989.

N 5

Leaf litter (%)	<b>F</b> actor of a state	Frequency of captures <sup>4</sup> (%)								
	Frequency sampled (%)	MI	MII	F	U	Total				
	N = 217	N = 71	N = 175	N = 200	N = 34	N = 480				
0	113 (52.1)	7 (9.9)	86 (49.1)	64 (32.0)	20 (58.8)	177 (36.9)				
1-25	44 (20.3)	18 (25.3)	36 (20.6)	44 (22.0)	13 (38.2)	111 (23.1)				
26-50	32 (14.7)	23 (32.4)	22 (12.6)	43 (21.5)	1 (3.0)	89 (18.5)				
51-75	23 (10.6)	20 (28.2)	26 (14.9)	36 (18.0)	0 (0)	82 (17.1)				
76-100	5 (2.3)	3 (4.2)	5 (2.8)	13 (6.5)	0 (0)	21 (4.4)				

Table 9. Observed frequencies of sex/classes of Orconectes illinoiensiscaptured in areas with differing amounts of leaf litter.

<sup>a</sup> MI = form I male; MII = form II male; F = female; U = undifferentiated juvenile

Table 10. Observed frequ	encies of sex/classes of <u>Orconectes illinoiensis</u>	
captured in ar (VEG).	eas with differing amounts of live vegetative cove	r

Percent VEG	_		Frequency of captures <sup>4</sup> (%)							
	Frequency sampled (9	) MI	MII	F	U	Total				
	N = 217	N = 71	N = 175	N = 200	N = 34	N = 480				
0	167 (77.0)	55 (77.5)	136 (77.7)	154 (77.0)	22 (64.7)	367 (76.5)				
1-25	27 (12.4)	1 (1.4)	21 (12.0)	26 (13.0)	2 (5.9)	50 (10.4)				
26-50	15 (6.9)	14 (19.7)	18 (10.3)	17 (8.5)	8 (23.5)	57 (11.9)				
51-75	7 (3.2)	1 (1.4)	0 (0)	3 (1.5)	1 (2.9)	5 (1.0)				
76-100	1 (0.5)	0 (0)	0 (0)	0 (0)	1 (3.0)	1 (0.2)				

<sup>a</sup> MI = form I male; MII = form II male; F = female; U = undifferentiated juvenile

three sex/classes indicated a preference (MI  $X^2$  = 62.5, F  $X^2$ = 47.8, U  $X^2$  = 13.3; df = 4; P < 0.01). Occurrence of form I males was disproportionately low in areas with no leaf litter and was high in areas with 1-75% litter. Occurrence of females was low in areas with no leaf litter, and high in areas with 26-100% leaf litter. Occurrence of undifferentiated juveniles was high in zero and 1-25% leaf litter and low or absent in 26-100% litter. Chi-square contingency table analysis also revealed a relationship between sex/class and amount of vegetative cover  $(X^2 = 33.9;$ df = 12; P < 0.01). Females and form II males exhibited an expected distribution (F  $X^2$  = 3.6, MII  $X^2$  = 9.4; df = 4; P > 0.05), but form I males and undifferentiated juveniles exhibited an affinity for specific amounts of vegetative cover (MI  $X^2 = 24.9$ , U  $X^2 = 19.2$ ; df = 4; P < 0.01). Occurrence of form I males and juveniles was higher than expected in areas of 26-50% vegetative cover and was lower in areas of 1-25%. Juvenile occurrence was also high in 76-100% vegetative cover.

Relationships between size (carapace length) and habitat variables were examined. A regression of carapace length on depth showed no significant relationship (F = 0.2; df = 1,547; P > 0.6). There were significant differences (ANOVA; F = 2.64; df = 6,542; P < 0.02) between the mean carapace length of crayfish found in different depth intervals (Figure 4). The mean carapace length in the 60+

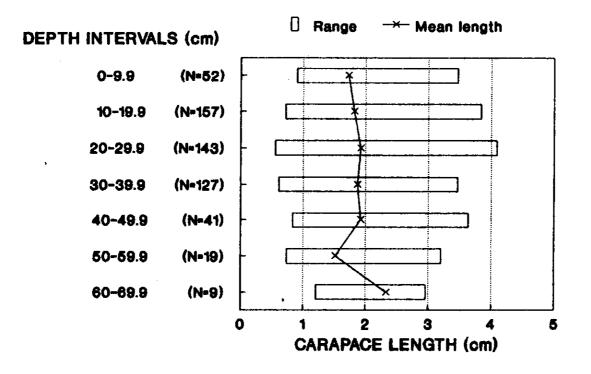


Figure 4. Range and means of <u>Orconectes illinoiensis</u> carapace lengths found in seven depth intervals.

cm depth interval was significantly greater than in all other depth intervals; carapace length in the 50-59.9 cm depth interval was significantly lower than in all other depth intervals except those which were in depths less than 20 cm (Duncan's; P < 0.05). There were no significant differences in mean carapace length among the remaining depth intervals.

Carapace length was positively correlated (P < 0.05) with the presence of boulder and bedrock (Table 11). However, the mean carapace lengths among the eight substrate groups (Figure 5) were not significantly different (ANOVA; F = 1.99; df = 7,541; P = 0.052). The mean and range of carapace length of <u>O</u>. <u>illinoiensis</u> found in different stream positions are presented in Figure 6; analysis indicated no significant difference in carapace length among these positions (ANOVA; F = 0.36; df = 5.530; P > 0.8).

Mean carapace length differed significantly according to the percentage of leaf litter present (Figure 7) (ANOVA; F = 4.93; df = 4,475; P < 0.01). Mean carapace length of crayfish found in areas covered with 76-100% litter was significantly less than from areas with no litter and with 26-75% litter cover (Duncan's; P < 0.05). Mean carapace length from areas of 1-25% litter cover differed significantly from areas with no litter and 51-75% leaf litter (Duncan's; P < 0.05).

Carapace lengths of O. illinoiensis were plotted

Substrate type	Г	
boulder	0.099*	
cobble	0.025	
pebble	- 0.071	
granule	0.080	
medium sand	- 0.049	
silt	- 0.075	
bedrock	0.089*	

Table 11.Correlations between Orconectes illinoiensis<br/>carapace length and substrates.

\* Statistically significant at P = 0.05.

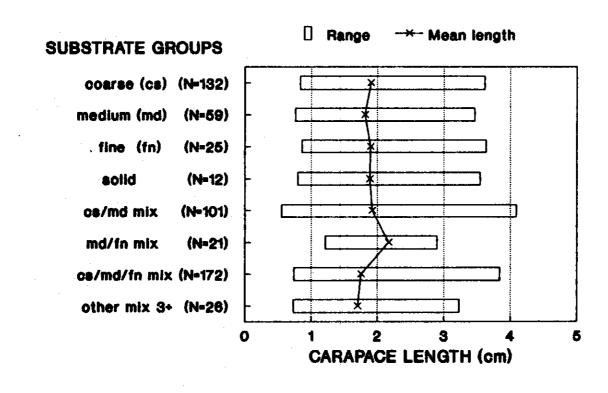


Figure 5. Range and means of <u>Orconectes illinoiensis</u> carapace lengths found in eight substrate groups

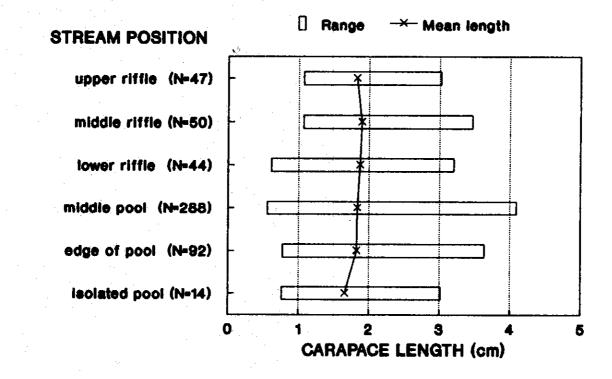


Figure 6. Range and means of <u>Orconectes illinoiensis</u> carapace lengths found in six stream positions.

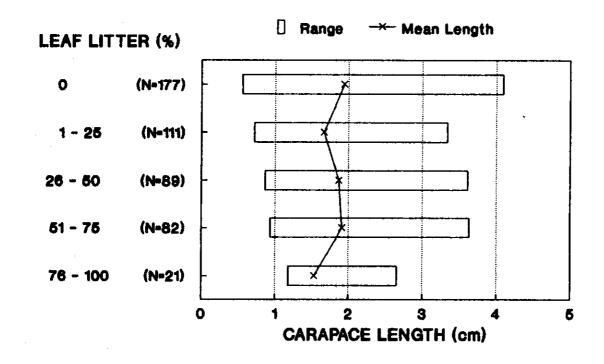


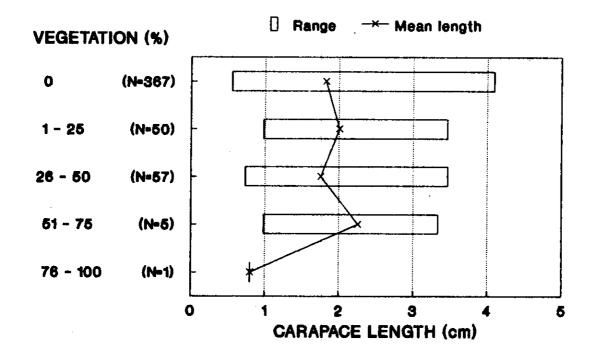
Figure 7. Range and means of <u>Orconectes illinoiensis</u> carapace lengths found in areas with differing amounts of leaf litter.

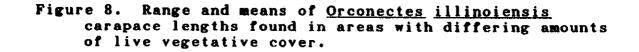
against the percentage of live vegetative cover (Figure 8), and no significant differences were found (ANOVA; F = 2.35; df = 4,475; P = 0.053). Only one crayfish was captured in an area where "other" cover (i.e., a tire) was present. There was no relationship between carapace length and water velocity (REGRESSION; F = 0.004; df = 1,516; P > 0.9).

Pairwise comparison of the first four principal component axes using sex/class to group individuals revealed apparently random distributions. Likewise, scatter plots of any two principal component axes using presence versus absence of captures to group microhabitats revealed no pattern.

## <u>Mussels</u>

Few mussels were found in either stream. Only one area (Big Grande Pierre station 21) was found with a concentration of mussels. In total, more shells (100 complete individuals and 22 half shells) were collected than live specimens (57). Eleven species were represented, nine species from Big Grande Pierre and seven from Lusk Creek (Table 12). In Big Grande Pierre, the most abundant live species and shell was <u>Lampsilis siloquoidea</u>. The most abundant live species from Lusk Creek was <u>Corbicula fluminea</u> and the most abundant shell was <u>L. siliquoidea</u>. Species of Sphaeriidae (fingernail clams) also were present in both streams but were not included in the study since their small





	Lusk	Cree	k	Big Gran	de P	ierr€
Species	Live	She	ells	Live	Sh	ells
Anodonta grandis	0	0		0	3	
<u>A. imbecillis</u>	1	0		0	0	
<u>Anodontoides</u> <u>ferussacianus</u>	2	2		0	1	
<u>Amblema plicata</u>	0	0		0		1/2
<u>Corbicula fluminea</u>	37	13	2/2	5	10	
<u>Lampsilis cardium</u>	0	0		0	6	2/2
L. siliquoidea	1	15	1/2	8	27	9/2
<u>Leptodea fragilis</u>	0	1		0	3	
<u>Ligumia subrostrata</u>	0	1		0	0	
<u>Potamilus alatus</u>	0	0		2	13	6/2
<u>Strophitus undulatus</u>	0	1		1	4	1/2
TOTAL # INDIVIDUALS	41	33	3/2	16	67	19/2
TOTAL # SPECIES	4	6		. 4	9	
TOTAL # SPECIES	•	7			9	

Table 12. Freshwater mussels collected from Lusk and Big Grande Pierre creeks, Nov 1989-Oct 1990.

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size precluded adequate sampling by the methods employed here.

Live mussels were found at stations 7, 13, 17, and 21 on Big Grande Pierre (see Figure 2). Stations 17 and 21 were in the upper reaches, and stations 7 and 13 were in middle reaches of the stream. The dissolved oxygen in these four stations ranged from 3-11 mg/L; the turbidity ranged from 0-20 JTU; total alkalinity was at least 15 mg/L.

Live mussels were found at stations 11, 13, 14, and 17 on Lusk Creek (see Figure 3). Stations 11 and 17 were in middle reaches, and stations 13 and 14 were in lower reaches of the stream. The dissolved oxygen at these stations ranged from 5-8 mg/L, and the turbidity ranged from 0-20 JTU. Bivalves from Lusk Creek inhabited waters with a total alkalinity ranging from 8-10 mg/L. All mussels were found predominantly in medium sand or pebble substrates, often mixed with cobble or silt.

### DISCUSSION

## Crayfish

Based on this research, <u>Orconectes</u> <u>illinoiensis</u> is the only open stream inhabiting crayfish of Big Grande Pierre and Lusk creeks. Even though <u>Cambarus diogenes</u> is abundant throughout southern Illinois, it is a burrowing species and is not as likely to be captured in an open stream situation (Page, 1985). The low number of <u>C</u>. <u>diogenes</u> captured is not indicative of their abundance in the stream corridors because many burrows were observed.

"Species of <u>Orconectes</u> are, with few exceptions, open stream inhabitants and they, more than other crayfishes, have found Illinois suitable for occupation" (Page, 1985). The Propinquus group of <u>Orconectes</u>, of which <u>O. illinoiensis</u> is a member, ranges throughout the northeastern United States (Fitzpatrick, 1967). <u>Orconectes illinoiensis</u> was described by Brown (1956) and is endemic to southern Illinois. It occupies the streams within and just north of the Shawnee Hills, and also occurs less abundantly in the rocky streams on the Coastal Plain of Illinois (Page, 1985). The species is little-studied other than for systematic review and state surveys (Brown, 1956; Page, 1985; Hobbs and Jass, 1988). Aside from descriptions of the types of streams from which specimens were collected (Brown, 1956; Page, 1985), there is little ecological information.

Orconectes illinoiensis used all sampled habitats within Big Grande Pierre and Lusk creeks, however, it showed affinity for certain habitat variables by size or sex/class.

Many crayfish species generally inhabit shallow waters and are rarely found in waters greater than one meter deep (Pennak, 1989; Black, 1963). However, in Missouri, adult O. punctimanus were found in water depths < 40 cm as frequently as from all other depths combined, while both adult and young-of-the-year (YOY) O. luteus were distributed evenly throughout depth intervals to 160 cm (Rabeni, 1985). Daniels (1980) found abundance of O. virilis and Pacifastacus fortis to be positively correlated with depth, and Gore and Bryant (1990) stated that O. <u>neglectus</u> showed equal preference for depths. Similarly, relationships between depth intervals and abundance of O. illinoiensis were revealed in this study. Mean carapace length of Q. illinoiensis was significantly greater in depths > 60 cm than in more shallow waters. Additionally, form II males were found in shallow areas (< 10 cm) in a proportion three times greater than expected and were not found at depths > 60 cm (Table 6). Females were most concentrated in depths from 30-39.9 cm and seemed to avoid depths > 50 cm. The sampling method employed in this study may have allowed crayfish greater escape time in deep areas (> 50 cm) and may account, in part, for the relatively low number of captures in these areas.

For stream inhabiting crayfish, selection of large substrates may be beneficial if there is a reduced current created by such irregular substrates, or if avoidance of predation is facilitated (Hynes, 1970). My results indicated that distribution of all sex/classes of O. <u>illinoiensis</u> was dependent upon substrate groups. Form I males and females were associated with coarse substrates or combinations of coarse, medium, and fine substrates; form I males also were associated with mixtures of medium and fine substrates (see Table 7). Form II males were highly associated with coarse/medium/fine mixtures and coarse/medium mixtures. Undifferentiated juveniles were commonly found in a mixture of coarse and medium substrates and in other various mixtures of three or more substrate types which often included bedrock. Carapace length was positively correlated with boulder and bedrock. As a species, Q. illinoiensis seems to prefer areas with at least some medium or coarse-grained substrates. This agrees with habitat descriptions for <u>O. illinoiensis</u> by others (Brown, 1956; Page, 1985).

Stream position was associated with distribution of Q. <u>illinoiensis</u> form II males and juveniles. Form II males selected upper riffles and edges of pool (Table 8). Undifferentiated juveniles, when found in riffles, were only at the lower end; perhaps these areas offered greater protection or food availability. Juvenile abundance also

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was disproportionately high near edges of pools. In a competitive situation, Boyd and Page (1978) found partitioning between <u>O. kentuckiensis</u>, which inhabited sluggish, rocky pools, and <u>O. placidus</u>, which was characteristic of gravel riffles.

Water velocity was not found to be important to the distribution of <u>O</u>. <u>illinoiensis</u>. However, other studies indicate water velocity as an important factor in <u>Orconectes</u> spp. distribution (Daniels, 1980; Maude and Williams, 1983; Rabeni, 1985; Gore and Bryant, 1990).

Since crayfish are opportunistic, leaf litter and live vegetation may serve as a food source and/or as protective cover. Relationships between percentage of leaf litter and occurrence of O. illinoiensis were revealed. Mean carapace length varied significantly among percentages of leaf litter present (Figure 7). Form I males were associated with 1-75% leaf litter cover; females with 26-100%, and undifferentiated juveniles with 0-25% (Table 9). Indeed, the molt to form I for male O. <u>illinoiensis</u> corresponds with the season of leaf-fall, which may account for their association with leaf litter, and the undifferentiated juveniles were only found from June to August which could account for their association with little or no leaf litter. Huryn and Wallace (1987) found litter processing by crayfish to be temperature regulated, and concluded that crayfish act as shredders of the more slowly processed leaf litter during

the summer months. If this is true of O. <u>illinoiensis</u>, then during the autumn months it likely is using leaf litter more for cover than for a food source.

Rabeni (1985) found the <u>O. punctimanus</u> YOY were clearly associated with aquatic macrophytes, but <u>O. luteus</u> YOY exhibited an inverse relationship with vegetative cover. Similar to <u>O. punctimanus</u>, this research found undifferentiated juveniles associated with 26-50% and 76-100% vegetative cover, and inversely associated with 1-25%. Form I males also were associated with 26-50% vegetative cover. Gore and Bryant (1990) also found adult <u>O. neglectus</u> associated with macrophyte beds. Shallow macrophyte beds were more difficult to sample with my methods which is the reason why only eight samples were taken in areas with 51-100% vegetation.

## Mussels

The high water quality and substrate stability of these streams provides good habitat for freshwater mussels. However, stable and abundant populations are more common in large streams and rivers, whereas small streams such as Big Grande Pierre and Lusk Creek support scattered, unstable populations (Pennak, 1989). Greater species diversity is usually found in downstream reaches of streams, rather than in headwaters and tributaries. This may explain why I found fewer species than Hunter/ESE (1989) did, since they were

able to sample (by diving) lower reaches of these streams where they found a more diverse mussel fauna.

Starret (1971) documented the disappearance of mussel species in the Illinois River resulting from pollution and habitat degradation. Cummings (1989b) reported the disappearance of many once widespread species from other major rivers in Illinois and suggested that remaining highquality habitats should be protected to prevent further extirpation of rare species. Lusk and Big Grande Pierre creeks are high-quality streams which support a small mussel fauna, as well as many other aquatic organisms.

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## CONCLUSIONS

<u>Orconectes illinoiensis</u> inhabits Big Grande Pierre and Lusk creeks without potential competition from other streamdwelling crayfish. Conspecific competition likely accounts for the resource partitioning shown by sex/classes and sizes of <u>O</u>. <u>illinoiensis</u>.

If preference exists, the species seems to prefer depths < 40 cm and least prefer or perhaps avoid depths > 50 It seems to favor predominantly coarse-grained (i.e., CM. boulder and/or cobble) substrates or mixtures which include coarse and/or medium (i.e., pebble and/or granule) sized particles. In fact, restriction to streams with medium and coarse substrates may be the barrier blocking species advancement to the north, and the large Ohio, Mississippi, and Wabash rivers provide barriers in other directions. These crayfish seem to show favoritism for upper or lower ends of riffles or edges of pools and seem to avoid midportions of riffles. All sex/classes prefer to be associated with leaf litter when it is present (i.e., Autumn months). The apparent preference of undifferentiated juveniles for areas with no or small amounts (1-25%) of leaf litter (Table 9) is misleading because by the time leaves begin to fall, the crayfishes are mature enough for differentiation into sex/classes. In fact, these juveniles were found primarily in June and July when only modest

amounts of leaf litter were present. If a preference for live vegetation exists, they show partiality for areas with > 26% live vegetative cover.

Carapace length of O. <u>illinoiensis</u> was positively correlated with boulder and bedrock and was related to depth intervals and leaf litter. Mean length of crayfish in depth intervals > 60 cm were significantly higher than means at lesser depths. Conversely, mean length in the next lowest depth interval (50-59.9) was, in general, less than means at other depth intervals. This inconsistency precludes any generalizations about relationships between size and depth. Similarly, the data will not allow for any generalizations about relationships between size and percentages of leaf litter. The data showed no relationships between crayfish size and substrate groups, stream positions, or percentages of live vegetative cover.

Representatives of 11 species of mussels were collected (nine from Big Grande Pierre and seven from Lusk creek) at localities scattered throughout both streams. Mussels in both streams were located in stable substrates of medium sand and pebble, and were not present in less stable, silt substrates. Alkalinity was higher (> 15 mg/L) at mussel sites in Big Grande Pierre than in Lusk Creek (8-10 mg/L). Mussels occupied waters with concentrations of dissolved oxygen as low as 3 mg/L in Big Grande Pierre and 5 mg/L in Lusk Creek.

Both Big Grande Pierre and Lusk creeks are high-quality streams which support a relatively diverse mussel fauna as well as populations of a crayfish which is endemic to the region. I support the inclusion of these two streams into the National Wild and Scenic River System in order to preserve the outstanding habitat they provide for <u>O</u>. <u>illinoiensis</u>.

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# APPENDICES

intervals.						
ANOVA						
Source	<u>ar</u>	<u>Sum of Squares</u>	<u>Mean</u> Square	<u>F</u> .		
depth group	6	5.91	0.99	2.54*		
erfor	542	202.05	0.37			

207.97

Appendix A. ANOVA and Duncan's results used to determine relationships between carapace length and depth intervals.

Duncans.

<u>Depth interval</u>	Mean
0-9.9 cm	1.73 ab <sup>1</sup>
10-19.9 cm	1.82 ab
20-29.9 cm	1.92 a
30-39.9 cm	1.87 a
40-49.9 cm	1.92 a
50~59.9 cm	1.52 b
60+ cm	2.33 c

548

TOTAL

\* Statistically significant at P < 0.05

<sup>1</sup> Means followed by the same letter are not significantly different at P < 0.05</p> Appendix B. ANOVA and Duncan's results used to determine relationships between carapace length and percentage of leaf litter present.

		ANOVA		
Source	dſ	Sum of Squares	<u>Mean Square</u>	<u>F</u>
leaf litter	4	7.83	1.96	4.93*
error	475	188.84	0.40	
TOTAL	479	196.67		

# **Duncans**

Percentage of litter	Mean
0	1.94 a <sup>1</sup>
1-25%	1.66 bc
26-50%	1.87 ab
51-75%	1.91 a
76-100%	1.53 c
26-50% 51-75%	1.66 bc 1.87 ab 1.91 a

\* Statistically significant at P < 0.05

<sup>1</sup> Means followed by the same letter are not significantly different at P < 0.05</p>