EVALUATION OF CRAYFISH AS A KEYSTONE ORGANISM

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Evaluation of crayfish as a keystone organism.

A Thesis

Presented for the

Master of Science Degree

Austin Peay State University

Robert B. Brinkman

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DEDICATION

This thesis is dedicated to my family and my fiancee Susan.

Their support and encouragement was essential

for me to complete this project.

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ABSTRACT

A keystone species is critical to a community and has the ability to affect more than one trophic levels. Crayfish are ecologically important to small stream ecosystems and have been described as keystone species. They occupy numerous trophic levels and are instrumental in making coarse particulate organic material (CPOM) into fine particulate organic material (FPOM) and available for other macroinvertebrates in the stream.

Two enclosure/exclosure experiments were conducted in 2002 to test the role of *Orconectes placidus* as a keystone species. An experiment to determine the impact crayfish have on processing leaf litter was started in February and lasted 28 days. A second experiment, lasting 21 days, to study possible impacts crayfish have on the abundance of other macroinvertebrates was started in May. The 16S ribosomal RNA gene from the mitochondrial DNA from twenty five specimens of *O. placidus* and four *O. durelli* was used to genetically distinguish them from each other. The DNA fragment was amplified using polymerase chain reaction (PCR) and treated with TaqI and VspI restriction enzymes. The digests were visually analyzed using agarose gel electrophoresis. *Orconectes placidus* significantly reduced the weights of leaf packets they were enclosed with. There was no significant differences in the abundances or richness of macroinvertebrates between crayfish enclosure and exclosure channels used as controls. The restriction enzyme TaqI produced different banding patterns between *O. durelli* and *O. placidus*. Banding patterns resulting from digestion with VspI were not visually distinguishable using this procedure.

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CHAPTER I

INTRODUCTION

Keystone species

A keystone species has been described as an organism that has the ability to influence or may be critical to a community (Krebs, 2001). Paine (1966) showed that the removal of *Pisaster* (starfish), the main predator, in a marine environment resulted in a decrease in diversity. A stable and diverse community is dependent upon the keystone species (Momot et al., 1978). Equilibrium and stability occurs when the top predator prevents a single species from monopolizing an ecosystem (Momot et al., 1978; Paine, 1966). Keystone species affect an ecosystem both directly and indirectly. Paine (1966) showed that *Pisaster* directly affected the ecosystem by feeding on barnacles, thus preventing them from dominating the ocean substrate and indirectly increasing the diversity of other organisms that could colonize the substrate.

Many biologists and ecologists describe freshwater crayfish and their behaviors as that of a keystone species (Charlebois & Lamberti, 1996; Creed, 1994; Lodge et al., 1994; Parkyn et al., 1997; Whitledge & Rabeni, 1997). Crayfish are an intricate link between trophic levels making energy from detritus, such as coarse particulate organic material (CPOM), available to lower trophic levels (DiStefano, 1993; Momot et al., 1978). Crayfish are generalist feeders that consume fine and coarse detritus, living macrophytes, macroinvertebrates, and periphyton (Charlebois & Lamberti, 1996; Lodge et al., 1994; Whitledge & Rabeni, 1997). Different studies have shown crayfish to have

a significant, direct influence on algae and periphyton (Creed, 1994; Lodge, 1994), snails (Hanson et al., 1990; Lodge et al., 1994; Parkyn et al., 1997), and macroinvertebrates (Charlebois & Lamberti, 1996; Hanson et al., 1990). It has been documented that crayfish are able to affect macroinvertebrates, macrophytes, and the energy flow to other trophic levels, making them effective keystone species in streams (Chambers et al., 1990; Charlebois & Lamberti, 1996; Momot et al., 1978; Parkyn et al. 1997; Whitledge & Rabeni, 1997).

Allochthonous Material

Streams rely on large inputs of allochthonous (originating outside the system) organic material, including large amounts of leaf litter, as the major energy source for the entire community (Minshall, 1967). In-stream leaf litter begins as CPOM but through natural processes it is effectively processed into fine particulate organic material (FPOM) and made available to other smaller macroinvertebrates (Baer et al., 2001; Charlebois & Lamberti, 1996; Parkyn et al., 1997). Crayfish assist in energy transfer by processing the leaf litter and other detritus (Parkyn et al., 1997).

Feeding Habits

Freshwater crayfish are often more active at night, and seek refuge during daylight hours. As a result, feeding occurs most frequently at night, with one study showing significantly lower numbers of empty stomachs at night (Whitledge & Rabeni, 1996). Crayfish are more carnivorous while younger and become more effective detritivores as they become larger. This idea is supported by Whitledge & Rabeni

(1996) who reported that 35-50% of young of the year (YOY) crayfish stomachs contained animal matter, while only 20% of adult *Orconectes luteus* stomachs contained animal matter. A significant portion of the crayfish diet is comprised of vegetation and plant detritus (Chambers et al., 1990; Whitledge & Rabeni, 1997). Creed (1994) suggests that large crayfish may consume 30% of their body weight in a 24-hour period. Parkyn et al. (1997) found obvious leaf processing after three weeks of freshwater crayfish *(Paranephrops planifrons)* exposure to leaf packs and complete processing in channels with high densities of crayfish.

Crayfish negatively affected macroinvertebrate communities, including taxa richness and biomass, when placed in controlled enclosures (Chambers et al., 1990; Charlebois & Lamberti, 1996; Parkyn et al., 1997). Some of the hypotheses for the decline in macroinvertebrate populations caused by crayfish include direct predation, dislodgment by foraging crayfish, and possible inhibition of colonization (Charlebois & Lamberti, 1996). Crayfish might also cause mobilization of sediment in streams by excavating, walking, foraging, or flipping of the telsons for swimming (Parkyn et al., 2000). The disturbance of sediment and small rocks in the substrate may alter or dislodge habitat for macroinvertebrates. Statzner et al. (2000) consider crayfish effective ecosystem engineers because they could change the physical habitat conditions at baseflow.

Life History

Crayfish are crustaceans of the order Decapoda. All crayfish east of the Rocky Mountains are classified in the family Cambaridae. Crayfish are the largest and

longest-living freshwater crustacean found in North America (Momot et al., 1978).

Crayfish inhabit a variety of environments. Lakes, streams, rivers, cave streams, swamps, ditch lines, ponds, and even underground burrows are all specific habitats in which crayfish might live. Crayfish are categorized as primary, secondary or tertiary burrowers. Primary burrowers live exclusively in underground burrows and usually only come out to feed at night. Primary burrowers can be found away from an apparent body of water. The primary burrowers burrow down to the water table. The secondary burrowers often have tunnels along the banks of a water source, directly connecting with the water. The tertiary burrowing crayfish make burrows in the substrate of the water source that they live in for short-term protection or aestivation. The morphology of a crayfish is often indicative of the habitat it lives in. A small, streamlined crayfish is best suited for lotic systems (flowing water), while large, bulky crayfish more often dig burrows or live in lentic systems (still water).

Limited research has been conducted to analyze the role crayfish have in stream ecosystems (Boyd & Page, 1978; DiStefano, 1993; Huryn & Wallace, 1987; Mitchell & Smock, 1991; Momot et al., 1978). There are approximately twenty life-history studies for crayfish species (DiStefano, 1993), so only generalized information is given for breeding parameters and life expectancy.

Young of the year crayfish hatch from eggs carried on the pleopods of female crayfish. Eggs often hatch from May through August (Smart, 1962). The YOY remain attached for the first two molts and then separate from the females and continue to molt numerous times during the first summer (Pflieger, 1996). There are no molts that occur during the winter months (Mitchell & Smock, 1991; Pflieger, 1996; Smart, 1962). A

life expectancy is one to three years for most crayfish (Pflieger, 1996; Rabeni, 1985; Smart, 1962).

Crayfish do not have a continuous growth pattern, but rely on molts for size increases (DiStefano, 1993). Growth is primarily achieved during spring and summer months, with the largest growth occurring in the May and June (Huryn & Wallace, 1987; Mitchell & Smock, 1991). Mature male crayfish are sexually dimorphic and molt twice per year. The first pair of pleopods of male crayfish are elongated and is the sexual organs or gonopods (Figure 1). Males molt from the sexually inactive form II stage into the sexually active form I stage before they are capable of mating. Form I characteristics include gonopods that are corneous and more sharply defined, while the form II gonopods are blunted and pliable at the apex (Figures 2a & 2b). The longer and sharpened corneous tips of the form I gonopods are necessary for the transfer of sperm to the annulus ventralis (Figure 3) of females during breeding (DiStefano, 1993). A molt from the non-reproductive form II stage to the sexually active form I often occurs during the summer and a molt converting back to the non-reproductive form II occurs the following spring (Weagle & Ozburn, 1972). Adult females molt once per year, after the young leave in early summer (Boyd & Page, 1978).

Study Animals

Tennessee has over 78 species of crayfish, at least 27 of them in the genus *Orconectes* (Williams & Bivens, 2001). *Orconectes* (*Procericambarus*) *durelli* (Bouchard & Bouchard) has a Tennessee range including the Highland Rim and

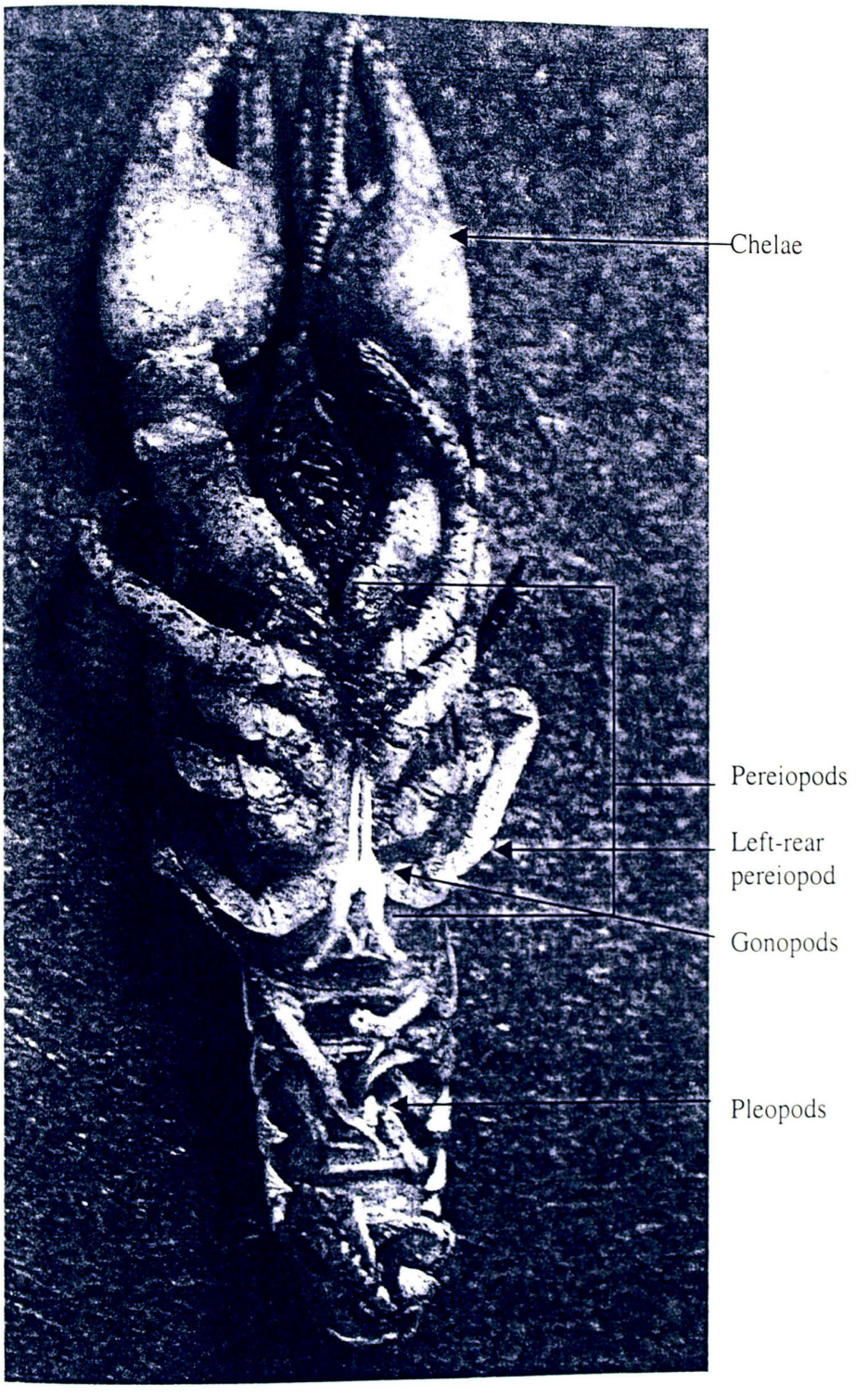

Figure 1. Ventral view of a form I *Orconectes placidus*.

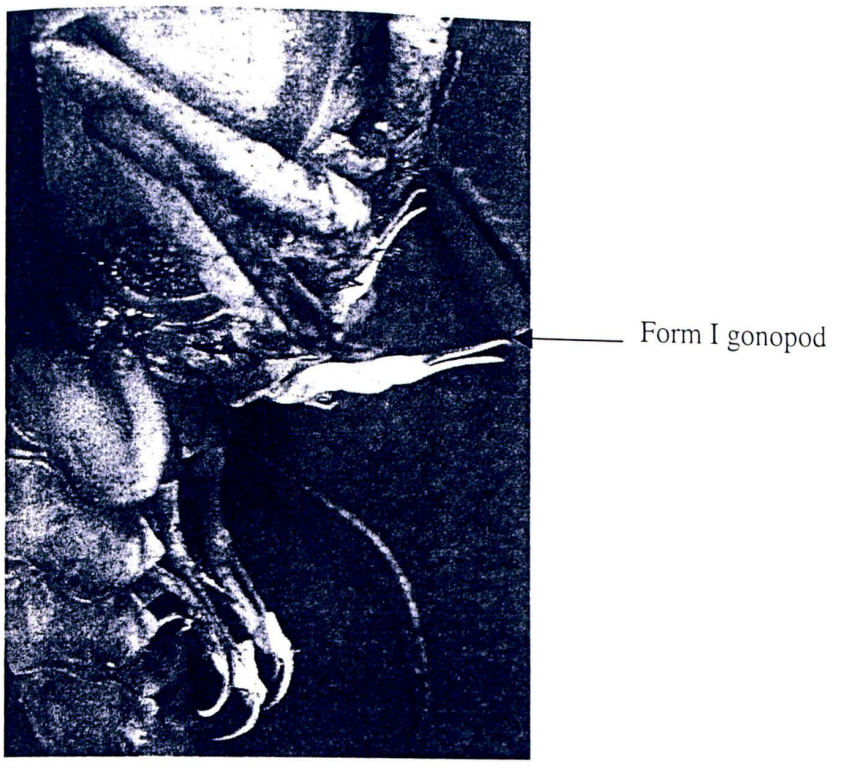

Figure 2a. Form I *Orconectes* male gonopod.

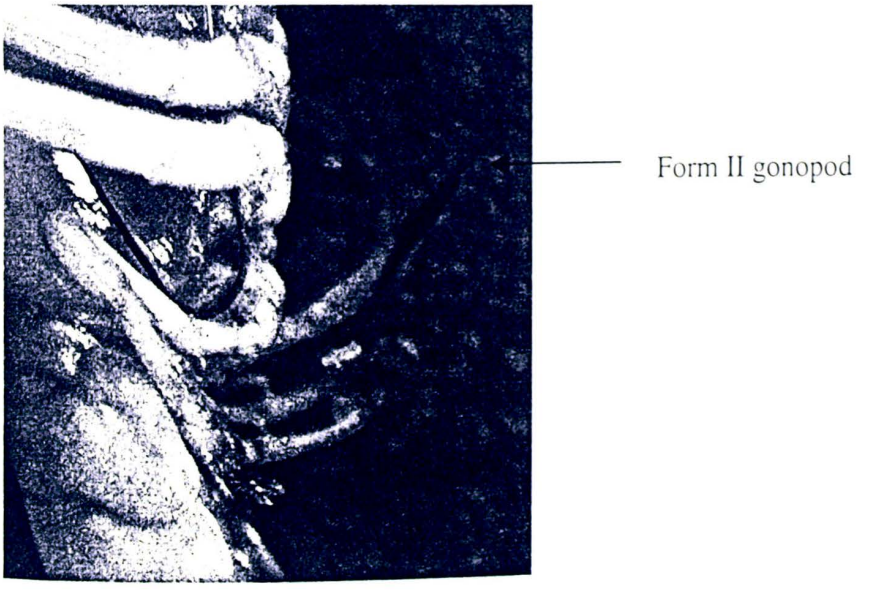

Figure 2b. Form II *Orconectes* male gonopod.

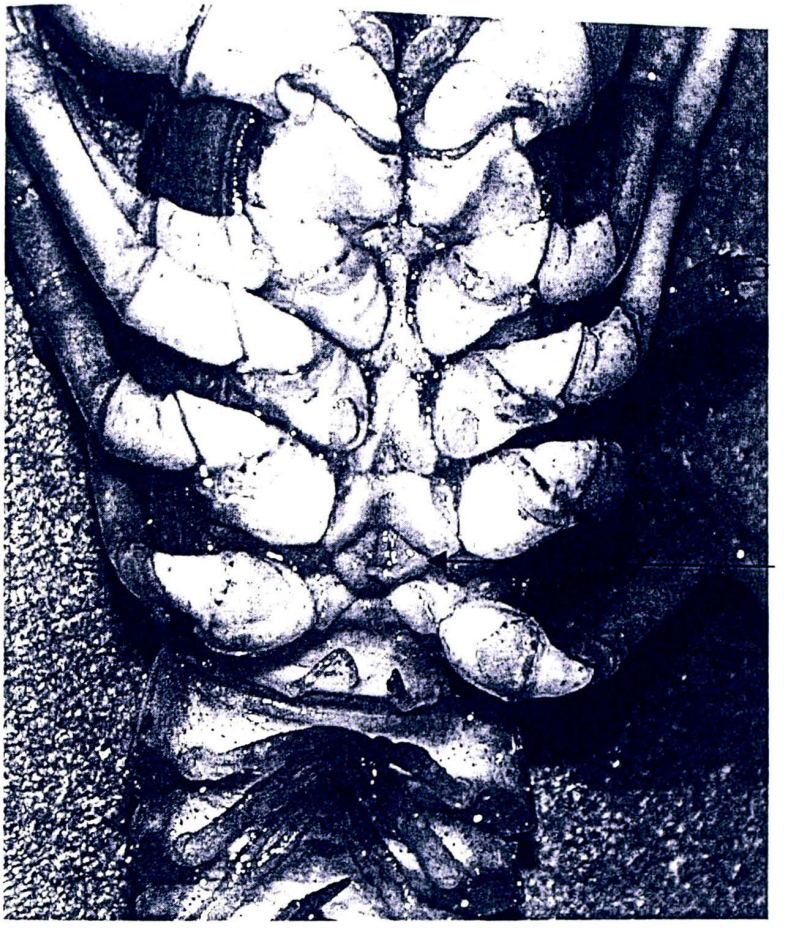

Figure 3. Ventral view of a female *Orconectes* crayfish.

Nashville Basin provinces. It is frequently found in small, gravel streams and often occurs in leaf litter, root masses, riffles, pools, shorelines, and under rocks (Williams & Bivens, 2001). *Orconectes durelli* often has a pale band running horizontally across the lateral surface of the cephalothorax (Williams & Bivens, 2001). *Orconectes (P.) placidus* (Hagen) has a Tennessee range including the Western edge of the Cumberland Plateau, Nashville Basin, and Highland Rim provinces, excluding Cypress Creek and other eastern tributaries of the Tennessee River (Williams & Bivens, 2001). *Orconectes placidus* frequently has a dactyl length greater than 2.5 times the mesial length of the margin of the palm (Figure 4) (Williams & Bivens, 2001).

Genetic Identification

Crayfish are of high ecological importance and are increasingly being studied by biologists. Understanding genetic variation within crayfish species is essential for conservation biology, systematics, and population genetics (Crandall, 1996). Identification of crayfish using morphological characteristics is sometimes difficult for many reasons. In most cases, form II males, females, and juvenile crayfish cannot be identified with keys alone (Pflieger, 1996). Only males that have molted to the sexually reproductive form can be reliably identified to species by slight differences in the gonopods and other details (Crandall & Fitzpatrick, 1996; Pflieger, 1996). Many of the characteristics used for identification are associated with the gonopods and chelae or "claws" of crayfish (Figure 4). Female and juvenile crayfish lack the decisive morphological properties to allow for accurate identification. Crayfish that lose chelae

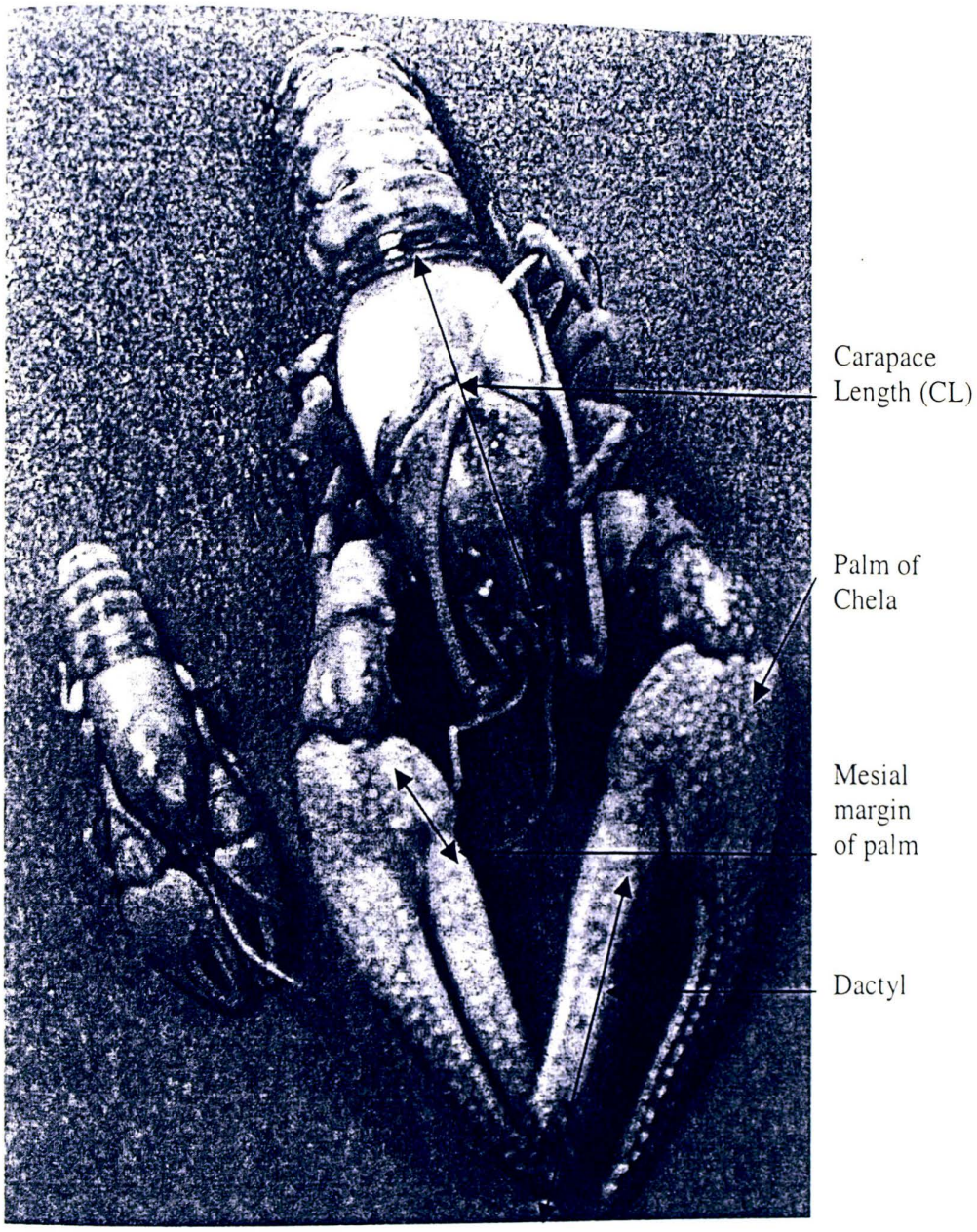

Figure 4. Dorsal view of *Orconectes durelli* (left) and *Orconectes placidus* (right) showing chela differences.

regenerate smaller and less characteristic chelae than the original. Colors and external morphologies are often variable within species and result in different species that may appear morphologically similar.

Crayfish are found worldwide, with the number of species approaching 450 (Pflieger, 1996). North America has more than 320 species of crayfish documented (Pflieger, 1996), with the potential for new species to be described (DiStefano, pers. comm, 2002). The Cumberland drainage hosts the largest diversity of crayfish species in the world, leaving most watersheds with numerous species of crayfish. There are few crayfish taxonomists, and this decreases the opportunity for biologists to verify species in their watershed. Genetic identification would supplement the field biologist's ability to describe species and their life history.

Genetic analysis using mtDNA has been successful in distinguishing species in other organisms (Routman, 1993; Shaffer & McKnight, 1996). Higher levels of genetic variation in crayfish may be obtained by restriction mapping of mtDNA, but such studies are lacking in crayfish (Crandall, 1996).

Study Area

Spring Creek in Robertson County, Tennessee was the study site for this experiment. Spring Creek is a third-order stream found in northern Middle Tennessee that flows south from Cedar Hill into Sulphur Fork Creek three to four hundred meters downstream from the study site. The lower-most reach of Spring Creek provided easy access, deep pools, good solar panel placement, and *Orconectes* crayfish. This site is at the Anderson Ranch on the east bank of the stream.

Spring Creek is located in the Interior Plateau, which is a level III ecoregion described by the Environmental Protection Agency (EPA) (Griffith et al., 1997). The Interior Plateau has an impressively diverse fish, mussel, and crayfish fauna attributed to the complex geology and numerous semi-independent drainage systems (Etnier & Starnes, 1993; Griffith et al., 1997). The Western Pennyroyal Karst sub-ecoregion is a more specific hierarchical classification for the area encompassing Spring Creek. The Western Pennyroyal Karst sub-ecoregion covers 2,220 square kilometers within northern Middle Tennessee, with most of Robertson County falling within its boundaries (Griffith et al., 1997).

Soils described in this sub-ecoregion are mainly Pembroke, Crider, and Baxter. This sub-ecoregion is mostly comprised of agricultural facilities including tobacco, grain crops, and livestock farms. The Western Pennyroyal Karst sub-ecoregion is between 165 and 200 meters in elevation, receives mean annual precipitation around 125 centimeters, has a freeze-free period in Tennessee of about 200 days, and has potential natural vegetation of oak-hickory forests and bluestem prairie. The karst ecoregion has numerous sinkholes and shallow depressions that connect and form underground drainage systems resulting in few permanent surface streams (Griffith et al., 1997).

Goals and objectives

There have been few studies to understand the relationship between crayfish and macroinvertebrate biomass and taxa richness (Charlebois & Lamberti, 1996). The goals of this study were to obtain data to better understand the role of crayfish as keystone

species in an aquatic community, in particular, its role in leaf litter processing and macroinvertebrate community composition. Also, to genetically distinguish the two species of *Orconectes* crayfish in Spring Creek. The specific objectives of this study were:

1) To construct a quadrat sampler and use it to estimate crayfish densities in Spring Creek.
2) To construct a multi-channeled apparatus approximating stream environments for crayfish.
3) To conduct two crayfish enclosure/exclosure experiments to determine the role of crayfish in leaf litter breakdown and composition of stream macroinvertebrates.
4) To determine whether the 16S ribosomal RNA gene from mtDNA of *O. durelli* and *O. placidus* can be differentiated genetically using restriction enzymes.

These goals and objectives were met by testing the following null hypotheses: a) There is no difference between leaf litter breakdown between channels with crayfish and channels with out crayfish; b) There is no difference between macroinvertebrate composition in channels with crayfish and channels with out crayfish; c) Restriction of the 16S ribosomal RNA gene from mtDNA would not produce different banding patterns between *O. placidus* and *O. durelli.*

CHAPTER II

METHODS AND MATERIALS

Crayfish Collection and Identification

The Form I sexual stage of male crayfish was initially used for identification. Females that were found with the males and morphologically similar were assumed to be the same species (G. Schuster pers. comm.). Once morphological differences were known, all individuals collected could be identified. *Orconectes placidus* and *Orconectes durelli* were the two species of *Orconectes* collected and used for this study.

Crayfish Preparation for Enclosure/Exclosure Experiments

Once collected, crayfish were stored in containers without food for one week prior to introduction to the density manipulation experiment. The crayfish were collected by seining, hand picking, or during density experiments. All crayfish collected for use in these experiments were weighed and carapace length (CL) measured before and after the experiments were conducted. Crayfish were weighed to the nearest 0.1 gram (g) using a Scout II portable scale (OHAUS, Florham Park, NJ). Carapace length is measured from the tip of the rostrum to the base of the cephalothorax, at the midline (Figure 4). Calipers were used to measure CL to the nearest 0.1 mm (Manostat, Switzerland). Only crayfish with a CL of 20-25 mm were used in this experiment. Crayfish were uniquely marked on the telson fin by snipping out pieces at different locations such that they could be identified later. If a crayfish died during the

experiment, a replacement crayfish was measured, weighed and marked as the original, and the time they were introduced to the apparatus was noted. Marked crayfish could be measured and weighed after the experiment and the results compared to pre-experiment measurements. Any crayfish that perished, escaped from the apparatus, or became gravid during the experiment were replaced as soon as possible. At the end of the experiment, the crayfish were removed from the apparatus and frozen for later studies.

Crayfish Enclosure/Exclosure Design

An apparatus was designed and built to contain nine channel enclosures that served as artificial streams. The apparatus was built on a 121.9 centimeter (cm) by 243.8 cm piece of 1.9 cm thick plywood. The individual channels were 15.2 cm deep and 10.6 cm wide by 198.6 cm long with an area of 0.21 m^2. The apparatus was covered with a wooden frame wrapped with 0.635 cm vinyl mesh to prevent escape by and predation of crayfish. The channels were lined with clear plastic to avoid any possible toxic affects from the treated lumber used in constructing the channels.

During daylight hours, a SWT-50 solar-powered water pump (Solar Water Technologies, Inc., Portsmouth, VA. USA) pumped water from Spring Creek into a 757-liter reservoir. Water from the reservoir was then metered into the channels at a constant flow.

This apparatus held four replicates of two different crayfish treatments, giving a total of eight channels for each experiment. The center channel was not used. The two treatments for these experiments included a crayfish exclosure treatment and a crayfish

enclosure treatment. The exclosure treatment channel contained no crayfish and the enclosure treatment channels contained three crayfish (approximately 15 crayfish/m^2). Each channel was randomly assigned a crayfish treatment before the experiment using a random numbers table. Male and female crayfish were grouped according to sex to prevent mating during the experiments.

Density estimates for crayfish in run and pool areas of Spring Creek were measured using a quadrat sampler (DiStefano, 2000). Densities were measured at various times from Fall 2001 through Spring 2002. DiStefano (2000) describes the quadrat sampling method as effective method of making density estimates because of its known area, high-enclosed sides and bottom flaps minimizing escapement. Also, it could be used in turbid water since visibility was not required. Runs and pools were sampled because they have slower flow and produce higher densities of crayfish than higher flow riffle areas (DiStefano, 2000). Crayfish densities in two Missouri streams averaged 21.64 crayfish/m^2 and 30.6 crayfish/m^2 in run and pool microhabitats, respectively (DiStefano, 2000). Based on studies in New Zealand streams, Parkyn et al. (1997) used 16-22 crayfish/m^2 in a study similar to this one. Based on previous density reports (DiStefano, 2000; Parkyn et al., 1997), this study used a density of 15 crayfish/m^2, resulting in three crayfish placed in each channel designated as a high-density treatment.

Leaf Litter Experiment

Leaves from a Sugar Maple (*Acer saccharum*) were collected on November 9, 2001. The leaves were air dried and stored until the experiments began.

Six leaf packets per channel were produced and attached to rough ceramic tiles in each of the eight enclosures. The ceramic tiles were 10 cm X 9.3 cm X 1.3 cm. Each leaf packet was between 0.9 g and 1.1 g in weight. The leaf packets were leached in water seven days before initiation of the experiment. Only leaf packs and ceramic tiles were added to the channels for this experiment.

The leaf packets were placed in the apparatus on February 26, 2002 and crayfish were introduced to the enclosure channels on March 5, 2002. A multiparameter sonde unit (YSI 600 XLM, Yellow Springs Instruments, Yellow Springs, OH) was deployed March 12, 2002 and removed April 2, 2002. The YSI unit collected basic water quality parameters in the unused middle channel of the apparatus. The leaves and crayfish were removed April 2, 2002. The leaves were placed in individual bags and stored in a 4°C cooler until drying began. The leaf packets were dried April 4, 2002 at 55°C for twenty-six hours. Dry leaf weights before and after the experiment were compared to quantify the role of *Orconectes* in leaf litter breakdown.

Macroinvertebrate Experiment

April 30, 2002, one week prior to the second experiment, gravel substrate was added to the apparatus directly from Spring Creek, and left to stabilize before introduction of crayfish. An attempt was made to evenly colonize each channel with gravel and macroinvertebrates before the experiment began. In addition to the macroinvertebrates added with the gravel, a kick-net sample was taken for each channel and the resulting macroinvertebrates were added to each channel. Macroinvertebrate densities were determined at the beginning and end of the enclosure/exclosure study by

taking three 0.0064 m^2 core type samples from each channel (Parkyn et al., 1997). All the macroinvertebrates were identified to family level, except oligochaetes, aquatic mites, and copepods, which were not identified further.

Samples of macroinvertebrates were taken prior to the addition of crayfish, on May 7, 2002. Crayfish were added to channels one, four, seven, and eight. The YSI sonde was deployed on May 8. The sonde unit was placed in the unused middle channel, which also had a gravel substrate to approximate conditions in the other channel. The crayfish were removed and the post-experiment macroinvertebrate samples were collected on May 28, 2002. The YSI sonde was removed and the water quality information downloaded to a computer.

Statistical Analysis of Experiments I and II

A one-way ANOVA was run on the parametric data collected from the leaf litter and the macroinvertebrate experiments. The data from the weight-gain of crayfish for experiments I and II were non-parametric, and a Wilcoxon test was run. All analyses were done using JMP-IN 4 (SAS, 2000), with an alpha level of 0.05.

DNA Isolation

At the conclusion of experiments I, the left-rear pereiopod (Figure 1) was removed for a tissue sample from the twelve remaining crayfish. Tissue samples were taken from another thirteen crayfish that were in captivity; twelve of which were later used in experiment II. Tissue samples were also taken from four *O. durelli* for comparison with *O. placidus*. The tissue samples were immediately frozen at –80°C.

DNA was isolated using the AquaPure Genomic DNA Kit (BIO RAD, Hercules, CA).

The frozen tissue samples were crushed to a powder using a mortar and pestle set. Genomic DNA Lysis solution (300 µl) was added to the powdered tissue. The lysis solution containing the crayfish DNA (300 µl) was transferred into a clean 1.5 ml microfuge tube and placed on ice. The samples were mixed thoroughly by inverting the tube 25 times and then incubated at 55°C for 2-3 hours (periodically inverting during incubation). RNA was removed by adding 1.5 µl of RNase Solution (4 mg/ml) and incubating at 65°C for 45 minutes.

The samples were cooled to room temperature and 100 µl of a protein precipitation solution was added to each cell lysate. The samples were vortexed at high speed for 20 seconds to insure uniform mixture. The precipitated protein was removed by centrifugation at 13,000-16,000 X g for 3 minutes. The supernatant containing the DNA was poured into a clean 1.5 ml microfuge tube containing 300 µl of 100% isopropanol (2-propanol). The tube was inverted gently 50 times and then centrifuged at 13,000-16,000 X g for 1 minute. The DNA produced a small white pellet. The supernatant was discarded and the tube containing the DNA pellet was briefly drained on a clean absorbent paper. The pellet was washed with 300 µl of 70% isopropanol by inverting the tube several times. The DNA was collected by centrifugation at 13,000-16,000 X g for 1 minute and the supernatant was carefully poured off. The tube with the DNA pellet was then inverted on an absorbent paper and left to air dry for 15 minutes. The DNA was hydrated in 100 µl of DNA Hydration Solution by incubating

for 5 minutes at 65°C. The solution was then vortexed for 5 seconds and then stored at 4°C until used.

Agarose Gel Electrophoresis

Agarose gel electrophoresis was performed to verify DNA isolation. A one percent (1%) agarose gel was used. The gel was run in 1X TBE buffer and stained with ethidium bromide. DNA was mixed with loading dye and added to the gel. The first and the last lanes were loaded with Lambda HindIII marker. The samples were electrophoresed at 100 volts until the samples migrated down approximately two-thirds of the gel. The DNA was placed on an ultraviolet transilluminator to be visualized. Any samples void of DNA proceeded through the DNA isolation steps and re-tested until DNA was secured.

Polymerase Chain Reaction (PCR)

Two microliters of the extracted DNA was amplified using the AccuPrime Super Mix II kit (Invitrogen, Carlsbad, CA). Amplification of the 16S ribosomal RNA gene from mtDNA required primers 1471 (5'-CCTGTTTANCAAAAACAT-3') and 1472 (5'-AGATAGAAACCAACCTGG-3') (Crandall & Fitzpatrick, 1996). The 25 µl PCR reaction contained 12.5 µl of AccuPrime Supermix, 0.5 µl of 1471 primer, 0.5 µl of 1472 primer, 5 µl of template DNA, and 6.5 µl of DNase-free water. The contents were mixed in a PCR tube and placed in the thermocycler at 95°C for 3 minutes for initial denaturation. The solution went through 30 cycles of amplification using the

following parameters: 95°C denaturation for 1 minute, 42°C annealing for 1 minute, and 72°C extension for 1.5 minutes (Crandall & Fitzpatrick, 1996). There was an additional extension of seven minutes at 72°C. The products were held at 4°C in the thermocycler until analyzed.

Agarose gel electrophoreses was performed, as described above, to verify successful completion of PCR. Any samples that were not amplified were discarded, and new reactions were run until they were successfully amplified.

Digestion with Restriction Enzymes

The amplified 16S ribosomal RNA gene fragment was digested with TaqI (Invitrogen, Carlsbad, CA) or VspI (Promega, Madison, WI) restriction enzymes (Perry et al., 2001). The restricted DNA fragments were analyzed by agarose gel electrophoresis using a two percent (2%) gel in 1X TBE buffer and stained with ethidium bromide. Photographs were taken of the resulting banding patterns.

CHAPTER III

RESULTS

Crayfish

Crayfish of the genus *Orconectes* were collected February and April of 2002 from Spring Creek and identified as *Orconectes placidus* and *O. durelli*. Dr. Guenter Schuster, Professor of Biology, Eastern Kentucky University (EKU) confirmed species identification. No other *Orconectes* species were collected from Spring Creek during these experiments. Only *O. placidus* was used for the leaf packet and macroinvertebrate experiments, while *O. placidus* and *O. durelli* were both used in the genetic experiment.

Density estimates were taken on two separate days from Spring Creek. Six samples were taken on December 11, 2001 and four samples were taken on December 18, 2001. The six quadrat samples taken on December 11 captured 19 crayfish, resulting in an estimate of 3.2 crayfish per square meter. The sex ratio was 47% female (9 individuals) and 53% males (10 individuals). There were 15 crayfish collected in four quadrat samples on December 18 resulting in an estimate of 3.8 crayfish per square meter. The sex ratio for this day was 73% female (11 individuals) and 27% male (4 individuals). The combined density estimate (10 samples and 34 crayfish) for Spring Creek was 3.4 crayfish per square meter with a sex ratio of 59% females and 41% males.

Leaf Packets

Crayfish in experiment I had a mean increase in CL of 7% (n = 12) and a mean weight-gain (wet- weights) of 13% (n = 12) (Table 1). Four crayfish died and one crayfish escaped during experiment I; all five were replaced. There was no significant difference in the increase of CL (P = 0.1168) or weight-gain (P = 0.877) among the crayfish in experiment I and experiment II (Figures 5 & 6).

Leaf packets in the exclosure channels (n = 6/channel) had a mean cumulative weight of 6.05 g at the onset. Those in the enclosure channels (n = 6/channel) had an average cumulative weight of 5.90 g. The crayfish enclosure channels had a mean leaf litter loss of 1.98 g/channel and the crayfish exclosure channels had a mean leaf litter loss of 1.18 g/channel (Table 2). The loss in exclosure channels is assumed to be natural loss due to leaching and microbial decay, which is experienced in both treatments. The average increase in leaf litter loss (0.8 g/channel) found in enclosure channels is assumed to be crayfish induced. Based on these assumptions, exposure to crayfish accounted for 40% of the weight loss of the leaf packets. The average loss in leaf weight for enclosure channels was significantly different than exclosure channels (P = 0.0055) (Figure 7).

Macroinvertebrates

Crayfish in experiment II had a mean increase in CL of 3% (n = 11) and a mean weight-gain (wet-weight) of 15% (n = 11) (Table 3). Seven crayfish died and were replaced during experiment II. An eighth crayfish, number 48, was found dead and partially devoured at the termination of experiment II. The CL and weight of

Table 1. Carapace length (mm) and weight (g) of crayfish as recorded before and after the leaf packet experiment (Exp I).

Individual	Channel #	CL-before	CL-after	WT-before	WT-after
7	1	21.55	24.50	2.50	3.20
15	1	22.20	24.20	3.00	3.50
18	1	21.75	21.70	2.70	2.60
2	4	21.30	25.00	2.60	3.40
4	4	22.20	24.00	2.80	3.30
3-r(12)	4	22.70	22.40	3.40	3.30
17-r(20)	7	23.40	26.60	3.40	4.40
16-r(10)	7	23.85	23.70	3.40	3.60
23	7	21.65	23.70	2.30	2.90
8	8	23.10	24.00	2.90	2.90
14-r(27)	8	23.05	26.00	3.30	4.10
22	8	24.10	25.80	2.90	3.60
Total	-	270.85	291.60	35.20	40.80
Percent increase		-	7.12%	-	13.73%

WT – Weight
r - replacement crayfish
(#) - number of days included in experiment

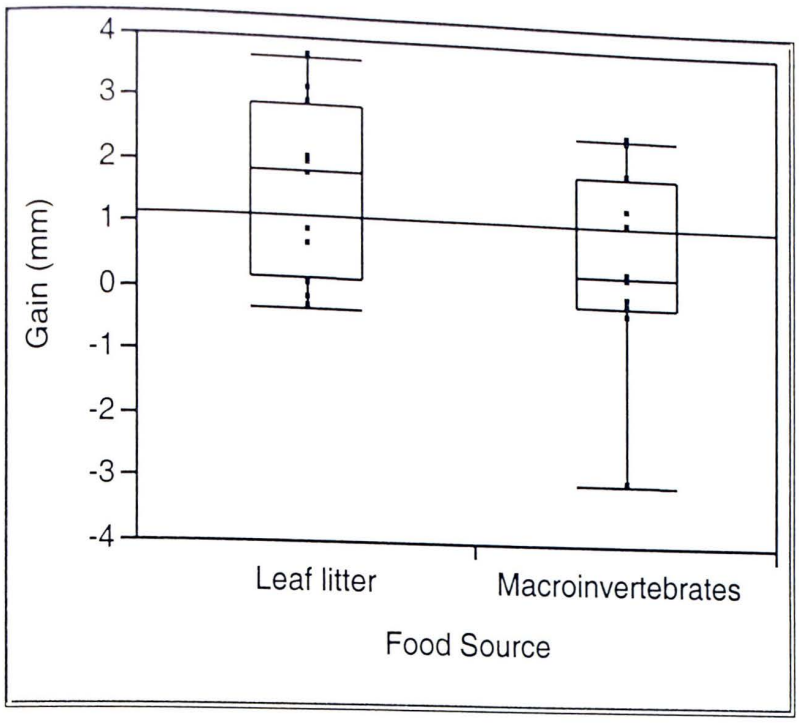

Figure 5. Carapace length (mm) of crayfish exposed to leaf litter and crayfish exposed to macroinvertebrates. Box plot showing a non-significant difference (P = 0.1168).

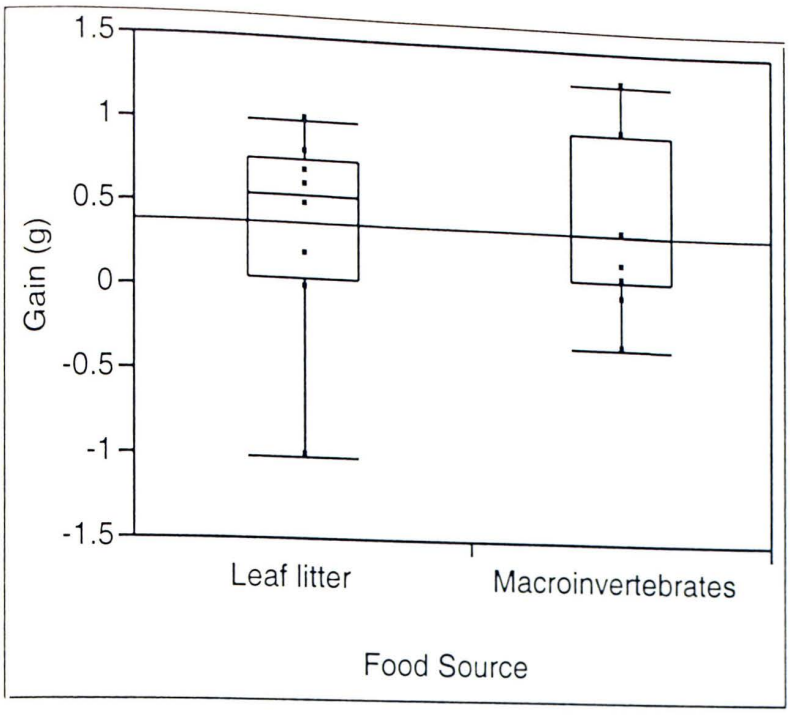

Figure 6. Weight gain (g) of crayfish exposed to leaf litter and crayfish exposed to macroinvertebrates. Box plot showing a non-significant difference (P = 0.877).

Table 2. Weights (g) of leaf packets before experiment, after experiment and weight lost during the leaf packet experiment (Exp I).

	Crayfish Exclosure Channels				Crayfish Enclosure Channels			
	C-2	C-3	C-6	C-9	C-1	C-4	C-7	C-8
LP-1	1.00	1.00	1.00	1.00	1.00	0.90	1.00	1.00
LP-2	1.00	1.10	1.00	1.00	1.00	1.00	0.90	1.00
LP-3	1.00	1.10	1.00	1.00	0.90	1.00	1.10	1.00
LP-4	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
LP-5	1.10	1.00	1.00	1.00	0.90	0.90	1.00	1.00
LP-6	0.90	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Total	6.00	6.20	6.00	6.00	5.80	5.80	6.00	6.00
LP-1A	0.80	0.80	0.90	0.70	0.70	0.50	0.50	0.70
LP-2A	0.80	0.80	0.80	0.70	0.70	0.60	0.70	0.60
LP-3A	0.80	1.00	0.70	0.70	0.50	0.50	0.80	0.80
LP-4A	0.90	0.80	0.80	0.90	0.70	0.70	0.70	0.70
LP-5A	0.90	0.80	0.90	0.80	0.60	0.60	0.60	0.70
LP-6A	0.80	0.70	1.00	0.70	0.70	0.60	0.70	0.80
Total	5.00	4.90	5.10	4.50	3.90	3.50	4.00	4.30
LP-1 loss	0.20	0.20	0.10	0.30	0.30	0.40	0.50	0.30
LP-2 loss	0.20	0.30	0.20	0.30	0.30	0.40	0.20	0.40
LP-3 loss	0.20	0.10	0.30	0.30	0.40	0.50	0.30	0.20
LP-4 loss	0.10	0.20	0.20	0.10	0.30	0.30	0.30	0.30
LP-5 loss	0.20	0.20	0.10	0.20	0.30	0.30	0.40	0.30
LP-6 loss	0.10	0.30	0.00	0.30	0.30	0.40	0.30	0.20
Total loss	1.00	1.30	0.90	1.50	1.90	2.30	2.00	1.70
% loss	16.66	20.97	15.00	25.00	32.75	39.66	30.00	28.33

C – channel
LP - leaf packet
A - weight after experiment

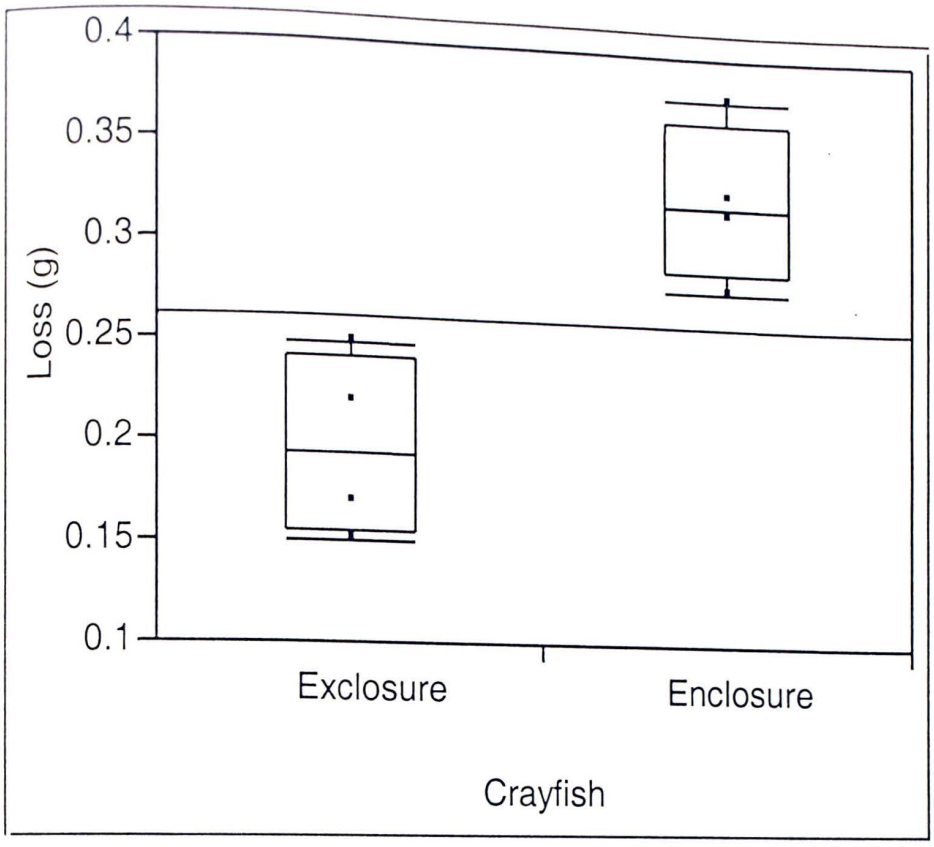

Figure 7. Leaf loss (g) between exclosure channels and enclosure channels. Box plots showing significant difference (P = 0.0055).

Table 3. Carapace length (mm) and weight (g) of crayfish as recorded before and after the macroinvertebrate experiment (Exp II).

Individual	Channel #	CL-before	CL-after	WT-before	WT-after
44-r(20)	1	22.80	25.30	2.50	3.50
52-r(19)	1	22.60	19.60	2.50	2.90
50-r(20)	1	23.50	26.10	2.50	3.80
31-r(13)	4	25.00	26.40	5.30	5.30
37	4	24.00	26.00	2.80	3.80
40	4	23.40	23.30	2.60	2.80
53-r(19)	7	20.00	21.20	1.80	1.90
45	7	23.10	23.50	3.00	2.70
48*-r(14)	7	21.30	Dead	1.80	Dead
27-r(20)	8	25.10	25.10	3.40	3.80
46	8	21.40	22.70	2.00	3.00
49	8	21.90	21.60	2.20	2.40
Total	-	252.80	260.80	30.60	35.90
Percent increase		-	3.07%	-	14.76%

WT – Weight
r - replacement crayfish
(#) - number of days included in experiment
* - crayfish 48 was not included in calculations

crayfish 48 were not measured or used in the analysis (Table 3).

The percent of macroinvertebrate change was analyzed between enclosure and exclosure channels (Table 4). The data from experiment II showed no significant difference ($P = 0.1481$) in the macroinvertebrate populations between enclosure and exclosure channels (Figure 8). The presence of crayfish in channels caused no significant difference in the percent decline of Pleuroceridae, Oligochaetes, or Ephemeroptera. There was also no significant difference in copepod colonization in enclosure channels versus exclosure channels. The remaining taxa did not occur in channels frequently enough to be statistically analyzed.

Total macroinvetebrate abundances collected in samples for all eight channels decreased from the start of the experiment (331 individuals) to the conclusion of the experiment (147 individuals). Samples collected prior to the beginning of experiment II contained 19 taxa and samples taken at the conclusion of the experiment contained 14 taxa. There was no significant change in taxa richness caused by the presence of crayfish in channels. The before and after samples were both dominated by Pleuroceridae (gilled snails) with 225 individuals (68%) and 110 individuals (75%), respectively. Oligochaetes were the next most abundant taxa found in the before and after samples with 55 individuals (17%) and 18 individuals (12%), respectively (Table 5). All channels at the end of the experiment contained copepods (83 individuals), which were absent from the before samples. Analyses did not include copepods as macroinvertebrates because they colonized the channels after the experiment began (Tables A-1 & A-2).

Table 4. The percent loss of macroinvertebrates in all eight channels from experiment II (Exp II).

	Crayfish Enclosure				Crayfish Exclosure			
Channel number	1	4	7	8	2	3	6	9
Before Average	49 (16.3)	31 (10.3)	33 (11)	24 (8)	36 (12)	36 (12)	17 (5.7)	105 (35)
After Average	18 (6)	11 (3.7)	16 (5.3)	6 (2)	17 (5.7)	23 (7.7)	20 (6.7)	36 (12)
Percent loss	62.27	64.52	51.52	75.00	52.78	36.71	0.00	15.71

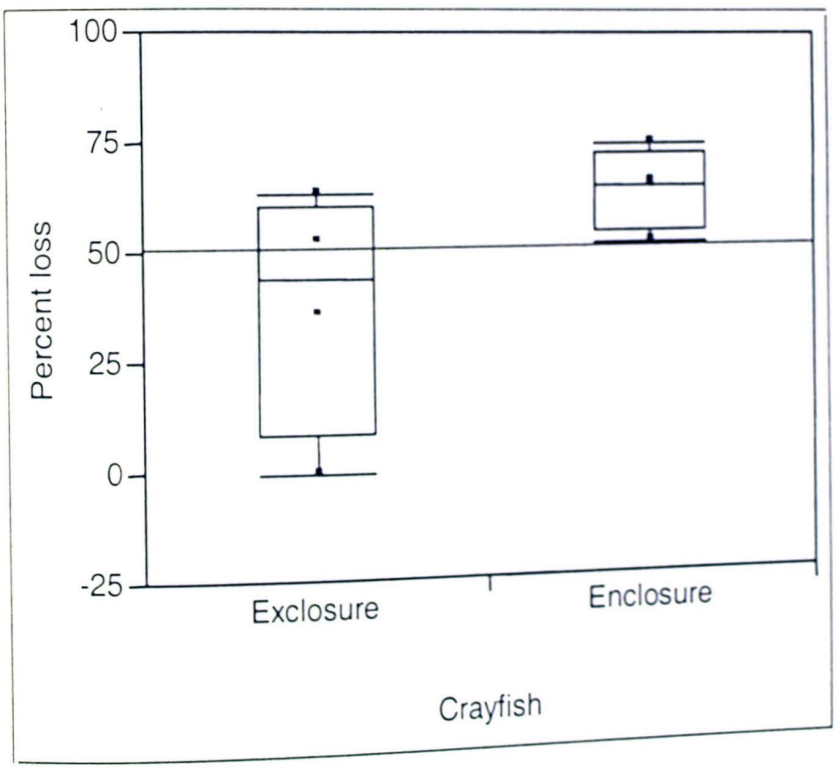

Figure 8. Percent loss of macroinvertebrates between exclosure and enclosure channels. Box plot showing a non-significant difference (P = 0.1481).

Table 5. Summary of macroinvertebrate data before and after the course of experiment II.

	Before	After
Total individuals in all 24 samples	331	147
Average macroinvertebrates collected/channel	41.38	18.38
Range of macroinvertebrates collected/channel	17-105	6-36
Estimated density of macroinvertebrates /m^2	2155	957
Number of taxa found/sample	19	14
Total Pleuroceridae in all samples	225	110
Percent of Pleuroceridae	67.98	74.85
Total Oligochaetes in all samples	55	18
Percent of Oligochaetes	16.62	12.24

Genetic Analysis

All crayfish samples had a 520 base pair fragment amplified. The TaqI restriction enzyme gave obviously different banding patterns between the two species (Figure 9). *Orconectes placidus* produced three bands of DNA and *O. durelli* produced two bands, when restricted with TaqI. Figure 9 is a photograph representing the banding patterns typical of DNA fragments from *O. placidus* and *O. durelli* that are not digested with restriction enzymes, digested with TaqI restriction enzyme, and digested with VspI restriction enzyme. The banding patterns resulting from VspI were not distinguishable by visual inspection (Figure 9).

Water Parameters

Water parameters were logged every thirty minutes during experiment I totaling 1008 samples. The water parameter averages in channel five from March 12, 2002 until April 2, 2002 were as follows: water temperature = 11°C, dissolved oxygen saturation = 75%, and ph = 8.25. Water parameters for experiment II were logged every 15 minutes (2015 total samples) in channel five from May 8, 2002 until May 28, 2002. The averages for experiment II were as follows: water temperature = 17°C, dissolved oxygen saturation = 74%, and pH = 7.71 (Table 6).

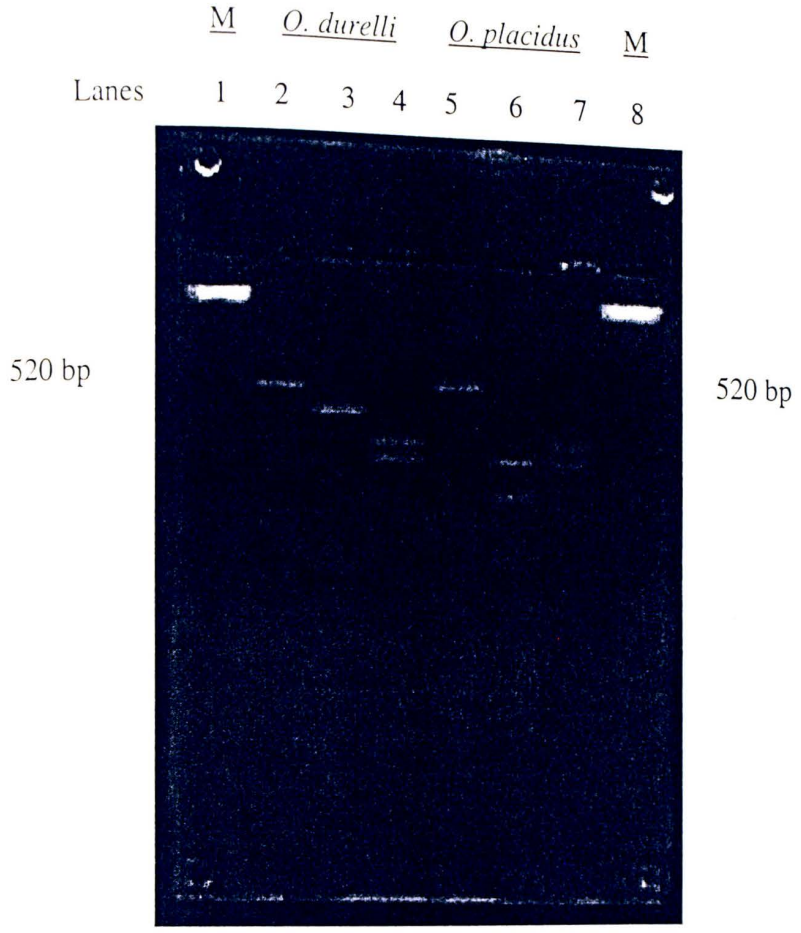

Figure 9. Agarose gel displaying digests of *Orconectes placidus* and *Orconectes durelli* with TaqI and VspI. Lanes 2 & 5 are not digested samples, Lanes 3 & 6 were digested with Taq I, Lanes 4 & 7 were digested with Vsp I, and Lanes 1 & 8 contained Lambda marker (M)

Table 6. Water quality parameters for experiment I and experiment II.

	Experiment I.			Experiment II		
	Temperature (C)	Dissolved Oxygen (%)	pH	Temperature (C)	Dissolved Oxygen (%)	pH
Minimum	-0.12	24.10	7.59	6.71	16.30	7.35
Maximum	25.53	110.30	9.74	30.19	148.10	8.31
Mean	10.92	74.71	8.25	17.21	74.28	7.71
Standard Deviation	4.75	16.46	0.44	4.30	28.75	0.24

CHAPTER IV

DISCUSSION

Design

Density estimates during one week of one season might be displaying extreme values instead of the streams normal values. Samples from both days were collected during the afternoon, and no attempts were made to sample after dark. Since some species of crayfish are known to feed more actively after dark (Whitledge & Rabeni, 1996), density estimates should include samples at that time. Daytime densities estimates of 3.4 crayfish/m^2 might not be representative of the true population densities in Spring Creek. Also, ten quadrat samples may not be sufficient to assign a reliable density estimate for Spring Creek. To obtain a more accurate density estimate, multiple samples during all seasons and samples during light and dark hours may be necessary.

The channels of the apparatus had an area of 0.21 m^2. A crayfish density of 5/m^2 would be necessary to include one crayfish per channel. Since a higher density estimate was desired, a density of 15/m^2 was used. Fifteen crayfish/m^2 is within the density range previously reported in runs and pools of streams and used in a study similar to this one (Parkyn et al., 1997; DiStefano, 2000). This density resulted in three crayfish per channel, used in this study.

Throughout this study, there appeared to be no toxic effect from the treated lumber. The plastic liner extended the entire length of the channels. The apparatus was large and heavy, but durable. The lid was covered with a plastic mesh with 0.635 cm

openings. Since the apparatus was not shadded, a sheet of foam insulation covered the lid to buffer against diurnal temperature fluctuation. The foam covered a large portion of the mesh surface and a likely prevented new macroinvertebrates from colonizing in the channels. The recruitment of new macroinvertebrates would have added additional and undesired factors to the experiment.

Placement of the solar-powered water-pump caused difficulty during the study. The pump was covered with a "sock" filter and placed in wooden frame box wrapped with a 0.635 cm plastic mesh and again with fine mesh screen (window screen), to protect the pump from debris. Silt was able to pass through the mesh and occasionally clogged the "sock" filter.

Leaf Litter Experiment

The data indicate that the *O. placidus* increased leaf processing in enclosure channels by 40%. These data suggest that *O. placidus* play an important role in processing of leaf litter and other detritus in Spring Creek. These results agreed with other research on leaf processing suggesting that crayfish primarily feed on detritus (Whitledge & Rabeni, 1996). Parkyn et al. (1997) showed a significant difference in leaf material processed in crayfish enclosure channels.

Leaf packets removed from the exclosure channels were covered by a film of periphyton. Leaf packets removed from the enclosure channel showed little or no sign of periphyton growth. Quantitative periphyton samples were not taken. Once the leaf packets were dried, the periphyton residue was removed from some random leaf packets and weighed. There was an insufficient amount of dried periphyton to influence the

weight of the leaf packets. As a result, periphyton residue was not removed from leaf packets before they were weighed. The absence of periphyton appeared to be related to the presence of crayfish. Crayfish may have impacted periphyton directly by consumption or indirectly by their activity.

Macroinvertebrate Experiment

Samples collected for this study produced small numbers of macroinvertebrates (331 before and 147 after). Crayfish did not significantly influence the abundance of macroinvertebrates in channel enclosure experiments. Total abundance was used because the family-level groups did not have enough individuals for statistical analyses.

The core samples taken from the channels may not have been representative of the overall macroinvertebrate community abundance. The sample area was small and resulted in a small number of macroinvertebrates to analyze. The small sample area was potentially biased toward smaller or slower macroinvertebrates. Larger or more mobile macroinvertebrates might have escaped the sample area. Any error in such a small sample size would have a significant impact. The small number of macroinvertebrates causes difficulty interpreting any trends that may have resulted from crayfish feeding activities.

There are several possible variables that may be partially responsible for this outcome. *Orconectes placidus* may not actively prey upon macroinvertebrates as intensively as documented in other crayfish. Parkyn et al. (1997) found no affect on Oligochaeta, Chironomidae, other Diptera, Elmidae, and Leptophlebiidae densities in a crayfish enclosure study, which partially supports my conclusions. During this

experiment the exclusion of leaf litter, a typical macroinvertebrate habitat and food resource, may have resulted in a loss in macroinvertebrate abundance obscuring any crayfish induced effects. Baer et al. (2001) found significant reduction in abundance of invertebrates when litter was excluded from a section of stream. Loss of some macroinvertebrates could have been due to emergence of adult insects, insufficient habitat, death, or any combination of these variables. The uncontrolled channel conditions appeared to have a substantial impact on macroinvertebrate abundances. Diurnal changes in temperature, pH, DO, and water flow could have individually or cumulatively been responsible for altering macroinvertebrate assemblages in the apparatus. The effects of these variables could have masked any potential effects the presence of crayfish would have caused.

Another possibility is that macroinvertebrate death could have provided food resources for the crayfish, thus crayfish would not have to expend energy preying upon the living organisms to obtain food. Lodge et al. (1994) suggested that nonsnail macroinvertebrates might be mobile enough to escape crayfish predation. This would suggest that many macroinvertebrates might be consumed only after death.

And finally, there may have been enough organic material in the channels for the crayfish to feed upon. The gravels that were placed in the channels were not cleaned and only large organic debris was removed. Large filamentous algae frequently bloomed in the reservoir during this experiment, flowed into the channels, and possibly provided a food source for the crayfish. Thus, the crayfish had food resources in the channels other than macroinvertebrates.

Crayfish

Some crayfish molted during experiment I and II and this must be the cause of any difference in mean increase of CL. Crustaceans must molt to grow (DiStefano, 1993), and since the molts appear to be dependant on environmental factors and not food resources, CL is not a good indicator of growth for this short-term study. The average weight increase between crayfish from experiment I (13.73%) and experiment II (14.76%) is potentially deceiving. Crayfish may retain more water after a molt, to help fill their new exoskeleton, thus any weight increase might not be completely due to the consumption of food resources available in the channels during the experiments.

Crayfish molts were not recorded during this experiment. Since crayfish can only increase CL by molting, the increases in CL recorded during experiments I and II (Tables 1 & 2) must be from a molt. Nine out of twelve crayfish from experiment I increased in weight and only one did so without molting. Nine of eleven crayfish from experiment II increased in weight, but four did so without molting. Since there was an overall increase in weight for crayfish from experiment II, this would suggest that there was some food resource in the channels that the crayfish were able to utilize. The energy could have come from dead macroinvertebrates, fine particulate organic material (FPOM) that was collected with the gravel, or periphyton that may have grown on the plastic lining and gravel of the channels.

Genetic Analysis

When the 16S ribosomal RNA gene from mtDNA was digested with TaqI, *O. placidus* and *O. durelli* both produced a small band of DNA of similar size suggesting a

conserved restriction site for both species. *Orconectes placidus* had an additional restriction site, producing two medium sized bands (Figure 9). Digestion with VspI produced similar banding patterns in both species. The similar banding pattern would suggest the recognition site for VspI is at or near the same location along the DNA fragment from either species. Analysis with agarose gel is not specific enough to distinguish fragments that differ by a small margin of bases. Polyacylamide gel could be used to test for subtle differences in banding patterns when *O. durelli* and *O. placidus* fragments were digested with VspI. Polyacrylamide gel has smaller pore spaces and can distinguish molecules that differ by one nucleotide, while agarose gel is not as specific.

Water Parameters

Diurnal patterns were observed in the data logged during experiment I (Figure 10). Temperature and percent dissolved oxygen (DO) displayed similar peaks and valleys for a 24-hour period, while pH appeared to respond opposite of DO. Samples 400-500 (ca. days 11, 12, & 13) decreased sharply in temperature and DO and increased in pH. The water in the channels had a layer of ice covering them. The ice was broken out, but the apparatus was without water flow until temperatures warmed above freezing. This resulted in a period of low DO and increased pH.

The data logged during experiment II displayed more distinct diurnal patterns. The data displayed expected diurnal patterns in temperature, DO, and pH corresponding with each other. Sunlight increased water temperature and provided energy for photosynthetic periphyton and algae in the stream and reservoir. The increase in

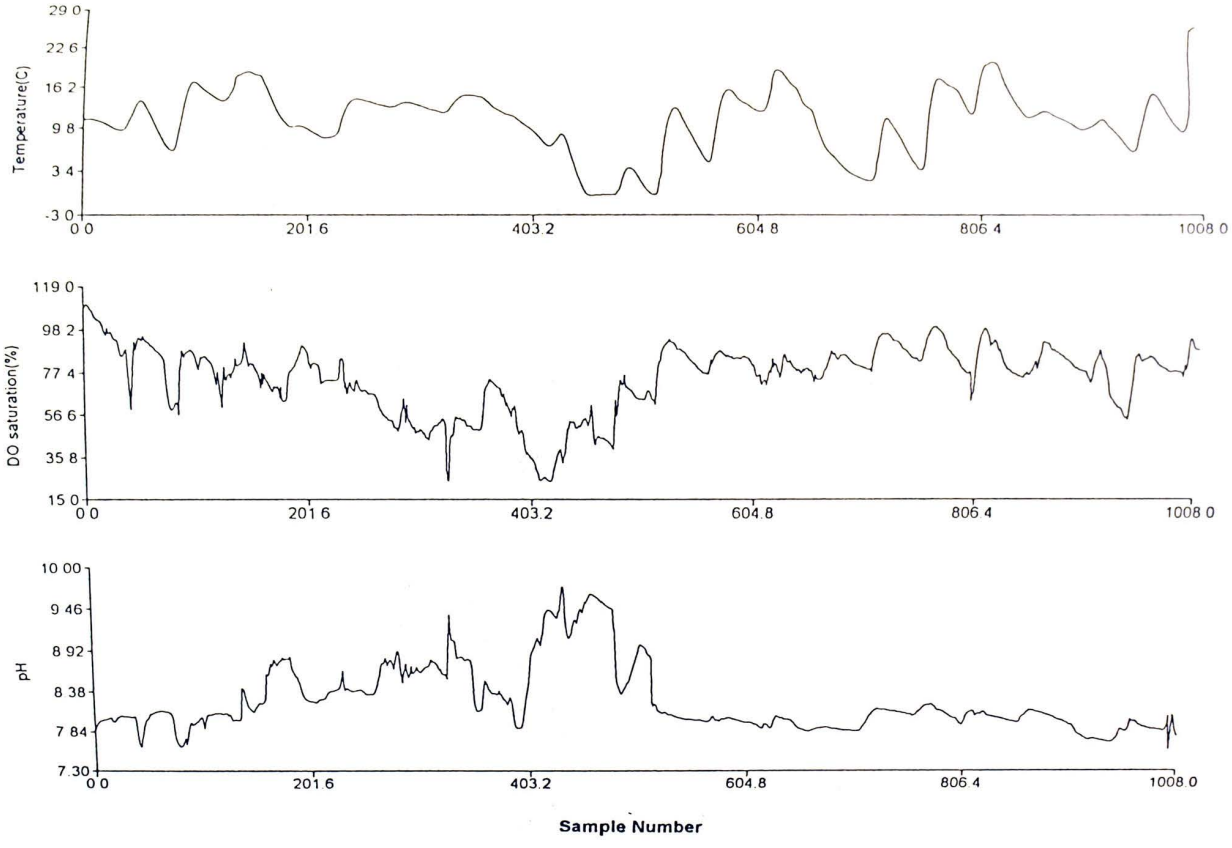

Figure 10. Continuous measurements of water quality parameters logged in apparatus from March 5 – April 2.

photosynthesis increased the amount of available oxygen in the water, thus increasing DO. The photosynthetic activity also used available CO_2 that resulted in the increase of pH in the water. Samples 930-1240 (ca. days 10, 11 & 12) had a fairly constant low reading of DO and pH (Figure 11). This represents cloudy and rainy days with little or no water being pumped into the channels. During experiment II the water was, on average, 6.28°C warmer than the water in experiment I. This increased temperature could have impacted the macroinvertebrates survival in the apparatus or influences the behavior of the crayfish.

Conclusions

The following observations were made: a) Leaf litter processing was significantly increased in crayfish enclosure channels; b) Macroinvertebrate abundances were not significantly impacted by the presence of *O. placidus* in these experiments. Because of uncontrolled variables confounding the study, these results are inconclusive; c) Digesting the 16S ribosomal RNA gene of mitochondrial DNA with TaqI is an effective way to identify *O. placidus* and *O. durelli*. This study has provided some baseline data on the impact that *O. placidus* has on leaf litter and macroinvertebrates in enclosure channels. The 16S ribosomal RNA gene region is A+T rich, and restriction enzymes that are specific for A+T regions could also be effective in producing distinguishable banding patterns among other *Orconectes* species. *Orconectes placidus* may serve as a keystone species in Spring Creek by processing detritus and making detrital energy available to other trophic levels.

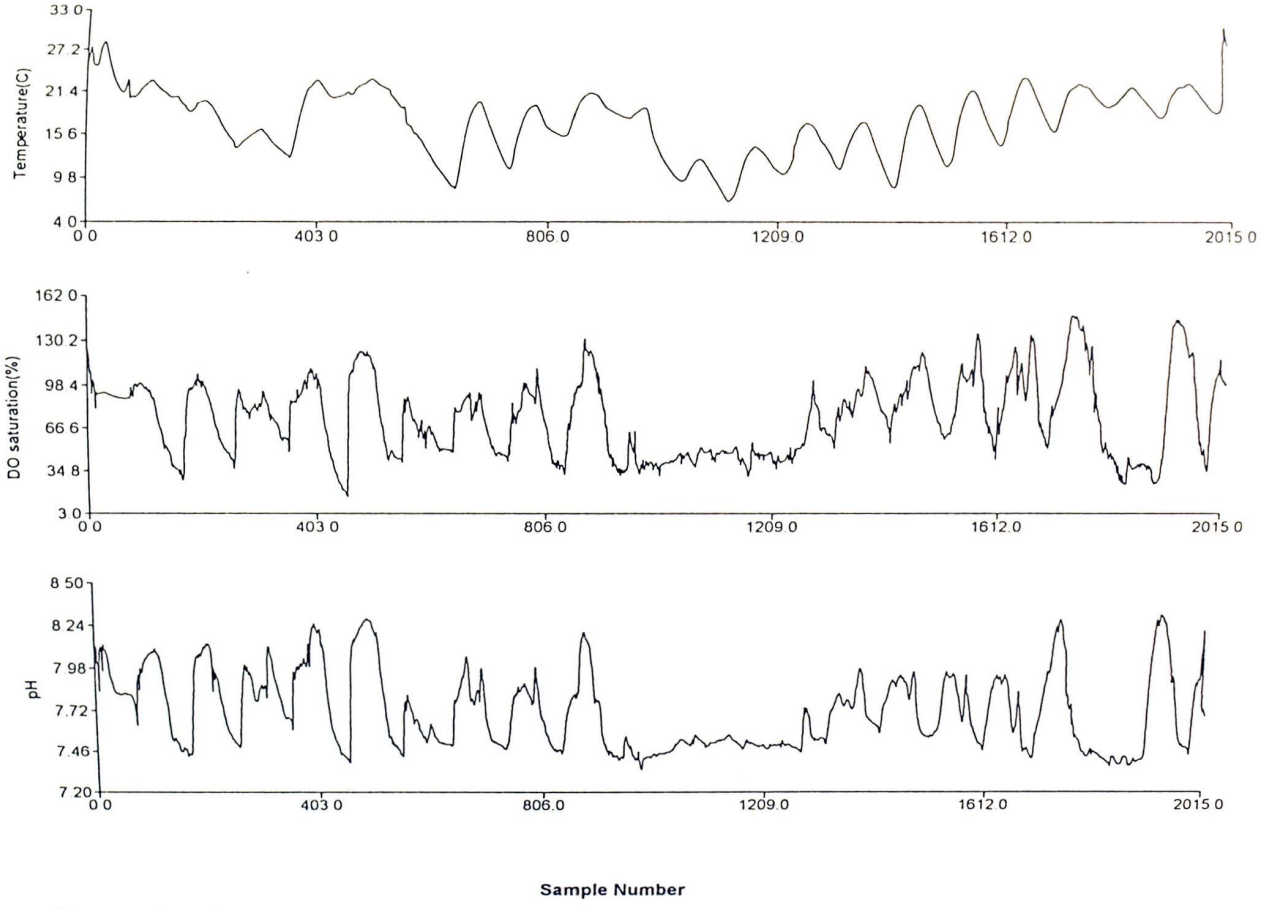

Figure 11. Continuous measurements of water quality parameters logged in apparatus from May 8 – May 28.

Suggestions for improvements

There are things that have been recognized that may have improved this study or may help to improve any future study. A solar-powered water-pump worked for this experiment, but was inconsistent and could not produce water during dark hours and prolonged periods of cloud cover. The pump would have worked better if placed in a well or dug into a gravel-bar. A gravel-bar would act as a natural filter for the water reducing the clogging of the "sock" filter and lengthening the life of the pump. A more constant flow regime would have been desired. If available, gravity-fed flow from stream would be more reliable, easier to maintain and more cost effective. If a solar powered water pump is used, then it would be more effective in providing continuous flow if coupled to a battery storage system. In a less remote location, utility power might be used to run the pump continuously.

The variables of this study could be changed to help develop future studies. The impact of the size or species of crayfish on leaf litter and/or macroinvertebrates could be analyzed. A similar study could be done to test for differences in feeding patterns caused by seasons. Different species of leaves might impact the feeding preference of crayfish and would be an interesting study.

Further studies to understand whether crayfish play a role in periphyton growth directly by grazing or indirectly by foraging through the leaf packets are necessary. An experiment including leaf packets and macroinvertebrates would provide necessary information in understanding the role of crayfish as keystone species.

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APPENDIX

Table A-1. Comprehensive table of samples collected from all channels prior to the beginning of the macroinvertebrate experiment.

	Channels and consecutive samples																								
	channel 1			channel 2			channel 3			channel 4			channel 6			channel 7			channel 8			channel 9		
Family/Group	1-1	1-2	1-3	2-1	2-2	2-3	3-1	3-2	3-3	4-1	4-2	4-3	6-1	6-2	6-3	7-1	7-2	7-3	8-1	8-2	8-3	9-1	9-2	9-3
Pleuroceridae	14	2	4	4	14	14	4	13	8	2	11	2	3	2	2	10	10	2	5	-	5	15	79	
Oligochaetes	11	12	3	-	-	-	1	1	-	2	4	2	5	-	-	-	5	-	1	3	3	2	-	
Chironomids	2	-	-	-	-	-	1	1	-	-	1	-	-	1	-	-	-	-	1	1	-	1	-	
Corbiculidae	-	-	-	1	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	
Sphaeriidae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	
Elimidae	-	-	-	1	-	-	-	-	-	2	1	-	-	-	-	-	-	-	-	-	1	-	-	
Psephenidae	-	-	-	-	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Dytisidae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	
Caenidae	-	-	1	-	-	-	-	1	-	-	-	-	-	-	-	2	2	-	-	-	-	1	1	
Baetidae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	
Leptophlebiidae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Ephemeridae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	
Heptageniidae	-	-	-	-	-	-	3	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	
Ephemerellidae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	1	1	-	-	-	-	-	
Polycentropodidae	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Helicopsychidae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	
Ephydridae Diptera	-	-	-	-	-	-	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	
Leuctridae Capniidae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	
Perlidae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Haliplidae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-
Cambaridae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Copepods	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Aquatic Mites	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

There were two samples labeled 9-3, neither were included in the statistics.

Table A-2. Comprehensive table of samples collected from all channels at the conclusion of the macroinvertebrate experiment.

	Channels and consecutive samples																								
	channel 1			channel 2			channel 3			channel 4			channel 6			channel 7			channel 8			channel 9		
Family/Group	1-1	1-2	1-3	2-1	2-2	2-3	3-1	3-2	3-3	4-1	4-2	4-3	6-1	6-2	6-3	7-1	7-2	7-3	8-1	8-2	8-3	9-1	9-2	9-3
Pleuroceridae	1	3	5	3	5	7	3	14	2	5	-	2	11	4	2	7	1	6		1	1	9	16	2
Oligochaetes	2	3	-	-	-	-	-	3	-	2	2	-	1	-	-	-	-	-		-	4	1	-	-
Chironomids	-	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-		-	-	1	-	-
Corbiculidae	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	-	-		-	-	-	-	-
Sphaeriidae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	5	-	-
Elimidae	-	1	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-
Psephenidae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-
Dytisidae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
Caenidae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-
Baetidae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	1	-
Leptophlebiidae	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	1	-
Ephemeridae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-
Heptageniidae	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-
Ephemerellidae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-
Polycentropodidae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-
Helicopsychidae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-
Ephydridae Diptera	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-
Leuctridae Capniidae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-		-	-	-	-	-
Perlidae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-
Haliplidae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-
Cambaridae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	1	-
Copepods	19	-	1	2	-	6	-	27	-	-	4	-	15	-	-	7	-	-		-	-	-	-	-
Aquatic Mites	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-

Channels 1,4,7 & 8 were crayfish enclosure channels

Sample 8-1 was absent

Table A-3. Macroinvertebrates found in channels before and at the conclusion of experiment II.

	C-1	II C-1	C-2	II C-2	C-3	II C-3	C-4	II C-4	C-6	II C-6	C-7	II C-7	C-8	II C-8	C-9	II C-9
Pleuroceridae	20(6.7)	9(3)	32(10.7)	15(5)	25(8.3)	19(6.3)	15(5)	7(2.3)	7(2.3)	17(5.7)	22(7.3)	14(4.7)	10(3.3)	2(0.7)	94(31.3)	27(9)
Oligochaetes	26(8.7)	5(1.7)	-	-	2(0.7)	3(1)	8(1.6)	4(1.3)	5(1.7)	1(0.3)	5(1.7)	-	7(2.3)	4(1.3)	2(0.7)	1(0.3)
Chironomids	2(0.7)	1(0.3)	-	1(0.3)	2(0.7)	-	1(0.3)	-	1(0.3)	-	-	-	2(0.7)	-	1(0.3)	1(0.3)
Corbiculidae	-	-	1(0.3)	-	-	-	1(0.3)	-	-	1(0.3)	-	1(0.3)	-	-	-	-
Sphaeriidae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4(1.3)	5(1.7)
Elimidae	-	1(0.3)	1(0.3)	-	2(0.7)	1(0.3)	3(1)	-	-	-	-	-	1(0.3)	-	-	-
Psephenidae	-	-	1(0.3)	-	-	-	1(0.3)	-	-	-	-	-	-	-	1(0.3)	-
Dytisidae	-	-	-	-	-	-	-	-	-	-	-	-	1(0.3)	-	-	-
Caenidae	1(0.3)	-	-	-	1(0.3)	-	-	-	2(0.7)	-	2(0.7)	1(0.3)	-	-	2(0.7)	-
Baetidae	-	-	-	-	-	-	-	-	-	-	1(0.3)	-	-	-	-	-
Leptophlebiidae	-	-	-	1(0.3)	-	-	-	-	-	-	-	-	-	-	-	1(0.3)
Ephemeridae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1(0.3)
Heptageniidae	-	1(0.3)	-	-	3(1)	-	1(0.3)	-	-	-	-	-	1(0.3)	-	-	-
Ephemerellidae	-	-	-	-	-	-	-	-	2(0.7)	-	2(0.7)	-	-	-	-	-
Polycentropodidae	-	-	1(0.3)	-	-	-	-	-	-	-	-	-	-	-	-	-
Helicopsychidae	-	-	-	-	-	-	-	-	-	-	1(0.3)	-	-	-	-	-
Ephydridae/Diptera	-	-	-	-	1(0.3)	-	1(0.3)	-	-	-	-	-	-	-	-	-
Leuctridae/Capniidae	-	-	-	-	-	-	-	-	-	-	-	-	1(0.3)	-	-	-
Perlidae	-	-	-	-	-	-	-	-	-	1(0.3)	-	-	-	-	-	-
Haliplidae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1(0.3)	-
Cambaridae	-	-	-	-	-	-	-	-	-	-	-	-	1(0.3)	-	-	-
Copepods*	-	20(6.7)	-	8(1.6)	-	27(9)	-	4(1.3)	-	15(5)	-	7(2.3)	-	1(0.3)	-	1(0.3)
Mites	-	1(0.3)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Totals	49	18	36	17	36	23	31	11	17	20	33	16	24	6	105	36
Average Totals	16.3	6	12	5.7	12	7.7	10.3	3.7	5.7	6.7	11	5.3	8	2	35	12

C - channels sampled before experiment

II C - channels sampled at the end of experiment

* - Copepods were not included in the totals

( ) - Average macroinvertebrates per channel

VITA

Robert Brinkman was born in New Tazewell, Tennessee on December 18, 1975. In 1994, he graduated from Reed City High School in Reed City, Michigan. After taking a year off from school, he began college at Kalamazoo Valley Community College in Kalamazoo, Michigan. After one semester, Robert moved back to East Tennessee, where he earned his Bachelor of Science degrees in Biology and Environmental Science from Lincoln Memorial University in May of 2000. Robert moved to Clarksville, Tennessee in August of 2000 to work on his Master of Science degree at Austin Peay State University where he received his degree August of 2002.