# Results of a Pilot Freshwater Mussel Survey in King County

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King County

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# EXECUTIVE SUMMARY

In 2004, a pilot freshwater mussel survey was conducted to better understand the density, age and size distribution of the western pearlshell mussel, *Margaritifera falcata*, in three King County streams. One study site was located in Bear Creek, a stream in the Lake Washington Watershed known to support at least one large mussel bed thought to be in decline. The other two study sites were located in Covington Creek in the Green River Watershed and Stossel Creek in the Snoqualmie River Watershed. Concern regarding freshwater mussels in upper Bear Creek has increased over the past two decades with increasing reports of dead and dying mussels and a general overall decline in numbers observed by local residents and County staff.

The goals of the survey were to locate three relatively dense beds of *M. falcata* and document size and age distribution within each bed, in addition to documenting in-stream and riparian habitat associated with the bed. The second goal was to determine the general condition of the mussels in these streams. This survey addressed four general questions regarding distribution and size, sex ratios, shell erosion, and histology.

25-cm wide transects were established at 5 m intervals across a 100 meter reach in each of the three streams. Each transect was divided into 25 cm by 25 cm quadrats. Mussels within the 25-cm-wide transects were hand picked from the stream bottom, counted, photographed and measured. To evaluate the accuracy of enumeration methods and to identify any sampling bias toward collection of larger animals, 10 percent of the sample quadrats were sieved (in addition to hand picking). Various habitat variables were also noted for each quadrat, including water type, substrate type, habitat structure, and riparian cover. An average of 22 mussels from each stream reach were sacrificed for sex determination, age determination, and shell characteristics. An additional 16 mussels were sacrificed for histological examinations.

Several results of this study suggest that the mussels within the Bear Creek study reach are declining and in poor health relative to Stossel and Covington creeks. First, Bear Creek had the lowest mussel density ($11/m^2$) compared to Stossel Creek ($35/m^2$) and Covington Creek ($19/m^2$), and comparison of these results to a previous study suggests a substantial decline in the mussel density in upper Bear Creek over the last 7 years. Second, sex ratios in Bear Creek mussels were dominated by males (4:1), whereas sex ratios at Covington (1.2:1) and Stossel (1.4:1) creeks were somewhat similar to what would be expected (near 1:1). Third, histological examination indicated that Bear Creek animals were in poor condition and severely stressed, as evidenced by gill lesions and severe loss of gill tissue. Fourth, while empty mussel shells were not quantified in this study, many empty shells (hundreds) were observed in the upper Bear Creek study reach, while few if any were observed in Stossel and Covington creeks.

This study did not attempt to identify a specific cause for the decline in freshwater mussels in upper Bear Creek. Multiple factors may affect mussels in small streams. The results of this and other studies, combined with our professional judgment, suggest that the decline is likely caused by some combination of changes in hydrology, changes in water quality, pathogenic infection, lack of filterable food, or clogging of filtering mechanisms, as opposed to predation, lack of reproductive success, or poor physical habitat quality.

# 1.0 INTRODUCTION

Freshwater mussels are one of the most endangered groups of animals in North America. Of the nearly 300 North American species, 35 have gone extinct in the last 100 years and 25% are listed as endangered or threatened under the Federal Endangered Species Act, and 75% are listed as endangered or threatened at the state level (Nedeau et al. 2005). During the past 30 years, both numbers and species diversity of native mussels have declined throughout the US and Canada (Williams et al. 1993). Multiple potential causes have been suggested for this decline, including habitat destruction from dams, channel modification, siltation, and introduction of non-indigenous mollusks, changes in fish populations, water quality impairment, and stormwater runoff. Freshwater mussels have been observed in multiple streams and rivers in King County, including upper Bear Creek.

In 2004, a pilot freshwater mussel survey was undertaken to begin to better understand the density, and age and size distribution of the western pearlshell mussel, *Margaritifera falcata*, in three King County streams. One study site was located in Bear Creek, a stream in the Lake Washington Watershed known to support at least one large mussel bed thought to be in decline. The other two study sites were located in Covington Creek in the Green River Watershed and Stossel Creek in the Snoqualmie Watershed.

The western pearlshell mussel is one of only three species of native freshwater mussels in Western Washington and was the only species presumed to be observed during the surveys. Populations of *M. falcata*, like the other species in the region, are thought to be in decline (Frest 2002; Frest and Johannes 1995; Williams et. al 1993). However, few studies have been conducted to examine changes in density or causes of potential population decline. This survey will serve as an initial assessment to evaluate mussels in these three study streams.

There were 2 key goals for this survey. The first was to locate three relatively dense beds of *M. falcata* and document the size and age distribution within each bed, in addition to documenting the in-stream and riparian habitat associated with the bed. The second goal was to determine the general condition of mussels in these streams. The basic approach used to conduct this survey was based on addressing five general questions:

1. **Distribution and Size -** How are mussels distributed within a bed or stream reach in the three study streams? What size classes are present, and how are they distributed within the study reach; do animals group themselves by size within each reach? Is there a relationship between length and age within a reach, and if so, is the relationship different among reaches?

2. **Sex Ratios -** Are sex ratios at the study sites different; are they within the range of an expected sex ratio (approximately 1:1, Baird 2000)?

3. **Shell Erosion -** Does shell erosion differ among and/or within the study reaches and if so, are the differences related to available habitat?

4. **Histology -** What is the general histological condition of mussels in the study streams?

5. **Use of Passive Integrated Transponder (PIT) tags –** Can PIT tags be used as a mark and recapture technique to determine mussel migration over time and to determine annual growth?

## 1.1 Overview of *Margaritifera falcata* Ecology

*Margaritifera falcata*, the most common species of freshwater mussel in the Pacific Northwest, inhabits cold, clean creeks and rivers used by salmon and native trout. These mussels are typically found in moving water in depths less than 2 meters, but will populate waters as deep as 7 meters.

*M. falcata* is one of four North American species that may produce hermaphroditic individuals (having both male and female reproductive organs). However, this condition is rare, and unless mussels are exposed to stressors, most populations of this species have separate sexes and typically exhibit a sex ratio of 1:1, male to female (Baird 2000). Fertilization typically occurs in early spring depending on water temperature (Nedeau et al. 2005). *M. falcata*, like many freshwater mussel species, requires a salmonid fish host to complete their life cycle. The mussel larvae, called glochidia, clamp onto the gill filaments of the fish where they become encysted for a few weeks, after which time they emerge and fall to the stream bottom where, if conditions are favorable, they continue their growth. The primary host species are chinook salmon (*Oncorhynchus tshawytscha*) and coho salmon (*Oncorhynchus kisutch*) salmon, but they have also been reported to use rainbow trout (steelhead) (*Oncorhynchus mykiss*) and cutthroat (*Oncorhynchus clarki*) trout (Nedeau 2005). Host species population fluctuations can directly impact the spawning success of the mussels. Unsuitable host fish will reject glochidia, sloughing them off after encystment (McMahon 1991). In general, the likelihood of their survival is very low; less than 1 in 100,000 is expected to survive the juvenile stage (McMahon 1991; Toy 1998). To offset this high mortality rate, mussels have a long reproductive lifespan and produce high numbers of glochidia each year (Bauer 1987).

After the glochidia drop from the fish, *M. falcata* spend their first few years buried in the sediment where they grow relatively fast as a protection against predators and the crushing and erosive force of rocks and water. Once mature, they spend their lives partially buried and prefer sand, gravel and cobble substrates. *M falcata* can live for over 100 years; this life span makes them one of the longest lived animal species on earth (Nedeau et al. 2005).

Mussels are important as indicators of general environmental conditions in the streams in which they reside because they are sensitive to changing water quality, physical habitat conditions, and fish communities. Low dissolved oxygen, chemical contaminants, and sedimentation are just a few of the numerous stressors that can adversely impact mussel communities Nedeau et al. (2005).

Delayed maturity, low effective fecundity, reduced powers of dispersal, high habitat selectivity, and poor juvenile survival make mussels very susceptible to both human and natural perturbations. As a result of these life history traits, populations do not rapidly recover once impacted (McMahon 1991).

## 1.2 Summary of other Local Mussel Evaluations

Freshwater mussels have been observed in multiple streams and rivers in King County, including upper Bear Creek. Concern regarding freshwater mussels in upper Bear Creek has increased over the past two decades, with increasing reports of dead and dying mussels and a general overall decline in numbers observed by local residents and County staff. Partly in response to these concerns, a series of surveys have been conducted to assess the general status of mussels in upper Bear Creek. The following provides a summary of studies completed to evaluate mussels in upper Bear Creek.

In 1998, a University of Washington graduate student (Kelly Toy) completed her master's thesis on "Growth, Reproduction and Habitat Preference of the Freshwater Mussel *Margaritifera falcata* in Western Washington" (Toy 1998). This study evaluated mussel populations in two locations in King and Snohomish counties, including Bear Creek. Toy found that age distribution in Bear Creek was skewed toward older individuals with a maximum age exceeding 90 years. Toy observed Bear Creek mussels to exhibit a male to female sex ratio of 1.5:1 and observed that they had a dioecious mode of reproduction with less than one percent hermaphrodites.

In 2001, King County conducted stream habitat assessments in Bear and Cottage Lake creeks to characterize habitat quality, primarily for salmonids. During these assessments, notations were made of freshwater mussel bed locations, including estimates of the number and size of mussels present, in addition to a survey of available mussel-bed substrate. A report, "Freshwater Mussels found in Bear and Cottage Lake creeks during Habitat Assessments in 2001" (King County 2002) was prepared to document these surveys. This study identified eleven relict mussel beds in upper Bear Creek that appeared identical to the live beds in other parts of the reach, except that only the empty shells were remaining. These findings indicate that the mussels died in place and not from some type of predation.

During the spring of 2002, Washington Trout conducted an evaluation of "Freshwater Mussel Bed Size, Mussel Density and Population Age Structure in upper Bear Creek, King County Washington" (Washington Trout 2002). This study provided a baseline survey of population characteristics of 10 mussel beds in upper Bear Creek. Three of the 10 mussel beds studied had lower densities of live mussels and higher proportion of dead mussels, although no cause for the observed higher proportion of dead mussels was identified from the many possible causes hypothesized. This study also raised concerns regarding whether mussels in upper Bear Creek are able to reproduce effectively and recommended additional study of this issue.

During summer 2002, King County hired a nationally-recognized freshwater mussel expert, Dr. Terry Frest of Deixis Consultants, to prepare a "Report of Freshwater Mussel Populations in Bear Creek on the Walsh Property (RM 10.32 to RM 10.47), August 12, 2002" (Frest 2002). Dr. Frest concluded that freshwater mussels at the Walsh property had experienced substantial decline as the result of increased beaver activity at the site (predation on mussels, dam-building, and changes to forest canopy near the stream). Dr. Frest recommended relocation of the beavers, and restoration of the site to pre-beaver-activity condition. Dr. Frest was effusive in his praise of the extent of the population on the Walsh property but is concerned about the numbers of young mussels.

During spring 2003, Washington Trout conducted an evaluation of "Freshwater Mussel Bed Re-surveys, Juvenile Recruitment and Predation Reconnaissance in Upper Bear Creek, King County Washington" (Washington Trout 2003). This study concluded that freshwater mussels in upper Bear Creek are likely stable on a short-term basis, with some beds showing a decline from the previous year and others showing an increase. Nevertheless, the authors reiterated their concern regarding dead and dying mussels in specific beds in upper Bear Creek, as well as concerns regarding the reproductive effectiveness of freshwater mussels in upper Bear Creek.

In the fall of 2003, King County, Washington Trout and The Bear Creek Water Tenders engaged in a cooperative effort to evaluate bioaccumulation of a limited number of chemicals in upper Bear Creek mussels (Applied Biomonitoring 2004). The results of this limited effort were somewhat inconclusive; no single contaminant, or group of contaminants were identified as obviously causing the observed mussel decline; however, the data were too limited and insufficient to rule out the possible role of chemical contaminants as a stressor to mussels in Bear Creek. One of the recommendations of this work was that future efforts include a more detailed assessment of contaminant concentrations in water and sediment.

# 2.0 METHODS

This section summarizes the methods used in this study. A more detailed description of methods can be found in the Sampling and Analysis Plan (SAP) (King County 2004a) which is included in Appendix A. A variety of equipment was used to conduct the sampling and analysis associated with this survey, and a full list of equipment can be found in Appendix B. Sampling was conducted between August and September 2004.

## 2.1 Site Selection

Mussel beds in three creeks were selected for the survey: Bear Creek, Stossel Creek, and Covington Creek (Figure 1). The stream reaches examined for potential inclusion in this survey had been previously identified by field staff and were revisited in early 2004 to verify the presence of mussel beds. The following subsections provide an overview of the three sites selected for inclusion in this study. Selection of study stream reaches was based on the presence of relatively dense mussel beds within the reach. Three additional criteria were used for selection of study reaches: (1) if possible, located downstream from a wetland, (2) mussels are visually present and (3) the reach is accessible by field staff.

**Figure 1. Map of Study Site**

### 2.1.1 Bear Creek

Bear Creek is located in the Greater Lake Washington Watershed, and the dominant land use in the vicinity of the study area is rural residential. A reach of Bear Creek located at the Walsh property (RM 9.5) was identified for this assessment. This site was selected because of the previous work conducted in the area, as well as a suspected population decline. The Bear Creek mussel beds in the selected study reach had been previously observed by King County staff while conducting salmon surveys (Fevold and Vanderhoof 2002) and also by Washington Trout staff who were studying a segment of the population below Paradise Lake in the uppermost reach of the main stem Bear Creek (Washington Trout 2002). Local residents have observed a general decline in mussels in this area.

### 2.1.2 Upper Covington Creek

The Covington Creek survey reach is located in Lake Sawyer Park in the Green River Watershed. In general, the dominant land use in the study area vicinity is 2$^{nd}$ and 3$^{rd}$ growth forest, with some suburban development. The mussel population in Upper Covington Creek had been previously identified by the author during a stream habitat survey in 1993. Staff had observed the presence of a considerable number of empty shells, which suggested a possible die off of mussels. However, the presence of dense groups of living mussels suggested the observed shell accumulations might be current-sorted assemblages of shells from a period of several years. Field checks by the author in 2003 and 2004 at nine locations between Ravensdale Lake and Lake Sawyer verified that a population still was present.

### 2.1.3 Stossel Creek

Stossel Creek is located within the Tolt River Watershed, most of which is owned and managed by WDNR. While conducting salmon habitat surveys in 2002 (King County 2004b), King County staff observed several dense mussel beds within Stossel Creek between its confluence with the Tolt River near Duvall and the upper reaches of the stream, approximately 3.0 miles upstream. A field check by King County staff in 2004 confirmed that mussels were still present in relatively high numbers.

## 2.2 Study Parameters

This section provides an overview of the methods used to address the study questions previously outlined in the Introduction.

### 2.2.1 Mussel Distribution, Age, and Length

To better understand the general distribution and size of mussels in the study streams, mussels in each study stream reach were enumerated and measured. Transects were established in 100 meter reaches in each of the three streams; within each of these reaches 20 transects were placed at 5 m intervals perpendicular to the stream channel. Each transect was divided into 0.0625 $m^2$ quadrats. Mussels within the 25 cm-wide transects were located, counted and measured. Beginning at the left bank, mussels were enumerated in each adjoining quadrat until the right

bank was reached. Because of the variable nature of channel width in each reach, the number of quadrats in each transect varied. Obstructions such as gravel bars and large logs along each transect were noted by field staff; these areas were not sampled. A more detailed description of these methods can be found in the project SAP (King County 2004a) (Appendix A).

All mussels were removed from each quadrat to determine length, which was measured along the longest axis of the shell to the nearest mm. Each mussel was photographed and returned to the stream. These data were later used to identify any statistical differences in mussel size between reaches, and also to determine whether mussels within a reach are distributed by size, and if so, if the observed grouping is related to available habitat.

To examine the relationship between length and age, 25 mussels were collected (and sacrificed) from each of the 3 study reaches. Five mussels were collected from each of five size groups (<40mm; 40-55 mm; 56-70 mm; 71-85 mm and >85 mm). For each group of 25 mussels, the first 5 individuals encountered in each of the five size ranges were collected. Two aging methods were utilized in an attempt to increase accuracy. The first method (Havlik 2004, personal communication) entailed counting the individual rings on the outside of the shell after the loose periostracum (the tough chitinous outer covering of the shell that protects it from the erosive action of water) had been sanded from the posterior ridge (similar to counting the rings of a tree). Those growth rings are formed during the period of low growth in the late spring (April and May) each year. At the same time, mussels also develop rest marks in the ligaments that hold their shells together. Since it is presumed that these marks and rings are only formed once a year, the marks in the ligament are called annuli, or annual rings. In addition to growth rings, mussels also develop rest marks during periods of limited growth. Enumeration of these rest marks (Hendleberg 1960) was the second method used to determine age. A drop of glycerin was applied to the ligament before the shell was opened with a scalpel. The ligaments were examined under 40X magnification with a dissecting microscope and the rest marks were enumerated.

The mussels were located visually and by touch (picking) in most cases because even a slight disturbance of sediment within the quadrat reduced visibility in the stream to near zero. Others have found that this method can underestimate the number of smaller organisms in a study area (Hastie 2000). As such, to estimate the efficacy of locating the animals by picking, and to determine if the sampling method was biased toward collection of larger animals, substrate material in every tenth quadrat was sieved. The sieving process included removing and enumerating mussels initially located by picking, and then a scoop with an attached 6 mm mesh bag was used to excavate the top 10 cm of sediment within each quadrat. The material retained by the sieve was evaluated for the presence of mussels; all additional mussels identified by the sieve method were measured for length, photographed, and retuned to the stream.

To better understand if habitat variables influence distribution, age, and size of mussels in each of the study reaches, a variety of stream habitat data were collected including water type (riffle, pool, and glide), substrate type, habitat structure, and riparian cover. Habitat data were collected for the area within each of the individual quadrats where mussels were enumerated. Substrate and structure characteristics were classified as outlined below in Tables 1 and 2. All habitat classifications were based on observations by field staff and based on the dominant characteristic

within a quadrat. If two or more types of structure or substrate were present within an individual quadrat, the type that comprised the majority of the area was used as the habitat classification.

**Table 1. Characteristics used to classify substrate types.**

| Substrate Type | Characteristics |
|---|---|
| Litter | Peaty, unconsolidated organic debris such as bark or twigs and leaves |
| Mud | Very loosely consolidated, very fine-grained material. Individual particles are not visible without magnification |
| Sand | Particle size ranges from barely visible up to 2 mm in diameter |
| Gravel | Material mm to 75 mm |
| Cobble | Material 76 mm to 150 mm. |

**Table 2. Characteristics used to classify habitat structure types**

| Habitat Structure Type | Characteristics |
|---|---|
| Boulders | Rock > 300mm in diameter |
| Logs | Tree trunks or branches >75mm in diameter that were imbedded in the streambed |
| Twigs and Sticks | Accumulations of twigs and sticks with little or no fine material |
| Herbaceous Vegetation | Type of vegetation in quadrat was noted |
| Roots | Roots of living trees such as Douglas Fir and Big Leaf Maple, or their stumps, undercut by the stream (root balls of shrubs such as Red Osier Dogwood and Ninebark were included in the herbaceous vegetation category) |
| Cobble | Material >76 mm to 150 mm |
| None | No structure present |

Riparian cover was also estimated for each quadrant and was classified as either presence or absence. If vegetation was present between the quadrat and the streambed and within 2 feet of the water surface, it was assumed to provide cover. A more detailed description of the methods used to collect habitat data is presented in the project SAP (King County 2004) (Appendix A).

### 2.2.2 Sex Ratios

As previously indicated *M. falcata* can exhibit hermaphroditism, however, they typically exhibit a 1:1 sex ratio of males to females (Baird 2000). To better understand the distribution of males and females, sex ratios were determined on 25 mussels in each of the three 100 meter stream reaches. To determine sex ratio, gamete smears were made from each of the animals collected for age analysis. The upper posterior portion of each animal was pierced through both sides and the gamete material was transferred onto a microscope slide. The material was then dried for 30 minutes and stained using a mixture of hematotoxylin and eosin dyes. The slides were then rinsed, dried, and cover slips were applied. The slides were examined under 100X magnification for the presence of eggs and sperm. If neither type of tissue was present, the gender was documented as "undetermined."

### 2.2.3 Shell Erosion and Characteristics

Shell erosion is a relatively common characteristic of some mussels including *M. falcata*; however, some researchers (Kinney 2003, Kat 1982) attribute increased erosion to water quality, particularly ph and hardness. If erosion is extensive, the shell can become perforated and the soft tissue may be exposed to the environment. This condition may result in a greater survival risk by small predators gaining accesses to the soft tissue and potential desiccation during low water periods (Kinney 2003). However, little work has been conducted to evaluate the ecological significance of shell erosion on overall mussel health. To determine if any correlation exists between degree of shell erosion and habitat characteristics, percent shell erosion was determined on a subset (approximately 10%) of the organisms from each study reach.

All mussels in each transect were photographed with a digital camera with 3.1 mega pixel resolution. The camera was attached to a rigid frame and the mussels were laid on a black background. To evaluate the degree of erosion, 10 percent of the images from each study stream were examined with photo editing software. The software enabled the user to draw polygons on the photos and provided the number of pixels contained in the polygon. A polygon was drawn around the entire shell margin and another around only the eroded portion. The number of pixels in each polygon was recorded and the percent shell erosion was calculated using the following equation:

**%Erosion= (# pixels in eroded polygon / # pixels in whole shell polygon) X 100**

In addition to evaluating shell erosion, other shell characteristics were noted including nacre color, distribution of color, and condition of nacre on the inside of each valve. In addition, the presence of thin areas and perforations were determined by holding the shell in front of bright light. Information on these shell characteristics can be used to provide some general insight on the health of the organism. The nacre of a healthy *M. falcata* typically appears to be polished and iridescent in some areas. The nacre color is usually white, purple, or salmon and is evenly distributed (Nedeau et al. 2005). Generally, the evenness of the nacre distribution is a distinguishing characteristic when the mantle of an organism is fully functional.

### 2.2.4 Histological Assessment

Previous observations (Washington Trout, 2002, 2003) have suggested that mussels in upper Bear Creek may be impacted by disease, as well as a variety of other possible stressors. Mussels have previously been observed lying and gaping on the stream bottom, and numerous dead mussels and empty shells have also been observed. To better understand the general health of the mussels, 16 live organisms from two of the three study streams (Bear and Stossel creeks) were collected for histological assessment. A necropsy to evaluate potential disease was performed on all 16 mussels and a histological examination to evaluate the microscopic structure of the tissues was conducted on 10 mussels. Mussels used for the histological assessment were collected on November 8, 2004.

### 2.2.5 Use of Mark and Recapture method to Determine Migration and Growth

Mark-recapture models are widely used in ecology, but have rarely been used to assess mussel populations (Strayer and Smith 2003). In mark-recapture designs, investigators collect a number of animals from the target population, mark them and release them back in their habitat. Later the target population is re-sampled and desired measurements are made. Passive integrated transponder (PIT) tags are a small externally mounted or implanted Radio Frequency Identification products used to positively identify study animals over extended periods of time. Each tag is a read-only tag that is programmed to transmit a unique code when activated. The tag transmits a unique digital code back to the transceiver/reader where the code is displayed and/or stored. PIT tags are typically used to track fish; however, we decided to evaluate the effectiveness of using this technology as a mark and recapture method for mussels.

PIT tags were attached to 25 mussels collected from a single point in each study stream reach. Tags were attached between September 30 and October 1, 2004. The tags were attached to track mussel migration over time and to evaluate if recapture of tagged mussels on an annual basis was feasible. Each PIT tag was affixed to the posterior portion of the mussels. A small area next to and parallel to the posterior ridge was cleared of periostracum by sanding and the PIT tag was attached to the shell with 5-minute epoxy glue. Each PIT tag code was recorded and the animal was measured and photographed. The animals were left overnight in trays with the posterior portions exposed to the air so that the glue could set without harming the animals. The animals were returned to the capture site the next morning and released.

# 3.0 RESULTS AND DISCUSSION

## 3.1 Mussel Distribution, Age, and Length

A number of measurements and statistical analyses were conducted to better understand the distribution and size of mussels in the three study streams. Statistical analyses were also conducted to better understand the influence of habitat and substrate characteristics on these distributions. The remainder of this section provides a description of the statistical methods and the results of these analyses.

### 3.1.1 Sieved/Un-sieved Comparison

As previously discussed, ten percent of the quadrats were sieved (in addition to picking) to evaluate the accuracy of enumeration methods and to determine if the method was biased toward collection of larger animals. Table 3 provides a summary of these data.

**Table 3. Number of mussels in sieved and un-sieved (picked) quadrats. All quadrats 0.0625 $m^2$**

| | Total | Sieved Quadrats | | | Un-sieved Quadrats (Picked Only) |
|---|---|---|---|---|---|
| | | Picked Only | Additional Sieved | Total | |
| **All sites combined** | | | | | |
| Number of Quadrats | 1804 | N/A | N/A | 174 | 1630 |
| Number of Mussels | 1807 | 190 | 82 | 272 | 1617 |
| Mean # per Quadrat | 1.00 | 1.09 | 0.47 | 1.56 | 0.99 |
| Average length mm | 75.9 | 77.5 | 66.6 | 74.2 | 75.7 |
| **Bear Creek** | | | | | |
| Number of Quadrats | 863 | N/A | N/A | 83 | 780 |
| Number of Mussels | 606 | 66 | 11 | 77 | 540 |
| Mean # per Quadrat | 0.70 | 0.80 | 0.13 | 0.93 | 0.69 |
| Average length mm | 84.6 | 84.8 | 72 | 83.0 | 84.6 |
| **Covington Creek** | | | | | |
| Number of Quadrats | 761 | N/A | N/A | 73 | 688 |
| Number of Mussels | 836 | 66 | 39 | 105 | 770 |
| Mean # per Quadrat | 1.10 | 0.90 | 0.53 | 1.44 | 1.12 |
| Average length mm | 67.6 | 64.7 | 63.1 | 64.1 | 67.9 |
| **Stossel Creek** | | | | | |
| Number of Quadrats | 180 | N/A | N/A | 18 | 162 |
| Number of Mussels | 365 | 55 | 32 | 87 | 310 |
| Mean # per Quadrat | 2.03 | 3.06 | 1.78 | 4.83 | 1.91 |
| Average length mm | 80.7 | 81.6 | 72 | 78.1 | 80.5 |

Of the 1804 quadrats sampled, 174 were sieved (approximately 10 percent). The mean number[1] of mussels in all un-sieved (picked only) quadrats was 0.99, while the mean number of animals in the sieved (picked plus additional sieved) quadrats was 1.56. The data were not normally distributed; therefore, it was most appropriate to use a non-parametric statistical test to evaluate whether sieving statistically increased the number of mussels per quadrat. The Mann-Whitney test ($p<0.10$) was applied to determine if among the 174 sieved quadrats, there were statistical differences between the number of mussels in the sieved quadrats that were initially identified by picking and the additional organisms identified by sieving. The data were evaluated by site, and across all sites. The statistical analysis indicated that when all sites were combined and when sites were individually evaluated that the number of mussels per quadrat were statistically different. This finding suggests that the picking method statistically underestimates the density of mussels in the study reaches.

Based on all site data combined, the "picked only" quadrats contained approximately 30% fewer mussels than the picked plus the sieved mussels; on an individual site basis there was a 14% difference at Bear Creek, 40% at Covington Creek and 37% at Stossel Creek. However, to avoid bias in the data analysis, all additional data analysis associated with mussel density presented in the remainder of this report was conducted using only the picked data. It is the author's opinion that the comparison between sites where mussels were counted using different methods is a "false" comparison. Thus, excluding the sieved mussels allowed comparisons based on one method. These sampling artifacts should be taken into account when interpreting the remainder of the data presented here.

### 3.1.2 Mussel Distribution and Density by Stream Reach

The distribution of mussels within each 100 m study reach was visually evaluated by plotting transect/quadrat data as shown in Figures 2-4. Based on visual observation of data presented in these figures, mussel distribution appears heterogeneous within each reach. Animals appear to have grouped themselves within distinct beds. However, based on field observations, no clear determination of the specific habitat conditions preferred by mussels could be visually identified.

[1] While the data were not normally distributed, we report mean number of mussels. The median is a better statistical measure for a non-normal distribution however, the medians were zero and do not provide a description of the relative densities.

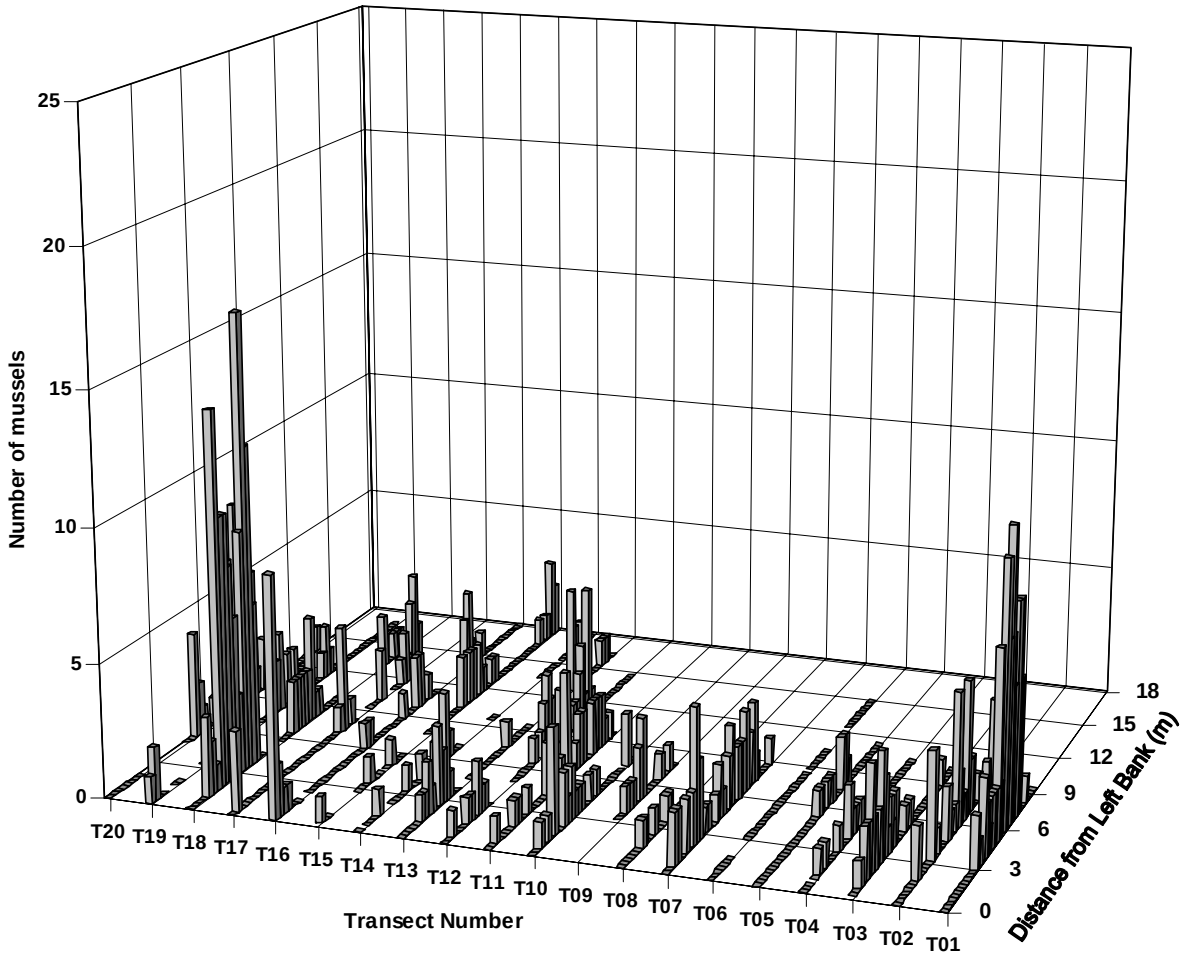


**Figure 2. Distribution of mussels in the Bear Creek study reach by quadrat and transect.**

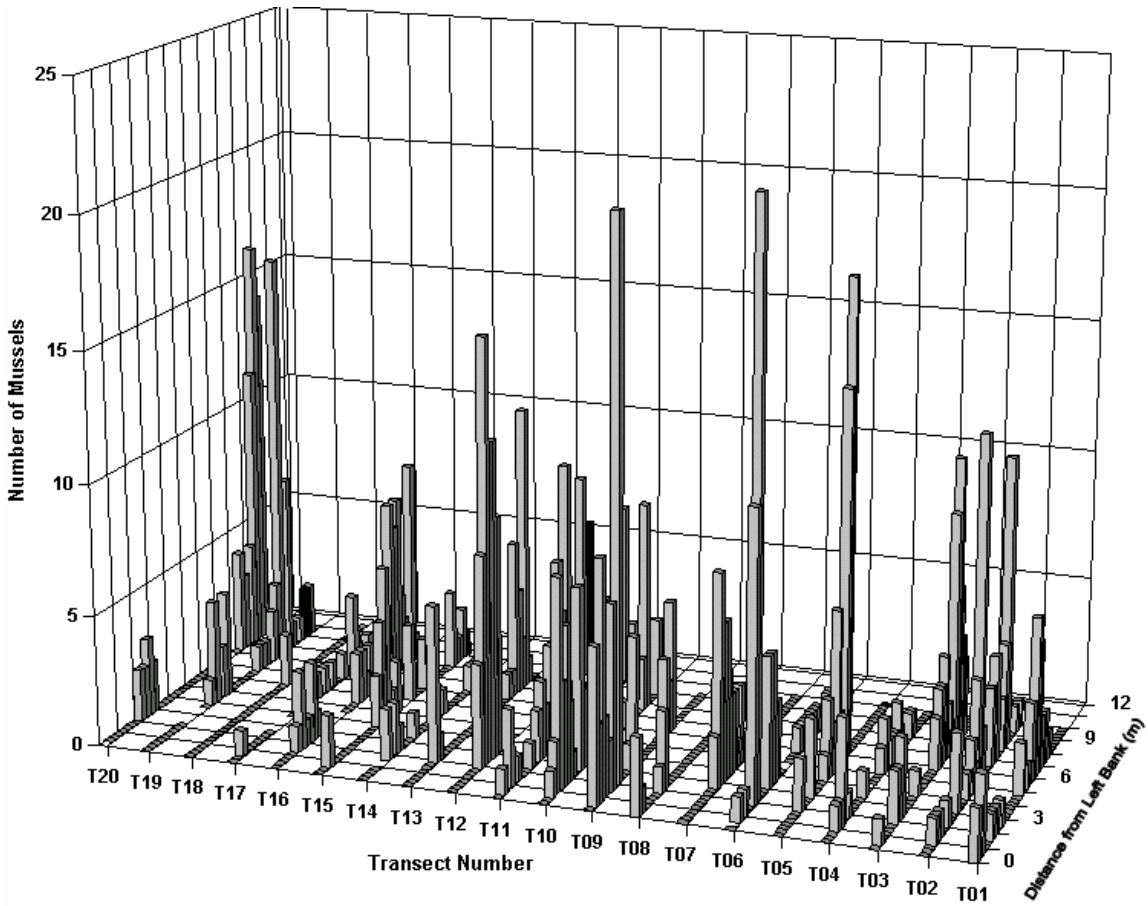


**Figure 3. Distribution of mussels in the Covington Creek study reach by quadrat and transect.**

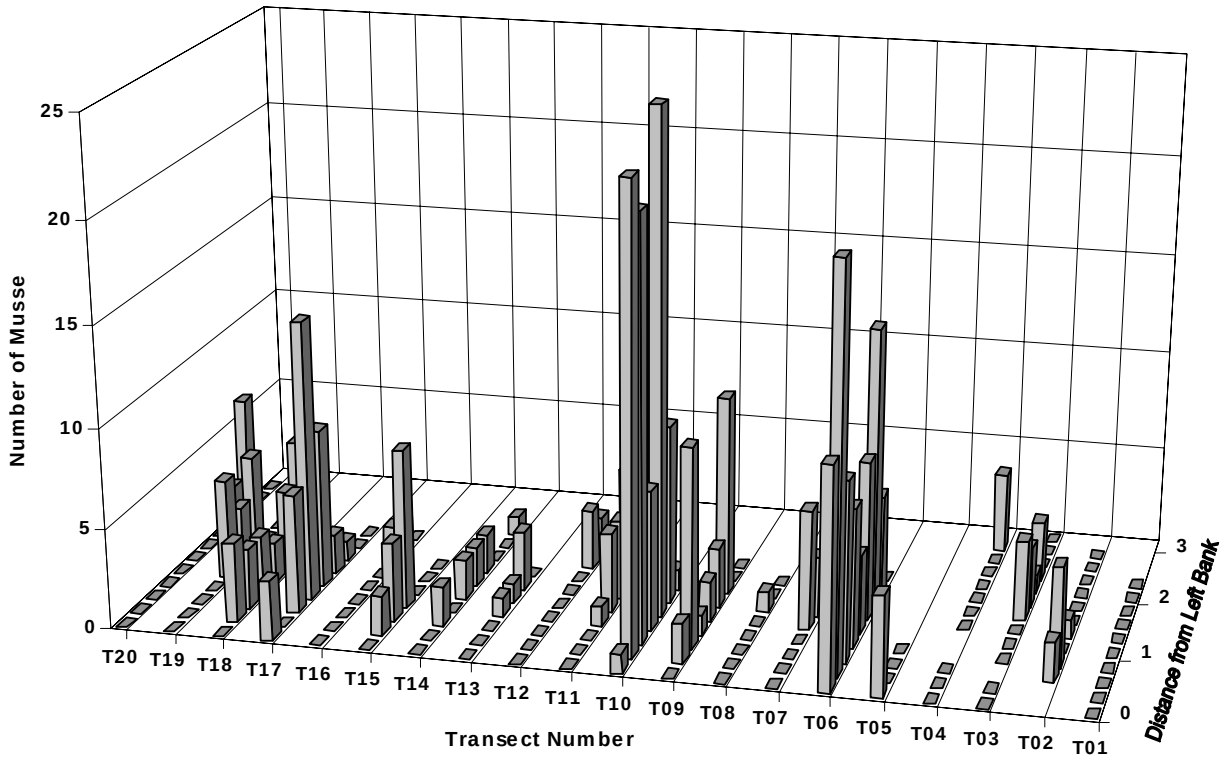


**Figure 4. Distribution of mussels in the Stossel Creek study reach by quadrat and transect.**

Table 5 provides a tabular summary of the distribution of mussels within the quadrats. More than 60 percent of the quadrats in which mussels were enumerated did not contain mussels. The majority of quadrats in which mussels were present contained from one to five organisms. Very few quadrats contained greater than 10 mussels per quadrat. These data suggest a heterogeneous distribution of mussels throughout the three study reaches.

**Table 5. Number of quadrats containing the specified number of mussels and percentage of total quadrats with that number of mussels.**

| | Number of Quadrats | | | | | |
|---|---|---|---|---|---|---|
| **Number of Mussels** | **0** | **1-5** | **6-10** | **11-15** | **16-20** | **>21** |
| All Sites | 1221 (67.5%) | 497 (27.5%) | 59 (3.3%) | 16 (1%) | 6 (0.3%) | 5 (0.3%) |
| Bear Creek | 599 (69.4%) | 244 (28.3%) | 16 (2%) | 3 (0.3%) | 1 (0.1%) | 0 (0%) |
| Covington Creek | 512 (67.3%) | 204 (26.8) | 29 (3.8%) | 10 (1.3%) | 4 (0.5%) | 2 (0.3%) |
| Stossel Creek | 110 (61.1%) | 49 (27.2%) | 14 (7.8%) | 3 (1.7%) | 1 (0.6%) | 3 (1.7%) |

The estimated mussel density for each study stream reach and all sites combined is presented in Table 6. Mussel density in the study stream reaches was greatest in Stossel Creek (35/m$^2$) and lowest in Bear Creek (11/m$^2$). Toy (1998) estimated the average density of Bear Creek mussels within a 340 m study reach at a location in the same general vicinity as this study to be 56/m$^2$. Although Toy's methods and specific sampling location were slightly different than in this study, these data suggest a decline in mussel density in this general area over the last 7 years. Toy (1998) suggested that if local mussel density (avg. density in a 0.05 mile radius) is less than 10/m$^2$, reproductive success may be too low to sustain a population.

**Table 6. Average mussel density and estimated total number of mussels in the three survey stream reaches.**

| Stream Reach (100 m) | Average Density (mussels/m2) | Estimated Number of Mussel in Each 100 m Reach[1] |
|---|---|---|
| All Reaches Combined | 17 | N/A |
| Bear Creek | 11 | 10,764 |
| Covington Creek | 19 | 15,411 |
| Stossel Creek | 35 | 6,188 |

[1] The authors realize that some readers may be interested in an estimate of the total number of mussels in the surveyed reaches. This estimate has been provided, but with significant reservations. As can be seen from Figures 2-4 the number of mussels in each quadrat is highly variable, with the most frequent occurrence being zero. In addition, the sampling method was not selected with the intention of providing such an estimate. Had we intended to do so, the sampling scheme would have been very different. See Strayer, D.L. and Smith, D.R, A Guide to Sampling Freshwater Mussel Populations, American Fisheries Society Monograph 8, 2003.

### 3.1.3 Observations of Empty Shells

Although not specifically quantified, a large number (100's) of empty shells were observed in the Bear Creek study reach. Examination of shells and the presence of organic/tissue matter attached to the shell, suggested that some of the organisms had recently died (2-4 weeks prior to observation). These finding are supported by the findings of Fevold and Vanderhoof (2002) and Washington Trout (2003) who also observed a substantial number of empty shells in upper Bear Creek. These observations suggest that whatever has caused the observed mussel decline in Bear Creek continues to impact the population. In contrast, very few, if any empty shells were observed in the other 2 study streams.

## 3.2 Influence of Habitat Factors on Mussel Distribution

To better understand the potential variables that may influence mussel distribution in a study reach, a number of habitat variables were evaluated. Water type, substrate, habitat structure and riparian cover were the variables examined in this portion of the study. As previously indicated, the habitat classifications were based on the dominant habitat in each individual quadrat.

The water type (i.e., pool, riffle, and glide) available in each of the 100 m study reaches varied (Figure 5). Bear Creek had the greatest amount of glide habitat (51%), whereas Stossel Creek had the lowest (27%). Covington Creek contained the greatest amount of riffle habitat (42%), and Stossel Creek had the lowest (12%). Stossel Creek contained the greatest amount of pool habitat (61%), and Covington Creek had the lowest (15%).

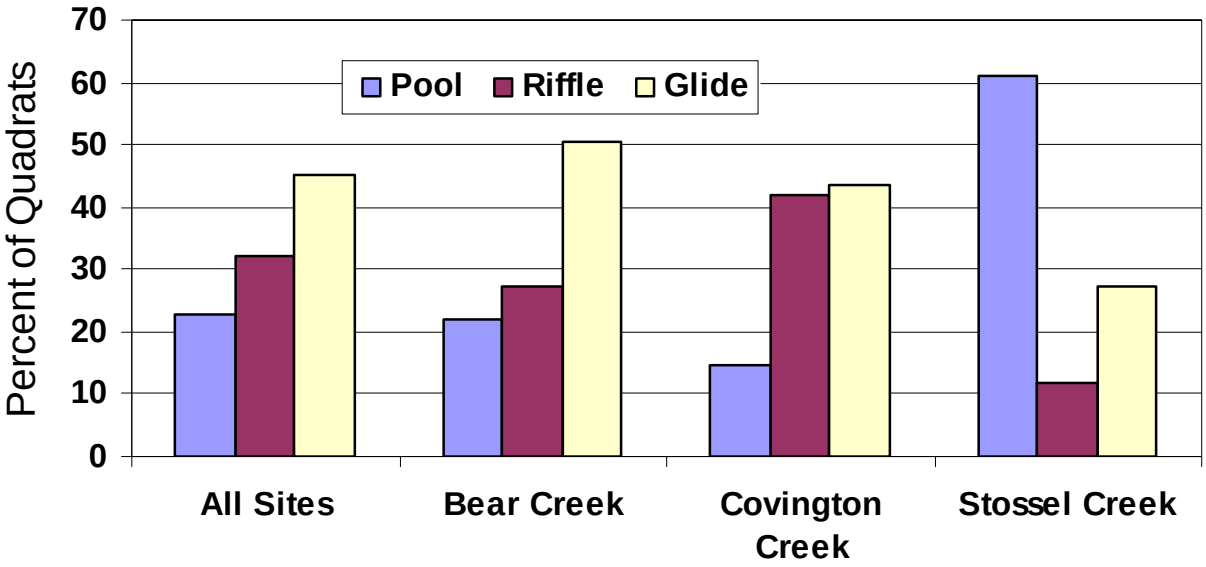


**Figure 5. Percentage of available pool, riffle and glide habitat in quadrats within each 100 m survey reach and all sites combined.**

Figure 6 presents the percentage of mussels found in each of the water types. When all sites are combined, greater than 80% of the mussels were found in either riffle or glide habitat. When evaluated on a site specific basis, the distribution of mussels within water types were similar at Bear and Covington creeks, where more than 50% of the mussels were present in riffle habitat, and more than 30 % were found in glide habitat. In Stossel Creek more than 50% of the mussels were found in pool habitat, while more than 40% were present in glide habitat; this is likely due to the lack of riffle habitat (Figure 5) available in Stossel Creek.

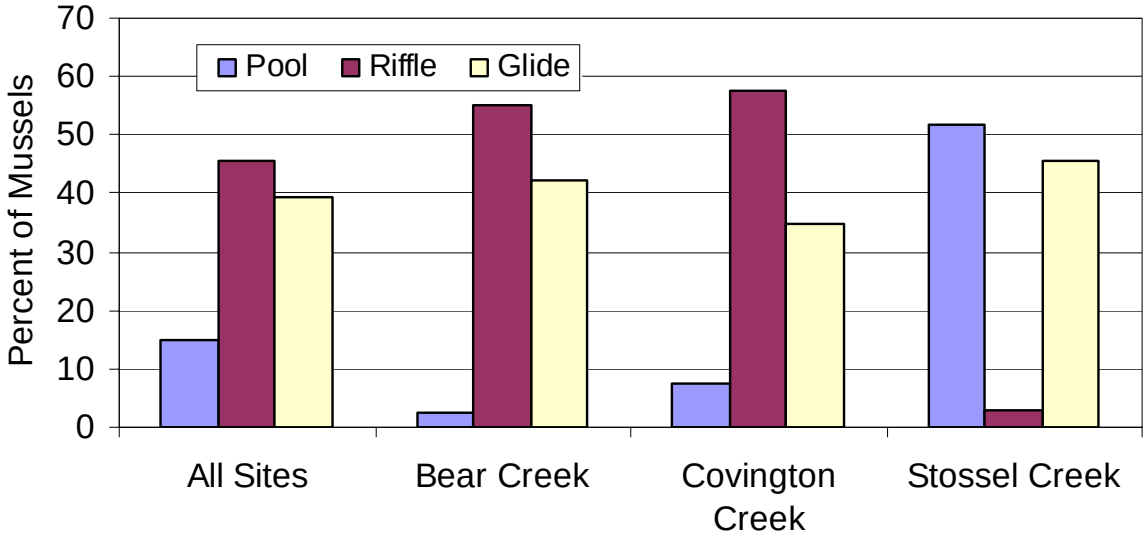


**Figure 6. Distribution of mussels in different water types (glide, pool and riffle) within each 100 m survey reach and all sites combined.**

Substrate type in each quadrat was classified as gravel (2 - 75 mm), cobble (76 - 150 mm), sand (0.1 - 2 mm), mud (<0.1 mm), and litter (plant and detrital material at the bottom of the stream bed). In some cases, where substrate type within a transect could not be identified (i.e., where there were braided channels, gravel bars, or large boulders etc.), substrate was classified as "not available" (NA). Since these quadrats were not sampled, they were not used in any calculations or statistical analyses. The percent substrate type present in each of the study stream reaches is presented in Figure 7. The dominant substrate type available in each study reach was found to be variable. In Bear and Covington creeks, gravel was the dominant available substrate, whereas sand was dominant in Stossel Creek. Litter layers generally represented less than 10% of the substrate.

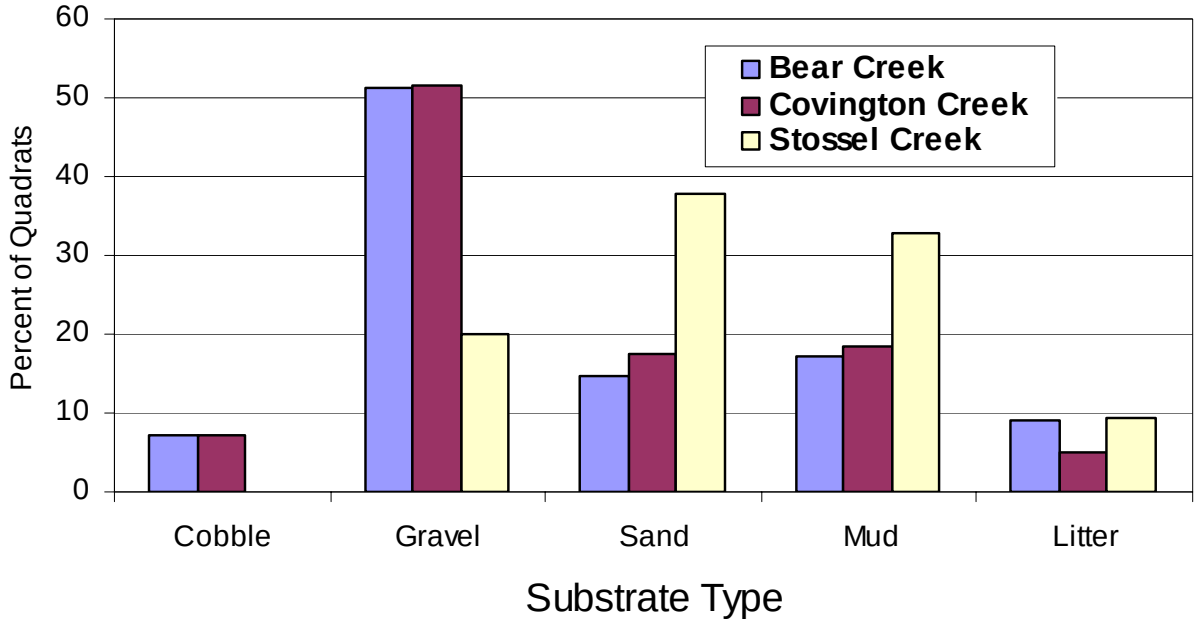


**Figure 7. Substrate type in study streams presented as percent of quadrats for all sites combined and individual sites.**

Greater than 50 percent of the mussels in the three study streams were present in gravel substrate (Figure 8). The distribution of mussels within the different substrate types in Bear and Covington creeks was similar to the distribution of available substrate (Figure 7) in these study reaches. In these two streams, it appears that mussels were relatively evenly distributed within the available substrate types. However, mussel distribution in Stossel Creek did not follow this pattern; although only 20 percent of the available substrate in Stossel Creek consisted of gravel, more than 50 percent of the mussels in this reach were present in this substrate; no mussels were identified in cobble substrate.

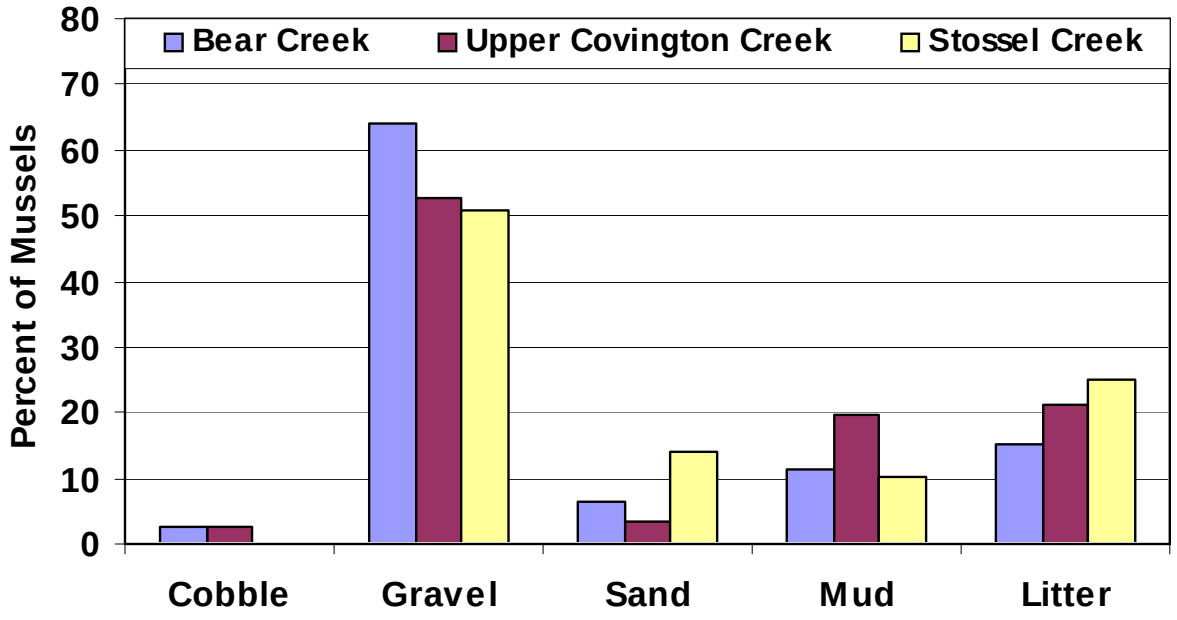


**Figure 8. Percent of mussels present in different substrate types for all sites combined and individual sites.**

Habitat structure within the three study sites was variable (Figure 9). There was no habitat structure present in more than 55 percent of the Bear Creek quadrats and more than 80 percent of the Stossel Creek quadrats. Stossel Creek contained the greatest diversity of habitat structure, while Stossel contained the least.

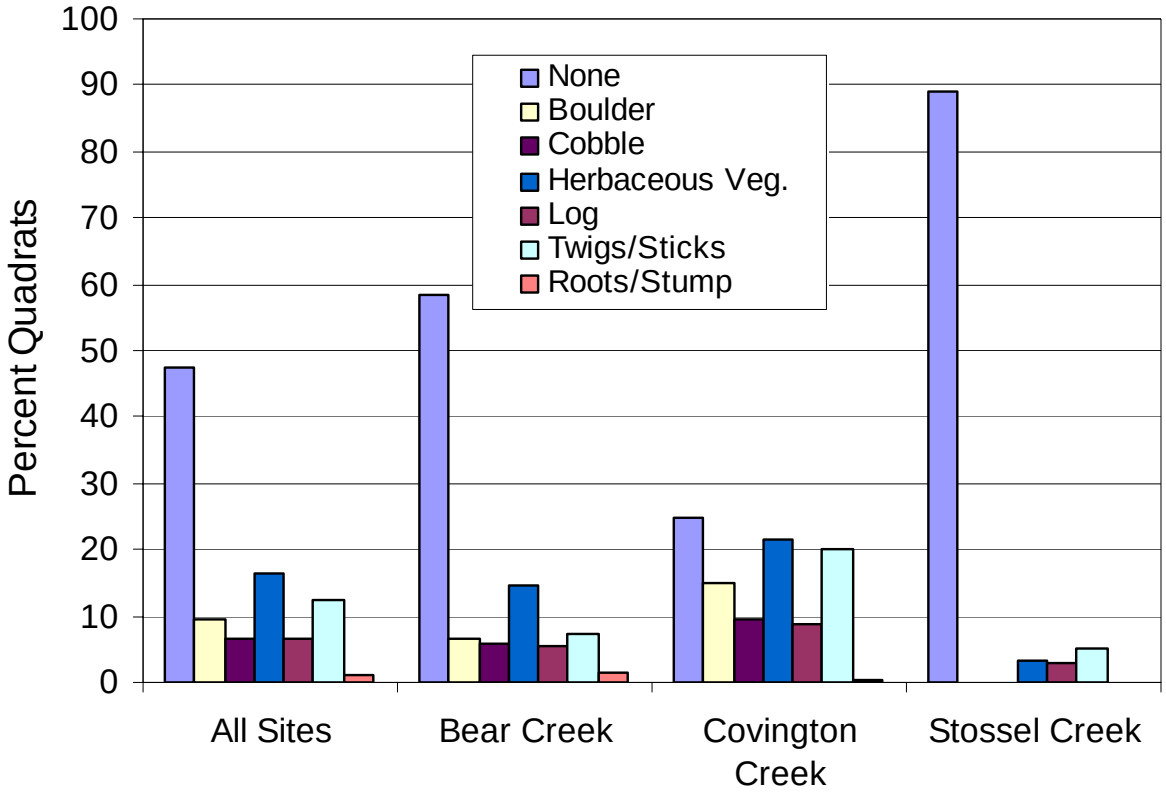


**Figure 9. Percentage of habitat structure in quadrats within each 100 m survey reach and all sites combined.**

More than 70 percent of the mussels in Bear and Stossel creeks were found in quadrats where no habitat structure was present (Figure 10). In Covington Creek mussels were relatively evenly distributed between the available habitat structure types.

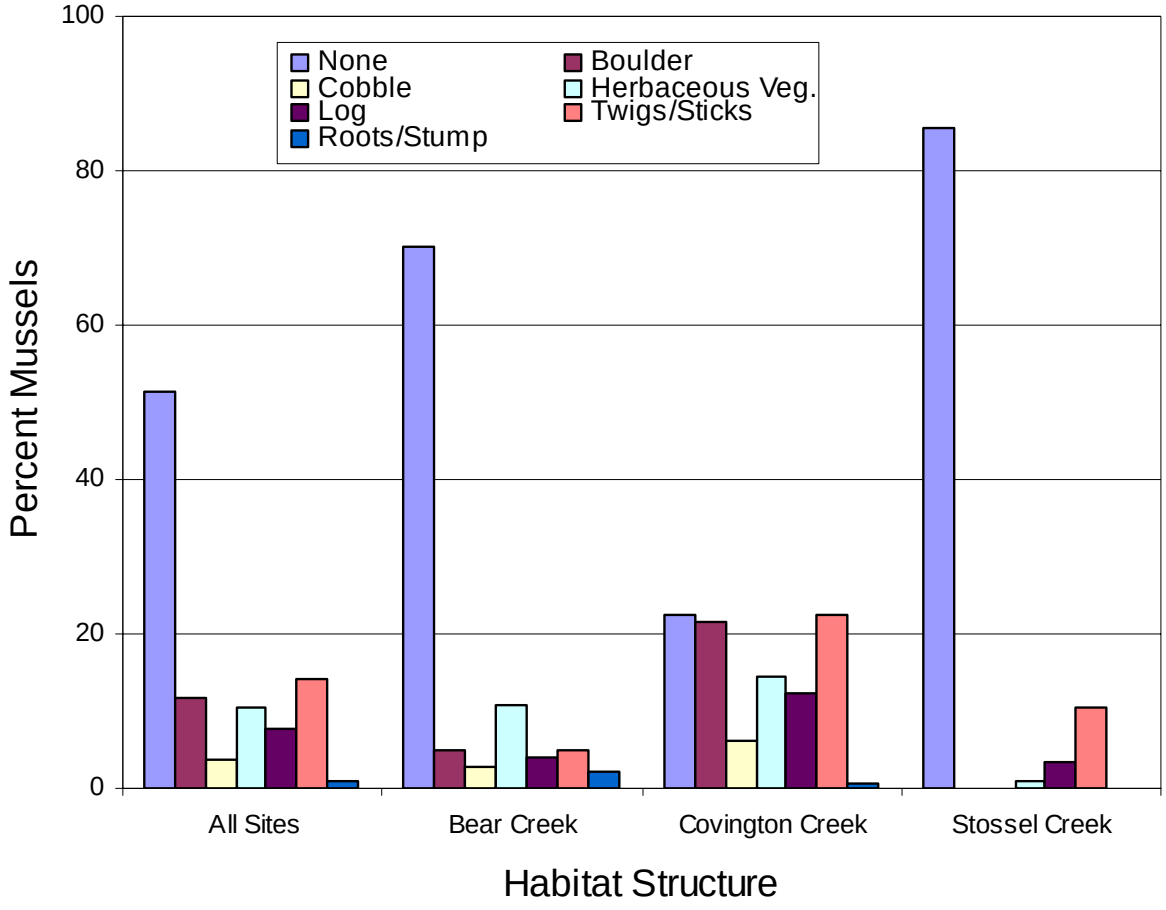


**Figure 10. Percent of mussels present in different habitat structure types for all sites combined and individual sites.**

Riparian cover was somewhat similar across all three study sites (Figure 11). The percent of quadrats with cover ranged from 76 percent at Stossel Creek to 57 percent at Covington Creek (Figure 11). The distribution of mussels was generally greater in quadrats where riparian cover was present (Figure 12).

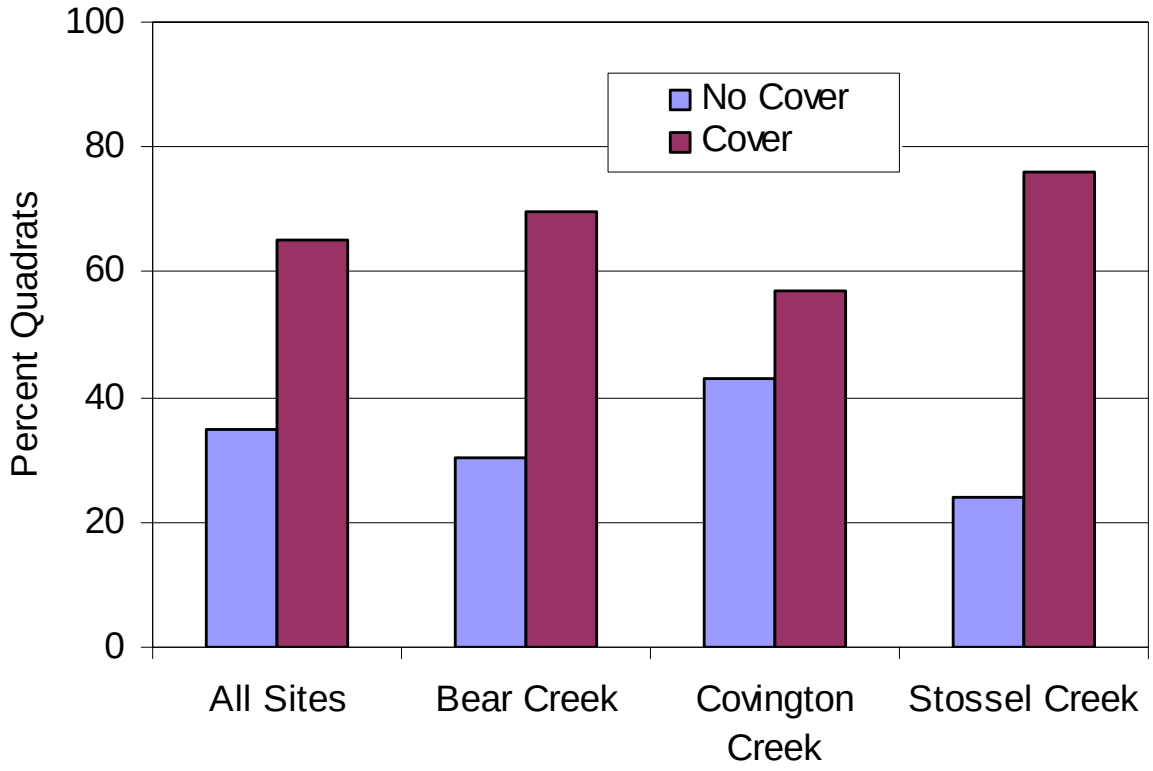


**Figure 11. Percentage of riparian cover in quadrats within each 100 m survey reach and all sites combined.**

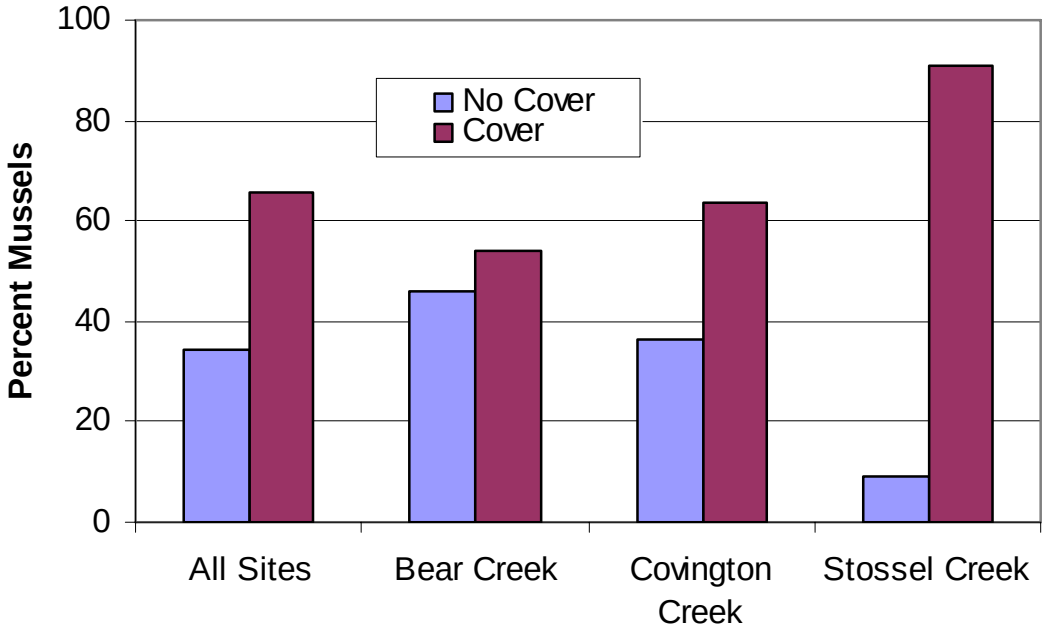


**Figure 12. Percent of mussels present in the presence or absence of riparian cover.**

To better understand the distribution of mussels present within the available habitat types at each of the three study streams, data were statistically evaluated to determine if there were significant differences between sites and the available habitat within each study reach.

Q-Q plots were developed to determine if the abundance data were normally distributed (Tables 1a-g, Appendix C). The plots indicated that the data were not normally distributed. Therefore, a Kruskal-Wallis (K-W) test using a 90 percent confidence level was used to determine it mussel density was statistically different between streams and if mussels show a preference for specific habitat variables (water type, substrate, structure, or riparian cover). Mann Whitney tests with Bonferroni adjustments were then used to identify specific differences identified by the K-W tests.

The results of the K-W tests (Table 7) indicate there are statistical differences in mussel density between the three streams and based on the presence of different habitat features within the streams. The K-W also indicates that water type, substrate type and habitat structure are

significant variables in determining mussel density per quadrat in the three study streams with the exception of Bear Creek when compared to habitat structure. When all site data were combined, mussel density was not significantly different when evaluated against the riparian cover conditions. However, when evaluated by individual site, there were significant differences in density when evaluated against the different riparian conditions.

**Table 7. Results of analysis using Kruskal Wallis with a confidence interval of 90% to determine statistical differences between mussel density per quadrat by site and habitat characteristics.**

**Water type** characteristics include riffle, glide and pool, **Substrat**e types include cobble, gravel, litter, mud and sand; **Habitat Structure** types includes boulder, cobble, logs, twigs, herbaceous vegetation, roots and none present; and **Riparian Cover** is represented as presence or absence. Live plants providing riparian cover are presented in Appendix G.

| | Statistically Different? | | | |
|---|---|---|---|---|
| | All N=1807 | Bear Creek N= 606 | Covington Creek N=836 | Stossel Creek N=365 |
| **Stream** | Yes | N/A | N/A | N/A |
| **Water Type** | Yes | Yes | Yes | Yes |
| **Substrate Type** | Yes | Yes | Yes | Yes |
| **Habitat Structure** | Yes | No | Yes | Yes |
| **Riparian Cover** | No | Yes | Yes | Yes |

N/A – Not applicable

To determine which specific habitat variables influence mussel density, a Mann-Whitney test with a Bonferroni adjustment was used. Results of this test indicate mussel density per quadrat in Stossel Creek was statistically greater than in Covington Creek which was greater than density in Bear Creek (Table 8). When all site data were combined, mussel density was statistically different in the water types (riffle, glide, and pool) evaluated; density was highest in glides and lowest in pools. A similar density pattern was detected in Bear and Covington creeks. However, in Stossel Creek density was greatest in riffles followed by pools and then glides. This result is interesting in that Stossel Creek contained a limited amount of available riffle habitat (12%). Quadrats with no riparian cover in both Bear and Covington creeks supported a significantly greater mussel density than was observed in Stossel Creek.

**Table 8. Result of Mann-Whitney test with Bonferroni adjustment to determine statistical differences between mussel density by quadrat and specific habitat features.**

**Stream characteristics include riffle, glide and pool, Substrate types include cobble, gravel, litter, mud and sand, and Habitat structure includes boulder, cobble, logs, twigs, herbaceous vegetation, roots and none present and Riparian Cover is represented as presence or absence (P<0.10).**

| | Statistical Differences | | | |
|---|---|---|---|---|
| | All (N=1807) | Bear Creek (N=606) | Covington Creek (N=836) | Stossel Creek (N=365) |
| **Stream** | ST>CV>BR | N/A | N/A | N/A |
| **Water Type** | G>R>P | G>R>P | G>R>P | R>P>G |
| **Substrate Type** | (GR,L,S) >(C,M) | (GR,S)>L>C>M | (GR,M,S)>(L) >C | (GR,L)>(M,S) No CB |
| **Habitat Structure** | (N,T)>(B,LG,RT)>CB,HV | No | B>(N, LG,T,RT,C,HV) | T>(HV,L)>N No CB or RT |
| **Riparian Cover** | N/A | No>Yes | No>Yes | No<Yes |

ST – Stossel Creek, BR – Bear Creek, CV- Covington Creek.
R-riffle, G-glide, P-pool
C-cobble, GR-gravel, L-litter, M-mud, S-sand
B-boulder, CB-cobble, LG-log, T-twigs, HV-herbaceous vegetation, RT-roots, N- none
No – no riparian cover; Yes – riparian cover present
N/A not applicable

Gravel substrate supported some of the greatest mussel densities. When all mussel density data were combined, substrate consisting of gravel, litter, and sand supported statistically greater mussel densities per quadrat than cobble or mud. In Bear Creek, gravel and sand substrate supported statistically greater mussel densities than litter, cobble, and mud substrates. Mussel density distribution in Covington Creek was statistically higher in gravel, sand, and mud and lowest in cobble. In Stossel Creek, mussel density in gravel and litter substrate was significantly greater than densities measured in mud and sand substrates.

In general, the data collected by this survey indicate that mussels prefer gravel substrates. However, it was difficult to determine if this was a function of the available substrate or a true preference. Statistical analysis indicated there were no statistical differences between mussel density in gravel and sand substrates in Bear Creek and gravel and litter in Stossel Creek. At two of the survey sites mussels were evenly distributed across the available substrate; only at Stossel Creek were the presence of mussels in a particular substrate greater than the substrate available.

This conclusion is supported by the statistical analysis which indicated there were no statistical differences between gravel and sand in Bear Creek and gravel and litter in Stossel Creek. In Covington Creek, mussel density was statistically greater in sand. In addition, Toy (1998) found that Bear Creek mussel density was greatest in a mixture of sand and gravel.

When all density per quadrat data were combined, the statistical analysis indicated mussel density associated with habitat structure that included twigs and no substrate was significantly greater than mussel density when structure included boulders, logs, or roots. Preference for a particular habitat structure in the three study reaches was highly variable. In Covington Creek, mussel density in quadrats that contained boulders was statistically greater than those that contained the five other structure types evaluated. In Stossel Creek, mussel density was statistically greater in habitat containing twigs, and lowest where no habitat structure was present. No statistically significant differences in mussel density were detected between mussel density and the presence of the five different structure types evaluated in Bear Creek.

When all site data were combined, mussel density was statistically higher in glides and lowest in pools. However, when individual streams were evaluated, the greatest mussel density in Stossel Creek was found in riffle habitat. This inconsistency is likely the result of the limited riffle habitat in this stream. Of the three study streams, Stossel had the lowest percentage of riffle habitat at 12 percent. Based on the available data, habitat structure features (roots, twigs, logs, cobble, boulder, herbaceous vegetation) are not having a strong influence on mussel density in the three study streams. There were no statistical differences in density associated with any of the habitat features in Bear and Stossel creeks, whereas density was statistically greater in boulder habitat in Covington Creek when compared to other habitat structure features. For Bear Creek these data contrast with the findings of Toy (1998) who found mussel density was greater in areas associated with large woody debris and areas with small plunge pools.

In summary, with the possible exception of gravel substrate, there does not appear to be a consistent trend in the influence of physical habitat on mussel density in the three study streams. Based on this analysis, there does not appear to be an obvious in-stream physical characteristic that is influencing mussel density in the three study streams.

## 3.3 Mussel Age and Size

Mussel age was to be determined by counting the “rest” bands, or growth annuli, in the ligaments. It is assumed that one rest band is laid down by the mussel each year. However, enumerating these rest marks proved to be an unreliable method because rest marks laid down after the spawning season and those laid down when the animal was under other stress could not be differentiated. Abrupt changes in the distance between the rest marks suggested that a factor other than annual climate fluctuations may be impacting the growth of some mussels.

If the ligament is intact, mussel age could also be determined by examining only the ligament. However, because the older part of the ligament of a *M. falcata* can typically be eroded, the number of annuli lost in that portion of the shell must be estimated by counting the number of annuli in ligaments of similar lengths from younger mussels. This method assumes that young mussels currently present are growing at the same rate as mussels were growing historically in the streams.

Two obstacles to using the ligament method to determine age became apparent. First, annuli were more difficult to identify in older mussels. The annuli in the ligaments produced when the mussel was young were easily identified; however, marks in the older portion became more difficult to enumerate due to presence of lighter bands, or false annuli occurring between the thicker annuli; in some cases, they appeared very similar. The difficulty in differentiating between real and false annuli did not diminish with higher magnification. To better understand this issue, other mussel specialists are being contacted for advice and recommendation on how to more accurately determine age. As a result of these technical difficulties, it was not possible to determine age of the mussels at this time. Other mussels researchers have been contacted and additional methods of age analysis are currently being explored. These data and any associated statistical analysis will be presented in a future document.

Mussel size was determined by measuring the long axis of the shell of each animal to the nearest millimeter. Because mussel size is not always directly correlated with age, and mussels of the same age from different water bodies may exhibit different length-age distributions simple length measurements cannot necessarily be used to estimate age (T. Frest, 2005 personal communication).

### 3.3.1 Analysis of Sampling Methodology and Length

As previously discussed, when enumerating mussels, 10% of the quadrats were sieved (in addition to picking) to evaluate any potential enumeration bias in the sampling method. An additional purpose for sieving a portion of the quadrats was to determine if the sampling method was biased to larger organisms. To make that determination, mussel length in sieved plus picked and picked only quadrats was analyzed. Quartile-Quartile (Q-Q) plots (Appendix C, Tables 2a-g) indicated the data were normally distributed with the exception of Stossel Creek. Therefore, application of a T-test to determine statistical difference was appropriate except for data from Stossel Creek, for which a Mann-Whitney test was used. T-test and Mann-Whitney test results suggested that mussels in picked quadrats were not significantly larger than those found in quadrats that were sieved and picked. The average length of the 1630 mussels in the un-sieved quadrats was 75.8 mm and the average length of the 272 mussels recovered by sieving was 74.2 mm (Table 9).

**Table 9. Analysis of statistical differences between mussel length in sieved plus picked and picked only quadrats by individual site and all sites combined using a T-Test (P>0.1) for all sites except Stossel Creek for which the Mann-Whitney test was used.**

| Data Set | Mean Length of Mussels per Quadrat | | Statistically Different? |
|---|---|---|---|
| | Picked Only | Picked and Sieved | |
| **All Sites** | 75.8 (N=1630) | 74.2 (N=272) | No |
| **Bear Creek** | 84.4 (N=780) | 83.0 (N=83) | No |
| **Covington Creek** | 67.5 (N=688) | 66.3 (N=73) | No |
| **Stossel Creek** | 80.5 (N= 162) | 76.9 (N=18) | No |

Based on this statistical analysis it does not appear that the sampling method was biased toward a particular size classification. However, to better evaluate if there were statistical differences, the data for only the sieved plus picked quadrats were statistically analyzed using a t- test with a confidence level of 90%. Based on this analysis there were statistical differences in length; the mussels that were identified by the additional sieving step were significantly smaller than the mussels identified by picking only. As such it appears that the picking only method was somewhat biased towards identification of larger organisms.

Although the sieving step resulted in identification of significantly smaller mussels, it was decided that these data (the sieved plus picked quadrats) would be used in further statistical analysis of length data. The reasoning behind this decision was that the smaller lengths identified in the sieved only quadrats indicate the probability that in the remaining 90% of the quadrats, some of the smaller mussels were missed. As a result, the average mussel length was likely overestimated. However, elimination of the sieved portion of the sample from any statistical analysis would increase any error associated with that estimation.

### 3.3.2 Influence of Habitat Factors on Length

To determine if mussel length was influenced by habitat variables, a series of univariate ANOVAs using a $p < 0.10$ were applied to the length data. Length data from all sites combined and individual sites were compared to a number of habitat variables including water type, substrate type, habitat structure, and riparian cover (Table 10).

**Table 10. Results from a series of univariate ANOVAs to identify statistical differences between mussel length within the three study streams and any differences in mussel length within the different habitat types in the individual study streams (P< 0.1).**

| | Statistically Significant? | | | |
|---|---|---|---|---|
| | **All** | **Bear Creek** | **Covington Creek** | **Stossel Creek** |
| **Stream** | Yes | N/A | N/A | N/A |
| **Water type** | No | Yes | No | No |
| **Substrate Type** | Yes | Yes | Yes | Yes |
| **Habitat Structure** | Yes | No | No | No |
| **Riparian Cover** | No | Yes | No | No |

N/A - Not Applicable

When all site data were combined, the statistical results indicated that mussel length was significantly different between the three study streams, within the different substrate types, and within the different habitat structures. When the individual site data were evaluated by the results were less consistent. Mussel length observed in the different water types and riparian cover types were only statistically different in Bear Creek; no statistical differences were detected between water type or riparian cover and length in the other two study streams. Length was not significantly different in the different habitat structure types at any of the three study sites. Substrate type seems to have the greatest influence on length; there were statistical differences in length at all three locations when compared to substrate type. To further evaluate these data a series s of univariate ANOVAs were applied to the data (Table 11).

**Table 11. Results from a series of univariate ANOVAs to evaluate statistical differences between size of mussels and the different habitat features.**

| | Statistical Significance | | | |
|---|---|---|---|---|
| | **All Sites Combined** | **Bear Creek** | **Covington Creek** | **Stossel Creek** |
| **Stream** | BR>ST>CV | N/A | N/A | N/A |
| **Water type** | N/A | (G,R)>P | N/A | N/A |
| **Substrate Type** | L>GR>(C,M,S) | L>(GR,M,S)>C | No | (GR,M)>(L,S) |
| **Habitat Structure** | (RT,N) >(CB,HV)>(B,L,T) | N/A | N/A | N/A |
| **Riparian Cover** | N/A | N>Y | N/A | N/A |

ST – Stossel Creek, BR – Bear Creek, CV- Covington Creek
R-riffle, G-glide, P-pool
C-cobble, GR-gravel, L-litter, M-mud, S-sand
B-boulder, CB-cobble, LG-log, T-twigs, HV-herbaceous vegetation, RT-roots, N- none
N – No Riparian Cover, Y – Riparian Cover

Statistical analyses indicated that mussel length is significantly different in the three study streams; with Bear Creek having the largest and Covington having the smallest mussels. This finding is not surprising as a number of site specific variables can influence growth. Temperature is a key factor for growth, and food availability is another factor; however, mussels have a relatively low metabolic rate and are able to grow in water bodies with low food availability (Toy 1998).

In Bear Creek, the only site where mussel size was significantly different in the three water types, mussel length in glide and riffle area were significantly greater than in pool areas. Areas in Bear Creek with no riparian cover contained significantly larger mussels than areas with riparian cover. Although the different substrate types supported significantly different lengths of mussels, there was not a consistent pattern in the substrate types that contained significantly larger or smaller organisms. When all sites are combined, litter substrate contained significantly larger mussels than gravel substrate; whereas length of mussel in gravel substrate were significantly larger than organisms found in either cobble, mud or sand. In Bear Creek, length of mussels in litter substrate were significantly greater than those in gravel, mud or sand; whereas length of mussels in gravel, mud, or sand were significantly larger than those found in cobble substrates. In Stossel Creek mussels found in gravel and mud substrates were significantly larger than those in litter and sand. No statistical differences in length associated with the different substrates were detected in Covington Creek.

In summary, although there were statistical differences in mussel length found in the different substrate types, no consistent habitat influences were observed in the three study streams. Mussel length in Bear Creek appears to be more influenced by the various habitat features than was observed in the other two study streams, and mussel length in Stossel Creek did not seem to be significantly influenced by any of the habitat factors evaluated.

## 3.4 Sex Ratios

The expected ratio of male to female mussels in a healthy adult population is assumed to be about 1:1 (Baird 2000). *M. falcata* are generally sexually differentiated at the age of 7-15 yrs (Nedeau et al. 2005; T. Frest, 2005. personal communication). As previously discussed, mussel collection was stratified by size class with the intent of collecting an even size and age distribution of organisms across all sample locations. To account for organisms that may not have reached sexual maturity, size class information was used to interpret the gender ratio assessment. However, the size range present in the three study creeks varied, and in some cases did not overlap at all sites. The gender of each of the 67 mussels collected from the three study sites was determined by examining gamete material and is presented in Table 12.

**Table 12. Gender data and sex ratios for mussels collected in the three study streams.**

| | All Sites N=67 | Bear Creek N=21 | Covington Creek N=23 | Stossel Creek N=23 |
|---|---|---|---|---|
| **Male** | 34 (51%) | 12 (57%) | 11 (49%) | 11 (49%) |
| **Female** | 20 (30%) | 3 (14%) | 9 (39%) | 8 (35%) |
| **Undistinguished** | 13 (19%) | 6 (29%) | 3 (13%) | 4 (17%) |
| **M:F** | 1.7 : 1 | 4 : 1 | 1.2 : 1 | 1.4 : 1 |

The percent males (51%) at all study sites combined was greater than the percent females (30%). This was especially the case in Bear Creek, where the male to female ratio was 4:1. Sex ratios in Covington and Stossel Creeks were similar to what would be expected; ratios in these streams were 1.2:1 and 1.4:1 respectively.

In Stossel and Covington creeks the “undistinguishable” mussels were small (<40mm) and it is likely that these animals had not sexually differentiated at the time of collection. In future studies, it is recommended that mussels less than 50 mm not be included in gender determination and sex ratio evaluations.

The Bear Creek results were quite different from those of the other two study creeks. Of the 21 mussels evaluated, 12 were male, 3 were female, and 6 were undetermined. Four of the undetermined animals were between 73 and 82 mm and the two were 96 and 101mm long.

Based on the size of these organisms, it is unlikely that the absence of sex cells can be attributed to age. Due to the relatively small sample size, statistical analysis was not conducted on the sex ratio data.

Although the sample size was small (n=6), the histological assessment may support the Bear Creek findings (see "Histological Assessment" below). All six of the mussels collected from Bear Creek for the histological assessment showed only development of male reproductive structures. In contrast, three of the four mussels collected from Stossel Creek for histological assessment were female; one mussel was classified as hermaphroditic. Sex could not be determined in six of the Bear Creek mussels; four of these were large enough to assume they should have been sexually differentiated. It is possible that these mussels could be exhibiting hermaphroditic characteristics; however, this could not be verified. The cause of this observed ratio is unknown; and could be caused by a variety of stressors. Lack of females would likely influence the reproductive capacity of the population, and may in part, be contributing to the decreasing mussel density observed in Bear Creek. In 1997 Toy (1998) observed a 1.5:1 ratio of M: F in bear Creek, and found that hermaphrodites made up less than 1 percent of the population. Although the sex ratio observed by Toy (1998) is slightly skewed toward males, it is much less so than the 4:1 ratio observed in this study. However, Toy (1998) had a much larger sample size (n=233) than was used in this study (n=21-23); this difference in sample size may account for some of the observed differences.

To better understand the differences in sex ratios between sites, the probability of achieving the observed sex ratio was estimated (Table 13). Organisms where sex could not be determined were not included in this analysis. The probabilities presented in the last column in Table 13 below indicate the chances (percent chance) of identifying the number of females that were observed if the population actually had 50% females. If there really were 50% females present in Bear Creek the probability that you would observe a sample with only 3 of 15 being female is 1.4%.

**Table 13. Probability of seeing the sex rations observed in the three study streams determined by using a binomial probability test; analysis assumes an equal likelihood of both sexes occurring in the population (sex ratio 1:1).**

| Stream | Observed No. of Females | Observed No. of Males | Total No. Mussels[1] | Probability of sample draw if population is 50% Female |
|---|---|---|---|---|
| **Bear Creek** | 3 | 12 | 15 | 1.4% |
| **Stossel Creek** | 8 | 11 | 19 | 14% |
| **Covington Creek** | 9 | 11 | 20 | 16% |

[1] Does not include mussels where sex could not be determined.

## 3.4 Shell Erosion and Characteristics

As previously indicated, all mussels were digitally photographed; 10 percent of the photos from each study reach were used to estimate degree of shell erosion. Table 14 provides a summary of these data. The highest degree of shell erosion was observed on mussels from Covington Creek (maximum - 45.2%, mean -16.9%) and the lowest was observed in Stossel Creek (maximum = 10.4%, mean 3.3%).

**Table 14. Summary of percent shell erosion data at all sites combined and individual sites.**

| | Percent Shell Erosion | | | |
|---|---|---|---|---|
| | **N** | **Min %** | **Max %** | **Mean % (SD)** |
| **All Sites** | 192 | 0.0 | 45.3 | 12.0 (9.0) |
| **Bear Creek** | 64 | 1.2 | 36.4 | 15.7 (9.2) |
| **Stossel Creek** | 40 | 0.0 | 10.4 | 3.3 (2.7) |
| **Covington Creek** | 88 | 7.1 | 45.2 | 16.9 (8.6) |

SD – Standard deviation

The Q-Q plots (Figure 3. Appendix B) indicated the data were normally distributed and therefore could be statistically evaluated using a univariate statistical analysis with a confidence level of 90 percent, comparing percent erosion to stream reach, substrate type, water type, habitat structure and mussel length. Riparian cover was not statistically evaluated, as it was not expected to be a contributing factor to shell erosion. Table 15 outlines the results of these statistical analyses.

**Table 15. Results of a series of multiple analyses of variance, (MANOVA) to determine statistical differences between shell erosion, study stream, habitat factors and length groups (p<0.10).**

| | Statistically Different? | | | |
|---|---|---|---|---|
| | All Data Combined | Bear Creek | Covington Creek | Stossel Creek |
| **Stream** | Yes | N/A | N/A | N/A |
| **Water Type** | Yes | No | Yes | Yes |
| **Substrate Type** | No | No | No | No |
| **Habitat Structure** | No | No | No | No |
| **Length Group[1]** | Yes | Yes | No | Yes |

[1] Mussels were sorted into 10 mm length groups ranging from <30 mm to 110 mm.

When all site data are combined, significant differences in percent shell erosion in the three streams , water types, length groups and riparian cover were identified but none among the sediment types or habitat structures. When each stream was evaluated individually, it was shown that erosion was significantly influenced by water type in Covington and Stossel Creeks and that there were significant differences among the shell length groups Bear-Evans and Stossel Creeks. Shell erosion was not significantly influenced by riparian cover in any of the sites

To identify specific causes for differences in shell erosion, Tukey's multiple comparison test was applied (Table 16). When all three sites were compared for percent erosion, each was significantly different from the others. The Covington Creek mussels were significantly more eroded than the mussels from the other two and the Bear Creek mussels were more eroded than those from Stossel Creek. The percent erosion was significantly greater on mussels from the fastest moving water, riffles. The mussels from the slow-moving pools were significantly less eroded than either of the other water types. The percent erosion on mussels in some length groups seemed to be significantly different from the others. Mussels less than 40 mm long were significantly less eroded than the other groups while those between 50 mm and 70 mm and those over 99 mm were significantly more eroded than those from other groups. In general, larger mussels were more eroded than smaller mussels, presumably because they are older. The Tukey results from the individual sites identified the same significant differences but not at all sites. The erosion increased with water velocity only at Covington Creek. The 100-110 mm mussels at Bear Creek were more eroded than the 70-80 mm mussels.

**Table 16. Results of a Tukey's multiple comparison test to identify significant differences in shell erosion and habitat elements and length group (P<0.01).**

| | Statistical Differences | | | |
|---|---|---|---|---|
| | **All Data Combined** | **Bear Creek** | **Covington Creek** | **Stossel Creek** |
| **Stream** | CV>BR>ST | N/A | N/A | N/A |
| **Water Type** | R>G>P | No | R>G>P | No |
| **Substrate Type** | N/A | No | No | No |
| **Habitat Structure** | No | No | No | No |
| **Length Group[1]** | (60,100) > (50,110s)>(70-90's)>(less than 50mm) | 100's>70's | No | No |

[1] Mussels were sorted into 10 mm groups ranging from <30mm – 110 mm
ST – Stossel Creek, BR – Bear Creek, CV- Covington Creek.
R-riffle, G-glide, P-pool
C-cobble, GR-gravel, L-litter, M-mud, S-sand
B-boulder, CB-cobble, LG-log, T-twigs, HV-herbaceous vegetation, RT-roots, N- none
N – No Riparian Cover, Y – Riparian Cover
N/A – Not applicable

Statistical analysis of the shell erosion data indicate the degree of erosion between the three sites is significantly different, with mussels in Bear and Covington creeks having a greater degree of erosion than those in Stossel Creek. Analysis of the influence of habitat variables and the degree of erosion indicated that most habitat variables did not have a significant influence on erosion; there were no differences observed in Stossel Creek. As previously indicated, water quality parameters such as pH and hardness may influence the degree of erosion observed in *M. falcata*. Unfortunately there are no co-located water quality data available for the study sites. In addition, mussel age will likely influence the degree of erosion; however, as previously discussed, age could not be reliably determined at this time. Stream velocity can also influence the degree of erosion; visual field observations suggest that velocity in Stossel Creek is less than that observed at both Bear and Covington creeks. This physical characteristic, may in part, have influenced the low degree of erosion observed on Stossel Creek mussels.

The nacre color of each of the sacrificed mussels was also recorded. Twenty of the Bear Creek shells were purple and one was white. Other features of the Bear Creek shells indicated potential signs of ill health. The nacre on all shells was dull and the color on the inside surface was mottled on one or both valves of nineteen, but was uniform on only two of the shells. The inside surfaces of ten of the shell pairs were generally dull-white. There were soft and chalky areas at various locations along the ventral edge, suggesting that the mantle was unable to produce sufficient, if any, nacre. The periostracum on many of those shells could be easily chipped off because the erosion undercut the periostracum. Rather than maintaining an oval configuration starting at the umbo, the eroded patch has serpentine, tunnel-like extensions beneath the

periostracum. This condition exacerbates the loss of the protective periostracum. Three of the Bear Creek shell pairs were nearly perforated at the site of attachment of the adductor mussels and one was quite thin at the umbo. In general, the Bear Creek animals appeared to be in poor condition.

All of the Covington Creek shells had purple nacre and the surface was generally shiny but the distribution was irregular, or mottled on 18 of the 23 mussels were colleted. Blisters on the interior surface were noted in nine of the shells. Blisters are formed by the mussel when a foreign body becomes embedded between the mantle and the inner surface of the shell. The animal lays down several layers of nacre in order to cover the irritant. If the irritating body remains lodged against the shell, a blister is formed, but if the body is loose but remains under the mantle, all sides of the body will be covered with nacre, forming a pearl. While the shells in Covington Creek are generally in better condition than those collected from Bear Creek, visual observation indicates they do show some signs of stress.

Usually, nacre color is consistent within a population of mussels and is sometimes used to differentiate among groups. The nacre color of 16 of the 23 Stossel Creek mussels was deep purple, six were salmon colored, and one was white. In all but the white shell, color was very evenly distributed and the nacre surface in all shells was bright. These shells show no obvious defects, which is consistent with the low level of outer shell erosion observed for the Stossel creek mussels.

## 3.6 Histological Assessment

In general, mussels from Bear Creek were in poor condition. These mussels exhibited lesions that are consistent with morbidity and mortality. The observed gill lesions were associated with bacterial colonies that could have contributed to loss of gill tissue. The severe loss of gill epithelium suggests that these lesions may be important as a causation factor in the observed morbidity and mortality. Examination of the digestive gland also indicated a condition of stress. A lack of ovarian development was also observed in the Bear Creek mussels; of the six mussels evaluated, only development of male reproductive structures was observed.

The condition of Stossel Creek mussels was generally normal; however, there was some evidence of chitin hyperplasia (an abnormal increase in the number of cells in an organ or tissue) and cysts. This finding is consistent with the infection process, and a few specimens had cellular structure that suggested microsporidian parasites.

The poor condition of these organisms is not necessarily a response to a single stressor but possibly the result of exposure to a variety of chemical and physical stressors, and possibly over a long period of time. Mussels in a weakened condition such as these would be expected to be more susceptible to opportunistic bacterial infections which appear to be associated with the loss of essential gill structure and brown deposits on the internal gill structure. In contrast, histological evaluation of four mussels from Stossel Creek indicated they were relatively healthy.

A more detailed account of the histological examination can be found in Appendix D (Elston 2004). In summary, the histological assessment suggests that the mussels examined from Bear Creek are stressed. The particular cause of this stress is unknown, but is likely to be a

combination of chemical and physical factors. Because of its location in a relatively intact watershed, Bear Creek is not likely being impacted by a point source or single source of pollution or stress. Increasing development pressure in the basin, in addition to non-point source associated contaminants (storm water, septic systems etc), are likely combining to cause the observed stress.

## 3.7 Analysis of Mark and Recapture Methods

Tagged mussels were retrieved between September 12 and 24, 2005. However, the data analysis has not been completed at this time and therefore is not included here. Results of this portion of the assessment will be presented in a future document.

# 4.0 CONCLUSIONS

This pilot survey successfully assesses the relative health of freshwater mussels in three stream reaches in King County: Bear, Covington, and Stossel creeks. Freshwater mussels in one stream reach in particular, on upper Bear Creek, have been previously studied and have been identified as being of concern for increased mortality and population decline. While many possible questions remain, this study confirms these concerns regarding the decline in freshwater mussels in upper Bear Creek. Key findings of this study are summarized below.

## 4.1 Mussel Density and Distribution

Assessment of mussel distribution in the three study streams indicated that in general, distribution is heterogeneous and more than 60 percent of the quadrats evaluated did not contain mussels. Mussel density in the three study reaches was variable and averaged 17/$m^2$. Stossel Creek supported the greatest density of mussels (35/$m^2$), followed by Covington Creek (19/$m^2$) and Bear Creek (11/$m^2$). Based on the findings that the sampling method may underestimate mussel abundance, these values may underestimate the actual mussel density at these locations. Based on all site data combined, the quadrats where mussels were located by hand picking from the stream bottom had approximately 30 percent fewer mussels than those quadrats where mussels were first hand-picked from the bottom and additional mussels were collected by sieving the bottom; on an individual site basis there was a 14 percent difference at Bear Creek, 40 percent at Covington Creek and 37 percent at Stossel Creek.

## 4.2 Changes in Mussel Density over Time

Toy (1998), who enumerated mussels by visual observation, estimated that mussel density in a reach of Bear Creek in the general vicinity of this study was 56/$m^2$. Even taking into account the average 30 percent underestimate of mussels in this study, mussel density in Bear Creek estimated by this study suggests a decline in the mussel density in this general area over the last 7 years.

## 4.3 Habitat Preference

Comparison of mussel density with a variety of habitat variables suggests that habitat does not consistently influence density across the three study reaches. In Covington and Bear creeks mussel density was significantly greater in glides and lowest in pools. Stossel Creek supported a significantly greater density in riffle habitat even though this study reach contained the lowest amount (11 percent) of available riffle habitat.

In general, the data indicate that mussels in the three study reaches prefer gravel substrate. However, it was difficult to determine if this was a function of available substrate or a true preference. Toy (1998) found that Bear Creek mussel density was greatest in a mixture of sand and gravel.

Preference of mussels for a particular habitat structure type was highly variable. No statistical differences in mussel density were detected between the five different habitat structures types evaluated in Bear Creek. The assessment of the influence of riparian cover on density also resulted in variable results. In two of the study reaches density was greater when riparian cover was not present; only density in Stossel Creek was significantly greater in the presence of riparian cover.

## 4.4 Mussel Size and Age

This study was unsuccessful in estimating mussel age. Other mussels researchers have been contacted and additional methods of age analysis are currently being explored. These data and any associated statistical analysis will be presented in a future document.

Mussel length was statistically different between the three study stream reaches; Bear Creek contained the largest mussels, whereas Covington Creek supported the smallest mussels. In general, although there were statistical differences in mussel length associated with the various habitat variables evaluated, no consistent habitat influences were identified. Mussel length in Bear Creek appeared to be more influenced by habitat than the other two study streams

## 4.5 Sex Ratios

Sex ratios in a healthy mussel population are expected to be approximately 1:1, male to female. Sex ratios at Covington (1.2:1) and Stossel (1.4:1) creeks were somewhat similar to what would be expected in a healthy population, while the ratio at Bear Creek was dominated by males (4:1). Although the sample size was limited, the data from the histological assessment support this finding: all six of the mussels examined form Bear Creek only exhibited male reproductive structures. In 1997, Toy (1998) observed a 1.5:1 (M:F) sex ratio. Lack of females would likely influence the reproductive capacity of the population and may be contributing to the decreasing mussel density of Bear Creek mussels.

## 4.6 Shell Erosion and Condition

The degree of shell erosion was statistically greater at Bear and Covington creeks than at Stossel Creek. Analysis of the influence of habitat variables on erosion indicted that most habitat variables measured did not have a significant influence on the degree of erosion. Because flow and water quality (pH, hardness, alkalinity) are likely to have the greatest influence on the degree of erosion, any future efforts to evaluate erosion, should also include evaluation of these parameters. Visual assessment of the shell condition indicated that Bear Creek animals appear to be in poor condition, while those in Covington and Stossel are in relatively good condition.

## 4.7 Histology

The histological examination of mussels from Stossel and Bear creeks indicated that Bear Creek animals were in poor condition and severely stressed. Bear Creek mussels had gill lesions and loss of gill tissue. The severe loss of gill epithelium may be a cause for the observed mortality at this site.

## 4.8 Causes for Declines in Upper Bear Creek Mussels

This study did not attempt to identify a specific cause for the decline in freshwater mussels in upper Bear Creek. Multiple factors may affect mussels in small streams. The results of this and other studies, combined with our professional judgment, suggest that the decline is likely caused by some combination of changes in hydrology, changes in water quality, pathogenic infection, lack of filterable food, or clogging of filtering mechanisms, as opposed to predation, lack of reproductive success, or poor physical habitat quality.

## 4.9 Lessons Learned

Assessment of the mussel sampling protocols determined that hand-picking mussels from the streambed (as opposed to sieving the streambed) can underestimate the number of mussels identified in a quadrat or study reach. Addition of the sieving step in 10 percent of the samples indicated that a significantly greater number of mussels are identified when this step is included in the enumeration process. The sieving method also identified significantly smaller organisms. Based on these findings future work should include the use of the sieving step and/or an additional assessment step that can assure that the sampled mussels accurately represent the density and size of mussels within the study area.

Empty mussel shells were not quantified in this study. Based on the observed number of empty shells in the upper Bear Creek reach studied, it is recommended that future studies also quantify this parameter. It is believed that this information would support the overall description of mussel health in upper Bear Creek.

The study was designed to quantify mussels in 5 percent of the area within a 100-meter stream reach. Based on the heterogeneous distribution of mussels in each reach (with over 60 percent of the quadrats having no mussels), a stratified-random design that covers a representative area of the reach and also targets relatively large beds for quantification is recommended for any future work.

## 4.10 Summary

Several results of this study suggest that the mussels within the Bear Creek study reach are declining and in poor health relative to Stossel and Covington creeks. First, Bear Creek had the lowest mussel density ($11/m^2$) compared to Stossel Creek ($35/m^2$) and Covington Creek ($19/m^2$), and comparison of these results to a previous study suggests a substantial decline in the mussel density in upper Bear Creek over the last 7 years. Second, sex ratios in Bear Creek mussels were dominated by males (4:1), whereas sex ratios at Covington (1.2:1) and Stossel (1.4:1) creeks were somewhat similar to what would be expected (near 1:1). Third, histological examination indicated that Bear Creek animals were in poor condition and severely stressed, as evidenced by gill lesions and severe loss of gill tissue. Fourth, while empty mussel shells were not quantified in this study, many empty shells (hundreds) were observed in the upper Bear Creek study reach, while few if any were observed in Stossel and Covington creeks. While overall declines in

freshwater mussel populations through out North America are well documented, we are not aware of other studies that have observed the high mortality and poor histological condition that has been observed in Bear Creek. In contrast, mussels in Stossel and Covington Creeks appear to be relatively unstressed at this time.

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# Appendix A

## Sampling and Analysis Plan for a Pilot Freshwater Mussel Survey in King County

# Sampling and Analysis Plan for a Pilot Freshwater Mussel Survey in King County

August 2004

King County

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# Sampling and Analysis Plan for a Pilot Freshwater Mussel Survey in King County

## Submitted by:

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Table 1. Project Schedule

# 1.0. PROJECT BACKGROUND / INTRODUCTION

## 1.1 Purpose of this project, its general location, timeline and budget

The purpose of this project is to answer questions about the geographic distribution; population density and age distribution of freshwater mussels selected reaches of a few of the streams and rivers of western King County. The target species is *Margaritifera falcata*, the Western Pearlmussel. This animal has been recently sighted in several south King County, or WRIA07 streams (upper Covington creek, Jenkins creek, Newaukum Creek and in the Green River near Flaming Geyser State Park). There are large populations in several tributaries to the Snoqualmie river, WRIA09 (Cherry, Harris and Stossel creeks as well as the Snoqualmie River). In WRIA08, Lake Washington drainage, mussels have been sighted in Taylor Creek, Issaquah Creek and particularly in the upper reaches of Bear and Cottage Creeks. Residents near the beds in upper Bear and Cottage Creek feel that the large mussel populations there are diminishing at an alarming rate. Recent studies of the population in Bear Creek where low numbers of juvenile mussels were encountered indicate that the population is not being replenished and large numbers of empty shells indicate that they may be are dying off at an accelerated rate.

*M. falcata*, like many freshwater mussel species requires an anadromous fish host species to complete their life cycle. The mussel larvae, or glochidia, parisitize the fish by clamping onto the gill filaments where they become encysted for a few weeks after which they emerge and fall to the stream bottom where, if the conditions are favorable, they continue their growth. The likelihood of the conditions being favorable is very low – fewer than one in a ten thousand is expected to survive the juvenile stage (Toy, 1998).

The primary host species are juvenile Chinook and Coho salmon but they have been reported to use rainbow trout (steelhead) and cutthroat trout to a limited degree. Fluctuations in the populations of these host species directly affect the spawning success of the mussels. As in the eastern part of the United States, dams, water pollution and habitat destruction have dramatically affected the populations of host fish species here. Large groups of empty shells being found in-situ in our streams suggest that some contaminant, disease or periodic natural occurrences of lethal conditions are threatening the adult mussels. Reports of dense groups of mussels being found in shaded areas along the banks of streams suggests that removal of riparian vegetation may have an adverse effect on these animals. While these animals do not seem to have light-sensitive organs, they apparently are able to sense heat and apparently will move to avoid open areas of the streambeds.

These animals are important as indicators of general conditions in the streams in which they occur. As effective filter feeders, they positively impact water clarity in both the

streams in which they occur but also in the water bodies in which those streams drain – rivers and lakes. Muskrats, otters and raccoons utilize the adult pearlmussel for food while fish will consume the larvae and juveniles.

The goal of this project is two-fold. First is to locate a few dense beds of the freshwater mussel, *Margaritifera falcata;* document the size and age distributions in each bed and to document the in-stream and adjacent riparian habitats of each bed. The second goal is to determine whether the populations in those beds are stable.

## 1.2 Reference to regulatory program

WDFW is concerned about the population and made it illegal to harvest freshwater mussels beginning in January, 2003.

## 1.3 Brief discussion of project goals and objectives

### 1.3.1 Goal 1: Describe the populations and habitats at three or more sites

The first objective is to locate freshwater mussel beds. Three beds have been located and access has been secured.

- A reach on upper Bear Creek that has been recently surveyed by Washington Trout. (Washington Trout, July 2002).
- Stossel Creek in the Tolt River watershed near Carnation Washington ().
- Ravensdale, or Upper Covington Creek in the Soos Creek Basin.

The second objective is to locate, count, measure and photograph each mussel located in a systematic random sampling scheme appropriate for the population distribution of each mussel bed.

The third objective is to tag some percentage of the animals in a manner that permits the identification of the individual animals collected at a later date.

The fourth objective is determine the ages of several of the animals by counting the "growth lines" in the ligaments of several animals of differing lengths and then estimating the growth rates of those animals, assuming that one "growth line" is produced each year.

Assemble a photographic record of: the distribution of mussels in each quadrat sampled, the surface of the substrate in each quadrat sampled, each of the animals measured.

Sample other nearby in-stream habitats for the presence of juvenile (<20mm) mussels. (It is possible that juvenile and adult mussels occupy different habitats).

Determine the sampling error attributable to animals' burying themselves when sampling is underway. The substrate of a number of quadrat will be sieved after the animals visible at the surface have been counted and removed.

### 1.3.2 Goal 2: Determine whether the mussel populations at the sites sampled are stable

Objective 1: re-sample each site after one or two years.

Objective 2: verify whether that population estimate (density and size) differs significantly from the one made initially.

Objective 3: recover the tagged mussels, re-measure them and photograph each.

Objective 4: verify the growth rate estimates made earlier by comparing the two length measurements.

Objective 5: use the images of each animal to determine the extent of shell erosion initially and when recovered. Those estimates can be used to whether the extent rate of erosion varies among sites.

Geographic distribution? (Among streams) We will begin to measure geographic distribution among WRIA's by observing conditions in one mussel bed in each of three streams, each stream being in a different WRIA.

- WRIA07 (Snoqualmie River) – Stossel Creek
- WRIA08 (Lake Washington) – Bear Creek
- WRIA09 (Green River) – Ravensdale Creek

### 1.3.3 The questions we hope to answer

Which mussels are present? We will identify the mussel species found at the surface of the substrate and return them to the beds. We will examine the mussels found in the sieved samples including the fingernail clams (*Sphaerium* sp. And *Musculium* sp.) and the peaclams (*Pisidium* sp). Population density estimates will not be made for these species.

How many are there in each bed? We will count mussels in an appropriate number of quadrats in each bed and from those counts, estimate the number of animals in each bed. (Only is *Margaritifera falcata* and *Anadonta* sp. will be counted.) Some quadrats will be sieved to determine the proportions of animals that are visible and buried. Note: Each animal found will be measured and photographed.

How old are they? We will take enough live animals from each stream to construct a length/age curve for that stream. The ligaments of those animals will be sectioned and the "growth rings" will be counted. The sections will be photographed.

Tagged animals recovered in later surveys will be re-measured in order to determine their growth rates. Growth rings laid down during that period will be examined in order to verify whether: (a) that one ring is laid down per year and (2) mussels grow at similar rates in each bed.

Are their numbers stable? We will repeat counts over time to determine whether the populations are declining, remaining stable or even increasing. Age distribution of the population in each stream will shed light on that.

Is shell erosion abnormally high? The total surface area of the shell in each digital image and the area of the eroded portion will be determined and the percentage of the total shell that has been affected by erosion will be determined. The percentage of shell erosion calculated for each bed will be compared to the pH and the velocity of the water to determine whether there a connection is apparent.

## 1.4 Project history

This will be a new study.

# 2.0. PROJECT MANAGEMENT

## 2.1 Roles and responsibilities

Bob Brenner will be the principle investigator with support from Tom Georgianna and Jon Frodge.

## 2.2 Time line / project schedule

Field surveys will be conducted July through September during low-flow

conditions.

Maps will be created in the fall. Aging the mussels will be conducted in the fall.

Findings will be presented in the late winter.

Table 2 describes the sampling schedule.

Table 1: Project Schedule - attached

## 2.3 Project deliverables

- Maps depicting the reaches surveyed and *Margaritifera falcata* population densities found in each.
- A report of the estimate of the extent of shell erosion will be produced.
- Lists of other bivalve species found in each stream will be provided to interested parties.
- A report on the comparison of pattern of growth bands in mussels from each stream and age distribution.

# 3.0. STUDY DESIGN

## 3.1 Statistical Data Quality Objectives

### 3.1.1 Specific study questions to be answered by the data from this project, hypotheses to be tested

- Are animal populations significantly different among streams; by size and by age?
- Are population densities significantly different among stream reaches?
- Does the extent of shell erosion vary significantly among streams and among reaches within steams?
- Are these animals dying at an accelerated rate?

### 3.1.2 Methods to be used in analyzing the data to provide answers

The following questions will be answered by analyzing the data with either t-tests of means or Mann Whitney tests:

- Are animal populations significantly different among streams; by size and by age?
- Among habitats?
- Are population densities significantly different among stream reaches?
- Does the extent of shell erosion vary significantly among streams and among reaches within steams?

Analysis of geographic range will be addressed with mapping software. We will map mussel beds. Beds will be located with GPS and tape measure. The corners of each bed will be located with GPS and the actual length and breadth will be measured by tape measure.

The method for determining the death rate of the animals is not yet determined. It will likely take several years of sampling to have enough data to analyze.

### 3.1.3 Data requirements of the statistical methods

The data requirements of the mussel data are; independence of data points. Additionally t-tests require the data to be normally distributed.

### 3.1.4 Estimated Confidence Level and Power in the statistical tests

There is no data with which to estimate power and confidence levels. The first data collected will allow us to make such estimates. We do; however, have data from other studies that indicate what the relationships of coefficient of variance (CE) are to quadrat size. We will attempt to sample so as to produce CE's of 0.3 or smaller.

Mapping data will require a level of precision, which is discussed below.

### 3.1.5 Sampling strategy to obtain representativeness

Mussel sampling strategy will follow Strayer and Smith, "A Guide to Sampling Freshwater Mussel Populations," American Fisheries Society Monograph 8, Bethesda, Maryland. The likely strategy will be a form of stratified random sampling.

## 3.2 Spatial data quality objectives

### 3.2.1 Methods for choosing locations

- Field notes from previous habitat surveys describing dense mussel beds
- Citizen input
- The existence of all sites will be verified with a field visit

### 3.2.2 Methods used to identify the locations to be included in the sampling

Arcview maps will be used to plan the surveys of each stream. Stream reaches will be identified by WRIA Identification Numbers and River Mile

### 3.2.3 Precision and completeness

This remains to be determined.

### 3.2.4 Spatial resolution

If the landmarks on Arcview maps are correctly located, then the accuracy will be within 5 meters.

## 3.3 Laboratory Data Quality

Not applicable

# 4.0. FIELD METHODS AND TECHNIQUES

## 4.1 Station positioning methods

Hand held GPS units will be used to establish location of landmarks and quadrat corners. Hipchain and compass bearings will be used between landmarks.

## 4.2 Field measurement procedures/protocols if applicable

Protocols in Srayer and Smith will be followed.

### 4.2.1 Sampling scheme

The criteria for selection of a reach will be 1) if possible, down stream from a wetland, 2) mussels are visually present, 3) the reach is accessible. A 100-meter reach in each stream will be established. Each reach will have 20 transects numbered 1-20 in the downstream direction. Adjoining 25cmX25cm quadrats across the width of the stream at each transect will be examined. Each quadrat will be identified by the transect in which it occurs and the distance from the left bank. For mapping purposes, the **uncorrected** magnetic bearing between left-bank ends of each sequential pair of transects will be determined and recorded.

The first transect will be randomly placed within the first five meters of the reach. Thereafter; transects will be five meters apart.

The mussels visible within each quadrat will be counted, measured, photographed and then replaced. Although it is likely that a few other mussel species will be encountered, *Margaritifera falcata*, Western Pearlmussel, is the target species. The presence of other animals will be noted and a few retained as voucher specimens, otherwise, they will be ignored.

The sampling will commence at transect 10. Five mussels in each of five size ranges (25 mussels total) will be retained and sacrificed in order to determine the gender and age distribution of the population. Those size ranges are: <40mm, 40-55mm, 56-70mm, 71-85mm, >85. If all 25 mussels are found at transect 10, then we will move downstream to transect 20, wait for the water to clear and then sample the transects in descending order. If not all of the target mussels are collected at transect 10, the sequence of transects sampled will be 11, then 9, then 12, then 8, etc. until the 25 are collected.

All quadrats located in areas with emergent vegetation will be sieved since the mussels are very difficult to see there regardless of size. The plants will be set aside and the sieved material will be retained so that both can be replaced after sampling has been completed. The names of the dominant plant(s) will be recorded.

### 4.2.2 Habitat Measurements

Stream Configuration. There are three conditions. Riffle is moving water that has a rippled surface as it passes from one pool to the next. A run is moving water with a smooth surface and no accumulation of fine sediment on the bottom. A pool has slowly moving water and has an accumulation of mud and organic material on the bottom.

Sediment size. The quadrat frame is divided into sixteen squares by intersecting wires. The particle size category of the sediment beneath the lower right corner of each square will be determined visually. If the individual particles are too small to distinguish, they will be called mud. Visible particles smaller than 2mm are sand. Particles larger than 2mm will be called gravel.

Shade. Estimate the percent of the day that direct sunlight might be expected to reach the quadrat.

Vegetation. This refers to in-stream vegetation and emergents. The dominant plant in each transect will be identified and recorded.

### 4.2.3 Mussel Measurements

The length of each mussel will be measured to the nearest millimeter.

### 4.2.4 Images

All of the mussels located in each quadrat will be laid out on a template in the order they were measured and a digital image will be taken and the image number will be recorded. The transect number and quadrat location will be included in the image.

### 4.2.5 Sieved quadrats

Some species of mussels can bury themselves so that they are not visible at the surface of the sediment and might be missed during a visual search. In order to determine whether *Margaritifera falcata* exhibit that behavior, at every tenth quadrat, the sediment to a depth of 7.5cm will be passed through a 6mm sieve in order to collect buried mussels. The *Margaritifera falcata* found will be counted, measured, and replaced or retained as are the visible mussels.

### 4.2.6 Sacrificed Mussels

These animals will be kept cool and damp so as to keep them alive until they are delivered to the laboratory. There, the gamete material will be collected and the ligaments split. The soft parts will be discarded but the shells retained. No preservative chemicals will be used in the field.

Sex determination. Twenty-five mussels from each reach will be sacrificed in order to meet this objective. Gamete smears will be made, stained with a mixture of hematotoxylin and eosin dyes and sealed with coverslips. The slides will be examined at 100-400X magnification for the presence of eggs. Slides will be identified by stream, transect, quadrat and mussel number. All slides will be retained.

Age determination. The ligament of each of the retained mussels will be split by sawing with a rotary carving tool and the cut surfaces coated with glycerine. The surfaces will be examined with a dissecting microscope at about 20X magnification and the "growth rings", rest stages, will be counted. The distance between the umbo and the ligament will be recorded. The number of years represented by the missing ligament will be read from a graph as in Hendelberg and Toy. The age will be determined by adding the count and the estimate of the missing ligament. Images will be made of the ligaments.

General Condition. First, the animals will be "towel dried" and immediately weighed to the nearest 0.1g. Then the animal will be suspended in a container of water and the weight change of the container noted. That weight change in grams is equal to the volume of water in cubic centimeters displaced by the mussel. The "towel dry" weight divided by the volume is the density, or specific gravity, of the animal.

After the mussels have been shucked, the liquid will be drained off and the tissue and shells will be weighed separately.

Nacre color. In some areas, the color of the nacre is a distinguishing characteristic of a population. Three colors are common here in the Pacific Northwest: white, purple and salmon.

Shell erosion. This is a concern to some workers in this region but generally appears to be characteristic of this species. It appears that the extent of the erosion is a function of pH, hardness and stream velocity. Erosion is exacerbated by low pH, low hardness and high stream velocity.

The aerial extent of the erosion can estimated by using various photo editing programs. Trace a polygon around the entire shell (W) and another around the eroded area (E). Usually, a histogram function will count the numbers of pixels in each polygon.

The eroded area estimate = 100(E/W).

### 4.2.7 Tagged Mussels

In order to determine how far mussels move over time, twenty mussels in each reach will be tagged by having a BioMark PIT tag affixed to the posterior end of each mussel. The mussels will be chosen from those located at transect number five. Four animals from each of the five length ranges identified above will be collected, tagged and then replaced in the quadrat in which they were found. If not all of the representatives can be collected from transect 5, the data sheets will be scanned for acceptable candidates closest to transect 5. Those animals will be located, tagged and replaced.

Fast-drying Epoxy glue will be used to affix the PIT tags. A narrow strip (1cm X 3cm) of periostracum parallel to the posterior ridge will be removed by sanding until the calcium carbonate shell is exposed. The glue adheres better to the roughened surface while periostracum has a tendency to flake off. The orientation of the tag parallel to the posterior ridge minimizes drag when the animal is moving through sediment or from water during higher flows.

During the summer of 2005 and subsequent years, a PIT tag reader will be used to locate the surviving tags in each reach and the location of each tag will be determined by both GPS technology and distance along the reach measured with a hip-chain or tape.

## 4.3 Field Measurement QC procedures/protocols, requirements, qualifiers

Field Measurement Replicate - A field measurement replicate is defined as a separate in-situ measurement made following all procedures typically done between individual measurements. Ten percent of the quadrats counted will also be sieved after the visible mussels have been extracted in order to determine whether a significant number escape detection by burrowing into the sediment. In order to minimize the likelihood of having to return to the site to collect more data, the coefficient of variance will be calculated on-site to determine whether enough samples have been collected to yield statistical significance.

## 4.4 Future SAP Changes

Some of the techniques of finding, locating, and sampling mussel beds, especially for the animals, which are buried, may change after the first year's experience.

# 5.0. SAMPLE COLLECTION

## 5.1 Station locations, coordinates, access provisions for private property

Permission to access the properties will be secured from the owners or managers before each visit.

Stossel Creek. Site is located in the NW1/4, NW1/4, Sec. 24,R8,T25. From state highway 203 in Duvall, proceed east on NE Big Rock Road to Kelly Rd. NE. Turn left (north). Proceed about 2 miles to Stossel Creek Rd. Turn right (NE). Continue about 1 mile east of 351st Ave. NE. Walk about ¼ mile downhill on an overgrown road east of Stossel Creek Road. Road ends at Stossel Creek. Proceed about 50m downstream to reach upstream end of the sample reach.

Bear Creek. Site is locared approximately in the center of the SW1/4, NE1/4, NE1/4, R6, T26. Site is accessed from the last property on the Bear Creek Farm Road, north of the Woodinville-Duvall Rd.

Upper Covington Creek – Site is located about in the center of NW1/4, Sec. 10, R5, T21. Site is on King County Property adjoining and east of Lake Sawyer near Black Diamond road. Downstream end of the reach is about 50m upstream of the equestrian trail crossing.

+Other site descriptions will be added as sites are selected.

## 5.2 Sampling equipment, maintenance and calibration

Not applicable.

## 5.3 Decontamination procedures

None required.

## 5.4 Sample storage containers, holding times and sample storage requirements

Voucher specimens will be stored in denatured ethyl alcohol to prevent shell erosion.

## 5.5 Sample collection methods and sample compositing procedures/protocols

Not applicable

## 5.6 Field QC sample collection

See 4.3 – "Field Measurement Replicate - A field measurement replicate is defined as a separate in-situ measurement made following all procedures typically done between individual measurements. Ten percent of the quadrats counted will also be sieved after the visible mussels have been extracted in order to determine whether a significant number escape detection by burrowing into the sediment. In order to minimize the likelihood of having to return to the site to collect more data, the coefficient of variance will be calculated on-site to determine whether enough samples have been collected to yield statistical significance."

## 5.7 Sample delivery to lab and/or other transportation issues

Not applicable

## 5.8 Field documentation and logbook procedures

WDFW logbook for the mussels retained will be kept current.

## 5.9 COC procedures

Not applicable.

## 5.10 Data qualifiers related to field procedures

Not applicable

# 6.0. ANALYSIS (LABORATORY OR OTHER)

## 6.1 Organics and Conventionals

Not applicable

## 6.2 Sorting requirements, level of taxonomic detail

Larger mussels, *Andonta* sp. and *Margaritifera falcata*, will be identified by Brenner to species level.

## 6.3 QC requirements and data qualifiers

Not applicable

## 6.4 Corrective action procedures

Not applicable

## 6.5 Documentation / record keeping

Not applicable

## 6.6 Sample archiving requirements

Any mussels collected will be kept in a voucher collection.

## 6.7 Sample disposal

Not applicable

# 7.0. LABORATORY AND FIELD DATA REVIEW AND REPORTING

## 7.1 Interpretation of Chemical Data

Not applicable

## 7.2 Quality Assurance Reviews

Not applicable

## 7.3 Laboratory Data Qualifiers

Not applicable

## 7.4 Sample Archiving and Disposal

Not applicable.

## 7.5 Record Keeping and Reporting

Not applicable

# 8.0. DATA MANAGEMENT PLAN

## 8.1 Type, frequency and amount of data

## 8.2 Storage plan

The data will be stored in Access.

Front ends for I/O have not yet been identified.

## 8.3 Security of the data

To be determined

## 8.4 Reporting needs

To be determined

## 8.5 Data validation and qualification by data management staff

To be determined

# 9.0. HEALTH AND SAFETY PLAN

See standing Science, Health and Safety Plan

# 10.0. REFERENCES

Strayer, David L. and Smith, David R.. 2003. A Guide to Sampling Freshwater Mussel Populations. Ameerican Fisheries Society Monograph 8.

# Appendix B

# Lab and Field Equipment

# 1.0 FIELD EQUIPMENT

## 1.1 Mussel Data Collection:

25cm X 25cm frames to define boundaries of sampling quadrats

Rope marked in 25cm and 1m increments

18” lengths of ½” rebar for identifying ends of transects

Scoop for collecting material for sieving – Metal frame 20cm X 10cm to which a bag of 1/8” mesh netting had been attached

Sieve – wood frame box (35cm X 35cm X 10cm) with a 6mm X 6mm hardware cloth screen

Micrometer accurate to 0.1 mm

Zip Loc bags to hold mussels

Flagging tape for making labels to include in bags with mussels

Black marking pen for writing on flagging tape

## 1.2 Mussel Tagging

PIT tags

Biomark PIT tag reader

5-minute Epoxy Glue

Flexible shaft engraving tool with wire brush disk – remove periostracum so that the glue has a surface to which it can adhere.

## 1.3 Mussel Gender

Paring knife for shucking

Ice cube tray for holding animals

Xacto knife for piercing mussels to extract sample

Microscope slides and cover slips

Sealant for slides

Microscope

## 1.4 Mussel Aging

Glycerin for lubricating knife blade

Xacto-knife for splitting the ligament

40X binocular dissecting microscope

## 1.5 Mussel Photography

Olympus 370C digital camera

Frame to hold camera

Sheet of black foam-core for background

Black paper for labels in the photographs

Marking pen with aluminum pigment for writing the quadrat locations on the black labels

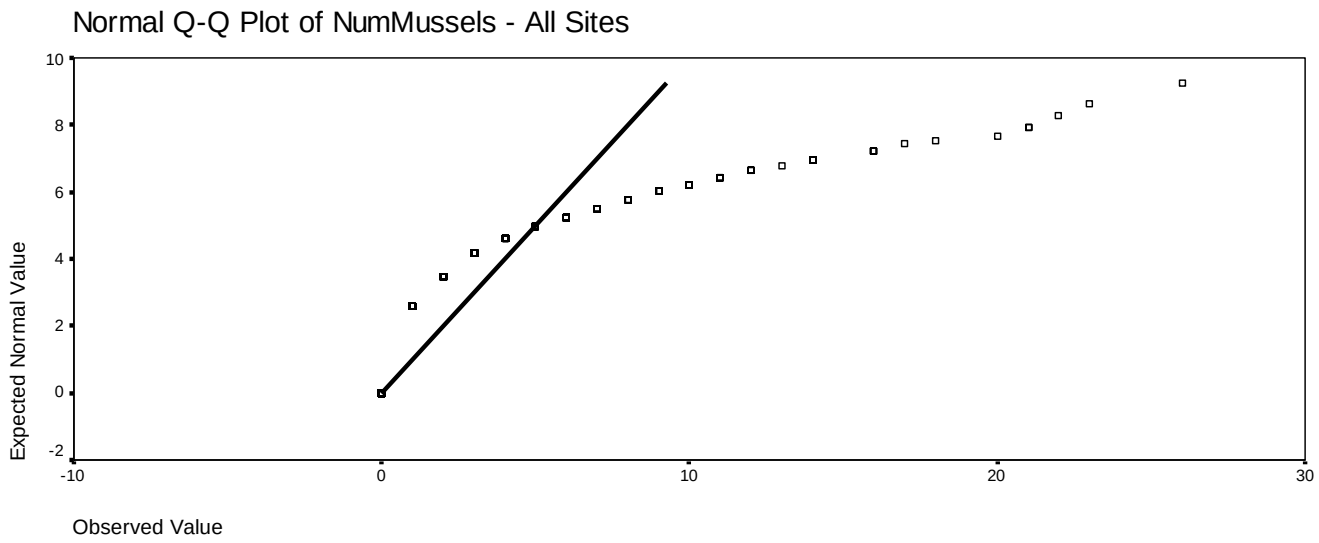
## 1.6 Software

Access for data storage and retrieval

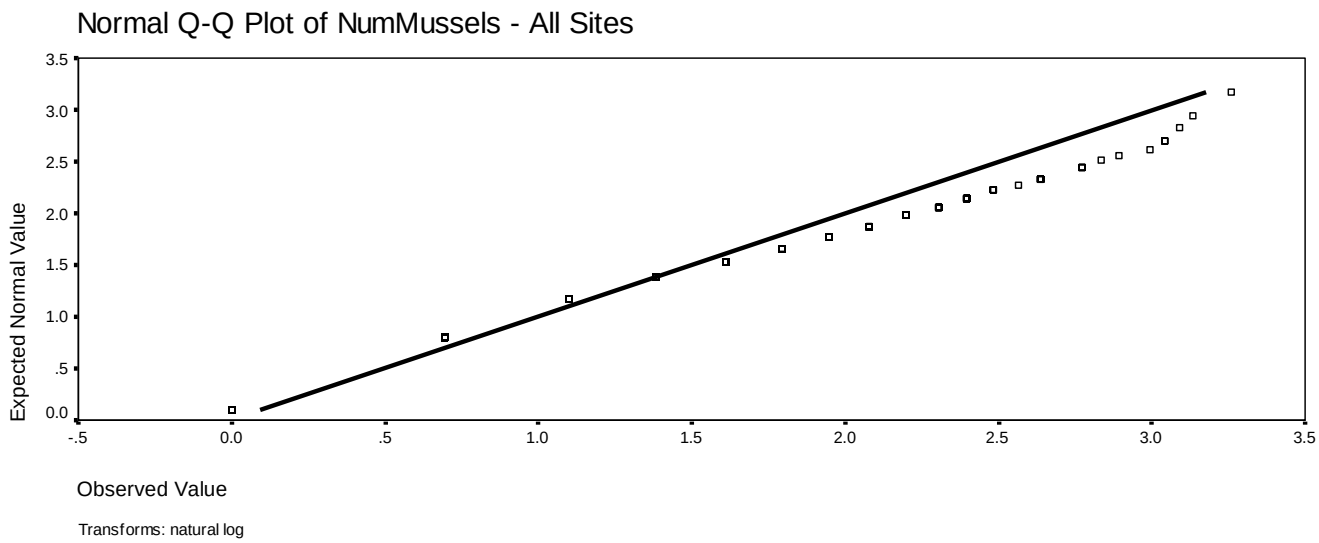
- S-Plus for statistical analysis
- SPSS
- Photoshop for enhancing, manipulating, and interpreting images

# Appendix C

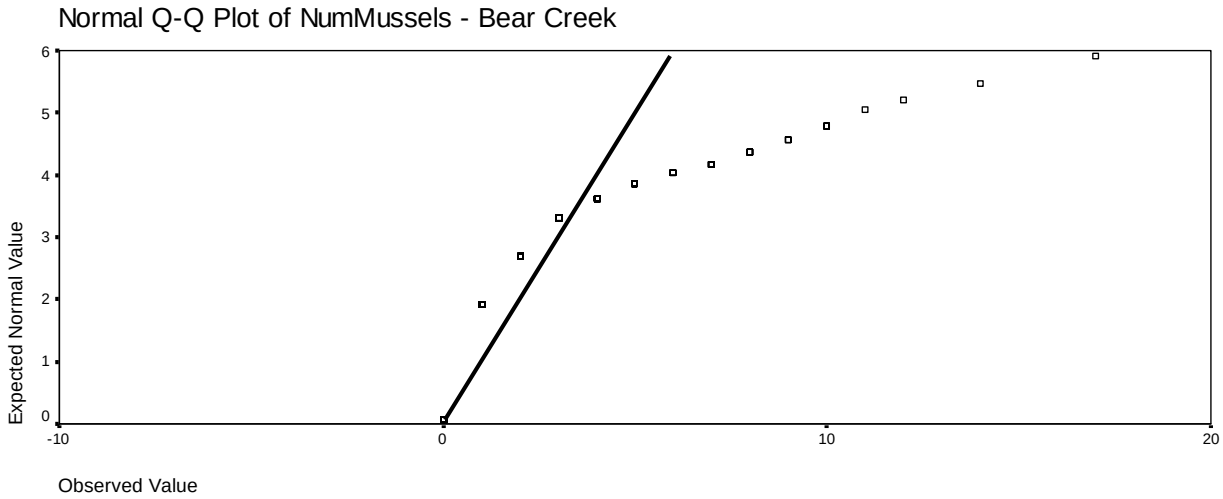
# Q-Q Plots



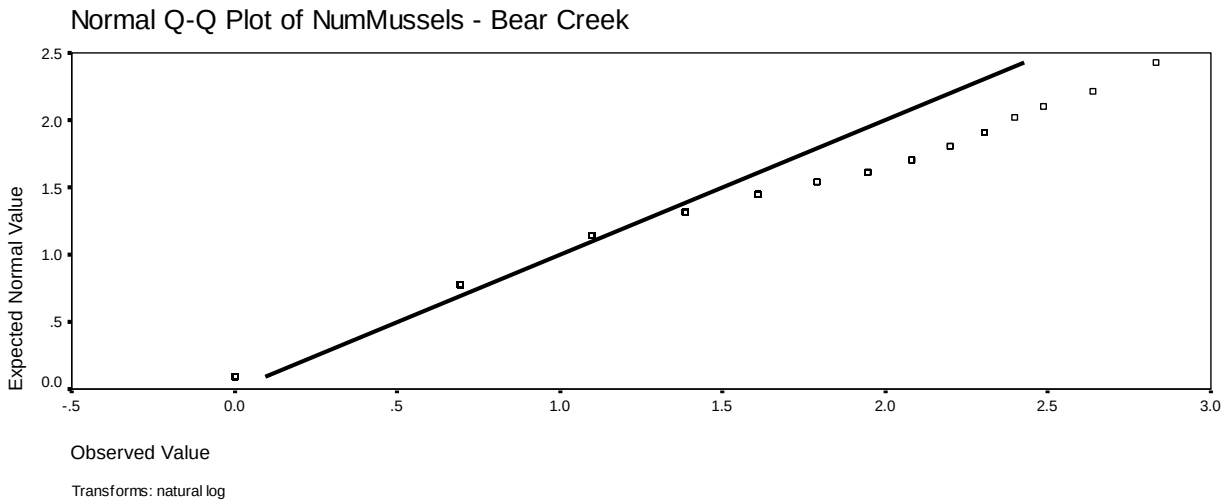

**Appendix C, Figure 1a**. Quartile-Quartile plots of mussel abundance for all sites to evalute data distribution



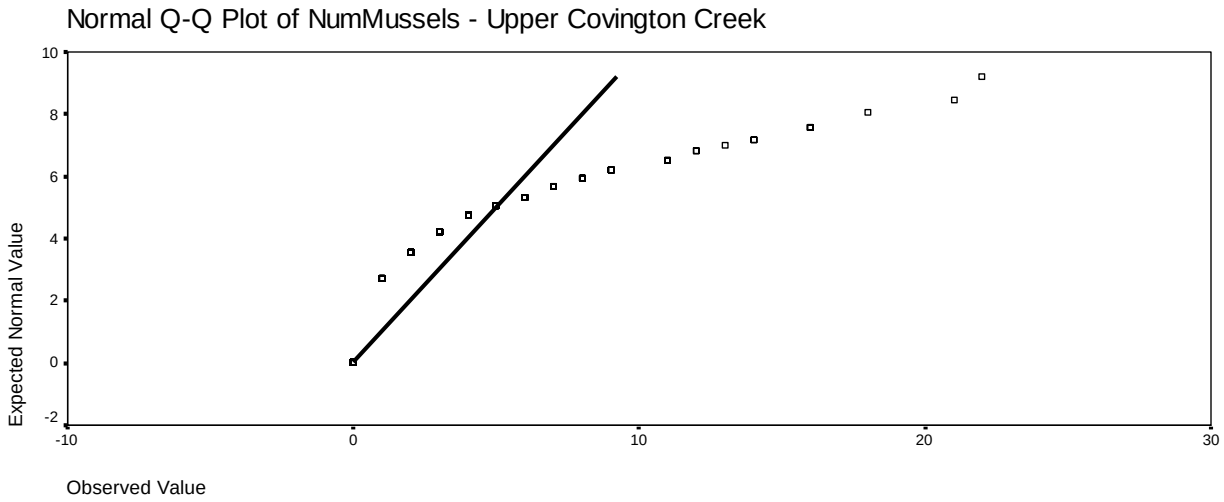

**Appendix C, Figure 1b**. Quartile-Quartile plots of log-transformed mussel abundance data for all sites to evalute data distribution.



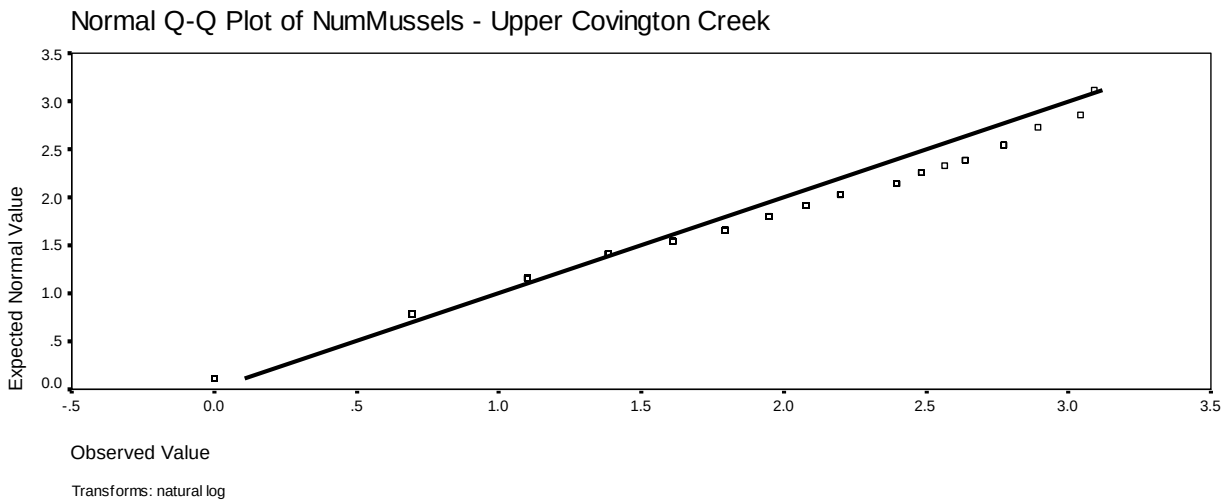

**Appendix C, Figure 1c**. Quartile-Quartile plots of mussel abundance data for Bear Creek to evalute data distribution.



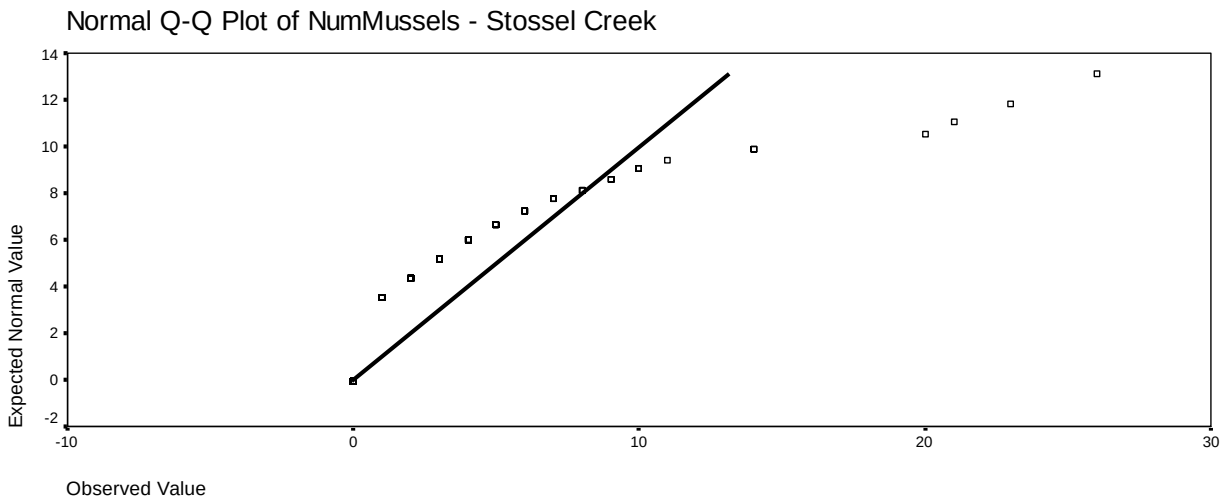

**Appendix C, Figure 1d**. Quartile-Quartile plots of log-transformed mussel abundance data for Bear Creek to evalute data distribution.



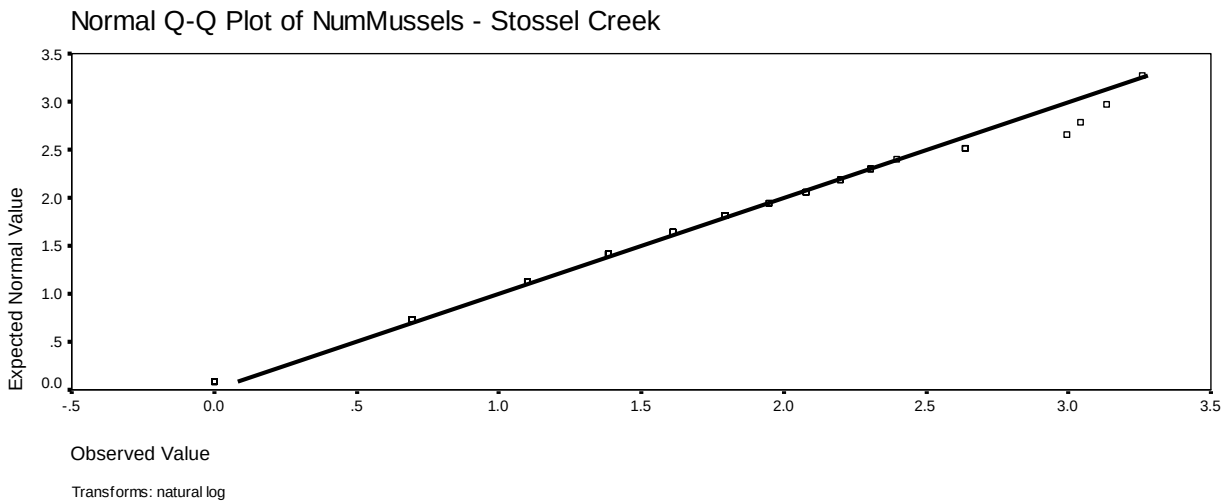

**Appendix C, Figure 1e**. Quartile-Quartile plots of mussel abundance data for Covington Creek to evalute data distribution.



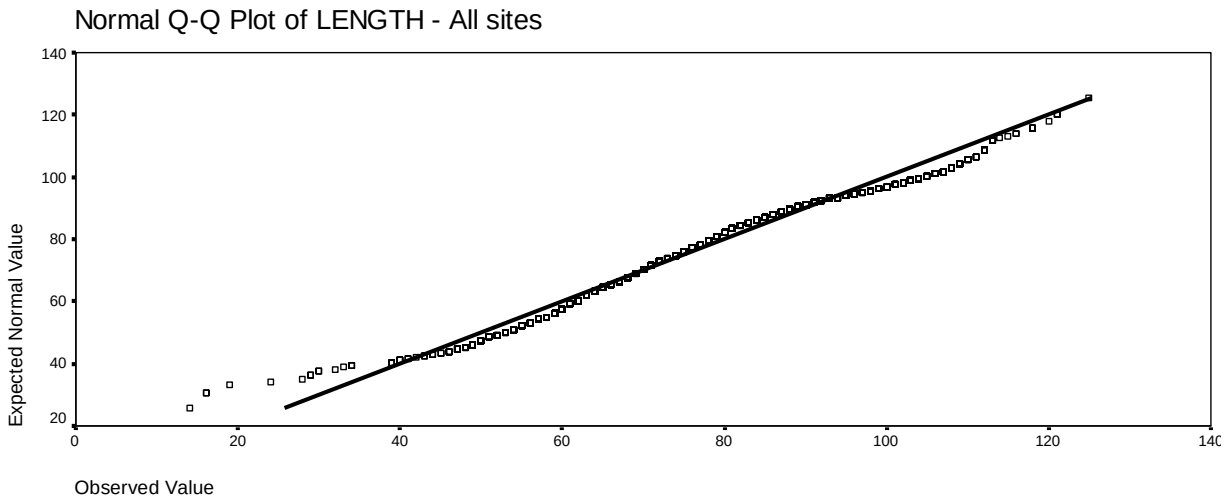

**Appendix C, Figure 1f**. Quartile-Quartile plots of log-transformed mussel abundance data for Covington Creek to evalute data distribution.



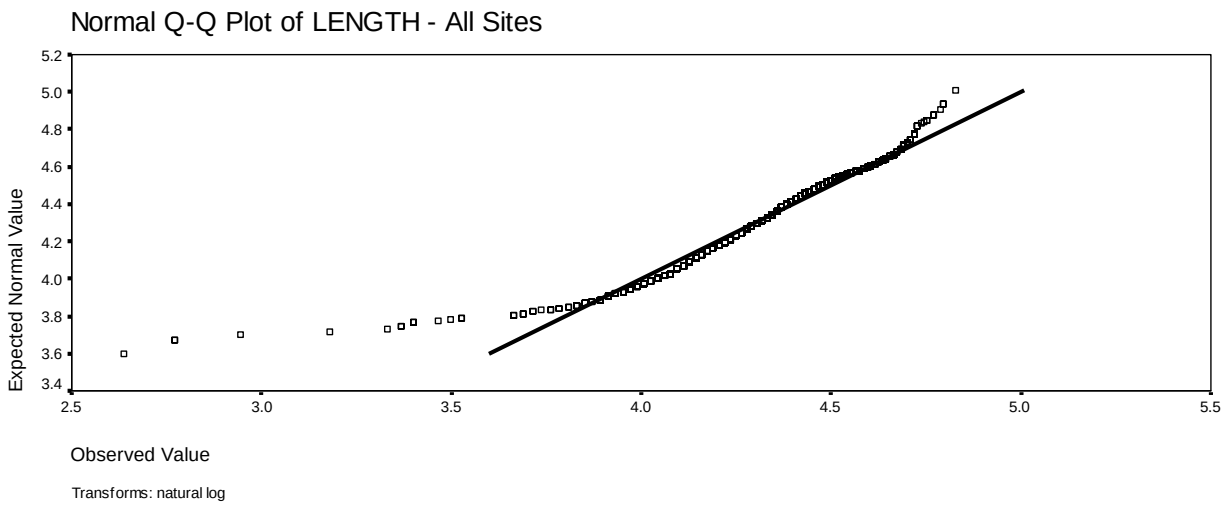

**Appendix C, Figure 1g**. Quartile-Quartile plots of mussel abundance data for Stossel Creek to evalute data distribution.



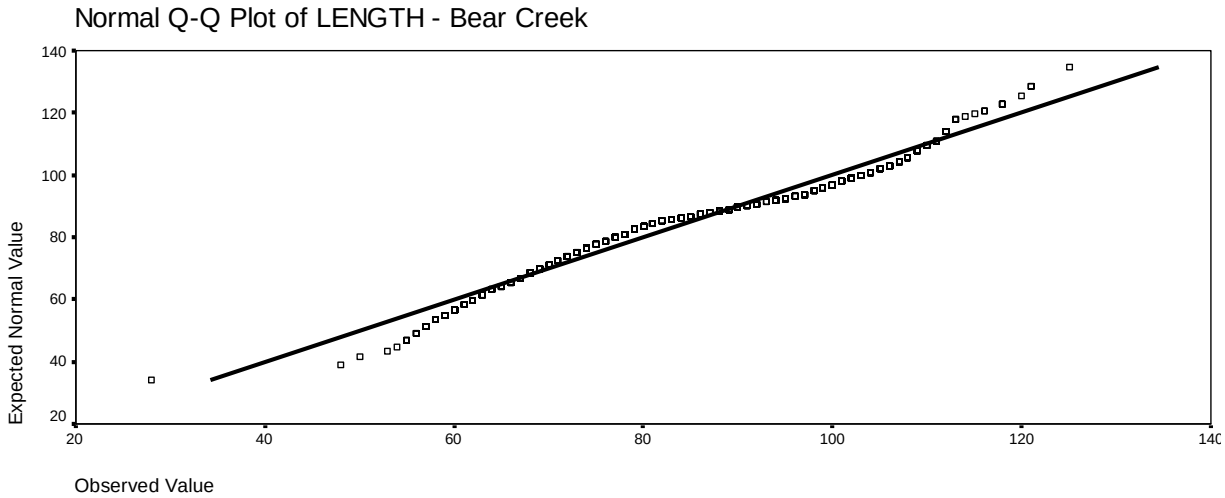

**Appendix C, Figure 1h**. Quartile-Quartile plots of log-transformed mussel abundance data for Stossel Creek to evalute data distribution.



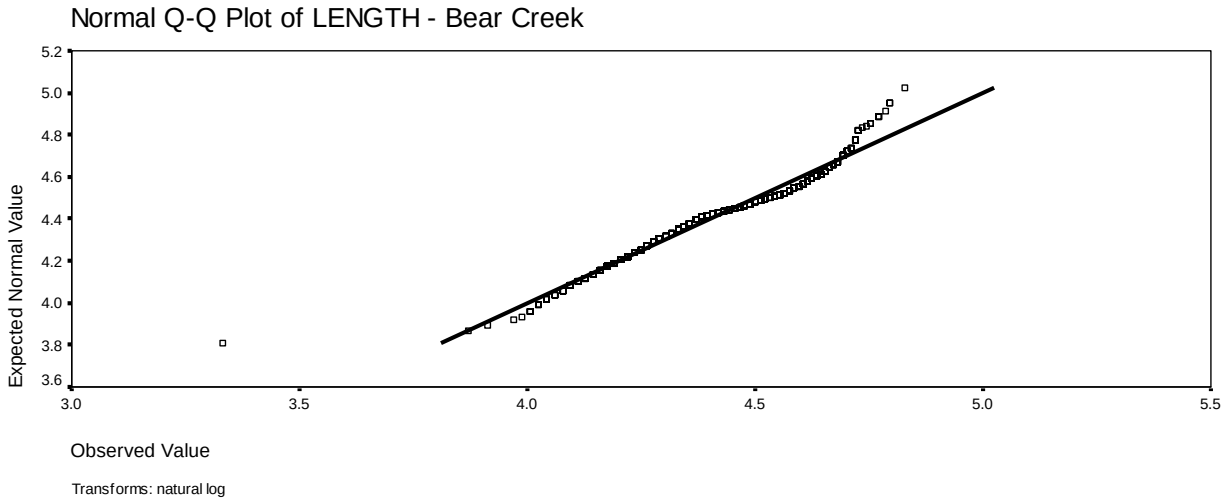

**Appendix C, Figure 2a**. Quartile-Quartile plots of mussel length for all sites to evalute data distribution.



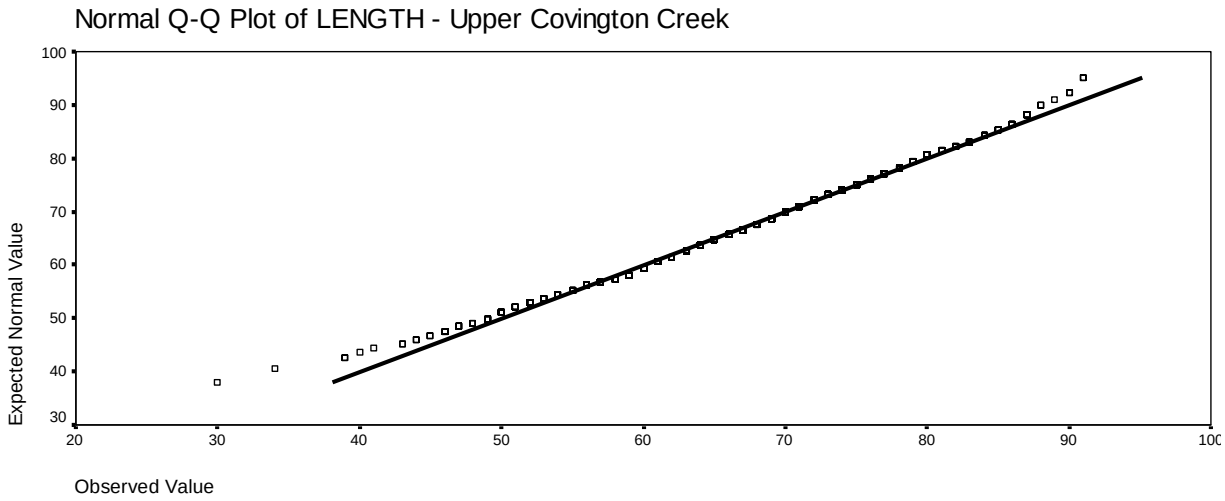

**Appendix C, Figure 2b**. Quartile-Quartile plots of log-transformed mussel length data for all sites to evalute data distribution.



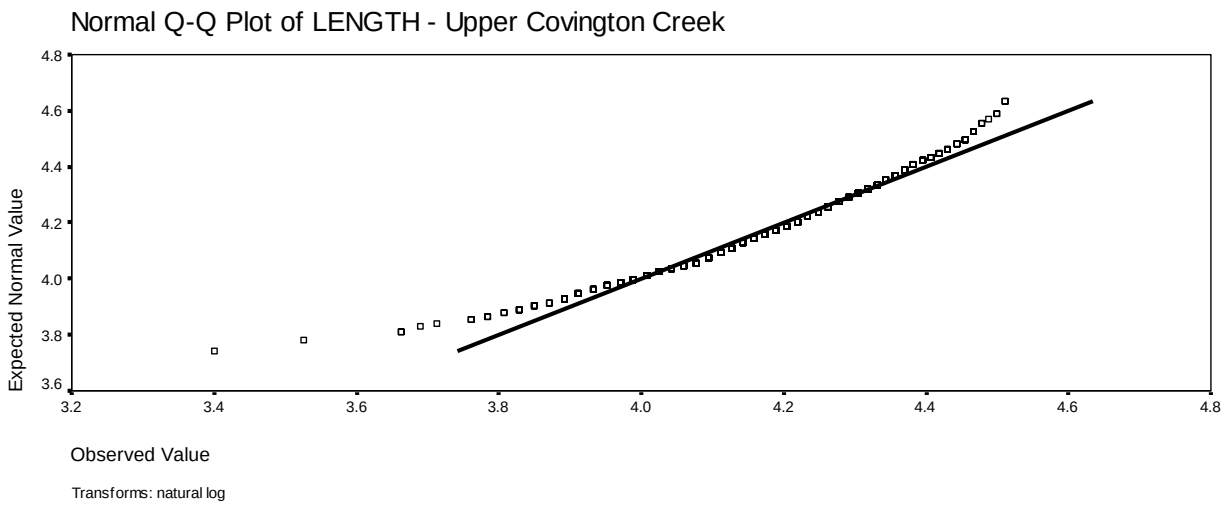

**Appendix C, Figure 2c**. Quartile-Quartile plots of mussel length data for Bear Creek to evalute data distribution.



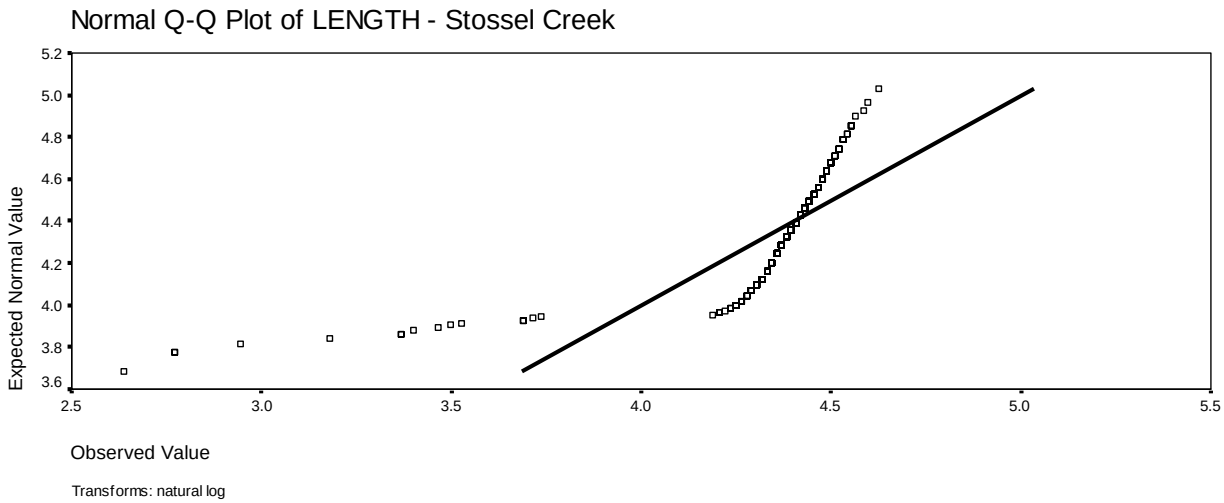

**Appendix C, Figure 2d**. Quartile-Quartile plots of log-transformed mussel length data for Bear Creek to evalute data distribution.



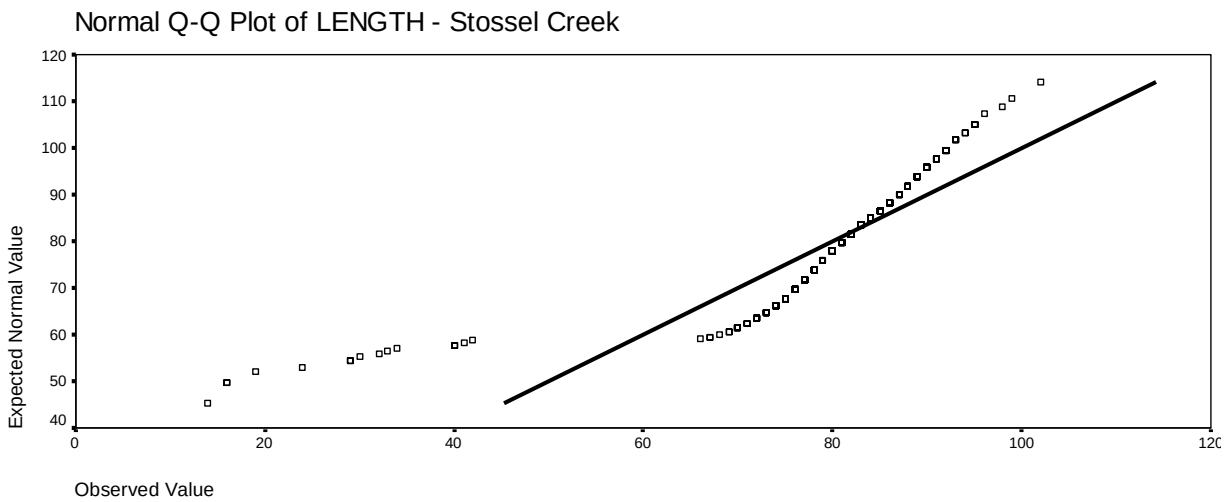

**Appendix C, Figure 2e**. Quartile-Quartile plots of mussel length data for Covington Creek to evalute data distribution.



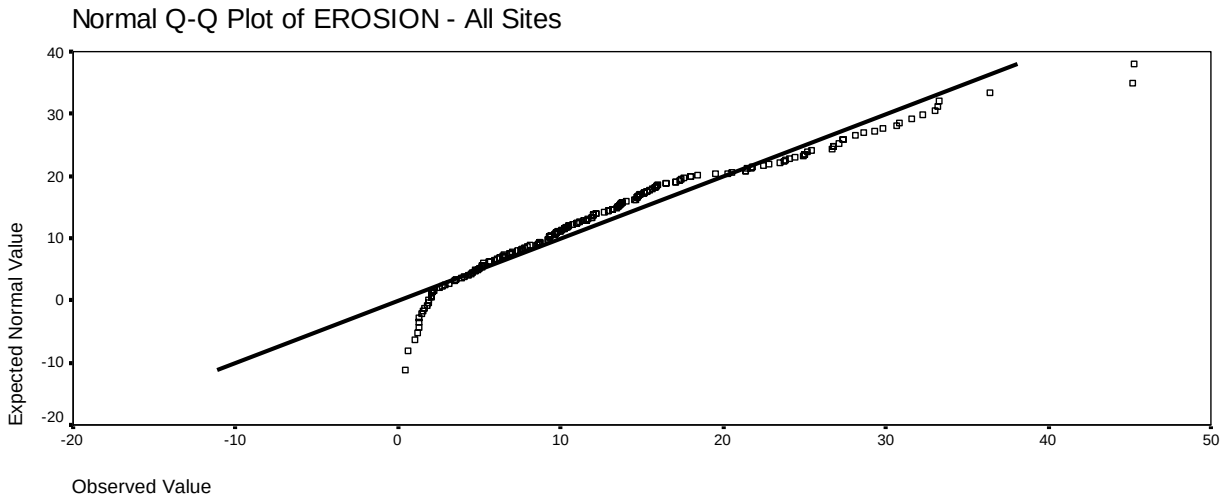

**Appendix C, Figure 2f**. Quartile-Quartile plots of log-transformed mussel length data for Covington Creek to evalute data distribution.



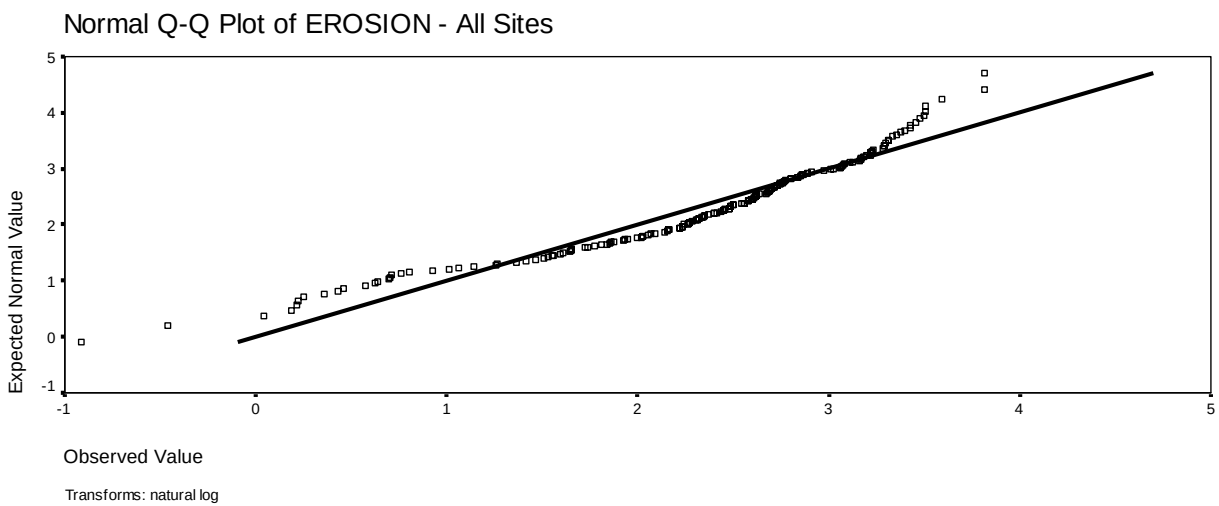

**Appendix C, Figure 2g**. Quartile-Quartile plots of mussel length data for Stossel Creek to evalute data distribution.



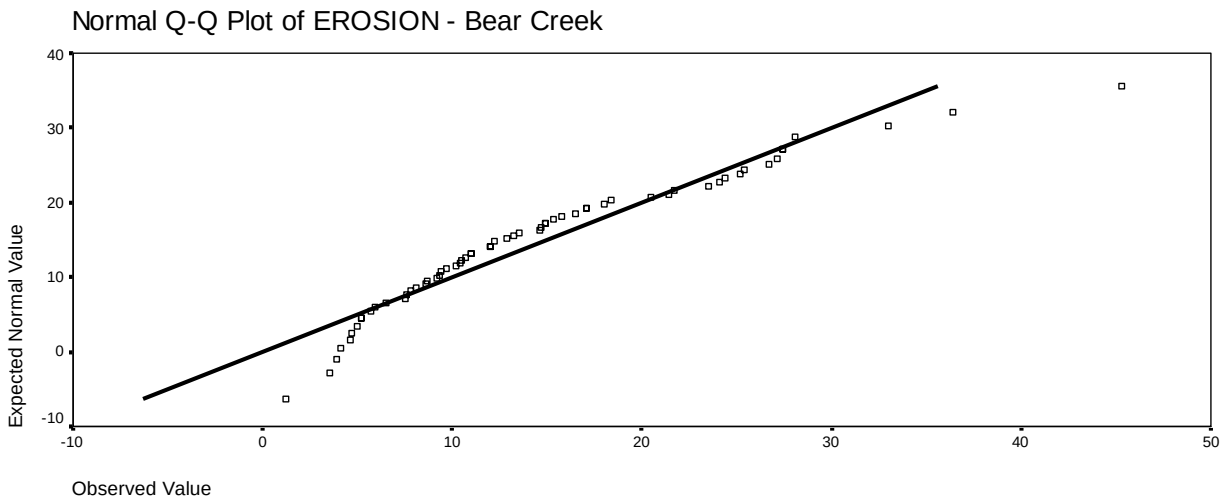

**Appendix C, Figure 2h**. Quartile-Quartile plots of log-transformed mussel length data for Stossel Creek to evalute data distribution.



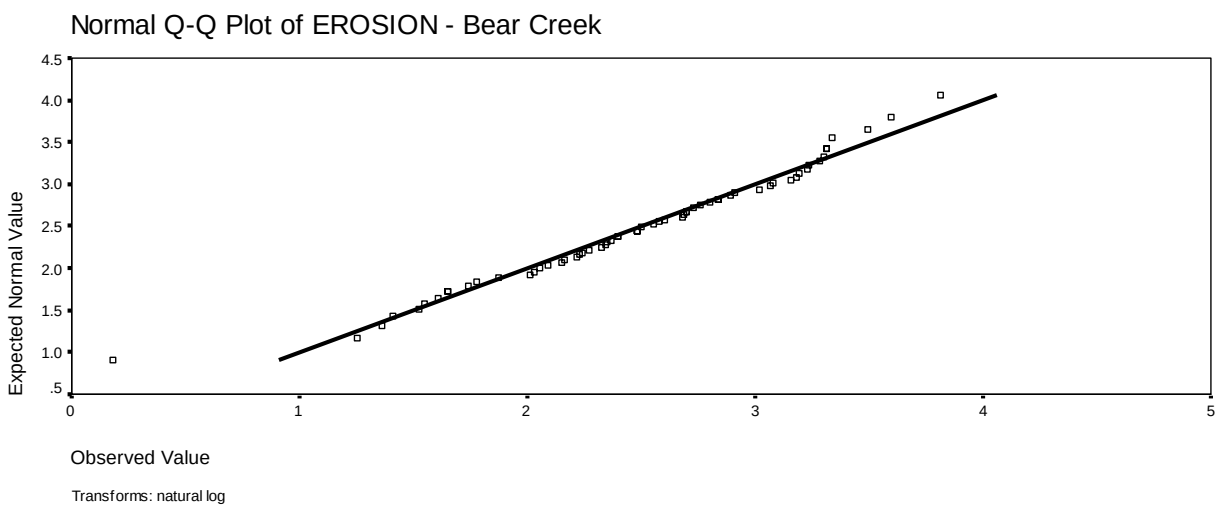

**Appendix C, Figure 3a**. Quartile-Quartile plots of percent shell erosion for all sites to evalute data distribution.



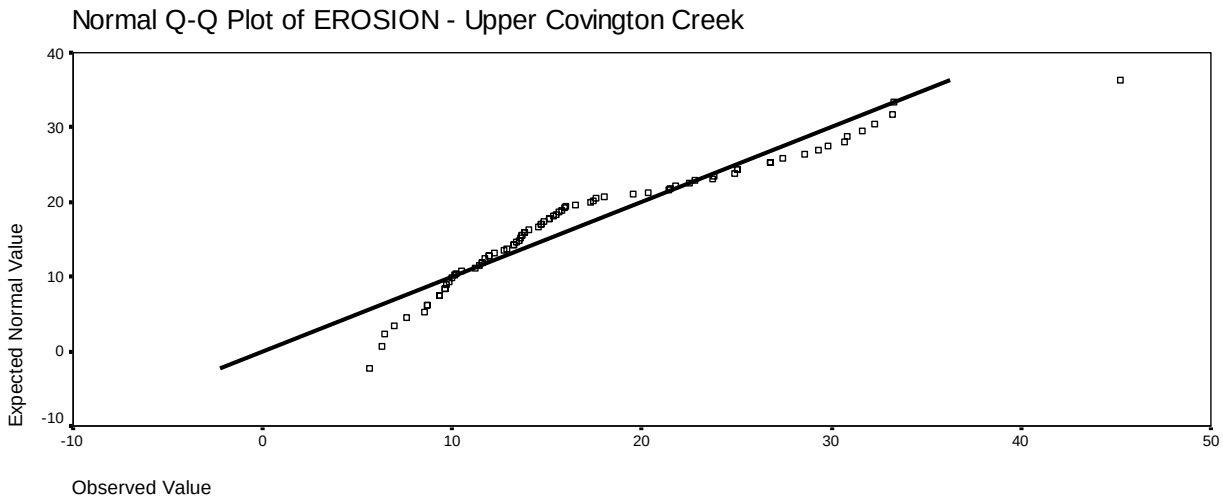

**Appendix C, Figure 3b**. Quartile-Quartile plots of log-transformed percent shell erosion data for all sites to evalute data distribution.



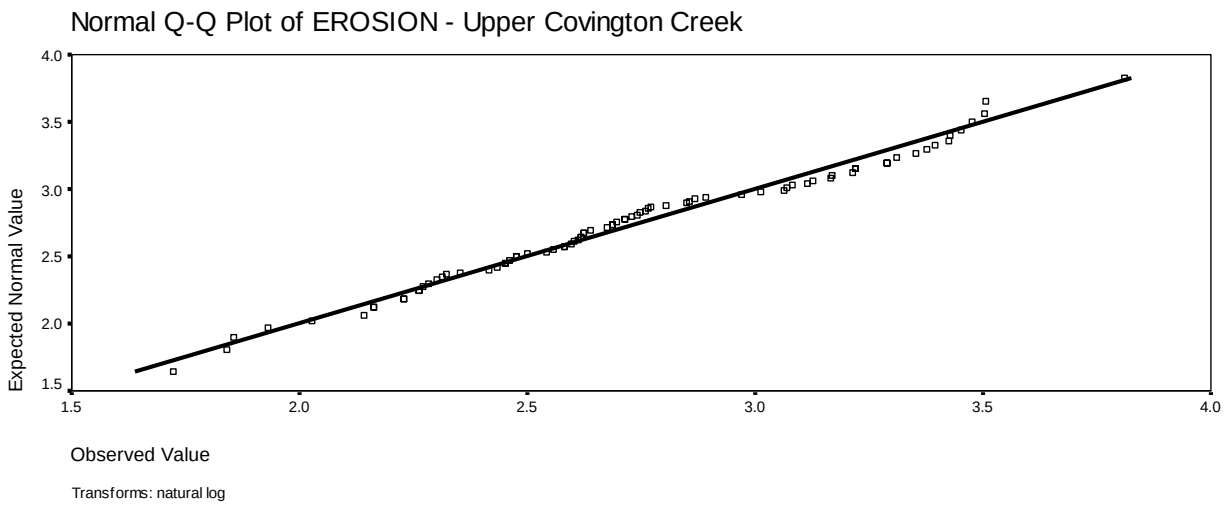

**Appendix C, Figure 3c**. Quartile-Quartile plots of percent shell erosion data for Bear Creek to evalute data distribution.



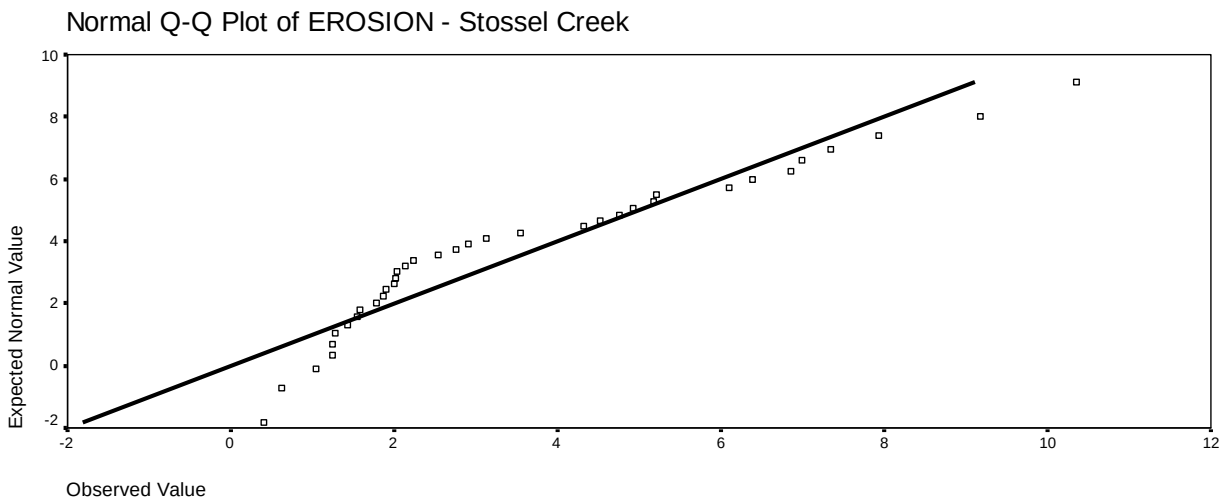

**Appendix C, Figure 3d**. Quartile-Quartile plots of log-transformed percent shell erosion data for Bear Creek to evalute data distribution.



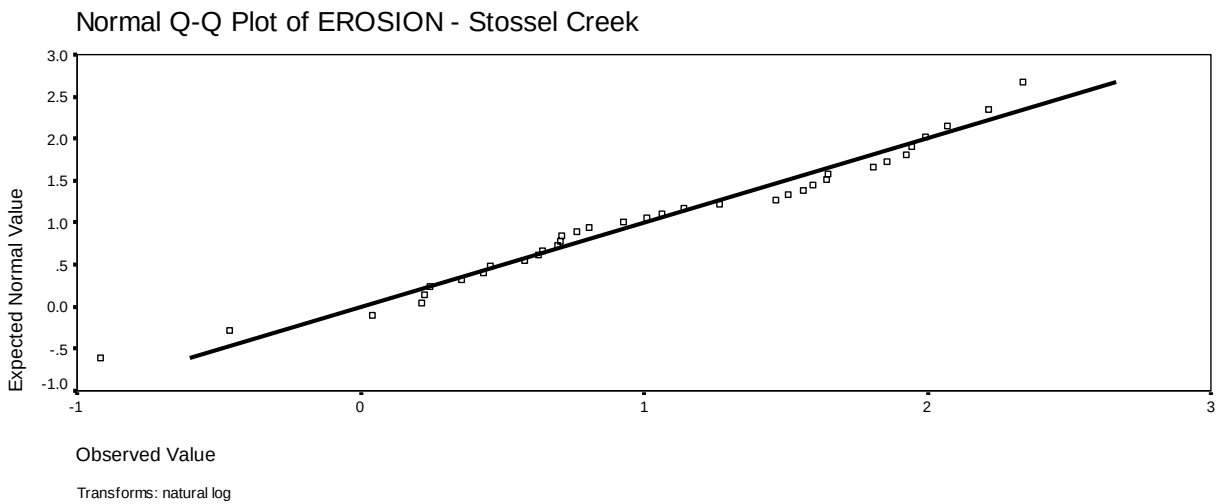

**Appendix C, Figure 3e**. Quartile-Quartile plots of percent shell erosion data for Covington Creek to evalute data distribution.




**Appendix C, Figure 3f**. Quartile-Quartile plots of log-transformed percent shell erosion data for Covington Creek to evalute data distribution.




**Appendix C, Figure 3g**. Quartile-Quartile plots of percent shell erosion data for Stossel Creek to evalute data distribution.




**Appendix C, Figure 3h**. Quartile-Quartile plots of log-transformed percent shell erosion data for Stossel Creek to evalute data distribution.

# Appendix D

## Shellfish Examination Summary

**Prepared for:** **Mr. Robert N. Brenner**
**King County**
**Department of Natural Resources and Parks**
**201 South Jackson Street, Suite 600**
**Seattle, WA 98104-3855**

***History:*** Three groups of freshwater mussels (*Margaritifera falcata*) were delivered to the laboratory by Mr. Bob Brenner on November 8, 2004. These were collected on November 7. The groups were identified as follows:

*Group 1:* Healthy or relatively healthy mussels from Stossel Creek, in North King County near the town of Duval, n=8.

*Group 2:* Sick mussels from Bear Creek, north of Redmond, Washington, n=4.

*Group 3:* Dead mussels, also from the Bear Creek location, n=4.

All mussels were examined by necropsy. Four mussels from Group 1 were processed for histological examination, as were four from Group 2 and two from Group 3.

***Necropsy and Histological Results:***

A detailed description of findings is given in the following text. A summary of a portion of the results is shown in the following table:

| Group No. | Animal ID | Shell Length (mm) | Sex | Reproductive Stage[1] | Digestive Gland Epithelial Cell Height[2] |
|---|---|---|---|---|---|
| 1 | 1-1 | 81 | M | 2+ | M/H |
| 1 | 1-2 | 94 | M-F | 3 | H |
| 1 | 1-3 | 82 | F | 3 | H |
| 1 | 1-4 | 80 | F | 3 | H |
| 2 | 2-1 | 90 | M | 3 | H |
| 2 | 2-2 | 82 | M | 2+ | VL |
| 2 | 2-3 | 101 | M | 3 | L |
| 2 | 2-4 | 87 | M | 3 | L |
| 3 | 3-1 | 95 | M | 3 | VL |
| 3 | 3-2 | 97 | M | 3 | L |

**Table key:**
[1]*Key to digestive gland condition:* The height of the digestive gland epithelium is rated as (1) **high** (indicating normal active metabolism and ingestion), (2) **medium** (indicating a condition at the lower end

of the normal range and an animal at risk from insufficient nutrition), (3) **low (**a pathological condition indicating insufficient nutrition or a toxic dietary effect, but a recoverable condition) and (4**) very low** (a distinctly pathological condition indicating insufficient nutritional intake or a toxic dietary effect, that may be unrecoverable in some cases).

[2]*Key to reproductive condition codes:* ? = shellfish with inactive or very limited activity in reproductive follicles or tubules of indeterminate sex; M=male; F= female.

Stage 0: quiescent follicles or tubules of indeterminate sex; Stage 1: very early development, often of indeterminate sex; Stage 2: indicates developing reproductive follicles or tubules. - indicates very early development. no mark indicates mid-stage development and + indicates nearly fully developed follicles or tubules. Stage 3: indicates fully developed reproductive products. Stage 4: partially or fully spawned reproductive follicle or tubule. Stage 5: resorbing reproductive follicle or tubules, may or may not be of determinate sex. Stage 2/5 or 3/5: indicates prerelease resorbtion of developing or fully developed follicle or tubule.

Mussels from Bear Creek which were sick or dead showed a greater degree of brown staining and raised deposits on the internal shell surface than did the mussels from the relatively healthy Stossel Creek site.

Fig. 1. The upper left photograph shows internal shell surface of mussels from Stossel Creek, the site with relatively healthy mussels. The upper right figure shows shells of sick mussels from Bear Creek. The lower photograph shows shells of dead mussels from Bear Creek.

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*laboratory location and courier address: 455 W. Bell Street, Sequim, Washington 98382 USA*

*Group 1:* Histological evaluation showed that the gill and digestive gland were in normal condition microscopically in contrast to specimens from group 2s and 3. However, 1 of 4 individuals from Group 1 had multifocal chitinous cysts and chitin hyperplasia. This condition was most severe near the base of the chitinous support rods of the gills. The finding is consistent with an infectious process and a few specimens had structures that suggested encysted microsporidian parasites. The digestive gland condition was normal in all four individuals as noted in the table.

*Group 2:* In this group, 4 of 4 individuals had severe or complete sloughing or loss of gill epithelium. This was associated with a diffuse distribution of bacterial cells, primarily coccoid in morphology. In addition, 2 of 4 mussels had mulfocal cysts and chitin hyperplasia of the chitinous support structures of the gill, consistent with the condition found in one animal from Group 1. As noted in the table, the condition of the digestive gland in these mussels indicated a condition of stress in three of the four sick mussels.

*Group 3:* In this group, both mussels showed nearly complete loss of the epithelial covering of the gill filaments. In addition, 1 of 2 mussels showed mulfocal cysts and chitin hyperplasia of the chitinous support structures of the gill, consistent with the condition found in Group 1 and 2 mussels. The digestive gland was in poor condition in both mussels in this group.

*General comment:* This species is presumed to be hermaphroditic, based on the condition of animal number 1-2. However, it is interesting that the mussels from Stossel Creek were 75% female or hermaphroditic while all the mussels from Bear Creek showed only development of male reproductive structures.

***Comments, Interpretation and Recommendations***

The mussels from Bear Creek showed lesions that were clearly consistent with near-term morbidity and mortality. These lesions were (1) loss of gill epithelium and (2) low and very low height of the digestive gland epithelium. The gill lesions were associated with bacterial colonies that could have contributed both to the loss of gill tissue and to the deposition of brown material on the internal shell surface. The presence of chitin hyperplasia is interesting and may be significant to the health of some mussels but did not appear sufficient to be linked to the observed morbidity and mortality. The lack of ovarian development in the mussels from Bear Creek could be an additional indicator of stress.

Loss of gill epithelium is not a response to a specific insult but a generalized response to a variety of chemical and physical stresses. It can also be a post mortem artifact.

However, the severe loss of gill epithelium in Group 2 (moribund but live mussels from Bear Creek) suggests that this lesion is important as a causation factor in the observed morbidity and mortality. In addition, the decline of digestive gland condition indicates that the mussels experienced some stressful stimulus from the environment. Such changes in the digestive gland are more chronic (longer term in development) than the loss of gill epithelium which is often an acute lesion (occurring over a short period of time).

The results suggest an environmental stressor which reduces the condition of the mussels as manifested by the poor digestive gland condition. Mussels in this weakened condition would be expected to be more susceptible to opportunistic bacterial infections which appear to be associated with the loss of essential gill structure and deposition of brown deposits on the internal gill structure.

The chitin hyperplasia is a lesion that could cause significant negative health effects if the condition became more severe than found in these mussels. It may be advisable to evaluate the condition further by special staining of the histological sections and electron microscopy of new specimen material to confirm whether or not it is infectious in nature. If the condition is infectious, as suggested by this evaluation, it may have significance in regard to spread of infectious disease by either intentional or inadvertent transfer of mussels from one watershed to another.

In addition, examination of mussels that are apparently healthy or in a less severe stage of morbidity from Bear Creek would help elucidate the primary cause of morbidity and mortality and sequence of pathological events leading to the observed morbidity and mortality.

January 13, 2005

Ralph Elston, PhD

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# Appendix E

## Numbers of Quadrats with Live Cover and the Plants that Provided that Cover

**Numbers of Quadrats with Live Cover and the Plants that Provided that Cover**

| American Name | *Generic name* | Total | Bear-Evans | Upper Covington | Stossel |
|---|---|---|---|---|---|
| **Trees** | | | | | |
| Big-leaf Maple | *Acer macrophyllum* | 4 | 1 | 3 | |
| Red Alder | *Alnus rubra* | 14 | | | 14 |
| Black Hawthorn | *Crataegus douglasii* | 29 | | 29 | |
| Douglas Fir | *Pseugotsuga menziesii* | 6 | | 1 | 5 |
| Cascara | *Rhanmus purshiana* | 50 | | 6 | 44 |
| Red Cedar | *Thuga plicata* | 7 | 3 | 4 | |
| Western Hemlock | *Tsuga heterophylla* | 1 | 1 | | |
| **Shrubs** | | | | | |
| Vine Maple | *Acer circinatum* | 16 | 10 | 6 | |
| Dogwood | *Cornus nutalli* | 8 | 4 | 4 | |
| Red Osier Dogwood | *Cornus stolonifera* | 256 | 229 | 11 | 16 |
| Salal | *Gaultheria shallon* | 10 | | 10 | |
| Twinberry | *Lonicera involucrata* | 15 | | | 15 |
| Klamath Plum | *Oemleria cerasiformis* | 8 | 7 | 1 | |
| Ninebark | *Physocarpus capitatus* | 186 | | 152 | 34 |
| Salmon Berry | *Rubus spectabilis* | 10 | 1 | 9 | |
| Hardhack | *Spirea douglasii* | 41 | | 7 | 34 |
| Red Huckleberry | *Vaccinium parvifolium* | 4 | | 4 | |
| **Vines** | | | | | |
| Blackberry | *Rubus discolor* | 7 | 5 | 2 | |
| Nightshade | *Solanum sp.* | 10 | | | 10 |
| **Terrestrial Herbs** | | | | | |
| Skunk Cabbage | *Lysichitum americanum* | 2 | | 2 | |
| Fringecup | *Tellima sp.* | 5 | 5 | | |
| **Aquatic Herbs** | | | | | |
| Starwort | *Callitriche sp.* | 15 | 5 | 10 | |
| Touch-me-not | *Impatiens capensis(?)* | 71 | 71 | | |
| Monkey flower | *Mimulus sp.* | 11 | | 11 | |
| Forget-Me-Not | *Myosotis sp.* | 23 | | 23 | |
| Water Parsley | *Oenenthe sarmentosa* | 32 | 29 | 3 | |
| Vallisneria | *Vallisneria sp.* | 42 | 42 | | |
| **Sedges/Rushes** | | | | | |
| Sedge | *Carex sp.* | 6 | | | 6 |
| **Ferns** | | | | | |
| Maidenhair Fern | *Adiantum pedatum* | 1 | | 1 | |
| Lady Fern | *Athyrium filix-femina* | 143 | 108 | 33 | 2 |
| Licorice Fern | *Polypodium glycyrrhiza* | 1 | | 1 | |
| Sword Fern | *Polystichum munitum* | 7 | 2 | 5 | |
| **Moss** | | | | | |
| Moss (Fontinalis) | *Fontinalis sp.* | 6 | | 6 | |
| **Total number of quadrats with live cover:** | | 1047 | 523 | 344 | 180 |
| **Number of quadrats evaluated:** | | 1804 | 863 | 761 | 180 |
| **Percent covered:** | | 58.0 | 60.6 | 45.2 | 100.0 |