Developing a Long-Term Monitoring Protocol for the Pygmy Sculpin (*Cottus paulus*), a Spring Dwelling Imperiled Species

by

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Abstract

Long-term monitoring protocols require adequate levels of precision in order to detect temporal changes in abundance or density. However, the ability to detect changes can be reduced by sampling variation, seasonal population fluctuations and environmental factors, such as habitat type. Using underwater observation, a monitoring protocol for Pygmy Sculpin (*Cottus paulus*) was developed and three sources of sampling variation were investigated: habitat type, seasonal variation, and observer effects. The Pygmy Sculpin is federally listed, threatened and is found in one just spring locality. The species is threatened by rising concentrations of trichloroethylene (TCE), which has contaminated the water supply, thus there is a need for a precise monitoring protocol. Pygmy Sculpin utilize habitat patches disproportionately, which causes an increase in sampling variation if habitat patch type is not accounted for in the sampling scheme. Pygmy Sculpin counts also differed among observers, and in most cases, experience did not influence counts. The resulting monitoring protocol was used over the course of a year to establish baseline data and to investigate potential seasonal fluctuations on the population.

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Introduction

Monitoring animal and plant populations to determine which species are in decline or at risk of extinction (Shea & Mengal 2001) and also to evaluate the efficacy of management strategies (Lindley et al. 2000) is one of the primary activities of conservation biology (Marsh & Trenham 2008). However, sampling animal and plant populations incurs many impediments for precision. Protocols must take into account the life history of the organism, as well as difficulties presented by the environment (Krebs 1999). Season, time of day, habitat heterogeneity and personnel changes may influence the precision of sampling protocols and monitoring programs must evaluate such factors. Additionally, when establishing a monitoring protocol ecologists must establish specific objectives and determine an appropriate sample size and sampling schedule (Gibbs et al. 1998). Regardless of the sampling method used, long-term monitoring protocols are established to provide standardized, repeatable procedures that can detect statistically significant changes in populations (Davis et al. 2011). Thus, precision must be maximized in order to detect changes (Thompson & Mapstone 1997). A strict and detailed protocol should be developed to ensure that any changes detected in a monitoring program reflect true changes occurring in the environment, and not error associated with sampling error, observation error, or annual stochastic variation (Lyons et al. 2008, Oakley et al. 2003). Monitoring protocols ensure that data meet standards of quality by establishing a known level of confidence, bolstering credibility by standardizing sampling, and are necessary to detect changes over time despite changes in personnel (Oakley et al. 2003).

In aquatic systems, there is a wide variety of sampling techniques that can be used for long term monitoring protocols including underwater visual observation, mark recapture studies, aerial counts, electrofishing, netting, trapping, and hook and line sampling. Regardless of the technique, numerous factors such as habitat type, temporal variation in populations, sampling design, and differences among sampling personnel can all affect precision. An initial investment of time spent defining objectives, optimizing sampling design, and determining how monitoring results will be used will make a monitoring protocol more likely to succeed in the long term (Oakley et al. 2003). Managers should evaluate different sampling techniques and sampling schemes before implementing a long-term monitoring protocol to determine which methods are most suitable for the specific objectives of a program. This study focused on developing a precise, long-term monitoring protocol for the Pygmy Sculpin (*Cottus paulus*), a threatened Alabama fish species with an entire range confined to Coldwater Spring and its associated run in Calhoun County, Alabama (33°36'11.25"N, 85°55'32.93"W). The Alabama Department of Conservation and Natural Resources (ADCNR) intends to monitor Pygmy Sculpin densities because rising concentrations of toxic trichloroethylene (TCE), reduced spring discharge, and stochastic events all have potential to affect the Pygmy Sculpin population. The specific objectives of the protocol were to: (1) determine if Pygmy Sculpin Densities are decreasing over time, (2) minimize the potential to harm Pygmy Sculpin while monitoring (3), and ensure that counts among multiple observers are comparable.

This study used underwater visual observation to monitor Pygmy Sculpin. Underwater visual observation refers to both snorkel and SCUBA surveys, and is a widely used technique for determining abundance, distribution, presence/absence, size, behavior (Stein et al. 1972, Laughlin & Warner 1980), and microhabitat use (e.g. Campbell et al. 1988, Cunjak & Power 1987) of fishes. Snorkel surveys are commonly used to monitor salmonids in streams and rivers, but they have also been used to study other groups of fishes. Nursall (1973) extended their use to include behavioral studies of non-game fish such as the Spottail Shiner (*Notropis hudsonius*). Snorkel surveys are also effective in determining distribution (Freeman & Grossman 1993), movement (Johnston 2000), and abundance (Scoppettone et al. 1992) of Cyprinids. Killian et al. (2010) used snorkel surveys to monitor the Maryland Darter (*Etheostoma sellare*) over the course of two years. Snorkel surveys have also proven useful in monitoring benthic cottids in lotic environments, such as the Bear Lake Sculpin (*Cottus extensus*) (Ruzycki et al. 1998), but no studies have used snorkel surveys to monitor cottids in spring habitats.

In this study, snorkel surveys were used as an alternative to electrofishing, anesthetizing, and seining for several reasons. First, toxicants and electrofishing both have potential to harm fish. Indeed, Henry et al. (2004) found that susceptibility to electrofishing-induced mortality varies among species and suggested avoiding habitats and locations with sensitive species. Electrofishing for threatened and endangered species should only be attempted if there is no other practical alternative (Nielsen 1998). The effects of electrofishing on Pygmy Sculpin are unknown, so it is prudent to avoid this technique. Second, much of the spring pool is too deep to be properly seined or electrofished. In contrast, the clear water of the spring allows for excellent visibility, and is not too deep for snorkeling. Third, Coldwater Spring is used as a water supply for the city of Anniston, so using toxicants is prohibited. Fourth, snorkeling requires less investment of time and money than electrofishing and seining (Hankin & Reeves 1988). Finally, snorkeling allows observers to make specific observations about microhabitat use and fish behavior that can be useful to future conservation efforts. Additionally, studies have shown that if water conditions are suitable, snorkeling surveys can be equally or even more effective at measuring abundance and species richness than other techniques. For example, Goldstein (1978) found that snorkel surveys identified more species, and typically more individuals than seining surveys.

Despite these advantages, studies have shown that there are some problems associated with snorkel surveys. First, sites to be surveyed must have clear, non-turbid water to ensure that observers can see target fishes. However, this can at least partially be addressed by considering the hydrology and seasonal weather patterns of an area and sampling when optimal water conditions will occur. Researchers should consider alternative methods if lack of visibility will be a recurring issue. Second, the precision of snorkel surveys varies greatly among systems and species, but pilot studies can help researchers determine if snorkel surveys are suitable for a particular system or species (Samoilys & Carlos 2000, Thompson & Mapstone 1997). Third, habitat heterogeneity can decrease precision (i.e. increase variance) of snorkel surveys (Distefano et al. 2003, Schill & Griffith 1984), but stratifying a site by habitat patch is one method that can reduce variance created by habitat heterogeneity. For example, Schill and Griffith (1984) stratified habitats into river channel and shoreline zones while estimating abundance of Cutthroat Trout (*Oncorhynchus clarkii*). Lamontagne and Rasmussen (1993) noted differences in densities of crayfish at various depths and recommended stratifying by depth. Similarly, Distefano et al. (2003) stratified streams into riffles, pools and runs to reduce variation in crayfish surveys. Fourth, inter-observer variation can reduce precision of snorkel surveys, but the degree of inter-observer variation differs by species and site (Thompson & Mapstone 1997). For example, Thompson and Mapstone found that inter-observer variation biased their density estimates of reef fish (1997), but Edgar et al. found that inter-observer variation was inconsequential and much smaller than variation among sites or months (2004). Clearly, inter-observer variation is not consistent among systems and species so it should be evaluated prior to establishing a monitoring protocol.

The Pygmy Sculpin is restricted to a Coldwater Spring and the associated spring run by a thermal gradient (Williams 1968). In a laboratory study, Pygmy Sculpin exposed to warm temperatures were lethargic and reduced feeding, and less than one fourth of Pygmy Sculpin exposed to 25 °C water survived (Walsh et al. 1997). The spring run flows into Dry Creek, a tributary of Choccolocco Creek, which is warmer than Coldwater Spring. Regardless of the cause, the species is restricted to a 5,800m^2 spring pool and the associated run, making it highly vulnerable to stochastic events.

Similar to most cottids, the Pygmy Sculpin is a benthic, cryptic fish; however, the Pygmy Sculpin is smaller than most sculpin, reaching a maximum size of 38mm (SL) (Boschung & Mayden 2004). Studies have examined pygmy sculpin feeding (McCaleb 1973), nesting habits (Johnston 2000, Johnston 2001), coloration and mate choice (Kierl & Johnston 2014), and sound production (Kierl & Johnston 2010), but studies focusing on the population abundance and density of Pygmy Sculpin are lacking. McCaleb (1973) used published densities of Mottled Sculpin in a Montana stream (from McCleave 1964) to approximate the abundance of Pygmy Sculpin in the spring run, but his estimate was based on the assumption that Pygmy Sculpin in the spring run would occur at similar densities to Mottled Sculpin in a Montana Stream. No effort was made to determine actual densities of Pygmy Sculpin *in situ*. The Alabama Department of Conservation and Natural Resources (ADCNR) has conducted some population studies, but they remain unpublished (J Stiles and S Rider, personal communication). A U.S. Fish and Wildlife Service report recommended developing a precise, long-term monitoring protocol for the species (USFWS Report 2008), but to this date no program has been implemented.

The dearth of population studies represents a substantial gap in knowledge relating to conservation of the Pygmy Sculpin amidst multiple threats to the population. The small range of the Pygmy Sculpin makes it vulnerable to stochastic events and recently concentrations of a toxic, volatile organic compound, trichloroethylene (TCE), have increased from 4.0 micrograms per liter (μg/L) in 2003 to 5.7μg/L in 2010 in Coldwater Spring (SAIC 2011). No toxicology studies have been performed on Pygmy Sculpin, however rainbow trout exposed to 5μg/L of TCE increased their ventilation frequencies, suggesting that even low concentrations of TCE can be detrimental to fishes (Kaiser et al. 1995). Additionally, the population of Calhoun County is growing rapidly, placing more demands on the groundwater. Although, the City of Anniston, AL owns the spring and has limited the amount of water they will withdraw from the spring, urbanization and changing land use in the spring recharge area have the potential to influence spring discharge. Studies of other springs have shown that water permanence and changing levels of discharge are factors that affect spring species (Barquin & Scarsbrook 2008). A thorough, long term monitoring program for the Pygmy Sculpin will help managers determine how these threats influence the Pygmy Sculpin population and how the population responds to management actions.

This study used quadrat and transect count snorkel surveys to develop a monitoring protocol for the Pygmy Sculpin and then collect baseline data on Pygmy Sculpin densities. Quadrat and transect sizes are typically negatively associated with variance (Distefano et al. 2003) and positively correlated with the cost or effort. Thus, there is a tradeoff between decreasing variance and increasing the cost as quadrat size increases. Variable detection rates can also influence the accuracy of quadrat and transect counts, but detection was not evaluated in this study. Detection was assumed to remain constant because Coldwater Spring is a stable habitat with little variation in visibility, water level, and water temperature.

This study also compared a simple random sampling scheme to a stratified random sampling scheme to determine how stratifying by habitat type influences precision. Coldwater Spring consists of three habitat types, rocks, a *Nostoc* algae sand mixture, and vegetation, which contribute to sampling variation. In addition, the spring run functions as its own unique habitat. Initial observations indicated that densities of Pygmy Sculpin in Coldwater spring varied among habitat types. Pygmy sculpin were most abundant in cobble habitats, followed by sand and vegetation (CVM personnel observation). A preference for rocks and cobbles has also been documented in a related species, the European bullhead (*Cottus gobio*) (Knaepkens et al. 2002), and pygmy sculpin have been shown to utilize cobbles as nesting habitats (Johnston 2001). Stratifying by habitat type was used as a variance reduction method.

Traditional belt transects are often used in underwater visual observation censuses and consist of a centerline pathway of fixed length that is searched on both sides up to a predetermined width. Belt transects are commonly used, but in heterogeneous habitats transects are likely to intersect multiple habitat patches, making it difficult to stratify by habitat patch. Thus, an alternative quadrat based sampling scheme was compared with traditional transects to determine which will be more efficient for monitoring the pygmy sculpin.

Long-term monitoring protocols must produce repeatable estimates of population densities. Increasing the sample size will reduce variance, but the reduction in variance is not linear. That is, the reduction in variance decreases as the sample size increases. At some point, increasing the sample size will not result in an appreciable reduction in variance. Increasing the sample size beyond this point will not decrease the variance enough to offset the additional cost and effort of increasing the sample size (Angermeirer & Smoger 1994). A bootstrapping technique described by Samoilys and Carlos (2000) was used to determine what sample size would yield repeatable density estimates for each stratum, without expending unnecessary effort that would not appreciably reduce variance.

It is unlikely that observers will remain constant for years to come, thus an evaluation of inter-observer variation was also necessary. This study compared density estimates from multiple observers to determine how observer effects bias Pygmy Sculpin counts. Density estimates from five sampling events were also analyzed to determine how experience levels of observers affect inter-observer variation and precision. The final goal of this study was using the protocol to monitor sculpin on a monthly basis. Monthly monitoring established baseline data for future managers and, monthly data were used to determine if Pygmy Sculpin densities are changing overtime.

The specific objectives of this study were to: (1) determine the quadrat size that is most precise for counting Pygmy Sculpin, (2) determine if a quadrat based, stratified random sampling scheme that accounts for habitat heterogeneity is more precise than a transect based sampling scheme that does not account for habitat heterogeneity (3) Determine the sample size that maximizes the amount of precision gained per sampling effort, (4) determine if Pygmy Sculpin densities are changing over time, (5) examine seasonal variation in Pygmy Sculpin densities, and, (6) investigate potential inter-observer variation bias in Pygmy Sculpin counts and (7) examine the effects of experience on inter-observer variation.

Materials and Methods

This study was conducted at Coldwater Spring and its associated run in Calhoun County, AL (33°36'11.38"N, 85°55'33.26"W). Coldwater Spring is a large spring that is owned and managed by Anniston Water Works and harbors the endemic Pygmy Sculpin and the endemic snail species, *Stioba nana* (Williams 1968, Thompson and McCaleb 1978). Unlike other springs in the area, Coldwater Spring flows from a breccia formation in the Jacksonville Fault (Thompson & McCaleb 1978) instead of solution channels. The spring discharges approximately 32 million gallons of clear, colorless water per day (Robinson 2004, McCaleb 1973). Daily flows range from 29.1 million gallons per day (mgd) to 33.9 mgd (SAIC 2011). During the course of this study, the temperature remained 17 °C, which is consistent with the 16 – 18 °C temperature range reported by Warman and Causey (1961).

The pool substrate is composed of patches of vegetation, cobbles, boulders, gravel and sand (Figures 1-4). Cobble and gravel habitats are in the swiftest shallowest parts of the spring pool (Johnston 2001). Aquatic vegetation occurs in deep areas of the spring pool with low flow, and consists of watermilfoil (*Myriophyllum sp.*), coontail (*Ceratophyllum sp.*), colonial algae (*Nostoc sp.*), and aquatic mosses (*Fissidens sp.* and *Fontinalis sp.*). A 6.9m weir impounds the spring discharge and forms a 5,800m^2 pool. Excess water flows over the weir into the 130m spring run which empties into Dry Creek, a tributary of Choccolocco Creeek (Coosa River Drainage). Pygmy Sculpin density decreases downstream from the weir. Forty-eight meters of the spring run are on Anniston Water Works property and were included in this study. The remainder of the spring run was not sampled.

To determine which quadrat size to use, 0.25m^2, 0.50m^2, 0.75m^2, and 1.00m^2 quadrats were constructed out of 2.54cm diameter PVC cut to length and joined at the corners with four 90° PVC couplers and standard PVC cement. Holes were drilled in the quadrats to allow them to sink to the bottom of the spring. Preliminary trials suggested that quadrats larger than 1m^2 were difficult to count because observers could not keep tack of individuals and might have difficulty holding their breath long enough to complete a count in deeper water, so no sizes larger than 1m^2 were tested.

First counts were conducted to estimate the mean and variance of all four quadrat sizes. Preliminary observations indicated that Pygmy Sculpin were most abundant and most difficult to count in the rock habitat; therefore the quadrats were optimized for the rock habitat patches. Two snorkelers each surveyed 20 random sites with rock substrate for each quadrat size in Coldwater Spring. To randomly select sites, a grid was overlaid on Google Earth satellite imagery, and an integer number was assigned to any grid cell that contained rock habitats. A random number generator (Program R) determined which cells would be sampled. A GPS coordinate was assigned to each cell, and snorkelers used a handheld GPS (DeLORME Earthmate PN-40) to locate the sites within the spring. The GPS unit was not precise enough to determine exactly where to place the quadrats, so quadrats were haphazardly placed within the area determined by the GPS coordinates.

Snorkelers wore wetsuits and hoods to prevent hypothermia and applied antifog solution to their masks to maintain visibility. To reduce any differences in counts caused by differences throughout the day changes or activity in the spring, quadrats of different sizes were counted in a random order. Observers placed quadrats, allowed them to sink and then immediately began counting, because observations indicated that sculpin were attracted to prey items that were dislodged during disturbance. Beldade and Gonçlave (2007) noted a similar problem and used small quadrats so that observers could count fish in the quadrat before fish entered from nearby areas. If there was any current, snorkelers approached the quadrat from down current side of the quadrat to prevent disturbed sediment from reducing visibility. Weight belts helped observers maintain their position and count quadrats in deeper water. Quadrats were counted from the outside in to reduce the chance of counting sculpin that entered the quadrat after the counts were started (Beldade and Gonçalve 2007). Rocks, vegetation and other debris were gently removed to reveal hidden sculpin. Beldade and Gonçalve (2007) demonstrated that this disturbance method was as effective as toxicants at finding benthic fish. Data were recorded on dive slates while underwater and transferred to notebooks immediately after exiting the spring.

Data were standardized to 1m^2 to allow for comparisons among the multiple sizes using the following formulas in which x denotes the size of the quadrat (Krebs 1999). Relative cost (C_r), and relative variance (s^2_r) were calculated using time as a proxy for cost.

Standardizing Mean Quadrat Counts to 1m^2

$$\frac{mean\ count}{\text{m}^2} = \frac{\left(\frac{mean\ count}{x\ \text{m}^2}\right)}{x}$$

Standardizing Variances of Quadrat Counts to 1m^2

$$\frac{Variance}{\text{m}^2} = \frac{\left(\frac{variance}{x\ \text{m}^2}\right)}{x^2}$$

Calculating Relative Cost, C_r, for a Given Quadrat Size x

$$C_r = \frac{C_x}{minimum\ C}$$

Calculating Relative Variance, s^2_r, for a Given Quadrat Size x

$$s_r^2 = \frac{s_x^2}{minimum\ s^2}$$

The program, Ecological Methodology (Program Group – Quadrat Sampling) calculated these values, and determined that 0.5m^2 was the optimal quadrat size (see results). For the rest of this study, any quadrat that was used was a 0.5m^2 quadrat.

Coldwater Spring was surveyed in December 2012 to February 2013 to compare transects sampling techniques to quadrat sampling techniques. Transect sites in the spring were randomly selected using a grid overlaid on Google Earth Satellite imagery (Figure 5) of Coldwater Spring using the program GEPath 1.4.6. Each cell in the grid was assigned an integer number and then the program R was used to select random integers within that range. In contrast, quadrats were stratified by habitat type. The quadrat samples were divided into 3 strata. Two strata were allocated to the spring pool based on habitat type (i.e. rock and *Nostoc*/sand) and one stratum was assigned to the run. The vegetation habitat was not sampled because pilot studies revealed that Pygmy Sculpin were uncommon in the vegetation. Quadrat sites were selected in a method similar to transect sites, except each cell in the grid was assigned to a habitat classification using Google Earth images (rock substrate, *Nostoc* /sand, vegetation), and then each cell within a habitat type was assigned an integer number (e.g. rock 1, rock 2 …, *Nostoc* / sand 1, *Nostoc* sand 2…). The program R was used to select 20 random sites from within each habitat classification. One day of sampling effort was dedicated to each method to simulate the amount of time state workers in the future will spend at the spring on a sampling trip, and observers sampled the maximum amount of transects and quadrats that could be sampled in the day. This ensured that the comparison between transects and quadrats compared an equivalent amount of sampling time.

Transects were marked at 1m increments with neon colored cable ties and colored flagging tape. Belt transects were 10m long and intended to be 1m wide, so the observer carried a 1m long piece of PVC pipe marked in the center (0.5m) to use as a reference of width. To prevent transects from intersecting each other, all transects were laid in a north/south direction with the aid of a compass. The observer followed the transects and counted sculpin within 0.5m on either side of the chain. Rocks, vegetation and debris were moved to reveal concealed sculpin. The number of sculpin in each transect was recorded on a dive slate while surveying and immediately transferred to a field notebook when the dive slate was full. Sculpin less than 5mm were considered young of the year sculpin and not included in the count. The procedure for counting quadrats was identical to the procedure used for determining quadrat size except all strata were sampled and only 0.5m^2 quadrats were used.

After sampling, the coefficients of variation (hereafter CVs) for transects and each quadrat strata were calculated and then compared. The CV ($CV = \sigma / \mu$) was used to compare the precision of transects and quadrats because it is a unit-less measurement of precision, and the quadrats and transects were not the same size. A lower CV indicated greater precision. CV's from quadrat counts varied among habitat, but overall they were an improvement compares to the CV's from transect counts, so quadrats were used for the rest of the study.

The next step in developing the monitoring protocol was determining the minimum sample size that could produce a repeatable estimate of the mean for each stratum. The sample size was determined using a bootstrapping technique described by Samoilys & Carlos (2000). The spring was over-sampled by conducting 100 quadrat counts in each strata (i.e. rock, *Nostoc*/sand, vegetation, and run). Next, the program R randomly selected 2 quadrat counts from the original 100 quadrat counts and calculated the mean. This process was iterated 1,000 times resulting in 1,000 estimates of the mean density of Pygmy Sculpin based on a sample size of 2. The coefficient of variation of the means was then calculated to determine how variable the mean estimates of Pygmy Sculpin density were. Next, the program R randomly selected 3 quadrat counts from the original 100 quadrats and calculated the mean. This was iterated 1,000 times resulting in 1,000 estimates of the mean density of Pygmy Sculpin from a sample size of 3. The coefficient of variation of the means was calculated to again determine how similar the mean estimates of Pygmy Sculpin density were. This process was repeated for every consecutive sample size ranging from 2 to 45. The coefficients of variation of the means were then plotted against sample size resulting in a curve that represents the influence of sample size on precision. Because the same 100 samples were used for the bootstrapping process, the curve shows the degree of similarity between independent estimates of means at each sample size when the actual density of Pygmy Sculpin in the spring remains constant. The minimum sample size was determined by selecting the sample size at which the curve levels off; i.e. the rate of the reduction in the coefficient of variation is drastically reduced (Bros & Cowell 1987).

Coldwater Spring was then sampled using the newly developed monitoring protocol. Sampling occurred every month (except January to weather and logistical constraints) from May 2013 until March 2014 with .5m^2 quadrats. Sample sizes were 25, 25 and 30 quadrat counts in rock, *Nostoc*/sand and the run respectively. Quadrats were counted using the same methodology as described for determining a quadrat size. The sampling date was converted to the number of days since sampling began to more accurately describe the change in time.

A generalized linear model was fit in the program R using the package MASS. Count data can only be 0 or positive integers (i.e. they are bound at 0) so a normal distribution is not the best representation of the data. Poisson distributions are often recommended for count data, however these data were slightly over dispersed (i.e. the variance was greater than the mean) so models were fit to a negative binomial distribution. Four *a priori* hypotheses were established and compared using the corrected Akaike Information Criterion (AIC_c). Observations suggested that Pygmy Sculpin densities differed among habitats so a term for habitat was included in all of the models. However, it was unclear weather Pygmy Sculpin would shift habitats seasonally, so a model with a habitat by sampling day term was also tested. The rock habitat was used as the references in all of the models. The four models below were fit to the monthly monitoring data and the AIC_c values were calculated and compared to select the best model using methods described by (Anderson et. al 2000, Anderson and Burnham 2002).

(1) Count ~ Sampling Day + *Nostoc*/sand + Run + Error

(2) Count ~ Sampling Day + *Nostoc*/sand + Run + Sampling Day : *Nostoc*/sand + Sampling: Day: Run + Error

(3) Count ~ Sampling Day + *Nostoc*/sand + Run + Sampling Day : *Nostoc*/sand + Error

(4) Count ~ Sampling Day + *Nostoc*/sand + Run + Sampling: Day: Run + Error

Models that were within 2 AIC_c units were not considered to be competitive with the top supported model if they also had an additional parameter because the penalty for an additional parameter is 2 AIC units, so a model with an additional parameter within 2

AIC_c units does not reduce the model deviance enough to outweigh the penalty (Arnold 2010).

ANOVA was used to test for differences among months to determine if Pygmy Sculpin counts vary seasonally. One ANOVA was run for each habitat type. A Tukey honest significant (HSD) difference test was used to conduct pairwise comparisons if the ANOVA indicated that there was a significant.

To compare among multiple observers, two naïve observers who had not surveyed the spring accompanied the experienced observer on five sampling trips. Observers were considered naïve if they had no experience conducting snorkel surveys to simulate the typical level of experience of ADNCR employees who will be responsible for monitoring Pygmy Sculpin in the future. All observers followed the monthly monitoring protocol developed earlier in the study. Each observer surveyed his or her own unique quadrat sites. The following negative binomial regression was used in each habitat to determine how Pygmy Sculpin counts differed among observers and to see if agreement among observer counts improved as the observers gained experience surveying.

$$\text{Count} \sim \text{Observer} + \text{Number of Sampling Trips} + \text{Observer} : \text{Number of Sampling Trips}$$

The number of sampling trips was used as a proxy for experience and used instead of time because it explains how the experience level of an observer affected Pygmy Sculpin counts. CVs were used an indicator of precision and evaluated using the following linear model in each habitat.

CV ~ Observer + Number of Sampling Trips + Observer : Number of Sampling Trips

Results

Standardized variance was highest (1.55) in the 0.25m^2 quadrat and then decreased (Figure 6). Standardized variances for the 0.50m^2, 0.75m^2, and 1.00m^2 quadrats were very similar and close to 1 (1.02, 1.07, 1.00 respectively) (Figure 6). Standardized cost had an opposite trend. Standardized cost was lowest for the 0.25m^2 quadrat (1.00) and increased exponentially (Figure 6). The product of standardized variance and standardized cost was lowest for the 0.50m^2 quadrats (1.09).

Seventeen transects were sampled in Coldwater Spring and had a mean of 55.7 (± 17.86, ± 95% C.I.) Pygmy Sculpin per transect or 5.57 (± 1.786, ± 95% C.I.) Pygmy Sculpin per m^2 and a CV of 0.84. Counts ranged form 9 to 235 Pygmy Sculpin per transect. Random site selection resulted in all habitats types (rock, *Nostoc*/sand, vegetation and run) being sampled. In one day of sampling with 0.5m^2 quadrats, I completed twenty quadrat counts in each stratum. Counts in the cobble had a CV of 0.39. The mean count was 5.35 (± .98, ± 95% C.I.) sculpin per quadrat and the range was 1-12 sculpin per quadrat. Counts in the *Nostoc*/sand habitat had a CV of.89. *Nostoc*/sand counts were lower than those in cobble, with a mean of 1.88 (± 0.56, ± 95% C.I.) and a range of 0-6. Six of the quadrat counts had 0 sculpin. Counts in the run had the lowest precision with a CV of 1.10. The mean count in the run was 1.28 (± 0.42, ± 95% C.I.) and counts ranged from 0-4. Nine of the 20 quadrat counts had 0 sculpin.

In all habitats, the coefficient of variation of bootstrapped means decreased with sample size. The most rapid reduction in CVs occurred at low sample sizes, and the rate of reduction decreased as sample size increased. The rock habitat consistently had the lowest CV of bootstrapped means (Figure 7). At a sample size of 2, the CV was .376. At a sample size of 25, the CV had decreased to .106. Increasing the sample size by 35 only reduced the CV to .088. A sample size of 25 was used for the cobble because the CV of bootstrapped means was relatively low (0.106) and increasing the sample size beyond this point did not appreciably reduce the CV of bootstrapped means. The *Nostoc*/sand habitat had an intermediate level of CVs of bootstrapped means (Figure 8). At a sample size of 2, the CV of bootstrapped means was .564 and it had decreased to .156 at a sample size of 25. Adding 10 additional samples only decreased the CV to .133. A sample size of 25 was used for the *Nostoc* because the CV of bootstrapped means was relatively low (.156) and adding additional samples did not appreciably reduce the CV. The run had the most variability (Figure 9). At a sample size of 2, the CV of bootstrapped means was 1.102. At a sample size of 25, it had only decreased to .329. At a sample size of 30 it had decreased to an acceptable level of .286 and adding additional samples did not decrease the CV enough to justify the increased sampling effort. Thus a sample size of 30 was selected for the run.

The negative binomial distribution fit the monthly monitoring data well. Model 3 was the best-supported model ($\omega_i = 3.14$) and described counts as a function of habitat, sampling day and a sampling day by habitat interaction. The deviance ratio was 1.16 and the dispersion parameter was 10.14. Pygmy Sculpin densities differed among habitat types. The rock habitat had 5.37 (4.82-5.98, 95% C.L.) Pygmy Sculpin per quadrat on the first sampling day, the highest density of all of the habitats that were monitored (Figure 10). The *Nostoc*/sand habitat had 0.42 (.35-.51, 95% C.L.) times as many

Pygmy Sculpin per quadrat as the rock habitat ($p < .001$) on the first sampling day (Figure 11). The run habitat had .23 (20-26, 95% C.L.) times as many Pygmy Sculpin as the rock habitat ($p <.001$), the lowest density of Pygmy Sculpin of the habitats that were monitored (Figure 12). There were significant differences in Pygmy Sculpin densities due to time such that for each 1 month increase in time, there were .96 (.95-.99, 95% C.L.) times as many Pygmy Sculpin in the rock habitat as the previous month ($p = .0014$). However, the best model had a marginally significant sampling day by *Nostoc*/sand habitat interaction term, such that for each passing month the decrease of Pygmy Sculpin densities in the *Nostoc*/sand habitat was 2.7% (-0.8 – 6.4%, 95% C.L.) less than the decrease observed in the rock habitat (i.e. the reference)(.,95% C.L.) ($p = .126$). Model 1 and model 4 were also competitive and only differed in the interaction term. Model 1 lacked the interaction term and model 4 had a sampling day by run habitat interaction term instead of a sampling day by *Nostoc*/sand habitat interaction term. Although model 2 was within 2 AIC_c units of the best-supported model, it was not considered competitive because it also had one more parameter than the best-supported model (Arnold 2010).

The ANOVA indicated that there were moderately significant (i.e. $p < .1$) differences between months in the rock habitat, $F(9,235) = 1.604$, $p = .09$. Tukey honest significant difference post hoc tests showed that 2.24 (-0.167 4.65, 95% C.I.) more Pygmy Sculpin were counted in November than in February ($p = .092$) in the rock habitat. No other pairwise differences between months were significant at an alpha of 0.10 (Table 4). There were significant differences between months in the *Nostoc*/sand habitat, $F(9,246) = 2.136$ ($p =.027$). According to the Tukey (HSD) post hoc tests, surveys in November had

1.56 (0.102 - 3.018, 95% C.I.) more Pygmy Sculpin per quadrat than surveys in February (p = .025), 1.52 (0.048 - 2.995, 95% C.I.) more Pygmy Sculpin per quadrat than surveys in July (p = .037), and 1.52 (0.034 - 3.013, 95% C.I.) more Pygmy Sculpin per quadrat than surveys in March (p = .040). There was also a marginally significant difference between surveys in November and May such that 1.40 (-0.041 - 2.847) more Pygmy Sculpin per Quadrat were counted in the November survey (p = .065). No other pairwise comparisons were significant (Table 5). The ANOVA suggested there was at least one marginally significant difference in mean counts between months in the run habitat, F(9,286) = 1.761 (p = .075). There were 0.987 (-0.067 - 2.040, 95% C.I.) more Pygmy Sculpin per quadrat in the October survey than the March survey (p = .082). No other pairwise comparisons were significant (Table 6).

The negative binomial model for the rock habitat fit the multiple observer data well. The deviance ratio was 1.14 and the dispersion parameter was 32.74. The additional experience gained over the five surveys with multiple observers had no significant effect on the experienced observer's counts. With each successive trip the experienced observer counted .97 (.91 – 1.03, 95% C.L.) as many sculpin as the previous trip (p = .308) (Figure 13). There were no significant differences in the counts between the experienced observer and the naïve observer 1. Naïve observer 1 counted 1.01 (.77-1.34, 95% C.L.) times as many Pygmy Sculpin as the experienced observer on the first sampling trip (p = .932) (Figure 13). Naïve observer 1 counted 1.04 (.96 – 1.13, 95% C.L.) times as many sculpin with each successive trip, but the interaction was not significant (p = .348) (Figure 13). In contrast, naïve observer 2 counted .60 (.44-.82, 95% C.L.) times as many Pygmy Sculpin as the experienced observer (p < .001), and .59 (.44 -

.81, 95% C.L.) times as many sculpin as the naïve observer 1 observer ($p < .001$) (Figure 11). Naïve observer 1 counted 1.09 (.96 – 1.13, 95% C.L.) more Pygmy Sculpin with each successive trip, but this interaction was not significant (p = .07).

The negative binomial model fit to the *Nostoc*/sand count data was a good fit. The deviance ratio was 1.14 and the dispersion parameter was 3.56. The additional experience gained over the five surveys with multiple observers did not significantly influence the experienced observer's counts. With each successive trip, the experienced observer counted 1.00 (.89 – 1.11, 95% C.L.) times as many sculpin as the previous trip (p =.968) (Figure 14). On the first trip, the experienced observer counted 1.74 (1.00 – 3.02, 95% C.L.) times as many Pygmy Sculpin as naïve observer1 (p = .046), and 4.33 (2.24 – 8.58, 95% C.L.) times as many Pygmy Sculpin as naïve observer 2 ($p < .001$ $1.49e^{-5}$) (Figure 14). The naïve observer's counts were also significantly different. Naïve observer 1 counted 2.49 (1.24 – 5.09, 95% C.L.) times as many sculpin as naïve observer 2 (p = .001) (Figure 14). Naïve observer 1 counted 1.08 (.91 – 1.27, 95% C.L.) times as many Pygmy Sculpin with each successive trip (p = .361) and naïve observer 2 counted 1.14 (.94 – 1.39, 95% C.L.) as many Pygmy Sculpin with each successive trip (p = .165) (Figure 14); however, neither interaction was significant.

A negative binomial model also explained the data from the run habitat. The deviance ratio was 1.13 and the dispersion parameter was 6.77. Experience gained over the course of the fiver surveys did not affect the experienced observer's Pygmy Sculpin counts. With each successive trip, the experienced observer counted .95 (.85 – 1.06, 95% C.L.) times as many Pygmy Sculpin as the previous trip (p = .328) (Figure 15). On the first trip, the counts of the experienced observer and naïve observer 1 were different, but the counts of the experienced observer and naïve observer 2 were not significantly different. The experienced observer counted 2.08 (1.18 – 3.70, 95% C.L.) times as many sculpin as naïve observer 1 (p = .012), and 1.32 (.78 – 2.24, 95% C.L.) times as many sculpin as naïve observer 2 (p = .304) (Figure 15). The naïve observers' counts were not statistically different. Naïve observer 2 counted 1.57 times as many Pygmy Sculpin as Naïve observer 1 (p = .132) (Figure 15). Both naïve observers counted more Pygmy Sculpin with each successive trip, but neither of the interactions was significant. Naïve observer 1 counted 1.12 (.94 1.32, 95% C.L.) times as many Pygmy Sculpin (p =.204), and naïve observer 2 counted 1.03 (.88 – 1.22, 95% C.L.) times as many Pygmy Sculpin (p = .686) with each successive trip (Figure 15).

The linear model showed that the number of trips did not affect the CV's of the experienced observer in the cobble habitat. For each successive trip, there was a .030 (± .034, ± 95% C.I.) decrease in the experienced observers CV (p = .08) (Figure 16). Naïve observer 1's CV was smaller than the experienced observer's by .010 (± .160, ± 95% C.I.) on the first trip, but the difference was not significant (p =.192) (Figure 16). Naïve observer 2's CV was greater than the experienced observer's by .047 (± .160, ± 95% C.I.) on the first trip, but the difference was not significant (p = .526) (Figure 14). Naïve observer 1's CV was smaller than naïve observer 2's CV by .150 (± .159, ± 95% C.I.) on the first trip (Figure 16). The interaction between naïve observer 1 and the number of sampling trips was insignificant, such that the slope of the relationship between naïve observer 1's CV and sampling trip was .024 (± .048, ± 95% C.I.) greater than the experienced observer's (p = .217) (Figure 16). The interaction between naïve observer 2 and the number of sampling trips was insignificant, such that the slope of the relationship between naïve observer 2's CV and sampling trip was .003 (± .049, ± 95% C.I.) less than the experienced observer's (p = .893) (Figure 16).

In the *Nostoc*/sand habitat, the experienced observer non-significantly counted .046 (± .151, ± 95% C.I.) fewer sculpin per trip (p = .511) (Figure 17). Naïve observer 1's CV was larger than the experienced observer's by .283 (± .706, ± 95% C.I.) on the first trip (Figure 17), but the difference was not significant (p =.252). Naïve observer 2's CV was greater than the experienced observer's by .95 (± .523, ± 95% C.I.) on the first trip (p < .0001) (Figure 15). Naïve observer 2's CV was .671 (± .075, ± 95% C.I.) greater than naïve observer 1's CV on the first rip (p = .017) (Figure 17). The interaction between naïve observer 1 and the number of sampling trips was insignificant, such that the slope of the relationship between naïve observer 1's CV and sampling trip was .051 (± .213, ± 95% C.I.) less than the experienced observer (p = .602) (Figure 17). The interaction between naïve observer 2 and the number of sampling trips was insignificant, such that the slope of the relationship between naïve observer 2's CV and sampling trip was .183 (± .213, ± 95% C.I.) less than the experienced observer's (p = .084) (Figure 15).

In the run, the number of previous trips did not significantly affect the CV of the experienced observer. With each successive trip, the experience observer's CV decreased by .089 (± .237, ± 95% C.I.) (p = .418) (Figure 18). Naïve observer 1's CV was smaller than the experienced observer's by .163 (± .820, ± 95% C.I.) on the first trip (Figure 19), but the difference was not significant (p =.664). Naïve observer 2's CV was smaller than the experienced observer's by .067 (± .819, ± 95% C.I.) on the first trip (p < .0001) (Figure 18). The interaction between naïve observer 1 and the number of previous sampling trips was insignificant, such that the slope of the relationship between naïve observer 1's CV and previous sampling trip was .015 (± .335, ± 95% C.I.) greater than the experienced observer's (p = .924) (Figure 18). The interaction between naïve observer 2 and the number of sampling trips was insignificant, such that the slope of the relationship between naïve observer 2's CV and sampling trip was .026 (± .335, ± 95% C.I.) greater than the experienced observer's (p = .349) (Figure 18).

Discussion

These data suggest that the protocol developed for monitoring Pygmy Sculpin was effective at detecting small changes in density. Mangers should be aware of an increase in counts due to recruitment in the fall. Some observer bias was detected, so observers should remain as consistent as possible. Additional monthly sampling could continue for a few more years to help managers better understand seasonal fluctuations in Pygmy Sculpin densities, but is not necessary to detect long term changes in Pygmy Sculpin densities. The full protocol is outlined in Appendix 1.

The primary concerns when selecting the quadrat size for the monitoring protocol was maximizing precision per unit effort. As predicted, the variance decreased with increased quadrat size because larger quadrats sampled more area and captured more variability among sculpin spatial distributions. Thompson and Mapstone (1997) also noted that sampling larger spatial scales resulted in increased precision and suggested that differences in counts were largely stochastic. The dimensions of sampling areas should consider the behavior, distribution, and density of fish (Samoilys & Carlos 2000). Pygmy Sculpin are relatively sedentary, so they do not typically leave quadrats once they are placed. Occasionally Pygmy Sculpin were attracted to the activity of counting quadrats, perhaps because prey items such as annelids, gastropods, and insect larvae were dislodged, but even with the largest quadrat size tested in this study ($1m^2$), observers were able to complete quadrat counts before Pygmy Sculpin moved into the quadrats. All quadrats sizes were suitable for the behavior and distribution of the benthic Pygmy Sculpin, so the important variable was the product of variance and cost (i.e. time). The $0.5m^2$ quadrats had the lowest product of variance and cost, so they were used for the rest of the study.

Although the quadrat based sampling scheme was the most effective sampling scheme, not all quadrat strata had lower CVs than the transect scheme. The CV of the run stratum was .26 greater than that of the transect scheme, and the CV of the *Nostoc*/sand stratum was .05 greater than the transect scheme. However, this was offset by the improvement that the quadrat scheme offered in the cobble habitat. The CV in the cobble was .45 less than the CV of the transect scheme, so the overall variation was lower in the quadrat sampling scheme. In addition, the quadrat sampling scheme allows for sample sizes of individual strata to be increased to reduce variation, which the transect scheme does not. These differences in CVs are based on 17 transects and 20 quadrat counts in each stratum, but it was possible to allocate more samples to just the run stratum to reduce its CV. Adding 10 more quadrats, a notable increase in sample size, takes less time than adding one more transect, a very minor increase in sample size. The quadrat scheme also required less equipment because observers were only counting $0.5m^2$ as a time. When water depth prevented observers from using their snorkels, observers could still complete a quadrat count with a short breath hold. In contrast, completing transect counts required the use of SCUBA gear. Quadrat counts also provide managers with more information about habitat use. For example, the generalized linear model was able to determine relative abundances among the various habitats.

The bootstrapping method was effective at determining sample sizes. The CVs of bootstrapped means decreased with sample size as predicted. The bootstrapped coefficients of variation of means indicated that the run was more variable than the *Nostoc*/sand habitat and the rock habitat. Two factors probably contributed to the high variation in the run habitat. First is habitat heterogeneity. Due to its small size relative to the rest of the study area, the run was not stratified despite having different habitat types including vegetation, gravel and cobble. Second is a mathematical artifact of whole numbers (Thompson & Mapstone 1993). When densities are low, inclusion or exclusion of one fish is a proportionally large difference compared to the mean. In Thompson and Mapstone's (1993) study, the mathematical artifact resulted in high variances among observers, but the same artifact could increase the variance among quadrats in this study. Regardless of the cause, more samples were allocated to the run to reduce the variation.

The protocol that was developed showed that Pygmy Sculpin densities have decreased over the past year, but the effect size of .97 times as many Pygmy Sculpin with each increasing month is small and may not be biologically significant. Monitoring only lasted for 1 year and it is likely that Pygmy Sculpin densities decrease in some years and increase in others. In addition, the positive sampling day by *Nostoc*/sand interaction term indicates that even though Pygmy Sculpin densities decreased in the rock habitat, there was a slight increase in Pygmy Sculpin densities in the *Nostoc*/sand habitat. This could be due to recruitment in the *Nostoc*/sand habitat, or a habitat shift. The ability to detect the small change in density demonstrates the precision of this protocol. More importantly, managers now have baseline data to compare to future Pygmy Sculpin densities. As of 2011, TCE concentrations were still rising in Coldwater Spring (SAIC 2011) and it is possible that the increasing concentrations could have population level effects on Pygmy Sculpin. Furthermore, stochastic events and the potential for reduced spring discharge are also threats that could effect the Pygmy Sculpin population. Monitoring should continue as long as these threats exist.

The negative binomial model also demonstrated Pygmy Sculpin do occur at different densities among habitats. Pygmy Sculpin densities were highest in the rock substrate. This finding is consistent with other studies that suggest sculpin select patches by substrate type and prefer to nest in cobble (Bateman & Li 2001, Johnston 2000, Ruzycki et al. 1998). In contrast, mottled sculpin in Appalachian streams select patches by prey abundance, not habitat type (Petty & Grossman 1996). The *Nostoc*/sand mixture had intermediate levels of Pygmy Sculpin, which tended to be juveniles. Ruzycki and Wurtsbaugh (1999) found that Bear Lake Sculpin undergo ontogenetic habitat shifts and it is possible that different size classes of Pygmy Sculpin utilize habitat types differently, but this hypothesis was not tested. Pygmy Sculpin densities were lowest and most variable in the run. The lower density in the run could be a result of increased temperature, habitat, or biotic interactions. Walsh et al. (1997) showed that Pygmy Sculpin are susceptible to warm temperatures and Pygmy Sculpin densities do decrease downstream from the weir. The highest densities of Pygmy Sculpin in the run occur just below the weir in swift water. Overall, counts within strata were homogeneous, but counts among strata were heterogeneous. Thus, adding the term for each stratum in the model removed the variance between strata and increased precision.

There is also evidence of surveys counting more Pygmy Sculpin in the fall. In both of the spring pool habitats, rock and *Nostoc*/sand, Pygmy Sculpin counts were greater in November than in other months. The *Nostoc*/sand habitat had more significant differences, while the rock habitat only had marginally significant differences. Pygmy Sculpin spawn year round, but spawning peaks in July (Johnston 2001), so recruitment is one probable cause for the increase in Pygmy Sculpin counts in the fall. In the run habitat, the differences were not as great, but there was still a marginally significant increase in October. Managers should consider the seasonal increase in Pygmy Sculpin counts when monitoring. Observations also suggest that Pygmy Sculpin in the *Nostoc*/sand habitat were smaller suggesting that the increase the greater increase in the *Nostoc*/sand is a result of new recruits, but this hypothesis was not explicitly tested.

The generalized linear model comparing multiple observers demonstrated that the degree of observer biases differed among habitat types. For example naïve observer 2 consistently undercounted when compared to the other observers in the rock habitat, but in the run habitat it was naïve observer 1 who undercounted. In the *Nostoc*/sand habitat, there were significant differences among all pairs of observers. The differences in Pygmy Sculpin counts among observers were not consistent among habitats suggesting that observers have unique biases depending on the habitat type. However, it is important to note that almost all quadrats in the *Nostoc* habitat were too deep to count while breathing through the snorkel. It is likely that the differences in counts among observers were largely a result of different abilities to hold breath, not just differences in the ability to count sculpin. An airline, or “hookah”, diving rig is a low cost solution that requires little training, but a more practical alternative may be to only use observers who are capable of holding their breath for at least a minute, because counts in the *Nostoc*/sand habitat rarely took longer than 1 minute to complete.

The non-significant term for the number of sampling trips suggests that experience does not play a significant role in Pygmy Sculpin counts, or that the experienced observer had already reached a saturation point at which increasing experience no longer affected Pygmy Sculpin counts. The observer by number of sampling trips interaction terms explain how experience influences the agreement between the experienced and naïve observers counts. If experience played no role, the naïve observer's counts would not differ from the experienced observer's counts, or if counts were different, there would be no significant interaction between observers and the number of sampling trips. If experience did play a role, the naïve observer's counts would differ from the experienced observer's on the first trip, but an interaction term would indicate that their counts were approaching that of the experienced observers with each successive trip. In this study, experience only improved agreement in counts between naïve observer 2 and the experienced observer in the rock habitat, but the interaction was only marginally significant ($p = .07$).

In general, the precision of observers increased with experience but the change was not significant. A study on coral reef fishes also found that variation within observers was not affected by experience (Williams et al 2006). The higher densities of Pygmy Sculpin in the cobble resulted in greater precision and less variation among observers. Thompson and Mapstone (1997) demonstrated that inter-observer variation is likely to be lower when densities of target fish are higher. In the *Nostoc*/sand habitat and the run, there were significant differences in the CVs among observers. The lower densities of these habitats may have contributed to greater variation among observers. Additionally, the *Nostoc*/sand habitat was more difficult to count because it often required observers to hold their breath and locate Pygmy Sculpin buried in *Nostoc*. Experience did not significantly affect the differences in CVs among observers, except there was a marginally significant (p = .08) interaction when naïve observer 2's CV was significantly larger than that of the experienced observer, suggesting that experience can reduce the CV. This only occurred in the *Nostoc*/sand habitat. It is important to note that observers were only compared over 5 sampling trips and the CVs of both experienced and naïve observers decreased slightly over time. It is possible that 5 trips were not enough to detect a significant decrease in CVs.

There is potential for differences among observers to bias results, but there are some ways to reduce this risk. Keeping observers constant among monthly and yearly surveys would reduce inter-observer differences because fewer observers would be involved. However, observers will inevitably leave and be replaced. Replacement observers and the leaving observer should overlap on some sampling trips so the inter-observer differences in counts can be determined. Even with biases due to observers, the parameter of interest is the estimate of how time affects Pygmy Sculpin densities, which was consistent among observers except naïve observer 2 in the cobble. If managers need a more precise method, a negative binomial regression with observer as a random effect could be used, but for the objectives of this study it was more appropriate to characterize the differences among observers rather than account for them using a mixed effects model. Characterizing the differences among observers by including observers as fixed effects makes managers aware of the biases, but does not complicate statistical testing or interpretation like incorporating observers as random effects would. More extensive studies on inter-observer differences in Pygmy Sculpin counts would be useful to managers.

One limitation of this study is detection was not evaluated. It is likely that detection varied among observers, which could have contributed to observer biases. However, it is also possible that detection varied temporally within individual observers due to changing conditions in the spring and the run. Conditions in the spring such as temperature, visibility, and habitat remain relatively constant so for this study assumed detection was constant but a more thorough examination of detection would be useful to future managers.

This study also demonstrated the importance of developing species-specific protocols. Standardized fisheries sampling protocols across states and regions have allowed biologists to remove variation caused by differences in sampling protocols and compare fish populations, growth rates, and assemblages among various water bodies (Bonar and Hubert 2002). However, for fishes that are threatened and have very limited ranges, the objective is not to compare data over a wide geographic area or among systems, but rather to determine how the population changes over time within a system. Thus, a species-specific protocol that reduces variance inherent within the system can be much more precise and valuable than a protocol that is standardized at the state or regional level.

Although this exact protocol was designed for Pygmy Sculpin, the method outlined here can be used to create snorkeling protocols for other spring species. Springs are unique habitats with high rates of endemism occurring at three way ecotones where the terrestrial environment, surface water and groundwater converge (Barquin & Scarsbrook 2008). Springs can serve as refuges from geological time and are often host to relict species (Botosaneau 1995). However, they are also highly susceptible to degradation because they occur at ecotones. Any changes to terrestrial, surface water, or groundwater environments have potential to affect springs. Like, Coldwater Spring, many other springs are threatened by pollutants (Barquin & Scarsbrook 2008). The problems of groundwater contaminants, stochastic events and changing spring discharge are widespread among spring habitats, thus this study can be useful to managers developing monitoring protocols for other endemic spring species. Furthermore, this study demonstrates some techniques that can be applied beyond snorkel surveys such as the bootstrapping method for determining sample sizes and stratifying by habitat type as a variance reduction method.

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Table 1. Descriptive Statistics for mean Pygmy Sculpin Counts for four quadrat sizes

Quadrat Size (m^2)	Mean Sculpin Count (sculpin / quadrat)	Variance	Standardized Cost	Standardized Variance	Product of Standardized Cost and Standardized Variance
0.25	3.9	4.2	1	1.54	1.55
0.50	7.1	10.5	1.07	1.02	1.09
0.75	4.73	14.2	1.23	1.07	1.31
1.00	4.98	19.9	1.78	1	1.78

Table 2. Descriptive Statistics for mean Pygmy Sculpin counts from transect and quadrat sampling methods

Sampling	Mean	Standard Deviation	CV
Transect	55.7	48.57	0.87
Quadrat - Rock	5.35	3.05	0.57
Quadrat - *Nostoc*/Sand	1.88	1.69	0.89
Quadrat - Run	1.16	1.28	1.1

Table 3. AIC Selection of four models describing change in Pygmy Sculpin densities in rock, *Nostoc*/sand, and run habitats over an 11 month period

Model	k	AIC	Delta AIC	Log Likelihood	ω_i
(3) Count ~ Sampling Day + Nostoc/sand + Run + Sampling Day : Nostoc/sand	6	2913.7	0	-1450.838	0.314
(1) Count ~ Sampling Day + Nostoc/sand + Run	5	2914	0.3	-1451.991	0.270
(4) Count ~ Sampling Day + Nostoc/sand + Run + Sampling Day : Run	6	2914.2	0.5	-1451.124	0.244
(2) Count ~ Sampling Day + Nostoc/sand + Run + Sampling Day : Run + Sampling Day:Nostoc	7	2914.9	1.2	-1450.452	0.172

Table 4. Differences in mean Pygmy Sculpin per quadrat between months in the rock habitat and Tukey honest significant difference pairwise comparisons

Months	**Difference**	**Lower 95% C.L.**	**Upper 95% C.L.**	**p - value**
December-August	0.08	-2.189	2.349	1.000
February-August	-0.68	-3.087	1.727	0.996
February-December	-0.76	-3.167	1.647	0.992
July-August	0.96	-1.309	3.229	0.940
July-December	0.88	-1.389	3.149	0.965
July-February	1.64	-0.767	4.047	0.476
June-August	1	-1.269	3.269	0.924
June-December	0.92	-1.349	3.189	0.954
June-February	1.68	-0.727	4.087	0.439
June-July	0.04	-2.229	2.309	1.000
March-August	-0.28	-2.549	1.989	1.000
March-December	-0.36	-2.629	1.909	1.000
March-February	0.4	-2.007	2.807	1.000
March-July	-1.24	-3.509	1.029	0.768
March-June	-1.28	-3.549	0.989	0.733
May-August	0.76	-1.509	3.029	0.987
May-December	0.68	-1.589	2.949	0.994
May-February	1.44	-0.967	3.847	0.661
May-July	-0.2	-2.469	2.069	1.000
May-June	-0.24	-2.509	2.029	1.000
May-March	1.04	-1.229	3.309	0.905
November-August	1.56	-0.709	3.829	0.462
November-December	1.48	-0.789	3.749	0.541
November-February	2.24	-0.167	4.647	0.092
November-July	0.6	-1.669	2.869	0.998
November-June	0.56	-1.709	2.829	0.999
November-March	1.84	-0.429	4.109	0.228
November-May	0.8	-1.469	3.069	0.982
October-August	0.28	-1.989	2.549	1.000
October-December	0.2	-2.069	2.469	1.000
October-February	0.96	-1.447	3.367	0.958
October-July	-0.68	-2.949	1.589	0.994
October-June	-0.72	-2.989	1.549	0.991
October-March	0.56	-1.709	2.829	0.999
October-May	-0.48	-2.749	1.789	1.000
October-November	-1.28	-3.549	0.989	0.733
September-August	0.24	-2.029	2.509	1.000
September-December	0.16	-2.109	2.429	1.000
September-February	0.92	-1.487	3.327	0.968

September-July	-0.72	-2.989	1.549	0.991
September-June	-0.76	-3.029	1.509	0.987
September-March	0.52	-1.749	2.789	0.999
September-May	-0.52	-2.789	1.749	0.999
September-November	-1.32	-3.589	0.949	0.697
September-October	-0.04	-2.309	2.229	1.000

Table 5. Differences in mean Pygmy Sculpin per quadrat in the *Nostoc*/sand habitat between months and Tukey honest significant difference pairwise comparisons

Months	**Differences**	**Lower 95% C.L.**	**Upper 95% C.L.**	**p - value**
December-August	-0.231	-1.662	1.200	1.000
February-August	-0.191	-1.622	1.240	1.000
February-December	0.04	-1.418	1.498	1.000
July-August	-0.153	-1.599	1.294	1.000
July-December	0.078	-1.395	1.552	1.000
July-February	0.038	-1.435	1.512	1.000
June-August	0.122	-1.245	1.490	1.000
June-December	0.353	-1.043	1.749	0.998
June-February	0.313	-1.083	1.709	0.999
June-July	0.275	-1.137	1.687	1.000
March-August	-0.155	-1.617	1.308	1.000
March-December	0.077	-1.413	1.566	1.000
March-February	0.037	-1.453	1.526	1.000
March-July	-0.002	-1.506	1.503	1.000
March-June	-0.277	-1.706	1.152	1.000
May-August	-0.034	-1.451	1.382	1.000
May-December	0.197	-1.247	1.641	1.000
May-February	0.157	-1.287	1.601	1.000
May-July	0.119	-1.341	1.578	1.000
May-June	-0.156	-1.538	1.225	1.000
May-March	0.12	-1.355	1.596	1.000
November-August	1.369	-0.062	2.800	0.074
November-December	1.6	0.142	3.058	0.019
November-February	1.56	0.102	3.018	0.025
November-July	1.522	0.048	2.995	0.037
November-June	1.247	-0.149	2.643	0.126
November-March	1.523	0.034	3.013	0.040
November-May	1.403	-0.041	2.847	0.065
October-August	0.12	-1.297	1.536	1.000
October-December	0.351	-1.093	1.795	0.999
October-February	0.311	-1.133	1.755	1.000
October-July	0.272	-1.187	1.732	1.000
October-June	-0.003	-1.384	1.379	1.000
October-March	0.274	-1.201	1.750	1.000
October-May	0.154	-1.276	1.584	1.000
October-November	-1.249	-2.693	0.195	0.156
September-August	0.329	-1.102	1.760	0.999
September-December	0.56	-0.898	2.018	0.968
September-February	0.52	-0.938	1.978	0.980

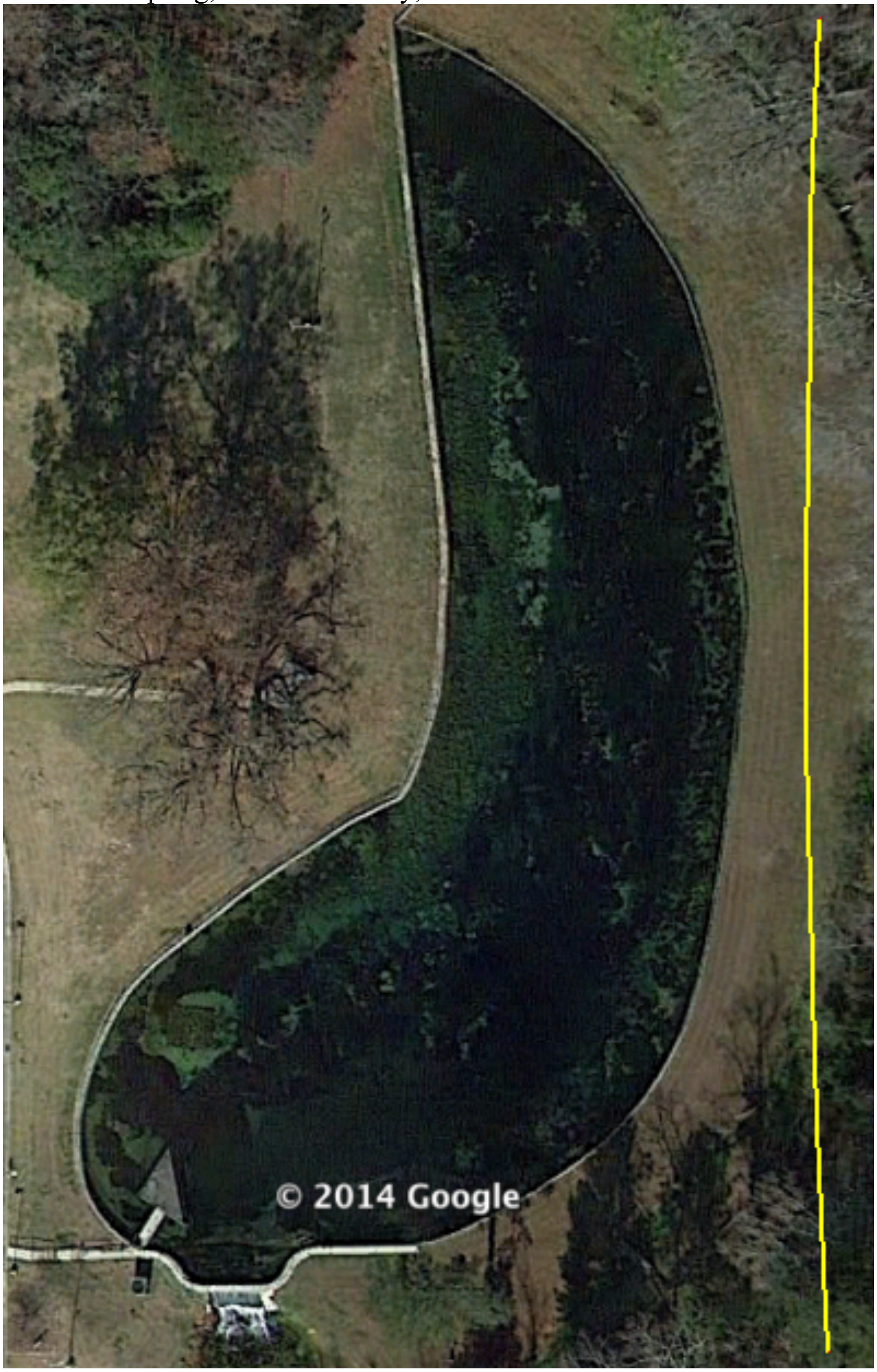
September-July	0.482	-0.992	1.955	0.989
September-June	0.207	-1.189	1.603	1.000
September-March	0.483	-1.006	1.973	0.990
September-May	0.363	-1.081	1.807	0.998
September-November	-1.04	-2.498	0.418	0.408
September-October	0.209	-1.235	1.653	1.000

Table 6. Differences in mean Pygmy Sculpin per quadrat in the run habitat between months and Tukey honest significant difference pairwise comparisons

Months	**Difference**	**Lower 95% C.L.**	**Upper 95% C.L.**	**p - value**
December-August	-0.233	-1.238	0.771	0.999
February-August	-0.467	-1.471	0.538	0.899
February-December	-0.233	-1.238	0.771	0.999
July-August	0.200	-0.805	1.205	1.000
July-December	0.433	-0.571	1.438	0.934
July-February	0.667	-0.338	1.671	0.518
June-August	-0.332	-1.329	0.664	0.988
June-December	-0.099	-1.096	0.898	1.000
June-February	0.134	-0.862	1.131	1.000
June-July	-0.532	-1.529	0.464	0.793
March-August	-0.767	-1.771	0.238	0.310
March-December	-0.533	-1.538	0.471	0.799
March-February	-0.300	-1.305	0.705	0.994
March-July	-0.967	-1.971	0.038	0.071
March-June	-0.434	-1.431	0.562	0.930
May-August	-0.333	-1.338	0.671	0.988
May-December	-0.100	-1.105	0.905	1.000
May-February	0.133	-0.871	1.138	1.000
May-July	-0.533	-1.538	0.471	0.799
May-June	-0.001	-0.998	0.996	1.000
May-March	0.433	-0.571	1.438	0.934
November-August	-0.167	-1.171	0.838	1.000
November-December	0.067	-0.938	1.071	1.000
November-February	0.300	-0.705	1.305	0.994
November-July	-0.367	-1.371	0.638	0.977
November-June	0.166	-0.831	1.162	1.000
November-March	0.600	-0.405	1.605	0.666
November-May	0.167	-0.838	1.171	1.000
October-August	0.220	-0.834	1.274	1.000
October-December	0.453	-0.600	1.507	0.935
October-February	0.687	-0.367	1.740	0.545
October-July	0.020	-1.034	1.074	1.000
October-June	0.552	-0.494	1.598	0.804
October-March	0.987	-0.067	2.040	0.088
October-May	0.553	-0.500	1.607	0.809
October-November	0.387	-0.667	1.440	0.976
September-August	-0.333	-1.338	0.671	0.988
September-December	-0.100	-1.105	0.905	1.000
September-February	0.133	-0.871	1.138	1.000

September-July	-0.533	-1.538	0.471	0.799
September-June	-0.001	-0.998	0.996	1.000
September-March	0.433	-0.571	1.438	0.934
September-May	0.000	-1.005	1.005	1.000
September-November	-0.167	-1.171	0.838	1.000
September-October	-0.553	-1.607	0.500	0.809

Figure 1. Satellite imagery showing rock, vegetation and *Nostoc*/sand habitat types in Coldwater Spring, Calhoun County, AL



Yellow line represents 150m.

Figure 2. Underwater image of rock habitat in Coldwater Spring

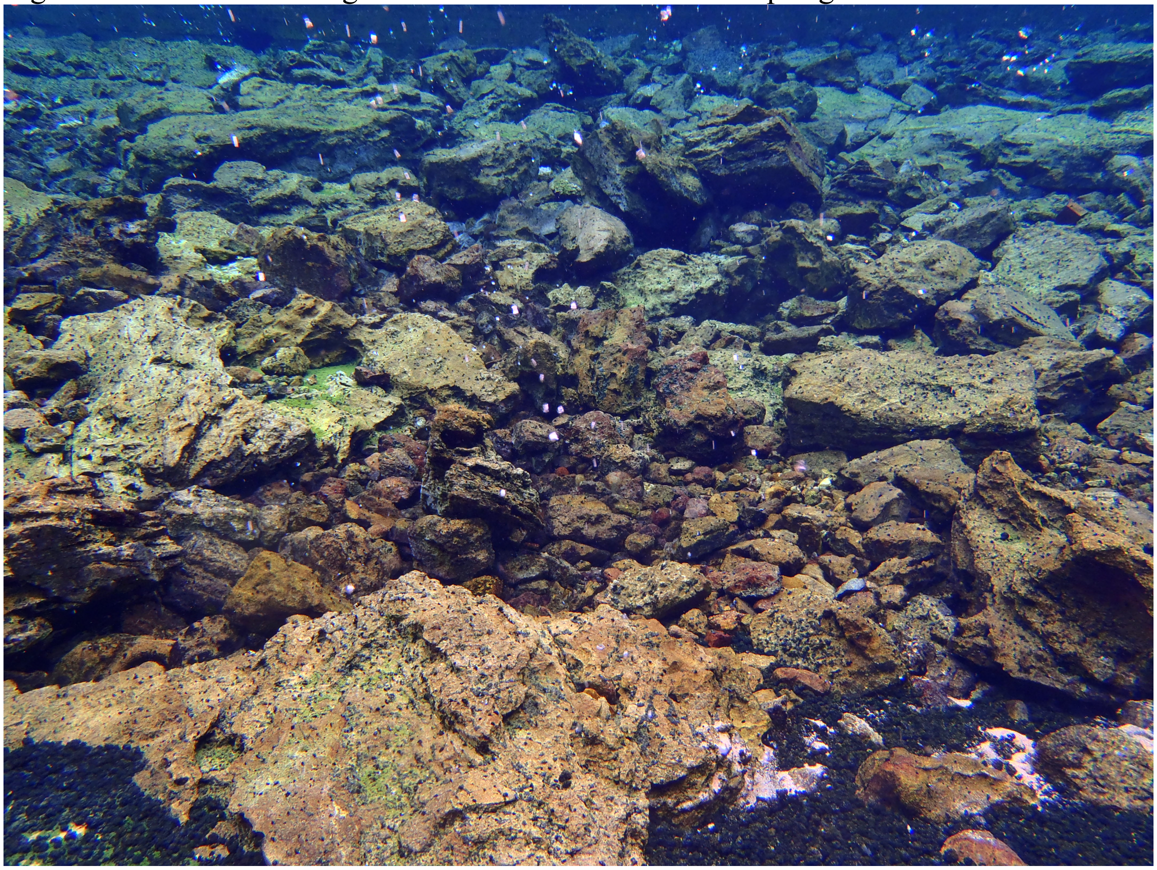


Figure 3. Underwater image of *Nostoc*/sand habitat in Coldwater Spring

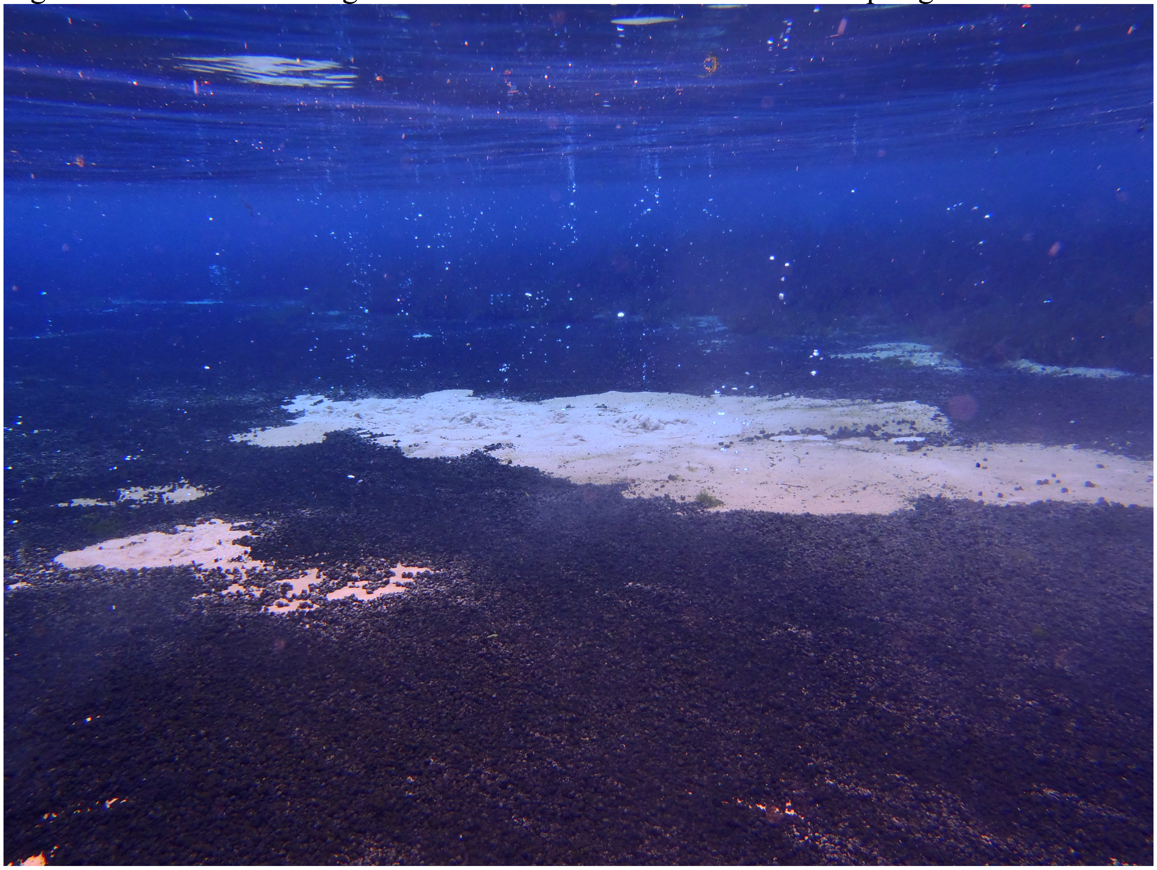


Figure 4. Underwater image of Coldwater Spring Run

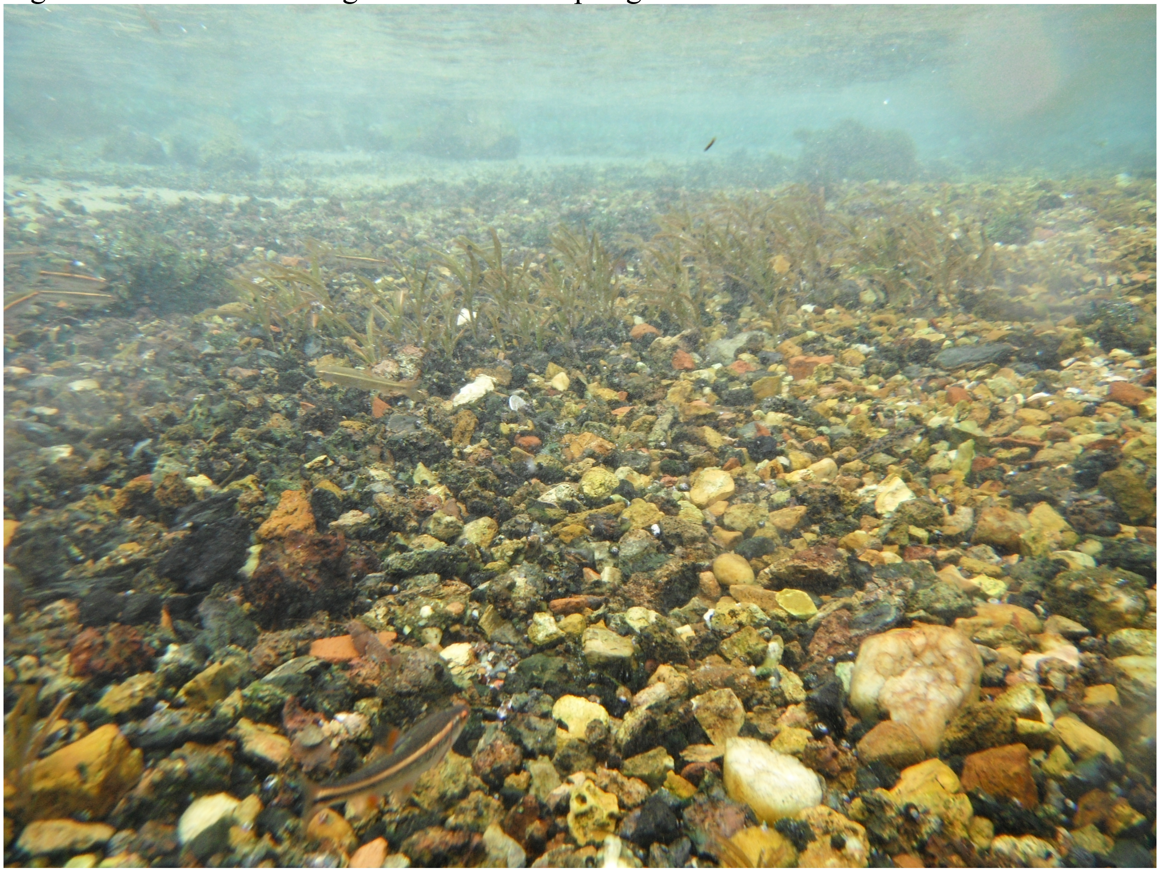
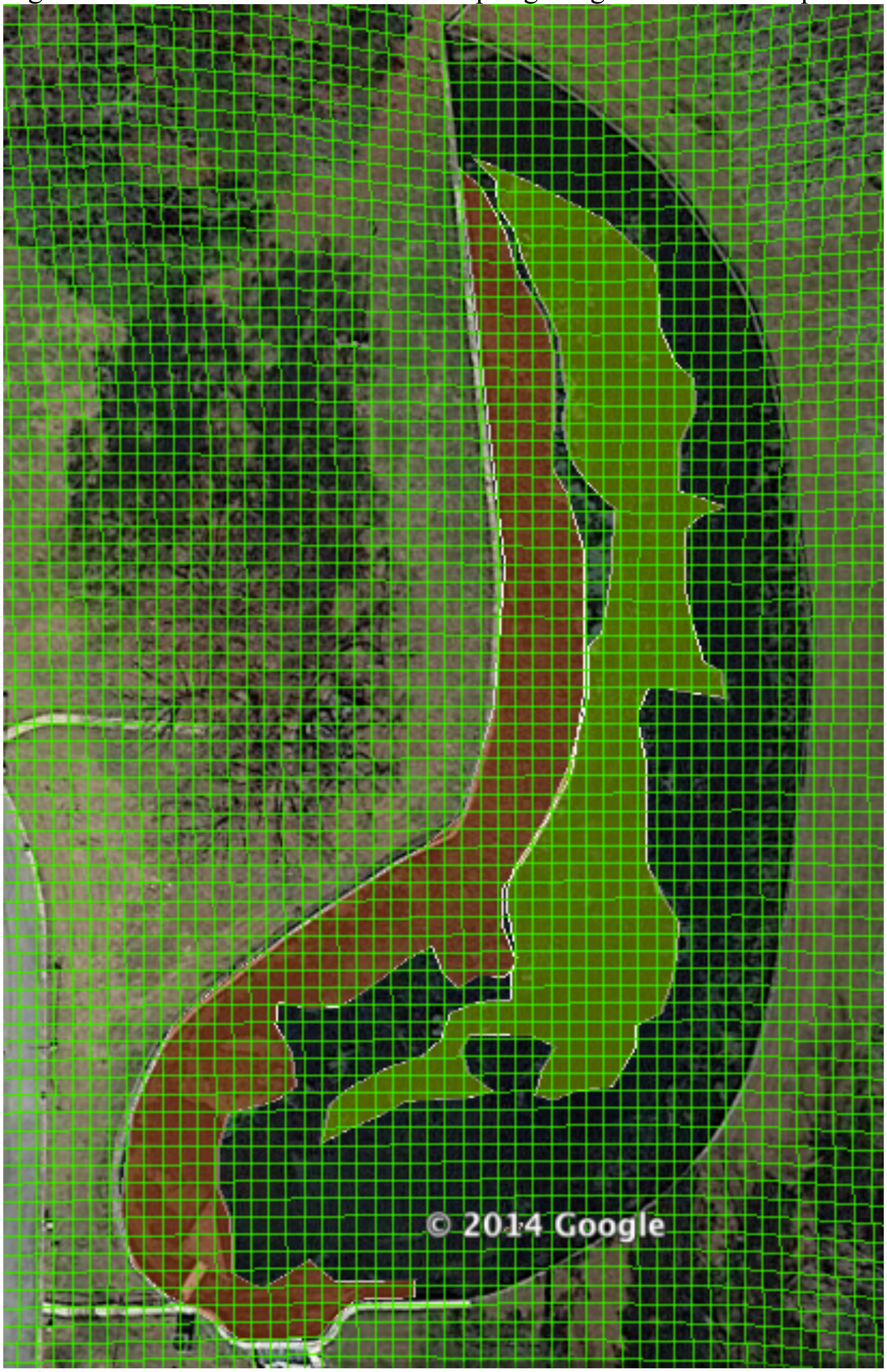


Figure 5. Grid overlaid on Coldwater Spring image used to select quadrat sampling sites



Orange represents rock habitat and green represents *Nostoc*/sand habitat. Numbers are assigned to each cell in each habitat starting in the upper left and counted to the right and down. If any portion of a cell is shaded, it is included in that habitat.

Figure 6. Relative Cost, Relative Variance, and Relative Cost times Relative Variance for four Quadrat Sizes

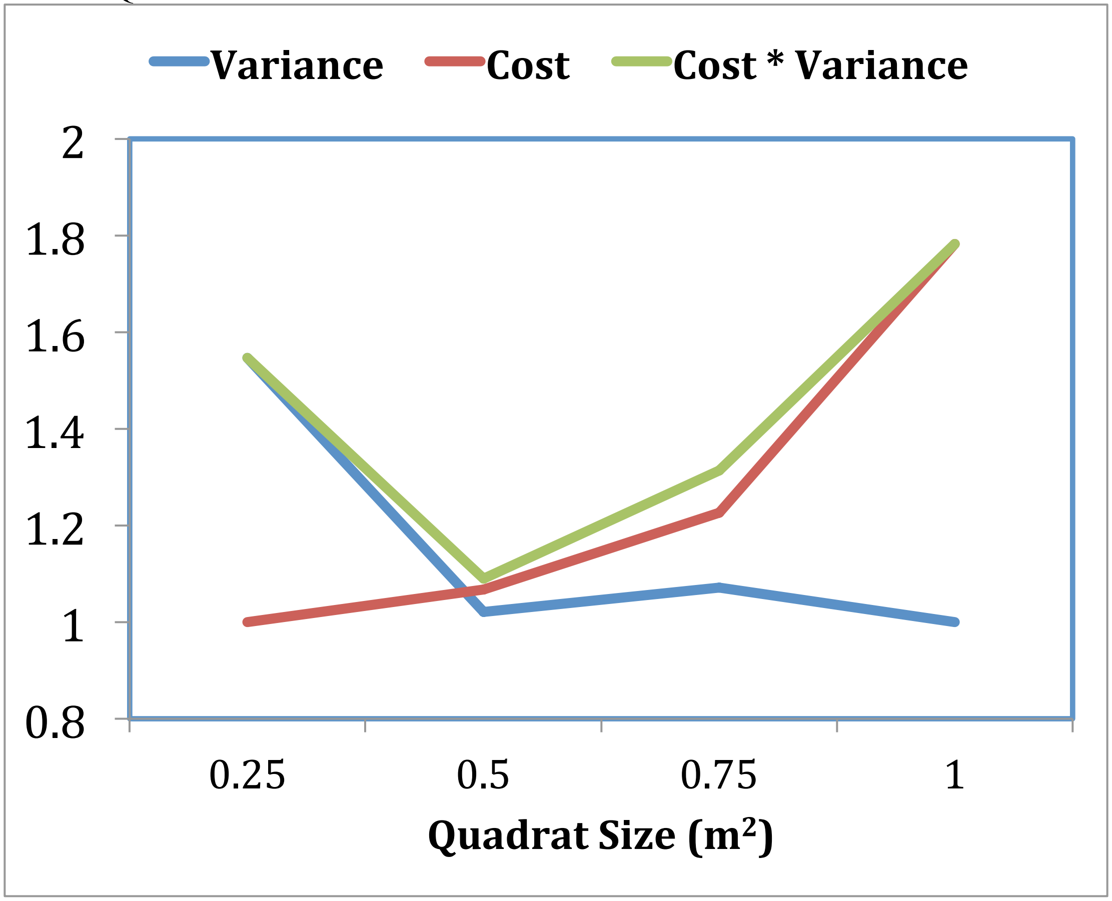


Figure 7. Coefficient of variation of bootstrapped mean Pygmy Sculpin counts in the rock habitat

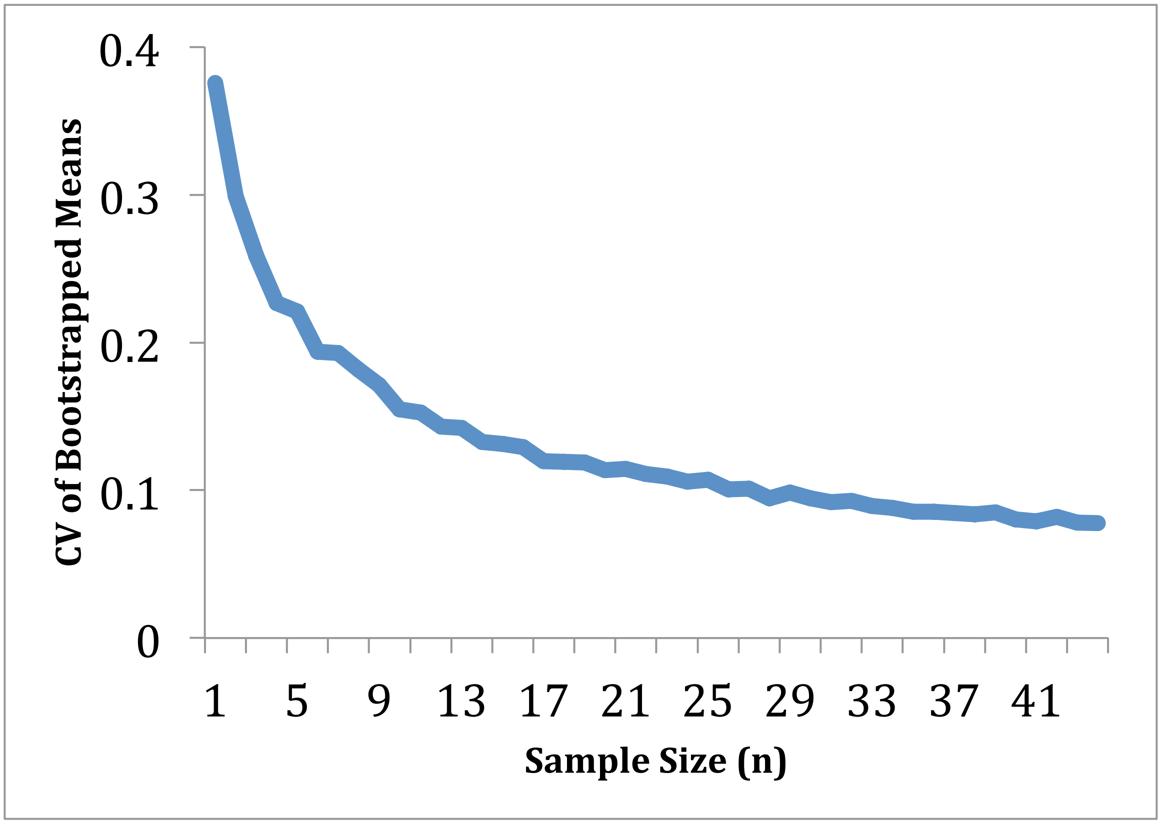


Figure 8. Coefficient of variation of bootstrapped mean Pygmy Sculpin counts in the *Nostoc*/sand habitat

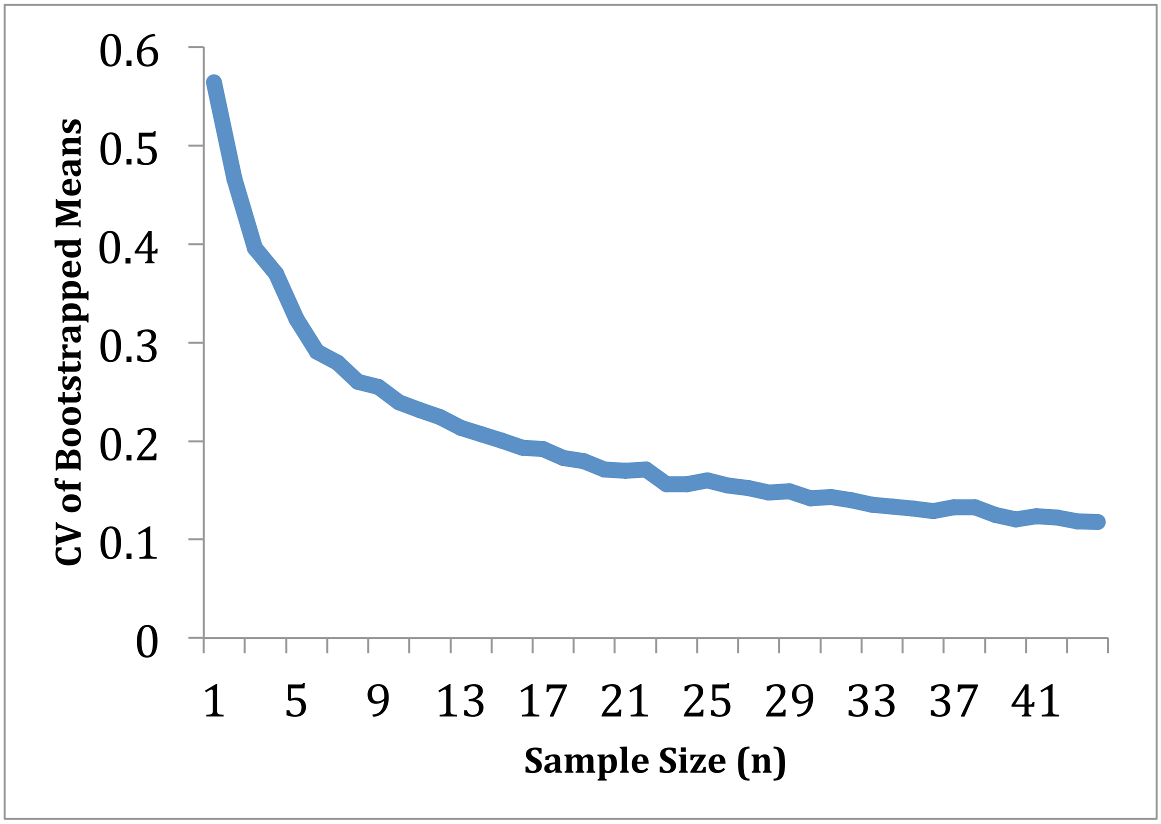


Figure 9. Coefficient of variation of bootstrapped mean Pygmy Sculpin counts in the run Habitat

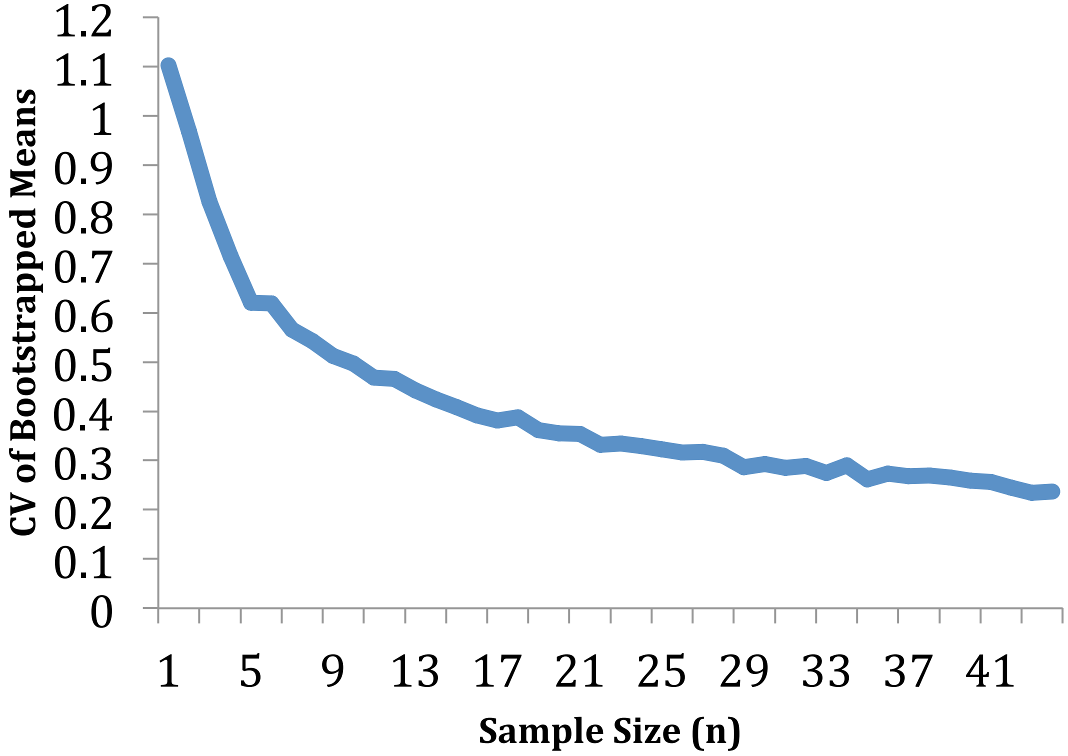


Figure 10. Mean Pygmy Sculpin densities from May 2013 through March 2014 in the rock habitat

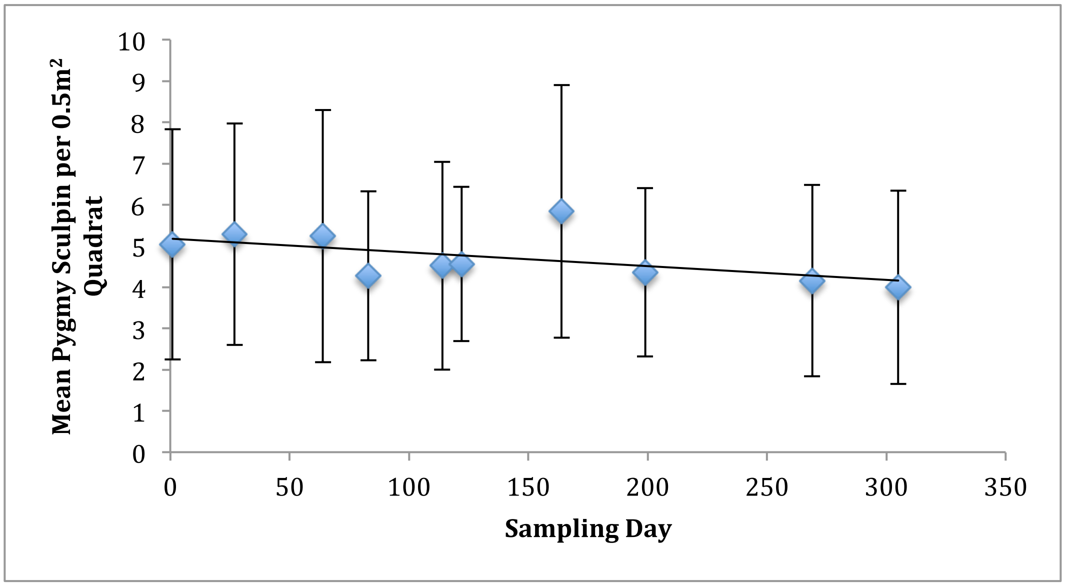


Figure 11. Mean Pygmy Sculpin densities from May 2013 through March 2014 in the *Nostoc*/sand habitat

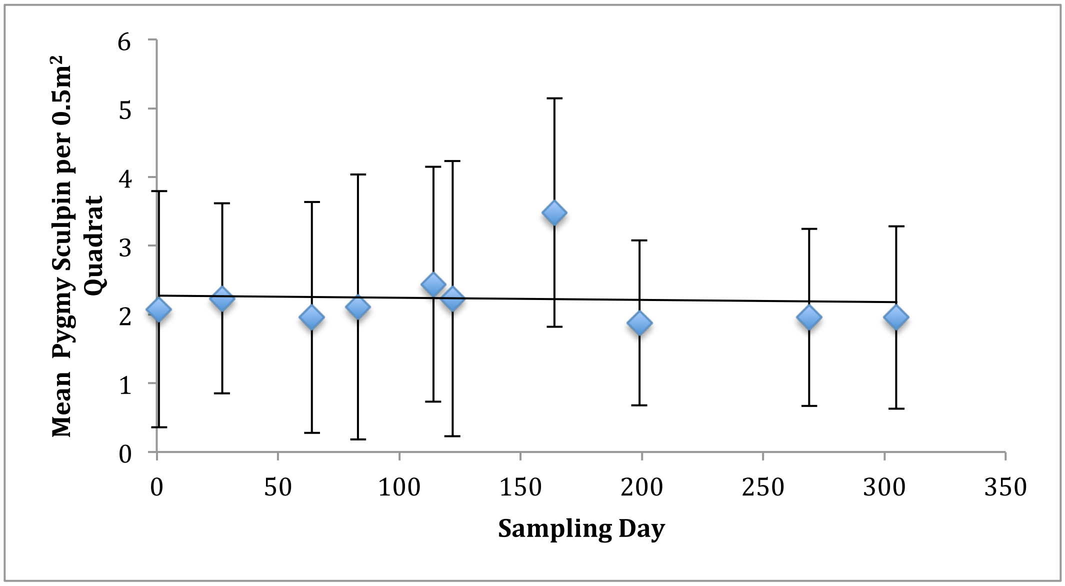


Figure 12. Mean Pygmy Sculpin densities from May 2013 through March 2014 in the run habitat

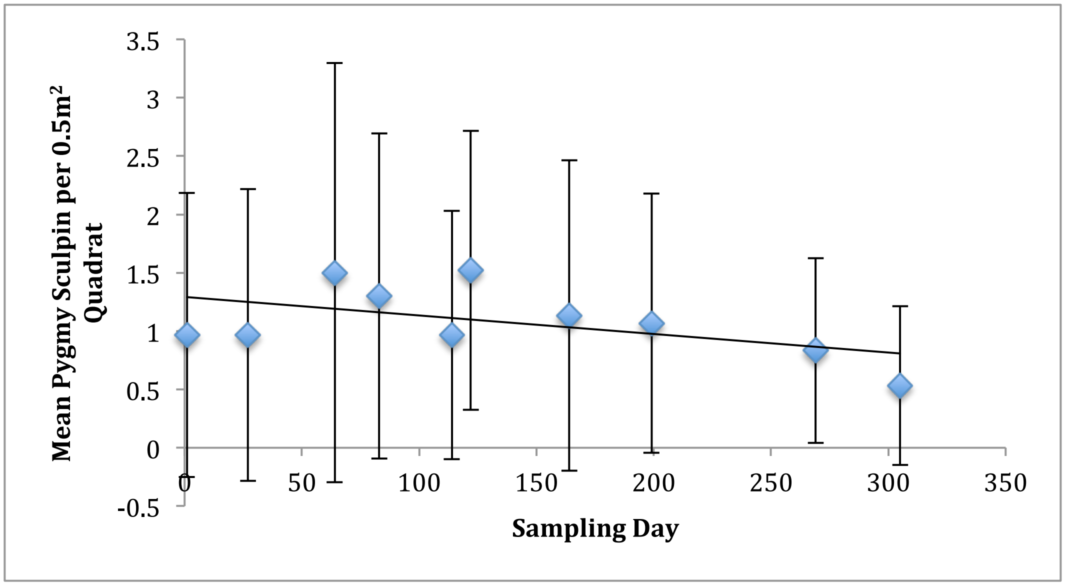


Figure 13. Comparison of experienced and naïve observer's pygmy sculpin counts in the rock habitat

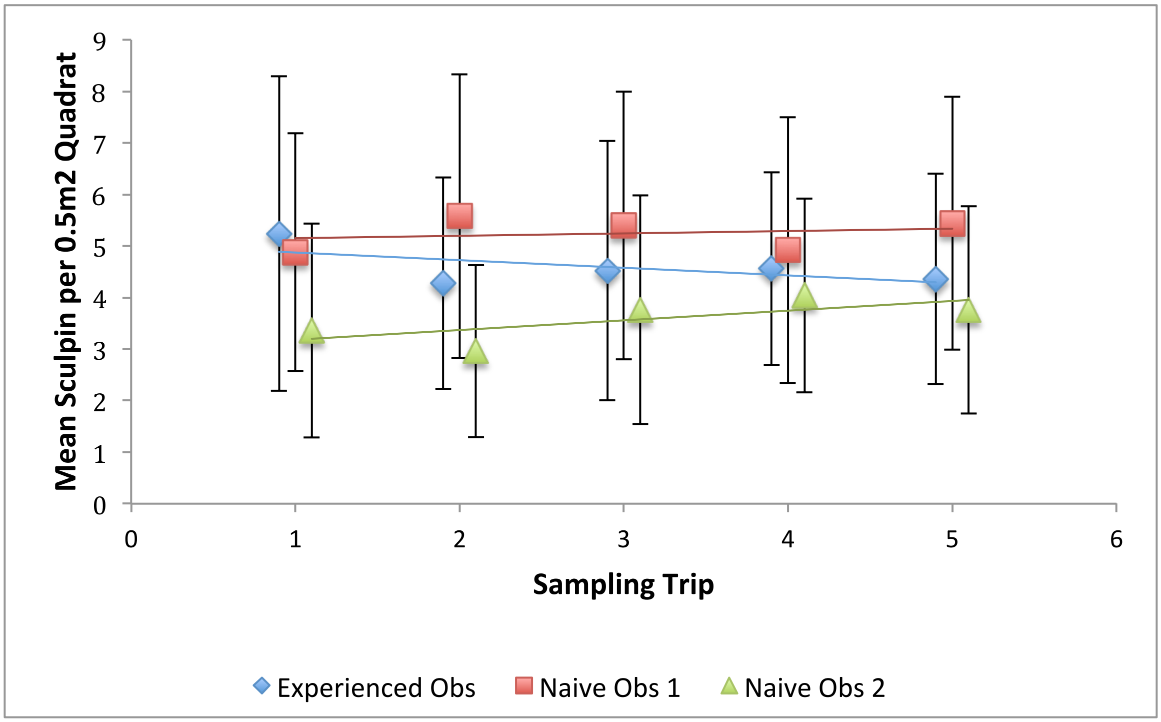


Figure 14. Comparison of experienced and naïve observer's pygmy sculpin counts in the *Nostoc*/sand habitat

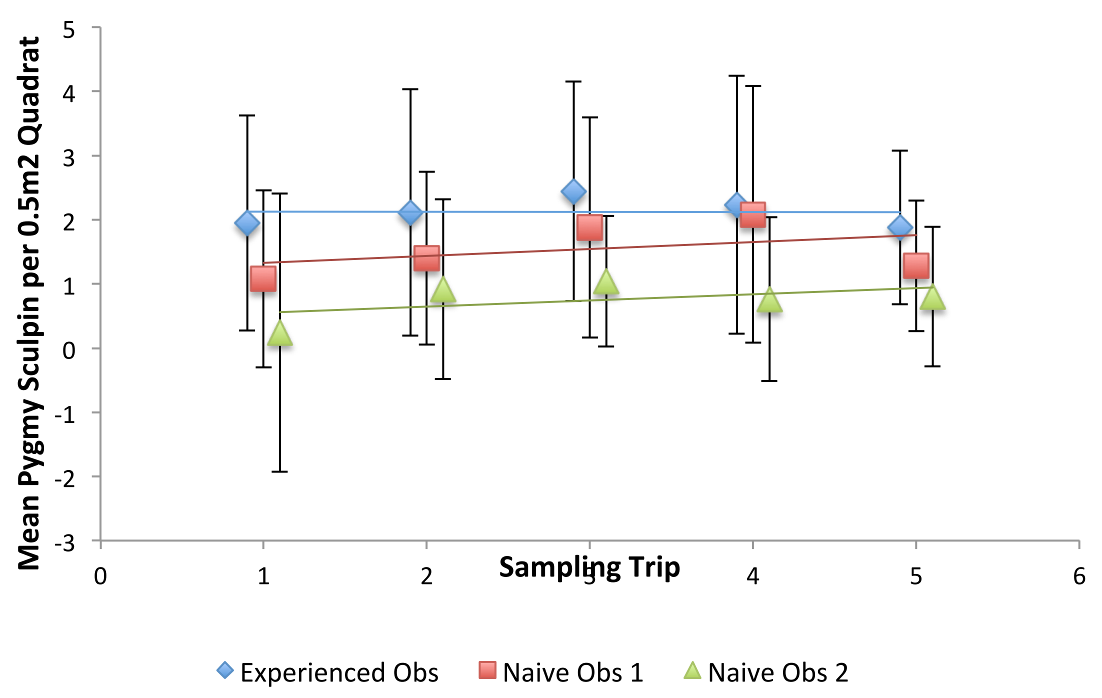


Figure 15. Comparison of experienced and naïve observer's pygmy sculpin counts in the run habitat

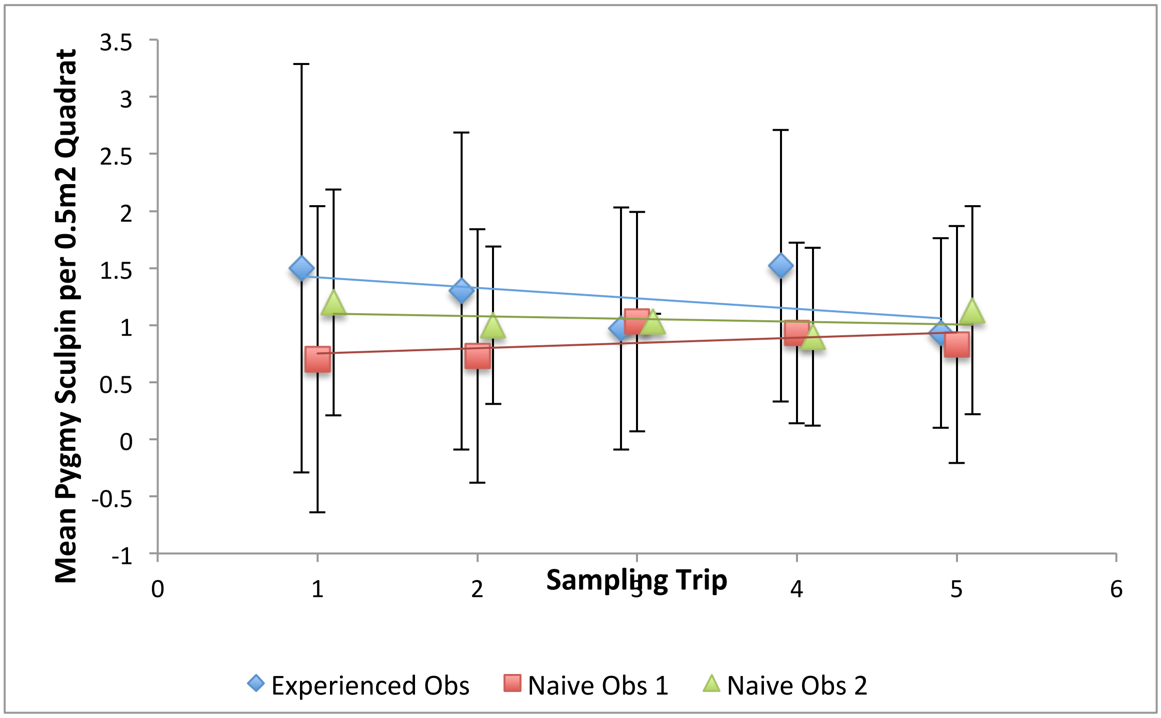


Figure 16. Precision of experienced and naïve observers in the rock habitat over multiple sampling trips

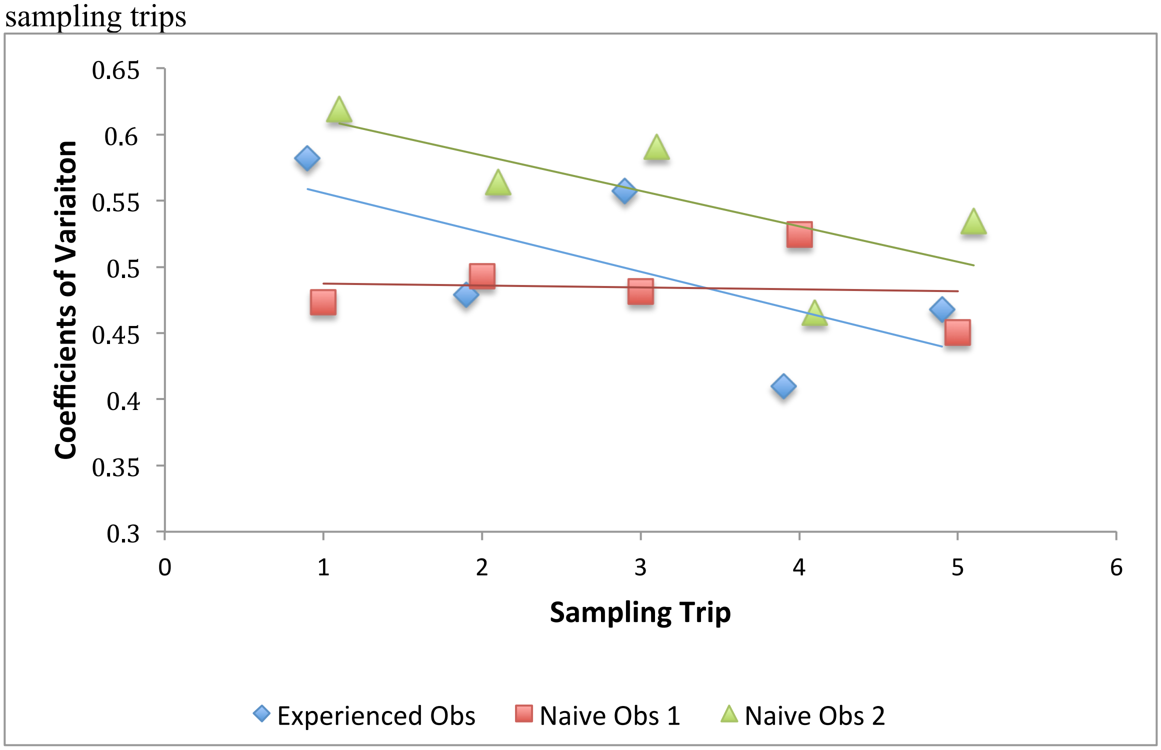


Figure 17. Precision of experienced and naïve observers in the *Nostoc*/sand habitat over multiple sampling trips

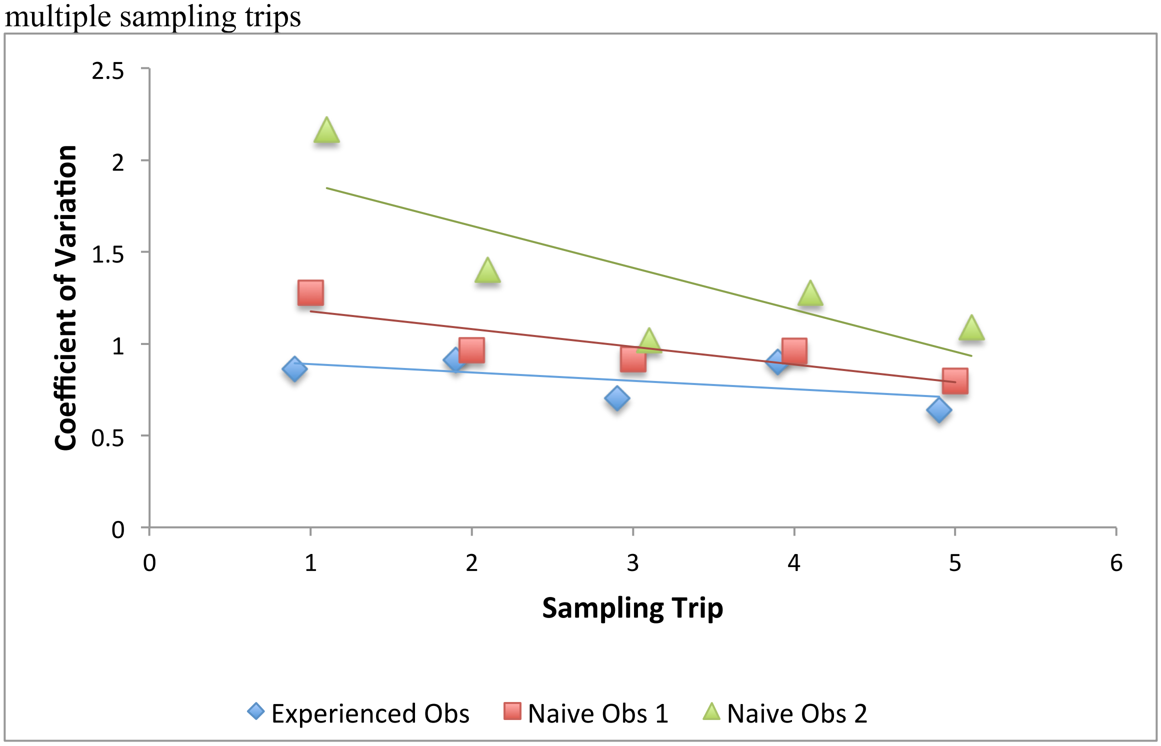
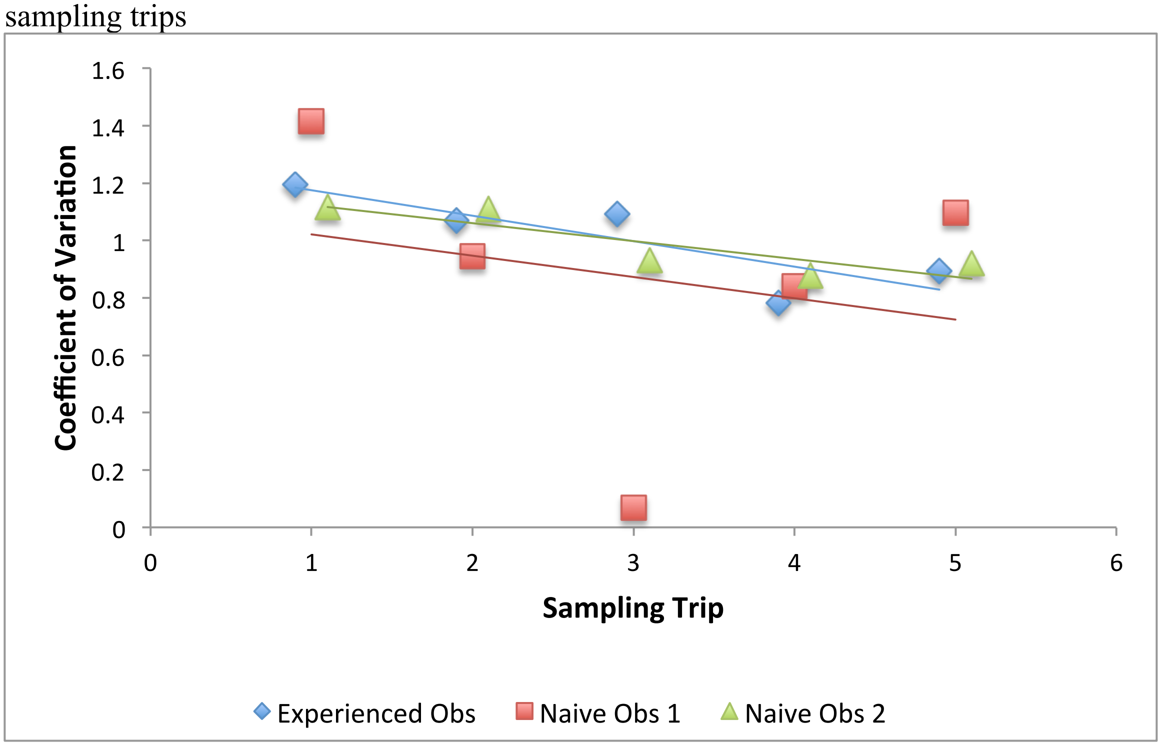


Figure 18. Precision of experienced and naïve observers in the run habitat over multiple sampling trips



Appendix – Sampling protocol for Pygmy Sculpin in Coldwater Spring, AL.

(1) Prior to departing for Coldwater Spring, quadrat sites should be determined by using a random number generator in conjunction with a digital map of the spring (Figure 5). Twenty-five quadrat sites can be selected by choosing 25 integers between 1 and 341, which correspond to each cell over the rock habitat starting at the upper left and counting towards the right and down. Twenty-five quadrat sites in the *Nostoc*/sand habitat can be selected in the same manner except the integers will range from 1-370. Thirty quadrat sites in the run should be selected using integers between 1 and 67. There were too many cells to label them all on the map, but each cell is labeled on the digital, Google Earth version of the map. Labels for sites for individual sampling trips can be made visible on the digital map and then the map can be printed. The map can then be laminated so observers can carry it in the spring. (2) Observers should check that weather conditions are safe for snorkeling activities. If thunderstorms are expected, sampling should be delayed until weather clears. Snorkelers can sample in light rain, but sunny weather is preferable because visibility will be better. (3) Observers should arrive at the spring by 10:00 am to ensure that there is adequate time to complete sampling during the day, and that sampling will occur during the brightest time of day. Upon arrival, observers should check again to make sure that the weather is clear and there are no safety hazards. (4) If the weather is clear and there are no hazards, observers can put on equipment and prepare sampling. Wetsuits should be worn to prevent hypothermia and antifog solution should be applied to masks to maintain visibility. Weight belts should be adjusted before

observers start sampling. (5) Observers should approach the quadrat site by visually referencing the map of sites and then haphazardly place the quadrat. If current exists, observers should approach from down current to prevent silt from hindering visibility. (6) Counts should begin as soon as the quadrat sinks. Sculpin less than 5mm are considered young of the year and should be not be counted, but observers should note their presence. Quadrats should be counted from the outside in, and any rocks, vegetation, wood or debris should be removed to check for hidden Pygmy Sculpin. Any object that is removed should be replaced after counting is complete, with special care given to objects that were concealing Pygmy Sculpin nests. In the *Nostoc*/sand habitat, observers should gently brush away any *Nostoc* to reveal Pygmy Sculpin hidden in the *Nostoc*. Observers should look for Pygmy Sculpin that dart out of the *Nostoc* when it is disturbed. (7) The number of Pygmy Sculpin and habitat type should be recorded on a data cuff immediately after the quadrat is sampled. Any young of the year Pygmy Sculpin or other interesting notes should also be recorded. (8) Steps 5 - 7 should be repeated until all quadrats sites are sampled. (9) Data should be transferred from the data cuffs to a field notebook as soon as observers exit the water. (10) A negative binomial regression should be used to test for changes in Pygmy Sculpin densities.