Missouri
Department of
Natural Resources

2008 Big River Sediment Sampling and Mussel Survey

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INTRODUCTION

The Old Lead Belt is a historic lead and zinc mining district within the Southeast Missouri Lead Mining District (SEMOLMD), which had some of the highest production of lead worldwide. There are six major chat piles and tailings impoundments (chat and tailings are mill waste produced by separating ore from host rock) in the Old Lead Belt within the Big River watershed. Releases of mill waste have been documented into the Big River and tributaries. These releases have contaminated sediment in over 90 miles of the Big River and its tributaries (MDNR, 2007) with lead and zinc in excess of Probable Effects Concentrations (PEC) established by MacDonald et al. (2000).

Freshwater mussels are important indicators of toxicological stressors on the aquatic benthic community due to their dependence on sediment for most of their lifecycle. Their abundance, diversity and species richness have been demonstrated to be negatively correlated with heavy metal contaminated sediment in the Tri-State Mining District of Missouri, Kansas, and Oklahoma (Angelo, 2007). Buchanon et al. (1979) surveyed naiads (freshwater mussels) in the Meramec River basin, including the Big River. Buchanon noted reduction of mussel numbers and diversity, presumably due to release of lead tailings into the Big River. Schmitt et al. (1987) found elevated metal concentrations in the gut and tissues of freshwater mussels in the Big River below lead mining sites. Roberts et al. (1999) surveyed some of the same locations as Buchanon (1979) and noted additional declines in mussel populations. The United States Fish and Wildlife Service's (Service) 2007 screening level survey of mussel populations and sediment metal concentrations in the Big River demonstrated that mussels are less abundant and less diverse in sampling locations below mining impacts where sediment concentrations exceed the PEC for Pb and/or Zn. Other anecdotal surveys have noticed further declines to mussel populations in locations further downstream of previous studies.

STUDY OBJECTIVES

This study will seek to:

1. provide a full characterization of the downgradient extent of heavy metal contamination of sediment; and
2. define the richness, distribution, and abundance of freshwater mussel species in the Big River.
3. evaluate the relationship between heavy metal concentrations in sediment and mussel populations

METHODS

1. Extent of Heavy Metal Contamination in Sediment

The Service (2007) identified elevated heavy metals (particularly Pb) in sediments throughout the entire extent of the Big River below the Old Lead Belt. However, the extent to which contaminated sediment in the Big River is impacting the Meramec River below their confluence

has not been adequately characterized. To ensure that the leading edge of contamination is characterized, field screening of metals in sediment will be conducted with an X-ray Fluorescence (XRF) meter.

1.1 Field screening of metals with XRF

Field screening will be used to identify the portion of the stream that represents the leading edge of sediment contamination near the Big River/Meramec River confluence. The leading edge of contamination will be defined as sediment concentrations above the Threshold Effects Concentrations (TEC), according to MacDonald et al. (2000), but below the PEC. Samples will be collected at the confluence with the Meramec River and proceed downstream at approximately 1 mile intervals until no metal concentrations are above the TEC. If concentrations of any of the metals of concern are above their respective TEC, a downstream sample location will be selected and the procedure will be repeated. Downstream sampling will be discontinued when sediment concentrations of all of the metals of concern are found below their respective TEC.

Sediment will be collected from relatively slack water near physically adequate mussel habitat, riffle/shoot complexes with relatively stable gravel sized particles. Composite samples will be collected from shallow water less than 15 cm (6 inches) deep. Each sample will contain no less than five aliquots collected within approximately a ten square meter area. Sediment subsamples 7 cm (3 inches) deep will be collected using a Teflon or plastic scoop (or large spoon) and placed on a paper plate or other non-metallic vessel in the open air for short-term drying to approximately 20% moisture or less based on visual estimation. (Twenty percent moisture is approximately equivalent to a moist soil.)

Samples will be analyzed in the field by placing the sample in contact with the portable XRF detector analytical window for approximately one minute after air drying. Concentrations of Pb, Zn, Cd, Ba, and Ni will be recorded in a log book and stored in the meter using a discreet sample number. After analysis with the XRF, the sample will be placed in a plastic zip-lock bag and labeled with the discreet sample number previously recorded in the log book.

A GPS reading and one or more photographs will be taken at every sample location. The GPS reading will be recorded in the log book.

1.2 Sediment sample collection

As mentioned above, field analyzed sediment samples will be collected in plastic bags for further XRF and laboratory analysis. In addition to the field screening sediment samples, field sediment samples will be collected at all of the sites identified in Table 1. Sampling collection techniques will be the same as the description in 1.1 above. Sediment will be collected from relatively low velocity, shallow water areas adjacent to riffles or other suitable mussel habitat and labeled as described above. Approximately 0.5 kilograms of sediment will be collected at each location. GPS readings and photographs will be taken and recorded as described above.

Additional sediment material will be collected at certain sampling locations for the purpose of quality control/verification. One quality control (QC) sample will be collected for every tenth sample, or one QC sample will be collected by each team per day, whichever number is greater.

For these samples additional sample volume will be required: approximately 1.5- 2.0 kilograms. Two separate bags should be collected with alternating spoonfuls of sample placed in each bag.

1.3 Sediment sample analysis

Sediment samples will be analyzed using an XRF meter and quality control samples will be analyzed by both XRF and by Inductively Coupled Plasma or Atomic Adsorption in a laboratory. Samples will be thoroughly mixed within a bag by shaking and/or hand manipulation. Samples will be analyzed by XRF after drying at room temperature for at least seven days or until less than 20% moisture has been achieved. A portion of each sediment samples will be sieved to less than 250 microns. Both the less than 250 micron and the bulk samples will be analyzed by XRF. Each sample will be analyzed for one minute with the XRF by placing the instrument directly against the bag with the sediment in full contact with the portion of the bag in contact with the XRF window. Three separate readings will be collected for each sample. These results will be recorded in a log book and stored electronically in a database spreadsheet.

A suite of calibration verification check samples will be used to check the accuracy of the XRFinstrument and to assess the stability and consistency of the analysis for the analytes of interest. Check samples will be analyzed at the beginning of each working day, during active sample analyses, and at the end of each working day. The measured value for each target analyte should be within ±20 percent (%D) of the true value for the calibration verification check to be acceptable. If a measured value falls outside this range, then the check sample should be reanalyzed. If the value continues to fall outside the acceptance range, the instrument should be recalibrated, and the batch of samples analyzed before the unacceptable calibration verification check will be reanalyzed (USEPA 1998).

Quality control samples will be analyzed by XRF as described above. In addition, QC samples will be submitted to a laboratory for analysis of total Pb, Zn, Cd, Ba, and Ni using Inductively Coupled Plasma or Atomic Adsorption following EPA method 3050b "Acid Digestions of Sediment, Sludges, and Soils". The QC samples will be analyzed in bulk and samples will also be wet sieved using site water to determine metals content and the percentage of sediments that fall within the following fractions: <62 µm, 62-250 µm, 250 µm-2mm, and >2 mm. A summary of the analytical parameters and methods are provided below:

Table 1. Analytical Parameters

Sample Type	Analytical Method	Analyte	Fraction analyzed
Leading Edge Definition	Field XRF	Pb, Zn, Cd, Ni, Ba	Bulk and <250 micron
Extent of contamination characterization	Office/laboratory XRF	Pb, Zn, Cd, Ni, Ba	Bulk and <250 micron
QC samples	Office/laboratory XRF	Pb, Zn, Cd, Ni, Ba	Bulk and <250 micron
QC samples	Laboratory ICP-MS	Pb, Zn, Cd, Ni, Ba	<62 µm, 62-250 µm, 250 µm-2mm, >2 mm, and Bulk fractions

1.4 Surface water quality

Surface water quality analyses (i.e., temperature, pH, conductivity, dissolved oxygen, turbidity) will be measured in situ at each riffle within a site with a multi-parameter water quality instrument (e.g. YSI 556).

2. Mussel Population and Diversity Evaluation

Timed, qualitative mussel surveys will be preformed in the Big River to evaluate current species richness and distribution and permit comparisons between present mussel assemblages and those reported by previous researchers (Buchanan 1979, Oesch 1995, Roberts and Bruenderman 2000, Missouri Department of Conservation Database). Timed search sampling methods have been chosen because Buchanan (1979) and Roberts and Bruenderman (2000) conducted timed searches, and methods between the surveys need to be similar in order to assess changes over time. Timed searches also produce a more complete list of species (including rare species) at a given site than quantitative (quadrat) sampling (Strayer *et al.* 1997, Vaughn *et al.* 1997). Furthermore, timed search sampling requires less effort than quadrats but still provides accurate relative abundance data (Obermeyer 1998, Vaughn *et al.* 1997).

Most sites selected for study will be those at which listed mussel species were previously reported (Buchanan 1979). New sites will be surveyed as deemed necessary to gain a better understanding of present conditions. All timed search surveys will be performed by at least two biologists experienced with mussel sampling and familiar with the regional fauna. Searches will be conducted during periods of low flow when aquatic habitats are accessible for visual and tactual searches. Timed searches for live mussels will be conducted by hand while snorkeling or wading. All habitats will be searched within the same sampling reach until at least 1.5 person-hours of additional searching failed to increase the number of confirmed mussel species. However, sampling times will always least match or exceed past sampling times for a given site.

Mussels will be identified and recorded as they are found. Representative collections of dead shell material will be retained for voucher purposes. Dead shells will be gathered along the shore, from muskrat and raccoon piles, and from the bottom of the stream. Dead specimens of species not represented by live individuals will be classified as either fresh-dead, dead, or subfossil. Fresh-dead shells represent individuals in which the soft anatomy has not fully decomposed. Dead shells and subfossil shells will be classified as described in Buchanan (1979, 1980). Dead shells have some luster to the nacre (innermost layer of the shell) and have a relatively intact periostracum (outermost layer of the shell). Subfossil shells have a chalky and lusterless nacre and the periostracum has peeled off considerably. The rate at which shell material decomposes following the death of a mussel depends on a variety of factors, including whether the shell was above or below the substrate, whether the shell was in the water or immersed, species of mussel, and shell thickness. In general, dead shells represent mussels that have been dead for less than a year and subfossil shells represent mussels that have been dead for more than a year.

A photo will be taken of each site, and the following information will be recorded: date, location, names of collectors, area sampled, sampling method(s), total sampling effort, the number of living specimens of each species found, and species represented by shell material only. Subjective descriptions will also be made of the habitat in which mussel species are found. If a distinct concentration of individuals ("bed") is encountered, the dimensions of the concentration and general water depth will be estimated and the location described.

Quantitative mussel sampling will be conducted to produce statistically rigorous estimates of mussel and Asian clam densities. Reference sites will be selected that are biologically representative of less contaminated reaches based on species composition and species richness reported from previous surveys of the Meramec River Basin (Buchanan 1979 and Roberts and Brunderman 2000). Reference sites will also have similar surrounding land uses (with the exception of mining) as sites influenced by mining. These will be compared to downstream locations that receive hydrological inputs from former mining areas, yet still have an evident bivalve community.

At each quantitative site, a predetermined number of 1.0 m^2 quadrats will be randomly placed in the reach sampled for mussels based on previous sampling (Strayer and Smith 2003). Water depth and current velocity (at 60% depth) will be measured above the center of the quadrat using a digital current meter. Principal substrate composition will be recorded. Sediment will be excavated to a depth of 10 cm and transferred to a floating 2 mm mesh screen to be searched manually for mussels.

The physical habitat will be evaluated at each timed search and quantitative survey site using the scoring method developed by Barbour et al. (1999) as modified by USEPA (2004). From this method a numerical score is generated representing habitat quality by rating various stream attributes on a scale of 0 to 20. Ratings for each attribute are calculated in the field by averaging the values assigned by three biologists following visual inspection of the targeted stream reach. The final physical habitat score is the sum of the averaged ratings of habitat attributes. Together with reach-specific environmental chemistry data (sediment samples), these scores will provide a general basis for distinguishing between potentially contaminant-limited and physical habitat-limited mussel populations.

DATA ANALYSIS

1. *Sediment Chemistry*

The three laboratory replicate XRF readings of metal concentrations will be combined into a mean metal concentration for each sample location.

Laboratory XRF analysis will be compared to ICP-MS laboratory analysis. If the XRF metals analysis for a bulk whole sediment sample is + or – 30% of the laboratory value, the XRF sample will be considered valid and the XRF sample will be used for further data evaluation. If the XRF sample for the bulk sediment sample is not within 30% of the bulk laboratory value, the laboratory sample will be substituted for the XRF value and used for further data analysis. In the

less than 250 micron samples, a sample will be considered in the acceptable data quality range, if the laboratory sample is within 15% of the XRF value. If greater than 20% of the comparative laboratory analyses are outside of the data quality precision range, an additional 10 % of the samples will be submitted for laboratory analysis. Samples rejected on the basis of QA review will be flagged and noted in data reports. A statistical trend analysis will be performed comparing laboratory and XRF data. If there appears to be a biased trend in the correlation between the XRF and laboratory analysis, the XRF values may be adjusted to account for the bias.

The metals concentrations in various size fractions will be compared with the bulk metal concentration data to determine any trends that may exist. In general the finer fraction is considered the more biologically available fraction. The 250 micron fraction may also be useful in future risk assessments. Other fractions will be useful to help guide future response or restoration efforts and will aid understanding in metal transport mechanisms.

2. *Mussel Population Analysis*

The timed search mussel survey data will be evaluated to determine species richness, relative abundance, and catch per unit effort for each sample location. Mussel diversity and density will be determined for each quantitative site. Mussel density among sites will be evaluated using the Mann-Whitney procedure.

3. *Mussel/Sediment Correlation*

Ultimately the sediment and the mussel data will be analyzed together to determine whether there are correlations between mussel diversity and/or population and metals concentration.

4. *Experimental Design or Methodological Approach:*

Sampling sites will be selected based on data collected in previous studies that characterized lead concentrations in sediment (USFWS, 2007). Samples will be collected during base flow (August-September) from a maximum of thirty sites.

5. *Listing of Critical Data:*

Collection location (including latitude and longitude determined by GPS); date; time; physical variables (i.e., current velocity, depth, substrate particle size); water quality; sediment metals concentrations; mussel diversity and abundance.

6. *Statistical Treatment:*

Data will be analyzed using Microsoft Office's Excel or other programs designed for statistical analysis. Data will be analyzed for normality, and appropriate statistical transformations will be made, if needed. Summary statistics for each endpoint will be computed and compared using parametric and non-parametric methods. Analysis of variance, linear regression, bivariate

correlation, and multivariate techniques will be conducted to ascertain the nature of relationships among variables.

7. *Acceptance or Rejection Criteria for Results:*

Each endpoint will have its own quality assurance program that includes standards, reference materials, and blanks. Data outside the range of acceptable criteria will be clearly noted and discussed.

8. *Special Safety Requirements:*

Department of Interior (DOI) Regulations state that all personnel should wear floatation devices when near water. A first aid kit should also be present in all field vehicles and boats.

9. *Quality Assurance Requirements:*

To the extent practicable, all analyses will comply with Good Laboratory Practices (GLPs). This includes descriptions of maintenance, inspections of instruments, and acceptance testing of instruments, equipment, and their components, as well as the calibration of such equipment and the maintenance of all records relating to these exercises. Documentation to be included with the final report(s) from each study will include field logs for the collection or generation of the samples, chain of custody records, and other QA/QC documentation as applicable.

10. *Endpoint of Study:*

The study will conclude when all chemical, biological, and statistical analysis; project completion report are finalized.

11. *Schedule of Study and Expected Outputs:*

Field collections will be conducted in July, August, and September 2008. Laboratory analyses will be completed by March 2009 with a draft report in review by May 2009.

12. *Place where Data will be Stored and Archived:*

U.S. Fish and Wildlife Service's Columbia, Missouri Field Office, 101 Park DeVille, Suite A, Columbia, MO 65203.

13. *Relationship to Cooperator Needs:*

The Department of Interior and the State of Missouri seek to demonstrate injury to biological resources. Freshwater mussels play an important role in Ozark streams because of their role in indicating the status of sediment and water quality. In addition, two freshwater mussel species, formerly found in the Big River, are considered endangered, and a third mussel is a candidate for listing under the Endangered Species Act. The research conducted within this study plan has been specifically requested by the Missouri Department of Natural Resources and U.S Fish and

Wildlife Service as a part of an NRDAR case. Data will be used in various regulatory and management programs related to the effects of mining on aquatic ecosystems.

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Table 1: List of probable study sites for sediment and mussel sampling

NAME	River Mile*	Sediment	Mussels	QC Sample	Sieved Sample	Lead Risk	Pb	Zn	Lat	Long
Above Irondale-Below Cedar Creek (Reference)	-21	x	x	x	x	LOW	19	0	37.82	-90.71
Below Hwy 8-Above Leadwood (Reference)	-15	x	x			LOW	22	32	37.87	-90.64
Below Leadwood	-5	x	x	x	x	UNKNOWN				
Above Flat River @ Hwy 67	3	x	x			HIGH	813	952	37.89	-90.51
Below Flat River @ Hwy K	6	x	x	x	x	HIGH	927	607	37.93	-90.50
Hwy 67 North of Bonne Terre	14	x	x			HIGH	495	501	37.96	-90.55
Hwy E Below St. Francois State Park	16	x	x	x	x	HIGH	598	314	0.00	0.00
Above Mill Creek	26	x				UNKNOWN				
Mill Creek near confluence		x	x							
Big River Hwy CC (Below Mill Creek)	27	x	x			HIGH	229	110	0.00	0.00
Washington State Park (above Mineral Fork)	33	x	x			UNKNOWN				
Mineral Fork near confluence		x	x	x	x	UNKNOWN				
Mammoth Access	38	x	x			HIGH	672	403	38.12	-90.68
Brown's Ford	49	x	x			HIGH	399	137	0.00	0.00

Above Morse Mill	61	x		x	x	HIGH	339	133	0.00	0.00
Below Morse Mill	62	x	x	x	x	HIGH	259	85	0.00	0.00
Below Belew Creek		x	x			UNKNOWN				
Above Cedar Hill Mill Dam	74	x				HIGH	285	111	0.00	0.00
Below Cedar Hill Mill Dam	75	x	x			MEDIUM	113	70	38.35	-90.64
Byrnesville Above Mill Dam	80	x				MEDIUM	68	42	38.39	-90.64
Below Byrnesville Mill Dam		x				UNKNOWN				
Above House Spring's Mill Dam/Rockford Beach	84	x			x	HIGH	327	123	38.42	-90.59
Below House Spring's Rockford beach	86	x	x			MEDIUM	84	42	38.42	-90.59
Above Byrne's Mill Dam	87	x				HIGH	382	135	38.44	-90.58
1 mi below Byrne's Mill Dam	88	x	x			MEDIUM	98	34	38.46	-90.59
Hwy W	94	x	x			MEDIUM	76	34	38.45	-90.62
1/4 mi above confluence w/Meramec	95	x		x	x	HIGH	134	61	38.47	-90.62
Confluence Meramec	96	x				MEDIUM	88	35	38.47	-90.62
Meramec Above Confluence	98	x				LOW	19	0	38.48	-90.66
Times Beach	100	x	x			MEDIUM	110	72	0.00	0.00

Meramec Below Times Beach (leading edge)		x		x	x	LOW				
Bourbeuse Reference Site		x	x	x		LOW				

* Mileage measured from the Desloge Pile.

Table 2: Requirements for accuracy, precision and detection limits.

Parameter	Estimated Accuracy for each matrix	Estimated Precision for each matrix	Precision Protocol for each matrix	Estimated Detection Limit
Chemical	Measure Values within 95% of CI or 10% of Mean	Replicate Values within ± 25%	Analyze duplicate at least once per run	Temperature (0.3°C)
				pH (0.1 unit)
				Conductivity (100 μmhos/cm)
				Dissolved oxygen (0.1 mg/L)
				Metals (varies)
				Particle size analysis (10% by class)
				GPS (10 m)

Procedures for calculating QC statistics are as follows:

Percent Relative Standard Deviation (%RSD) = SD/Mean x 100
Relative Percent Difference or RPD = (D1-D2)/Mean x 100
% Spike Recovery = (Total Measured – Background)/Spike Amount x 100
Method Limit of Detection = $3 \times (SD_b^2 + SD_s^2)^{1/2}$ where

SD_b = standard deviation of a blank or low level standard and
Sd_s = standard deviation of a low level sample.

Table 3: Proposed quality assurance samples for various matrices.

Type	Matrix	Frequency	Analysis	Rationale
Field Duplicates	Water	1 per run	YSI or Hydrolab®, water quality	Measures precision of sample collection and degree of environmental variability
Blanks	Sediment	1 per field samples	Metals	Monitors procedural contamination
Analytical duplicate	Sediment	1 per 20 analyses	Metals	Monitors instrumental precision
Analytical Spike	Sediment	1 per analytical run per matrix	Metals	Monitors instrumental accuracy
Laboratory Control Sample	Sediment	2 per analytical run	Metals	Monitors instrumental accuracy
Calibration Standard	Water, Sediment	1 per analytical run	Metals, YSI or Hydrolab® water quality	Monitors accuracy

Table 4: Proposed water quality, sediment, and biotic variables to be measured.

Matrix	Variable	No. Reps / Site	Where measured
Mussel	Richness, Distribution	1.5 hrs survey	In situ
Mussel	Density	Varies by site	In situ
Sediment	Leading Edge Metals	1	In situ
Sediment	Sediment particle size characterization	At select sites	Lab
Sediment	Selected metals	1	Lab
Site	GPS	1	In situ
Surface Water	Temperature	1	In situ
Surface Water	pH	1	In situ
Surface Water	Conductivity	1	In situ
Surface Water	Dissolved Oxygen	1	In situ
Surface water	Current velocity	1 riffle	In situ
Surface water	Depth	1 riffle	In situ

Appendices

Appendix 1: Surface Substrate Composition, Current Velocity, and Depth at Riffles

Objectives: To characterize microhabitats of riffles. Data will be used to determine whether surface substrate composition, current velocity, and depth help explain densities of mussels.

Data to be recorded: Site name; site number; lateral distance between measurements for each transect (e.g., measurements obtained at left and right wetted margin and at points along transects); distance of entire riffle (e.g., downstream to upstream distance or longitudinal length); GPS coordinates for each riffle (taken at downstream end of riffle); and surface substrate size, current velocity; and depth at points along transects in each riffle.

Methods: Transects will be set up across each riffle, and measurements will be taken along each transect (see below). Distance between transects and within transects will be determined by the riffle length and width. Start at the downstream end of Riffle 1 (the furthest downstream riffle at each site). Mark each transect with numbers, starting with "1" at the most downstream end of each riffle (i.e., renumber in each riffle).

Distance between stations on each transect:
Measure wetted width of stream.
If width is <5 m, take velocity/depth measurements at 1-m intervals.
If width is 5< x <10 m, take velocity/depth measurement at 2-m intervals.
If width is 10< x <15 m, take velocity/depth measurements at 3-m intervals.
If width is 15< x <20 m, take velocity/depth measurements at 4-m intervals.

Distance to next transect:
If riffle length is <= 50 m; place next interval 10 m upstream.
If riffle length is 50< x <100 m; place next interval 20 m upstream.
If riffle length is >100 m; place next interval 30 m upstream.

Velocity Measurements:
For water depths <75 cm, measure velocity once at 0.6 of the depth from the water surface (e.g., if water is 50 cm deep, measure velocity at 30 cm from the water surface; 40 cm from the bottom surface). For water depths >75 cm, measure velocity twice at 0.2 d and 0.8 of the depth. Average these two readings to determine the velocity for that cross section. Velocity will also be measured at 2 cm above the substrate surface. Record velocity in m/sec; depth in cm.

Depth measurements:
Water depth will be measured using a standard depth gauge. Record depth in cm.

Surface substrate composition measurements:
A grid (e.g., a piece of rebar welded into an ‘X’ with each length measuring 0.5 m) will be used to characterize substrate at each point along each transect. The five-pointed grid will be haphazardly dropped down on the substrate at the point where depth and velocity readings were taken. Substrate will be classified at each of the four ends of the grid (or "X") as well as the center point (5 points in total), using the following categories (from a modified Wentworth scale):

Sand/silt (<2 mm diameter), gravel (2 mm to 16 mm diameter), pebble (17 mm to 64 mm diameter), cobble (65 mm to 250 mm diameter), boulder (> 256 mm diameter), flat bedrock and irregular bedrock.

Each of those categories is assigned a numerical value (Bain et al. 1985):

Sand/silt = 1.0, bedrock = 1.0, gravel = 2.0, pebble = 3.0, cobble = 4.0, boulder = 5.0, irregular bedrock = 6.0

The five numerical values (from each of the five grid contact points) are recorded and averaged to obtain a mean substrate value (to the tenths decimal place) for that location along each transect.