King County Freshwater Mussel Survey, 2004

Goals

- Determine whether survey method works.
- Estimate population density at each site.
- Determine whether habitat influences mussel distribution.
- Determine whether habitat influences size.
- Rough estimate of age/length relationship
- Determine growth rates
- Assess health of the populations
- Determine whether mussels move significant distances

Survey Sites

- Bear Creek on Walsh property
- Upper Covington Creek upstream of Lake Sawyer
- Stossel Creek four miles upstream of confluence with the Tolt River

Survey Method

- Twenty transects at each site
- Transects 5 meters apart
- Quadrats (25cm x 25cm) adjoining along each transect
- Ten Percent of the quadrats were excavated and sieved with 6mmx6mm screen
- Most mussels were photographed
- All returned to the stream where collected.
- 20-25 sacrificed from each site.
- 25-30 were tagged and released in each reach

Information Collected about Mussels

- Numbers - Located by touch and counted
- Length - measured to nearest mm
- Photographs – used to document percent erosion
- Age
- Gender
- Internal shell condition (nacre color, nacre condition, perforations)

Habitat Data

At each quadrat location:

Stream Configuration (Pool, Riffle, Glide)

Sediment type (Cobble, Gravel, Sand, Mud, Litter)

Obstructions (Boulder, Cobbles, Roots, Logs, Twigs/sticks, Herbaceous vegetation, None

Overhead cover within two feet of surface

Un-sieved/sieved Comparison

Numbers of mussels in un-sieved and sieved quadrats

		Total	Sieved	Unsieved
All Sites	Quadrats	1808	174	1634
	Mussels	1886	280	1606
	% with mussels	32.5	40.2	31.7
Bear	Quadrats	866	84	782
	Mussels	615	77	538
	% with mussels	30.5	42.2	29.3
Covington	Quadrats	762	72	690
	Mussels	874	116	758
	% with mussels	32.8	33.3	32.8
Stossel	Quadrats	180	18	162
	Mussels	397	87	310
	% with mussels	41.1	61.1	38.9

Non-parametric Statistical Tests

- Data distribution was neither normal nor lognormal (Q-Q plot)
- Used T-test and Mann-Whitney test to determine significance

Results

Statistical significance of differences between the average numbers of mussels in un-sieved and sieved quadrats

	Means		Significance	
Dataset	**Un-sieved**	**Sieved**	**T-test**	**Mann Whitney**
All sites	0.98	1.61	Yes	Yes
Bear	0.69	0.93	No	Yes
Covington	1.1	1.61	No	No
Stossel	1.91	4.83	No	No

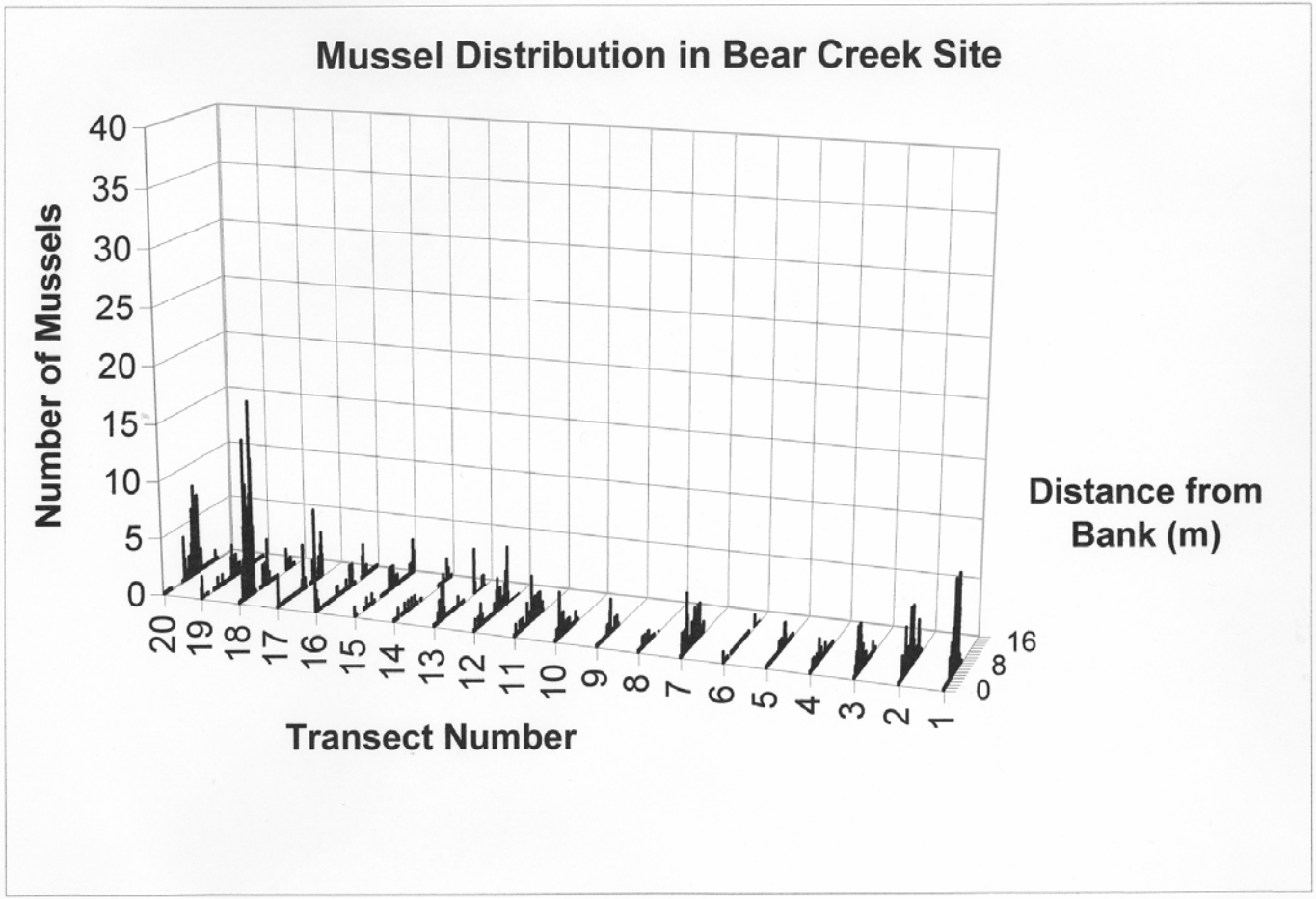
Mussel size in the sieved and un-sieved fractions

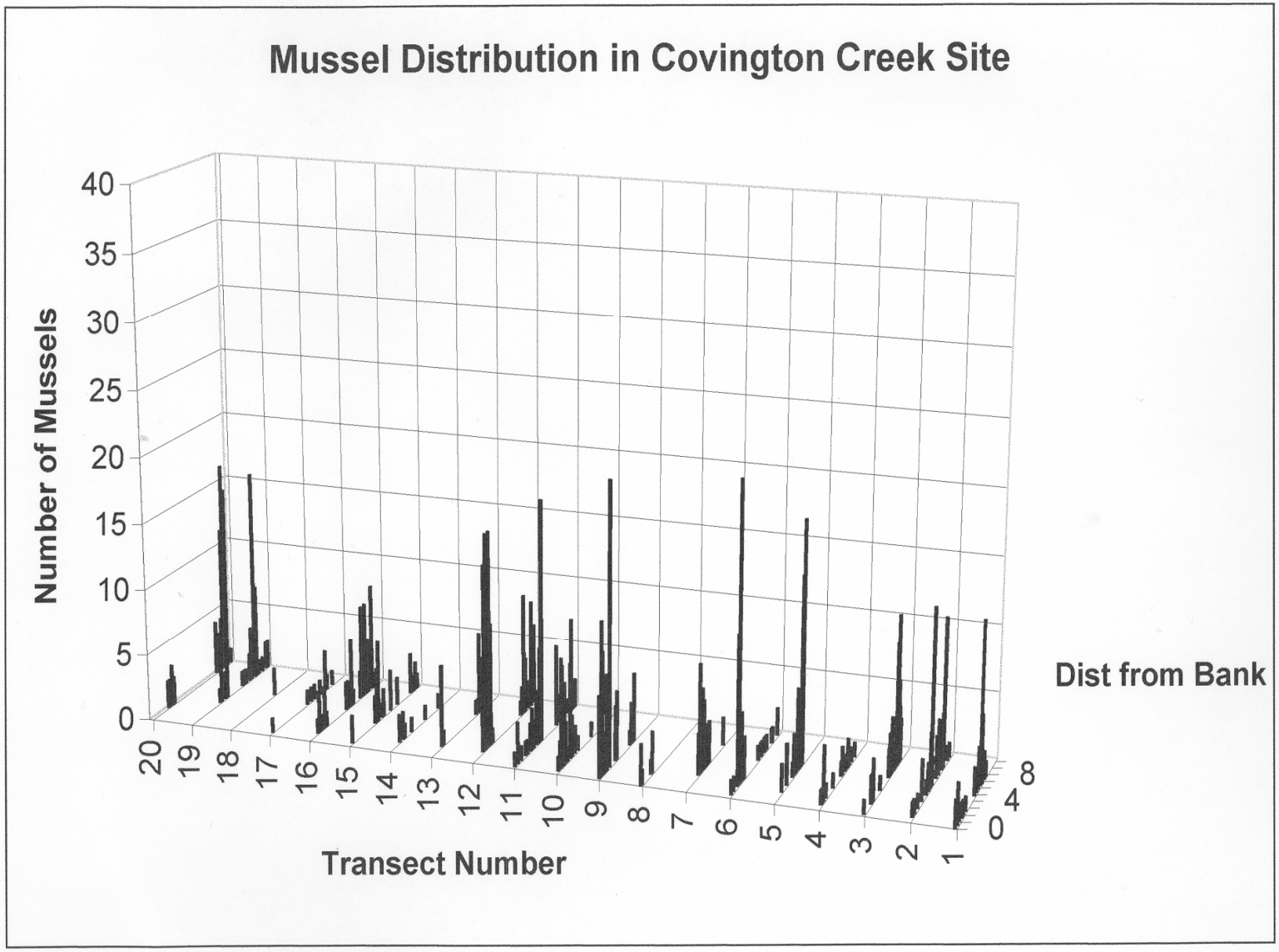
- Length data were normally distributed
- Applied T-test
  - Un-sieved average = 75.7mm
  - Sieved average = 74.2
  - Difference is not significant.

Apparently, juveniles not being missed by either method.

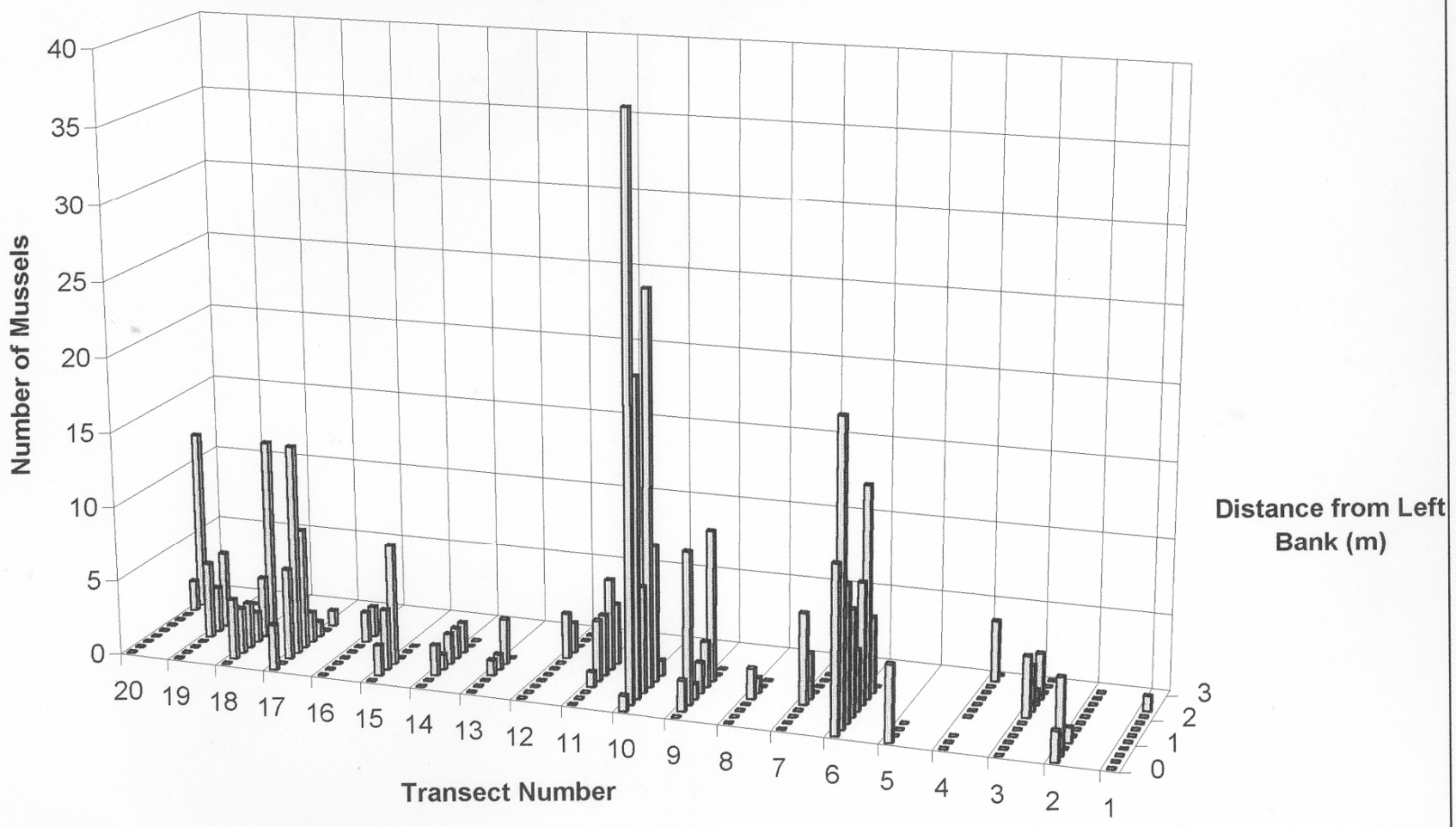
Illustrations of Mussel Distribution

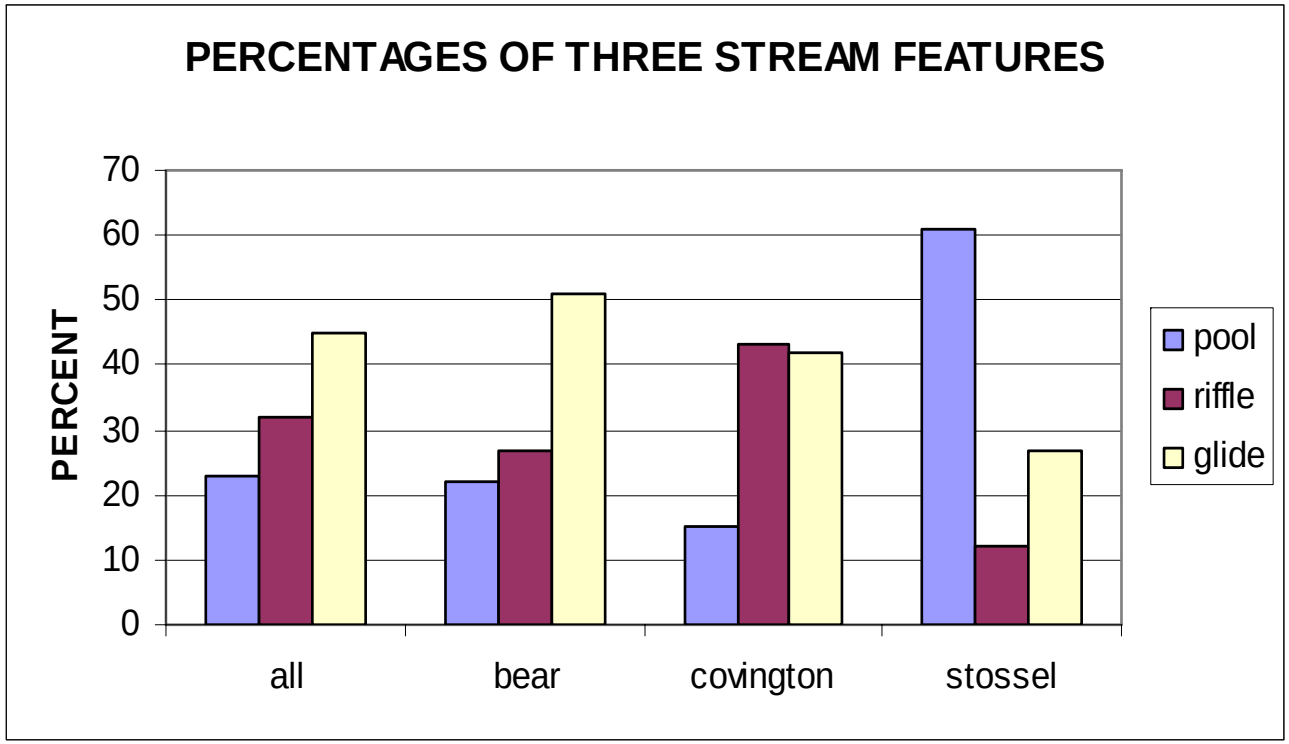
- Mussels seem to clump rather than evenly distribute themselves throughout favorable habitat.
- Locations of beds are suggested
- Density is suggested

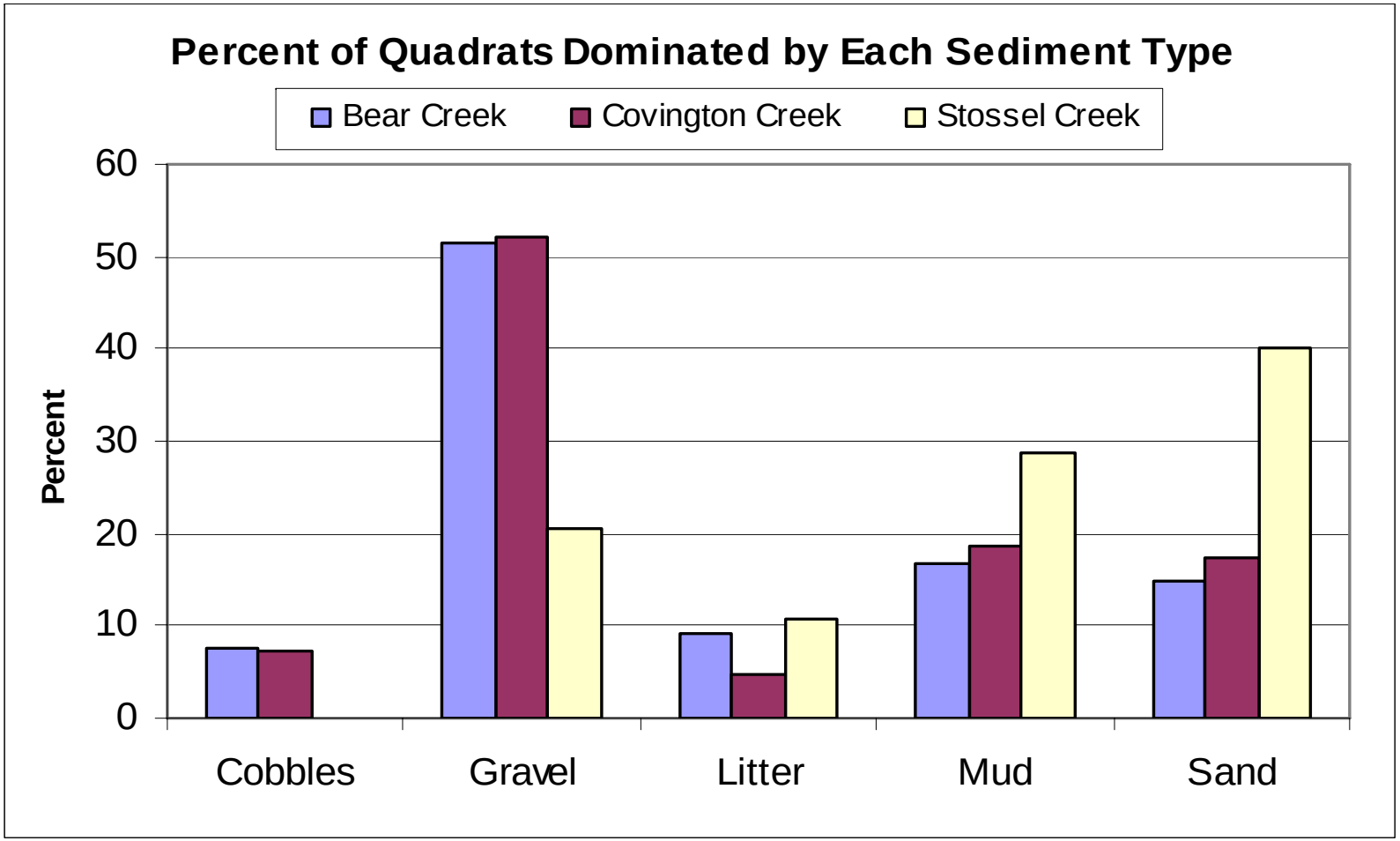

Mussel Distribution in Bear Creek Site
Number of Mussels
40
35
30
25
20
15
10
5
0
20
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
Transect Number
16
8
0
Distance from Bank (m)


Mussel Distribution in Covington Creek Site
Number of Mussels
40
35
30
25
20
15
10
5
0
20
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
Transect Number
0
4
8
Dist from Bank

Mussel Distribution at Stossel Creek Site




PERCENTAGES OF THREE STREAM FEATURES
PERCENT
70
60
50
40
30
20
10
0
all
bear
covington
stossel
pool
riffle
glide


Percent of Quadrats Dominated by Each Sediment Type
Bear Creek
Covington Creek
Stossel Creek
Percent
60
50
40
30
20
10
0
Cobbles
Gravel
Litter
Mud
Sand

Mussel Abundance

- Abundance data are usually neither normally nor log normally distributed but the Q-Q plots suggest that they are.
- ANOVA was used to determine whether there are significant differences within the categories.
- Tukey’s post hoc test was used to identify which subsets were different.

Results of Statistical Analyses of Mussel Abundance Data

ANOVA

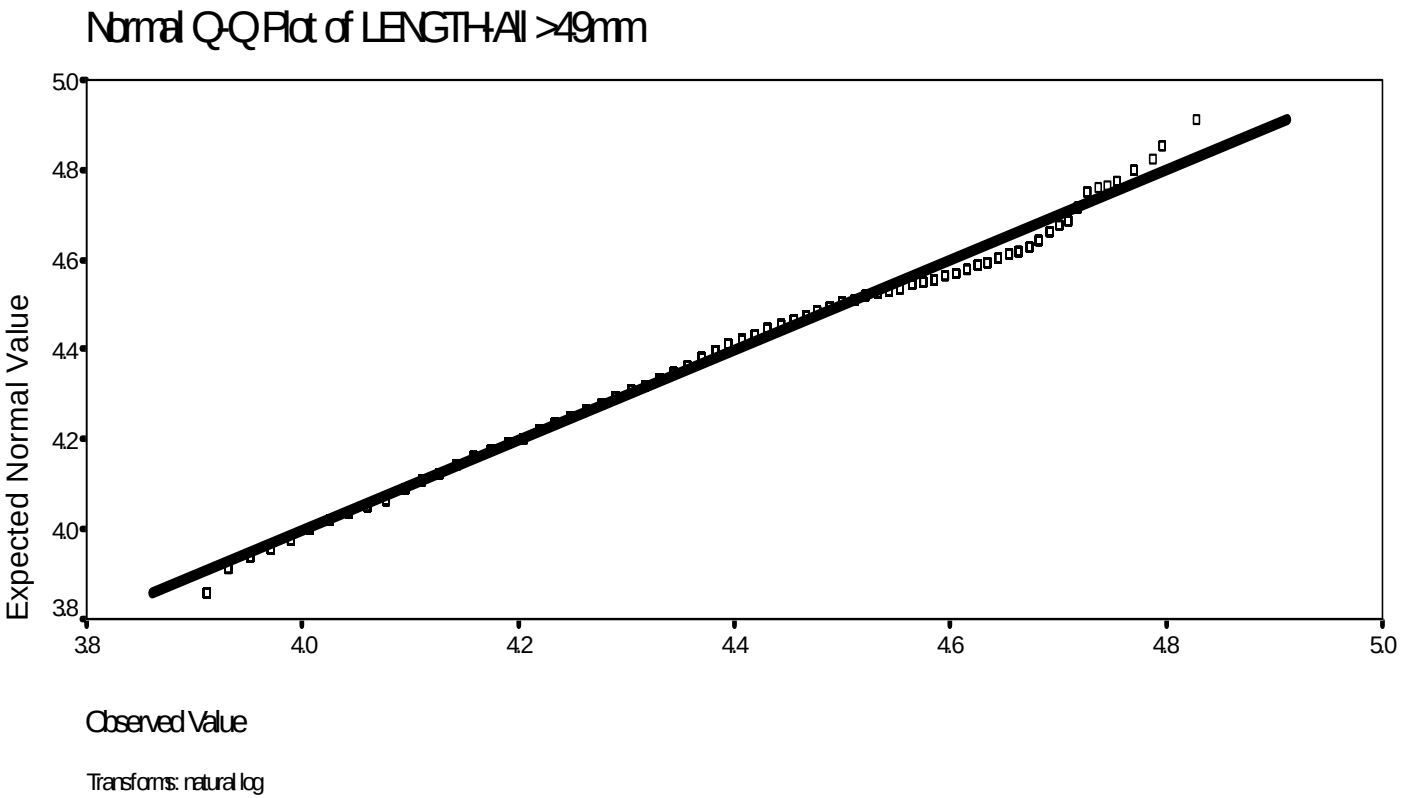
	All	Bear	Covington	Stossel
Stream Configuration	Yes	Yes	No	No
Sediment Type	Yes	Yes	No	Yes
Obstruction/Structure	No	No	No	No

Post Hoc

Stream Configuration	No	R>P>G	No	G>R>P
Sediment Type	M>(C=L)>(G=S)	M>(C=G=S=L)	No	G>M>(L=S)
Obstruction/Structure	No	No	No	No

Configuration: P=Pool, G=Glide, R=Riffle
Sediment: C=Cobbles, G=Gravel, L=Litter, M=Mud, S=Sand
Structure: B=Boulder, C=Cobbles, L=Log, N=None, T=Twigs,
V=instream herbaceous Vegetation, R=Roots

Mussel Size


Normal Q-Q Plot of LENGTH-All >49mm
Expected Normal Value
Observed Value
Transforms: natural log
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.8
4.0
4.2
4.4
4.6
4.8
5.0

Results of Statistical Analyses of Mussel Length Data

ANOVA

	All	Bear	Covington	Stossel
Stream Configuration	No	Yes	No	No
Sediment Type	Yes	Yes	No	Yes
Obstruction/Structure	Yes	No	Yes	No

Post Hoc

Stream Configuration	No	(R=G)>P	No	G>R>P
Sediment Type	L>G>C>(M=S)	No	No	G>M>(L=S)
Obstruction/Structure	(R=N)>V>(C=T)>L>B	No	No	No

Configuration: P=Pool, G=Glide, R=Riffle
Sediment: C=Cobbles, G=Gravel, L=Litter, M=Mud, S=Sand
Structure: B=Boulder, C=Cobbles, L=Log, N=None, T=Twigs,
V=instream herbaceous Vegetation, R=Roots

Streams ranked by mussel length

- Bear > Stossel > Covington
- 84.6mm > 81.7mm > 68.1mm

Sediment ranked by mussel length

- Litter>Gravel>Cobbles>(Sand=Mud)
- 81.2mm>77.3mm>74.7mm>74.2mm>73.3mm

Structures ranked by mussel length

- (Roots=None)>Vegetation>(Cobbles=Twigs) >Log>Boulders
- (84.5mm=80.3mm)>74.7>(73.1mm=72.2mm)>72.4>68.3mm

Shell Erosion Estimates

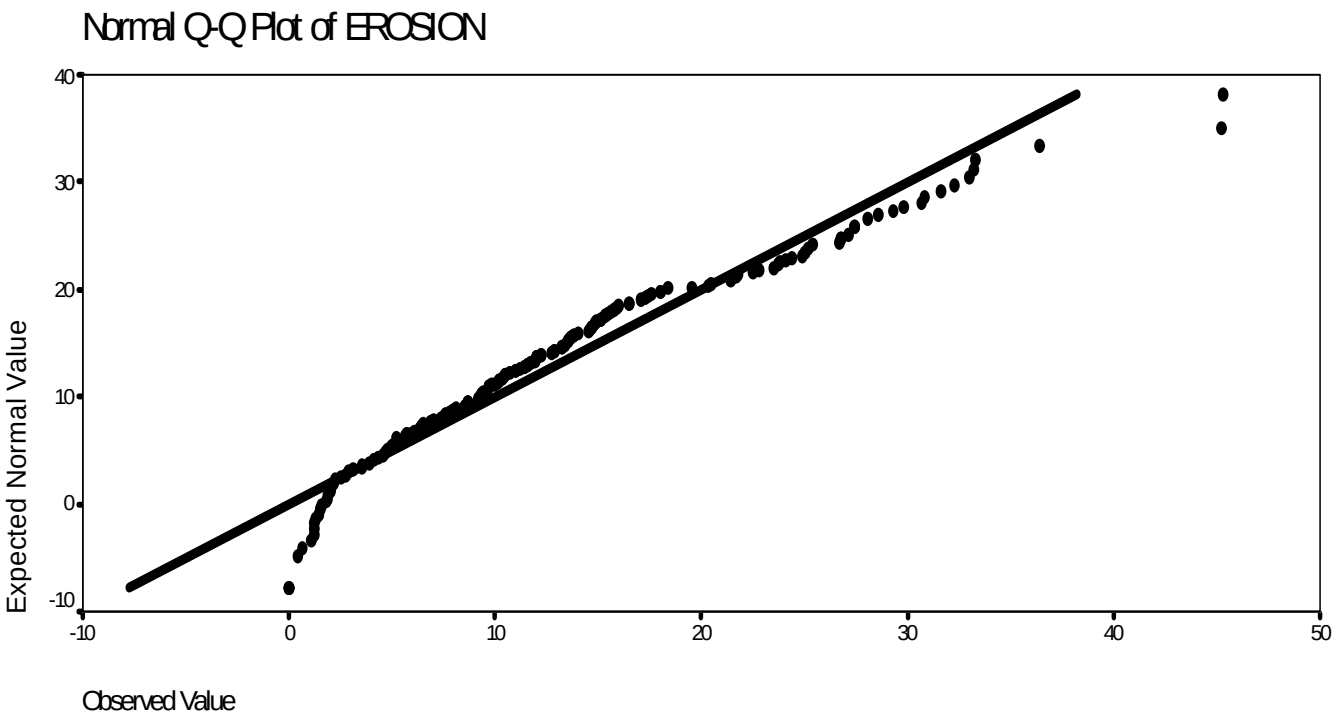
Method

The percent of the shell surface eroded was estimated for ten percent of the mussels photographed.

Using photo editing software, polygons were drawn around outer perimeter of the shell and eroded area. The area of each polygon, in pixels was recorded.

%eroded=100*(eroded area/total area)

Q-Q plot of percent-erosion



Statistical Analyses of Percent of Shell Eroded Data

ANOVA

	All	Bear	Covington	Stossel
Stream Configuration	Yes	No	Yes	No
Sediment Type	No	No	Yes	No
Obstruction/Structure	No	No	Yes	No
Length (Grp)	Yes	No	No	No

Post Hoc

Stream Configuration	R>G>P	No	R>G>P	No
Sediment Type	NO	No	(G=S)>L>M>C	No
Obstruction/Structure	NO	No	N>(C=L=V)>(B=T)	No
Length Group	(60's=100's)>(50's=90's=110's)>70's>80's>40's>30's	No	No	No

Configuration: P=Pool, G=Glide, R=Riffle
Sediment: C=Cobbles, G=Gravel, L=Litter, M=Mud, S=Sand
Structure: B=Boulder, C=Cobbles, L=Log, N=None, T=Twigs, V=instream herbaceous Vegetation, R=Roots

ANOVA Results for Stream Reach

- Bear n=62 Average %=14.6
- Covington n=80 Average%=17.0
- Stossel n=40 Average%= 3.3

(Bear=Covington)>Stossel

Covington Creek Mussels

Stossel Creek Mussels

Male:Female Ratio

- 21-23 mussels from each stream were sacrificed
- Gamete smears on microscope slides were prepared from stained body fluids
- Sex determined by identification of eggs or sperm on each slide.

- Three categories: male, female, neither

Results

Stream	Female	Male	Neither
Bear	3	12	6
Covington	9	11	3
Stossel	8	11	4

Probabilities

- **Used only male and female counts.**
- **“Neither” category was ignored**

- **Stream**
  - **Bear 3 or fewer females of 15 = .0176**
  - **Covington 9 or fewer females of 20 = .4119**
  - **Stossel 8 or fewer females of 19 = .3238**

Interpretation

- Expect about a 50:50 ratio if population is not stressed
  - Covington and Stossel ratios are “normal”
  - Bear creek ratio is not normal. Chance of that occurring randomly are less than 1 in 50. Animals are stressed.

Shell Condition

- The empty shells from the animals sacrificed for gamete smears were examined for:
  - Nacre Color
  - Nacre condition
  - Distribution of color
  - Perforations

Nacre Color and Condition

- Bear 20 purple, 1 white; 11 dull to chalky on inner surface; 19 of 21 mottled on one or both valves
- Covington 23 purple; dull; 19 of 23 mottled on one or both valves.
- Stossel 14 purple, 5 salmon, 1 white; surface bright and shiny; only the right valve of one mussel showed mottling

Stossel Creek Nacre Colors

Bear Creek

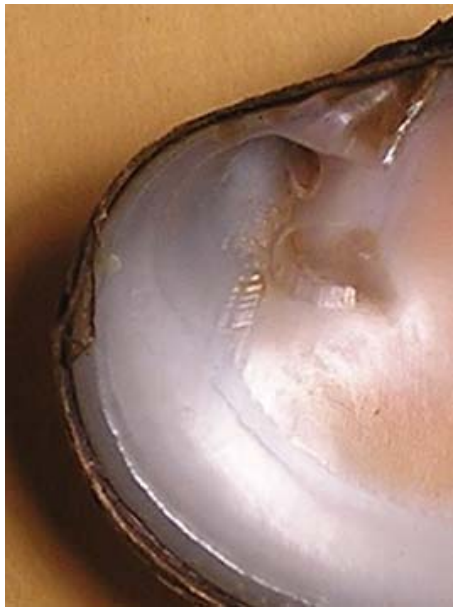
Perforations

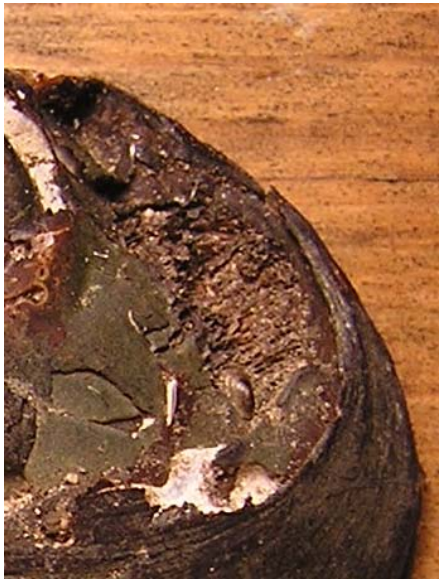
- Perforations are rare in live animals since the condition is fatal; consequently, finding one alive in Covington Creek is unusual.

- 9 of the mussels from Bear Creek had chalky areas along the ventral margin on the inside of shell.

Mottling and Chalky Areas

Erosion at Anterior Adductor Mussel Attachment Point





Interpretation or shell characteristics

- If the mantle is fully functional, the nacre is shiny, and there is no evidence of mottling
- If mantle functions are impaired, nacre becomes dull, periostracum layers are visible through the thin nacre. Shell can become chalky.

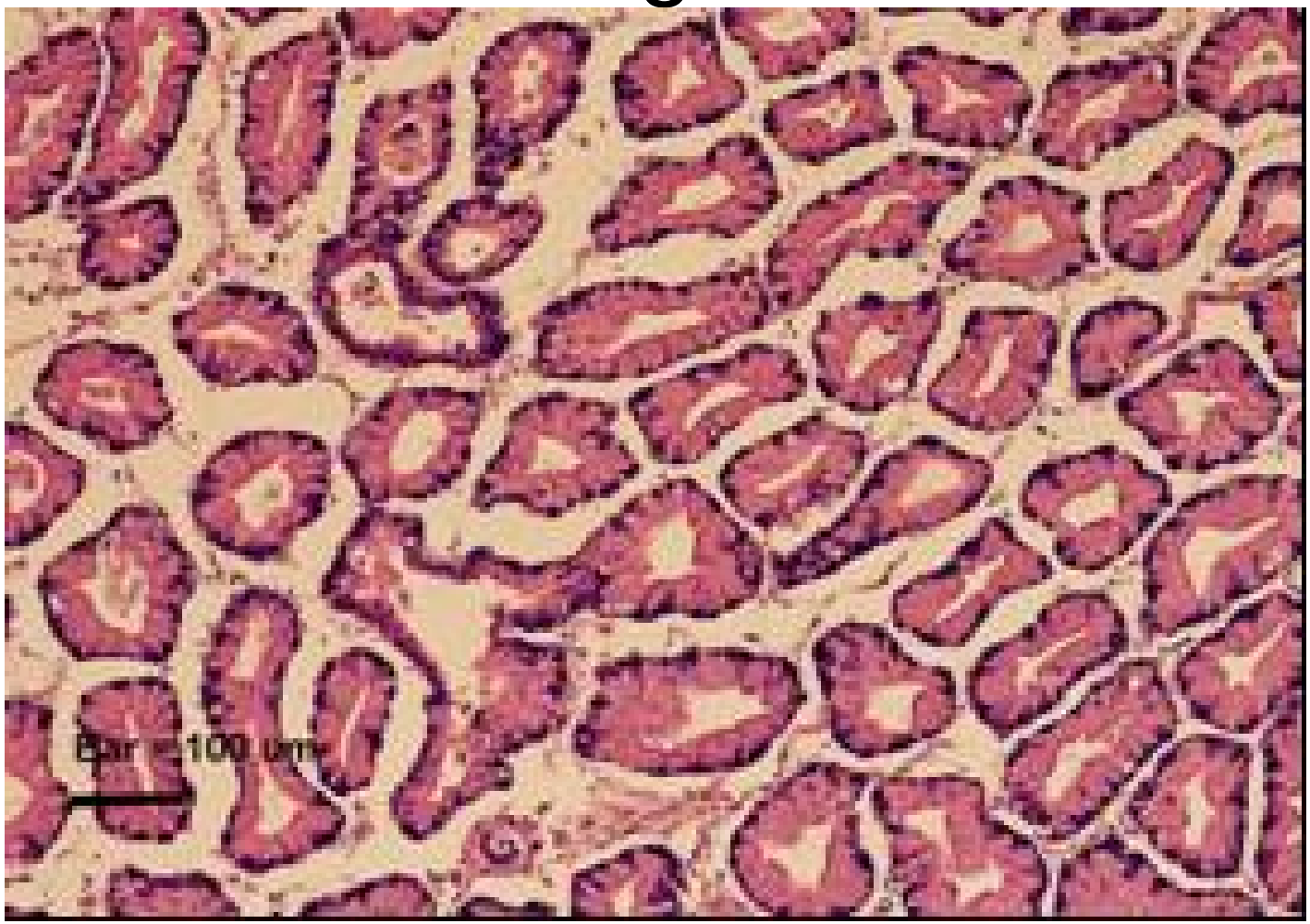
Histology

- Many dead or dying animals were sighted during the survey. That unusual occurrence prompted further study.
- Four dug-in animals, four gaping and 2 dead were collected from Bear Creek. Four dug-in animals were collected from Stossel.
- Thin sections were made from ten.

Histology Conclusions

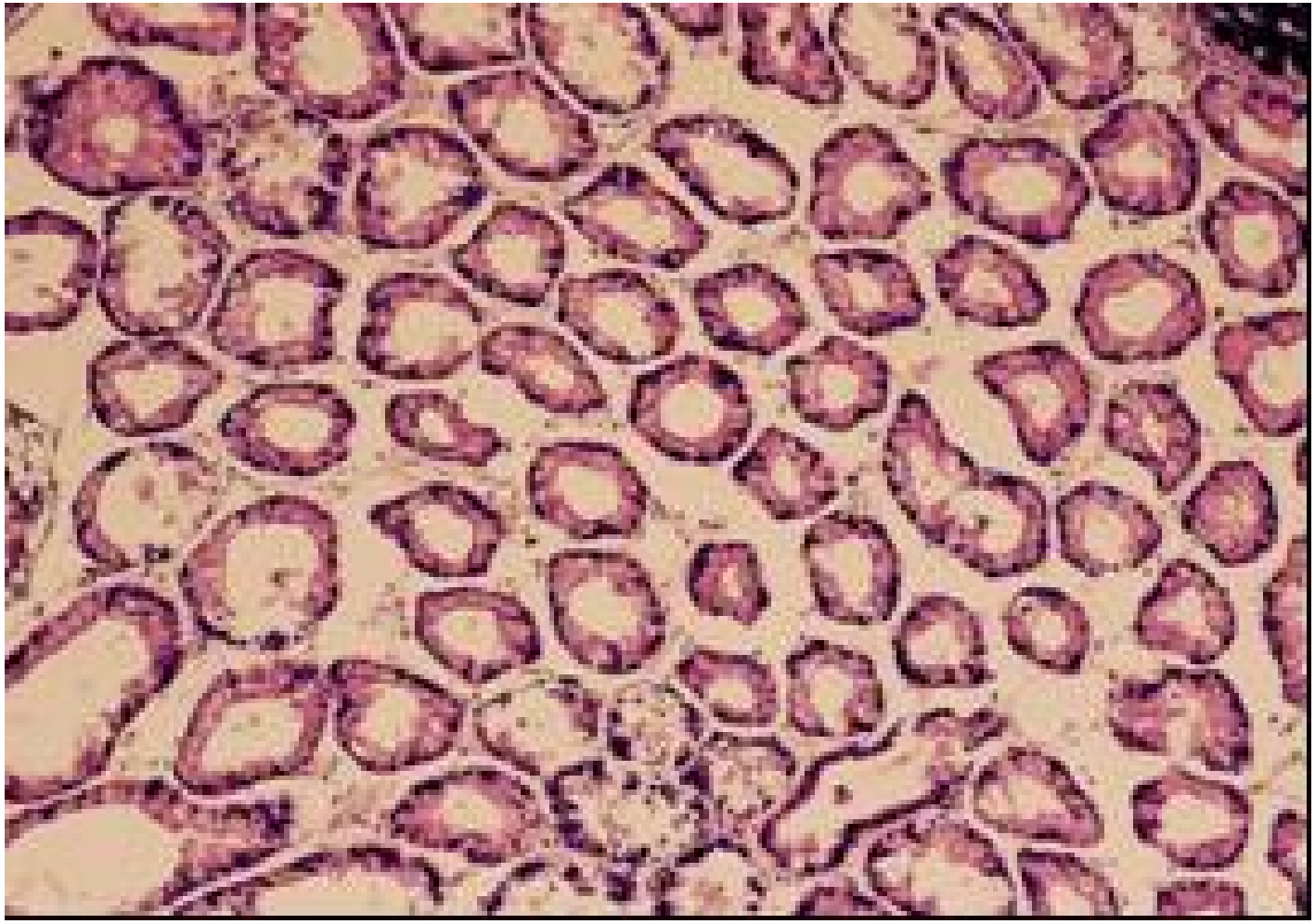
- Decline in the digestive gland condition is indicative of a chronic condition – stress has been long term
- Loss of gill epithelium may be a response to a variety of chemical and physical stresses.
- Bacterial infection may have finished them.

Normal dig gland tubule epithelium height



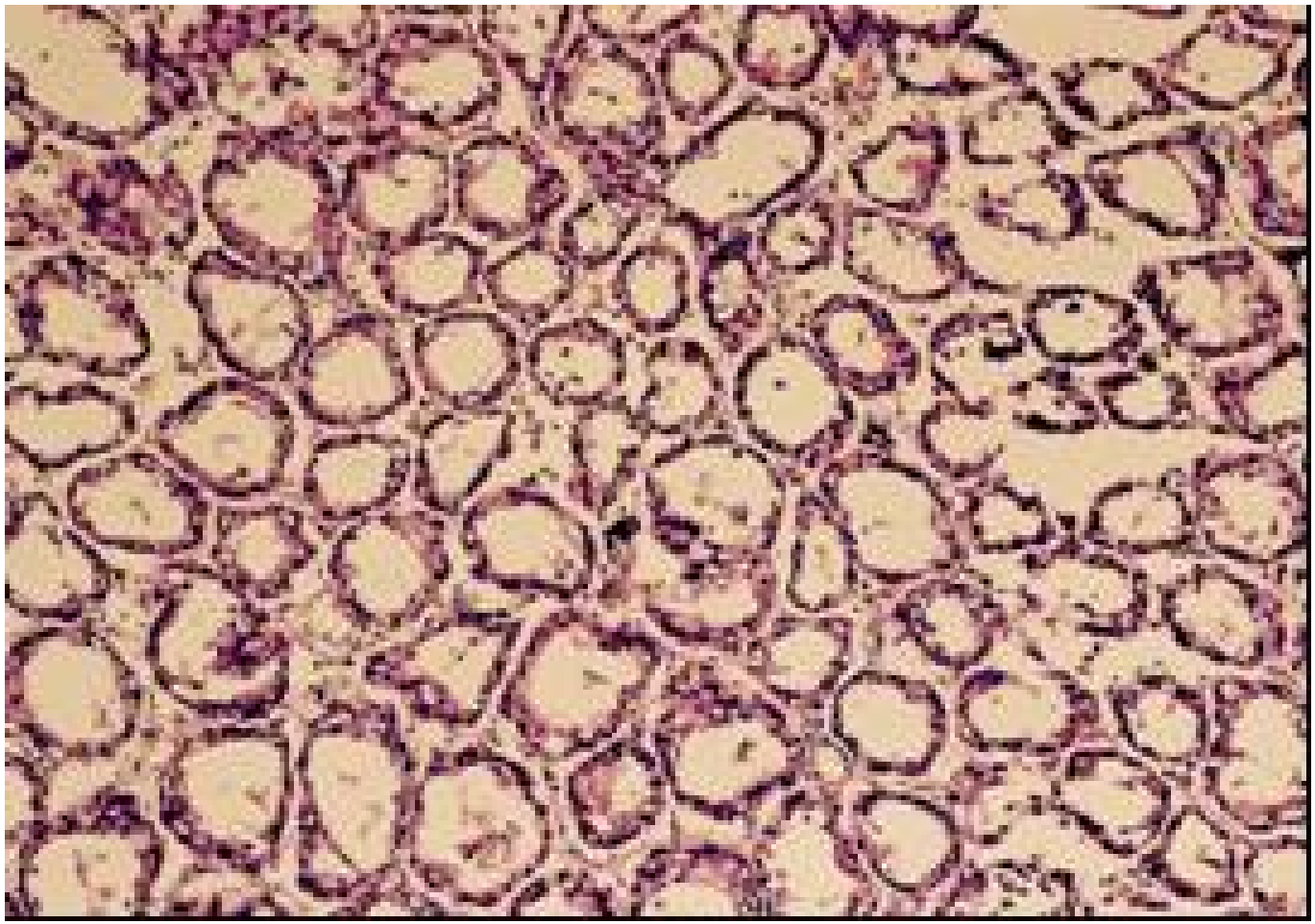
Reduced dig gland tubule epithelium height

This condition is probably recoverable

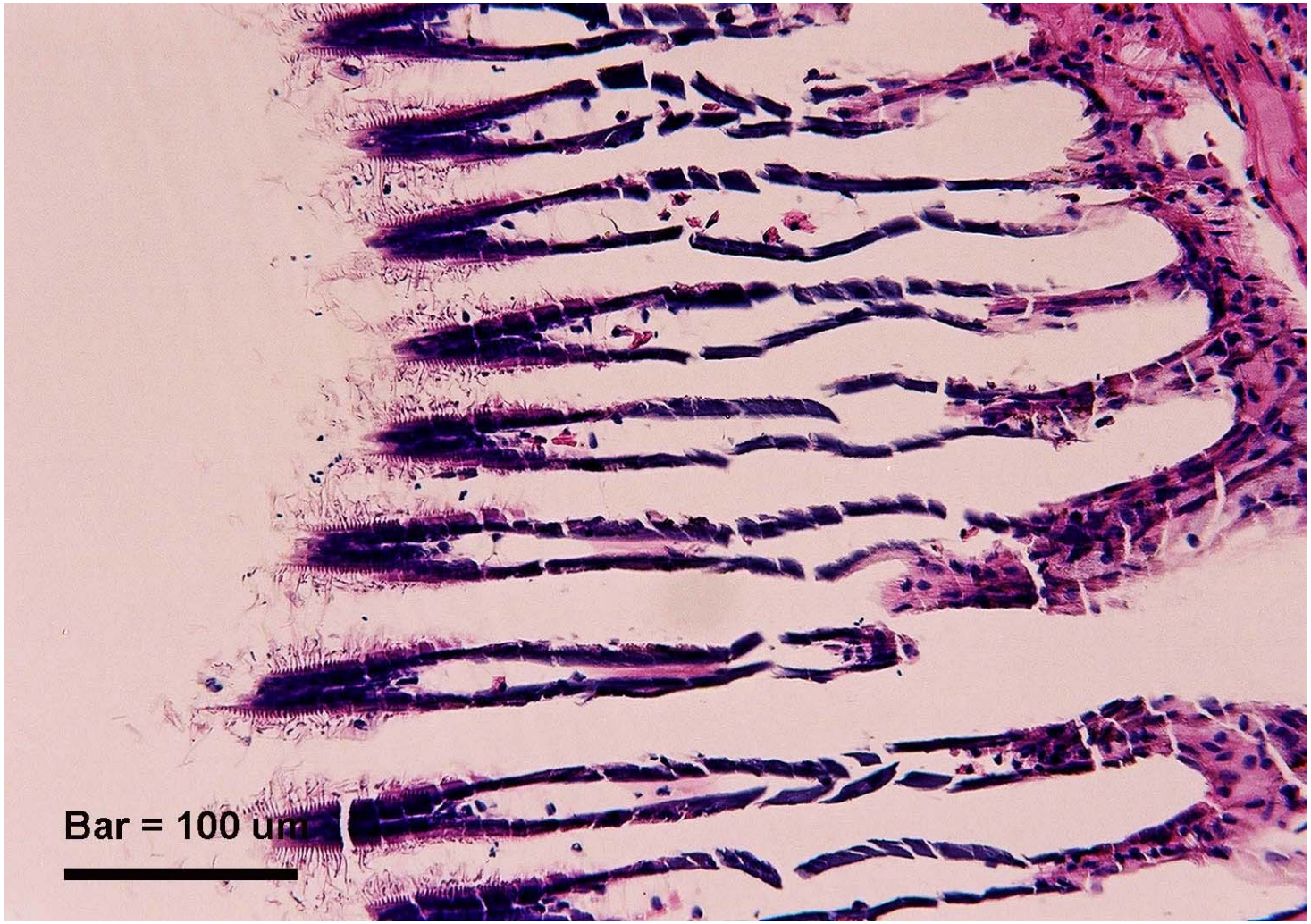


Low dig gland tubule epithelium height.

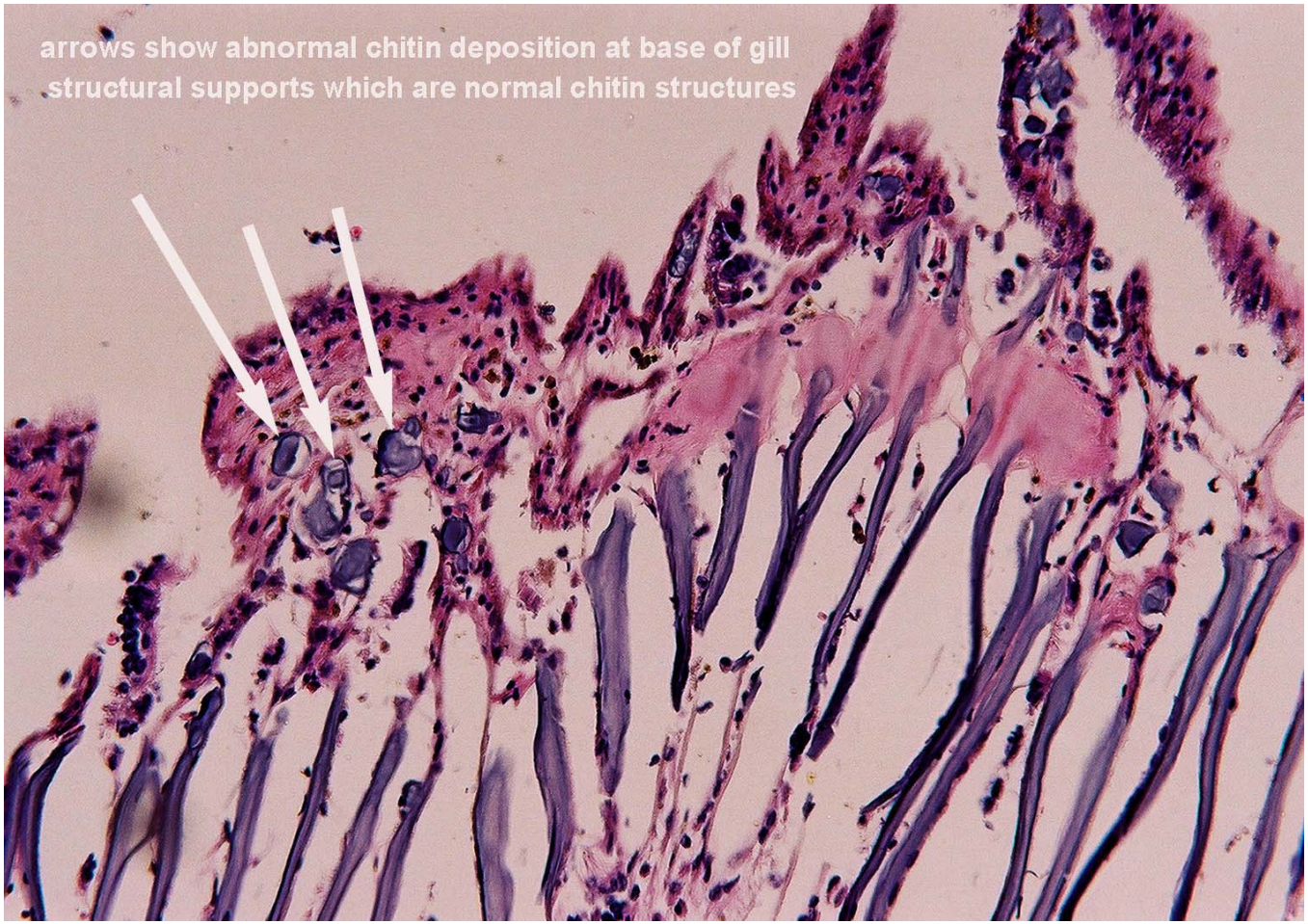
This condition is probably not recoverable



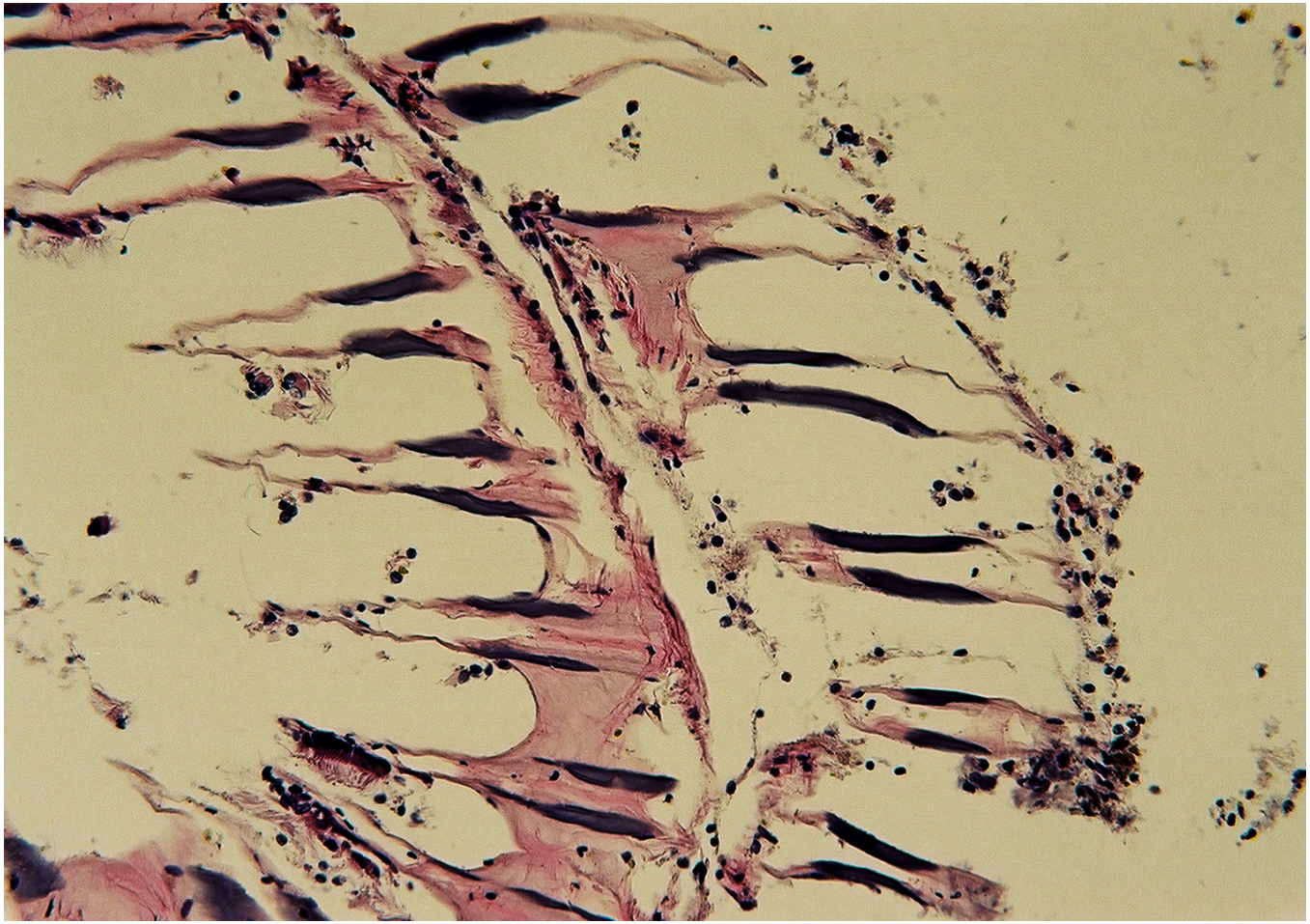
Normal gill with epithelium intact



Chitin anomaly in Bear Creek mussel



Loss of much of the epithelium. Not recoverable



Conclusions and Recommendations

- The sieved:un-sieved comparison suggests that collecting mussels by touch is not an accurate method if determining population density is the objective. Screening all sites may be more accurate.
- Juvenile mussels were probably not overlooked within the quadrats.
- If assessing condition, average size or habitat preference, locating by touch will be effective.

Habitat - Abundance

- Density varies significantly between streams
- Geology affects preferences
- Stream configuration affects numbers
- Numbers are higher in gravel or mud
- Not affected by structure in the stream

Habitat – Mussel Size

- Size varies significantly among streams
- Seems to vary with sediment type in some streams
- Mussels larger in moving water than in pools.
- Relationship to structure is not clear
- No statistically significant link to cover

Percent Erosion – Habitat and Size

- Covington and Bear mussels significantly more eroded than Stossel mussels.
- Link between size and percent erosion unclear. Stream by stream analysis may be more appropriate.
- Erosion does not always appear to be linked to stream configuration, sediment type nor structure.

Stossel Creek Mussel Health

- Histology indicates excellent condition
- Shells in excellent condition both inside and out
- If land in the drainage basin remains in the current condition with little or no improvement, this population might remain stable.

Covington Creek Mussel Health

- Covington
  - No histology
  - Mottling on inside of shells suggests diminished efficiency of the mantle
  - Small size but high percent of erosion suggests that they might be dying at an early age

Animals may be stressed

Bear Creek Mussel Health

- Stresses suggested by:
  - Male:Female ratio
  - Shell condition suggests that mantle is not fully functioning (dull surface, mottling, chalky areas)
  - Numbers of dead and dying mussels noted
  - Histology (decline over time)

Future Work in Bear Creek Basin

- Locate mussel populations upstream of this site.
- Assess shell condition
- Examine rest marks in ligaments
- Histology