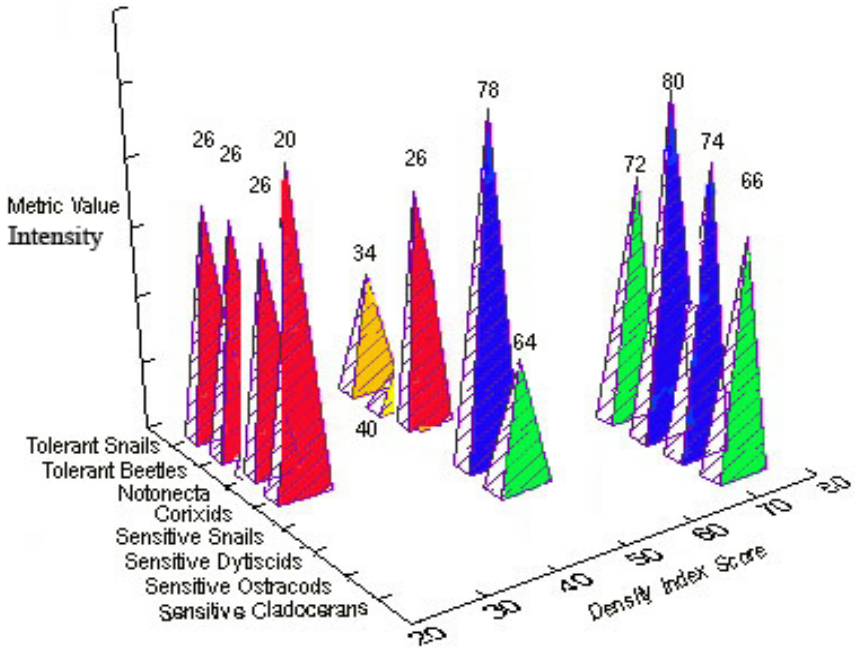
Density-based Invertebrate Community Index (DICI) of Ohio Wetlands

Final Index Report to:
Integrated Wetland Assessment Program
Part 8: Initial Development of Wetland Invertebrate Community Index for Ohio
and
Investigations of Invertebrate Communities of Wetlands in the Huron/Erie Lake Plains Ecoregion (2003) and Mitigation Banks (2004)

Ohio EPA Technical Report WET/2007-5



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Introduction

A density based invertebrate community index (DICI) was developed for Ohio wetlands. Densities of invertebrates collected in 24-hr activity traps from Ohio wetlands between 1996 to 2006 were used to develop 18 metrics (Table 1 and 2). These metrics were based on invertebrate densities which were statistically associated (p-values < .05) with wetland quality.

Densities of invertebrate taxa are associated with the physical characteristics of habitat, chemical composition of soil and water, and the trophic level and food chain present in a wetland ecosystem. Large dytiscid beetle genera Acilius, Cybister, Dytiscus have been associated with high wetland quality (Appendix B) and along with salamander and amphibians have been observed to be the main predators in many high quality wetlands. Many wetland mitigation projects have vegetative and design flaws that limit the habitat potential for these organisms to thrive. In many mitigation banks the wetland resembles more of a lake ecosystem or a mere retention basin with large areas of open water providing little or no shade, and steep banks and dikes which are kept mowed providing little vegetative opportunity and limited near margin habitat. Top predators at these banks many times are predatory fish. Food sources will also contribute to the densities of organisms present. Higher densities of the herbivorous adult beetle genera Berosus, Haliplus, Peltodytes, and Tropisternus, and some corixid genera were observed in growths of filamentous algae during qualitative sampling.

Previous metric selection was at the family and order level. Analyses of invertebrate data has shown associations with wetland quality at the generic and specific level. High densities of the ostracod species Cypridopsis vidua and Cypria maculata were found to be statistically associated with poor wetland quality (Appendix B).

In earlier reports use of landscape attributes were used to develop metrics for amphibians and plants (VIBI and AmphIBI). The distributions of many of the amphibian and plant species have been described as landscape driven. The communities were affected to a large degree by anthropogenic change over the past couple of centuries (Mack 2001a, Micacchion 2002), The relative abundance of the invertebrate communities appear to be driven more by the water and soil characteristics of the wetland community. The landscape features indirectly affect these characteristics. Reduced forest canopy and open water with no groundwater surcharge will have a higher water temperature which has a direct effect on invertebrate communities. Invertebrate data evaluated together with plant and amphibian data gives a total picture (holistic) of wetland quality.

Previous Ohio EPA invertebrate reports

Ohio EPA has been collecting invertebrate data from wetlands from1996 to 2007. Crustacean and dipteran species were selected as potential attributes based on wetland type (HGM classification) and habitat assessment (ORAM v. 5.0) in a report from 2000 (Gray, et. al., 2000). An initial index employing relative abundances of oligochaetes, microcrustaceans, dytiscid beetles, sensitive snails, corixids, tolerant beetles, tolerant snails, and odonates (Wetland Invertebrate Community Index, WICI) was developed in 2004 (Knapp, 2004). Using refined taxonomy of oligochaetes and microcrustaceans to the species level, individual sensitivities and tolerances separated out tolerant varieties out of the original metrics from 2004 (Knapp 2006). These earlier indexes developed metrics by quadrisecting relative abundance (percent of population) and assigning scores based on sensitive and tolerant organism groups. The density based approach developed in this report developed metrics using raw densities of invertebrates collected. Large densities of a specific organism/group will affect the percentage of the other organisms in the sample. The density based approach gives the same metric value to an organism group from one sample with the same density as another sample with the same organism density, independent of relative abundance.

Sampling Methods

Ohio EPA began evaluating wetland macroinvertebrate and amphibian sampling methods in 1996. A variety of sampling methods including artificial substrate samplers, several types of funnel traps, and qualitative sampling with dip nets were evaluated (see Fennessy *et al.* 1998). The qualitative dipnet method was used from 1996 to 2003, but discontinued from 2004 due to the low performance of collecting additional taxa (Knapp 2006). The use of funnel traps as a method of sampling has been used extensively for amphibians and more recently as a protocol for macroinvertebrate collections in wetlands. The funnel traps are similar in design to commercially available minnow traps. A more detailed description of methods is provided in Appendix A.

Statistical Analyses

Systat 9.0 and 10.0 were used to perform all statistical tests. Least squares means graphs and density metric 3-D plots were created in Systat. P-values from analysis of variance and subsequent Tukeys multiple comparison tests were used to separate density groups of characteristic tolerant and sensitive invertebrates. Adobe Photoshop CS and Microsoft Paint were used to edit text in graphs and for the images on the front cover.

1.0 Density Based Invertebrate Community Index (DICI)

A Density-based Invertebrate Community Index (DICI) was developed for Ohio wetlands. Invertebrate data was analyzed from 265 samples collected from 24-hour activity traps between 1996 and 2006. Eighteen metrics based on invertebrate densities were statistically associated (p-values < .05) with wetland quality (Tables 1 and 2).

Disturbance scale/Environmental Gradient

Metrics were chosen based on associations with an invertebrate measure of wetland quality, the WICI (Knapp, 2004 and 2006), and a disturbance scale based on ORAM 5.0 (Mack 2001b). The WICI score and the disturbance scale were transformed to a 0 to 100 scale. These transformed scales are named WICI100 and DIST100 in the data analyses and graphs in the Appendix. Metric values assigned were the median WICI score for each higher organism density group (absent, >0, >20,>100, e.g.) statistically different from lower densities. The analyses for the eighteen metrics are shown in Appendix B and a summary of the metric values are provided in Tables 1 and 2. The metric values were between 20 and 40 for tolerant associations and 60 to 80 for sensitive associations. The DICI index score is the average of the individual metric values.

Metric value analyses

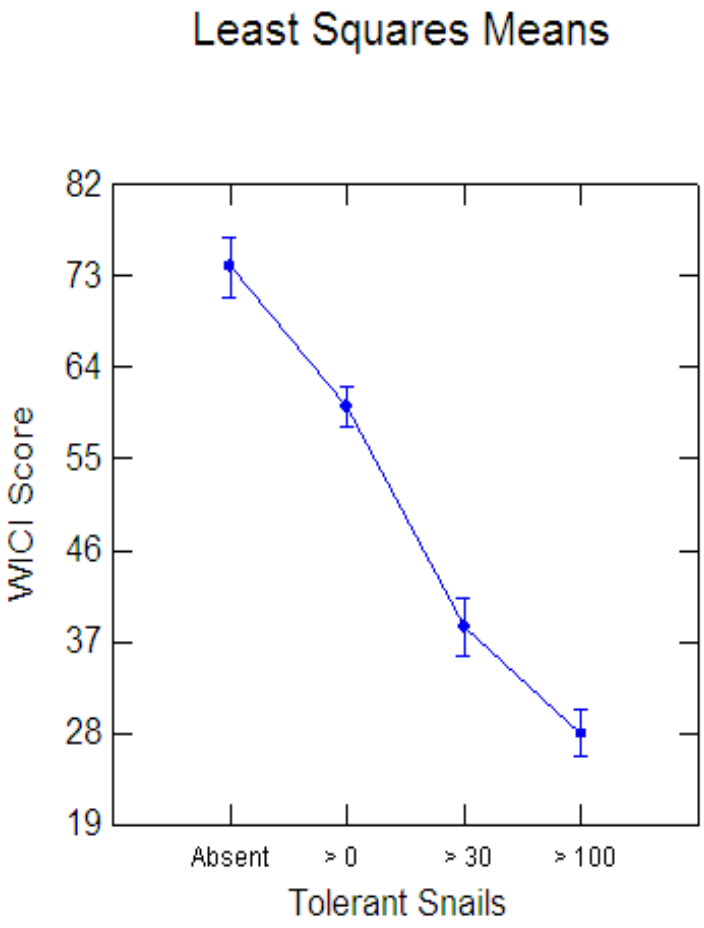
1. Assignment of density groups.
   Taxa were grouped in ascending density clusters from absent (0), present (>0 but < n1), to succeeding larger clusters based on breakpoints observed in a stem and leaf diagram. Care was taken to include a least a minimum of 10 observations (20 preferable) in each cluster for statistical power (assure valid comparison results). The whole database was used including 265 records from 1996-2006.

```
Stem and Leaf Plot of variable:     Tolerant Snails, N = 265
         Minimum:        0.000
         Lower hinge:        3.000
         Median:        24.000
         Upper hinge:      115.000
         Maximum:     2058.000

               0 H 00000000000000000000000000000000000000000000000000000000*
               0 M 22222222222233333333333333
               0   4444445555555
               0   666677777
               0   8888888999
               1 H 00000011111
               1   2222333
               1   4444445
               1   6666677
               1   899
               2   00011
               2   223
               2   45
               2   667
      * * * Outside Values * * *
               2   899
               3   112236
               4   12568
               5   016
               6   0
               7   05
               9   99
              11   2
              13   8
              15   0
              18   1
              19   7
              20   55
```

2) ANOVA statistical test for differences between density groups followed by Tukey's multiple comparison test for differences among groups.

```
Categorical values encountered during processing are:
Tolerant Snails (4 density levels)
   0, 1-30, 31-99, >100

Dep Var: WICI   N: 265   Multiple R: 0.660   Squared multiple R: 0.436

                              Analysis of Variance

Source             Sum-of-Squares   df  Mean-Square     F-ratio       P

Tolerant Snails        78162.138     3    26054.046      67.236       0.000

Error                 101137.877   261      387.501
------------------------------------------------------------------------------
```

The output shows that there is a significant difference in WICI scores among groups with different density levels at least at .05 probability level.



The SYSTAT graph shows a plot of the average value of the dependent variable for each level of the factor. ANOVA tests for significance the differences illustrated in this plot.

3) Those density groups with significant differences in WICI scores between lower densities and higher densities were used as metrics.

```
Post Hoc test of WICI
-------------------------------------------------------------------------------
Tukey HSD Multiple Comparisons.
Matrix of pairwise comparison probabilities:
                        Absent      > 0         >30         > 100
                        1           2           3           4
              1         1.000
              2         0.001       1.000
              3         0.000       0.000       1.000
              4         0.000       0.000       0.025       1.000
-------------------------------------------------------------------------------
```

The output for pairwise comparisons includes a table of probabilities. To determine significant differences, examine the pairs and their probability level. From the output it is evident that there is a significant difference between absent and all other groups at least at the 0.05 level.

4) The mean and median WICI scores were computed for those groups with significant differences in WICI scores and the median used as metric values in the DICI index. Since these density levels represent changes in wetland quality based on increasing density levels, no metric values are assigned to densities that are absent or at lower density levels not significantly different that absent. See Appendix B for analyses of all 18 metric groups.

	WICI score	
	>30	>100
n	46	82
median	**36**	**26**
avg	38.3	27.7

5) Eighteen metrics were chosen based on their significant differences (p values < .05) of WICI scores among density groups (Tables 1and 2) Sensitive metrics had values between 60 and 80, and tolerant metrics had values between 20 and 40.

Table 1. Sensitive Metric Values based on Invertebrate Densities in 10 Activity Traps

Organism Group	Total Density of Species/Species Groups	Density Level (total 10 traps)		
		Metric value assigned		
Cladocerans	Bunops sp., Daphnia sp., Kurtzia sp., Simocephalus expinosus	> 30		
		66		
Ostracods	All ostracods excluding Cypridopsis vidua, Cypria sp, Cypria maculata.	≥ 20	> 100	
		62	**80**	
Copepods	Calanoids, Harpacticoids	≥ 10	≥ 30	
		62	**74**	
Crayfish	All species	≥ 10		
		60		
Megaloptera	Chauliodes sp.	Present		
		62		
Caddisflies	Limnephilid caddisflies: Linmephilus sp, Ironoquia sp.	Present		
		66		
Dytiscids	Acilius, Cybister, Dytiscus	≥ 10	> 20	
		64	**74**	
Diptera	Machlonyx sp. (Not Chaoborus sp.)	Present		
		74		
Snails	Aplexa elongata, Planorbella armigua, Promonetus exacuous	> 10	> 30	> 100
		62	**72**	**78**

Table 2. Tolerant Metric Values based on Invertebrate Densities in 10 Activity Traps

Organism Group	Total Density of Species/Species Groups	Density Level (total 10 traps)
		Metric value assigned
Cladocerans	Simocephalus vetulus, Ceriodaphnia reticulata, Scapholebris sp.	> 30
		33
Ostracods	Cypridopsis vidua, Cypria maculata	≥ 30 > 100
		34 28
Copepods	Cyclopoids	> 60
		40
Mayflies	Callibaetis sp., Caenis sp.	Present
		32
Hemiptera	All corixids excluding the genus Hesperocorixa	≥ 20 ≥ 100
		28 20
Hemiptera	Notonecta sp.	> 40
		26
Coleoptera	Haliplidae: Haliplus sp and Peltodytes sp Hydrophilidae: Berosus and Tropisternus	> 30 > 60
		40 26
Dytiscids	Hygrotus sp, Laccophilus sp.	≥ 20
		32
Snails	Physella sp, Gyraulus sp, Planorbella pilsbryi	> 30 > 100
		36 26

6) A proportion of the sites do not have high enough densities of any of the 18 metric organism groups to score a metric value. They may have low overall densities of scoring taxa, or have high densities of organisms not included the 18 metrics. For example no DICI score is shown in Table 6 for Big Run Vernal. It is suggested that the data for these sites be analyzed from another pass that may have more metric scores. Some sites only score on 1 or 2 metrics and BPJ must be used on these sites for a valid assessment. As more data is collected, associations with other organism groups may be calculated, specifically for oligochaetes, and may aid in the invertebrate analyses for these sites.

Index Score Calibration

Reference sites in the database were based on ORAM 5.0 classification in the database. Based on this dataset classification the 25th percentile and 75th percentile of DICI scores were 43.5 and 70, respectively. . The reference database was reevaluated for inclusion into an invertebrate reference database. The 25th percentile and 75th percentile of DICI scores were 55 and 70, respectively. The upper scores did not change, but lower scores that were not representative of invertebrate reference sites were dropped.

Criteria for sites to be dropped:

1) Sites with not enough metrics (only 0,1, or 2) were dropped as mentioned earlier in this report
2) Only one record per site was used. The pass chosen was the one with the most number of metrics used.
3) Two sites, Ballfield and Tinkers Creek, were dropped. Although they scored high AmphIBI and VIBI scores for amphibians and plants, they consistently scored low on the DICI score. Upon further examination they had had high chloride values. An analyses of chloride data is provided in Appendix C.

Good DICI scores are taken to be greater than 55 and very good as 70 and above. No exceptional category is being proposed at this time. Some sources use the 75th percentile of the reference sites as the exceptional limit. The 75th percentile of the reference data may not necessarily be characteristic of exceptional communities. As with the Ohio stream invertebrate ICI program, new thoughts for recalibration may include a reevaluation of metrics for determining exceptional communities. A detailed analyses of exceptional wetland community attributes has not been conducted at the time of this report.

A poor category was obtained by taking all sites with only tolerant metric values (no sensitive metric values) and taking the 75th percentile of the DICI scores as the upper limit of poor. The sites were unique records and the pass chosen was the one with the most metrics. All sites with only one metric value were excluded. The total number of unique sites used was 55. The 75th percentile was 32.25. Scores of 20 to 32 are considered poor and 33 to 45 fair.

Scores between 46 and 54 have an even average of both sensitive and tolerant metrics. These sites should be judged on an individual basis. Another pass from these sites may be needed to evaluate it for invertebrate assessment.

2.0 Refined taxonomy of "Microinvertebrates"

Oligochaetes, cladocerans, and ostracods were previously identified only to order for most of the Ohio EPA wetland invertebrate data from 1996 to 2002. These orders were identified to the generic and specific taxonomic levels in laboratory analyses for most of the 2003 and 2004 samples**,** and all data for 2005 and 2006. Additional samples with high densities, greater than 200 per site, of these groups from the previous years 1996 to 2002 were revisited and identified to generic and specific levels.

From this refined taxonomic level, sensitive and tolerant species of cladocerans and ostracods were associated with wetland quality (Appenix B). The sites were grouped into increasing levels of density (0-10, 11-100, 101-300, and >300 organisms per site, e.g.) and ANOVA used to test for differences between density levels based on the invertebrate WICI inesx scores. Significant differences between lower and higher density levels were determined by Tukey pairwise comparisons. The tolerant ostracod species associated with lower WICI scores were *Cypridopsis vidua* and *Cypria maculata*.

More data analyses is necessary to make statistical statements for oligochaetes and chironomids. Many of the species in these two groups are not active swimmers and their occurrence in the funnel traps may be incidental. Preliminary observations appear to show tolerant oligochaete species identified as Tubificids, and the Naididae genera *Nais, Ophidonais*, and *Pristina*, plus other Naididae, and tolerant chironomid species as the genus *Cricotopus*. Sensitive oligochaetes appear to be the genera *Dero* and *Stylaria* (Knapp 2006). Some of these observations were made on as little as 10-15 occurrences in the database.

3.0 Wetlands in the Huron/Erie Lake Plains (HELP) Ecoregion, 2003

In 2003, 30 sites were evaluated for invertebrates in the Huron/Erie Lake Plains (HELP) Ecoregion with an emphasis on sites in the Oak Openings subecoregion were selected for study. The Oak Openings area is unique. It is situated on the old lake plain so there is a high content of clay in the soils underlying the area. Overlaying this highly impermeable soil are deep deposits of sand left from the old lake beach ridges that developed as lake waters retreated over time. In depressions wetlands develop where surface and ground water is retained. Wet prairies form in the depressions with upland prairies on the dunes and swales. The wetlands in this subecoregion have sandy soils with organic deposits. This is found nowhere else in the state and is rare globally.

Table 3 shows the densities of organisms for the eighteen metrics and DICI scores from the 2003 sampling year. The DICI scores from the HELP ecoregion were similar to the reference sites from other areas in the state. Many of the sites in the study area from 2003 were on protected lands such as the Oak Openings MetroPark, Maumee State Forest, and other natural areas and preserves. Twenty-seven of the 31 sites sampled had DICI scores in the good and very good range. Twenty-five of these sites did not have high densities of any tolerant invertebrates and scored only on sensitve metrics. This is in stark contrast to the sites at the mitigation banks sampled in 2004 which were dominated by densities of tolerant invertebrates.

Blue Heron Marsh was the only site to score in the poor range. This site had a high percentage of the tolerant ostracod, *Cypridopsis vidua* . Bike Trail, Nasa #100 and Steidtman Marsh had DICI scores in the 46-54 region. Scores between 46 and 54 have an even average of both sensitive and tolerant metrics. These sites should be judged on an individual basis. Invertebrates collected at Blue Heron Marsh, Lou Campbell and Nasa #100 only had densities of organism groups to score on 1 of the 18 metrics. Another pass from these sites may be needed to evaluate the invertebrate assemblages.

Table 3. Invertebrate densities (total collected in ten funnel traps) during 2003 in the Oak Openings and selected others from NW Ohio. Organism groups shown correspond to the DICI Metrics from Tables 2 and 3. Densities in red and blue represent tolerant and sensitive densities, respectively, which have metric values associated with them. The DICI score is the average of the metric values.

WETLAND	MO/DY	DICI score	Abund.per trap	Tol. Clad.	Tol. Ostr.	Cyclo.Cope.	Tol. Mayfl	Corixid	Notonecta	Tol. Beetle	Tol. Dytis	Tol. Snails	Sens. Clad.	Sens. Ostr.	Non-Cyclo	Cray-fish	Chaul iodes	Limn Caddis	Sens. Dytis	Mach lonyx	Sens. Snails
Bike Trail	6/25	54.7	58	0	.	.	0	1	0	0	0	191	2	.	.	18	0	0	8	0	137
Blue Heron M	7/1	30.0	33	4	140	1	1	1	27	23	7	5	0	0	0	0	0	0	0	0	0
Blue Heron W	5/13	64.0	36	0	15	15	0	0	0	0	13	0	187	0	3	12	0	2	16	0	0
Blue Oxbow	5/6	71.2	212	0	.	0	0	1	0	3	1	3	221	.	612	0	0	0	17	27	234
D4	6/25	61.2	121	47	0	67	0	0	2	0	10	0	16	689	0	0	1	0	78	0	235
Derby	5/6	62.0	409	0	0	69	0	0	0	57	1	8	3062	500	0	0	0	0	6	2	68
Garden Road	5/14	70.0	70	1	.	10	0	0	1	0	0	1	7	.	92	6	0	0	13	0	59
Green Oxbow	6/25	71.3	152	16	17	35	0	5	1	1	1	0	27	441	9	1	6	0	3	0	100
Hiltner	5/14	69.0	31	29	1	2	0	0	2	4	0	3	93	2	2	0	0	0	6	0	69
Irwin Pin Oa	5/6	73.0	78	3	.	7	0	0	0	1	1	1	44	.	110	8	0	0	3	15	109
Irwin Prarie	5/6	68.0	10	2	.	1	0	0	0	4	0	3	0	.	0	2	0	0	2	1	18
Irwin Vernal	5/6	68.0	44	.	.	.	0	0	0	2	1	8	.	.	.	15	0	2	2	91	44
Kinglet	5/6	71.6	113	0	0	17	0	0	0	1	0	8	0	211	5	10	0	10	5	239	254
Lodge	3/25	73.3	57	0	0	29	0	0	1	0	0	25	0	198	152	2	0	1	2	0	2
Lou Campbell	6/25	66.0	29	0	.	0	0	0	12	18	1	26	0	.	0	14	0	0	1	0	33
Lucas	6/25	76.0	52	0	.	23	0	0	0	0	0	0	6	.	1	3	0	0	66	0	321
Mancy Tract	5/14	64.0	25	0	4	1	0	0	0	30	6	0	5	0	2	0	0	1	2	0	28
Marie North	7/1	66.4	108	4	4	11	0	0	0	0	0	0	373	41	7	3	4	0	16	0	200
Muck Farm	5/6	68.0	29	0	.	2	0	0	1	2	1	6	51	.	19	7	0	11	0	3	47
Nasa #100	5/13	51.0	27	.	.	.	0	0	0	27	0	73	.	.	.	0	0	8	0	0	0
Nasa #3	5/13	66.0	44	.	.	.	0	1	5	4	2	7	.	.	.	0	0	35	4	0	0
Nasa #8	5/13	62.3	117	17	126	12	0	1	2	7	3	4	0	508	28	2	0	10	14	1	0
Old State Li	5/14	65.2	148	2	5	3	0	0	0	4	1	1	21	20	18	1	0	1	4	1	16
PattonTractN	5/14	67.0	21	0	.	0	0	0	0	5	0	6	16	.	29	1	0	0	0	0	69
PattonTractS	5/14	68.7	25	0	.	0	0	0	0	5	2	5	10	.	16	0	0	1	2	0	137
Ranger	6/25	71.3	83	12	.	0	0	0	4	0	0	15	148	.	83	9	0	0	43	0	9
Reed Road	3/25	70.0	27	.	.	2	0	0	0	1	0	5	.	.	81	0	0	10	4	0	0
Rudolph Sava	7/1	69.0	31	4	1	2	0	0	0	0	2	1	46	3	0	1	0	0	4	0	66
Skull #2	5/6	64.7	19	0	.	2	0	0	0	0	2	11	40	.	13	1	0	5	0	0	7
Steidtman Bu	7/1	61.2	100	28	9	46	8	0	4	7	5	13	44	3	100	0	2	0	2	0	35
Steidtman Ma	7/1	51.0	118	0	1	2	1	0	17	4	2	109	41	176	6	0	0	0	0	0	0

4.0 The Mitigation Bank Study 2004

In 2004, invertebrates from 20 sites at seven banks were analyzed. The bank sites included were Cherry Valley, Chippewa, Grand River Lowlands, Panzner, Sandy Ridge, Three Eagles, and Trumbull Creek. Additionally, six sites from three banks samples in 2001 were analyzed from the Hebron, Little Scioto, and Slate Run banks. Many wetland mitigation projects have vegetative and design flaws that limit the habitat potential for sensitive organisms to thrive. Some mitigation banks resemble more of a lake ecosystem or a mere retention basin with large areas of open water providing little or no shade, and steep banks and dikes which are kept mowed providing little vegetative opportunity and limited near margin habitat.

The DICI scores were in the poor and fair range. Invertebrate assemblages at the banks had high densities of tolerant species of ostracods, hemipterans, beetles, and physid snails (Table 4). The adult beetle genera *Berosus, Haliplus, Peltodytes*, and *Tropisternus* are mostly herbivores and have been found in dense mats of aquatic vegetation or algal mats (Knapp 2006). Corixid density, especially the genera *Sigrara* and *Trichocorixa,* was high at the bank sites and the tolerant snail genera *Physella* and *Gyraulus* were also frequently present in high densities associated with poor wetland quality. Two species of tolerant ostracods (*Cypridopsis vidua*, *C. maculata*) were present in high densities at many bank sites. *C. vidua* is a cosmopolitan, opportunistic species often found at high densities (Altinsacli et. al. 2000) and both species have been associate with low quality Ohio wetlands (Appendix B).

Few sensitive invertebrates were present at densities characteristic of good wetland quality (Tables 1 and 2) and some sensitive groups (caddisflies, sensitive dytiscids, sensitive diptera) were not present at all (Table 4). Densities of more sensitive taxa of ostracods and dytiscid beetles were lower at the bank sites than at high quality wetlands.

Table 4. Invertebrate densities (total collected in ten funnel traps) during 2004 in the Mitigation Bank Study, Ohio. Organism groups shown correspond to the DICI Metrics from Tables 2 and 3. Densities in red and blue represent tolerant and sensitive densities, respectively, which have metric values associated with them. The DICI score is the average of the metric values.

WETLAND	MO/DY	DICI score	Abund.per trap	Tol. Clad.	Tol. Ostr.	Cyclo.Cope.	Tol. Mayfl	Corixid	Notonecta	Tol. Beetle	Tol. Dytis	Tol. Snails	Sens. Clad.	Sens. Ostr.	Non-Cyclo	Cray-fish	Chaul iodes	Limn Caddis	Sens. Dytis	Mach lonyx	Sens. Snails
3Eagle Mdw	5/20	26.4	115	1	**31**	9	0	**232**	**54**	**68**	15	**252**	7	4	0	0	0	0	6	0	0
3Eagle NE Ma	6/24	33.8	290	**108**	**256**	**118**	**152**	13	4	14	2	**36**	0	0	0	2	0	0	0	0	0
Cherry V 1N	6/29	28.0	83	9	7	.	**30**	2	3	**100**	13	**144**	0	0	.	0	0	0	2	0	0
Cherry V 1S	6/29	30.6	125	**131**	**40**	.	**53**	**66**	0	**103**	5	12	0	0	.	0	0	0	0	0	0
Cherry V 3	6/29	33.0	181	16	**49**	.	**152**	1	10	**53**	17	**115**	0	0	.	0	0	0	1	0	3
Chippewa Mid	6/16	31.0	295	2	**1344**	**99**	**69**	**31**	3	13	**41**	**998**	5	3	4	0	0	0	0	0	0
Chippewa Nor	6/16	35.6	362	**138**	**606**	**159**	**115**	**26**	28	19	**70**	**1816**	**95**	0	0	0	0	0	1	0	0
Chippewa Sou	6/16	36.7	318	5	**812**	**110**	**6**	18	14	20	**168**	**1127**	24	0	0	1	**5**	0	0	0	0
Grand Riv AD	6/29	35.0	390	2	**544**	0	**9**	5	0	**106**	**40**	**2058**	**64**	0	0	3	0	0	4	0	0
Grand Riv BC	6/29	31.5	121	1	**209**	11	**20**	7	1	**49**	3	**426**	0	0	0	0	0	0	8	0	0
Grand Riv Ft	6/29	45.0	192	12	**144**	59	**2**	0	2	**38**	12	**80**	3	0	3	0	9	0	1	0	**60**
Panzner A	6/16	31.3	356	**380**	**228**	**281**	0	**729**	12	**38**	**69**	**1506**	0	0	0	0	0	0	0	0	1
Panzner B	6/16	33.8	174	**41**	12	**71**	**5**	4	6	**50**	**110**	**509**	0	0	0	0	0	0	0	0	0
Panzner C	6/16	33.8	287	**404**	24	**212**	**9**	18	3	**56**	**46**	**1384**	0	0	0	0	0	0	0	0	0
Sandy Rdg 1	6/23	29.8	965	**69**	**7008**	**112**	**16**	**164**	20	**62**	1	0	0	0	9	0	0	0	0	0	0
Sandy Rdg 2	6/23	44.0	91	5	**517**	28	**24**	0	0	8	1	19	0	0	0	0	0	0	0	0	**67**
Sandy Rdg 3	6/23	28.0	472	18	**3238**	40	**42**	9	17	**64**	10	**111**	0	0	0	0	0	0	1	0	0
Trumbull C5	6/30	31.1	126	8	**50**	29	**9**	**26**	**143**	**58**	**45**	**198**	14	0	1	0	0	0	1	0	0
Trumbull C7	6/30	36.4	137	**91**	2	8	**15**	1	**94**	**53**	**75**	**125**	**47**	0	1	2	0	0	2	0	0
Trumbull For	6/30	41.0	142	1	**32**	25	**1**	10	**54**	21	19	**148**	**268**	0	0	0	**1**	0	3	0	0

5.0 Cuyahoga Watershed Wetland Assessment

In 2005, 19 sites were evaluated for invertebrates in Cuyahoga River Watershed. The study was part of a wetland watershed project in which over 200 sites were randomly picked and evaluated using habitat assessment (ORAM 5.0). A smaller subset was randomly picked for intensive biological study.

DICI scores in the good range (55-69) included Aquilla, Bath Road, Black Road, Goodyear Bog, Hasbrook, Lake Rockwell Vernal, Miller, Quail Hollow, and Twinsburg. Old Forge had a DICI score above the 75th percentile of invertebrate reference sites characteristic of very good. Aquilla was located in the headwaters of the West Branch of the Cuyahoga River in the middle of a large wetland complex. Hasbrook was located in Hudson township in a remnant woodlot at least 100 acres, however, while sampling there were signs that part of this woodlot was being earmarked for housing development. Black Road is interesting in that it is located in a 50+ acre woodlot that use to be farm field 30+ years ago. However, since it is separated from other woodlots, natural colonization of amphibian populations has been slow to occur.

The two lowest DICI scores were 39.5 and 35.5 (both fair) at Bartholomew and Wingfoot Lake, respectively. These sites were wetland areas along small inlet areas of Lake Rockwell and Wingfoot Lake.

Brecksville, Marsh Wetlands, Oak Knolls, Rhinehart, Tare Creek, Thut, and Ward had DICI scores in the 46-54 range. Scores between 46 and 54 have an even average of both sensitive and tolerant metrics. These sites should be judged on an individual basis. Invertebrates collected at Bath Road only had densities of organism groups to score on 1 of the 18 metrics. Another pass from these sites may be needed to evaluate the invertebrate assemblages. The Marsh Wetlands site sampled for amphibians and invertebrates was located near an abandoned railroad/multipurpose recreation trail and differs in location from the Marsh Wetlands vegetation site sampled the year before.

Table 5. Invertebrate densities (total collected in ten funnel traps) during 2005 in the Cuyahoga River basin, Ohio. Organism groups shown correspond to the DICI Metrics from Tables 2 and 3. Densities in red and blue represent tolerant and sensitive densities, respectively, which have metric values associated with them. The DICI score is the average of the metric values.

WETLAND	MO/DY	DICI score	Abund.per trap	Tol. Clad.	Tol. Ostr.	Cyclo.Cope.	Tol. Mayfl	Corixid	Notonecta	Tol. Beetle	Tol. Dytis	Tol. Snails	Sens. Clad.	Sens. Ostr.	Non-Cyclo	Cray-fish	Chaul iodes	Limn Caddis	Sens. Dytis	Mach lonyx	Sens. Snails
Aquilla	5/17	56.0	71	8	**30**	42	0	1	5	6	0	5	3	**84**	**22**	0	0	**2**	2	0	0
Bartholomw	6/21	39.5	140	**84**	**60**	**69**	**12**	4	4	5	0	**78**	1	**39**	7	0	0	0	0	0	0
Bath Rd	4/6	62.0	43.6	4	0	16	0	13	31	5	0	1	14	18	**19**	0	0	0	6	0	2
Black Rd	4/12	66.0	49.9	0	1	52	0	1	3	11	3	0	**157**	**55**	**99**	0	**1**	**21**	7	0	0
Brecksvill	5/11	49.6	56.4	2	0	6	0	2	18	**36**	**38**	**54**	**84**	1	2	0	0	0	**26**	0	1
Goodyear	5/10	61.7	378	0	0	**67**	0	0	1	0	4	4	**38**	**49**	**1227**	0	**1**	**1**	0	0	**17**
Hasbrook	5/11	68.4	101	0	2	19	0	0	15	5	2	5	**663**	10	**14**	4	0	**42**	**23**	**4**	8
LakeRock	4/6	68.0	26.7	0	0	17	0	0	33	0	3	0	0	10	**60**	0	**8**	**1**	**17**	**3**	4
MarshWet	5/7	54.2	69.2	**45**	0	33	0	11	14	15	11	**52**	**47**	6	**20**	0	0	**17**	0	0	**25**
Miller	4/5	67.3	65	0	0	3	0	0	0	0	0	1	0	**31**	**137**	0	0	**32**	2	0	0
OakKnoll	5/10	54.3	103	0	13	33	0	0	**58**	1	3	**147**	28	**53**	**102**	0	0	**20**	1	0	**68**
OldForge	5/11	70.7	271.2	0	25	9	0	0	0	0	0	17	**31**	**152**	**927**	0	0	**40**	**11**	**40**	1
QuaillHolw	4/5	66.5	56.6	0	0	39	0	2	11	3	1	0	0	1	**84**	0	**4**	**54**	**18**	0	4
Rhinehart	5/11	46.0	19	0	1	21	**1**	0	1	0	0	5	8	2	1	**19**	0	0	7	0	0
Tare Ck	5/17	54.5	41.3	5	0	35	0	**33**	3	23	10	0	0	**21**	**19**	0	0	**2**	1	0	0
Thut	6/21	49.0	112	0	1	31	0	0	2	8	4	**49**	0	0	1	0	**8**	0	2	0	0
Twinsburg	5/10	65.0	27	0	0	25	0	2	31	3	1	5	27	9	7	0	0	**33**	**14**	0	4
Ward	4/5	51.0	28	0	0	0	0	0	0	0	0	**35**	0	5	0	3	0	**69**	1	0	0
Wingfoot	6/21	35.5	136.4	16	**45**	24	0	**202**	3	30	3	**327**	4	**25**	7	0	0	0	1	0	2

6.0 Urban Wetland Assessment Project

In 2006, 18 sites were evaluated for invertebrates in the Columbus Metropolitan area. The study was part of a urban wetland assessment project in which a number of wetland sites were randomly picked and evaluated using biological, chemical, habitat, and morphometry factors. Overall, about half of the sites sampled for wetland invertebrates were below expectations characteristic of reference conditions.

Invertebrates collected from Airoport Plaza, Easton, Hill, Reflections, Somerset, Watkins, and Wilson had DICI scores in the good range and Woodward in the very good range. Many of these are vernal pools, where, although the surrounding land use has been drastically reduced, there is little or no drainage from surrounding residential or commercial areas entering the pools. In other words the water quality of the wetland has not been affected by the development around it.

Invertebrate densities collected at Alum Creek, Ridenour, Sunbury and Towne Center had DICI scores in the poor range. 3 Creeks, Big Run Vernal and Quarry had DICI scores in the fair range. Communities were dominated by tolerant cladocerans, ostracods, corixids and snails. ATV and ISG had marginal scores with a fairly equal mix of sensitive and tolerant densities of invertebrates. Problems from some of these sites include drainage from residential areas.

Big Run Dugout did not have densities of metric organisms high enough to score any values. Airport Plaza and ISG had only two metric values and should be evaluated on an individual basis. Another pass from these sites may be needed to evaluate the invertebrate assemblages.

Table 6. Invertebrate densities (total collected in ten funnel traps) during 2006 in the Urban Wetland Assessment Project. Organism groups shown correspond to the DICI Metrics from Tables 2 and 3. Densities in red and blue represent tolerant and sensitive densities, respectively, which have metric values associated with them. The DICI score is the average of the metric values.

WETLAND	MO/ DY	DICI score	Abund. per trap	Tol. Clad.	Tol. Ostr.	Cyclo. Cope.	Tol. Mayfl	Corixid	Noto necta	Tol. Beetle	Tol. Dytis	Tol. Snails	Sens. Clad.	Sens. Ostr.	Non-Cyclo	Cray-fish	Chaul iodes	Limn Caddis	Sens. Dytis	Mach lonyx	Sens. Snails
3 Creeks	6/13	35.3	261.1	**125**	**50**	**111**	**1**	**553**	3	10	11	**751**	21	4	1	2	**5**	0	0	0	0
ATV	5/9	45.4	176	**278**	10	**210**	0	**44**	0	4	0	2	**576**	0	4	**12**	0	0	0	0	0
Airport Plaz	4/11	64.0	38.9	0	0	8	0	11	10	23	0	2	**64**	19	**13**	7	0	0	0	0	0
Alum Ck	6/13	31.0	86.4	**255**	**72**	17	0	10	10	18	0	**106**	22	0	6	0	0	0	0	0	0
BigRun Dug	6/13		22.9	0	10	3	0	0	22	11	1	12	13	0	0	1	0	0	0	0	0
BigRun Ver	6/13	36.0	150.5	4	**423**	9	**1**	**28**	10	30	1	**203**	**441**	0	0	0	0	0	2	0	0
Easton	5/11	63.6	219.1	0	**50**	28	0	1	1	5	0	0	**95**	**615**	**586**	0	0	0	**10**	0	0
Hill	6/13	61.6	144.4	0	28	13	0	4	6	2	6	**90**	**196**	**424**	0	0	**6**	0	**20**	0	0
ISG	6/13	47.0	47.8	3	28	31	0	**26**	5	11	2	28	**54**	0	2	3	0	0	1	0	0
Quarry	6/13	40.4	163.8	**45**	**152**	**67**	0	**38**	0	3	5	**299**	**68**	6	0	1	**5**	0	0	0	1
Reflection	5/17	66.0	468.8	0	8	**108**	0	0	0	0	0	1	**3450**	**664**	**12**	0	0	0	**24**	**1**	0
Ridenoor	6/13	32.2	69	**95**	12	**61**	0	**166**	1	15	1	**84**	8	1	0	1	0	0	0	0	0
Somerset	5/11	57.1	310.6	24	**68**	**184**	0	8	7	11	2	0	**401**	**24**	**213**	**15**	0	0	**13**	0	0
Sunbury	6/13	30.6	96.5	**105**	**185**	13	0	14	**58**	**45**	9	**245**	0	0	0	0	0	0	0	0	0
Towne Cntr	5/11	29.8	241	**100**	**317**	50	**1**	1	0	1	0	**315**	4	3	5	4	0	0	0	0	0
Watkins	4/6	64.7	86.1	0	0	**85**	0	0	0	5	1	0	16	**184**	**36**	4	0	0	0	0	0
Wilson	4/6	64.7	122.3	4	0	**84**	0	3	2	6	0	0	4	**138**	**742**	0	0	0	0	0	0
Woodward	5/1	73.3	340.1	0	2	40	0	0	0	0	0	0	**2729**	**164**	0	7	0	0	7	**22**	0

7.0 Other Sampling Methods

Density and Relative Abundance Based Methods

The density based approach to invertebrate analyses is specific to the use of the funnel trap sampling method. However, the approach can be applied to other methods as well. For relative abundance data, certain miminum densities can be established before percentage metrics are employed. For SQM and other "semi-quantitative" methods, the decision criteria for picking out a certain number of taxa could be modified to include say 100 ostracods, 100 oligochaetes, 100 cladocerans, 100 corixids, 100 beetles, etc, such that predominant organisms are fairly represented throughout the invertebrate spectrum

.Qualitative Dipnet Sampling Method

A qualitative dipnet method was used for sampling from 1996 to 2004. Evaluation of the organisms collected using this method showed little information gained in the number of taxa collected compared to the quantitative funnel trap method (Figure 2). The overall efficiency of the dipnet method to collect taxa was low (average of 14.0 taxa per site) compared to the funnel trap method (average of 34.9 taxa per site). There was only an average of 3.1 additional taxa per site when the dipnet taxa were added to the funnel trap taxa.

The qualitative dipnet method does not estimate densities or relative abundance of invertebrates. Densities of tolerant and sensitive invertebrate species were used to discriminate between low and high quality wetland sites with the funnel trap method. Based on the low numbers of taxa collected and the inability to estimate densities, the qualitative dipnet method is suggested at this time to be dropped from the program.

Other methods used by various wetland researchers use a dipnet method with a quantitative approach. The dipnet is sweeped for a specified distance and the combined substrates are either picked of organisms in the field or sorted later in a laboratory. These methods produce relative abundances that can be used for quantitative results.

Macroinvertebrate Demo Sheets (Appendix D)

A simplified method patterned after the density based approach was developed for use with demonstration groups studying wetlands in the field. Macroinvertebrate densities observed in the field from ten funnel traps placed in a wetland for 24 hours are assessed in a similar manner as the DICI index. The resolution of the assessment is not as precise as the DICI score, but can give a general impression between poor communities and fair to good communities.

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Appendix A

Sampling Methods

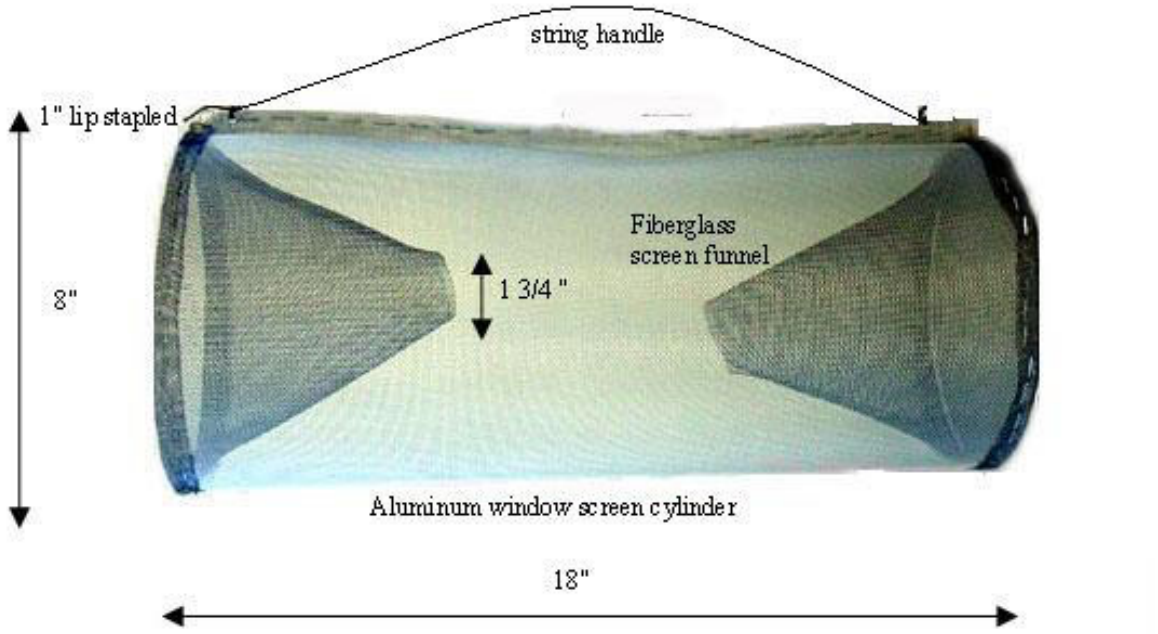
Quantitative Collection Protocol

Ohio EPA began evaluating wetland macroinvertebrate and amphibian sampling methods in 1996. A variety of sampling methods including artificial substrate samplers, several types of funnel traps, and qualitative sampling with dip nets were evaluated (see Fennessy *et al.* 1998a). The use of funnel traps as a method of sampling has been used extensively for amphibians and more recently as a protocol for macroinvertebrate collections in wetlands. A number of different kinds of funnel traps have been described ranging from modified two liter pop bottles to custom-made designs of PVC or clear acrylic plastics to using different types of metal meshes. In addition to the sampling method, the time of year to sample, the intensity, frequency, and duration of sampling were evaluated. Since 1997, field collection techniques have become standardized and the same protocols are used at each wetland sampled.

Funnel traps are constructed of aluminum window screen cylinders with fiberglass window screen funnels at each end (Figure 1). The funnel traps are similar in design to commercially available minnow traps. However, the use of window screen, with its smaller mesh, makes the traps better able to collect a wide range of sizes of larval amphibians and macroinvertebrates. Aluminum screening is used for the cylinders to provide maximum structure and fiberglass screening is used for the funnels to allow flexibility to ease funnel inversion and eversion.

For most wetland surveys, 10 funnel traps are placed evenly around the perimeter of the wetland. The perimeter around the wetland is measured by pacing. The perimeter total is then divided by 10 and a trap is placed each time that amount is paced off while traversing the perimeter for the second time. Alternatively, for large wetlands or where the placement around the entire perimeter is not feasible (slopes too steep, water too deep, etc), transects along one or several sides of the wetland are used. Care is taken to assure that all habitat types within the wetland are represented proportionally within the transect. Each funnel trap location is marked using flagging tape both at the standing water/saturated soil interface and in vegetation above or near the trap. Flagging is numbered sequentially using a permanent marker and traps are set at the same locations throughout the sampling season. When vegetation is extremely dense a hand held GPS unit can be used to record and navigate to trap locations.

Figure 1. Funnel Trap Design



Aluminum window screening 28” x 18” is rolled into a cylinder 18” long and stapled through a 1” lip to form a tube 8” in diameter. Fiberglass screening is cut out and stapled to form a funnel with an opening of 9” on the wide end and 1 ¾” on the narrow end. The narrow end of the funnel is placed inside the cylinder as indicated in the figure. The wide end of the funnel is rolled over the outside edge of the cylinder and stapled every ½”. A string handle is attached to the lip. The trap is emptied by everting the fiberglass funnel and dumping and shaking the contents into a pan.

Wetlands were typically sampled three times between March and early July spaced approximately six weeks apart. The late winter/early spring (March-early April) sample allows monitoring of adult ambystomid salamanders, early breeding frog species and macroinvertebrates such as fairy shrimp, caddisfly larvae, and other early season taxa which are often present for a limited time in some wetlands. A middle spring sample (late April-mid May) is conducted in order to collect some adult frog species entering the wetland to breed, to sample early-breeding amphibian larvae and to sample for macroinvertebrates. A late spring/early summer (early June-early July) sampling is performed to collect relatively well developed amphibian larvae and macroinvertebrates.

The traps are placed on the substrates of the wetland and the trap is almost completely submersed. Traps are placed to allow some exposure of air into the upper part of the cylinder. Placement to allow organisms access to atmospheric oxygen becomes more important as the season progresses, water temperatures rise and oxygen levels in the water decrease. Traps can be placed in shallower water as long as the funnel openings remain immersed during the sampling period. In all cases, the traps are left in the wetland for twenty-four hours in order to ensure unbiased sampling for species with diurnal and nocturnal activity patterns. Limiting trapping time to twenty-four hours also works to minimize the potential for mortality due to individuals being in the traps for extended periods.

Upon retrieval, the traps are emptied by everting the funnel and shaking the contents into a white collection and sorting pan. Organisms that can be readily identified in the field (especially adult amphibians and larger and easily identified fish) are counted and recorded in the field notebook and released. The remaining organisms are transferred to wide-mouth one liter plastic bottles by washing them out of the collection and sorting tray into the bottles using a plastic squeeze bottle filled with 95% ethanol. The collection pan is then thoroughly rinsed with water from the wetland to remove any trace of alcohol that might adversely affect amphibians to be released from the next trap collection.

Laboratory analysis of the funnel trap macroinvertebrate and fish samples follows the standardized Ohio EPA procedures (Ohio EPA 1989).

Appendix B

Organism Profiles

CLADOCERAN ANALYSES

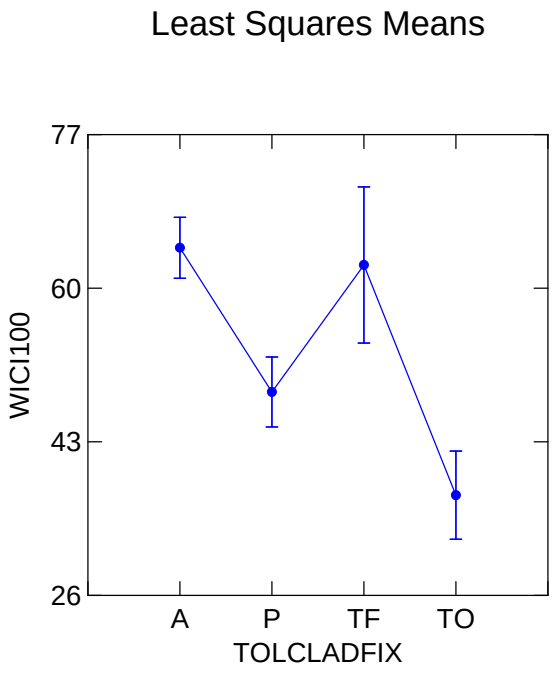
Tolerant Cladocerans
- *Ceriodaphnia reticulata*, *Scapholebris* sp., *Simocephalus vetulus*

```
Stem and Leaf Plot of variable:   Tolerant Cladocerans, N = 110
         Minimum:        0.000
         Lower hinge:        0.000
         Median:         2.100
         Upper hinge:       23.000
         Maximum:      404.000

               0 M 000000000000000000000000000000000000000000000001111222222334*
               0   5555889
               1   1223
               1   66678
               2 H 334
               2   89
               3   00
               3
               4   014
               4   557
      * * * Outside Values * * *
               6   9
               8   4
               9   15
              10   0258
              12   5
              13   18
              21   3
              25   5
              27   8
              38   0
              40   4
155 cases with missing values excluded from plot.
```

```
Effects coding used for categorical variables in model.

Categorical values encountered during processing are:
Tolerant Cladocerans (4 levels)
   A, P, TF, TO
155 case(s) deleted due to missing data.

Dep Var: WICI100   N: 110   Multiple R: 0.434   Squared multiple R: 0.189
```

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
TOLCLADFIX$	12903.846	3	4301.282	8.219	0.000
Error	55471.754	106	523.318		




```
Durbin-Watson D Statistic     2.075
First Order Autocorrelation  -0.039
COL/
ROW TOLCLADFIX$
  1  A  absent
  2  P >0
  3  TF >20
  4  TO >30
Using least squares means.
Post Hoc test of WICI100

-------------------------------------------------------------------------------

Using model MSE of 523.318 with 106 df.
Matrix of pairwise mean differences:
                          1           2           3           4
              1         0.000
              2       -15.964       0.000
              3        -1.907      14.057       0.000
              4       -27.387     -11.423     -25.481       0.000

Tukey HSD Multiple Comparisons.
Matrix of pairwise comparison probabilities:
                       Absent       > 0         >20         >30

                          1           2           3           4
              1         1.000
              2         0.013       1.000
              3         0.997       0.451       1.000
              4         0.000       0.263       0.056       1.000
```

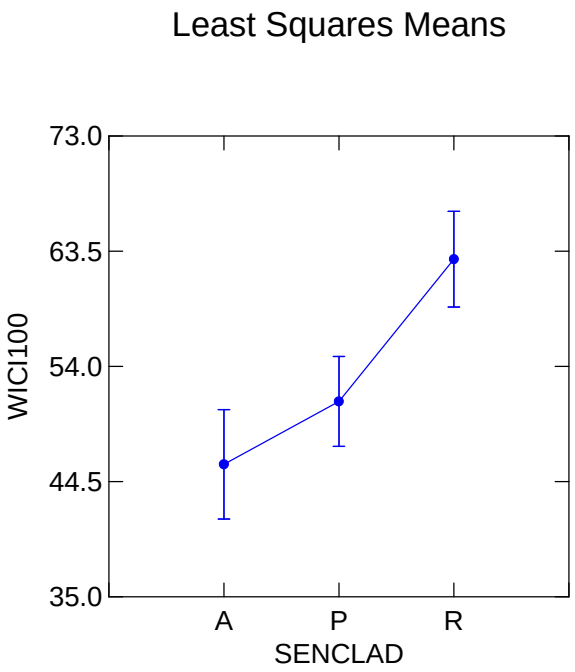
The output shows that there is a significant difference among density groups with different levels of WICI scores at least at .05 probability level.

The output for pairwise comparisons include a table of mean differences and another table of probabilities. To determine significant differences, examine the pairs and their probability level. From the output it is evident that there is a significant difference between group 1 (absent) and density levels > 30.

	TOLCLAD>30 WICI100	All CLAD DATA WICI100
N of cases	22	110
Minimum		
Maximum		
Median	**33**(32,34)	54
Mean	37.1	53.8
Standard Dev		

Sensitive Cladocerans
- *Bunops* sp., *Daphnia* sp., *Kurtzia* sp., *Simocephalus expinosus*

```
Stem and Leaf Plot of variable:      SENCLAD, N = 110
         Minimum:        0.000
         Lower hinge:        0.000
         Median:       14.000
         Upper hinge:       51.000
         Maximum:    3450.000

               0 H 00000000000000000000000000000122333444
               0   556667777889
               1 M 023444
               1   66667
               2   11234
               2   7778
               3   001
               3   8
               4   0144
               4   6777
               5 H 14
               5
               6   44
               6   8
               7
               7
               8   4
               8
               9   3
               9   55
      * * * Outside Values * * *
              14   38
              15   7
              18   7
              19   6
              22   1
              26   8
              37   3
              40   1
              44   15
              57   6
              66   3
              84   5
             103   5
             147   6
             272   9
             306   2
             345   0
155 cases with missing values excluded from plot.

Categorical values encountered during processing are:
SENCLAD$ (3 levels)
   A, P, R
155 case(s) deleted due to missing data.

Dep Var: WICI100   N: 110   Multiple R: 0.276   Squared multiple R: 0.076
                           Analysis of Variance
Source             Sum-of-Squares   df  Mean-Square     F-ratio       P

SENCLAD$                 5212.267     2     2606.133       4.415       0.014

Error                   63163.333   107      590.312
-------------------------------------------------------------------------------
```




```
Durbin-Watson D Statistic     1.884
First Order Autocorrelation   0.052
COL/
ROW SENCLAD$
  1  A
  2  P >0
  3  R >30
Using least squares means.
Post Hoc test of WICI100
```

```
Using model MSE of 590.312 with 107 df.
Matrix of pairwise mean differences:

                    1           2           3
             1      0.000
             2      5.185       0.000
             3     16.911      11.726       0.000
```

```
Tukey HSD Multiple Comparisons.
Matrix of pairwise comparison probabilities:
                        Absent      >0          >30
                        1           2           3
              1         1.000
              2         0.649       1.000
              3         0.016       0.081       1.000
--------------------------------------------------------------------------------
```

The output shows that there is a significant difference among density groups with different levels of WICI scores at least at .05 probability level.

The output for pairwise comparisons include a table of mean differences and another table of probabilities. To determine significant differences, examine the pairs and their probability level. From the output it is evident that there is a significant difference between group 1 (absent) and density levels > 30.

	Sens Clad WICI100	Total Clad WICI100
n	n=38 of 110	n = 110
median	**66**	54
average	62.8	53.8

OSTRACODS ANALYSES

Sesnitive Cladocerans- everything but Cypridopsis vidua vidua, Cypria maculate and Cypria sp.

```
Stem and Leaf Plot of variable:       SENOST, N = 106
         Minimum:        0.000
         Lower hinge:        0.000
         Median:         3.000
         Upper hinge:       55.000
         Maximum:     1534.000

               0 M 00000000000000000000000000000000000000000001111222223333344
               0   556699
               1   00
               1   89
               2   0114
               2   59
               3   1
               3   9
               4   01
               4   9
               5   3
               5 H 5
               6
               6
               7   2
               7
               8   4
     * * * Outside Values * * *
              13   8
              15   2
              16   4
              17   6
              18   45
              19   48
              20   6
              21   1
              42   4
              44   11
              47   6
              48   5
              50   08
              51   7
              61   5
              65   9
              66   4
              68   9
              80   3
             153   4
```

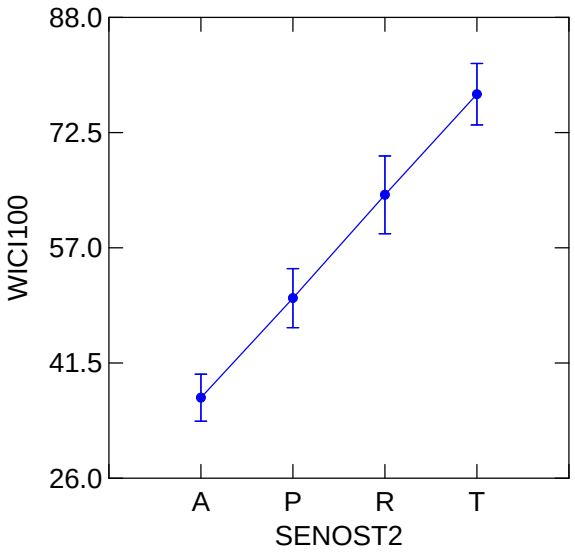
Categorical values encountered during processing are:
SENOST2$ (4 levels)
   A, P, R, T
159 case(s) deleted due to missing data.

Dep Var: WICI100   N: 106   Multiple R: 0.629   Squared multiple R: 0.396

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
SENOST2$	27377.079	3	9125.693	22.284	0.000
Error	41771.487	102	409.524		

Least Squares Means



Durbin-Watson D Statistic   2.035
First Order Autocorrelation -0.018
COL/
ROW SENOST2$
  1 A
  2 P
  3 R
  4 T
Using least squares means.

```
Post Hoc test of WICI100


--------------------------------------------------------------------------------

Using model MSE of 409.524 with 102 df.
Matrix of pairwise mean differences:

                        1           2           3           4
             1          0.000
             2         13.402       0.000
             3         27.304      13.903       0.000
             4         40.837      27.436      13.533       0.000

Tukey HSD Multiple Comparisons.
Matrix of pairwise comparison probabilities:
                        Absent      > 0         ≥ 20        > 100
                        1           2           3           4
             1          1.000
             2          0.046       1.000
             3          0.000       0.154       1.000
             4          0.000       0.000       0.183       1.000
--------------------------------------------------------------------------------
```

The output shows that there is a significant difference among density groups with different levels of WICI scores at least at .05 probability level.

The output for pairwise comparisons include a table of mean differences and another table of probabilities. To determine significant differences, examine the pairs and their probability level. From the output it is evident that there is a significant difference between group 1 (absent) and density levels ≥ 20.

Sensitive ostracods		WICI 100	
	n	avg	median
All ostracod data	106	53.2	54
Ostracod >= 20	15	64.1	**62**
Ostracod >100	24	77.7	**80**

Tolerant Ostracods

- *Cypria maculata* and *Cypridopsis vidua vidua*

```
Stem and Leaf Plot of variable:       TOLOST, N = 106
         Minimum:        0.000
         Lower hinge:        1.000
         Median:       17.000
         Upper hinge:       73.000
         Maximum:     7008.000

               0 H 000000000000000000000000111111122223444455789
               1 M 000223577
               2   045567888
               3   0129
               4   059
               5   0007
               6   0268
               7 H 2358
               8   0
               9   0
              10
              11
              12   6
              13
              14   04
              15   2
     * * * Outside Values * * *
              18   5
              20   9
              21   6
              22   8
              25   46
              31   7
              42   3
              49   2
              51   7
              54   4
              60   6
              81   2
             134   4
             145   4
             249   8
             323   8
             700   8
159 cases with missing values excluded from plot.
```

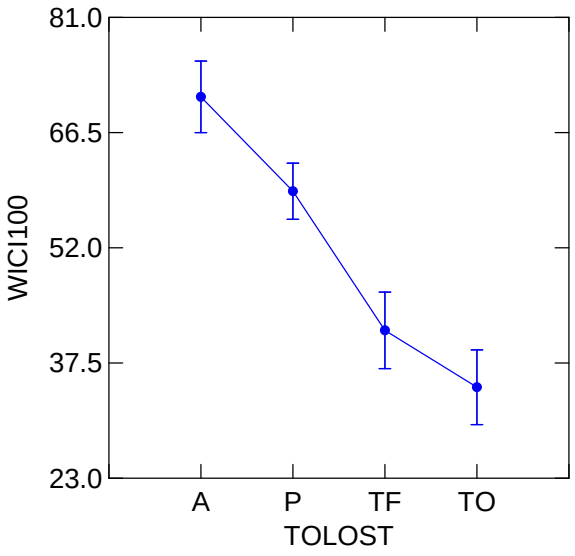
Categorical values encountered during processing are:
TOLOST$ (4 levels)
   A, P, TF, TO
159 case(s) deleted due to missing data.

Dep Var: WICI100   N: 106   Multiple R: 0.531   Squared multiple R: 0.282

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
TOLOST$	19521.800	3	6507.267	13.375	0.000
Error	49626.766	102	486.537		

Least Squares Means



Durbin-Watson D Statistic   1.826
First Order Autocorrelation   0.084
COL/
ROW TOLOST$
  1 A
  2 P
  3 TF>=30
  4 TO>100
Using least squares means.

```
Post Hoc test of WICI100


--------------------------------------------------------------------------------

Using model MSE of 486.537 with 102 df.
Matrix of pairwise mean differences:

                        1           2           3           4
             1          0.000
             2        -11.872       0.000
             3        -29.381     -17.509       0.000
             4        -36.545     -24.674      -7.165       0.000

Tukey HSD Multiple Comparisons.
Matrix of pairwise comparison probabilities:
                        Absent      > 0         ≥ 30        > 100
                        1           2           3           4
             1          1.000
             2          0.168       1.000
             3          0.000       0.021       1.000
             4          0.000       0.000       0.712       1.000
--------------------------------------------------------------------------------
```

The output shows that there is a significant difference among density groups with different levels of WICI scores at least at .05 probability level.

The output for pairwise comparisons include a table of mean differences and another table of probabilities. To determine significant differences, examine the pairs and their probability level. From the output it is evident that there is a significant difference between group 1 (absent) and density levels ≥ 30.

	>=30		>=30-100	>100
	WICI100	Dist100	WICI100	WICI100
n	43	12	21	22
median	32	78-81	**34**	**28**(26-30
avg	38.0	66	41.6	34.5

COPEPOD ANALYSES

Noncyclopoid copepods

- Calanoids and Hapacticoids

Stem and Leaf Plot

```
Stem and Leaf Plot of variable:       NONCYC, N = 104
         Minimum:        0.000
         Lower hinge:        0.000
         Median:         2.000
         Upper hinge:       18.500
         Maximum:     1227.000

               0 H 00000000000000000000000000000000000000000000001111111
               0 M 22222333
               0   4455
               0   66677777
               0   99
               1
               1   233
               1   4
               1   6
               1 H 8999
               2   0
               2   2
               2
               2
               2   89
               3
               3
               3
               3   6
               3
               4
               4
               4
               4   6
      * * * Outside Values * * *
               6   0
               8   134
               9   29
              10   02
              11   0
              13   7
              15   2
              21   3
              58   6
              61   2
              74   2
              92   7
             122   7
161 cases with missing values excluded from plot.
```

265 cases and 343 variables processed.

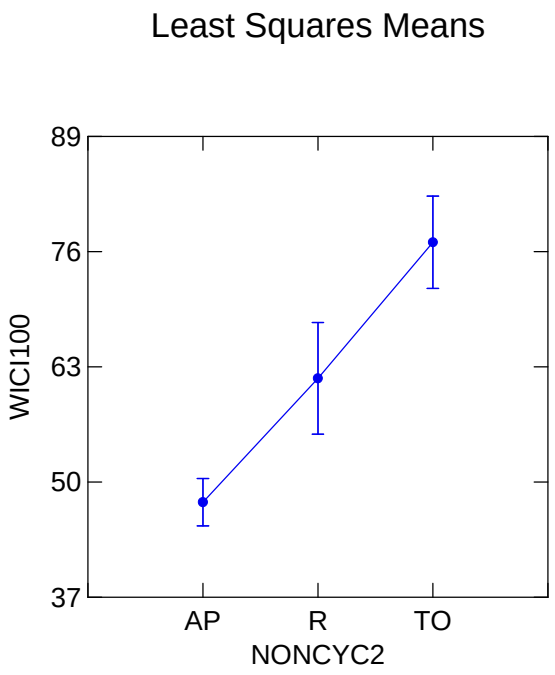
Effects coding used for categorical variables in model.

Categorical values encountered during processing are:
NONCYC2$ (3 levels)
   AP, R, TO
161 case(s) deleted due to missing data.

Dep Var: WICI100   N: 104   Multiple R: 0.456   Squared multiple R: 0.208

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
NONCYC2$	13632.724	2	6816.362	13.224	0.000
Error	52060.161	101	515.447		



Durbin-Watson D Statistic   1.302
First Order Autocorrelation   0.339
COL/

```
ROW NONCYC2$
  1  AP
  2  R>10
  3  TO>30
Using least squares means.
Post Hoc test of WICI100

-------------------------------------------------------------------------------

Using model MSE of 515.447 with 101 df.
Matrix of pairwise mean differences:

                          1           2           3
              1           0.000
              2          13.970       0.000
              3          29.330      15.360       0.000

Tukey HSD Multiple Comparisons.
Matrix of pairwise comparison probabilities:
                  Absent/Present      > 10         > 30
                          1           2           3
              1           1.000
              2           0.108       1.000
              3           0.000       0.150       1.000
-------------------------------------------------------------------------------
```

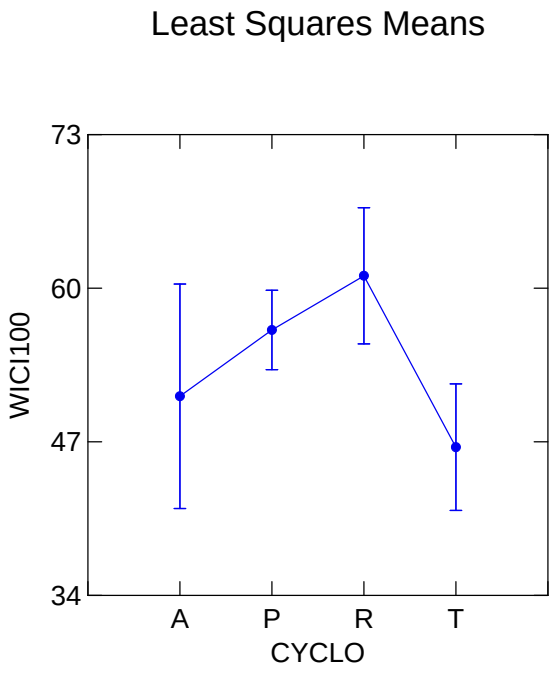
The output shows that there is a significant difference among density groups with different levels of WICI scores at least at .05 probability level.

The output for pairwise comparisons include a table of mean differences and another table of probabilities. To determine significant differences, examine the pairs and their probability level. From the output it is evident that there is a significant difference between group 1 (absent/present) and density levels > 10.

	>10	>30
	WICI100	WICI100
n	13	19
median	62.000	74.000
avg	61.692	77.053

Cyclopoid Copepods – significant only when > 100 with DIST (not with WICI), but with new data median of 40 WICI for > 60

```
Stem and Leaf Plot of variable:        CYCLO, N = 104
         Minimum:        0.000
         Lower hinge:        4.800
         Median:        20.000
         Upper hinge:       48.000
         Maximum:      281.000

               0 H 000000011112222222233344445667888999
               1   011123334567779
               2 M 1344558899
               3   011335569
               4 H 00124679
               5   029
               6   177799
               7   1
               8   45
               9   9
              10   8
              11   012
      * * * Outside Values * * *
              11   8
              15   9
              18   4
              21   02
              23   6
              24   6
              28   1
161 cases with missing values excluded from plot.



Effects coding used for categorical variables in model.

Categorical values encountered during processing are:
CYCLO$ (4 levels)
   A, P, R, T
161 case(s) deleted due to missing data.

Dep Var: WICI100   N: 104   Multiple R: 0.195   Squared multiple R: 0.038


                           Analysis of Variance

Source             Sum-of-Squares   df  Mean-Square     F-ratio       P

CYCLO$                   2505.697     3      835.232       1.322       0.272

Error                   63187.188   100      631.872

-------------------------------------------------------------------------------
```




```
Durbin-Watson D Statistic     2.173
First Order Autocorrelation  -0.096
COL/
ROW CYCLO$
  1  A
  2  P
  3  R
  4  T
Using least squares means.
Post Hoc test of WICI100
```

```
Using model MSE of 631.872 with 100 df.
Matrix of pairwise mean differences:
```

	1	2	3	4
1	0.000			
2	5.607	0.000		
3	10.195	4.588	0.000	
4	-4.312	-9.919	-14.507	0.000

Tukey HSD Multiple Comparisons.
Matrix of pairwise comparison probabilities:

	Absent	> 0	>30	>60
	1	2	3	4
1	1.000			
2	0.945	1.000		
3	0.796	0.902	1.000	
4	0.979	0.401	0.260	1.000

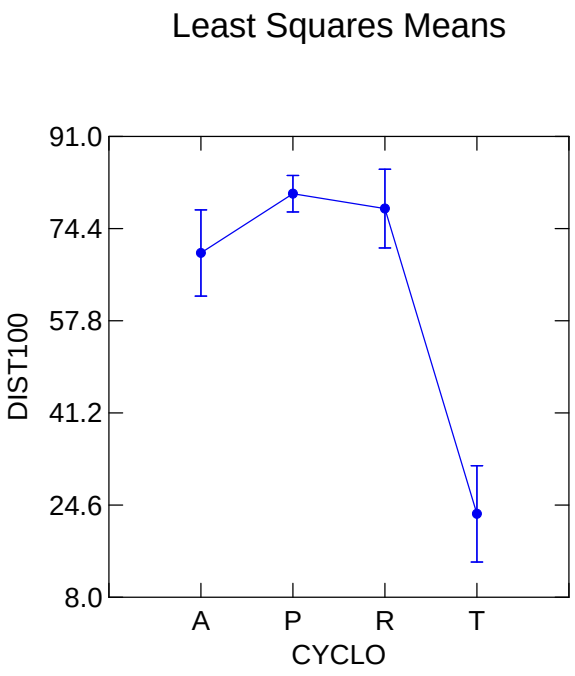
Effects coding used for categorical variables in model.

Categorical values encountered during processing are:
CYCLO$ (4 levels)
A, P, R, T
222 case(s) deleted due to missing data.

Dep Var: DIST100 N: 43 Multiple R: 0.709 Squared multiple R: 0.502

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
CYCLO$	11824.963	3	3941.654	13.110	0.000
Error	11726.107	39	300.669		



```
Durbin-Watson D Statistic     2.131
First Order Autocorrelation  -0.109
COL/
ROW CYCLO$
  1  A
  2  P
  3  R
  4  T
Using least squares means.
Post Hoc test of DIST100


--------------------------------------------------------------------------------

Using model MSE of 300.669 with 39 df.
Matrix of pairwise mean differences:

                          1           2           3           4
              1         0.000
              2        10.679       0.000
              3         8.000      -2.679       0.000
              4       -47.000     -57.679     -55.000       0.000

Tukey HSD Multiple Comparisons.
Matrix of pairwise comparison probabilities:
                       Absent       > 0         >30         >60
                          1           2           3           4
              1         1.000
              2         0.588       1.000
              3         0.871       0.986       1.000
              4         0.001       0.000       0.000       1.000
--------------------------------------------------------------------------------
```

Decision criteria: This is one of the few organism groups that show a very strong relationship with the disturbance scale (based on ORAM 5.0), for densities greater than 60. A relationship with the invertebrate index, WICI, does not show as strong a relationship, but the median value for densities greater than 60 is used for a metric value. It may be that the cyclopoid copepods as a group should be identified to species and then reanalyzed at a later time.

The output shows that there is a significant difference among density groups with different levels of DIST scores at least at .05 probability level.

The output for pairwise comparisons include a table of mean differences and another table of probabilities. To determine significant differences, examine the pairs and their probability level. From the output it is evident that there is a significant difference between group 1 (absent/present) and density levels > 60.

	WICI100
	> 60
N of cases	22
Median	40.000
Mean	46.545

CRAYFISH ANALYSES

Total Crayfish

```
Stem and Leaf Plot of variable:      TOTCRAY, N = 265
         Minimum:        0.000
         Lower hinge:        0.000
         Median:         0.000
         Upper hinge:        3.000
         Maximum:      145.000

               0 M 0000000000000000000000000000000000000000000000000000000000000*
               1   0000000000000000000000000
               2   000000000000000
               3 H 00000000000
               4   000000000
               5   00000
               6   00000
               7   0000
      * * * Outside Values * * *
               8   0
               9   000000
              10   00000
              12   000
              13   000
              14   0
              15   00
              17   0
              18   0
              19   0
              20   0
              22   0
              23   00
              25   0
              32   0
              40   0
              42   0
              48   0
              55   0
              60   0
              61   0
              75   0
              96   0
             145   0
```

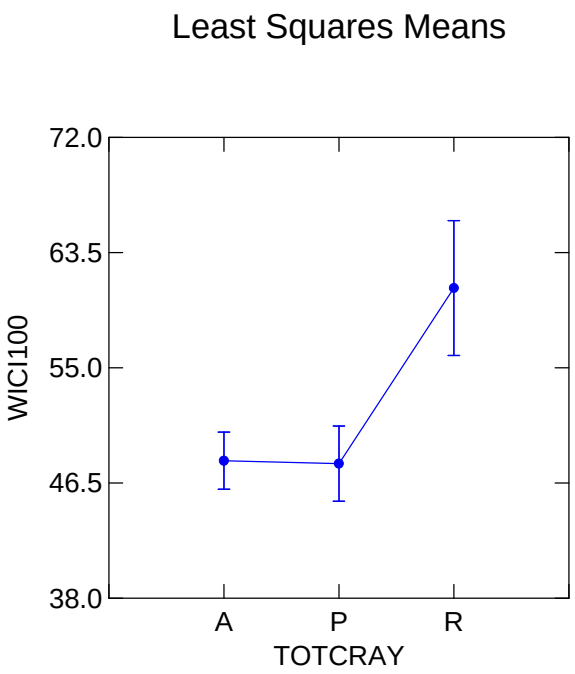
Effects coding used for categorical variables in model.

Categorical values encountered during processing are:
TOTCRAY$ (3 levels)
   A, P, R

Dep Var: WICI100   N: 265   Multiple R: 0.149   Squared multiple R: 0.022

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
TOTCRAY$	3988.968	2	1994.484	2.981	0.052
Error	175311.048	262	669.126		




```
Durbin-Watson D Statistic     1.754
First Order Autocorrelation   0.120
ROW TOTCRAY$
  1  A
  2  P
  3  R>10
Using least squares means.
Post Hoc test of WICI100
```

```
Using model MSE of 669.126 with 262 df.
Matrix of pairwise mean differences:
                          1           2           3
              1         0.000
              2        -0.215       0.000
              3        12.743      12.958       0.000

Tukey HSD Multiple Comparisons.
Matrix of pairwise comparison probabilities:
                        Absent       > 0        ≥ 10
                          1           2           3
              1         1.000
              2         0.998       1.000
              3         0.048       0.060       1.000
```

The output shows that there is a significant difference among density groups with different levels of WICI scores at least at .05 probability level.

The output for pairwise comparisons include a table of mean differences and another table of probabilities. To determine significant differences, examine the pairs and their probability level. From the output it is evident that there is a significant difference between group 1 (absent) and density levels $\geq$ 10.

	WICI100>=10	DIST100>=10
N of cases	30	29
Minimum	6.000	6.000
Maximum	100.000	96.000
Median	**60.000**	80.000
Mean	60.933	71.310
Standard Dev	24.628	27.802

MAYFLY ANALYSES

Tolerant Mayflies
- *Caenis* sp. and *Callibaetis* sp.

```
Stem and Leaf Plot of variable:       TOLMAY, N = 265
         Minimum:        0.000
         Lower hinge:        0.000
         Median:         0.000
         Upper hinge:        2.000
         Maximum:      450.000

               0 M 0000000000000000000000000000000000000000000000000000000000000*
               1   00000000000000000000000
               2 H 000000
               3   000000
               4   0000
               5   000
      * * * Outside Values * * *
               6   000
               7   0000
               8   000
               9   00000
              10   0
              11   0
              12   000
              13   0
              14   0
              15   000
              16   0
              18   00
              20   0
              21   0
              22   0
              23   0
              24   000
              26   0
              27   0
              30   0
              42   0
              49   0
              51   0
              53   0
              69   0
              70   0
              81   0
             115   0
             137   0
             144   0
             152   00
             231   0
             450   0
```

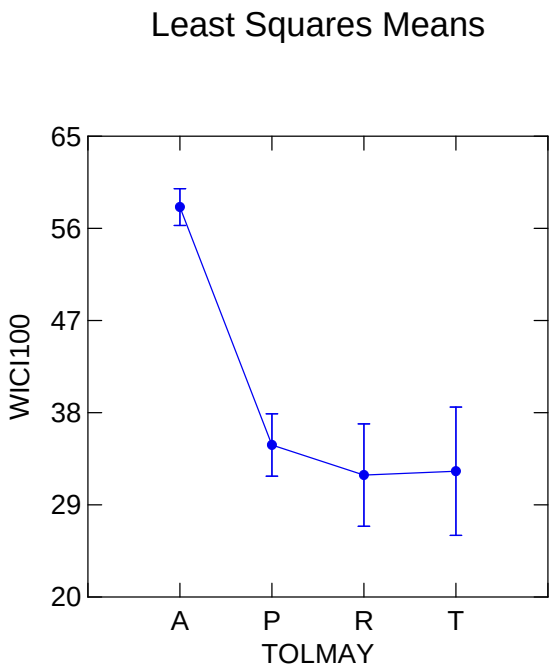
Effects coding used for categorical variables in model.

Categorical values encountered during processing are:
TOLMAY$ (4 levels)
   A, P, R, T

Dep Var: WICI100   N: 265   Multiple R: 0.449   Squared multiple R: 0.202

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
TOLMAY$	36140.866	3	12046.955	21.963	0.000
Error	143159.150	261	548.502		



Durbin-Watson D Statistic   1.690
First Order Autocorrelation   0.150
COL/
ROW TOLMAY$
  1 A absent
  2 P>0
  3 R>10
  4 T>30
Using least squares means.
Post Hoc test of WICI100

```
------------------------------------------------------------------------------

Using model MSE of 548.502 with 261 df.
Matrix of pairwise mean differences:

                        1           2           3           4
             1        0.000
             2      -23.235       0.000
             3      -26.173      -2.938       0.000
             4      -25.797      -2.562       0.377       0.000

Tukey HSD Multiple Comparisons.
Matrix of pairwise comparison probabilities:

                        1           2           3           4
             1        1.000
             2        0.000       1.000
             3        0.000       0.959       1.000
             4        0.000       0.983       1.000       1.000
```

The output shows that there is a significant difference among density groups with different levels of WICI scores at least at .05 probability level.

The output for pairwise comparisons includes a table of mean differences and another table of probabilities. To determine significant differences, examine the pairs and their probability level. From the output it is evident that there is a significant difference between group 1 (absent) and and all other density levels > 0.

	1996-2006 n=265			
	Absent WICI100	Absent DIST100	Present WICI100	Present DICI100
N of cases	170	130	95	50
Minimum				
Maximum				
Median	80	73	**32**	46
Mean	58.1	67.4	33.8	46.8
Standard Dev				

CORIXID ANALYSES

Corixids
- All corixids except the genus *Hesperocorixa*

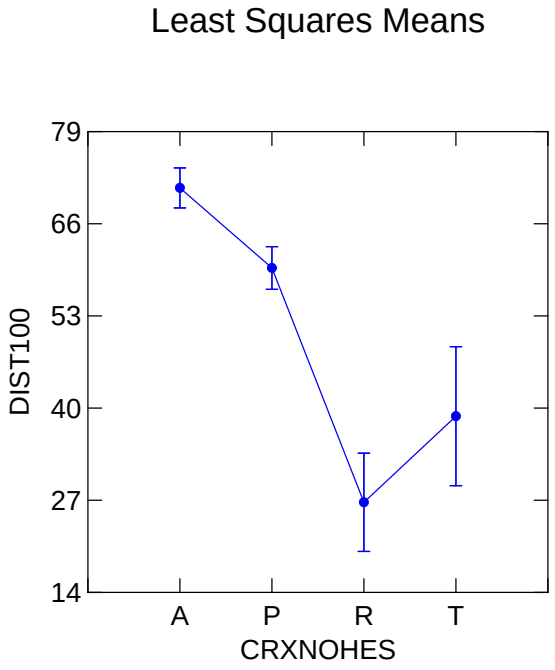
```
Stem and Leaf Plot of variable:     CRXNOHES, N = 265
         Minimum:        0.000
         Lower hinge:        0.000
         Median:         1.000
         Upper hinge:       11.000
         Maximum:    72175.000

               0 M 00000000000000000000000000000000000000000000000000000000000*
               0   22222222222222222222233333333333
               0   44444455555
               0   66667
               0   88888999999
               1 H 0000111
               1   2222233
               1   444455
               1   67
               1   889
               2   1
               2   2
               2   5
               2   6667
      * * * Outside Values * * *
               2   8
               3   0112388
               4   14
               5   0035
               6   268
               9   07
              10   08
              13   3
              15   1
              16   46
              19   8
              20   2
              23   2
              28   5
              54   2
              55   3
              59   3
              72   99
             103   8
             105   0
             184   0
             317   4
             949   9
            7217   5
```

Effects coding used for categorical variables in model.
Categorical values encountered during processing are:
CRXNOHES$ (4 levels)
   A, P, R, T
85 case(s) deleted due to missing data.

Dep Var: DIST100   N: 180   Multiple R: 0.440   Squared multiple R: 0.194

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
CRXNOHES$	28509.035	3	9503.012	14.120	0.000
Error	118449.515	176	673.009		



Durbin-Watson D Statistic   1.872
First Order Autocorrelation   0.064
COL/
ROW CRXNOHES$
  1 A
  2 P
  3 R
  4 T

Using least squares means.
Post Hoc test of DIST100

Using model MSE of 673.009 with 176 df.
Matrix of pairwise mean differences:

	1	2	3	4
1	0.000			
2	-11.289	0.000		
3	-44.345	-33.056	0.000	
4	-32.202	-20.913	12.143	0.000

Tukey HSD Multiple Comparisons.
Matrix of pairwise comparison probabilities:

	1	2	3	4
1	1.000			
2	0.032	1.000		
3	0.000	0.000	1.000	
4	0.009	0.174	0.743	1.000

Effects coding used for categorical variables in model.

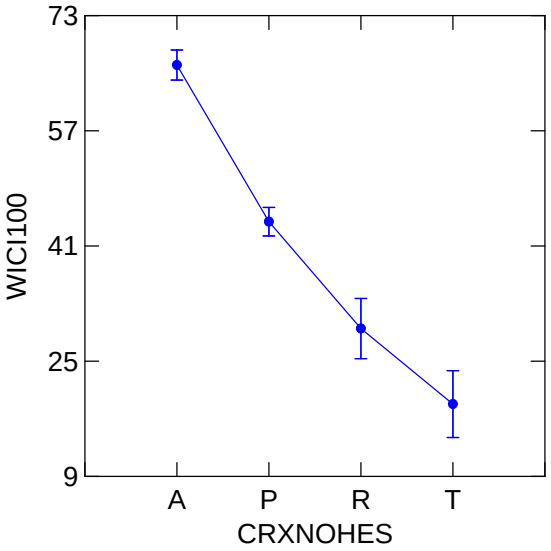
Categorical values encountered during processing are:
CRXNOHES$ (4 levels)
   A, P, R, T

Dep Var: WICI100   N: 265   Multiple R: 0.585   Squared multiple R: 0.342

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
CRXNOHES$	61347.339	3	20449.113	45.249	0.000
Error	117952.676	261	451.926		

Least Squares Means




```
Durbin-Watson D Statistic     2.012
First Order Autocorrelation  -0.009
COL/
ROW CRXNOHES$
  1  A absent
  2  P > 0
  3  R ≥ 20
  4  T ≥ 100
Using least squares means.
Post Hoc test of WICI100
```

```
Using model MSE of 451.926 with 261 df.
Matrix of pairwise mean differences:
                          Absent      > 0          ≥ 20         ≥ 100
                          1           2            3            4
              1           0.000
              2         -21.753       0.000
              3         -36.597     -14.844        0.000
              4         -47.088     -25.335      -10.491        0.000
```

```
Tukey HSD Multiple Comparisons.
Matrix of pairwise comparison probabilities:

                    1           2           3           4
          1         1.000
          2         0.000       1.000
          3         0.000       0.007       1.000
          4         0.000       0.000       0.333       1.000
--------------------------------------------------------------------------------
```

The output shows that there is a significant difference among density groups with different levels of WICI scores at least at .05 probability level.

The output for pairwise comparisons include a table of mean differences and another table of probabilities. To determine significant differences, examine the pairs and their probability level. From the output it is evident that there is a significant difference between group 1 (absent) and density levels $\geq$ 20.

	WICI100	
	>=20	>=100
n	27	21
median	**28**	**20**
avg	29.6	19.0

NOTONECTA ANALYSES

Notonecta

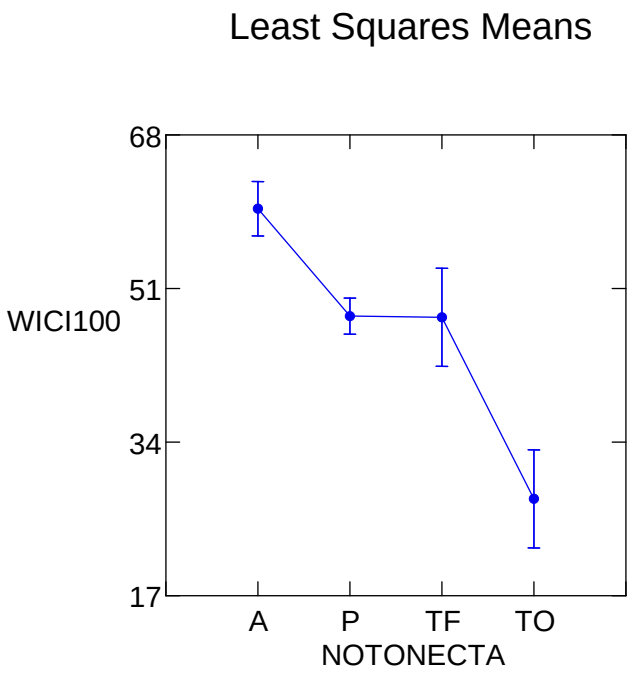
Previous analyses (n=228) wici..t>20 was used. New updated analyses (n=265) at end shows to use only >40 (median 26) for index, … but could still use >20 (median = 46) for demo sheet since many of the >40 groups includes juveniles that would not normally be recognized by untrained personnel or without use of scope onsight.

```
Stem and Leaf Plot of variable:         NOTO, N = 265
         Minimum:        0.000
         Lower hinge:        0.000
         Median:         4.000
         Upper hinge:       13.000
         Maximum:      178.000

               0 H 000000000000000000000000000000000000000000000000000000000000*
               0   22222222222222233333333333333
               0 M 444444444444445555555555
               0   6666666777
               0   8888889999
               1   000000000000000011111111
               1 H 2233
               1   44444445
               1   667777777777
               1   88
               2   00111
               2   233
               2   55
               2   6677
               2   88
               3   1111
               3   2
     * * * Outside Values * * *
               3   34
               4   257
               5   44588
               6   2
               8   39
               9   47
              12   8
              14   239
              15   46
              16   8
              17   8
```

Dep Var: WICI100 N: 265 Multiple R: 0.314 Squared multiple R: 0.098

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
NOTOTEST$	17649.254	3	5883.085	9.499	0.000
Error	161650.761	261	619.352		




```
Durbin-Watson D Statistic     2.058
First Order Autocorrelation  -0.029
COL/
ROW NOTOTEST$
  1  A
  2  P
  3  TF>20
  4  TO>40
Using least squares means.
Post Hoc test of WICI100
-------------------------------------------------------------------------------

Using model MSE of 619.352 with 261 df.
Matrix of pairwise mean differences:

                    1           2           3           4
          1         0.000
          2       -11.888       0.000
          3       -12.014      -0.126       0.000
          4       -32.109     -20.221     -20.095       0.000

Tukey HSD Multiple Comparisons.
Matrix of pairwise comparison probabilities:

                    1           2           3           4
          1         1.000
          2         0.006       1.000
          3         0.214       1.000       1.000
          4         0.000       0.003       0.044       1.000
-------------------------------------------------------------------------------
```

The output shows that there is a significant difference among density groups with different levels of WICI scores at least at .05 probability level.

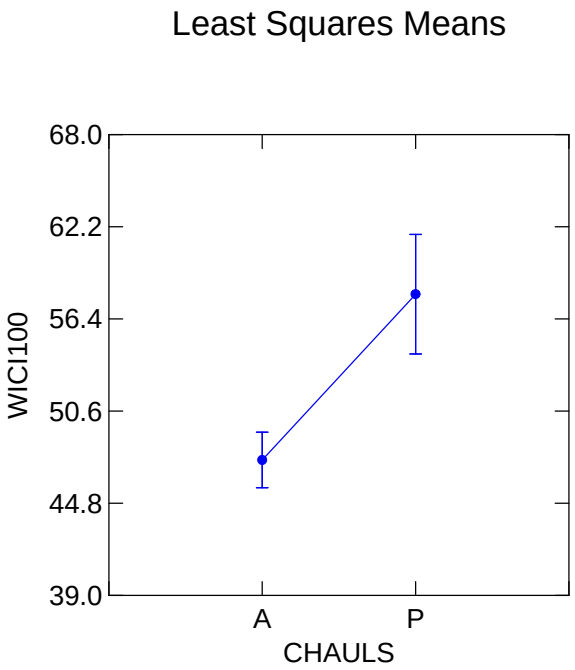
The output for pairwise comparisons include a table of mean differences and another table of probabilities. To determine significant differences, examine the pairs and their probability level. From the output it is evident that there is a significant difference between group 1 (absent) and density levels > 40.

	WICI100
N of cases	21
Median	**26.000**
Mean	27.714

MEGALOPTERA ANALYSES

Chauliodes sp.only

```
Stem and Leaf Plot of variable:       CHAULS, N = 265
         Minimum:        0.000
         Lower hinge:        0.000
         Median:         0.000
         Upper hinge:        0.000
         Maximum:       17.000

               0 M 0000000000000000000000000000000000000000000000000000000000000*
               0   11111111111111111111
                0   222222233
               0   444555555
               0   66
               0   88889
               1   0
               1
               1
               1  7

Categorical values encountered during processing are:
CHAULS$ (2 levels)
   A, P

Dep Var: WICI100   N: 265   Multiple R: 0.153   Squared multiple R: 0.023


                            Analysis of Variance

Source              Sum-of-Squares   df  Mean-Square     F-ratio       P

CHAULS$                  4209.715     1     4209.715       6.323       0.013

Error                  175090.300   263      665.743

-------------------------------------------------------------------------------
```




```
Durbin-Watson D Statistic     1.763
First Order Autocorrelation   0.117
COL/
ROW CHAULS$
  1  A
  2  P
Using least squares means.
Post Hoc test of WICI100
-------------------------------------------------------------------------------

Using model MSE of 665.743 with 263 df.
Matrix of pairwise mean differences:
                          1           2
              1         0.000
              2        10.435       0.000

Tukey HSD Multiple Comparisons.
Matrix of pairwise comparison probabilities:

                          1           2
              1         1.000
              2         0.012       1.000
-------------------------------------------------------------------------------
```

The output shows that there is a significant difference among density groups with different levels of WICI scores at least at .05 probability level.

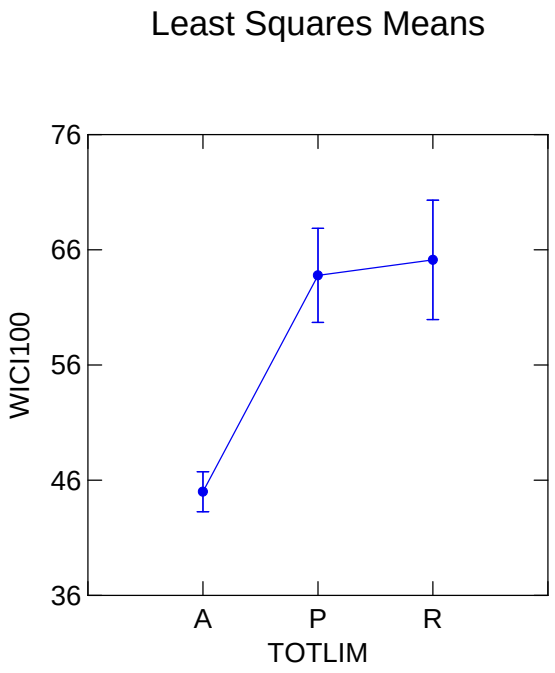
The output for pairwise comparisons includes a table of mean differences and another table of probabilities. To determine significant differences, examine the pairs and their probability level. From the output it is evident that there is a significant difference between group 1 (absent) and and all other density levels > 0.

WICI100 (47 pts)		DIST100 (35 pts – no mitigation)	
Avg.	Median	Avg.	Median
58	**62**	66	75

CADDISFLY ANALYSES

Limnephilids only

```
Stem and Leaf Plot of variable:       TOTLIM, N = 265
         Minimum:        0.000
         Lower hinge:        0.000
         Median:         0.000
         Upper hinge:        0.000
         Maximum:       99.000

               0 M 000000000000000000000000000000000000000000000000000000000000*
               0   5578899
               1   000124
               1   7
               2   013
               2   88
               3   23
               3   59
               4   0223
               4
               5   014
               5
               6
               6   9
               7
               7
               8
               8
               9   2
               9   9



Effects coding used for categorical variables in model.

Categorical values encountered during processing are:
TOTLIM$ (3 levels)
   A, P, R

Dep Var: WICI100   N: 265   Multiple R: 0.311   Squared multiple R: 0.097


                           Analysis of Variance

Source             Sum-of-Squares   df  Mean-Square     F-ratio       P

TOTLIM$                17306.141     2     8653.071      13.995       0.000

Error                 161993.874   262      618.297

-------------------------------------------------------------------------------
```




```
Durbin-Watson D Statistic     1.565
First Order Autocorrelation   0.217
COL/
ROW TOTLIM$
  1  A  ABSENT
  2  P > 0
  3  R > 10
Using least squares means.
Post Hoc test of WICI100
```

```
Using model MSE of 618.297 with 262 df.
Matrix of pairwise mean differences:

                    1           2           3
             1      0.000
             2     18.779       0.000
             3     20.126       1.347       0.000
```

```
Tukey HSD Multiple Comparisons.
Matrix of pairwise comparison probabilities:
                        Absent      > 0          > 10
                         1           2           3
              1          1.000
              2          0.000       1.000
              3          0.001       0.977       1.000
--------------------------------------------------------------------------------
```

The output shows that there is a significant difference among density groups with different levels of WICI scores at least at .05 probability level.

The output for pairwise comparisons includes a table of mean differences and another table of probabilities. To determine significant differences, examine the pairs and their probability level. From the output it is evident that there is a significant difference between group 1 (absent) and and all other density levels > 0.

	VAR00355
N of cases	60
Median	66.000
Mean	64.300

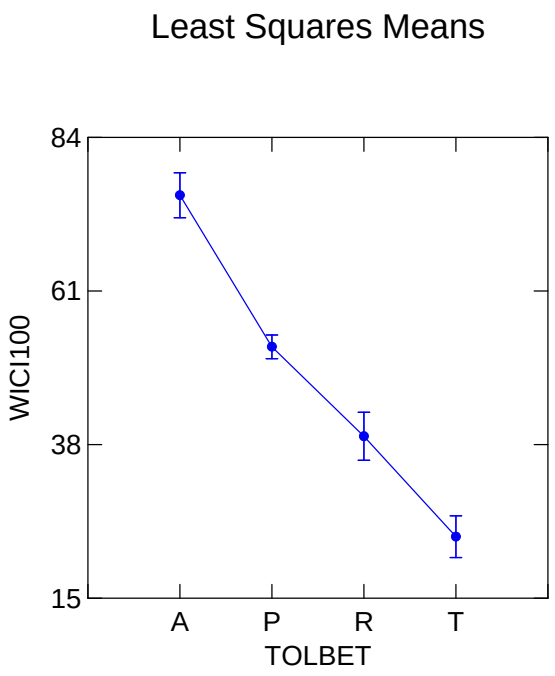
COLEOPTERA ANALYSES

Tolerant Beetles

- Total number of *Haliplus* sp., *Peltodytes* sp., *Berosus* sp., and *Tropisternus* sp.

```
Stem and Leaf Plot of variable:       TOLBET, N = 265
         Minimum:        0.000
         Lower hinge:        2.000
         Median:        10.000
         Upper hinge:       44.000
         Maximum:      308.000

               0 H 00000000000000000000000000000000000000000011111111111111111*
               0   55555555555555666666667778888999
               1 M 000000111112334
               1   55566666788999
               2   0111122333334
               2   556777778
               3   000001222233
               3   555889
               4 H 344
               4   5579
               5   001112333
               5   667788
               6   224444
               6   67899
               7   1
               7   788
               8   24
               8   788
               9   002
               9   567
              10   023
              10   6
     * * * Outside Values * * *
              11   11699
              12   9
              13   4
              14   2
              15   8
              17   0
              20   028
              27   29
              30   8
```

```
Effects coding used for categorical variables in model.

Categorical values encountered during processing are:
Tolerant Beetles (4 levels)
   A, P, R, T

Dep Var: WICI100   N: 265   Multiple R: 0.584   Squared multiple R: 0.342

                          Analysis of Variance

Source             Sum-of-Squares   df  Mean-Square     F-ratio       P

TOLBET$               61231.764     3    20410.588      45.119       0.000

Error                118068.251   261      452.369
```




```
Durbin-Watson D Statistic     1.778
First Order Autocorrelation   0.109
COL/
ROW TOLBET$
  1  A  absent
  2  P > 0
  3  R > 30
  4  T > 60
Using least squares means.
Post Hoc test of WICI100
```

```
Using model MSE of 452.369 with 261 df.
Matrix of pairwise mean differences:

                1           2           3           4
 1          0.000
 2        -22.697       0.000
 3        -36.093     -13.396       0.000
 4        -51.133     -28.435     -15.040       0.000
```

Tukey HSD Multiple Comparisons.
Matrix of pairwise comparison probabilities:

	Absent	> 0	> 30	> 60
	1	2	3	4
1	1.000			
2	0.000	1.000		
3	0.000	0.005	1.000	
4	0.000	0.000	0.009	1.000

The output shows that there is a significant difference among density groups with different levels of WICI scores at least at .05 probability level.

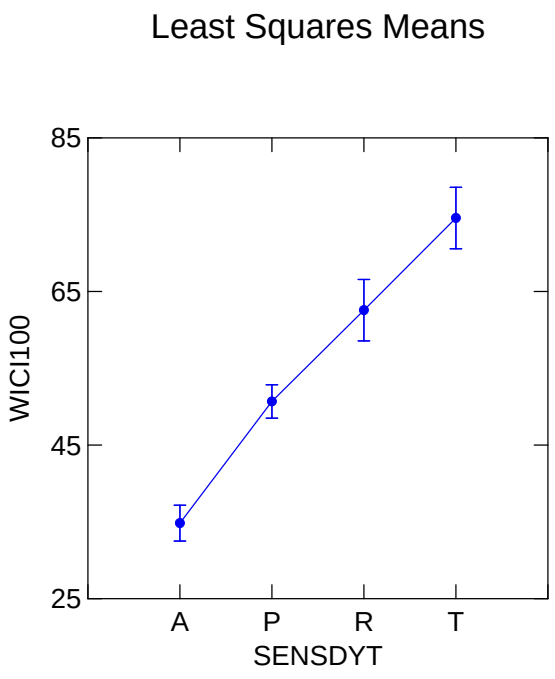
The output for pairwise comparisons include a table of mean differences and another table of probabilities. To determine significant differences, examine the pairs and their probability level. From the output it is evident that there is a significant difference between group 1 (absent) and density levels $\geq$ 30.

	DICI 100	
	> 30	> 60
N of cases	35	46
Median	**40**.000	**26**.000
Mean	39.257	24.217

Sensitive Dytiscids

- Total number of *Acilius* sp., *Cybister* sp. and *Dytiscus* sp.

```
SENSDYT$ (4 levels)
   A, P, R, T

Dep Var: WICI100   N: 265   Multiple R: 0.506   Squared multiple R: 0.256

                          Analysis of Variance

Source              Sum-of-Squares   df  Mean-Square     F-ratio       P

SENSDYT$                 45933.437    3    15311.146      29.964       0.000

Error                   133366.578  261      510.983

-------------------------------------------------------------------------------
```




```
Durbin-Watson D Statistic     1.883
   First Order Autocorrelation   0.058

COL/
ROW SENSDYT$
```

```
  1  A - absent
  2  P – > 0
  3  R - ≥ 10
  4  T - > 20
Using least squares means.
Post Hoc test of WICI100


-------------------------------------------------------------------------------

Using model MSE of 510.983 with 261 df.
Matrix of pairwise mean differences:

                        1           2           3           4
             1          0.000
             2         15.843       0.000
             3         27.733      11.890       0.000
             4         39.733      23.890      12.000       0.000

Tukey HSD Multiple Comparisons.
Matrix of pairwise comparison probabilities:

                        1           2           3           4
             1          1.000
             2          0.000       1.000
             3          0.000       0.045       1.000
             4          0.000       0.000       0.146       1.000
-------------------------------------------------------------------------------
```

The output shows that there is a significant difference among density groups with different levels of WICI scores at least at .05 probability level.

The output for pairwise comparisons include a table of mean differences and another table of probabilities. To determine significant differences, examine the pairs and their probability level. From the output it is evident that there is a significant difference between group 1 (absent) and density levels ≥ 10.

	Density ≥ 10		Density > 20	
	WICI100	DIST100	WICI100	DIST100
N of cases (total =228)	25	24	29	28
Minimum	26.000	6.000	26.000	6.000
Maximum	100.000	96.000	100.000	96.000
Median	62.000	80.500	**74.000**	83.500
Mean	60.880	73.792	74.621	78.143
Standard Dev	23.145	22.292	18.001	21.355

	Density ≥ 10	Density > 20
	WICI100	WICI100
N of cases (total =265)	32	32
Median	**64**	76
Mean	62.6	74.6

Decision criteria. Since adding 28% more sites (7 to 25) increases both the median and average by 2 use 64 for >= 10, But since adding only 10% (3 to 29) increases the median by two but leaves the average unchanged stay with the old value of 74 for > 20.

Tolerant Dytiscids

- Total of *Hygrotus* sp. and *Laccophilus* sp.

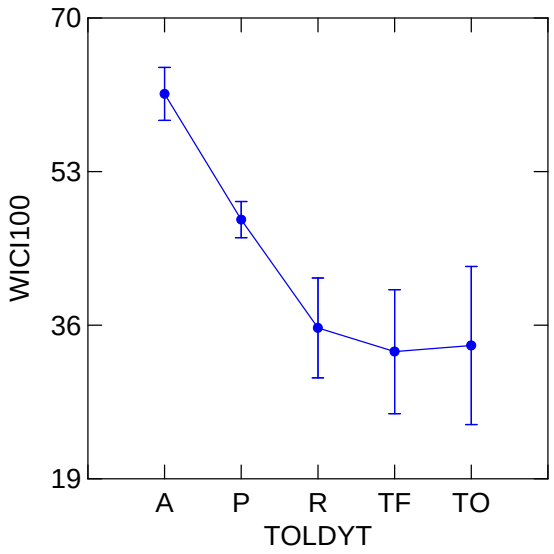
TOLDYT$ (5 levels)
   A, P, R, TF, TO

Dep Var: WICI100   N: 265   Multiple R: 0.336   Squared multiple R: 0.113

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
TOLDYT$	20203.493	4	5050.873	8.254	0.000
Error	159096.522	260	611.910		

Least Squares Means



Durbin-Watson D Statistic 1.465
First Order Autocorrelation 0.262
COL/
ROW TOLDYT$
  1 A - absent
  2 P - >0
  3 R - ≥ 20
  4 TF - > 40
  5 TO - ≥ 70
Using least squares means.
Post Hoc test of WICI100

```
-------------------------------------------------------------------------------

Using model MSE of 611.910 with 260 df.
Matrix of pairwise mean differences:

                        1           2           3           4           5
             1        0.000
             2      -13.919       0.000
             3      -25.906     -11.986       0.000
             4      -28.529     -14.609      -2.623       0.000
             5      -27.856     -13.936      -1.950       0.673       0.000

Tukey HSD Multiple Comparisons.
Matrix of pairwise comparison probabilities:
                        Absent      > 0         ≥ 20        > 40        ≥ 70
                        1           2           3           4           5
             1        1.000
             2        0.001       1.000
             3        0.000       0.248       1.000
             4        0.001       0.245       0.998       1.000
             5        0.021       0.528       1.000       1.000       1.000
-------------------------------------------------------------------------------
```

The output shows that there is a significant difference among groups with different levels of WICI scores at least at .05 probability level.

The output for pairwise comparisons include a table of mean differences and another table of probabilities. To determine significant differences, examine the pairs and their probability level. From the output it is evident that there is a significant difference between group 1 (absent) and the rest of the density groups). Since there is no apparent difference between groups greater than and equal to 20, they were combined into one group and the median used for a metric value.

	Density ≥ 20	
	WICI100	DIST100
N of cases	41	22
Minimum	0.000	0.000
Maximum	86.000	96.000
Median	**32.000**	46.000
Mean	34.488	43.818
Standard Dev	19.879	28.011

Machlonyx - note present at only Shrub and Forest sites

```
Stem and Leaf Plot of variable:        MACHL, N = 265
         Minimum:        0.000
         Lower hinge:        0.000
         Median:         0.000
         Upper hinge:        0.000
         Maximum:      298.000

               0 M 00000000000000000000000000000000000000000000000000000000000*
               0   2223
               0   44
               0
               0   9
               1   0
               1   3
               1
               1
               1
               2   0
               2   3
               2
               2
               2   89
```

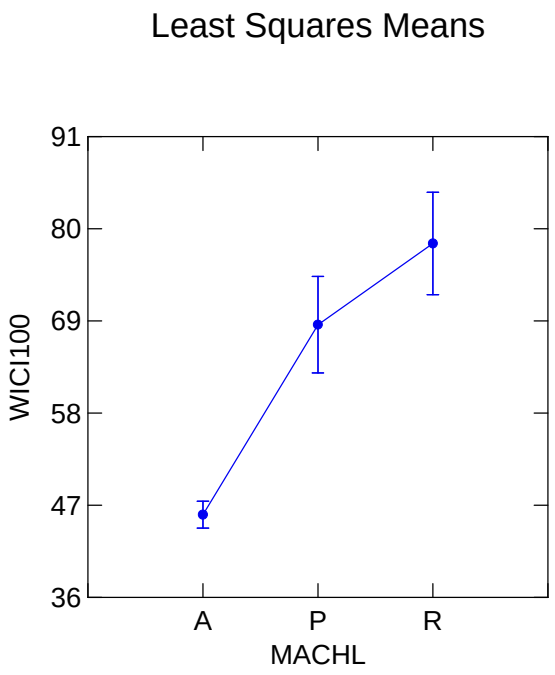
Effects coding used for categorical variables in model.

Categorical values encountered during processing are:
TOTLIM$ (3 levels)
   A, P, R
MACHL$ (3 levels)
   A, P, R

Dep Var: WICI100   N: 265   Multiple R: 0.356   Squared multiple R: 0.127

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
MACHL$	22785.965	2	11392.982	19.072	0.000
Error	156514.051	262	597.382		




```
Durbin-Watson D Statistic     1.586
First Order Autocorrelation   0.207
COL/
ROW MACHL$
  1  A
  2  P
  3  R
Using least squares means.
Post Hoc test of WICI100
```

```
Using model MSE of 597.382 with 262 df.
Matrix of pairwise mean differences:

                1           2           3
1           0.000
2          22.677       0.000
3          32.371       9.694       0.000
```

Tukey HSD Multiple Comparisons.
Matrix of pairwise comparison probabilities:

	1	2	3
1	1.000		
2	0.000	1.000	
3	0.000	0.481	1.000

The output shows that there is a significant difference among density groups with different levels of WICI scores at least at .05 probability level.

The output for pairwise comparisons includes a table of mean differences and another table of probabilities. To determine significant differences, examine the pairs and their probability level. From the output it is evident that there is a significant difference between group 1 (absent) and and all other density levels > 0.

	VAR00354
N of cases	34
Minimum	32.000
Maximum	100.000
Median	75.000
Mean	73.118
Standard Dev	15.948

Present in 34 of 265 records, median is avg of 74 & 76, avg is73.1, assign same metric value of 74 as earlier analyses with only n=225 records.

SNAIL ANALYSES

Sensitive Snails

- *Aplexa* sp., *Planorbella armigua*, *promenetus* sp.

```
Stem and Leaf Plot of variable:       SENSNL, N = 265
         Minimum:        0.000
         Lower hinge:        0.000
         Median:         0.000
         Upper hinge:        4.000
         Maximum:      321.000

               0 M 0000000000000000000000000000000000000000000000000000000000*
               1   0000000000000001
               2   000000
               3   00
               4 H 00000000
               5   00000
               6   0000
               7   00000
               8   00
               9   0
              10   0
     * * * Outside Values * * *
              11   0
              16   00
              17   0
              18   00
              19   0
              20   0
              21   00
              22   00
              23   0
              25   0
              26   0
              28   0
              29   0
              33   0
              35   0
              44   0
              47   0
              49   00
              59   0
              60   0
              62   0
              66   0
              67   0
              68   00
              69   00
              72   0
              83   0
             100   0
             109   0
             137   00
             144   0
             175   0
             182   0
             200   0
             234   0
             235   0
             254   0
             321   0
```

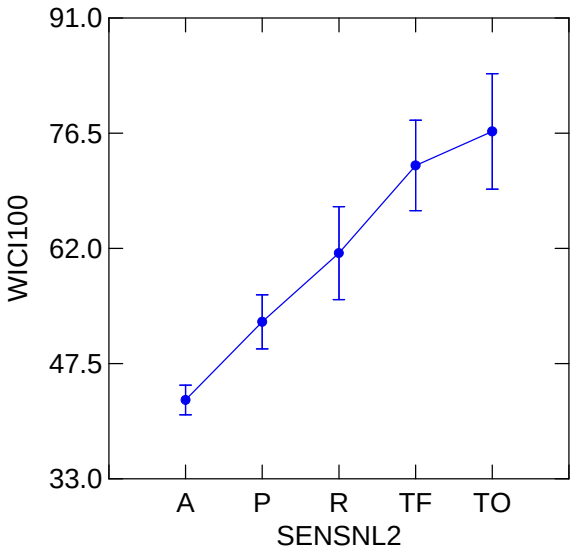
SENSNL2$ (5 levels)
   A, P, R, TF, TO

Dep Var: WICI100   N: 265   Multiple R: 0.394   Squared multiple R: 0.155

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
SENSNL2$	27880.151	4	6970.038	11.968	0.000
Error	151419.864	260	582.384		

Least Squares Means



Durbin-Watson D Statistic 1.651
First Order Autocorrelation 0.174
COL/
ROW SENSNL2$
  1 A
  2 P > 0
  3 R > 10
  4 TF > 30
  5 TO >100
Using least squares means.
Post Hoc test of WICI100

```
--------------------------------------------------------------------------------

Using model MSE of 582.384 with 260 df.
Matrix of pairwise mean differences:

                    1           2           3           4           5
          1         0.000
          2         9.837       0.000
          3        18.489       8.652       0.000
          4        29.521      19.684      11.033       0.000
          5        33.804      23.967      15.316       4.283       0.000

Tukey HSD Multiple Comparisons.
Matrix of pairwise comparison probabilities:

                    1           2           3           4           5
          1         1.000
          2         0.084       1.000
          3         0.022       0.705       1.000
          4         0.000       0.025       0.659       1.000
          5         0.000       0.024       0.472       0.991       1.000
--------------------------------------------------------------------------------
```

The output shows that there is a significant difference among density groups with different levels of WICI scores at least at .05 probability level.

The output for pairwise comparisons include a table of mean differences and another table of probabilities. To determine significant differences, examine the pairs and their probability level. From the output it is evident that there is a significant difference between group 1 (absent) and density levels ≥ 30

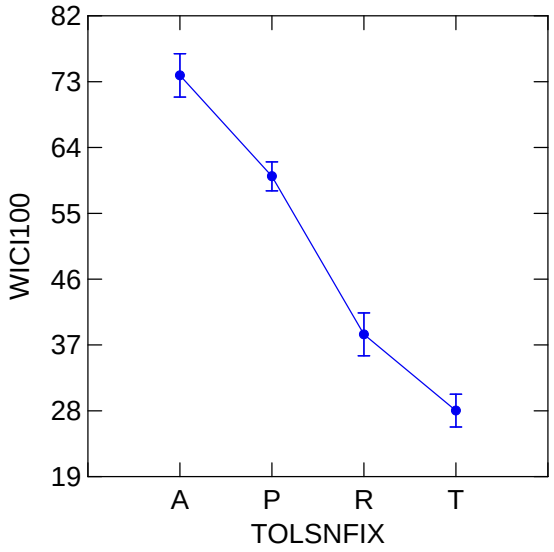
	>10		>30		>100	
	WICI100	DIST100	WICI100	DIST100	WICI100	DIST100
n	17	15	18	15	11	11
MEDIAN	64(54,84)	66	72	75	**80**	81
AVERAGE	**61.4**	70.9	72.4	73.7	76.7	78.5
CRITERIA	62		72		78 **(same as previous)**	

Decision criteria: Based on difference between median and average criteria revised per last line of table above.

Tolerant Snails
- *Gyraulus* sp., *Physella* sp., *Planorbella pilsbrii*

```
Stem and Leaf Plot of variable:     TOLSNFIX, N = 265
         Minimum:        0.000
         Lower hinge:        3.000
         Median:        24.000
         Upper hinge:      115.000
         Maximum:     2058.000

               0 H 00000000000000000000000000000000000000000000000000000000000*
               0 M 22222222222223333333333333
               0   4444445555555
               0   666677777
               0   8888888999
               1 H 00000011111
               1   2222333
               1   4444445
               1   6666677
               1   899
               2   00011
               2   223
               2   45
               2   667
      * * * Outside Values * * *
               2   899
               3   112236
               4   12568
               5   016
               6   0
               7   05
               9   99
              11   2
              13   8
              15   0
              18   1
              19   7
              20   55



Effects coding used for categorical variables in model.

Categorical values encountered during processing are:
SENSNL2$ (5 levels)
   A, P, R, TF, TO
TOLSNFIX$ (4 levels)
   A, P, R, T

Dep Var: WICI100   N: 265   Multiple R: 0.660   Squared multiple R: 0.436


                            Analysis of Variance

Source             Sum-of-Squares   df  Mean-Square     F-ratio       P

TOLSNFIX$              78162.138     3    26054.046      67.236       0.000

Error                 101137.877   261      387.501

-------------------------------------------------------------------------------
```

Least Squares Means




```
Durbin-Watson D Statistic     1.565
First Order Autocorrelation   0.217
COL/
ROW TOLSNFIX$
  1  A
  2  P > 0
  3  R > 30
  4  T > 100

Using least squares means.
Post Hoc test of WICI100
```

```
Using model MSE of 387.501 with 261 df.
Matrix of pairwise mean differences:
```

	1	2	3	4
1	0.000			
2	-13.803	0.000		
3	-35.419	-21.616	0.000	
4	-45.838	-32.035	-10.418	0.000

```
Tukey HSD Multiple Comparisons.
Matrix of pairwise comparison probabilities:
                        Absent        > 0         > 30        > 100
                          1           2           3           4
              1           1.000
              2           0.001       1.000
              3           0.000       0.000       1.000
              4           0.000       0.000       0.025       1.000
--------------------------------------------------------------------------------
```

The output shows that there is a significant difference among density groups with different levels of WICI scores at least at .05 probability level.

The output for pairwise comparisons include a table of mean differences and another table of probabilities. To determine significant differences, examine the pairs and their probability level. From the output it is evident that there is a significant difference between group 1 (absent) and density levels > 30

	WICI	
	>30	>100
n	46	82
median	**36**	**26**
avg	38.3	27.7

Appendix C

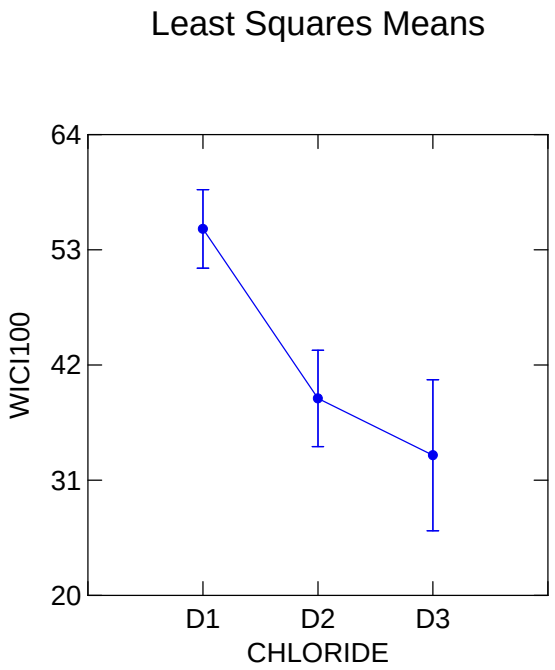
Chloride Analyses

CHLORIDE ANALYSES

The output shows that there is a significant difference among WICI, DIST, and DICI scores with different levels of chloride at least at .05 probability level.

The outputs for pairwise comparisons include a table of mean differences and another table of probabilities. To determine significant differences, examine the pairs and their probability level. From the output it is evident that there is a significant difference between group D1 (≥ 0mg/l, but < 10mg/l) and other concentration levels for the WICI and DICI scores. However, the DIST score detects only a difference between lower concentration groups (D1, D2) and the higher concentration group D3 (≥ 50mg/l)

```
Stem and Leaf Plot of variable:   CHLORIDE_M (mg/l), N = 93
         Minimum:        0.010
         Lower hinge:        2.500
         Median:         9.800
         Upper hinge:       17.300
         Maximum:     1440.000

               0   00
               0 H 22222222222222222222222222222222
               0   55555
               0   67777
               0 M 99
               1   000000111111
               1   22233
               1
               1 H 66677
               1   899
               2   00000
               2   3
               2   4
      * * * Outside Values * * *
               7   999
               8   0
               9   00
              21   66
              22   66
              37   66
             144   0
Categorical values encountered during processing are:
CHLORIDE$ (3 levels)
   D1, D2, D3
172 case(s) deleted due to missing data.

Dep Var: WICI100   N: 93   Multiple R: 0.333   Squared multiple R: 0.111


                            Analysis of Variance

Source              Sum-of-Squares   df  Mean-Square     F-ratio       P

CHLORIDE$                7594.521     2     3797.261       5.612       0.005

Error                   60897.952    90      676.644

------------------------------------------------------------------------------
```




```
Durbin-Watson D Statistic     1.553
First Order Autocorrelation   0.218
COL/
ROW CHLORIDE$
  1  D1 ≥ 0
  2  D2 ≥ 10
  3  D3 ≥ 50
Using least squares means.
Post Hoc test of WICI100
```

```
Using model MSE of 676.644 with 90 df.
Matrix of pairwise mean differences:

                          1           2           3
              1           0.000
              2         -16.187       0.000
              3         -21.615      -5.428       0.000
Tukey HSD Multiple Comparisons.
Matrix of pairwise comparison probabilities:
                          ≥ 0         ≥ 10        ≥ 50
                          1           2           3
              1           1.000
              2           0.021       1.000
              3           0.025       0.802       1.000
```

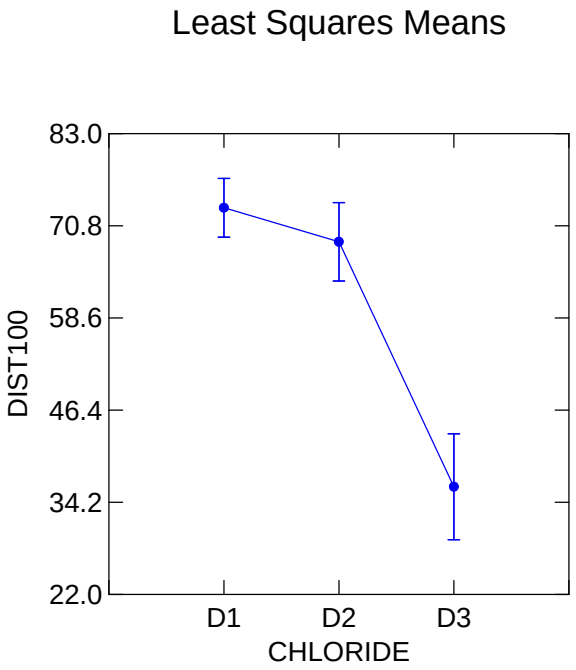
Effects coding used for categorical variables in model.

Categorical values encountered during processing are:
CHLORIDE$ (3 levels)
   D1, D2, D3
192 case(s) deleted due to missing data.

Dep Var: DIST100   N: 73   Multiple R: 0.486   Squared multiple R: 0.236

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
CHLORIDE$	12784.795	2	6392.398	10.814	0.000
Error	41378.766	70	591.125		



Durbin-Watson D Statistic   1.409
First Order Autocorrelation   0.290

COL/
ROW CHLORIDE$
  1 D1 ≥ 0
  2 D2 ≥ 10
  3 D3 ≥ 50
Using least squares means.
Post Hoc test of DIST100

Using model MSE of 591.125 with 70 df.
Matrix of pairwise mean differences:

	1	2	3
1	0.000		
2	-4.498	0.000	
3	-36.929	-32.432	0.000

Tukey HSD Multiple Comparisons.
Matrix of pairwise comparison probabilities:

	≥ 0	≥ 10	≥ 50
	1	2	3
1	1.000		
2	0.768	1.000	
3	0.000	0.001	1.000

Effects coding used for categorical variables in model.

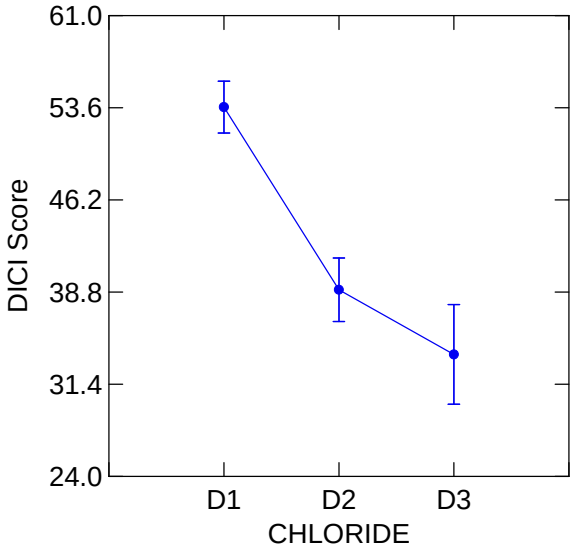
Categorical values encountered during processing are:
CHLORIDE$ (3 levels)
   D1, D2, D3
172 case(s) deleted due to missing data.

Dep Var:DICI   N: 93   Multiple R: 0.502   Squared multiple R: 0.252

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
CHLORIDE$	6318.839	2	3159.419	15.192	0.000
Error	18717.033	90	207.967		

Least Squares Means




```
Durbin-Watson D Statistic     1.777
First Order Autocorrelation   0.100
COL/
ROW CHLORIDE$
  1   D1 ≥ 0
  2   D2 ≥ 10
  3   D3 ≥ 50

Using least squares means.
Post Hoc test of DICI
-------------------------------------------------------------------------------
Using model MSE of 207.967 with 90 df.
Matrix of pairwise mean differences:
                          1           2           3
              1         0.000
              2       -14.665       0.000
              3       -19.859      -5.194       0.000

Tukey HSD Multiple Comparisons.
Matrix of pairwise comparison probabilities:
                          ≥ 0         ≥ 10        ≥ 50
                          1           2           3
              1         1.000
              2         0.000       1.000
              3         0.000       0.520       1.000
-------------------------------------------------------------------------------
```

Decision criteria: Based on the above analyses sites with greater than 50 mg/l chloride were dropped from the invertebrate reference database analyses.

Appendix D

Demo Sheets

Wetland Macroinvertebrate Taxa Groups

Assessment to be used for demonstrational purposes only

Instructions:

Set 10 funnel traps, leaving air in the top of the traps, for 24 hours.

Shake contents into a pan.

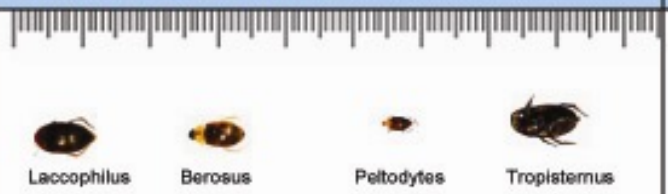
Identify macroinvertebrates which are greater than 3mm with this sheet.

Tally total number of macroinvertebrates which fall into each category for all ten traps.

Score according to guidelines on the back of this sheet.

TOLERANT

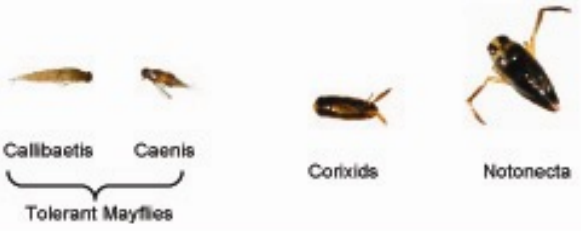
Beetles < 12mm



Tolerant Snails - Physella

NOTE: do not confuse Physella with Aplexa (other snails) which is common in northern Ohio

Other Tolerant Invertebrates

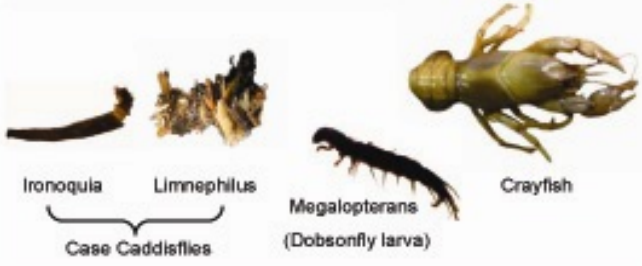


SENSITIVE

Beetles > 12mm

Other Snails

Other Sensitive Invertebrates



Assessment

Tolerant	Sensitive
Beetles < 12mm	**Beetles > 12mm**
Total Beetles<12mm ☐ ≥30…..-1 → Score A (0 or -1) ☐	Total Beetles>12mm ☐ ≥10…..+1 → Score D (0 or +1) ☐
Tolerant Snails - Physella	**Other Snails**
Total Tolerant Snails ☐ ≥30…..-1 → Score B (0 or -1) ☐	Total Other Snails ☐ ≥10…..+1 → Score E (0 or +1) ☐
Other Tolerant Invertebrates	**Other Sensitive Invertebrates**
Total Notonecta ☐ Total Caenis & Callibaetis ☐ Total Corixids ☐ Notonecta ≥20…..-1 Tolerant Mayflies Caenis, Callibaetis Present…-1 Corixids ≥20…..-1 → Score C (0 to -3) ☐	Total Case Caddisflies ☐ Total Crayfish ☐ Total Megalopterans ☐ Case Caddisflies Present…+1 Crayfish ≥5…..+1 Megalopterans-Dobsonfly Present…+1 → Score F (0 to +3) ☐

Score A ☐ + Score B ☐ + Score C ☐ + Score D ☐ + Score E ☐ + Score F ☐ = **Final Score** ☐