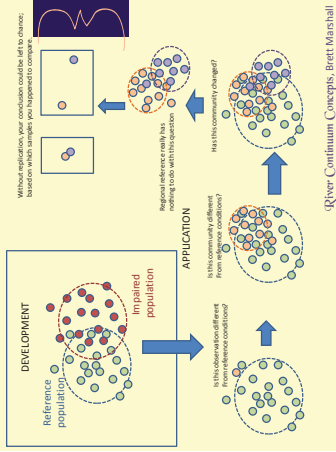




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## Part I: Elements of Design



## Part II: The Myth of Variance Homogeneity

This type of comparison is only statistically or scientifically valid if the within-site variation equals zero, or is sufficiently close to zero to be considered negligible.

- More variation among metric scores within a site than expected...
- Too much within site variation to assume one composite sample can represent the condition of a site....
- Why? This seems counter-intuitive if you understand sampling theory!

A: Because regardless of how much beneficial material you collect in a composite sample, the lab will only find count.

## Part III: The Myth of Taxonomic Completeness

Another common misconception is that composite samples are "more taxonomically complete" than smaller replicate samples... This is untrue... Observe:

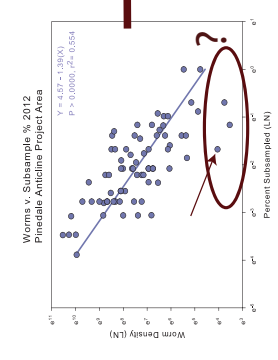
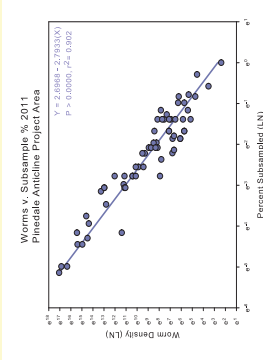
When we electronically "composited" smaller samples, richness was much greater than in the field-composited samples processed at 500-ct. This was true compared to our own field composites at these sites, and true compared to Terra Tech's (Stribling et al., 2000) estimates of richness.

Therefore, it appears that electronically compositing 8 small replicates is more taxonomically representative than field compositing.

## Part IV: Standard-Unit Effort

**What is the Problem?**  
Occasionally, a few species become very abundant. This phenomenon causes some complications with the interpretation of response variables including metrics, biomass, and densities.

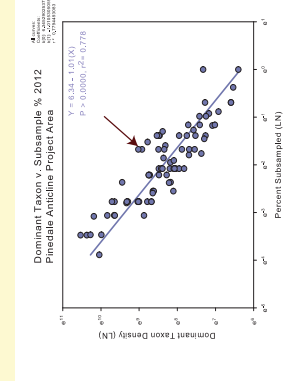
We observed that changes in the density of small worms (*Meis* sp.) influenced the amount of the sample that had to be used in the laboratory to reach the fixed count subsampling target. This was true for 200, 300, and 500 count samples. Closer examination indicated that the relationship was quantifiable and statistically significant:



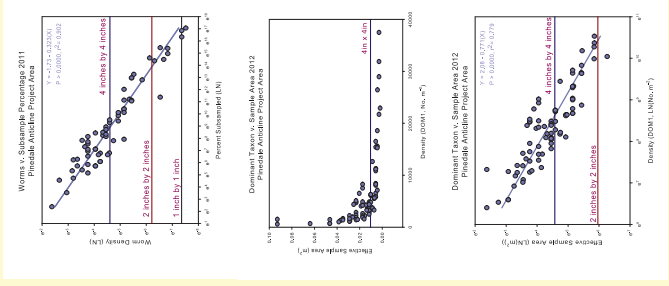
In 2012, the relationship was weaker. This was because of two factors. First the variation in worm abundances was not as great as observed in 2011 (and the variation among subsample percentages was reduced). This caused the data to span a smaller range than in the previous year, which reduced the strength of the linear relationship somewhat (lower left).

The second factor reducing the strength of the relationship was the fact that we had some samples with low worm density which only used a small percentage of the sample to reach the subsample target. This was caused by an abundance of black flies (>8,000/sample).

Thus the process is driven by the density of the dominant taxon, not *Nais* per se.



**Why is this important?**  
When studies involve inference of invertebrate density or biomass, there is a critical assumption of standard unit effort among the samples. When the data are standardized to the relationship between the effective area sampled (after subsampling), we see that the actual area represented by the samples is not constant. Rather, it varies by several orders of magnitude (below)



**When is this a problem?**  
Often, people are eventually interested in questions related to density, biomass, production, or biodiversity. If you are interested in questions related to these topics or think you will eventually be interested in one or more of these topics, you need to be concerned about the lack of appropriately standardized samples.

Thus, you should never use a fixed count subsample for the following investigations:

- Fisheries Investigations,
- Hydropower Recertifications,
- Environmental Impact Statements,
- Scientific studies not related to bioassessment...

**When is this NOT a problem?**  
If your only purpose for collecting data is to calculate an MMI or a RIVPACS-type model then you should not worry about this. The multivariate and multivariate tools of bioassessment are calibrated using fixed counts to calculate percent abundances and richness estimates per 100, 200, 300, or 500 (etc.) organisms. If your only desire is to use these bioassessment tools, then ask your bioassessment expert.

The misuse and over application of bioassessment methods, for non-bioassessment purposes, has become a systemic problem in aquatic resource management.

**What Alternative Laboratory methods circumvent these issues?**

- No subsampling
- Proportional subsampling
- Very high subsampling criteria (~3000-organism)
- Nested sieves fixed count subsampling
- Nested sieves proportional subsampling
- Nested sieves hybrid subsampling
- OR,
- USE A LARGER MESH SIZE!

(Large rare search always introduces bias... ask me to explain)