Objectives

- **Cytochrome P4501A1 Expression in Liver, Lung, and Urinary Bladder of Cetaceans as Indication of Persistent Organic Pollutant Exposure**

- **The goal of this project to look for relationships between groups and CYP1A1 scores in different tissues and also see if there is difference of how tissue score are oriented in multivariate space.**

- **My objective was to run a PCA or NMDS as Lopez et al 2012 and 2014 performed on Hawaiian monk seal contaminant data.**

- **I believe age class will be a driving factor in how the samples will be grouped and oriented based on CYP1A1 scores.**

Dataset Description

- **Data file: Matrix1.wk1**
- Raw data → 35 cetaceans & 12 tissues
- Clean data → 20 samples and 3 variables
- Samples are cetaceans of 9 different species
- Variables: Organs with CYP1A1 scores (0- 9)

• Liver

• Lung

• Urinary Bladder

Main - Matrix1.wk1

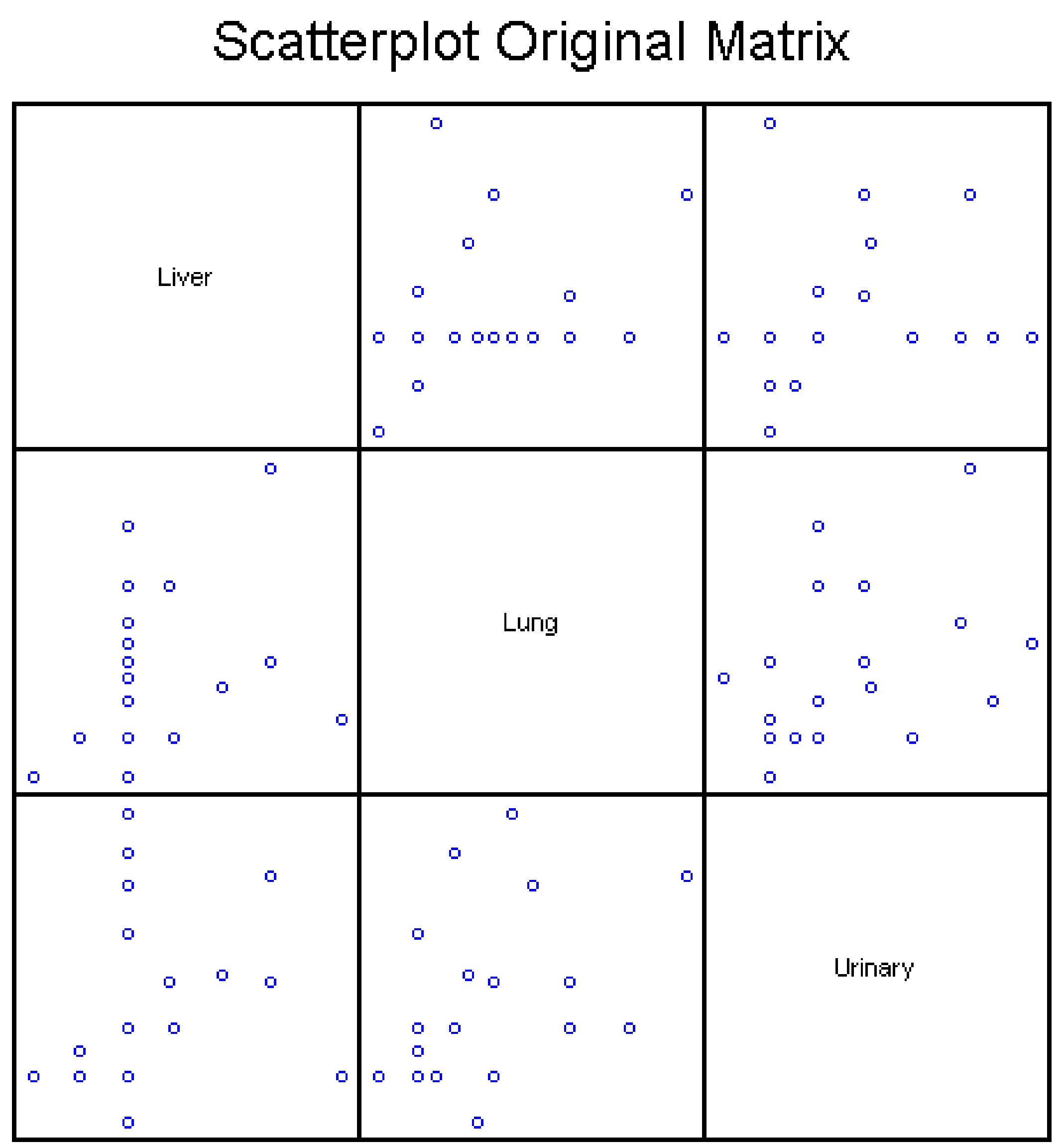
20	Cetaceans		
3	Tissues		
	Q	Q	Q
	Liver	Lung	Urinary Bla
6-171	7.5	2.5	2

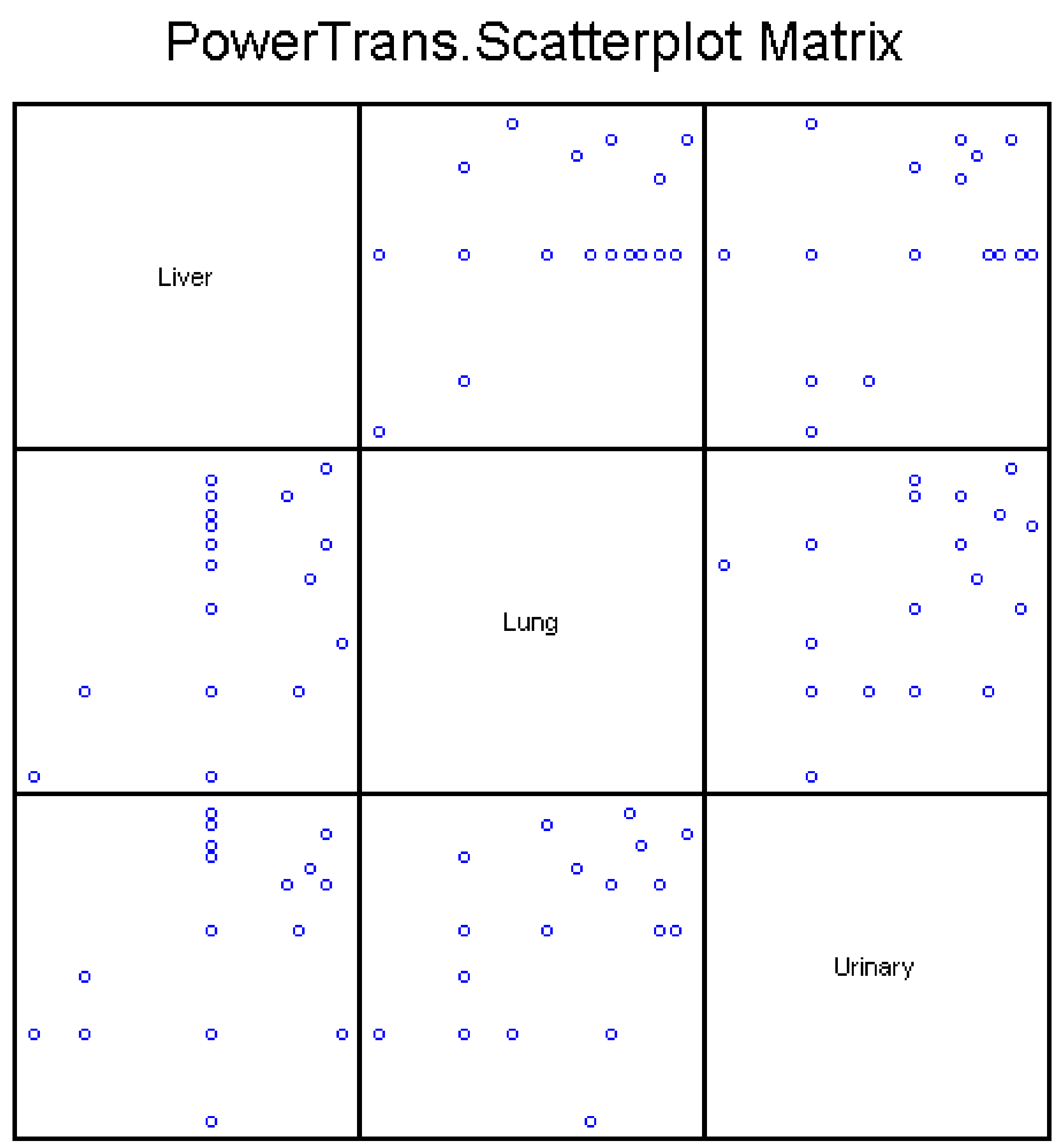
Dataset Processing

- I did not discard any samples after starting with 35 and going down to 20 cetaceans.
- My data had one outlier cetacean 06-171 with SD 2.5 and skewness between -1 & +1
- I performed log and power transformations without removing the outlier.
- General relativization and relativization by maximum did not solve the outlier problem
- Relativization by rank resulted in no outliers, so I decided to use this matrix for my exploratory analysis

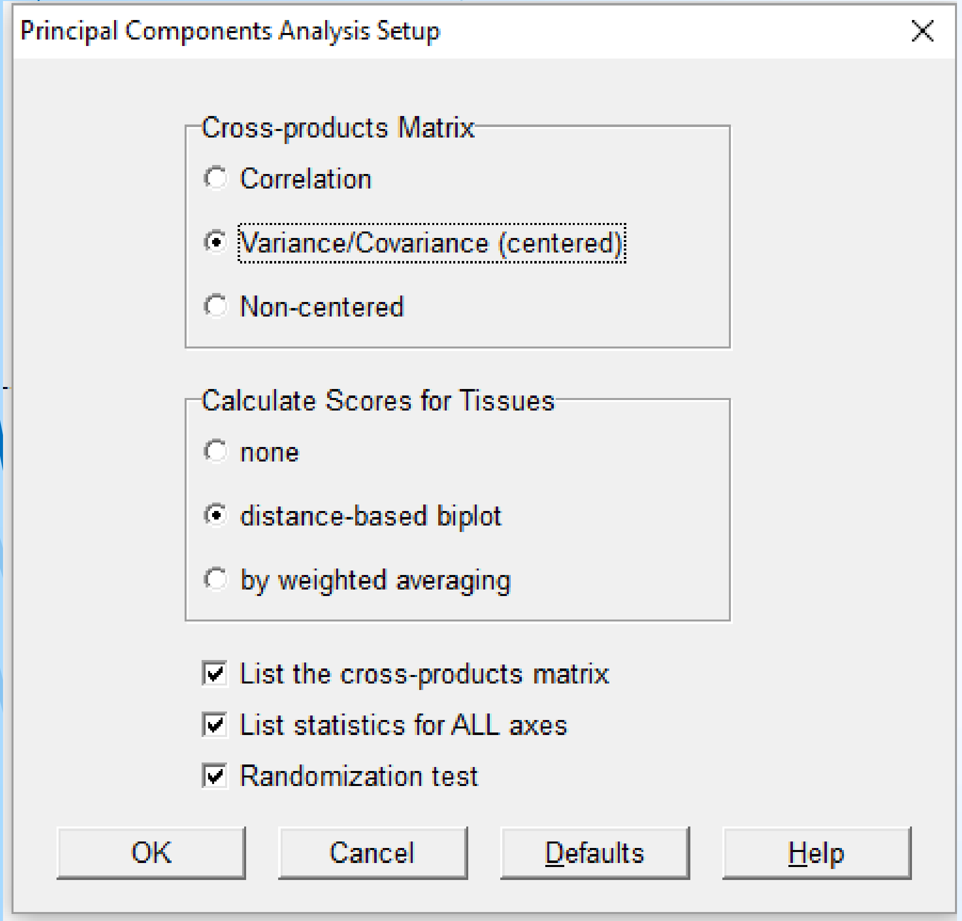
➢ However, after running several PCA's and NMDS's the power transformed data provided the only useful ordination with a PCA

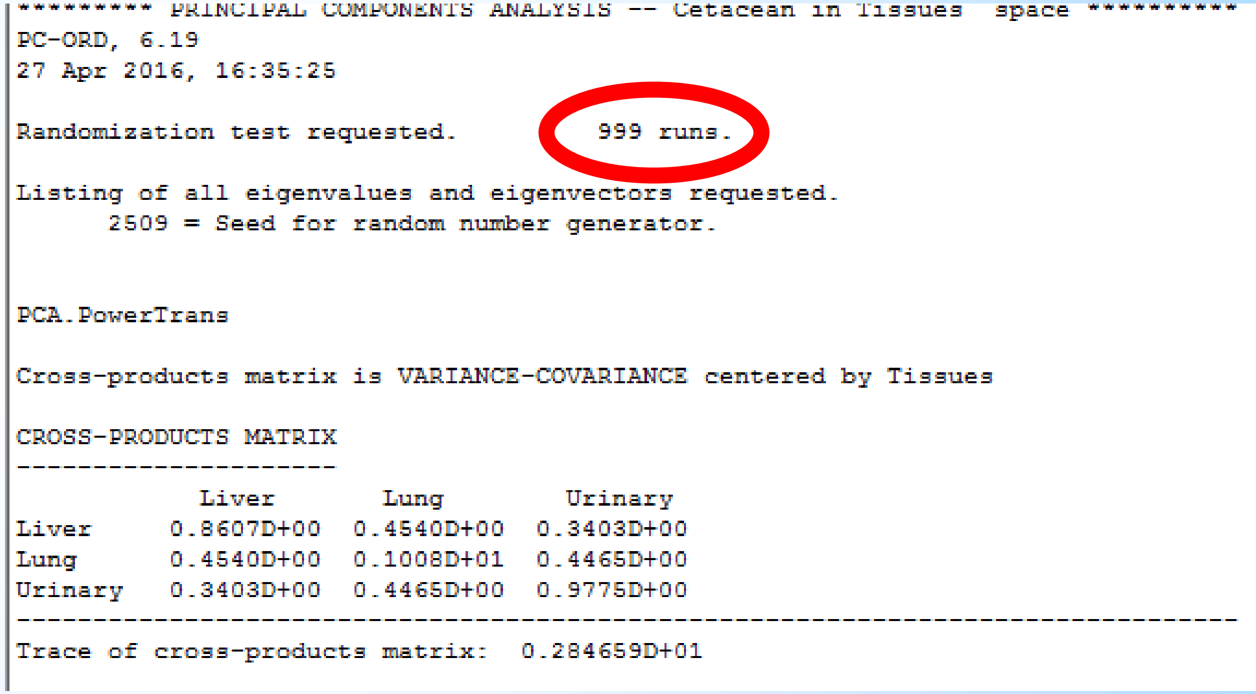
Dataset Exploration





Dataset Analysis





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********** PRINCIPAL COMPONENTS ANALYSIS -- Cetacean in Tissues  space **********
PC-ORD, 6.19
27 Apr 2016, 16:35:25

Randomization test requested.        999 runs.

Listing of all eigenvalues and eigenvectors requested.
      2509 = Seed for random number generator.


PCA.PowerTrans

Cross-products matrix is VARIANCE-COVARIANCE centered by Tissues

CROSS-PRODUCTS MATRIX
---------------------
             Liver        Lung         Urinary
Liver     0.8607D+00  0.4540D+00  0.3403D+00
Lung      0.4540D+00  0.1008D+01  0.4465D+00
Urinary   0.3403D+00  0.4465D+00  0.9775D+00
--------------------------------------------------------------------------------
Trace of cross-products matrix:  0.284659D+01
```

Results Interpretation

Criterion 1: Eigenvalue > Broken-stick Eigenvalue

Criterion 2: P value < 0.05

VARIANCE EXTRACTED, FIRST 3 AXES

AXIS	Eigenvalue	% of Variance	Cum.% of Var.	Broken-stick Eigenvalue
1	**1.784**	**62.683**	**62.683**	**1.740**
2	0.593	20.833	83.516	0.791
3	0.469	16.484	100.000	0.316

RANDOMIZATION RESULTS

999 = number of randomizations

Axis	Eigenvalue from real data	Eigenvalues from randomizations: Minimum	Average	Maximum	p *
1	**1.7843**	**1.0118**	**1.3001**	**1.8692**	**0.005000**
2	0.59304	0.55805	0.92935	1.2477	0.999000
3	0.46922	0.22253	0.61717	0.85919	0.876000

Results Interpretation

FIRST 3 EIGENVECTORS, **scaled to unit length.**

Eigenvector

Tissues	1	2	3
Liver	-0.5219	0.5186	0.6773
Lung	**-0.6338**	0.2956	-0.7147
Urinary	-0.5708	-0.8023	0.1744

Correlations of axes with main matrix

N = 20

Axis:	1		
	r	r-sq	tau
Liver	-.751	.565	-.524
Lung	**-.843**	.711	-.643
Urinary	-.771	.595	-.621

Coefficients of determination (% of Variance Explained):

R Squared

Axis	Increment	Cumulative
1	**.734**	**.734**
2	.161	.895
3	.105	1.000

Increment and cumulative R-squared were adjusted for any lack of orthogonality of axes.

Axis pair	**r**	**Orthogonality,% = 100(1-r^2)**
1 vs 2	0.000	100.0
1 vs 3	0.000	100.0
2 vs 3	0.000	100.0

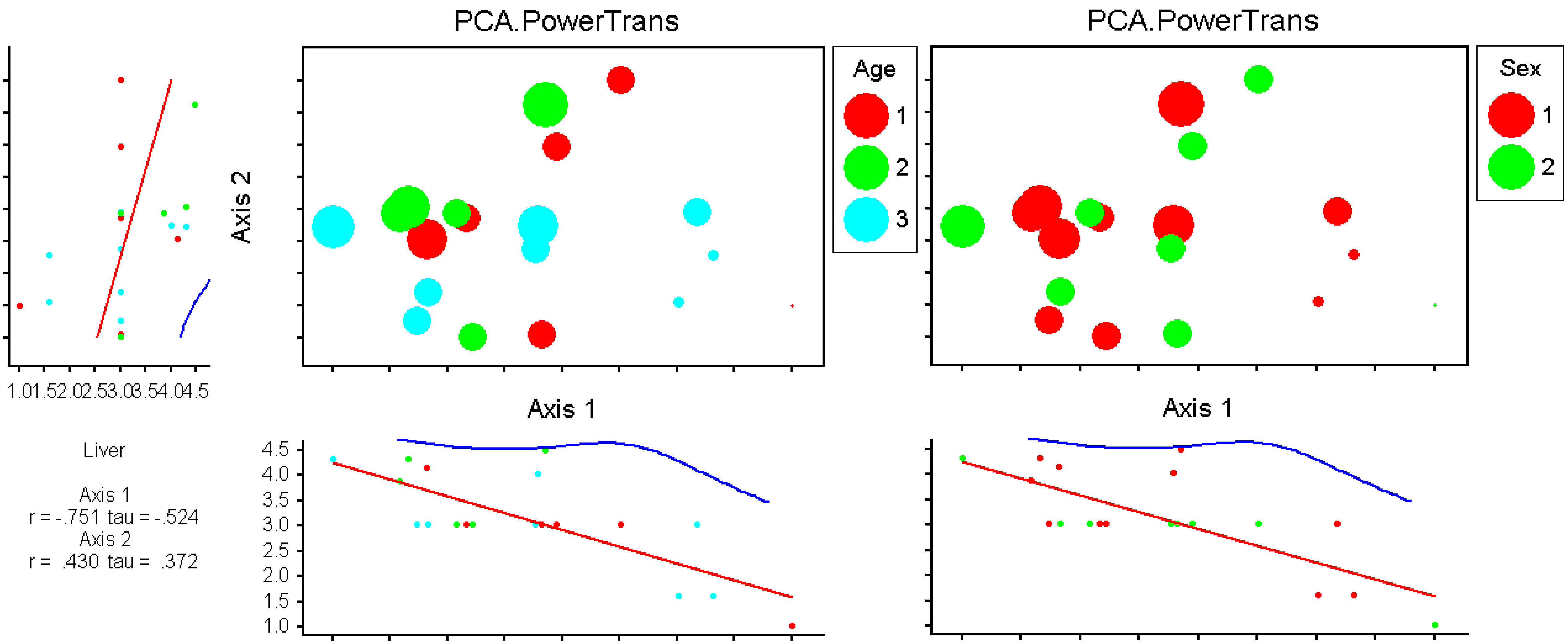
Number of entities = 20

Number of entity pairs used in correlation = 190

Distance measure for ORIGINAL distance: Euclidean (Pythagorean)

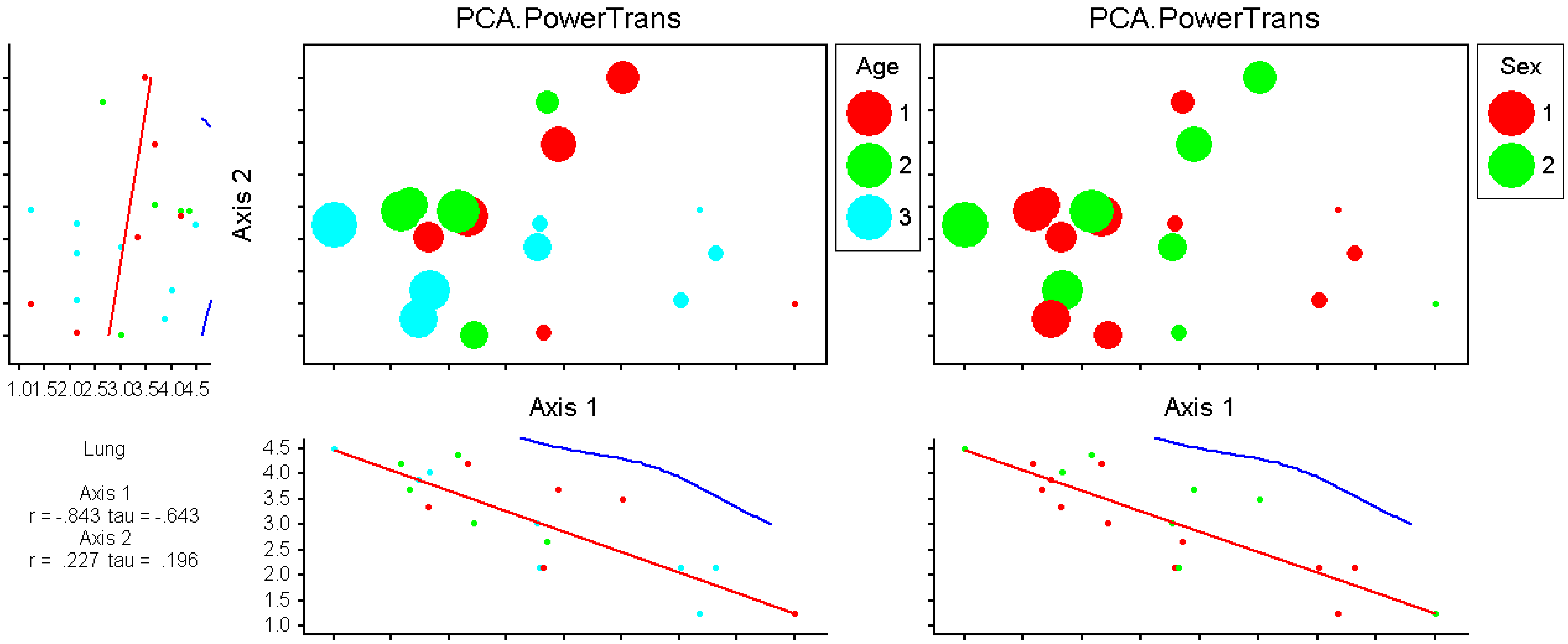
Results Interpretation—Liver

Overlay with second matrix of age classes and sex.
Age → 1= calf 2= subadult 3= adult
Sex → 1= male 2= female



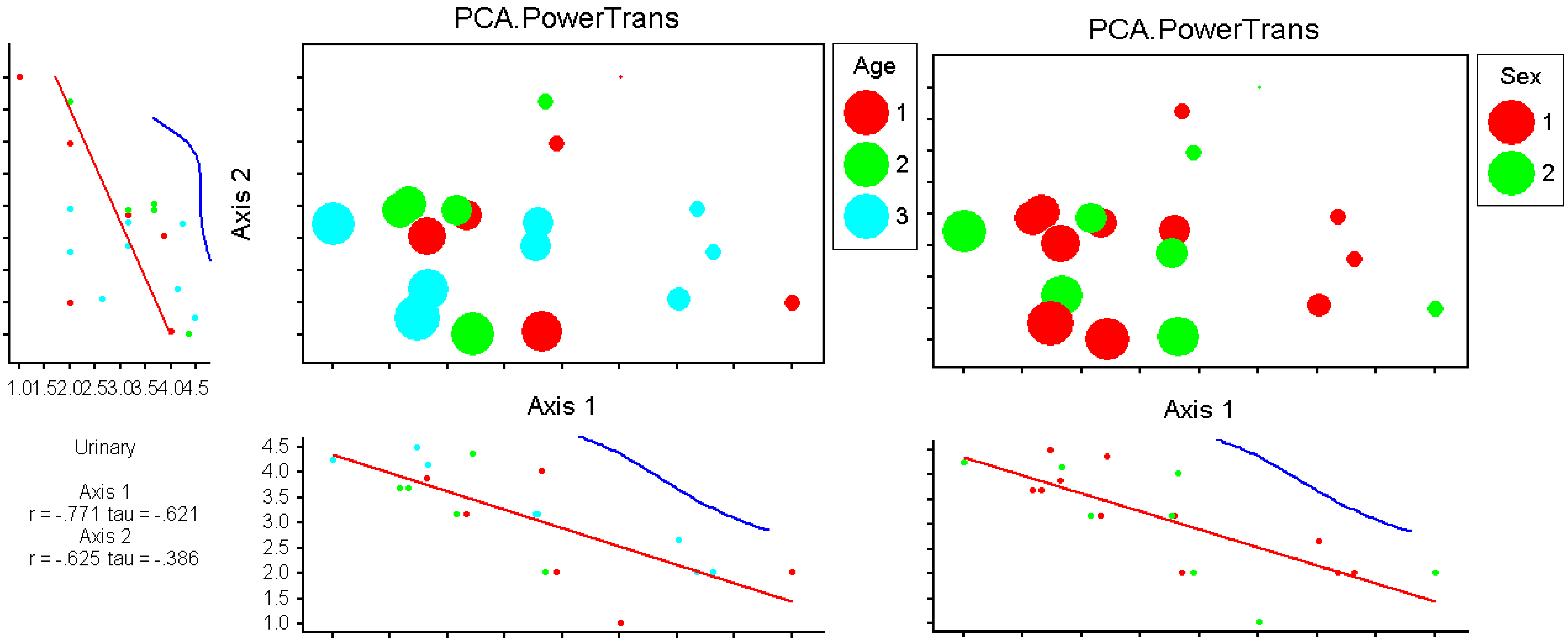
Results Interpretation—Lung

Overlay with second matrix of age classes and sex.
Age → 1= calf 2= subadult 3= adult
Sex → 1= male 2= female



Results Interpretation—Urinary Bladder

Overlay with second matrix of age classes and sex.
Age → 1= calf 2= subadult 3= adult
Sex → 1= male 2= female



Discussion—the Method

- When I compared the cetaceans' loadings in axis 1 to their CYP1A1 scores, cetaceans with higher scores for their organs had smaller or more negative loadings compared to cetaceans with low CYP1A1 scores.
- After examining second main matrix overlay with the first axis, there does not seem to be any differences between males and females nor the different age classes.
- The cetaceans were not separated into any specific groups. Running an MRPP did not result in any significant differences among groups.
- I think to find a more meaningful ordination to see how the age classes and sexes could differ I would need more cetaceans.
- To represent each species, I would need females, males, and animals of each age class.

Discussion – Next Steps

➢ For my re-analysis, I think I should run a polar ordination to see how the cetaceans are organized along a gradient based on their CYP1A1 scores of the liver, lung, and urinary bladder.

➢ If I were to continue this study, I would collect more animals of each species and more tissues, such as integument, heart, and brain.

Species Rows	Tissues Columns			
Species ID	**Liver**	**Lung**	**Urinary Bladder**	**PCA Axis 1 loadings**
06-171 (S.coeruleoalba)	7.5	2.5	2	0.1953
06-172 (S. coeruleoalba)	3.89	6	4	-1.4223
06-173 (M.novaeangliae)	5	3.33	4.13	-1.1170
06-174 (S. coeruleoalba)	3	3	3	0.0756
06-176 (S. coeruleoalba)	4	2	3	0.1106
06-177 (S. attenuata)	3	5	6	-1.1067
06-178 (S. longirostris)	3	2	5	0.1543
06-179 (S. attenuata)	3	4	2	0.3117
06-181 (S. attenuata)	3	6	3	-0.6744
06-217 (F. attenuata)	**6**	**9**	**6.22**	**-2.1533**
07-295 (K. breviceps)	3	4.5	7.5	-1.2255
07-296 (S. longirostris)	3	3	6.67	-0.6075
08-018 (S. longirostris)	3	3	3	0.0756
08-019 (M. densirostris)	3	1	2	1.8643
08-203 (M. novaeangliae)	**1**	**1**	**2**	**2.9081**
08-206 (S. longirostris)	2	2	2.5	1.6679
08-283 (O. orca)	6	4	4	-1.3231
09-053 (S. longirostris)	3	3.56	1	1.0157
09-054 (S. coeruleoalba)	3	7.5	3	-0.7857
09-132 (F. attenuata)	2	2	2	2.0365