Appendix B

Data Screening

Non-temporal sources of variation (contamination, leaching, etc.) can have a profound effect on the AAR data. Understanding the source of this variation can identify outliers that are truly aberrant, as opposed to older, reworked shells. Identification of AAR outliers is on an empirical basis, but no universal standard for recognizing and rejecting outliers exists. A set of screening criteria for one taxon may not be applicable to others. Kaufman (2003) used a cutoff of L-Ser/L-Glu ratios > 1.0 to reject ostracod AAR data, whereas values of L-Ser/L-Asp > 0.8 (> 1.5 in degraded samples) resulted in rejection of foraminiferal AAR data (Kaufman, 2006). Kosnik and Kaufman (2008) discuss outlier analysis in detail, by comparing transformed marine mollusk AAR data to linear models and flagging residuals greater than a specified cutoff value.

I followed the suggestions of Kaufman (2000), Kaufman (2006), and Kosnik et al. (2008) to systematically identify outliers in *Succinea*, *Catinella*, and *Helicodiscus*. The remaining taxa consisted of too few individuals to provide reliable estimates. The outlier screens presented here are a modification of those described in the literature (e.g. Kaufman, 2006; Kosnik and Kaufman, 2008) based on empirical analysis of the Big Platte and Kulas Quarry data sets. To reduce the influence of screening on the inferred age-population of the shells, I only flagged samples whose residuals were more than three standard deviations outside the mean for a normal distribution. Samples that were flagged by more than one screening test were rejected.

Tests for outliers included: 1) the covariance of L-Ser/(L+D Asp) versus D/L Glu. Values of the labile L-Ser should be small; samples with abnormally high L-Ser may

indicate modern contamination (e.g. Kaufman, 2000). 2) Covariance of L-Ser/(L+D Glu) versus D/L Asp is a quasi-independent test compared to 1 (*sensu* Kosnik and Kaufman, 2008). Both tests used values calculated from the peak areas measured during HPLC. 3) The concentration of [Asp] and [Glu] should covary as a function of time. Departures from this relationship may indicate aberrant behavior. Concentration was calculated as the sum of the peak D+L areas within each sample, calibrated to an internal spike of the non-protein amino acid L-*h*Arg (Kaufman, 2000). 4) Finally, D/L Asp and D/L Glu should both increase over time (D/L Asp at a faster rate) and samples that do not display this well-documented covariance may indicate an unusual diagenetic pathway (e.g. Kaufman, 2003). 5) In addition to univariate tests, I used the "Outlier Analysis" option in PC-ORD 5.0 (McCune and Mefford, 2006) to analyze the entire data matrix for outliers within rows (samples). This method creates a frequency distribution for the calculated average distances between all entities in the matrix and flags multivariate outliers from this distribution at user-defined cutoffs. I transformed the data matrix by subtracting the mean from each variable and dividing by its standard deviation. This transformed matrix represents the total variation within each column. The distances between individual samples were measured using the Euclidean distance measure. I flagged samples whose average distance was more than three standard deviations above the mean average distance for all samples.

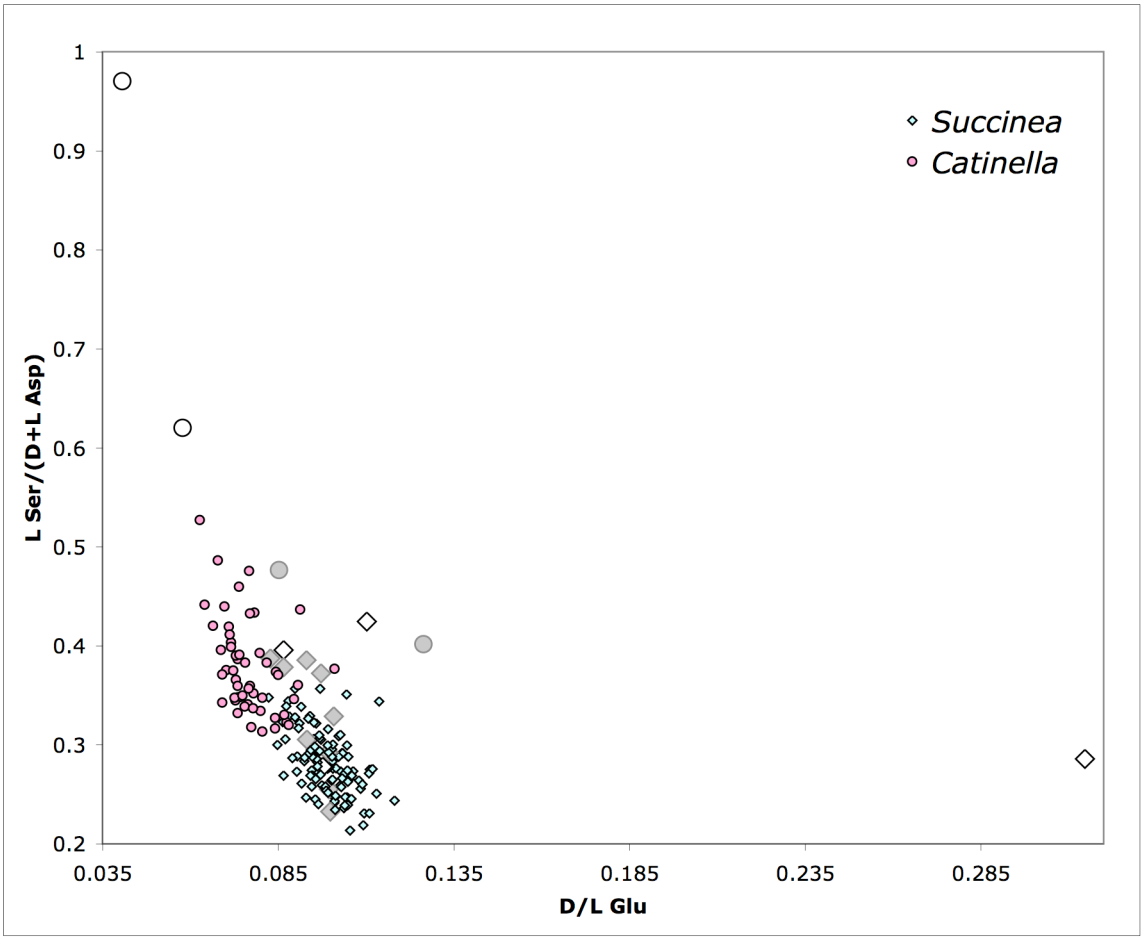

Figure B1. Covariance of D/L Glu versus L-Ser/(D+L Asp) for succineid shells. Outliers in gray, rejected outliers are open symbols.

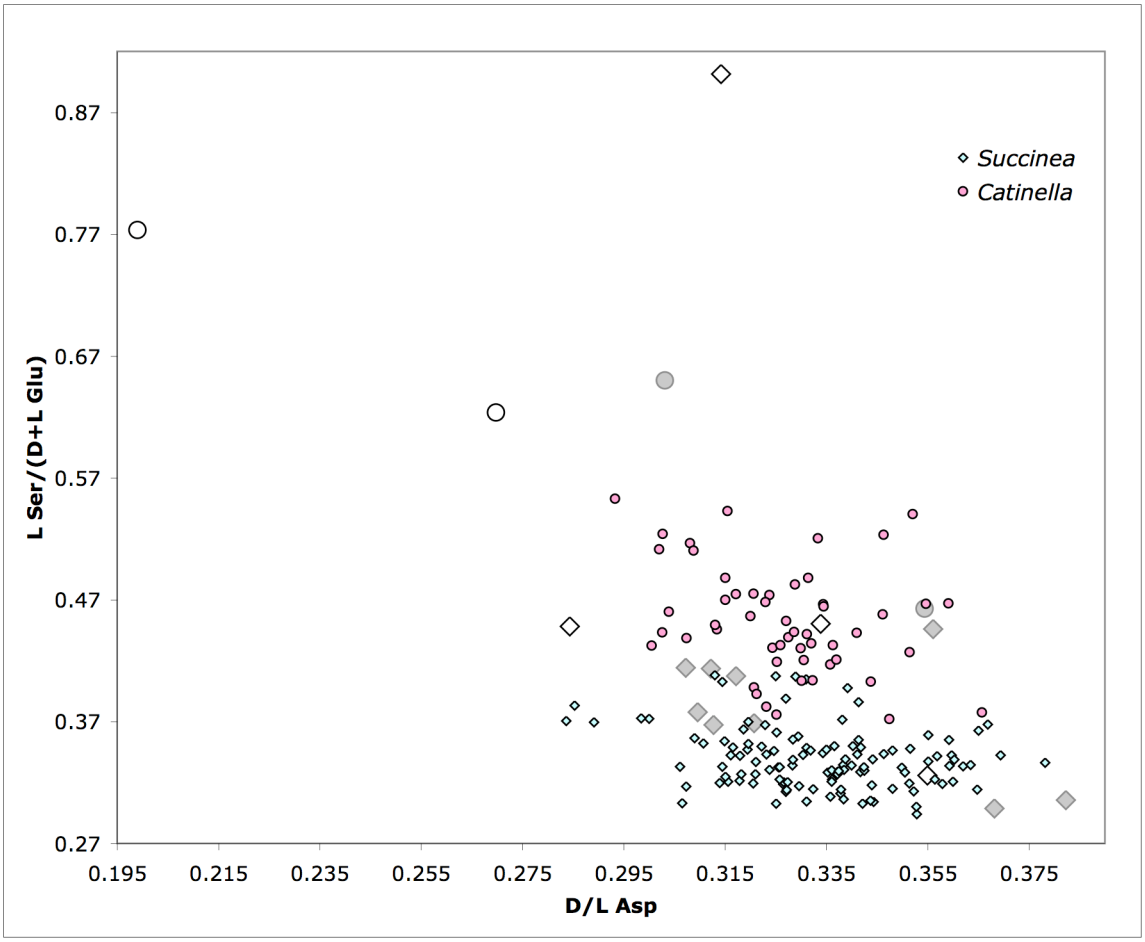

Figure B2. Covariance of D/L Asp versus L-Ser/(D+L Glu) for succineid shells. Outliers in gray, rejected outliers are open symbols.

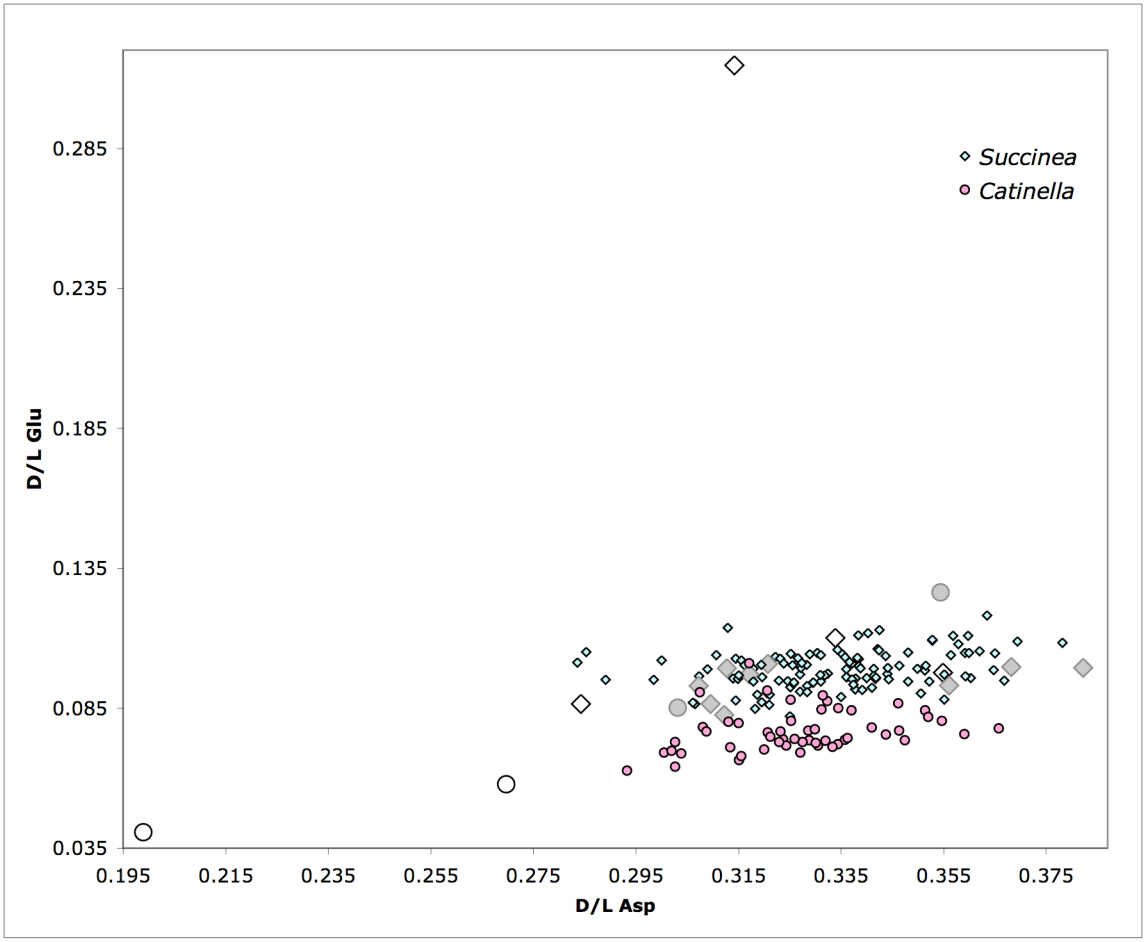

Figure B3. Covariance of D/L Asp versus D/L Glu for succineid shells. Outliers in gray, rejected outliers are open symbols.

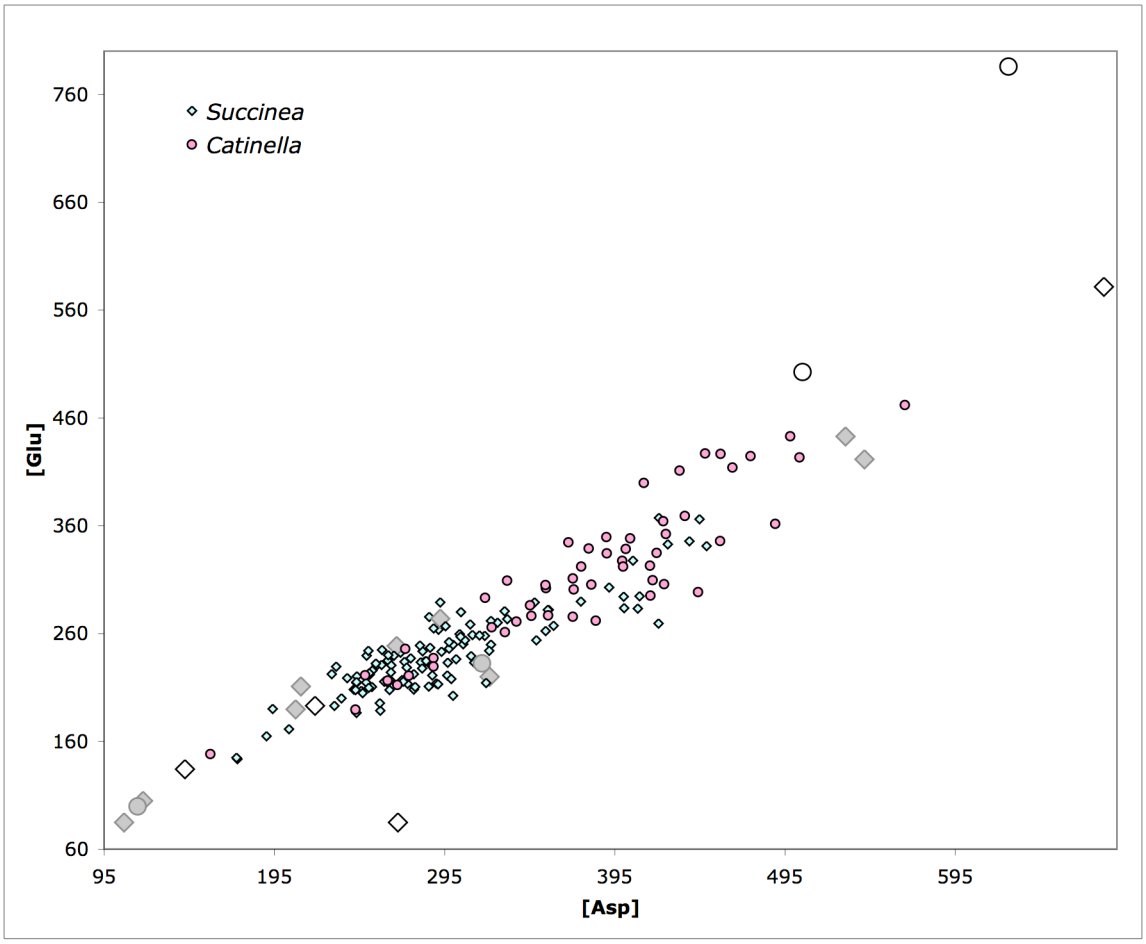

Figure B4. Covariance of L-Ser/(D+L Asp) for succineid shells. Outliers in gray, rejected outliers are open symbols.

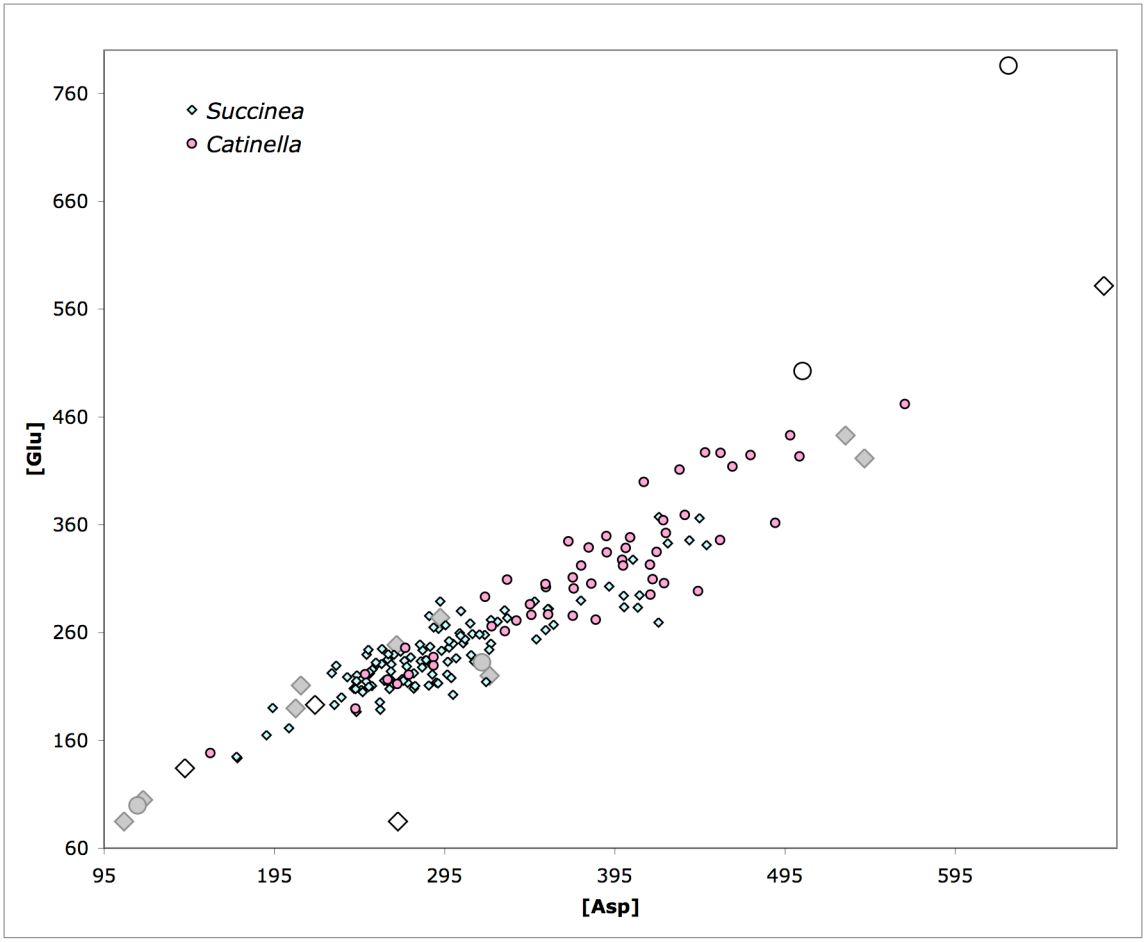

Figure B5. Covariance of [Asp] versus [Glu] for succineid shells. Outliers in gray, rejected outliers are open symbols.

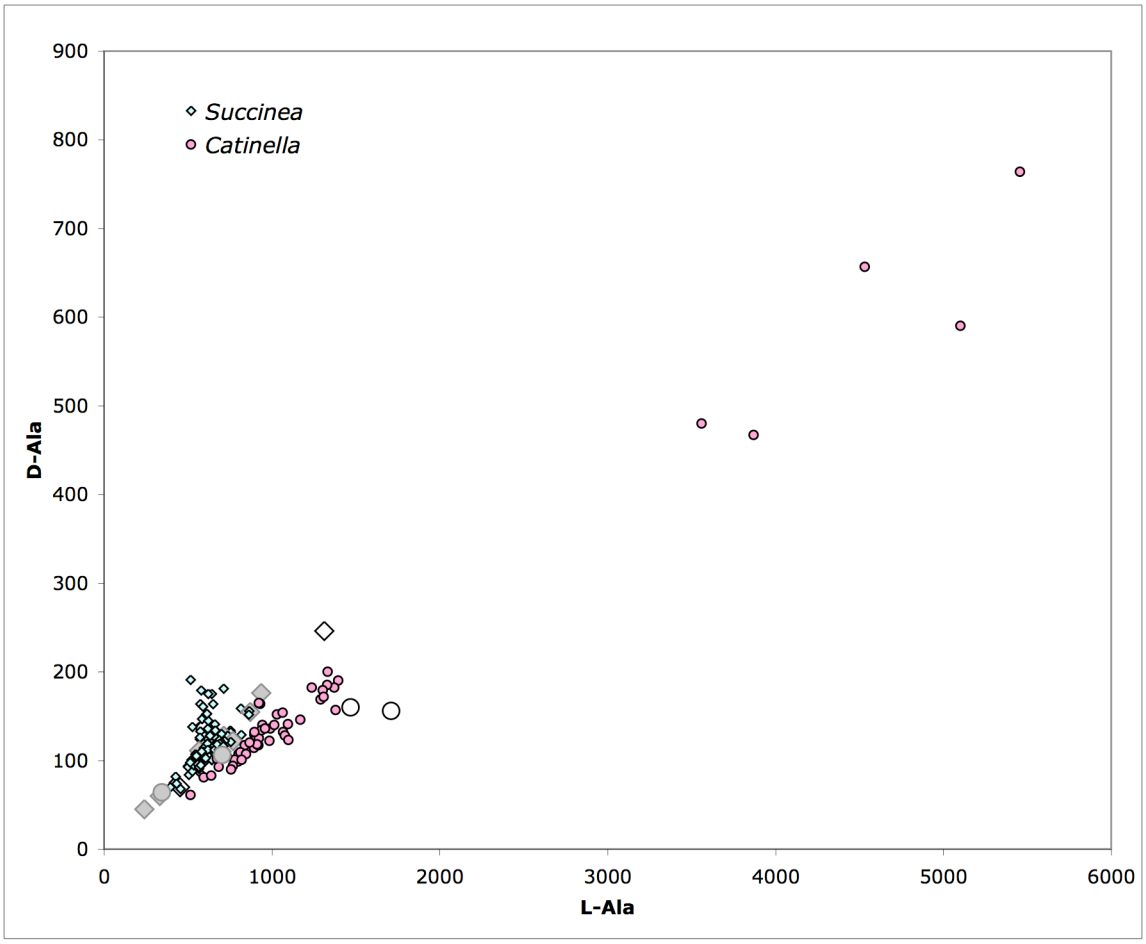

Figure B6. Covariance of L-Ala versus D-Ala for succineid shells. Outliers in gray, rejected outliers are open symbols. This analysis was not used in data screening. Note that several shells ("Succinea" represented with blue diamonds) that do not fit the general linear covariance of L- and D-Ala are not flagged as outliers by these screening tests.