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Supplementary Information for

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Non-destructive Raman Spectroscopic Determination of Freshwater Mollusk Composition, Growth, and Damage Repair

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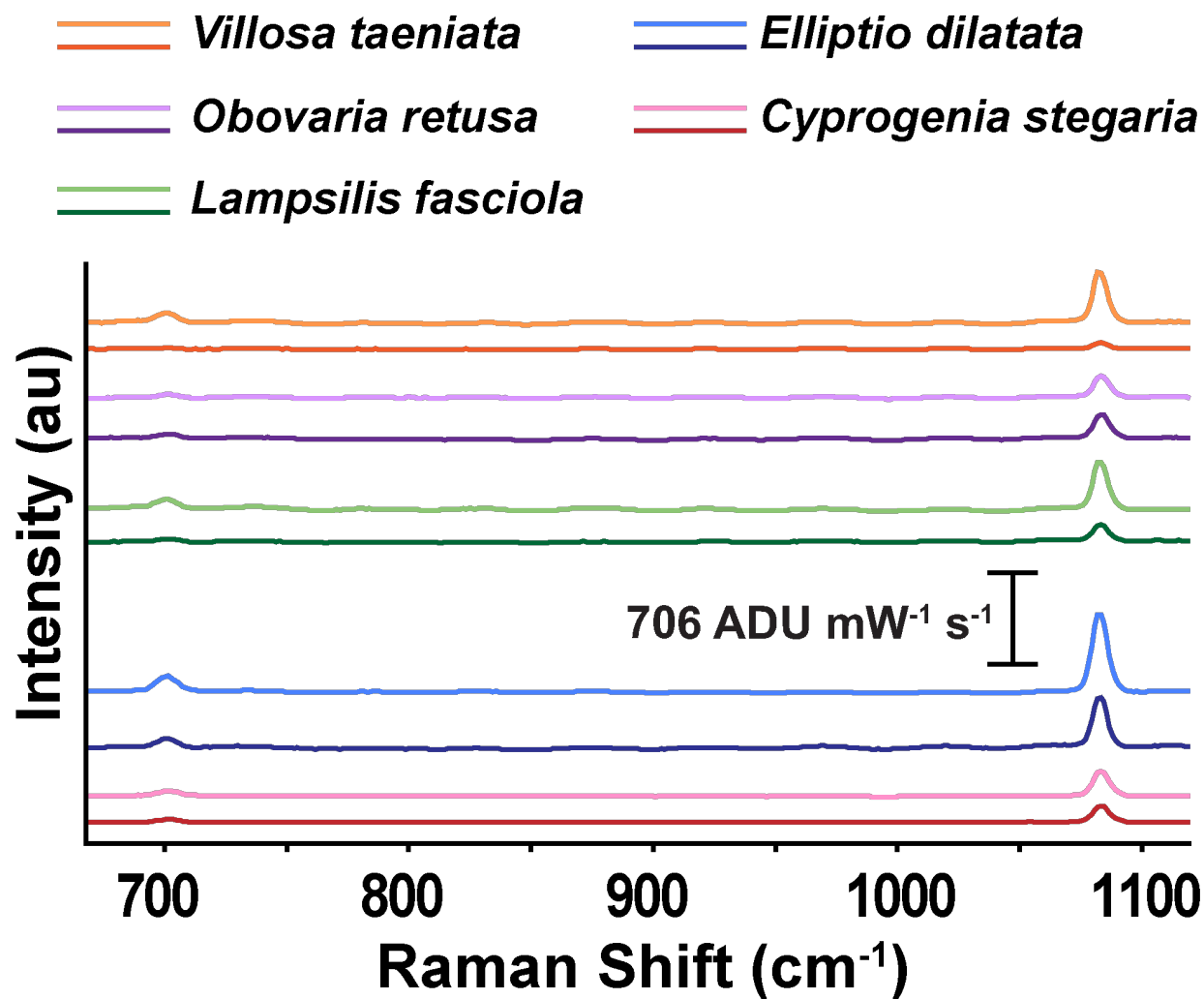Bhavya Sharma, University of Tennessee, Knoxville, TN, United States

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1. Representative Raman spectra of the light and dark regions on the exterior surface of the mollusk shells.

Figure S1. Raman spectra of the light and dark regions on the exterior surface of the mollusk shells. The dark version of each color correlates with the spectra for dark regions of the shell, while the lighter version of the color represents spectra taken in the light regions. The Raman bands were consistently found at 701 and 1084 cm⁻¹, however the Raman intensity varied due to the color of the collection region of the shell. λ_{ex} = 785 nm, P = 7 mW, t = 60 sec, 20X objective.

2. *Cyprogenia stegaria*: Averaged Raman spectra for PCA-LDA and PCA loadings
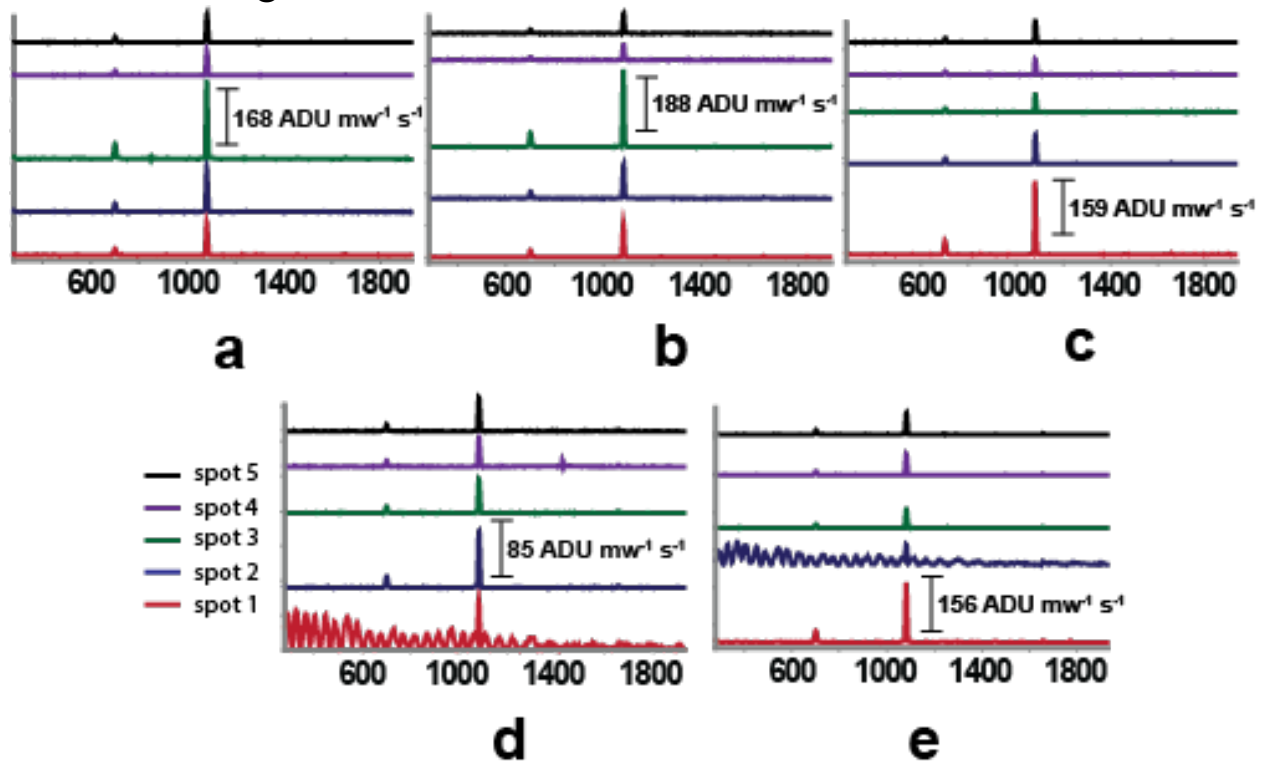

Figure S2. *Cyprogenia stegaria* averaged Raman spectra collected at each spot on the shell, which were input into PCA-LDA. λ_{ex} = 785 nm, *P* = 20 mW, *t* = 1 s, 20X objective.
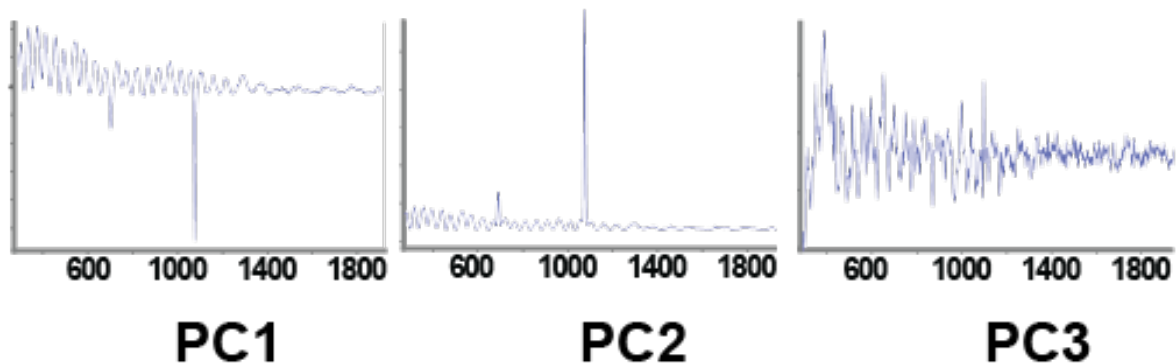

Figure S3. PCA loadings for *Cyprogenia Stegaria* Raman spectra. PC1 is dominated by etaloning, which arises from the CCD chip at 785 nm. PC2 has the characteristic aragonite peaks. PC3 is primarily comprised of background noise in the spectra.

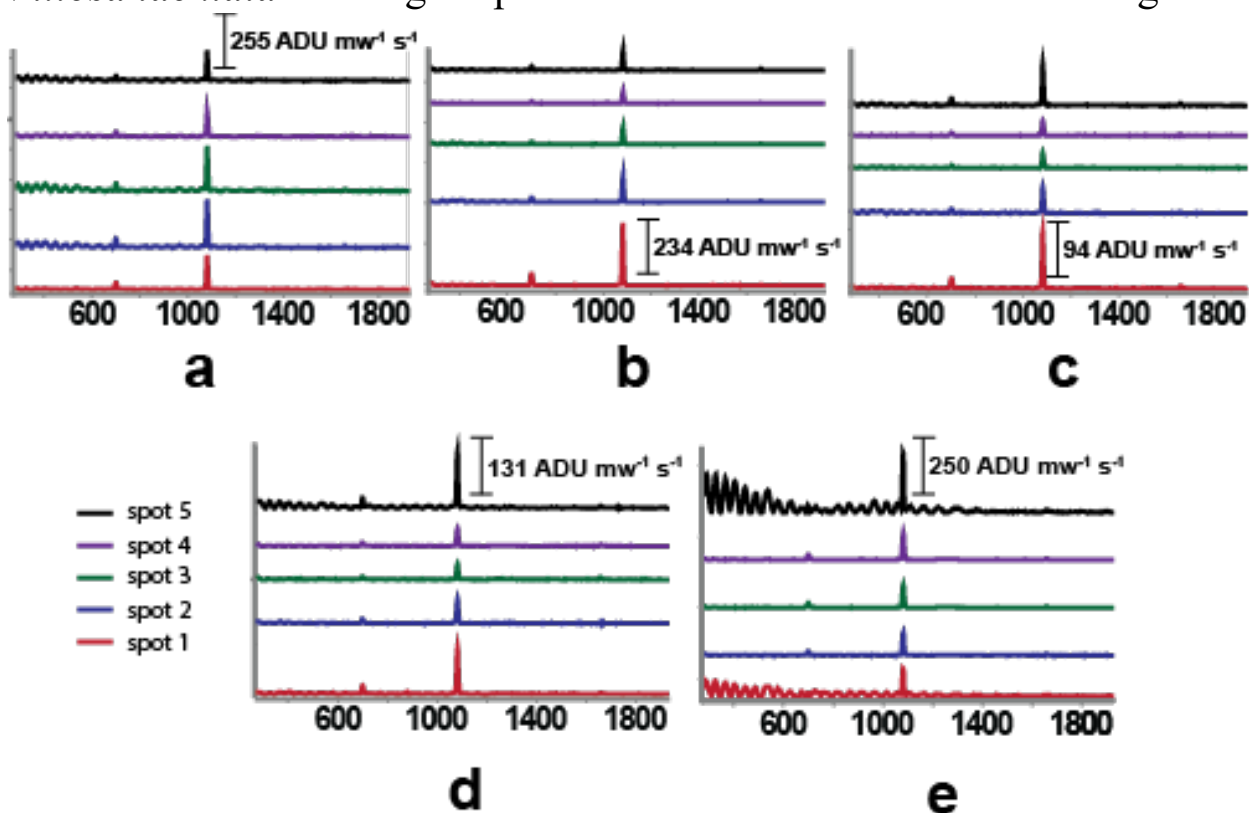## 3. *Villosa taeniata*: Averaged spectra for PCA-LDA and PCA loadings

Figure S4. *Villosa taeniata* averaged Raman spectra collected at each spot on the shell, which were input into PCA-LDA. λ_{ex} = 785 nm, P = 20 mW, t = 1 s, 20X objective.
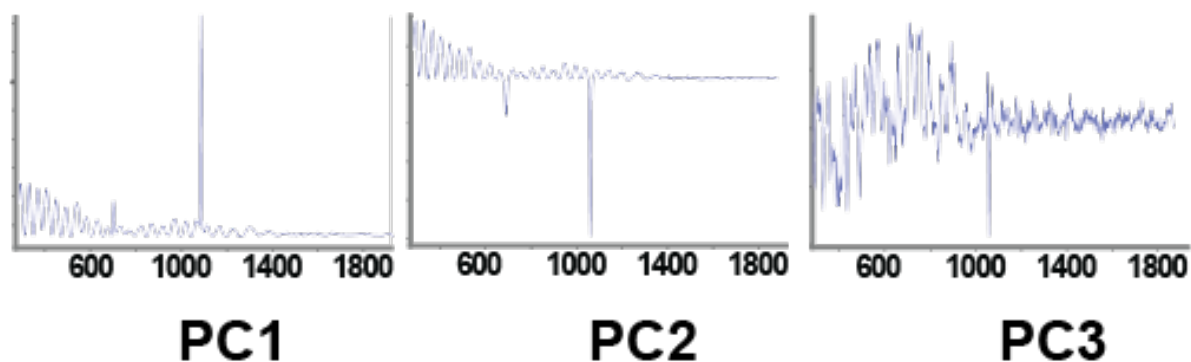

Figure S5. PCA loadings for *Villosa taeniata* Raman spectra. PC1 is primarily composed of the aragonite Raman peaks and the etaloning signature. PC2 shows the inverse of PC1, while PC3 includes noise and the negative of the 1084 cm^{-1} peak.

4. *Elliptio dilatata*: Averaged spectra for PCA-LDA and PCA loadings
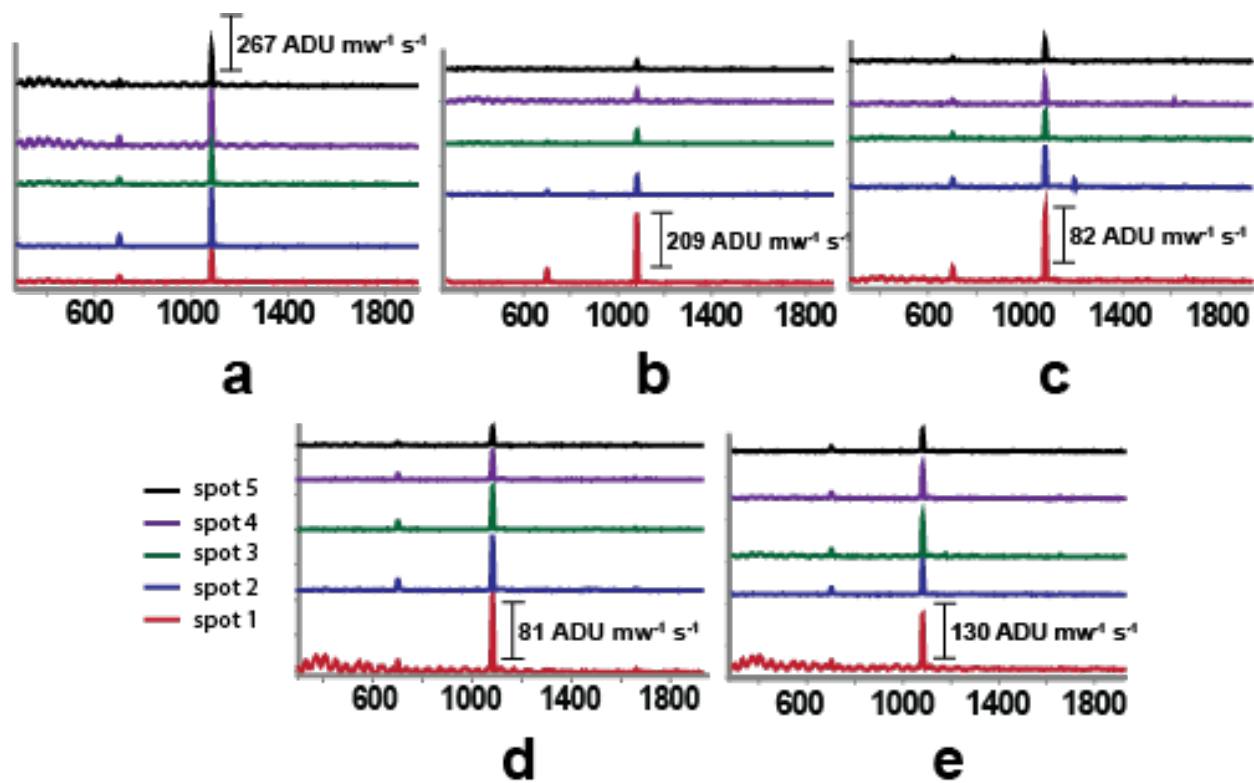

Figure S6. *Elliptio dilatata* averaged Raman spectra collected at each spot on the shell, which were input into PCA-LDA. λ_{ex} = 785 nm, P = 20 mW, t = 1 s, 20X objective.
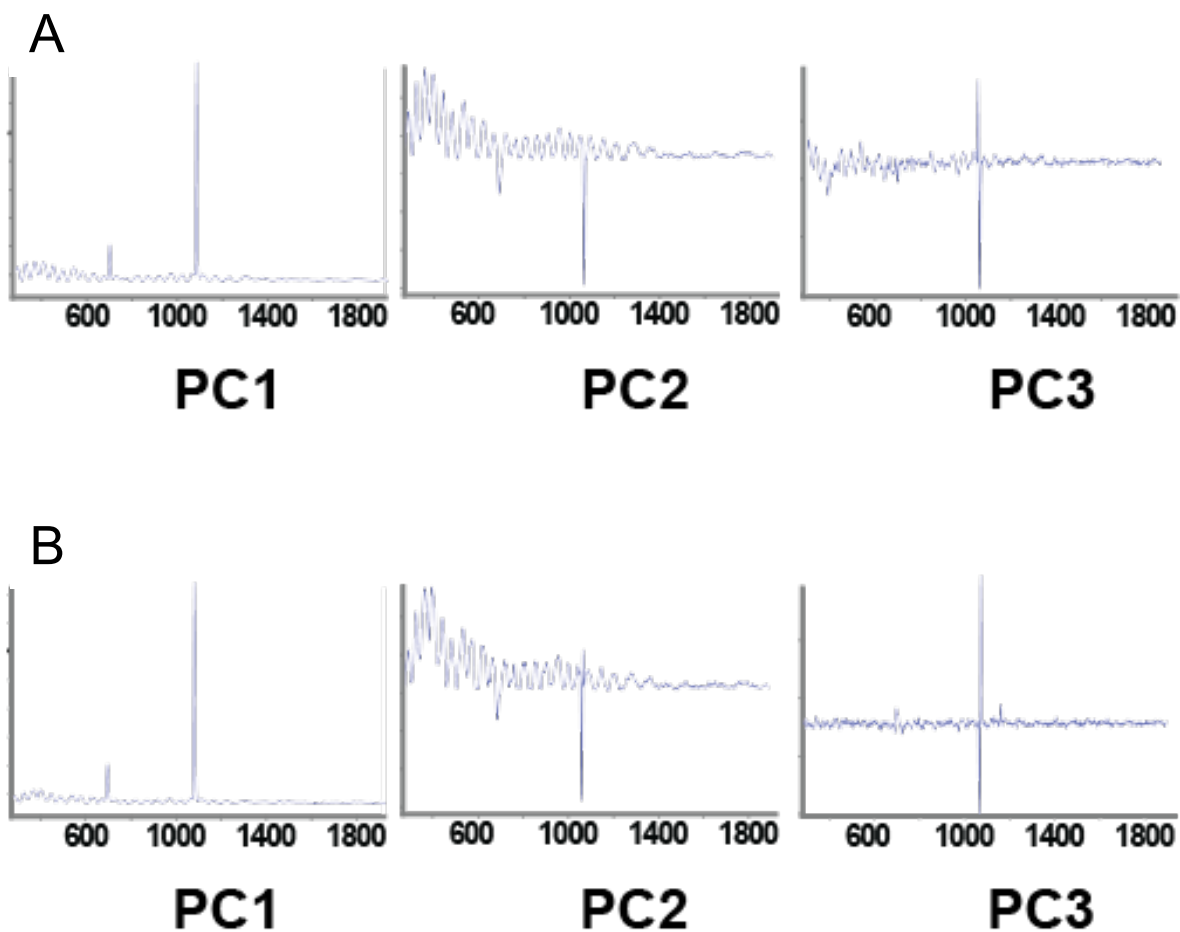
Figure S7. PCA loadings for *Elliptio dilatata* Raman spectra. (A) PC loadings for data included in PCA-LDA analysis of all regions of the shell in Figure 8B. (B) PC loadings for regions b-e (not including a) of the shell shown in Figure 8C. Both sets of loadings show similar patterns, with PC1 composed of the Raman peaks for aragonite, PC2 the inverse of PC1 with increased etaloning, and PC3 showing a first derivative shape at the 1084 cm^{-1} peak.