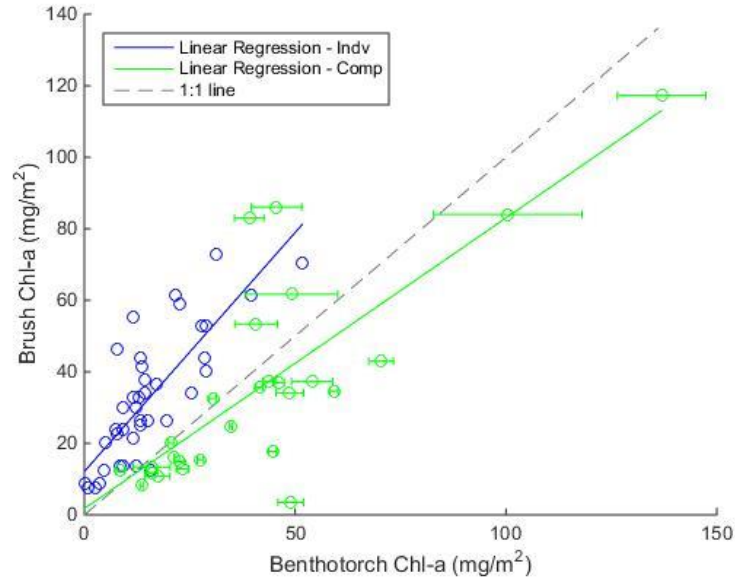


Benthic Chl-*a* measurements made with the BenthoTorch were compared against the standard brush sampling/ethanol extraction/spectrophotometric analysis method in order to assess the ability to compare the in-situ data collected in this study against the standard methods used by others. Comparisons were made against both 40 measurements on individual substrate particles [e.g. *Biggs et al.*, 1999] and 27 composite samples consisting of three particles [e.g. *Segura et al.*, 2011]. Sediment particles were removed from a riffle upstream from the sampling grid and analyzed using the BenthoTorch. Care was taken not to disturb the algal community during this measurement. The same location on the sediment particle was then sampled using the brush sampling method. The location was covered with a cap of similar diameter to the measurement surface of the BenthoTorch (3 cm vs 1 cm for the cap and BenthoTorch respectively) and the remainder of the rock was scrubbed with a nylon brush and rinsed. Following the rinsing procedure, the cap was removed and the area below it was scrubbed vigorously with a nylon brush. This procedure was repeated two additional times for the composite samples. The removed material was placed into a 250 mL bottle and topped off to 250 mL with stream water. The samples were kept cold prior to transport to the laboratory. In the laboratory, the samples were filtered in the dark and collected onto 0.7  $\mu\text{m}$  glass fiber filters. The filters were stored in centrifuge tubes at -20°C for 18 days prior to extraction using sonication and hot 95% ethanol. Chl-*a* concentrations of the extractant were measured using a spectrophotometer and not corrected for phaeophytin as the BenthoTorch cannot distinguish between photoactive pigments.

The comparison between Chl-*a* measurements using both the BenthoTorch and the brush method demonstrates that the same relative result to characterize contrasting Chl-*a* concentrations can be achieved using either method. Furthermore, the comparison also highlights the utility of replicating Chl-*a* measurements when using either method (Figure **Error! No text of specified style in document.**1). The slopes and  $R^2$  values of both regression lines (0.81 vs 1.34 and 0.61 vs 0.64 for the composite and individual respectively) demonstrate strong linear relationship between measurements made by both analytical methods. Variability in the measurements can be reduced through replication as indicated by the slope of the linear regression of the composite samples being closer to 1 than the individual samples. The individual samples were consistently greater than the 1:1 line while there was a random dispersion of the

composite samples around the 1:1 relationship. This comparison underscores the replication that we employed in our sampling scheme.



**Figure Error! No text of specified style in document..1 - Chlorophyll-a Measurement Method Comparison:** Relationship between Chlorophyll-*a* concentrations measured using both the Benthotorch and a standard brush method followed by ethanol extraction and spectrophotometry. Results are shown for both individual (indv) and composite (comp) brush samples. The individual linear regression corresponds to the equation  $[\text{Chl-}a]_{\text{Brush-indv}} = 1.33 * [\text{Chl-}a]_{\text{Benthotorch}} + 12.12$  ( $R^2 = 0.64$ ). The composite linear regression corresponds to the equation  $[\text{Chl-}a]_{\text{brush-comp}} = 0.81 * [\text{Chl-}a]_{\text{Benthotorch}} + 1.76$  ( $R^2 = 0.61$ ). Error bars on the composite samples correspond to the standard deviation of the three Benthotorch replicates. The data is also plotted against the 1:1 agreement line.