Validating a digital photogrammetric method for non-invasive estimates of limpet growth in the field

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Abstract

The use of digital photography provides the potential for making non-invasive measurements of many organisms, including organisms that require minimal disturbance during ongoing experiments. As part of a long term field experiment involving intertidal gastropod limpets, we used a standardized photography method at monthly intervals to estimate limpet growth rates by measuring shell projected area with ImageJ software. To estimate the repeatability of our measuring protocol, multiple people analyzed the same images, and we repeated measurements on the same images to assess within-person and between-person variation. Variation among multiple measurements of the same image was 3.54 ± 0.09 mm² (mean ± 1SE) while the variation among multiple estimates of shell growth between sampling periods was 3.17 ± 0.20 mm² (mean ± 1SE). This non-invasive measurement method produces good repeatability for individual estimates of limpet shell size, but our results indicate that the standardization provided by a single trained measurer provides the most consistent estimates of growth, particularly when growth rates are low.

Introduction

While we expect average environmental temperatures to climb in many habitats as a result of climate change, we also expect that variance in temperatures may also increase, particularly as rare events such as heat waves become more frequent, intense, and longer in duration. As part of an intertidal field experiment that sought to manipulate temperature variation for grazing limpets and their microalgae food resource, we monitored limpet growth through time on a series of standardized plates (Miller et. al. 2015; LaScala-Gruenewald et al. 2016).

Generating growth estimates required a non-destructive, non-invasive method to assess changes in limpet body size repeatedly at monthly intervals. Our experimental design utilized aluminum plates covered with rubber grip tape to provide a standardized habitat for limpets and microalgae. We took advantage of the flat, even surface of these experimental arenas to use a camera framer and 10 megapixel digital camera to record overhead photographs of individually tagged limpets without disturbing the animals. Those photographs could then be measured on a computer using a standardized protocol in the laboratory. Here, we assess the repeatability of this photogrammetry method.

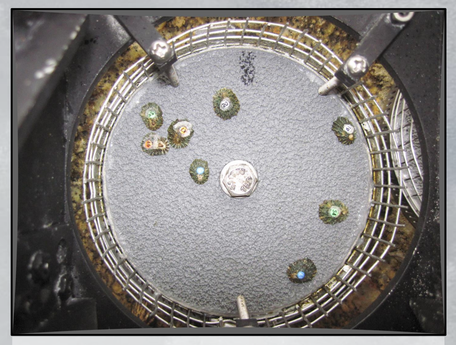
Methods

We used ImageJ (Rasband 2016) to measure the overhead projected area of each limpet.

1. The size scale for each image was set by drawing a straight line between the two framer feet at the top of each image, which had a known distance of 51.5 mm between them. At this scale, a single image pixel represented an area of approximately 0.04 mm².
2. The program was then set to measure area based on the number of pixels enclosed in a painted region generated by the user.
3. The user used the paintbrush tool to carefully trace the shell outline of the limpet. Once a continuous line was traced around the shell, ImageJ measured the area within the painted region, including the painted pixels, and converted this value to an area estimate, mm².
4. Estimates of growth for an individual limpet were generated by subtracting the initial shell area measurement from the area measurement of the following month's photograph, resulting in an estimate of the change in area, mm².
5. To estimate repeatability, we carried out repeated measurements of limpets in two ways: 1) One user repeatedly measured the same limpet photograph 3 times on separate days to assess within-person variation 2) Four users measured the same limpet one time each to assess among-person variation.
6. The variance among repeated measurements by one person or between four persons was calculated as $s_A^2 = \frac{MS_{groups} - MS_{error}}{n}$ where n was the number of repeated measurements of a limpet.
7. Repeatability was calculated as variance among repeated measurements divided by the total measurement variance (including variation among limpets): $\text{Repeatability} = \frac{s_A^2}{s_A^2 + MS_{error}}$.

Analyses of individual patterns of measurement differences were carried out with fixed factor ANOVA using the residuals of each person's area estimates or growth estimates versus the mean of the 4 repeated measures.

Experimental plates deployed in the intertidal zone at Hopkins Marine Station (Pacific Grove, CA) with 4 or 8 tagged limpets. A photograph of each plate was taken each month to track individual limpet size.



Example photograph taken with the framer. The distance between the upper two framer feet provided a fixed distance reference at the plate surface in each photograph.

For this experiment, we used two species of common intertidal limpets found along the coast of California: *Lottia scabra* (left) and *L. austrodigitalis* (right)

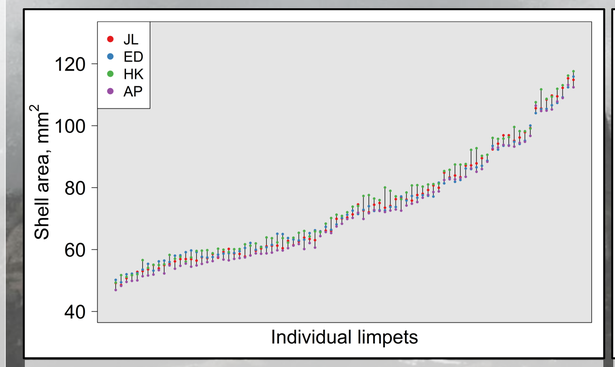


Figure 1. Repeated area measurements for individual limpets (arranged by limpet size) when measured by 4 different people. Measurements of each individual limpet are connected by black lines. n = 86 limpets, dots represent individual measurers.

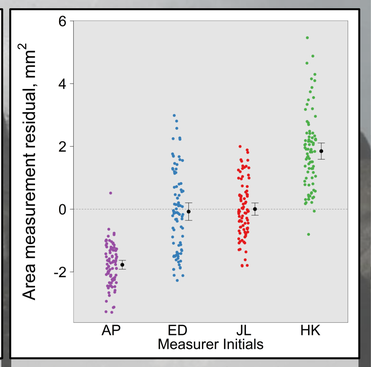


Figure 2. Raw residuals and average residual (black dots ± 95% CI) for each measurer relative to the group average area for individual limpets. n = 86 limpets.

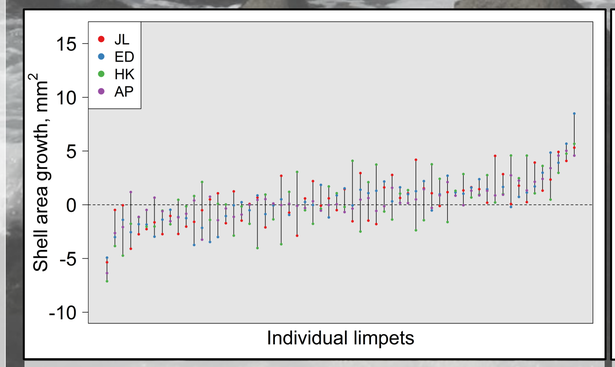


Figure 3. Estimates of growth (net change in shell area month-to-month) for individual limpets. Growth estimates for each individual limpet are connected by black lines. n = 60 limpets, colored dots represent individual measurers.

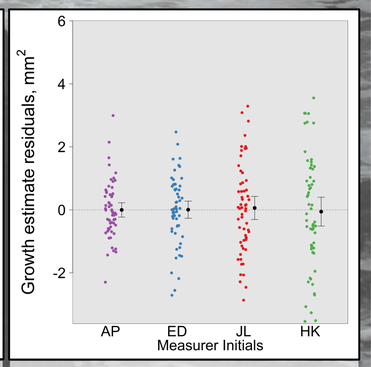


Figure 4. Raw residuals and average residual (black dots ± 95% CI) for each measurer's estimate of shell growth relative to the group average estimate. n = 60 limpets.

Results

Repeatability in area measurements using 1 person

- The repeatability for measurements of shell area by a single measurer was > 99%.
- The mean difference in measured area for repeated measures of the same limpet by the same person was 0.51 ± 0.08 mm² (mean ± 1 SE). The maximum difference for a single limpet was 1.44 mm² (n = 22 limpets)

Repeatability in area measurements among 4 people

- Repeatability in limpet shell area measurements was >98% among 4 different measurers using the same protocol (Figure 1).
- The mean difference between the largest and smallest measurements by multiple people for any single limpet was 3.54 ± 0.09 mm² (mean ± 1 SE).
- There were consistent differences among measurers in shell area measurements ($F_{3,340}$ = 169.1, P<0.001, Figure 2)

Repeatability in growth estimate

- Repeatability in growth estimates (net change in shell area month-to-month) declined to 53% when 4 people measured the same limpet (Figure 3).
- Limpets with 4 repeated growth estimates by different people had a mean range in growth values of 3.17 ± 0.20 mm² (mean ± 1SE).
- Individual measurers did not consistently under- or overestimate growth relative to the group ($F_{2,236}$ = 0.08, P = 0.97, Figure 4).

Growth repeatability by species

- *L. austrodigitalis* with three measurements by different people had a mean range of growth estimates for an individual limpet of 1.97 ± 0.17 mm² (mean ±1SE).
- For *L. scabra,* mean growth measurement range for repeated measures of the same individual by three different people was 2.62 ± 0.15 mm² (mean ± 1SE).

Conclusions

- Limpet measurement using ImageJ showed high repeatability when one person measured a set of limpets in the same photograph, but repeatability in area measures showed greater variation when different people measured the same photographs. For best results, use a single person to remove the effects of person-to-person variation.
- The effect of using multiple people was greater when calculating small growth increments, where a small variation in body size estimation can result in substantial variation in growth estimates. Shell area needed to increase by > 2 mm² to reliably be captured by this measurement method.
- The variation among repeated measurements changed when analyzing different limpet species. The more complicated shell margin of *L. scabra* gave rise to greater variation among repeated measurements by different people, compared to the smoother shell margin of *L. austrodigitalis*.

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Citations

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