Morphological Medley: Differentiating between *Asterias forbesi* and *Asterias rubens*

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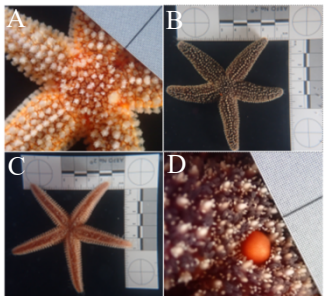

Figure 1. Photos of the (A) central disc, (B) aboral side, (C) oral side, and (D) madreporite to be used for morphological analysis.

In the Gulf of Maine and Northwest Atlantic, there are two congeneric species of sea star that differ in their distribution: *Asterias forbesi*, which typically has a more southern distribution, and *Asterias rubens*, which has a more northern distribution. Both are found intertidally and in shallow subtidal habitats. Possibly due to rising sea temperatures, the distribution of these two species has become less sharply delineated over the last few decades. In addition, hybridization, which is when individuals from different species reproduce to form offspring, is known to happen between *A. rubens* and *A. forbesi* (Harper and Hart, 2007). Traditionally, morphological characteristics have been used to differentiate between these two species of sea stars (Harper and Hart, 2007; Harper et al., 2007; Bateson, 2015). However, it has recently been difficult to determine the species or level of hybridization by relying on morphological phenotypes alone. This summer, we used the color of several body parts, the madreporite (Fig. 1d) and the dermis (skin), and genetics to tell the species apart more reliably.

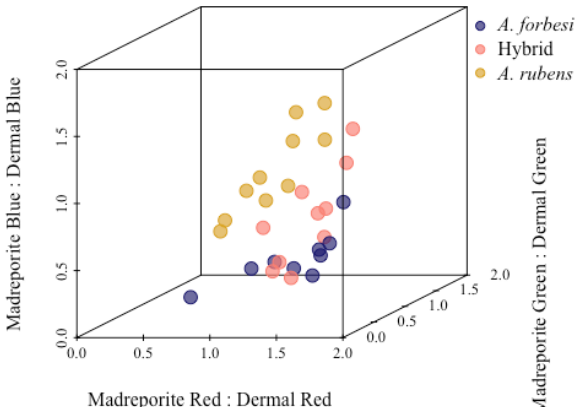

Figure 2. Proportion of red, green, or blue in the madreporite to the corresponding dermal color, for A. forbesi (blue), A. rubens (yellow), and hybrids (pink).

For morphological analyses, we photographed 30 sea stars underwater using underwater lights and an underwater camera, with an AFBO #2 grey color standard to adjust for variance in lighting in each photo. Sea stars were selected based on visual assessment to be either *A. forbesi*, *A. rubens*, or unknown (haphazardly selected). We took four types of photos of each sea star, including the: madreporite, central disk, whole aboral side, and whole oral side (Fig. 1). Morphometrics (body proportions) were measured using ImageJ. In ImageJ we measured: arm length, arm width from where they attach to the central disk, arm width from one third down the sea star's arm, area of the central disk, total area, and the podia area on the underside of the sea star. For our analysis we analyzed scaling of these measurements relative to each other for each individual sea star. Dermal and madreporite color (Fig. 2) were measured using the RGB color system in Photoshop. In the RGB color system, the colors red, green, and blue, are assigned intensities on a scale from 0 to 255, where 0 would mean none of that color, and 255 would be the maximum amount of color. We used photoshop to record the average RGB values of the madreporites and dermis. To measure the madreporite color, we used the lasso tool to outline the madreporite, and then recorded the RGB from the histogram. To measure dermal color, we used the lasso tool to outline the central disc. We also measured the RGB values of the grey standard and adjusted our RGB values of the madreporite and skin to account for slight differences in lighting.

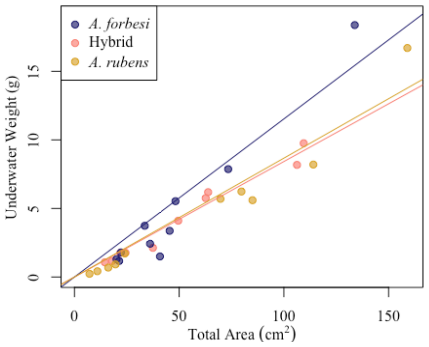

Figure 3. Underwater weights compared to total area of A. forbesi (blue), A. rubens (yellow), and hybrids (pink).

In terms of morphometrics, the only difference between species we found was the ratio of underwater weight to air weight, with *A. forbesi* having a higher density (Fig. 3). In terms of color analysis, the two species could be distinguished by both dermal and madreporite color, with the unknowns falling at intermediate values, indicating likely hybrids (Figs. 2, 4). Using both dermal and madreporite values simultaneously distinguished a non-overlapping group of *A. rubens* relative to the other sea stars in this study (Figs. 2, 4).

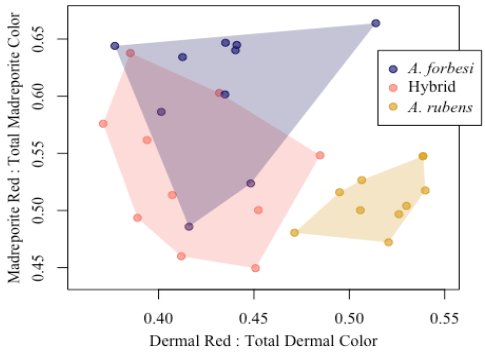

Figure 4. Proportion of red in the dermis, compared to the proportion of red in the madreporite, for A. forbesi (blue), A. rubens (yellow), and hybrids (pink).

At the end of the summer, we used our methods to be more certain of the species that are being used in the temperature locomotion studies also being done in the Johnson lab. By being able to say with certainty that an individual sea star is either *Asterias rubens*, *Asterias forbesi*, or a hybrid of the two, researchers will be able to better claim that the functional differences they find between sea stars are due to differences in the species. Our long-term goal is to combine morphometric, color and genomic variables in a principle components analysis to distinguish more clearly the species/hybrid status of each sea stars. Our initial DNA extractions from clipped sea star podia are promising and will be continued in future work in the Johnson lab.

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