**Patch Variation Analysis**

In this document, I will explain each part of the spectral analysis code, what each analysis tells us about the fish patch data, and the biological and statistical significance of the findings. I'll walk through each component in the order it appears in the code.

1. **Spectral Angle Calculation**

* Spectral angles provide a mathematically robust way to compare spectral signatures.
* Unlike simple Euclidean distance, this method focuses on the spectral "shape" rather than absolute reflectance values.
* Lower angle values indicate greater similarity between spectra.

1. **Organization by Patch Type and Individual**

* The data was organized into a nested structure to facilitate analysis by both patch type and individual.
* This organization allows us to easily compare:
  1. The same patch type across different individuals (within-patch variation).
  2. Different patch types on the same individual (between-patch variation).
* This structured approach allows systematic comparison across both dimensions (patch type and individual).
* Without this organization, it would be difficult to separate biological variation from measurement variation.

1. **Mean Spectra by Patch Type**

A graph of different colors

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The image shows:

* The mean spectral reflectance curves for each patch type (black, white, orange, blue) across the visible spectrum (400-700nm).
* Each line represents the average reflectance pattern for a specific patch type.

What it tells us:

* There are clear, distinctive spectral signatures for each patch type.
* White patches have the highest overall reflectance (70-90%), as expected.
* Orange patches show moderate reflectance (20-45%) with increasing reflectance at longer wavelengths (characteristic of orange coloration).
* Blue patches show specific reflectance patterns with a slight peak around 550-580nm.
* Black patches have the lowest reflectance overall (below 15%).

Importance:

* This confirms that different patch types have fundamentally different spectral signatures.
* These distinctive signatures form the basis for using hyperspectral analysis to identify and differentiate patches.

1. **Spectral Angles Between Patch Types**

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The figure above shows:

* A matrix of spectral angles between the mean spectra of different patch types.
* Values range from 0 (identical) to higher values (more different).

**What this tells us:**

* Black and orange patches are most similar to each other (angle = 0.1224).
* White and orange patches are most different (angle = 0.2131).
* Blue patches are most similar to orange patches (angle = 0.1065).
* White patches are consistently the most different from other patch types.

**Importance:**

* This quantifies exactly how different each patch type is from the others.
* Shows which patches might be most easily confused with each other.
* Provides a numerical basis for distinguishing patches in classification tasks.

1. **Within-Patch Variation**

A close-up of a code

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The figure above shows:

* Statistical summary of within-patch variation.
* Includes min, max, median, mean, quartiles, and standard deviation.

A number with numbers and symbols

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The figure above shows:

* The average spectral angle within each patch type across individuals.
* Lower values indicate more consistent spectral signatures within that patch type.

**What this tells us:**

* White patches show the least variation between individuals (0.0334).
* Blue patches show the most variation between individuals (0.1182).
* The median within-patch variation is relatively low (0.0577).
* Most within-patch angles fall between 0.033-0.065 (1st-3rd quartile).

**Importance:**

* Low values suggest that patches are relatively consistent across individuals.
* Higher variation in blue patches suggests these may be more variable biologically.

1. **Between-Patch Variation**

A computer code with numbers and symbols

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The figure above shows:

* Statistical summary of between-patch variation.
* Includes min, max, median, mean, quartiles, and standard deviation.

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The figure above shows:

* The average spectral angle between different patches for each individual.
* Higher values indicate greater differentiation between patches on the same individual.

**What this tells us:**

* Between-patch angles have a mean of 0.1832 (much higher than within-patch).
* Between-patch angles are fairly consistent across individuals (0.178-0.188).
* The minimum between-patch angle (0.1053) is still higher than most within-patch angles.

**Importance:**

* Shows that different patches on the same fish are consistently distinguishable.
* Provides basis for reliable patch identification.

1. **Within vs. Between Variation Comparison**

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The image above shows:

* Boxplots comparing within-patch vs. between-patch spectral angles.
* Visual representation of the distribution of angles in each category.

A computer code with black text

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The figure above shows:

* Results of a t-test comparing within-patch and between-patch angles.
* Extremely significant p-value (3.252e-11) confirming the difference.
* Mean values: within-patch = 0.0629, between-patch = 0.1832.

**What this tells us:**

* Within-patch variation is significantly lower than between-patch variation.
* The 95% confidence interval for the difference is between 0.092 and 0.148 radians.
* The difference is not due to chance (p << 0.001).

**Importance:**

* This is the key statistical evidence that patches are reliably distinguishable.
* The large gap between within and between variation confirms patches are biologically distinct.
* This validates the use of spectral analysis to identify patch types.

1. **Individual Variation by Patch Type**

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The image above shows:

* Four panels showing individual variation within each patch type.
* Each panel shows spectral curves for each repeat (individual) and the mean.

**What this tells us:**

* Black patches: Relatively consistent across individuals with slight variation around 600nm.
* White patches: Individual 01 shows lower reflectance than others; 02-04 are very similar.
* Orange patches: Individual 01 shows higher reflectance; Individual 03 shows lower reflectance.
* Blue patches: Most variable patch type; Individual 03 shows distinctive peak around 580nm.

**Importance:**

* Reveals detailed patterns of individual variation within each patch type.
* Shows which patch types are most consistent or variable across individuals.
* Helps identify potentially unusual individuals (e.g., Individual 01 for white patches).

1. **Heatmap of All Spectral Angles**

**A yellow and orange squares with black and white text

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The image above shows:

* Hierarchical clustering and heatmap of spectral angles between all individual measurements.
* Darker colors indicate higher similarity (lower spectral angle).
* The tree diagram shows hierarchical relationships based on spectral similarity.

**What this tells us:**

* Measurements cluster primarily by patch type, not by individual.
* All four orange patches cluster together, as do all black patches.
* Blue patches mostly cluster together (blue\_01 is an outlier).
* White patches form a distinct cluster.

**Importance:**

* Confirms that patch type is the primary determinant of spectral signature.
* Shows that the spectral angle method successfully groups similar patches.
* Identifies potential anomalies (like blue\_01) that may need further investigation.
* The hierarchical clustering provides an unbiased grouping method.

**Overall Conclusions**

1. The spectral analysis conclusively demonstrates that different patch types (black, white, orange, blue) have distinctive spectral signatures that can be reliably differentiated.
2. The variation within each patch type (across individuals) is significantly lower than variation between different patch types, confirming that patches are biologically distinct entities.
3. The t-test confirms with high statistical confidence (p < 0.001) that the within-patch and between-patch variations are fundamentally different.
4. There are subtle individual variations within each patch type, with some individuals showing distinctive patterns, particularly for blue and orange patches.
5. The spectral angle method successfully identifies and groups similar patches, validating this approach for rapid assessment of biological variation as described in the original piranha paper.

To this end, these findings support the use of hyperspectral analysis as a biodiversity screening tool, as the approach can clearly distinguish between different patch types even with natural biological variation across individuals.