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## **Sample size reflections of biodiversity in the Southern California intertidal**

### **Introduction:**

Developing of efficient ecological sampling methods that accurately reflect community composition and biodiversity is critical for effectively managing the dynamic intertidal system. The intertidal habitat is often left out of Marine Protected Area zoning, leaving it vulnerable to anthropogenic decimation associated with tourism, pollution, and climate change. In this regard, consistently monitoring intertidal biodiversity is crucial for developing plans for biodiversity conservation. A consistent sampling of habitats reflects biodiversity loss, resource availability, carbon capture, food web structure, and ecosystem function (Johnson, 2020). Emerging technologies in ecological monitoring include computer vision (analysis of digital photos and video), acoustic sensors, radar, and molecular methods (e.g., eDNA analysis) (Van Klink et al., 2022). Advances in data logging systems have created access to a wide range of both lab calibrated sensors, as well as customized microcontrollers such as Raspberry Pi, Arduino, Particle, and others that have increased the efficiency and accessibility of monitoring (Cannon et al., 2022). However, there has yet to be a technological equivalent for taxonomic specialists versed in identifying rare and difficult-to-distinguish species required for biodiversity monitoring (Van Klink et al., 2022).

Quadrat sampling is a widespread method of collecting ecological data both in marine and terrestrial landscapes that incorporates the benefits of taxonomic expertise ad hoc. Though it is one of the most efficient methods of human data collection, it is still labor intensive such that strategies of collecting the most amount of accurate data possible in the least amount of time are vital. One of its most limiting features is the time and labor required to conduct quadrat surveys. Quadrat sampling is used in different manners according to different ecological fields, such as entomology and botany, but it generally serves as a tool for determining the percent cover or species composition of an area (Van Klink et al., 2022; Chianucci et al., 2021). The amount of quadrat data required to represent an area is a debated topic, as previous studies have sought to determine the sample size necessary for effective resampling or the accuracy of quadrat arrangements in reflecting the population of an area (Johnson, 2020; Chianucci et al., 2021). Furthermore, the size of the quadrat varies according to the methodology of each study, and there is no agreed-upon standard for quadrat sample size that accurately reflects the biodiversity of an intertidal area.

Establishing a species accumulation curve (SAC) for the marine intertidal would increase understanding of appropriate quadrat sampling strategies for this ecosystem. A SAC represents the ecological richness of an area as a function of the sampling effort. SACs can be used to extrapolate data for how many species are likely to be present in an area (Dove & Cribb, 2006). Generally, SACs display a sharp increase in richness in small sample sizes followed by a “leveling off” of richness at larger sample sizes that is either asymptotic or logarithmic (Dove & Cribb, 2006). The sample size at which this leveling takes place depends heavily on the system. The applications of SACs are numerous, but they are primarily used as guides for when to stop sampling and predictors of the total richness of a system (Dove & Cribb, 2006). Both

applications would benefit intertidal ecological surveying and monitoring by increasing the efficiency and intentionality of data collection. Though more data provides the most accurate representation of a system, the vastness of the intertidal habitat and the rate at which it is changing necessitates efficient and effective sampling that minimizes the labor involved in data collection.

### Hypotheses:

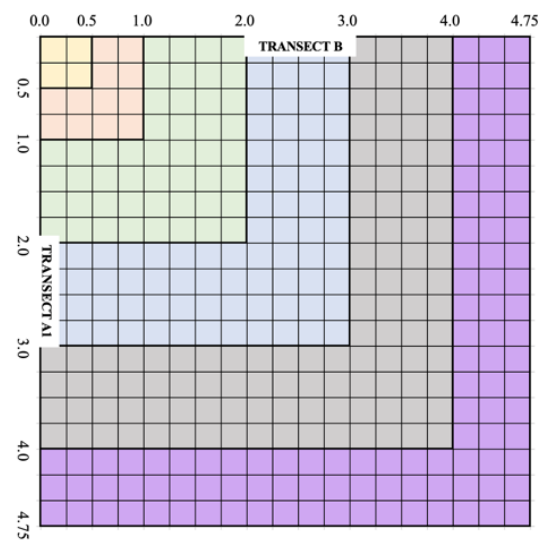
**(H1)** The quantitative biodiversity indicators (richness, Shannon's index, Simpson's index) in the Southern California rocky intertidal increase with plot sample size. **(H2)** The biodiversity reflected by sample size will follow a SAC and begin to plateau at a point such that increasing the plot size does not significantly increase the biodiversity reflected within the Southern California rocky intertidal. **(H3)** The quantitative biodiversity indicators will increase in lower intertidal zones, but this will also plateau at a point where plot size begins to include all three zones. **(H4)** The trends between sample size and biodiversity will not be affected by season.

### Methods:

In statistical data analysis, Shannon's and Simpson's biodiversity indices are the most common representations of the biodiversity of an area that take both richness (the number of unique species) and evenness (the spatial distribution of each species) into account (Nagendra, 2002). Previous studies have demonstrated that Shannon's index tends to be more sensitive to the richness component of biodiversity, while Simpson's index tends to be more sensitive to the evenness component (Nagendra, 2002). Both indices increase as the richness or evenness increase (Nagendra, 2002). Quadrat sampling can be used to determine both percent cover and percent composition, which can then be used to calculate these comparable indices. Both indices will be used in this experiment to analyze the similarities and differences in the trends between sample size and each index.

Four different sites of intertidal area along the coast of Malibu will be selected. Two sites will be marine protected areas, and two will be nonprotected areas. The sites will be selected according to similar ecological population composition and tourism popularity. Metadata will be collected at each site for each data collection session, including day and time, location, ambient weather, tide height, and water temperature. For each data collection session, species and their count will be recorded for each data point.

Data will be collected using a point-intercept quadrat method to address the hypotheses. Five 25-meter transects (hereafter referred to as transects A1–A5) will be laid perpendicular to the shoreline, with their origin 4.75 meters out from the beginning of the intertidal. Each transect will be 5 meters apart.



**Figure 1: Diagram of Data Collection and Plot Size (m) Organization** – Transect A1 runs perpendicular to shore, Transect B runs parallel to shore and perpendicular to A1. Data is taken every 0.25 meters (at each intersection point shown above) along each transect.

Another transect (hereafter referred to as B transect) will be laid perpendicular to the A1–A5 transects and parallel to the shore to determine the data points to be collected. Points will be collected every 0.25 meters along the B transect for 4.75 meters for a total of 21 points, then the B transect will be moved 0.25 meters down the A1 transect, and 21 more points will be collected along the B transect. This process will repeat for 4.75 meters along the A1 transect resulting in a 4.75-meter by 4.75-meter square of data points as shown in Figure 1. This process will be repeated for transects A2–A5 for a total area of 25 meters by 4.75 meters for a complete iteration of data collection at each site.

This data will then be split into six regions of plot size for transects A1–A5 (Figure 1). The sizes will be I 0.5 m<sup>2</sup>, II 1.0 m<sup>2</sup>, III 2.0 m<sup>2</sup>, IV 3.0 m<sup>2</sup>, V 4.0 m<sup>2</sup> and VI 4.75 m<sup>2</sup>. The species and population data will then be used to calculate richness and Shannon's and Simpson's biodiversity indices for each plot size I–VI. Plot size, richness, and the biodiversity indices will then be analyzed in R for correlation and trends between plot size and biodiversity to address **H1**, **H2**, and **H3**. Additionally, SACs will be created by plotting richness versus sample size for each 4.75 m by 4.75 m plot for transects A1–A5, and one will be created for the total 15 m by 4.75 m area to examine the species accumulation across the area. The work with R will allow a greater understanding of how sample size reflects biodiversity and whether there is a point in which biodiversity plateaus in a SAC. This collection will be completed twice per month at all four intertidal locations throughout Malibu for a period of a year to understand temporal trends to address **H4**. This temporal analysis will demonstrate whether the results vary seasonally.

### Anticipated Results and Preliminary Data:

Preliminary data was collected on a smaller scale within a 2.75-meter by 12-meter area of intertidal zone using the same methodology. Instead of six plot sizes, four plot sizes were used, measuring 0.5 m, 1.0 m, 2.0 m, and 3.0 m. Instead of 5 A transects (A1–A5), 4 A transects were used (A1–A4). The data was collected once at Latigo Beach, a non-marine protected area. Richness, Shannon's, and Simpson's diversity indices were calculated for each plot size for a total of 16 points. This data was inputted into R for correlation and trend analysis. According to the assumptions for correlation tests, the richness, Shannon's, and Simpson's raw data were checked for normalcy.

**Figure 2: Richness Shapiro-Wilk normality test**

```
data: spatial_intertidal$richness
W = 0.94931, p-value = 0.4789
```

Richness data is normal.

**Figure 3: Shannon's Shapiro-Wilk normality test**

```
data: spatial_intertidal$shannon
W = 0.91917, p-value = 0.1636
```

Shannon's data is normal.

**Figure 4: Simpson's Shapiro-Wilk normality test**

```
data: spatial_intertidal$simpson
W = 0.83861, p-value = 0.009303
```

Simpson's data is normal.

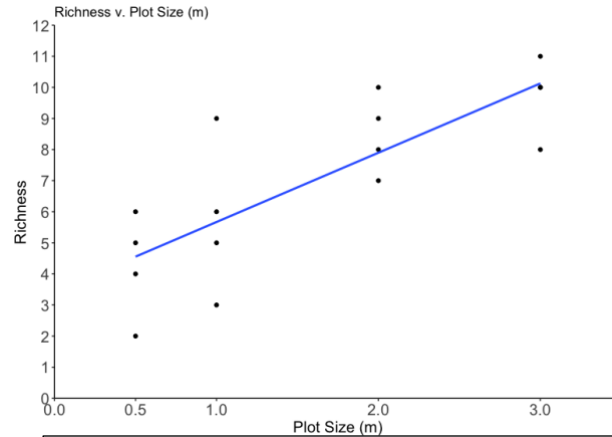
Initial tests showed that the data for richness and Shannon's index versus plot size are normally distributed (Figures 2, 3), while Simpson's index versus plot size is not (Figure 4). Since some of the data was not normal, the stricter Spearman's rank correlation test was run on all three categories of data which returned a richness rho of 0.817 and a p-value of 0.00011, meaning there is a significant positive correlation between plot size and richness. The Shannon's index rho was 0.182, and its p-value was 0.500 meaning there is not a significant positive correlation between plot size and Shannon's index. Finally, the Simpson's index rho was 0, and

**Figure 5: Richness  
Spearman's rank correlation  
rho**

data: spatial\_intertidal\$plot\_size\_m and  
spatial\_intertidal\$richness

$S = 124.22$ ,  $p\text{-value} = 0.0001107$

alternative hypothesis: true rho  
is not equal to 0  
sample estimates:  
rho  
0.8173163



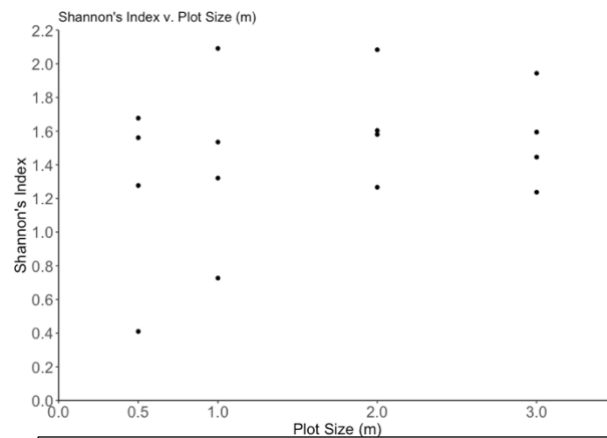
**Figure 6: Richness v. Plot Size (m) (above)** – This graph illustrates the positive linear correlation between richness versus plot size. ( $p\text{ value} = 0.0001$ ,  $\rho = 0.817$ )

**Figure 7: Shannon's  
Spearman's rank correlation  
rho**

data: spatial\_intertidal\$plot\_size\_m and  
spatial\_intertidal\$shannon

$S = 556.31$ ,  $p\text{-value} = 0.5002$

alternative hypothesis: true rho  
is not equal to 0  
sample estimates:  
rho  
0.1819017



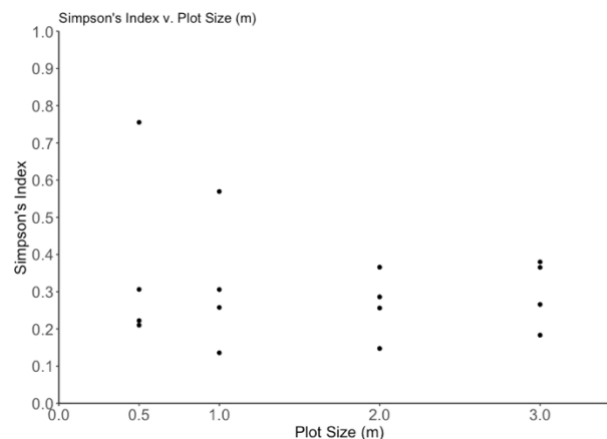
**Figure 8: Shannon's Index v. Plot Size (m) (above)** – This graph illustrates there is no significant correlation between the Shannon's index versus plot size. ( $p\text{ value} = 0.500$ ,  $\rho = 0.182$ )

**Figure 9: Simpson's  
Spearman's rank correlation  
rho**

data: spatial\_intertidal\$plot\_size\_m and  
spatial\_intertidal\$simpson

$S = 680$ ,  $p\text{-value} = 1$

alternative hypothesis: true rho  
is not equal to 0  
sample estimates:  
rho  
0



**Figure 10: Simpson's Index v. Plot Size (m) (above)** – This graph illustrates there is no significant correlation between the Simpson's index versus plot size. ( $p\text{ value} = 1$ ,  $\rho = 0$ )

its p-value was 1, meaning there is no significant correlation between plot size and Simpson's index.

This preliminary data shows that there is a significant positive correlation between increasing plot size and increasing richness (Figure 5). There is no significant correlation between increasing plot size and Shannon's index (Figure 7), and no significant correlation between increasing plot size and Simpson's index (Figure 9). The richness versus plot size data was then fit to a linear model (Figure 6). The visualizations of richness and biodiversity indices versus plot size show that as plot size increases, richness also increases. However, there does not appear to be a trend for the biodiversity indices (Figures 8, 10). Based on these results, it is anticipated that complete study results will also show a significant positive relationship between increasing plot size and increasing richness and no correlation between increasing plot size and Shannon's or Simpson's indices. However, further experimentation is needed to verify this as well as examine any SACs since there was not enough data with the preliminary analysis to complete this. Since this preliminary data was only taken at one site on one day, there is no data that reflects how biodiversity changes on a temporal scale. However, it is anticipated that biodiversity indices will not show temporal variance.

### **Limitations**

A notable limitation of this methodology is the difficulty in incorporating the lower intertidal into the sampled data. The intertidal range is generally larger than 5.0 meters, so the lower intertidal will not be well represented by the data. Additional spatial analyses that better incorporate the lower intertidal would be beneficial for determining whether the spatial representation of biodiversity depends on the intertidal zone. A preliminary understanding of **H3** can be obtained through the methodology described, but additional analyses should focus on **H3**.

Additionally, the shape and location of the plot sizes influence how the data will be represented in the smaller and larger sizes. Since the smaller sizes will be organized using data from lower in the intertidal, they may display a different level of biodiversity than if they were organized using data from a higher zone of the intertidal. Additional analyses and different plot sizes and shape organizations should be examined to determine the effects of this limitation.

### **Future Research**

Few studies discuss species accumulation trends in the intertidal. A greater understanding of this will allow for better ecological monitoring of this habitat. There is much more work to be carried out on this topic to determine whether species accumulation and biodiversity depend on geographical location and how it is affected by abiotic factors such as currents, wave action, and marine topography.

Another notable feature of these correlation graphs is that with increasing plot size, the variability of both Shannon's index and Simpson's index appears to decrease (Figures 8, 10). This trend makes sense because a larger plot size would provide more data to represent the biodiversity of an area accurately; however, it appears as though there may not be a significant difference in variation between a 2.0 m<sup>2</sup> and a 3.0 m<sup>2</sup> sample size, indicating that larger sample sizes may not have a significantly beneficial impact on the variation of data. However, more data is required to draw any conclusions about this phenomenon.

## References

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