Report_ExperimentalDesign&Reproducibility

Rita Eusébio

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Research Question

How do the invertebrate faunal communities of the coluvial MSS across Portugal vary seasonally (along one year) in terms of diversity and richness, and how are they affected by environmental conditions?

Independent variables

- Location

- Altitude

- Average temperature

- pH

- Conductivity

- Soil water content

- OC (Organic carbon)

- Nitrate

- Phosphate

- Ammonium

Dependent variables

- Richness (Species number)

- Abundance

Protocol

Faunal sampling

The sampling localities are situated in colluvial MSS areas (scree slopes) across Portugal: Serra da Estrela (40°17'24.7"N 7°35'20.4"W), Sicó (39°58'51.4"N 8°33'09.0"W), Alcobertas (39°25'46.2"N 8°54'59.5"W), Montejunto (39°10'11.3"N 9°04'12.6"W) and Arrábida (38°28'59.2"N 9°00'09.7"W).

The sampling localities were chosen within natural protected areas of continental Portugal.

Invertebrate specimens were collected using modified pitfall traps: 50 cm long PVC tube with 8 mm perforations (between 20 and 40 cm) and one baited trap, at the bottom of the tube, with propylene glycol as a preserving liquid and pork liver as bait. The traps were installed in the scree slopes by digging a narrow vertical hole, 60 cm deep, and placing the PVC tube 10 cm below the surface. Five traps were installed per site, 10 m away from each other and 0.5–1 m away from the surrounding trees/shrubs.

Specimens were collected every season (3 months) for a year (March 2022 – March 2023). Seasons were defined as such: spring (20th March - 20th June), summer (21st June - 22nd September), autumn (23rd September - 20th December), winter (21st December - 19th March).

Environmental variables

Sediment samples (250 g) were collected in each invertebrate sampling season, for each trap (soil was collected near each one of the traps, in all locations).

The following environmental variables were used to characterize each sampling locality:

1) altitude: data collected with a Garmin GPSMAP 64sc in each trap;

2) average temperature: recorded every 2 hours: in th MSS with one data logger installed at the bottom of each trap (TidbiT v2 Temp UTBI-001, Onset, MA, US), and at surface with one data logger installed at ground level near each trap, in a location where no tree or shrub would cast shade over it;

3) pH and conductivity: measured following standard procedures (Patriquin et al., 1993);

4) soil water content: measured by weight loss in percentage;

5) organic carbon (%): soil organic matter (SOM) was measured through loss on ignition (2 g of dried sediment placed in a crucible and ignited at 550◦C for 6 hours) and organic carbon as half of SOM value for each sampling site (Dean, 1974);

6) nitrate (NO3), phosphate (PO4) and ammonium (NH4) (µg/g sediment): measured using a FIAstar 5000 analyser unit with sediment samples being suspended in purified water.

Specimen sorting and identification

Specimens were sorted and identified to species level where possible using a Leica Wild M10 stereomicroscope and are deposited in the National Museum of Science & Natural History of Portugal.

Statistical analysis

All analyses have been performed in R software version 3.5.0.

In order to test significant differences between localities, for each environmental parameter we used a one-way ANOVA analysis alongside a Tukey's test. The five replicates of each locality were tested against the five replicates of each of the other localities, for every single parameter.

To assess the richest locality per season, we used the Shannon-Wiener diversity index for all orders. For each season we used the abundance of each species, while for each locality we summed the abundance values of all seasons for each species. We used this index to verify which locality and season within locality were the richest.

To test the correlation between environmental variables and total abundance, we used the Shapiro-Wilk test to verify if the data was normally distributed, and then either a Pearson correlation test for normally distributed samples or a Kendall correlation test for not normally distributed samples.

References

Dean, W.E., 1974. Determination of carbonate and organic matter in calcareous sediments and sedimentary rocks by loss on ignition; comparison with other methods. J. Sediment. Res. 44 (1), 242–248. https://doi.org/10.1306/74D729D2-2B21-11D7-8648000102C1865D

Patriquin, D.G., Blaikie, H., Patriquin, M.J., Yang, C., 1993. On-farm measurements of pH, electrical conductivity and nitrate in soil extracts for monitoring coupling and decoupling of nutrient cycles. Biol. Agric. Hortic. 9 (3), 231–272. https://doi.org/10.1080/01448765.1993.9754638.

Calculating the estimated sample size required for my protocol

```
library(pwr)
```

#Considering that I do not have a control group as my experiment is about diversity and abundance of a never studied before habitat, I feel that I cannot accuratelly do these calculations. I do not have an idea of the sample sizes I a going to collect neither about the variation between replicates.

#Still, I will make up some values ir order to do this calculation

```
delta<-5
sigma<-50
d <-delta/sigma

pwr.t.test(d=d, sig.level=0.05, power=0.80, type=c("two.sample"))

##
##      Two-sample t test power calculation
##
##               n = 1570.733
##               d = 0.1
##       sig.level = 0.05
##           power = 0.8
##     alternative = two.sided
##
## NOTE: n is number in *each* group
```

#According to these values, I will need approximatelly 1571 individuals per group, which seems reasonable if I consider the group each trap, which collects invrtebrates for 3 months at a time.