
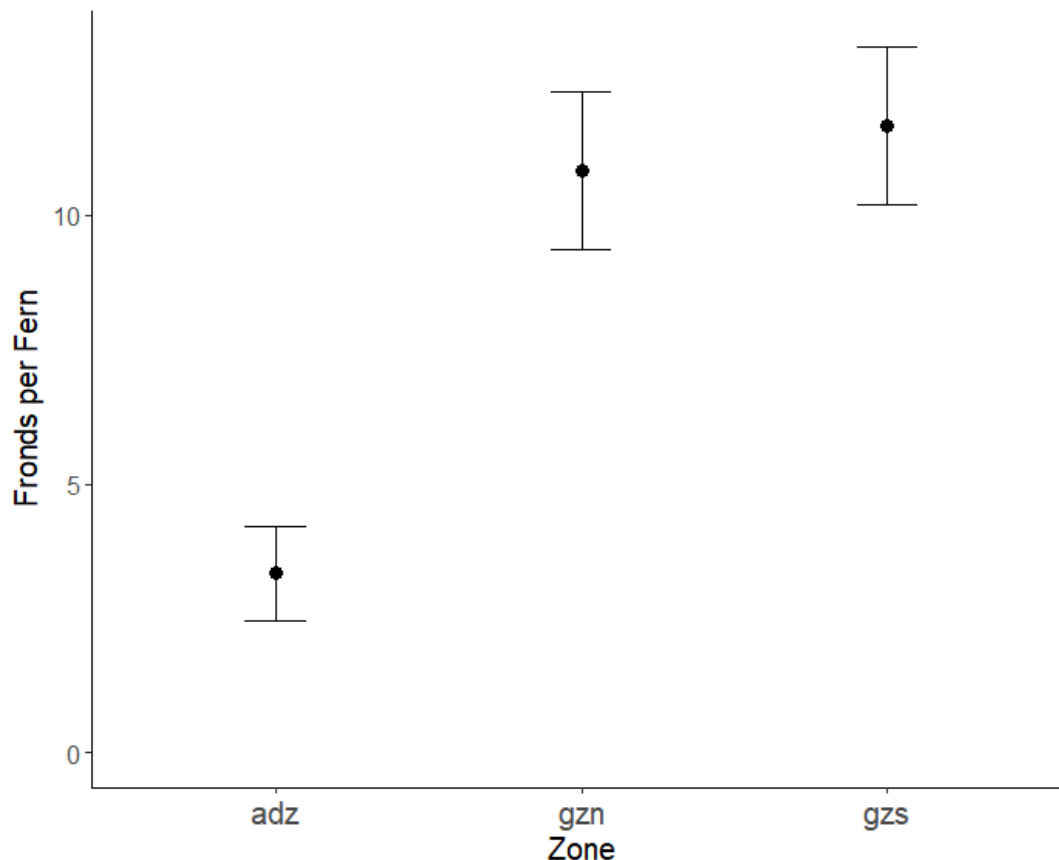


From: **Paul Shannon via groups.io** pshannon=systemsbiology.org@groups.io   
Subject: [swordFernDieOff] 5-year sword fern survival experiment: 26 months along, strong results  
Date: April 24, 2020 at 5:07 PM  
To: swordferndieoff@groups.io  
Cc: Paul Shannon pshannon@systemsbiology.org

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## 2020 Fiddlehead counts at Ground Zero and in the Active Die-off Zone



In February 2018, Suzanne Bouchard and I planted 3 lines of 12 nursery ferns:

**gzn:** Ground Zero, northern line  
**gzs:** Ground Zero, southern line  
**adz:** at the edge of the northward spreading active die-off zone

In my experiment proposal to Seattle Parks, I predicted that after five years adz ferns would be generally in ill-health (or dead), and that the gz ferns would be generally healthy.

I watered all 36 ferns weekly - 2 liters per plant - through 2 summers, to maximize chances of survival.

We assess fern mortality and morbidity by eye, counting new emerging fronds (fiddleheads) on April 20, 2020:

plant	notes	mean fiddleheads per
adz:	0 2* 0 8 0 0 7 5 2 7 4 5	3.3
mortality concentrated to north, where areal die-off is ongoing		
gzn:	10 16 5 1* 16 12 8 6 16 16 10 14	10.8
gzs:	16 21 12 5 18 12 8 8 12 15 5 8	11.7

\*adz fern #2 is barely surviving, has only a few very small fronds from last year; the 2 new fronds, now unfurled, are also very small.  
 \*gzn fern #4 has been in poor health for two years, the only one of the 24 gz ferns in obvious decline

Dylan Mendenhall did a more rigorous analysis of the data than I did: see his summary below.

Our analyses are consistent with, but of course do not prove that the agent/s of sword fern die-off are no longer active at Ground Zero (die-off was 2014), and have not been active for at least two years. The agent/s may return. We can similarly infer (but not prove) that the agent/s are active at the expanding edge of the die-off. Note that the 12 adz ferns were planted just 20 feet **beyond** the 2018 northern boundary of the die-off. I anticipated further northward spread to engulf this planting.

Simple t.tests suggest that adz and gzn+gzs counts come from different distributions - that is, that the different counts did not occur by chance variation. With similar ferns planted, and identical treatment attempted, it appears that different processes are at work in the two sites.

```
t.test(gzn, gzs)$p.value 0.69
t.test(gzn, adz)$p.value 0.00035
t.test(gzs, adz)$p.value 0.00013
t.test(c(gzn, gzs), adz)$p.value 1.5e-06
```

I obtained similar results last year, and learned that fiddlehead emergence (n=1 year) is complete by mid-April.

From Dylan Mendenhall:

I tested the data for normality and equal variance. It passed both tests so it meets the basic assumptions of parametric tests. For this kind of pairwise comparison, the Tukey test is most appropriate. The p values are in the right column:

Tukey multiple comparisons of means

```

95% family-wise confidence level
factor levels have been ordered

Fit: aov(formula = fiddleheads ~ zone, data = fid)

$zone
      diff      lwr      upr      p adj
gzn-adz  7.5000000  2.982826 12.017174 0.0007774
gzs-adz  8.3333333  3.816159 12.850508 0.0002133
gzs-gzn  0.8333333 -3.683841  5.350508 0.8935606

```

As you can see, the results agree with your t-tests. Because the data are normal, most people present the information as means +/- SE, so that's what I would share with the working group, whether as a graph or in text. Personally I think boxplots are more appropriate for small samples like this. Anyhow both graphs are attached.

For your question about presenting the results, I would state:

- This was a small sample size (n=12).
- All ferns were watered in the same manner.
- We assumed that each fern is an independent observation.
- We tested for normality and equal variance of the data.

If you want to be more formal you could say:

- Using one-way ANOVA, we found significant differences between the 3 zones ( $p < 0.001$ ,  $F(2,33) = 12.43$ ). We then applied a multiple-comparisons Tukey HSD test. Mean fronds per fern were similar between the Ground Zero North zone ( $10.8 \pm 1.5$  SE) and Ground Zero South zone ( $11.7 \pm 1.5$  SE). However, there were significantly fewer fronds in the Active Die-off Zone ( $3.3 \pm 0.9$  SE) compared to both the Ground Zero North zone ( $p < 0.001$ ) and the Ground Zero South zone ( $p < 0.001$ ).

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